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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

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Fresh produce, such as lettuce and spinach, serves as a route of food-borne illnesses. The U.S. FDA has approved the use of ionizing irradiation up to 4 kGy as a pathogen kill step for fresh-cut lettuce and spinach. The focus of this study was to determine the inactivation of poliovirus and rotavirus on lettuce and spinach when exposed to various doses of high-energy electron beam (E-beam) irradiation and to calculate the theoretical reduction in infection risks that can be achieved under different contamination scenarios and E-beam dose applications. The D_{10} value (dose required to reduce virus titers by 90%) (standard error) of rotavirus on spinach and lettuce was 1.29 (± 0.64) kGy and 1.03 (± 0.05) kGy, respectively. The D_{10} value (standard error) of poliovirus on spinach and lettuce was 2.35 (± 0.20) kGy and 2.32 (± 0.08) kGy, respectively. Risk assessment of data showed that if a serving (~14 g) of lettuce was contaminated with 10 PFU/g of poliovirus, E-beam irradiation at 3 kGy will reduce the risk of infection from >2 in 10 persons to approximately 6 in 100 persons. Similarly, if a serving size (~0.8 g) of spinach is contaminated with 10 PFU/g of rotavirus, E-beam irradiation at 3 kGy will reduce infection risks from >3 in 10 persons to approximately 5 in 100 persons. The results highlight the value of employing E-beam irradiation to reduce public health risks but also the critical importance of adhering to good agricultural practices that limit enteric virus contamination at the farm and in packing houses.

Viral infections transmitted through fresh produce are of growing concern, since food-borne viruses are responsible for a majority of food-borne illnesses in the United States (36). Health care costs associated with food-borne viruses are currently estimated at $6 billion (37). Though norovirus (NoV) is clearly the leading food-borne virus of concern, all enteric viruses can cause food-borne illnesses, with many such infections leading to chronic disease complications, such as myocarditis, meningitis, and chronic fatigue. The other enteric viruses associated with food-borne infections include adenovirus, astrovirus, rotavirus, and hepatitis A virus (3, 4, 12, 28). Salads, leafy greens, mixed-fruit dishes, frozen strawberries, tomatoes, melons, cruciferous vegetables, berries, and green onions/scallions are some of the primary vehicles for the transmission of food-borne viruses, including rotavirus (3) (OutbreakNet—Foodborne Outbreak Online Database [FOOD]). Fresh produce is vulnerable to enteric virus contamination in the field via feces-contaminated irrigation water and in packing houses with contaminated wash water (48). Wild-type poliovirus is a threat in many parts of the world, and its presence and spread through environmental waters have been documented (14, 42).

In 2008, to address the continuing food-borne illnesses associated with fresh produce and in response to petitions filed by the Grocery Manufacturers Association, the United States Food and Drug Administration (FDA) approved the use of ionizing irradiation (up to a dose of 4.0 kGy) for control of food-borne pathogens—and extension of shelf-life—in fresh iceberg lettuce and fresh spinach (9, 10). Ionizing radiation technology to control food-borne pathogens is more than 100 years old. However, the technology for delivering ionizing radiation is undergoing change. Today, ionizing radiation can be generated by either cobalt-60- or cesium-137-based radioisotopes, from high-energy (7.5-MeV) X-rays, or high-energy (10-MeV) electron beams (E-beams) generating linear accelerators (27). Though the basic mechanism by which the ionizing radiation inactivates microorganisms is through extensive damage to the nucleic acids either directly or by indirect effects caused by the radiolytic splitting of water molecules, there are differences in the different technologies in terms of the energy employed, the dose rate, etc. For example, gamma ray (cobalt-60)-based ionizing radiation uses photons in the energy range of about 1.6 MeV, whereas E-beam uses electrons at energies of about 10 MeV (27). Similarly, the dose rate of gamma rays from cobalt-60 is very often in the range of hundreds of grays per minute (40), while that of E-beam is around tens of millions of grays per minute (27). Thus, E-beam processing is a significantly higher-throughput process than gamma irradiation processes, and the technology has the potential to be deployed around the world since there are no associated issues, such as shipping, storing, and disposing of radioactive material.

The published literature comparing the inactivation of enteric viruses and that of bacteria when using ionizing radiation is limited (2, 5, 7, 23, 24, 30). Viruses are generally considered to be

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more resistant to ionizing radiation than bacteria due to the relatively smaller genome sizes (8, 15). Viruses have smaller genomes than bacteria or eukaryotes, such as fungi or protozoa, and hence, they are more resistant (19). The nucleic acid is the primary target of ionizing radiation due to its large G value compared to that of proteins or lipids. The G value is the number of radiolytic species produced per 100 eV of energy that is absorbed. The U.S. FDA approves the use of ionizing radiation as a hurdle technology for specific foods for reducing the exposure to pathogens in the food supply (9). Examples of irradiated foods that are currently available in grocery stores in the United States include meat products, papayas, mangoes, guavas, sweet potatoes, and spices.

The aim of this study was 2-fold. The first was to determine the sensitivity of poliovirus and rotavirus to E-beam irradiation on fresh-cut lettuce and spinach, and the second was to quantify the theoretical reduction in health risks if lettuce and spinach in different virus contamination scenarios were irradiated at various E-beam doses. The underlying hypothesis was that enteric viruses, such as poliovirus and rotavirus, are sensitive to E-beam irradiation when present on fresh produce and that a demonstrable reduction in potential health risks from enteric-virus-contaminated lettuce and spinach could be achieved at FDA-approved doses. Since the FDA has approved ionizing radiation for only lettuce and spinach, these studies were performed only on these two leafy greens.

MATERIALS AND METHODS

Propagation of viruses. The poliovirus type 1 Chat strain (VR-192) was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and propagated using the Buffalo green monkey kidney (BGMK) cell line (Diagnostic Hybrids Inc.). The cells were grown in minimal essential medium (MEM) (Invitrogen, Carlsbad, CA) containing 10% (vol/vol) fetal bovine serum (FBS). The virus stocks were harvested from infected BGMK monolayers by freeze-thaw lysis, low-speed centrifugation (400 × *g*, 5 min), and filtration through a 0.2-μm-pore-size syringe filter. Similarly, simian rotavirus strain SA-11 (VR-1565) was purchased from the ATCC and propagated in mammalian MA-104 cells (ATCC CRL-2378.1) grown in Dulbecco's modified Eagle's medium (DMEM) (high glucose) supplemented with 10% fetal bovine serum and 1.7 g liter^{-1} sodium bicarbonate.Virus stocks were harvested from infected MA-104 cells by freeze-thaw lysis, low-speed centrifugation (400 × *g*, 5 min), and filtration through a 0.2-μm-pore-size syringe filter. The virus suspensions were divided into aliquots and were stored at −80°C prior to use.

Inoculation of fresh produce. Iceberg lettuce and baby spinach samples were purchased from retail grocery stores. Five-gram samples of lettuce and baby spinach were placed separately in Ziploc plastic bags using aseptic practices. The samples were inoculated with the viruses by adding 10 spots on different leaves, with each spot containing 100 μl of a mixture of rotavirus SA-11 and poliovirus (approximately 10^5 PFU/ml). The inoculated samples were allowed to remain at room temperature overnight to allow maximum pathogen adsorption to the leaf surface and to roughly simulate natural contamination scenarios. The overnight holding of the inoculated samples allowed the moisture from the inoculation drops to evaporate, thereby avoiding any experimental artifacts due to the moisture film around the inoculum. Following the hold at room temperature, the plastic bags were heat sealed after elimination of all the excess air inside.

Electron beam irradiation. The E-beam irradiation was performed at the E-beam facility of the National Center for Electron Beam Research at Texas A&M University. A 10-MeV, 18-kW linear accelerator was used for delivering the E-beam dose. Alanine dosimeters were placed at strategic positions on the Ziploc bags to verify the delivered E-beam dose. Dosimetry was performed using alanine dosimetry that involved validated standards that were traceable to international standards. The dosimeters were read using the Bruker e-scan spectrometer (Bruker, Billerica, MA) to measure the delivered irradiation dose. An extensive set of preliminary studies was performed to ensure that the Ziploc bags containing 5 g of lettuce or spinach could be irradiated very 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 maximum dose and the minimum dose anywhere within the Ziploc bag were close to unity. The E-beam irradiation experiments were performed separately for lettuce and spinach. The target doses for virus inactivation were 0 kGy (unirradiated control), 1.5 kGy, 2.0 kGy, 3.0 kGy, 4.0 kGy, and 5.0 kGy. The measured doses (as determined using the alanine dosimeters) were 1.3 kGy, 2.07 kGy, 2.99 kGy, 4.01 kGy, and 5.04 kGy. The E-beam irradiation doses were calibrated by varying the speed of the processing table that moved underneath the E-beam. The E-beam had a fixed dose rate of approximately 10^3 Gy/second. All treatments were replicated at least 3 times.

Recovery of viruses from irradiated lettuce and spinach samples. After E-beam irradiation, the samples were transferred to a bag containing 50 ml of phosphate-buffered saline (PBS) and 1 M NaCl solution. The bags were placed on a shaker for 20 min to recover viruses from the leaves. This protocol of recovering viruses from lettuce and spinach was based on previous studies in our laboratory which demonstrated that the addition of 1 M NaCl enhances virus recovery from lettuce (44). The viruses in the washing solution were then recovered and concentrated using Centricon Plus-70 centrifugal filters with a molecular weight cutoff (MWCO) of 100,000 (Millipore) according to the manufacturer's instructions. The final concentrate of approximately 400 μl (containing both rotavirus and poliovirus) was stored at −20°C. Aliquots of the sample concentrates were neutralized with specific monoclonal antibodies for the different viruses prior to cell line infection to facilitate enumeration for the two different virus types. Thus, to enumerate polioviruses, the sample was treated with monoclonal antibodies against rotavirus (SC-58188; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). This prevented the rotaviruses in the sample from interfering with the poliovirus assay. Similarly, to quantify rotaviruses, the samples were treated with monoclonal antibodies against polioviruses (SC-57983; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The manufacturer's protocols were employed for the neutralization step. The surviving polioviruses and rotaviruses in the sample concentrates after the respective neutralization steps were assayed using BGMK and MA-104 cell lines, respectively. The presence of plaques was the endpoint for the presence of viruses, and the results are expressed in PFU per g.

Enumeration of rotavirus. Rotavirus SA-11 was enumerated using a modified PFU method based on MA-104 cytopathic effects (CPE) (17, 38). MA-104 cells were grown to 95 to 100% confluence with 5% CO_2 in 6-well cell culture plates with DMEM supplemented with 10% FBS. MA-104 cells were thoroughly washed prior to infection with serum-free DMEM. Serial dilutions of VR-1565 were made in DMEM and were then treated with 20 U of trypsin/ml for 60 min at 37°C. MA-104 culture plates that were infected with the serial dilution of VR-1565 were incubated under 5% CO_2 for 90 min, with periodic rocking at 15-min intervals for efficient adsorption. Following adsorption, an agar overlay maintenance medium consisting of 2× DMEM supplemented with 1 μg/ml of trypsin and 2% agar was added and allowed to solidify. The plates were incubated in the 5% CO_2 incubator for 3 days, and then 4 ml of 1% formaldehyde in normal saline solution was added to each well and the plates were kept for 12 h at room temperature. The solid overlay was gently removed from the wells without disturbing the cell monolayer. Three milliliters of a 0.1% crystal violet solution was added to each well to permit visualization of plaques. Plaques were quantified, and results from duplicate flasks were averaged to calculate the viable viral concentration.

Enumeration of poliovirus. The BGMK cell line has been suggested for use in enteric virus analysis (6, 11). The cells were grown to >95% confluence with 5% CO_2 in 6-well cell culture plates with minimal essen-

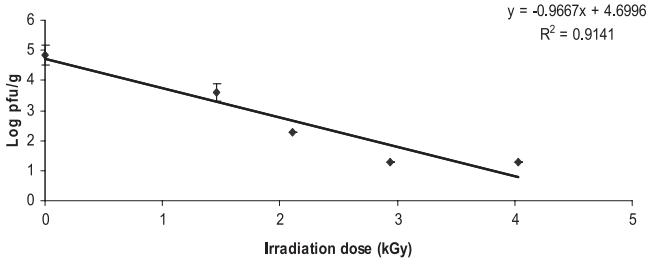

FIG 1 Inactivation of rotavirus SA-11 on lettuce when exposed to 10-MeV, 18-kW E-beam irradiation.

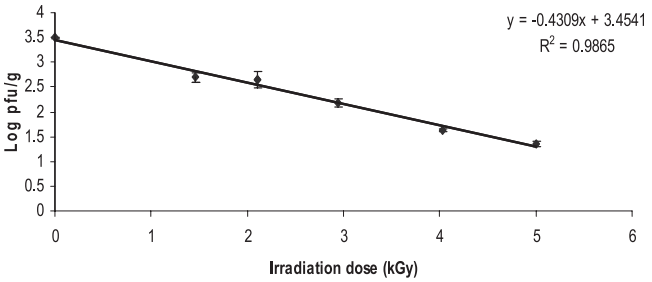

FIG 3 Inactivation of poliovirus type 1 Chat strain on lettuce when exposed to 10-MeV, 18-kW E-beam irradiation.

tial medium (MEM) containing 10% (vol/vol) FBS and were thoroughly washed. Serial dilutions of VR-192 were made in MEM and were used to infect BGMK culture plates, which were then incubated under 5% CO_2 for 90 min, with periodic rocking at 15-min intervals for efficient adsorption. Following adsorption, an agar overlay maintenance medium consisting of 2× MEM and 2% agar was added and allowed to solidify. The plates were incubated in the 5% CO_2 incubator for 48 h, and then 4 ml of 1% formaldehyde in normal saline solution was added to each well and the plates were kept for 12 h at room temperature. The solid overlay was gently removed from the wells without disturbing the cell monolayer. Three milliliters of a 0.1% crystal violet solution was added to each well to permit visualization of plaques. The plaques were quantified, and results from duplicate flasks were averaged to calculate the viable viral concentration.

Data analysis. The surviving rotavirus (log PFU/g) and poliovirus (log PFU/g) concentrations were plotted as a function of the measured dose (kGy). The slope of the curve was determined from the regression analysis, and the negative reciprocal of the slope was calculated to be the D_{10} value (dose required to reduce virus titers by 90%) (21). The data from the experimental replicates were used in plotting the virus inactivation curves (Fig. 1, 2, 3, and 4). Statistical analyses were performed using an unpaired two-sample *t* test to determine whether there were statistically significant differences between the D_{10} values (Table 1).

Quantitative microbial risk assessment. Single serving sizes of lettuce (14 g) and spinach (0.8 g) were used in estimating the reduction in health risks that can be achieved if lettuce and spinach are E-beam treated (33). These serving sizes were assumed to be hypothetically contaminated with starting poliovirus and rotavirus concentrations of 1 PFU/g, 10 PFU/g, 100 PFU/g, and 1,000 PFU/g. The reductions in these contamination levels at specified E-beam doses were based on the experimental results, and the resulting reductions in health risks were computed using the beta-Poisson model for rotavirus and the exponential model for poliovirus. The beta-Poisson model uses the equation $P_i = 1 - (1 + N/\beta)^{-\alpha}$, where P_i is the probability of infection and N is the number of viruses ingested. α (0.2531) and β (0.42) are parameters of the dose-response curve. The health risks accruing from a single exposure were determined with the assumption that all viruses on the produce were infectious. Infection risks were estimated for poliovirus using the exponential model $P_i = 1 - \exp(-N/k)$, where P_i is the probability of infection, N is the number of infectious viruses in the exposure, $1/k$ (0.009) refers to the fraction of viruses that survive and are capable of initiating an infection, and exp signifies that e is raised to the exponent of the value in parentheses. The parameter values were based on those used by Haas et al. (16).

RESULTS

Inactivation of rotavirus on lettuce and spinach during E-beam irradiation. Figures 1 and 2 show the reduction of rotavirus SA-11 on lettuce and spinach samples, respectively, when exposed to 10-MeV E-beams at various doses. The virus was susceptible to E-beam irradiation. The dose (standard error [SE]) required for achieving a 90% reduction (D_{10} value) of rotavirus SA-11 on lettuce was calculated to be 1.03 (± 0.05) kGy, while the dose (SE) for achieving the same reduction of the virus on spinach was 1.36 (± 0.64) kGy. There was no statistically significant difference ($P = 0.6$) between the D_{10} value of rotavirus on lettuce and the D_{10} value of rotavirus on spinach (Table 1).

Inactivation of poliovirus on lettuce and spinach during E-beam irradiation. Figures 3 and 4 show the reduction of poliovirus type 1 on lettuce and spinach when exposed to different E-beam doses. On both lettuce and spinach, there was a linear relationship between the reduction in virus titers and the delivered dose. On lettuce, poliovirus exhibited a D_{10} value (SE) of 2.32 (± 0.08) kGy, while on spinach, poliovirus exhibited a D_{10} value (SE) of 2.24 (± 0.20) kGy. There was no statistically significant difference between the sensitivity of poliovirus on lettuce and the sensitivity of poliovirus on spinach ($P = 0.8$) (Table 1).

However, when comparing the D_{10} values of rotavirus to those of poliovirus, there were significant differences between their inactivations on spinach ($P = 0.05$) and between their inactivations on lettuce ($P < 0.05$).

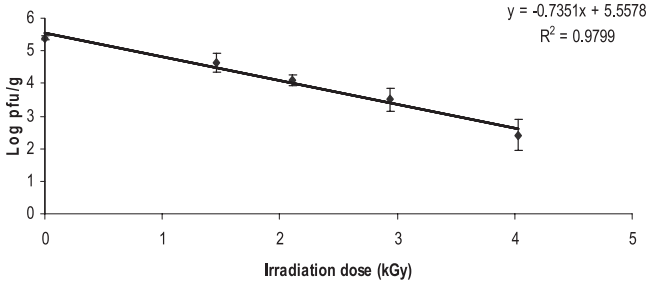

FIG 2 Inactivation of rotavirus SA-11 on spinach when exposed to 10-MeV, 18-kW E-beam irradiation.

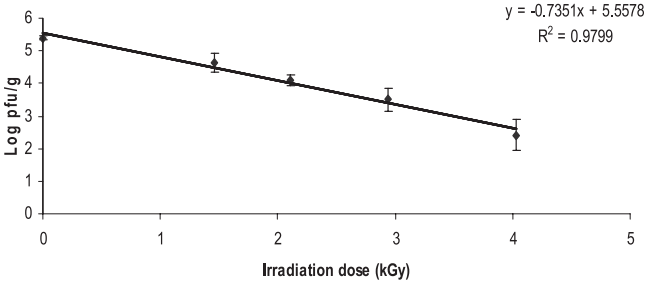

FIG 4 Inactivation of poliovirus type 1 Chat strain on spinach when exposed to 10-MeV, 18-kW E-beam irradiation.

TABLE 1 D_{10} values for poliovirus type 1 Chat strain and rotavirus SA-11 titers on lettuce and spinach when exposed to 10-MeV, 18-kW E-beam irradiation

Virus	D_{10} value (kGy) for virus on[a]:	
	Spinach	Lettuce
Rotavirus (SA-11)	1.36 ± 0.64 A	1.03 ± 0.05 A
Poliovirus (PV-1)	2.24 ± 0.20 B	2.32 ± 0.08 B

[a] Values are the means and standard error. D_{10} values with different letters indicate statistically significant ($P \leq 0.05$) differences.

Reduction in health risks achievable with E-beam irradiation. Table 2 and Table 3 represent the theoretical reductions in poliovirus-associated infection risks if E-beam irradiation is employed on lettuce and spinach, respectively, when they are contaminated with various amounts of poliovirus. At various doses of E-beam irradiation, ranging from approximately 1 kGy to 4 kGy (the FDA-mandated maximum dose), there is a discernible reduction in infection risks when the level of contamination is below 100 PFU/g. The risk of poliovirus infection is higher on lettuce than on spinach. The first log reduction in risk on lettuce occurs at around a 3-kGy dose. There is no significant reduction in poliovirus infection risks even at 4 kGy if the lettuce is contaminated at levels between 100 and 1,000 PFU per gram. However, if the level of contamination is <10 PFU/g, there is a log reduction in infection risks even at 3 kGy. However, even at 4 kGy, there is still a probability of approximately 2 in 1,000 persons becoming ill if the lettuce has a starting contamination level of 1 PFU/g (Table 2). However, when comparing 1.5-kGy and 4-kGy doses, the infection risks are reduced from 2 in 100 persons to approximately 2 in 1,000 persons. When considering poliovirus infection risks on spinach, however, the use of E-beam irradiation results in a significant reduction in infection risks (Table 3). Even if a serving size of spinach (0.8 g) is contaminated with 1,000 poliovirus particles per gram, the use of a 3-kGy dose results in a log reduction of risk. If spinach is contaminated with 1 PFU of poliovirus per gram, the use of a 4-kGy dose reduces the risk to 8 in 100,000.

Table 4 and Table 5 show the theoretical reductions in infection risks associated with decreasing amounts of rotavirus on lettuce and spinach, respectively, when exposed to E-beam irradiation. Even after a 4-kGy E-beam dose, if a serving size of lettuce is contaminated with 10 to 1,000 rotavirus particles per gram, the risk of infection still ranges between 2 in 100 persons and 5 in 10 persons. If the starting level of rotavirus is approximately 1 PFU/g per lettuce serving size, the risk of infection decreases from 2 in 10 to approximately 3 in 1,000 persons. If spinach is contaminated with rotavirus at 10 PFU/g, there is a log reduction in infection risks when E-beam irradiation at 3 kGy is used. However, if spinach is contaminated with 100 rotavirus particles per gram, a log reduction in risk occurs only when a 4-kGy dose is employed. Even after exposure to a 4-kGy dose, the risk of infection is still approximately 4 out of 100 persons. If a serving size of spinach is contaminated at levels between 1 and 1,000 PFU per gram, a quantifiable reduction of health risks can be achieved with a 1-kGy dose. The risk of infection drops from approximately 8 of every 10 individuals to approximately 7 out of every 100 persons.

TABLE 3 Infection risks associated with poliovirus-contaminated spinach after treatment with various E-beam irradiation doses

E-beam irradiation dose (kGy) (% poliovirus reduction)	Infection risk[a] for each initial poliovirus contamination level (PFU/g)			
	1,000	1000	10	1
1.15 (88.3)	5.73×10^{-1}	8.17×10^{-2}	8.48×10^{-3}	8.52×10^{-4}
2.04 (97.9)	1.42×10^{-1}	1.52×10^{-2}	1.53×10^{-3}	1.53×10^{-4}
3.05 (99.2)	5.66×10^{-2}	5.81×10^{-3}	5.82×10^{-4}	5.82×10^{-5}
4.0 (98.9)	7.70×10^{-2}	7.98×10^{-3}	8.01×10^{-4}	8.01×10^{-5}

[a] Probability of infection.

DISCUSSION

Today, fresh produce is viewed as highly vulnerable to microbial pathogen contamination. The risk of pathogen contamination and infection is high because produce is most often grown in the open and often consumed without any validated pathogen kill or cooking steps. Food-borne viruses are responsible for the majority of food-borne disease cases in the United States (36). Foods, especially fresh fruits and vegetables, are often contaminated with viral pathogens at preharvest stages. There have been large-scale disease outbreaks associated with the consumption of virus-contaminated fresh produce that was grown in another country (4).

The present study provides information on the use of E-beam irradiation as a final "hurdle" to prevent the consumption of enteric-virus-laden lettuce and spinach. The U.S. FDA has approved the use of ionizing irradiation, such as E-beam and gamma irradiation, up to 4 kGy for pathogen reduction in lettuce and spinach (9, 10). This study indicates that E-beam irradiation can inactivate poliovirus and rotavirus on lettuce and spinach at various efficiencies. There is a linear relationship between dose and level of virus inactivation. This linear relationship between virus inactivation and ionizing radiation has been reported earlier (2, 26, 35). Poliovirus exhibited greater resistance to E-beam irradiation than did rotavirus (Table 1). Though the literature related to

TABLE 2 Infection risks associated with poliovirus-contaminated lettuce after treatment with various E-beam irradiation doses

E-beam irradiation dose (kGy) (% poliovirus reduction)	Infection risk[a] for each initial poliovirus contamination level (PFU/g)			
	1,000	100	10	1
1.46 (83.99)	9.99×10^{-1}	8.73×10^{-1}	1.86×10^{-1}	2.04×10^{-2}
2.11 (85.39)	9.99×10^{-1}	8.48×10^{-1}	1.72×10^{-1}	1.87×10^{-2}
2.94 (95.19)	9.98×10^{-1}	4.62×10^{-1}	6.01×10^{-2}	6.18×10^{-3}
4.03 (98.62)	8.31×10^{-1}	1.63×10^{-1}	1.76×10^{-2}	1.78×10^{-3}

[a] Probability of infection.

TABLE 4 Infection risks associated with rotavirus-contaminated lettuce after treatment with various E-beam irradiation doses

E-beam irradiation dose (kGy) (% rotavirus reduction)	Infection risk[a] for each initial rotavirus contamination level (PFU/g)			
	1,000	100	10	1
1.46 (94.4)	8.52×10^{-1}	7.35×10^{-1}	4.56×10^{-1}	4.56×10^{-1}
2.11 (99.7)	6.90×10^{-1}	4.56×10^{-1}	1.62×10^{-1}	1.62×10^{-1}
2.94 (99.97)	4.56×10^{-1}	1.62×10^{-1}	2.41×10^{-2}	2.41×10^{-2}
4.03 (99.97)	4.56×10^{-1}	2.41×10^{-2}	2.54×10^{-3}	2.54×10^{-3}

[a] Probability of infection.

TABLE 5 Infection risks associated with rotavirus-contaminated spinach after treatment with various E-beam irradiation doses

E-beam irradiation dose (kGy) (% rotavirus reduction)	Infection risk[a] for each initial rotavirus contamination level (PFU/g)			
	1,000	100	10	1
1.46 (83.99)	7.72×10^{-1}	5.94×10^{-1}	3.13×10^{-1}	7.16×10^{-2}
2.11 (85.39)	6.86×10^{-1}	4.50×10^{-1}	1.57×10^{-1}	2.30×10^{-2}
2.94 (95.19)	5.61×10^{-1}	2.70×10^{-1}	5.45×10^{-2}	6.17×10^{-3}
4.03 (98.62)	2.37×10^{-1}	4.32×10^{-2}	4.76×10^{-3}	4.82×10^{-4}

[a] Probability of infection.

E-beam inactivation of enteric viruses is rather limited, the variability in virus susceptibility to ionizing radiation in general has been reported extensively (13, 20, 31, 32). The efficiency of virus reduction appears to depend not only on the virus type but also on whether the viruses are present on lettuce or spinach (Table 1). There were, however, no statistically significant differences between the D_{10} values of either virus on spinach and the D_{10} values of that virus on lettuce. There are other reports in the literature which suggest that the matrix in which the organisms are present influences the reduction kinetics (1, 2, 22, 35, 39). Researchers have recently reported that the human norovirus surrogate, murine norovirus, showed differential reduction when exposed to E-beam irradiation depending on whether the virus was present on cabbage or strawberries (35). The differential reduction of enteric viruses (though not statistically significantly different) on different fresh produce commodities highlights the importance of choosing an appropriate treatment dose to achieve the desired reduction.

The results also highlight the reduction in infection risks that can be achieved with various doses of E-beam irradiation. To the best of our knowledge, there has been no published information to date highlighting the infection risk reduction that can be achieved with specific irradiation doses. This reduction in risks of infection was calculated based on the empirical virus inactivation data that were obtained during the course of these studies. The serving sizes of lettuce (14 g) and spinach (0.8 g) used in these calculations were based on U.S. demographic data. This significant difference in serving sizes results in generally higher risks associated with virus contamination of lettuce than with virus contamination of spinach. However, it is important to highlight that though poliovirus was more resistant to E-beam irradiation, the infection risks from rotavirus exceeded those of poliovirus. This is primarily because of their inherent differences in infectivity which were factored into the risk calculations. The dose required to achieve a 90% reduction (D_{10}) of poliovirus (>2 kGy) was greater than that required for rotavirus (~1 kGy), regardless of whether the viruses were present on lettuce or spinach (Table 1). Thus, poliovirus contamination of lettuce poses a greater risk of infection than rotavirus contamination of spinach. This, along with the greater resistance to E-beam irradiation for poliovirus than for rotavirus, requires that careful attention be paid to agricultural on-farm practices (to reduce virus contamination) and the E-beam irradiation dose that is chosen for the irradiation treatment.

A previous study has reported that viral contamination of lettuce and spinach is generally less than 1,000 viral particles (18). However, according to the FDA, the upper dose limit for irradiation treatment of fresh-cut lettuce and spinach to control pathogens is 4 kGy (9, 10). The present study highlights the importance of the starting number of viral particles on fresh produce in terms of infection risks, even if E-beam irradiation is used. Rose et al. have reported that the current risk of acquiring a food-borne disease in the United States is estimated at a probability of 2.7×10^{-2} annually, which contributes to the >$3 billion health care burden (34). The use of E-beam irradiation can achieve a defined reduction in infection risks which will then translate to savings in health care costs (41).

Importantly, these results also highlight the extremely significant influence that starting concentrations of virus contamination can have (irrespective of E-beam dose) on the ultimate risks of infection. Postharvest technologies, such as gamma irradiation or E-beam irradiation, were never designed to be used as cleanup technologies (10, 39). These technologies are meant to be used only as the final step of a comprehensive food safety program that starts with good agricultural practices (GAPs) in the field and good manufacturing practices (GMPs) in packing sheds. Unless the fresh produce commodity has manageable levels of contaminants, the use of E-beam or other such postharvest technologies cannot be expected to make significant reductions in the numbers of infections in the general public.

In conclusion, this study shows that E-beam irradiation is capable of inactivating poliovirus and rotavirus on lettuce and spinach. Additionally, this study has shown the potential that E-beam irradiation technology has to reduce the risks of infections for the general public, provided that the starting levels of virus contamination are kept low by good agricultural and postharvest practices.

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