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To Be Submitted To: Microbial Risk Analysis

Research Paper

Farm to Fork Quantitative Microbial Risk Assessment for Norovirus on Frozen Strawberries

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Abstract (400 words or less)

Foodborne illness outbreaks have been increasingly linked to the consumption of fresh and frozen berries that were contaminated with pathogenic viruses, such as human norovirus (NoV). Contamination of berries is assumed to take place at harvest by the use of contaminated water for pesticide dilution, irrigation water source or by shedding berry pickers in the field. A quantitative microbial risk assessment simulation model was built to replicate the largest known NoV outbreak which sickened about 11,000 people over a 3-week period. The outbreak occurred in Germany in 2012 when contaminated frozen strawberries were served at nearly 400 schools and daycare centers. The risk model explicitly assumed that all contamination would arise from NoV contamination of surface water used for pesticide dilution. Input data was collected from the published literature, observational studies and assumptions. The model starts with contamination of the berries in the field, and proceeds through transportation to processing facility, washing, sanitizing, freezing, frozen transport to cargo ship, transport view of cargo ship, transport to distribution center, frozen storage at the distribution center, transport to the catering facility, food service preparation and consumption, dose response, and predicted illnesses. A total of 21 scenarios were chosen to evaluate the impact of model parameters on the number of illness associated with NoV contamination of berries. Scenarios evaluated include the initial level of NoV in surface water, the effect of seasonality on the prevalence of NoV in surface water, the strength of the pesticide used, the volume of water used to dilute the pesticide, temperature during transportation to processing facility, washing and sanitizing conditions at processing facility and preparation (heat-treatment) of berries prior to consumption. Scenarios were compared via the Factor Sensitivity technique where the logarithm of the ratio of mean illnesses was used to compare different assumptions. The input that had the greatest effect on increasing

in the number of illnesses was a high NoV concentration in the water (8 log Genome Copies/L) when compared to the baseline scenario with resulting mean illnesses of 7,964 illnesses and ~2 illnesses, respectively. This assumption about the concentration of virus in the pesticide makeup water was the only variable capable of producing an outbreak similar to that observed in Germany in 2012. Heat-treatment of the berries, use of a pesticide with strong antiviral effect, and assumption about the virus concentration in the pesticide make-up water had the largest impact on decreasing illnesses.

Introduction

Norovirus (NoV) is the leading cause of foodborne disease worldwide, causing an estimated 685 million cases of acute gastroenteritis annually [1-3]. Although most deaths occur in developing countries, NoV continues to be a significant burden to high-, middle- and low-income countries [4]. NoV are transmitted primarily from person-to-person via the fecal-oral route or from aerosolized vomit. The virus may also be transmitted indirectly through contaminated food, water, fomites and environmental surfaces.

The average incubation period for NoV-associated gastroenteritis is 12 to 48 hours and is typically followed by symptoms including nausea, vomiting and diarrhea with abdominal cramps. The average probability of infection for a single NoV particle was estimated to be near 50% (0.5), exceeding any other virus studied thus far [5]. Viral load from an infected person has been shown to range from 10^8 to 10^{12} viral particles per gram of feces [5-7]. Shedding of NoV can start in the pre-symptomatic phase as early as 3 to 14 hours before onset and those who are infected with NoV can continue to shed it in their feces for several months after initial infection [6, 8, 9]. NoV stability in the environment is thought to be due to its lack of a viral envelope; it can survive freezing and heating, can survive for weeks on surfaces and is resistant to many common chemical disinfectants that are effective for bacteria [10, 11].

The market for frozen berries has continued to succeed because of the availability to consume the product year-round [12], even though fresh and frozen berries have been linked to NoV and Hepatitis A virus (HAV) foodborne disease outbreaks around the world [13-21].

The risk factors for contamination of berry fruits at primary production with NoV are not well documented in the published literature due to limited data. Suggested risk factors based on what is known for other pathogens associated with fresh produce include (1) environmental factors such as heavy rainfall that increase the transfer of NoV from sewage runoff to irrigation water sources or fields (2) use of sewage-contaminated agricultural water as irrigation water or for the application of agricultural chemicals such as pesticides and (3) poor food handlers health and hygiene or contaminated equipment at harvest or post-harvest [14, 22].

Temperature is considered a major factor influencing virus persistence, although it is not considered an effective mitigation strategy for fresh berries because persistence of enteric viruses is higher at low temperatures and quality loss (e.g. decay) generally increases with an increase in temperature [23]. Some berries undergo washing (strawberries, blueberries) prior to freezing, while more fragile berries (e.g. raspberries and blackberries) may not get washed as it can lower product quality. The presence of NoV in frozen berries has been linked to many outbreaks of gastroenteritis throughout the world, which clearly shows these viruses survive and remain infectious after freezing [15-18, 20].

Quantitative microbial risk assessment (QMRA) is used to better understand and manage food safety risks. Models are developed to describe the transmission of pathogens over a specified food production chain. These models may cover the complete farm to fork pathway or only a portion of it. De Keucklaere et al. analyzed published risk assessments that studied viruses, fresh produce, irrigation and wash water from food safety and water management perspectives [24]. Several studies have presented quantitative risk assessments showing the impact of contaminated water on the spread of NoV on leafy greens and other crops consumed raw [25-27]. Other risk assessments and exposure assessments have focused on the spread of NoV by ill food handlers, highlighting the importance of hand hygiene measures in foodservice facilities [28-32]. A quantitative farm-to-fork exposure model was developed describing the spread of NoV and Hepatitis A during the harvesting and processing of leafy greens and berry fruits [33].

Here we consider the source of the contamination, NoV inactivation and survival on berries, as well as processing at the facilities and preparation of the berries prior to consumption. Our QMRA is designed to simulate the largest known outbreak arising from NoV-contaminated berries, which occurred in 2012 in Germany and was linked to frozen strawberries sourced from China.

Materials and methods

Overview of the development of the risk model. Data from the peer reviewed literature regarding NoV behavior in fresh and frozen fruit were used to develop the model. The model parameters and their corresponding probability distributions are described in Table 1. Inputs were assumed to be independent, although some inputs may have dependencies (e.g. strength of pesticide diluted in a specific volume of water). The risk model assumes that contamination of strawberries strictly arises from NoV contamination in the surface water. Other sources of contamination will be explored in subsequent research.

Contamination source. Berries are susceptible to contamination with NoV through spraying with pesticides mixed with contaminated water. Sources of water used for agriculture applications can be ranked by risk of microbiological contamination and are in order of increasing risk: rain water, ground water from wells, surface water, and raw or inadequately treated wastewater [34]. The main sources of NoV in surface and groundwater are sewage discharge and human fecal waste. Pesticides are often diluted in different volumes of water depending on the crop. Although NoV does not replicate in water, it can remain infectious in water for prolonged periods of time. Seitz et al. [35] found through human challenge studies that NoV remained infectious in water for at least 61 days.

We assumed the use of drip irrigation in our model. Drip irrigation itself is an unlikely point of microbial contamination because water is applied to the soil or directly at the roots of the plant, far from the edible fruit. Drip irrigation is a preferred method for berries since berries are particularly susceptible to mold growth which is likely to occur if overhead irrigation is used [28]. Limited information on NoV adherence to and persistence on strawberries exists, and therefore were not considered in this model.

Our model assumes that all of the pesticide applied adheres to the edible fruit, which implied no run-off (or pesticide drift). Pesticide drift occurs in many crops [36], including strawberries, and is complex and multi-faceted [37]. Given the complexity of modeling this aspect of agricultural production, we have chosen the simplifying assumption above.

Our model also assumes that contaminated the water is applied immediately before harvest. Many pesticides have a prescribed pre-harvest interval, which specifies the length of time after application that is required prior to harvest. During this pre-harvest interval any virus particles present on the berries would be subject to environmental stresses, including exposure to sunlight and drying, and thus would lose viability. Unfortunately, we have no knowledge of the pre-harvest interval period between pesticide treatment and picking of strawberries in China. Information on pre-harvest interval for strawberries in the US [38] shows that pre-harvest intervals of 0 to 1 days are quite common for many of the pesticides used on strawberries. Given the lack of the published data on pre-harvest intervals in China, common short pre-harvest intervals in the US, and minimal declines observed over these short intervals, we have chosen to make the simplifying assumption that no reduction in virus population occurs pre-harvest.

Effects of washing and chlorine application. Data from Butot et al. [39] and Predmore et al. [40] on the effect of washing and sanitizing berries prior to freezing were extracted from the scientific literature and analyzed for inclusion in the model. Wash water as a source of contamination was not considered in this model. Some berries (e.g. strawberries) are washed with water before freezing, but more fragile fruits (e.g. raspberries) are not [41]. Washing fruits or vegetables with water alone generally yields no more than a 2-log reduction in microbial concentration [42]. Excessive chlorine concentrations must be avoided as they can affect sensory quality [43]. It has been shown that prolonged treatment of berries with chlorinated water did not result in a significant increase in the effectiveness, although various surrogates have been shown to be affected differently [44-46].

Time and temperature during transportation and storage. Strawberries were assumed to be transported on a refrigerated truck after harvest to a processing facility. The baseline simulation used 4 °C as the temperature for transporting strawberries to the processing facility, as literature data showed that the fruit quality is not adversely affected at this temperature [47]. Data on NoV survival and inactivation at various storage temperatures was used to determine the concentration of NoV on strawberries over time [48-50]. The simulation assumed that once the strawberries arrived at the processing facility, they were exposed to washing and sanitizing steps, followed by individually quick freezing (IQF). The frozen strawberries were transported by cargo ship, assuming a transportation time of 25-30 days from the port in China to the port in Germany [51].

Process of freezing berries. Although all processing steps are important in maintaining the quality of berries, the freezing process in the most critical. The primary goal in freezing fruit is to maintain the original characteristic product quality. This is best achieved by freezing rapidly and careful handling before and after freezing. If freezing is slow, large ice crystals will form and can break down food structures. This results in high drip losses and a deterioration in product quality. Several factors that affect freezing rates include the type of freezing equipment used, initial berry temperature and product characteristics (e.g. size, shape and structure). Individually quick freezing (IQF) is one of the quickest ways of freezing small fruits. Advantages of the IQF process include short freezing times, efficient heat transfer and less product dehydration [52]. Freezing has no significant effect on the infectivity of NoV, and virus particles appear to retain their structural and genome integrity after freezing and during multiple freeze-thaw cycles [53]. Data from Butot et al. [39] were used to determine the log reduction of NoV during frozen storage.

Preparation at catering facility and consumption. The serving size was selected after an internet search of similar recipes and it was decided that ~4 strawberries per serving of strawberry compote was an appropriate serving size. While we could have used a more complex assumption regarding serving size, since our specific objective was to simulate the 2012 German outbreak (rather than for example all domestically consumed frozen strawberries in the United States), this simplified assumption suits our purpose. Should a future risk assessment need to address more complex scenarios, food consumption databases could be used to estimate variable serving sizes.

The baseline model assumed that strawberries were not heated prior to consumption. Different heat treatment scenarios were considered based on data available in the literature. The effect of mild heat treatment (30s at 65°C) was simulated with a normal distribution with mean log reduction and standard deviation of 1.86 ± 0.32 [54]. High heat treatment (15s at 75°C) resulted in a mean log reduction and standard deviation of 2.81 ± 0.39 [54, 55]. NoV inactivation data was based on NoV surrogates including feline calicivirus F9 (FCV) and the murine norovirus 1 (MNV-1).

Dose-response modeling. Dose-response models mathematically link exposure to probability of infection and/or illness, where exposure represents the dose ingested [56]. Illness (i.e. symptoms of vomiting and/or diarrhea) is the endpoint of this risk assessment. An existing dose response model for the probability of illness among infected subjects was used with parameters η and r as given by Teunis et al. [5]. The risk of illness is considering the dose as the sum of dose from GI and GII and the parameters, η and r, were assumed to be independent of the NoV genogroup (GI or GII), as well as secretor status [56, 57]. The values for η and r in this model are 2.55 x 10^{-3} and 0.086, respectively Equation 1:

$$P\,(\text{ill}/\text{dose}, \eta, r, \text{inf}) = 1 - (1 + \eta \times \text{dose})^{-r} \qquad (1)$$

Simulation modeling. Extracted data and user inputs were entered into an Excel (Microsoft, Redmond, WA) spreadsheet as described in Table 1, discussed in detail in the results and discussion section below. The Excel add-in @Risk (Palisade Corporation) was used to perform Monte Carlo simulations of 100,000 iterations for each scenario evaluated. Scenarios were constructed to reflect the best estimates of the number of servings (~100,000) believed to be involved in the outbreak. The baseline simulation condition is shown in detail in Table 1, but briefly: The concentration of NoV in water was modeled as a uniform distribution from 1.27 to 4.84 log genome copies (GC)/L. The volume of liquid used to apply pesticides was 200 L/ha. The seasonality for prevalence of NoV in surface water was represented by a triangular distribution assuming a minimum of 12%, a most likely value of 12% and a maximum value of 95% per L. The effect of pesticide on reduction in NoV concentration was described by a lognormal distribution with mean 0.35 and standard deviation of 0.56 log GC/L where the resulting is shifted by -0.21. The truck temperature for transport from the field to the freezing location was assumed to by 4 °C, with no change in NoV concentration at that temperature. We assumed that the strawberries were washed in cool (18 °C) water resulting in a reduction of NoV concentration following a normal distribution (mean 0.67, standard deviation 0.33) log GC NoV. We also assumed the strawberries were sanitized using 200 ppm chlorine, resulting in a reduction of NoV concentration following a normal distribution (mean 1.35, standard deviation 0.24) log GC NoV. The baseline assumed no heating step during foodservice preparation resulting in no change in NoV concentration prior to consumption.

Sensitivity analysis. A total of 21 scenarios were chosen to evaluate the impact of model parameters on the number of illness associated with NoV contamination of strawberries, and these are shown in Table 2. Scenarios evaluated include the initial level of NoV in surface water, the effect of seasonality on the prevalence of NoV in surface water, the strength of the pesticide used, the volume of water used to dilute the pesticide, temperature during transportation to processing facility, washing and sanitizing conditions at processing facility and preparation (heat-treatment) of strawberries prior to consumption. Each scenario was selected to explore variations around each of the 8 parameter baselines to test the individual impact of a given parameter on the change in the number of illnesses. Equation 2, adapted from Zwietering et al. [58], represents the scenario factor sensitivity (FS), which is the order of magnitude of the importance of each scenario relative to the baseline. High factor sensitivity values equate to a high sensitivity to the variations and reveal what factors have greater effects on the number of illnesses (N).

$$\mathrm{FS_k} = \log\frac{N_k(variation)}{N_k(baseline)} \qquad (2)$$

Results and Discussion

As noted above, Table 1 summarizes the Excel spreadsheet used for risk calculations and explains how the variables are linked in the risk assessment. The first column represents the spreadsheet cell designation for the variable on that line of the table. The next column is a description of the variable in words, with bold headers describing each section of the risk assessment. The third column is either a number, formula or an @Risk formula representing the value of a given cell. The fourth column shows the units of the variable in the third column. The last column represents the source of the information used to determine the value of the variable. The source can be either user input, from the published literature or calculated from other cells in the spreadsheet.

The first section of Table 1 (In field) represents variables describing the environmental factors that influence NoV contamination on strawberries in the field. Information involving conditions strawberries were exposed to during the outbreak in Germany from strawberries harvested in China is limited [17]. Because much of this information is unknown, important variables were included from the published literature or as user input. Key in-field variables include starting concentration and prevalence of NoV in surface water, fraction of positive water liters used for pesticide delivery and the ability of pesticides to reduce NoV concentration in the pesticide water. The NoV concentration in water is expressed as a uniform distribution [59] and the prevalence of NoV in the water is expressed as a triangular distribution based on the published literature [60]. Surface water (river water, lake water, canal water, etc.) is typically used for diluting the pesticide that will be sprayed onto strawberry plants [28, 61, 62]. The effect of pesticides on the reduction in NoV from water used to dilute pesticides is expressed as a log normal distribution based on data extracted from Verhaelen et al. [63]. Various pesticide strengths were evaluated in the sensitivity analysis. A binomial distribution was used to determine how many liters were positive which was used to calculate the effective concentration per liter, considering positive and negative liters. The level of NoV on the strawberries at harvest was determined by calculation of the effective concentration per liter, considering positive and negative liters multiplied by the volume of water sprayed on strawberries.

The next section (Transportation to processing facility) presents data extracted from Kurdziel et al. [48], Verhaelen et al. [49] and Dawson et al. [50] to estimate the effect of temperature on the persistence and survival of NoV during transportation to the facility. Relevant data extracted include mean log reduction and the standard deviation of the log reduction at 3 different temperatures (4, 10 and 21°C). Refrigeration temperature (4°C) was used as the baseline with no log reduction of NoV observed [48, 49].

The simulation assumes strawberries were washed and sanitized after receipt at the processing plant. Data were extracted from the peer reviewed literature [39] to estimate the degree to which washing reduces NoV contamination on strawberries (Table 1, Washing log reduction). Although the primary purpose of the washing step is to remove dirt and debris rather than achieve a microbial reduction, reductions in NoV concentration has been shown when berries were washed with warm or cold water [39]. The simulation assumed that sanitizer was applied to the strawberries after washing. The baseline used for spray sanitizer data on berries was a 200 ppm chlorine solution (Table 1, Sanitizer log reduction). Chlorine concentrations for produce and wash water are generally ≤ 200 ppm [42]. The variables in this section, as well as the washing section, express the variability in log reduction by using the RiskNormal function using the mean log reduction and standard deviation from the published literature [64]. Scenarios with varying sanitizers (5ppm ClO_2 and 10ppm ClO_2) using the RiskNormal function, as well as no application of sanitizers, were considered (Table 2). The washed and sanitized strawberries undergo the IQF method (Table 1, Freezing process). Although commercial IQF is generally thought to cause little change in the concentration of microorganisms, no peer reviewed data on survival of NoV during the IQF process was found. A single non-food related study that examined the effect of freeze-thaw cycles on NoV titers found that both capsid integrity and viral RNA titers remained stable through repeated freeze/thaw cycles [53], so we assumed that freezing had no effect on NoV concentration.

The next three sections of Table 1 (Truck to cargo ship, Transport via cargo ship and Transport to distribution center) model the expected change in NoV level on strawberries during these three phases of frozen storage. Data for log reduction after 90 days frozen storage was extracted and calculated from Butot et al. [39] using a normal distribution (mean 0.4, standard deviation 0.18), and this was adjusted to estimate the log reduction per day. A uniform distribution was used to model the variability in each leg of transport. Depending on the transportation step, the length of storage was either determined by data from the literature or user input. Ranges of 0.5 to 2 days during transport from China distribution center to cargo ship, 25 to 30 days on cargo ship from China to Germany [51] and 0.5 to 5 days on a truck from the cargo ship to distribution center in Germany, were selected from uniform distributions.

The Frozen storage at distribution center section estimates the time and corresponding log reduction during storage at the German frozen food distribution center. The time at the distribution center was expressed as a uniform distribution ranging from 0.5 to 90 days. The simulation then assumed the product was transported on a truck, frozen, to the catering facility. The time for transport from the frozen food distribution center to the catering facility was expressed as a uniform distribution ranging from 0.5 to 5 days. All the frozen transport time variables, except for the transport by cargo ship are designated as user inputs, as no good source for these data were readily available. Since frozen strawberries can maintain their quality for 14-18 months [65], the values selected here may underestimate the declines in NoV populations observed during frozen storage.

The next section of Table 1 (Foodservice preparation and consumption) represents the expected change in NoV level on frozen strawberries depending upon preparation method. Strawberry compote made with unheated or cold frozen strawberries was the food type associated with the large NoV outbreak in Germany [17, 18]. German kitchens not associated with the outbreak almost exclusively served the strawberries after “heating”, but the temperatures reached during that heating processes were unknown. Our baseline model assumes no heat step was applied to the strawberries prior to serving, and thus no thermal inactivation of NoV. Two different preparation steps were used to represent alternative scenarios where frozen berries were heated prior to consumption: mild heat treatment for 30s at 65°C by using the @Risk function RiskNormal (1.54, 0.32) and high heat treatment for 15s at 75°C with a log reduction of RiskNormal (2.81, 0.39).

The next section of Table 1 (Serving and dose response) includes the serving size of the number of strawberries consumed per dessert, calculations that convert the log genome copies (GC) per strawberry to the dose per serving, and the parameters of the dose-response model from Teunis et al. [5]. Based on the dessert implicated in the outbreak (strawberry compote) and extensive search of strawberry compote recipes for one serving, we assumed that 4 strawberries constituted a serving [17]. The model output was the probability of illness, which was used to calculate the number of illnesses. The probability of illness given the dose from the previous section was combined with the number of servings used per iteration in a binomial distribution to predict the number of illnesses arising from those servings.

Figure 1 shows a tornado plot representing a sensitivity analysis of the risk assessment. Since risk assessment models can be complex and may have intricate interactions between various inputs, it may be difficult to determine which model parameters contribute the most to variation in the output. Although there are many different approaches to sensitivity analysis, we used the method of Zwietering et al. [58] because the resulting Factor Sensitivity values distinctly show the sensitivity to individual variants. Figure 1 shows the log relative change in mean number of illnesses from alternative scenarios compared to the baseline scenario from 100,000 iterations. The scenario that had the greatest impact on the number of illnesses relative to the baseline was the assumption of a high level of NoV present in the water (8 log GC/L). This resulted in a mean of 7,694 illnesses (Table 2), whereas the baseline risk model resulted in a mean of only 1.89 illnesses. The top bar for Figure 1 shows a factor sensitivity of 3.6 calculated from these two values using equation 2: log(7,694/1.89). Mild- and high-heat treatment to strawberries had a significant reduction on the illnesses relative to the baseline, with 0.02 mean illnesses and 0 mean illnesses, respectively. Since it was not possible to calculate log(0/1.89), it was assumed that the minimum number of possible illnesses occurred in the high-heat treatment scenario (i.e. 1 illness in 100,000 iterations, log(0.00001/1.89) or a factor sensitivity of -5.3). Use of a pesticide with a strong antiviral effect also impacted the probability of illnesses (0.02 mean illnesses, for a factor sensitivity of 1.9). Relative to the other scenarios, seasonality of NoV prevalence in water and the truck temperature had the least effect on the outcome of illnesses, with factor sensitivities ranging from -0.5 to 0.2. Other key scenarios evaluated the volume of water used to dilute pesticides. Pesticides sprayed using large volumes of water may lead to a greater risk of viral contamination of the crop because the probability of contaminated water coming in contact with crops is higher and the concentration of pesticides is lower due to dilution, resulting in potentially greater viral persistence [63]. The baseline model assumed a volume of 200 L/ha was applied to the strawberries, and the sensitivity analysis showed that illnesses were 10-fold lower and higher (factor sensitivities of -1 and 1) vs. the baseline when 20 L/ha and 2,000 L/ha, respectively were used.

Figure 2 compares the distribution of predicted illnesses over the 100,000 iterations for the baseline and worst case (8 log GC/L in the surface water) scenarios, using both illnesses and log(illnesses). Figure 2a shows that for the baseline scenario most iterations (~70%) result in no illnesses, the average number of illnesses is ~2, and the distribution is highly skewed with one iteration resulting in over 400 illnesses. Figure 2b shows a much different picture for the 8 log GC/L in the surface water scenario. In this case the most frequent result is still no illnesses, but many more scenarios result in illness, with much less skewed distribution, mean illnesses over 7,000 and one scenario resulting in almost 40,000 illnesses. Figure 2c shows the baseline scenario on a log(illness) scale. Most iterations (~70%) result in no illnesses, but because log(0) is undefined, those iterations are indicated as such. Figure 2c makes it clear that when illnesses occur, the most common number of illnesses is 1, shown as 0 on the log illness scale, with frequency declining steadily. Figure 2d shows a similar log(illness) plot for the high illness scenario (8 log GC/L in the surface water). As with Figure 2b, the most common result is no illnesses. Figure 2d makes it clear that (as also seen in Figure 2c) that when illnesses occur, the most common number of illnesses is also 1 (shown as 0 on the log illness scale). The frequency of various illness rate declines and remains fairly constant from about 1.5 log (31 illnesses) to 3 log (1,000 illnesses), when the frequency increases to around the mean of 3.5 log, followed by a steady decline to the maximum number of illnesses (~4.5 log).

Figure 3 shows a comparison of the distribution of simulated virus particles per serving from 100,000 iterations of quantitative microbial risk assessment for Norovirus in frozen strawberries. The y-axis represents the logarithm of the relative frequency of observation of specific virus particle concentrations. A logarithmic transformation is used on this axis to better visualize frequency of low probability events, where zero represents 100% (i.e. all iterations of the simulation), -1 represents 10%, -2 represents 1%, etc. Panel (A) baseline scenario shows the baseline distribution of virus particles. As with all of the other scenarios, the most frequent prediction was for a serving to contain zero virus particles. The next most common prediction was for a serving to contain a single virus particle in about 4% of the iterations. as the predicted number of virus particles increases, the predicted frequency decreases. The highest predicted concentration of virus particles per serving in the baseline scenario was 17. Since that figure represents 100,000 iterations, those predictions showing a frequency of -5 represent a single iteration of the simulation. Panel (B) represents the baseline conditions plus high heat (15s at 75 °C) use during food service preparation. The highest number of virus particles predicted per serving in this scenario was only two, which occurred in less than 10 iterations of the simulation. Panel (C) shows the results from the scenario where highly contaminated (8 Log GC/L) water was used for pesticide application, and dramatically higher virus particle concentrations for serving were predicted, with the highest concentrations in excess of 80,000 virus particles. The pattern of contamination is however consistent with those shown in panel A and B, where the most frequent simulation result is still a serving containing zero virus particles. Panel (D) shows a scenario where the interaction between the use of highly contaminated (8 Log GC/L) water plus high heat (15s at 75 °C) use during food service preparation is presented. The most contaminated serving contains almost 800 virus particles, but this was only observed during one iteration of the simulation, and more than 97% of servings contained zero virus particles.

This risk assessment was undertaken to simulate the German 2012 NoV outbreak linked to frozen strawberries sources from China [18], but we also believe it can be adapted to other berry types. It was possible to develop a working QMRA model, which has identified available data and data gaps, and which is able to provide simulation results which approximate the German outbreak. The data gaps identified include information on persistence and survival of human NoV strains (instead of surrogates) in fresh and frozen strawberries and in response to heating. Our model shows that the German outbreak in 2012 could have resulted from the use of a highly contaminated water source applied to a large number of strawberries prior to harvest. Our model also predicts that thorough heating of frozen strawberries prior to serving would have a dramatic effect on risk. Following the outbreak that sickened ~11,000 people in Germany, the European Union (EU) regulation now requires 5% of consignments of frozen strawberries imported from China into the EU to be tested for norovirus, as well as recommending to the catering sector to heat-treat berries prior to consumption [18]. These two interventions appear to have prevented the recurrence of an outbreak the size of the German 2012 event. The use of a model-based risk assessment supports these risk management measures and would likely assist in comparison of the utility of additional intervention measures.

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Competing interests

The authors have no competing interests to declare.

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Table 1. Norovirus in frozen berries risk model using baseline parameters in @risk for farm to fork quantitative microbial risk assessment.

Cell	Variable	Value/Distribution	Units	Source
D2	**In field**			
D3	Starting concentration in water	=RiskUniform(1.2721,4.8428)	Log GC/L	[22, 63, 66-70]
D4	Log reduction of NoV in pesticide solution	=RiskLognorm(0.34575,0.5552,RiskShift(-0.21468))	Log GC/L	[63]
D5	concentration after mixing with pesticide	=D3-D4	Log GC/L	Calculated
D6	concentration after mixing with pesticide	=10^D5	GC/L	Calculated
D7	Prevalence of NoV in surface water	=RiskTriang(0.12,0.12,0.95301)	per L	[67-69, 71-79]
D8	Number of liters applied	200	L/ha	User input
D9	How many liters positive?	=RiskBinomial(D8,D7)	L/ha	Calculated
D10	Effective concentration per liter, considering pos. and neg. liters	=D6*D9/D8	GC/L	Calculated
D11	Conversion of hectare to acre	2.47105	Acre/Ha	User input
D12	Number of berry plants per acre	=ROUND((9113.22222),0)	Plants/acre	User input
D13	Number of berries per plant	10	Berries/plant	User input
D14	Volume of water sprayed per plant	=D8/(D11*D12)	L/plant	Calculated
D15	Volume of water sprayed per berry	=D14/D13	L/berry	Calculated
D16	concentration on berry after pesticide treatment	=D10*D15	GC/berry	Calculated
D17	Log concentration on berry after pesticide treatment	=LOG(D16)	Log GC/berry	Calculated
D18	**Transportation to processing facility**			
D19	Temperature, truck	4	C	User Input
D20	Time, truck	1	Days	User Input
D21	Log reduction at 4°C per day	0	Log reduction/day	[48] (fresh raspberries - poliovirus, 9 days), [49, 50]

D22	Log reduction at time of delivery	=D20*D21	Log reduction	Calculated
D23	concentration on berry at time of delivery	=D17-D22	Log GC/berry	Calculated
D24	**Washing log reduction (18°C, cold water)**			
D25	Mean log reduction on contaminated berries	0.667		[39]
D26	SD log red on contaminated berries	0.332		[39]
D27	Log reduction on contaminated berries	=RiskNormal(D25,D26)	Log GC/berry	Calculated
D28	concentration on contaminated berries	=D23-D27	Log GC/berry	Calculated
D29	**Sanitizing log reduction, 200ppm Chlorine concentration**			
D30	Mean log reduction on contaminated berries	1.35		[39, 40]
D31	SD log red on contaminated berries	0.24		[39, 40]
D32	Log red difference on contaminated berries	=RiskNormal(D30,D31)	Log GC/berry	Calculated
D33	concentration on contaminated berries	=D28-D32	Log GC/berry	Calculated
D34	**Freezing process**			
D35	Log reduction	0	Log GC/berry	User input
D36	concentration on berry after freezing	=D33-D35	Log GC/berry	Calculated
D37	**Transportation from truck to cargo ship, -21C**			
D38	Time, transport	=ROUND(RiskUniform(0.5,2),0)	Days	User input
D39	Mean log reduction at frozen storage	0.4	Log GC/berry	[39]
D40	SD log reduction at frozen storage	0.18		[39]

D41	Log reduction, frozen storage after 90 days	=RiskNormal(D39,D40)		Calculated
D42	Log reduction, frozen storage per day	=D41/90		Calculated
D43	Log reduction	=D42*D38	Log GC/berry	Calculated
D44	concentration on berry at port in China	=D36-D43	Log GC/berry	Calculated
D45	**Transport via cargo ship, -21C**			
D46	Time, transport	=ROUND(RiskUniform(25,30),0)	Days	User input
D47	Log reduction	=D42*D46	Log GC/berry	Calculated
D48	concentration on berry at port in Germany	=D44-D47	Log GC/berry	Calculated
D49	**Transport to distribution center, -21C**			
D50	Time, transport	=ROUND(RiskUniform(0.5,5),0)	Days	User input
D51	Log reduction	=D42*D50	Log GC/berry	Calculated
D52	concentration on berry upon arrival at distribution center	=D48-(D51)	Log GC/berry	Calculated
D53	**Frozen storage at distribution center, -21C**			
D54	Time, distribution center	=ROUND(RiskUniform(0.5,90),0)	Days	User input
D55	Log reduction	=D42*D54	Log GC/berry	Calculated
D56	concentration on berry	=D52-D55	Log GC/berry	Calculated
D57	**Transport, distribution center to catering facility, -21C**			
D58	Time, transport	=ROUND(RiskUniform(0.5,5),0)	Days	User input
D59	Log reduction	=D42*D58	Log GC/berry	Calculated
D60	concentration on berry	=D56-D59	Log GC/berry	Calculated
D61	**Foodservice preparation and consumption**			
D62	concentration on berry (antilog)	=10^D60	GC/berry	
D63	**Serving and dose response**			
D64	Serving size	4	Berries	User input
D65	concentration (non log)	=D62	GC/berry	Calculated

D66	concentration per serving	=D64*D65	GC/berry	Calculated
D67	Dose-response η	=2.55*10^-3	No units	[5]
D68	Dose-response r	0.086	No units	[5]
D69	Probability of illness	=1-(1+(D66*(0.00255)))^-0.086		Calculated
D70	**Illnesses**			
D71	Number of servings to consider per iteration	100000	Servings	User input
D72	Illness per number of servings per iteration	=RiskBinomial(D71,D69)	Illnesses	Calculated
D73	Was there illness?	=IF(D72>0,1,0)	No units	Calculated
D74	Log number ill	=IF(D73=1,LOG(D72),-5)	Calculated	Calculated

Table 2. A comparison of baseline conditions to other scenarios showing minimum, mean and maximum number of illnesses as well as factor sensitivities for different scenarios.

Parameter	Variations	Simulated illnesses					Factor Sensitivity
		Minimum	5th percentile	Mean	95th percentile	Maximum	(Log relative change)
Baseline conditions [1]		0	0	1.89	9	487	0.000
Concentration in surface water	8 Log GC/L	0	646	7,694	18,306	36,056	3.601
	5 Log GC/L	0	0	22	83	1,348	1.057
	2 Log GC/L	0	0	0.023	0	3	-1.924
Pesticide application	2000 L/ha	0	0	19	91	3,762	0.993
	20 L/ha	0	0	0.193	1	83	-1.000
NoV prevalence in surface water	Spring 57%	0	0	2.68	13	521	0.143
	Summer 33%	0	0	1.56	8	238	-0.092
	Fall 14%	0	0	0.684	4	263	-0.451
	Winter 70%	0	0	3.4	17	808	0.246
Pesticide strength	Strong	0	0	0.023	0	7	-1.930
	Weak	0	0	1.71	9	242	0.009
	No pesticide effect	0	0	1.97	1	398	-0.053
Truck temperature	10 °C	0	0	1.75	8	359	-0.043
	21 °C	0	0	1.57	8	415	-0.090
Washing step	Warm water	0	0	1.06	6	166	-0.260
	No wash	0	0	6.5	34	1,119	0.527
Sanitizing step	5ppm ClO2	0	0	8.28	41	1,165	0.633
	10ppm ClO2	0	0	6.67	33	814	0.539
	No sanitizer	0	0	35.8	180	4,270	1.268
Foodservice preparation	Mild heat (30s at 65 °C)	0	0	0.026	0	9	-1.871
	High heat (15s at 75 °C)	0	0	0.00001[2]	0	0	-5.300

1: Baseline conditions: Concentration of NoV in water as a uniform distribution, volume of liquid to apply pesticides 200 L/ha, seasonality for prevalence of NoV in surface water as a triangular distribution, the effect of pesticide on reduction in NoV concentration as a lognormal distribution, truck temperature for transport from the field by 4 °C, berries washed in 18 °C water, sanitized using 200 ppm chlorine, and no heating step during foodservice preparation.

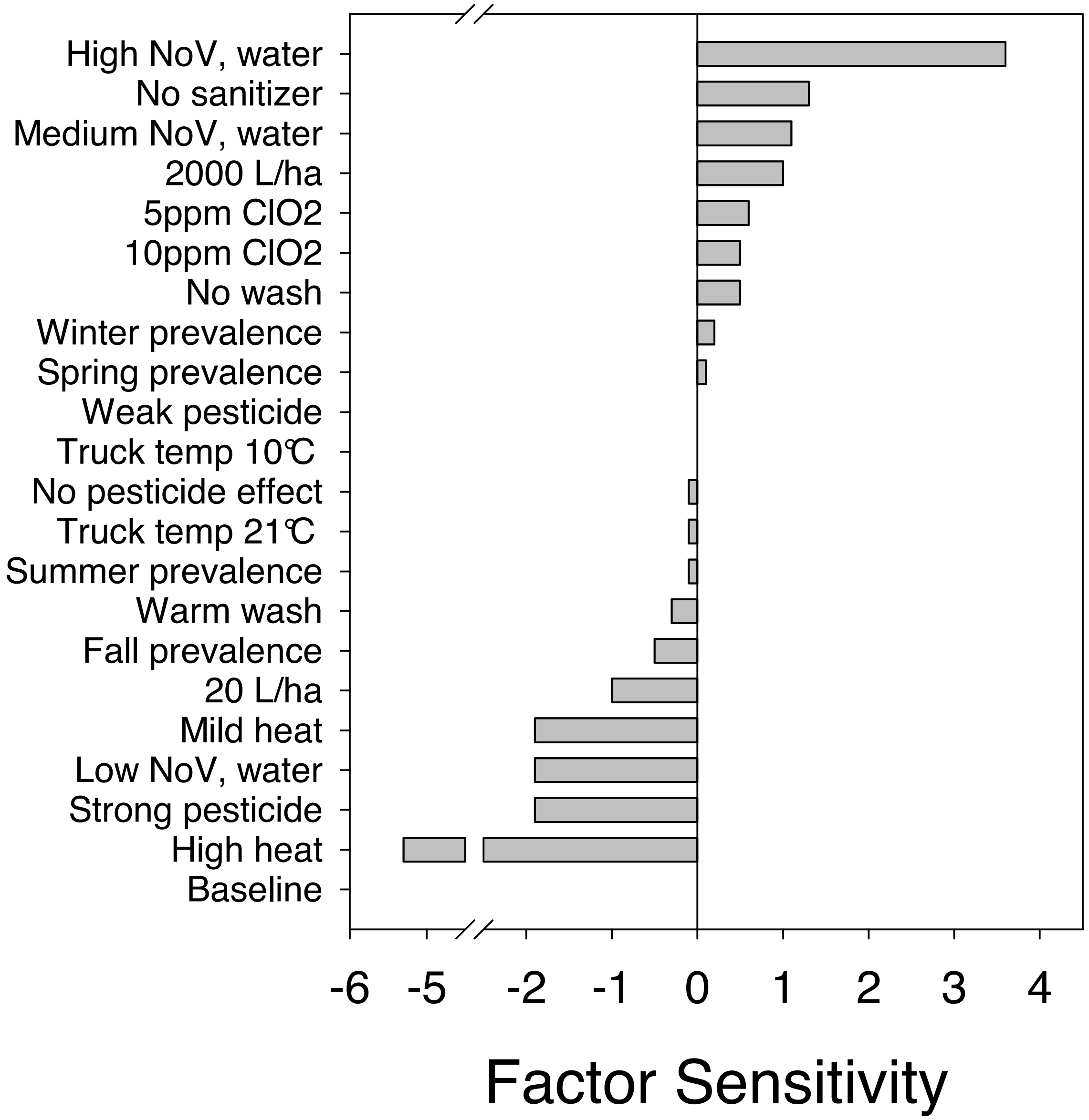
2: Since no illnesses were predicted under high heat conditions, and it is not possible to calculate the logarithm of zero, we assumed one iteration resulted in one illnesses for purposes of calculating factor sensitivity (log relative change)

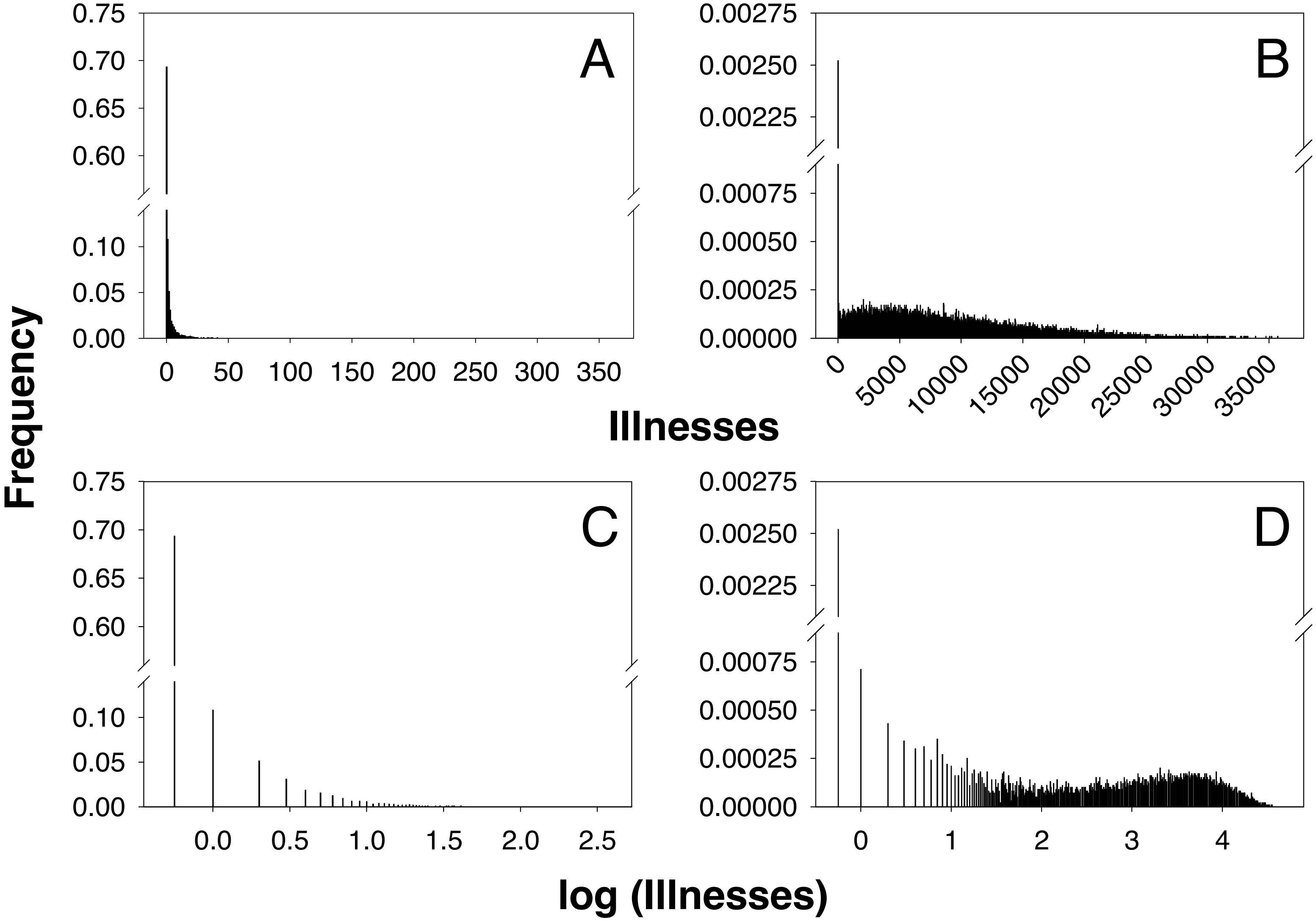
Figure legends

Figure 1. Comparison of Norovirus in frozen strawberry scenario assumptions on factor sensitivity. Factor sensitivity is defined as the logarithm of the ratio of the mean number of illnesses for the relevant factor versus the baseline scenario.

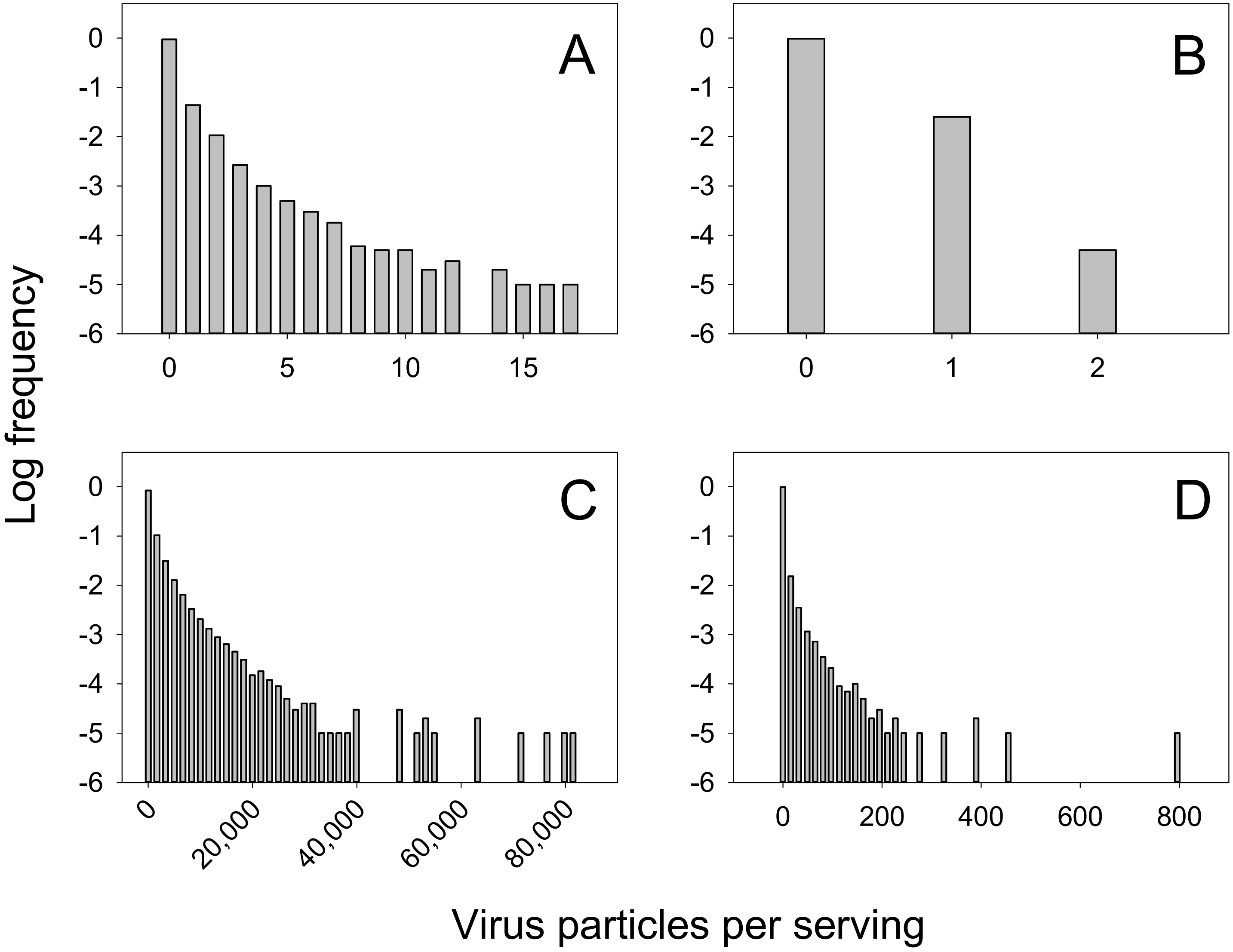
Figure 2. Comparison of distribution of predicted illnesses from 100,000 iterations of quantitative microbial risk assessment for Norovirus in frozen strawberries. Leftmost panels represent baseline scenario (A and C) versus highly contaminated (8 log GC/L) pesticide makeup water (B and D). Topmost panels (A and B) show data as illnesses, while bottommost panels (C and D) show the same data expressed as logarithm of the number of illnesses.

Figure 3. Comparison of distribution of simulated virus particles per serving from 100,000 iterations of quantitative microbial risk assessment for Norovirus in frozen strawberries. (A) baseline scenario, (B) baseline plus high heat (15s at 75 °C) use during food service preparation, (C) Highly contaminated (8 Log GC/L) water used for pesticide application, (D) Highly contaminated (8 Log GC/L) water used for pesticide application plus high heat (15s at 75 °C) use during food service preparation.


High NoV, water
No sanitizer
Medium NoV, water
2000 L/ha
5ppm ClO2
10ppm ClO2
No wash
Winter prevalence
Spring prevalence
Weak pesticide
Truck temp 10℃
No pesticide effect
Truck temp 21℃
Summer prevalence
Warm wash
Fall prevalence
20 L/ha
Mild heat
Low NoV, water
Strong pesticide
High heat
Baseline
-6
-5
-2
-1
0
1
2
3
4
Factor Sensitivity



A
B
C
D
Frequency
Illnesses
log (Illnesses)
0.75
0.70
0.65
0.60
0.10
0.05
0.00
0 50 100 150 200 250 300 350
0.00275
0.00250
0.00225
0.00075
0.00050
0.00025
0.00000
0 5000 10000 15000 20000 25000 30000 35000
0.0 0.5 1.0 1.5 2.0 2.5
0 1 2 3 4



A
B
C
D
Log frequency
0
-1
-2
-3
-4
-5
-6
0
5
10
15
0
1
2
0
20,000
40,000
60,000
80,000
0
200
400
600
800
Virus particles per serving