# **General Interest**

# Control of *Listeria monocytogenes* in the Food-Processing Environment

### **R. B. TOMPKIN\***

ConAgra Refrigerated Prepared Foods, 3131 Woodcreek Drive, Downers Grove, Illinois 60515, USA

MS 01-310: Received 28 August 2001/Accepted 30 November 2001

#### ABSTRACT

The purpose of this paper is to provide guidance to food processors in controlling *Listeria monocytogenes* in foodprocessing environments. Of particular concern are outbreaks of a few to several hundred scattered cases involving an unusually virulent strain that has become established in the food-processing environment and contaminates multiple lots of food over days or months of production. The risk is highest when growth occurs in a food before it is eaten by a susceptible population. The information presented in this paper provides the basis for the establishment of an environmental sampling program, the organization and interpretation of the data generated by this program, and the response to *Listeria*–positive results. Results from such a program, including examples of niches, are provided. Technologies and regulatory policies that can further enhance the safety of ready-to-eat foods are discussed.

The purpose of this paper is to provide information that food processors and regulators can use as a basis for strategies to control *Listeria monocytogenes* in food-processing environments. This paper is also a supplement to two previous papers on the control of listeriae (92, 93). A portion of this material has been published (91). While the material herein concerns the control of *L. monocytogenes*, some of the concepts can be applied to the control of other pathogens (e.g., salmonellae) and spoilage microorganisms. When this information is applied to other situations, the temperature of the environment in relation to the growth of the target organism should be considered. For example, salmonellae would not be expected among the resident flora of refrigerated work spaces.

The significance of listeriosis with regard to public health is well known. Although the disease is rare (i.e., about 1 to 9 cases per 1,000,000 people per year) and accounts for only about 0.02% of all foodborne illness, listeriosis accounts for about 28% of the deaths resulting from foodborne illness (10, 65, 83). This high degree of severity, particularly among those at higher risk (i.e., immunocompromised individuals, neonates), emphasizes the necessity to minimize the exposure of high-risk individuals to L. monocytogenes. It also has been established that the foods of the most concern are those in which L. monocytogenes can multiply. In general, foods that have been implicated in listeriosis contain >1,000 CFU/g or >1,000 CFU/ml (12, 46, 47). Consumer protection, then, largely depends on preventing contamination of those foods in which growth can occur.

Experience over the past 15 years points to recontam-

ination as the primary source of L. monocytogenes in many commercially prepared ready-to-eat (RTE) processed foods. This realization has led to significant changes in how the postprocessing environment is managed (21, 92, 93). Major modifications in plant layout and equipment design, procedures for cleaning and sanitizing, and personnel practices have been necessary. It is realistic to expect that L. monocytogenes will continue to be introduced into the environment in which RTE foods are exposed for further processing and packaging. By controlling the establishment and multiplication of L. monocytogenes in these environments, it is possible to minimize, and in some cases prevent, the risk of product contamination with sanitation procedures. Depending on the food and the environmental control program, however, it should be possible in most food processes that include a validated listericidal step (e.g., cooking) to keep the prevalence of product contamination at <0.5%. If this objective can be achieved, then, assuming random distribution, at a product contamination level of 0.5% there would be a 61% probability that a production lot would be accepted even if 100 samples were tested (49). Thus, end product testing becomes of little value for assessing and verifying control.

Another disadvantage of product testing is that if a product is found to be positive, no information will have been gained to indicate what the mode of contamination was or how to prevent further occurrences. Commercial experience has led to the determination that environmental testing is a better, more cost-effective measure for assessing control, particularly when approached from a process control viewpoint. Ideally, the data are used to detect trends indicating a potential loss of control and to enable timely corrective actions. As described, environmental sampling

<sup>\*</sup> Author for correspondence. Tel: 630-512-1031; Fax: 630-512-1124; E-mail: btompkin@crfc.com.

Species	No. of clones identi- fied	No. of clones commonly recovered from disease episodes	% of disease caused by common clones
Bordetella bronchiseptica	21	3	87
Bordetella pertussis	2	2	100
Bordetella parapertussis	1	1	100
Escherichia coli (neonatal			
invasive)	18 <sup>a</sup>	$5^a$	63
Hemophilus pleuropneumonia	32	2	47
Hemophilus influenza serotype b	182	9	81
Legionella pneumophila	50	5	52
Neisseria meningitis			
Serogroups B and C	192 <sup>a</sup>	$7^a$	85
Serogroup A	50	7	_
Shigella sonnei	1	1	100
Salmonella spp. (eight serotypes)	71	11	61-100
Yersinia ruckeri	4	1	89

TABLE 1. Numbers of clones of various bacterial species commonly causing disease (73)

<sup>*a*</sup> Clone families composed of several or many very closely related clones.

programs are intended to assess the control of the environment and not the probability that a specific lot of food has been contaminated. Such programs are not statistically designed sampling plans but are based on prior experience and familiarity with the given processing conditions. Similar programs have been used for over 40 years in a wide variety of food operations to assess the control of pathogens (e.g., salmonellae) and spoilage flora.

The following sections include discussions of the rationale for establishing an environmental sampling program for the control of *L. monocytogenes*; the concept of harborage sites, or niches; how to organize and use data to detect sources of contamination; examples of niches; and possible future directions to enhance the safety of RTE foods.

## ARE ALL *L. MONOCYTOGENES* STRAINS EQUALLY HAZARDOUS?

Variability in virulence within the species *L. monocy*togenes is slowly gaining recognition and acceptance. It has been confirmed through studies with mice that most, but not all, strains of *L. monocytogenes* can cause disease (8, 24, 43, 45, 74, 90). In preliminary studies involving pregnant rhesus monkeys, one strain previously linked to abortion in monkeys appeared to be the most virulent among the six strains tested (88). These and other studies (42, 50, 52, 71, 73, 76, 78, 96) show that some strains have greater potential to cause disease than others do. This finding should not be unexpected, since, as can be seen in Table 1, a limited number of clones account for the majority of disease caused by other pathogens (73). Pathogenicity also is limited to certain types of Yersinia enterocolitica and Escherichia coli (48, 68, 97). Of *L. monocytogenes* it has been said that "most strains are pathogenic, some strains may be pathogenic, some strains are non-pathogenic" (44). More recent research comparing different methods for assessing virulence has demonstrated that a plaque-forming assay using a HT-29 cell monolayer leads to three classifications: avirulent, hypovirulent, and fully virulent (78).

The virulence of *L. monocytogenes* is influenced by six genes on the chromosome in the PrfA-dependent virulence gene cluster and by other important virulence genes (e.g., internalin genes) located outside the gene cluster (56). Presumably, strains having a full complement of virulence genes would have greater potential to cause disease, but the prevalence of such strains in food-processing environments is not known. There has also been speculation that certain strains have greater potential to survive under adverse conditions and to multiply in the processing environment and/ or in certain foods.

Throughout the world, three serotypes (i.e., 4b, 1/2a, and 1/2b) account for 89 to 96% of cases of human listeriosis (30), providing additional evidence that certain strains are more likely to cause illness. More interesting is the realization that a small number of clonal lineages have been responsible for large documented outbreaks in different regions of the world. For example, researchers using a variety of typing methods have confirmed that one epidemic clone of serovar 4b having a phagovar identical or similar to 2389:2425:3274:2671:47:108:340 has caused several major outbreaks (4, 5, 11, 20, 22, 28, 50, 58, 73, 88, 98), such as an outbreak in Switzerland from 1983 to 1987 originating from soft cheese (Vacherin Mont d'Or) and involving 122 cases, resulting in 34 deaths; an outbreak in the United States in 1985 originating from Mexican-style queso fresco and involving 142 cases, resulting in 48 deaths; an outbreak in Denmark from 1985 to 1987 with an unknown origin involving 35 cases; an outbreak in the United States in 1989 with an unknown origin (Philadelphia "outbreak"; isolates from two patients); an outbreak in Denmark from 1989 to 1990 originating from blue-veined cheese; and an outbreak in France in 1992 originating from pork tongue in aspic and involving 279 cases, resulting in 22 abortions and 63 deaths. In addition, the same clone accounted for more than 25% of all human isolates in Sweden (29) and 20.7% of the isolates from patients and foods in Japan (67).

Two other genetically distinct clonal lineages were involved in outbreaks in North America (4, 20), one in the New England outbreak of 1983 (49 cases, 14 deaths) and the other in the frankfurter outbreak of 1998 to 1999 ( $\sim$ 100 cases, 21 deaths). Similar more-virulent clonal lineages of serovars 1/2a and 1/2b may occur.

Variability in virulence helps explain the small number of cases in situations involving frequent exposure to foods containing *L. monocytogenes*. For example, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) monitoring program for products sampled at FSIS-inspected establishments between 1989 and 1999 has shown *L. monocytogenes* prevalence rates of ca. 2 to 3% for cooked beef, ca. 2 to 5% for small-diameter sausages such as franks, ca. 1 to 3% for cooked poultry, and ca. 1 to 5% for RTE meat and poultry salads. The prevalence rate for sliced lunch meat ranged between 4.2 and 7.8% from 1994 to 1999. In France, the prevalence rate for RTE foods decreased from 9 to 8 to 6% for the years 1997 through 1999 (19), indicating a favorable trend of continued reductions. Prevalence rates of 1 to 10% and higher are typical for a wide variety of foods throughout much of the world (9, 30, 33, 34, 84), yet symptomatic listeriosis remains a rare illness.

In summary, the information presented here indicates that certain strains of *L. monocytogenes* are more virulent and much more likely to be involved in foodborne illness than others are. This information can help food processors understand why the foods from one establishment and not those from other establishments have been implicated as a source of listeriosis despite comparable rates of contamination at the establishments in question. Virulence is but one important factor involved in the complex events leading to disease that must be taken into account in developing strategies for the control of *L. monocytogenes*.

# RESIDENT AND TRANSIENT STRAINS OF L. MONOCYTOGENES IN THE PROCESSING ENVIRONMENT

Another important piece of the puzzle involves studies on the microbial ecology of the food-processing environment. Many researchers have demonstrated that certain strains of *L. monocytogenes* can become established in a food-processing facility and remain members of the resident microbial flora for months or years. Table 2 summarizes many of these reports. In general, a variety of strains were detected in each food-processing establishment, particularly in the postprocessing environment, but certain strains were found over repeated visits to an establishment.

Experience in cold-smoked-fish-processing establishments indicates that an array of strains can be found in the receiving and raw-fish-handling areas. As the fish is injected with brine and smoked, other strains become dominant, even though these steps are not listericidal. Another shift can then occur during slicing (31).

Similar investigations have been conducted in other types of food-processing establishments, but the data from these investigations are inadequate to demonstrate whether a change occurs in dominant strains when foods are subjected to different conditions. The methodology used to differentiate the isolates has continued to evolve from sero-typing or phage typing to more discriminating molecule-based methods. While serotype(s) have been reported and are included in Table 2, newer DNA-based methods such as random amplified polymorphic DNA, pulsed-field gel electrophoresis, and ribotyping are necessary to differentiate the strains recovered within each environment. These newer techniques provide much greater insight into the ecology of food operations and should provide guidance for improving the control of *L. monocytogenes*.

It is significant that certain food-processing establishments have been known to harbor *L. monocytogenes* for long periods but the foods processed in these establishments have not been implicated in illness. Considering the continued detection of *L. monocytogenes* in a variety of foods, the existence of a resident flora of *L. monocytogenes* in food-processing establishments is more common than previously thought. The risk of listeriosis appears to be at its highest when a more virulent strain becomes established in an environment in which RTE foods can become contaminated (e.g., between cooking and packaging) and growth occurs in these foods before they are eaten by a susceptible population. While this finding might explain how outbreaks occur, industry and government must continue to treat all *L. monocytogenes* strains as potentially pathogenic.

#### THREE SCENARIOS LEADING TO ILLNESS

The establishment of an effective environmental sampling program requires some understanding of the circumstances that lead to listeriosis. Foodborne listeriosis appears to generally follow a pattern of three scenarios. Scenario 1 consists of isolated cases for which information about the food of origin is seldom available. The long incubation period (i.e., days to weeks) that can elapse before symptoms develop makes it difficult to identify a specific food as the source (Table 3). Scenario 2 consists of an outbreak or a cluster of cases involving a single lot of contaminated food. These events typically involve errors in food handling that lead to the contamination of a food and an opportunity for growth before that food is eaten. Once the implicated lot of food is no longer available, additional cases cease to occur (Table 4). Scenario 3 consists of outbreaks involving a few to several hundred cases that are scattered with regard to time and location. These outbreaks typically involve an unusually virulent strain that has become established in the food-processing environment and contaminates multiple lots of food over days or months of production (Table 5).

Investigations of cooked meat and poultry operations indicate that a niche is commonly involved (Tables 2 and 6). A niche is a site within the manufacturing environment in which *L. monocytogenes* becomes established and multiplies. These sites may be impossible to reach and clean by normal cleaning and sanitizing procedures. In fact, in an establishment with an effective *Listeria* control program, the processing environment typically appears clean and acceptable. The sites serve as reservoirs from which the pathogen is dispersed during the processing operation and contaminates product contact surfaces and the food.

In all three scenarios, the growth of *L. monocytogenes* occurs before the contaminated food is eaten. This information can be used as a rationale for establishing control systems that may be more effective in reducing consumer risk. Specifically, the systems should be designed to prevent scenario 3, recognizing that the attainment of this objective should also minimize the risk of scenarios 1 and 2. A second priority is to comply with current regulatory policies, some of which may not be based on these considerations.

## SIGNIFICANCE OF A NICHE

Microbiological testing of the processing environment and the equipment in use is necessary to detect a niche. Examples of niches include hollow rollers on conveyors, cracked tubular support rods on equipment, the space between close-fitting metal-to-metal or metal-to-plastic parts,

TABLE 2. Examples demonstrating that certain strains of L. monocytogenes can become established and persist in the food-processing
environment

Type of food produced at plant	Time of persistence <sup>a</sup>	Country	Implicated in illness?	Serotype(s) <sup>b</sup>	Refer- ence(s)
Cheese	4 years	Switzerland	Yes	4b	5
Cheese, blue veined	7 years	Sweden	No	3b	94
Cheese, goat	11 months	United Kingdom	Yes	4b	3, 63
Fish, smoked	Months	Switzerland	No	Several	6
	14 months	Finland	No	1/2a (86%), 4b (14%)	53
	Months	United States	No	ND	70
Frankfurters	4 months	United States	Yes	1/2a	16, 95
Frankfurters (outbreak strain was not					
isolated from the plant)	Months	United States	Yes	4b	17
Ice cream	7 years	Finland	No	1/2	66
Meat, sliced lunch	4 years	Norway	No	ND	69
Mussels, smoked	3 years	New Zealand	Yes	1/2	7
Pâté (product from one plant was the source					
of an outbreak from 1987 to mid-1989)	2 years	United Kingdom	Yes	4b(x), 4b	64, 72
Pork tongue in aspic (outbreak strain recov-					
ered from the implicated plant)	Months	France	Yes	4b	50, 86
Poultry, cooked	1 year	Ireland	No	1/2	57
Poultry, cooked deli products (outbreak					
strain matched a strain previously isolated from the same plant (95))	12 years	United States	Yes	4b	89
Salmon, cold smoked	4 years	Denmark	No	40 ND	31
Salmon, smoked	8 months	Norway	No	ND	82
Seafood, smoked salmon	Months–2 years	Norway	Possibly	4,1	81
Shrimp, raw shelled frozen	NS	Brazil	No	1, 4b	25
Trout/salmon, gravad	1 month	Sweden	No	4b	<u> </u>
Trout, gravad and cold smoked	11 months	Sweden	Yes (gravad)	4b	28
Trout, smoked/salmon, gravad	>4 years	Sweden	Possibly	1/2a	<u>5</u> 9
Trout, cold smoked	NS	Finland	No	1/2	2

<sup>a</sup> NS, not stated.

<sup>b</sup> ND, not determined.

worn or cracked rubber seals around doors, on-off valves and switches for equipment, and saturated insulation. Table 6 provides an extensive list of examples of sites that have been found to be sources of listeriae in commercial facilities producing a wide variety of RTE meat and poultry products. It is significant that the source was often limited to very specific sites of growth that led to the contamination of product contact surfaces during production. The location of the niche was typically limited to a specific packaging line (i.e., a number of pieces of equipment, such as slicers, tables, conveyors, and packaging machines used in series for packaging RTE foods). Parallel packaging lines located within a few feet of the positive line consistently tested negative. This finding indicates that sampling plans should include all of the packaging lines at a frequency that is adequate to detect a loss of control. Furthermore, products being produced on packaging lines that are adjacent to a line that has tested positive should be considered acceptable as long as monitoring data support this assessment.

In some cases, extensive sampling was necessary before the ultimate source(s) could be detected. Furthermore, the sources were often not detectable unless the equipment was operating with product being processed. This requirement creates a dilemma for which there is no ready solution: a significant period may elapse from the time a problem is first detected to the time its source is discovered. Additional time is then needed to take the necessary corrective actions to eliminate the source and verify that the problem has been corrected. The period from the time the first positive sample is detected to the time the source is investigated can be reduced by analyzing all routine monitoring samples individually rather than compositing. However, analyzing all routine monitoring samples as individuals will significantly increase the analytical workload. This would be particularly burdensome for smaller establishments. In some cases, prior experience may point to certain equipment or sites as sources and indicate where corrective actions should be taken.

A rather common misconception is that air is a notable source of contamination. Throughout 14 years of investigation (unpublished data), the air in a room has never been found to be a chronic source of contamination of product contact surfaces. Others have reported similar findings (2, 51, 57, 86). Specific examples, however, can be cited in which air from compressed air lines has been implicated and has been traced to a niche near the point of use (e.g.,

Type of food	Year <sup>a</sup>	Country	Factors leading to case	Sero- type <sup>b</sup>	Reference(s)
Cheese, goat	1988	United Kingdom	An otherwise healthy 40-yr-old wom- an ate about 85 g about 24 h be- fore onset of symptoms. Four pack- ages from the same lot had <i>L</i> . <i>monocytogenes</i> at $30 \times 10^6$ to $50 \times 10^6$ CFU/g. Elevated display case temperature may have been a factor.	4b	3, 63
Chicken, cooked and chilled; purchased at a supermarket	1988(?)	United Kingdom	A 31-yr-old woman delivered a non- viable 23-wk-old fetus 5 days after eating leftover chicken that had been held in the refrigerator for 3 days.	4	55
Chicken nuggets	1989	United Kingdom	The patient was a 52-yr-old woman on steroids for lupus erythemato- sus. It was found that her healthy 29-yr-old son had milder symp- toms. Chicken from a take-away shop was the assumed source, but this conclusion may have been in- fluenced by a Centers for Disease Control and Prevention case control study implicating undercooked chicken as a risk factor. Other foods were not mentioned.	1/2a	54
Frankfurters	1988	United States	A consumer with cancer ate one frank heated in the bun for 45 s on high in a microwave oven each day for lunch.	1/2a	16, 95
Meat, sliced lunch Milk, raw	1999 Early 1950s	United States Germany	The patient was an elderly man. An infant drank milk from a cow with listerial mastitis.	NS NS	35 Cited in 87
Sausage, homemade	1988(?)	Italy	The patient was an apparently other- wise healthy man. Analysis of the remaining sausage yielded <i>L. mono-</i> <i>cytogenes</i> at $2.7 \times 10^6$ CFU/g. The sausage was made from cooked pork stuffed into raw natural casing and then held at 20–22°C for 24– 36 h before eating.	4	13
Sausage	1989	United States	The patient was a 94-yr-old man with a history of colon cancer. The sau- sage consisted of cooked pork, rice, etc., stuffed into raw natural casing and sold in a package labeled "Fully Cooked."	4b	36
Vegetable rennet	1988(?)	United Kingdom	A 29-yr-old woman miscarried at about 23 wk gestation. The bottle of rennet, held for 3 mo in the re- frigerator, was the only item to contain the same isolate as the fe- tus.	4	55

TABLE 3.	Examples	of isolated	cases (i.e.,	scenario 1	)
----------	----------	-------------	--------------	------------	---

 $^{\it a}$  A question mark indicates that the year listed is the year of publication.

<sup>b</sup> NS, not stated in the report.

Type of food	Year	Country	Factors leading to cluster/outbreak	Reference(s)
Coleslaw	1981	Canada	Cabbage was fertilized with manure from sheep with listeriosis and held in cold storage for months, allowing possible <i>L. monocytogenes</i> growth before the cabbage was used to make coleslaw.	87
Milk, chocolate (this outbreak also fits the scenario 3 definition because other cases occurred and multiple lots of milk were involved) <sup><i>a</i></sup>	1994	United States	The milk was poorly refrigerated after pasteurization and was temperature abused before being served at a July picnic.	
Salad, corn and tuna <sup>a</sup>	1997	Italy	A blend of canned corn and canned tuna prepared on May 20 was evi- dently contaminated during prepara- tion. Subsequent tests suggested that the time and temperature would have allowed growth.	1
Salad, rice <sup>a</sup>	1993	Italy	The salad was stored overnight at ambi- ent temperature in June.	85
Shrimp, cooked	1989	United States	Ten of 36 persons attending a party de- veloped listeriosis. Consumption of shrimp cooked on the day of the party remained a significant risk fac- tor after controlling for the consump- tion of other foods.	77

TABLE 4. Examples of clusters of cases involving a single lot of food that typically became contaminated and was held at conditions permitting the growth of L. monocytogenes before the food was eaten (i.e., scenario 2)

<sup>a</sup> Outbreaks of listerial gastroenteritis, not invasive listeriosis.

growth in a filter) or air from an automated bag opener located under a table that was not properly cleaned and maintained. On one occasion, the exhaust from a small pump near the floor was an unexpected source. Experience also indicates that equipment placed too close to floor drains is more difficult to control, perhaps because of aerosols created during sanitation or air currents that may come from the drains as water levels change in the drainage system. This latter possibility is speculative and has not been confirmed by testing. Previous research that predates concern about *L. monocytogenes*, however, has demonstrated that floor drains can be a source of microorganisms to the immediate surrounding air space (41).

Evidence indicates that construction in an area where

TABLE 5. Examples of outbreaks involving cases scattered with regard to time and location, typically involving an unusually virulent strain of L. monocytogenes that had become established in the environment and contaminated multiple lots of food over days or months (i.e., scenario 3)

Implicated food	ated food Year(s)		No. of cases	Reference(s)	
Butter <sup>a</sup>	1998–1999	Finland	18	61, 62	
Cheese	1983-1987	Switzerland	122	5, 72	
Cheese, Brie	1995	France	36	39, 79	
Cheese, Mexican-style	1985	United States	142	58	
Franks (lunchmeat?)	1998–1999	United States	$\sim 100$	17	
Milk, chocolate	1994	United States	53	23	
Pâté	1987-1988	United Kingdom	>300	63	
	2000	United States	11	15	
Pork tongue in aspic	1992	France	279	86	
	1999-2000	France	26	26, 27	
Pork rillettes	1993	France	39	80	
Poultry, cooked	2000	United States	29	18	
Trout, cold smoked/gravad	1994–1995	Sweden	6–8	28	
Unknown	1975-1976	France	≤167	14	
	1985-1987	Denmark	35	38	

<sup>a</sup> This outbreak involved an exceptionally sensitive population.

RTE products are exposed can increase the risk of product contamination. Some believe this increased risk is due to dust that is dispersed throughout the area. Such contamination may occur during construction, but of greater concern is the potential introduction of a new, more virulent strain of L. monocytogenes into the environment from an outside source or through the disturbance of a harborage site (e.g., the process of replacing floor drains, walls, or cooling units). Should these strains become established in a niche in the environment in which RTE products are processed, the potential for product contamination may increase. To address this concern, even greater attention is now being paid to separating construction zones from other areas where production continues to occur. In addition, the routine sampling program may be temporarily modified by increasing the number of samples or sample sites to verify that control is being maintained.

## SIX STRATEGIES FOR CONTROLLING L. MONOCYTOGENES

If the preceding information is considered, then the basic components of a Listeria control program become clearer and include the following strategies: (i) prevention of the establishment and growth of listeriae in niches or other sites that can lead to the contamination of RTE foods; (ii) implementation of a sampling program that can assess in a timely manner whether the environment in which RTE foods are exposed is under control; (iii) as rapid and effective a response as possible to each positive product contact sample; (iv) verification by follow-up sampling that the source has been detected and corrected; (v) a short-term assessment (e.g., involving the last four to eight samplings) to facilitate the detection of problems and trends; and (vi) a longer-term assessment (e.g., quarterly, annually) to detect widely scattered positive sites on a packaging line and to measure overall progress toward continuous improvement.

An earlier paper (93) provided guidelines for controlling listeriae in an environment in which RTE foods are exposed. Some guidance on environmental testing has also been provided (92, 93). This paper will provide further guidance on environmental testing and other information.

## ENVIRONMENTAL SAMPLING PROGRAMS

Two factors determine the effectiveness of a *Listeria* control program: (i) the design of the environmental testing program and (ii) the response to a positive finding. A routine environmental testing program is essential for providing a continuing assessment of control. In the event that a positive product contact sample is detected, corrective actions should be initiated to identify and control the source of contamination, thereby minimizing product contamination. A wide variety of schemes for sampling the environment are used throughout the food industry. Where possible, the samples should be analyzed individually. In plants that have few positive samples, however, compositing the sponge or gauze pad samples from each packaging line is an acceptable alternative. In addition, compositing may be necessary to minimize the cost to smaller operations.

Environmental sampling programs should reflect pre-

vious experience in each facility. The sampling sites should include areas that have been found to be good indicators of control. The sites sampled should include equipment surfaces to which the food is exposed, for example, between cooking and final packaging. Additional samples, such as saturated brine used for chilling cooked products, should be included. Other sampling sites may include floors near packaging machines and in coolers where exposed product is held for further processing. The program should be viewed as a routine investigative sampling program that targets selected sites to detect a loss of control (49). The sampling program is not based on a statistical sampling plan, and it is not possible to determine a specific level of confidence that a product will not become contaminated even though all the samples are negative. Despite this perceived weakness, experience has shown that a well-designed environmental sampling program can be remarkably sensitive in detecting a loss of control.

Experience has shown that the RTE-food-processing environment in many establishments should be sampled at least weekly from each packaging line, with emphasis on product contact surfaces (92). Sampling frequency should depend on risk to consumers if the food becomes contaminated. Specifically, there should be little need for an extensive sampling program if (i) it is known that contamination cannot occur after a lethal treatment (e.g., canned or cook-in-bag products) or (ii) growth cannot occur from the time the food is produced to the time it is eaten (e.g., frozen, dried, or acidified foods or certain fermented foods). Also, consideration must be given to how the food will be handled and prepared before it is eaten (46).

The data must be organized and reviewed as they become available. One simple method is to review the results for the last four to eight samplings. This approach provides a moving window that can help to detect patterns and trends over the past 1 to 2 months, assuming samples are collected on a weekly basis. Ideally, the results also should be reviewed annually, if not quarterly, to obtain a longer-term perspective and identify problems that might otherwise go undetected. While it would be preferable to analyze and control specifically for *L. monocytogenes*, some companies may limit the analyses to *Listeria*-like colonies on modified Oxford agar or colonies that have been confirmed to belong to the genus *Listeria*.

A successful sampling program will be aggressive in attempting to detect listeriae. In addition, an effective *Listeria* control program must take into account the human element as well as the scientific basis for control. It is important to recognize that even with an effective control program, extensive testing will periodically detect positive samples. Such a finding should be viewed as a "success" because it indicates that the monitoring program has been effective, the problem can be corrected, and consumer protection can be ensured. Recrimination against plant management for the presence of this ubiquitous bacterium invariably proves counterproductive in the long term. It is part of human nature to avoid problems, and it is also fairly easy to generate negative results when testing for listeriae. In recognition of this important human element, the best

Product	Equipment or area	Source(s) of contamination (i.e., niches or other sites of growth)	Corrective action(s) taken
Franks	Continuous brine chill chamber for product sus- pended from smoke sticks	Sponge rubber seals around edge of doors at top and side of chill unit	Rubber seals were removed; doors were redesigned so that seals were not needed
	Hopper that catches franks after peeling	Cinder blocks around opening in wall between peeler room and packaging room	Cinder blocks were sealed to prevent moisture from accumulating in the blocks; stainless steel lip was in- stalled around top of opening to di- vert moisture down the side
	Continuous brine chill chamber for product on racks with wheels	Doors made of rubber-coated fabric, large metal hinges extending the width of the door, and hollow bump guards at bottom of door	Doors were replaced with rigid clean- able plastic material; large hinges and bump guards were removed
	Ammonia unit used to chill brine solution	Fiberglass insulation on ammonia line to brine chilling unit became satu- rated with brine splashing from chilling unit	Contaminated insulation was removed pipe and area were cleaned and sanitized; Insulation was not placed too close to pipe to brine chiller
	Refrigeration unit near ceiling of holding cooler before peeling	Condensate from refrigeration unit	Refrigeration unit was cleaned and sanitized
	Area of brine chill exit and peeler	Hoses and spray nozzles at exit end of brine chill tunnel used to spray down franks for easier peeling	Hoses and nozzles were replaced; dai- ly cleaning was initiated
	Collator and conveyor	Undetermined	Equipment was covered with large tarp and steam was injected
	Peeler area	Overhead on/off valves for steam and water lines near peeling equipment	Area was included in daily sanitation program
Franks and similar linked products	Peeler area (multiple events)	Peeler	Peelers were modified for ease and ef- fectiveness of cleaning; centralized casing removal systems were in- stalled to avoid operator contact with spent casings; metal boxes with steam ports were built so that peelers could be steamed each day before start of operation
Franks	Incline conveyor leading out of peeler room into packaging area	Two-ply Plexiglas shield guard on un- derside of conveyor had a crack where meat particles became en- trapped	Plexiglas was replaced with stainless steel guard
	Brine chill	Construction of brine chill tunnel had stainless steel framing with metal touching metal, causing an unclean- able space	Framing was modified to facilitate cleaning and to prevent material from getting into the space
	Incline conveyor leading from peeler room to packaging area	Contaminated liquid was discovered within a hollow split sprocket	Hollow sprocket was replaced with solid sprocket
	Wall in peeler room	Insulation behind fiberglass wall was contaminated by condensate from overhead pipe(s)	All fiberglass/insulation was removed from wall; concrete wall was cleaned with an acid base cleaner, sanitized, and sealed: overhead pipes were rerouted to be closer to the floor
	Casing removal system (a long pipe through which vacuum conveys casings from the peeler to a can- ister in another room)	Design made cleaning difficult; inade- quate cleaning and sanitizing	System was rebuilt to shorten length, replace existing pipe with stainless steel, and remove deadends and 90° angles; training and education were provided to supervisor and cleaning person

TABLE 6. Examples of sources of contamination by Listeria species or Listeria-like organisms in RTE-food-processing operations and corrective actions that were taken (1989–2000)

#### TABLE 6. Continued

Product	Equipment or area	Source(s) of contamination (i.e., niches or other sites of growth)	Corrective action(s) taken
Sliced lunch meats	Slicer	Worn hydraulic seals at base of slicer, oil with water and product residue	Slicer was stripped, cleaned, sanitized, and placed into oven, where moist heat was applied; seals were replaced; slicer was put on preventive mainte- nance schedule; oil was used with lis- tericidal additive (sodium benzoate)
Sliced ham from cans	Slicing/packaging line	Can opener with heavy wire safety cover	Cover was modified so that it could be removed daily for cleaning (OSHA had required that it not be removable for employee safety)
Sliced pepperoni	Slicer	Buildup inside safety cover over gear and drive belt; material from this site contaminated product conveyor locat- ed below	Cover was changed so that it could be removed for cleaning each night
Diced cooked meat or poultry	Dicer (multiple events)	Undetermined	Dicer was placed into oven and moist heat was applied, or dicer was cov- ered with tarp and steam was applied
Cooked sausage	Packaging machine	Crack in stainless steel covering on top edge of the packaging machine near loading area	Area was cleaned, sanitized, and welded
Cooked products	Conveyors (multiple events)	Hollow rollers	Hollow rollers were replaced as de- tected; where possible, conveyors were replaced with sloping stainless steel slides
Hams	Brine chill tunnel for prod- uct on hanging racks	Damaged rubber seals on stainless steel door at exit end of tunnel	Damaged door seals were replaced; cleaning procedure was modified
Cooked turkey prod- ucts	Conveyor between shrink tunnel and boxing	Worn conveyor made of rubber-coated fabric	Conveyor was replaced with one of new material
Cooked turkey breast	Conveyor leading to pack- aging machine	Fabric conveyor belt material	Belt was replaced with stainless steel slide
	Cooked product stripping area	Hand-held knives for opening product	Knives were cleaned and sanitized daily in an automatic washer and were not stored in lockers
Large cooked products	Bagging table	Air duct at base of table for blowing bags open	Table was modified to make duct ac- cessible for nightly cleaning
Breaded products	Exit conveyor from spiral freezer	Wheel bearings for conveyor belt	Wheel bearings were removed and replaced
	Spiral freezer	Undetermined	Cleaning frequency was increased and equipment was allowed to defrost be- fore cleaning
Cooked meat patties and links	Between freezer and pack- aging machine	Overhead conveyor	Safety ladder was provided so that conveyor could be cleaned from above rather than from below
	Wire mesh conveyor be- tween oven and freezer	Hollow support rods for conveyor	Hollow support rods were replaced with solid support rods
Cooked sausage links	Packaging machine	Stainless steel rods for pushing prod- uct into carton	Push rods were removed, cleaned, and sanitized on daily basis

approach is to provide sufficient technical assistance and laboratory support to help restore control. The information gained can be used to reduce, perhaps prevent, additional positive samples. Sharing experiences with others can be very helpful and should be encouraged.

For the reasons just mentioned, corporate and regulatory policies should encourage environmental sampling programs and consider positive findings more as a success of the monitoring program and less as a failure of control. Working cooperatively, industry and regulatory agencies may be more successful in preventing the likelihood of scenario 3 events and minimizing the occurrence of scenarios 1 and 2.

# DEGREE TO WHICH PACKAGING LINES CAN BE CONTROLLED

Results from 10 to 12 plants producing a wide variety of RTE meat and poultry products indicate that listeriae can be controlled but not eliminated from the cooked-productprocessing environment. For example, 50 to 68% of 79 to 106 packaging lines tested negative throughout the year 6

≥7

Total

% of the total of 148 positive sample sets 1

1

0.7

0

0

No. of samples	No. of sample sets with:						
collected per packaging line	1 positive	2 positives	3 positives	4 positives	5 positives	6 positives	≥7 positives
2	9						
3	19	1					
4	2	1					
5	28	3		1	1		

5 3

8

5.4

1

2

1.4

8

6

19

12.8

TABLE 7. Distribution of samples from product contact surfaces that tested positive for Listeria-like organisms over a 1-year period<sup>a</sup>

<sup>a</sup> All samples were analyzed separately (i.e., not composited).

33

26

117

80.4

when these lines were monitored weekly from 1990 through 1999. Another 20 to 29% of the lines had only one or two positive weekly samplings, and 12 to 22% had three or more positives in a year. The routine methods employed throughout this period were designed to detect *Listeria*-like organisms or *Listeria* species, not *L. monocytogenes* specifically. Colonies that appeared to be typical for *Listeria* on modified Oxford agar plates were recorded as *Listeria*-like. If the typical colonies were streaked onto standard plate count agar and yielded characteristic colonies, they were reported as *Listeria* species. Questionable colonies on standard plate count agar were examined microscopically for small rods and tested for positive catalase and negative oxidase activity before being recorded as *Listeria* species.

# NUMBER OF POSITIVE SAMPLES IN A SAMPLE SET

The number of samples collected from a packaging line should be adequate to assess control and might range from 2 to 10 samples. The number selected should reflect the prior history of control and the complexity of the system. In a few cases, a list of up to 20 sites may be identified and a fixed number of samples may be randomly collected each week from among sites on the list. To provide information on the minimum number of samples that would be adequate to assess control, the data for 1 year from approximately 200 packaging lines were tabulated.

TABLE 8. Number and frequency of product contact surfacesample sets that tested positive for Listeria-like organisms on con-secutive weekly samplings over 2 years<sup>a</sup>

	No. of posi- tive sets	% of posi- tive sets
1 positive set, with the next set negative	483	66.1
2 consecutive positive sets	136	18.6
3 consecutive positive sets	36	4.9
4 consecutive positive sets	32	4.4
$\geq$ 5 consecutive positive sets	44	6.0

<sup>*a*</sup> The total number of sample sets collected and analyzed was 15,778, and the number of sets that tested positive was 731.

For the following discussion, a sample set consists of all samples collected from a packaging line at one time. The number of samples collected from each packaging line was determined according to the complexity of the operation and the equipment. The numbers of samples testing positive in the sample sets are summarized in Table 7. For example, for all of the packaging lines from which six samples were collected each week, there were 33 instances in which one of the samples tested positive, 8 instances in which two of the samples tested positive, and 5 instances in which three of the samples tested positive. A positive sample was determined by the presence of *Listeria*-like colonies on modified Oxford agar plates.

1

0.7

During the year, 148 positive sample sets yielded one or more positive samples. On 117 (80.4%) occasions, only one sample in the sample set tested positive. On 19(12.8%)occasions, two of the sample sites tested positive. Moreover, there were 8, 2, and 2 occasions on which 3, 4, and  $\geq$ 5 samples tested positive, respectively. These data indicate that when listeriae were detected on product contact surfaces, the distribution normally was limited and either the level was low or listeriae were not widespread across the surfaces being sampled. The data could be interpreted to suggest that increasing the number of sample sites would increase the probability of detecting listeriae within a sample set. There are limitations, however, to the number of samples that can be collected. Some packaging lines are lengthy and complex, while other lines consist of a single table for bulk packaging of the product into boxes. The cost impact also must be considered when establishing a routine monitoring program.

# FREQUENCY OF CONSECUTIVE POSITIVE RESULTS

Another factor to consider is the number of times a packaging line is found to be positive on consecutive samplings. The data in Table 8 summarize results for 2 years during which 15,778 sample sets were collected from about 200 packaging lines producing a wide variety of products. On 731 (4.6%) occasions, a packaging line was positive for listeriae. On 483 (66.1%) of those occasions, the packaging line was positive one week and negative the next (i.e., an

isolated positive). On 136 (18.6%) occasions, a positive sample was detected from a packaging line for two consecutive weeks. On 112 (15.3%) occasions, certain packaging lines were positive for three or more consecutive weeks. Again, these data indicate that when a positive packaging line is detected, the degree of contamination is normally limited to an isolated positive finding or two consecutive positive findings. These events accounted for about 85% of the positive findings.

When the data in Tables 7 and 8 are considered together, it is evident that listeriae are usually very limited in both distribution and frequency, in part because every positive finding is pursued to eliminate the contamination. This process involves a variety of corrective actions and a level of sampling beyond that required by the basic monitoring program.

These data support a policy with an initial emphasis on implementing corrective actions and not on product testing in the event that a product contact surface sample gives a positive test result. If the initial corrective actions are not effective, then product testing becomes more appropriate. It is a matter of judgment whether product testing, which involves holding all the product produced from the positive line, should be applied after the first or the second positive finding. The data indicate a 33.9% probability that a line will again test positive on the next consecutive sampling.

Of the greatest concern are lines with repeated positives over a prolonged period. Some of these events reflect difficulty in finding the source (i.e., niche). Hundreds of samples may be necessary to detect the source before effective corrective actions can be implemented. It is important that the monitoring program be capable of identifying these events, particularly so that appropriate safeguards (e.g., placing a product on hold and testing it) can be implemented until the problem is resolved. At its best, a monitoring system will reveal the extent of a problem so that resources that are typically limited can be directed where attention is most needed.

# THE RELATIONSHIP BETWEEN LISTERIA SPECIES AND L. MONOCYTOGENES

What is the likelihood that a sample testing positive for *Listeria* species would be confirmed to be positive for *L. monocytogenes*? During 1990 and 1991, approximately 18,000 environmental samples were analyzed from 12 plants producing a variety of RTE meat and poultry products (92). It was found that 44% of the samples yielded black modified Fraser broth, 15% yielded suspect colonies on modified Oxford agar plates, and 13% were determined to be positive for *Listeria* species. Subsequent tests found that 40% of the samples with *Listeria* species were confirmed to contain *L. monocytogenes*.

The likelihood that a sample containing *Listeria* species would contain *L. monocytogenes* varied with each plant (Table 9). From 1987 through 1991, this likelihood remained relatively stable within each plant. Thus, the significance of a finding of *Listeria* species was highly dependent on the unique ecology that was characteristic of each plant.

TABLE 9. Relationship between environmental samples testing
positive for Listeria species and the presence of L. monocytogeness

Plant	No. of positive <i>Listeria</i> samples	% of positive samples with <i>L. monocytogenes</i>
1	115	96
2	90	71
3	128	62
4	328	57
5	237	54
6	204	47
7	46	41
8	85	38
9	90	34
10	219	27
11	241	23
12	318	5

Some advantages of testing only for *Listeria* species or *Listeria*-like organisms rather than testing for *L. monocytogenes* are that the results become available sooner and at a much lower cost, there is greater latitude in methodology, and the concern that *Listeria innocua* or other species will mask the presence of *L. monocytogenes* is avoided. Moreover, a program that is based on the control of *Listeria* species is more conservative and will control *L. monocytogenes*. It is important, however, to respond to all positive *Listeria* species results as though they are *L. monocytogenes*.

#### SEASONALITY

Experience has shown a higher prevalence rate in the processing environment during the summer months (92). This general pattern persisted for over 10 years, with the degree of fluctuation being reduced through a process of continuous improvement, application of the recommendations previously reported, and reduced response times in the event of positive samples (91–93). The higher prevalence in the summer months was likely related to increased production and greater difficulty in maintaining control of the processing environment. Similar experience has been noted in the cold-smoked fish industry, with more positive samples being detected during periods of intensive production (i.e., in November and December) just before the holiday season (40). Thus, high throughput can be an important factor in influencing control.

The above findings must be interpreted with caution. The relative influence of seasonality as a function of throughput, warmer temperatures, and level of control may depend on the plant, the type of food being processed, and other factors yet to be identified. For example, a lowthroughput plant that is not in control would be more likely to yield a higher percentage of positive environmental samples than a high-throughput plant that maintains an aggressive program of sampling and responding to the results.

## **RESPONSE TO A POSITIVE FINDING**

The ultimate goal of a sampling program is to control the environment so that all of the product contact surface

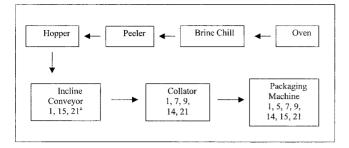


FIGURE 1. Example showing how positive results for samples collected from 1 to 21 August can be mapped for the steps from cooking to packaging along a frankfurter line. <sup>a</sup> Numerals indicate the dates in August on which positive samples were detected on the incline conveyor.

samples consistently yield negative results. If a positive sample is detected, however, corrective action is necessary. In general, the equipment that is the source of the positive sample should be dismantled (while suspicious sites are sampled), cleaned, and sanitized. This procedure is normally adequate, and it is the preferred corrective action. Occasionally, extensive dismantling and cleaning will prove ineffective. For smaller equipment with many parts, cleaning in a recirculating bath of hot water with detergent will be effective, particularly because of the heat. Often, for larger equipment that can be moved, sensitive electronics, oil, and grease can be removed, and the equipment can be moved into an oven (e.g., a smokehouse) for heating with moist heat. If this is not possible, the equipment can be covered with a heat-resistant tarp, and steam can be introduced from the bottom. When steam heating is carried out in an oven or under a tarp, the target is to achieve an internal temperature of 160°F (71°C) and to hold this temperature for 20 to 30 min. Thermocouples placed within the equipment can be used to monitor the temperature.

Even in the best of operations, listeriae can become established in a niche and lead to positive product contact surface samples. To regain control, it is necessary to determine the source of the contamination so that appropriate corrective actions can be taken. A simple map showing the layout of the rooms and the equipment can be beneficial. As positive sites are detected, these sites should be marked on the map with the dates. A very simple schematic drawing such as that in Figure 1 or a blueprint of the facility can be used. By organizing the results to show which sites test positive more frequently and where the positive samples first occur, the source of contamination can be more easily located. In an environment that has been in control, this will often lead to specific equipment that is harboring the bacterium. In general, contamination flows down along or through processing equipment with the flow of the product.

An abbreviated analytical method for listeriae can be used in investigating a source of contamination. For example, it is faster and much cheaper to stop the analysis following incubation of the modified Fraser broth tubes. By striving for samples that do not yield black tubes, samples from more sites and from different times can be processed, and more information can be obtained sooner.

# FACING THE REALITY THAT LISTERIAE WILL CONTINUE TO BE INTRODUCED INTO THE RTE-FOOD-PROCESSING ENVIRONMENT

Despite the best efforts, listeriae will continue to be reintroduced into food-processing environments. Failure to control listeriae on floors increases the likelihood that packaging lines will eventually test positive. One method to control listeriae on floors is to scrub the floors with caustic powder, rinse them, sanitize them with a high concentration of sanitizer (e.g., 800 to 1,000 ppm quaternary ammonium compound), and dry them. Maintaining clean, dry floors can be effective in most situations. A fine application of crystalline citric acid to maintain a pH of  $\leq 4.5$  (as indicated by pH paper) can improve control in certain areas, but the flooring material must be able to withstand this treatment. Other methods (e.g., frequent application of sanitizer) may prove necessary in areas where the floor remains wet because of the type of operation.

Cleaning and sanitizing procedures should be directed toward *Listeria* control. Cleaning more frequently during production, at midshift, or between shifts is counterproductive and detrimental to *Listeria* control and must be avoided. A clean, dry environment is preferable to a wet environment during production. Contamination is normally limited to a single packaging line, with adjacent lines not being affected. Random contamination from air, people, packaging materials, and so forth, is minor. In a facility with a controlled environment, growth within a niche is the major concern. It should be apparent that an assertion that *Listeria* contamination is due to poor sanitation indicates a lack of understanding of this difficult issue.

Extensive research has shown that bacteria adhering to surfaces in biofilms are more resistant to sanitizers. This may lead some to believe that biofilms are a key factor influencing the survival and growth of listeriae in the environment and on or in equipment. This may be true in certain closed systems that rely on clean-in-place technology. In open systems, however, available chemical agents are very effective for removing listeriae, provided that adequate mechanical action (e.g., scrubbing) is applied to the surfaces, before rinsing and sanitizing. Exposed surfaces, however, are seldom sources of listeriae. Of greater concern are enclosed areas (e.g., within a hollow roller on a conveyor) where food deposits and moisture accumulate and cannot be removed by normal cleaning, scrubbing, and disinfecting. These harborage sites are not biofilms per se, but rather niches in which a variety of bacteria become established and multiply.

### **FUTURE DIRECTIONS**

Some future changes will be necessary to improve and maintain control of the food-processing environment. Continued improvements in equipment design are needed to facilitate cleaning, to eliminate potential harborage sites, and to minimize breakdowns and repairs during operation. A certification program is being developed for equipment to be used in meat- and poultry-processing operations. There will likely be more extensive use of steam as described above for sanitizing certain equipment at some routine frequency (e.g., weekly). For this purpose, equipment must be designed so that electronic components can be protected when steam is applied. More durable floors are needed to withstand the increased use of chemicals. Improved control is needed for recirculating brine solutions for chilling foods (e.g., frankfurters, hams) after cooking. One method that shows promise is to acidify the brine solution to a pH of  $\leq 3.5$  with citric acid. There will be increased use of postpackaging pasteurization with irradiation, hot water, steam, high pressure, and so forth.

New food additives that inhibit *L. monocytogenes* will be introduced and will become more widely used in foods in which growth can occur. Current USDA-FSIS policies, however, provide few options for inhibiting *L. monocytogenes* in RTE meat and poultry products. The most widely used additives include sodium lactate, sodium diacetate, and combinations of the two (e.g., 2% sodium lactate on a dry weight basis and 0.1 to 0.15% sodium diacetate). Other methods (e.g., the addition of peptides or live lactic acid bacteria cultures) are being investigated as additional means to prevent growth during refrigerated storage of a variety of perishable foods.

USDA-FSIS policy now requires the addition of a critical control point when new ingredients are added for the control of listeriae. This requirement places an unnecessary burden on companies that are willing to pay the extra cost of the additive(s) and use them to enhance consumer protection. Instead, their use should be encouraged and not treated differently from that of traditional inhibitory additives such as salt, sodium nitrite, acidifiers, and smoke. For foods in which *L. monocytogenes* can multiply, the primary line of defense is to prevent product contamination; inhibitory additives can be viewed as additional insurance in the event that contamination occurs.

Likewise, restrictive regulatory requirements for validation of postpasteurization treatments that may not offer a 5 or 6D reduction should be avoided. Some postpasteurization methods may offer incremental protection that can be beneficial for enhancing consumer protection, and their use should be encouraged.

One of the outcomes of the Food and Agriculture Organization-World Health Organization risk assessment for L. monocytogenes in RTE foods is that risk increases with the number of cells consumed (32). Thus, consumer protection is not a presence-or-absence issue, but rather a number-of-cells-consumed-per-serving issue. While this may have been obvious to some, this information provides guidance to the RTE food industry as it strives to minimize risk. To date, the industry has been seeking technologies that prevent or eliminate L. monocytogenes in RTE foods, but for some foods this is an unachievable goal. The industry should consider technologies that can minimize risk by reducing the number of cells likely to be consumed. This could be achieved, for example, through the use of additives that extend the lag phase but may not prevent eventual growth or through postpackaging technologies that can significantly reduce but may not eliminate L. monocytogenes in a food. For certain products, until the ideal technology

becomes available, combinations of partially effective control measures may be the preferred method to minimize risk while still producing products of acceptable quality. Admittedly, producers operating in a regulatory environment of zero tolerance for *L. monocytogenes* would still be in jeopardy if their product tested positive, but they would be closer to achieving the greater goal of consumer protection.

## CATEGORIZATION OF FOODS ACCORDING TO RISK TO CONSUMERS: FOODS THAT DO NOT SUPPORT GROWTH

Certain types of food are of low risk to consumers because they do not support the growth of L. monocytogenes. Such foods include products with a low pH and/or low water activity (e.g., barbecued products, fermented dry sausage, jerky, dry cured meats, precooked bacon) and frozen products that are typically heated before serving (e.g., frozen dinners, entrees, pizza). Many other types of products are of no apparent risk because they are cooked in the container in which they are sold or they are hot filled at a temperature that will preclude the presence of L. monocytogenes. The policies of Canada and legislation currently being considered by the Commission of the European Communities, for example, place foods that do not support growth into a lower-risk category. More stringent criteria are applied to those foods that do support growth or that are intended for higher-risk populations.

Regulatory policy should reflect differences in risk (46). Current FDA and USDA tolerances should be changed to recognize a food safety objective of no more than 100 CFU/g at the time the food is eaten. Such an objective acknowledges the lower likelihood that small numbers of cells will be involved in foodborne listeriosis, the widespread distribution of *L. monocytogenes* in our environment, and the difficulty in producing products that will consistently test negative for *L. monocytogenes*. It has been estimated that foods containing no more than 100 CFU/g when they are eaten would provide a level of consumer protection comparable to that of foods that meet a criterion of "absence in 25 or 50 g" (32).

# THE NEGATIVE IMPACT OF A ZERO TOLERANCE POLICY ON EFFORTS TO CONTROL L. MONOCYTOGENES

Several practical reasons have already been given for testing for *Listeria* species or *Listeria*-like organisms and not specifically for *L. monocytogenes*. Additional reasons stem from the FDA and USDA-FSIS zero tolerance policy for *L. monocytogenes* in food and from changing FSIS policies. Current FSIS policy requires the recall of a product in which *L. monocytogenes* has been found, because such a product is considered adulterated.

Current USDA-FSIS policy also assumes that all of the food produced on a packaging line is adulterated if *L. monocytogenes* has been found on a product contact surface. The amount of the product implicated includes all of the food that came in contact with the equipment from the start of production until the equipment was cleaned and sanitized.

J. Food Prot., Vol. 65, No. 4

In late 1998, a major recall of sliced lunch meats and franks totaling about 1,800,000 lb occurred after a plant's testing program detected *L. monocytogenes* on product contact surfaces. The product had not been linked to any known cases of listeriosis, but subsequent testing of products from retail outlets by the FSIS yielded *L. monocytogenes*. This event and numerous others since 1987 have discouraged the RTE food industry from confirming the presence of *L. monocytogenes*.

The detection of *L. monocytogenes* in the food-processing environment also is considered evidence by the FSIS that the pathogen is "reasonably likely to occur" and therefore must be addressed in the hazard analysis critical control point (HACCP) plan. This is a requirement that ignores the different roles of the HACCP plan and prerequisite programs in managing the microbiological safety of food. In reality, the requirement cannot be met because postprocessing contamination is not controlled through the HACCP plan but through a wide variety of prerequisite programs (*93*). Again, this regulatory conflict can be avoided by not testing specifically for *L. monocytogenes*.

Finally, if a product from a given establishment is known to have had a chronic problem with regard to positive samples or is suspected of having been the source of human listeriosis, the USDA-FSIS will obtain, through court order if necessary, all existing environmental and product test results from the establishment. In addition, all isolates that the establishment may have recovered from the environment or from the product and maintained in a culture collection for validation tests or other purposes must be provided to the agency along with any pulsed-field gel electrophoresis files that may exist for isolates.

The current regulatory policy is, in essence, a "zero presence" policy that does not encourage testing for *L. monocytogenes* per se. Yet, there is general agreement among industry and agency personnel on the importance of maintaining an aggressive sampling program to assess control of the environment. At present, many industry programs strike a balance between providing maximum consumer protection and working under the constraints of regulatory policy. This situation has stymied research on the ecology of *L. monocytogenes* in the environment and has led to the use of indicators.

Among the changes proposed by the USDA-FSIS in February 2001 (37) is one that would require a product produced on equipment on which *Listeria* species or *Listeria*-like organisms have been detected to be tested for *L. monocytogenes*. The implementation of this proposal would require holding all product produced on a sampled line until the results became available and it was known whether the product could be released. According to the proposal, if subsequent testing of the product revealed *L. monocytogenes*, then the product would be recalled from the market and an accompanying public announcement would be issued. If adopted, this policy would shift the balance away from industry's desire to implement aggressive environmental testing programs, because the industry would find it very difficult to hold all of the product involved and would also frequently fail to meet the shipping times expected by its customers.

The prevalence of product samples testing positive for *L. monocytogenes* in the USDA-FSIS monitoring program suggests the magnitude of the risk posed by the proposed policy to industry as a whole, bearing in mind that some would test for an indicator (i.e., *Listeria* species or *Listeria*-like organisms). Thus, the 1 to 5% prevalence rate mentioned earlier for *L. monocytogenes* presumably would be higher if an indicator were used. The impact of the proposed policy must be weighed against the estimated reduction in cases of foodborne listeriosis from the current estimate of 2,493 cases per year in the United States (65). Very likely, better consumer protection would result from the establishment of a policy that encourages frequent, aggressive testing for *Listeria* species or *Listeria*-like organisms, followed by appropriate corrective actions for positive results.

Clearly, regulatory policy can have a profound influence on industry's willingness to test for *L. monocytogenes* per se and to generate the information needed to better understand the ecology of this potential pathogen in foodprocessing operations. Unfortunately, after more than a dozen years, neither the FSIS nor the industry has gained meaningful insight into the ecology of *L. monocytogenes* in the environment of RTE meat- and poultry-processing operations. The majority of this information must be gleaned from European research.

# ADDITIONAL INFORMATION

A new book from the International Commission on Microbiological Specifications for Foods (49) provides additional guidance on environmental testing and the development of food safety management systems for the control of microbiological hazards in foods. An excellent review of *L. monocytogenes* from Health Canada is available for additional information on this important pathogen (30). A risk evaluation and recommended control measures for cold-smoked fish have become available (9, 33). In addition, two *L. monocytogenes* risk assessments are nearing completion (32, 34). Each will be available through its respective organization's Web site.

#### REFERENCES

- Aureli, P., G. C. Fiorucci, D. Caroli, G. Marchiaro, O. Novara, L. Leone, and S. Salmoso. 2000. An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. New Engl. J. Med. 342:1236–1241.
- Autio, T., S. Hielm, M. Miettinen, A.-M. Sjöberg, K. Aarnisalo, J. Björkroth, T. Mattila-Sandholm, and H. Korkeala. 1999. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Appl. Environ. Microbiol. 65:150–155.
- Azadian, B. S., G. T. Finnerty, and A. D. Pearson. 1989. Cheeseborne *Listeria* meningitis in immunocompetent patient. Lancet i: 322–323.
- 4. Bibb, W. F, B. G. Gellin, R. Weaver, B. Schwarz, B. D. Plikaytis, M. W. Reeves, R. W. Pinner, and C. V. Broome. 1990. Analysis of clinical and food-borne isolates of *Listeria monocytogenes* in the United States by multilocus enzyme electrophoresis and application of the method to epidemiologic investigations. Appl. Environ. Microbiol. 56:2133–2141.
- 5. Bille, J. 1990. Epidemiology of human listeriosis in Europe, with

special reference to the Swiss outbreak, p. 71–74. *In* A. J. Miller, J. L. Smith, and J. G. A. Somkuti (ed.), Foodborne listeriosis. Elsevier, Amsterdam.

- Boerlin, P., F. Boerlin-Petzold, E. Bannerman, J. Bille, and T. Jemmi. 1997. Typing *Listeria monocytogenes* isolates from fish products and human listeriosis cases. Appl. Environ. Microbiol. 63:1338–1343.
- Brett, M. S. Y., P. Short, and J. McLauchlin. 1998. A small outbreak of listeriosis associated with smoked mussels. Int. J. Food Microbiol. 43:223–229.
- Brosch, R., B. Catimel, G. Milon, C. Buchrieser, E. Vindel, and J. Rocourt. 1993. Virulence heterogeneity of *Listeria monocytogenes* strains from various sources (food, human, animal) in immunocompetent mice and its association with typing characteristics. J. Food Prot. 56:296–301, 312.
- Busta, F. F., G. E. Bledsoe, G. J. Flick, L. Gram, D. Herman, M. L. Jahncke, and D. R. Ward. 2001. Processing parameters needed to control pathogens in cold-smoked fish. J. Food Sci. 66(Suppl.): S1055–S1134.
- Buchanan, R., and R. Lindqvist. 2000. Hazard identification and characterization of *Listeria monocytogenes* in ready-to-eat foods. Preliminary Report prepared for the Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods, 17–21 July. Food and Agriculture Organization of the United Nations, Rome.
- Buchreiser, C., R. Brosch, B. Catimel, and J. Rocourt. 1993. Pulsedfield gel electrophoresis applied for comparing *Listeria monocytogenes* strains involved in outbreaks. Can. J. Microbiol. 39:395–401.
- CAC Drafting Group. 1999. Management of *Listeria monocytogenes* in foods. Draft document prepared by the Codex Drafting Group and submitted in preparation for the meeting of the Codex Committee on Food Hygiene to be held in 1999.
- Cantoni, C., C. Balzaretti, and M. Valenti. 1989. Episodio di listeriosi da consuma di insaccato. A case of *L. monocytogenes* human infection associated with consumption of "testa in cascetta" (cooked meat pork product). Arch. Vet. Ital. 40:141–142.
- Carbonelle, B., J. Cottin, F. Parvery, G. Chambreuil, S. Kouyoumdjian, M. L. Lirzin, G. Cordier, and F. Vincent. 1978. Epidemic of listeriosis in western France (1975–1976). Rev. Epidemiol. Sante Publique 26:451–467.
- Carter, M. (Baltimore, Md.) 2000. Final report: investigation of outbreak 99-372. Unpublished data.
- Centers for Disease Control and Prevention. 1989. Listeriosis associated with consumption of turkey franks. Morb. Mortal. Wkly. Rep. 38:267–268.
- Centers for Disease Control and Prevention. 1999. Update: multistate outbreak of Listeriosis—United States, 1998–1999. Morb. Mortal. Wkly. Rep. 47:1117–1118.
- Centers for Disease Control and Prevention. 2000. Multistate outbreak of listeriosis—United States, 2000. Morb. Mortal. Wkly. Rep. 49:1129–1130.
- Cerf, O., and M. Sanaa. 2001. Prevalence of *L. monocytogenes* in ready-to-eat foods at the retail level in France, 1997–1999. Personal communication (O. Cerf).
- Clark, E. E., I. Wesley, F. Fiedler, N. Promadej, and S. Kathariou. 2000. Absence of serotype-specific surface antigen and altered teichoic acid glycosylation among epidemic-associated strains of *Listeria monocytogenes*. J. Clin. Microbiol. 38:3856–3859.
- Cox, L. J., T. Kleiss, J. L. Cordier, C. Cordellana, P. Konker, C. Pedrazzini, R. Beumer, and A. Siebenga. 1989. *Listeria* spp. in food processing, non-food and domestic environments. Food Microbiol. 6:49–61.
- Czajka, J., and C. A. Batt. 1994. Verification of causal relationships between *Listeria monocytogenes* isolates implicated in food-borne outbreaks of listeriosis by randomly amplified polymorphic DNA patterns. J. Clin. Microbiol. 32:1280–1287.
- Dalton, C. B., C. C. Austin, J. Sobel, P. Hayes, W. F. Bibb, L. M. Graves, B. Swaminathan, M. E. Proctor, and P. M. Griffin. 1997. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. New Engl. J. Med. 336:100–105.
- 24. del Corral, F., R. L. Buchanan, M. M. Bencivengo, and P. Cooke.

1990. Quantitative comparison of selected virulence associated characteristics in food and clinical isolates of *Listeria*. J. Food Prot. 53: 1003–1009.

- Destro, M. T., M. F. F. Leito, and J. M. Farber. 1996. Use of molecular typing methods to trace the dissemination of *Listeria monocytogenes* in a shrimp processing plant. Appl. Environ. Microbiol. 62: 705–711.
- de Valk, H. 2000. Outbreak of listeriosis linked to the consumption of pork tongue in jelly in France. Dairy Food Environ. Sanit. 20: 356.
- Dorozynski, A. 2000. Seven die in French *Listeria* outbreak. Br. Med. J. 320:601.
- Ericsson, H., A. A. Eklow, M.-L. Danielsson-Tham, S. Loncarcvic, L. O. Mentzing, I. Persson, H. Unnerstad, and W. Tham. 1997. An outbreak of listeriosis suspected to have been caused by rainbow trout. J. Clin. Microbiol. 35:2904–2907.
- Ericsson, H., P. Stålhandske, M.-L. Danielsson-Tham, E. Bannerman, J. Bille, C. Jacquet, J. Rocourt, and W. Tham. 1995. Division of Listeria monocytogenes serovar 4b strains into two groups by PCR and restriction enzyme analysis. Appl. Environ. Microbiol. 61:3872– 3874.
- Farber, J. M., and P. I. Peterkin. 2000. *Listeria*, p. 1178–1232. *In* B. M. Lund, A. C. Baird-Parker, and G. Gould (ed.), The microbiology of food. Chapman and Hall, London.
- Fonnesbech Vogel, B., H. H. Huss, B. Ojeniti, P. Ahrens, and L. Gram. 2001. Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNAbased typing methods. Appl. Environ. Microbiol. 67:2586–2595.
- 32. Food and Agriculture Organization of the United Nations/World Health Organization. 2001. Joint FAO/WHO expert consultation on risk assessment of microbiological hazards in foods. Risk characterization of *Salmonella* spp. in eggs and broiler chickens and *Listeria monocytogenes* in ready-to-eat foods, 30 April–4 May. Food and Agriculture Organization of the United Nations, Rome, [Internet, WWW], ADDRESS: http://www.fao.org/WAICENT/ FAOINFO/ECONOMIC/ESN/pagerisk/reportSL.pdf.
- 33. Food and Drug Administration, 2001, "Processing Parameters Needed to Control Pathogens in Cold Smoked Fish," Food and Drug Administration, [Internet, WWW], ADDRESS: http://www. cfsan.fda.gov/~comm/ift2-toc.html.
- 34. Food and Drug Administration–U.S. Department of Agriculture, 2001, "Draft Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* among Selected Categories of Ready-to-Eat Foods," Center for Food Safety and Applied Nutrition, Food and Drug Administration, U.S. Department of Health and Human Services and the Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, D.C., [Internet, WWW], ADDRESS: http://www.foodsafety.gov/~dms/ Imrisk.html.
- Food Safety and Inspection Service, 1999, Press release and Recall Notification Report dated January 15, Food Safety and Inspection Service, [Internet, WWW], ADDRESS: http://www.fsis.usda.gov.
- Food Safety and Inspection Service. 1989. Press release dated December 20 and information provided by Earl Montgomery, Emergency Programs Staff, Washington, D.C. Personal communication.
- Food Safety and Inspection Service. 2001. Performance standards for the production of processed meat and poultry products: proposed rule. Fed. Regist. 66:12590–12636.
- Frederiksen, W. 1991. *Listeria* epidemiology in Denmark 1981– 1990. Proceedings of the International Conference on *Listeria* and Food Safety, June 1991, Laval, France. ASEPT Editeur, Rue des Docteurs Calmette et Guérin, Laval, France.
- Goulet, V., C. Jacquet, V. Vaillant, I. Rebiére, E. Mouret, C. Lorente, E. Maillot, F. Stäiner, and J. Rocourt. 1995. Listeriosis from consumption of raw-milk cheese. Lancet 345:1581–1582.
- 40. Gram, L. (Danish Institute for Fisheries Research, Lyngby). Personal communication.
- Heldman, D. R., T. I. Hedrick, and C. W. Hall. 1965. Sources of airborne microorganisms in food processing areas—drains. J. Milk Food Technol. 28:41–45.

- 42. Herd, M., and C. Kocks. 2001. Gene fragments distinguishing an epidemic-associated strain from a virulent prototype strain of *Listeria monocytogenes* belong to a distinct functional subset of genes and partially cross-hybridize with other *Listeria* species. Infect. Immun. 69:3972–3979.
- Hof, H. 1984. Virulence of different strains of *Listeria monocy-togenes* serovar 1/2a. Med. Microbiol. Immunol. 173:207–218.
- Hof, H., T. Nichterlein, and M. Kretschmer. 1994. When are Listeria in foods a health risk? Trends Food Sci. Technol. 5:185–190.
- 45. Hof, H., and J. Rocourt. 1992. Is any strain of *Listeria monocy-togenes* detected in food a health risk? Int. J. Food Microbiol. 16: 173–182.
- International Commission on Microbiological Specifications for Foods. 1994. Choice of sampling plan and criteria for *Listeria* monocytogenes. Int. J. Food Microbiol. 22:89–96.
- International Commission on Microbiological Specifications for Foods. 1996. Annex to Codex document on establishment of sampling plans for *L. monocytogenes* in foods in international trade. Submitted by ICMSF to the Codex Food Hygiene Committee, September 1996.
- International Commission on Microbiological Specifications for Foods. 1996. Microorganisms in foods. 5. Characteristics of microbial pathogens. Blackie Academic & Professional, London (available through Aspen Publishers, Gaithersburg, Md.).
- International Commission on Microbiological Specifications for Foods. 2002. Microorganisms in foods. 7. Microbiological testing in food safety management. Aspen Publishers, Gaithersburg, Md.
- Jacquet, C., B. Catimel, R. Brosch, C. Buchrieser, P. Dehaumont, V. Goulet, A. Lepoutre, P. Veit, and J. Rocourt. 1995. Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. Appl. Environ. Microbiol. 61:2242–2246.
- Jacquet, C., J. Rocourt, and A. Reynaud. 1993. Study of *Listeria* monocytogenes contamination in a dairy plant and characterization of the strains isolated. Int. J. Food Microbiol. 20:13–22.
- Jeffers, G. T., J. L. Bruce, P. L. McDonough, J. Scarlett, K. J. Boor, and M. Wiedmann. 2001. Comparative genetic characterization of *Listeria monocytogenes* isolates from human and animal listeriosis cases. Microbiology 147:1095–1104.
- Johansson, T., L. Rantala, L. Palmu, and T. Honkanen-Buzalski. 1999. Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. Int. J. Food Microbiol. 47:111–119.
- 54. Kacsmarski, E. B., and D. M. Jones. 1989. Listeriosis and readycooked chicken. Lancet 8637:549.
- 55. Kerr, K. G., S. F. Dealler, and R. W. Lacey. 1988. Materno-fetal listeriosis from cook-chill and refrigerated food. Lancet ii:1133.
- Kuhn, M., and W. Goebel. 1999. Pathogenisis of *Listeria mono-cytogenes*, p. 97–130. *In* E. T. Ryser and E. H. Marth (ed.), *Listeria*, listeriosis, and food safety, 2nd ed. Marcel Dekker, New York.
- Lawrence, L. M., and A. Gilmour. 1995. Characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Appl. Environ. Microbiol. 61:2139–2144.
- Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, L. Yonekura, P. Hayes, R. Weaver, A. Audurier, B. D. Plikaytis, S. L. Fannin, A. Kleks, and C. V. Broome. 1988. Epidemic listeriosis associated with Mexican-style cheese. New Engl. J. Med. 319:823–828.
- Loncarevic, S., M.-L. Danielsson-Tham, P. Gerner-Schmidt, L. Sahlstrom, and W. Tham. 1998. Potential sources of human listeriosis in Sweden. Food Microbiol. 15:65–69.
- Loncarevic, S., W. Tham, and M.-L. Danielsson-Tham. 1996. The clones of *Listeria monocytogenes* detected in food depend on the method used. Lett. Appl. Microbiol. 22:381–384.
- Lyytikäinen, O., T. Autio, R. Maijala, and 11 other collaborators. 2000. An outbreak of *Listeria monocytogenes* serotype 3a infection from butter in Finland. J. Infect. Dis. 181:1838–1841.
- 62. Lyytikäinen, O., P. Ruutu, J. Mikkola, A. Siitonen, R. Maijala, M.

Hatakka, and T. Autio, 1999, "An Outbreak of Listeriosis due to *Listeria monocytogenes* Serotype 3a from Butter in Finland," Eurosurveillance Weekly 3 (11 March), [Internet, WWW], AD-DRESS: http://www.euroserv.org/.

- McLauchlin, J., M. H. Greenwood, and P. N. Pini. 1990. The occurrence of *Listeria monocytogenes* in cheese from a manufacturer associated with a case of listeriosis. Int. J. Food Microbiol. 10: 255–262.
- McLauchlin, J., S. M. Hall, S. K. Velani, and R. J. Gilbert. 1991. Human listeriosis and paté: a possible association. Br. Med. J. 303: 773–775.
- Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerging Infect. Dis. 5:607–625.
- Miettinen, M. K., K. Björkroth, and H. J. Korkeala. 1999. Characterization of *Listeria monocytogenes* from an ice cream plant by serotyping and pulsed field gel electrophoresis. Int. J. Food Microbiol. 46:187–192.
- Nakama, A., M. Terao, Y. Kokubo, T. Itoh, T. Maruyama, C. Kaneuchi, and J. McLauchlin. 1998. A comparison of *L. monocytogenes* serovar 4b isolates of clinical and food origin in Japan by pulsed-field gel electrophoresis. Int. J. Food Microbiol. 42:201– 206.
- Nesbakken, T. 2000. *Yersinia* species, p. 1363–1393. *In* B. M. Lund, A. C. Baird-Parker, and G. Gould (ed.), The microbiology of food. Chapman and Hall, London.
- Nesbakken, T., G. Kapperud, and D. A. Caugant. 1996. Pathways of *Listeria monocytogenes* contamination in the meat processing industry. Int. J. Food Microbiol. 31:161–171.
- Norton, D. M., M. A. McCamey, K. L. Gall, J. M. Scarlett, K. J. Boor, and M. Wiedmann. 2001. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry. Appl. Environ. Microbiol. 67:198–205.
- Norton, D. M., J. M. Scarlett, K. Horton, D. Sue, J. Thimothe, K. J. Boor, and M. Wiedmann. 2001. Characterization and pathogenic potential of *Listeria monocytogenes* isolates from the smoked fish industry. Appl. Environ. Microbiol. 67:646–653.
- O'Donoghue, K., K. Bowker, J. McLauchlin, D. S. Reeves, P. M. Bennett, and A. P. MacGowan. 1995. Typing of *Listeria monocy-togenes* by random amplified polymorphic DNA (RAPD) analysis. Int. J. Food Microbiol. 27:245–252.
- Piffaretti, J.-C., H. Kressebuch, M. Aeschbacher, J. Bille, E. Bannerman, J. M. Musser, R. K. Selander, and J. Rocourt. Genetic characterization of clones of the bacterium *Listeria monocytogenes* causing epidemic disease. Proc. Natl. Acad. Sci. USA 86:3818–3822.
- Pine, L., S. Kathariou, F. Quinn, V. George, J. D. Wenger, and R. E. Weaver. 1991. Cytopathogenic effects in enterocytelike Caco-2 cells differentiate virulent from avirulent *Listeria* strains. J. Clin. Microbiol. 29:990–996.
- Proctor, M. E., R. Brosch, J. W. Mellen, L. A. Garrett, C. W. Kaspar, and J. B. Luchansky. 1995. Use of pulsed-field gel electrophoresis to link sporadic cases of invasive listeriosis. Appl. Environ. Microbiol. 61:3177–3179.
- Rasmussen, O. F., P. Skouboe, L. Dons, L. Rossen, and J. E. Olsen. 1995. *Listeria monocytogenes* exists in at least three evolutionary lines: evidence from flagellin, invasive associated protein and listeriolysin O genes. Microbiology 141:2053–2061.
- Riedo, F. X., R. W. Pinner, M. L. Tosca, M. L. Cartter, L. M. Graves, M. W. Reeves, R. E. Weaver, B. D. Plikaytis, and C. V. Broome. 1994. A point-source foodborne listeriosis outbreak: documented incubation period and possible mild illness. J. Infect. Dis. 170:693–696.
- Roche, S. M., P. Velge, E. Bottreau, C. Durier, N. Marquet-van der Mee, and P. Pardon. 2001. Assessment of the virulence of *Listeria monocytogenes*: agreement between a plaque-forming assay with HT-29 cells and infection of immunocompetent mice. Int. J. Food Microbiol. 68:33–44.
- Rocourt, J., and J. Bille. 1997. Foodborne listeriosis. World Health Stat. Q. 50:67–73.

- Rocourt, J., C. Jacquet, J. Rebiere, and 32 other collaborators. 1993. Epidemie de listeriose a lysovar 2671-108-312 en France. Resultats preliminaires de l'enquete epidemioloique coordonnee par le reseau national de sante publique. Bull. Epidemiol. Hebd. 34:157–158.
- Rørvik, L. M., B. Aase, T. Alvestad, and D. A. Caugant. 2000. Molecular epidemiological survey of *Listeria monocytogenes* in seafoods and seafood-processing plants. Appl. Environ. Microbiol. 66:4779–4784.
- Rørvik, L. M., D. A. Caugant, and M. Yndestad. 1995. Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and smoked salmon processing plant. Int. J. Food Microbiol. 25:19–27.
- 83. Ross, T., E. Todd, and M. Smith. 2000. Exposure assessment of *Listeria monocytogenes* in ready-to-eat foods. Preliminary report prepared for the Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods, 17–21 July. Food and Agriculture Organization of the United Nations, Rome.
- 84. Ryser, E. T., and E. H. Marth. 1999. *Listeria*, listeriosis and food safety. Marcel Dekker, New York.
- Salamina, G., E. Dalle Donne, A. Niccolini, and 11 collaborators. 1996. A foodborne outbreak of gastroenteritis involving *Listeria monocytogenes*. Epidemiol. Infect. 117:429–436.
- Salvat, G., M. T. Toquin, Y. Michel, and P. Colin. 1995. Control of *Listeria monocytogenes* in the delicatessen industries: the lessons of a listeriosis outbreak in France. Int. J. Food Microbiol. 25: 75–81.
- Schlech, W. F., III, P. M. Lavigne, R. A. Bortolussi, and 8 other collaborators. 1983. Epidemic listeriosis—evidence for transmission by food. New Engl. J. Med. 308:203–206.
- Smith, M. A. (Department of Environmental Health Science, University of Georgia). Personal communication.

- Swaminathan, B. (Centers for Disease Control and Prevention, Atlanta). Personal communication.
- Tabouret, M., J. de Rycke, A. Audurier, and B. Poutrel. 1991. Pathogenicity of *Listeria monocytogenes* isolates in immunocompromised mice in relation to listeriolysin production. J. Med. Microbiol. 34:13–18.
- Tompkin, R. B. 2000. Managing *Listeria monocytogenes* in the food processing environment. Can. Meat Sci. Assoc. News December:4–8.
- Tompkin, R. B., L. N. Christiansen, A. B. Shaparis, R. L. Baker, and J. M. Schroeder. 1992. Control of *Listeria monocytogenes* in processed meats. Food Aust. 44:370–376.
- Tompkin, R. B., V. N. Scott, D. T. Bernard, W. H. Sveum, and K. S. Gombas. 1999. Guidelines to prevent post-processing contamination from *Listeria monocytogenes*. Dairy Food Environ. Sanit. 19: 551–562.
- Unnerstad, H., E. Bannerman, J. Bille, M.-L. Danielsson-Tham, E. Waak, and W. Tham. 1996. Prolonged contamination of a dairy with *Listeria monocytogenes*. Neth. Milk Dairy J. 50:493–499.
- Wenger, J. D., B. Swaminathan, P. S. Hayes, S. S. Green, M. Pratt, R. W. Pinner, A. Schuchat, and C. V. Broome. 1990. *Listeria monocytogenes* contamination of turkey franks: evaluation of a production facility. J. Food Prot. 53:1015–1019.
- Wiedmann, M., J. L. Bruce, C. Keating, A. E. Johnson, P. L. McDonough, and C. A. Batt. 1997. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infect. Immun. 65: 2707–2716.
- Willshaw, G. A., T. Cheasty, and H. R. Smith. 2000. *Escherichia coli*, p. 1136–1177. *In* B. M. Lund, A. C. Baird-Parker, and G. W. Gould (ed.), The microbiology of food. Chapman and Hall, London.
- Zheng, W., and S. Kathariou. 1995. Differentiation of epidemic-associated strains of *Listeria monocytogenes* by restriction fragment length polymorphism in a gene region essential for growth at low temperatures (4°C). Appl. Environ. Microbiol. 61:4310–4314.