Quantitative Microbial Risk Assessment for *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in Leafy Green Vegetables Consumed at Salad Bars

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ABSTRACT

Fresh vegetables are increasingly recognized as a source of foodborne outbreaks in many parts of the world. The purpose of this study was to conduct a quantitative microbial risk assessment for *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* infection from consumption of leafy green vegetables in salad from salad bars in The Netherlands. Pathogen growth was modeled in Aladin (Agro Logistics Analysis and Design Instrument) using time-temperature profiles in the chilled supply chain and one particular restaurant with a salad bar. A second-order Monte Carlo risk assessment model was constructed (using @Risk) to estimate the public health effects. The temperature in the studied cold chain was well controlled below 5°C. Growth of *E. coli* O157:H7 and *Salmonella* was minimal (17 and 15%, respectively). Growth of *L. monocytogenes* was considerably greater (194%). Based on first-order Monte Carlo simulations, the average number of cases per year in The Netherlands associated the consumption leafy greens in salads from salad bars was 166, 187, and 0.3 for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, respectively. The ranges of the average number of annual cases as estimated by second-order Monte Carlo simulation (with prevalence and number of visitors as uncertain variables) were 42 to 551 for *E. coli* O157:H7, 81 to 281 for *Salmonella*, and 0.1 to 0.9 for *L. monocytogenes*. This study included an integration of modeling pathogen growth in the supply chain of fresh leafy vegetables destined for restaurant salad bars using software designed to model and design logistics and modeling the public health effects using probabilistic risk assessment software.

Scientists, public health officials, and consumers recognize that fresh fruits and vegetables play an important role in a healthy diet, providing important vitamins, minerals, and nutrients (50). The increased consumer demand for healthy food and the year-round availability of these products has resulted in an increase in consumption of fresh produce in the United States and Europe (2, 52). Most of the microorganisms that are naturally present on fruits and vegetables are nonpathogenic epiphytic bacteria. However, because most fresh produce is grown in a natural environment, these products are vulnerable to contamination with pathogens. Contamination also may occur during harvest, transportation, and further processing and handling of the produce.

Foodborne illness associated with fresh produce appears to be increasing in the United States and Europe (2, 49, 58, 75). Surveys have revealed the presence of human pathogens on produce (7, 33), and experiments have revealed the ability of human pathogens such as *Escherichia coli* O157 and *Salmonella* to colonize crops grown in contaminated substrate (29, 41, 42, 61). Thus, plants may be more important carriers for enteric pathogens than previously thought (10). Leafy green vegetables that are consumed raw, such as lettuce, spinach, and endive, are the most commonly implicated produce types associated with foodborne disease outbreaks, especially disease resulting from *E. coli* O157:H7 and *Salmonella* contamination (58). In the United States and Europe, several outbreaks associated with this product-pathogen combination have occurred in recent years (30, 36, 60, 65). *Listeria monocytogenes* also is of concern with respect to fresh vegetables because this pathogen is psychrotrophic and has the potential to grow under freezing conditions (27).

Temperature is one of the most important environmental parameters affecting both food quality and food safety. The temperature of fresh produce should be maintained below approximately 5°C to reduce the proliferation of spoilage organisms and human pathogens. Temperature abuse was identified as the most important contributing factor in foodborne disease outbreaks, responsible for more than 32% of the total number of outbreaks (70). Maintenance of the chilling chain is of particular importance for fresh produce because of the absence of thermal treatment before consumption. Temperature control is important for both supermarkets and out-of-home foods, which can be found at all possible locations where people eat outside the home (e.g., restaurants, catered events, and cafeterias). Out-of-home food services are the most frequently cited sources of sporadic foodborne infection cases and outbreaks. During 2007, 74% of the registered...
foodborne infections in The Netherlands were associated with food consumption at an out-of-home food service venue (restaurants and cafeterias) (21). On a larger scale, consumption at out-of-home food service venues accounted for 59% of the foodborne disease incidences in Europe between 1993 and 1998 (70). The increase in the consumption of fresh fruits and vegetables is reflected in the increased popularity of salad bars, especially in work canteens and company restaurants (3). In the United Kingdom, foodborne disease outbreaks associated with prepared salads were more likely to occur in commercial food service premises than were other foodborne disease outbreaks, with restaurants and hotels accounting for almost 75% of the outbreaks (48). Several outbreaks of foodborne illness have been associated with consumption of products from salad bars (24, 37, 38, 40, 53).

The purpose of the present study was to develop a quantitative risk assessment model to estimate the risk of acquiring an *E. coli* O157:H7, *Salmonella*, or *L. monocytogenes* infection from consumption of a single portion of leafy green vegetable salad from salad bars in The Netherlands. Pathogen growth was modeled based on measured time-temperature profiles in the chilled supply chain of leafy green vegetables and in one particular restaurant with a salad bar. Prevalence level, contamination level, and consumption data allowed for an estimation of the likelihood that an individual or a population would be exposed to a microbial hazard as a result of consuming leafy green vegetable salad (exposure assessment). This estimate then served as input for dose-response models to evaluate the risk of infection associated with the consumption of leafy green vegetables from salad bars (risk characterization).

**MATERIALS AND METHODS**

**Hazard identification.** The microbial hazards considered in the present study were *E. coli* O157:H7, serovars of *Salmonella*, and *L. monocytogenes*. *E. coli* O157:H7 and *Salmonella* are gram-negative, facultative, anaerobic, non–spore-forming bacteria. *E. coli* O157:H7 is a particular serotype of the group referred to as enterohemorrhagic *E. coli*. This group constitutes a subset of the so-called verocytotoxin-producing *E. coli* that has been firmly associated with severe clinical symptoms and disease in humans, such as bloody diarrhea (hemorrhagic colitis) and hemolytic uremic syndrome (13). The verocytotoxin-producing *E. coli* group is a highly diverse group of pathogens with over 200 serotypes, of which more than 100 serotypes have been associated with human disease (6). In The Netherlands, *E. coli* O157:H7 causes on average of 42 registered infection cases each year (2.6 cases per 1 million inhabitants per year). However, the real number of infections was estimated to be 2,111 per year (over 130 cases per 1 million inhabitants per year) for the period 1990 to 2000 (34).

In The Netherlands, the most commonly isolated *Salmonella* serovars from human infection cases during the period 1984 through 2001 were Typhimurium and Enteritidis (72). The disease caused by *Salmonella* is called salmonellosis, and the two main manifestations of salmonellosis are typhoid or typhoidlike fever and gastroenteritis. In The Netherlands, the incidence is around 1,600 laboratory-confirmed cases per year (26). The true number of cases was estimated at more than 4.3 × 10^7 per year (35). Both *E. coli* O157:H7 and *Salmonella* can be transmitted from animals and are increasingly associated with foodborne disease associated with the consumption of fresh vegetables (28, 58).

*L. monocytogenes* is an opportunistic pathogen that most often affects those with an impaired immune system. Although listeriosis is a relatively rare disease, the severity of the disease and the very frequent involvement of industrially processed foods, especially during outbreaks, mean that the social and economic impact of listeriosis is among the highest of the foodborne diseases (39). The average incidence of *L. monocytogenes* infection in The Netherlands before 2005 was 3.0 cases per million inhabitants per year (22). During 2005 and 2006, the incidence increased to 3.9 cases per million inhabitants per year (63 patients in 2006) (22). *L. monocytogenes* is widely distributed in the environment and has been isolated from a variety of food products, especially ready-to-eat products (25). *L. monocytogenes* is more resistant to various environmental conditions than many other non–spore-forming foodborne pathogenic bacteria, which allows longer survival under adverse conditions (25). A particular concern associated with foodborne *L. monocytogenes* is its ability to grow at refrigeration and even freezing temperatures when given sufficient time (74).

**Exposure assessment: pathogen level and growth.** The growth of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* was modeled by a modification of the growth model of Baranyi and Roberts (4, 5). The original model contains a term that characterizes the physiological state of the cells, enabling a time delay in the transition to the exponential growth phase (i.e., lag phase). In the present study, we made the fail-safe choice of a null lag hypothesis, assuming the cells are in the exponential growth phase and the temperature changes in the supply chain are so small that the growth rate adjusts virtually instantaneously in response to changes in temperature (5, 31, 64). When removing the lag phase term from the Baranyi and Roberts model (and fixing the curvature parameter m as 1), the model reduces to a standard logistic growth function:

\[
\frac{dx}{dt} = \mu_{\text{max}} \left( 1 - \frac{x}{x_{\text{max}}} \right) x
\]  

where \(x\) is the cell level (CFU per gram), \(t\) is time (hours), \(\mu_{\text{max}}\) is the maximum specific growth rate (1 per hour), and \(x_{\text{max}}\) is the maximum population density (CFU per gram). In a discrete-event model, usually from time to time the entire new situation is calculated. For this reason, it is common to work with analytical equations rather than with differential equations. The analytical solution of equation 1 reads

\[
x_{t+\Delta t} = \frac{x_{\text{max}} x_{t} e^{\mu_{\text{max}} \Delta t}}{x_{\text{max}} + x_{t} (e^{\mu_{\text{max}} \Delta t} - 1)}
\]

The Ratkowsky square root model (with temperature \(T\), conceptual minimal temperature for microbial growth \(T_{\text{min}}\), and fitting parameter \(b\)) was used as a secondary model for the temperature dependence of the maximum specific growth rate \(\mu_{\text{max}}\) (5, 36):

\[
\sqrt{\mu_{\text{max}}} = b (T - T_{\text{min}})
\]

Values for the parameters \(x_{\text{max}}\) and \(T_{\text{min}}\) were adopted from experimental inoculation studies with *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* (mixture of serovars Typhimurium and Enteritidis) on fresh-cut leafy green vegetables under constant temperatures ranging from 5 to 25°C (43, 44). An overview of all parameter values used is given in Table 1.

The initial levels (C0) of *E. coli* O157, *Salmonella*, and *L. monocytogenes* were adopted from a recently conducted large-scale survey on the microbial contamination of fresh produce for raw
consumption in The Netherlands (54). From October 2006 to October 2007, samples from raw produce and derived products were taken in two major processing plants in The Netherlands. Approximately 94% of the samples were leafy green vegetables (different lettuce types and endive). The reported most-probable-number (MPN) estimates (CFU/g) for E. coli O157 and L. monocytogenes on endive were used for the present study. For Salmonella, the MPN estimate for iceberg leafy green vegetables was adopted. We assumed a Poisson distribution with the reported MPN estimates as mean (Table 1).

### Exposure assessment: time-temperature profile.

Time-temperature data were collected in two steps. First, information and data were collected about the supply chain, i.e., activities required to distribute fresh-cut leafy green vegetables from the processing plant to the restaurant (Restaurant of the Future, Wageningen University & Research Center, Wageningen, The Netherlands). Second, data were collected about the restaurant itself, i.e., activities needed to make salads available at the salad bar during lunch time.

For collecting supply chain data, I-buttons (small recording buttons with wireless transmission of data to a recorder) (Maxim Integrated Products Inc., Sunnyvale, CA) were used for collecting time-temperature data every 5 min. These I-buttons were added to several individual products at one particular processing plant. Data collection started at time 1416 h, when products were stored at the processing plant. That same evening transportation to the regional warehouse took place, and the morning after products were delivered at the restaurant. Here, only short cold storage took place until salad preparation time. Collecting time-temperature data about the supply chain took place during 1 week, resulting in 19 samples at every time step of 5 min.

For collecting time-temperature data in the restaurant, three salad bowls were provided with three I-buttons (top, medium, and bottom) just before being filled with salad. These I-buttons collected time-temperature data every minute. After the bowls were filled with salad, a needle sensor was added to the salad. This sensor could be moved freely within the salad according to consumer’s influences. Data collection started at the time of salad preparation. The prepared salad was placed in cold storage until needed and then transported to the salad bar. Data collection ended at the end of lunch (1400 h). Time-temperature data were collected...
in the restaurant for 1 day, resulting in four samples per bowl at 1-min sampling intervals.

**Exposure assessment: pathogen prevalence.** The probability of exposure to a nonzero amount of pathogen was adopted from the survey on the microbial contamination of fresh produce for raw consumption in The Netherlands (54). The prevalence of contaminated leafy green vegetable samples was determined by summing the results for the raw produce and the derived products. The prevalences were 1 in 2,593 for *E. coli* O157:H7, 6 in 2,611 for *Salmonella*, and 1 in 2,641 for *L. monocytogenes*. The uncertainty in the prevalence was expressed with a beta distribution ($\beta = s + 1, n - s + 1$, where $s$ is the number of positive samples and $n$ is the total number of samples) (Table 1).

**Exposure assessment: consumption.** To estimate the number of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* infections in the Dutch population as a result of consumption of contaminated leafy green vegetables from salad bars, consumption data were collected. In The Netherlands, approximately 3.5 million people make daily use of the lunch facilities in a restaurant at work (3). An estimated 15% of these restaurants offer salads from a salad bar, resulting in a point estimate of $5.3 \times 10^8$ people visiting a restaurant with a salad bar each day ($N$). The uncertainty around this estimate was described by a triangular distribution (Table 1).

To be able to estimate the fraction of people buying leafy green salad ($fN_p$), the fraction of salad containing more than 20% leafy green vegetables (lettuce, vegetables, endive, spinach) ($f$), and the portion size ($M$), point-of-sale data were used from a particular restaurant (Restaurant of the Future). Each day about 300 customers visit this restaurant for lunch.

The fraction of visitors buying salad ($fN_p$) was estimated using point-of-sale data from the months April, May, and June 2008. The variation in the fraction of visitors buying leafy green salad between days was described with a normal distribution ($n = 125$; Anderson-Darling statistic, 0.290; $P > 0.9$), with an average of 0.371 (Table 1). Usually a proportion is described by beta distribution. However, in this case we wanted to describe the variability in fractions over the days, not the uncertainty per day or over the whole period. When the parameters of the beta distribution are sufficiently large (>10), the beta distribution can be approximated by the normal distribution. The small standard deviation of the fitted normal distribution (0.05) prevents the likelihood of generating negative values or values larger than 1.

During the measurement period, 87 different types of salad were offered at the salad bar. For each type of salad, the chief cook estimated the percentage (weight) of leafy green vegetables in each salad; 48 salads consisted of more than 20% leafy green vegetables (55%). The mean fraction of sold salads with more than 20% leafy green vegetables ($f$) relative to the total number of sold salad consumptions was 0.58. The variation in this fraction between days was described by a uniform distribution (Table 1).

The estimation of the portion size was based on measurements (difference between salad weight offered and the weight of nonconsumed salad divided by the number of people buying salad) that took place during 8 weeks (2 September to 24 October 2008). The variation (between days) in the portion size of salad containing more than 20% leafy green vegetables ($M$) was described with a log-logistic distribution ($n = 49$; Anderson-Darling statistic, 0.175; $P > 0.25$), with an average of 115.3 g (Table 1).

The number of selling days per year ($d$) was fixed at 250 days. The total number of consumed portions per year ($N_p$) was calculated with the parameters described above: $(N \times fN_p \times f \times d)$.

**Dose-response assessment.** Dose-response models were used to estimate the probability of infection ($P_{inf}$) resulting from an exposure to a single consumed portion with a certain pathogen dose. The most commonly used models are single hit models, where only one organism ingested is required to cause infection even though the probability of this occurring may be very small. The simplest form of this model is the exponential model, which assumes that the number of organisms ingested (dose, $D$) is randomly distributed and that each microorganism has an equal and independent probability ($r$) of causing infection to the host:

$$P_{inf} = 1 - e^{-rD} \quad (4)$$

Dose-response relationships for *E. coli* O157:H7 have been developed based on experimental infection in rabbits (32) and on surrogate pathogens *Shigella dysenteriae* and enteropathogenic *E. coli* (55). However, these models seem to underestimate the human risk of *E. coli* O157:H7 infection (66). Recently, exponential dose-response relations have been parameterized based on human outbreaks (51, 63). We adopted the value for $r$ (best fit parameter derived from fitting the exponential dose-response model to binomially distributed data applying the maximum likelihood method) from Strachan et al. (63) ($r = 1.13 \times 10^{-3}$) because this study included data from eight *E. coli* O157:H7 infection outbreaks, including the highly infectious Morioka outbreak used by Nauta et al. (51). Few studies have included dose-response modeling for *Salmonella* (68, 76). Latimer et al. (45) developed weighted composite models for *Salmonella* using previously reported data from human feeding studies and distinguishing different virulence classes ($r = 6.85 \times 10^{-2}$). In the present study, we used the average value of the exponential parameter $r$ as estimated for the low, moderate, and high *Salmonella* virulence class. For *L. monocytogenes*, we adopted the values for $r$ of the combined exponential model as reported by Chen et al. (17) ($r = 1.91 \times 10^{-10}$). The values for the exponential parameter $r$ for the different pathogens considered in the present study are summarized in Table 1. We did not consider different subpopulations with different levels of vulnerability to illness following exposure. We also limited ourselves to calculating the probability of infection (not the probability of illness as a result of infection).

**Risk characterization.** The risk of infection associated with the consumption of leafy green vegetables from salad bars was estimated using the predictions of the exposure assessment as inputs for the dose-response model (Table 1). The probability of infection from a single portion $P_{inf}(D)$ was calculated with the dose-response model using the estimated dose per portion $D$. The final risk of infection $P_{inf}$ was calculated as the product of the probability of exposure $P_E$ and the risk of infection from a single portion $P_{inf}(D)$. An estimate of the annual number of infection cases in the Netherlands $Y$ was made as the product of the final risk of infection $P_{inf}$ and the annual number of consumed portions in The Netherlands $N_p$.

**Modeling procedure: modeling environment.** Pathogen growth as a function of the time-temperature profile was modeled in Aladin (agro logistics analysis and design instrument), a visual interactive object-orientated simulation environment for simulating, analyzing, and visualizing the behavior of fresh product chains. Aladin is built in the Logistics Suite of the simulation package Enterprise Dynamics (ED), which is an object-oriented, graphical discrete-event simulation package that enables users to perform animations on the basis of the (discrete) model equations. An important feature of this approach is that apparently black-box simulations are made transparent to the user, which enhances the
Modeling procedure: temperature trajectories. For modeling purposes, the temperature at time $t$ was assumed to be normally distributed with mean $\mu_t$ equal to the average of all 19 samples at time $t$ and standard deviation $\sigma_t$ equal to

$$\frac{\max(T_{i,t}) - \min(T_{i,t})}{6}$$

where $i$ is the sample at time $t$. This expression reflects the assumption that the highest and lowest measured temperatures really represent the extremes that can be expected to occur. Time-temperature profiles of the supply chain and the restaurant were combined with a short period (30 min) of a constant and relatively high temperature (16.5°C) inserted right before filling the dish and putting it into cold storage because no data were available for this period.

Modeling procedure: exposure assessment. The time-temperature profile as obtained above was used as an input in modeling the concentration of pathogens on leafy green vegetables at the moment of consumption. An initial temperature was sampled from the normal distribution at time 0, representing the variability in temperature at that time point. Subsequently, the temperature at time $t$ was determined by taking the temperature value that corresponded to the same relative location within the temperature profile as obtained above. This expression reflects the assumption that the highest and lowest measured temperatures really represent the extremes that can be expected to occur. An initial temperature was sampled from the normal distribution at time 0, representing the variability in temperature at that time point. The temperature profile as obtained above was used as an input in modeling the concentration of pathogens on leafy green vegetables at the moment of consumption.

Modeling procedure: risk characterization and sensitivity analysis. The output distribution of the growth modeled in Aladin was exported to Microsoft Excel 2003 (Microsoft, Redmond, WA). A spreadsheet model using @Risk 4.5.7 (Palisade Corporation, Ithaca, NY) was constructed to calculate the probability of infection and the estimated number of infections per year in The Netherlands as a result of eating salad containing leafy green vegetables from a salad bar. A summary of the model parameters is given in Table 1. The simulation was run with 10,000 iterations of the model using Latin hypercube sampling and run initially without any separation of uncertainty and variability.

A basic analysis of the sensitivity of the estimated annual number of $E. coli$ O157:H7, Salmonella, and $L. monocytogenes$ infection cases as a result of consumption of leafy green vegetable salad consumed from a salad bar to model input distributions was performed using tornado graphs and Pearson rank order correlations.

A more advanced sensitivity analysis was performed by separating variability and uncertainty in the model. The uncertainty in the daily number of people visiting a restaurant with a salad bar ($N$) and pathogen prevalence ($P_E$) was modeled by triangular and beta distributions, respectively. The effect of this uncertainty was assessed by running 20 simulations with the value for these parameters fixed on one random value from the uncertainty distribution and sampling from the variability distributions for each iteration with the simulation (i.e., second-order modeling).

Modeling procedure: scenario analysis. For each considered pathogen ($E. coli$ O157:H7, Salmonella, and $L. monocytogenes$), a reference situation was simulated based on the temperature profile as measured in the supply chain and at the restaurant. In addition, the impact on pathogen growth and public health resulting from a breakdown of the salad bar’s cooling unit was simulated (scenario 1). From the moment that the salad bar was filled (1128 h), we assumed that the temperature was 18°C.

RESULTS

Temperature profiles and temperature trajectories. The temperature profile of leafy green vegetables was fairly consistent at around 4°C in the supply chain from the production plant to the restaurant (Fig. 1). The temperature of the leafy green vegetables was higher and fluctuated more between 1100 and 1130 h (transport from cold storage to salad bar) and in the restaurant, with temperature peaks occurring around 1000 h (filling of the salad dishes) (Fig. 2). During display, the temperature of the salad was approximately 5 to 6°C on average, with a minimum below 2°C and a maximum up to 9°C.

Based on the temperature profile and temperature variation at each time point, temperature trajectories were constructed (Fig. 3). The temperature trajectories reflect the slightly higher temperature variation just before putting the product into cold storage at the restaurant (around 0900 h) and the period of higher temperature during transport to the salad bar (between 1100 and 1130 h).
Pathogen growth in the supply chain and restaurant. Growth of E. coli O157 and Salmonella was minimal under the conditions encountered in the chilled catering chain (Table 2). E. coli O157 and Salmonella levels at time of consumption were respectively 0.061 CFU/g (95% confidence interval [CI], 0.048 to 0.074 CFU/g) and 0.323 CFU/g (95% CI, 0.294 to 0.353 CFU/g), which is on average a relative growth of 17 and 15%, respectively. Growth of L. monocytogenes was more profound and increased to 735 CFU/g (95% CI, 690 to 780 CFU/g), which is on average a relative growth of 194%. The mean estimated numbers of cells of E. coli O157, Salmonella, and L. monocytogenes per contaminated portion of leafy green vegetable salad were 7 (95% CI, 2 to 17), 37 (95% CI, 10 to 89), and 85,000 (95% CI, 23,000 to 200,000), respectively (Table 2). Simulating a hypothetical breakdown of the salad bar cooling resulted in a relative growth of 88% for E. coli O157, 80% for Salmonella, and 265% for L. monocytogenes (Table 2). As a result, the average dose per portion was 11 cells (95% CI, 3 to 27 cells), 58 cells (95% CI, 16 to 140 cells), and 105,000 cells (95% CI, 28,500 to 251,000 cells) of E. coli O157, Salmonella, and L. monocytogenes, respectively.

Risk characterization. Based on the estimated dose, the prevalence of contaminated portions, and the dose-response relations, the probability of infection resulting from a single consumption of salad (containing more than 20% leafy green vegetables) obtained from a salad bar was estimated. Without separating variability and uncertainty, the average probability of infection was $6.04 \times 10^{-6}$ (95% CI, $5.84 \times 10^{-7}$ to $1.81 \times 10^{-5}$) for E. coli O157:H7, $6.83 \times 10^{-6}$ ($1.40 \times 10^{-6}$ to $1.78 \times 10^{-5}$) for Salmonella, and $1.23 \times 10^{-8}$ ($1.23 \times 10^{-9}$ to $3.75 \times 10^{-9}$) for L. monocytogenes (Table 3). By multiplying the probability of infection by the estimated number of annual consumptions, the number of infection cases as a result of consumption of salad (with >20% leafy green vegetables) from a salad bar could be estimated. The model estimated an average of 166 (13 to 544) E. coli O157:H7 infection cases per year, 187 (29 to 520) Salmonella infection cases per year, and 0.34 (0.03 to 1.06) L. monocytogenes infection cases per year (Table 3). Temperature abuse during display in the salad bar (18°C) resulted in 1.6 times more cases of E. coli O157:H7 and Salmonella infection (273 and 292 cases per year, respectively) and 1.2 times more cases of L. monocytogenes infection (0.42 cases per year) (Table 3).

The correlation analysis revealed different results for the different pathogens. The probability of E. coli O157:H7 infection was most highly correlated with portion size ($M$) ($r = 0.74$), pathogen prevalence (probability of exposure, $P_E$) ($r = 0.62$), and the dose per gram ($C_{con}$) ($r = 0.13$). The probability of Salmonella infection also was mostly highly correlated with portion size ($r = 0.84$) and pathogen prevalence ($r = 0.51$). For L. monocytogenes infection, the

FIGURE 2. Average temperature profile of leafy green vegetables in the restaurant.

FIGURE 3. Modeled time-temperature trajectories ($n = 50$) based on the measured time-temperature profiles. The time-temperature trajectories are constructed in such way that the temperature at time $t$ corresponds to the same relative location within the normal distribution of temperatures for that particular time point $t$, $N(\mu_t, \sigma_t)$, as the initial temperature had within the initial temperature distribution $N(\mu_0, \sigma_0)$. 
highest correlations were found with pathogen prevalence ($r = 0.75$) and portion size ($r = 0.62$).

The effect of uncertainty in the model (pathogen prevalence and number of restaurant visitors) on the probability of infection and the estimated number of annual cases was evaluated by simulating the model 20 times, each time with a fixed different (random) value from the uncertainty distributions (Fig. 4). The mean probability of infection with E. coli O157:H7 ranged from $1.39 \times 10^{-6}$ to $1.79 \times 10^{-3}$ (42 to 551 cases per year) (Table 3). For L. monocytogenes, the mean probability of infection ranged from $3.70 \times 10^{-5}$ to $3.05 \times 10^{-6}$ (0.10 to 0.85 cases per year). For Salmonella, the effect of uncertainty with respect to the true prevalence on the probability of infection was smaller than the effect of uncertainty. The mean probability of infection with Salmonella ranged from $5.42 \times 10^{-6}$ to $1.20 \times 10^{-2}$ (81 to 281 cases per year) (Table 3). These results indicate that for Salmonella the model outcomes are less dominated by uncertainty than were the outcomes for E. coli O157:H7 and Listeria because of the lower number of positive samples for E. coli O157:H7 and Listeria.

**DISCUSSION**

Temperature profiles and pathogen growth in the supply chain and restaurant. In this study, temperature measurements were conducted to evaluate the potential for growth of E. coli O157:H7, Salmonella, and L. monocytogenes on leafy green vegetables and salad based on this type of produce (salads containing more than 20% leafy green vegetables). The temperature in the supply chain from the processing plant to the restaurant was generally properly maintained between 2 and 5 °C. During display in the salad bar, the temperature fluctuated widely (between 0 and 13 °C), indicating potential for pathogen growth. However, because of the relative short display time (maximum 2 h), growth of E. coli O157:H7 and Salmonella was limited to 17 and 15% increase in population density, respectively. In contrast, substantial growth of L. monocytogenes was observed (194%). One specific characteristic of L. monocytogenes that appears to be critical for its ability to cause human foodborne illness is its capacity to grow at low and even freezing temperatures (16). The reported growth rates of L. monocytogenes (and those used in this study) on leafy green vegetables were higher at temperatures up to 12°C than were growth rates of E. coli O157:H7 and Salmonella (43, 44). In the present study, we made the fail-safe choice of modeling pathogen growth without the occurrence of a lag phase, assuming that the cells are in the exponential growth phase and that the temperature changes within 1-min intervals (as used in the present study) are so small that the growth rate adjusts virtually instantaneously to changes in temperature (5, 31, 64). Experimental work revealed the absence of a lag phase for L. monocytogenes at temperatures as low as 13, 7, 4, and 2°C, depending on the pH and water activity (15, 62). Large temperature fluctuations that may result in lag times were avoided in the current model by assigning temperature trajectories to each model run. The fail-safe option of no lag phase also was applied in an assessment of L. monocytogenes in chilled ready-to-eat foods in school catering facilities (57).

Risk characterization and relation to epidemiological data. The risk assessment model estimated an average of 166 cases of E. coli O157 infection per year due to the consumption of leafy green salad from salad bars in the Netherlands. For the period 1990 through 2000 the total number of E. coli O157 infections was estimated to be around 2,000 per year, of which half were associated with contaminated food as the vehicle of transmission (34, 35). The number of estimated cases associated with salmonella consumption from salad bars is approximately 8% of all E. coli O157 infections and 17% of food-associated E. coli O157 infections. In The Netherlands, an average of 48 cases of E. coli O157 infections were officially reported (2.4%), which corresponds to 4 reported cases per year as a result of consumption of leafy green salad from salad bars. A recently conducted Dutch risk assessment estimated 740 E. coli O157 infections per year associated with the consumption of ready-to-eat mixed salads (54), i.e., 4.5 times higher than the estimated number of cases associated with consumption of leafy green salad from salad bars in the present study.

For Salmonella, the current risk assessment estimated an average of 187 infection cases per year associated with consumption of leafy green salad from salad bars. In 2006, a total of 43,381 Salmonella infections in The Netherlands...
FIGURE 4. Cumulative probabilities of the log probability of illness resulting from ingestion of (A) E. coli O157, (B) Salmonella, and (C) L. monocytogenes from a single consumption of a leafy green vegetable salad from a salad bar in The Netherlands. The heavy lines represent the result of a single simulation without separating uncertainty and variability; the thin lines represent the results of 20 successive simulations with separation of uncertainty and variability. The separate curves indicate the true variability within a simulation, and the difference between curves is the effect of uncertainty in the estimated probability of infection.

TABLE 3. Probability of infection associated with the consumption of leafy green vegetable salad from salad bars in The Netherlands as estimated by first- and second-order Monte Carlo simulations

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Pathogen</th>
<th>First order</th>
<th>Second order</th>
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<td></td>
<td>Probability of infection ($P_{inf}$)</td>
<td>Estimated no. of cases ($Y$)</td>
<td>Probability of infection ($P_{inf}$)</td>
</tr>
<tr>
<td></td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
</tr>
<tr>
<td>Baseline</td>
<td>E. coli O157:H7</td>
<td>6.04 x 10^-6 5.84 x 10^-7-1.81 x 10^-5</td>
<td>166 13-544</td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
<td>6.83 x 10^-6 1.40 x 10^-7-1.78 x 10^-5</td>
<td>187 29-520</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes</td>
<td>1.23 x 10^-8 1.23 x 10^-7 3.75 x 10^-8</td>
<td>0.34 0.03-1.06</td>
</tr>
<tr>
<td>Scenario 1</td>
<td>E. coli O157:H7</td>
<td>9.82 x 10^-6 9.54 x 10^-7 2.92 x 10^-5</td>
<td>273 216-862</td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
<td>1.06 x 10^-6 2.24 x 10^-6 2.68 x 10^-5</td>
<td>292 48-803</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes</td>
<td>1.50 x 10^-8 1.53 x 10^-9 4.58 x 10^-8</td>
<td>0.42 3.3 x 10^-2 1.34</td>
</tr>
</tbody>
</table>

* Mean range of 20 simulations.
was estimated, and 63% of these cases were associated with food (35). The number of cases estimated in the present risk assessment covers only 0.7% of the total cases and 0.4% of the food-associated *Salmonella* cases, which is a considerable lower fraction than that for *E. coli* O157.

For *L. monocytogenes*, the present model estimated 0.34 cases per year as a result of consumption of leafy green salad from Dutch salad bars (1 case in 3 years). For 2006, the total number of *L. monocytogenes* infection cases was estimated to be 80, of which 64 were associated with food (35). In 2006, 63 cases of *L. monocytogenes* infection were reported (22). The estimated number of cases in the present study covers approximately 0.5% of the total number of cases. However, during 2008 only three cases of *L. monocytogenes* infection were officially reported. Thus, based on the 2008 epidemiological data, the number of *L. monocytogenes* infections due to consumption of leafy green salad from Dutch salad bars covers 11% of the total number of cases.

Based on comparison with epidemiological data, we conclude that the number of *E. coli* O157, *Salmonella*, and *L. monocytogenes* infections, as estimated with the model presented in this study, are in proportion to the total number of foodborne infections associated with these pathogens in The Netherlands.

**Limitations of the model: pathogen prevalence and level.** In the present risk assessment study, we assumed that no reduction in pathogen level occurs due to washing, cutting, and partitioning. The effects of size reduction processing steps (e.g., cutting, slicing, shredding, chopping) on the growth of pathogens and the diluting effects on the average pathogen level are not sufficiently quantified. When contaminated crops are cut, washed, and packed, the prevalence of contaminated portions may increase because of repartitioning and cross-contamination from mixing uncontaminated product with contaminated product (23). However, the pathogen level in a prepacked bag of leafy green produce may be lower than that of the original contaminated crop because of a dilution effect. Although shredding and cutting of plant tissue could increase the probability of pathogen growth, no difference in the growth of *L. monocytogenes* was observed on cut and whole lettuce (8). Cutting the tissue of lettuce released antilisterial compounds, which may reduce pathogen growth (20). However, the pathogen level in a prepacked bag of leafy green produce may be lower than that of the original contaminated crop because of a dilution effect. Although shredding and cutting of plant tissue could increase the probability of pathogen growth, no difference in the growth of *L. monocytogenes* was observed on cut and whole lettuce (8). Cutting the tissue of lettuce released antilisterial compounds, which may reduce pathogen growth (20). Because of strong attachment of bacteria to plant tissue, the formation of biofilms, and the possible presence of pathogens in protected sites (e.g., pores or cut surfaces), washing with water without a sanitizing agent is not likely to reduce the contamination level significantly. Reduction of the level of background flora also might result in pathogen outgrowth from elimination of competing organisms (14). In some studies, *E. coli* O157 and *Salmonella* have been internalized into the lettuce plants, making these pathogens inaccessible to the effects of washing and sanitizing (29, 41, 61). The effects of different processing steps on pathogen prevalence and level in fresh leafy green produce require further investigation and quantification in order to be used in risk assessments.

**Limitations of the model: pathogen growth.** The pathogen growth model used in this risk assessment was parameterized with growth data on lettuce tissue as published by Koseki and Isobe (43, 44). However, this growth rate for *E. coli* O157:H7 is relatively high compared with that previously published elsewhere. Although Koseki and Isobe (44) reported stable population sizes at 5°C over 5 days, others reported a decline in *E. coli* O157:H7 numbers under 5°C (1, 46, 69). Koseki and Isobe (44) reported 1-log growth over 5 days at 10°C, whereas others reported no growth over 14 days at 10°C (19). The limited amount of survival data points reported in the other studies prevented their use for model fitting and subsequent application in the present study. However, by using only the Koseki and Isobe data and no lag phase, pathogen growth might be slightly overestimated. In addition, within this risk assessment, pathogen growth was based on temperature data from one specific supply chain in The Netherlands, which was subsequently generalized for the entire country.

In reality, salads contain dressing and substances such as mayonnaise that will usually enhance pathogen death (9, 59). Given the limited amount of time that the pathogen was present in this prepared salad (a few hours maximum), it is unlikely that the dressing had a drastic effect on pathogen levels. However, the physical characteristics of the food item in which the pathogen is transmitted is thought to have major consequences for dose-response relations (12). Entrapment of bacterial cells in fat droplets may protect the cells from exposure to gastric fluid, thereby enhancing the probability of survival and colonization of the site of replication and subsequently increasing the probability of infection (12). However, the extent to which pathogens from the lettuce tissue can be entrapped in fat droplets of salad dressing in currently unknown. Further research into this matter may lead to more food matrix-specific dose-response relations.

**Limitations of the model: dose-response modeling.** In the current study we used exponential dose-response curves to model the probability of infection associated with a single consumption of a leafy green vegetable salad from a Dutch salad bar. The choice of the dose-response model and parameter values highly influence the model outcomes. The most commonly used models are single hit models, where only one ingested organism is required to cause infection, even though this probability might be very low. The simplest form of this model is the exponential model, used in the present study, which assumes that each of the organisms has an equal and independent probability of causing infection of the host. However, because each host may respond differently, the survival probability of the pathogen may be better described by a probability distribution such as the beta-distribution, resulting in a beta-Poisson dose-response model. Although the beta-Poisson model in many cases provides a statistically significant improvement in fit over the exponential model (18, 63), this model also may lead to an overestimation of risks at low doses (<2 log units) (67). For pathogens that are believed to be highly infectious, such as *E. coli*...
O157:H7, the choice of the dose-response model can profoundly influence the risk outcome because pathogen doses involved in sporadic cases of foodborne disease or outbreaks are generally below 2 log CFU per portion. Recently, human dose-response models for E. coli O157:H7 were constructed based on quantitative data from eight different human disease outbreaks (63), and the binomial exponential model was used for the current risk assessment. This model assumes lower E. coli O157 virulence than does the model of Nauta et al. (51), which was based on a single outbreak with a highly infectious strain, but higher virulence than the models based on surrogate pathogens (55) or studies with mice (32).

With respect to L. monocytogenes, a recently developed exponential dose-response model was used in the present risk assessment (17). This model combines the virulence of different L. monocytogenes lineages. The value for r adopted from this model (log r = −9.72) is similar to previous exponential dose response parameter estimations by Buchanan et al. (11) (log r = −9.93) and Lindqvist and Westoo (47) (log r = −9.25), which corresponds to the apparent lower virulence of L. monocytogenes compared with other foodborne pathogens (73).

The present study included an integration of modeling pathogen growth in the supply chain of fresh leafy vegetables destined for restaurant salad bars using software designed to model and design logistics (ALADIN) and modeling the public health effects using probabilistic risk assessment software (@Risk). Instead of using distributions of average temperatures and length of stay in the supply chain, we used time-temperature profiles to model microbial growth throughout the supply chain. Although the modeling of microbial growth on chilled ready-to-eats foods in catering establishments using time-temperature profiles has been described (57), this study is unique in using time-temperature profiles of the supply chain to assess public health effects of pathogens associated with salads consumed from salad bars. The temperature in the studied cold chain was fairly well controlled, and growth of E. coli O157:H7 and Salmonella was minimal. Growth of L. monocytogenes was greater but was not considered problematic because of the low virulence of this pathogen. The estimated number of annual infection cases were considered reasonable in relation to epidemiological data and in proportion to an earlier risk assessment based on the total consumption of fresh vegetables.

The current risk assessment included time-temperature profiles with fixed time-location trajectories. However, these time-location trajectories are variable (weekend delay or nondaily delivery), which was determined by the logistic concept (planning and scheduling) within the supply chain. The effect of this variation could be assessed by modeling these time-location trajectories, which would enable assessment of the effect of different logistic scenarios on the product microbial safety.

REFERENCES


