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Probabilistic risk model of norovirus transmission during handling and preparation of fresh produce in school foodservice operations

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ABSTRACT

Human noroviruses (NoV) are recognized worldwide as important pathogens and the primary cause of foodborne disease outbreaks from contaminated food in the U.S. They are often transmitted by infected food handlers manipulating foods during preparation, such as fresh fruits and vegetables. This paper provides a study to model the transfer of NoV between food handlers and vegetables during salad preparation in school food services based on direct observation data. Three transfer pathways were modeled by considering different initial contamination sources (environment, handlers and contaminated produce). The probability of infection by NoV was also estimated based on the NoV levels at consumption obtained from each simulated transfer pathway. A scenario analysis ranging a wide concentration from 10^2 to 10^7 NoV infective particles was performed to represent different levels of NoV in the initial contamination sources. In addition, a sensitivity analysis was applied to identify the most important model inputs and determine the safest handling practices to be implemented in school food service operations. The pathway describing transfer from contaminated surfaces or handlers to foods indicated that initial levels of $\leq 10^4$ NoV particles/fomite resulted in < 0.5% cases per serving of NoV infection. When initial levels were higher, % cases of NoV infection was estimated to be ca. 3%. This rise in % cases of infection was linked to higher doses (5% serving with ≥15 NoV particles/serving) and prevalence levels at consumption (> 0.2). In the pathway modeling cross contamination from contaminated vegetables to non-contaminated vegetables, all scenarios could lead to infected individuals, although number of cases of infection were lower (< 1.3%), despite concentration levels were higher. On the contrary, for this pathway, prevalence was 2-fold lower than that observed in the pathways describing transfer from contaminated surfaces and hands. Based on the sensitivity analysis, NoV transfers to fresh produce may be minimized by improving hand washing, and therefore effective training programs need to be carried out specifically addressing hand washing. Moreover, the produce's washing step showed to be an effective control measure, depending on the desinfectant efficacy, by reducing % cases of NoV infection from 6 to 1%. The model in this study might be used, in the future, to evaluate the impact on the risk associated with NoV transmission of specific and effective training programs, aimed at food chain operators.

1. Introduction

In 2015, the Centers for Disease Control and Prevention (CDC) reported 902 foodborne outbreaks (CDC, 2015) with human norovirus (NoV) listed as the pathogen most frequently responsible for those outbreaks (37%). In a separate study, Scallan et al. (2011) estimated that NoV causes 58% of the estimated 9.4 million episodes in the U.S. Both of these studies indicate that foodborne illeness as a whole remains a major public health problem in the U.S. despite many interventions over the years and NoV is a major cause that needs to be thoroughly addressed.

NoVs are mostly transmitted by the fecal-oral route and are highly

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contagious, with studies in humans suggesting very low infectious dose (ID_{50} of 18 viruses) (Teunis et al., 2008). Thus, infected food handlers pose a significant risk for infecting those consuming any prepared ready-to-eat (RTE) food, including fresh fruits and vegetables. These food items have no control step to eliminate or reduce pathogens, and thus are more prone for NoV transmission and subsequent infections. In fact, fresh produce was associated with 11–20% of all outbreaks each year from 2011 to 2013 (CDC, 2015, 2014a, 2014b). Since the demand for fresh fruits and vegetables remains high in the U.S. and other countries, the importance of safe produce handling throughout the entire production, processing, and service system cannot be overemphasized.

Most of the foodborne disease outbreaks reported in U.S. occur in restaurants, which have been identified as the origin of 60% all outbreaks from known locations (CDC, 2015, 2014a, 2014b). Schools are rarely involved in foodborne disease outbreaks in the U.S. (approximately 1–2%) (CDC, 2015, 2014a, 2014b) but children, specially those aged < 5 years, comprise a portion of the highly susceptible population to NoV disease (Ahmed et al., 2014; Hall et al., 2013). Furthermore, with 30 million children eating at U.S. schools every day (USDA, 2018), the average number of cases associated with each foodborne disease outbreak was greater at schools (36.2 persons per outbreak) than those at restaurants (12.9 persons per outbreak) (CDC, 2015).

Schools have unique challenges to provide healthy and safe food for large numbers of children each day. Since the Healthy Hunger-Free Kids Act of 2010 (USDA, 2010) has become effective, U.S. school foodservice operators had challenges to provide an increased amount of fresh produce while reducing the food waste. Many school foodservice operators have utilized self-service salad bars where students can serve themselves. While providing self-service salad bars increases students' fresh produce selection and consumption, such system exposes them to additional food safety risks related to contamination by fellow students and staff.

Since lack of proper employee hygiene and unsanitary hand contact have been identified as the largest contributing factors for foodborne disease outbreaks, specially for the viral infections, recent studies have attempted to quantify transfer rates of NoV cross-contamination during preparation of fresh produce items, such as Romaine lettuce (Bidawid et al., 2004; Grove et al., 2015) or globe tomatoes (Shieh et al., 2014), and fresh produce-containing RTE foods, such as delicatessen sandwiches (Rönnqvist et al., 2014). Quantitative microbial risk assessment is a useful tool that estimates relative risks associated with certain scenarios (Hamilton et al., 2006). By applying mathematical modeling for characterizing microbial risks for the fresh produce, researchers and food operators are able to focus their efforts to reduce the greatest risk factors during food handling. Therefore, data derived from these studies are key to quantify the NoV risk posed by different food handling practices.

To date, few risk assessment models have specifically treated the risk of transmission by NoV (Bouwknegt et al., 2015; Duret et al., 2017; Jacxsens et al., 2017; Mokhtari and Jaykus, 2009; Sales-Ortells et al., 2015; Stals et al., 2015). In order to develop accurate risk models, it is paramount to assess food handling behaviors and potential contamination pathways. Only when the effect of food handling practices relating to human behaviors is considered, can suitable models be developed explaining pathogen transfers and further pathogen reduction strategies. Therefore, the purpose of the study was to develop a probability model describing cross-contamination through food handlers and vegetables (i.e. lettuce) during salad preparation in school foodservices for NoV based on direct observation data in order to determine the safest produce handling practices.

2. Material and methods

Food preparation in school food services can be related to different behavioral practices, including the use of barriers (hand washing, use of soap, gloves, etc.) and the application of other control measurements (food contact surface cleaning and disinfection). A previous work by Kwon et al. (2014) has shown how often food handlers fail to correctly wash their hands during vegetable preparation in school foodservice facilities. The study also reported the frequency of food-contact cleaning and disinfection among other aspects. The current research built on that study with a focus on school foodservice operation by developing a probabilistic model based on observations and microbiological analysis to determine the risk of NoV infection associated with three specific transfer pathways: NoV transfers from kitchen environment and handlers as initial source of contamination (Pathway 1 and 2, respectively) and transfer from contaminated produce as initial source of contamination (Pathway 3).

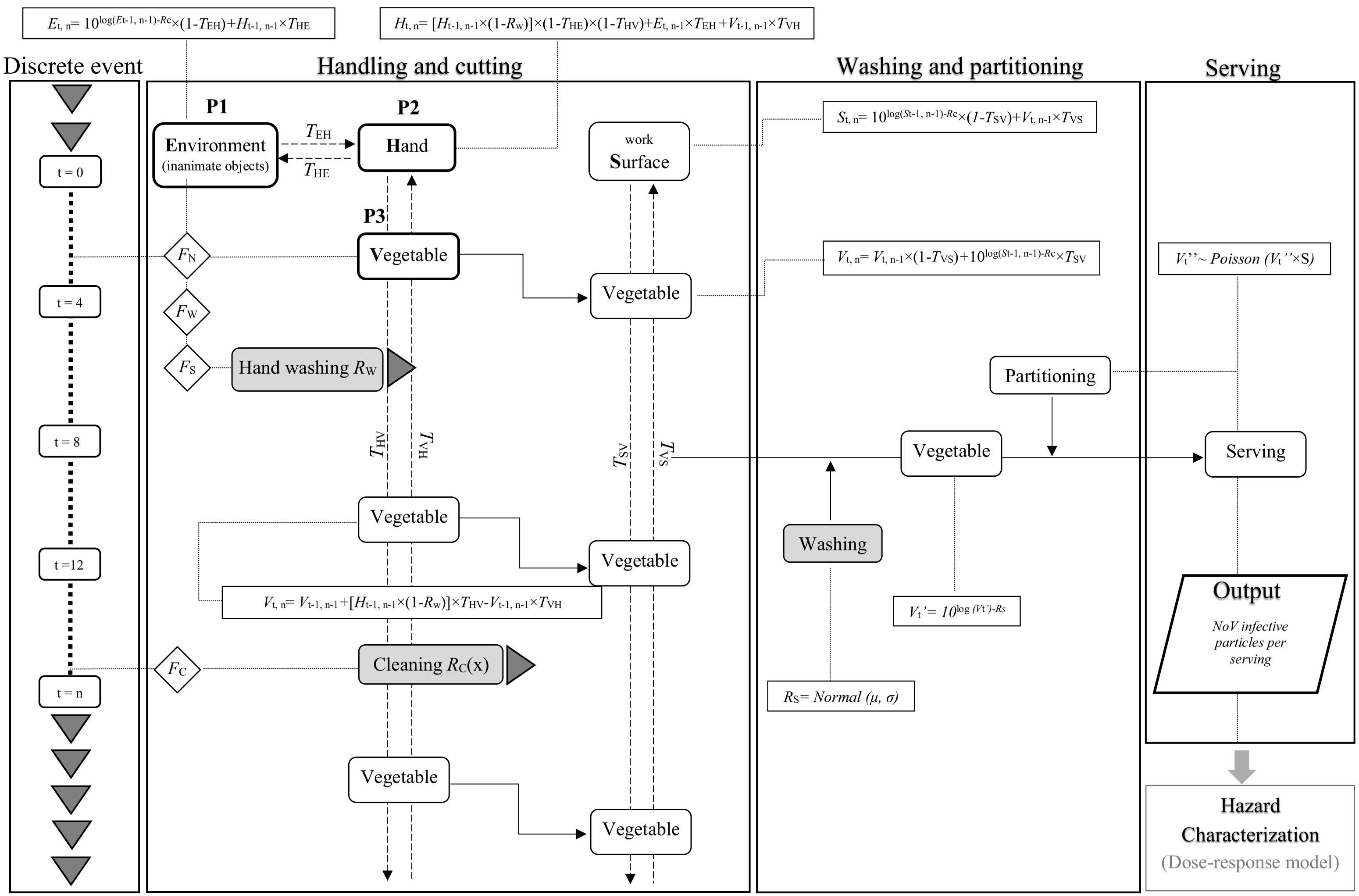
2.1. Norovirus transfer pathways

Different handling scenarios or transfer pathways were modeled by considering population decreases through disinfection and environmental stress and transfer between product, environment and handlers. The transfer pathways depict contamination from different initial sources (i.e. environment, worker hands and product) and by considering three different transmission barriers (i.e. hand washing, work surface cleaning and vegetable washing and disinfection). The model did not consider transmission through cough or sneeze due to the frequent use of food shield in school cafeteria, which would greatly reduce their impact in comparison to transmission by hand.

In Pathway No. 1, the environment is considered the initial contamination source and the starting point of the sequence of events, where the first transfer event occurs from surface of contaminated inanimate objects such as faucets, spigots, door handles or equipment to hands of food workers and then from their hands to produce items during their preparation. Vegetables can also become contaminated, by NoV, through work surfaces that are previously contaminated by contaminated workers during food preparation. Pathway No. 2 represents transfer from contaminated hands of workers, directly infected by the pathogen or through indirect contact with a contaminated object or person (i.e. starting point in the sequence of events), to produce items during their preparation. As in Pathway No. 1, vegetables can get contaminated through work surfaces that have been previously contaminated by infected contaminated workers during surface handling. These two pathway scenarios are similar but in the second one, the dose on the hands could be higher with an infected person suffering from vomiting or diarrhea. Pathway No. 3 simulates transfer from a contaminated produce item (e.g., leafy greens) to other produce items during preparation. In this case, transfer occurs from produce to handler and work surfaces and then from these to the final products. For this pathway, no environmental contamination is considered, so no transfer between inanimate objects and hands occurs. In this pathway, servings derived from the initially contaminated produce (i.e. contamination source) are included in the output containing the remaining contamination after transfer and washing. The output was expressed in NoV infective particles (NoVP) per serving of the produce item (lettuce). A graphical scheme illustrating the transfer pathways and reduction processes is shown in Fig. 1.

2.2. Initial contamination

Since available data concerning NoV levels are scarce, and if published they are not suitable for modification to our models, or fail to include important sources of uncertainty, a scenario analysis was preferred to represent the different levels of NoV in the initial contamination sources such as environment, handler and produce. Generic scenarios S1 to S6 encompassed a wide concentration range from 10^2 to 10^7 NoVP per produce/hand or fomite.



Solid lines represent a different step during vegetable manipulation. Dashed lines represent transfer routes and dotted lines indicate an event, variable or input parameter. Shaded boxes indicate a NoV reduction process. Rhombus symbol represents a decision event determined by a specific occurrence frequency. NoV infective particles at time t and contact number n on hand ($H_{t,n}$), vegetable ($V_{t,n}$), work surface ($S_{t,n}$) and inanimate objects ($E_{t,n}$). V_t' and V_t'' stand for NoV levels on vegetable at product washing and partitioning steps, respectively. T_{ij} represents NoV transfer rate between sources i and j, where i and j are selected among inanimate objects (E), hand (H), vegetable (V) and work surface (S). R_W: NoV reduction on hand during hand washing, considering use of soap or no; R_C: NoV reduction on work surface due to cleaning as function of different variable (x); S: Serving size (g); F_N: Frequency of contacting soiled objects; F_W: Frequency of washing hand; F_S: Frequency of soap use; F_C: Frequency of cleaning work surfaces. Values, units, probability distributions and predictive models used for the different variables and parameters in the risk model are included in the Material and Methods.

Fig. 1. Schematic overview of the exposure modeling process describing the cross-contamination pathways during leafy greens manipulation from the source of contamination (P1 = kitchen environment; P2 = worker hands; P3 = produce) to the vegetable to be served.

2.3. Transfer rates and reduction rate

To simulate virus transfer during handling and reduction due to washing, different data sources were consulted incorporating those more significant and representative for the simulated scenario. Data concerning NoV from the literature were preferred where these were available over those of surrogates, feline calicivirus (FCV) and murine norovirus (MNV). However, most data were obtained from surrogate virus strains since NoV cannot be grown in existing cell lines. The difference in units recorded in different studies was also an important issue because these are not equivalent since virus counts can be given in infective viral particles (plaque forming units, PFU or median tissue culture infectious doses, $TCID_{50}$) and genome units (not specifically infective particles). In practice, $TCID_{50}$, infective virus particles and genome units are not identical, but these were considered equivalent for our purpose since we assumed that particle transfer characteristics are not affected by their infectivity. The most representative transfer and reduction rate distributions used for describing above-mentioned three cross-contamination scenarios are presented in Table 1.

Many of the model parameters (inputs) used in the model to describe transfer and reduction rates were taken from the study of Bidawid et al. (2004). These authors used the finger pad protocol to simulate virus transfer from hands to food and metal disks. Another study has been published more recently (Grove et al., 2015) reporting transfer coefficients of NoV during the preparation of lettuce, but transfer scenarios between lettuce and hands were performed using palm and fingers of bare hands. Although the protocol can be useful for capturing experimental and natural variability in virus transfer of specific food handling scenarios (Ansari et al., 1989), reported data by Bidawid et al. (2004) were preferred, in our study, since this enabled us to develop a more analytical model including factors such as number of contacts and number of fingers touching food (Table 1). The work by Stals et al. (2013) was selected for representing NoV cross-contamination between lettuce and work surfaces since they applied a transfer protocol similar to that used by Bidawid et al. (2004).

2.4. Food employee practices

Occurrence of different tasks or practices such as washing, contact with soiled surfaces or use of soap were considered in the model by using a discrete distribution and a binary decision tree (i.e. 0 and 1) based on data and frequency collected in the Kansas school cafeteria survey (Kwon et al., 2014). In this study, food handler behavior was monitored in different food school services during vegetable food preparation, specifically focused on tasks related with cross-contamination events and hand washing practices. For any iteration during a simulation, the specific action or practice carried out was scored as 1, whereas if the action did not occur it is scored as 0. Washing frequencies are reported in Table 2 for such purpose. As an illustrative example in this table, if the frequency with which workers washed hands after handling soiled equipment, etc. was 13%, it means that during simulation 13 out of 100 iterations, the value for the decision tree was 1 and therefore, the model simulated washing hand by applying the corresponding reduction rate on hands.

2.5. Handling level

The way of handling produces during preparation influences transfer levels. The model includes, therefore, two parameters, number of contacts and number of fingers touching food. In the case of contact between product and work surfaces, only the number of contact was utilized. The values for these parameters can be defined by the user. In the model herein simulated, values were described by discrete distributions returning values between 1 and 5 for finger and 1 and 4 for contacts (Table 2).

2.6. Disinfection or washing step

Vegetables were washed before serving them according to the survey data. Little information was found in literature data regarding the effect of washing step on virus reduction. The studies on NoV, MNV and hand washing procedures by Bae et al. (2011), Baert et al. (2009) and Bloomfield et al. (2007), respectively were used as basis to construct a distribution defining the log decrease in leafy green vegetables submitted to a washing process. The distribution corresponded to Normal (μ, σ) truncated at 0 and 2 log NoVP as minimum and maximum values. The maximum was defined based on the maximun log reduction values observed in the above-mentioned studies for NoV after washing leafy green vegetables with chlorinated water. The normal distribution parameters were also described by uniform distribution: μ = Uniform (0.26, 1.06) and σ = Uniform (0.03, 0.34) which accounted for variability between studies.

In the case of the same water used for washing different products, a survival model depicting NoV survival in washing water would be needed. To this end, an exponential model (Eq. (1)) was fitted to data taken from the study by Shin and Sobsey (2008) based on experiments in water containing 1 ppm free chlorine, which is expected to simulate washing conditions in school food services. This model allowed to predict NoV reduction in chlorinated water as a function of time (min). Observations obtained in the Kansas school cafeteria (Kwon et al., 2014) were used to input the model with a mean washing time, which corresponded to 8.6 min.

$$\log(N/N_0) = -0.58\cdot\ln(t) - 1.54 \quad (1)$$

where t (> 0.07) stands for time (min), N is the levels of NoV (infective particles) at time t, and N_0 is the initial level of NoV at time 0.

The obtained survival model is represented in Fig. 2 together with the equation and goodness-of-fit index (R^2).

2.7. Work surfaces cleaning

In Pathway 2, work surfaces also act as contamination source after a product is handled on them. However, during preparation, surfaces could be submitted to a cleaning procedure, thus reducing the viral load on surfaces. To consider this scenario, a polynomial disinfection model (Eq. (2)) for stainless steel surfaces was implemented based on the work by Kim et al. (2012) which used MNV as surrogate to simulate NoV.

$$\log TCID_{50} = a + b\cdot X_1 + c\cdot X_2 + d\cdot X_1\cdot X_2 + e\cdot X_1^2 + f\cdot X_2^2 \quad (2)$$

where X_1 = concentration of free chlorine (0–5000 ppm), X_2 = disinfection time (0–5 min) and a–f are regression coefficients, with $a = 0.0471$, $b = 0.0807$, $c = 0.0001$, $d = 0.0001$, $e = -0.0910$ and $f = -0.0001$.

Probability distributions were used to represent variability on X_1 and X_2. X_1 was equal to Pert (0.1, 1, 3) and X_2 = Pert (0, 1, 2).

2.8. Partitioning effect

The distribution of NoV in servings was based on the assumption that viral particles were distributed homogeneously over the vegetable product. This fact can be indirectly observed in results in residual water from the study by Baert et al. (2009). Therefore, Binomial and Poisson distributions were used to represent partitioning effect on NoV distribution in servings. Serving size (g) variability was also modeled by including a Uniform distribution (25, 50).

2.9. Dose-response modeling

A number of dose-response models are available in literature for NoV (Messner et al., 2014; Schmidt, 2015; Teunis et al., 2008; Van Abel et al., 2017). To date, dose-response models have been developed by using a variety of data sets obtained from clinical human challenge

Table 1
Input parameters associated with transfer and reduction rates used for describing cross-contamination scenarios in the probabilistic risk model for NoV.

Notation	Definition of input parameter	Unit	Value or distribution	Mean	SD[a]	Distribution parameters	Source
Inputs associated with NoV transfer during handling and cutting of vegetables (*lettuce*)							
T_{HV}	Transfer rate from hands to lettuce	/	Lognormal (truncated)[b]	0.187	0.057	Min: 0; Max: 1	Bidawid et al. (2004)
T_{VH}	Transfer rate from lettuce to hands	/	Lognormal (truncated)	0.14	0.035	Min: 0; Max: 1	Bidawid et al. (2004)
T_{HE}	Transfer rate from hands to environment (inanimate objects)[c]	/	Lognormal (truncated)	0.13	0.036	Min: 0; Max: 1	Bidawid et al. (2004)
T_{EH}	Transfer rate from environment (inanimate objects) to hands	/	Lognormal (truncated)	0.07	0.019	Min: 0; Max: 1	Bidawid et al. (2004)
T_{SV}	Transfer rate from work surface to lettuce	/	Lognormal (truncated)	0.48	0.53	Min: 0; Max: 1	Stals et al. (2013)
T_{VS}	Transfer rate from lettuce to work surface	/	Lognormal (truncated)	0.8	0.25	Min: 0; Max: 1	Stals et al. (2013)
Inputs associated with hand washing efficiency							
/	Recovery before washing (1)	%	Lognormal (truncated)	71.6	8.9	Min: 0; Max: 100	Bidawid et al. (2004)
/	Recovery after washing (2)	%	Lognormal (truncated)	8.6	1.4	Min: 0; Max: 100	Bidawid et al. (2004)
/	Recovery after washing + soap (3)	%	Lognormal (truncated)	5.6	1.3	Min: 0; Max: 100	Bidawid et al. (2004)
R_W	Reduction in NoV levels after washing	%	(2)/(1)				Calculated
R_W	Reduction in NoV levels after washing + soap	%	(3)/(1)				Calculated
Inputs associated with work surface cleaning and vegetables disinfection							
R_C	Reduction in NoV levels after cleaning work surfaces	$\log_{10}$ NoV	Polynomial equation[d]				Kim et al. (2012)
R_S	Reduction in NoV levels after washing vegetables	$\log_{10}$ NoV	Normal (truncated)	Uniform (0.26, 1.06)	Uniform (0.03, 0.34)	Min: 0; Max: 2	Bae et al. (2011), Baert et al. (2009), Bloomfield et al. (2007)

[a] SD: standard deviation.
[b] X ~ lognormal(a, b) with mean(X) = a and SD(X) = b.
[c] Inanimate objects and work surfaces are represented by stainless steel surfaces.
[d] See Section 2.7 in Material and methods.

Table 2
Model parameters associated with different tasks or actions during food preparation in school food services based on observations in Kansa's survey (Kwon et al., 2014).

Notation	Definition of event/input parameter	Distribution	p	$q = 1 - p$
F_W	Frequency of washing hand after handling soiled environment (inanimate objects)	Discrete (0, 1, q, p)[a]	0.13	0.87
F_W	Frequency of washing hand before handling different foods	Discrete (0, 1, q, p)	0.33	0.67
F_S	Frequency of washing hand with soap	Discrete (0, 1, q, p)	0.72	0.28
F_C	Frequency of cleaning work surfaces	Discrete (0, 1, q, p)	0.1	0.9
F_N	Frequency of contacting soiled environment (inanimate objects)	Discrete (0, 1, q, p)	0.05	0.95
/	Number of fingers contacting food or working surface	Uniform (1, 5)		
/	Number of contacts between hand and food or working surface	Uniform (1, 4)		

[a] Discrete distribution corresponding to Bernoulli distribution with p (probability of occurrence of the event) = 1 and q (probability of non-event) = 0.

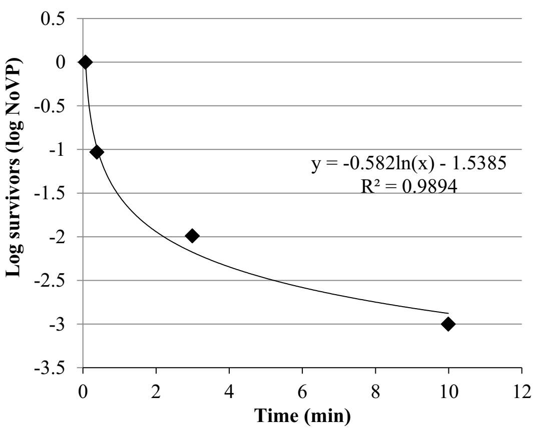


Fig. 2. Best fit exponential model to data from Shin and Sobsey (2008) describing NoV survival in water containing 1 ppm free chlorine.

studies and numerous assumptions should be made when the models are applied (Van Abel et al., 2017). Thus, no one dose-response model can be identified as being best for estimating infection probability by ingestion of NoV through food consumption. Nonetheless, the mayority of published risk analysis studies use the $_1F_1$ hypergeometric dose–response model (Van Abel et al., 2017). In our study, the $_1F_1$ hypergeometric was simplified to the conditional dose-response function, the beta-binomial, considering that the dose received was not the mean but a discrete dose (Duret et al., 2017; Haas, 2002; McBride et al., 2013). The beta-binomial form (Eq. (3)) was computed in Microsoft Excel to estimate the probability of infection (P_{inf}) for each simulated serving using the software Excel Add-in @Risk (Palisade ©, NY).

$$P_{inf} = 1 - \mathrm{B}(\alpha, \beta + \text{dose})/\mathrm{B}(\alpha, \beta) \tag{3}$$

where B is the standard Beta function with parameters α and β written in Excel as $B(\alpha, \beta) = \exp(\text{gammaln}(\alpha) + \text{gammaln}(\beta) - \text{gammaln}(\alpha + \beta))$. Since no information is available about the secretor status of the targeted populations, a conservative approach was applied by assuming that all individuals were susceptible, i.e. positive secretors (Se +). Furthermore, in our study, virus aggregation was unknown, hence probabilities of infection were calculated making no assumption about aggregation state of NoV particles. Taking in consideration these aspects, dose-response model parameters were defined with $\alpha = 0.04$, and $\beta = 0.055$ according to Teunis et al. (2008).

2.10. Risk characterization

To assess the risk associated with each concentration scenario, simulated doses at the time of consumption were used to input the dose-response model in each simulation iteration resulting in a probability distribution accounting for NoV infectivity probability. This distribution was integrated numerically to derive the total number of NoV cases for each scenario. Risk output was expressed as the percentage of cases in relation to the total number of servings consumed in the school (i.e. iterations), assuming that each individual ingested one single serving.

2.11. Risk model simulation

The sequence of events, variables and parameters are represented in Fig. 1 for the three simulated transfer pathways. This model pictogram shows how the different elements, variables and processes are integrated mathematically in the simulation, however, it does not include a complete representation of the algorithms used in the model simulation. Assumptions related to model simulation are shown in Table 3.

Each model simulation was performed with 10,000 iterations using @Risk software. Each iteraction was assumed to represent a serving consumed by an individual in the school population. The model parameters were defined based on observation and data obtained from Kansas's survey as well as scientific literature and was named **Baseline**

Table 3
Main assumptions in the probabilistic exposure model for NoV.

Related to data
- NoV surrogates (i.e. MNV) have similar properties as human NoV strains (survival, transfer, hand washing and disinfection).
- Transfer rates (%) are not affected by virus infectivity, hence transfer percentages calculated with different virus quantification units (e.g. infective particles and genomic copies) can be combined to estimate virus transfer through the different pathways.
- The contact area between vegetable, hands (finger tips) and surfaces used in literature to estimate transfer and reduction rates are representative for modeling the virus transfer in the different scenarios and events under study.

Related to model simulation
- Each discrete event corresponds with one iteration in the simulation in which a different vegetable (i.e. lettuce) is processed.
- The number of contacts (n) for vegetable-hand, vegetable-surface and hand-objects is simulated sequentially within each discrete event.
- The variable number of contacts (n) is not included for calculating NoV levels on vegetable at product washing and partitioning steps.
- Reductions (hand washing, vegetable washing and work surface cleaning) are exclusively applied at $n = 0$.
- Biomass balance is kept through the simulation, i.e. the number of NoV particles coming into the system is equal to the number of virus particles coming out the system minus the number of virus particles inactivated as result from the different reduction processes applied.
- During product washing step, no transfer of NoV particles from wash water to vegetable is considered.
- In Pathway 3, no cross-contamination between hands and inanimate objects is considered.

model (defining current risk status) which are included in Table 2. The distributions in the model represent uncertainty and variability for the different variables. The events reflected in the model (transfer routes, washing, serving, etc.) were simulated sequentially, using the Discrete-Event Simulation (DES) method to mimic the observed processes occurring over time during food preparation and serving. One discrete event corresponded to one produce item to be processed. Frequencies for certain processes such as cleaning, hand washing, and use of soap were integrated in the DES, using probability trees (Fig. 1). Since modeling transfer routes involved complex processes like loops, multiple contamination sources and interrelation between transfer routes, a programming subroutine was written in Visual Basic for Excel to enable modeling such elements, and was called (invoked) after each iteration during simulation.

2.12. Sensitivity analysis

In order to identify risk factors or crucial thresholds in inputs concerning microbial risk, a scenario analysis was performed based on a multiple simulation approach with the same RNG (Random Number Generators) seed in order to make results comparable. These fixed values were plotted against different statistics of outputs, that is, mean and 95th percentile of the final concentration of the pathogen in the food at the time of consumption.

3. Results and discussion

3.1. Norovirus transfer from environment and handlers as initial source of contamination: Pathway 1 and 2

Results obtained for Pathway 1 and 2 were similar, reflecting equivalent risk scenarios and trends, which could result from the similar sequence of events and transfers used in both pathways (Fig. 1). The only difference was the initial contamination source (i.e. starting point in the sequence of events), which corresponded to the kitchen environment (surface of inanimate objects) in Pathway 1 and hands in Pathway 2. Therefore, only data obtained from Pathway 1 will be presented for further discussion. The statistics for the NovV doses (i.e. NoVP/serving at the moment of consumption) simulated at the different concentration scenarios for Pathway 1 are shown in Fig. 3 and Table 4. Results indicated that Scenario 1 (i.e. initial concentration of 10^2 NoVP/fomite) was the only one that did not produce any contaminated serving. This result suggests that hand washing and treatments applied in the baseline model (work surface cleaning and washing step) were effective interventions to reduce NoV transmission when NoV contamination was very low. Although the present model did not include the effect of gloves, it is expected that gloving when combined with hand washing would further reduce the risk of NoV cross-contamination from hands to food (Mokhtari and Jaykus, 2009). It should be pointed out that gloves can also lead to non-hygienic practices, such as the use the same gloves for handling ready to eat and raw products or wearing the same gloves for other activities than food handling. Therefore, if gloves are recommended, specific educational programs should be implemented to avoid the misuse of gloves and promote an effective application to further reduce cross-contamination risk. The inclusion of gloves in risk models should also consider these aspects to be correctly assessed with regards to their final contribution to microbial risk. For that, more specific studies are needed providing suitable data to represent these glove use scenarios.

Simulation of Scenario 2, corresponding to 10^3 NoVP/fomite, allowed contaminated products but at very low levels through the different subsequent steps and reaching a maximum level of 3 NoVP/serving. These low NoVP levels resulted in 0.1% cases of infection, assuming that each invidual consumed a single serving. Scenario 3 (10^4 NoVP/fomite) also resulted in contaminated servings but the number of cases of infection was around 0.5%. In Fig. 3, NoV doses simulated for Scenario 3 are represented, showing that values were lower than 10 NoVP/serving and the 95% servings contained either none virus or only one virus particle. These results would suggest that environmental contamination below 10^4 NoVP/fomite would allow to keep the percentage of infected individuals below 0.5%, if baseline conditions are given. On the contrary, Scenarios from 4 to 6 with increasing initial contamination levels up to 10^7 NoVP/fomite led to higher number of infections ($\geq$3%) as result from the high doses (5% servings with $>$ 15 NoVP/serving) and prevalence levels ($>$ 0.2) obtained in these scenarios (Table 4). NoV can be excreted in very high numbers in human feces (e.g, 10^{8-10} viral particles/g) (Henke-Gendo et al., 2009; Lee et al., 2007; Tu et al., 2008); hence, the high levels simulated in these scenarios could be representative for cases in which products are handled by infected worker. For Pathway 1, the 95th percentile values corresponded to 142 and 1418 NoVP/serving for Scenarios 5 and 6, leading to a percentage of expected number of infected individuals of 3.25% and 3.53%, respectively (Table 4 and Fig. 3).

3.2. Norovirus transfer from contaminated produce as initial source of contamination: Pathway 3

In this cross-contamination pathway, transfer between contaminated vegetables and non-contaminated vegetables during handling at school food services was modeled by considering hands and work surfaces as transmission vehicles. Since NoV can only come from human feces or vomitus, it is important to point out the possiblity of NoV contamination during pre-harvest manipulations of the lettuce (e.g. NoV transfer from sewage or sewage effluents to irrigation water sources or fields of leafy greens). According to Baert et al. (2011), sewage or water was the most likely contamination source of leafy greens originating from U.S.

Few studies have published data related to the contamination levels of NoV on lettuce at harvest (Baert et al., 2011; Kokkinos et al., 2012; Mattison et al., 2010). The concentrations reported by these studies ranged from 1 to 7 log NoV genomic copies/g produce. The noticeable difference between concentrations in these studies can be explained by the fact that the contamination levels on the produce at harvest depend on multiple factors that are highly variable, such as climatic conditions, the volume of water retained by food crops and the concentration of norovirus therein (Bouwknegt et al., 2013). Furthermore, the level of handling during harvesting, which is often high for produce items harvested by hand (e.g., green onions, strawberries, lettuce heads), contributes to increase the variability of enteric pathogens levels at this step (Fiore, 2004). For this reason, the initial concentration was modeled, in our study, with different scenarios (S1 = 10^2 to S6 = 10^7 NoV particles/produce) in order to represent all possible NoV levels ocurring on lettuce handled in school foodservices. The model elements were similar to those used for Pathway 1 and 2, even though adequate variables, values and structure were used to describe events occurrring in Pathway 3. The simulation output for this pathway is shown in Table 5. The percentage of cases of NoV infection estimated in Pathway 3 was lower ($<$ 1.3%) than that obtained for Pathway 1 and 2 ($<$ 3.5%), specially for S4–S6. Although in Pathway 3 higher mean and maximum values were obtained for all contamination scenarios, the prevalence values for the most relevant scenarios in terms of risk, that is S4–S6, were at least 2-fold lower than those recorded for Pathway 1 (Tables 4 and 5). This fact could be the main cause of the lower risk obtained in Pathway 3.

As stated in lines above, higher NoV concentration on lettuce was obtained for Pathway 3 in comparison to Pathway 1 and 2. This finding could be related to the transfer coefficients used for describing NoV transfer between unprocessed product (lettuce) and work surface (stainless steel), which were considerably high (i.e. $\geq$50%) according to study by Stals et al. (2013). Noteworthy is that the transferability observed by these authors for the surrogate MNV was much lower ($<$ 5%) at the same experimental conditions. This could suggest that

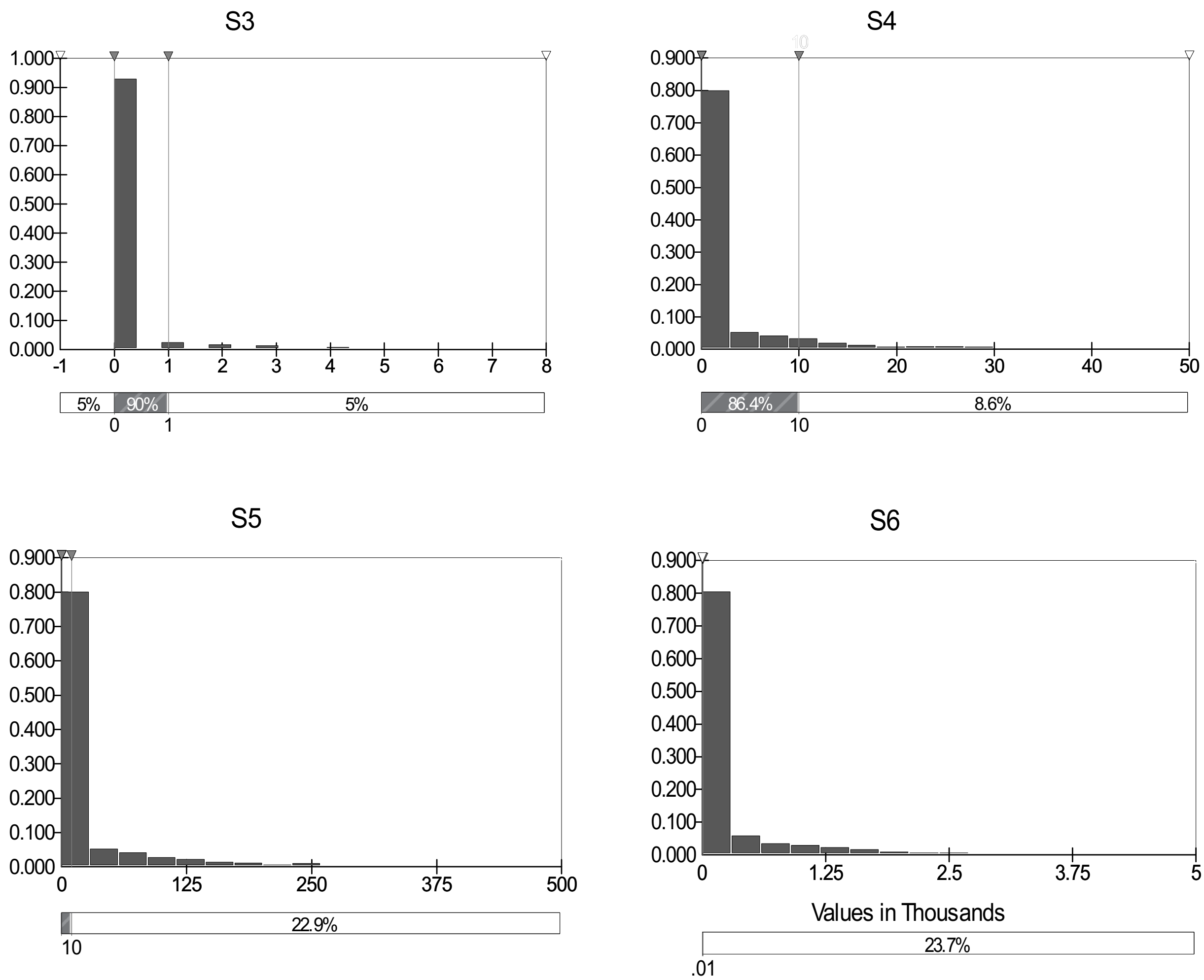


Fig. 3. Output from the simulated cross-contamination model of NoV at school food services, for Pathway 1 "*Norovirus transfer from environment as initial source of contamination*", showing the levels of NoV at the time of consumption (NoVP/serving) associated with the initial contamination scenarios: S3 = 10^4; S4 = 10^5; S5 = 10^6; S6 = 10^7 NoVP/fomite. The axis X and Y in graphs represents for simulated concentration (NoVP/serving) and corresponding frequency, respectively. The vertical line in graph corresponds to simulated concentrations of > 1 NoVP/serving for Scenario 3 and of > 10 NoVP/serving for Scenarios 4, 5 and 6.

Table 4
Statistics of the doses (NoVP/serving), prevalence (per one) at the time of consumption and percentage of infection cases (%) over 10,000 servings simulated at the different concentration scenarios (S1 to S6) in Pathway 1 "*Norovirus transfer from environment as initial source of contamination*".

Statistical parameter	S1 (10^2 NoVP/fomite)	S2 (10^3 NoVP/fomite)	S3 (10^4 NoVP/fomite)	S4 (10^5 NoVP/fomite)	S5 (10^6 NoVP/fomite)	S6 (10^7 NoVP/fomite)
Minimum	0	0	0	0	0	0
Maximum	0	3	10	176	1603	15,525
Mean	0	0.01	0.2	2.7	27.3	273.7
SD	0	0.2	1.1	9.8	94.5	939.0
Variance	0	0.03	1.2	95.2	8932.0	881,631.4
Skewness	N/C[a]	26.8	12.1	9.7	9.1	8.9
Kurtosis	N/C	758.8	208.5	135.9	118.3	112.3
Mode	0	0	0	0	0	0
80th	0	0	0	3	34	318
85th	0	0	0	5	54	541
90th	0	0	0	8	87	869
95th	0	0	1	15	142	1418
Prevalence	0.00	0.01	0.06	0.23	0.24	0.25
Cases (%)	0.00	0.11	0.43	2.97	3.27	3.53

[a] N/C: not computed.

behavior of surrogates is not always representative for NoV strains. While MNV has so far proven to be a reliable surrogate for human NoV due to their close genetic relationship (Wobus et al., 2006), direct comparisons with human NoV strains in terms of transfer between environmental surfaces are still challenging due to the inability to culture NoV in vitro (Grove et al., 2015). However, our finding could be also explained by the differences in the sequence of events, in the DES, derived from each contamination source (i.e. starting point in the sequence of events). New observational studies, further focused on how workers handled or prepare the produce (number of contacts, order in which events take place, etc.), could reduce uncertainty on these aspects, increasing model accuracy.

3.3. Sensitivity analysis

In order to evaluate the importance of variables and factors on the degree of NoV decontamination by the frequency and efficacy of hand washing, vegetable washing, and food contact surface cleaning, a sensitivity analysis was performed on key inputs in the risk model. This sensitivity analysis was carried out on the Scenario 4, corresponding to 10^5 NoVP; hence, results should not be extrapolated to higher initial contamination levels such as Scenario 5 and 6. The results for reduction rates associated with hand washing (with soap) showed that extreme

Table 5
Statistics of the doses (NoVP/serving), prevalence (per one) at the time of consumption and percentage of infection cases (%) over 10,000 servings simulated at the different concentration scenarios (S1 to S6) in Pathway 3 "*Norovirus transfer from contaminated produce as initial source of contamination*".

Statistical parameter	S1 (10^2 NoVP/produce)	S2 (10^3 NoVP/produce)	S3 (10^4 NoVP/produce)	S4 (10^5 NoVP/produce)	S5 (10^6 NoVP/produce)	S6 (10^7 NoVP/produce)
Minimum	0	0	0	0	0	0
Maximum	80	680	6430	64,253	645,320	6.5×10^6
Mean	0.3	3.1	31.0	310.6	3108.5	31,092.9
SD	2.7	27.5	275.9	2763.9	27,656.7	276,623
Variance	7.5	754.0	76,107.7	7.6×10^6	7.7×10^8	7.7×10^{10}
Skewness	14.0	13.8	13.8	13.8	13.8	13.8
Kurtosis	245.8	230.6	230.6	231.2	231.4	231.5
Mode	0	0	0	0	0	0
80th	0	0	0	0	0	0
85th	0	0	0	0	0	0
90th	0	0	0	0	0	9
95th	0	0	10	95	944	9421
Prevalence	0.02	0.04	0.06	0.08	0.09	0.12
Cases (%)	0.13	0.14	0.53	0.57	0.61	1.31

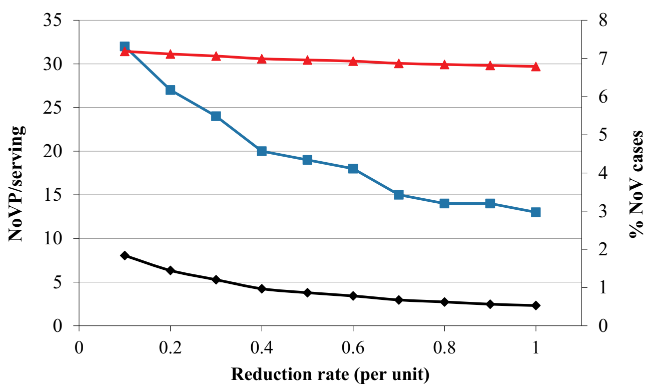


Fig. 4. Statistical results of the concentration of NoV in the food at the time of consumption (dose) describing the effect of increasing reduction rate derived from hand washing with a washing-soap procedure simulated at Scenario 4 (initial contamination level of 10^5 NoVP/hand). The mean is represented with (◆), 95th percentile with (■) and % cases of NoV infection per total number of servings with (▲).

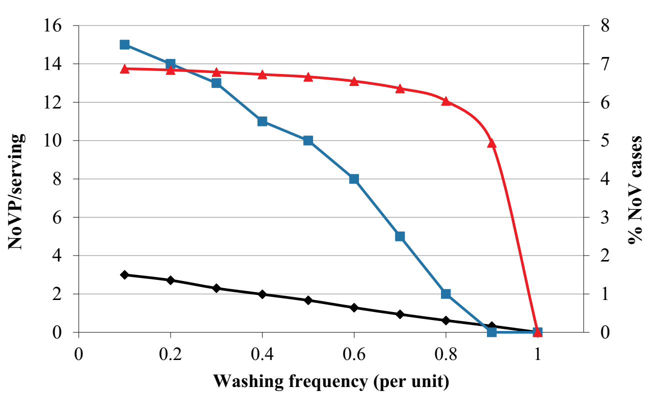


Fig. 5. Statistical results of the concentration of NoV in the food at the time of consumption (dose) describing the effect of increasing hand washing frequency simulated at Scenario 4 (initial contamination level of 10^5 NoVP/hand). The mean is represented with (◆), 95th percentile with (■) and % of NoV cases of NoV infection per total number of servings with (▲).

concentration levels represented by the 95th percentile were quite sensitive to a washing-soap procedure, particularly for the reduction rate values between 0 and 0.4 (Fig. 4). However, the simulated values did not show any substantial effect on reducing the percentage of cases of NoV infection, which only descended from 7.2 to 6.8% cases of NoV infection. On the other hand, the washing frequency, as shown in Fig. 5, compared with hand washing with soap, had a greater impact on NoV contamination risk. Importantly, the line corresponding to cases of NoV infection (%) (red line) indicates that only a strict compliance (of 100%) with hand washing can reduce to "0" the risk of cases of NoV infection. Other authors have also highlighted the importance of hand washing as an intervention step to reduce the risk linked to norovirus transmission. Duret et al. (2017) suggested that efficient hand washing would reduce to 58% the mean number of infected customers. Grove et al. (2015) found that hand washing was effective in removing > 2.5 log of MNV from hands, but additional intervention strategies are needed to adequately prevent the transfer of viruses from hands.

The analysis of cleaning frequency of work surfaces confirms the importance of performing cleaning often during vegetable preparation to reduce NoV contamination but also evidenced that frequency levels above 50% for cleaning work surfaces did not produce significantly more decrease of NoV contamination levels (Fig. 6). Nevertheless, increasing cleaning frequency of work surfaces did not yield any evident decrease in % cases of NoV infection. For produce's washing step, according to Fig. 7, a decrease of 1.5 log NoVP would result in reducing the number of infections by NoV from > 6% to < 1%.These values would be only reached if a sanitizer solution is applied to washing water like for example hypochlorite (Kennedy et al., 2011; Kim et al., 2012; Park et al., 2010; Shin and Sobsey, 2008; Singh et al., 2012). Although no data are available to accurately relate these reduction levels with specific disinfectant concentrations (e.g. chlorine), reductions

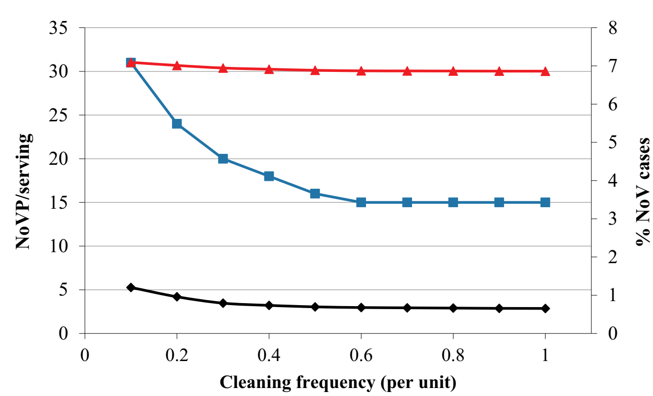


Fig. 6. Statistical results of the concentration of NoV in the food at the time of consumption (dose) describing the effect of increasing cleaning frequency of work surfaces simulated at Scenario 4 (initial contamination level of 10^5 NoVP/hand). The mean is represented with (◆), 95th percentile with (■) and % of cases of NoV infection per total number of servings with (▲).

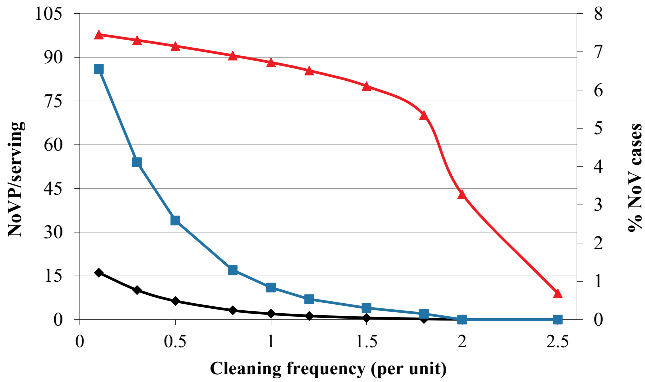


Fig. 7. Statistical results of the concentration of NoV in the food at the time of consumption (dose) describing the effect of increasing efficacy of produce's washing simulated at Scenario 4 (initial contamination level of 10^5 NoVP/hand). The mean is represented with (◆), 95th percentile with (■) and % of cases of NoV infection per total number of servings with (▲).

of ~1 log virus particles are expected to be associated with relatively high concentrations of disinfectant (i.e. > 50 ppm free chlorine) due to the high content of organic matter present in the washing process, which reduces the efficiency of oxidizing agents such as hypochlorite. Nevertheless, results for the washing step demonstrate that its reducing effect can be crucial to remove NoV contamination but if previous steps fail.

4. Conclusions

Results indicated that when the contaminated produce was the initial contamination source, this led to higher NoV doses on final product during school foodservice operations compared with infected food handlers and contaminated food contact surfaces. On the other hand, when workers or work surfaces were the initial contamination source, a higher prevalence was obtained. These differences in the contamination patterns resulted in a higher risk (% cases of Norovirus infection) when originated from food handlers infected with NoV (and contaminated food contact surfaces) in school cafeterias. Based on the sensitivity analysis, NoV transfers to fresh produce may be minimized through effective training programs specifically addressing hand washing. The model in this study should be able to be incorporated into future evaluations of the impact of specific and effective training programs, aimed at food chain operators, on the risk associated with NoV transmission.

The model in this study might be used, in the future, to evaluate the impact of specific and effective training programs, aimed at food chain operators, on the risk associated with NoV transmission.

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