Appendix 9:

Using Outbreak Investigations in Quantitative Risk Assessment

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The dose-response model developed for the revised FDA/FSIS risk assessment can be used to make predictions about the public health impact of *L. monocytogenes* isolated from a food source. For example, in the best case, a clinical isolate may be traced to the source food. If the source of the food is known or suspected, the number of servings of the source food that are likely to be consumed can be estimated. If the isolate strain can or has been tested in a manner consistent with the LD_{50} mouse experiment, the virulence potential can be established. With these inputs, the dose-response model can predict, with acceptable uncertainty, the median number of deaths for different populations. If not all of this information is available, the dose-response model still can predict the probability of death for different populations and different consumption scenarios, and provides an estimate of the uncertainty for that prediction.

There have been many foodborne listeriosis outbreaks and reports of sporadic individual cases in the past 15 years, with a variety of foods implicated as vehicles of infection. Those outbreak and individual case reports that identify the food vehicle for infection have been very helpful to the hazard identification phase of the *L. monocytogenes* risk assessment. However, identification of the food source of *L. monocytogenes* contamination is infrequent and estimation of cfu/g at consumption even less frequent.

To be informative for quantitative risk assessment, outbreak reports must include quantitative exposure data linked to individuals. Many reports have provided information about the food source, serotype, and contamination in food, but the amount of food consumed by each person and the number of people exposed is usually not reported. For example, an outbreak of listeriosis occurred in Massachusetts in 1983 involving 49 cases (Fleming *et al*., 1985). Illness was strongly associated with the consumption of pasteurized milk. The investigation revealed attack rate, food source, and serotype data, but information was not provided about the level of contamination and amount of milk consumed. In addition, outbreak reports with information about the dose and attack rate are rare. The total number of people exposed (number of servings consumed) and immune status of those exposed is also rarely known.

Tables A9-1 and A9-2 list examples of outbreak and sporadic case reports where data on contamination level in the implicated food were reported. These tables also provide the location and year of occurrence, the implicated food source(s), *L. monocytogenes* serotype, level of contamination of implicated food(s), amount of food consumed, number of persons affected, and the attack rate. Of the outbreaks listed in Table A9-1, only the report of the outbreak in 1994 associated with chocolate milk contained the critical details necessary to estimate the dose-response relationship. However, the primary endpoint of this outbreak (gastrointestinal illness) makes it of minimal usefulness in characterizing outbreaks of severe listeriosis. None of the other reports contained information on the amount of either the food or *L. monocytogenes* consumed per serving by individuals or the attack rate. However in two cases, the Mexican-style soft cheese and the Finnish butter outbreaks, an attack rate and dose range could be estimated. The Mexican-style soft cheese would be similar to the Fresh Soft Cheese food category used in this risk assessment. Butter is one of the foods included in the High Fat and Other Dairy Products category.

Table A9-1. Location, Year of Occurrence, and Epidemiologic Data for Outbreaks of Listeriosis

Location, Year (Reference)	Food Source	Serotype	Contamination Level (cfu/g)	Amount Consumed	No. Ill	Attack Rate
LA County, 1985 (Linnan *et al.,* 1988)	Mexican-style soft cheese	82% 4b	$1.4x10^4$ to $5x10^4$	NA [a]	142	NA
Switzerland, 1983-87 (Bula *et al.,* 1995)	Soft smear-ripened cheese	75% 4b	$1x10^4$ to $1x10^6$	NA	122	NA
IL, MO, WI, 1994 (Dalton *et al.,* 1997)	Chocolate milk	1/2b	$1x10^9$ (cfu/mL)	240 mL	45	45/60 (median)
Italy, 1993 (Salamina *et al.,* 1996)	Cream cheese fruit tart Rice Salad [b]	1/2b 1/2b NA	460 0.93 NA	NA NA NA	18	18/39
Finland, 1998-99 (Lyytikäinen *et al.,* 2000)	Butter	3a	<100 [c]	NA	25	NA
Multistate, 1998-99 (CDC, 1998b)	Frankfurters	4b	<0.3	NA	101	NA

[a] NA = Not available
[b] Rice salad implicated by epidemiology; $p<0.001$
[c] One sample contained 11,400 cfu/g

Table A9-2. Location, Year of Occurrence, and Epidemiologic Data for Sporadic Cases of Listeriosis not Reported in Outbreaks

Location (Reference)	Year	Confirmed Food Source	Serotype	Contamination Level (cfu/g)	Amount Consumed	Number Ill	Attack Rate
England (Azadian *et al.,* 1989)	1988	Goat Cheese	4b	4×10^7	85 g	1	NA [a]
Oklahoma (Barnes *et al.,* 1989)	1988	Frankfurter Sausage	NA	>1100[b]	1 per day	1	NA
Italy (Cantoni, 1989)	1989	Homemade Sausage	NA	2.7×10^6	NA	1	NA
New Jersey (Ryser, 1999b)	NA	Ricotta Cheese	NA	100 to 10^6	NA	1	NA
Finland (Juntilla and Brander, 1989)	1989	Salted Mushrooms	4b	1×10^6	NA	1	NA
Belgium (Andre *et al.,* 1990)	1989-90	Commercial Ice Cream	4b	1×10^4	NA	1	NA
Tasmania (Brett *et al.,* 1998)	1991	Smoked Mussels	NA	1.6×10^7	90 g	2	NA
Canada (Farber, 1997)	1997	Imitation Crabmeat	NA	2.1×10^9	NA	2	NA

[a] NA= Not Available
[b] >1100 cfu/g, in opened package, patient refrigerators; <0.3 cfu/g in closed package, retail.

The 1985 Los Angeles Mexican-Style Soft Cheese Outbreak

Between January 1 and August 15, 1985, 142 cases of listeriosis were reported in Los Angeles County. There were 48 deaths (including 19 fetal and 10 neonatal). The overall case fatality rate was 33%. Of the 142 cases, 93 (65%) were perinatal. The mean age of mothers was 26 years and the mean gestational age of fetus or neonate was 32 weeks. Eighty-six percent of the cases were Hispanic individuals. Of the remaining non-perinatal cases, the mean age was 58 years and only 29% were Hispanic individuals. Mexican-style soft cheese was epidemiologically and bacteriologically associated with the occurrence of disease.

At the time the Los Angeles County Department of Public Health reported on the outbreak, their report did not contain information on either the amount of cheese consumed per serving by individuals or the attack rate. Fortunately, consumption data by individuals were collected and records from the outbreak were saved. Therefore, in 1998, an attack rate was estimated (Buchholz,

2000) based upon information in the outbreak records and Los Angeles County demographics. Table A9-3 shows the calculation of the attack rate for the listeriosis outbreak among pregnant Hispanic females in Los Angeles County in 1985 associated with consumption of Mexican-style soft cheese.

There were a total of 81 listeriosis cases among pregnant Hispanic females. Only cases infected with the outbreak phage type were used in the analysis (63 of 142). Two matched case-control studies (n=39) were conducted during the outbreak (Linnan *et al*., 1988). The total number of controls (31) and the number of controls that were exposed to the implicated food (11) were determined by re-construction of the odds ratio table. This allowed for an estimate of the proportion of the population that consumed the implicated food (11/31=35%).

To estimate the dose-response for pregnant females, it was assumed that the same percentage of pregnant Hispanic females as in the case-control studies ate the implicated cheese. The total number of pregnant Hispanic females within the marketing area during the time interval of interest (33,642) was multiplied by the calculated proportion of the population that consumed the implicated food (35%), providing an estimate of the number of pregnant Hispanic women who ate the implicated food (11,775).

Two studies were used to determine the Mexican-style soft cheese contamination rate. Laboratory data from one study were examined to determine the total number of food samples qualitatively tested (85) and the number of samples that were positive (22) outbreak (Linnan *et al*., 1988). The number of positive tests divided by the number of foods sampled yielded an estimate of the proportion of food contaminated (22/85 = 26%). That proportion multiplied by the estimate of the number of pregnant women who ate the implicated food (11,775), provides one estimate of the total number of pregnant women who were exposed to *L. monocytogenes* (3,061).

A second estimate of the proportion of food contaminated was determined based on the second contamination study (Ryser, 1999a). Samples (665) were tested, with 56 positive results, to give an estimated contamination frequency of 8.4%. It should be noted that these outbreak related cheese samples were recovered from a landfill after disposal, which had an unknown impact on the results. The low estimate of pregnant Hispanic women who ate contaminated cheese (989) was derived by multiplying this contamination rate (8.4%) by the total number of Hispanic females who ate the implicated cheese (11,775).

For the high estimate, the estimated attack rate (2.1%) is equal to the number of cases that developed listeriosis from the outbreak phage type (63) divided by the high estimate of the total number of pregnant Hispanic females who were exposed (3,061). The proportion of actual cases that were identified during the outbreak was then estimated based on 100% of cases identified (best case scenario) and 75% of cases identified (based on estimates from health care workers in Los Angeles at the time of the outbreak).

For the low estimate, the estimated attack rate (6.4%) is equal to the number of cases that were infected with the outbreak phage type (63) divided by the low estimate of the total number of exposed persons in the population (989). The proportion of actual cases that were identified during the outbreak was again estimated based on 75% and 100% of cases identified.

Using this strategy, the estimated attack rate during the Mexican-style soft cheese outbreak ranges between a low of 2.1-2.7% and a high of 6.4 to 8.5%.

From the outbreak records, it was possible to estimate the one-day consumption of the implicated cheese from 39 of 63 pregnant Hispanic females infected. The consumption ranged from 0.5 ounces/day to 21 ounces/day (median about 5.5 ounces/day). In addition to reporting consumption for one day, about 38% of the females reported their usual consumption of cheese for more than one day. The effect of cumulative doses on the attack rate and pathogenesis is not well understood and was not estimated.

Listeria monocytogenes contamination levels in this outbreak were reported twice, at 1,000 to 10,000 cfu/g (NACMCF, 1991) and 14,000 to 50,000 cfu/g (Ryser, 1999a). The dose of *L. monocytogenes* consumed in the contaminated cheese in one day was calculated to range between 15,000 cfu/day (0.5 oz/day X 30 g/oz X 1,000 cfu/g) and 31,500,000 cfu/day (21 oz/day X 30 g/oz X 50,000 cfu/g). It was therefore estimated that approximately 2.1%-8.5% of pregnant Hispanic females who consumed between 1.5×10^4 and 3.15×10^7 *L. monocytogenes* 4b organisms in a single day became ill.

Table A9-3. Calculation of Attack Rate for an Outbreak of *Listeria monocytogenes* 4b in Pregnant Hispanic Females Using Data from the 1985 Mexican-style Soft Cheese Outbreak

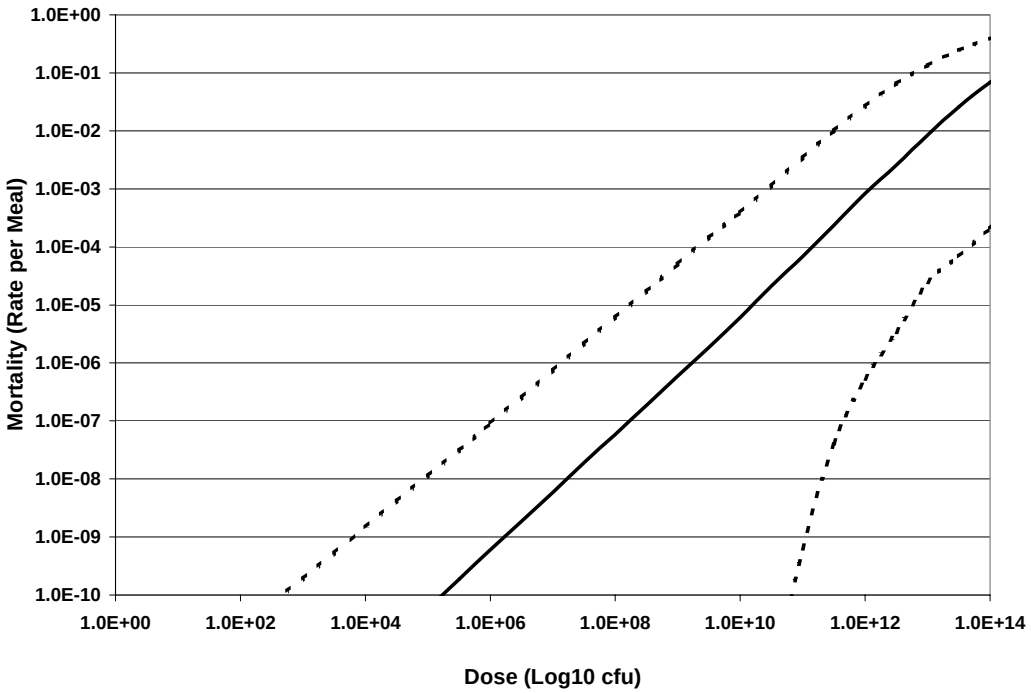
Hispanic births (January - June, 1985), LA County	33628
Hispanic fetal and neonatal deaths (January - June, 1985)	+ 350
Proportion of multigestational births (1%)	- 336
Population giving rise to cases (Total Hispanic pregnant females, January - June, 1985)	33642
Total Hispanic pregnant females who ate the implicated cheese (based on an estimate that 35% of controls ate implicated cheese)	11775
High estimate of Hispanic pregnant females who ate contaminated cheese (based on a estimate of 26% product contamination x 11775)	3061
Total Listeriosis cases among Hispanic pregnant females	81
Cases with outbreak phage type	63
Attack rate if all cases were identified (63 x 100 /3061)	2.1%
Attack rate if 75% of cases identified (63 x 100/3061)/0.75	2.7%
Low estimate of Hispanic pregnant females who ate contaminated cheese (based on an estimated rate of 8.4% product contamination x 11775)	989
Attack rate if all cases were identified (63 x 100 /989)	6.4%
Attack rate if 75% of cases identified (63 x 100/989)/0.75	8.5%

A dose-response model of this outbreak was developed using the same structure as the dose-response model for the national estimates (Figure A9-1). However, two of the components of the outbreak dose-response model were modified to reflect specific information from the outbreak.

First, a distribution ranging from 10^2 to 10^4 was used as an estimate of the *L. monocytogenes* concentration in the contaminated cheese. Because the cheese samples were obtained from consumer refrigerators, it is reasonable to assume that the measurements producing these estimates included growth during storage. Therefore, no additional growth was modeled.

Second, the strain virulence model was modified to include only mouse LD_{50} values for the single strain associated with the outbreak. Attribution of all the cases to a single strain implies that strain virulence is no longer a source of variability in the cause of illness. The frequency distribution in the national model is therefore replaced by a single uncertain value. Since mouse LD_{50} values are available, the uncertainty in the virulence is much less than it otherwise would be (e.g., as shown in

FigureA9-1). A normal distribution of the three LD_{50} values was used to represent the uncertainty; since the doses were measured in logs and the distribution is essentially Lognormal.



FigureA9-1. *Listeria monocytogenes* Dose-Response with Single Strain Virulence Derived from 1985 Los Angeles Mexican-style Soft Cheese Outbreak

Without a dose-response scaling factor, the Los Angeles model also overestimated the number of cases expected in the epidemic. However, a much smaller scaling factor (3.5 to 4.5 logs) was required to produce an estimate that roughly corresponded to the observed number of cases than was required for the national model for neonatal (6 to 10 logs). Since the main difference between the national and Los Angeles outbreak models are the estimates of *L. monocytogenes* concentrations and strain virulence, it appears that a major portion of the uncertainty described by the dose-response adjustment factor may be attributed to these two model components. More specifically, the national estimates for *L. monocytogenes* concentrations may be too high or the Los Angeles estimates are low, and/or the number of low-virulence *L. monocytogenes* strains may be underrepresented in the frequency distributions for strain virulence. The Los Angeles model, based on our limited data, primarily provides assurance that the population-based model can be useful as a predictive tool that is reasonably accurate (within a several log range) when *L. monocytogenes* concentrations and strain virulence information are known. The dose response model using the 1985 Mexican-style soft

cheese outbreak represents an application of the model to a specific set of circumstances. Therefore, those data were not used in this *L. monocytogenes* risk assessment to develop the dose-response relationship.

The 1999 Finland Pasteurized Butter Outbreak

The Finland pasteurized butter outbreak attack rate and dose was calculated in a manner similar to the method used to calculate these parameters for the Mexican-style soft cheese outbreak. Between December 1998 and February 1999, an increase in cases of listeriosis due to *L. monocytogenes* serotype 3a in Finland was recognized (Lyytikäinen *et al.*, 2000). Review of national laboratory surveillance data from June 1, 1998 to March 31, 1999 identified 25 *L. monocytogenes* 3a cases, which included six deaths. Cultures of blood, cerebrospinal fluid, and samples from other sterile sites were used to identify cases of listeriosis. Most of the cases were hematological or organ transplant patients. The median age of cases was 53 years (range 12-85). Ten males and no pregnant females or newborns were identified as listeriosis cases. The average annual number of hematological and organ transplant patients admitted to the hospital is 410. Therefore, the number of persons at risk for the 9-month period of concern was approximately 308.

Butter was implicated as the vehicle of infection. Isolates of *L. monocytogenes* 3a from the butter and from the 25 cases were indistinguishable by PFGE. At the tertiary care hospital where a majority of the cases (15/25) occurred, only one brand of butter was consumed during the outbreak period. The hospital is the only site in Finland for organ transplants and is where most bone marrow transplants are performed.

Thirteen butter samples obtained from the hospital kitchen and 139 butter samples obtained from the dairy and a wholesale store were analyzed for the presence of *L. monocytogenes.* The outbreak strain was detected in all thirteen hospital-kitchen samples. The outbreak strain was also detected in several lots from the dairy and wholesale store. The level of *L. monocytogenes* contamination was <100 cfu/g (range 5 to 60 cfu/g) for all positive butter samples, except for one sample that contained 11,000 cfu/g. A complete description of the environmental investigation is described in Lyytikäinen *et al*. (2000).

It was possible to estimate butter consumption for five case patients based on interviews about dietary practices (number of times per week and per day). Researchers assumed that patients ate one package of butter per meal (7 g). The estimated consumption was divided by 31 days (median hospital stay) to estimate daily butter consumption. To determine the median dose range, the minimum butter consumption (1.1 g/day) was multiplied by the minimum contamination level for the hospital kitchen samples (5 cfu/g) and the maximum butter consumption (55 g/day) was multiplied by the maximum contamination level for the hospital samples (60 cfu/g). Using the hospital samples, the consumed dose would be 5.5 to 3,300 cfu/day. By using the maximum contamination level found in the wholesale samples (11,000 cfu/g), then the daily dose consumed would range between 1.21 x 10^4 to 6.6 x 10^5 cfu/day.

Table A9-4 shows the calculation for the attack rate of the 1999 Finland butter outbreak. Approximately 6.4% to 10.7% of the hematological/transplant patients at the hospital who consumed between 5.5 cfu/g and 6.6 x 10^5 cfu/g developed listeriosis. We assumed that hospitalized patients ate the implicated butter on each of 31 days (median hospital stay) while hospitalized. The majority of the illnesses were associated with severe symptoms. The effect of cumulative doses on the attack rate and pathogenesis was not estimated.

Table A9-4. Calculation of Attack Rate for an Outbreak of *Listeria monocytogenes* Serotype 3a Infections from Butter in Finland for Hematological and Transplant Patients

Annual number of new diagnoses for acute leukemias/lymphomas plus annual number of kidney/liver transplants performed at the hospital.	410
Total persons at risk (time interval x annual new diagnoses, time interval: June 1998 to February 1999, 9/12 months)	308
Estimated number of hematological and transplant patients in the population that ate the butter (estimated proportion of controls that ate implicated butter, 76%)	234
Number of cases during the outbreak	25
Number of cases at tertiary care hospital	15
High estimate of product contamination (100%)	
Population at risk (100%)	234
Attack rate (15 x 100/234)[1]	6.4%
Mid-estimate of product contamination (80%)	
Population at risk 80%	187
Attack rate (15 x 100/187)	8.0%
Low estimate of product contamination (60%)	
Population at risk 60%	140
Attack rate (15 x 100/140)	10.7%

The Finland outbreak demonstrates the serious consequences of even low dose exposure when the severely immunocompromised are exposed. Because this outbreak currently lacks strain information and the relative susceptibility of transplant patients is unknown, it is not possible to calculate a new dose-response curve for this population. However, intensive outbreak investigations such as this one are applauded. They help to explain the sources of uncertainty in our dose-response model and they provide controlled environments in which accurate dose and attack rate data may be collected.