ABSTRACT: The possibility of modeling the cross-contamination of Listeria species, total L. monocytogenes, or specific L. monocytogenes strains using a quantitative mathematical model using Monte Carlo simulation techniques is proposed. This article illustrates this approach using 2 different models: one that tracks L. monocytogenes number and prevalence for 4 different strains (Model I) and one that tracks only prevalence for a single strain (Model II). These models have been developed to provide a starting framework for predictive modelers and scientists studying L. monocytogenes to begin research together with the ultimate goal of understanding and controlling L. monocytogenes in food-processing plants.

Keywords: Listeria monocytogenes, modeling, risk assessment, simulation, processing plant

Introduction

L. monocytogenes is an important foodborne pathogen responsible for more than an estimated 2500 cases and almost 500 deaths per year in the United States (Mead and others 1999). This organism is a problem in cooked ready-to-eat products that are contaminated with L. monocytogenes after pasteurization. Foods commonly linked to listeriosis outbreaks include milk and dairy products (for example, soft cheeses made from raw milk), meat and meat products (for example, deli meats and hot dogs), vegetables, and seafood (for example, cold smoked fish) (Rocourt and Cossart 1997). Efforts to control this organism have led to a large body of published research and to new regulations proposed by the U.S. Dept. of Agriculture-Food Safety and Inspection Service (USDA-FSIS 2003).

The tools of predictive modeling (McMeekin and others 2002) and quantitative microbial risk assessment (Lammerding and Paoli 1997) are being used more and more to guide food-safety decision making and risk management, including decisions regarding L. monocytogenes. Many L. monocytogenes predictive models have been published in the last few years alone (Augustin and others 2000; Gill and Holley 2000; Ross and others 2000; Buchanan and Phillips 2001; Devlieghere and others 2001; Pleasants and others 2001; Baty and others 2002; Chhabra and others 2002; LeMarc and others 2002; Whiting and Bagi 2002; Juneja 2003; Lihono and others 2003) and the USDA (Gallagher and others 2003), the Food and Drug Administration (FDA) (Elliot and Kvenberg 2000; Hitchins and Whiting 2001), the Food and Agriculture Organization/World Health Organization (FAO-WHO 2001), and others (Bemrah and others 1998; Lindqvist and Westoo 2000) have developed or are in the process of developing quantitative risk assessments for L. monocytogenes.

Despite these scientific advances, and the apparent importance of cross-contamination in leading to contaminated finished products, little is known quantitatively about the movement of L. monocytogenes in food-processing plants. This is due in part to a limited ability to track the movement of specific strains of L. monocytogenes from raw product or environment to finished product.

Hypothesis

Modeling the movement of Listeria species, total L. monocytogenes, or specific L. monocytogenes strains using a quantitative mathematical model using Monte Carlo simulation techniques is proposed. This article will illustrate this approach using 2 different models: one that tracks L. monocytogenes number and prevalence and one that tracks only prevalence. Although a model tracking concentration would be preferred, most data commonly available today are for prevalence only.

By having this framework as a starting point, interested individuals will see new ways to use existing data to reduce L. monocytogenes risk and to improve L. monocytogenes risk-management practices in food-processing plants. This framework may also spur additional data collection to confirm or refute certain aspects of the model design and model predictions. The Microsoft Excel (Microsoft Corp., Redmond, Wash., U.S.A.) file used for these at-risk simulations is available at http://foodsci.rutgers.edu/schaffner/files.htm.

General Model Features

Figure 1 illustrates the general features of the model. It is important to note that this model represents L. monocytogenes behavior in a highly abstract manner. Such a simplification is necessary because it would be unrealistic (and computationally very difficult) to represent all the different activities occurring in an actual food-processing plant. This model is similar, but not identical, to that used by USDA-FSIS for the risk assessment for L. monocytogenes in deli meats (Gallagher and others 2003).

The initial number (colony-forming units, CFU) or prevalence of L. monocytogenes cells coming into the plant on the raw product are represented by the box in the upper left corner. Some of those bacteria contaminate the environment (at a rate represented by the variable “xc”). The number of bacteria in the environment is represented by the next box. Some bacteria in the environment are able to persist (at a rate represented by the variable “p”) and become part of the environmental reservoir for L. monocytogenes, represented by the box in the upper right corner of Figure 1. Bacteria
Modeling *Listeria* cross-contamination . . .

### Table 1—Simulation Model I parameters for *Listeria monocytogenes* initial number, persistence, and transferability in a food-processing plant

<table>
<thead>
<tr>
<th><em>Listeria monocytogenes</em> strain number</th>
<th>Nr (CFU/product)</th>
<th>Standard deviation (log_{10} CFU)</th>
<th>Transferability* (per area)</th>
<th>Persistence (per area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.0003</td>
<td>1</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>II</td>
<td>0.003</td>
<td>1</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>0.03</td>
<td>1</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>IV</td>
<td>0.3</td>
<td>1</td>
<td>30</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Transferability simulation results greater than 100% were truncated at 100%. CFU = colony-forming units.

the environmental reservoir may cross-contaminate a product contact surface (again, at a rate \( x_c \)), represented by the box in the lower right corner. Just as some cells may persist in the environment, some *L. monocytogenes* cells may persist on the product contact surface (again, at a rate represented by the variable \( p \)), becoming part of the product contact surface reservoir. Finally, some of the cells on the product contact surface reservoir may cross-contaminate the finished product. The number of bacteria in the finished product is represented by the box in the lower right corner of the diagram. The model can be expressed by the following 5 simple equations:

\[
\text{Raw product CFU} \times \text{Cross-contamination rate} = \text{Environmental CFU}
\]

\[
\text{Environmental CFU} \times \text{Persistence rate} = \text{Environmental reservoir CFU}
\]

\[
\text{Environmental reservoir CFU} \times \text{Cross-contamination rate} = \text{Product contact surface CFU}
\]

\[
\text{Product contact surface CFU} \times \text{Persistence rate} = \text{Product contact surface reservoir CFU}
\]

\[
\text{Product contact surface reservoir CFU} \times \text{Cross-contamination rate} = \text{Finish product CFU}
\]

**Model design—Tracking Number of Organisms and Subtypes**

**Model design**

Model I includes data on *L. monocytogenes* CFU and describes the movement and persistence of 4 different “virtual” strains (I, II, III, and IV). Four strains are used to show that strains with initially low numbers, but high abilities to persist, will come to dominate the population of *L. monocytogenes* contaminating the finished product. Table 1 shows the initial values for the model characteristics of the 4 strains used in the 1st demonstration. For simplicity sake, in the current model, all 4 strains have an approximately equal ability to transfer from raw product to the environment, environment-to-product contact surface, and from product contact surface to finished product. The average transfer rate is set at 30%, so 30% of the cells present are transferred (that is, if 10 cells are present, 3 are transferred).

As noted previously, several strains are used to demonstrate that strains with initially low numbers, but high abilities to persist, will come to dominate the population of *L. monocytogenes* contaminating the finished product. Strain I is such a strain. Strain I has a very low number on the incoming raw material (an average of 0.0003 CFU/product, or about 1 in every 3333 units containing 1 cell of *L. monocytogenes*). This strain also has a very high ability to persist (100% on average) should it come into the plant on the raw material and transfer to the environment or a product contact surface. Conversely, strain IV is present on average in about 1 in 3 incoming raw products but has a very limited ability to persist, such that only about 0.1% or 1 in every 1000 cells present will persist in the environment or on food contact surfaces. Strains II and III are designed to show intermediate initial numbers and abilities to persist.

Because information regarding the model parameters may be uncertain, and the behavior of different *L. monocytogenes* strains may be variable, the conceptual model also includes an uncertainty/variability component in the form of standard deviations shown in Table 1. The computer simulation considers this parameter as it calculates the behavior of the *L. monocytogenes* cells in each iteration of the simulation. Again, for the sake of simplicity, the standard deviations for each parameter have been fixed at 1 log_{10} CFU or 1 log percent. The mathematical reasons for expressing these values on a log scale are beyond the scope of this article, but these are reasonable assumptions given what is known about the distribution of microbial populations (Jarvis 1989) and transfer rates (Chen and others 2001). Because an average transfer rate of 30% that varies by \( \pm \) on the log percent scale may occasionally result in transfer rates more than 100%, the distribution was truncated, such that predictions above 100% were not allowed. Persistence values were not truncated to account for the possibility that some of the time, a strain might be able to grow in the food-processing plant environment.

The simulation is constructed using the at-risk add-in (Palisade Corp., Newfield, N.Y., U.S.A.) for Microsoft Excel. The simulation tracks the number of cells present, and that number is expressed on a log scale. In this simulation, cells are always present, albeit sometimes at very low numbers (that is, \( 10^{-5} \) CFU/surface or \( 10^{-5} \) log CFU/surface). Because real-world methods for *L. monocytogenes* are not able to detect cells present at such low numbers, the simulation also includes an assumed “detection limit.” The detection limit is fixed at 1 CFU/product or per surface sampled.

**Model I results**

When the simulation results for the number of each *L. monocytogenes* strain are summed, the results are as shown in the top panel of Figure 2. Almost 40% of the raw material is positive for I of the 4 *L. monocytogenes* strains. A little more than 30% of the simulated environmental samples are positive for *L. monocytogenes*, whereas less than 5% of the product contact surfaces are similarly positive. Finally, only about 2% of the finished products are positive for...
L. monocytogenes. The prevalence falls throughout the simulation because at each step, only some of the L. monocytogenes present are transferred and because many of the L. monocytogenes do not persist (Table 1).

What is not shown in Figure 2 is the variability in the L. monocytogenes concentrations with each iteration in the simulation. Although about 40% of the raw material is positive for L. monocytogenes, in 7% of the iterations the concentration exceeds 10 CFU, and in less than 1% of the iterations, the concentration exceeds 100 CFU. Similarly, about 8% of the environmental samples exceed 10 CFU, and almost 2% of the environmental samples exceed 100 CFU. The product contact surface exceeds 10 CFU less than 1% of the time and exceeds 100 CFU only 0.15% of the time. Finally, the finished product exceeds 10 CFU less than 1% of the time and exceeds 100 CFU only 0.27% of the time.

Because the simulation is capable of tracking the behavior of each of the 4 individual strains, it is also possible to show the data from the top panel of Figure 2, with the data from the individual strains highlighted separately. This more detailed data is shown in the bottom panel of Figure 2. When the data are shown in this way, a much different picture emerges because of the differences in the starting numbers and ability of the simulated strains to persist in the environment. Although almost 40% of the raw product is still contaminated, it is clear from the bottom panel of Figure 2 that most of that contamination is due to L. monocytogenes strain IV. Less than 10% of raw product is contaminated by L. monocytogenes strain III, whereas a very small fraction is contaminated by strain II.

Although it is not obvious from the bottom panel of Figure 2, about 0.1% of the raw product is also contaminated by L. monocytogenes strain I. The next bar shows the breakdown of contamination levels in the environmental samples, and the trends are similar to those seen in the raw product samples, except that the overall contamination percent is lower, while the fraction contaminated by L. monocytogenes strain IV is less and the amount contaminated by strains II and III have increased. The fraction of samples contaminated by L. monocytogenes strain I is still not visible on the bar graph, but it is about 0.25% of all the simulated samples. In the simulated product contact surface samples, the 4 strains now constitute approximately equal fractions of the overall number of contaminated samples, while in the finished product samples, L. monocytogenes strain I predominates.

The results shown in the top and bottom panels of Figure 2 illustrate the ability of the conceptual framework outlined in Figure 1 to model the behavior and movement of 4 L. monocytogenes strains in a food-processing plant environment. Unfortunately, detailed data on the actual number of L. monocytogenes on products or various surfaces are seldom available.

Model II—Tracking Prevalence

Listeria monocytogenes data obtained from food-processing plants very often will include only prevalence, that is, the fraction of samples that are positive for Listeria, L. monocytogenes, or a particular strain. The 2nd simulation model was designed for these situations. Because the simulation of this situation is inherently more complex, this simulation will track the movement of only 1 strain of L. monocytogenes (but there are no practical reasons why more could not be added in the future).

β distribution

The beta distribution is commonly used to represent the uncertainty about the prevalence of a positive sample in a population, based on the total number of samples collected and the number of positive samples observed in that total. For example, if 9 positive samples are observed in 10 total samples, the number of positive samples in the population can be expressed as Beta (positive + 1, negative + 1) or Beta (9 + 1, 1 + 1), and is shown graphically in the top panel of Figure 3. The distribution shows a peak at 0.9, which is the most likely value for the fraction of positive samples, but the peak is also quite broad, which means that it is certainly possible that the fraction of positive samples is lower (for example, 0.7% or 70%) and that only 10 samples failed to include the expected number of negative samples. The bottom panel shows the output for Beta (91,11), which represents the uncertainty around the estimate if 100 samples are collected and 90 are positive and 10 are negative. In this case, the peak stays in the same location, but the distribution narrows, reflecting that fact that certainty regarding the estimate has increased.

Other modeling considerations

Modeling cross-contamination from prevalence data is also complicated by the need to infer contamination rates that are not measured from those that are. Assume that collected data indicate that 50% of the raw material is contaminated and 25% of the environmental samples are contaminated. If the 25% figure is used directly, this will lead us to an incorrect conclusion. If the probability of 1 event is 50% and the probability of another event is 25%, then the chance of those 2 events occurring together is 0.50 × 0.25 = 0.125 or 12.5%. In this case, the 12.5% is the fraction of environmental samples that are contaminated. But the collected data shows that the real fraction of contaminated environmental samples is 25%. The
error comes from using the 25% figure directly in the calculations, when it should be a result of those calculations. To solve this problem, the fraction of contaminated raw materials that transfer its contamination to the environment, such that the final level of environmental samples that become contaminated is 25%, needs to be calculated:

\[
\text{Fraction of raw materials contaminated} \times \text{Fraction spreading contamination} = \text{Fraction of contaminated environmental samples}
\]

Therefore, the correct value to use in the simulation for the “fraction spreading contamination” is 0.50% or 50%.

Because representing the uncertainty regarding the “fraction spreading contamination” with a beta distribution is desirable and this fraction was not directly observed, its value must be calculated, and then the number of observations from what was observed (that is, the environmental samples) in the beta distribution must be used.

Table 2 shows the simulation Model II parameters for *Listeria monocytogenes* prevalence and persistence in a food-processing plant. Although these are fictitious data, they were developed to approximately mimic those of Hoffman and others (2003). The simulation works by calculating a series of probabilities (that is, the raw material is positive, the environment becomes positive, the contamination persists, and so on). Only when each of these probabilities is true does the finished product become contaminated with *L. monocytogenes*. This series of calculations can be expressed by this equation:

\[
\text{Probability raw material positive} \times \text{Probability environment transfer} \times \text{Probability of environmental persistence} \times \text{Probability food contact surface transfer} \times \text{Probability food contact surface persistence} \times \text{Probability finish product transfer} = \text{Probability finish product positive}
\]

In is important to note that the values shown in Table 2 are the expected values, and in each of the 10000 iterations of the simulation, they will take on different values. For example, although 39% of the raw material samples are positive (on average), in 5% of the cases, the actual number of positive samples was less than 30%; in another 5% of the cases, the actual number of positive samples was almost 50%. Similarly, in the case of food-contact surface samples, in 5% of the cases the actual number of positive samples was about 3%, whereas in another 5% of the cases, the actual number of positive samples was more than 20%. Similar variability is seen with the other parameters in Table 2.

Model II results

The results of this simulation model are presented in Table 3. When the default settings (Table 2) are used, the simulation predicts 281 of 10000 finished products to be positive for *L. monocytogenes* (or almost 3%). Four different alternative scenarios that can be investigated by the simulation are depicted in Table 3. These scenarios are intended to illustrate the implications of loss of control in several areas of plant sanitation. These scenarios are (1) all incoming products are positive for *L. monocytogenes*, (2) all environmental samples are positive, (3) all food-contact surfaces are positive, and (4) all incoming products, all environmental samples, and all food-contact surfaces are positive. Clearly the last scenario is the worst, and in this virtual processing plant, more than 80% of all products are positive.
products produced will be contaminated with \textit{L. monocytogenes}. The only reason why all finished products are not positive in this scenario is that in some iterations of the simulation, the \textit{L. monocytogenes} does not persist in the environment or on the food-contact surface, or get transferred to the finished product.

The next most severe scenario is the one in which all food-contact surfaces are positive. In this scenario, 2690 (almost 27\%) of 10000 surface, or get transferred to the finished products. In this scenario, 2690 (almost 27\%) of 10000 finished products are positive for \textit{L. monocytogenes}. It is not surprising that this scenario produces these dramatic results because, as shown in Table 2, the original fraction of food-contact surface positive samples was quite small (2/31 or 6\%), so increasing this variable to 100\% would have a dramatic effect on the simulation output. Raising the fraction of positive incoming product or environmental samples has some effect, but it is not nearly so dramatic because these values are higher (>30\%) to begin with, and in these 2 scenarios, the low rate of food contact prevalence still serves as the bottleneck for \textit{L. monocytogenes} contamination to the finished product.

Other uses for the simulation

It is possible to use this simulation to investigate other scenarios as well. For example, if a plant is faced with choices about allocation of resources to improve raw material quality, environmental sanitation, or finished product contact-surface sanitation, the simulation could be used to evaluate the cost and predicted benefit for different intervention strategies.

Conclusions

Monte Carlo simulation techniques can be used to model the persistence and spread of \textit{L. monocytogenes} in a virtual food-processing plant. This article has demonstrated this approach using 2 different models, 1 that tracks \textit{L. monocytogenes} number and prevalence for 4 different strains (Model I), and 1 that tracks only prevalence for a single strain (Model II). The Model I results show that it is possible to set simulation parameters such that different virtual strains of \textit{L. monocytogenes} predominate in different areas of the virtual processing plant. Simulation parameters can be set such that overall prevalence levels match those reported in the literature. Because \textit{L. monocytogenes} CFU data are not always available, another model that tracks only prevalence for a single strain (Model II) has also been developed. Model II used the beta distribution to represent the uncertainty about the prevalence of a positive sample in a population, based on the total number of samples collected and the number of positive samples observed in that total. This model can also be calibrated to give results that match those reported in the literature, although this process can be quite complex because of the need to infer contamination rates that are not measured from those that are. These models have been developed to provide a starting framework for scientists and predictive modelers studying \textit{L. monocytogenes} to begin research with the ultimate goal of understanding and controlling \textit{L. monocytogenes} in food-processing plants. The Microsoft Excel file used for these at-risk simulations is available at: \url{http://foodsci.rutgers.edu/schaffner/files.html}.

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References


