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Public Health Relevance of Cross-Contamination in the Fresh-Cut Vegetable Industry

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ABSTRACT

Although quantitative studies have revealed that cross-contamination during the washing stage of fresh produce occurs, the importance of cross-contamination in terms of public health relevance has rarely been assessed. The direct distribution of initially contaminated leafy vegetables to a multitude of servings by cutting and mixing also has not been addressed. The goal of this study was to assess the attribution of both contamination pathways to disease risk. We constructed a transparent and exploratory mathematical model that simulates the dispersion of contamination from a load of leafy greens during industrial washing. The risk of disease was subsequently calculated using a Beta-Poisson dose-response relation. The results indicate that up to contamination loads of 10^6 CFU the direct contamination route is more important than the indirect route (i.e., cross-contamination) in terms of number of illnesses. We highlight that the relevance of cross-contamination decreases with more diffuse and uniform contamination, and we infer that prevention of contamination in the field is the most important risk management strategy and that disinfection of washing water can be an additional intervention to tackle potentially high ($>10^6$ CFU) point contamination levels.

Although fruits and vegetables are clearly considered part of a healthy diet, foodborne illness associated with the consumption of such produce has been widely reported *(17)*. In 2008, the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO-WHO) *(7)* categorized leafy green vegetables as the highest priority in terms of fresh produce safety from a global perspective. These products are often grown in an open environment where they are vulnerable to microbial contamination and often are not treated to reduce or eliminate pathogens *(8)*. Although preharvest strategies such as the application of good agricultural practices during growing and harvesting are general recognized as critical to reduce the risk of contamination, the processing industries in various countries still rely on disinfection strategies *(9)*.

Washing procedures applied to fresh produce can reduce microbial contamination from the surface of the product to meet quality and safety goals *(22)*. However, the main effect of sanitizing treatments for washing fresh-cut produce is reduction and control of the microbial load in the water used in processing and thus prevention of cross-contamination rather than a decontamination or preservative effect on the produce itself *(12)*. Chlorine is widely used by the produce industry because of its ability to reduce microbial levels, its low cost, and its minimal adverse effect on product quality. However, in some European member states (e.g., The Netherlands, Denmark, and Germany) the use of antimicrobial agents in the fresh-cut processing industry is in principle prohibited, and strict criteria apply for possible approval.

Maintaining the quality of produce processing water with disinfectant agents that provide a barrier to cross-contamination is essential to prevent spread of contamination *(3, 14, 15, 18, 20)*. Although quantitative studies have revealed that cross-contamination in the washing stage of fresh-produce occurs *(11, 12, 19, 24)*, the importance of cross-contamination in terms of public health relevance has rarely been assessed *(2)*. The direct distribution of initially contaminated leafy greens to a multitude of servings by cutting and mixing has not been addressed by industry or researchers. The goal of this study was to compare the contribution of this direct contamination pathway with that of the indirect (cross-contamination) pathway. We constructed a transparent and intuitive mathematical model that may serve as a tool for making a science-based assessment of the public health relevance of water disinfection in the fresh-cut produce industry.

MATERIALS AND METHODS

Leafy greens processing line. A typical leafy greens processing line contains some or all of the following steps: supply of raw materials, manually trimming of raw materials, shredding, prewash rinsing, washing, postwash rinsing, moisture removal, packaging, and storage. The complete process was assumed to occur at an average temperature of 4 to 6°C. In this study, only the washing step was modelled because this is the primary step associated with cross-contamination events. We considered

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TABLE 1. *Parameters of the model*

Variable	Description	Value or calculation
b	Batch size	2.2×10^7 g
m	Serving size	100 g
N	Local contamination load	10^3, 10^6, or 10^9 CFU/batch
f_{dw}	Fraction of N to water	0.78^a
$1 - f_{dw}$	Fraction remaining on product	0.22
S_d	No. of directly contaminated servings	10, 100, or 1,000
D_d	Mean dose (CFU) per directly contaminated serving	$N(1 - f_{dw})/S_d$
$E(\text{Ill}_d)$	Direct cases of illness	$S_d\left[1-\left(1+\frac{D_d}{\beta}\right)^{-\alpha}\right]$
f_{wi}	Fraction CFU from water to product	0.012^a
D_i	Mean dose (CFU) per indirectly contaminated serving	$N^*f_{dw}f_{wi}(m/b)$
$E(\text{Ill}_i)$	Indirect cases of illness	$\frac{b}{m}\left[1-\left(1+\frac{D_i}{\beta}\right)^{-\alpha}\right]$
α	Dose-response parameter	*Salmonella*: 0.1324; *E. coli* O157: 0.248
β	Dose-response parameter	*Salmonella*: 51.45; *E. coli* O157: 48.8

[a] Average of previously published data; see Table 2.

commonly consumed fresh-cut leafy greens that are typically processed in large-scale processing lines, including vegetables such as romaine lettuce, iceberg lettuce, spinach, endive, and chicory. Large-scale processing lines in The Netherlands normally continuously replace the water in the wash tank, and the total size of the wash water system can be more than 4 m^3. The processing lines are operational full time and are extensively cleaned at least once per day. A batch of ready-to-eat leafy greens was defined as the total of all vegetables processed between two cleanings.

Model description. The model describes the dispersion of a point microbial contamination event from entrance of the processing line to individual consumer servings during washing of a batch of fresh-cut leafy greens in a typical large-scale processing line. Based on a Beta-Poisson dose-response relationship *(10),* this contamination was translated into the number of illnesses caused by this batch of leafy greens. It was assumed that the first part of the batch to be processed contained the contamination load. This approach can be considered a worst-case scenario, because contamination at the start of the batch has the maximum potential for cross-contamination. Wash water replacement also was not included in the model to allow the maximum potential for cross-contamination. Model calculations were executed for *Escherichia coli* O157 and *Salmonella* because these pathogens are well known causes of outbreaks associated with leafy greens. The model was implemented in MS Excel 2010 (Microsoft, Redmond, WA). The variables of the model are described in Table 1, and a schematic diagram of the model is shown in Figure 1.

Direct contamination. The contamination load (N) is the number of CFU present as a local contamination in a batch of leafy

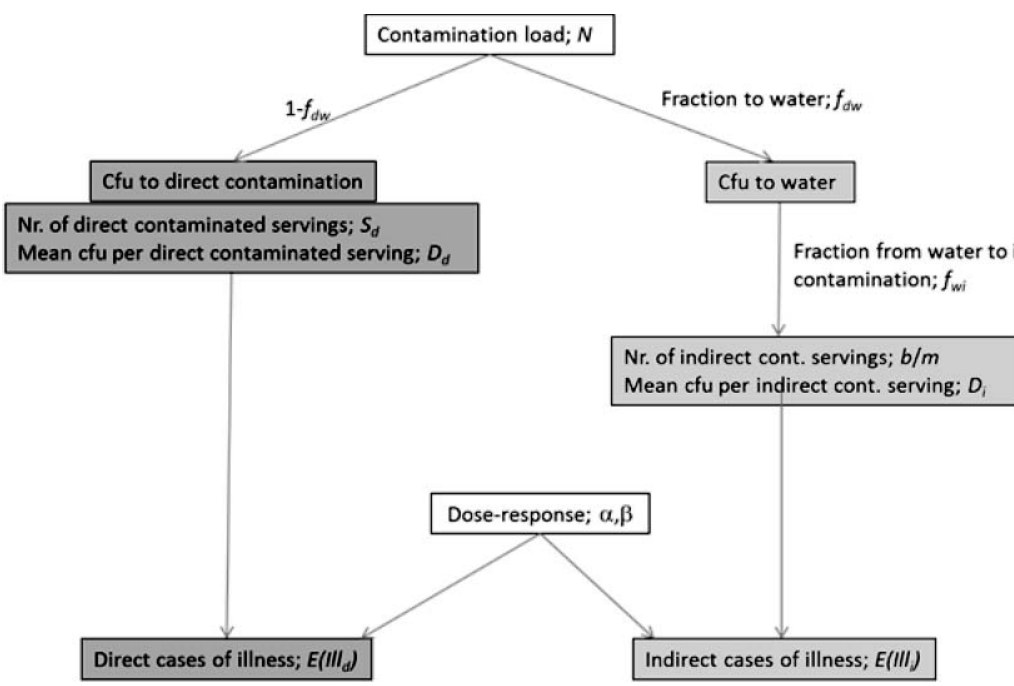

FIGURE 1. *Schematic model overview.*

TABLE 2. *Overview of transfer coefficients from literature*

Characteristic of study	Buchholz et al. *(1)*	Holvoet et al. *(11)*
Microorganism	*E. coli* O157:H7	*E. coli, E. coli* O157, MS2 phage, murine norovirus
Leafy green type	Baby spinach, iceberg lettuce, romaine lettuce	Lettuce
Study dimension	Pilot plant scale: 890-liter water tank, 22.7-kg batch	Laboratory scale: 4-liter washing tank; 0.2-kg batch
Processing steps	Cutting, washing, dewatering (centrifuge)	Cutting, washing (2×), dewatering (spinner)
Inoculation method and level	All greens dip inoculated, centrifuged, drained, air dried for 1 h at 22°C; 1E6, 1E4, 1E2 CFU/g	Lettuce: *E. coli* submersion 1E4 CFU/g Water: *E. coli* 1E1, 1E2, 1E3 CFU/ml; *E. coli* O157 1E2.8, 1E3.6, 1E4.7 CFU/ml; MS2 1E2.0, 1E3.1, 1E4.5 PFU/ml; NoV 1E4.5 PFU/ml
Initial contamination	All leafy greens inoculated before shredding	Lettuce: inoculated after slicing, before washing bath Water: spiked
Water	Sanitizer-free tap water	Lettuce: sanitizer-free potable water Water: produce processing water and potable water
Parameter estimates	From leafy greens to flume water, 71%; to centrifugation water, 19% (total 90% to water); to surfaces, 1%; left on leafy greens, 9%	From lettuce (*E. coli*) to water, 43%; left on lettuce, 57% From water to lettuce: *E. coli*, 0.6% ± 0.2%; *E. coli* O157, 1.0% ± 0.3%; MS2, 0.5% ± 0.2%; norovirus, 0.5% ± 0.1% (average 0.65%)
Remarks		Data from only one tank used for parameter estimates
f_{dw} (%)[a]	90	43
f_{wi} (%)[b]		0.65

[a] Fraction of N transferred to water.
[b] Fraction CFU transferred from water to product.

greens entering the washing step in a food processing facility. All leafy greens (except for baby spinach) are shredded just before entering the wash tank. In the wash tank, the shreds are distributed spatially by the turbulence in the water. A fraction f_{dw} of N will transfer from the initially contaminated leaves to the wash tank. As a consequence of shredding and mixing, part of the contamination $(1 - f_{dw})$ will be distributed to a number of consumer servings, referred to here as directly contaminated servings (S_d). Hence, the mean CFU in a serving due to direct contamination is

$$D_d = \frac{N}{S_d}(1 - f_{dw})$$

Following the Beta-Poisson dose-response model *(10),* the expected number of cases of illness due to direct contamination is

$$E(\text{Ill}_d) = S_d\left[1 - \left(1 + \frac{D_d}{\beta}\right)^{-\alpha}\right]$$

where α and β are the dose-response parameters.

Indirect contamination. In this section we consider the indirect route of transmission: cross-contamination via the washing water. We assume that the contamination entering the wash tank ($N \times f_{dw}$) will disperse immediately and completely due to turbulence in the washing water. A fraction of the contamination (f_{wi}) causes indirect contamination by transferring to initially uncontaminated leaves. We assume that the relevance of transferring contamination from the water to an initially contaminated leaf is negligible. When the wash water is not replenished, the total number of CFU due to indirect contamination (N_i) is

$$N_i = f_{dw} f_{wi} N$$

Given a serving size m (grams) and a batch size b (grams), the number of servings per batch is b/m, and the mean indirect dose per serving (D_i) is

$$D_i = \frac{N_i}{\frac{b}{m}}$$

Following the Beta-Poisson dose-response model, the expected number of cases of illness due to indirect contamination is

$$E(\text{Ill}_i) = \frac{b}{m}\left[1 - \left(1 + \frac{D_i}{\beta}\right)^{-\alpha}\right]$$

Parameter values. To our knowledge, no data are available on the size (in CFU) of a typical point contamination (N) event. In the absence of data, three contamination loads were defined: 10^3, 10^6, and 10^9 CFU per batch. A literature search was used to obtain evidence-based parameterization of the bacterial fraction transferred from the product to the water (f_{dw}) and vice versa (f_{wi}). From six studies, the values for these parameters were extracted and the average was used for the model (Table 2). The value for f_{dw} is 0.78, and the value for f_{wi} is 0.012. We assumed a serving size for leafy greens (m) of 100 g. To our knowledge, no data are available on the size (in terms of grams of contaminated leafy greens) of a

TABLE 2. *Extended*

Luo et al. *(16)*	Pérez-Rodríguez et al. *(19)*	Jensen et al. *(13)*	Deng et al. *(4)*
E. coli O157:H7	*Salmonella* Enteritidis	*E. coli* O157:H7	*E. coli*: 8 O-types and 2 surrogates
Baby spinach, iceberg lettuce	Iceberg lettuce	Romaine lettuce, romaine spring mix	Romaine lettuce
Pilot plant scale: 3,200-liter water tank; 540-kg batch	Laboratory scale: 12-liter washing tank; 3-kg batch	Laboratory home scale: 0.1-liter washing tank; 0.015-kg batch	Laboratory scale: 0.03-liter washing volume; 16-cm^2 batch
Cutting, washing (2×), dewatering	Cutting, washing, dewatering (centrifugation), packing	Cutting, washing	Cutting, washing
Spinach: spray inoculation 2E5 CFU/g Water: maximum 8.1 CFU/ml	Lettuce: immersion 10, 1E4, 1E6, 1E7 CFU/g	Lettuce: spot inoculation 1E6 CFU	Lettuce: suspension 1E4 CFU/cm^2 Water: 3.2E3 CFU/ml
Baby spinach inoculated, spread separately onto conveyer belt Water: microorganisms from baby spinach	Of a 3-kg lot, only one 150-g lettuce head was inoculated; introduction during cutting in the following order: ⅔ of the lot, contaminated head, remaining ⅓ of the lot	Inoculation of only one lettuce leaf piece after cutting but before washing	Lettuce: inoculation of one leaf piece (8 cm^2) after cutting but before washing Water: microorganisms from lettuce
Tap water with sodium hypochlorite and partly T128 added	Tap potable water, no chlorine added	Sterile potable city water	Deionized water, partly chlorine added
From spinach to water, 68%; left on spinach, 32%	From lettuce to water, 74%; left on lettuce, 26%	From lettuce to water, 99.1% (as derived from amount left on lettuce); left on lettuce, 0.9%	From lettuce to water, 92%; left on lettuce, 8%
Only water tank 1 measurements available			Only measurements without chlorine used
68	74	99	92
2.1			0.8

typical point contamination event. In the absence of data, we explored respectively 100 g, 1 kg, and 10 kg of directly contaminated material. According to industry data (personal communication), a rough average of leafy green snip size can be set at 1 g. The interval of S_d (number of directly contaminated servings) for a 100-g contamination event, given a serving size (m) of also 100 g will then span from 1 serving (all contaminated shreds in one serving) to 100 servings (all contaminated shreds in different servings). Based on this rationale, the intervals for S_d are [1, 100], [10, 1,000], and [100, 10,000] directly contaminated servings. For our model, we chose representative values of these ranges (10, 100, and 1,000 servings) as the values for S_d.

The batch size (b) of leafy greens differs depending on the size of the production line, type of leafy green, and general condition of the leafy green batch. Given a typical full-time operational production line at 1,000 kg/h, with an overnight extensive cleaning of 2 h, b is 2.2×10^7 g (personal communication). We used dose-response parameters (α and β) from FAO-WHO *(6)* for *Salmonella* and from Teunis et al. *(21)* for *E. coli* O157.

TABLE 3. *Cases of illness due to direct contamination at three different levels (*N*)*

N	S_d	No. of *Salmonella* cases	No. of *E. coli* O157 cases
10^3	10	0.5	0.9
	100	0.6	1.1
	1,000	0.6	1.1
10^6	10	6	8
	100	39	61
	1,000	198	345
10^9	10	8	10
	100	76	93
	1,000	669	876

RESULTS

Attribution of exposure. Given the selected default values for f_{dw} (78%) and f_{wi} (1.2%), 22% (100% − 78%) of the contamination load N will be ingested via directly contaminated servings and 0.9% (78% × 1.2%) of N will be ingested via indirectly contaminated servings. The remaining 77.1% of the contamination load N will be discharged with the wash water. The attribution of exposure therefore is 96% (22%/[22% + 0.9%]) for directly contaminated servings and 4% for indirectly contaminated servings.

Cases of illness via direct contamination. The number of cases of illness attributed to direct contamination at $N = 10^3$ CFU did not exceed one (Table 3). However, the number of cases of illness ranged from 6 to 345 for $N = 10^6$ CFU and from 8 to 876 for $N = 10^9$ CFU.

Cases of illness via indirect contamination. For the disease burden due to indirect contamination, the number of directly contaminated servings was not relevant. Hence, Table 4 presents the results for only three scenarios of

TABLE 4. *Cases of illness due to indirect contamination at three different levels (*N*)*[a]

	No. of infection cases	
N	*Salmonella*	*E. coli* O157
10^3	0.02	0.05
10^6	24	48
10^9	16,872	31,678

[a] Total number of servings in the batch was 220,000.

contamination load *N*. Contamination loads up to 10^3 CFU resulted in very low expected number of illnesses, i.e., on average 10 times lower than for the direct contamination route. Contamination loads of 10^6 CFU give a burden of disease via indirect contamination of the same order of magnitude as 100 directly contaminated servings. Extremely high contamination loads ($N = 10^9$) resulted in a large increase of cases of illness; up to 14% (100% × [31,678/220,000]) of the servings from the batch would cause illness. All calculations were conducted under the worst-case assumptions of a contamination load at the start of the batch and no water replacement.

Comparison between indirect and direct contamination. The direct and indirect cases of illness for all possible contamination loads of 10 to 10^9 CFU given that the number of directly contaminated servings (S_d) is 100 are shown in Figure 2. The indirect pathway (cross-contamination) causes more illness than the direct contamination pathway only at pathogen levels exceeding 10^6 CFU for both *Salmonella* and *E. coli* O157.

DISCUSSION

Although cross-contamination (i.e., indirect contamination) can occur during the washing step in leafy green

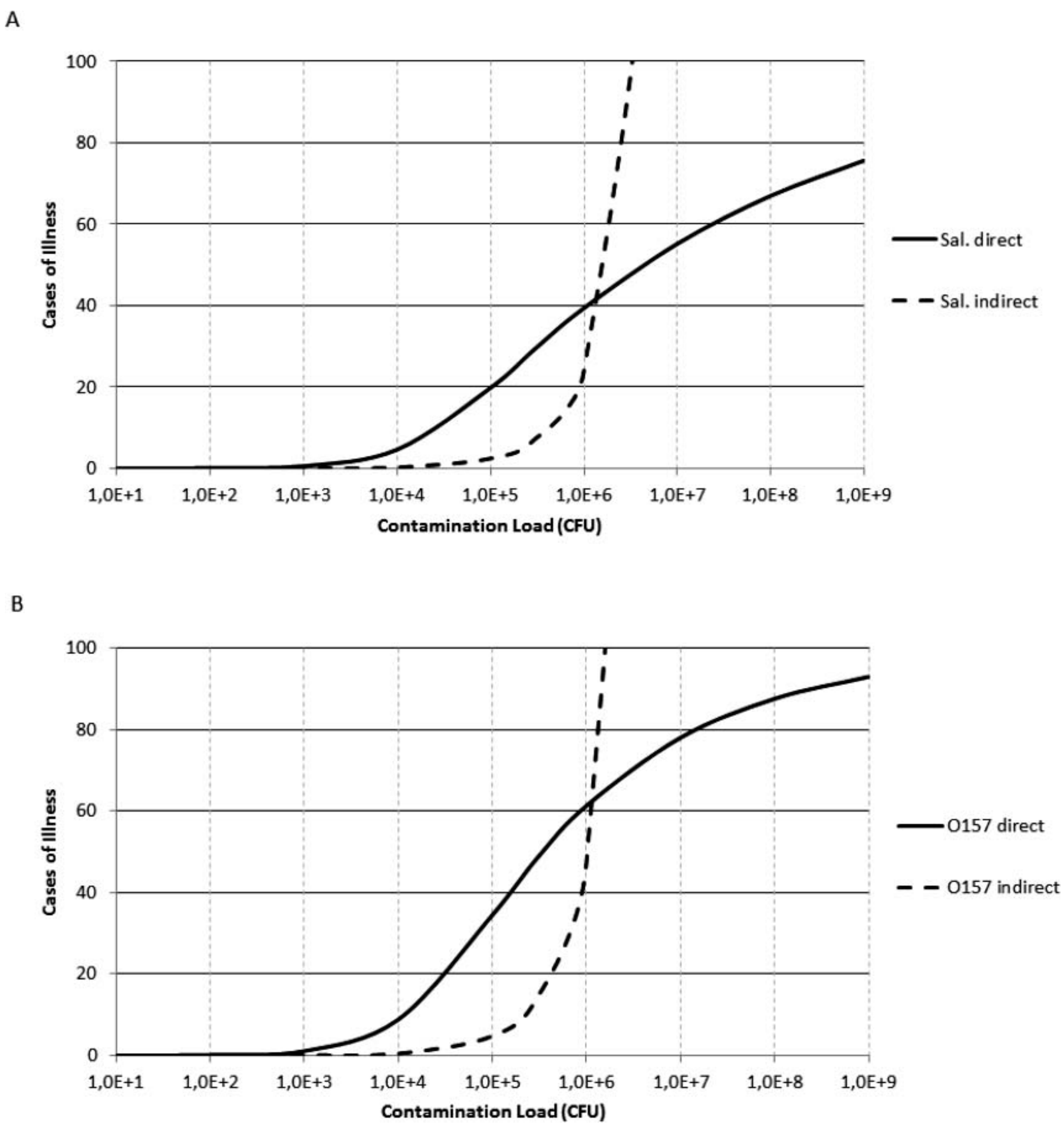

FIGURE 2. *Disease burden as a function of the contamination load (CFU per batch) of* Salmonella *(A) and* E. coli *O157 (B) as a result of direct (solid line) and indirect (dashed line) contamination of servings of leafy greens. The number of directly contaminated servings is set at 100.*

processing facilities and is intuitively important *(11, 12, 24)*, quantification of the public health relevance of this contamination has rarely been assessed *(2)*. The results of our modelling approach revealed a number of insights concerning the public health relevance of indirect contamination during the washing step of leafy green processing.

Direct contamination route usually is the dominant contamination route in terms of number of disease cases. The number of illness cases via direct contamination, no matter how high the load, will never exceed the number of servings to which a point contamination can be partitioned. However, given the low transfer rate from water to product, it takes a very high point contamination load ($>10^6$ CFU) for the number of cases via indirectly contaminated servings to exceed the number of cases via directly contaminated servings (Fig. 2). Such contamination loads have never been found in surveillance programs and monitoring studies. In those studies, estimated *E. coli* O157 levels on leafy green vegetables ranged from 0.018 to 0.052 CFU/g *(2, 23)*. These estimates would correspond to approximately 18 to 52 CFU/kg (1.3 to 1.7 log CFU/kg), which is far below the limit above which the indirect contamination route becomes dominant (in terms of number of disease cases) over the direct route. The direct contamination route cannot be managed by preventing cross-contamination during industrial washing; therefore, our results suggest that managing risks during primary production should have priority.

For contamination loads $<10^6$ CFU per batch, we found that the direct contamination route was responsible for more illness cases. In contrast, Danyluk and Schaffner *(2)* hypothesized that 95 to 100% of the illness cases caused by *E. coli* O157:H7 in the 2006 spinach outbreak could be explained by occurrence of cross-contamination. They developed a stochastic model where one iteration represents the fate of an entire batch of spinach from farm to fork. Storage time abuse then results in iterated batches in which almost all servings cause illness. Consequently, because the majority of servings are cross-contaminated (which is also the case in our model but with much lower bacterial levels), these iteration results skew the outcome of the simulation toward a high percentage of cases of illness attributable to cross-contaminated servings. If variability in storage between bags or servings were modelled rather than variability between batches, the results of both models would be more similar.

Relevance of cross-contamination decreases with more diffuse and uniform contamination. In the case of a more uniform contamination event, which can occur when contaminated irrigation water is used, all servings coming from a single batch will be contaminated, and cross-contamination will not be relevant. Consequently, preventing cross-contamination, e.g., by adding chlorine to the washing water, is not effective in such a situation. This finding highlights the importance of field level prevention of contamination *(5, 8)*.

No single risk management strategy is appropriate for all purposes. Given the diverse scenarios for contamination of fresh produce with pathogens, no single risk management strategy is appropriate for all purposes. Proactive minimization of the risk of contamination in the field should be the primary focus when the major contamination scenario is point contamination with loads up to 10^6 CFU (e.g., due to intrusion of wildlife) or when contamination is expected to be very diffusely distributed over the batch (e.g., due to the use of contaminated irrigation water). Wash water disinfection is a suitable risk management option when contamination exceeding 10^6 CFU is considered possible or has occurred.

Limitations of the model. In contrast to the model of Danyluk and Schaffner *(2)*, we did not include storage times. However, in The Netherlands leafy greens are typically stored for a few days. Levels of pathogens in both directly contaminated and cross-contaminated leafy green servings generally are in the low and linear part of the dose-response curve, even after pathogen growth has occurred. Consequently, the attribution of illness to the direct and cross-contamination routes will remain unaffected. However, to get better estimations of absolute numbers of illnesses when storage times are longer than 1 week, stochastic storage characteristics should be included in the model.

The present model is a deterministic model and does not account for variability in transfer coefficients. However, we assumed that the variability between batches will be very low and can be ignored. Variability in transfer rates between individual shreds might be high but can also be ignored because servings consist of many shreds, which will level out the variation in number of CFU per shred. The transfer coefficients used (Table 2) originated from a variety of experimental designs, but the calculated values were similar and are therefore considered robust parameter estimations.

Future research. Our model indicates the importance of the direct contamination pathway in contrast with the indirect (cross-contamination) pathway in foodborne illness associated with leafy greens. Further research is necessary to validate this claim, to identify potential preventive strategies, and to better estimate the public health burden. Three clearly defined data gaps are (i) the actual distribution of contamination loads (N), (ii) the relative occurrence of point contamination events versus uniform contamination events, and (iii) a data-based estimation of the number of directly contaminated servings (S_d) following a contamination event.

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