

Use of Risk Assessment to Reduce Listeriosis Incidence

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Quantitative risk assessment can help lower risk of foodborne illness resulting from *Listeria monocytogenes* infection

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INGESTION OF FOODBORNE *LISTERIA MONOCYTOGENES* BY AT-RISK humans (Table 1) may result in listeriosis. The bacterial pathogen resides frequently in the gastrointestinal tracts of many animals and enters the food chain either indirectly by the shedding of feces into the environment or directly by food contamination.

Environmental sources of *L. monocytogenes* include rotting vegetation, soil, sewage, water, and effluents. These sources, in turn, can contaminate animal feeds, which can lead to reinfection of domestic animals. *L. monocytogenes* can enter food processing plants on raw materials, including meat, poultry, and produce. The ability to grow below 5°C and at a comparatively low water activity explains the pathogen's frequent presence in food manufacturing plants, particularly in biofilms on food preparation surfaces, floors, and drains.

Quantitative risk assessment can provide estimates of how remedial actions can lower listeriosis risks. The approach can be useful to establish raw material specifications, food handling and processing practices, and plant sanitation regimes.

Microbial Modeling

The development of microbial pathogen modeling has been documented in reviews by McMeekin et al. (1993), Skinner et al. (1994), Whiting and Buchanan (1994), and Whiting (1995) and in a workshop proceedings (Buchanan et al., 1993). Growth models for *L. monocytogenes* were first published by Buchanan and Phillips (1990), with temperature, pH, NaCl, and sodium nitrite as the environmental factors under an aerobic or anaerobic atmosphere. The growth curves were fitted to the sigmoidal Gompertz equation. The values of the parameters of that equation were described by polynomial regression equations.

Buchanan and Whiting incorporated models for *L. monocytogenes* and other pathogens into an easily used personal computer software package. This Pathogen Modeling Program is available from the U.S. Dept of Agriculture's Eastern Regional Research Center, 600 E. Mermaid Ln., Wyndmoor, PA 19038 (phone 215-233-6620). A similar predictive program called Food MicroModel was developed by the United Kingdom Ministry of Agriculture, Fisheries and Food and the Leatherhead Research Laboratory. It is available from Leatherhead Food Research Association, Randalls Rd., Leatherhead, Surrey, UK KT22 7RY (phone 44-1372-376761).

Survival models for *Listeria* in aerobic and anaerobic environments were subse-

quently developed for the Pathogen Modeling Program (Buchanan et al., 1994), and the Food MicroModel contains a thermal death model that includes the factors temperature, pH, and NaCl. Research continues to improve the scope, precision, and applicability of these models. Specific areas being refined are the calculation of the lag phase, predictions after a series of sequential conditions, and determination of the error ranges about a prediction.

The ultimate question being asked of pathogen modeling is whether consumption of a particular food will cause illness. This requires quantitative knowledge in four areas: the numbers of pathogens present in the raw ingredients or at the start of the modeled process, changes in microbial numbers in the food during the process, amount of food (number of pathogens) ingested, and infectious doses. This general approach to hazard assessment of *L. monocytogenes* was demonstrated in bovine milk by Peeler and Bunning (1994). They determined that the probability was less than 2 in 100 that one cell occurs in nearly 40 billion gallons of milk pasteurized at 74.4°C for 20 sec. Cassin et al. (1996) questioned some of Peeler and Bunning's calculated probabilities but concluded that "probabilistic analysis of hazards is an important improvement to the true understanding of the risks."

Quantitative information about *L. monocytogenes* on animal carcasses and meat and poultry products is being collected by the Food Safety and Inspection Service (FSIS, 1994). Using these data, the growth potential of the organism can be predicted by various growth, thermal death, and survival models. Infectious dose models (Haas, 1983; Regli et al., 1991) have been developed for several microbial pathogens, primarily those which are waterborne. Infection is the establishment of the pathogen in the intestinal tract of the host, which may be followed by illness and death. Studies have not yet been performed with *L. monocytogenes* on strain virulence and host susceptibility to create an infectious dose model. Yet, Hof

Table 1—U.S. populations with probable sensitivity to foodborne pathogens

Population category (year)	No. of individuals	% of U.S. population	Reference
Pregnant women (1988)	6,341,000	2.5	U.S. Census Bureau (1994), Table 108
Children <5 years (1992)	19,512,000	7.7	U.S. Census Bureau (1994), Table 13
Elderly 65 years (1992)	32,284,000	12.7	U.S. Census Bureau (1994), Table 13
Residents in nursing and related care facilities (1991)	1,729,000	0.7	U.S. Census Bureau (1994), Table 192
Cancer cases under care (1992)	4,081,312	1.6	ACS (1992), Table p. 15
Organ transplant patients (1992)	415,458	0.02	U.S. Census Bureau (1994), Table 190
Total HIV (AIDS) infections (January 1993)	630,000–897,000 ^a	0.03–0.04	Rosenberg (1995)
U.S. population (1992)	255,082,000	25.5	U.S. Census Bureau (1994), Table 13

^aThere were 45,472 new HIV infections in 1992 (Census, Table #204). The AIDS surveillance case definition was changed in 1993, and new AIDS cases increased 127% to 103,691 (CDC, 1993)

et al. (1994) indicated that while *L. monocytogenes* infections in humans occur frequently, development of listeriosis is rare. Therefore, a working morbidity threshold of approximately 100 viable cells/g has been suggested for this pathogen and will be used here (ICMFS, 1994; IFST, 1995).

Buchanan et al. (1997) estimated the probability of listeriosis from German epidemiologic and consumption data. The exponential dose-response model estimates that a single consumption of 10^4 *L. monocytogenes* (100-g serving with 100 cfu/g) has a risk probability on the order of 2×10^{-5} .

To demonstrate how a risk assessment model could work and to illustrate the potential effect of environmental contamination by *L. monocytogenes*, a prototype was created for a cooked-refrigerated meat product, such as meatballs (Fig. 1). The initial contamination by *L. monocytogenes* was adapted from the FSIS (1994) data by converting from bacteria/cm² to bacteria/g of meat by assuming that all surface bacteria were mixed into the entire cut-up carcass. To avoid underestimating the risk, in the 65% of the instances when less than 10^{-3} cfu/g were found, the value of 10^{-3} was used. In 24% of the samples, 10^{-2} cfu/g were present; 5% 10^{-1} cfu/g; and 6% 10^0 cfu/g. This distribution had an overall average of 6.8×10^{-2} cfu/g (the model uses the log values when making the calculations, and the average was log -2.42).

The temperature (7°C), pH (5.8), and salt levels (0.5%) are assumed, with 96 hr of storage. The growth equations of the Pathogen Modeling Program for *L. monocytogenes* estimate a lag duration of 28 hr and a growth rate of 0.052 log cfu/g/hr at these conditions, to yield an average log cfu/g of 1.22 at the end of this storage period.

Assume that the meat is then formed into meatballs with added acidulant (pH 5.2) and salt (3%) and cooked for 45 sec to a temperature end-point of 64°C. The thermal death time model was calculated from *L. monocytogenes* survival data in milk (Bradshaw et al., 1985) because no data on thermal survival in meat was found. The D-value at this temperature is 20.3 sec. Therefore, 45 sec gives a 2.2 log reduction to yield -1.0 log cfu/g.

Assume that after cooking, the meatballs are held for 8 hr at 21°C, given a nonlethal warming, and consumed. At this time, the growth model estimates the counts to be 1.0 log cfu/g, and consumption of 100 g of the meatballs would mean that 995 cfu of *L. monocytogenes* were ingested. This is below the target of consuming less than 100 cells/g (10^4 total) and therefore appears to be a "safe" process.

However, this analysis did not consider the various distributions and variations in these steps. To determine their effects, Monte Carlo analysis (@Risk, Palaside Corp., Newfield, N.Y.) was used. The model described above is repeatedly calculated (simulated), and, at each step where a variation or distribution is encountered, a value is selected or the mean is adjusted according to the variation specified for that step. For a series of processing steps, the final output will be a frequency chart showing the number of simulations that result in various outcomes—in this example, more than 10^4 *Listeria* (100 g containing 100 cfu/g) consumed.

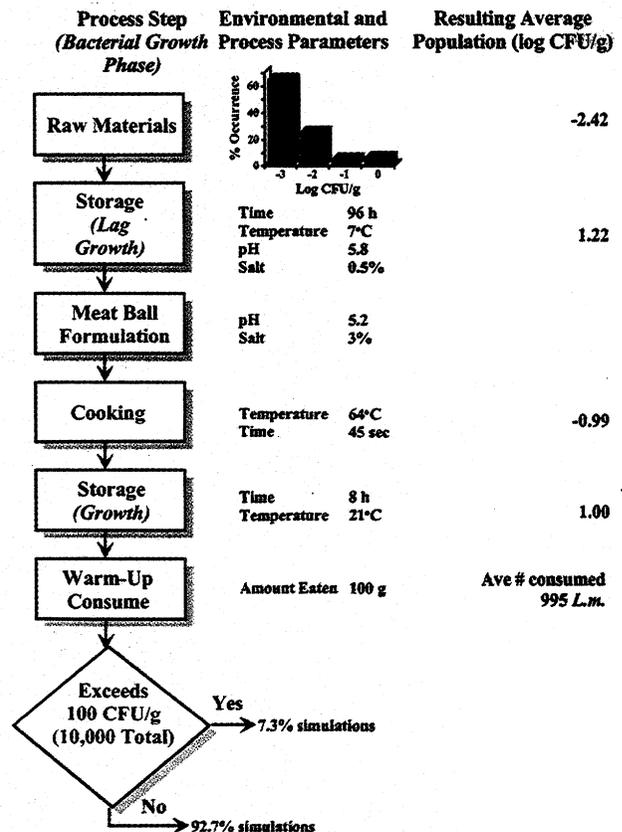
After adding the variation to the model, different simulations start with different initial populations of *Listeria*. Since standard deviations for the original growth models have not been calculated, however, an estimate of 0.15 was made for the first step of lag and growth. The second growth period was assumed to not have a lag, and the standard deviation was estimated to be smaller at 0.1. The D-values for the thermal death model had average coefficients of variation of 20%, so a standard deviation of 0.2 was used. When this process was simulated 1,000 times, with a value from each of these distributions chosen randomly for each simulation, there was a probability for some simulations with higher initial numbers, greater growth, and less thermal death to end with more *L. monocytogenes* than the simulations containing the average values. In effect, this is an estimate of the likelihood that the "worst case" will

occur. In this initial example, the numbers of *Listeria* consumed exceeded the 10^4 acceptance standard in 7.3% of the simulations. The safety of this process, which appeared adequate based on the average values and consumption of 995 *L. monocytogenes*, was less clear when the frequency distributions were considered.

The impact of a hypothetical intervention to reduce environmental *L. monocytogenes* levels was estimated by changing (lowering) the distribution of the initial counts. If the 6% of the time that the raw meat has 10^0 cell/g (see frequency distribution, Fig. 1) could be eliminated by improved sanitation, the impact on safety would be significant, based on a recalculation of the model. With 71% of the raw product now containing 10^{-3} cfu/g, 24% 10^{-2} , 5% 10^{-1} , and 0% 10^0 , and after running 1,000 simulations, the average log number of *Listeria* consumed has now decreased to only 729 cfu, not greatly different from earlier. However, only 0.94% of the simulations now yielded *Listeria* levels exceeding 100 cells/g.

Whether this reduction in initial contamination modeled in the second scenario (Fig. 2) would be considered to produce a tolerable risk or not requires the input of policy makers who are responsible for determining a risk acceptance threshold. If this level of contamination were still deemed an unacceptable frequency of high *Listeria* consumption, the model could be used to show the overall im-

Fig. 1—Prototype risk assessment model for *L. monocytogenes* in a refrigerated, cooked meatball. Distribution of organism is shown in the panel inset and ranges from -3 to 0 log cfu/g. The process flow diagram runs vertically on the left side of the figure, with corresponding parameter and modeling values in the center. The right side of the figure shows the average number of *Listeria* for the corresponding process steps. The critical threshold is assumed to be 100 *L. monocytogenes*/g of product. The lower left number is the percent of simulations where 100/g is exceeded



impact on *Listeria* reduction by increasing the cooking time or temperature, for example. The risk assessment model can indicate the contribution of various components of the process to the overall risk.

These examples illustrate that the most important occurrences for each process step are those at the extremes, i.e., the relatively few samples with high initial pathogen counts, the first cells to grow, the most heat-resistant portion of a bacterial population, or the few times the thermal process fails to reach the specified temperature. Without some knowledge of the ranges and distribution shape naturally present in the pathogen's parameter values and the processing steps, it is impossible to determine the safety of a product. Based on this recognition, risk assessment teaches that even if only a few packages out of a lot or one lot out of a week's production exceeds the acceptable risk for a pathogen, the process would have to be considered unsafe.

The magnitude of the variation present in each of the processing and modeling steps is relatively unknown. Part of this variation comes from the statistics of the experimental and modeling processes. However, an important source of variation is microbial strain differences. Barbosa et al. (1994) found that the lag phase duration of 39 strains of *Listeria* at 10°C ranged from 36.5 to 68.9 hr. Relatively large variations between strains were also found for the exponential growth rate, 47.4 ± 10.7 absorption units/hr. Kinetic differences arising from physiological state of individual cells, includ-

ing starved and biofilm cells, and inconsistencies in process operations also contribute to the overall increase in standard deviations and the likelihood of high listerial numbers.

Will Help Develop HACCP Programs

A risk assessment must underlie a hazard analysis critical control point (HACCP) program to provide the goals and targets for the control steps. Without an assessment of the frequency and extent of contamination, opportunities for growth, and the infectious dose, it is impossible to determine the severity of thermal inactivation or set values for other critical control points (CCPs) that are necessary to ensure safety. This becomes particularly relevant for heat-sensitive foods, such as liquid eggs, where additional heating may be linked with a loss in functionality. Criteria for each CCP cannot exist in isolation from the entire food process from raw materials to the consumer. Currently, CCPs are determined with information on each step, qualitative information on the entire process, and commercial experience with the food.

A risk assessment should not be viewed as providing a safe-not safe answer. The value of the risk assessment is in assembling and organizing the relevant information, and then testing the model by changing the input parameters. This testing process leads to a better understanding of how the processing steps and parameters interact to affect the food's safety. For example, a specified cooking temperature may be shown to be adequate with normal numbers of pathogen in the raw ingredients but insufficient with occasional high levels of contamination. The model will illustrate the importance of temperature control. Because of the log D vs temperature relationship, a thermal process temperature under that specified will markedly reduce the amount of microbial inactivation. The risk assessment can estimate the outcome of various abuse scenarios to evaluate the robustness of the process.

Implicit in the HACCP approach is that tighter control will be given to particular process parameters through better and more frequent monitoring. The risk assessment will show that safety problems usually arise from the outliers of a distribution, i.e., the times when a process is not in control. If process variation can be reduced, the CCPs may then be set closer to the edge. For example, if a thermal process must exceed 60°C 99.9% of the time to achieve an acceptable level of safety and the standard deviation for the temperature control is 0.5°C, then the CCP must be set at 61.5°C. If the standard deviation can be reduced to 0.2°C, then the CCP can be set at 60.6°C to achieve the same risk.

This information must then be forwarded to the risk manager, who considers the other factors such as technical achievability, quality, and cost in designing the process and specifying the HACCP plan. The risk assessment may show that inadequate information exists for a particular step and data collection is necessary to create a better HACCP plan.

L. monocytogenes remains a significant concern to the food industry because it has many opportunities to enter the food chain, establish itself in food manufacturing establishments, contaminate foods and processing wastes, then recontaminate the environment through waste streams. Yet, the industry has made remarkably swift progress toward the eradication of the problem. For example, Tappero et al. (1995) reported 44% and 48% reductions in illness and death, respectively, due to listeriosis in the U.S. between 1989 and 1993. This was accomplished by industry, regulatory, and educational efforts. Continued measures to prevent primary contamination and recontamination of environmental sources by *L. monocytogenes* will lower its occurrence on raw foods. In turn, use of risk assessment tools will point the way to the development of HACCP programs to lower *L. monocytogenes* levels and incidence on ready-to-eat foods. Taken together, these efforts will reduce further human foodborne listeriosis morbidity and mortality.

Fig. 2—Prototype model of Fig. 1 after reduction of initial bacterial load. Distribution of organism is shown in the center panel inset and differs from that in Fig. 1 by reduction of the percent occurrence of the highest levels of *L. monocytogenes* ranges (-1 to 0 log cfu/g). The percent of simulations where 100/g is exceeded is lower than that in Fig. 1

