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Susceptibility of Murine Norovirus and Hepatitis A Virus to Electron Beam Irradiation in Oysters and Quantifying the Reduction in Potential Infection Risks

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Consumption of raw oysters is an exposure route for human norovirus (NoV) and hepatitis A virus (HAV). Therefore, efficient postharvest oyster treatment technology is needed to reduce public health risks. This study evaluated the inactivation of HAV and the NoV research surrogate, murine norovirus-1 (MNV-1), in oysters (*Crassostrea virginica*) by electron beam (E-beam) irradiation. The reduction of potential infection risks was quantified for E-beam irradiation technology employed on raw oysters at various virus contamination levels. The E-beam dose required to reduce the MNV and HAV titer by 90% (D_{10} value) in whole oysters was 4.05 (standard deviations [SD], ±0.63) and 4.83 (SD, ±0.08) kGy, respectively. Microbial risk assessment suggests that if a typical serving of 12 raw oysters was contaminated with 10^5 PFU, a 5-kGy treatment would achieve a 12% reduction (from 4.49 out of 10 persons to 3.95 out of 10 persons) in NoV infection and a 16% reduction (from 9.21 out of 10 persons to 7.76 out of 10 persons) in HAV infections. If the serving size contained only 10^2 PFU of viruses, a 5-kGy treatment would achieve a 26% reduction (2.74 out of 10 persons to 2.03 out of 10 persons) of NoV and 91% reduction (2.1 out of 10 persons to 1.93 out of 100 persons) of HAV infection risks. This study shows that although E-beam processing cannot completely eliminate the risk of viral illness, infection risks can be reduced.

Currently, human noroviruses (NoV) and hepatitis A virus (HAV) are considered the principal viral pathogen threats to shellfish consumers. It is estimated that noroviruses are responsible for more than half of all reported outbreaks of gastroenteritis (1). Although normally associated with self-limiting gastroenteritis, NoV accounts for 25% of hospitalizations and 11% of deaths from food-borne illnesses in the United States (2). According to Scharff (3), the estimated cost of NoV illnesses to the U.S. economy is $5.8 billion per year. Due to recent vaccination campaigns and improved hygienic standards, HAV is less common in the United States and Europe. However, HAV remains a major public health threat around the world. Bivalves, such as oysters, filter large volumes of water and bioaccumulate NoV and HAV, as well as a variety of bacterial pathogens (4–8). The U.S. oyster production is approximately 27 million pounds per annum, 70% of which is consumed raw (9, 10). The prevalence of NoV contamination of U.S. market oysters has been estimated to be 3.9% (4) and greater than 70% for market oysters in the United Kingdom (11). The precise reasons for this disparity in NoV occurrence are presently unknown. Although there have only been two known HAV outbreaks associated with U.S. oysters in the last 20 years, a recent study found that approximately 4.4% of U.S. market oysters tested positive for HAV (4).

A variety of bacterial pathogen intervention technologies for oysters, such as depuration, relaying, flash pasteurization, high-pressure processing, individual quick freezing with extended frozen storage, flash freezing, and ionizing irradiation, have been recommended (12, 13). The utility of many of these approaches against viral pathogens in the context of reducing infection risks is still relatively limited or unknown (14). The U.S. FDA has approved the use of ionizing radiation of up to a maximum of 5.5 kGy as a pathogen intervention strategy to control naturally occurring *Vibrio vulnificus* in shellfish (15). Currently, only a small percentage of the commercial oysters sold in the United States are being processed by ionizing radiation, such as cobalt-60 (personal communication, Food Technology Services, Inc., Mulberry, FL [16]). Ionizing radiation can be generated using either radioactive isotopes (cobalt-60 or cesium-137) or linear accelerators to generate electron beams (E-beam). Cobalt-60 and cesium-137 generate gamma rays (photons), while E-beam is made up of a beam of high-energy electrons. Although the basic mechanism by which all types of ionizing radiation inactivate microorganisms is thought to be the same, i.e., DNA strand breakage, there are fundamental differences between E-beam and gamma irradiation with respect to energy and dose rate when used for commercial food processing. For example, the energy of gamma rays in cobalt-60 is approximately 1 MeV, while for E-beam-associated electrons it is usually around 10 MeV. The dose rate of gamma rays from cobalt-60 is often in the range of hundreds of grays per minute, while in the case of E-beam the dose rate is in the range of tens of millions of grays per minute (17). Thus, during commercial E-beam irradiation, microbial pathogens experience ionizing radiation at significantly higher dose rate (usually in seconds) conditions than pathogens experiencing commercial cobalt-60 irradiation.

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To the best of our knowledge, there are no published reports addressing the use of E-beam to reduce or eliminate viral pathogens, such as NoV and HAV, in whole oysters. Additionally, there is no information on the reduction in viral infection risks that can be expected when a pathogen-kill step, such as E-beam radiation, is used for raw oysters. The aims of this study were 2-fold. The first aim was to determine the sensitivity of the widely used NoV surrogate, murine norovirus (MNV-1), and a tissue culture-adapted HAV strain to E-beam irradiation in whole oysters. The second aim was to quantify the reduction in potential infection risks that would be achievable if raw oysters contaminated with different levels of virus were irradiated at various E-beam doses approved by the FDA. The underlying hypothesis was that NoV and HAV in oysters are susceptible to E-beam irradiation, and that defined reductions in health risks are achievable with the use of E-beam irradiation.

MATERIALS AND METHODS

Murine norovirus and hepatitis A virus. Murine norovirus (MNV-1) was kindly provided by Herbert W. Virgin IV, Washington University, St. Louis, MO. The virus was propagated in RAW 264.7 (ATCC, Manassas, VA) cells cultured in Dulbecco's minimum essential medium (DMEM) (Mediatech, Manassas, VA) containing 10% fetal bovine serum (FBS; Atlanta Biologicals, Atlanta, GA), 1% HEPES buffer, 1% penicillin-streptomycin (Mediatech). The MNV stocks were prepared and stored as previously described (18). The HAV strain obtained from the American Type Culture Collection (Manassas, VA) was strain VR-1402, a cell culture-adapted cytopathic clone of strain HM-175/18f. The virus was propagated in fetal rhesus monkey kidney (FRhK-4) cells in DMEM supplemented with 10% fetal bovine serum (Invitrogen Corp., Carlsbad, CA). The HAV stocks were prepared and stored as previously described (13).

Virus-contaminated oysters and oyster meat homogenates. The sensitivity of the two viruses to E-beam irradiation was studied in whole oysters and in oyster meat homogenates. Whole clutchless Eastern oysters (*Crassostrea virginica*) were obtained from the Auburn University Aquaculture Facility in Dauphin Island, AL, or local Maryland distributors. Oyster meat homogenates were prepared by blending the meat from around 30 medium-sized oysters in a laboratory blender (model 31BL91; Waring, New Hartford, CT) for 3 min at maximum speed. One-milliliter aliquots of HAV (8.5×10^6 PFU/ml) and MNV (7.9×10^6 PFU/ml) were mixed into the 10-ml oyster meat homogenate preparation. Live oysters were permitted to accumulate HAV and MNV under simulated natural conditions in an accumulation tank at the U.S. FDA Gulf Coast Seafood Laboratory, Dauphin Island, AL, as described previously (13, 18). The oysters were maintained for more than 3 weeks prior to being transferred to a flume which utilized single-pass UV-treated natural seawater (with salinity ranging between 5 to 20 ppt). To determine the sensitivity of MNV in the absence of an oyster matrix, MNV (7.9×10^6 PFU/ml) was also suspended in 10 ml phosphate-buffered saline (PBS) and used in the irradiation experiments. The oyster meat homogenates, the PBS-suspended viruses, and the virus-spiked whole oysters were placed on blue ice and shipped to Texas A&M University for the E-beam irradiation trials. The samples were shipped in containers that met the International Air Transport Association (IATA) Dangerous Goods Shipping Regulations (STP 100; SAF-T-PAK, Alberta, Canada).

Packaging samples for E-beam irradiation. In order to comply with the university biosafety regulations, all virus-spiked samples were placed in heat-sealed double-bagged Whirl Pak bags (Nasco, New York, NY). These heat-sealed bags were then placed inside specimen transport bags that were rated up to 95 kPa (Thermosafe, Arlington Heights, IL). Triplicate packages of such sealed bags of virus-spiked PBS (10 ml) and oyster homogenates (10 ml) were prepared. For whole oysters, similar heat-sealed bags containing three virus-spiked whole oysters each were also prepared for the E-beam irradiation trials.

E-beam irradiation and dosimetry. Experimental trials were performed to ensure that the samples (live oysters, homogenate, or PBS) were appropriately packaged and that they received uniform E-beam doses. These dose-mapping trials included dose measurements with live oysters. These trials were performed to ensure that the packages containing PBS, oyster meat homogenate, or whole oysters could be irradiated effectively with a dose uniformity ratio (DUR) of ~1.0. The DUR is an important criterion when performing irradiation experiments. A DUR of ~1.0 signifies that the dose is uniform within the package. Irradiation experiments have to minimize dose variation within the experimental bags. Dose delivery trials were performed to determine the appropriate conveyor speed and other specifications to achieve the target doses.

A 10-MeV E-beam linear accelerator was used for delivering E-beam doses. Defined doses were delivered by conveying the samples across the incident E-beam using commercial-scale computer processor-controlled conveyor system. Dosimeters (L-α-alanine pellet dosimeters; Harwell Dosimeters, Oxfordshire, United Kingdom) were placed at various positions on the packages to verify the delivered E-beam dose. As part of dose-mapping experiments, dosimeters (in water-proof pouches) were placed within the meat inside the oyster shell, except in cases where the samples contained live viruses. The alanine dosimetry system that was employed was traceable to international standards. The dosimeters were measured using the Bruker E-scan spectrometer (Bruker, Billerica, MA) to measure the delivered irradiation dose. Although the chosen target doses were 0.5, 1.5, 3.5, 4.5, and 5.5 kGy, the actual measured doses were used for data plotting and analysis. In order to determine the E-beam dose required for complete inactivation of HAV and MNV, irradiation trials were performed using higher doses, such as 5, 10, 20, and 30 kGy, with oyster homogenate spiked with HAV (1.02×10^6 PFU/ml) and MNV (4.07×10^6 PFU/ml). Nonirradiated samples (0 kGy) were used as controls for both HAV and MNV. The irradiated as well as nonirradiated samples were shipped under blue-ice conditions overnight back to the USDA-ARS laboratory in Dover, DE, for virus extraction and assays. The irradiation of samples and shipment to the USDA-ARS laboratory were completed within 24 h of receiving the samples for irradiation.

Virus extraction and enumeration. Three whole irradiated virus-contaminated oysters from the sample bags were shucked, and the contents of the oysters were pooled and placed in 50-ml conical tubes. The tubes were briefly centrifuged in a tabletop centrifuge to facilitate separation of oyster meat from oyster liquor. Virus extractions were performed as described by Calci et al. (18). Briefly, the oyster meat was placed in 200 ml of phosphate buffer (0.15 M Na_3PO_4, pH 9.5) and homogenized using a laboratory blender (Waring Inc., New Hartford, CT) for 3 min at the maximum speed setting. The homogenates were pelleted for 15 min at 15,000 × *g*, and the supernatant was retained and neutralized with 2N HCl. Tenfold serial dilutions of neutralized supernatant were made in Earle's balanced salt solution (EBSS) (Gibco-Invitrogen, Grand Island, NY). For oyster homogenates, 10 ml was mixed with 25 ml of phosphate buffer, followed by centrifugation, neutralization of the supernatant, and dilution in EBSS as described above.

HAV assays were performed using 2 ml of extract or 2 ml of 10-fold serial dilutions made in EBSS. Plaque assays were performed in triplicate using FRhK-4 cells as described by Richards and Watson (14). For MNV, plaque assays were performed in triplicate using confluent monolayers of RAW cells (19). The MNV plaque assay was essentially as described by Kingsley et al. (13), assaying 0.5 ml of extract or 0.5 ml of 10-fold serial dilutions. Due to oyster debris in the oyster homogenates, the plates were washed with 2 ml of EBSS after inoculation and incubation for 2 h. For homogenate dilutions of 1:100 or greater, 0.5 ml was used to infect individual wells of a 6-well dish and the washing step was omitted. The virus plaque assays were recorded as PFU per ml. In the case of MNV in PBS, samples were directly diluted in EBSS and used for plaque assay.

Quantitative microbial risk assessment. We estimated the infection risks that would arise from exposure to HAV-contaminated oysters and human norovirus-contaminated oysters. For these risk calculations, we

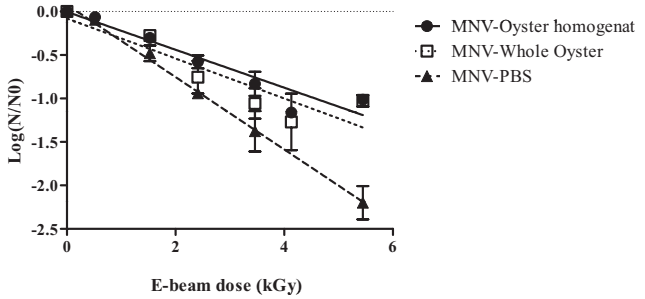

FIG 1 Inactivation of murine norovirus (MNV-1) under various 10-MeV E-beam doses when present in whole oysters, oyster homogenate, and phosphate-buffered saline. The apparent filled squares are those data points where the open squares overlap the solid circles.

assumed that the inactivation of human norovirus under E-beam irradiation was equal to that of MNV. The reductions of MNV were used as the basis for calculating the reduction of risks associated with human noroviruses. A standard U.S. serving size of 12 oysters, with each oyster containing approximately 13.68 g of oyster meat, was assumed (20). Reduction in infection risks associated with various levels of virus contamination loads and various doses of E-beam irradiation were estimated. Additionally, the infection risks associated with smaller serving sizes of only 6 and 3 oysters were calculated to determine whether infection risks could be reduced by a combination of E-beam processing and smaller serving sizes. The initial HAV and NoV virus loads were assumed to be 1, 10, 100, and 1,000 PFU/g. The infection risks were estimated using the beta-Poisson model, $P_i = 1 - (1 + N/\beta)^{-\alpha}$, where P_i is the probability of infection and N is the number of viruses ingested. α and β are parameters reflecting the dose-response curve for NoV and HAV. For NoV, α (0.04) and β (0.055) were based on the dose-response curve for human norovirus (21). For HAV, α (0.374) and β (186.69) were based on the dose-response curve published by Pinto et al. (22). We assumed that all viruses in the oysters were infectious and that all of the exposed individuals were susceptible to infection.

Data analysis. For MNV, three independent E-beam irradiation trials (with three replicates each) were performed for the whole-oyster and oyster homogenate samples, and two independent irradiation trials were done for PBS samples. In the case of HAV, two independent trials (with three replicates each) for whole oysters and oyster homogenates were performed. In order to determine complete inactivation of viruses by higher E-beam dose, three separate irradiation trials (with three replicates each) were conducted for oyster homogenate spiked with HAV. In the case of oyster homogenate spiked with MNV, two independent irradiation trials (with three replicates each) were conducted with higher doses of E-beam. The inactivation of the viruses by E-beam irradiation was assumed to be linear (23). The D_{10} value represents the dose that achieves a 90% (1-log) reduction of the target virus. The surviving HAV and MNV concentrations (log PFU/ml) were plotted as a function of the measured E-beam dose (kGy). Linear regression analysis was performed, and the negative reciprocal of the slope was calculated to be the D_{10} value. The Student's *t* test was performed to determine whether there was any statistically significant difference between the D_{10} values of the two viruses in the different matrices.

RESULTS

Inactivation of MNV in oyster homogenate and whole oysters. To evaluate MNV inactivation by E-beam, oyster homogenates were seeded with MNV and live oysters were contaminated under simulated natural bioaccumulation by placing them in MNV-contaminated seawater. The reduction of human norovirus surrogate, MNV spiked in PBS, oyster homogenate, and whole oysters when exposed to various doses of E-beam irradiation is shown in Fig. 1. The inactivation of MNV in PBS was greater than that in oyster homogenate and whole oysters. The dose required for achieving 90% reduction (D_{10} value) for MNV in PBS, oyster homogenate, and whole oysters was calculated to be 2.55 (± 0.42), 4.97 (± 0.65), and 4.05 (± 0.63) kGy, respectively (Table 1). The inactivation of MNV was significantly greater in PBS than in whole oysters ($P = 0.0373$) or the oyster meat homogenate ($P < 0.0001$). There was no statistically significant difference in MNV inactivation irrespective of whether the virus was present in the oyster homogenate or in whole oysters. At the maximum delivered dose of 5.45 kGy, MNV was reduced by approximately 90% in whole oysters.

TABLE 1 D_{10} values for MNV-1 and hepatitis A virus in PBS, oyster meat homogenate, and whole oysters when exposed to 10-MeV E-beam irradiation

Matrix	D_{10} value[a] (kGy)	
	MNV-1	HAV (VR-1402)
Phosphate-buffered saline	2.55 ± 0.42[A]	ND
Oyster homogenate	4.97 ± 0.65[B]	5.74 ± 0.86[B]
Whole oysters	4.05 ± 0.63[B]	4.83 ± 0.03[B]

[a] Values are means ± standard deviations. D_{10} values with different letters indicate statistically significant ($P \leq 0.05$) differences. ND, not determined.

Inactivation of HAV in oyster homogenate and whole oysters. Figure 2 shows the inactivation of HAV in oyster homogenate and whole oysters when exposed to various doses of E-beam irradiation. E-beam irradiation inactivates the virus in whole oysters as well as in the oyster homogenate. However, the dose required for achieving a 90% reduction (D_{10} value) of HAV in the oyster homogenate and whole oysters was calculated to be 5.74 (± 0.86) kGy in the homogenate and 4.83 (± 0.08) kGy in whole oysters (Table 1). There was no significant difference ($P = 0.142$) between the D_{10} values of HAV in the oyster homogenate and that in whole oysters (Table 1). At the maximum dose (~5.5 kGy) that was applied, approximately 94% of the original virus titer was inactivated within whole oysters.

Table 2 shows the results from the experiments conducted to determine the E-beam dose required for complete inactivation of viruses in the oyster meat matrix. Separate oyster meat homogenates were spiked with MNV and HAV and subjected to higher E-beam doses. A dose of ~23 kGy resulted in 4.2- and 4.5-log reductions of MNV and HAV, respectively. Delivery of

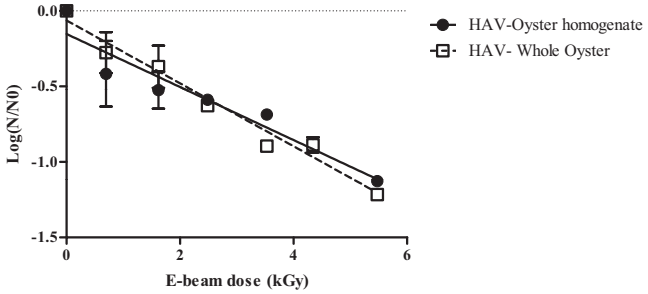

FIG 2 Inactivation of hepatitis A virus (ATCC VR-1422) under various 10 MeV E-beam doses when present in whole oysters and in oyster homogenate. The apparent filled squares are those data points where the open squares overlap the solid circles.

TABLE 2 Inactivation of MNV-1 and hepatitis A virus (VR-1402) in oyster meat homogenate exposed to various high doses of 10-MeV E-beam irradiation

Inactivation of:			
MNV-1		HAV (VR-1402)	
E-beam dose (kGy)	Log PFU/ml[a]	E-beam dose (kGy)	Log PFU/ml[b]
0.00	4.898	0.00	4.26
4.90	3.570	4.85	3.39
9.38	2.362	10.06	2.31
23.64	0.362	23.20	0.04
31.90	BD	32.17	BD

[a] Values refers to mean log PFU/ml from 6 replicates. BD, below detection limit of plaque assay for MNV-1 (23 PFU/ml) and HAV (6 PFU/ml).
[b] Values refers to mean log PFU/ml from 9 replicate trials.

a higher dose of ~32 kGy reduced viruses to below detectable levels (Table 2).

Reduction in infection risks achievable with E-beam irradiation. Table 3 represents the reductions in HAV-associated infection risks if E-beam irradiation is employed on whole oysters contaminated at various virus levels (10^2, 10^3, 10^4, and 10^5 PFU). As stated earlier, an average serving size of 12 oysters with 13.68 g of meat/oyster was used in these calculations (20). At various doses of E-beam irradiation, ranging from approximately 1.5 to 5.5 kGy, there is a corresponding reduction in infection risks. If a serving size (12 oysters at 13.68 g meat/oyster) had a total contamination level of 10^5 PFU, 9.21 out of 10 susceptible persons would become ill if the oysters were not treated with E-beam irradiation. If these oysters were E-beam irradiated at 5.5 kGy, the infection risks would be reduced by approximately 16% (from 9.21×10^{-1} to 7.76×10^{-1}). If the oysters had an initial contamination level of approximately 10^4 PFU, the use of 5.5 kGy of E-beam irradiation would reduce the risk by approximately 39% (from 8.13×10^{-1} to 4.99×10^{-1}). If the initial virus contamination in oysters is limited to 10^3 PFU, employing an E-beam dose of 5.5 kGy would theoretically achieve a health risk reduction of 74% (from 5.74×10^{-1} to 1.48×10^{-1}). At the maximum FDA-approved dose of 5.5 kGy and a total virus load of 10^2 PFU in a serving, the reduction of HAV would be approximately 91% (from 2.10×10^{-1} to 1.93×10^{-2}) (Table 3). The only logarithmic reduction of infection risk occurs when the oysters harbor viruses at low levels (~ 10^2 PFU) and the oysters are exposed to doses of ≥2.5 kGy (Table 3).

TABLE 3 Possible infection risks associated with hepatitis A virus-contaminated oysters after treatment with various E-beam doses

E-beam dose[a] (kGy) (% HAV reduction)	Infection risk[b] for consuming oysters[c] with various levels of HAV contamination			
	10^5 PFU	10^4 PFU	10^3 PFU	10^2 PFU
0 (0)	9.21×10^{-1}	8.13×10^{-1}	5.74×10^{-1}	2.10×10^{-1}
0.8 (44.5)	9.01×10^{-1}	7.68×10^{-1}	4.85×10^{-1}	1.38×10^{-1}
1.55 (55.1)	8.93×10^{-1}	7.50×10^{-1}	4.50×10^{-1}	1.17×10^{-1}
2.49 (76.4)	8.64×10^{-1}	6.84×10^{-1}	3.43×10^{-1}	6.81×10^{-2}
3.50 (87.3)	8.29×10^{-1}	6.08×10^{-1}	2.45×10^{-1}	3.89×10^{-2}
4.34 (86.9)	8.31×10^{-1}	6.11×10^{-1}	2.49×10^{-1}	3.99×10^{-2}
5.54 (93.9)	7.76×10^{-1}	4.99×10^{-1}	1.48×10^{-1}	1.93×10^{-2}

[a] Virus reduction at specified E-beam doses.
[b] Probability of infection.
[c] Serving size of 12 oysters containing 13.68 g meat per oyster.

TABLE 4 Possible infection risks associated with human norovirus-contaminated oysters after treatment with various E-beam doses

E-beam dose[a] (kGy) (% MNV reduction)	Infection risk[b] for consuming oysters[c] with various levels of NoV contamination			
	10^5 PFU	10^4 PFU	10^3 PFU	10^2 PFU
0 (0)	4.49×10^{-1}	3.96×10^{-1}	3.38×10^{-1}	2.74×10^{-1}
1.44 (46.8)	4.35×10^{-1}	3.81×10^{-1}	3.21×10^{-1}	2.55×10^{-1}
2.31 (78.5)	4.14×10^{-1}	3.58×10^{-1}	2.96×10^{-1}	2.28×10^{-1}
3.23 (89.7)	3.97×10^{-1}	3.39×10^{-1}	2.75×10^{-1}	2.05×10^{-1}
4.13 (91.3)	3.93×10^{-1}	3.34×10^{-1}	2.70×10^{-1}	1.99×10^{-1}
5.09 (90.4)	3.95×10^{-1}	3.37×10^{-1}	2.73×10^{-1}	2.03×10^{-1}

[a] Virus reduction at specified E-beam doses.
[b] Probability of infection.
[c] Serving size of 12 oysters containing 13.68 g meat per oyster.

Based on the assumption that MNV and NoV have similar sensitivities to E-beam treatments, Table 4 shows the reduction of infection risks that can be expected when NoV-contaminated whole oysters are treated with E-beam irradiation. At the maximum dose (5.0 kGy) that was tested, the MNV titers were reduced by about 90% (Table 4). However, there was no significant (i.e., logarithmic) reduction of potential NoV infection risks at any of the E-beam doses. The infection risks are reduced by approximately 12%, from 4.49×10^{-1} to 3.95×10^{-1} (if the initial virus titer per serving size was 10^5 PFU) and by approximately 26% (from 2.74×10^{-1} to 2.03×10^{-1}) if the initial virus titer per serving size was 10^2 PFU. We modeled the influence of reduced oyster serving size to further evaluate the effect E-beam irradiation would have on potential public health risks (Table 5). Using hypothetical serving sizes of 3 and 6 whole oysters in these calculations, it was evident that even with a reduced serving size, there would be no significant reduction (i.e., log reduction) in human norovirus infection risks (Table 5).

DISCUSSION

The focus of this study was to understand the inactivation kinetics of HAV and MNV-1 under E-beam irradiation and its potential application as a viral pathogen intervention technology for oysters. Since assessment of inactivation of human norovirus is not currently feasible without the use of human subjects, MNV-1 was used as an NoV surrogate (13, 24). A key secondary objective of

TABLE 5 Comparison of infection risks associated with consuming human norovirus-contaminated whole oysters of various serving sizes with and without E-beam irradiation

E-beam dose and serving size (no. of oysters)	Infection risk[a] for consuming oysters with various levels of NoV contamination			
	10^5 PFU	10^4 PFU	10^3 PFU	10^2 PFU
No irradiation				
12	4.49×10^{-1}	3.96×10^{-1}	3.38×10^{-1}	2.74×10^{-1}
6	4.34×10^{-1}	3.79×10^{-1}	3.19×10^{-1}	2.53×10^{-1}
3	4.18×10^{-1}	3.62×10^{-1}	3.00×10^{-1}	2.33×10^{-1}
5.09 kGy (90.4% NoV reduction)				
12	3.95×10^{-1}	3.37×10^{-1}	2.73×10^{-1}	2.03×10^{-1}
6	3.78×10^{-1}	3.18×10^{-1}	2.52×10^{-1}	1.80×10^{-1}
3	3.61×10^{-1}	2.99×10^{-1}	2.31×10^{-1}	1.58×10^{-1}

[a] Probability of infection.

this study was to determine the reduction in potential public health risks that could be realized with viral inactivation under E-beam irradiation conditions. To make this study relevant to the commercial oyster industry and to public health needs, E-beam treatments up to the currently approved maximum FDA-allowable doses were employed for both whole oysters and oyster homogenate. However, to determine the dose required for complete inactivation of the viruses in the oyster meat, higher E-beam doses were utilized.

Gamma radiation as a sanitization treatment for shellfish has been studied by different research groups (12, 16, 25–28). A variety of bacterial pathogens are susceptible to ionizing radiation, such as *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, with D_{10} values as low as 0.1 kGy (27, 29). Inactivation studies on *Salmonella* spp. with live oysters showed a relatively low D_{10} value of 0.45 to 0.55 kGy (16). Harewood et al. (12) reported that virus inactivation required a higher dose of irradiation than bacterial pathogens. A D_{10} value of 13.5 kGy was reported for F coliphages (12). Mallet et al. (27), when employing gamma irradiation, reported no significant difference in the inactivation of viral pathogens, reporting D_{10} values of 2.02, 3.1, and 2.4 kGy for HAV, poliovirus I, and rotavirus SA11, respectively. As stated earlier, we are not aware of any published studies on enteric viruses in whole oysters treated with E-beam irradiation.

This study demonstrates that E-beam irradiation is capable of inactivating HAV and MNV in whole oysters (Fig. 1 and 2 and Table 2). However, at the FDA-approved maximum dose of 5.5 kGy, the reduction of HAV and MNV in whole oysters does not exceed 94 and 90%, respectively. The matrix in which the virus is present does have an impact on MNV sensitivity to E-beam irradiation. The D_{10} value of MNV was significantly lower ($P < 0.05$) when present in PBS than when present in oyster meat homogenate or whole oysters (Table 1). This indicates that the oyster meat reduces the effectiveness of E-beam irradiation. A key mechanism of microbial inactivation by ionizing radiation involves reactive oxygen species which damage the nucleic acids (17). The presence of organic materials in the irradiation matrix is known to reduce irradiation effectiveness, as they scavenge the reactive species produced during treatment (30, 31). Reduction of water activity, which lowers the radiolytic splitting of water molecules, has also been reported to be responsible for attenuation of radiation effects (28). We do not know whether MNV or HAV accumulate in hemocytes, similar to human NoV, which is known to accumulate in hemocytes. The location of the enteric viruses within the oyster's tissue could have an influence on their sensitivity to ionizing radiation if it is protected from the reactive oxygen species. It has been reported that MNV shows differential inactivation depending on whether it is present on cabbage or strawberries (32). However, using poliovirus and rotavirus, we have recently shown that while there are inactivation differences on fresh produce, such as lettuce and spinach, these observed differences were not statistically significant (23). This suggests that factors other than matrix play a role in the inactivation of viruses during ionizing irradiation.

Assuming that the response observed with MNV-1 to E-beam is the best-case scenario of how NoV will respond to E-beam irradiation, these results suggest that virus infection risks arising from shellfish cannot be eliminated with E-beam processing at the current U.S. FDA-approved level of 5.5 kGy. The combined inherent resistance of enteric viruses to ionizing irradiation and the highly infectious nature of NoV indicate that E-beam would only be effective if the oysters had low initial levels of virus contamination (Table 4). The actual level of virus contamination required for oyster-borne virus transmission is unclear. Doré et al. (33) excised and tested oyster digestive tissue and found that oysters associated with NoV outbreaks contained ≥1,000 NoV genomic units/g. Also, Lowther et al. (34) and Le Guyader et al. (35) have reported two large outbreaks that were associated with NoV levels of >8,000 viral genome copies per g digestive tissue. Using human volunteers, Leon et al. (36) have shown that a total dose (injected into oysters) of 1×10^4 genome equivalent copies was capable of eliciting norovirus infection.

Overall, these studies show that the use of the U.S. FDA-approved E-beam at 5.5 kGy does inactivate MNV and HAV by greater than 90% in shellfish. However, the potential health risks associated with this reduced virus load still is not significantly different from the health risks associated with consuming unirradiated oysters. If E-beam irradiation (at the maximum FDA approved dose) is used, the NoV infection risks will be reduced by about 12% (from 4.49×10^{-1} to 3.95×10^{-1}) if the virus contamination in oysters was 10^5 PFU. However, if the average serving size of oysters contained approximately 10^4 or 10^3 PFU, the reduction in NoV infection risks will be approximately 15% (3.96×10^{-1} to 33.7×10^{-1}) and 19% (from 3.38×10^{-1} to 2.73×10^{-1}), respectively. It must, however, be emphasized that these predictions are based on murine norovirus, an experimental surrogate of human norovirus. At very low NoV contamination levels ($\sim 10^2$ PFU), there will be a 26% reduction (from 2.74×10^{-1} to 2.03×10^{-1}) in infection risks.

In the case of HAV, however, the use of E-beam processing could result in reduction of health risks. Assuming that the average serving size of oysters harbored a total of approximately 10^5 PFU of HAV, the use of E-beam irradiation would achieve a 16% reduction (from 9.21×10^{-1} to 7.76×10^{-1}) of infection risks. At lower levels of contamination (10^2 PFU), the reduction in infection risks is estimated to be 91% (from 2.10×10^{-1} to 1.93×10^{-2}) (Table 3). Calci et al. (18) reported that oysters in natural estuarine waters can concentrate HAV to quite high levels ($>10^5$) in a relatively short period of time (24 h). Thus, for HAV, 5.5-kGy treatment does result in ≥1 log reduction in infection risk but only at relatively low virus contamination levels. In fact, the potential HAV risk would reduce from 20 to 2% (from 2 in 10 persons to 2 in 100 persons) (Table 3) if a typical serving size of oysters contained approximately 10^2 PFU.

In conclusion, this study shows that both HAV and MNV are susceptible to E-beam irradiation. However, at FDA-approved doses, the reduction of HAV and MNV-1 in shellfish is limited to only around 90%. While E-beam irradiation does not completely eliminate shellfish-associated virus infection risks, some benefits can be expected. In 1995, the FDA estimated that shellfish-associated illnesses (bacterial and viral) cost the U.S. economy approximately $200 million (37). It is important to calculate the cost savings that could be realized with the reduction in virus-related health risks that were identified in this study. Given the large economic costs associated with norovirus infections, these reductions in infection risks with the use of E-beam irradiation technology could yield significant cost savings for countries such as the United States. It needs to be emphasized that a pathogen-kill step, such as E-beam processing, is meant to be part of a comprehensive HACCP plan that includes classification of growing areas and

monitoring of fecal coliforms and management strategies, such as closure during high-rainfall events. Lastly, this study shows that current commercial irradiation of shellfish to address *Vibrio* spp. contamination can have collateral reduction of virus levels, albeit modest. This study also emphasizes the need to develop new technologies that could be used in conjunction with E-beam irradiation to significantly reduce virus health risks. The sensory attributes of E-beam-processed oysters were not part of this study but warrant further studies.

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REFERENCES

1. **CDC.** 2012. Surveillance for norovirus outbreaks. http://www.cdc.gov/Features/dsNorovirus/. Accessed 23 June 2012.
2. **Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM.** 2011. Foodborne illness acquired in the United States–major pathogens. Emerg. Infect. Dis. **17**:7–15.
3. **Scharff RL.** 2012. Economic burden from health losses due to foodborne illness in the United States. J. Food Prot. **75**:123–131.
4. **DePaola A, Jones JL, Woods J, Burkhardt W, Calci KR, Krantz JA, Bowers JC, Kasturi K, Byars RH, Jacobs E, Williams-Hill D, Nabe K.** 2010. Bacterial and viral pathogens in live oysters: 2007 United States market survey. Appl. Environ. Microbiol. **76**:2754–2768.
5. **Le Guyader F, Haugarreau L, Miossec L, Dubois E, Pommepuy M.** 2000. Three-year study to assess human enteric viruses in shellfish. Appl. Environ. Microbiol. **66**:3241–3248.
6. **Lees D.** 2000. Viruses and bivalve shellfish. Int. J. Food Microbiol. **59**:81–116.
7. **Levin M.** 1978. Fish and shellfish associated disease outbreaks. J. Water Pollut. Control Fed. **50**:1377–1381.
8. **Potasman I, Paz A, Odeh M.** 2002. Infectious outbreaks associated with bivalve shellfish consumption: a worldwide perspective. Clin. Infect. Dis. **35**:921–928.
9. **Hardesty S.** 2001. Pacific coast shellfish growers association: marketing opportunities for Pacific coast oysters. Food Marketing and Economics Group, Davis, CA.
10. **Rippey SR.** 1994. Infectious diseases associated with molluscan shellfish consumption. Clin. Microbiol. Rev. **7**:419–425.
11. **Lowther JA, Gustar NE, Hartnell RE, Lees DN.** 2012. Comparison of norovirus RNA levels in outbreak-related oysters with background environmental levels. J. Food Prot. **75**:389–393.
12. **Harewood P, Rippey S, Montesalvo M.** 1994. Effect of gamma irradiation on shelf life and bacterial and viral loads in hard-shelled clams (*Mercenaria mercenaria*). Appl. Environ. Microbiol. **60**:2666–2670.
13. **Kingsley DH, Holliman DR, Calci KR, Chen H, Flick GJ.** 2007. Inactivation of a norovirus by high-pressure processing. Appl. Environ. Microbiol. **73**:581–585.
14. **Richards GP, Watson MA.** 2001. Immunochemiluminescent focus assays for the quantitation of hepatitis A virus and rotavirus in cell cultures. J. Virol. Methods **94**:69–80.
15. **Federal Register.** 2005. Irradiation in the production, processing, and handling of food, final rule. Fed. Regist. **70**(157):48057–48073.
16. **Jakabi M, Gelli DS, Torre J, Rodas MAB, Franco B, Destro MT, Landgraf M.** 2003. Inactivation by ionizing radiation of *Salmonella enteritidis*, *Salmonella infantis*, and *Vibrio parahaemolyticus* in oysters (*Crassostrea brasiliana*). J. Food Prot. **66**:1025–1029.
17. **Miller RB.** 2005. Food irradiation using electron beams, p 43–74. *In* Miller RB (ed), Electronic irradiation of foods: an introduction to the technology. Springer, New York, NY.
18. **Calci KR, Meade GK, Tezloff RC, Kingsley DH.** 2005. High-pressure inactivation of hepatitis A virus within oysters. Appl. Environ. Microbiol. **71**:339–343.
19. **Wobus CE, Karst SM, Thackray LB, Chang KO, Sosnovtsev SV, Belliot G, Krug A, Mackenzie JM, Green KY, Virgin HW.** 2004. Replication of norovirus in cell culture reveals a tropism for dendritic cells and macrophages. PLoS Biol. **2**:2076–2084. doi:10.1371/journal.pbio.0020432.
20. **Federal Registry.** 2005. Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters, notice of availability. Docket no. 1999N-1075. Fed. Regist. **70**:41772–41773.
21. **Teunis PFM, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J, Calderon RL.** 2008. Norwalk virus: how infectious is it? J. Med. Virol. **80**:1468–1476.
22. **Pinto RM, Costafreda MI, Bosch A.** 2009. Risk assessment in shellfish-borne outbreaks of hepatitis A. Appl. Environ. Microbiol. **75**:7350–7355.
23. **Espinosa AC, Jesudhasan P, Arredondo R, Cepeda M, Mazari-Hiriart M, Mena KD, Pillai SD.** 2012. Quantifying the reduction in potential health risks by determining the sensitivity of poliovirus type 1 chat strain and rotavirus SA-11 to electron beam irradiation of iceberg lettuce and spinach. Appl. Environ. Microbiol. **78**:988–993.
24. **Karst SM, Wobus CE, Lay M, Davidson J, Virgin HW.** 2003. STAT1-dependent innate immunity to a Norwalk-like virus. Science **299**:1575–1578.
25. **Di Girolamo RJ, Liston J, Matches J.** 1972. Effects of irradiation on the survival of virus in West Coast oysters. Appl. Microbiol. **24**:1005–1006.
26. **Dixon DW, Rodrick GE.** 1998. Effect of gamma radiation on shellstock, p 97–110. *In* Combination processes for food irradiation. Panel Proceedings Series (IAEA). Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria.
27. **Mallett JC, Beghian LE, Metcalf TG, Kaylor JD.** 1991. Potential of irradiation technology for improved shellfish sanitation. J. Food Saf. **11**:231–245.
28. **Song HP, Kim B, Jung S, Choe JH, Yun HJ, Kim YJ, Jo C.** 2009. Effect of gamma and electron beam irradiation on the survival of pathogens inoculated into salted, seasoned, and fermented oyster. LWT-Food Sci. Technol. **42**:1320–1324.
29. **Bandekar JR, Chander R, Nerkar DP.** 1987. Radiation control of *Vibrio parahaemolyticus* in shrimp. J. Food Saf. **8**:83–88.
30. **Jung PM, Park JS, Park JG, Park JN, Han IJ, Song BS, Choi JI, Kim JH, Byun MW, Baek M, Chung YJ, Lee JW.** 2009. Radiation sensitivity of poliovirus, a model for norovirus, inoculated in oyster (*Crassostrea gigas*) and culture broth under different conditions. Radiat. Phys. Chem. **78**:597–599.
31. **Sommer R, Pribil W, Appelt S, Gehringer P, Eschweiler H, Leth H, Cabaj A, Haider T.** 2001. Inactivation of bacteriophages in water by means of non-ionizing (UV-253.7 nm) and ionizing (gamma) radiation: a comparative approach. Water Res. **35**:3109–3116.
32. **Sanglay GC, Li J, Uribe RM, Lee K.** 2011. Electron-beam inactivation of a norovirus surrogate in fresh produce and model systems. J. Food Prot. **74**:1155–1160.
33. **Doré B, Keaveney S, Flannery J, Rajko-Nenow P.** 2010. Management of health risks associated with oysters harvested from a norovirus contaminated area, Ireland, February-March 2010. Eurosurveillance **15**:12–15.
34. **Lowther JA, Avant JM, Gizynski K, Rangdale RE, Lees DN.** 2010. Comparison between quantitative real-time reverse transcription PCR results for norovirus in oysters and self-reported gastroenteric illness in restaurant customers. J. Food Prot. **73**:305–311.
35. **Le Guyader FS, Le Saux JC, Ambert-Balay K, Krol J, Serais O, Parnaudeau S, Giraudon H, Delmas G, Pommepuy M, Pothier P, Atmar RL.** 2008. Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. J. Clin. Microbiol. **46**:4011–4017.
36. **Leon JS, Kingsley DH, Montes JS, Richards GP, Lyon GM, Abdulhafid GM, Seitz SR, Fernandez ML, Teunis PF, Flick GJ, Moe CL.** 2011. Randomized, double-blinded clinical trial for human norovirus inactivation in oysters by high hydrostatic pressure processing. Appl. Environ. Microbiol. **77**:5476–5482.
37. **GAO.** 2001. Report to the Committee on Agriculture, Nutrition and Forestry. Food safety: federal oversight of shellfish safety needs improvement GAO-01-702. GAO, Washington, DC.