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Quantitative farm-to-fork risk assessment model for norovirus and hepatitis A virus in European leafy green vegetable and berry fruit supply chains

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ABSTRACT

Fresh produce that is contaminated with viruses may lead to infection and viral gastroenteritis or hepatitis when consumed raw. It is thus important to reduce virus numbers on these foods. Prevention of virus contamination in fresh produce production and processing may be more effective than treatment, as sufficient virus removal or inactivation by post-harvest treatment requires high doses that may adversely affect food quality. To date knowledge of the contribution of various potential contamination routes is lacking. A risk assessment model was developed for human norovirus, hepatitis A virus and human adenovirus in raspberry and salad vegetable supply chains to quantify contributions of potential contamination sources to the contamination of produce at retail. These models were used to estimate public health risks. Model parameterization was based on monitoring data from European supply chains and literature data. No human pathogenic viruses were found in the soft fruit supply chains; human adenovirus (hAdV) was detected, which was additionally monitored as an indicator of fecal pollution to assess the contribution of potential contamination points. Estimated risks per serving of lettuce based on the models were 3×10^{-4} (6×10^{-6}–5×10^{-3}) for NoV infection and 3×10^{-8} (7×10^{-10}–3×10^{-6}) for hepatitis A jaundice. The contribution to virus contamination of hand-contact was larger as compared with the contribution of irrigation, the conveyor belt or the water used for produce rinsing. In conclusion, viral contamination in the lettuce and soft fruit supply chains occurred and estimated health risks were generally low. Nevertheless, the 97.5% upper limit for the estimated NoV contamination of lettuce suggested that infection risks up to 50% per serving might occur. Our study suggests that attention to full compliance for hand hygiene will improve fresh produce safety related to virus risks most as compared to the other examined sources, given the monitoring results. This effect will be further aided by compliance with other hygiene and water quality regulations in production and processing facilities.

1. Introduction

In the Netherlands, there were an estimated 680,000 cases of foodborne disease in 2009 due to 14 pathogens, of which 23% were attributed to viruses compared to 9% to non-toxin producing bacteria (Havelaar et al., 2012). When combining these incidence estimates with the severity of illness following infection, rotavirus and NoV were the third and fourth most important food-related pathogens, following *Toxoplasma gondii* and *Campylobacter* spp. (Havelaar et al., 2012). To provide safe food it is thus important to direct efforts at reducing the numbers of viruses on consumed food items, preferably by prevention as recommended by EFSA (2011). Reported foodborne virus outbreaks among humans are frequently associated with fresh produce such as raspberries, salad vegetables and sun-dried tomatoes (e.g., Ethelberg et al., 2010; Gallot et al., 2011; Sarvikivi et al., 2012).

The consumption of fresh produce does not involve preparation steps that inactivate viruses, and therefore the infection risks need to be reduced prior to retail. Inactivation processes applicable before retail,

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however, may not suffice to eliminate norovirus contamination and may lead to unacceptable food quality (Baert et al., 2009; Niemira, 2003).

As an alternative to inactivating treatments, effective prevention of contamination could reduce virus numbers on produce and thereby decrease consumer risks of viral foodborne infections and therefore illness. Therefore, knowledge on the contribution of potential contamination points to the overall virus-contamination of food, the effects of food handling on the virus contamination and virus-specific characteristics such as persistence and transfer proportions due to surface contact is required. Such data were gathered in the European FP7 project "VITAL", which focused on the integrated approach of data collection and data analysis for use in risk assessment and management. Potential contamination points along three raspberry, one strawberry and three lettuce supply chains were identified using HACCP-based questionnaires and site visits by HACCP experts (Table 1). Required sample sizes were then calculated and delivered in chain-specific sampling plans to the data gathering laboratories. Samples were collected longitudinally per chain (Kokkinos et al., 2012; Maunula et al., 2013) and analyzed for norovirus (NoV; genogroups 1 and 2) and hepatitis A virus (HAV) with optimized, standardized detection procedures (D'Agostino et al., 2011; Martinez-Martinez et al., 2011). In addition to these human pathogenic viruses, the presence of human adenoviruses (hAdV) was examined to demonstrate that a route of contamination existed from infected humans to the sampling point, which other enteric viruses could follow. These monitoring data are subsequently used in a quantitative microbiological risk assessment model to estimate the human health risks associated with the consumption of fresh produce and the contribution of potential contamination points to the overall virus contamination. The model consists of newly developed mathematical descriptions of the contamination points along supply chains to examine the most important contamination points among those considered. The model was parameterized using the raw data collected in VITAL, completed with literature data to fill remaining data gaps. That model and the model outcomes are presented in the current paper.

2. Materials and methods

2.1. Conceptual model

Food supply chains differed within and between countries. For instance, harvested produce was sold at farmers' markets without further processing, others were rinsed and further handled prior to transport to supermarkets. Each process step was represented by a specific module, with each module describing the net contribution to the overall virus concentration on the produce. The full conceptual model that was considered is shown in Fig. 1. The appropriate modules were selected per supply chain and linked in a fixed chronological order: irrigation, harvesters' hands, conveyor belt, food handlers' hands, rinsing, consumption and dose–response. Contamination occurring in kitchens of consumers was not considered in the current model. Other potential sources that might contribute to virus contamination, such as direct contamination with feces in the production fields and addition of viruses through pesticides that are prepared with surface water (Verhaelen et al., 2013b), were not included at present due to lack of data. Furthermore, potential intrinsic contamination of viruses through uptake via roots or leafs was not considered in the current model.

2.2. Supply chains

Three salad vegetable chains were studied. The practices in supply chain A could be represented by the irrigation module, the harvesters' hands module and the rinsing water module (Table 1). The type of lettuce produced was romaine lettuce. The second and third supply chains (B & C) could be represented by the irrigation module and harvester's hands module only. The type of lettuce produced was butterhead lettuce.

Two raspberry chains were studied. These chains were short with no processing of fruits involved, and spray irrigation was not used in the examined supply chains. One of the chains, chain D, involved mechanical harvesting and food handlers inspecting and touching the berries on the conveyor belt. Chain D was therefore represented by two modules: conveyor belt and food handlers' hands (Table 1). Chain E involved manual harvesting of berries, followed by transport on a conveyor belt. Chain E was therefore represented by two modules: harvesters' hands and conveyor belt.

The strawberry production chain (chain F) employed drip irrigation and manual harvest followed by immediate transport of berries to retail after harvest. This chain was therefore modeled using only the harvesters' hands module.

2.3. Modules

2.3.1. Irrigation

The production sites that were monitored in VITAL applied drip irrigation, spray irrigation or no irrigation. Drip irrigation supplies water at the branches or roots of crops and therefore was not considered for external contamination of produce in the current study. The irrigation water module thus considered contamination with virus through spray irrigation, which was used only in the lettuce head production chains.

Irrigation schemes are developed to provide each plant with a certain volume of water. It was therefore considered reasonable to assume a uniform distribution of the sprayed water across the crop field during

Table 1
Overview of potential contamination points modeled per production chain, including results (positive/total) from the production chain monitoring (Kokkinos et al., 2012; Maunula et al., 2013).

Chain	Product	Irrigation	Harvesters	Food handlers	Rinsing	Conveyor belt	Consumption & dose–response
A	Romaine lettuce	■ hAdV: 17/22 NoV: 1/5	■ hAdV: 31/87 NoV: 1/12		■ hAdV: 2/6 NoV: na[a]		■
B	Butterhead lettuce	■ hAdV: 0/17	■ hAdV: 3/66				■
C	Butterhead lettuce	■ hAdV: 0/22 HAV: 0/20	■ hAdV: 1/86 HAV: 2/87				■
D	Raspberries[b]			■ hAdV: 1/51		■ hAdV: 0/15	
E	Raspberries[b]		■ hAdV: 4/64			■ hAdV: 0/24	
F	Strawberries[b]		■ hAdV: 1/60				

[a] na: not available.
[b] No consumption and dose–response, because no human pathogenic viruses were found in the monitoring and only hAdV was modeled.

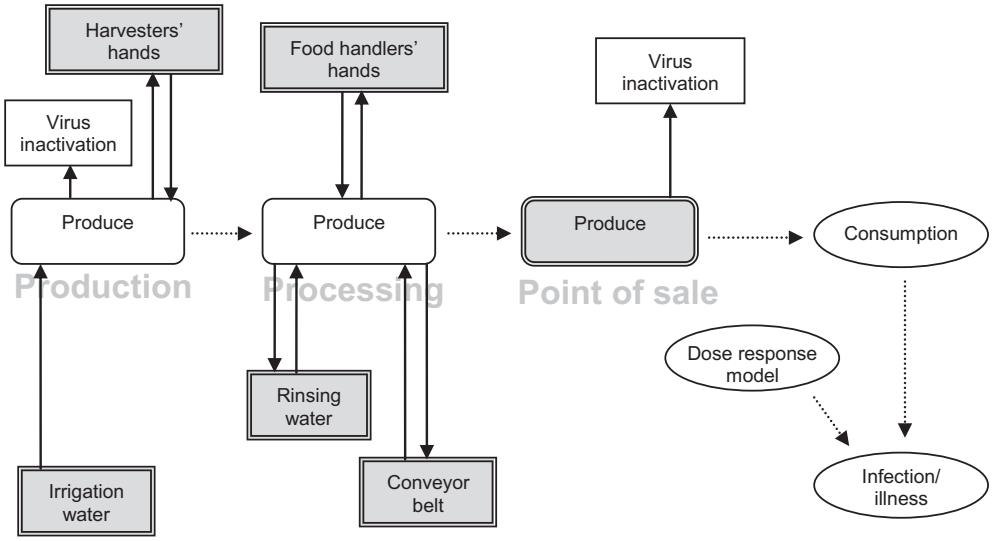

Fig. 1. Full conceptual model of the soft fruit and leafy green vegetable production chains. Each box represents a module. The actual models differ per production chain based on the practice applied in that chain. Double-lined, shaded boxes indicate where samples were collected in the monitoring. Ovals indicate processes that occur in the consumer phase.

irrigation. Under this assumption, the volume of water that falls on the lettuce head was estimated from the average intensity of irrigation across the field (I_{irw}; in L per m^2). Assuming the water falls onto the heads from one direction, and the lettuce head is a round sphere with diameter d (in m), then the surface area of the produce that is watered (ω_{prod}; m^2) can be estimated as half a sphere by $0.5 \times 4\pi(0.5d)^2$. The estimated number of viruses deposited per unit of product item due to spray irrigation (n_{irw}) was calculated as:

$$n_{irw} = C_{irw} I_{irw} \omega_{prod} \quad (1)$$

where C_{irw} is the estimated virus concentration per liter water. Data about the probability of human pathogenic viruses attaching to the produce are lacking. The worst-case situation involving the attachment of all viruses falling onto the produce was therefore modeled, which may lead to an overestimation of the risk.

Petterson et al. (2001) showed experimentally that for bacteriophages the last irrigation event before harvest is most determining for the virus contamination of lettuce heads by irrigation. Therefore the irrigation module considered one single irrigation event (although an extension to multiple irrigation events could easily be made).

If spray irrigation was not applied, then n_{irw} was considered to be zero.

2.3.2. Harvester's hands

The proportion of virus that transfers from harvesters' hands to produce depended on the surface concentration of viruses on the whole hand (C_{harv}; n per hand), the surface area of the hand that touches the product (ω_{harv}; in cm^2) and the proportion of viruses transferred from hand to product (f_{hand}). Furthermore, a proportion of viruses already present on the product may be transferred from the product to hands (f_{rasp} and f_{lett}, generally referred to as f_{prod}), reducing the number of viruses in this module. For products that are harvested manually, the number of viruses per product after harvest (n_{harv}) was calculated as:

$$n_{harv} = n_{irw} - f_{prod} \frac{\omega_{harv}}{\omega_{prod}} n_{irw} + f_{hand} \frac{\omega_{harv}}{\omega_{hand}} C_{harv} \quad (2)$$

with ω_{hand} being the total surface area of a harvesters' hand.

The total skin surface area of both hands, including palm, back and fingers, is reported to be on average 0.107 m^2 for males and 0.089 m^2 for females (USEPA, 2011). These estimates were further combined to a single mean estimate of 0.098 m^2, or 980 cm^2, for both hands, because the ratio between male and female harvesters was unknown. Lettuce heads were assumed to be picked using one hand only (the other hand is used for cutting) and therefore the palm surface area of 245 cm^2 was used for ω_{harv}. Do note that this reported skin surface area originates from studies on skin burns and covers the surface area of e.g. the fingers round, possibly overestimating the actual skin surface area that touches the lettuce head. Soft fruits are generally picked with three fingers. Verhaelen et al. (2013a) estimated the surface area of the thumb, index finger and middle finger for touching a raspberry at 0.7 cm^2 per finger and found no statistical difference between the surface area of the three finger types. The value for ω_{harv} of 2.1 cm^2 was therefore used for soft fruits in the current study.

The fraction of virus transfer from hands to product (raspberry, strawberry and lettuce) and product (raspberry and lettuce) to hand after contact was estimated experimentally for hAdV and NoV genogroups 1 and 2 by Verhaelen et al. (2013a). The values appropriate for the virus and transfer route modeled in the current study were used. These values include the mean transfer proportion and the associated uncertainties.

If produce is picked mechanically (e.g., by shacking bushes and collecting berries), as was done in one of the raspberry supply chains, then n_{harv} was assumed to equal n_{irw} (i.e., no handling of produce occurred).

2.3.3. Conveyor belt

The conveyor belt was only used in the soft fruit production chains and was modeled to contribute to the number of viruses per product after this stage (n_{belt}) by transfer of viruses from the conveyor belt to the produce and by transfer of previously introduced viruses from the produce to the conveyor belt according to Eq (3):

$$n_{belt} = n_{harv} - f_{prod} \pi_{belt} n_{harv} + f_{hand} \pi_{belt} \omega_{prod} C_{belt} \quad (3)$$

where π_{belt} is the proportion of the surface area of the fruit item that touches the conveyor belt, ω_{prod} is the total surface area of the fruit item (cm^2) and C_{belt} is the number of viruses per cm^2 of belt. Specific transfer proportions from conveyor belts to fruits could not be retrieved from literature and therefore were assumed to be equal to the transfer proportion from produce to hands (f_{hand}) as described in Section 2.3.2. The value for π_{belt} was unknown and modeled with a uniform distribution between 0.25 and 1 to simulate its uncertainty, from one side of a berry touching the belt up to all of the berry.

The value of C_{belt} was based on swab samples collected during the monitoring. The swab samples were collected from a recorded area of the conveyor belt, allowing for estimation of the virus contamination per cm^2 under the assumption that viruses are distributed homogeneously.

If a conveyor belt was not used, then n_{belt} equaled n_{harv}.

2.3.4. Food handlers' hands

Food handlers touching lettuce heads occurred in a single food supply chain, for cutting. For soft fruits, touching by food handlers occurred on occasion during transport on a conveyor belt to turn the berries for visual inspection. For these supply chains, the number of viruses per product after food handling (n_{touch}) was modeled with Eq. (4):

$$n_{touch} = n_{belt} - f_{prod}\pi_{food}n_{belt} + f_{hand}\frac{\omega_{food}}{\omega_{hand}}C_{food} \quad (4)$$

with C_{food} being the virus number per hand of a food handler's hand and π_{food} being the proportion of the surface area of the food handlers' hand touching the product. For lettuce heads, the touching surface of a hand (ω_{food}) was modeled similarly as for harvesters' hands. For soft fruits, π_{food} was unknown and modeled using a uniform distribution between 0 and 1, because part of the berries likely remain untouched whereas other berries made a full turn when touched as based on visual observations in one of the production chain.

If berries were untouched, then n_{touch} equaled n_{belt}.

2.3.5. Rinsing water

Rinsing was applied in a single salad vegetable supply chain. This process can affect the virus contamination by addition and removal of virus. The addition can result from viruses already present in the rinsing water before the start of washing, or due to contamination of formerly clean water by already-contaminated produce. Removal of virus can occur due to washing-off. The number of viruses after rinsing (n_{rinse}) was estimated using Eq. (5):

$$n_{rinse} = n_{touch}10^{-f_{rinse}} + C_{rinse}V_{rinse} \quad (5)$$

where f_{rinse} is the decimal removal rate of viruses due to rinsing, C_{rinse} is the virus concentration in the rinsing water and V_{rinse} is the volume of rinsing water that clings to the produce. C_{rinse} was estimated from the data obtained during monitoring. V_{rinse} was obtained from Shuval et al. (1997), who experimentally assessed the volume of water retained by long leaf lettuce after full immersion under water to be 10.8 mL per 100 g of lettuce. This figure was considered the maximum carrying capacity of water by lettuce. The examined lettuce type that was rinsed in the current study weighed on average 1.8 kg at retail, and thus would carry at most about 200 mL of water after rinsing when assuming that the 10.8 mL per 100 g is representative for each 100 g of the lettuce head. The virus concentration in the rinsing water was not modeled dynamically in this model.

Mokhtari and Jaykus (2009) synthesized several experimental studies on virus removal due to washing and concluded that the effect of rinsing with clean water (f_{rinse}) was best described by a uniform distribution of 1 to 2 $\log_{10}$ units removal. In the absence of more recent and virus-specific data for the current study, the same approach was used.

2.3.6. Virus inactivation

Virus inactivation for NoV and hAdV was modeled as exponential reduction using the general virus inactivation model presented by Verhaelen et al. (2012). This model estimates the temperature-dependent average daily reduction (ADR) in virus numbers. For viruses detected by PCR on fruits kept refrigerated at 5 °C, the mean predicted $\log_{10}$ ADR was 0.011 $\log_{10}$-units per day. For viruses on fruits at 20 °C, the mean predicted $\log_{10}$ ADR was 0.151 $\log_{10}$-units. For lettuce, the same values as for raspberries were used regarding ADR and temperature.

The inactivation rate of hepatitis A virus for exponential reduction was taken from Bertrand et al. (2012), who provided estimates for HAV inactivation at temperatures <50 °C. Using their results, the estimated time to first $\log_{10}$-unit reduction (TFL) of infectious HAV at 4 °C and 20 °C was 76 (95% prediction interval: 6–928) and 25 (2–302) days, respectively, with associated average ADRs of 1/76 = 0.013 and 1/25 = 0.040 $\log_{10}$ units per day. Note that these estimates are for culturable HAV. However, estimates based on PCR detection have not been reported.

The times of inactivation considered per module were: 14 days for irrigation (assuming the last irrigation event occurs two weeks before harvest) applied to n_{irw} and subsequently 7 days for one other module (assuming that fresh produce is consumed seven days after harvest) applied to either n_{harv}, n_{rin}, n_{touch} or n_{belt} (whichever is modeled last in the specific food production chains; only one variable is corrected to prevent double accounting for inactivation).

2.3.7. Consumption

The exposure and public health risks were estimated for consumption of lettuce only, because no human pathogenic viruses were found on berries. The amount of lettuce consumed was set to 200 g per event, which is the advised amount of vegetables to be consumed per day in the Netherlands.

2.4. Risk characterization

2.4.1. Estimation of virus concentrations per source

Virus counts in positive samples were expressed as PCR-detectable units (PDUs), with one PDU representing an unknown number of virus particles (1 or more) depending on the properties of the (RT-)PCR. By definition, the lower limit of detection for any (RT-)PCR is 1 PDU in a reaction vessel. The estimated virus concentration for each sample was based on the actual portion of the sample that was examined in the (RT-)PCR. This portion was back-calculated using the concentration and dilution factors in nucleic acid isolation and detection procedures. The PDUs were assumed to be distributed randomly within samples, and to be gamma-distributed between samples. The most likely parameter value for the Gamma distribution was obtained by maximum likelihood estimation as described by Bouwknegt et al. (2011). Briefly, the likelihood of an actual virus concentration per sample, c_k, was assessed using the presence/absence profiles for neat and serial 10-fold diluted RNA solutions for sample k according to:

$$g_k(c_k|V,p) = \prod_{i=1}^{j}\left(1-Exp\left[-c_k\cdot V_{pcr,i}\right]\right)^{p_i}\cdot\left(Exp\left[-c_k\cdot V_{pcr,i}\right]\right)^{1-p_i} \quad (6)$$

where $V_{pcr,i}$ represents the actual portion of the sample represented in solution i (e.g., 1 = neat, 2 = 10-fold dilution, etc.), and p_i denotes the presence ($p_i = 1$) or absence ($p_i = 0$) of ≥1 PDU in solution i. The likelihood for the parameters r and λ of a gamma distribution based on n samples per sampling point was subsequently assessed by

$$\ell(r,\lambda) = \prod_{k=1}^{n}\int_{c=0}^{\infty}\frac{c^{r-1}e^{-\frac{c}{\lambda}}}{\lambda^k\Gamma(r)}\cdot g_k(c). \quad (7)$$

When the likelihood function (Eq. (7)) did not converge, then the virus concentration was assumed to be homogeneously distributed between samples (i.e., all observations were treated as if obtained from a single sample k) and Eq. (6) was used. When no positive samples are found for a potential contamination point, then the most likely estimate of the PDU concentration is zero. The 95% upper limit of the virus concentration was subsequently estimated by solving Eq. (6) for a −2 Log transformation of the likelihood value of 3.84 (these transformed likelihoods are approximately chi-square distributed with 1 degree of freedom). This 95% upper limit of the virus concentration was included

in respective modules for estimating the approximate 95% upper limit of the total estimated virus contamination per product.

2.4.2. Dose–response models

The used hypergeometric dose–response model for norovirus (combined 8fIIa and 8fIIb) was developed by Teunis et al. (2008), who based the model on volunteer experiments with Norwalk virus, a particular NoV strain. This model allows for heterogeneity in host susceptibility to NoV infection. We kindly received a set of 10,000 parameter values for the hypergeometric dose–response model, representing parameter uncertainty, from Prof. Teunis (this set is available as Supplementary data). We randomly sampled one parameter set from this list per iteration of the Monte Carlo sampling.

The exponential dose–response model for hepatitis A virus was initially described by Haas et al. (1999), based on data from Ward et al. (1958) who inoculated institutionalized individuals with HAV and monitored the occurrence of jaundice (hence the prediction of jaundice cases for this dose–response model). The dose administered to the individuals was expressed as gram of feces. The ingested doses in the VITAL risk assessments were PDUs per consumption event. To harmonize the estimated dose in the model with the dose inoculated by Ward better, the likelihood function of the dose–response model using the original doses reported by Ward et al. (1958) was expanded with a likelihood function for the number of HAV genome copies per gram of feces as reported by Kamel et al. (2011). This model assumes that all individuals are equally susceptible to develop jaundice for a certain exposure dose.

2.4.3. Risk estimation

The estimated virus concentrations on the end product were estimated by Monte Carlo simulation in Mathematica version 8 taking 10,000 random samples from the uncertainty distributions. Table 2 lists all parameters and their values or distributions used in the risk assessment.

The estimates from the model were compared to the estimates based on the point-of-sale monitoring of produce. Virus numbers per food product were estimated as described in Section 2.4.1, and the exposure was assessed using the consumption data as described in Section 2.3.7.

2.4.4. Sensitivity analysis

The sensitivity analysis was done for two production chains to cover each module (lettuce supply chain A and raspberry chain D). The sensitivity of the model to each parameter was assessed multivariably using Monte Carlo simulation (10,000 iterations). For each parameter a value was randomly drawn from the distributions as listed in Table 3 per iteration and used to estimate the contamination level for the produce. Spearman rank correlation coefficients (SCCs) were subsequently assessed in SAS v9.3 (SAS Institute, Cary, USA) to rank the correlation between the estimated contamination levels and input parameter values (Mokhtari and Frey, 2005). The SCCs were used, because the risk assessment model is nonlinear and monotonic. Those parameters with the highest SCC were considered most influential.

Table 2
List of parameters, their values and/or uncertainty distributions used in the risk assessment models.

Parm.	Explanation	Value or distribution	Ref
C_{irw}	Virus PDU concentration in irrigation water (n per L water)	NoV, romaine lettuce (chain A): Gamma [0.084, 0.039] hAdV romaine lettuce (chain A): Gamma [0.577, 0.031] hAdV, butterhead lettuce (chain B): most likely 0; 95% upper limit: 0.12 HAV, butterhead lettuce (chain C): most likely 0; 95% upper limit: 0.12 hAdV, butterhead lettuce (chain C): most likely 0; 95% upper limit: 0.11	Estimated in this study
V_{irw}	Volume of water (V_{irw}) sprayed per unit surface	50,000 L/ha or 0.5 mL/cm^2 (provided by suppliers)	Determined in this study
ω_{prod}	Surface area of produce	Raspberry: normal [1064, 167] mm^2 Strawberry: normal [1064, 167] mm^2 Butterhead lettuce: 1400 cm^2 Romaine lettuce: 226 cm^2	Estimated in this study
f_{prod}	Transferred proportion per touch from produce to hand	NoV, lettuce: Beta [18.55, 49.05] HAV, lettuce: data for NoV used: Beta [18.55, 49.05] hAdV, lettuce: Beta [13.36, 430] hAdV, raspberry: Beta [15.64, 41.94] NoV, strawberry: data for raspberries used, Beta [7.42, 39.51]	(Verhaelen et al., 2013a)
f_{hand}	Transferred proportion per touch from hand to produce	NoV, lettuce: LogNormal [−2.22, 0.17] hAdV, lettuce: Beta [15.14, 46.72] hAdV, raspberry: LogNormal [−8.34, 0.58] NoV, strawberry: LogNormal [−2.32, 0.15]	(Verhaelen et al., 2013a)
ω_{harv}	Surface area of hands that touch produce	Lettuce: 245 cm^2 Raspberry and strawberry: 2.1 cm^2	(USEPA, 2011) (Verhaelen et al., 2013a)
ω_{hand}	Total surface area of one side of one hand	245 cm^2	(USEPA, 2011)
π_{food}	Proportion of the food handlers' hand touching produce	Uniform [0, 1]	Assumed in this study
C_{harv}	Virus PDU number on harvesters' hands (n)	NoV, romaine lettuce (chain A): Gamma [1.11, 4.46] hAdV, romaine lettuce (chain A): Gamma [1.22, 24.53] hAdV, butterhead lettuce (chain B): Gamma [0.06, 117] HAV, butterhead lettuce (chain C): Gamma [0.98, 1.55] hAdV, butterhead lettuce (chain C): Gamma [0.009, 141.2]	Estimated in this study
C_{harv}	Virus PDU number on harvesters' hands (n)	hAdV, raspberry (chain E): Gamma [0.14, 54.6] hAdV, strawberry (chain F): Gamma [0.002, 40135]	Estimated in this study
C_{food}	Virus PDU concentration on food handlers' hands (n)	hAdV, raspberry site A: Gamma [0.67, 1.62]	Estimated in this study
C_{belt}	Virus concentration on conveyor belts (n per m^2)	hAdV: most likely 0; 95% upper limit: 70	
π_{belt}	Proportion of the berry surface touching the conveyor belt	Uniform [0.25, 1]	Assumed in this study
f_{rinse}	Decimal removal rate of viruses due to rinsing	Uniform [1, 2]	(Mokhtari and Jaykus, 2009)
δ	Temperature-dependent virus decay ($\log_{10}$ units per day)	NoV, MCMC post., T was set at 20 °C for irrigation and 5 °C for all other modules HAV, for $T = 20$: normal [1.88,0.555], for $T = 5$: normal [1.40, 0.555]	(Verhaelen et al., 2012) (Bertrand et al., 2012)
$(\alpha,\beta)_{NoV}$	Set of parameters for the NoV dose–response model	MCMC post, see Supplementary data for the set and a graph with plotted parameter values showing their correlation. The most likely value for α was 0.040 (95% interval in the set: 8×10^{-6}–0.10), for β 0.055 (95% interval in the set: 5.1×10^{-6}–4.6).	(Teunis et al., 2008)
r_{HAV}	Infectivity of HAV in exponential dose–response model	MCMC post., mean: 4×10^{-6}; 95% interval: 1×10^{-6}–1×10^{-5}	Estimated in this study

Table 3
Alternative parameter values for the sensitivity analysis (see Table 2 for an explanation of the parameters).

Parameter	Probabilistic analysis	Scenario-based analysis				
	Distribution	Lowest	Low	Baseline	High	Highest
ω_{lett}	r ~ Uniform [6, 24][a]	57	127	226	509	905
I_{irw}	$0.5 \times 10^{\text{Uniform }[-2,2]}$	0.005	0.05	0.5	5	50
C_{irw}	$0.02 \times 10^{\text{Uniform }[-2,2]}$	2×10^{-4}	2×10^{-3}	2×10^{-2}	2×10^{-1}	2
ω_{harv}	Uniform [0.25 × 2.1, 4 × 2.1]	0.5	1.1	2.1	4.2	8.4
f_{lett}	Uniform [0, 1]	0	0.003	0.03	0.3	1
f_{hand}	Uniform [0, 1]	0	0.01	0.1	.	1
ω_{hand}	Uniform [123, 490]	123	184	245	368	490
C_{harv}	$30 \times 10^{\text{Uniform }[-2,2]}$	0.3	3	30	300	3000
f_{rinse}	Uniform [0, 1]	0	0.5	1.5	2.5	3.5
π_{belt}	Uniform [0, 1]	0	.	0.625	.	1
ω_{rasp}	Uniform [6.8, 14.5][b]	6.8	9.5	10.6	11.8	14.5
C_{belt}	$10^{\text{Uniform }[0,4]}$	1	10	100	1000	10,000
f_{rasp}	Uniform [0, 1]	0	0.03	0.3	.	1
π_{food}	Uniform [0, 1]	0	.	0.5	.	1
C_{food}	$10^{\text{Uniform }[0,4]}$	1	10	100	1000	10,000

[a] r is the radius of a lettuce head, ω_{lett} is calculated as $1/2\ 4\pi r^2$.
[b] Parameters are based on the 1% and 99% limits of the distribution for raspberry surface as described in Table 1.

The SCC does not provide insight in the magnitude of change of the outcome due to alternative parameter value. The relative change in estimated contamination of produce was therefore assessed using a scenario-based approach with alternative parameter values as listed in Table 3. Each parameter was adjusted individually per scenario. Alternative parameter values were based on modifications of the baseline value (virus concentrations based on hAdV), except for the hAdV concentration on the conveyor belt and food handlers' hands. The most likely values for the latter two parameters were 0, and therefore the sensitivity was based on the hypothetical concentration of 1 up to 10^4 PDUs. Note that the assumption that a whole hand touches a lettuce head removes the proportion $\omega_{harv}/\omega_{hand}$ from Eq. (2) for lettuce heads, and thus also from the sensitivity analysis.

3. Results

3.1. Salad vegetables

Human adenovirus was included in the VITAL study to demonstrate an existing route of human fecal contamination, and used to estimate the contribution of irrigation water and harvesters' hands to the overall produce contamination. Human adenovirus was found in none of 17 (chain B) and 22 (chain C) irrigation water samples, respectively, whereas hAdV was detected on 3 of 66 and 1 of 86 harvesters' hands for chains B and C, respectively (Table 1). In supply chain A, hAdV was detected in irrigation water and on harvesters' hands. Using the model, the estimated average contamination was 0.01 (6×10^{-4}–0.08) for chain A (romaine lettuce) and 0.08 (95% interval: ~0–8) and 5×10^{-5} (~0–7) for chains B and C, respectively (butterhead lettuce) (Table 4), with harvesters' hands contributing most to the contamination (Fig. 2A).

Human pathogenic viruses were detected in samples taken in supply chains A and C. In chain A, NoV was found in 1 of 5 irrigation water samples and in 1 of 12 harvesters' hand swabs (Table 1). Rinsing water was not examined for human pathogenic viruses. The most likely NoV concentrations in the water and on hands were 3 PDUs (95% interval: 0–34) per L water and 5 PDUs (95% interval: 0.2–17) per hand, respectively. The median estimated virus number per lettuce head at point of sale was 9×10^{-3} (3×10^{-4}–0.08), yielding an estimated infection risk of 3×10^{-4} (6×10^{-6}–5×10^{-3}) for the consumption of 200 g of romaine lettuce. Harvesters' hands contributed more to the estimated NoV contamination than irrigation water (Fig. 2B). The estimated infection risk based on point-of-sale data was 0, with a 97.5% upper limit of 0.5.

In chain C, HAV was found in none of 20 irrigation water samples and in 2 of 87 harvesters' hands swabs (Table 1). The most likely HAV concentrations in the water and on hands were 0 PDU per liter water (95% upper limit: 0.12) and 1 (95% interval: 0.07–18), respectively. The median estimated HAV PDU concentration at point of sale per lettuce head based on the model was 8×10^{-2} (5×10^{-3}–7), yielding an estimated risk for HAV jaundice of 3×10^{-8} (7×10^{-10}–3×10^{-6})

Table 4
Estimated virus concentration (mean PDU and 95% interval) per produce item, risk of infection after consumption or risk of jaundice after consumption for fresh produce. Estimates are either based on the production chain model or on the point of sale measurements as described in Section 2.4.3.

		Estimate based on	
Produce	Virus	Chain model	Point of sale monitoring
Contamination per item			
Romaine lettuce (chain A)	hAdV	0.01 (6×10^{-4}–0.08)	600 (42–3×10^3)
Butterhead lettuce (chain B)	hAdV	5×10^{-5} (~0[a]–7)	0.02 (3×10^{-3}–0.06)
Butterhead lettuce (chain C)	hAdV	0.08 (~0–8)	50 (~0–586)
Raspberry (chain D)	hAdV	1×10^{-5} (5×10^{-8}–0.1)	0.03 (0.003–0.08)
Raspberry (chain E)	hAdV	1×10^{-5} (~0–1×10^{-3})	0.01 (8×10^{-4}–0.07)
Strawberry (chain F)	hAdV	9×10^{-6} (0–1×10^{-7})	1.2 (~0–1.3)
Infection risk per serving			
Romaine lettuce (chain A)	NoV	3×10^{-4} (6×10^{-6}–5×10^{-3})	0 (0–0.5)
Jaundice risk per serving			
Butterhead lettuce (chain C)	HAV	3×10^{-8} (7×10^{-10}–3×10^{-6})	0 (0–2×10^{-6})

[a] Estimate $< 10^{-10}$

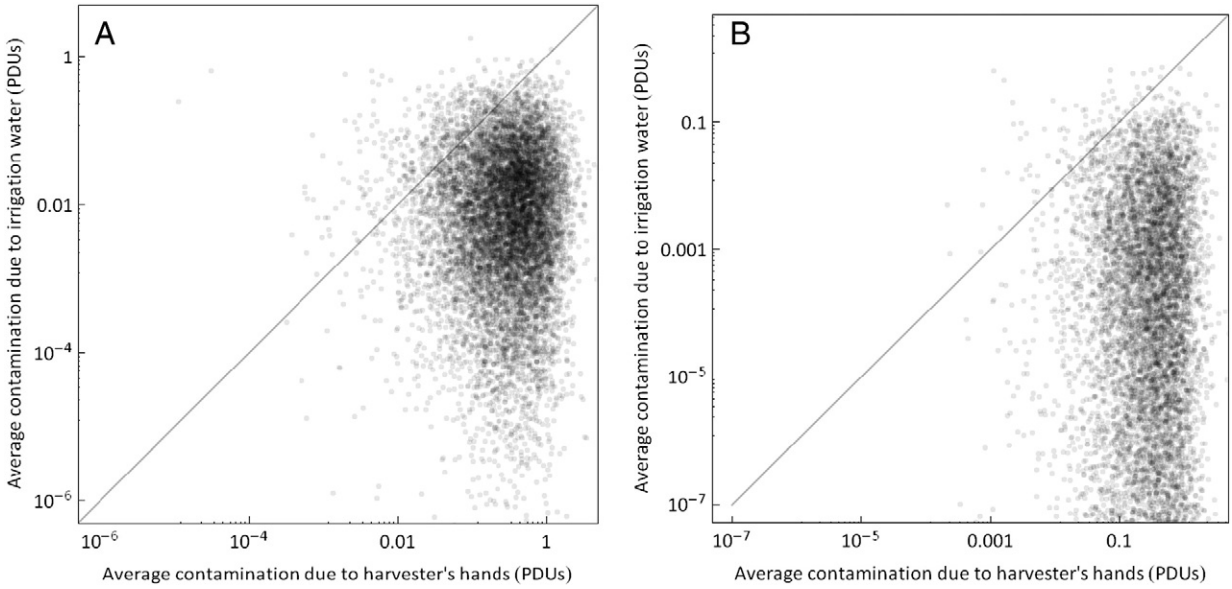

Fig. 2. Contribution of harvesters' hands and irrigation water to the estimated contamination of lettuce heads with hAdV (A) and NoV (B) for supply chain A. Each marker represents a single iteration from the Monte Carlo simulation. The gray line represents an equal contribution for both potential contamination points. Markers positioned above this line indicate a greater contribution for irrigation water (than hands) and markers below the line indicate a greater contribution for hands.

for the consumption of 200 g (Table 4). No HAV was found on lettuce heads at point of sale, leading to an associated 97.5% upper limit of 1×10^{-6} for the jaundice risk based on monitoring data.

3.2. Soft fruits

No human pathogenic viruses were detected in samples from either of the two raspberry supply chains, while hAdV was detected (Maunula et al., 2013). The estimated average number of hAdV per raspberry at point of sale using the model was 1×10^{-5} (95% interval: 2×10^{-7}–0.09) for chain D and 6×10^{-6} (95% interval: 8×10^{-7}–2×10^{-5}) for chain E (Table 4). At both chains, the majority of the virus contamination was estimated to originate from hands, because no hAdV was found on the conveyor belts. The monitoring at points of sale, with the locations linked to these two production sites, identified 2 of 28 and 1 of 37 raspberry samples to be contaminated with hAdV, leading to estimated virus concentrations of 0.04 (0.01–0.12) PDU and 0.01 (0.001–0.07) PDU per berry for sites D and E, respectively (Table 4).

In the strawberry monitoring (chain F), NoV was not detected on harvester hands' (Maunula et al., 2013), while hAdV was detected on 1 of 60 hands. The most likely hAdV PDU concentrations on hands was 70 PDUs (28–131) per hand. The estimated NoV PDU concentration at point of sale for strawberries was 2×10^{-4} PDUs (3×10^{-5}–5×10^{-4}) per strawberry. In the point of sale monitoring hAdV was detected on 1 of 51 strawberries obtained at point of sale, giving a most likely average contamination of 3 (1–5) PDUs per strawberry.

3.3. Sensitivity analysis

The most influential parameters for the lettuce head production chain was f_{rinse}, for the raspberry production chain C_{belt}. Overall, various parameters for estimated virus concentrations at potential contamination points (C_{irw}, C_{harv}, C_{food}) were among the top-ranked influential parameters (Table 5). The parameter π_{belt} had an overall important impact on the results (Table 5), but the effect on the estimated virus concentration was one-sided (Fig. 3). Alternative parameter values led to an increase or decrease up to two orders of magnitude on the final product within the range examined (Fig. 3). The parameters describing proportions, such as transfer and surface area proportions, generally had smaller effects on the estimated contamination levels than the concentration estimates.

4. Discussion

The magnitude of a public health risk posed by viruses in the food chain cannot be assessed solely from detected presence or absence of viruses in the products at retail. A single sampling is usually not representative of the actual public health risk due to e.g. temporal and geographic variation, virus levels below the detection limit that results in false-negative results, and a non-homogeneous distribution of viruses on the product. Ideally, data on the pathogen concentration in the contamination source, the fate and behavior of the pathogen, the level of eventual exposure of humans and the probability of an adverse health event associated with that exposure are integrated, and a QMRA provides a valuable tool for this purpose (Haas et al., 1999; Vose, 2008). The model presented in the current study is developed with that aim and is relatively easily applicable to other situations when quantitative data on virus contamination in sources are available.

Ranking the contribution of potential contamination points showed that contact with hands was the most dominant contamination source given the current monitoring data and the modeling used. Hand hygiene may thus be a prime starting point for prevention of contamination, as is the case for bacteria. Full (i.e., 100%) compliance at all times

Table 5
Spearman's rank correlation coefficients (SCCs) for the correlation between model parameters and estimated contamination level of lettuce and raspberries, as part of the sensitivity analyses (see Table 2 for an explanation of the parameters).

Lettuce head model		Raspberry model	
Parameter	SCC	Parameter	SCC
f_{rinse}	−0.88	C_{belt}	0.86
C_{harv}	0.22	f_{hand}	0.31
I_{irw}	0.11	π_{belt}	0.24
C_{irw}	0.10	N_{food}	0.09
f_{hand}	0.06	f_{rasp}	−0.09
ω_{lett}	0.03	π_{food}	−0.07
f_{lett}	−0.01	ω_{rasp}	0.06
		ω_{hand}	−0.01
		ω_{harv}	0.00

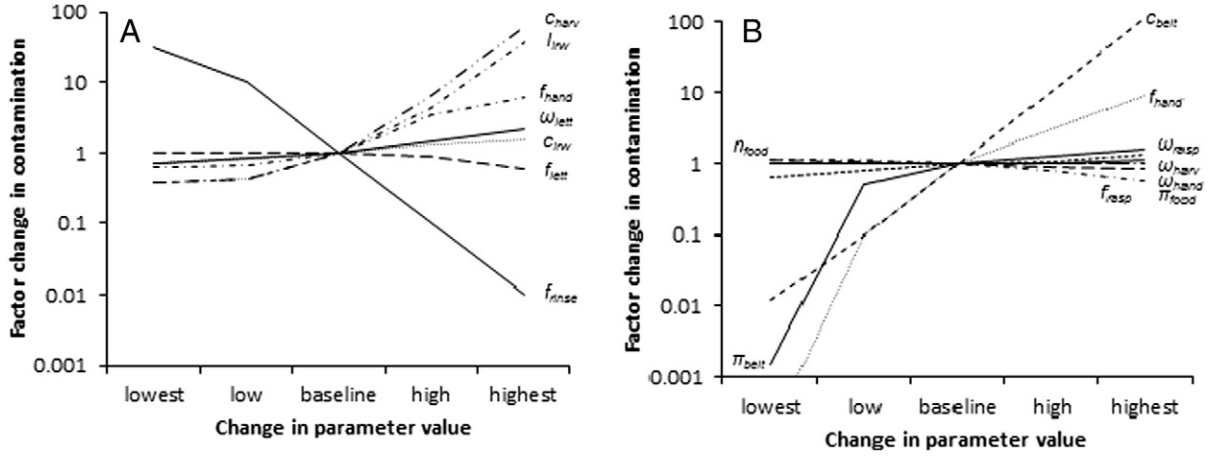

Fig. 3. Results of the sensitivity analysis using the irrigation, the harvester hand and the rinsing modules for lettuce (A) and conveyor belt and food handler hand modules for raspberries (B). Details of the scenarios represented on the *x*-axis are described in Table 2.

to current HACCP guidelines therefore likely contributes to virus-safe production of soft fruits and vegetables. The efficacy of proposed or implemented hygiene practices for viruses, however, needs to be assessed. For instance, the bactericidal activity of a hand rub is not per se similar to that for viruses (Sattar et al., 2002). Nonetheless, even washing with water is effective in reducing virus numbers on hands, as concluded from a quantitative meta-analysis using data from multiple experiments (Mokhtari and Jaykus, 2009), and therefore is a recommended preventive measure.

As always with QMRA, restrictions in the current parameterization and generalizations are present. Firstly, the applied parameters of the dose–response models cause large uncertainties in results due to the heterogeneity among hosts (including susceptibility) and pathogens and the small sample size associated with parameterization of the dose–response models (Haas et al., 1999; Teunis et al., 2008). Furthermore, an important aspect relates to the translation of estimated PDU concentrations into the ingested dose of infectious organisms. The detection of viral DNA or RNA by (RT-)PCR indicates that contamination has occurred in the production chain, but the presence of infectious viruses at the moment of sampling is not confirmed (Havelaar and Rutjes, 2008; Stals et al., 2013). Detected RNA could originate from defective virus particles. Furthermore, the exposure dose was estimated in PDUs per event. Applying this dose to the dose–response model implies that the ratio between infective and defective particles of the monitoring samples is identical to that of the dose–response samples. This ratio is however highly variable (De Roda Husman et al., 2009) and that assumption likely does not hold. However, no further information is available on this ratio and therefore it is not possible to indicate whether risks are over- or underestimated. Furthermore, no objective alternatives are currently available to include this ratio properly in risk assessment, but several methods are emerging for the determination of viability by PCR, including cell-culture PCR, long-template PCR and enzymatic pre-treatment with RNAses and propidium monoazide (Allain et al., 2006; Greening et al., 2002; Parshionikar et al., 2010; Sanchez et al., 2012; Schielke et al., 2011). When fully validated for foodborne viruses in food environments, such approaches might be used to more accurately estimate the (likely variable) fraction of infectious viruses among all viruses detected by PCR. Similar efforts also need to be made for data supporting dose–response models.

The estimated risks based on the production chain model and the point-of-sale samples agreed for the human pathogenic viruses. Yet the estimated contamination levels per produce item for hAdV differed several orders of magnitude with respect to most likely values. The uncertainty for the latter estimates, however, was large due to the relatively low sample sizes per sampling point and few positive samples and 95% intervals overlapped in four of five cases. Discrepancies between the model estimates and point-of-sale estimates can have several causes: 1) not all contamination points were included as modules in the monitoring and the model; 2) the modules poorly reflected the essence of the included contamination processes; and 3) the contamination of produce is episodic in nature and sampling was too limited in time and space to provide data on the likelihood and the extent of incidental contamination. The virus concentrations for potential contamination points were shown in the sensitivity analysis to be most influential on the risk outcome, possibly altering the risk estimates several orders of magnitude. The higher estimates based on the point of sale monitoring might be the result of an episodic contamination event in the production of that particular batch, whereas similar events might not have been encountered during the production chain monitoring. The discrepancies between model estimates and point-of-sale estimates, the model sensitivity, but also the observed variety of processes employed in the supply chains, impact the generalizability of our results and show the need for case specific parameterization of the risk model.

Given the relatively low risk estimates in the current study, but the occasional large extent of outbreaks, possibly the incidental contamination events contribute to a larger extent to the adverse public health effect than the general production practices. Episodic events may occur due to e.g. a single noncompliance event for hand hygiene or due to combined sewer overflows after heavy rainfall events. In other instances, prolonged outbreaks, such as for HAV in semi-dried tomatoes, suggest a structural contamination source along that food supply chain (Carvalho et al., 2012; Fournet et al., 2012). Gaining insights into the effect of structural as well as episodic contamination points is important for accurate estimation of the effectiveness of implemented intervention measures.

Human pathogenic viruses were found at low numbers in the monitoring. These low numbers provide limited information on the virus concentrations for contamination points and thus affect the uncertainty of the virus concentration estimates. Hence the effort in the current study to model explicitly the uncertainty. Human adenovirus was found in a larger number of samples, providing more robust estimates of the virus concentrations, the exposure levels and the larger contribution of hand hygiene compared to irrigation water to the virus contamination. Nevertheless, the uncertainty for hAdV was also large. Future monitoring efforts for human pathogenic viruses along food production chains would benefit from even larger sample sizes than those examined in VITAL, combined with highly sensitive detection methods.

In conclusion, the current study showed that viral contamination in each of the different food production chains occurred. In addition to large epidemiological studies that have been conducted and have shown a significant number of people falling ill due to the consumption of virus-contaminated foods, the use of model-based risk assessments

adds value to the development of targeted intervention measures. It was shown here that the estimated mean risks were in general low, although some 95% upper limits did not exclude the potential for considerable risks (i.e., 50% infection risk for NoV on romaine lettuce) per serving. Furthermore, hand transfer was found to be a more likely contamination source for lettuce than irrigation water, based on the monitoring data and subsequent modeling. Our study suggests that attention to full compliance for hand hygiene will improve fresh produce safety related to virus risks most as compared to the other examined sources, given the monitoring results. This effect will be further aided by compliance with other hygiene and water quality regulations in production and processing facilities.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijfoodmicro.2014.12.013.

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