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Survey of *Listeria monocytogenes* in Ready-to-Eat Foods

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ABSTRACT

The purpose of this study was to develop data on the risk of listeriosis to support a science-based strategy for addressing *Listeria monocytogenes* in foods in the United States. Eight categories of ready-to-eat foods were collected over 14 to 23 months from retail markets at Maryland and northern California FoodNet sites. The product categories included luncheon meats, deli salads, fresh soft "Hispanic-style" cheeses, bagged salads, blue-veined and soft mold-ripened cheeses, smoked seafood, and seafood salads. The presence and levels of *L. monocytogenes* in the samples were determined by rapid DNA-based assays in combination with culture methods. Of 31,705 samples tested, 577 were positive. The overall prevalence was 1.82%, with prevalences ranging from 0.17 to 4.7% among the product categories. *L. monocytogenes* levels in the positive samples varied from <0.3 MPN (most probable number) per g to 1.5×10^5 CFU/g, with 402 samples having levels of <0.3 MPN/g, 21 samples having levels of $>10^2$ CFU/g, and the rest of the samples having intermediate levels. No obvious trends with respect to seasonality were observed. Significant differences ($P < 0.05$) between the sampling sites were found, with higher prevalences for threes categories in northern California and for two categories in Maryland. Significantly ($P < 0.001$) higher prevalences were found for in-store-packaged samples than for manufacturer-packaged samples of luncheon meats, deli salads, and seafood salads, while 16 of the 21 samples with higher counts were manufacturer packaged. The data collected in this study help to fill gaps in the knowledge about the occurrence of *L. monocytogenes* in foods, and this new information should be useful in the assessment of the risk posed by *L. monocytogenes* to consumers.

Listeria monocytogenes has been recognized as a human pathogen for >70 years. Only within the past 2 decades, however, has *L. monocytogenes* been associated with food and classified as a foodborne pathogen. The Centers for Disease Control and Prevention (CDC) have estimated that up to 2,500 cases of listeriosis, resulting in 500 deaths *(17),* occur each year in the United States. Several large outbreaks in the early 1980s prompted the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) to establish a policy under which ready-to-eat foods contaminated with *L. monocytogenes* at a detectable level are deemed adulterated. This "zero tolerance" policy was established on the basis of very few data concerning the prevalence and control of the organism in food and represented a conservative approach based on the limited information available at the time. According to the policy, which assumes that all *L. monocytogenes* strains are pathogenic, the presence of the organism at a detectable level (e.g., 0.04 CFU/g) in a ready-to-eat (RTE) food renders that product adulterated *(23).* Since the establishment of the policy, more information about the prevalence of the organism in food-manufacturing plants and in foods has become available. Also, there exists a better understanding about which types of foods are more likely to be involved in illnesses, which foods support the growth of the organism, and what segments of the population are likely to be adversely affected by the consumption of food containing *L. monocytogenes.*

Despite efforts to eradicate the organism from RTE foods *(25, 26), L. monocytogenes* contamination continues to occur. Surveillance and monitoring activities of the FDA and the USDA have indicated that as much as 5% of some RTE foods, such as prepared deli-style salads and sliced luncheon meats, contain *L. monocytogenes (11, 15).* Such a prevalence of the organism in these frequently consumed products implies that consumers are exposed to detectable levels of *L. monocytogenes* billions of times each year. This finding appears inconsistent with the relatively low level of listeriosis cases reported by the CDC. There are several possible explanations for the discrepancy: (i) only some of the population are sensitive to *L. monocytogenes*; (ii) only exposure to high levels of *L. monocytogenes* causes listeriosis, and/or (iii) only some subtypes of *L. monocytogenes* cause listeriosis. We know that the first hypothesis provides a partial explanation; listeriosis occurs most frequently in immunocompromised individuals, pregnant women, neonates, and elderly people. However, this factor does not account for all of the discrepancy. A risk assessment is needed to reveal and rank the factors contributing to listeriosis.

In a risk assessment, one typically characterizes risk by correlating an exposure assessment for the hazard with a dose-response model *(13, 18).* A risk assessment for *L. monocytogenes* presents some difficulties. First, an accurate exposure assessment is unavailable. The FDA, the USDA,

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and the CDC undertook a risk assessment for *L. monocytogenes* in 1999 *(29)* and discovered that, while there are many data on *L. monocytogenes* prevalence in many RTE foods, there are almost no data on *L. monocytogenes* levels in foods. Because of the zero tolerance policy, any detectable level is unacceptable, so there has been no benefit to monitoring levels of *L. monocytogenes* in food products. Second, a dose-response relationship for the pathogen is unknown. The high case fatality rate (20% *(17)*) makes human feeding studies unacceptable. While several feeding studies involving animals have been undertaken *(7, 20, 24),* there is uncertainty associated with the extrapolation of animal results to humans.

In the mid-1990s, several studies were undertaken to estimate the likelihood of acquiring listeriosis from exposure to *L. monocytogenes* in foods *(1, 8, 11).* An exposure assessment was carried out with the use of survey data regarding *L. monocytogenes* in RTE foods (these data were mainly from the United Kingdom, but some were from the United States) and dietary intake data from the published literature, including data from surveys undertaken by the FDA and the USDA *(11).* Foods investigated included processed meats, cooked poultry, milk, cheeses, ice cream, and prepackaged salads. Hitchins *(11)* estimated that *L. monocytogenes* was being consumed about once every 3 or 4 days (on average, 100 times per year). Most of the times the organism was ingested, <10 cells would be consumed; 10% of foods would contain $>10^4$ CFU per 25 g, 5% would contain $>10^5$ CFU per 25 g, and 2% would contain $>10^7$ CFU per 25 g. Hitchins *(11)* estimated that 15% of the population was at high risk for listeriosis. Even in view of this low estimate, he could not reconcile the low listeriosis rate with the high rate of exposure to low levels of *L. monocytogenes.* He concluded that the frequency of the consumption of low levels of *L. monocytogenes* (1 to 10 CFU) was much higher than the frequency of the occurrence of foodborne listeriosis (ca. 7×10^{-6} cases per person per year). Exposure to higher doses ($>10^3$ CFU) could account for the observed rate of listeriosis.

Buchanan et al. *(1)* estimated a dose-response relationship for *L. monocytogenes* on the basis of food survey data for levels of *L. monocytogenes* on smoked fish combined with epidemiological data on listeriosis in Germany. These authors assumed that the dose-response relationship for foodborne human listeriosis fit the exponential model that had been used to describe the dose-response relationships for *Salmonella, Shigella,* and other pathogens. Other key assumptions were that all listeriosis cases in Germany were attributable to the consumption of smoked fish and that only at-risk individuals became ill. *L. monocytogenes* was absent (less than one organism in 25 g) from 93% of the smoked fish sampled. Seventy percent of the contaminated samples contained ≤100 cells, 15% contained 100 to 10,000 cells, and 15% contained >10,000 cells. The dose-response model predicted that the probabilities of acquiring listeriosis were 2.17×10^{-10} if 50 cells were ingested and 6.19×10^{-7} if 500,000 cells were ingested.

The present study was part of a larger National Food Processors Association (NFPA) Research Foundation (RF) risk assessment project that endeavored to build on the previous risk assessments to develop data on the risk of listeriosis to support a science-based strategy for addressing *L. monocytogenes* in foods in the United States. Specific objectives were (i) to quantify the levels of *L. monocytogenes* in certain foods in order to estimate actual consumer exposure to the organism, (ii) to use the quantitative exposure data in combination with illness data collected concurrently for consumers in the same geographical regions and conduct a risk assessment to determine the impact of the number of *L. monocytogenes* cells consumed on the risk of listeriosis, and (iii) to identify the subtypes of *L. monocytogenes* in those foods and the clinical subtypes from the same geographical areas and conduct a risk assessment to determine the impact of the subtype of the *L. monocytogenes* consumed on the risk of listeriosis. This paper describes results relative to the first objective; results relative to the second objective are described elsewhere *(3).* Studies with regard to the third objective are still under way.

MATERIALS AND METHODS

Product selection. Product selected for the first two groups (i) had relatively high prevalence rates of *L. monocytogenes* contamination, as indicated by historical data; (ii) were expected to be frequently consumed in the areas in which they were collected; and (iii) were ready to eat and not likely to be further treated in a way that would decrease the levels of *L. monocytogenes* after purchase.

In order to obtain reasonably high exposure levels, consumption patterns were used to narrow the food types selected. Consumption data for 1994 to 1996 were obtained from the Continuing Survey of Food Intakes by Individuals (CSFII), a national survey conducted by the USDA's Agricultural Research Service that provides information on 2-day food intakes for more than 15,000 individuals of all ages. The CSFII database was reviewed to identify the most frequently consumed foods previously identified as being likely sources of *L. monocytogenes*. The product categories selected were sliced luncheon meats (i.e., ham, bologna, and poultry) and prepared deli salads (i.e., potato salad, tuna salad, pasta salad, and coleslaw).

Concurrent with this study, the FDA-USDA-CDC undertook a risk assessment on *L. monocytogenes* in foods to estimate and rank the relative risk of illness associated with the consumption of RTE foods that may be contaminated with *L. monocytogenes (29).* One outcome of that risk assessment was the realization that data were often nonexistent and that much of the data available predated changes in production practices implemented to eliminate *L. monocytogenes.* In other cases, the only data available were non-U.S. data, potentially not reflecting current U.S. food production practices. In order to develop missing exposure data, the following product categories were added to this study: fresh soft "Hispanic-style" cheese, bagged precut leafy vegetable salad, blue-veined and soft mold-ripened cheeses, seafood salads (except tuna), and smoked seafood.

Number of samples. Because levels of contamination were expected to be low (<5%) for most products and because the cost of enumerating *L. monocytogenes* is high, we attempted to estimate the total sample size on the basis of the following equation:

$$n = \frac{z^2 P(1 - P)}{d^2}$$

where n is the number of positive samples, P is the actual value of the proportion or percentage positive for the population, d is the desired upper bound on the absolute error (i.e., margin of error), and $z = 1.96$, corresponding to a 95% confidence level for the probability that the estimate is within $\pm d$ of the population value *(9)*. For a fixed value of d, the sample size is at its maximum when $P = 0.5$ (50%). To be conservative, and since we did not know the values of the percentages to be estimated, we assumed that $P = 50\%$. For this approach, n values of 125, 250, and 500 correspond to upper absolute error bounds of 8.8, 6.2, and 4.4%, respectively. Assuming that 5% of the total samples tested would be positive for *L. monocytogenes,* the corresponding total sample sizes required would be 2,500, 5,000, and 10,000, respectively. On the basis of this analysis, we decided to collect 2,500 samples of each of two types of products, luncheon meats and deli salads, at each of two FoodNet sites (see "Sampling Site Selection" section), for a total of 5,000 samples per food type, or 10,000 samples. In the course of the study, we found that prevalence levels for *L. monocytogenes* in luncheon meats and deli salads were considerably lower than had been expected, and thus we decided to double the total number of luncheon meat and deli salad samples collected. For the other product categories, we targeted 2,500 to 3,000 samples per food type.

For luncheon meats and deli salads, we weighted the number of samples by consumption of the product. For example, with luncheon meats, the proportions of ham, bologna, and chicken-turkey samples were based on the frequency of consumption of these meats in the geographical area involved (West for California and South for Maryland) according to the CSFII. Thus, for Maryland, 50% of the luncheon meat samples were ham, 30% were bologna, and 20% were turkey-chicken; for California, 43% of the samples were ham, 30% were bologna, and 27% were turkey-chicken. Owing to the diverse nature of the additional product categories, there was no weighting within those categories.

Sampling site selection. The CDC conduct active surveillance for listeriosis at nine FoodNet sites *(2),* and this surveillance provides the most accurate estimate of listeriosis possible. In addition, while the present study was in progress, the CDC performed case-control studies for listeriosis at these sites, which provided a potential opportunity to obtain *L. monocytogenes* isolates from listeriosis patients and compare them with isolates from foods in the same geographical area. Thus, in order to relate exposure data and food isolates to illness, food samples were collected at the northern California and Maryland FoodNet sites. The rationale for selecting these sites was as follows. Although FoodNet data indicated that the incidence of listeriosis was similar for all sites (approximately 0.5 cases per 100,000 people), the 1997 FoodNet final report *(2)* indicated a slightly higher rate at the northern California site (0.7 cases per 100,000 people). The potential for a larger number of cases of listeriosis, and therefore more isolates for comparison with food isolates, at the northern California site was one factor in the selection of this site as one of our sampling sites. In addition, the limited geographical area (Alameda and San Francisco counties) simplified the sampling procedure (compared with a sampling area encompassing an entire state, such as Minnesota or Georgia). We selected Maryland because its FoodNet site was relatively small (comprising five counties plus Baltimore City) and because it was geographically far removed from the northern California site. However, in Maryland, listeriosis data for FoodNet are collected statewide, so we elected to sample all counties containing more than 2% of the population (10 counties) plus Baltimore City, covering 87.5% of the population. Counties in which products to be investigated (e.g., fresh soft Hispanic-style cheeses) were not available were omitted from the sampling list.

Selection of sampling locations within the FoodNet sites. Sampling within the sites was weighted by the populations in the counties involved (1 July 1998 estimate from www.census.gov). For example, since it was determined that approximately 65% of the population resided in Alameda County and 35% resided in San Francisco County, the study was designed so that 65% of the samples would be collected in Alameda County and 35% would be collected in San Francisco County. The sampling strategy for fresh soft Hispanic-style cheeses was based on the Hispanic population in the sampling area. In order to simplify sample collection and minimize travel costs, samples were generally collected in only one county on each sampling day. The order in which the counties were sampled within a site was determined with the use of a random number table *(12)*.

Selection of collection sites within counties. Given the ubiquitous nature of the organism, foods may be contaminated with *L. monocytogenes* at food service establishments or in the home, but we focused on evaluating levels of *L. monocytogenes* in foods purchased at retail stores. Purchasing products at retail stores allowed us to sample a variety of products (brand-name products and unbranded products) representative of what the consumer would purchase and consume in the areas of the study. Logistically, it was easier to sample at retail stores than at food service establishments or to obtain foods from consumers' homes, and the packages and containers for the samples obtained made these samples easier to ship to the laboratories than, for example, food service meals would have been. Furthermore, by collecting retail samples, we avoided the potential for cross-contamination via handling by consumers.

Lists of large and small retail markets were created with the use of current telephone directories accessed at the Library of Congress. For each county, the list of stores was divided into list A (major supermarkets) and list B (other grocers). It was assumed that list A stores would carry luncheon meats and deli salads. All list B stores were contacted by telephone to determine whether they carried the specific product to be sampled and to verify their addresses; stores were deleted from the list if they did not respond to three phone calls during business hours, if their phone number was incorrect, or if they did not carry the product to be sampled. For the additional product categories, list A stores were also contacted to verify product availability. The stores on the lists were numbered, and the random number table *(12)* was used to select stores for each collection week (5 major supermarkets and 10 other grocers). It was assumed (on the basis of our experiences and the experiences of others in the retail industry) that 75% of shopping is done at major supermarket chains and 25% is done at other grocers, and the number of samples from lists A and B were weighted accordingly. Supplementary lists of stores reported to have specific products (smoked seafood, seafood salads, soft cheeses, and bagged salads) were provided for use as needed to obtain the selected numbers of samples for these products.

Collection of samples. The NFPA RF contracted with an independent third party to collect samples of all products except fresh soft Hispanic-style cheese at retail markets; fresh soft Hispanic-style cheese samples were collected by a second independent third party.

Collection of samples: luncheon meats and deli salads. For luncheon meats and deli salads, 120 samples were collected in northern California and Maryland for approximately 90 weeks (each week, in alternating weeks) over 23 months. The NFPA RF

provided specific instructions for collectors and store lists for each collection week. The information specified the product category, specific types of products included in the category, the number of samples of each type of product to be obtained, the size of the sample to be purchased, and how to collect the sample. For example, for the sampling of luncheon meats in California, collectors were instructed to purchase 39 samples of ham, 27 samples of bologna, and 24 samples of poultry from stores on list A and 13 samples of ham, 9 samples of bologna, and 8 samples of poultry from stores on list B. Ham could include products made from pork or poultry (such as turkey) and could include regular, low-salt or low-sodium, low-fat, extra-lean, and fat-free varieties. Bologna could include products made from pork, beef, turkey, or mixtures of these meats and could include regular, low-salt or low-sodium, low-fat, and fat-free varieties. Turkey or chicken could be smoked or not smoked. All products were to be sliced (either presliced by manufacturers or sliced by the retail stores). Samples were to be obtained first from the delicatessen when a delicatessen was present (0.25- to 0.5-lb [113- to 227-g] samples of each different type of ham, bologna, and chicken or turkey luncheon meat) and then from the refrigerated cases. No attempt was made to prespecify the percentage collected from the delicatessen versus the percentage collected from the refrigerated case. For each type of manufacturer-packaged product, the top unit of each "facing" (hook or stack) of 4- to 16-oz (113- to 454-g) packages (except variety packs) was to be selected. If this strategy did not produce the specified number of samples, the back or bottom unit of every facing was to be selected, starting again at the top row.

Sample collectors were instructed to make purchases from at least two stores on list A and two stores on list B until the specified number of samples had been collected. If the required number of samples could not be purchased at two stores, shoppers were to go to additional stores on the list until the specified number of samples was obtained. To ensure that at least two stores of each type (major supermarkets and other grocers) were visited, shoppers were to collect no more than two-thirds of a category goal at the first store. In order to minimize shopping time and travel costs, shoppers were instructed to not go to more than five list B stores; if the required number of samples was not obtained from the five list B stores, shoppers were to select a store from list A that had not been sampled, if possible, to collect the remaining samples needed. Also, if shoppers were not able to obtain adequate numbers of samples of a particular type of product (e.g., sliced poultry), they could select the needed number of samples from other products in the category (e.g., ham or bologna).

Collection of samples: smoked seafood, seafood salad, blue-veined or soft mold-ripened cheeses, bagged salads, and fresh soft cheese. For approximately 50 weeks over 14 months, 28 samples of each of the five additional product types were collected in each of the two locations. Four product types (smoked seafood, seafood salad [except tuna], blue-veined or soft mold-ripened cheeses, and bagged salads) were collected from the same locations from which luncheon meats or deli salads had been obtained the previous day. Collectors were instructed to make purchases for each product type from at least two list A stores and two list B stores until the specified number of samples had been collected and to not collect more than two-thirds of a category goal at the first store. However, if shoppers were unable to find a sufficient amount of the product of the desired type in the stores they had visited the previous day, they were to select stores from the supplementary lists for the area and product type (with a maximum of eight stores [to minimize costs]) until the specified number of samples had been obtained.

Twenty-eight samples of fresh soft Hispanic-style cheese were collected in a similar manner from northern California and Maryland each week for approximately 50 weeks. The NFPA RF randomized the order of the counties for shopping and store lists (major supermarkets and other grocers) for counties in which the product was available (Alameda and San Francisco counties in northern California; Montgomery County, Prince George's County, and Baltimore City and County in Maryland). Shoppers were to select any of the stores from the lists but to ensure that all of the stores were visited during the study. Shoppers were instructed to make purchases from at least two major supermarkets and two other grocers (except in one county, where none of the major supermarkets reported carrying the product) until the specified number of samples had been collected. To ensure that at least two stores in a category were visited, shoppers were to collect no more than two-thirds of the samples from one store. If the required number of samples was not obtained from the four stores, shoppers were instructed to select any of the stores on either list to collect the remaining samples needed and to not go to more than eight stores altogether.

Shoppers were provided information on what was included in or excluded from each group and the sizes of the samples (8 to 16 oz [227 to 454 g], or two packages of the same code if only a <8-oz size was available). Shoppers were instructed to obtain samples from both the delicatessen (if there was one) and the refrigerated case, where applicable. They were to select one package of each type of product from the specific category from the deli and to select the top container and the bottom container of each stack or facing (hook) when they purchased manufacturer-packaged products.

Handling of samples after selection. Samples were placed in insulated shipping coolers containing frozen gel packs, which were placed on the sides, in the middle, and on the top of the product. A data logger (model SL100 or SX100, Dickson, Addison, Ill.) was placed between two products in the middle of the cooler to monitor temperature during product transportation to verify that the temperature remained within desirable limits. When the cooler was opened at the laboratory, a thermometer or other temperature-sensing device was inserted between packages, and the temperature was recorded. The temperature profile recorded by the logger was evaluated together with the temperature recorded at the laboratory, and samples would have been rejected if both the data logger temperature and the lab-measured temperature were >10°C. No such occasion occurred.

Selection of testing laboratories. Two testing laboratories were selected on the basis of (i) familiarity with procedures for detecting *L. monocytogenes* in foods at the laboratory, (ii) accuracy in detecting and quantifying *L. monocytogenes* in several NFPA-prepared test samples at the laboratory, and (iii) an on-site audit of the laboratory's standard operating procedures. The laboratories ultimately selected to test the product samples were in Green Bay, Wis., and Modesto, Calif. Samples collected in Maryland were shipped to the Green Bay laboratory overnight. Samples collected in California were sent to the Modesto laboratory by ground transportation on the day of collection or the next morning, and the cooler was held refrigerated overnight prior to further handling.

Handling of samples on receipt. The laboratories were instructed to discard any sample with package damage such that the microbiological integrity of the sample was compromised and any sample that was collected but did not meet the description for the product category. After culling unacceptable samples for luncheon

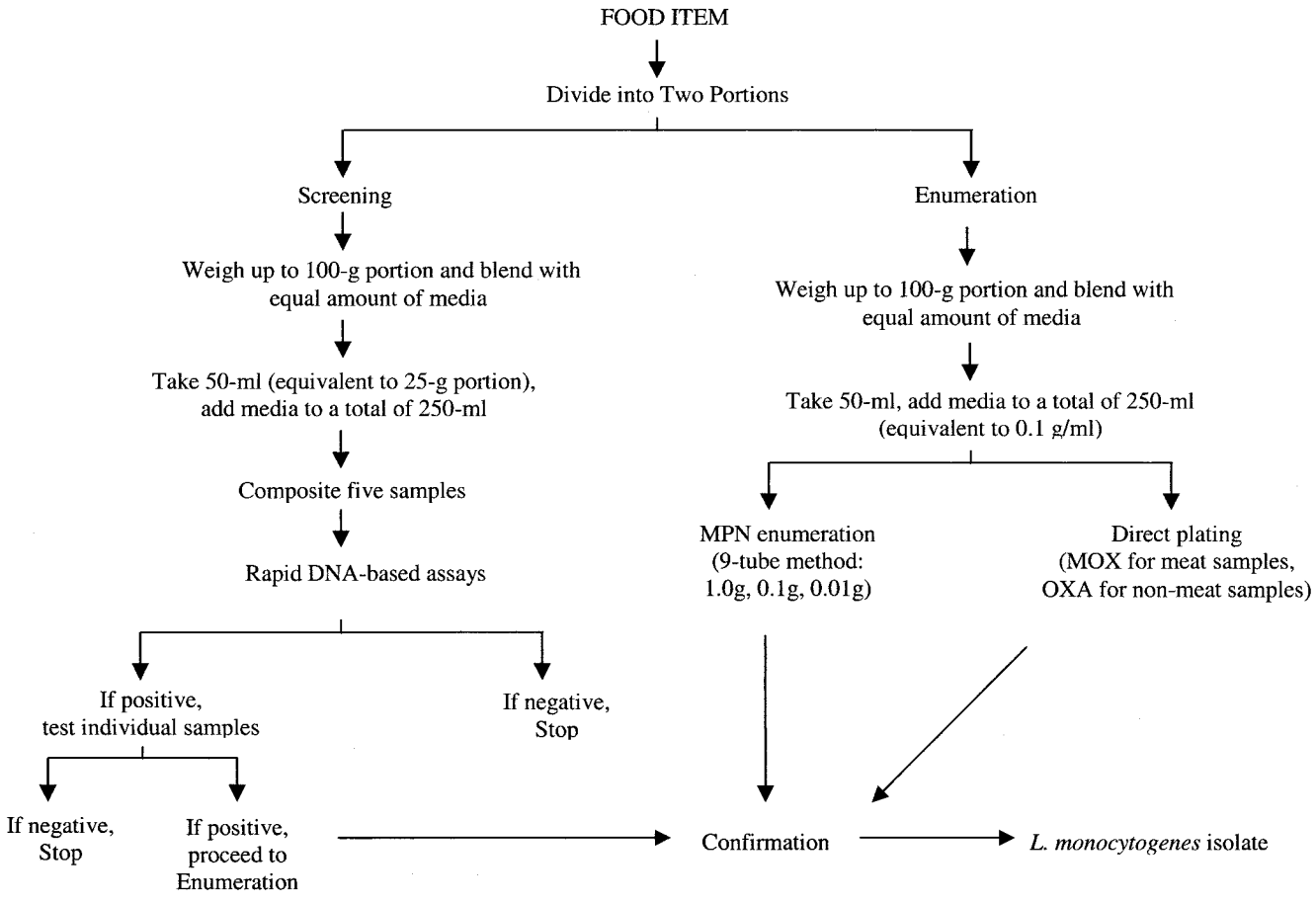

FIGURE 1. *Detection and enumeration of* L. monocytogenes *in RTE food samples.*

meats and deli salads, 100 samples were selected for testing. For the other product categories, 50 samples were selected for the first several weeks of sampling, and 25 samples were selected each week thereafter for testing. In a week when <100 or <25 samples were purchased (owing to product shortages in the stores), all acceptable samples were tested.

Samples were assigned codes, and the following product information was recorded for the luncheon meats and deli salad samples: sampling location (northern California or Maryland), date of receipt at the laboratory, and whether the sample appeared to be packaged in-store or in the original manufacturer's packaging. Additional product information was recorded for the other product categories; depending on the product, information included type of ingredient, whether pasteurized milk was listed as an ingredient, whether the product was domestic or imported, whether or not the product was vacuum packaged, and the use-by or sell-by date code, if present.

The laboratories were instructed to transfer samples aseptically into individual sterile plastic bags and discard the original retail packages. For Maryland samples, the selection of the 100 or 25 samples to be tested occurred after all samples had been transferred into plastic bags. For northern California samples, the selection occurred prior to the transfer of the samples. The selected samples were stored at 2 ± 2°C until they were used. Sample testing was initiated within 24 h of the receipt of samples.

Testing procedures. The general scheme for sample testing is shown in Figure 1. Four combinations of testing procedures were used to screen the samples for *L. monocytogenes*. The choice of screening procedure was based on product type and which laboratory performed the testing (see below). Samples were screened by recognized methods typically used by the laboratory for the detection and enumeration of *L. monocytogenes*. The Gene-Trak assay (Neogen, Lansing, Mich.) and the BAX assay (DuPont Qualicon, Wilmington, Del.) were used to screen samples collected in Maryland and northern California, respectively. In a prestudy evaluation of the laboratories, both DNA-based assays generated comparable results for the detection of *L. monocytogenes* in samples provided by the NFPA (data not shown). USDA or FDA testing procedures for *L. monocytogenes* were modified for use in enumeration and isolation. Methods described in chapter 8 of the revised *Microbiological Laboratory Guide (27)* were adapted for meat products, and those in the *Bacteriological Analytical Manual (30)* were adapted for nonmeat products.

Sample screening. Each sample was divided into two portions for screening and enumeration. For screening, approximately half (up to 100 g) of a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth. For Maryland samples, University of Vermont broth 2 (UVM-2 broth) was used as the enrichment broth for luncheon meats, smoked seafood, and seafood salads; phosphate-buffered *Listeria* enrichment broth was used for the other product types. For northern California samples, demi-Fraser broth was used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24 ± 2 h.

After incubation, individual Maryland sample enrichments were swabbed onto two plates of modified lithium chloride-ceftazidime agar (mLCA), which were incubated at 35°C for 24 ± 2 h. Cell growth was collected from one of the plates with a cotton swab for composite screening with Gene-Trak, and the other plate was held for individual screening if needed. For each California sample, 0.1 ml of the demi-Fraser enrichment culture was transferred to 10 ml of morpholinepropanesulfonic acid–*Listeria* enrichment broth and incubated at 35°C for 20 to 24 h. After the secondary enrichment, 1 ml of the broth culture was used for screening with the BAX assay as described below.

Up to five like-product samples were composited and screened. For Maryland samples, cells from five cotton swabs (representing five samples) were suspended in 5 ml of phosphate-buffered saline (PBS, pH 7.5). The suspension was centrifuged at 3,000 × *g* for 10 min; the cell pellet was resuspended in 1 ml of PBS and tested by the Gene-Trak assay according to the manufacturer's procedures. For California samples, five 1-ml samples of secondary enrichment broths were composited, and the 5-ml composite was tested by the BAX assay according to the manufacturer's procedures.

If a composite tested negative, all samples in the composite were considered negative (in 25 g) and were not tested further. If a composite tested positive, samples in the composite were tested individually by Gene-Trak assay with the PBS from the second mLCA plate or by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further. If an individual sample tested positive, the retained portion was subjected to an enumeration assay. The PBS suspension or the secondary enrichment broth was also streaked onto an agar plate (modified Oxford agar [MOX] for luncheon meats and Oxford agar [OXA] for the other product types), which was incubated at 35°C for 24 to 48 h and held for confirmation if needed.

Enumeration. Individual positive samples were enumerated by both the most probable number (MPN) method *(27, 30)* and the direct plate count. Up to 100 g of the retained portion of a sample was blended by the same procedure used in the preparation of samples for screening (see above). The media used in sample preparation and MPN enumeration for samples from both Maryland and northern California were UVM-1 broth for luncheon meats and phosphate-buffered *Listeria* enrichment broth for other product types. A 250-ml sample homogenate in an appropriate medium, representing 25 g of the original sample, was prepared.

Enumeration: MPN method. A nine-tube MPN method was used. The nine tubes were divided into three sets of three tubes. The second and third sets of tubes contained 10 ml of the appropriate broth medium. Three aliquots of the sample homogenate in volumes of 10, 1, and 0.1 ml were dispensed into the first, second, and third sets, representing 1.0, 0.1, and 0.01 g of the original sample, respectively. For a non–luncheon meat sample, the tubes were incubated at 30 ± 2°C for 48 h and subjected to confirmation. For a luncheon meat sample, the tubes were incubated at 30 ± 2°C for 22 ± 2 h, and 0.1 ml from each tube was transferred to a new tube containing 10 ml of Fraser broth. The tubes were incubated at 35 ± 2°C for 26 ± 2 h. Darkened Fraser tubes (an indicator of esculin hydrolysis and the potential presence of *L. monocytogenes*) were subjected to confirmation. If a Fraser broth tube did not darken, it was examined again after an additional 26 ± 2 h of incubation.

For Maryland samples, the MPN pattern was determined by the Gene-Trak assay. Darkened Fraser tubes for luncheon meat samples, and all nine tubes for samples of the other product types, were individually streaked onto mLCA plates, which were incubated at 35°C for 24 ± 2 h. Individual plates were swabbed, and cells were suspended in 1 ml of PBS and tested by the Gene-Trak assay. The MPN was determined on the basis of the number of positive tubes in each of the three sets and an MPN table *(27, 30)*. Each sample for which at least one MPN tube was found to test positive was tested further by biochemical assays and to obtain an *L. monocytogenes* isolate. If only one tube tested positive, the corresponding PBS suspension was subcultured onto an appropriate agar plate (MOX for luncheon meat samples and OXA for other samples) and incubated at 35 ± 2°C for 24 to 48 h. If tubes from more than one set tested positive, the PBS suspension from the highest MPN dilution was subcultured. The isolation of *L. monocytogenes* was carried out by the method described below.

For northern California samples, the MPN pattern was determined by culture methods and biochemical assays. Darkened Fraser tubes for luncheon meats, and all nine tubes for samples of the other product types, were individually subcultured onto an appropriate agar plate (MOX for luncheon meat and OXA for other product types) and incubated at 35 ± 2°C for 24 to 48 h. If suspected colonies on the plate were confirmed to be *L. monocytogenes* colonies (see below), the corresponding MPN tube was deemed positive. The MPN pattern for the sample was determined from the number of positive tubes yielding confirmed *L. monocytogenes* colonies.

The MPN method resulted in direct estimates of *L. monocytogenes* levels in the range of 0.3 to 110 MPN/g. For some samples, a value of <0.3 was also obtained through further calculation, for which a 10-tube MPN pattern (including the screening step as one tube with a 25-g test portion) was used in MPN determination.

Enumeration: direct plating. All positive samples from both Maryland and northern California were tested by direct plating as follows. The 250-ml sample homogenate that was used for MPN enumeration was also used for direct plating. A 0.2-ml volume of homogenate was evenly spread onto each of five agar plates (MOX for luncheon meat samples and OXA for other samples). The total number of colonies on the five plates represented a 0.1-g test portion. The homogenate was also diluted 10-fold, and 0.1 ml of the dilution was plated in duplicate. The average of the counts on these two plates represented a 0.001-g test portion. The plates were incubated at 35 ± 2°C for up to 48 h and examined for suspected *L. monocytogenes* colonies.

Up to 20 different individual colonies were subjected to confirmation analysis. Ten suspected colonies from each of the 0.001-g plates or a total of 20 colonies from the five 0.1-g plates were individually picked and point transferred onto a section of a horse blood agar plate. After incubation at 35 ± 2°C for 19 ± 3 h, the blood plates were examined for the presence of beta-hemolytic colonies. Up to three clearly isolated beta-hemolytic colonies were subjected to confirmation analysis (see below). If a colony was confirmed to be an *L. monocytogenes* colony, all of the beta-hemolytic colonies were considered *L. monocytogenes* colonies. The ratio of the number of beta-hemolytic colonies to the total number of colonies picked (up to 20) was calculated and used to determine the count (CFU/g) for the plate. The concentration of *L. monocytogenes* in a sample was determined by multiplying the ratio by the total count. For a few of the positive samples, the MPN and direct count methods provided different results. In these cases, the larger number was used in further calculations.

Isolation and confirmation of *L. monocytogenes*. For a sample testing positive during screening, an isolate was collected from plates obtained at the direct-plating, MPN, or screening step. If an isolated beta-hemolytic colony obtained at the enumeration

step was confirmed to be an *L. monocytogenes* colony, the isolate was retained. When no isolate for a sample was obtained from the MPN or the direct plating step, the MOX or OXA agar plate held from the screening step was used to recover an *L. monocytogenes* isolate.

The agar plate was examined for suspected *L. monocytogenes* colonies (on a MOX agar plate, distinctive 1- to 2-mm round colonies surrounded by darkened zones of esculin hydrolysis; on an OXA plate, distinctive 1- to 2-mm round colonies surrounded by a black halo) at 24 h and then at 48 h. If suspected colonies were present on a plate obtained from the MPN step or the screening step, up to 20 colonies were picked (by running a loop through them), and streaked onto a horse blood agar plate. Suspected colonies on a plate from the direct plating step were individually picked and point transferred onto a horse blood agar plate. This plate was incubated at 35 ± 2 °C for 19 ± 3 h and examined for the presence of translucent colonies surrounded by a small zone of beta hemolysis. When necessary, colonies from the horse blood plate were restreaked onto a second horse blood plate to obtain isolated colonies. A clearly isolated beta-hemolytic colony, if present, was subjected to further biochemical confirmation. If it was confirmed to be an *L. monocytogenes* colony, all of the beta-hemolytic colonies were considered *L. monocytogenes* colonies. If it was confirmed not to be an *L. monocytogenes* colony, up to two more beta-hemolytic colonies were subjected to biochemical confirmation analysis. If all three colonies were confirmed not to be *L. monocytogenes* colonies, none of the beta-hemolytic colonies were considered *L. monocytogenes* colonies.

Biochemical confirmation was carried out with the use of the API *Listeria* ID strip (bioMérieux, Inc., Hazelwood, Mo.) or the Micro ID *Listeria* kit (Organon Teknica Corp., Durham, N.C.) according to the manufacturer's procedures. Northern California samples collected prior to October 2000 were confirmed by the Micro ID method, and all other samples were confirmed by the API method. Confirmed isolates were retained on Trypticase soy agar with yeast extract slants and sent to the NFPA laboratory in Washington, D.C., for archiving.

Statistical analysis. Contingency table analysis *(15, 22)* was used to determine whether *L. monocytogenes* prevalence levels for the eight product categories differed significantly. The contingency table analysis was based on the chi-square distribution and tested the null hypothesis that percentages of positive samples did not differ significantly among the product categories. For luncheon meat, deli salad, and seafood salad samples, we used a similar approach, the chi-square test for homogeneity, to test the null hypothesis that prevalence did not differ between samples packaged by manufacturers and those packaged in-store. This analysis was performed for percentages for combined Maryland and northern California samples. Chi-square tests were also performed for each of the eight product types to compare prevalence levels for Maryland samples with those for northern California samples. The χ^2 statistic for the ≤5% level of significance was used for the tests.

RESULTS AND DISCUSSION

Sample collection and temperature control. Deli salad and luncheon meat samples were collected over 23 months, whereas samples of the other products were collected over 14 months. A total of 31,705 product samples were tested for *L. monocytogenes*. The desired numbers of seafood salad and smoked-seafood samples were not always available, reflecting the small market for these products in the sampling regions. Consequently, fewer samples of these products than planned were collected for the study. Data on blue-veined and soft mold-ripened cheese samples, which were collected as a single category, were tabulated and analyzed separately because the two cheeses have different characteristics that could have effects on *L. monocytogenes*.

Temperatures experienced by the samples during collection and transport to the laboratories were within the expected range and were, to a certain degree, consistent with what products might experience during a consumer's grocery-shopping trip. Typical temperatures during transportation and temperatures recorded at the laboratory were <5°C. During the first few hours of sample collection, temperatures recorded by the logger were typically <10°C. Occasionally, the logger indicated that the cooler's environment was at 10 to 15°C for a period (less than a few hours, mostly during shopping) but then cooled to <5°C during transport. Such circumstances applied to samples that tested negative as well as to those that tested positive. In the few events in which the temperature exceeded 10°C, the exposure time and the temperature were not likely to allow the growth of more than one generation of *L. monocytogenes* in the products, even if the organism had been present and in a physiological state beyond the lag phase *(5, 28)*. Therefore, the numbers reported here are likely to be equal to or lower than those experienced by consumers, given the home refrigerator temperatures reported in a 1999 Audits International survey (http://www.foodriskclearinghouse.umd.edu/).

Prevalence. Of the 31,705 samples analyzed, 577 tested positive (a 1.82% prevalence rate). Table 1 shows a breakdown of positive samples by product and sampling region. The highest rates of positive samples were those for seafood salads (4.7%) and smoked seafood (4.3%). The prevalence rate for smoked-seafood samples was similar to that reported by Loncarevic et al. *(16)*, who found that 4 of 92 smoked-fish samples tested positive. Higher prevalence rates have been reported for smoked fish obtained from processing plants (7.3% *(19)* and 79% *(6)* in the United States, 34 to 60% in Denmark *(14)*, and about 20% in Italy *(4)*). A prevalence rate of about 22% was reported for smoked fish from retail outlets in Spain *(33)*. For seafood salads, a prevalence rate of 16% was reported for samples from markets in Iceland *(10)*, and a rate of 27% was reported for samples from supermarkets in Belgium *(31)*. In these studies, fewer samples (about 50 to 400 samples) were analyzed. In addition to the fact that the RTE products in our study and the products used in these studies were collected from different regions and at different times, differences in food production and handling practices as well as differences in detection methods may also account for some of the differences in prevalence rates.

The lowest prevalence rates were those for fresh soft cheese (0.17%) and bagged salads (0.74%). Percentages of *L. monocytogenes*–positive samples of deli salads and sliced luncheon meats were considerably lower than expected, at 2.4% and 0.89%, respectively. This compares

TABLE 1. *Prevalence of* L. monocytogenes *in ready-to-eat food samples tested in 2000 and 2001*[a]

Product category	No. of positive samples/no. of samples (%)		
	MD	CA	Total
Fresh soft cheeses	4/1,450 (0.28)	1/1,481 (0.07)	5/2,931 (0.17)
Bagged salads	8/1,465 (0.55)	14/1,501 (0.93)	22/2,966 (0.74)
Blue-veined cheeses	6/956 (0.63)	17/667 (2.55)	23/1,623 (1.42)
Mold-ripened cheeses	1/517 (0.19)	13/830 (1.57)	14/1,347 (1.04)
Seafood salads	88/1,225 (7.18)	27/1,221 (2.21)	115/2,446 (4.70)
Smoked seafood	43/1,281 (3.36)	71/1,363 (5.21)	114/2,644 (4.31)
Luncheon meats	54/4,599 (1.17)	28/4,600 (0.61)	82/9,199 (0.89)
Deli salads	103/4,293 (2.40)	99/4,256 (2.33)	202/8,549 (2.36)
Total	307/15,786 (1.94)	270/15,919 (1.70)	577/31,705 (1.82)

[a] MD, Maryland FoodNet site; CA, northern California FoodNet site.

with reported rates of 4.2 to 8.0% for sliced luncheon meat samples (about 2,300 samples) collected by the FSIS in U.S. federally inspected establishments between 1990 and 1999 *(15)* and about 6% for sliced cooked ham and poultry products (about 900 samples) collected from Belgium supermarkets in 1997 and 1998 *(31)*.

Significant differences in prevalence were found among the eight product types (for Maryland and northern California samples combined) by a contingency table analysis performed to compare the eight percentages ($P < 0.001$). Significant differences between prevalence rates for the northern California and Maryland sites were found for seafood salads ($P < 0.001$), luncheon meats ($P < 0.01$), blue-veined cheeses ($P < 0.01$), soft mold-ripened cheeses ($P < 0.05$), and smoked seafood ($P < 0.05$). For example, there were four times as many positive samples of blue-veined cheese and eight times as many positive samples of soft mold-ripened cheese from northern California as there were from Maryland. On the other hand, there were more than three times as many positive samples of seafood salads from Maryland as there were from northern California, and twice as many positive luncheon meat samples.

Contamination levels. In 402 of the 577 *L. monocytogenes*–positive samples, the numbers of cells were below enumeration levels (<0.3 MPN/g). Because the levels were so low and because the products were screened by the Gene-Trak or the BAX assay, it was not always possible to culture an *L. monocytogenes* isolate from the product. However, isolates were collected from 330 of these 402 below-enumeration-level samples. For the remaining 175 *L. monocytogenes*–positive samples, levels ranged from 0.3 MPN/g to a maximum of 1.5×10^5 CFU/g. A breakdown of *L. monocytogenes* levels by product type and sampling site is presented in Table 2. The majority of the positive samples were contaminated at levels of <10 CFU/g. The largest

TABLE 2. *Levels of* L. monocytogenes *contamination detected in various products*

Product category	Total no. of samples positive	No. of positive samples in concn (CFU/g) range							
		0.04[a]–0.1	>0.1–1	>1–10	>10–10^2	>10^2–10^3	>10^3–10^4	>10^4–10^5	>10^5–10^6
California									
Deli salads	99	78	14	4	2	0	1	0	0
Luncheon meats	28	10	8	6	1	2	1	0	0
Fresh soft cheeses	1	0	0	0	1	0	0	0	0
Bagged salads	14	12	1	0	1	0	0	0	0
Blue-veined cheeses	17	13	3	0	1	0	0	0	0
Mold-ripened cheeses	13	11	0	2	0	0	0	0	0
Seafood salads	27	26	1	0	0	0	0	0	0
Smoked seafood	71	44	5	11	5	4	1	0	1
Maryland									
Deli salads	103	84	14	5	0	0	0	0	0
Luncheon meats	54	32	12	4	1	5	0	0	0
Fresh soft cheeses	4	2	0	0	2	0	0	0	0
Bagged salads	8	5	0	1	1	1	0	0	0
Blue-veined cheeses	6	5	0	1	0	0	0	0	0
Mold-ripened cheeses	1	1	0	0	0	0	0	0	0
Seafood salads	88	56	18	10	2	2	0	0	0
Smoked seafood	43	23	6	8	3	2	0	0	1

[a] A 25-g sample tested positive.

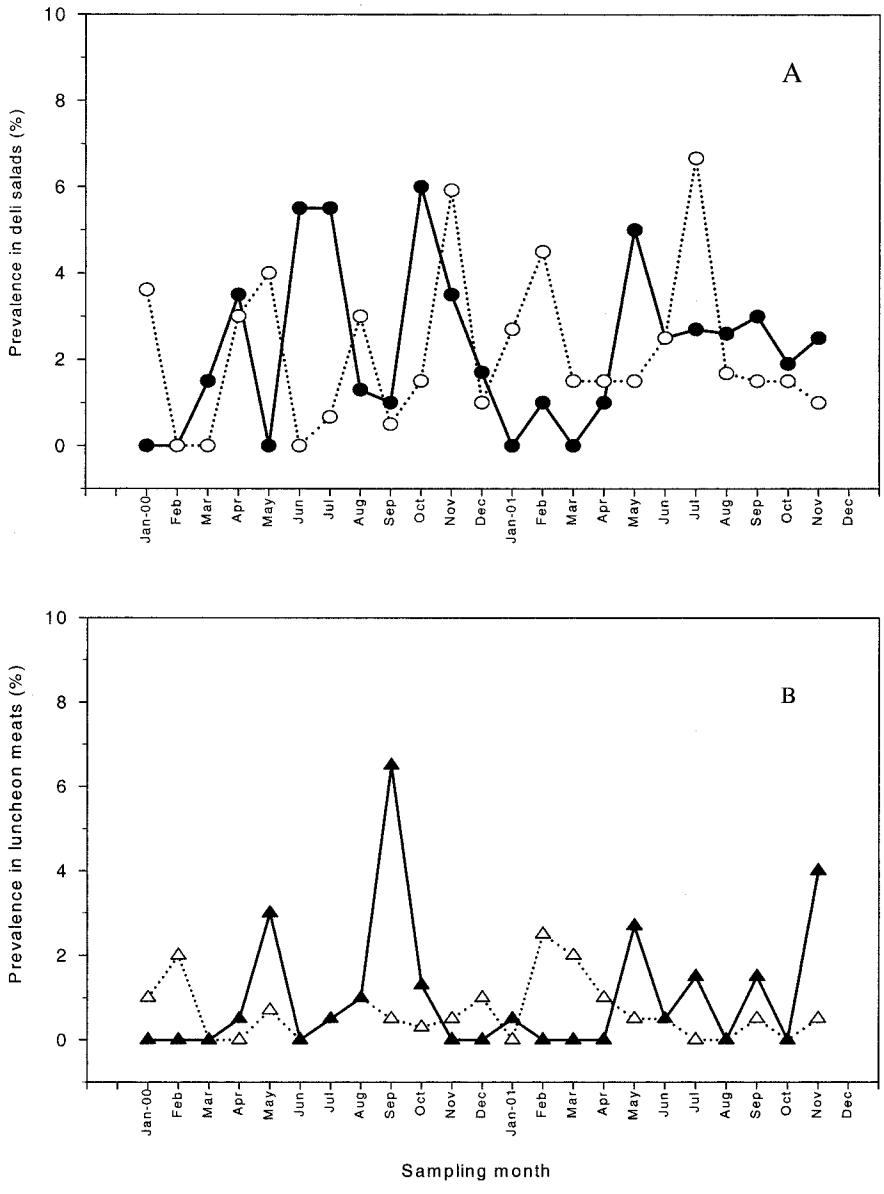

FIGURE 2. *Prevalence of* L. monocytogenes *in deli salads (A) and luncheon meats (B) by month. In both panels, solid and open symbols represent samples collected from the Maryland and northern California FoodNet sites, respectively.*

numbers of positive samples with levels of $>10^2$ CFU/g were those for luncheon meats and smoked seafood (eight and nine samples, respectively). The deli salads, seafood salads, and bagged salads accounted for four more positive samples with levels of $>10^2$ CFU/g. Of the 21 samples with concentrations of $>10^2$ CFU/g, 10 were from northern California and 11 were from Maryland. Only 2 of the 31,705 samples, one smoked-seafood sample from Maryland and one smoked-seafood sample from northern California, had levels of $>10^4$ CFU/g.

In previously published studies, most often only prevalence levels have been reported. For studies in which enumeration was carried out, Uyttendaele et al. *(31)* reported that *L. monocytogenes* was generally detected in small numbers (<10 CFU/g) for processed meat products, while larger numbers of *L. monocytogenes* (>10 CFU/g) were reported for fish and shrimp salads from supermarkets in Belgium. Levels of $>10^2$ CFU/g were reported for 14 of 199 *L. monocytogenes*–positive RTE products from retail displays in Northern Ireland *(32)*. For several studies, levels of contamination under various storage conditions have been reported. In a study on ready-to-use vegetables obtained from a processor in Canada *(21)*, levels of $>10^2$ CFU/g were found for 8 of 120 samples stored at 10°C for up to 11 days, while 5 of these samples had levels of $<10^2$ CFU/g. In the same study, none of 175 samples stored at 4°C after 7 days contained levels of $>10^2$ CFU/g. Contamination levels of $>10^3$ CFU/g were reported for vegetables stored at 10°C *(21)*, and levels of $>10^4$ CFU/g were reported for cooked meat products *(32)*. Jørgensen and Huss *(14)* reported that of 76 positive smoked-fish samples, 12 contained 10^2 to 10^3 CFU/g and 4 contained $>10^3$ CFU/g after 14 days of storage at 5°C. *L. monocytogenes* levels as high as 10^5 CFU/g have been reported for smoked fish *(16)*.

Seasonality. The present study provided an opportunity to examine how seasonality affects the occurrence of *L. monocytogenes* in RTE foods. Figure 2 shows a breakdown of *L. monocytogenes* prevalence in deli salads and luncheon meats by month. No obvious seasonality was ob-

TABLE 3. *Influence of packaging location on prevalence of* L. monocytogenes

Product category	% of samples packaged:		*L. monocytogenes* prevalence (%) for samples packaged:	
	By manufacturer	In-store	By manufacturer	In-store
Luncheon meats	77	23	0.4	2.7
Deli salads	48	52	1.4	3.6
Seafood salads	40	60	1.4	6.9

served with regard to prevalence for any of the product categories tested when these categories were considered individually or in combination (all data not shown). We also evaluated whether higher levels ($>10^2$ CFU/g) were seasonal. Among the 21 samples found in the $>10^2$-CFU/g range, four occurred in samples collected in May, August, and October 2000; 17 occurred throughout 2001 except that none occurred in samples collected in January and August 2001. Collectively, six higher-count samples were collected in May, but there was no obvious trend in seasonality (data not shown). This finding is consistent with the apparent lack of seasonality among 2000 listeriosis cases as reported by the CDC *(2).* Wilson *(32)* also reported that no seasonal pattern was found in the recovery of *L. monocytogenes* from about 8,000 retail RTE food samples collected in Northern Ireland in 1994.

Packaging. Although the present study was not designed to compare prevalence rates between manufacturer-packaged and in-store-packaged products, data collected for several products revealed a trend for in-store-packaged deli salads, luncheon meats, and seafood salads to have higher frequencies of *L. monocytogenes*–positive samples than manufacturer-packaged products (Table 3). Approximately three-quarters of the luncheon meat samples and less than half of the deli salad and seafood salad samples were packaged at manufacturers' processing plants, while the rest of the samples appeared to have been packaged in-store. A significant difference between the prevalence levels of manufacturer-packaged and in-store-packaged samples was found; for all three product categories, the value of χ^2 exceeded the critical value for the 0.001 level ($P < 0.001$). In other words, there is a <1-in-1,000 chance that the prevalence of *L. monocytogenes* did not differ between the two packaging locations for luncheon meats, deli salads, and seafood salads. There were 6.8, 2.6, and about 5 times as many positive in-store packaged samples as positive manufacturer-packaged samples for luncheon meats, deli salads, and seafood salads, respectively.

There could be several reasons for these findings; for example, the additional handling at retail stores may have resulted in more samples being contaminated, or refrigeration temperatures may have differed in the retail deli cases in which these products were held, resulting in growth to detectable levels in store-packed samples. It should be noted that we did not see a significant difference for smoked seafood (with 4.4% positive in-store-packaged samples versus 4.3% positive manufacturer-packaged samples), for which 95% of the samples collected were packaged by manufacturers. It was interesting to note that for luncheon meats, a significantly higher prevalence level was found for in-store-packaged Maryland samples (4.2% versus 0.19% for manufacturer-packaged samples), but no significant difference was found for northern California samples (0.70% compared to 0.55%). Moreover, although the prevalence of *L. monocytogenes* tended to be higher for in-store-packaged products than for manufacturer-packaged products, *L. monocytogenes* concentrations tended to be higher in the latter. Of the 21 samples with counts of $>10^2$ CFU/g, 16 were manufacturer packaged. The influence of packaging location on *L. monocytogenes* contamination warrants further investigation.

The data collected in this study help to fill gaps in the knowledge concerning the occurrence of *L. monocytogenes* in RTE foods, and this new information should be useful in the assessment of the risk posed by *L. monocytogenes* to U.S. consumers. The raw data and attendant analytical methods will be made available to the risk assessment community and to other stakeholders through the JIFSAN Food Safety Risk Analysis Clearinghouse website (http://www.foodriskclearinghouse.umd.edu/).

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