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The Development of a Quantitative Microbial Risk Assessment Model to Evaluate the Efficacy of Produce Rule Interventions to Reduce Norovirus and Hepatitis A Virus Contamination of Fresh Produce on Farms and Packing Facilities

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By

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Bachelor of Science
Arkansas State University
2013

Thesis Committee Chair: Juan Leon, PhD MPH

An abstract of
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Abstract

The Development of a Quantitative Microbial Risk Assessment Model to Evaluate the Efficacy of Produce Rule Interventions to Reduce Norovirus and Hepatitis A Virus Contamination of Fresh Produce on Farms and Packing Facilities
By Zachary Marsh

From 1998 to 2008 in the United States, over 100,000 foodborne illnesses, 1,000 hospitalizations, and 13 deaths were attributed to norovirus (NoV) and hepatitis A virus (HAV). To mitigate the impact of foodborne pathogens like NoV and HAV and grant regulatory authority over intervention implementation, the United States Food and Drug Administration enacted the Food Safety and Modernization Act and established a set of minimum food safety interventions in the Produce Rule. The goal of this study was to evaluate the effectiveness of Produce Rule interventions at reducing the consumer NoV and HAV risk of infection (hereafter referred to as risk of infection) for four produce commodities in the pre-harvest, harvest, and packing stages of produce production. To achieve this goal, quantitative microbial risk assessment (QMRA) models were developed to estimate and evaluate the NoV and HAV risks of infection before and after the implementation of Produce Rule interventions. The QMRA models were designed to follow a produce item through a number of harvest and packing stages on US farms and packing facilities. The parameter estimates and distributions used to populate the model equations were obtained from publicly available literature data. For all produce commodities and scenarios, the QMRA models demonstrated the average NoV (8.28×10^{-2}) risk of infection was significantly higher ($p < 0.001$) than that of HAV (5.81×10^{-7}). There was a general trend observed that shorter harvest and packing operations and machine harvesting, instead of hand harvesting, resulted in lower risks of infection. Finally, it was found that all Produce Rule interventions reduced NoV and HAV risk of infection (range: 0.1- 70.0%), yet the magnitude of risk reduction was greatest for glove use (range: 36.4- 70.0%) and handwashing (range: 17.2- 40.3%). The NoV and HAV QMRA models were successful in demonstrating the ability of Produce Rule interventions to reduce NoV and HAV risk of infection on farms and packing facilities. This QMRA modeling approach could be used to identify and evaluate current and future food safety interventions on farms and packing facilities to ensure the United States maintains a safe and reliable produce production system.

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Literature review

Foodborne disease is a common public health concern that results in a burden to the United States economy and health infrastructure. Foodborne diseases annually result in an estimated 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths in the United States alone, which result in worker furloughs and increased medical expenses (1). The USDA Economic Research Service has stated that based on the above figures, foodborne diseases result in approximately $15 billion of economic loss annually (2). From 1998 to 2008, there were approximately 13,000 foodborne disease outbreaks of which roughly 8,000 had known or suspected etiologies in the United States (3). Thus, the magnitude of foodborne disease is tremendous, and it negatively impacts both economic and worker health.

Despite the amount of food produced in the United States, the country continues to import a growing percentage of produce to ensure a variety of produce year-round, yet this practice also increases the risk of foodborne outbreaks (4). The number of foodborne outbreaks in the United States has doubled since the inception of the Foodborne Disease Outbreak Surveillance System in 1973 (5). Much of this increase was attributed to an increased focus on improved surveillance, yet this may also be due to globalization of the food industry (reviewed in (6)). Increasingly, food is imported throughout the year in order to provide the consumer with a consistent assortment of food regardless of local availability. This increased importation of foods from countries with less strict agricultural safety policies may account for the increased number of food related outbreaks (reviewed in (7)). Therefore, this globalization of the food production industry necessitates the evaluation and implementation of safety practices to prevent foodborne

outbreaks, which, in the current economic climate, have the potential for wide ranging effects.

The food industry is also faced with a large number of food vehicles that may facilitate pathogen transmission. Almost all foodborne disease outbreaks originate from one of three food groupings: aquatic animals, land animals, and plants (1). Moreover, these exposures occur through a wide variety of food commodities such as fish, crustaceans, mollusks, dairy, eggs, beef, game, pork, poultry, grains/beans, oils/sugars, and produce (8). DeWaal et al have shown produce to be the second most reported source of foodborne outbreaks behind meat. During the 10 year period from 1998 to 2008, around 700 produce outbreaks resulted in 28,000 illnesses compared to 1,200 meat outbreaks with the same number of illnesses (1). These numbers demonstrate the potential for produce-based outbreaks to infect a large number of people per outbreak due to factors such as minimal processing and raw consumption (4). This variety of food products, though a benefit to consumer choice, increases the means through which foodborne pathogens may be transmitted.

There is also a diverse set of foodborne pathogens with which the food industry must contend (1, 5, 6). There are currently 25 pathogens for which laboratory surveillance data is currently collected, yet this number does not encompass the full spectrum of potential etiologies (3). As mentioned previously, around half of all foodborne outbreaks reported from 1998 to 2008 had a known etiology (5). Among these, bacteria were implicated in 45%, viruses another 45%, parasites 1%, and chemical or toxic agents the remaining percentage (5). While bacteria have been the focus of much attention, our knowledge of the impact viruses have on foodborne disease continues to grow as detection methods

improve (reviewed in (9)). This assortment of pathogens creates a unique challenge for the food industry as it works to contend with a large number of potential pathogen and commodity combinations.

The virus most commonly implicated among these viral foodborne outbreaks was norovirus (NoV), which accounted for around 75,000 illnesses, 800 hospitalizations, and 5 deaths during the 10 year period (5, reviewed in (10)). Following NoV in impact was hepatitis A virus (HAV) that, despite causing only 2,100 illnesses and 268 hospitalizations, resulted in the death of 8 people over the same 10 year period (5). As a result of NoV being the most common foodborne pathogen and HAV resulting in chronic complications, the World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) established a committee to evaluate the risk posed to the global food industry by foodborne viruses, specifically NoV and HAV (11). The impact of these two viruses on human health continues to grow as improved detection and surveillance methods attribute an increasing number of foodborne outbreaks to these pathogens.

The first of these major viral, foodborne pathogens of interest is NoV. NoV is a positive-sense, single-stranded RNA virus, and it is the only member of the *Norovirus* genus within the *Caliciviridae* family. There are currently 3 genogroups (G) known to infect humans: GI, GII, and GIV. GI, GII, and GIV are further subdivided into at least 32 genotypes. Among the three genogroups, GI and GII have the largest impact on human health with GI most commonly implicated in food and waterborne outbreaks and GII in person-to-person transmitted outbreaks (reviewed in (12)). NoV is highly infectious with modeling estimates claiming as few as 18 virions are required to cause an infection (13).

The appearance of symptoms generally occurs within 24 to 48 hours following infection and subsides within the same time frame. Symptoms most frequently exhibited are vomiting and profuse, watery diarrhea. The disease course is most severe in young, old, and immunocompromised individuals. Currently, the only treatment for NoV is supportive therapy such as maintenance of proper hydration and salts, and there is no available vaccine to protect from infection if exposed (reviewed in (14)). It is this lack of vaccine and highly infectious nature of NoV that makes prevention of exposure of paramount importance to avoid rampant outbreaks and disease.

The second most commonly implicated viral, foodborne pathogen is HAV. HAV is a positive-sense RNA virus that belongs to the *Picornaviridae* family. There is only a single HAV serotype, which has facilitated the development of a successful vaccine (reviewed in (15)). HAV tends to be an asymptomatic infection, when encountered at a young age, which results in lifelong immunity. The average incubation period for the virus is 28 days after which time the main symptom, jaundice, becomes apparent. Despite the availability of a vaccine, many individuals living in more developed countries are never exposed to HAV and suffer a more severe disease course due to a later exposure (reviewed in (16)). This makes the protection of unvaccinated populations, such as those found in the United States, particularly important since much of the population is unexposed to the virus, thus susceptible to severe disease if exposed.

Another obstacle to food safety is the challenge of detection of some pathogens and the assessment of their infectivity. Although viruses are known to cause a large number of foodborne outbreaks, the true number of outbreaks is likely underestimated due to the many obstacles in viral testing methods (3, reviewed in (17)). For example, current

detection of viruses in food is difficult due to certain inhibitory factors within the food matrix (17). The presence of these inhibitory factors in the food matrix results in false negatives during diagnostic testing, which leads the investigator to believe the pathogen is absent in the sample. Additionally, current technology is unable to determine the infectivity of important foodborne viruses (i.e. NoV) since reliable assays or cell culture methods are unavailable (reviewed in (18)). This challenge in pathogen detection and infectivity assessment is a major hindrance to obtaining accurate estimates, and it is an area that must be addressed if reliable evaluation of food safety is to be conducted.

This challenge to evaluate the safety of the produce industry is exacerbated by the complexity of the farm-to-fork continuum. In its journey from the farm to the consumer, produce encounters many potential contamination sources throughout the major stages of production, most importantly in the pre-harvest, harvest, and processing stages. The United States Department of Agriculture (USDA) regularly monitors farming practices to identify risks of produce contamination. USDA and other studies have highlighted soil quality (reviewed in (19)), irrigation (reviewed in (20)) and rinse water quality (21), harvest and packing workers (22), and harvest and packing equipment (23) as important potential sources of contamination (24). While the USDA has provided guidance on how to prevent introduction and transmission of foodborne pathogens, this guidance did not have the authority to require their implementation by the produce industry (25-28). Consequently, the United States government needed to address its lack of regulatory power in order to provide an assurance of the quality and safety of the United States produce industry.

In an effort to address this rising concern, the United States Congress and Food and Drug Administration (FDA) took action to combat the risk of both accidental and intentional contamination of the food industry. On January 4, 2011, the Food Safety and Modernization Act (FSMA), the first major overhaul to food safety law in 70 years, was enacted (29). Two years following the establishment of the FSMA, a set of explicit produce-specific interventions, referred to as the Produce Rule, were released. One of the major advances of the Produce Rule was its requirement for all of the produce industry, with minor exceptions, to adhere to interventions established by the Produce Rule (30). As a result of increasing produce-associated infectious disease, the FSMA was enacted and established a set of produce-specific interventions referred to as the Produce Rule.

The Produce Rule was written in a way to encompass the entire U.S. produce industry; therefore, certain government-mandated interventions may not pertain to all industries. For example, the requirement to treat irrigation water may not be appropriate for farming operations that use drip irrigation. Since the drip irrigation water will never come in contact with the edible portion of the plant, the irrigation water treatment regulation is not important to these operations. In order to accommodate the diversity of the food industry, the Produce Rule allows for variances to be administered that exempt certain industries from implementing specific interventions (29). In order to be awarded a variance, the specific industry must demonstrate that waiving the requirement to implement the specified intervention(s) would not result an unnecessary increase in the risk of produce contamination. In consideration of the diversity of the produce industry, the Produce Rule awards variances when the industry provides scientifically-based evidence that the absence of intervention does not increase produce contamination risk.

Currently available detection and evaluation methods preclude select industries from obtaining the scientifically-based evidence required for the variance application. Additionally, direct sample methods only consider the pathogen contamination level at a particular moment with an explicit set of environmental conditions. Since conditions, such as water quality, can and are likely to change throughout the food production chain, it would be beneficial for industries to consider this fluctuation as they decide whether the interventions are appropriate for their operations. Moreover, there are typically conditions besides pathogen prevalence that contribute to produce contamination. Therefore, both the FDA and produce industry require a robust method to demonstrate the Produce Rule interventions are effective or to provide evidence that the new interventions are not applicable to their respective industry.

An alternative method to strictly using direct sample testing for risk estimation is a risk assessment. To date, many risk assessments have been used to evaluate a variety of public health concerns where traditional methods were not warranted. The National Research Committee defines a risk assessment as "the use of a factual base to define the health effects of exposure of individuals or populations to hazardous materials and situations" (31). In conducting a standard risk assessment some or all of the following four stages are implemented: hazard identification, dose response assessment, exposure assessment, and risk characterizations. In hazard identification the researcher determines whether there is a causal linkage between the potentially hazardous agent and the adverse disease outcome. Dose-response assessment allows one to evaluate the impact that different estimates of exposure has upon the probability of developing the outcome of interest. Exposure assessment determines the magnitude of human exposure to the agent

of interest following the implementation of regulations. Finally, risk characterization defines the magnitude of risk posed to populations based on estimates from the previous stages of assessment. In conducting these steps, researchers or government agencies are provided with a framework, which they may adapt to meet the needs of their question of interest.

A particular type of risk assessment framework that has been used increasingly to evaluate risks due to microbial pathogens is a quantitative microbial risk assessment (QMRA). In conducting a QMRA, one develops a mathematical model, which applies scientifically-derived parameters to a dose-response distribution to obtain a risk of infection or illness. Typically a researcher will use a computer package to run a large number of iterations of the model to generate a probability distribution of the risk estimate (32). By using this QMRA modeling strategy, a researcher is able to consider a large number of variables that may contribute to the overall estimation of risk. For this reason, this approach lends itself to addressing a number of different scenarios where traditional sampling or evaluation methods may not be able to provide an accurate risk estimate.

Since the produce industry is a complex environment with challenges in the diversity of food, pathogens, and potential contamination entry points, a more holistic approach, such as QMRA modeling, would be more effective at risk estimation than risk estimation at a single time point (reviewed in (33)). With a QMRA model, parameter estimates for contamination entry points at each stage of produce production could be fit to the model for a variety of pathogens and produce commodities. Moreover, QMRA modeling can apply pathogen die off, removal, and transfer events that provide more representative

estimates of the actual risk posed to the consumer. For instance, there is generally a low prevalence of viruses in food, yet they are problematic due to the high infectivity and persistence in a variety of environmental conditions (34). Traditional sampling methods would fail to account for these factors and conclude that the risk to the consumer is low since the viral prevalence is low. QMRA modeling would likely come to a different conclusion since the risk calculation would consider the many different elements such as transfer of new virus, its survival, and the low ID_{50} of many pathogens. Finally, QMRA models are able to evaluate the impact of different scenarios on contamination risk unlike direct sample testing, which is tied solely to the specific conditions in which the samples were collected (35). This ability to account for the complexity of the produce industry underscores the value in conducting a QMRA to determine the overall risk to the consumer.

Due to the more complete consideration of risk factors, many researchers have used QMRA modeling to estimate risk of infection from produce consumption (36-39). While the approach was initially used for bacterial pathogens, emerging interest in foodborne viruses has led to QMRA models being developed for these pathogens (40-46). Most of the models have focused on the impact of using alternative irrigation sources including rainwater, highly treated wastewater, and household greywater (36-38, 40-41). One study looked to use a QMRA model to establish microbial water quality standards based on the risk from irrigating produce with varying qualities of water (42). Two recent publications have investigated the impact of enteric viruses on the contamination of produce during its production. Initially, Bouwknegt et al looked to evaluate the contamination risk throughout the entire food production chain for leafy greens and berries (43), described

below. More recently, Newman et al evaluated the impact of handwashing compliance, improved handwashing efficacy, and worker furlough on NoV contamination levels of produce during the harvest and processing stages (47). What all of these models fail to consider is the impact that the US-mandated interventions have on contamination risk during the pre-harvest, harvest, and processing stages of production.

In response to the call to action by the European Food Safety Authority (EFSA), Bouwknegt et al developed QMRA models to assess the consumer viral risk of infection (hereafter referred to as risk of infection) posed to the consumer due to contamination by NoV and HAV in the raspberry, strawberry, and lettuce production chains (48). This study used data collected in cooperation with European produce growers to provide parameter estimates that could be fit to a model for the specific produce commodity and pathogen combination. Potential sources of contamination considered were irrigation water quality, harvester's hands, conveyer belt use, food handler's hands, rising water quality, consumption, and dose response (43). This model was the first use a QMRA model to quantify the risk due to NoV and HAV contamination in commercial produce production. Although the authors took a comprehensive look at the pre-harvest, harvest, and processing stages of produce production, they failed to consider the impact that different preventive interventions have on reducing viral levels. Since the viral levels on produce is dynamic, an evaluation of the addition and removal of virus would provide a more complete picture of the risks posed to consumers.

The most recent QMRA model to consider NoV contamination of produce on farms and packing facilities was conducted by Newman et al who evaluated the impacts of hand hygiene and worker furloughs on NoV contamination of produce. The QMRA model

used fecal contamination on hands in conjunction with multiple contact events to determine the level of hand hygiene necessary to reduce viral levels below 10 virions/cm^2. In addition to hand hygiene the authors evaluated whether barring workers from working while ill would reduce viral levels below the 10 virions/cm^2 level. While this study indirectly considered select interventions presented in the FSMA, it focused solely on interventions related to food worker contamination. Potentially important interventions not considered are irrigation and rinse water quality, the irrigation water delivery mechanism, farmworker and processing worker glove use and handwashing, and cleaning of harvest equipment and conveyer belts. Additionally, the published model considered NoV, yet it failed to evaluate any other important enteric pathogens such as HAV. Therefore, our QMRA model aims to expand upon this work by considering the impact of additional stages of produce production and Produce Rule interventions on the final risk of infection to the consumer.

A QMRA modeling strategy that builds upon the work conducted by Bouwknegt et al and Newman et al could be used to address the need of the FDA and produce industry to identify the most effective interventions at reducing viral risk in the farm-to-fork continuum (43). First of all, QMRA modeling would allow for the estimation of NoV and HAV risk of infection due to the number of contamination entry points that contribute to viral contamination of fresh produce. Our work would rely on sample estimates obtained from a variety of publications after an exhaustive literature search. In using many different publications, we will be able to provide more accurate parameter estimates compared to the limited number of samples collected by Bouwknegt et al. Second, a sensitivity analysis allows for the most problematic contamination entry points to be

determined. This could be highly beneficial to the FDA and produce industry since it would provide scientifically-based evidence to direct the focus of regulatory and intervention efforts. Third, QMRA models could be used to assess the effect of newly-mandated Produce Rule interventions (30). Not only does the FDA need to evaluate whether their interventions provide a meaningful improvement in produce safety, but growers also need to evaluate if the intervention(s) benefit the safety of their produce. By using a QMRA model, produce stakeholders could evaluate interventions in a number of scenarios to decide on the most effective set to implement. Finally, growers would be able to use evidence generated from QMRA models to apply for variances from the FDA (29). Therefore, the goal of this thesis is to develop QMRA models to evaluate the efficacy of Produce Rule interventions to reduce the risk of NoV and HAV contamination of produce in the pre-harvest, harvest, and processing stages of production.

To achieve this goal, this thesis aims to 1) select parameters estimates of common contamination entry points during produce production from publicly available data, 2) estimate the NoV and HAV risk of infection in the pre-harvest, harvest and packing stages of fresh produce production, 3) identify parameter estimates most contributory to final risk of infection, and 4) use the QMRA models to evaluate the efficacy of Produce Rule interventions to prevent or reduce viral contamination of fresh produce on farms and packing facilities.

As a result of successfully completing the proposed QMRA analysis, the FDA and produce industry will have updated estimates of the NoV and HAV risk of infection from consuming fresh produce harvested and packed on US farms and packing facilities. Additionally, these stakeholders will know the most problematic NoV and HAV

contamination entry points on farms and packing facilities. By using the QMRA models developed in this thesis, the produce industry will have the capacity to generate scientifically-based evidence to use in future variance applications. The produce industry, government policy makers, and foodborne disease researchers will also be provided with a tool to which they can apply future contamination and intervention parameter estimates in order to evaluate a number of scenarios on farms and packing facilities. Finally, these stakeholders will have validation of Produce Rule intervention efficacy on farms and packing facilities. The ability to efficiently evaluate a number of scenarios on produce farms and packing facilities across the country will prove invaluable to government regulators and the produce industry as they both strive to ensure our food is healthy and safe for years to come.

The Development of a Quantitative Microbial Risk Assessment Model to Evaluate the Efficacy of Produce Rule Interventions to Reduce Norovirus and Hepatitis A Virus Contamination of Fresh Produce on Farms and Packing Facilities

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Statement of Contribution

All authors contributed extensively to this study. Juan S. Leon and Anna M. Aceituno initially conceived the study and obtained the major funding. Zachary Marsh obtained additional funding to complete the study, developed the initial QMRA models, refined and executed the final QMRA models, identified parameter estimates and their distributions from the literature, performed the data management and analysis, and wrote all sections of the manuscript. With input from Juan S. Leon, Zachary Marsh wrote and revised the manuscript.

Introduction

Foodborne diseases annually result in an estimated 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths in the United States (1). 13,000 foodborne outbreaks were reported from 1998 to 2008 where 45% of the outbreaks with a known etiology were caused by viruses (5). The proportion of viruses attributed to foodborne disease continues to grow, yet our understanding of the viruses and their overall disease burden is still limited (17).

In the United States, the most commonly implicated foodborne virus is norovirus (NoV), which accounted for around 100,000 illnesses, 1,000 hospitalizations, and 5 deaths over a 10 year period (5, 10). Following in impact is hepatitis A virus (HAV) that caused around 2,100 illnesses, 300 hospitalizations, and killed 8 people over the same 10 year period in the United States (5). NoV and HAV are responsible for a large proportion of foodborne disease, so there is a need to identify contamination mechanisms and develop control measures.

Despite awareness of these viruses, little is known about their behavior and burden (17). Due to many reported fresh produce outbreaks, an area of growing interest is the farm-to-fork continuum (1, 3, 5, 49-58). A challenge in ensuring fresh produce safety is that many produce items are consumed raw or undercooked, yet preventing contamination on farms and packing facilities is challenging (reviewed in (24), 59). Government agencies and other researchers have highlighted a number of potential contamination entry points including: soil (19), irrigation (20) and rinse water (21), harvest and packing workers (22), and harvest and packing equipment (23). To add to the

challenges, the Food and Drug Administration (FDA) has lacked the authority to enforce existing control and prevention measures (25-28).

In response to numerous food safety issues and lack of regulatory power, the FDA enacted the Food Safety and Modernization Act (FSMA) in 2011 (3, 5, 29). Because fresh produce is implicated in many outbreaks, the FSMA required the development of the *Standards for Growing, Harvesting, Packing, and Holding of Produce for Human Consumption* (referred to hereafter as the Produce Rule) (1, 5, 25-28, 60). Since its release, FDA has received requests from the produce industry to provide supporting evidence of the effectiveness of these interventions against common foodborne pathogens (60). Additionally, the Produce Rule only provides bacterial - not viral – produce safety standards, yet the fresh produce disease burden of these pathogens is immense (5, 60). Therefore, the ability of Produce Rule interventions to reduce foodborne virus contamination on farms and packing facilities should be evaluated.

To evaluate the Produce Rule interventions, a robust method, like a risk assessment, is needed to estimate the consumer viral risk of infection (hereafter referred to as the risk of infection) (31). A particular type of risk assessment is a quantitative microbial risk assessment (QMRA) (32). A QMRA estimates viral contamination with a series of mathematical equations, and the output of these equations is fit to a dose-response model to calculate risk of infection (32). With a QMRA approach, multiple variables (referred to hereafter as parameters) are fit to uncertainty distributions and applied to models representing a number of scenarios.

The applicability of QMRA models to address public health problems, such as the ones presented in this study, has already been demonstrated (36-39). While the approach

was initially used for bacterial pathogens, emerging interest in foodborne viruses has led to the development of viral QMRA models (40-46). To date, two publications have investigated enteric virus contamination of fresh produce (43, 47). Bouwknegt et al was the first to evaluate the NoV and HAV risk of infection throughout leafy green and berry production chains (43). More recently, Newman et al evaluated the impact of handwashing compliance, improved handwashing, and worker furlough on NoV contamination of fresh produce (47). What both of these models failed to consider was the impact that Produce Rule interventions have on contamination risk during the pre-harvest, harvest, and processing stages of production (43, 47).

As a result of the growing number of produce outbreaks and the limitations of traditional laboratory methods, there is a need for a way to identify the most effective interventions at reducing fresh produce risk of infection in the farm-to-fork continuum. To address this need, the goal of this study is to use QMRA models to evaluate the ability of Produce Rule interventions to reduce NoV and HAV risk of infection. To achieve this goal, this study aims to 1) select parameters estimates of contamination entry points during produce production from publicly available data, 2) estimate the NoV and HAV risk of infection in the pre-harvest, harvest and packing stages of fresh produce production, 3) identify parameter estimate contribution to risk of infection, and 4) evaluate the efficacy of Produce Rule interventions to reduce risk of infection on farms and packing facilities. Upon completing the QMRA analysis, these results will serve as an evidence-base for produce stakeholders to reduce the NoV and HAV risk of infection from fresh produce consumption thereby improving the health of the American people.

Methods

a. Model overview

The models were developed based on fresh produce industry documents (61-69) and existing fresh produce QMRA models (43, 47, 70). The overall approach resembled the QMRA models of both Bouwknegt et al and Newman et al (43, 47). The major difference between the models developed in these two published studies and the models presented in this study was the inclusion of Produce Rule intervention parameter estimates to evaluate the effect of Produce Rule interventions. Additionally, the models in this study considered two types of equipment use (container storage and conveyer belt transfer) whereas Bouwknegt et al only considered container storage (43). Finally, this study also considered irrigation and rinse water use in addition to farmworker contact, container storage, conveyer belt transfer, and packing worker contact modeled by Newman et al (47).

The models in this study were designed to follow a produce item through a variety of stages (Figure 1). For this study, a stage is defined as the equipment, human, or water contact with the produce item on farms and packing facilities. The general model consisted of six individual equations that represented six harvest and packing stages (irrigation water, farmworker contact, container storage, rinsing, conveyer belt transfer, and packing worker). The different equations were modeled in many different combinations in order to best reflect the standard growing practices (13, 43, 56, 61-65, 67, 69).

Six different combinations of stages were used to represent all possible harvest and packing scenarios (hereafter referred to as scenarios) for four different produce

commodities (berries, leafy greens, melons, and vine/stalk grown) (Figure 1). A scenario is defined as the order of stages involved in produce production. The first and second combination of stages in figure 1 were used to represent the hand (first combination) and machine (second combination) harvest and packing scenarios of berries. Hand harvest and packing is defined as the contact of farmworker and/or packing worker hands with the produce. The hand scenarios were modeled using the farmworker contact and/or packing worker equations (Table 1). Machine harvest and packing is defined as the absence of farmworker and/or packing worker hand contact with produce. The machine scenarios were modeled by omitting farmworker contact and/or packing worker equations (Table 1). The third combination of stages in Figure 1 was used to represent the leafy green hand harvest and packing scenarios (Figure 1). The fourth combination of stages was used to represent the melon hand harvest and packing scenarios (Figure 1). The fifth and sixth combination of stages were used to represent the hand (fifth combination) and machine (sixth combination) harvest and packing scenarios of vine/stalk grown.

b. Parameter estimates and assumptions

Table 2 displays all parameter estimates, the parameter estimate distributions, and assumptions, obtained from an extensive literature review, used to conduct the baseline risk assessments. The parameters were either fit to a statistical distribution where the value selected for inclusion in the model was randomly selected, or the parameter was a single, defined value used for every iteration of the model. For each equation, the final concentration of the produce item depended both upon the value of the equation parameters and the existing produce contamination obtained during the previous stage of harvest or packing.

It was assumed that all parameters behaved independently, yet this may not mirror the reality of their interactions. The produce item was assumed to maintain a constant contamination level throughout harvest and packing unless virus was transferred during a contact event or rinsed off. For the irrigation module, the produce item was modeled to have uniform water contact across half of its total surface area. The irrigation water (C_{irw}) was assumed to have a homogenous concentration of NoV and HAV. The harvest and packing workers were assumed to contact the produce once with one hand for all produce groups. The contact surface area of hands (w_{harv}) was held constant at the minimum hand surface area except for berries for which the surface area of three finger pads was used (43). Items were assumed to contact containers ($cont_{reuse}$) and conveyer belts ($belt_{reuse}$) between 0 and 5 times depending on equipment reuse frequency. Finally, after passing through the final stage the produce items were assumed to be held for seven days prior to consumer access allowing for viral decay (71, 72).

c. Model execution

The model equations were designed and written in Microsoft Excel® (Seattle, WA). The parameter distributions were fit and the Monte Carlo simulations were conducted with the Excel® add-in Crystal Ball® developed by Oracle (Redwood City, CA). For each model simulation, 10,000 iterations of the model were performed using the forecast function in Crystal Ball®. With each iteration of the model, a value was randomly selected from the parameter distribution described in Table 2 by the Crystal Ball® add-in and placed in the equation written in the Excel® cell. The equations written in Excel® were used to represent all possible scenarios (Table 1). The output for each of the equations were used in the calculation of the next equation in the harvest or packing

scenario until the output of the equation preceding the dose response was used to estimate the risk of infection (Figure 1 & Table 1). The dose response model used to calculate the risk of infection was a fractional Poisson model developed by Messner et al, which appears to perform equally as well as the beta-Poisson model developed by Teunis et al (13, 73). The exponential dose response model used for HAV was originally described by Haas et al and recently used by Bouwknegt et al (32, 43). Using Crystal Ball®, the mean and standard errors were calculated for the viral contamination of each equation and the final risk of infection to fresh produce consumers. Using these estimates, 95% confidence intervals were calculated for the average contamination and risk of infection estimates. Due to a lack of normally distributed data, a Mann-Whitney U test was conducted to compare the average NoV and HAV risks of infection in R 3.2.2 (74). In order to compare the average NoV and HAV risk of infection for each of the four produce commodities, a Kruskal-Wallis test was conducted in R 3.2.2. To determine which produce commodity groups were significantly different for the average NoV and HAV risk of infection, a Nemenyi pair-wise multiple comparisons test was conducted using the PMCMR package in R 3.2.2 (75).

d. Sensitivity analysis

In an effort to understand the parameters of each scenario most contributory to the final risk of infection, a sensitivity analysis was performed using Crystal Ball®. In performing a sensitivity analysis, Crystal Ball® selects a parameter from the equations involved in the risk calculation and sets the value of the selected parameter to a defined value preventing it from varying. Crystal Ball® then compares the risk calculated as a result of fixing the select parameter value to when it was allowed to vary. Crystal Ball®

repeats this process for every parameter involved in the risk calculation for a number of values for each parameter. The add-in uses the fluctuation of the risk to evaluate which of the parameters has the strongest influence on the final risk calculation. The combined values for the sensitivity analysis were exported to a new Excel® sheet to compare the effect of different parameters for both enteric viruses.

e. Intervention assessment

To evaluate the Produce Rule, six interventions were evaluated for all scenarios. All six interventions were evaluated for all four produce commodities. To evaluate the interventions, the risks of infection after implementing the intervention were compared to the baseline risk of infection. The baseline risk of infection is defined as the probability a consumer becomes infected from eating a produce item contaminated during normal harvest and packing operations where Produce Rule interventions were not implemented. The six interventions considered were drip irrigation use (assumes no irrigation water contact with the produce item), well water use (assumes irrigation water quality is equivalent to rinse water quality, which is a municipal water source), glove use (reduced hand to produce and increased produce to hand transfer rates), handwashing (initial viral hand contamination [C_{irw}] reduced by 0 to 2 $\log_{10}$), container cleaning (no container reuse without cleaning), and conveyer belt cleaning (no conveyer belt reuse without cleaning). Additionally, two combinations of interventions (1] drip irrigation use, glove use, container cleaning, and conveyer belt cleaning and 2] drip irrigation use, handwashing, container cleaning, and conveyer belt cleaning) were considered and compared to the single interventions previously mentioned. The values for the six single intervention parameter estimates are listed in Table 3.

In order to evaluate the effect of the intervention, the intervention parameter estimate was substituted for the nonintervention (baseline) parameter estimate from Table 1, and the model was executed identically as with the baseline parameter estimate. The risk of infection after implementing the intervention was compared to the risk of infection for the baseline parameters for each respective scenario for all four produce commodities. In order to quantify the effect of the intervention, the risk reduction was calculated by dividing risk after implementing by the risk without any interventions. The percent reduction was calculated by subtracting the ratio of the risks from 1 and multiplying by 100.

Results

a. Viral risk of infection

To determine the baseline (produce harvested and packed without any interventions; see Methods *subsection e* for full definition) NoV and HAV risk of infection for the four produce commodities, a number of different scenarios were considered (Figure 1). Overall, the risk of infection was calculated by fitting the distribution of NoV or HAV contamination levels after the final stage (equipment, human, or water contact; see Methods *subsection a* for full definition) to a dose response model. The average baseline risk of infection was calculated by conducting 10,000 Monte Carlo simulations of each harvest and packing scenario. Each harvest and packing scenario was represented by a specific combination of equations and parameter estimates in Tables 1-3, and the specific combination of equations are described in Figure 1.

For all produce commodities and scenarios, the average NoV baseline risk of infection (8.28×10^{-2}) was significantly higher than HAV (5.81×10^{-7}; p-value < 0.001, Table 4). When comparing the risk of infection for each of the four produce commodities, the average NoV baseline risk of infection for at least one produce commodity was found to be statistically significantly different (Kruskall-Wallis $\chi^2 = 9.84 \times 10^4$; p-value < 0.001). When the average NoV risk of infection for all scenarios was pooled, the Nemenyi pairwise analysis indicated that all produce commodities were significantly different from the others (p-value < 0.001) where berries had the lowest NoV risk of infection and melons the highest. Among the average HAV baseline risks of infection for the four produce commodities, there was also at least one produce commodity statistically significantly different (Kruskal-Wallis $\chi^2 = 8.18 \times 10^4$; p-value <

0.001). When the average HAV risk of infection for all scenarios was pooled, the Nemenyi pairwise analysis indicated that all produce commodities, except for leafy greens and melons (p-value = 0.65), were significantly different from the others (p-values < 0.001) where berries had the lowest HAV risk of infection and leafy greens and melons the highest.

There was a general trend observed that fewer harvest and packing stages resulted in a lower average risk of infection. Therefore, if one follows the risks of infection from full to short scenarios, it is expected that the risk gradually decreases regardless of virus and produce commodity. For example, the NoV risk of infection for melons was 1.63×10^{-1} for full, 1.05×10^{-1} for long, and 1.03×10^{-1} for short scenarios. Despite the general trend, there were exceptions such as the slight increase in NoV risk of infection between full hand harvest and long hand harvest of leafy greens and vine/stalk grown. The reason for this slight increase may be due to the inclusion of a rinsing step that decreases the risk of infection in the full compared to long and short scenarios. For example, for leafy greens, the full scenario contained a rinsing step that reduced the risk of infection from 1.04×10^{-1} to 8.88×10^{-2} (Table 4). It appears that a rinsing stage, after a conveyor belt stage, in the full hand harvested leafy greens reduces NoV contamination to 1.94 $\log_{10}$ virions, yet long hand harvested leafy greens, ending at the conveyor belt stage, never drop below 2.59 $\log_{10}$ virions (Figure 2B). Therefore, the magnitude of viral contamination is likely higher for the scenarios, like long and short harvest, that don't include a rinsing stage. For produce commodities with both hand and machine harvesting scenarios considered, the machine harvesting scenarios consistently demonstrated a lower NoV and HAV risks of infection (Table 4). In summary, it appears that the fewer stages

involved in a scenario and the harvesting scenarios without worker contact resulted in a lower risk of infection.

b. Viral contamination

In order to better understand how viral contamination fluctuated as the produce item passed through the different scenarios, the average contamination level of NoV and HAV was calculated during the Monte Carlo simulations for each of the stages. Overall, the contamination fluctuation pattern was similar for NoV (Figure 2) and HAV (Figure 3). One of the first large increases in viral contamination followed farmworker contact (Figure 2 & 3). For the scenarios that included a packing worker, packing worker contact resulted in a second large increase in contamination levels. Rinsing consistently resulted in around one $\log_{10}$ of viral contamination reduction. The viral decay that resulted over the simulated seven day holding period generally led to a 1-2 $\log_{10}$ reduction for NoV and HAV. There was a similar pattern of viral increase and decrease observed for the two viruses, yet the magnitude of viral contamination overall was much lower for HAV compared to NoV.

Despite the fluctuation similarity for the two viruses, there were differences observed within and between the four produce commodities. For the same produce commodity, the length of the scenario dictated the magnitude of viral contamination. For example, Figure 2D (NoV vine/stalk grown) demonstrated that at the end of the field packing scenario there were lower viral levels compared to short, long, and full scenarios. When comparing the four produce commodities across all scenarios, berries had the lowest average NoV contamination (4×10^0 to 4.7×10^1 GEC) and leafy greens had the highest (3.9×10^2 to 1.1×10^7 GEC) (Figures 2A & 2B). Berries were also the produce

commodity with the lowest average HAV contamination (1.0×10^{-5} to 2.0×10^{-5} GEC) and vine/stalk grown the highest (1.1×10^{-3} to 2.0×10^{-3} GEC) (Figure 3A & 3B).

c. Sensitivity analysis

A sensitivity analysis was conducted for NoV and HAV for the longest scenarios of each produce commodity group. In the sensitivity analysis, the longest scenarios for each commodity were selected as representative scenarios because they included the most parameters. For all sensitivity analyses, five parameters had a consistently high effect on the NoV risk of infection (Figure 4). It was shown that hand contamination, C_{hand}, (rho > 0.74) for all four produce commodities, hand-to-produce transfer, f_{hand}, (rho > 0.19) for leafy greens and vine/stalk grown, irrigation water concentration, C_{irw}, (rho > 0.38) for berries, and rinse water concentration, C_{rinse}, (rho > 0.50) for melons were most positively strongly correlated with NoV risk of infection. In contrast, hand surface area, w_{hand}, was most strongly negatively correlated with NoV risk of infection. Hand contamination, C_{hand}, (rho > 0.91) for all four produce commodities was also strongly positively correlated with the HAV risk of infection. In contrast, viral decay, $decay_{HAV}$, (rho < -0.13) for berries and leafy greens and rinsing rate, f_{rinse}, (rho < -0.15) for melons and vine/stalk grown were strongly negatively correlated with HAV risk of infection.

d. Intervention assessment

For all four produce commodities, six Produce Rule interventions were evaluated to assess their ability to reduce NoV and HAV risk of infection (Figures 5 & 6). Among the single interventions, harvest and packing worker interventions (glove use [f_{prodg} & f_{handg}] (36 to 70%) and handwashing [hw_{eff}] (17 to 40%) reduced risk of infection by a percentage comparable to the combined interventions (Combined 1 [47 to 82%] and

Combined 2 [23 to 63%]). In fact, glove use reduced NoV risk of infection for leafy greens by 68.6% compared to 69.3% for Combined 1 and 37.9% for Combined 2 (Figure 5). For NoV, the most effective single intervention was glove use (Figure 5). The single interventions with the least effective risk reduction were container cleaning alone (≤ 2.4%) and conveyer belt cleaning alone (≤ 1.7%). Among the combined interventions, combined 1 (drip irrigation, glove use, container cleaning, and conveyer belt cleaning) had the largest risk reduction (82.6%) compared to combined 2 (drip irrigation use, handwashing, container cleaning, and conveyer belt cleaning, 62.8%). The largest HAV risk reduction was also due to glove use (range: 28.9- 48.2%) (Figure 6). For HAV, the least effective single intervention was drip irrigation (range: 0.2- 5.1%). Overall, all six single Produce Rule interventions and two combined interventions reduced the risk of infection across all four produce commodities.

In addition to comparing the interventions across produce commodities, the intervention risk reduction was evaluated between produce commodities. For both NoV and HAV and regardless of produce commodity, it was found that overall intervention risk reduction was similar (NoV: 0.1- 70.0%; HAV: 0.1- 48.2%) (Figures 5 & 6). The only exception was a 30% higher NoV risk reduction by drip irrigation and a 27% higher NoV risk reduction by well water for berries compared to other commodities (Figure 5). In summary, regardless of commodity, interventions reduced NoV and HAV risk of infection by the same magnitude.

To assess the intervention effect on different scenario lengths, the risk reduction was calculated for all possible scenarios. It was found for both viruses and all produce groups that the intervention risk reduction increased as the scenario had fewer stages. For

example, well water use reduced NoV risk for vine/stalk grown produce harvested with a machine by 0.2% (full scenario), 4.7% (long scenario), and 73.6% (short scenario) (data not presented). Additionally, the intervention risk reduction was always higher for machine harvest scenarios than hand harvest. For example, substituting well water for untreated surface water reduced risk by 4.7% for machine harvest but only 1.7% for hand harvest. Overall, it appears the shorter scenarios and machine harvesting increases the efficacy of interventions.

Discussion

The goal of this study was to evaluate the effectiveness of the United States Food and Drug Administration (FDA) Produce Rule interventions at reducing NoV and HAV contamination of fresh produce on United States farms and packing facilities. NoV and HAV on fresh produce posed different risks of infection to the consumer. The QMRA models also suggested that shorter, compared to longer, scenarios, and machine, compared to hand, harvesting resulted in lower risks of infection. Glove use and handwashing were the most effective single interventions at reducing NoV and HAV risks of infection.

The NoV and HAV QMRA models suggested that the overall risk of infection from fresh produce consumption was different for the two viruses. One hypothesis to explain the different NoV and HAV risks of infection was the difference in NoV and HAV prevalence on fresh produce on farms and packing facilities. At each of the major harvest and packing stages (i.e. irrigation water, farmworker contact, and rinse water) used in this QMRA model, the NoV prevalence estimates were higher than HAV (Table 2). For example, the irrigation water NoV concentration (C_{irw}) was around six fold higher than the HAV C_{irw}, and the NoV hand contamination concentration (C_{hand}) was around four fold higher than C_{hand} for HAV. Additionally, NoV was also shown to be consistently more prevalent than HAV in different farming environments (43, 44, 72, 76-89). For example, in a study of South Korean groundwater samples by Jung et al, the authors found NoV in seven and HAV in zero of the thirty-nine samples (83). Therefore, it appears the difference in NoV and HAV risk of infection may result from the different prevalence of the viruses in the farming environment. In further support of the hypothesis

that NoV is more prevalent that HAV, there are more NoV outbreaks reported than HAV (5). Over the 10 year period in the United States, there were 3,444 NoV and only 76 HAV reported outbreaks.

Fewer harvest and packing stages and use of machine harvesting reduced the risk of infection from consuming fresh produce. One hypothesis to explain this finding may be that fewer produce contamination entry points along the shorter scenarios lead to lower risk of infection from consuming fresh produce. On farms that field pack leafy greens (short scenarios), there are two potential contamination entry points (irrigation water and farmworker contact), yet on farms that use packing facilities (full scenario), there are six contamination entry points (irrigation water, farmworker contact, container storage, conveyer belt transfer, rinsing, and packing worker, Figure 1) (90, 91). Each of these additional harvest and packing stages add additional viral contamination to the produce. For example, researchers have identified multiple viral contamination points on farms and packing facilities such as farmworker and packing worker contact (22). Studies have shown that every time a produce item is touched by hand there is bidirectional transfer of virus (76, 77, 80, 92). Overall, the magnitude of virus removal from produce during hand contact is less than virus addition during the same hand contact (76, 77, 80, 92). Therefore, it appears that having fewer harvest and packing stages, like farmworker and packing worker contact, may reduce risk of infection. A hypothesis for the reduced risk of infection associated with machine, compared to hand, harvesting is that machine harvesting eliminates the farmworker hand contamination. Since the sensitivity analysis showed hand contamination (C_{hand}) to be the highest contributor of risk of infection, the removal of hand contact from the harvest and packing scenario resulted in a much lower

risk of infection compared to scenarios that included hand contact. In support of this observation, Principle 3 of *Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables* stated the major sources of produce contamination are human contact and animal feces (25). Additionally, the leafy green industry has discussed the advantages and disadvantages of using machinery instead of human to harvest the produce (61, 90, 91, reviewed in (93)). Based on the reports, if properly maintained, machine harvesting results in less contamination, yet when contamination does occur with machine harvesting, it is generally more widespread. As a result, using properly maintained machine harvesting in place of farmworker hand harvesting could dramatically reduce the risk of infection.

Glove use for farmworkers and packing workers and handwashing (hw_{eff}) were found to be the two most effective single interventions at reducing risk of infection for both NoV and HAV. One hypothesis for the efficacy of glove use is that it mitigates the transfer of virus from farmworker and packing worker hands to the fresh produce. In the QMRA models in this study, the transfer parameter from produce to gloved hands (f_{prodg}) was much higher than the transfer parameter from gloved hands to produce (f_{handg}). The higher produce to hand transfer rate in gloved hands is the opposite of what was modeled with bare hand contact (76, 77, 92). These different transfer rates reflect the observation that more virus may cling to the gloves compared to bare hands, thus reducing viral transfer to the produce item (92). One hypothesis for the consistently high effect of handwashing on risk of infection is that handwashing reduces viral hand contamination (C_{hand}), which contributes the most to the final risk of infection for NoV and HAV. Since hw_{eff} directly affects the magnitude of C_{hand}, the intervention had a large reductive effect

on the risk of infection. Bidawid et al demonstrated that handwashing with any hand antiseptics they tested significantly reduced the amount of NoV present on hands and the NoV transfer rate to lettuce (77). Since glove use and handwashing were shown to be the most effective at reducing risk of infection, farms and packing facilities should prioritize the implementation of these two Produce Rule interventions.

There were a number of strengths and limitations to this study. The first strength of using QMRA modeling in this study was the ability to quickly estimate the risk of infection in a number of harvest and packing scenarios that would otherwise cost a tremendous amount of money, time, and manpower. The second strength of this modeling strategy was it provided the ability to estimate the change in viral contamination, at a number of harvest and packing stages, required to reduce the risk of infection below acceptable levels. The third strength of this study was the ability to assess the health impact of different foodborne pathogens in identical farm and packing facility conditions and to evaluate the ability of interventions to reduce the risk of infection of the pathogens. One limitation of this approach was large amount of uncertainty surrounding many of the parameter estimates since certain estimates may not perfectly represent the behavior of the parameters being modeled. The second limitation of this study was the inability to include an infectivity parameter in the QMRA models to determine the proportion of infectious virus present at the end of harvest and packing due to a knowledge gap in the literature. The third limitation of this study is it only considered FDA Produce Rule interventions, yet there are many other potential interventions, some of which may provide a larger risk of infection reduction than the ones considered in this study.

The QMRA models presented in this study provide great insight into the effect of Produce Rule interventions on risk of infection, yet the value of these models will increase once more robust parameter estimates of viral hand contamination, hand transfer rates, and irrigation water concentration have been obtained. Using currently available data, these models suggested that hand interventions, like glove use and handwashing, are most effective single interventions. With this information, government regulators and farms and packing facilities now have proof that implementing hand hygiene measures can greatly improve fresh produce safety. Additionally, the models suggested that farms and packing facilities should limit the number of harvest and packing stages in order to reduce the risk of viral produce contamination and improve efficiency. This means the fresh produce could go from the farm to consumer quicker – all while ensuring the produce is safer. Moreover, the QMRA models suggested that substituting machine harvesting for hand harvesting may reduce the risk of viral produce contamination. As with reducing the number of harvest and packing stages, machine harvesting could both improve produce safety and cut costs. Therefore, farms and packing facilities should modify their operations to limit the number of potential contamination entry points through reducing the number of harvest and packing stages or utilizing machine harvest methods. These QMRA models have wide-ranging applications for not only estimating food safety risk but also evaluating the effectiveness of current and future food safety interventions. As a result, the NoV and HAV QMRA models presented in this thesis provide valuable information to policy makers and the produce industry and will lead to a safer, more dependable produce supply.

Overall, the QMRA models in this study were able to quantify NoV and HAV risk of infection and evaluate the efficacy of Produce Rule interventions. The models suggested consuming fresh produce posed a significantly different risk of infection between NoV and HAV. The length of the harvest and packing scenario appeared to affect the risk of infection since shorter harvest and packing scenarios have fewer viral contamination entry points. Machine harvesting was also found to result in a lower risk of infection compared to farmworker hand harvesting. Finally, glove use and handwashing were found to be the two most impactful single interventions. Based on the QMRA models presented in this study, the produce industry and government regulators, like the FDA, now have a better evidence base on the efficacy of Produce Rule interventions on US farms and packing facilities. This evidence can be used to reduce NoV and HAV contamination of fresh produce on farms and packing facilities thus improving the health and wellbeing of the American people.

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Tables

Table 1: Equations for each harvest and packing stage in the QMRA models.

Stage	Equation
Irrigation water (irrigation water use)	$n_{irw} = C_{irw} I_{irw} w_{prod}$
Farmworker contact (hand harvest)	$n_{harv} = n_{irw} - f_{prod} \frac{w_{harv}}{w_{prod}} n_{irw} + f_{hand} \frac{w_{harv}}{w_{hand}} C_{hand}$
Container storage (*after hand harvest[1] and conveyer belt use[2]*)	$n_{cont} = n_{harv} - f_{cont} \pi_{cont} n_{harv} + f_{cont} \pi_{cont} n_{harv} cont_{reuse}$ [1] $n_{cont} = n_{belt} - f_{cont} \pi_{cont} n_{belt} + f_{cont} \pi_{cont} n_{harv} cont_{reuse}$ [2]
Conveyer belt transfer (*after irrigation water[1], farmworker contact[2], and container storage[3]*)	$n_{belt} = n_{irw} - f_{belt} \pi_{belt} n_{irw} + f_{belt} \pi_{belt} n_{cont} belt_{reuse}$ [1] $n_{belt} = n_{harv} - f_{belt} \pi_{belt} n_{harv} + f_{belt} \pi_{belt} n_{cont} belt_{reuse}$ [2] $n_{belt} = n_{cont} - f_{belt} \pi_{belt} n_{cont} + f_{belt} \pi_{belt} n_{cont} belt_{reuse}$ [3]
Rinsing (rinse water use [*after container storage[1] and conveyer belt transfer[2]*])	$n_{rinse} = n_{cont} 10^{-f_{rinse}} + C_{rinse} V_{rinse}$ [1] $n_{rinse} = n_{belt} 10^{-f_{rinse}} + C_{rinse} V_{rinse}$ [2]
Packing worker (packing worker contact [*after container storage[1] and rinsing[2]*])	$n_{touch} = n_{cont} - f_{prod} \frac{w_{food}}{w_{prod}} n_{cont} + f_{hand} \frac{w_{food}}{w_{hand}} C_{hand}$ [1] $n_{touch} = n_{rinse} - f_{prod} \frac{w_{food}}{w_{prod}} n_{rinse} + f_{hand} \frac{w_{food}}{w_{hand}} C_{hand}$ [2]
Decay (norovirus decay [*after farmworker contact[1], container storage[2], conveyer belt transfer[3], and packing worker[4]*] and hepatitis A virus decay [*after farmworker contact[5], container storage[6], conveyer belt transfer[7], and packing worker[8]*])	$n_{decay} = 10^{(log_{10} n_{harv} - 1.057)}$ [1] $n_{decay} = 10^{(log_{10} n_{cont} - 1.057)}$ [2] $n_{decay} = 10^{(log_{10} n_{belt} - 1.057)}$ [3] $n_{decay} = 10^{(log_{10} n_{touch} - 1.057)}$ [4] $n_{decay} = 10^{(log_{10} n_{harv} - decay_{HAV} * 7)}$ [5] $n_{decay} = 10^{(log_{10} n_{cont} - decay_{HAV} * 7)}$ [6] $n_{decay} = 10^{(log_{10} n_{belt} - decay_{HAV} * 7)}$ [7] $n_{decay} = 10^{(log_{10} n_{touch} - decay_{HAV} * 7)}$ [8]
Dose response (*after decay for norovirus and hepatitis A virus*)	$risk = 0.722 * (1 - e^{\frac{-n_{decay}}{1106}})$

n_{irw} is the virus contamination on the produce item following irrigation water use.
n_{harv} is the virus contamination on the produce item following farmworker contact.
n_{cont} is the virus contamination on the produce item following container storage.
n_{belt} is the virus contamination on the produce item following conveyer belt transfer.

Table 2: QMRA model parameter estimates and uncertainty distributions.

Parameter (Units), *notation*	**Value(s), *virus or produce***	**Distribution**	**Source**
Irrigation water concentration (GEC/L), C_{irw}	551.55 (57, 1494.6), *NoV** (8.53×10^{-4}, 7.94×10^{-3}), *HAV*	Triangular Uniform	(1-4) (5)
Irrigation flow rate (L/cm^2), I_{iw}	0.0025		(6)
Produce surface area (SA) (cm^2), w_{prod}	226, *leafy green* $\mu = 258.55$, $\sigma = 23.25$, *melon* $\mu = 10.64$, $\sigma = 1.67$, *berry* $\mu = 101.45$, $\sigma = 15.35$, *vine/stalk*	Point Normal Normal Normal	(6) (7) (6) (7)
Hand contamination (GEC/hand), C_{hand}	3779.99 (0, 28183.83), *NoV** $\alpha = 0.98$, $\theta = 1.55$, *HAV*	Triangular Gamma	(8) (6)
Produce-to-hand transfer (%), f_{prod}	(0.105, 0.175), *NoV* 0.092 (0.083, 0.101), *HAV**	Uniform Triangular	(9, 10) (11)
Hand-produce contact (cm^2), w_{harv}	225, *non-berry* 2.1, *berry*	Point	Assumed (6)
Total hand SA (cm^2), w_{hand}	267.5 (225, 327.5)*	Triangular	(12)
Hand-to-produce transfer (%), f_{hand}	(0.123, 0.237), *NoV* 0.092 (0.083, 0.101), *HAV**	Uniform Triangular	(9) (11)
Container and produce transfer (%), f_{cont}	(0.027, 0.041)	Uniform	(13)
Proportion of produce SA that touches container (%), π_{cont}	0.25 (0, 1)*	Triangular	Assumed & (6)
Number of container reuses, $cont_{reuse}$	(0, 1, 2, 3, 4, 5)	Discrete uniform	Assumed
Rinse water concentration (GEC/L), C_{rinse}	0.6 (0, 116), *NoV** 0, *HAV*	Triangular Point	(14) (15, 16)
Rinse removal (%), f_{rinse}	(1, 2), *NoV* (0.05, 1.25), *HAV*	Uniform	(17) (18)
Volume of water retained on produce (L), V_{rinse}	0.2, *leafy green* 0.1, *melon* 4.32×10^{-4}, *berry* 0.025, *vine/stalk*	Point	(19) Assumed & (19)
Conveyer belt and produce transfer (%), f_{belt}	(0.024, 0.027)	Uniform	(13)
Proportion of produce SA that touches the conveyer belt (%), π_{belt}	(0.25, 1)	Uniform	(6)
Number of conveyer belt reuses, $belt_{reuse}$	(0, 1, 2, 3, 4, 5)	Discrete uniform	Assumed
Hepatitis A virus decay, $decay_{HAV}$	0.29 (0.273, 0.307)*	Triangular	(18)

*Value outside parentheses is most likely value and values within are range for triangular distribution.

Table 3: Produce Rule interventions, parameter estimates, and uncertainty distributions.

Intervention	Parameter (Units), *notation*	Value(s), *virus*	Distribution	Source
Avoid direct produce contact	Drip irrigation use (GEC/L), C_{irw}	0	Point	Assumed
Improved untreated water quality	Well water concentration (GEC/L), C_{irw}	μ=1.69, σ=1.40, *NoV* 0, *HAV*	Lognormal Point	(14) (20)
Measures to prevent worker contamination	Viral transfer from produce to gloves, f_{prodg}	(0.18, 0.26)	Uniform	(10)
	Viral transfer from gloves to produce, f_{handg}	(0.002, 0.098)	Uniform	(10)
Using hygienic worker practices	Handwashing removal efficacy ($\log_{10}$(GEC)), hw_{eff}	0.5 (0, 2)*	Triangular	(21-24)
	Handwashing probability, *P(hw)*	0.29	Point	(25)
Standards for equipment to prevent contamination	Container cleaning, $cont_{clean}$	0	Point	Assumed
	Conveyer belt cleaning, $belt_{clean}$	0	Point	Assumed

*Value outside parentheses is most likely value and values within are range for triangular distribution.

Table 4: Norovirus and hepatitis A virus consumer risk of infection.

Virus	Commodity	Scenario[1]	Risk of infection[2]
NoV	Berry	1, long	3.00E-02
		1, short	2.13E-02
		2, long	2.14E-02
		2, short	1.23E-02
	Leafy green	3, full	8.88E-02
		3, long	1.04E-01
		3, short	1.01E-01
	Melon	4, full	1.63E-01
		4, long	1.05E-01
		4, short	1.03E-01
	Vine/stalk	5, full	8.66E-02
		5, long	1.38E-01
		5, short	9.13E-02
		6, full	8.46E-02
		6, long	8.98E-02
		6, short	1.04E-02
HAV	Berry	1, long	1.37E-08
		1, short	7.35E-09
		2, long	7.23E-09
		2, short	7.48E-10
	Leafy green	3, full	9.21E-07
		3, long	7.55E-07
		3, short	7.22E-07
	Melon	4, full	9.88E-07
		4, long	7.46E-07
		4, short	7.28E-07
	Vine/stalk	5, full	1.03E-06
		5, long	1.32E-06
		5, short	7.48E-07
		6, full	8.49E-07
		6, long	7.30E-07
		6, short	7.15E-09

[1]Scenario is defined in Methods section I. Model overview

[2]Risk of infection is the probability of becoming infected after consuming the produce item. The risks are baseline, which is defined as the probability a consumer becomes infected from eating a produce item contaminated during normal harvest and packing operations where Produce Rule interventions were not implemented.

Figures

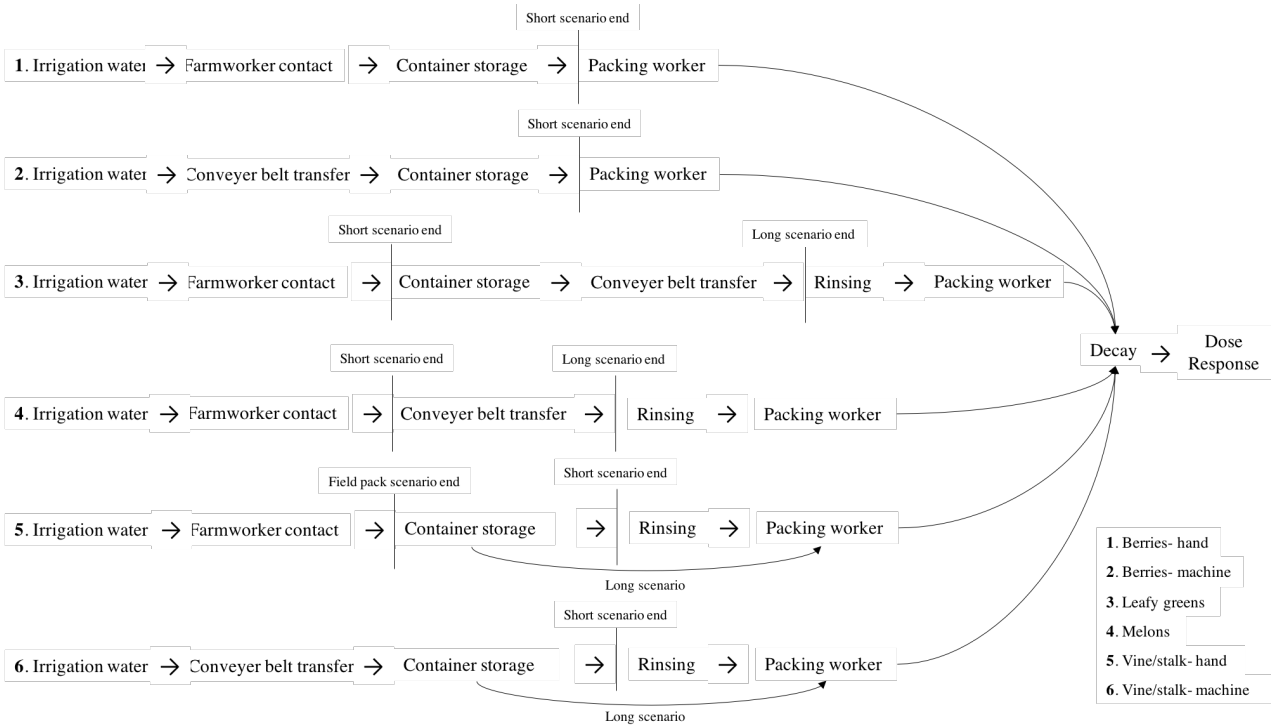

Figure 1: Overview of six possible combinations of harvest and packing stages by produce commodity. Four possible scenario lengths (full, long, short, and field pack) were considered. While the exact scenario length definitions vary by produce commodity, berries were modeled with long and short scenarios, leafy greens and melons with full, long, and short scenarios, and vine/stalk grown with full, long, short, and field pack. In general, full scenarios are defined as the longest of the four scenarios, long scenarios are defined as second longest of the four scenarios, short scenarios are defined as the second shortest of the four scenarios, and field pack scenarios are defined as the shortest possible of the four scenarios. The full scenario is represented by the entire row while the vertical lines signify the final stage for each of the other scenario lengths prior to decay. The long scenario for vine/stalk grown is represented by the entire row except for rinsing (as indicated by the arrow).

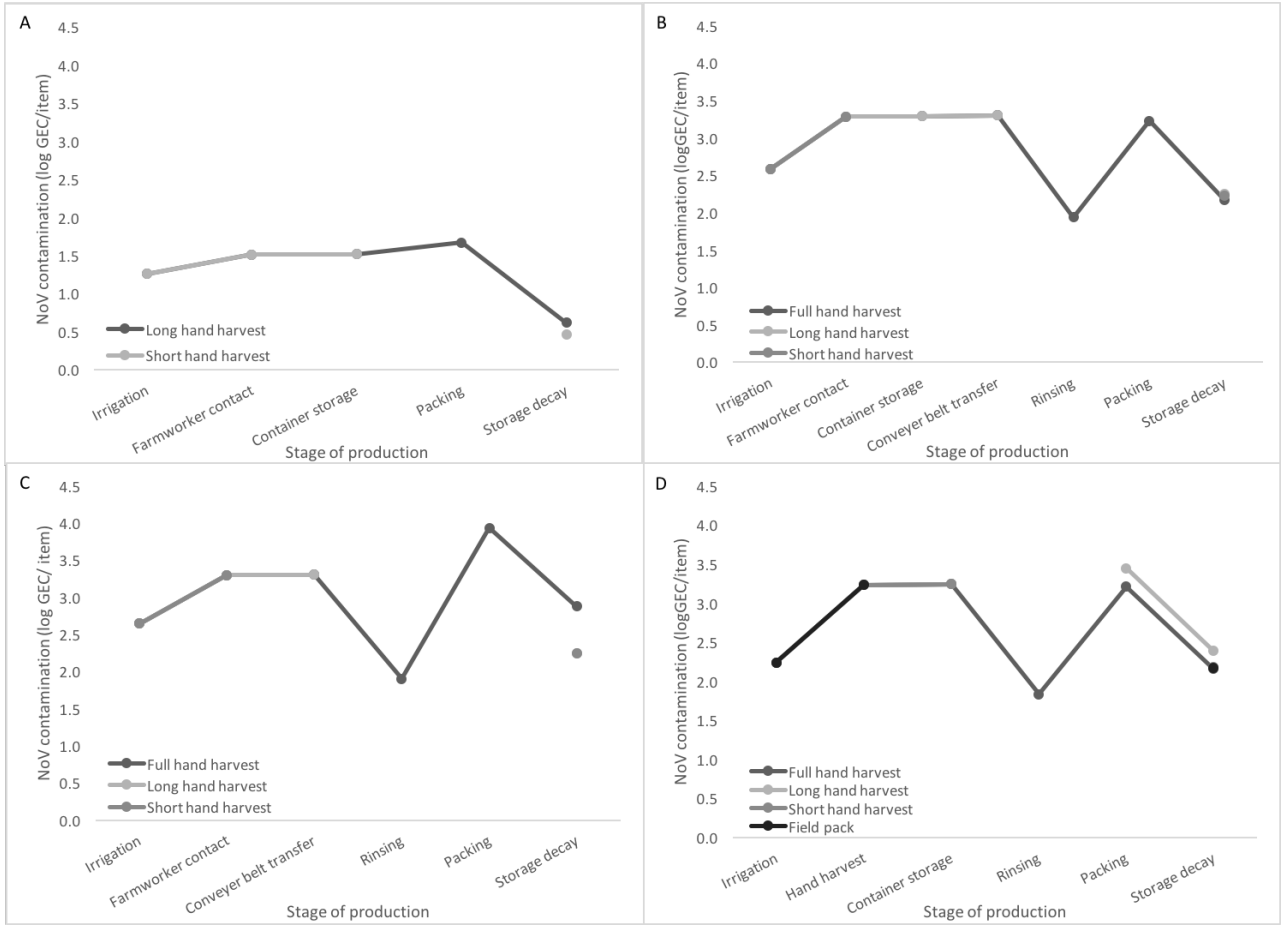

Figure 2: $\log_{10}$ norovirus (NoV) contamination fluctuation throughout harvest and packing. Four produce commodities (berries [A], leafy greens [B], melons [C], and vine/stalk [D]) were compared for all possible harvest and packing scenarios. The change in line color indicates the end of a shorter harvest and packing scenario and the new color is the continuation of the next longest harvest and packing scenario. For all produce items, NoV contamination after irrigation water was the first point displayed. The estimated final viral contamination after seven days of decay is represented with the final single point above storage decay stage for each scenario.

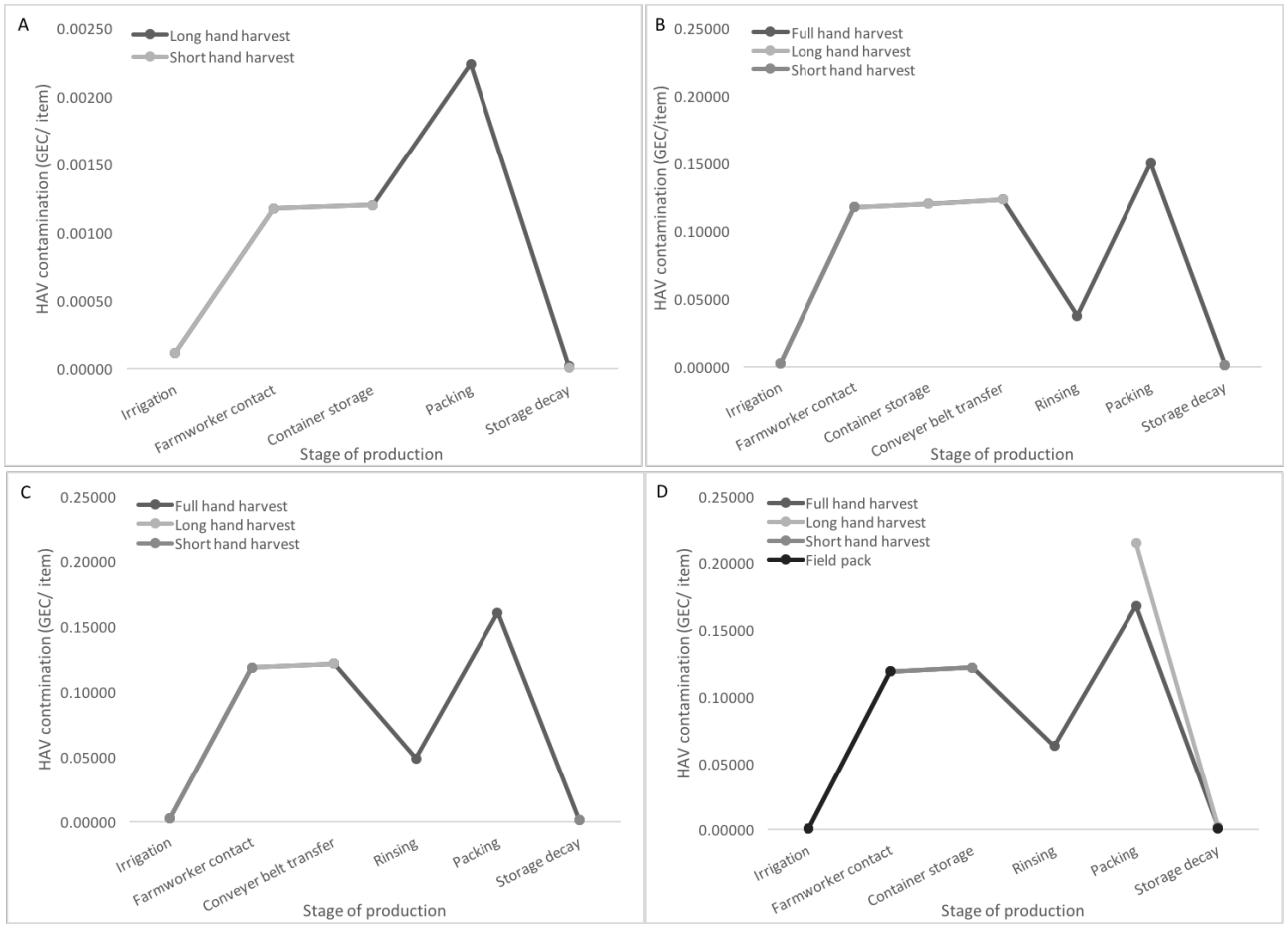

Figure 3: Hepatitis A virus (HAV) contamination fluctuation throughout harvest and packing. Four produce commodities (berries [A], leafy greens [B], melons [C], and vine/stalk [D]) were compared for all possible harvest and packing scenarios. The change in line color indicates the end of a shorter harvest and packing scenario and the new color is the continuation of the next longest harvest and packing scenario. For all produce items, HAV contamination after irrigation water was the first point displayed. The estimated final viral contamination after seven days of decay is represented with the final single point above storage decay stage for each scenario.

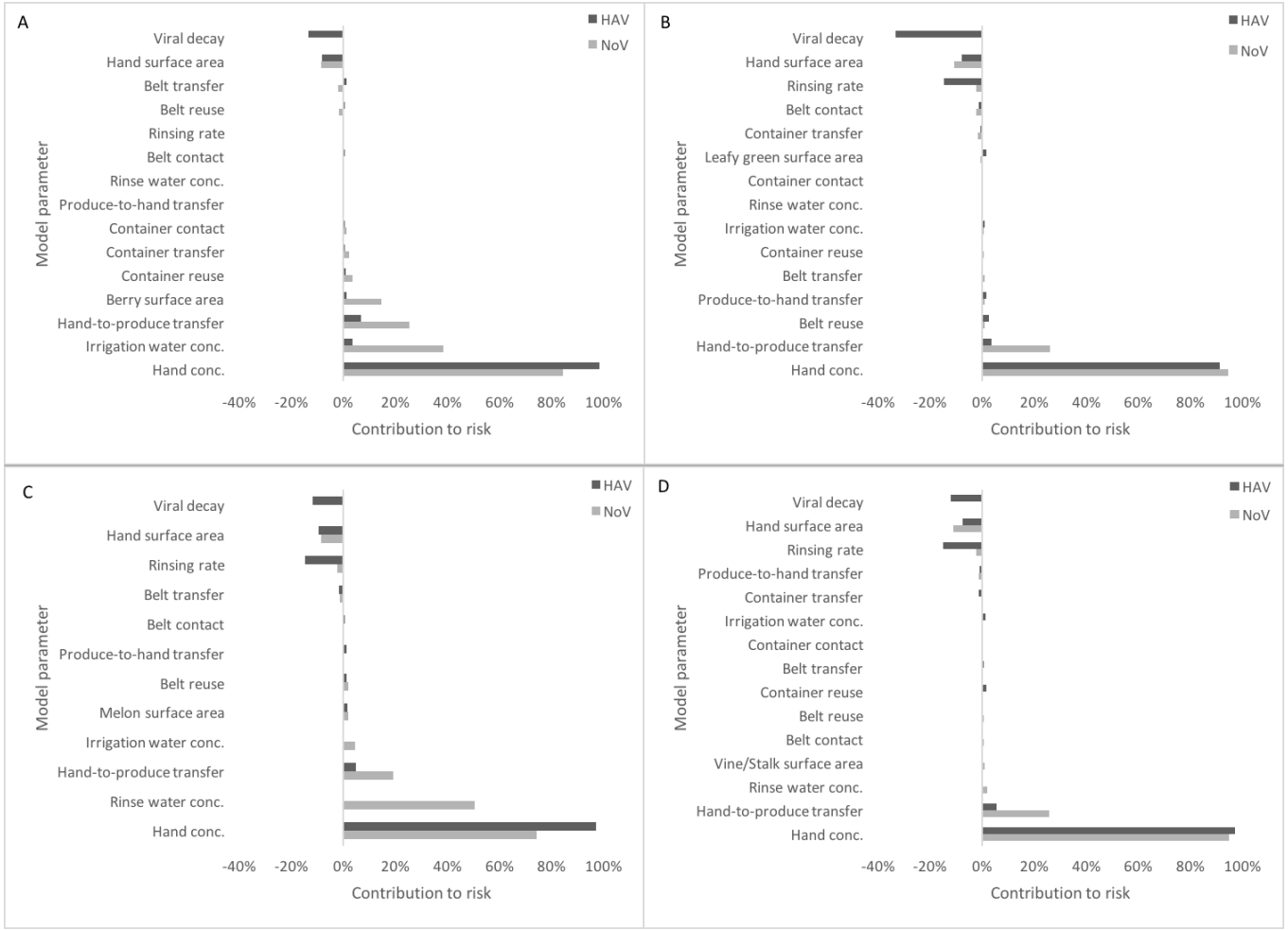

Figure 4: Sensitivity analysis of norovirus and hepatitis A virus consumer risk of infection for the four produce commodities (berries [A], leafy greens [B], melons [C], and vine/stalk grown [D]) for the longest harvest and packing scenarios without any interventions.

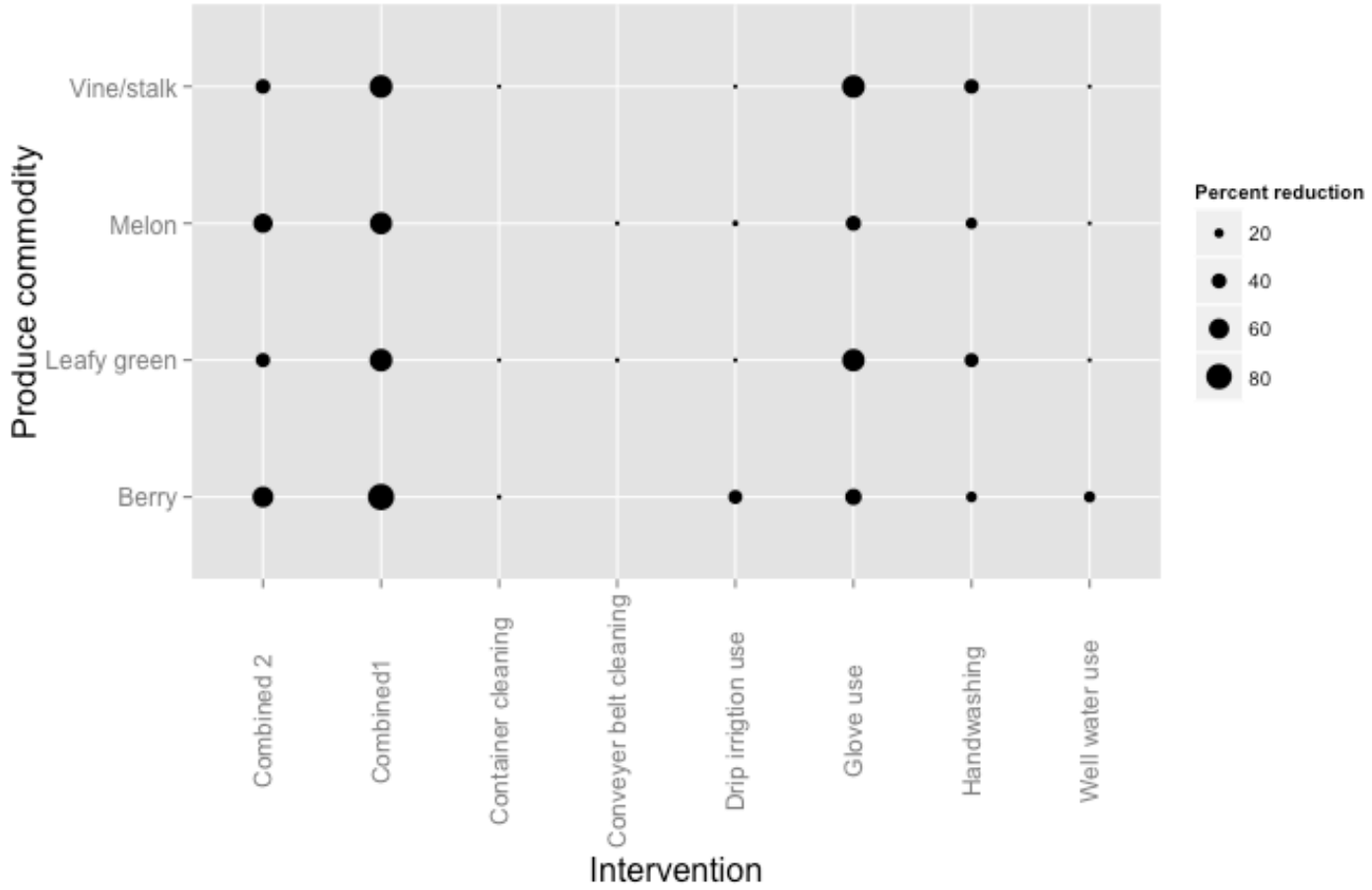

Figure 5: Percent reduction of norovirus consumer risk of infection due to the application of single and combined interventions for the four produce commodities. Combined 1 – drip irrigation, glove use, container cleaning, and conveyer belt cleaning and Combined 2 – drip irrigation, handwashing, container cleaning, and conveyer belt cleaning.

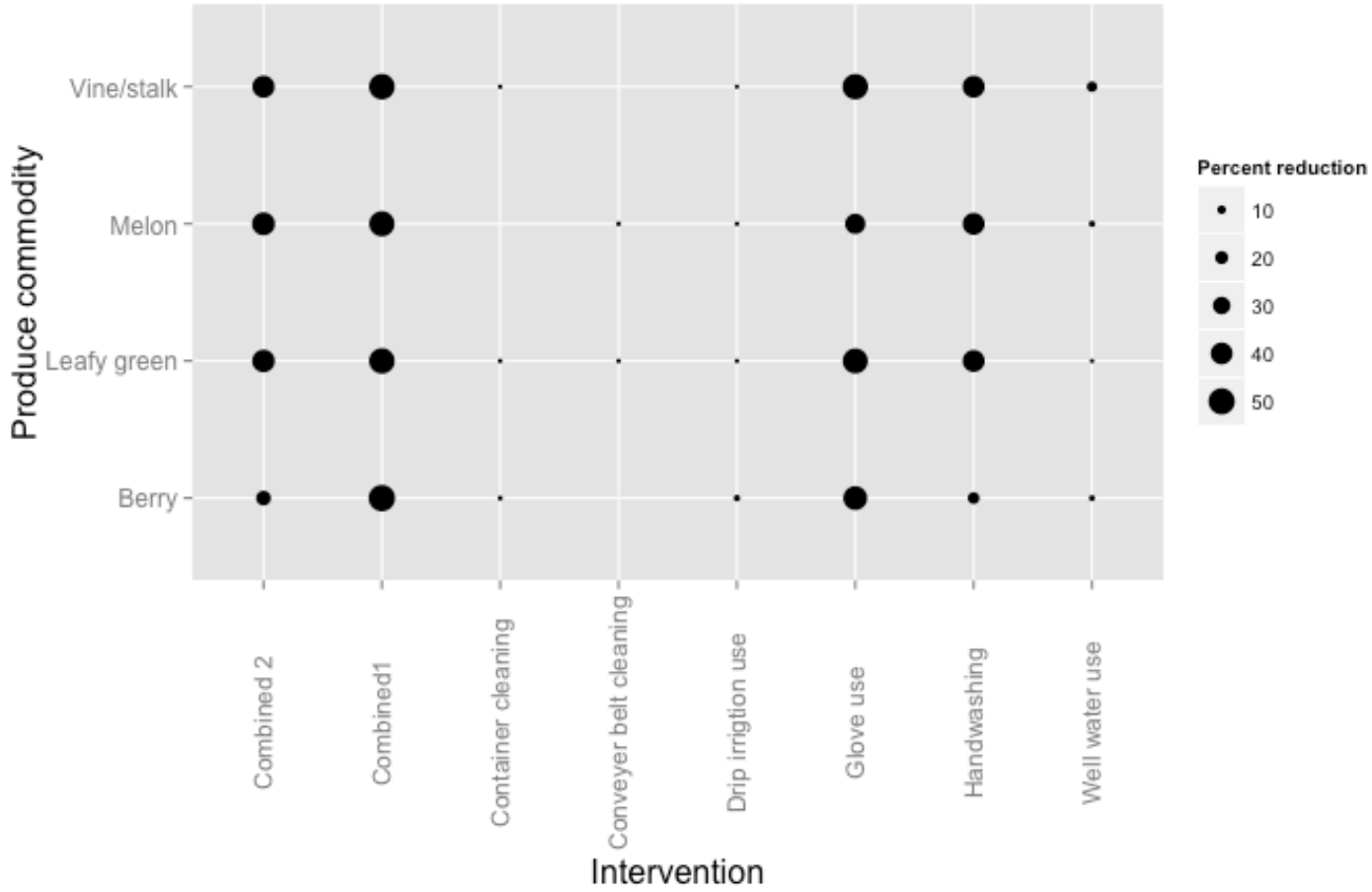

Figure 6: Percent reduction of hepatitis A virus consumer risk of infection due to the application of single and combined interventions for the four produce commodities. Combined 1 – drip irrigation, glove use, container cleaning, and conveyer belt cleaning and Combined 2 – drip irrigation, handwashing, container cleaning, and conveyer belt cleaning.