Quantitative Microbial Risk Assessment for *Escherichia coli* O157 on Lettuce, Based on Survival Data from Controlled Studies in a Climate Chamber

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ABSTRACT

The aims of the study were to determine the survival of *Escherichia coli* O157 on lettuce as a function of temperature and light intensity, and to use that information in a screening-level quantitative microbial risk assessment (QMRA) in order to evaluate risk-reducing strategies including irrigation water quality guidelines, rinsing, and holding time between last irrigation and harvest. Iceberg lettuce was grown in a climate chamber and inoculated with *E. coli* O157. Bacterial numbers were determined with the standard plate count method after inoculation and 1, 2, 4, and 7 day(s) postinoculation. The experiments were carried out at 11, 18, and 25°C in light intensities of 0, 400, and 600 mmol (m²)⁻¹ s⁻¹. There was a significant effect of temperature and light intensity on survival, with less bacteria isolated from lettuce incubated at 25 and 18°C compared with 11°C (*P < 0.0001*), and in light intensities of 400 and 600 mmol (m²)⁻¹ s⁻¹ compared with 0 mmol (m²)⁻¹ s⁻¹ (*P < 0.001*). The average log reductions after 1, 2, 4, and 7 day(s) were 1.14, 1.71, 2.04, and 3.0, respectively. The QMRA compared the relative risk with lettuce consumption from 20 scenarios. A stricter water quality guideline gave a mean fivefold risk reduction. Holding times of 1, 2, 4, and 7 day(s) reduced the risk 3, 8, 8, and 18 times, respectively, compared with harvest the same day as the last irrigation. Finally, rinsing lettuce for 15 s in cold tap water prior to consumption gave a sixfold risk reduction compared with eating unrisned lettuce. Sensitivity analyses indicated that variation in bacterial inactivation had the most significant effect on the risk outcome. A QMRA determining the relative risks between scenarios reduces uncertainty and can provide risk managers with decision support.

Verocytotoxin-producing *Escherichia coli* (VTEC) can cause severe disease, with hemorrhagic diarrhea, hemolytic uremic syndrome, and neurological symptoms (29). Many *E. coli* serotypes have been isolated from human VTEC cases, but the most common serotype associated with outbreaks is O157 (18). Ruminants are reservoirs of VTEC, and most foodborne outbreaks are caused by consumption of meat and dairy products (17). However, outbreaks caused by contaminated water (1) and vegetables (for example, lettuce (12) and spinach (8)) are problems of increased relevance worldwide (28), with the largest documented outbreak to date in Japan, 1996, from contaminated radish sprouts (13).

Iceberg lettuce (*Lactua sativa*) is the most common free-land cultivated vegetable in Sweden, and overhead (ramp, spray) irrigation is normally used. Although irrigation with reclaimed wastewater on food crops is prohibited by Swedish law, surface water used for irrigation can be fecally contaminated. In areas with contaminated cattle herds, excreted VTEC end up in manure and on pasture. During rainfall, VTEC can be flushed into surface water and can later contaminate lettuce via irrigation (5). This was the case in which 135 people were infected by VTEC from contaminated lettuce in Halland County (in the southwestern part of Sweden) in 2005 (37). Presently, there are European guidelines neither on irrigation water quality nor on holding time between irrigation and harvest. However, after the lettuce outbreak in 2005, a governmental commission recommended that irrigation water should at least comply with bathing water quality of <100 CFU *E. coli* ml⁻¹ (3). Another risk-reducing strategy that can be used by the risk manager is to regulate when irrigation prior to harvest can take place. With a holding time between irrigation and harvest, pathogenic bacteria can be reduced in numbers by UV radiation, drying, or competition with commensal microbiota (7). The abovementioned commission recommended that irrigation 48 h prior to harvest should be of drinking water quality, i.e., *E. coli* not detected in 100 ml, until better knowledge has been acquired (3).

Several studies on *E. coli* inactivation on lettuce have been reported (22, 38, 40), but there is a lack of data from
experiments where inactivation has been determined as a function of important physical inactivating factors such as temperature and light intensity. These data are needed as input for microbial risk assessment models evaluating holding time as a barrier. Therefore, one of the aims of the present study was to determine the inactivation rate of \textit{E. coli} O157 on lettuce, depending on temperature and light intensity in a controlled environment, e.g., a climate chamber. The inactivation data was used in a quantitative microbial risk assessment (QMRA) approach, with the objective to provide a basis for recommendations on risk mitigation strategies e.g., irrigation water quality, consumer rinsing, and holding time between irrigation and harvest.

**MATERIALS AND METHODS**

**Inactivation of \textit{E. coli} O157 on lettuce.** Cultivation was performed under controlled conditions at the Phytotron Climate Chamber at the Swedish University of Agricultural Sciences, Uppsala, Sweden (http://www-fytootronen.slu.se/index.html). Lettuce (\textit{L. sativa}) seeds were planted in autoclaved soil and were allowed to germinate at 18°C. The sprouts were kept at 18°C and were drip irrigated with nutrient solution until the start of the inactivation studies, 8 weeks from planting, when the inner leaves were folded to form a head. The inactivation studies were performed at 11, 18, and 25°C in light intensities of 0, 400, and 600 (only at 18°C) mmol (m²)⁻¹ s⁻¹ and a constant relative humidity of 78%.

\textit{E. coli} O157 strain CCUG 42901 (VT\textsubscript{x1}, VT\textsubscript{x2}, eae\textsuperscript{A}, hly\textsuperscript{A}, and flb\textsuperscript{C}}: from the culture collection of the University of Gothenburg, Uppsala, Sweden) was grown on blood agar base (National Veterinary Institute production) overnight at 37°C. A loopful of bacteria was suspended in 1 liter of tap water (free chlorine concentration <0.01 mg l⁻¹). The prepared water, having \textit{E. coli} levels between 10\textsuperscript{3} to 10\textsuperscript{8} CFU ml⁻¹, determined by plate count on blood agar base (National Veterinary Institute production), was sprayed onto the lettuce plants to final densities between 10\textsuperscript{5.2} and 10\textsuperscript{6.7} CFU g⁻¹. Three individual plants from each regime were sampled for bacterial enumeration directly after inoculation and 1, 2, 4, and 7 day(s) postinoculation. As a complement, the inactivation rate in saline solution at 25°C, and light intensities of 0 and 400 mmol (m²)⁻¹ s⁻¹ were determined in duplicate at sample days, as above. The rationale of this study was to get an experiment more controlled on the effect of light intensity.

**Bacterial enumeration.** \textit{E. coli} O157 was enumerated with the Nordic Committee on Food Analysis (Oslo, Norway) method no. 164 (31). In brief, the lettuce head was cut into small pieces, and 10 g of cut leaves was mixed with 90 ml of peptone salt diluent (0.1%) and homogenized in a stomacher (AES Chemunex, Bruz Cedex, FR) for 1 min at medium speed. The supernatant was serially diluted, and 0.1 ml from the dilutions was spread on sorbitol MacConkey agar with cefixime and tellurite (National Veterinary Institute production). The plates were incubated at 37°C for 21 ± 3 h. Because the bacterial background on the agar was limited, confirmation of typical colonies was not necessary.

**Microbial risk assessment.** Exposure of the general population to VTEC bacteria via consumption of contaminated lettuce was evaluated. The hazard was defined by VTEC from positive herds ending up in surface water used for irrigation of lettuce. All VTEC are not associated with illness because of a lack of virulence factors other than those responsible for verocytotoxin production, for example the locus of enterocyte effacement-mediated type III secretion system (15). Thus, basing the evaluation on all VTEC in manure will lead to an overestimation of the risk in the QMRA model. However, this is considered of minor importance, as risk-reducing strategies were compared in relative terms.

**Hazard characterization.** The health-based target outcome was the probability of illness, \( P_{\text{ill}} \), derived from the simplified beta-Poisson model by Furumoto and Mickey (14) (equation 1), with \( \alpha = 15.045 \) and \( \beta = 0.16 \), data fitted from human feeding trials of \textit{Shigella} spp. validated with quantitative data obtained from outbreaks (41) and the dose, e.g., viable cells (in CFU), modeled from the respective scenarios described in the exposure assessment.

\[
P_{\text{ill}} = 1 - \left( \frac{1 + \text{Dose}}{\beta} \right)^{-\alpha}
\]

**Exposure assessment.** The pathway and processes described in the screening-level QMRA was the concentration of VTEC in surface water (depending on total \textit{E. coli} concentration, i.e., water quality, and ratio of VTEC to \textit{E. coli}), transfer to lettuce via irrigation, the change in concentration of VTEC on the lettuce with time (depending on holding time), and assumed temperature and sunlight intensity, rinsing, and consumption of lettuce. Because the guideline suggestions are based on surface water quality, processes occurring prior to contamination of surface water, e.g., transport via rainfall, VTEC excretion rate of cattle, dilution, and inactivation of bacteria in the receiving surface water used for irrigation, were not included in the model.

Several scenarios were simulated in order to evaluate the relative risk of: (i) two water quality guidelines, (ii) five different holding times, and (iii) rinsing or not rinsing lettuce prior to consumption (Table 1). These assessments were made based on the VTEC prevalence in Halland County, a region with high cattle density and 23.3% VTEC herd prevalence (11).

**Irrigation water quality guidelines.** The former Swedish guideline (cited in (3)) allowed a maximum limit of 100 CFU of \textit{E. coli} 100 ml⁻¹ in compliant bathing water. To comply with the European Union (EU) bathing water directive (2006/7/EC), water should be analyzed four times a year for a 4-year period, with an upper limit of 1,000 CFU of \textit{E. coli} 100 ml⁻¹, based upon a 95th percentile evaluation for inland waters. In the risk models, water qualities were defined by uniform distributions according to Table 1. These distributions were chosen to reflect situations with intermittent fecal contaminations, however still in compliance with the respective guidelines.

As guidelines are based on CFU \textit{E. coli} 100 ml⁻¹ in the surface water used for irrigation, the VTEC:E. coli ratio in VTEC containing manure, the prevalence of VTEC among herds in the investigated area and total \textit{E. coli} concentrations in surface water were needed as model inputs to estimate concentration of VTEC in irrigation water. This ratio was calculated from [concentration of VTEC in manure (positive herds)/total \textit{E. coli} in manure] × VTEC prevalence in herds, using manure data from Muniesa et al. (30) and Hutchison et al. (21), and prevalence data from Eriksson et al. (11), respectively. To the latter, a beta-distribution was fitted (Table 1). The ratio of VTEC:E. coli was defined by a lognormal distribution of \( 10^{0.19 ± 0.60} \), with the exponent truncated at 0 (e.g., the ratio cannot be higher than 1).

From the irrigation water, the number of bacteria on the lettuce is dependent on the bacterial attachment rate to lettuce. This figure was taken from Shuval et al. (35), who measured the water holding capacity of lettuce. The attachment rate of 0.108 was used...
in a QMRA by Hamilton et al. (19), who assigned a normal distribution to the parameter, which was also used in the present study (Table 1).

**VTEC inactivation.** Inactivation of *E. coli* O157 on lettuce was calculated from the climate chamber study (results), and five scenarios were modeled: lettuce harvested directly after irrigation and 1, 2, 4, and 7 day(s) post-irrigation (i.e., length of environmental exposure). The log changes for each holding time were calculated by subtracting the log concentration of each replicate at a specific time from the mean log concentration at t(0), to estimate a normal distribution for the log change at each holding time (Table 1).

**Rinsing.** Studies of the effect of washing methods, with and without antimicrobials, on *E. coli* O157 reduction on lettuce have been reported (26, 32, 36, 43). Data from the study by Pangloli et al. (32), reporting log removal after rinsing lettuce for 15 s in cold tap water, defined by a normal distribution, with the parameter, was chosen for the QMRA. For the nonrinsed lettuce scenario, there was no postharvest removal included in the model (Table 1).

The probability of illness (*P*ill) was calculated from the ingested dose of a serving size of 100 g, previously used by Peterson et al. (33) and Shuval et al. (35). The serving size does not influence the relative risk between scenarios and it was kept constant.

**Risk characterization.** Risk modeling was performed with @Risk 5.5 software (Palisade Corp., Ithaca, NY). The *P*ill for every scenario was derived from 10,000 Latin hypercube iterations, a single iteration representing the *P*ill from eating 100 g of lettuce. By summing the *P*ill from 10,000 iterations within a scenario, the number of illnesses per 10,000 servings for that scenario was determined. The relative risk was calculated by dividing the number of illnesses from each scenario with number of illnesses in the best-case scenario; lettuce irrigated with water complying with the former Swedish bathing water guidelines, harvested 7 days after last irrigation and rinsed before consumption. Further, the relative risk reduction was calculated by dividing the number of illnesses 10,000 servings−1 from comparative scenarios with and without the mitigation option, which effect was to be determined. To determine the influence of variation in distribution inputs on *P*ill, sensitivity analyses were performed in @Risk by using the Spearman rank-order correlation.

**Statistical analysis.** A general linear model (GLM) was used to determine the effects of light intensity levels and temperature, with time as a covariate, on the inactivation of *E. coli* O157 on lettuce by using Tukey’s all-pairwise comparison. The GLM analysis was performed with Minitab 15 software (Minitab Inc., State College, PA). Figures were created in SigmaPlot 2001 software (SPSS, Inc., Chicago, IL).

**RESULTS**

There was a significant effect of temperature on survival (*P* < 0.0001, GLM), with less bacteria isolated from lettuce at 18 and 25°C than at 11°C (Figs. 1 and 2). The inactivation was further affected by the light intensity (*P* < 0.001, GLM) with fewer bacteria isolated from lettuce in light intensities of 400 and 600 than 0 mmol (m²)−1 s−1 (Fig. 3). Inactivation on lettuce data was not well described by a log-linear decay model (*P* > 0.05, GLM). Instead, inactivation was described as log change over time with mean log reductions of 1.14, 1.71, 2.04, and 3.00 after 1, 2, 4, and 7 day(s) of holding time respectively. These data

![FIGURE 1. Escherichia coli O157 numbers (error bars equal standard deviations) on lettuce as a function of time in 0 mmol (m²)−1 s−1 light intensity at 11°C (●), 18°C (○), and 25°C (▼). Reference line represents the inactivation rate in saline solution at 25°C, *N*₁ = 5.1 − 0.18t, R² = 0.87.](image-url)
were used as input in the QMRA models (Table 1). The inactivation rate in saline solution at 25°C was log-linear though, with a rate almost three times faster in 400 mmol ($m^2$)$^{-1}$ $s^{-1}$ compared with 0 mmol ($m^2$)$^{-1}$ $s^{-1}$ light intensity, and $-0.48$ log day$^{-1}$ compared with $-0.18$ log day$^{-1}$ (Figs. 1 and 2).

For lettuce produced in a VTEC endemic region in Sweden, 20 scenarios were modeled, and $P_{ill}$ (95th percentile), illnesses 10,000 servings$^{-1}$, and the relative difference due to risk-mitigation strategies are presented in Table 2. Because the approach taken in the present study is a screening-level QMRA to evaluate the potential effects of water quality and rinsing as risk management options, the estimated $P_{ill}$ and illness per 10,000 servings are only indicative and should not be considered as predictions of risk in absolute terms. A QMRA approach more sophisticated and additional data are needed for that objective. From an estimated 1,390 illnesses from 10,000 servings in the worst-case scenario, a stricter water quality guideline could lead to a mean fivefold reduction in illnesses (minimum of 3.2 and a maximum of 7.0). Irrigation cessation resulted in mean risk reductions of 2.6, 8.3, 7.8, and 18 times for 1, 2, 4, and 7 day(s) of holding time, respectively, compared with lettuce harvest the same day as irrigation. Rinsing lettuce for 15 s in cold water could further reduce the risk sixfold (3.6 to 8.5) compared with eating un-rinsed lettuce.

Significant correlation between $P_{ill}$ and parameter variation is shown in the sensitivity analyses (Table 2). When there was a holding time included in the scenario, it had the most significant effect on risk outcome with correlation coefficient ($-0.42 < R < -0.16$). For scenarios with harvest the same day as irrigation, the $P_{ill}$ was most significantly correlated to the ratio VTEC:E. coli in manure.

**DISCUSSION**

In the present approach, QMRA was used to estimate the $P_{ill}$ of VTEC from lettuce contaminated via irrigation water, taking into account the effect of microbiological barriers, e.g., holding time and rinsing. However, the main objective was to evaluate the mitigation effect of water quality guidelines, irrigation restrictions, and rinsing in relative terms enabling a simple, transparent model to be communicated objectively to the risk manager (44).

**Water quality guidelines.** Guidelines are tools for competent authorities managing public health protection. There is neither guideline for irrigation water quality in the EU nor in Sweden, but according to EU legislation, irrigation water for growing vegetables should be suitable for the purpose. Adopting the levels for bathing waters is one suggested option (3). In the present study, the former Swedish and current European bathing water directives were evaluated in relative terms.

QMRA indicates that allowing 100 CFU E. coli 100 ml$^{-1}$ in surface water used for irrigation of lettuce would lead to a fivefold reduction (3 to 7) in VTEC illnesses compared with water complying with good EU bathing water standards (1,000 CFU 100 ml$^{-1}$, based on the 95th percentile value). The lower guideline is still 50 times higher than the allowed levels of fecal coliforms in the State of California’s wastewater reclamation criteria (4). On the other hand, reclaimed wastewater is more likely than manure to contain human pathogens present in higher numbers than VTEC, e.g., enteric viruses, Giardia cysts, and Campylobacter (4, 42).

The hazard identification and the present risk model considered surface water contaminated with manure. In Sweden, there is temporal variation, with cattle shedding more VTEC during late summer and autumn (2), as well as a spatial variation in the prevalence of VTEC among cattle herds, with the highest prevalence in Halland (23%) and a few herds being VTEC carriers in the northern sections of the land (11). However, neither the spatial nor the temporal variation of prevalence was taken into account in the scenario simulation. The variation in VTEC numbers in manure (21, 30) leads to a large variation in the VTEC:E.coli ratio in

**FIGURE 2.** Escherichia coli O157 numbers (error bars equal standard deviations) on lettuce as a function of time in 400 mmol ($m^2$)$^{-1}$ $s^{-1}$ light intensity at 11°C (●), 18°C (○), and 25°C (▲). Reference line represents the inactivation rate in saline solution at 25°C, $N_t = 5.2 - 0.48t$, $R^2 = 0.98$.

**FIGURE 3.** Escherichia coli O157 numbers on lettuce (error bars equal standard deviations) as a function of time at 18°C and light intensities of 0 (●), 400 (○), and 600 (▲) mmol ($m^2$)$^{-1}$ $s^{-1}$.
manure and, as a consequence, in the irrigation water. Thus, more data on the occurrence of VTEC in manure and the spatial and temporal variation would be useful in order to evaluate the effect of a variable proportion of VTEC in the surface water. This information could also be used to develop improved models with the capability to simulate risk estimates on a more refined, temporal, and geographical scale. This variability was mainly manifested in scenarios without irrigation cessation with a significant correlation between ratio in manure and \( P_{\text{ill}} \) with the correlation coefficient \( 0.64 < R < 0.90 \) (Table 2).

**Holding time–survival study.** As microorganisms are inactivated on the crop after irrigation, cessation of irrigation before harvest can be deployed as a barrier. The aim of the experimental setup was to determine VTEC inactivation models at different temperatures and light intensities. There was a large variation between replicate samples, and a simple linear inactivation was not observed. The nonlinear inactivation and large variation could be a result of a bacterial adaptation and/or an adsorption phase, leading to faster inactivation in the beginning of the trial and then by a tailing phase. Adsorption to material has, for example, been described as an important factor for the initial reduction of viruses during inactivation studies (16). Further, bacterial growth on individual plants or microsites on a plant can increase the bacterial numbers on some of the lettuce heads, which might have been the case between days 2 and 4 in the present experiment (Figs. 1 through 3). *E. coli* O157 growth on lettuce has been demonstrated and was correlated to leaf age, with younger leaf exudates being richer in nutrients and thus more growth promoting (16). Therefore, another approach was used in the QMRA model, where the log changes instead were calculated at every time point as the difference between

<table>
<thead>
<tr>
<th>Guideline (CFU 100 ml(^{-1}))</th>
<th>Holding time (day[s])</th>
<th>( P_{\text{ill}} ) (95th percentile (log)(^a))</th>
<th>Illnesses 10,000 (^{-1}) servings(^a)</th>
<th>Relative risk</th>
<th>Sensitivity analysis (R)(^b)</th>
<th>Attachment rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Unrinsed lettuce</td>
<td>0</td>
<td>(-1.9 (-0.7))</td>
<td>436</td>
<td>130</td>
<td>NA(^c)</td>
</tr>
<tr>
<td></td>
<td>Lettuce rinsed by the consumer</td>
<td>0</td>
<td>(-3.0 (-1.4))</td>
<td>74</td>
<td>21</td>
<td>NA(^c)</td>
</tr>
<tr>
<td>Unrinsed lettuce</td>
<td>1</td>
<td>(-3.0 (-1.0))</td>
<td>160</td>
<td>46</td>
<td>(-0.42)</td>
<td>0.37</td>
</tr>
<tr>
<td>Lettuce rinsed by the consumer</td>
<td>1</td>
<td>(-4.2 (-2.0))</td>
<td>29</td>
<td>8.3</td>
<td>(-0.28)</td>
<td>0.22</td>
</tr>
<tr>
<td>Unrinsed lettuce</td>
<td>2</td>
<td>(-3.6 (-1.6))</td>
<td>52</td>
<td>15</td>
<td>(-0.33)</td>
<td>0.32</td>
</tr>
<tr>
<td>Lettuce rinsed by the consumer</td>
<td>2</td>
<td>(-4.7 (-2.7))</td>
<td>6.8</td>
<td>2.0</td>
<td>(-0.21)</td>
<td>0.19</td>
</tr>
<tr>
<td>Unrinsed lettuce</td>
<td>4</td>
<td>(-3.9 (-1.7))</td>
<td>53</td>
<td>15</td>
<td>(-0.33)</td>
<td>0.25</td>
</tr>
<tr>
<td>Lettuce rinsed by the consumer</td>
<td>4</td>
<td>(-5.1 (-2.7))</td>
<td>8.2</td>
<td>2.4</td>
<td>(-0.21)</td>
<td>0.14</td>
</tr>
<tr>
<td>Unrinsed lettuce</td>
<td>7</td>
<td>(-4.9 (-2.2))</td>
<td>24</td>
<td>6.9</td>
<td>(-0.27)</td>
<td>0.15</td>
</tr>
<tr>
<td>Lettuce rinsed by the consumer</td>
<td>7</td>
<td>(-6.0 (-3.3))</td>
<td>3.4</td>
<td>1.0</td>
<td>(-0.16)</td>
<td>0.09</td>
</tr>
<tr>
<td>1,000</td>
<td>Unrinsed lettuce</td>
<td>0</td>
<td>(-1.0 (-0.4))</td>
<td>1390</td>
<td>400</td>
<td>NA(^c)</td>
</tr>
<tr>
<td></td>
<td>Lettuce rinsed by the consumer</td>
<td>0</td>
<td>(-2.0 (-0.7))</td>
<td>390</td>
<td>110</td>
<td>NA(^c)</td>
</tr>
<tr>
<td>Unrinsed lettuce</td>
<td>1</td>
<td>(-2.0 (-0.5))</td>
<td>570</td>
<td>160</td>
<td>(-0.52)</td>
<td>0.51</td>
</tr>
<tr>
<td>Lettuce rinsed by the consumer</td>
<td>1</td>
<td>(-3.1 (-1.1))</td>
<td>140</td>
<td>41</td>
<td>(-0.39)</td>
<td>0.35</td>
</tr>
<tr>
<td>Unrinsed lettuce</td>
<td>2</td>
<td>(-2.6 (-0.8))</td>
<td>250</td>
<td>73</td>
<td>(-0.44)</td>
<td>0.46</td>
</tr>
<tr>
<td>Lettuce rinsed by the consumer</td>
<td>2</td>
<td>(-3.7 (-1.7))</td>
<td>47</td>
<td>14</td>
<td>(-0.31)</td>
<td>0.29</td>
</tr>
<tr>
<td>Unrinsed lettuce</td>
<td>4</td>
<td>(-2.9 (-0.9))</td>
<td>230</td>
<td>67</td>
<td>(-0.46)</td>
<td>0.38</td>
</tr>
<tr>
<td>Lettuce rinsed by the consumer</td>
<td>4</td>
<td>(-4.0 (-1.7))</td>
<td>48</td>
<td>14</td>
<td>(-0.32)</td>
<td>0.24</td>
</tr>
<tr>
<td>Unrinsed lettuce</td>
<td>7</td>
<td>(-3.8 (-1.3))</td>
<td>100</td>
<td>29</td>
<td>(-0.40)</td>
<td>0.25</td>
</tr>
<tr>
<td>Lettuce rinsed by the consumer</td>
<td>7</td>
<td>(-5.0 (-2.3))</td>
<td>20</td>
<td>5.9</td>
<td>(-0.25)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^a\) Data on \( P_{\text{ill}} \) and illnesses per 10,000 servings are indicative only and should be interpreted with caution.

\(^b\) The sensitivity of \( P_{\text{ill}} \) to variation in input distributions in the simulation was determined by Spearman rank-order correlation.

\(^c\) NA, not applicable.

\(^d\) —, not significantly correlated.
the actual time point and \( t(0) \). In this way, potential growth on individual heads as well as daily temperature and light fluctuations were included in the model.

The limited competition in the climate chamber might have underestimated \( E. \) coli O157 reduction. On the other hand, a <2-log reduction in 4 weeks was recorded by Islam et al. (22) on lettuce grown in field. On persley, the inactivation was even slower (22). In addition, Solomon et al. (38) suggested that \( E. \) coli O157 persisted well on lettuce plants in field-like conditions after exposure to contaminated water, and growth was indicated in some of the trials, whereas Stine et al. (40) reported linear decay of \( E. \) coli O157 on lettuce, with an inactivation rate of 4.9 log day\(^{-1}\). In the present study, holding times of 1, 2, 4 and 7 day(s) resulted in reductions in numbers of \( E. \) coli O157 according to Table 1, but as already mentioned, linear models were not able to explain inactivation over time (\( P > 0.05 \), GLM). Holding time as a barrier would be easy to implement. However, the effect will vary, not only with environmental factors, but also because of competing microbiota (9). In the study in saline solution, the inactivation was three times faster in light intensity of 400 mmol (m\(^2\))\(^{-1}\) s\(^{-1}\) compared with a dark environment. A suggestion is to perform controlled studies in order to develop predictive models taking into account temperature, light intensity, rain events, repeated irrigations, etc., that can be validated in the field.

In terms of risk, the reduction was twofold after 1 day and eightfold after 2 days of holding. After 4 days of holding, however, the estimated risk did not decrease, because there was a higher variability in the log change, which can be an effect of increased survival in biofilms and stomata (39) or bacterial growth between days 2 and 4. Thus, even if the mean \( P_{ill} \) was lower in scenarios with 4 days' holding time, the 95th percentile values and illnesses per 10,000 servings were equal to 2 days' holding time. After 7 days of holding, an 18-fold risk reduction was estimated. However, irrigation cessation will lead to production losses in terms of quality as well as quantity (23). Based on the present inactivation study, 2 days' holding time might be optional from a best-management practice point of view. From a public health point of view, there was no difference between 2 and 4 days' cessation. Prolonging the holding time to 7 days gave a mean 54% risk reduction compared with 2 days, but will require large volumes of high-quality water to retain the same lettuce quality.

**Rinsing.** VTEC attached to the edible parts of vegetables after irrigation can be inactivated if a holding time between harvest and consumption takes place. An additional fraction of bacteria can be removed mechanically by rinsing lettuce before consumption. Often, the consumer does rinse vegetables that are to be consumed raw; however, the main reason is often to remove traces of pesticides. In organic farming, pesticides are banned, and therefore, the consumer might be less likely to rinse organically cultured foods and vegetables (27). These might, however, be contaminated from manure and/or irrigation water and should therefore be rinsed before consumption to reduce the risk of gastrointestinal disease.

A quick rinse under cold water is a normal home washing procedure (25, 32) and, according to the present QMRA model, rinsing for 15 s in cold tap water led to an average sixfold risk reduction. It has been suggested that enterohemorrhagic \( E. \) coli strains exploit EspA filaments for attachment to lettuce leaves (34), perhaps making them harder to remove than other bacteria. For example, Kilonzo-Nthenga et al. (25) reported a 1.41-log reduction of \( Listeria innocua \) after the same treatment, compared with the 1.16 used in the present study. Other reported removals of \( E. \) coli O157 range between 0.8 and 1.9 (26, 36, 43). Except for the method of rinsing, the inoculum method, biofilm formation, and tissue damage have shown to effect removal (24, 26, 36).

**Model.** The risk in absolute terms was presented in Table 2 as illnesses per 10,000 servings, median, and the 95th percentile of \( P_{ill} \). However, the main objective for the present study was to compare different risk-mitigation strategies in relative terms, thereby decreasing the influence of variation (variability and uncertainty). To rectify conclusions from the absolute probabilities, the QMRA needs to be developed further. For example, the assumption that all consumers eat 100 g is conservative. During the Swedish lettuce outbreak, there were no deaths and relatively fewer hemolytic uremic syndrome cases than during other outbreaks caused by the same strain, probably because children do not eat as much lettuce (37). In a QMRA by Hamilton et al. (19), the amount of food consumed was the most significant determinant of the risk of infection. After consumption, virus concentration in water correlated most with the annual probability of infection (\( P_{ill} \)) in the study by Hamilton et al. (19). In the present study, \( P_{ill} \) was most sensitive to variation in holding times, and then to the ratio VTEC:\( E. \) coli in manure (Table 2). However, all VTEC might not be pathogenic, and the absolute risk estimates should be interpreted with caution. Considering the great uncertainties and the potentially large effects of assumptions on the risk assessment outcome, looking at scenario analyses in relative terms might be sufficient, considering the objective and the available data (44).

This study looked at risk reduction strategies for lettuce harvested and sold as whole heads. However, there is an increasing trend toward buying prepacked spinach, rocket, and shredded lettuce, with shelf lives up to 2 weeks. Postharvest managing such as keeping the temperature, disinfectant efficacy, and cross-contamination events during the washing process are then further parameters to take into account in a quantitative approach (10). The barriers assessed in the present study will be important for prepacked products, minimizing the risk of introducing \( E. \) coli O157 to the individual bag. Even though barriers closer to the consumer could have the largest effect the importance of control during the whole chain, from healthy animals and manure management to rinsing and kitchen hygiene practices must be emphasized (27).

The recommendation of the Swedish governmental commission on irrigation water quality that irrigation water should have the (former) Swedish bathing water standards applied no later than 48 h prior to harvest (3) is reasonable.
Information, not only specifically to restaurants that serve many portions, etc., but also to the consumers on the importance of proper rinsing should be communicated. According to the QMRA, these three barriers offered a mean reduction of 400 times for the risk of VTEC illness from consumption of lettuce contaminated by manure-contaminated irrigation water.

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