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Listeria monocytogenes, a Food-Borne Pathogen

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INTRODUCTION

In 1966, Gray and Killinger (181) published their classic review of *Listeria monocytogenes* and listeric infections in humans and other animals. Since then, the organism has been implicated as the causative agent in several outbreaks of food-borne listeriosis in North America and in Europe (429), and an understanding of its mechanisms of pathogenicity is growing rapidly (76). It seemed an appropriate time to review the many aspects of *L. monocytogenes* as a

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TABLE 1. Differentiation of *Listeria* spp.

Characteristic	Result for:				
	L. monocytogenes	*L. innocua*	*L. ivanovii*	*L. welshimeri*	*L. seeligeri*
β-Hemolysis	+	−	+	−	+
CAMP test (*S. aureus*)	+	−	−	−	+
CAMP test (*R. equi*)	−	−	+	−	−
Acid production from:					
α-Methyl-D-mannoside	+	+	−	+	−
Rhamnose	+	v[a]	−	v	−
Xylose	−	−	+	+	+
Mouse virulence	+	−	+	−	−

[a] v, variable.

food-borne pathogen, including its epidemiology, its incidence and growth in foods, and the virulence factors involved in the human disease.

L. monocytogenes was first described by Murray et al. (304), who named it *Bacterium monocytogenes* because of a characteristic monocytosis found in infected laboratory rabbits and guinea pigs. It was renamed *Listerella hepatolytica* by Pirie in 1927 and given its present name by him in 1940 (181). The first confirmed isolations of the bacterium from infected individuals, following its initial description, were made in 1929 by Gill from sheep and by Nyfeldt from humans (181). Since then, sporadic cases of listeriosis have been reported, often in workers in contact with diseased animals (52). As a result of food-borne outbreaks, interest in the organism grew rapidly in the 1980s among food manufacturers and government bodies, with a concomitant increase in the published literature (76, 157, 243, 338, 438). The present review is based largely on current papers and does not cover the extensive body of literature on the effect of the organism on the immune system.

CHARACTERISTICS OF THE ORGANISM

Microbiology

L. monocytogenes is a gram-positive, nonsporeforming, facultatively anaerobic rod which grows between −0.4 and 50°C (210, 425). It is catalase positive and oxidase negative and expresses a β-hemolysin which produces zones of clearing on blood agar. The hemolysin acts synergistically with the β-hemolysin of *Staphylococcus aureus* on sheep erythrocytes; the substance mediating this effect is known as the CAMP factor after Christie, Atkins, and Munch-Petersen (70), the workers who first described the phenomenon in group B streptococci. The organism possesses peritrichous flagella, which give it a characteristic tumbling motility, occurring only in a narrow temperature range. When the organism is grown between 20 and 25°C, flagellin is both produced and assembled at the cell surface, but at 37°C flagellin production is markedly reduced (324). The colonies demonstrate a characteristic blue-green sheen by obliquely transmitted light (193). *L. monocytogenes* is widely present in plant, soil, and surface water samples (431), and has also been found in silage, sewage, slaughterhouse waste, milk of normal and mastitic cows, and human and animal feces (278). *L. monocytogenes* has been isolated from cattle, sheep, goats, and poultry, but infrequently from wild animals (181).

In tryptic soy broth supplemented with 0.6% yeast extract, incubated at 30°C, *L. monocytogenes* F5027, F5069, S4b, and Scott A grew at pH values from 4.5 to 7.0, with no growth at pH 4.0 and lower (319). Of several acids (acetic, lactic, citric, and hydrochloric acids) used to lower the pH of brain heart infusion broth before using it as the growth medium for four *L. monocytogenes* strains, acetic acid was the most effective growth inhibitor (2, 130). The authors found that the minimum pH required for initiation of growth ranged from 5.0 to 5.7 at 4°C and from 4.3 to 5.2 at 30°C. Recently, Buchanan and Phillips developed a mathematical model describing the effects of temperature (5 to 37°C), pH (4.5 to 7.5), NaCl (5 to 45 g/liter), $NaNO_2$ (0 to 1,000 μg/ml), and atmosphere (aerobic or anaerobic) on the growth kinetics of *L. monocytogenes* Scott A in tryptone phosphate broth (46).

Studies on carbohydrate fermentations by *Listeria* spp. were reported by Pine et al. (330). Under anaerobic conditions only hexoses and pentoses supported growth; aerobically, maltose and lactose, but not sucrose, also supported growth. *L. monocytogenes* and *L. innocua* utilize glucose, lactose, and rhamnose under aerobic conditions; *L. grayi* and *L. murrayi* also utilize galactose. *L. ivanovii* and *L. seeligeri* are the only *Listeria* spp. to ferment xylose. These, and other reactions used in the differentiation of *Listeria* spp., are listed in Table 1.

The incidence of cryptic plasmids in *L. monocytogenes* strains is low, ranging from 0 to 20% (144, 326, 326a). This may be due to the use of acriflavine, a known plasmid-curing agent, in the isolation media. Recently, a 37-kbp plasmid carrying genes for resistance to chloramphenicol, erythromycin, streptomycin, and tetracycline was isolated from a clinical strain of *L. monocytogenes* (336). The plasmid was self-transferable to other *L. monocytogenes* strains.

Taxonomy

Although *L. monocytogenes* was classified for a time by *Bergey's Manual of Determinative Bacteriology* in the family *Corynebacteriaceae* (398), it is listed in the latest edition of *Bergey's*, together with *Lactobacillus*, *Erysipelothrix*, *Brochothrix*, and other genera, in a section entitled Regular, Nonsporing Gram-Positive Rods (376). Both the intra- and intergeneric taxonomy of bacteria of the genus *Listeria* have been problematical for a number of years. *L. monocytogenes* was the only recognized species within the genus until 1961; *L. denitrificans*, *L. grayi*, and *L. murrayi* were added to the genus in 1961, 1966, and 1971, respectively (347). All serovar 5 strains showed a strong β-hemolysis and were proposed as a separate species, *L. bulgarica*, by Ivanov in 1975 (347). This species was officially named *L. ivanovii* in 1984 (376). Nonpathogenic strains of *L. monocytogenes* belonging to serovar 6 were recognized as a new species, *L. innocua* (373,

TABLE 2. Serovars of *L. monocytogenes*

Designation		O antigens										H antigens		
Paterson (322)	Seeliger (372) and Donker-Voet (105)													
1	1/2a	I	II	(III)								A	B	
	1/2b	I	II	(III)								A	B	C
2	1/2c	I	II	(III)									B	D
3	3a		II	(III)	IV							A	B	
	3b		II	(III)	IV					(XII)	(XIII)	A	B	C
	3c		II	(III)	IV					(XII)	(XIII)		B	D
4	4a			(III)		(V)		VII	IX			A	B	C
	4ab			(III)		V	VI	VII	IX	X		A	B	C
	4b[a]			(III)		V	VI					A	B	C
	4c			(III)		V		VII				A	B	C
	4d			(III)		(V)	VI		VIII			A	B	C
	4e			(III)		V	VI		(VIII)	(IX)		A	B	C
	7			(III)							XII XIII	A	B	C

[a] Also 4b(x), [(III) V VI VIII] (289).

376). *L. welshimeri* and *L. seeligeri* were added in 1983 (376). Reviews on the topic include those by Jones (205, 206), Seeliger and Finger (374), and McLauchlin (282).

Stuart and Pease (398) concluded from a numerical taxonomic study of 123 strains of *Listeria* and nine other genera, that *Listeria* and *Erysipelothrix* are distinct genera that are not closely related, that *L. denitrificans* is quite different from other *Listeria* strains and that these other *Listeria* strains constituted a single monospecific genus. The numerical taxonomic, DNA base composition, and DNA-DNA hybridization studies of Stuart and Welshimer (399, 400) led them to conclude that *L. denitrificans* should be reclassified and to propose that *L. grayi* and *L. murrayi* be transferred to a new genus, *Murraya*, as *M. grayi* and *M. grayi* subsp. *murrayi*, respectively. The moles percent G+C content of the DNA of the 19 strains studied varied from 37 to 39, except for that of *L. denitrificans*, which was 56. An extensive numerical taxonomic survey (193 strains, 143 unit characters [440]) was performed on 49 *Listeria* strains, as well as on representatives of the genera *Erysipelothrix*, *Brochothrix*, *Lactobacillus*, *Streptococcus*, *Corynebacterium*, and *Kurthia*.

The present taxonomic position of the genus *Listeria* as concluded from these numerical taxonomic and chemical studies, as well as the more recent DNA homology and 16S rRNA cataloging results (349), is as follows (207, 342, 373): (i) it includes the species *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, *L. grayi*, and *L. murrayi*; (ii) *L. denitrificans* is excluded from the genus and transferred to a new genus, *Jonesia*, as *J. denitrificans*; and (iii) the genus is closely related to the genus *Brochothrix*; both of these genera occupy a position between *Lactobacillus* and *Bacillus* and are more distantly related to *Streptococcus*, *Lactococcus*, *Enterococcus*, *Staphylococcus*, *Kurthia*, *Gemella*, and *Erysipelothrix*.

Antigenic Structure

The biochemistry of the cell structure of *L. monocytogenes* and other *Listeria* spp. was studied by Fiedler (142), who proposed a macromolecular model of the organization of the *Listeria* cell wall. Electron micrographs of the cell wall showed it to be that typical of gram-positive bacteria, i.e., a thick homogeneous structure surrounding the cytoplasmic membrane and without the outer membrane characteristic of gram-negative bacteria. Isolated dry cell walls are composed of about 35% peptidoglycan, consisting of cross-linked *meso*-diaminopimelic acid. The remaining carbohydrate consists of cell wall teichoic acids, which are polymers covalently linked to a specific site on the peptidoglycan. They are usually composed of glycerol or ribitol, neutral sugars, *N*-acetylamino sugars, and phosphate. Structurally, two types of cell wall teichoic acids exist amongst *Listeria* serotypes. In the first, ribitol residues are covalently linked by phosphodiester bonds between C-1 and C-5 and are sometimes found with *N*-acetylglucosamine substituted at C-2; this type is found associated with serotypes 1/2a, b, and c, 3a, b, and c, and 7. In the second, *N*-acetylglucosamine is integrated into the chain; this type is found associated with serotypes 4a, b, and d. *Listeria* cell walls also consistently contain lipoteichoic acids, in which a glycolipid moiety, such as a galactosyl-glucosyl-diglyceride, is covalently linked to the terminal phosphomonoester of the teichoic acid. This lipid region anchors the polymer chain to the cytoplasmic membrane. These lipoteichoic acids resemble the lipopolysaccharides of gram-negative bacteria in both structure and function, being the only amphipathic polymers at the cell surface.

The serovars of *L. monocytogenes* (Table 2) were classified by Paterson (322) and later modified by Seeliger (372) and Donker-Voet (105). A revision has recently been proposed by Garcia et al. (155), who found factor IX in some strains of serovar 4b.

LISTERIOSIS

Human Infections

Most cases of human listeriosis appear to be sporadic, although a portion of these sporadic cases may be previously unrecognized common-source clusters (72). The source and route of infection are usually unknown. However, the recent association of *L. monocytogenes* with several large foodborne outbreaks suggests that contaminated food may be the primary source of the organism.

The majority of human cases of listeriosis occur in individuals who have an underlying condition which leads to suppression of their T-cell-mediated immunity. In this re-

TABLE 3. Recent sporadic cases of focal listeriosis

Patient (age, gender)[a]	Case	Comment	Reference
73Y,F	Prosthetic valve endocarditis	This patient as well as five others with similar infection not immunocompromised	340
69Y,M	Persistent corneal defect	Alcoholic	118
50Y,F	Peritonitis complicating chronic ambulatory peritoneal dialysis	History of SLE[b];[a] *L. monocytogenes* 4b cultured from dialysis fluid	235
47Y,M	Encephalitis	Healthy individual	445
64Y,M	Rhabdomyolysis and acute renal failure	Healthy individual	404
86Y,F	Necrotizing ring ulcer of the cornea	Diabetic; smears from corneal ulcer contained *L. monocytogenes* 4b	197
66Y,M	Prosthetic hip infection	Diabetic and alcoholic; source of infection traced to contaminated cheese	126
48Y,M	Ondine's curse caused by brain stem encephalitis	Alcoholic	202
66Y,M	Cryptogenic liver abscess	Diabetic	4
76Y,F	Cholecystitis	Diabetic	175
28Y,M	Indwelling intravenous catheter	AIDS patient	217
42Y,M	Pneumonia	Healthy individual, vegetarian	437
69Y,M	Septic arthritis with peritonitis	Rheumatoid arthritis, cirrhosis of liver	82
56Y,M	Recurrent meningitis (4 and 8 mo after heart transplant)	Both episodes followed increased levels of corticosteroid administration	244
64Y,F	Prosthetic knee joint	Severe rheumatoid arthritis, no preceding systemic symptoms	35
43Y,M	Osteomyelitis (finger)	Leukemia	260
40Y,M	Anal listeriosis	AIDS patient	96

[a] Y, years; M, male; F, female.
[b] SLE, Systemic lupus erythematosus.

gard, a recent study of listeriosis in the United States has estimated a minimum case rate of 90 per 100,000 AIDS patients, a rate which is 150 times that of the general population in the same age group (158). There are, however, instances in which apparently normal healthy individuals have become ill with listeriosis in both food-borne epidemics (363) and sporadic cases (16). Some of the predisposing conditions which are often associated with listeriosis include neoplastic disease, immunosuppression, pregnancy, extremes of age, diabetes mellitus, alcoholism, cardiovascular and renal collagen diseases, and hemodialysis failure (307). The clinical syndromes associated with adult listeriosis include mainly central nervous system infections and primary bacteremia, but can also include endocarditis. In addition, sporadic cases of focal listeriosis have been reported with increasing frequency in the literature, with many different parts of the body being affected (Table 3).

Although the highest incidence of listerial infection is usually seen in neonates, followed by those older than 60 years, the proportion of cases not associated with pregnancy appears to be on the increase (285, 365). In fact, in a recent summary of listeriosis cases in 1989 from 16 countries, it was shown that 31 and 22% of the total cases occurred in patients older than 60 years and younger than 1 month, respectively (343). The same report stated that of 782 cases of listeriosis reported from 20 countries, 43% were maternal and neonatal infections, 29% were septicemic infections, 24% were central nervous system infections, and 4% were atypical forms. Meningitis, a common manifestation of listeriosis in adults, is seen mainly in the elderly and in immunocompromised patients (259). Central nervous system infection with *L. monocytogenes* is typically meningitic or encephalitic and usually presents with prodromal symptoms including headache, vomiting, fever, and malaise before the appearance of focal signs of central nervous system infection. Although only some 14 cases have been reported, *L. monocytogenes* can cause brain abscesses in predisposed individuals, especially in leukaemia patients or in renal transplant recipients (94). Meningitis cases in adults and elderly people are generally associated with a high mortality rate (315). In view of the strong tendency of *L. monocytogenes* to infect the meninges, the organism should be included in the differential diagnosis of meningitis in high-risk groups such as neonates, cancer patients, and immunocompromised individuals (259). Mortality rates for patients from various countries, and for a select group of patients having a common disease syndrome, are shown in Table 4. Mortality rates calculated worldwide by using data obtained for the year 1989 ranged between 13 and 34% (343). It has recently been suggested that listeriosis may well be the leading fatal food-borne infection in the United States (158).

Neonatal listeriosis. Pregnancy, while predisposing to listeriosis, does not seem to predispose to carriage of the organism (242). In a perinatal infection, the pregnant women usually but not always (302) contracts a mild, self-limited influenzalike illness. Only rarely does she contract a full-blown case of listeriosis. Diarrhea, abdominal cramps, and lower-back pain, although less common, have also been reported. Maternal listeriosis can be associated with abortion late in the third trimester of pregnancy (although cases of listeriosis have been found in both the first and second trimesters), but, more commonly, infection presents as preterm labor (22). Maternal listeriosis does not inevitably lead to infection of the fetus, as has been noticed in several instances in which only one case of infant listeriosis was noted in a twin delivery (387). In addition, a case of first-trimester maternal sepsis due to *L. monocytogenes*, in which a healthy infant was delivered at term, was recently described (81). Healthy pregnant women may be carriers of *L. monocytogenes* and still give birth to healthy infants.

TABLE 4. Mortality rates of selected groups of disease or patients with listeriosis

Condition or country involved[a]	No. of patients studied	No. (%) of patients dead	No. of healthy individuals with no underlying condition	Reference
Endocarditis	44	21 (48)	2	62
Cancer	11	1 (9.1)	0	224
Listerial brain abscess	14	8 (57)	4	94
CNS infection	54	28 (52)	23	334
Meningitis	16	7 (43.8)	3	188
Endocarditis (1950–1986)	34	17 (50)	4	152
Neuromeningeal	63	21 (33.3)	NC[b]	69
Adult listeriosis (1980–1982, United States)	660	123 (19.1)	NK[c]	72
GDR (1980–1986)	56	21 (37.5)	41	315
Belgium (1967–1987) meningitis	23	3+3[c] (13.0)	7	325
Britain (1967–1985) nonpregnant adults and juveniles	371	164 (44)	76/337 (23%)	285

[a] CNS, central nervous system; GDR, German Democratic Republic.
[b] NC, not clear from manuscript.
[c] NK, not known.
[d] Three died from underlying disease.

Besides influenzalike symptoms, the pregnant woman may present with decreased fetal movements or early labor. Listeriosis has recently been reported in immunocompromised pregnant women, with two cases resulting in intrauterine death at 22 to 24 weeks gestation (100, 339) and two other cases being reported, one in an AIDS patient (who actually died of listeriosis) in which the infant survived and another in a women being treated with immunosuppressive drugs for lupus nephritis in which the infant died (122, 436). These cases have led to suggestions that listeriosis may be an important unrecognized disease in pregnant women with impaired immunity (122).

Two clinical forms of neonatal listeriosis, early- and late-onset forms, are known (Table 5). The mean incubation time for onset of symptoms for the former disease is 1.5 days and presumably occurs in infants infected in utero. The disease is known as granulomatosis infantisepticum. The organism is widely disseminated in the body, with lesions being found most typically in the liver and placenta. The poorest prognosis appears to occur in the early-onset group, with the highest mortality rate being found in infants born earliest in gestation (284). In late-onset neonatal listeriosis, the mean onset of symptoms is 14.3 days, with meningitis as the predominant form of the disease. The source of the organism in these late-onset cases is unclear, although the infection may be acquired either from the mother's genital tract during birth or from environmental sources after birth. Up to one-quarter of the late-onset cases may be due to cross-infection (284).

Manifestations of neonatal listeriosis include respiratory distress syndrome, rash, purulent conjunctivitis, pneumonia, hyperexcitability, vomiting, cramps, shortness of breath, shock, hematologic abnormalities, and either hyper- or hypothermia (22, 49, 119, 223). Most neonatal deaths from congenital listeriosis appear to be due to pneumonia and respiratory failure. The organisms can be readily isolated from the cerebrospinal fluid, placenta, meconium, gastric aspirate, blood, and skin of the newborn baby, although culture results can be received too late to influence the outcome of the infection. Possible diagnostic features of neonatal listeriosis include bright green meconium, the presence of miliary granulomata and microabscesses in the placenta, and the presence of gram-positive bacilli in clinical specimens.

Early diagnosis and effective antibiotic treatment are crucial to the survival of the infected neonate. Recent work

TABLE 5. Perinatal listeriosis

Characteristic	Clinical syndrome for:	
	Early (0–2 days) and intermediate (3–5 days) onset	Late onset (>5 days)
Onset of disease	Infected in utero	Infant colonized at birth, with delayed onset of infection or due to cross-infection
Major symptoms observed	Septicemia	Meningitis
Mortality rate	15–50%	10–20%
Infant status	Usually occurs in premature child with low birth weight	Usually occurs in infants apparently healthy at birth
Conditions	Respiratory distress, cyanosis, apnea, pneumonia, widespread microabscesses	Fever, poor feeding, irritability, leukocytosis, diarrhea
Possible contaminated sites	External ear, nose, throat, meconium, amniotic fluid, placenta or blood, lung, gut	Cerebrospinal fluid; blood and superficial cultures may be negative
Maternal fever and isolation of *L. monocytogenes* from maternal sites	About 50% of cases	0–5% of cases

TABLE 6. Mortality rates in neonates

Country and/or period	No. of patients	No. with:		No. (%) of deaths	No. with sequelae	Reference
		Early onset	Late onset			
Switzerland (1972–1987)	35	31	4	5 (14.3)	7	49
Netherlands (neonatal meningitis, 1976–1982)	12	3	9	2 (16.7)	1	302
New Zealand (1969)	13	11	2	7 (53.8)	0	22
France (children)[a]	56[b]	NK[c]	NK	13 (23.2)	NK	407
Michigan (1974–1988)	17	NK	NK	0	1	223
California (1974–1978)	12	9	3	3 (25.0)	3	187
United States (meningitis, 1962–1976)	25		22[d]	1 (4.0)	2	423
Los Angeles (1985)	23	–[e]		5 (21.7)	NK	228
GDR[f] (1960–1972)	117	–[g]		55 (47.0)	NK	95
Netherlands (1985–1986)	4	4		2 (50.0)	1	411
Kuwait (1985–1986)	9	5	4	3 (33.3)	2	99
Dresden (1981–1986)	18	18		3 (16.7)	6	371
Halifax (1981)	15	15		7 (46.7)	2	119
Canada (1988)	12	10	2	3 (25.0)	NK	416
Britain (1967–1985)	248[h]	118	42	89 (50.9)[i]	NK	284

[a] Children (1 month to 5 years).
[b] 26 children had an underlying condition; there were 11 deaths in this group.
[c] NK, not known.
[d] Age at onset given for only 22 patients.
[e] The majority of cases were infected in utero and ill at birth; five neonates apparently healthy at birth developed sepsis or meningitis 1 to 8 days after birth.
[f] GDR, German Democratic Republic.
[g] Two-thirds of patients were premature babies; the rest were mature infants.
[h] Includes 42 intrauterine deaths, 8 cases of intermediate onset (3 to 5 days), 29 cases with no details on time of onset of infection, and 9 cases of maternal bacteremia without infection of the fetus.
[i] Outcome known for only 175 neonates.

has demonstrated that Gram staining of the gastric aspirate may be a reliable test for early diagnosis of listerial infection (49). Febrile pregnant women and/or those whose blood cultures were positive for *L. monocytogenes* have been treated successfully with antibiotics; this has enabled some pregnancies to proceed to term successfully (119, 198). Infection of the placenta without obvious fetal infection may also occur. However, because of the rarity of neonatal listeriosis (3) and because features in both the mother and baby can be nonspecific, delays in diagnosis can occur. Factors associated with a fatal outcome include low birth weight, early gestational age, and a long interval between onset of symptoms and delivery of antibiotics (49). Sequelae to the original infection can occur both in listeriosis acquired during pregnancy (121) and in late-onset neonatal listeriosis (49). The relative risk of abortion or stillbirth due to *L. monocytogenes* is unknown, and there is no concrete evidence that listeriosis is associated with repeated abortions or infertility. Although listeriosis infections beyond the neonatal period are rare and occur mainly in children with underlying conditions, infections have been reported in otherwise healthy infants and children (111, 223, 423).

Despite modern antibiotic therapy, neonatal listeriosis still has a mortality rate of about 36% (Table 6). This high rate could be related to the prematurity of many of the affected infants and/or to the advanced stage of illness often seen at birth (119).

Epidemiology

Incidence. The incidence of listeriosis appears to be on the increase worldwide, with the number of cases rising especially in Europe (34, 54, 282). The annual endemic disease rate varies from 2 to 15 cases per million population, with published rates varying from 1.6 to a high of 14.7 in France for 1986 (Table 7). Whether this reflects a true increase in numbers or is due to better diagnosis and/or increased awareness of the disease is unclear. However, there is no doubt that the susceptible population is increasing, as are the numbers and types of foods in which *L. monocytogenes* is able to survive and grow.

Serotypes involved in listeriosis. Although human listeriosis may be caused by all 13 serovars of *L. monocytogenes*, serovars 1/2a, 1/2b, and 4b cause most of the cases (Table 8). Geographic differences in the global distribution of serotypes apparently exist. Although serovar 4b predominates in most of Europe, there appears to be an even distribution of serovars 1/2a, 1/2b, and 4b in Canada and the United States (Table 8). No direct links have been made between particular forms of listeriosis and certain serotypes, but recent work has shown an epidemiologic association between perinatal listeriosis and serovars 1/2b, 3b, and 4b (158).

Carrier status. *L. monocytogenes* appears to be a normal resident of the intestinal tract in humans; this may partially explain why antibodies to *Listeria* spp. are common in healthy people (374). The number of human carriers of *L. monocytogenes* as assessed by the examination of fecal samples ranges from a low of 0.5% (9 of 1,732) to a high of 69.2% (36 of 52) or 91.7% (11 of 12 female laboratory technicians) (338). At any one time, around 5 to 10% of the general population could be carriers of the organism. The use of newer methods, however, may show the carrier rates to be significantly higher. However, Kampelmacher and Van Noorle Jansen (212) found that pregnant women with stools positive for *L. monocytogenes* never delivered an infant with listeriosis. Thus, because of the high rate of clinically healthy carriers, the presence of *L. monocytogenes* in the feces is not necessarily an indication of infection.

In a study of the duration of fecal excretion, Ortel (314) reported that of 12 people examined over 16 months, 11

TABLE 7. Incidence of listeriosis worldwide[a]

Country	Year (no. of cases)	Incidence/10^6 population	Reference
United States	1986	7.0	369
United States	Estimated annual figure (1,600)	8.3	44
Canada	1988 (60)	2.3	416
Australia (Western)	1989 (13)	7.6	343
New Zealand	1989 (21)	7.0	343
Belgium	1989 (48)	4.8	343
Denmark	1987 (27)	4.7–5.3	360
	1989 (32)	6.0	343
Finland	1989 (29)	5.9	343
France	1984 (630)	11.3	178
	1986 (811)	14.7	177
	1989 (416)	8.0	343
Norway	1987	4.0[b]	
	1989 (7)	1.6	
Scotland	1987 (40)	7.0[c]	54
	1988 (35)		
	1989 (29)	5.7	343
Sweden	1987	8.0[b]	
	1989 (32)	3.8	343
Switzerland	1988	6.0[b]	
	1989 (34)	5.0	343
United Kingdom	1988	5.8	54
	1989	4.3[d]	343
FRG[e]	1989 (14)	5.8	343
Yugoslavia	1989 (29)	3.0	343

[a] Some data taken from reference 343, with permission.
[b] Data from World Health Organization Informal Working Group, Geneva, 15 to 19 February 1988. See reference 34.
[c] Rate for 1987–1988.
[d] England and Wales only.
[e] FRG, Federal Republic of Germany.

excreted *L. monocytogenes* on one or more occasions: one for 6 months, one for 4 months, three for 3 months, four for 2 months, and two for 1 month. However, no one excreted the same serotype of *L. monocytogenes* in the feces for a consecutive period of longer than 2 months. It is apparent that although shedding patterns tend to be erratic among different individuals, carriers in some cases can shed the organism for long periods.

Although among animals the carrier rate is generally considered to be 1 to 5% (range, 1 to 29% [212, 338]) recent studies involving newer methods for isolating *Listeria* spp. have indicated that much higher carriage rates may also occur. Skovgaard and Morgen (383) found that 39 of 75 samples of bovine feces (52%) examined from seven dairy farms contained *L. monocytogenes*, with an additional 12 samples containing other *Listeria* spp.

Strain Typing

Because cases of human listeriosis are caused mainly by only three serotypes (4b, 1/2a, and 1/2b [Table 8]), serotyping is of limited value in epidemiological investigations. Investigators have therefore looked for alternate means of typing strains. Biotyping, although useful for species identification, cannot be used to discriminate among strains (376).

Phage typing. Phage typing has proven to be a valuable epidemiological tool in investigations of outbreaks of many infectious diseases. Since the initial discovery of phages specific for *Listeria* spp. in 1945 (368), several groups have assessed the usefulness of phage typing of *L. monocytogenes* (13, 15, 287, 344). Although phage typing appears to be reproducible and discriminatory (287, 288), it is limited by the low percentage of typable strains of serogroup 1/2 (around 50%) and by the lack of phages for other serovars (serovars 3, 4a, 4ab, 4c, 4d, 4e, and 7), which rarely cause disease (13, 344, 345). The overall percent typability of strains of *L. monocytogenes* has ranged from 52 to 78% in various studies (13).

Recently, however, a new set of phages derived from both environmental sources and lysogenic strains has been described (258). More than 90% of serotype 1/2 strains were typable, in addition to all isolates of serotypes 4a (one strain), 4ab (four strains), 4c (two strains), 4d (one strain), and 4e (one strain) and the majority of isolates of serotype 4b (33 of 34 strains) (258). Serovars 3 (2 of 20 strains) and 7 (0 of 1 strain) appeared to be resistant to the phages. Despite its limitations, phage typing has been a useful tool in studies of listeriosis outbreaks (13, 15, 345). There is currently an international phage-typing system for *L. monocytogenes*, and an International centre for *Listeria* phage typing has been established at the Pasteur Institute (207).

Isoenzyme typing. In isoenzyme typing, bacteria are differentiated by the variation in the electrophoretic mobility of any of a large number of metabolic enzymes. Piffaretti et al. (329) examined 175 *L. monocytogenes* isolates recovered from various sources and found 45 distinctive allele profiles or electrophoretic types (ETs), whereas Bibb et al. (32) examined 310 strains and found 56 ETs. Interestingly, all of the major food-borne listeriosis outbreaks appear to have been caused by strains of the same or similar multilocus genotype (329). By comparing the genetic relationships or genetic distance among the ETs of *L. monocytogenes*, two primary divisions were delineated along flagellar antigen lines, with serotypes 4b and 1/2b (and 3b) falling into one group and serotypes 1/2a and 1/2c (and 3a) falling into the other (32, 329). The data obtained with *L. monocytogenes* were consistent with work done with other pathogenic organisms (mainly by Selander's group), which has found most disease to be caused by only a few of the existing clones (329).

The technique appears to be useful in either confirming or eliminating a common source as the cause of an outbreak of food-borne listeriosis. Bibb et al. (32) point out that results must be interpreted cautiously if an isolate pair (patient-food) is of a commonly occurring ET, in contrast to a situation in which a less common ET is involved and a causal link or association can be made with more confidence.

DNA fingerprinting. Restriction enzyme analysis (REA) has recently been used to characterize strains of *L. monocytogenes* causing outbreaks of listeriosis associated with Mexican-style soft cheese in Los Angeles, as well as the Nova Scotia and Switzerland outbreaks (309, 435). Nocera *et al.* (309), using REA and phage-typing methods on the

TABLE 8. Serovar distribution from human listeriosis cases worldwide[a]

Country (yr)	No. (%) of serovar:										Reference
	1	1/2a	1/2b	1/2c	4	3a	3b	4b	4c	Unknown	
Canada (1988)		4 (12.9)	13 (41.9)			2 (6.6)	1 (3.2)	9 (29)	2 (6.6)		416
Belgium (1989)		5 (10.4)	5 (10.4)					37 (77.1)		1[b] (2.1)	343
Finland (1989)		11 (42.3)						15 (57.7)			343
France (1989)		84 (20.2)	50 (12)					266 (63.9)		16[b] (3.8)	343
GDR[c] (1969–1985)		56 (18.2)	32 (10.8)					196 (66.2)		14 (4.7)	365
Scotland (1987–1988)		6 (8.3)	4 (5.6)		7 (9.7)[d]			41 (56.9)		4 (5.6)	54
Switzerland (1989)		1 (3.5)	9 (31)					19 (65.5)			343
United Kingdom (1967–1990)		207 (15)	140 (10)	49 (4)	77 (6)	15 (1)		872 (64)		3 (?)	286
Yugoslovia (1989)		2 (6.9)	1 (3.4)					26 (89.7)			343
New Zealand (1989)		4 (19)	4 (19)					12 (57.1)		1 (4.9)[b]	343
Argentina (1970–1985)	8				1						352
Brazil (1989)		3 (25)						9 (75)			343

[a] Some data taken from reference 343, with permission.
[b] Other strains including 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b (x), 4c, 4d, and 4e.
[c] GDR, German Democratic Republic.
[d] One strain 4ab; four strains 4b(×).

strains associated with the Swiss listeriosis outbreak, found a single clone among 10 *L. monocytogenes* strains isolated from the incriminated soft cheese and 40 isolates from patients. REA however, allowed for the characterization of some non-phage-typeable strains. REA typing has also recently been used to show that *L. monocytogenes* isolates from the Nova Scotia, Los Angeles, and 1983 Massachusetts outbreaks each exhibit a unique restriction enzyme pattern (435). In addition, two different cases of cross-infection with *L. monocytogenes*, one in Canada and one in Italy, have recently been confirmed by DNA fingerprinting (120, 129).

rRNA typing involving both radioactive and nonradioactive methods has also been evaluated recently and, along with DNA fingerprinting, was found to be more discriminatory than either serotyping or phage typing, and equivalent to isoenzyme typing, for subtyping strains of *L. monocytogenes* (311a). Recent ribotyping analysis of *L. monocytogenes* strains by using a digoxigenin-labeled DNA probe has shown that ribotyping can in some cases be less discriminating than REA, but more discriminating than multilocus enzyme electrophoresis (180).

Plasmid typing. Although plasmid typing was recently used in conjunction with DNA fingerprinting to confirm a case of cross-infection with *L. monocytogenes* (120), it is not likely to be of much value as a typing tool, since most strains of *L. monocytogenes* do not appear to carry plasmids (326). However, plasmid profile analysis recently proved useful in tracing sources of environmental contamination with *L. innocua* in dairy plants; more than 90% of *L. innocua* strains were shown to contain plasmids ranging in size from 3 to 55 MDa (336a).

Monocine typing. Monocine typing has recently been evaluated as a typing tool for *L. monocytogenes* (439). In one instance a pair of *L. monocytogenes* strains isolated from a mother and a newborn, which could not be phage typed, proved to be identical by monocine typing. Although this technique is potentially promising as an epidemiological tool, only 59 and 56% of serovars 1/2a and 4b, respectively, were found to be producers of monocines, thus limiting the usefulness of the technique (439).

MECHANISMS OF VIRULENCE

Many factors affecting the pathogenicity of *L. monocytogenes*—its capacity for intracellular growth, iron compounds, catalase and superoxide dismutase, surface components, hemolysins—have been proposed over the years, indicating that its virulence is multifactorial (66). The virulence of the organism may be affected by its growth temperature. Growth of *L. monocytogenes* at a reduced temperature (4°C) increased its virulence in intravenously inoculated mice, although it did not seem to affect mice which had been infected orally (84). This phenomenon may increase the virulence of the organism in refrigerated foods.

Iron Compounds

Iron compounds reduced the dose that killed 50% of mice and improved the in vitro growth of the organism (66, 402), suggesting a possible involvement of host iron metabolism in the infection process. The synthesis of the *L. monocytogenes* hemolysin increases with decreased iron concentration in the growth medium (403), perhaps with the result in vivo of increased lysis of erythrocytes as a source of iron (78, 161). Conversely, as discussed below, superoxide dismutase activity is increased by a higher iron concentration in the medium (433). Recently, a protein of ca. 10,000 Da present in *L. monocytogenes* culture supernatants was found to mobilize iron from transferrin. It requires NADH, flavin mononucleotide, and Mg^{2+} as cofactors (78). The organism binds Fe(II) and also ferric citrate and does not take iron up from ferric ferroxamine, ferric EDTA, or $FeCl_3$ (1). This suggests that iron is acquired principally as the ferrous ion, but that a citrate-inducible uptake system also exists.

Attachment and Intracellular Growth

Many pathogenic bacteria have the ability to invade host tissues by inducing their own endocytosis, with subsequent transport across normally protective barriers. This phenomenon, called parasite-directed endocytosis, seems to be operative in the attachment and entry of *L. monocytogenes* into intestinal cells and macrophages (281, 337). Endocytosis was demonstrated with the human colon carcinoma cell line Caco-2, which expresses enterocytic differentiation (150). In the presence of cytochalasin D, a drug which inhibits microfilament function and hence endocytosis, bacterial entry was inhibited. However, electron micrographs showed the presence of the bacteria inside vacuoles. In contrast, nonvirulent *Listeria* spp. were not able to induce their own phagocytosis.

The presence of a parasite-directed endocytosis of the organism in a mouse embryo fibroblast cell line was confirmed by Kuhn et al. (238). The uptake of a virulent, hemolytic strain of the organism was inhibited by cytochalasin B. Strains of other *Listeria* species, including the hemolytic avirulent *L. seeligeri* and the strongly hemolytic *L. ivanovii*, did not penetrate the fibroblast cells, even though *L. ivanovii* is pathogenic in mice.

A virulent strain of the organism which bound to the cells of a hepatocarcinoma cell line having a well-characterized α-D-galactose receptor was found to possess a surface α-D-galactose residue (79). This residue was lacking in two nonvirulent strains. The binding was abolished by pretreatment of the cell line with the sugar or with neuraminidase. The authors proposed that the mechanism of attachment of virulent *L. monocytogenes* cells to eucaryotic cells is mediated by the interaction of the surface sugar in the microbial cell with the eucaryotic galactose receptor.

The entry of the organism into macrophages does not seem to depend on listeriolysin O (238). Lack of listeriolysin synthesis in transposon-induced nonhemolytic (Hly^-) mutants of *L. monocytogenes* did not reduce the entry of these organisms into mouse peritoneal macrophage cells, although their subsequent survival was reduced significantly (238). The Hly^- mutants were demonstrated to be avirulent in the mouse pathogenicity test, in contrast to the parent strain and the Hly^+ mutant. Although the Hly^- mutants were taken up by the mouse spleen cells, they failed to multiply and were eliminated from the animals within 1 day (215). Kuhn et al. (238) concluded that the hemolysin is required for the intracellular survival of the organism, but not its initial entry. Later, Kuhn and Goebel (237) identified a major extracellular protein apparently involved in the entry of the organism. Hly^- mutants lacking this 60,000-Da protein (p60) lost their ability to invade mouse fibroblast cells and formed long chains of bacterial cells. These disaggregated to normal-sized single cells, which again showed invasiveness, when incubated with p60 at 37°C. Laboratory strains of the organism which had been stored on synthetic media showed a variable ability to invade intestinal epithelial cells. This invasive ability can be enhanced by animal passage (334a).

The requirement of hemolysin for intracellular growth was confirmed by Portnoy and coworkers (53, 335), who developed transposon Tn*916* mutants which were nonhemolytic, lacked a secreted 58,000-Da protein, and were avirulent. These mutants were defective in intracellular growth. Revertants were hemolytic, secreted the 58,000-Da protein, were virulent, and were able to grow intracellularly. This intracellular growth was demonstrated in cell lines of mouse bone marrow macrophages J774, primary mouse fibroblasts CL7, and human epithelial cells Henle 407.

Intracellular survival and growth of *L. monocytogenes* were demonstrated by Mackaness (267), using electron microscopy. There are two aspects of intracellular survival—the virulence of the *L. monocytogenes* strain and the state of activation of the macrophages. Among the virulence factors, secretion of the hemolysin seems to be crucial for growth of *L. monocytogenes* in host tissues (24, 25). Following phagocytosis of the organism, the membrane surrounding the phagosome undergoes cytolysis, presumably mediated by the hemolysin, allowing growth within the cytoplasm (335). Within 2 h of infection, actin filaments coat the *Listeria* cells (406) and then become reorganized to form polar tails, which seem to be associated with intracellular movement and intercellular spread (86, 405). Nonvirulent mutants of *L. monocytogenes* did not move intracellularly, although actin polymerization was induced. The actin coat was not reorganized, and the bacterial cells did not spread (239). The use of the bacterial protein synthesis inhibitor chloramphenicol showed that the material inducing actin assembly is secreted by the *Listeria* cell, and not by the macrophage (405). Thus, the infecting organism can spread from cell to cell, apparently bypassing the humoral immune system of the host. The organism has even been found within cell nuclei, where it may be protected from cellular enzymes (341). The organism seems to stimulate host cell actin assembly in a directional manner, leading to its rapid movement through the cytoplasm (86). Cytochalasin D treatment prevents the formation of the actin filaments, and bacterial intra- and intercellular movement stops.

Donnelly et al. (110) developed a useful in vitro system to study intracellular growth, by using bovine phagocytes harvested from mastitic milk. Once ingested, the organism was resistant to killing by the phagocytes. Czuprynski et al. (85), on the other hand, demonstrated the ability of bovine phagocytes (blood polymorphonuclear leukocytes, monocytes, and milk leucocytes) to ingest the organism, produce an oxidative response, and kill the intracellular listeriae. When tested with human neutrophils, *L. monocytogenes* F5380, Scott A, Murray B, and EGD were more resistant to killing when grown at 4 than at 37°C (395). This decreased killing did not appear to be related to poor ingestion by the neutrophils.

Defense against Activated Phagocytes

Facultative intracellular pathogens such as *L. monocytogenes* must possess means of overcoming the nonspecific immune responses mediated by activated phagocytes. The organism survives inside nonactivated cells of the mononuclear phagocyte system, but is killed in activated macrophages (218). The formation of a toxic free radical, superoxide ($O_2^{\cdot -}$), is an important part of the sequence in the phagocytic killing of bacteria. The presence of bacterial superoxide dismutase offers a defense against this toxic molecule and hence is a possible virulence factor of the organism. The virulence of five strains of the organism as measured by the 50% lethal dose was also lower in catalase-positive strains, and the 50% lethal dose roughly paralleled the superoxide dismutase activity (432). Increased O_2 consumption and catalase activity during successive passages of *L. monocytogenes* (strains 1/2a and 4b) in monkey kidney epithelial cells (185) was correlated to intracellular multiplication of the bacterial cells. Dallmier and Martin (87) demonstrated that the strains with the highest catalase activity also had the highest superoxide dismutase activity. Bortolussi et al. (37) studied the sensitivity of *L. monocytogenes* to oxidative antibacterial agents such as the hydroxyl radical, H_2O_2, and hypochlorous acid, which may be present in phagocytic cells. They found that the organism is resistant to these products during log phase growth when the catalase concentration is higher than in the stationary phase, perhaps contributing to its intracellular survival.

Hemolysins

The hemolysin of *L. monocytogenes* is recognized as a major virulence factor (76, 348), and its secretion is essential for promoting the intracellular growth and T-cell recognition of the organism (21, 23, 24, 406). The hemolysin, designated listeriolysin O (161) (analogous to streptolysin O [SLO]) was first isolated from *L. monocytogenes* culture supernatants

and shown to be a sulfhydryl (SH)-activated cytolysin, sharing properties with other proteins of this group, such as SLO (162, 308). Hof and Hefner (196) demonstrated that only *L. monocytogenes* and *L. ivanovii*, both of which possess a β-hemolysin, were able to multiply within mice after intravenous injection. All strains of *L. innocua* and *L. welshimeri*, both nonhemolytic species, were avirulent. *L. seeligeri*, however, is weakly hemolytic but avirulent. In a recent report on the hemolysins of the genus *Listeria*, it was shown that all strains of *L. monocytogenes* examined produced listeriolysin O (molecular mass, 60,000 Da) (163). *L. ivanovii* and *L. seeligeri* strains also produced thiol-dependent exotoxins, at about 10 times and 1/10 the level respectively, as that found in *L. monocytogenes*. Hemolysin was not found in *L. innocua* or *L. welshimeri* strains.

A second hemolysin, present in some *L. monocytogenes* strains and immunologically distinct from listeriolysin O, was first reported by Parrisius et al. (321). Two types of hemolysins were identified in clones from an *L. monocytogenes* gene bank constructed in *Escherichia coli* (65, 170). The first was a 23,000-Da protein, possibly the CAMP factor, which was not SH activated and did not cross-react with antilisteriolysin or anti-SLO antibodies. The other cross-reacted with anti-SLO, but activation by SH groups was not tested (247). Vicente et al. (421, 422) identified 12 recombinants expressing β-hemolytic activity after the cloning of *L. monocytogenes* genomic DNA into *E. coli* host cells. Deletions of one of these clones resulted in the preparation of a stable hemolytic clone with an 8.3-kbp insert. Clones whose hemolytic activity was detectable only after sonication were prepared by further subcloning. Gel filtration of the sonicated preparation led to the elution of two peaks of hemolytic activity, corresponding to proteins of 22,000 and 48,000 Da, suggesting the existence of two hemolysins. Genetic evidence of an additional hemolytic determinant to *hlyA* was obtained from hemolytic recombinants of an *L. monocytogenes* gene bank by restriction mapping and hybridization to Southern blots (326a). *L. ivanovii* also secretes two cytolytic factors. One is a thiol-activated hemolysin of 61,000 Da, termed ivanolysin O, and the other is a 27,000-Da sphingomyelinase C found to be involved in the activity of the CAMP factor (418).

Biochemistry of hemolysin. Most of the work on the purification and characterization of listeriolysin has been done by Seeliger's and Goebel's groups at the University of Würzburg. Listeriolysin from *L. ivanovii* was isolated in its membrane-associated form and shown to possess properties similar to those of SLO. The listeriolysin within the membranes generated large transmembrane pores, which are probably related to the cytolytic properties of this molecule (321). Listeriolysin isolated from these membranes, with a monomeric molecular mass of 55,000 to 60,000 Da, was used as the antigen for the preparation of rabbit polyclonal antibodies. Immunoblots of membrane-bound listeriolysin of 28 β-hemolytic *L. monocytogenes* strains with these antibodies led to the unexpected finding that only 2 strains produced a positive reaction. This suggested the production of at least two immunologically distinct hemolysins by human pathogenic *Listeria* strains. The authors proposed that the SLO-related toxin (listeriolysin O) be named α-listeriolysin and that the other(s) be named β-listeriolysin.

Listeriolysin O was purified to homogeneity from a medium containing peptone and yeast extract, which had been treated with a chelating resin (Chelex) (161, 162). The resulting 20-fold increase in toxin production was presumably due to the very low iron concentration resulting from the use of the chelate. The lytic activity of this protein (molecular mass, 60,000 Da) was inhibited by cholesterol and oxidizing agents, was activated by thiols, and showed antigenic cross-reactivity with SLO. The in vitro inactivation by cholesterol is thought to be due to competitive binding with the membrane-binding site of listeriolysin O, in common with other SH-activated cytolysins (403). There is evidence that different domains are involved in cytolytic activity and cholesterol binding. A truncated listeriolysin O lacking a 48-amino-acid C-terminal oligopeptide lacked hemolytic activity but still bound to the membrane receptor cholesterol (417). Listeriolysin O differed from these toxins (e.g., pneumolysin, perfringolysin, alveolysin, SLO), however, in that its optimum pH was 5.5 and it was inactive at pH 7.0. Its activity was restored by again lowering the pH to 5.5. The authors suggest that this optimization of its lytic activity in an acidic environment such as exists in macrophages might promote intracellular growth of the organism (162). It has been demonstrated that under conditions of stress such as heat shock or oxidative stress, at least five heat shock proteins are coinduced with listeriolysin O in *L. monocytogenes* strains, but not in the other *Listeria* species (393, 394).

Hemolysins from *L. monocytogenes* and *L. ivanovii* were characterized and partially sequenced (171, 236). They showed the characteristics typical of listeriolysin O, namely activation by SH reagents, inhibition by cholesterol, cross-reactivity with SLO antibodies, and molecular mass of 58,000 Da. In *L. ivanovii*, a protein with a molecular mass of 24,000 Da copurified with this protein and was separated from it by gel filtration in the presence of SDS. This smaller protein was strongly hemolytic against sheep erythrocytes when combined with culture supernatants from *Rhodococcus equi*, and not with supernatants from *S. aureus*. It may therefore represent the *L. ivanovii* CAMP factor. Determination of the N-terminal sequences of the 58,000- and 24,000-Da proteins showed no homology with the N termini of other SH-activated cytolysins. Listeriolysin O is secreted by all virulent strains of *L. monocytogenes*, but it could not be demonstrated in the supernatants of *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. grayi*, and *L. murrayi* by cross-reaction with anti-listeriolysin O or anti-SLO antibodies (171).

Genetics of hemolysin. In further attempts to identify the role of hemolysin in the virulence of *L. monocytogenes*, transposon mutagenesis was used to inactivate the genetic determinant for hemolysin production (214, 215). Three nonhemolytic (Hly^-) transconjugants and a hemolytic (Hly^+) transconjugant were chosen from mutants produced by using transposon Tn*916* in matings with a serotype 1/2a *L. monocytogenes* strain. The nonvirulent Hly^- mutants either lacked the 58,000-Da extracellular protein (listeriolysin O) or produced a truncated protein of 49,000 Da. Hly^+ revertants regained the hemolytic phenotype, virulence, and production of the 58,000-Da protein (215). Gene complementation studies were used by Cossart et al. (77) to exclude the hypothesis that a polar effect of the transposon insertion was causing the production of the Hly^- mutants. A transposon-induced Hly^- mutant was generated, and the insertion was localized in *hlyA* by DNA sequence analysis. The mutant was transformed with a plasmid carrying only *hlyA* to a stable, hemolytic phenotype identical to that of the wild type. Transposon mutagenesis with Tn*1545* resulted in the production of an Hly^- mutant which produced a 52,000-Da SH-dependent hemolysin, lacking the COOH-terminal por-

TABLE 9. Food-borne outbreaks due to *L. monocytogenes*

Location (yr)	No. of cases (no. of deaths)	No. perinatal/no. nonperinatal	Foods associated	No. of immunocompromised individuals	Reference
Boston (1979)	20 (5)[a]	0/20	Raw celery, tomatoes, lettuce[b]	10	195
New Zealand (1980)	29 (9)	22/7	Shellfish, raw fish[b]	0	253
Maritime Provinces (1981)	41 (17)	34/7	Coleslaw	0	363
Massachusetts (1983)	49 (14)	7/42	Pasteurized milk[b]	42	145
California (1985)	142 (48)	93/49	Jalisco cheese	48	256
Canton de Vaud, Switzerland (1983–1987)	122 (31)	63/59	Raw milk, cheese	–[c]	34
Philadelphia (1986–1987)	36 (16)	4/32	Ice cream,[b] salami[b]	24	370
Connecticut (1989)	9 (1)	2/7	Shrimp[b]	0	341a
United Kingdom (1987–1989)	>300 (?)	NK[d]	Paté[b]	NK	286a

[a] For two of these five deaths, an underlying disease, not listeriosis, was apparently the cause of death.
[b] Foods only epidemiologically linked.
[c] Not stated in manuscript.
[d] NK, not known.

tion of listeriolysin O, with an abnormal regulation by iron (403).

It was not possible to demonstrate a direct relationship between virulence and the amount of hemolysin produced. By using a hyperhemolytic (Hly^{++}) strain, which had a titer of 96 hemolytic units compared with 12 units in the parent strain, Kathariou et al. (216) demonstrated increased levels of production of a protein of 58,000 Da in the Hly^{++} strain. Despite the increased hemolysin production, virulence—as measured by the number of cells required to infect, number of cells isolated from the spleen during infection, and time course to death—remained unaffected.

Transposon mutagenesis has also been used to prepare Hly^{-} mutants useful in studying the sequence of the hemolysin determinant of this organism (151). The conjugative 26-kb transposon Tn*1545*, encoding kanamycin, tetracycline, and erythromycin resistance, was transferred with a frequency of 10^{-8} to *L. monocytogenes* NCTC 7973, a hemolytic virulent strain. The resulting nonhemolytic mutant also was nonvirulent to mice. The ability to infect mice and to grow in spleen and liver cells was restored by spontaneous loss of the transposon. The Hly^{-} mutant secreted a truncated protein of 52,000 Da, which was detected by immunoblotting with an antiserum raised against listeriolysin O, thus demonstrating the insertion of Tn*1545* in the structural gene for this protein. The insertion region of the transposon was then cloned and sequenced (293, 294, 296). The transposon had inserted in an open reading frame (ORF). The deduced amino acid sequence of this ORF revealed homology with SLO and pneumolysin. DNA-DNA hybridization showed that *L. monocytogenes* is the only *Listeria* species in which this *hlyA* sequence is present (75, 296). The *hlyA* gene was cloned into *Bacillus subtilis* host cells, which then expressed hemolysin and were able to grow intracellularly (33). The change of a common bacterium into a virulent organism by cloning of an *L. monocytogenes* hemolysin determinant was also observed by Peterkin (326a) in the β-hemolytic clones of an *L. monocytogenes* gene bank in *E. coli*. These clones were lethal to mice, whereas nonhemolytic clones were not. This work demonstrates the significance of the hemolysin as an essential virulence factor of the organism and the only bacterial gene product known to be absolutely required for intracellular growth (76).

The *hlyA* gene region has been studied to learn how the gene is regulated and whether silent copies of it exist in nonhemolytic species. The 5′ adjacent regions have sequences which show homology to *L. ivanovii* and *L. seeligeri*, but the downstream regions appear specific to *L. monocytogenes* (176). A spontaneous 450-bp deletion located 1.6 kbp upstream from an intact *hlyA* gene resulted in the production of a nonhemolytic, avirulent mutant, indicating an area involved in controlling the expression of the gene (248). The mutant had its hemolytic activity restored by the introduction of a recombinant plasmid expressing a 27-kDa protein (?49). The gene expressing this polypeptide, *prfA*, positively regulates transcription of the *hlyA* gene. Sequence analysis of the gene region revealed the presence of two ORFs. ORF D is located downstream from *hlyA*, and ORF U is located upstream and in the opposite direction; *hlyA* and ORF U are transcribed in opposite directions from promoters which are adjacent (297). These two promoter regions are separated by a 14-bp palindromic sequence. This palindrome was also found upstream of the ORF D promoter, suggesting that all three genes are similarly regulated (297). The ORF located immediately downstream of *hlyA* was sequenced, and its putative amino acid translation product was deduced (102, 295). The amino acid sequence was highly similar to that of a family of secreted metalloproteases, of which the *Bacillus* thermolysin is the prototype. The gene, *mpl*, was species specific to *L. monocytogenes* (102).

LISTERIA SPP. IN FOODS

Food-Borne Outbreaks

The vehicle of infection by *L. monocytogenes* in both sporadic and epidemic listeriosis was unknown as recently as 10 years ago, though direct transmission from infected animals to farm workers and veterinarians was well documented for cutaneous lesions. However, an outbreak involving 41 cases (34 perinatal and 7 adult) (Table 9) occurred in the Maritime Provinces of Canada between March and September 1981 (363). Of the 34 perinatal cases, there were 9 stillbirths, 23 live births of an ill infant with a subsequent 27% mortality rate, and 2 live births of a well infant. The adult mortality rate was 28.6%. The epidemiological study of these cases is a model of its kind. As a result of analyses of case-control surveys, coleslaw consumption was associated with illness. Coleslaw obtained from the refrigerator of one of the patients was shown to contain *L. monocytogenes* type 4b, the epidemic strain. The coleslaw had been prepared by a regional manufacturer, and distribution was confined to the

Maritime Provinces. Environmental cultures taken at the implicated plant failed to reveal *L. monocytogenes*. A review of the sources of raw vegetables for the plant identified a farmer who raised cabbage and kept a flock of sheep, two of which had died of listeriosis, one in 1979 and one in March 1981. The cabbage was grown in fields fertilized by both composted and raw manure from this flock of sheep. Following the cabbage harvest each October, the crop was stored in a large cold-storage shed. As *Listeria* spp. are able to grow at temperatures so low that other organisms either die or enter a stationary phase, the period of cold storage acted essentially as a period of selective enrichment for this species.

An outbreak of listeriosis associated with *L. monocytogenes* serotype 4b occurred among adult patients in eight Boston hospitals in the fall of 1979 (20 cases), although it was not reported until several years later (195). Case-control studies tentatively identified three foods as being preferred by cases as compared to controls: tuna fish, chicken salad, and cheese. The common feature among these was the serving of the foods with raw vegetables such as celery, tomatoes, and lettuce. Three patients died of listeriosis (15% mortality rate). Ten (50%) of the patients were immunosuppressed as a result of chemotherapy or steroid treatment. The vegetables were not available for testing.

In the summer of 1983, an outbreak of listeriosis associated with *L. monocytogenes* type 4b occurred in Massachusetts (145) (Table 9). Of the 42 adult and 7 perinatal cases, a total of 14 (29%) died. Case-control studies showed that the outbreak was strongly associated with drinking a specific brand of pasteurized milk. The case for an association with milk was strengthened by linkage of a specific phage type with the milk-associated disease. The milk associated with the outbreak came from a group of farms where bovine listeriosis was known to have occurred at the time of the outbreak. *L. monocytogenes* was isolated from raw milk from these farms. There was no evidence of improper pasteurization procedures at the plant. This report was the first to indicate a possible increased heat resistance of *L. monocytogenes*, with the suggestion that its intracellular location gave it added protection.

The outbreak which raised listeriosis to a higher level of concern among food manufacturers and regulatory agencies occurred in California from January to August 1985 (256) (Table 9). Of the 142 cases, 93 were perinatal and 49 were adult, with a total of 48 deaths (34% mortality rate) involving 30 fetuses and newborn infants, and 18 nonpregnant adults. Among the 49 adult cases, 48 were immunosuppressed or elderly or had a severe chronic illness. Case-control studies implicated Mexican-style soft cheese of a certain brand. The presence of *L. monocytogenes* type 4b, the epidemic phage type, in this cheese was confirmed. Environmental samples taken from the plant producing the implicated cheese were positive for the organism of the epidemic phage type. Also, although the pasteurizer was found to be in good operational order, 11% of test samples of the cheese were positive for phosphatase and several occasions were documented of delivery of more raw milk than could be handled by the pasteurizer. The factory was closed, and the cheese of the implicated brand was recalled. No *Listeria* organisms were found in raw milk samples from the 27 dairy farms supplying the cheese plant. This is the first recorded listeriosis outbreak in which the food causing the epidemic was identified and recalled during the outbreak.

Since food manufacturers had become aware of the problems which could arise from the presence of *L. monocytogenes* in food (as a result of the above-described outbreak), Kraft Inc. voluntarily recalled Polar B'ar ice cream bars as a precautionary measure in July 1986 when the organism was detected during routine sampling. Of the 330 exposures reported in the United States, 42 involved pregnant women. No significant symptoms developed in any of the women or their fetuses (276).

Another outbreak due to the consumption of soft cheese (34) occurred in the western part of Switzerland (canton de Vaud). During the period 1983 to 1987, 122 listeriosis cases with 31 deaths were recorded (Table 9). More than 85% of the strains isolated during the epidemic period were of a similar serotype (serotype 4b) and phage type. Initially, a source of infection or route of spread could not be found. However, following the outbreak due to Jalisco cheese, an extensive survey of dairy products for the presence of *Listeria* spp. was carried out in Switzerland. When the *Listeria* dairy isolates from this survey were serotyped and phage typed, only the isolates recovered from the surface of a Vacherin Mont d'Or soft cheese were identical to the outbreak strains. A follow-up investigation of the entire Vacherin production also turned up strains of a similar phage type and serotype. Clinical cases were observed mainly during the winter period because the cheese is produced exclusively during the winter season and eaten in the area where the cases occurred. In late November 1987, all products were recalled from the market. This turned out to be an effective control procedure since the number of new cases dropped substantially in the area and no case due to an epidemic strain was recorded in 1988 (34). Interestingly, in a study of 25 of the cases during a 15-month period from January 1983 to March 1984, 10 of 14 adult cases occurred in healthy individuals. In addition, 6 of these 10 previously healthy patients presented with a brain stem encephalitis, suggesting a possible organism tropism for the brain stem of healthy adults (271).

Listeriosis outbreaks in which there was epidemiological association only. Beside the outbreaks which have been linked to a certain food item, other listeriosis outbreaks have occurred in which one or more foods were linked only epidemiologically (Table 9). Some salient points regarding these outbreaks are as follows. (i) An episode in New Zealand in 1980 (253) was the first one to implicate fish and/or fish products in a listeriosis outbreak. (ii) In the Boston outbreak implicating raw vegetables as a possible source of infection (195), antacid consumption was a risk factor for acquisition of listeriosis. (iii) In the Philadelphia outbreak (370), there was lack of a single implicated product, multiple serotypes (4b, 1/2a, 1/2b, and 3b) and isoenzyme types (11 types) were found, and *L. monocytogenes* was not isolated from any of the foods eaten by the patients. Because of all these factors, along with the fact that a high rate of enteric symptoms was experienced by the patients, it was hypothesized that an infecting organism (bacterium and/or virus) may have precipitated the multiplication of *Listeria* spp. present in the gut (370).

In the Boston outbreak, the majority of patients also experienced gastrointestinal tract symptoms (195). In this latter outbreak, however, a single serotype (serotype 4b) appeared to be involved. In some instances factors other than a single contaminated food may contribute to community-acquired outbreaks of listeriosis (80, 346, 370).

Sporadic food-borne listeriosis. Cases of sporadic food-borne listeriosis are listed in Table 10. It should be noted that in some instances doubts remain as to the true source of the infection, because unopened packages of the food involved

TABLE 10. Sporadic food-borne cases of listeriosis

Food	Patient (age, gender)[a]	Health status	Confirmation	Serotype	Reference
Fish	54Y,F	NK[b]	Phage, DNA typing	4	120
Cooked chicken nuggets	52Y,F	Lupus, steroids	None	1/2a	211
Cheese	66Y,M	Heart disease, diabetes, alcoholic	Isoenzyme typing	1/2b	126
Alfalfa tablets	55Y,M	Chronic hepatitis, steroids, antacids	Isoenzyme typing	4b	126
Turkey frankfurter	61Y,F	Cancer	Isoenzyme typing	1/2a	20
Cheese	36Y,F	Healthy	Phage typing	4b	19
Whey cheese	40Y,F	Healthy	Phage typing	4b	16
Cook-and-chill chicken	31Y,F	Pregnant	Phage typing	4	220
Vegetable rennet	29Y,F	Pregnant	Phage typing	4	220
Human breast milk	24D,F	Healthy	Serotyping	NK	401
Homemade sausage	NK	NK	Serotyping	4	56
Salted mushrooms	80Y,M	Healthy	Phage typing	4b	208
Cajun meat and rice sausage	>55Y	Healthy	Isoenzyme typing	4b	12
Raw milk	76Y,F	Chronic renal failure	Isoenzyme typing, ribosomal rRNA typing	1/2a	424
Cod roe (smoked)	38Y,F	Underlying disease	Phage typing	4b	343
Ice cream, fresh cream	64Y,M	Healthy	Phage, isoenzyme typing	4b	343
Pork sausage	43Y,M	Healthy	Serotyping	4b	320

[a] Y, year; M, male; F, female.
[b] NK, not known.

in the case either did not contain the same strain or were unavailable for testing.

Several of the cases provided some interesting background information. In a case involving chicken nuggets (211), the son of the woman involved also contracted a mild, brief gastrointestinal illness. Culture of the son's stools yielded several serotypes of *L. monocytogenes*, one of which was the same as that isolated from his mother (serotype 1/2a). It is unknown how many cases of food-borne listeriosis occur in which only a mild upper gastrointestinal-type illness is seen, but the numbers may be substantial.

In a sporadic illness involving whey cheese (16), a follow-up study (290) suggested that many people in the United Kingdom were likely to have ingested this contaminated cheese. Cheese samples from the incriminated plant were frequently contaminated with large numbers of *L. monocytogenes* ($>10^5$ CFU/g). However, phage-typing data showed that only 3% of the listeriosis cases occurring in Britain in 1987 to 1988 belonged to this particular phage type. Thus, many people were exposed to doses of *L. monocytogenes* in excess of 10^5 CFU/g and did not become seriously ill (290).

A sporadic listeriosis case in which turkey frankfurters were implicated was the first well-documented one involving a meat product (20). The woman heated the product in a microwave oven for 45 s to 1.0 min on high before eating; this suggests that this microwave treatment was not sufficient to inactivate any *Listeria* cells present (20). As a result of this case, 600,000 lb of turkey hot dogs was recalled by the hot dog manufacturer (10). Again, many other individuals probably consumed this product without becoming seriously ill. The case involving the human breast milk was interesting in that some of the incriminated milk had been given to three Doberman puppies, all of which became sick with vomiting, diarrhea, and blood in the stools. *L. monocytogenes* was isolated from the stools of one of the two surviving dogs (401). It is noteworthy that of the 15 sporadic listeriosis cases in which the health status of the individual was known, 7 occurred in normal healthy individuals (Table 10).

A retrospective study conducted in 1986 to 1987, involving 154 listeriosis patients in six regions in the United States, found that patients with sporadic cases of listeriosis were significantly more likely than controls to have eaten either uncooked hot dogs or chicken meat that was still pink (369). The authors found 20% of the sporadic cases of listeriosis to be linked to the above two food products. Therefore, 80% of sporadic infections were not linked to a specific food. Some foods with high incidences of *L. monocytogenes* such as shellfish, however, were not included on the questionnaire. It is quite possible that not all sporadic cases of listeriosis are food borne. The study did not identify any nondietary risk factors.

There have been many other outbreaks of listeriosis in which no foods have been implicated (Table 11). One of the latest recorded outbreaks occurred in the United Kingdom in 1987 (289). In this outbreak, an unusual serotype of 4b, called 4b(x) by the authors, was found to be responsible. The strains, which reacted strongly with the O factor VII antiserum, had been previously identified in only 12 of 842 cases in Britain between 1967 and 1986. The worldwide prevalence of this unusual serovar is unknown; it appears that it could easily be mistaken for a normal 4b serovar. In an outbreak in Strasbourg in 1989 (346), seven different strains appeared to be involved, ruling out a common source (Table 11).

For the incubation periods of some of the sporadic and outbreak cases, see Table 19. There is a wide discrepancy in reported incubation periods. The World Health Organization (438) has suggested an incubation period in adult disease of 1 to several weeks. Obviously the dose of organism ingested, host immune system, and possible intercurrent viral or bacterial infection all play some role in determining the period of onset of the illness after initial exposure to the organism.

Incidence and Survival in Foods

Incidence and growth in dairy products. As can be seen from Table 12, *L. monocytogenes* has been found in a wide range of dairy products. Among these products, cheese has

TABLE 11. Non-food-related outbreaks of listeriosis[a]

Location	Time of outbreak	No. of cases			Major serogroup involved	Suggested agent(s) of transmission	Reference
		Total	Adults	Perinates			
Prague, Czechoslovakia	Aug–Nov 1955	41	0	41	1/2	NK	292
Bremen, Germany	1960–1961	81	NK[b]	NK	NK	NK	375
Bremen, Germany	1963	20	NK	NK	NK	NK	375
Halle, Germany	Apr–Dec 1966	279	0	279	1/2	NK	313
Auckland, New Zealand	Apr–Sep 1969	20	6	14	NK	NK	146
Greenville, United States	Mar–Oct 1975	6	0	6	4b	NK	143
Anjou, France	Jan–Jun 1976	162	36	126	4b	NK	59
Johannesburg, South Africa	Aug 1977–Apr 1978	14	5	9	4b	NK	199
Perth, Australia	Jan 1978–Oct 1979	12	0	12	NK	NK	254
San Juan de Dio's, Chile	Aug–Sep 1980	5	0	5	4	NK	154
Christchurch, New Zealand	Feb 1981–May 1982	18	10	8	4b[c]	NK	123
Saxony, Germany	1983	25	12	13	4b	NK	305
Houston, United States	May–July 1983	10	0	10	1/2b	NK	55
Lausanne, Switzerland	Jan 1983–Mar 1984	25	14	11	4b	NK	271
Austria	May–Oct 1985	17	1	16	4b	NK	315
Kuwait	Oct–Dec 1985	6	0	6	4b	Raw milk, cheese, vegetables?	377
Linz, Austria	1986	28	4	24	1/2a[d]	NK	5
United Kingdom	May–Oct 1987	23	13	10	4b(X)	NK	289
Denmark	Nov 1985–Mar 1987	35	20	15	NK	NK	360
Strasbourg, France	Mar–July 1989	14	6	8	—[e]	NK	346
Costa Rica	June 1989	9	0	9	4b	Mineral oil	367

[a] Adapted from reference 288, with permission.
[b] NK, not known.
[c] Ten cases 4b; one mother-baby pair 4a.
[d] 24 strains, 1/2a; 4 strains, 4b.
[e] Seven different strains involved.

been the most intensively examined because of its known association with food-borne listeriosis. Levels of *L. monocytogenes* as high as 10^7 CFU/g have been found in some naturally contaminated cheeses (Table 12). With soft cheese the contamination is localized almost exclusively on the surface of the rind (298, 354). This phenomenon appears to be due to a pH effect, since a wide pH gradient develops in these cheeses during ripening and *L. monocytogenes* growth has been shown to parallel the increase in pH of cheese during ripening. Excluding data from Spain, the overall incidence worldwide of *L. monocytogenes* in raw milk appears to be around 2.2% (Table 13). Thus, raw milk must be considered by the dairy processor as a source of contamination coming into the plant.

L. monocytogenes has the ability to survive the manufacture and ripening of many different cheeses, surviving best in cheeses such as Camembert and worst in products such as cottage cheese (Table 14). *L. monocytogenes* is usually concentrated in the curd, with only small numbers of organisms being found in whey. The growth of the organisms appears to be slowed but not totally inhibited by the lactic starter culture used in cheese making. The organism has also been shown to survive in products such as cultured buttermilk, butter, and even yoghurt (Table 14). Conflicting results have been obtained on the length of time that *L. monocytogenes* can survive in stored yoghurt, mainly owing to differences in the methods of recovering injured organisms, in the strains used, and in the solids content and pH of the product (183). It appears, however, that *L. monocytogenes* can survive in some instances for up to 30 days after the manufacture of yoghurt, at pH values as low as 4.0. *L. monocytogenes* generally appears to be hardier than coliforms in buttermilk, yoghurt, and probably cheese (68). Thus, coliform-free dairy products are not necessarily free of *L. monocytogenes* contamination.

L. monocytogenes grows well in both naturally and artificially contaminated fluid dairy products (including soymilk) at temperatures ranging from 4 to 35°C (134, 141, 272, 323, 353). The organism obviously grows in the presence of common psychrotrophic bacteria in milk; it has been shown that the presence of pseudomonads in milk may enhance the growth of *L. monocytogenes* (272).

L. monocytogenes also survives the fermentation of skim milk with *Streptococcus cremoris* or *S. lactis* and during a period of refrigerated storage of the product. Fermentation with the thermophilic bacterium *Lactobacillus bulgaricus* was found to be more detrimental to the growth and survival of *L. monocytogenes* than were fermentations with mesophilic lactic starter cultures (361).

L. monocytogenes growth in whey appears to be enhanced over its growth in other fluid dairy products (356). Its growth in whey at 6°C was markedly influenced by two factors, pH and the presence or absence of a *Penicillium camemberti* culture. The organism grew faster in cultured whey and at the higher pH values tested (pH 5.6, 6.2, and 6.8). Growth generally ceased in cultured whey at pH values of 5.4 or lower, although two of the four strains tested grew in cultured whey at pH 5.4 (356).

Incidence and growth in meats. A wide variety of meats are contaminated with *L. monocytogenes* (Table 15), with the incidence of contamination (CFU per gram) varying greatly. This variation is partly due to differences in methods of detection including such factors as the method used, the sample size, the number of single-colony isolates taken to confirm the presence of hemolytic colonies, and the source from which the samples were purchased (retail or commercial outlets). Most of the observed contamination is on the surface. However, Johnson et al. (204) recently found *L. monocytogenes* in the interior muscle cores of 5 of 110 total

TABLE 12. Incidence of *L. monocytogenes* in dairy products

Product type	No. of samples	No. (%) *L. monocytogenes* positive	Major serotype isolated (%)	*L. monocytogenes* CFU/g	Reference
Hard cheese	88	0 (0)	NK[a]		42
Semihard cheese	205	4 (2.0)			42
White-mold-cured cheese	261	7 (2.7)	NK		42
Red-smear cheese	343	33 (9.6)			42
Other cheeses	107	6 (5.6)			42
Soft cheeses[b]	222	23 (10.0)	—[c]	$<10^2$–10^5	332
Soft cheeses	338	6 (1.8)	NK		420
Various cheeses	100	2 (2.0)	1/2a		350
Soft cheeses	121	2 (1.6)	1		274
Butter	20	0 (0)			
Soft and semisoft cheese	374	2 (0.5)	NK	10^4–10^5	128
Cheese	509	29 (5.7)	1 (76)		428
Cheese	140	1[d] (0.7)			73
Cheese	89	8 (9.0)	1 (100)		366
Cheese[e]	23	20 (87.0)	1/2a	10^4–10^7	298
Cheese	350	31 (8.9)	NK	10^3–10^7	414
Soft ripened cheese[f]	769	63 (8.2)	1/2 (71)		182
Soft unripened cheese	366	4 (1.1)			182
Hard cheese	66	1 (1.5)			182
Goat milk cheese[f]	476	22 (4.6)	4b (55)		182
Ewe milk cheese	141	1 (0.7)			182
Yoghurt	180	4 (2.2)			182
Ice cream	394	1 (0.3)	NK		131
Ice cream mix	85	0 (0)			131
Ice cream novelties	51	1 (1.9)			131
Ice cream	150	3 (2.0)	4		182

[a] NK, not known.
[b] Includes cheeses from seven countries.
[c] 13 serotype 1/2; 6 serotype 4b; 3 both serotypes 1/2 and 4b.
[d] Homemade goat cheese.
[e] All samples from one cheese dairy.
[f] Most samples contained <500 CFU/g.

samples of beef, pork, and lamb roasts. These organisms were probably present in the muscle at the time of slaughter.

It is interesting that serotype 1 is the prominent serovar found in meats worldwide (Table 15). One exception is paté in Britain, where serotype 4b was the most frequently isolated during a survey in 1989 (301). A drop in the number of listeriosis cases in Britain from the second half of 1989 and continuing into 1990, may have been associated with an increased awareness of the dangers of eating contaminated paté (163a, 286a). Since most cases of human illness worldwide appear to be caused by serotype 4b (Table 8), some investigators contend that meats are not involved in foodborne listeriosis outbreaks. However, this cannot be completely true, since there have been many sporadic and epidemic episodes of listeriosis involving serotype 1 (Tables 10 and 11), although it is true that, generally speaking many of the contaminated meat products appear to contain lower levels of *L. monocytogenes* than do many soft-cheese products (Tables 12 and 16).

Chicken also seems to be heavily contaminated with *L. monocytogenes* as surveys show contamination rates ranging from 12 to 60% (Table 17). Few studies have examined the serotype predominance in chickens, but it appears that, as for beef, serotype 1 is the most prevalent (see Table 17). Bailey et al. (18) have recently examined the factors influencing colonization of broiler chickens with *L. monocytogenes*. Although *L. monocytogenes* (orally inoculated) does not colonize chickens as easily as do salmonellae or *Campylobacter jejuni*, younger birds were more susceptible to colonization than older birds, and there was a dose-related colonization response. For example, in 1-day old chickens, a challenge of 10^2 and 10^6 *L. monocytogenes* cells resulted in the colonization of 20% (3 of 15) and 73% (11 of 15) of the infected birds, respectively. It is evident that poultry can

TABLE 13. Incidence of *L. monocytogenes* in raw milk

Country	No. of samples	No. (%) *L. monocytogenes* positive	Major serotype involved (%)	Reference
Switzerland	317	4 (1.3)	NK[a]	42
Australia	206[b]	1 (0.5)	NK	420
United States	200	8 (4.0)	1 (71.4)	255
United States	650	27 (4.2)	1 (61.5)	264
Canada	256	4 (1.6)	NK	93
Canada	315	17 (5.4)	1 (82.4)	386
Canada	455	6 (1.3)	NK	132
Scotland	540[c]	14 (2.6)	1 (76.9)	140
New Zealand	71	0 (0)		397
Spain	95	43 (45.3)	NK	104
Hungary	80[d]	3 (3.8)	1 (100)	350
Italy	40	0 (0)		274
United Kingdom	361	13 (3.6)	1/2 (85.0)	182
United Kingdom	1,039[b]	11 (1.1)	4b (63.6)	182
United Kingdom	480[e]	4 (0.8)	1/2 (100)	182
United Kingdom	56[f]	1 (1.8)	NK	182

[a] NK, not known.
[b] Pasteurized milk.
[c] Exact number not clear from manuscript.
[d] Four of the 80 samples were from Czeckoslovakia, of which 1 was positive for *L. monocytogenes*.
[e] Goat milk.
[f] Ewe milk.

TABLE 14. Survival and/or growth of *L. monocytogenes* in various types of dairy products

Product type	Manufacture		Ripening or storage		Reference
	Survival[a]	Growth[b]	Survival[a]	Growth[b]	
Gouda, Maasdam	+	+	+	−	310
Blue cheese	+	+	+	−	318
Cold-pack cheese	+	−	±[c]	−	357
Cheddar cheese	+	+	±	−[d]	355
Brick cheese	+	++[e]	+[e]	+	358
Colby cheese	+	−	±	−	442
Butter	+	+	+	++	312
Feta cheese	+	++	±	+[f]	317
Camembert cheese	+	++	+	++[g]	354
Cottage cheese	+	−	±	−	359
Yoghurt[h]			−	−	380
Yoghurt[i]	+	+	±[j]	−	361
Yoghurt			+[k]	−	68
Cultured buttermilk			+[l]	−	68

[a] ±, numbers decreased during ripening.
[b] −, no growth; +, limited growth; ++, good growth.
[c] Survival period varied depending on preservative and acidifying agent used.
[d] Some growth of the organisms used may have occurred during early stages of ripening in Cheddar cheese at pH 5.0 to 5.1.
[e] Some strains only.
[f] Limited growth occurred during the early ripening stage with some strains only.
[g] After 18 days of ripening.
[h] Results shown are for an initial inoculum level of 10^2 CFU/g of yoghurt; with a high inoculum (10^7 CFU/g), *L. monocytogenes* could be recovered up to 9 days after manufacture.
[i] Initial inoculum, 1×10^3 to 5×10^3 cells per ml.
[j] Survival ranged from 1 to 12 days depending on strain and trial number.
[k] Survival ranged from 18 to 26 days depending on strain.
[l] Survival ranged from 13 to 27 days depending on strain.

become contaminated either environmentally during production or from healthy carrier chickens in the processing plant (18, 159).

Although studies by several groups (203, 378) have shown that *L. monocytogenes* may be unable to grow on meat stored at 4 or 25°C, other researchers have shown that the organism is definitely capable of growing on meat (71, 101, 168, 179, 219). Growth, however, appears to be highly dependent on the temperature and the pH of the meat, the type of tissue, and the type and amount of background microflora present. At 7°C or below, *L. monocytogenes* was unable to grow in meat with a low initial background microflora (10^5 CFU/g) present, whereas at 25°C, no growth of *L. monocytogenes* was observed with a background of 10^7 CFU/g or higher (219, 251). Lactobacilli and not pseudomonads appeared to be the major organisms exerting an antilisterial effect. *L. monocytogenes*, however, grew well in sterile beef stored at temperatures ranging from 4 to 20°C (219).

Glass and Doyle (168) found that growth of *L. monocytogenes* on meat was highly dependent on product type and pH. The organism tended to grow well on meat products with a pH value near or above 6.0, whereas it grew poorly or not at all on meats near or below pH 5.0. Poultry supported the growth of *L. monocytogenes* better than other meats, and roast beef, summer sausage, and hot dogs supported it the least. For roast beef, summer sausage, and hot dogs the inhibitory factors appeared to be pH, combined pH and water activity (a_w), and liquid smoke, respectively (168). *L. monocytogenes* also grew better at 0°C on vacuum-packaged beef of pH 6.0 than on meat of pH 5.6 (179). Grau and Vanderlinde (179) found that regardless of storage temperature or pH, *L. monocytogenes* grew to higher levels on fat than on lean meat, probably as a result of a much shorter lag phase. In contrast, other workers have found similar growth patterns of *L. monocytogenes* on both lean and fat beef tissue, with slightly longer lag periods occurring with the fatty tissue (101).

In general, the organism appears to be quite capable of survival on meat regardless of treatment. For example, freezing, surface dehydration, and simulated spray chilling do not appear to adversely affect its survival (101, 219). Conflicting results have been obtained on the effects of vacuum packaging of meats on the growth of *L. monocytogenes*. The organism has been shown to grow equally well on both vacuum-packaged and air-stored beef (101), but was found to grow more slowly on vacuum-packaged chicken breasts than on film-overwrapped samples (61). Gill and Reichel (165) found *L. monocytogenes* capable of growth on vacuum-packed meat stored at 0, 2, 5, and 10°C; however on high-pH beef packaged under 100% CO_2, it did not grow below 5°C. Also, it does not appear to survive well on raw chicken stored in an anaerobic modified atmosphere, although it grows well if some O_2 (5%) is present (441).

Most investigators studying the fate of *L. monocytogenes* in fermented sausages have found at least a 100-fold reduction in the level of the organism during the manufacture of fermented sausages (27, 169, 209, 410). An exception was beaker sausage prepared without starter culture and held at 32.2°C for 6 h; under these conditions *L. monocytogenes* numbers increased approximately 100-fold (169). In con-

TABLE 15. Incidence of *L. monocytogenes* in meat products

Meat product	Source[a]	No. of samples	No. (%) of samples positive for *L. monocytogenes*	No. of serotypes:					Reference
				4b	1/2a	1/2b	1/2c	Other	
Minced pork	C	30	24 (80)	7[b]	9	5	15		364
Fresh mettwurst	C	30	17 (59)						364
Frozen beef patties	B	149	39 (26.2)		6		25		306
Dry and fresh sausages	B	157	20 (12.7)	1	10	1	5		306
Minced beef	R	67	19 (28)						383
Raw beef	C	658	41 (6.2)						60
Salami and pressed pork	C	243	4 (0.2)						58
Beef, salami, mettwurst		99	4 (4.0)	1	1		2		409
Seasoned sausage mix	NK	156	20 (12.8)						57
Mixed minced meat	NK	85	19 (22.4)	18[c]	3	31	16	2 (4ab, 4d)	40–42
Beef		18	3 (16.7)						40–42
Pork		31	4 (12.9)						40–42
Air-dried meat		44	4 (9.1)						40–42
Uncooked ham		19	0 (0)						40–42
Salami		63	4 (6.3)						40–42
Smoked sausage		55	3 (5.5)						40–42
Mettwurst		19	4 (21.1)						40–42
Beef	R	25	23 (92)						265
Pork	R	25	17 (68)						265
Boneless beef	C	25	5 (20)						265
Boneless lamb	C	15	9 (60)						265
Paté	R	73	37 (50.7)	25				16[d]	301
Ground pork	B	15	6 (40)	1	2	2	3	1[e]	430
Ground beef	B	21	11 (52.4)	2	3	1	4	1	430
Ground meat	C	100	36 (36)						43
Mettwurst	C	100	23 (23)						43
Minced pork	C (mainly)	51	6 (11.8)						384
Ground beef	R	59	27 (46)[f]						213
Ground pork	R	58	23 (40)[f]						213
Onion mettwurst	R	11	1 (9)						213
Raw beef	C	450	31 (6.9)						8
Raw beef	C	1,294	84 (6.5)						9
Cooked beef	C	844	23 (2.7)						9
Sliced canned ham	C	205	3 (1.5)						9
Jerky	C	116	0 (0)						9
Minced pork	Butchershop	90	15 (16.7)	2	10	9	6	1 (4d)	229
Minced pork and beef	Butchershop	48	10 (20.8)						229
Minced pork, beef, and veal	Butchershop	19	3 (15.8)						229
Fermented sausages	C	96	5 (5.2)						138

[a] Abbreviations: C, commercial; R, retail; B, both; NK, not known.
[b] Combined results from 36 strains of *L. monocytogenes*.
[c] Seventy strains of *L. monocytogenes* examined in total from a variety of meats.
[d] Serotype 4b(x).
[e] Nine isolates in total from six samples.
[f] Includes both *Listeria* spp. and *L. monocytogenes*.

TABLE 16. Numbers of *Listeria* spp. or *L. monocytogenes* in various meat products

Food	No. of *Listeria*-positive samples	No. of samples with following concn of *Listeria* spp. (CFU/g):						Reference
		<20	$\geq 10^1$–10^2	$\geq 10^2$–10^3	$\geq 10^3$–10^4	$\geq 10^4$–10^5	$\geq 10^5$–10^6	
Minced pork	16	8		7	1			364
Mettwurst	29	24		5				364
Salami, beef, mettwurst	206	206						409
Paté	37	18	5	4	3	4	3	301
Ground beef	65	30	23	12				43
Ground beef or pork	50	37[a]	25[a]	10[a]				213
Seasoned sausage mix	20		20[b]					57
Ground meat	19	8		11[c]				42
Sausage emulsion	36	17		19[c]				42
Raw meat products	18	11		7[b]				42

[a] Includes both *Listeria* spp. and *L. monocytogenes*.
[b] All samples <100 CFU/g.
[c] From 10 to 10^3 CFU/g.

trast, Berry et al. (27) observed no growth and a slight decrease of *L. monocytogenes* numbers in summer sausage made without starter culture. These different observations most probably result from physical and chemical differences between sausages, e.g., pH, a_w, salt and nitrate levels, and background flora.

Besides the sporadic cases of listeriosis involving meat products (Table 10), there have been recalls in the United States, Canada, and the United Kingdom of meat products containing *L. monocytogenes*. Some of the products recalled have included turkey frankfurters; farm sausages; cooked ham; prepared hamburger sandwiches; hot dogs; chicken spread; vacuum-packaged sliced ham; sausage sandwiches; chicken, egg, and ham salads; frozen heat-and-serve chili dog and chili and cheese dog sandwiches; and Cajun pork sausages. The total economic loss to the respective companies has run well into the millions of dollars.

Incidence and growth in egg products. *L. monocytogenes* was recently found at low levels (1 and 8 CFU/ml) in 2 of 42 samples of commercially broken raw liquid egg (245). It appears that it can survive in refrigerated raw egg and grow well in cooked eggs. Generation times for *L. monocytogenes* growing in egg yolks, cooked whole eggs, cooked yolks, and cooked albumen were 1.7, 1.9, 2.3 and 2.4 days at 5°C. These latter generation times are slightly longer than previously reported for dairy products. *L. monocytogenes* was unable to grow at 5°C in whole egg, maintaining a steady population at levels of around 10^6 CFU/ml over a period of 22 days. In raw albumen, levels of *L. monocytogenes* decreased approximately 5 logs within 22 days at 5°C (379).

Incidence and growth in vegetables. Although many different types of vegetables have been analyzed for the presence of *L. monocytogenes* (42, 131, 192, 241, 381), only potatoes and radishes appear to be regularly contaminated (192). Recent work has also shown that *L. monocytogenes* can be found in individual salad ingredients (2 of 108 samples) and in an even higher proportion of prepacked mixed salads (8 of 42 samples). This implies that further spread of the organism probably occurs during salad preparation. Low levels of *L. monocytogenes* (<200 CFU/g) were found in positive samples (419). As with meats, it appears that serotype 1 is the predominant *L. monocytogenes* sero-

TABLE 17. Incidence of *L. monocytogenes* in poultry products

Product	Source[a]	No. of samples	No. (%) of samples positive for *L. monocytogenes*	No. of samples with serotype			Reference
				4	1	Other	
Cook-chill chicken	R	21	5 (24)				220
Poultry	NK	56	14 (25)				40
Poultry portions	R	25	12 (48)				265
Precooked ready-to-eat poultry	R	527	63 (12)				164
Chilled meats (mainly poultry)	R	74	13 (18)				164
Fresh chicken parts	R	130	19 (14.6)				159
Fresh and frozen chickens	R	35	20 (57)				240
Fresh packaged chicken parts	C	75	37 (49.3)				240
Poultry	R	68	10 (14.7)	10	5		167
Chicken legs	R	16	9 (56.3)	1	7	1	131
Marked broilers	C	90	21 (23)		27[b]	6[c]	17
Fresh turkey parts	R	180	27 (15)				160
Chicken	NK	56	14 (25)				42
Fresh chicken	R	50	33 (66)	11	50	15[d]	332
Frozen chicken	R	50	27 (54)				332
Frozen chicken	R	80	12 (15)	3	9		415
Precooked chicken	R	102	27 (26.5)	6	20	NK	221

[a] Abbreviations: C, commercial; R, retail; NK, not known.
[b] Twenty-one 1/2b; six 1/2c.
[c] Two 3b; four not known.
[d] Twelve 3; three nontypable.

var. Sources of contamination of vegetables include soil, water, animal manure, decaying vegetation, and effluents from sewage treatment plants (29).

Although *L. monocytogenes* appears to grow quite well in lettuce juice stored at 5°C (299, 396), it could grow in heat-sterilized cabbage juice only when stored at 30°C and containing ≤2.0% NaCl. At 4°C, although the organism did not grow in the cabbage juice, it was able to survive for long periods (74). Steinbreugge et al. (396) found that *L. monocytogenes* grew on shredded lettuce stored at 5, 12, and 25°C, although the increase was only about 1 log after 14 days at 5 and 25°C and about 3 logs after 14 days at 12°C. In addition, in several trials the organism did not grow or had died after 14 days. The variation in results could not be fully explained, but was partially attributable to differences of pH and competition from the bacterial flora. Various salads left at 4°C for 4 days supported the growth of *L. monocytogenes* (roughly twofold increase), indicating that the organism can survive and multiply during storage of refrigerated pre-prepared salads (381).

Berrang et al. (26) found that *L. monocytogenes* grew well at 15°C on fresh vegetables stored in air or under a controlled atmosphere, increasing in number by about 4 logs within 6 days on asparagus, broccoli, and cauliflower. It did not grow nearly as well at 4°C, however, increasing by only about 1 log after 14 days on asparagus and actually decreasing by about 0.5 log after 14 days on broccoli and cauliflower. The observation that storage under a controlled atmosphere did not influence the rate of growth of *L. monocytogenes* is significant, since the storage life of the vegetables was considerably increased by the controlled-atmosphere treatment. For vegetables on which the organism is capable of growing at 4°C (e.g., asparagus), the increased shelf life of the products allows extra time for *L. monocytogenes* to grow to significantly higher levels.

L. monocytogenes does not appear to be able to grow well on carrots unless they are cooked (30). Indeed, an anti-*Listeria* effect has been observed, with raw carrots stored at 5 or 15°C spoiling before *L. monocytogenes* could grow. Broth culture medium containing as little as 1% raw-carrot juice substantially inhibited the growth of the organism, as compared with the control. The component(s) toxic to *L. monocytogenes* has not yet been isolated (30).

Incidence and growth in seafood. Fish products have received less study than other foods. Weagant et al. (427), upon examining 57 samples of frozen seafood products, found 15 samples, including shrimp, crabmeat, lobster tail, fin fish, and surimi-based seafood, to be positive for *L. monocytogenes*. Jemmi (201) tested 377 samples of smoked and marinated fish and found 47 to be positive for *L. monocytogenes*. Of 100 smoked samples, 24% were positive for the organism. Tropical fish and fish products including dried-salted fish were found to be free of *L. monocytogenes*, although *L. innocua* was found in 3 of 10 and 5 of 14 fresh and frozen samples, respectively (149).

Very little work has been done to examine the growth of *L. monocytogenes* in seafoods. Lovett et al. (263) examined the growth of *L. monocytogenes* in shrimp, crabmeat, surimi, and white fish stored at 7°C. *L. monocytogenes* inoculated into samples of these products, which had been sterilized prior to inoculation, increased in number by about 5 logs within 14 days. It has been demonstrated that the organism can also grow in nonsterile fish products including cooked shrimp, cooked lobster, and cold smoked salmon (124a).

As indicated above, fish products have been epidemiologically implicated in two listeriosis outbreaks and have been thought to be the cause of one case of sporadic listeriosis (Tables 9 and 10). In addition, many fish products in North America including frozen cooked shrimp; canned frozen, fresh, and imitation crab meat; smoked salmon; imitation scallops; frozen canned lobster; and surimi products have been found to be contaminated with *L. monocytogenes*, and have been recalled from the market.

Thermal Resistance

The question of whether *L. monocytogenes* is unusually thermotolerant arose following the 1983 Massachusetts outbreak, where epidemiological evidence strongly implicated the drinking of a specific brand of pasteurized whole or 2% milk. No evidence of faulty pasteurization was found in the implicated dairy. The suggestion was made at that time (145) that the intracellular location of the organism provided a protective milieu. Doyle's group (114, 359) reported that *L. monocytogenes* inoculated into skim milk at a level of 10^5/ml survived the heating that occurred during the manufacture of nonfat dry milk and of cottage cheese. Because it was assumed that these artificially inoculated organisms were not intracellular, the conclusion was drawn that, indeed, an increased heat resistance might pose a potential problem in the survival of the organism during the pasteurization of naturally contaminated dairy products. At the same time, however, Bradshaw et al. (39) reported the results of a series of determinations over a 2-year period of the thermal resistance of *L. monocytogenes* Scott A suspended in raw milk and heated in sealed glass tubes to determine *D* values. These authors concluded that the organism would not survive 71.7°C (161°F) for 15 s, which are the conditions for high-temperature, short-time (HTST) pasteurization. Further, this heat resistance was a stable characteristic. These conflicting results led to a series of studies on the thermal resistance of *L. monocytogenes* present in artificially or naturally contaminated milks and other foods. Doyle et al. (113) infected four cows with *L. monocytogenes* Scott A and performed pasteurization trials on the milk obtained from these cows, which contained the organism within polymorphonuclear leukocytes. Their study indicated that these intracellular organisms could survive HTST pasteurization conditions. They suggested that the discrepancy between this result and that of Bradshaw et al. (39) was due mainly to the intracellular location of the bacteria. However, after an extensive study involving sterile whole milk in which *L. monocytogenes* was either freely suspended or located within bovine milk phagocytes, Bunning et al. (50) reported that the intracellular position of the organism did not significantly increase thermal resistance. However, they did demonstrate that the HTST conditions (71.7°C for 15 s) did not provide an adequate *D* process, suggesting that *L. monocytogenes* could survive commercial pasteurization, whether located extracellularly or intracellularly. In addition, Garayzabal et al. (153) showed that although no *L. monocytogenes* cells were isolated from inoculated raw milk immediately after thermal treatment in a pilot plant size pasteurizer at temperatures ranging from 69 to 73°C for 15 s, they could be isolated from samples heated from 69 to 72°C after incubation for 2 days to 3 weeks. This emphasizes the importance of the isolation techniques used for studying heat-stressed organisms. In a study again involving both extra- and intracellularly located *L. monocytogenes* cells in naturally contaminated milk, Farber et al. (135) reported that although the organism was recovered from milk heated to 60

to 66°C, no viable *Listeria* cells were recovered after treatment at 69°C and above for 16.2 s. An interesting aspect of this study is that in contrast to the results of Doyle et al. (113), Farber et al. (135) found the organism mainly within macrophages and not polymorphonuclear leukocytes.

The heat resistance of *L. monocytogenes* in dairy products has been reviewed recently (107, 124, 184, 268). Mackey and Bratchell (268) neatly summarized the data and found that according to their models, HTST and vat pasteurization of milk would achieve a 5.2 and 39 *D* reduction in the numbers of *L. monocytogenes*, respectively. *z* values calculated from heating in both sealed tubes and slug flow heat exchanger were 6.1 and 7.4°C, respectively. These values were close to the overall (all foods and broths) calculated *z* value of 6.7 to 6.9°C (268) and are close to those values found for *L. monocytogenes* in meats (see Table 18). Recent assessment of the problem (112, 124, 268, 270) show that there is still disagreement on the question of the thermal resistance of *L. monocytogenes*. Two recent developments may help to explain some of the discrepancies in the literature. The first is the phenomenon called the heat shock response. Several investigators have found that if *L. monocytogenes* is exposed to sublethal temperatures of around 44 to 48°C before being subjected to the final test temperature, the cells acquire an enhanced thermotolerance (125, 139, 233). This has been demonstrated in both a milk (139) and a meat (125) system. In the meat system, heat-shocked cells shifted to 4°C appeared to maintain their thermotolerance for at least 24 h after heat shock (125). In addition, and perhaps more importantly, cells grown at high temperatures appear to become more heat resistant. For example, Knabel et al. (233) found that *L. monocytogenes* cells grown at 43°C were more thermotolerant than cells grown at lower temperatures or cells that had been heat shocked at 43°C (for 5, 30, or 60 min). This implies that *L. monocytogenes* grown in a refrigerated food may acquire enhanced thermotolerance if that food is temperature abused.

The second development concerns the methods for recovering heat-stressed organisms. It has been recently discovered that the use of strict anaerobic techniques when enumerating heat-stressed *L. monocytogenes* cells can lead to recovery of significantly more cells than are recovered in the presence of oxygen (233). For example, $D_{62.8°C}$ values for cells grown at 43°C and recovered anaerobically were at least sixfold greater than those obtained previously by using cells grown at 37°C and enumerated aerobically (233). The oxygen sensitivity of heat-stressed *L. monocytogenes* was believed to be due to the inactivation of the enzymes catalase and superoxide dismutase during heating (233). The authors suggest that if growth temperature and anaerobic recovery are taken into account, high levels of *L. monocytogenes* would survive the minimum HTST treatment required by most countries. In this regard, we recently conducted experiments similar to our previous studies (135) and found that although *L. monocytogenes* cells (10^5 CFU/ml) grown at 30°C and suspended in raw milk do not survive an HTST treatment, those grown at 39 and 43°C can survive pasteurization (124a). Questions that remain to be answered include the following. (i) How "anaerobic" are the heated food environments in which one can find *L. monocytogenes*? (ii) Would organisms which are maintained at around 39°C in the cow's udder, not multiplying, acquire an enhanced thermotolerance, and if so, how long would this last? (iii) Do conditions or compounds such as H_2O_2, within the phagocytes, induce additional heat resistance within *Listeria* cells?

Heat resistance in meats. Although much of the previous work on the thermal resistance of *L. monocytogenes* has been done with dairy products, some work has recently been done on the heat resistance of *L. monocytogenes* in meat and meat products (Table 18). Some interesting points that have been found include the following. (i) Although the addition of beef fat does not appear to enhance the heat resistance of *L. monocytogenes*, the presence of curing salt substantially increases it (127, 270). (ii) The heat shock phenomenon must be taken into account when heating meats, especially for products heated slowly to a final internal temperature (125, 269). (iii) Survival of *L. monocytogenes* on chicken breasts heated to internal temperatures as high as 82.2°C is not consistent with published *D* and *z* values (61, 270).

TABLE 18. Heat resistance of *L. monocytogenes* in meat, chicken, and fish

Product	Temp (°C)	*D* value (min)	*z* value (°C)	Reference
Beef	60	3.8	7.2	270
	70	0.14		
Chicken leg	60	5.6	6.7	270
	70	0.11		
Chicken breast	60	8.7	6.3	270
	70	0.13		
Ground beef	60	3.12	5.3	127
Fermented sausage mix	60	16.7	4.6	127
Meat slurry	60	2.54		38
	70	0.23		
Chicken	60	5.29,[a] 5.02[b]	6.72,[a] 7.39[b]	156
	70	0.16, 0.20		
Beefsteak	60	8.32, 6.27	5.98, 5.98	156
	70	0.20, 014		
Liver sausage slurry	60	2.42	6.2	31
Meat extract broth	60	3.9		7
Meats (predicted value)	60	3.82	6.8	270
	70	0.13		
Raw liquid whole egg	60	1.46[c]	6.6[c]	147
Crabmeat	60	2.61	8.4	189

[a] Strain Scott A.
[b] Strain 11994.
[c] Average of five strains.

Mackey et al. (270) have formulated an equation for calculating processing times based on a "7*D*" inactivation of *L. monocytogenes* in meat, which takes into account the pronounced shoulders which appeared on their survivor curves. The equation, $\log_{10}$ processing time = $10.3943 - 0.14618t$, where t is the heating temperature and D is the decimal reduction time in minutes, predicted, for example, that 70°C for 1.45 min would be equivalent to a 7*D* kill. The authors agreed with Gaze et al. (156) that for the majority of cases, heating at 70°C for 2 min would be sufficient to inactivate any *L. monocytogenes* present in raw meat.

The heating of hot dogs to an internal temperature of 160°C (71°C) resulted in an approximate 3*D* reduction in numbers of *L. monocytogenes* (444), whereas studies of the heat resistance of *L. monocytogenes* in liquid whole egg demonstrated that the minimal pasteurization schedule (60°C for 3.5 min) would result in a 2 to 3 log reduction in numbers of *L. monocytogenes* (147).

There have been few, if any, detailed studies comparing the heat resistance of different *Listeria* species or *L. monocytogenes* serotypes. It appears, however, that *L. innocua* may possess a similar heat resistance to *L. monocytogenes* (270). In addition, one report suggests that serotype 1 strains

TABLE 19. Minimum infectious dose and incubation period in food-borne listeriosis

Patient description[a] or outbreak	Status	Food	Dose	Symptoms	Incubation period	Reference
59Y,F	Healthy	Cheese	2.7×10^6	Mild	NK	124a
40Y,F	Healthy	Whey cheese	3.4×10^9	Meningitis	<24 h	16
Jalisco cheese outbreak	See Table 9	Cheese	10^2–10^4	Severe, varied	31 to 35 days (range, 1–91 days)	256
NK	NK	Home-made sausage	2.7×10^6/g	Meningitis	16–18 h	56
80Y,M	Healthy	Salted mushrooms	3.8×10^6/g	Septicemia	NK	208
61Y,F	Cancer	Frankfurter sausage	$>1.1 \times 10^3$/g	NK	NK	20
64Y,M	NK	Ice cream, fresh cream	1×10^6/g	NK	2 days	343
F (two)	Pregnant	Shrimp[b]	NK	Mild	19–23 days	341a
52Y,F	Steroids, lupus	Chicken nuggets	NK	Severe	3–5 days	211
29Y,M	Healthy	Chicken nuggets	NK	Mild	3–5 days	211

[a] Abbreviations: Y, years; M, male; F, female; NK, not known.
[b] Shrimp only epidemiologically implicated. Nine individuals met the case definition for illness. Incubation period calculated only for two pregnant women.

may be more heat resistant than those belonging to serotype 4 (252).

Heat-induced injury. Little work has been done on the repair of heat-injured *L. monocytogenes* cells. From what is known, it is apparent that selective media currently in use for the isolation of *L. monocytogenes* are not satisfactory for the recovery of injured cells (389). An optimal repair period appears to be 6 to 9 h in a nonselective medium at 20 to 40°C; lower temperatures (5 and 12°C) lead to decreased recovery (388). Of the compounds that have been studied, sugars, salts and polyols such as glycerol or mannitol decreased the extent of heat injury, whereas fructose or NH_4Cl enhanced cell death (391). Surprisingly, the use of carbohydrates by *L. monocytogenes* for growth and metabolism was not related to the ability of the compounds to protect the cells against heat injury. The addition of catalase or pyruvate to selective media does not appear to enhance repair of heat-injured *L. monocytogenes* cells (391). Obviously, much more work is needed in the whole area of heat- and/or stress-induced injury.

Minimum Infectious Dose

Animals. The infective route of orally ingested cells appears to be via the Peyer patches of the intestine and then into phagocytes, passing into the mesenteric lymph nodes by way of the lymphatic pathways. Mice infected orally have shown variable responses, with 50% infectious doses ranging from 1.7×10^3 to 9.9×10^6 (14, 174). Although previous publications have shown intraperitoneal infection to be much more effective than intragastric inoculation, recent work by Pine et al. (331) has shown 50% lethal doses for both intragastric and intraperitoneal challenge to be comparable, with death actually occurring earlier with intragastric feeding. The optimum weight for intragastric testing of mice was 15.0 g. Golnazarian et al. (174) found that the response of pregnant mice, beige mutants, or cimetidine-treated mice was comparable to that of the normal control mice. Only animals receiving large doses of hydrocortisone acetate (2 mg/day for 3 days prior to infection) were considerably more infected than controls, with an average $\log_{10}$ 50% infectious dose of 0.41.

Other studies on oral feeding of mice with *L. monocytogenes* have shown that very high ($\geq 2.5 \times 10^8$ cells) levels of organisms are required to cause invasion of the Peyer's patches (266) or death in normal mice (14). In addition, Miller and Burns (300) found that a dose of $\geq 4 \times 10^7$ cells caused fetal death in 6 of 10 pregnant mice. Numerous studies with various animal models have shown that only the administration of a large oral inoculation or the presence of a diminished normal microbial flora could lead to listerial colonization (362).

Recent feeding trials of *L. monocytogenes* in which a nonhuman primate model was used showed that only animals receiving a dose of 10^9 *L. monocytogenes* cells became noticeably ill, with symptoms of septicemia, irritability, loss of appetite, and occasional diarrhea. Smaller numbers of cells did not result in noticeable symptoms of disease (126a).

Humans. The minimum number of pathogenic *L. monocytogenes* cells which must be ingested to cause illness in either normal or susceptible individuals is not known. Table 19 lists cases in which the numbers causing illness have been approximated. It is to be expected that the number of cells causing illness will vary tremendously depending upon a variety of factors, the most important of which are bacterial strain differences and host susceptibility. It is apparent, however, that many healthy susceptible individuals consume foods containing *L. monocytogenes* in small numbers daily, without becoming ill. This is at least partly because most normal individuals carry T cells with reactivity to *Listeria* spp. (303), probably as a result of subclinical infection with either *Listeria* spp. or other gram-positive bacteria sharing antigens with *Listeria* spp. (303).

METHODS OF DETECTION IN FOODS

Isolation of *L. monocytogenes* from environments such as food, which can be heavily contaminated with other organisms, often proves challenging. Various selective agents including potassium tellurite, nalidixic acid, and acriflavine have been proposed (106). Refrigeration of the sample in a nonselective medium for prolonged periods (up to 6 months) can improve recovery, owing to the psychrotrophic nature of the organism. McBride and Girard (277) developed a selective agar medium which, coupled with the oblique illumination of the colonies suggested by Henry (193), contributed to successful isolations of *L. monocytogenes* from food. Many conventional culture methods have been developed recently (63, 106), and alternative methods involving monoclonal antibodies (117, 133) and DNA probes (89, 227, 234, 328) are being reported.

TABLE 20. Selective and indicator agents in some *L. monocytogenes* plating media

Medium[a]	Acriflavine	Glycine anhydride	Phenylethanol	LiCl	Esculin	K tellurite	Antibiotic[b]	Reference
MMA	−	+	+	+	−	−	Cyclo	261
LPM	−	+	+	+	−	−	Mox	246
Mod V.J.	−	+	−	+	−	+	Mox, Nal, Bac	47
RAPAMY	+	−	+	−	+	−	Mox, Nal	45
PALCAM	+	−	−	+	+	−	Cz, Poly	413
ACA	+	−	−	−	−	−	Cz	45
Oxford	+	−	−	+	+	−	Ctt, Fos, Col	83
Mod Oxford	−	−	−	+	+	−	Mox, Col	280
ASLM	+	−	+	+	+	−	Mox, Cz	6

[a] Abbreviations: MMA, modified McBride's agar; LPM, lithium chloride-phenylethanol-moxalactam agar; Mod V.J., modified Vogel Johnson agar; ACA, acraflavine-ceftazidime agar; Mod Oxford, modified Oxford medium; ASLM, Al-Zorecky-Sandine *Listeria* medium.

[b] Cyclo, cycloheximide; Mox, moxalactam; Nal, nalidixic acid; Bac, bacitracin; Cz, ceftazidime; Poly, polymyxin B; Ctt, cefotetan; Fos, fosfomycin; Col, colistin.

Conventional Methods

Plating media. Many isolation media have been developed to recover *Listeria* spp. from foods. Direct-plating procedures do not reliably isolate *Listeria* spp. and typically are used in conjunction with a prior enrichment. The selective medium developed by McBride and Girard (277) was among the first solid media suitable for recovering *L. monocytogenes* from mixed cultures. Another early medium contained nalidixic acid, polymyxin B and acriflavine as selective agents (98). A modification of McBride's agar (MMA) was developed which contained phenylethanol, glycine anhydride, lithium chloride, and cycloheximide as selective agents (261). After incubation on MMA plates, *Listeria* spp. appear as small bluish granular colonies when the plates are examined by oblique (45°) transmitted light under a stereomicroscope (193).

An improved selective medium was developed by Lee and McClain (246) for the isolation of *L. monocytogenes* from meats. This medium, the first reported to contain moxalactam (a broad-spectrum antibiotic inhibitory to many gram-positive and gram-negative bacteria), also contains lithium chloride and phenylethanol (LPM agar). It improves the recovery of the organism from mixed cultures, over the recovery on MMA. Other useful formulations include a modification of Vogel Johnson agar (MVJ [47]); two media, RAPAMY and PALCAM, developed by Van Netten et al. in the Netherlands (412, 413), Oxford agar (83), and a modified Oxford agar (280). The recovery of heat-injured *L. monocytogenes* cells on MVJ was recently improved 100-fold by the addition of Tween 80, fetal bovine serum, or egg yolk emulsion (390). The selective and indicator agents used in some of these media are shown in Table 20.

There are several reports evaluating different selective media for isolation and enumeration of *Listeria* spp. in foods (191). Dominguez et al. (103) compared their medium with the original McBride agar for the recovery of five *Listeria* spp. inoculated into raw milk and cheese. They found their medium to be superior, a result which is not surprising in view of the nonselectivity of McBride agar. Golden et al. (172, 173) compared six media, McBride's original agar, MMA, the gum-based medium of Martin et al. (273), Despierres agar (98), an agar based on the *Listeria* enrichment broth of Donnelly and Baigent (LEB) (108), and Dominguez medium (104), for their ability to recover heat- and freeze-injured *L. monocytogenes* from foods. Loessner et al. (257) compared seven plating media, including the original McBride agar, MMA, LPM agar, Dominguez medium, and MVJ agar, for their suitability to enumerate *Listeria* spp. Recently, Buchanan et al. (48) compared LPM and MVJ agars for detection of *Listeria* spp. in meat, poultry, and seafood both by direct plating and in conjunction with a most-probable-number enrichment. Cassiday et al. (64) compared the enumeration of *L. monocytogenes* from artificially inoculated hams and oysters on 10 different direct plating media, including MMA, Dominguez medium, LEB agar, Despierres agar, LPM agar, and MVJ. Jatisatienr and Busse (200) compared the recovery of the organism from inoculated cheese on MMA and Oxford agars as the selective media. In summary, these studies indicated that (i) the performances of the media were affected by the menstruum; (ii) although MMA was not sufficiently selective, the other media were about equal for enumeration of *L. monocytogenes*; (iii) LPM agar was the best medium overall in enumerating *L. monocytogenes* in foods, as only it inhibited the growth of organisms of other species while supporting the growth of all *L. monocytogenes* strains; (iv) MVJ and Oxford agars had an advantage over LPM agar in that *Listeria* spp. could be visually differentiated from other bacteria without the need for Henry's illumination test; and (v) although the recovery of freeze-injured *L. monocytogenes* was not affected by the plating medium used, heat-injured cells were recovered in larger numbers when modified Despierres agar was used (173). Although no one medium has clearly emerged as superior, PALCAM medium appears to be preferred in Europe, whereas LPM and Oxford media are the most widely used in North America.

Enrichment procedures. Among the earliest methods used to recover *L. monocytogenes* from food and environmental samples was one that used cold enrichment (181). Samples were diluted in nutrient broth and stored at 4°C. After 24 h, and once a week thereafter, portions of the enrichment broth were plated onto selective media which were incubated at 35°C. With this procedure, detection of the organism can take up to 3 months. Incubation at 4°C suppresses the growth of most microorganisms, but *Listeria* spp. multiply slowly with a generation time of 1.5 days (353).

More recently, the incorporation of specific selective agents into enrichment media has shortened the time required to isolate the organism. Klinger et al. (231) discussed the selective agents recommended by various authors. Donnelly and Baigent (108) modified Dominguez medium in developing the widely used LEB by adding nalidixic acid and acriflavine as selective agents.

Nalidixic acid and acriflavine have also been used in the U.S. Food and Drug Administration (FDA) enrichment broth (EB) (138a, 264, 333), which also contains cycloheximide, an antifungal agent used to suppress the yeasts and molds often present in foods. This broth forms the basis of the commonly used FDA *Listeria* detection method (262, 264), which is recommended for the detection of the organism in foods other than meats. The method originally used MMA as the selective medium. As presently modified (194, 426), samples are inoculated into EB and incubated at 30°C for 2 days. At 1 and 2 days, samples are plated onto Oxford and LPM agars and then incubated for 24 to 48 h at 35 and 30°C, respectively, for detection and identification (138b).

The highly selective LPM agar formed the basis of a U.S. Department of Agriculture detection procedure for use with meat and poultry (246, 279). The primary enrichment broth, consisting of LEB modified to contain 20 mg of nalidixic acid $liter^{-1}$ was modified further to form the secondary enrichment broth containing 25 mg of acriflavine $liter^{-1}$. The primary broth containing the sample was incubated for 24 h at 30°C, after which 0.1 ml was transferred to 10 ml of secondary enrichment broth. As modified by the Health Protection Branch (426), this was incubated for 24 to 48 h at 30°C before being plated onto Oxford and LPM agars for detection.

Other enrichment procedures have used (i) an enrichment broth for use with cheese samples, in which 2% sodium citrate is added to tryptose broth (443); and (ii) the addition of lithium chloride to the secondary broth of McClain and Lee (279) to inhibit the growth of enterococci and the addition of ferric ammonium citrate to detect the hydrolysis of the esculin already present in this broth (148). The use of the latter medium, known as Fraser broth, as a secondary enrichment broth increases the recovery of *Listeria* spp. by about 6% (426). The current international Dairy Federation procedure uses a selective enrichment in EB at 30°C, with plating onto Oxford agar after 48 h (11).

Several investigators have compared enrichment procedures for the isolation of *Listeria* spp. from dairy products and meats. Doyle and Schoeni (116) compared the detection of *L. monocytogenes* in cheese by (i) cold enrichment in tryptose broth at 4°C over a period of 8 weeks, (ii) the FDA enrichment procedure; and (iii) the selective enrichment procedure of Doyle and Schoeni (115). *L. monocytogenes* was isolated from 41 of 90 (46%) samples of soft, surface-ripened cheese, with 21 of these isolations being made by using the cold enrichment procedure. In most cases, the organism was isolated from a cheese sample by only one of the three procedures. Slade and Collins-Thompson (385) compared the isolation of *Listeria* spp. from milk by (i) the FDA enrichment procedure and (ii) the two-stage enrichment procedure of Hayes et al. (190), the latter consisting of a cold enrichment in tryptose broth prior to a selective enrichment in thiocyanate-nalidixic acid-acriflavine broth, followed by plating on McBride *Listeria* agar. *L. innocua* and *L. monocytogenes* were isolated from 19 of 34 (56%) raw milk samples, both procedures being equally effective. Pini and Gilbert (332) compared the detection of *L. monocytogenes* in chicken or soft cheese by a cold enrichment in tryptose phosphate broth at 4°C over a period of 12 weeks and by the FDA enrichment procedure. *L. monocytogenes* was isolated from 70 of 160 (44%) chicken samples and from 23 of 222 (10%) cheese samples. Neither method alone yielded all isolates from the two food types. Truscott and McNab (408) compared the recovery of the organism from ground beef by LEB and by an enrichment broth (*Listeria* test broth [LTB]) containing Tween 80, acriflavine, and moxalactam as selective agents, as well as horse serum and esculin. *L. monocytogenes* was isolated from 29 of 50 (58%) ground-beef samples, but neither broth alone recovered all of the positive samples and the differences in recovery were not statistically significant.

The use of commercially available identification systems for the confirmation of presumptive *Listeria* isolates to the species level has been examined. Kerr et al. (222) found, in a comparison between the Mast ID and API 50CH systems for the detection of carbohydrate fermentation patterns, that although both gave accurate results within 24 h, the Mast ID system was less expensive and time-consuming. The API-ZYM system, using the detection of 19 constitutive enzymes in a total of 65 strains of *Listeria* spp., was evaluated by del Corral and Buchanan (97). After analysis of the enzyme patterns shown by the species examined, they concluded that the API-ZYM system could be used for the rapid (4-h) confirmation of *L. monocytogenes*.

Tests for hemolytic activity. Since *L. monocytogenes* was first isolated, workers in the field have stated the need for simple and rapid procedures to differentiate pathogenic from nonpathogenic *Listeria* strains, without resort to animal inoculation (181, 348). Several procedures to demonstrate hemolysis have been proposed to meet this need. The CAMP phenomenon was adapted for use with *Listeria* spp. (338). Groves and Welshimer (186) used three in vitro reactions to identify *Listeria* spp. that were pathogenic: a positive CAMP reaction, fermentation of rhamnose, and nonfermentation of xylose. Skalka et al. (382) improved the testing of *Listeria* spp. for the CAMP reaction by using horse blood agar and by adding a streak of *R. equi*, which enabled *L. ivanovii* to be differentiated from *L. monocytogenes* and *L. seeligeri* by their opposite reactions with the hemolysins of the two test organisms. More recently, Smola (392) found that when the purified exosubstances of the *S. aureus* and *R. equi* strains were applied to the surface of the blood agar plate in place of cultures of these organisms, *L. monocytogenes* showed increased β-hemolysis with both substances, whereas *L. ivanovii* showed synergism only with the *R. equi* factor.

The simplest test for the presence of β-hemolysis is the appearance of zones of clearing on horse blood agar plates. However, these are often difficult to interpret with *L. monocytogenes* and *L. seeligeri* as strains vary in the intensity of their reaction. A microplate technique proposed by Rodriguez et al. (351) for the routine determination of hemolytic activity with erythrocyte suspensions is a reliable method yielding semiquantitative results. This method, combined with D-xylose, L-rhamnose, and α-methyl-D-mannoside fermentation tests, allows the differentiation of *L. monocytogenes*, *L. seeligeri*, and *L. ivanovii*. Continuous cell lines have also been suggested as a test vehicle for pathogenic *Listeria* spp. Farber and Speirs (137) tested culture filtrates from 18 *Listeria* strains for presence of hemolysis on blood agar, a cytotoxic effect against tissue culture cells, and pathogenicity to mice. *L. monocytogenes* and *L. ivanovii* were positive in all three tests, whereas *L. innocua*, *L. murrayi*, and *L. grayi* were negative. Of the eight cell lines tested, Chinese hamster ovary cells were the most sensitive.

Alternative Methods

To monitor the incidence of *L. monocytogenes* in foods, reliable methods must be developed for the rapid detection of the organism. Conventional methods are tedious and are

TABLE 21. Immunological and genetic methods for the detection of *L. monocytogenes* or *Listeria* spp.

Method (target)	Commercial	Specificity	Tested in foods	Reference
Immunological				
MAb (flagellar antigen)	No	*Listeria* spp.	Yes	136
MAb (heat-stable antigen, 30–38 kDa)	Organon Teknika (Listeria-Tek)	*Listeria* spp.	Yes	275
Polyclonal Ab	Bioenterprises (Tekra ELISA)	*Listeria* spp.	Yes	221
DNA probes				
Natural probes				
msp, 500-bp fragment	No	*L. monocytogenes*	Yes	89
hlyA region, 3.1 kb	No	*L. monocytogenes*	No	91
hlyA, 650-bp fragment	No	*L. monocytogenes*	No	91
16S rRNA	Gene-Trak	*Listeria* spp.	Yes	232
Delayed-hypersensitivity factor gene	No	*L. monocytogenes*	No	311
hlyA, 651-bp fragment	No	*L. monocytogenes*	No	67
β-Hemolysin, 1,610 bp	No	*L. monocytogenes*	No	328
Synthetic probes				
msp, 20-bp sequence	No	*L. monocytogenes*	Yes	92
16S rRNA	Gene-Trak	*Listeria* spp.	Yes	226
Polymerase chain reaction				
hlyA, 606-bp segment	No	*L. monocytogenes*	No	28
hlyA, 702-bp segment	No	*L. monocytogenes*	No	36

variable in their results. Once repair and selective enrichment procedures have been optimized, methods for the rapid identification of food isolates must be designed (283). Suggested techniques have included fluorescent-antibody assay, enzyme immunoassay (EIA), flow cytometry (FCM), and DNA hybridization (230). Because *Listeria* spp. other than *L. monocytogenes* have been recovered from a variety of food products, commercial alternative methods are usually genus specific, rather than specific for *L. monocytogenes* (230, 275). However, the value of using other, nonpathogenic *Listeria* species as indicator organisms for the presence of *L. monocytogenes* has never been demonstrated.

An unusual application of a physical detection method to food microbiology was reported by Donnelly and Baigent (108) in their use of FCM for the detection of *L. monocytogenes* in raw-milk samples. Fluorescently labeled bacterial populations were passed rapidly through a laser beam and analyzed by FCM, allowing the characterization of a population of cells on the basis of parameters such as morphology, DNA content, and surface antigenicity. The DNA content was measured by its fluorescence intensity following propidium iodide staining. The FCM detection was combined with a selective enrichment in LEB for the organism isolated from raw milk. FCM detected *L. monocytogenes* in artificially and naturally contaminated milk, with no interference from streptococci or staphylococci. A study comparing the microbial analysis of 939 raw-milk samples by FCM following selective enrichment in LEB and by a cold enrichment procedure showed that for the 15 samples positive culturally for *L. monocytogenes*, FCM analysis showed a 5.9% false-positive rate and a 0.5% false-negative rate (109).

Alternative methods which use immunological or genetic techniques for the detection of *Listeria* spp. or *L. monocytogenes* are listed in Table 21. The production of monoclonal antibodies (MAbs) for use in the identification of *Listeria* spp. in EIA methods was first reported by Farber and Speirs (136). MAbs were developed which reacted in the presence of either H antigen A, B, or C. These H antigens are found on *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, and *L. seeligeri*. There was no cross-reactivity with 30 cultures of other genera, including staphylococci and streptococci. When one of the anti-B flagellar antigen MAbs was incubated with colony blots of *L. monocytogenes* on nitrocellulose membrane, its binding was demonstrated by an enzyme-labeled goat anti-mouse immunoglobulin A procedure, thus allowing a presumptive identification of *Listeria* spp. in 2 days from naturally contaminated foods. The MAbs were also tested in preliminary experiments involving colony blotting and microtiter plate EIA procedures on naturally contaminated ground meats (133).

The production of other MAbs specific to the genus *Listeria* was reported by Butman et al. (51). Tested by EIA and Western immunoblots for specificity, the 15 immunoglobulin G MAbs showed no cross-reactivity when screened against a panel of 21 other species, including streptococci and staphylococci. The genus-specific antigen was identified as a heat-stable protein with a molecular mass in the range of 30 to 38 kDa. Two of the MAbs are used in a commercial EIA method (Listeria-Tek) for detection of *Listeria* spp. in foods following a 40- to 44-h enrichment procedure (275). In a test of 136 samples of meats and dairy products, the EIA method showed no false-positives or false-negatives when compared with the FDA procedure. McLauchlin and Pini (291) have recently reported the use of MAbs in the detection of *L. monocytogenes* in soft cheeses by using a rapid immunofluorescent test without an enrichment step. *L. monocytogenes* was detected by this direct test in 7 of 35 samples; the organism was present at $>10^3$ CFU/g in these 7 samples.

The detection of *L. monocytogenes* by colony hybridization was first reported by Datta et al. (89), using a radiolabeled DNA probe consisting of some 500 bp of the β-hemolysin gene. The *Listeria* cells were irradiated by microwaves while in contact with the NaCl-NaOH lysing solution in order to break the gram-positive cell wall and denature the DNA. The method was tested against 52 pure cultures of *Listeria* spp., and homology was detected only with β-hemolytic (CAMP-positive) strains. This DNA probe was used to identify *L. monocytogenes* in naturally contaminated dairy products (90). Although β-hemolytic *L. monocytogenes* represented only 1/100 to 1/1,000 of the cells recovered from the food samples after direct plating, they were easily enumerated by colony hybridization. Isolates recovered from the plates after identification on the autoradio-

grams were CAMP positive. This probe has also been chromogen labeled and used in a colony hybridization procedure for species-specific detection of *L. monocytogenes* (225). The 500-bp fragment was cloned into M13 bacteriophage vectors and sequenced by the dideoxynucleotide technique (88, 91). From this sequencing information, several oligodeoxynucleotides were synthesized and used as synthetic probes in a colony hybridization method to identify *L. monocytogenes*. The probes were specific when tested against 10 strains of the organism and did not react with 9 strains of 5 other *Listeria* spp. or with cultures of 13 other organisms. Two of the synthetic probes, AD07 and AD03, were tested with artificially contaminated dairy products plated directly on MMA and also LPM agar, which was more effective than the MMA in suppressing the background flora. The number of spots on the autoradiograms of colony blots from LPM plates equaled 70 to 100% of the colonies that grew. Use of the synthetic probe AD07 for detection of the organism in cheese was also reported by Van Renterghem et al. (414), who noted both the specificity and the speed of the method.

The possibility of developing specific probes based on unique regions of rRNA was exploited in the development of a commercial hybridization assay (Gene-Trak) for *Listeria* spp. in foods (226, 227, 231, 232). The target nucleic acids are genus-specific regions of 16S rRNA. Although the bulk of rRNA is highly conserved, small unique regions exist which form excellent targets as a result of their presence as multiple copies, thus increasing assay sensitivity. Sequence information on potentially variable regions in 16S rRNA was obtained, and oligodeoxynucleotides complementary to unique regions were synthesized as candidate probes. The procedure involves the use of two probes which hybridize to adjacent regions of the target *Listeria* 16S rRNA. The detector probe also contains a ligand, fluorescein, for subsequent enzyme detection, and the capture probe contains a polydeoxyadenylate (dA) domain. The dA portion serves to bind the target-probe complex onto plastic dipsticks coated with polydeoxythymidine. The presence of the target-probe complex is then detected by using a horseradish peroxidase-labeled anti-fluorescein antibody, followed by visualization by means of a substrate. Although *Brochothrix thermosphacta* showed significant homology to the probes, it was negative in the final procedure because of its inability to grow at 35°C. The method gave a positive reaction with 290 strains of *Listeria* spp. and did not react with 59 species of other genera. In a study involving more than 350 dairy, meat, and seafood samples, as well as environmental samples, the method showed false-positive rates of 0 to 3.5% and false-negative rates of 2.7 to 5.6%, at levels as low as 1 cell per 25 g of food.

Four procedures to detect *Listeria* spp. in food, i.e., the two commercially available alternative methods for detection of *Listeria* spp. (Listeria-Tek and Gene-Trak), the DNA probe for *L. monocytogenes* that involved LPM agar (FDA-LPM [99]), and the FDA procedure, were compared on a total of 309 food samples (71 milk and 238 vegetables) (191). A sample was considered positive if confirmed *Listeria* isolates were detected by at least one method. All four procedures yielded positive results with either 58 or 59 of the 59 positive milk samples. With the 44 positive vegetable samples, the FDA-LPM method yielded 38 positive results (86%) and the other methods performed more poorly. The Listeria-Tek procedure produced 22 false-positive results, whereas the others produced none. The authors attributed the higher recovery of the FDA-LPM procedure partly to the superior performance of LPM agar.

Notermans and coworkers (311, 434) used a DNA probe to the *L. monocytogenes* delayed-hypersensitivity factor to detect pathogenic serotypes from among 284 strains of *Listeria* spp. Sequence homology to the probe was demonstrated for all 177 *L. monocytogenes* strains tested, except those of serogroup 4a, and for the *L. ivanovii* type strain. The hybridization reaction was negative for *L. seeligeri*, *L. welshimeri*, *L. innocua*, *L. grayi*, and *L. murrayi*. The authors suggested that this probe would prove useful in the detection of *L. monocytogenes* in food and environmental samples. Chenevert et al. (67) have shown that the internal 651-bp *Hind*III fragment of the cloned listeriolysin O gene has potential usefulness as a DNA probe. Peterkin et al. (327, 328) have reported on the specificity for *L. monocytogenes* of a β-hemolysin determinant used as a chromogen-labeled DNA probe in a direct colony hybridization procedure on hydrophobic grid-membrane filters.

Detection of *L. monocytogenes* by means of polymerase chain reaction amplification, followed by either agarose gel electrophoresis or dot blot analysis with a ^{32}P-labeled internal probe, has been reported (28, 36). The method was positive for all 95 *L. monocytogenes* strains tested, but negative with 12 *Listeria* strains of other species and 12 strains of other genera.

The methodology for the detection of *L. monocytogenes* in foods is in a state of flux, with an abundance of new and sometimes contradictory information currently being published. However, as the results of more critical comparative studies become available, it is hoped that procedures suitable for the rapid detection of the organism in all foods will emerge.

CONCLUSION

Methods used to control *L. monocytogenes* are not new, but involve the implementation of basic quality assurance systems such as hazard analysis critical control point procedures, from raw-material acquisition through to finished-product handling. In food plants, many of the problems linked to contamination of the final product with *L. monocytogenes* have been due to postprocessing contamination. It is known that once *L. monocytogenes* contaminates a food-processing plant, it can survive there for a long time if the temperature is low and the organism is protected by food components (316). Environmental and in-line samplings have played a large role in pinpointing trouble areas and revealing plant conditions that may have contributed to the problem. With this knowledge in hand, much effort has been expended by the dairy, meat, and fish industries to improve sanitation, hygiene, and general cleanliness inside food-processing operations. Although much of the early effort aimed at controlling *L. monocytogenes* occurred in the dairy industry, both the meat and seafood industry are now providing information to individual processors on ways to control the organism. However, even though the increased attention to sanitation, hygiene, and hazard analysis critical control point procedures appears to have reduced the potential for contamination, it remains a difficult, if not impossible, task to eliminate *L. monocytogenes* from all products.

It is important to remember that control is required at all stages in the food chain, not just during processing; hazard analysis critical control point procedures should be applied equally enthusiastically at all levels along the food chain. Because *L. monocytogenes* can grow, albeit slowly, at chill

temperatures, control of the organism may be of added concern with respect to new-generation refrigerated foods with extended shelf life. For these foods it becomes necessary to add additional barriers or hurdles to control the growth of *L. monocytogenes* (250). Controls at both the retail and consumer levels include prevention of cross-contamination, and maintaining chill cabinets and refrigerators at as low a temperature as possible.

Unfortunately, control measures for *L. monocytogenes* in foods are also influenced by differing worldwide government policies regarding the presence of the organism in food—a problem compounded by the lack of knowledge regarding the numbers of cells which must be ingested to cause illness. Whether or not worldwide food regulatory agencies should tolerate low levels of *L. monocytogenes* in foods in which the organism cannot grow is debatable. However, every effort should be made to inhibit multiplication of the organism when it is present in a food in which it can grow. Despite the unknowns, the hazard analysis critical control point approach as a total quality control system should be used to reduce or eliminate *L. monocytogenes* in foods.

The last 5 years have brought tremendous advances in our knowledge of the applied biology of *L. monocytogenes*. An obvious benefit of this has been a new awareness of control procedures, knowledge which has benefited the whole food industry through increased product safety and shelf life. It is hoped that the next decade will bring the knowledge to a point at which we can eliminate the "politics" and provide the benefits of safety to both consumers and the food industry alike.

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