Kor. J. Appl. Microbiol. Bioeng.
Vol. 17, No. 6, 634-643 (1989)

총설 (Review)

Listeriosis and *Listeria monocytogenes*

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리스테리아증(症)과 *Listeria monocytogenes*

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***Listeria monocytogenes*, one of five species in the genus *Listeria* and the only one currently believed to be pathogenic for humans, is a small gram-positive, nonsporeforming, aerobic, motile and hemolytic rod-shaped bacterium. The bacterium is widespread in the environment, having been isolated from soil, dust, animal feed, water, sewage, almost every type of animal that has been cultured, and asymptomatic humans. *L. monocytogenes* causes listeriosis, a disease which most often affects humans with a compromised immune system. Included are pregnant woman, infants and adults suffering from such diseases as cancer, cirrhosis of liver or AIDS or are being treated with drugs such as corticosteroids. Listeriosis is manifested by such syndromes as pregnancy infections, granulomatosis infantiseptica, sepsis, meningoencephalitis, and focal infections. Infections, can be treated successfully with penicillin, ampicillin, or erythromycin. However, a mortality rate of about 30% has occurred in outbreaks of listeriosis. Food-associated outbreaks of listeriosis have been attributed to coleslaw (Canada, 1981), pasteurized milk (U.S., 1983), and soft cheese (U.S., 1985). Presence of *L. monocytogenes* in various dairy foods has prompted recall of such products from the U.S. market-place. *L. monocytogenes* also has been found in raw meats and seafood.**

Listeriosis was recognized years ago, but it is among the least understood of bacterial infections of man, animals and wildlife. More than six decades have passed since Murray *et al.* (65) first described the organism known today as *Listeria monocytogenes*. During the ensuing time, we have learned that the host range of the organism includes at least 42 kinds of domestic and wild animals, 22 avian species, fish, ticks, flies and crustaceans (34). Even though the organism apparently is distributed widely, the sporadic occurrence of the various forms of listeric infection suggests that its distribution in nature is actually restricted by unknown factors (92).

Still undetermined are the carriers that distribute and perpetuate the disease, and the factors that determine that in certain species infection usually results in encephalitis, septicemia, abortion or some other disorder. Also undetermined is the possible role of the organism in syndromes such as mononucleosis, habitual abortion in women, and chronic mental disorders.

This paper will deal with (a) history of *Listeria* and listeriosis, (b) *L. monocytogenes*, (c) outbreaks of listeriosis and pathogenicity of *L. monocytogenes*, (d) transmission of *L. monocytogenes* and food, (e) control of *L. monocytogenes* and listeriosis, and (f) future direction.

History of *Listeria*

Some papers described an organism closely resembling *Listeria monocytogenes* as early as 1891 (34) and 1911 (42). However, in 1926 Murray *et al.* (65) isolated the organism from the liver of stick rabbits and guineapigs, and named it *Bacterium monocytogenes*. The following year, Pirie (75) isolated an identical bacterium from the liver of several gerbilles and named it *Listeria hepatolytica*. The

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disease new known as listeriosis was seen in sheep in Germany as early as 1925 and Matthews (62) reported an outbreak of encephalitis of unknown etiology in cattle, which very likely was listeriosis. Gill (28) observed a disease among sheep in Wales which he called "circling disease", a name still often applied to listeric encephalitis of ruminants. Two years later he succeeded in isolating a bacterium from the brain of affected animals. However, six more years passed before the true identity of the bacterium was determined.

The first confirmed report of listeric infection in man was made by Nyfeldt in 1929 (69). He isolated the bacterium from three patients with an infectious mononucleosis-like disease. Burn (10, 11) established *L. monocytogenes* as a cause of infection in the perinatal period. Since that time, the bacterium has been isolated from varios kinds of animals, and it seems to be everywhere. The first case of listeric meningitis in the United States was reported by Burns (11).

The bacterium now known as *L. monocytogenes* was given other epithets by early authors. Included are *Listerella bovine, L. cuniculi, L. gallinarum, L. gallinarium, L. gerbilli,* and *L. suis,* but they are seldom encountered today. Other names which appeared in the literature describing an organism that presumably was *L. monocytogenes* are *Bacterim hepatitis* (42), *B. monocytogenes* (65), *Corynebacterium infantisepticum* (72, 73), *C. parvulum* (34), *Erysipelothrix monocytogenes, Listerella hepatolytica* (74), *L. monocytogenes hominis* (34), *L. ovis* (29), and *Listeria infantiseptica* (72). With an increased awareness of the disease stimulated by the number of recent case reports, listeriosis may eventually be found to be of far greater importance than was previously suspected (6, 31).

Characteristics of *L. monocytogenes*

Five species of *Listeria* are currently recognized (78). Three of these (86), *L. innocua, L. welshimeri,* and *L. seeligeri,* are considered to be avirulent. There is some evidence to suggest that *L. ivanovii* may produce disease under certain conditions (86). Only *L. monocytogenes* is currently belived to be pathogenic for humans.

L. monocytogenes is small (1.0-2.0×0.5), gram-positive, non-sporeforming, non-acid-fast, diphtheroid-like rod with round ends. It is aerobic, motile at room temperature and hemolytic. In gram stains of cerebrospinal fluids, *L. monocytogenes* may appear coccoid or in pairs and be mistaken for a gram-positive coccus, especially the pneumococcus (6). In 24- to 36-hr-old colonies, cells are definitely gram-positive, but examination of older cultures often reveals gram-negative cells. Within the cell wall, the plasma membrane has three dense layers, each varying in width from 15 to 35 Å, which alternate with two light zones (bridged and unbridged layers), each with an average thickness of 30 Å (23, 35, 45, 68). The cytoplasm may be packed with dense granules less than 100 Å in diameter. The nuclear apparatus has a low density as compared with the cytoplasm and contains fibrils 25 to 50 Å in diameter, which appear as rows of beads or twisted filaments. The bacterium is catalase-positive and Voges-Proskauer-positive, and can produce beta-hemolysin on blood agar (34). Esculin is hydrolyzed. The cell wall of the bacterium has been partially characterized. Peptidoglycan (89), teichoic acid (44), lipoteichoic acid (38), lipopeptidopolysaccharide (58), and endotoxin-like material (93) have been detected in cell walls of different serotypes. There are at least 11 serotypes (6), but three cause 90% of the clinical infections: types Ia, Ib, and IVb. One recent study has shown that types Ia and Ib are more common in neonates infected in utero (early neonatal onset), whereas type IVb is more common in those assumed to be infected at or after birth (late neonatal onset).

On colorless solid media, colonies of *L. monocytogenes* are round, translucent, and slightly raised and are blue-green when viewed with obliquely transmitted light (47, 78).

Culturing of *L. monocytogenes*

After initial growth on artificial media, *L. monocytogenes* usually grows well on most commonly employed bacteriological media. Tryptose agar (Difco) is an excellent substrate for cultivation and preservation of the bacterium. *L. monocytogenes* grows best in a neutral to slightly alkaline medium and will grow at pH values from 5.2 (in cheese) to 9.6 (34, 81, 79). However, survival of the organism for 49days in cabbage juice at pH 4.8 also has been shown (14). Carbohydrates are essential for growth of *L. monocytogenes.* Glucose is irreplaceable as a

source of carbon and energy (34, 78). The bacterium is very salt-tolerant. It can survive for 4months in a solution of 25.5% NaCl held at 4°C (63). The psychrotrophic nature of the organism has been documented (16, 36, 40, 46, 78, 94). Although *L. monocytogenes* grows best at 30 to 37°C, the organism thrives at refrigeration temperatures. It grows at temperatures as low as 3°C in tryptose-phosphate broth (40), 4°C in milk (16, 34, 63), and 0°C in sterile meat after 16 to 20days (46). It also can withstand freezing temperatures, as is evident from its recent discovery in bulk ice cream and ice cream novelties (4, 5). Although growth is slow at 3 to 4°C, turbidity in broth or growth on a solid medium can be observed in 5 to 8days. At 6°C, the log phase is reached in 10 to 11days or less (91).

The organism grows better when the atomosphere contains about 5% of O_2 and 5 to 10% of CO_2 (18, 48). The D-value at 71.7°C is about 1 second, based on U.S. Food and Drug Administration results. The data suggest that about 10^{12} freely suspended *Listeria* /ml of raw milk are needed for the organism to survive 71.7°C for 12 seconds (19). The organism can occur and grow within leukocytes in milk (18). Under such circumstances, survival after 12 seconds at 71.7°C is possible (19). Noticeable degradation of leukocytes with intracellular listeriae was detected in unpasteurized milk after 3days of storage at 4°C, and after 4days of storage leukocytes had deteriorated to cellular debris, suggesting that holding unpasteurized milk refrigerated for 4 or more days would eliminate the protective effect leukocytes may provide to increase the heat resistance of *L. monocytogenes* (19).

Three methods to isolate and identify *L. monocytogenes* are currently being used although others have been suggested. The oldest and simplest is the cold enrichment method (34, 48). Suspect material is macerated and added to tryptose broth, followed by incubation at 4°C. Storage at this temperature retards growth of many organisms but allows growth of the psychrotrophic *L. monocytogenes*. Furthermore, it is thought that incubation at 4°C increases recovery of the organism by enhancing repair of stressed or injured cells. The culture is then sampled for 4 to 8 weeks and plated on McBride's agar (55), a medium which contains the selective agents of glycine anhydride and lithium chloride. These compounds inhibit many gram-positive bacteria. The phenylethanol agar base of the McBrid's medium inhibits growth of many gram-negative organisms. Sheep blood is also added to detect beta-hemolysis. Plates are then incubated 48 hours at 35°C. At atmosphere of 10% CO_2, 5% O_2, and 85% N_2 may be used for incubation. However, *L. monocytogenes* produces well-formed colonies with normal aerobic incubation (78). After incubation, colonies may be screened biochemically for metabolic profile, catalase reaction, and tumbling motility. Serological testing also may be done.

The U.S. Food and Drug Administration has developed a method for isolating *L. monocytogenes* from foods (54). Here, trypticase soy-yeast extract enrichment broth is supplemented with three selective agents: acriflavin-hydrochloride, naladixic aicd, and cycloheximide. Cultures are stored at 30°C and plated within 24 hours and 7 days on modified McBrids's agar (without blood). Colonies thought to be *L. monocytogenes* are then transferred to blood agar to determined hemolysis and later the isolates are confirmed as with the cold enrichment method.

A shortened selective enrichment procedure (SEP) developed by Doyle and Shoeni (21) uses the antibiotic polymaxin B in addition to acriflavin-hydrochloride and naladixic acid for selectivity. Cultures are sampled after 24 hours of storage at 37°C.

A comparison of these three procedures (22) for testing cheese revealed the cold enrichment method to be slightly superior to the SEP and FDA methods. Some of the other suggested methods (61) include use of a gum-based naladixic acid medium (GBNA), and a modified MCBride's agar with moxalactam, but these are not cited often.

L. monocytogenes produces a soluble, filterable hemolysin capable of attacking most mammalian erythrocytes. It is most pronounced in freshly isolated cultures and may be completely absent in old laboratory-maintained strains. Kleikamp (48) found no increase in hemolysin production by increasing the CO_2 content of cultures incubated at 37°C or room temperature. Girard *et al.*(30) found the bacterium produced a soluble hemolysin which could be precipitated from culture filtrates by 60% saturated $(NH_4)_2SO_4$ at 5°C. It was proteinaceos in nature and migrated electrophoretically as a gamma-type globulin. Jenkins *et al.* (43) further purified the hemolysin after ammonium sulfate precipitation

from calcium phosphate gels by a series of adsorption and elutions steps. Cysteine, sodium hydrosulfite, and a number of reducing agents markedly increased the potency of the purified hemolysin. Separation of hemolysin, by chromatography, from a virulent strain of *L. monocytogenes* yielded fractions with diphosphopyridine nucleotidase, hemolytic, or platelet-damaging activity. Lipids from the cell, possibly phospholipids, produce marked monocytosis and depression of lymphocyte activities (60). The toxin was inactivated by heating at 70°C for 30 minutes; it was not precipitated with 30% saturated ammonium sulfate, but was precipitated by three volumes of ethyl alcohol at -5°C.

Outbreaks of Listeriosis and Pathogenicity of *L. monocytogenes*

L. monocytogenes is widespread in the environment, and so humans can be exposed to the bacterium in various ways. The organism has been isolated from soil, dust, animal feed, water, sewage and almost every type of animal cultured (12, 34), including asymptomatic humans. Animals from which positive cultures have been obtained include 42 domestic and wild mammalian species, 22 avian species and others. The bacterium also has been isolated from an assortment of plants including corn, cereals, soybeans, clover, weeds (63), and cabbage (82). Schlech (82) indicated that in Wastern Canada cattle graze on Ponderosa pine needles and suffer from frequent abortion (1). When such pine needles were fed to mice, they developed listeriosis. Limited evidence suggests that cells of the bacterium harbored in dust and dirt can become airborne, inhaled by humans then cause the pneumonic form of listeriosis (85).

There is ample evidence that transmission of *L. monocytogenes* from one human to another not only is possible, but, in fact, does occur. Persons suffering from listeriosis may spread the causative bacterium through nasal and throat secretions, urine, feces, conjunctivial secretions, epidermal pus and blood (75). Some reports indicate that anywhere from 1 to 10% of healthy humans excrete the organism in their feces (18). The bacterium has appeared in raw foods such as milk, red meats, poultry, seafood, vegetables and fruits (20, 26, 29, 78, 81, 83, 90).

Prevalence of listeriosis

In Denmark, there have been 2.3 cases of listeriosis per million persons per year. Similar reports have come from Holland, Sweden, and Germany (12). During 33years (1933-1966), there were 731 bacteriologically confirmed cases in the United States; in 3years (1967-1969), 255 cases were reported to the Centers for Disease Control, and they were from only 35 states (52). In Denmark and in the United States, about one-third of the cases occurred in woman during pregnacy or during the neonatal period, while in Sweden and Germany, two-thirds of the cases occurred in woman during these periods (7). The fecal excretion rate of the bacterium from normal persons is estimated at a minimum of 1%, whereas it is 26% from symptomatic patients and 4.8% from workers in slaugther houses (7). Although the infection has been regarded among the zoonosis, most cases in the United States occur in urban areas in persons without a history of animal contact. Seasonally, the incidence is higher in humans in the summer, and in animals in winter (6).

The distribution of *L. monocytogenes* serotypes has not yet shed much light on the epidemiology of listeriosis (2). One recent report has shown that serotypes Ia and Ib are more common in neonates infected in utero (early neonatal onset), whereas type IVb is more common in those assumed to be infected at or after birth (late neonatal onset) (6). The first major reported outbreak of listeriosis in Noth America occurred in Canada (82). Coleslaw was implicated as the source of *L. monocytogenes* type IVb in this outbreak of 41 cases of listeriosis (83). The second reported outbreak of listeriosis occurred in Massachusetts in 1983. There were 49 confirmed cases, of which 7 were newborns, and 42 were adults; 14 of those patients died. The third and most recent major outbreak of listeriosis occurred in 1985 in Los Angeles County, California, where there were more than 100 confirmed cases, of which more ethan 90 were infants. At least 40 patients died

Mortality varies considerably according to the syndrome. Granulomatosis infantiseptica and meningitis in immunosuppressed patients are associated with the highest mortality, ranging from 33 to 100% in the former (7, 34, 64) and from 12.5 to 43% in the latter (3, 9, 13, 53). Survival rates of up to 100% in meningitis listeriosis have been associated with a normal cerebrospinal fluid glucose level (51, 53).

Pathogenicity of *L. monocytogenes*

L. monocytogenes can invade the eye and the skin of humans after direct exposure. This has been observed in laboratory accidents and by veterinarians (34). The bacterium also can cross the placenta during maternal bacteremia and infect the placenta, amniotic fluid, and the fetus. In most human cases, however, the portal of entry is not evident. If the portal of entry is the gastrointestinal tract and more than one person in 100 is at least a transient carrier, then why are only certain people affected ? Many patients with listeriosis are immuno-supressed, either by a basic disease such as a lymphoma or by administration of various agents usually including adrenocorticosteroids (9, 27, 53, 88). From clinical observations of the types of patients infected, and from studies in laboratory animals and *in vitro,* it is apparent that the mononuclear phagocyte is very important in response of the host to *L. monocytogenes.* In mice, resistance was correlated with development of a delayed hypersensitivity skin reaction and inactivation of bacilli by macrophages (mononuclear phagocytes) (56).

The cell wall of *L. monocytogenes* also is involved in its pathogenicity, although a variety of constituents seems to contribute to the total effect. A water-soluble toxic polysaccharide from the cell wall is able to induce lymphopenia and granulocytosis (37). Protein and carbohydrates appear in extracts and are antigenic, pyrogenic, or able to induce lymphopenia and granulocytosis. A fractionated glycine lysate which increased virulence of the bacterium, also was pyrogenic and caused granulocytosis (75).

Symptoms of listeriosis

Results of an infection with *L. monocytogenes* can be manifested in a number of different syndromes which Armstrong (6) prefers to divide into five categories. They are pregnancy infections, granulomatosis infantiseptica, sepsis, and meningoencephalitis, and focal infections. However, the most common result of contact with the organism appears to be a transient, asymptomatic carrier state.

Pregnancy infections may occur anytime during pregnancy, but most often in the third trimester. The patient usually complains of chills, fever, back pain, headaeche, and discolored urine. In some instances, pharyngitis, diarrhea and pyelitis have been noted. Only occasionally has listeric meningitis been observed in pregnant women (87). When the "flu-like" symptoms are evident, *L. monocytogenes* can be isolated from blood, umbilical cord blood, lochia, tissue obtained by currettage, vaginal mucus, urine, and placental tissue (87). Infection of the pregnant woman leads to infection of the fetus either via the transplancental route or during delivery. As already mentioned, most cases of listeriosis in the fetus occur after the fifth month of pregnancy; however, some have occurred before the fouth month. Earlier infections may cause damage to the embryo (85). The interval between maternal and fetal infection is poorly defiend.

As just mentioned, infection of the pregnant women with *L. monocytogenes* can lead to transplacental infection of the fetus. This infection is likely to be a bacteremia, which can lead to presence of *L. monocytogenes* in fetal urine. The fetal is discharged into the amniotic fluid, causing it to become infected. This contaminated aminotic fluid is then aspirated by the fetus, leading to the widespread involvement of the respiratory and gastrointestinal tracts, as commonly observed at necropsy. Symptoms of listeriosis of the newborn commonly include respiratory distress, heart failure, difficult and forced respiration, cyanosis, refusal to drink, vomiting, convulsions, soft whimpering, early discharge meconium and mucus in stools. Also common is the presence of small cutaneous granulomas in the posterior pharyngeal wall. Histologically, there may be pronounced leukocytosis and monocytosis. Pathologically, listeriosis of the newborn is characterized by involvement of numerous organs which develop nodules. Major involvement of the liver is common and numerous grayish yellow nodules appear on this organ. Similar findings are associated with the spleen, adrenal gland, lung, esophagus, posterior pharyngeal wall and tonsils. Necrosis of granulomas located subepithelially is common. Granulomas also may be found in lymph nodes, thymus, bone marrow, myocardium, testes and skeletal muscles. Intestinal involvement of most commonly is associated with the lymphatic structures of the small intestine and appendix (60).

The third category includes infections that manifest themselves as sepsis of unknown origin. Patients are adults or neonates and have chills, fever, severe pharyngitis, and a leukocytosis accompanied by mononucleosis (3). Adults in most instances are

immunosuppressed; the neonates become symptomatic after 3days of age, and it appears that the infection is contracted during or after birth rather than in utero. The mother is almost always asymptomatic. Although recovery from this form of listeriosis is common, evidence suggests that sometimes it turns into the meningitic form of disease (87).

Meningitis and meningoencephalitis caused by *L. monocytogenes* develop in newborns and in older persons, usually males more than 50years old. clinically, listeric maningitis cannot be distinguished from meningitis caused by other bacterial infections of the meninges. The disease comes on suddenly and the fatality rate is approximately 70% for patients who are either untreated or treated too late in the course of the disease (87). Symptoms of this form of listeriosis in newborn or very young infants include shallow and rapid breathing, slight cyanosis, lethargy, fever, failure to thrive, anorexia, convulsions, and irritability. The disease usually is fatal, with the infant being lethargic or delirious at the end (85). Eight survivors of fetal or neonatal listeriosis were examined at 16months of age by Evans *et al.* (24) to determined if they were suffering from any long-term medical difficulties. Two of the eight had a neurodevelopmental handicap. These two were born prematurely with severe perinatal disease and had complications of the central nervous system in the newborn period.

In adults, this form of listeriosis often begins with "flu-like" symptoms, which are followed by headache, pain in the legs, chills, pyrexia, increasing rigidity of the neck, nausea, vomiting, photophobia, and cirrhosis. Victims become increasingly somnolent, have intermittent bouts of convulsions and delirium and finally die in a coma (85). Principal changes in blood include an accelerated sedimentation rate, leukocytosis with predominant granulocytosis, and occasional monocytosis. Spinal fluid shows pathological changes with elevated pressure, positive protein reactions, decreased values for sugar, and in most instances appears turbid to purulent.

Encephalitis caused by *L. monocytogenes* has two phases; the first lasts about 10days and includes such symptoms as headache, backache, vomiting, conjunctivitis and rhinitis. The second phase begins with a high fever, which is followed by disturbances in the central nervous system. Death usually follows in 2 to 3 days if the patient does not receive appropriate treatment, which consists of administering the antibiotics ampicillin, penicillin or erythromycin.

Ocular infections may be seen as a part of granulomatosis infantiseptica or as a solitary manifestation of an infection by *L. monocytogenes,* sometimes after direct inoculation. Conjunctivitis sometimes accompanies the septicemia which was just described. Localized listeria-conjunctivitis sometimes can be followed by a purulent meningitis which can end fatally (88).

The other forms of listeriosis include the cutaneous form (85), granulomatosis septica, cervicoglandular form, and the pneumonic form (87). Focal infections with *L. monocytogenes* also can result in arthritis, osteomyelitis, spinal or brain abscesses, peritonitis, and cholecystitis (6).

Transmission of Listeriosis and Food

The first recorded probably milk-mediated outbreak of the disease occurred in post-WWII Europe when milk was rationed and sold on the black market (31). The first major food-borne outbreak of listeriosis that was recorded in Canada occurred in 1981. There were 41 confirmed cases, of which 7 were adults and 34 were perinatal. The mortality rate for cases in this outbreak was about 30% and most of those were infants who died following abortion. The causative organism was identified as *L. monocytogenes* serotype IVb and the probable vehicle was determined to be coleslaw. The second outbreak that was reported occurred in 1983 in Massachusetts. The Centers for Disease Control throughly investigated this outbreak and determined that there were 49 confirmed cases of which 7 were newborn, 42 were adults, and 14 of these patients died. The causative agent was *L. monocytogenes* serotype IVb and the probably vehicle was pasteurized milk. However, the vehicle was not confirmed at that time. The third and most recent major outbreak of listeriosis occurred in 1985 in Los Angeles County, California, where there were more than 100 confirmed cases of which more than 90 were infants. At least 40 patients died. The causative agent was again determined to be the same serotype mentioned above. This time the vehicle was determined to be the Mexican-style cheese. Although *L.*

monocytogenes was isolated from cheese, the bacterium also was found in environmental samples and ants from the factory. Within the last five years or so, *L. monocytogenes* was found in several dairy products in Texas, in chocolate milk in Michigan, in chocolate milk and ice cream in Wisconsin, in ice cream in Iowa, in ice milk mix and casein in California, in Mexican style soft cheese in Arizona and in similar dairy foods from other locaitons. When this was written, newspaper accounts appeared which indicated illness and death from listeriosis in some Swiss citizens who had consumed a locally-produced mold-ripened specialty soft cheese.

There is evidence to suggest that improperly fermented silage can serve as a vector for the bacterium (25, 32, 33). This and other environmental sources can lead to contamination of raw milk with freely suspended cells of *L. monocytogenes.* Infection of the udder of cattle also can occur and is of special concern because contamination of the milk of mastitic animals is then probable. Occurrence *L. monocytogenes* in raw milk has been noted in several countries. European data reveal that from 0.9 [in Germany (84)] to 45.3% [in Spain (76)] of raw milk samples tested positive for the bacterium. An overall incidence of 4.2% (54) has been reported for samples taken from three areas of the United States. Of the 650 samples that the U.S. Food and Drug Administration examined, 27 were positive. Of the 27 isolates obtained from these samples, 25 were pathogenic according to results of the mouse test.

The ability of *L. monocytogenes* to grow in fluid dairy products (skim milk, whole milk, chocolate milk and cream) has been determined at several temperatures (17, 19, 20, 77). The lag phase under these circumstances for several strains of *L. monocytogenes* was about 5days at 4°C. Then the bacterium started growing and grew at essentially the same rate in all of these fluid products. At 15days or two weeks, there were close to a million cells of *L. monocytogenes* per milliliter of the products except chocolate milk. In chocolate milk, the population reached somewhere between 10^8 and 10^9 *L. monocytogenes* per milliliter.

The work demonstrated another noteworthy point-at 65days of storage at 4°C *L. monocytogenes* still was surviving with no apparent decrease in numbers. An average of about 35 hours, a little over a day, was required for the population of *L. monocytogenes* to double at 4°C once growth had been initiated. At 13°C, it took about 4.5 to 6 hours, at 21°C about 2 hours, and at 35°C about 41 minutes for the population to double.

In non-fat dry milk (20), the drying process caused the loss of roughly 1 log of population-a 90% decrease in numbers as a consequence of drying. During room temperature storage, the population decreased over time in non-fat dry milk made from concentrated or regular skim milk. However, between 84 and 96days were needed before the population was down to some non-detectable number using the cold enrichment procedure.

In cottage cheese (81), during the time that *L. monocytogenes* was in the milk with the starter culture, awaiting acid production- about 5 hours at 90°F-there was no appreciable growth of the bacterium. Results indicate that the organism in small numbers did indeed survive the cheesemaking process and for a 28-day period in the cottage cheese stored at about 3°C, regardless of whether it was or was not creamed. Lack of growth of *L. monocytogenes* in cottage cheese probably resulted because the pH was too low for growth to be initialted by cells that probably were injured.

Cheddar cheese (80) was made from pasteurized milk that was inoculated with *L. monocytogenes* and starter culture. Coagulant also was added. There was no appreciable growth of *L. monocytogenes* during the manufacturing process, neither was there destruction of the bacterim. When Cheddar cheese was ripened at 6 or 13°C there was a die-off of the bacterim, but survial exceded the 60-day period reequired in the U.S. when cheese is made from raw or heat-treated milk. In one instance, *L. monocytogenes* survived for more than 434 days.

Camembert cheese (79) made from pasteurized milk inoculated with *L. monocytogenes,* was ripened at 6°C after 10 days of storage at 15-16°C to allow proper growth of *Penicillium camemberti. Listeria* counts for strains Scott A, CA and OH decreased to less than 10 to 100 CFU/g in all cheese samples taken during the first 18 days of ripening. In contrast, numbers of strain V7 remained unchanged during this period. All *L. monocytogenes* strains initiated growth in cheese after 18 days of ripening. Maximum *Listeria* counts of ca. 1×10^6 to 5×10^7 CFU/g were attained after 65 days of ripening. Generally, a 10- to 100- fold increased in numbers of *Listeria* occur-

red in wedge or surface as compared to the interior cheese samples taken during the latter half of the ripening period (79).

Control of Listeriosis

Studies with mice indicate several phases of anti-*Listeria* resistance, which appear in response to an intravenous injecion of a sublethal dose *L. monocytogenes.* Within 10 minutes after the injection, approximately 90% of the cells are captured by the liver, and most of the remainder by the spleen (49). Six hours later, the number of viable bacteria in the liver has decreased by 90%, indicating that this organ rapidly destroys most cells of *Listeria* that it initially captures. Destruction of the bacterium is attributed to resident tissue macrophages which are designated Kupffer cells (49). The macrophages are activated by soluble products (lymphokines) of specifically sensitized t-lymphocytes (thymus-derived) (49).

Resistance of mice to infection by *L. monocytogenes* can be affected by conditions other than their genetic constitution. For example, treatment of mice with the trichothecene mycotoxin designated as T-2 and synthesized by molds in the genus *Fusarium,* increased resistance of the animals to infection by *L. monocytogenes* (15). Another report (66) indicates that intravenous injection of heat-killed cells of *Lactobacillus casei* into mice increased their resistance to infection by *L. monocytogenes.*

From clinical observations of the types of patients infected (9, 27, 53, 88) and from studies in laboratory animals and *in vitro* (50, 57, 70, 71), it is apparent that the mononuclear phagocyte is very important in the host response to *L. monocytogenes.* Studies have shown that macrophages kill *L. monocytogenes* more efficiently in the presence of immune serum than in the presence of normal control serum (67). It is also apparent that complement is important in opsonization of *L. monocytogenes* by both mouse peritoneal macrophages and polymorphonuclear leukocytes. Furthermore, it is evident from observations both on experimental animals and humans, that the thymus-derived lymphocyte and mononuclear phagolyte are of prime importance in host defenses against the bacterium and the immunoglobulins and complement for opsonization are also important. If resistance fails, disease may result. Hence some brief comments will be made about control of human listeriosis.

First, prompt diagnosis and treatment of a person with listeriosis is essential for saving the patient's life, particularly if the person is suffering from certain types of listeriosis. Ampicillin or penicillin appear to be the best drugs for treating listeriosis (6). Treatment failure may result if the antibiotic is administered too late during the course of the disease. Treatment failure with penicillin has been reported (85), and limited *in vitro* penicillin resistance has been demonostrated (9, 85). Most strains of the bacterium also are sensitive to tetracycline, erythromycin, chloramphenicol and cephalothin (6). The second phase of control is too keep healthy susceptible people healthy by minimizing their exposure to the bacterium. The prudent individual can take some steps to reduce the risk of infection. The susceptible person should avoid contact with animals that might be infected with or shedding *L. monocytogenes,* places in the environment likely to be contaminated with the bacterium, and humans suffering from listeriosis, and should avoid consuming foods that might contain *L. monocytogenes.*

For prompt diagnosis, an enzyme-linked immunosorbent assay to detect antibody (IgG: ELISA-G and IgM: ELISA-M) microagglutination and complement fixation tests are available (41). The specificity of the tests ranges from 78 to 91%. However, the sensitivity of each test was more variable; varying from 56% for the microagglutination test to 78% for IgM. The complement fixation test was better than the other tests with sensitivity of 78% and a specificity of 91%. The serotype IVb specific antibody response was predominantly of the IgM class based on differential reactivity between serotype Ia and IVb.

How can the food industry respond to prevent *Listeria* contamination in food production areas and thus minimizing the likelihood of offering contaminated foods to the consumer (8)? One of the facts is to allow no refrigerated return product to come into the plant. Through refrigerated storage these products probably have undergone cold enrichment that is necessary for cells to perhaps repair the heat damage they suffered from pasterization. Milk haulers, raw milk handlers and other unauthorized persons shold be kept out of processing areas. The

milk hauler, in particular, has been in the milk house on the farm,, and possibly has been in contact with bovine feces which may be brought into the factory on shoes and boots. Handlers of raw milk in a factory should be segregated from areas where finished products are found. Also, they should be required to follow appropriate sanitary practices. Cleaning should not be relegated to the newest employee in the plant. The employee must be given the needed training and guidance for proper cleaning and sanitizing of equipment. Laboratory personnel should collect samples from the filler, first product through and last product throgh during a day's operation to determine the sanitary status of the operation.

If possible in plant layout, clarifiers and separators should be isolated from the pastueurization area. particular attention should be given to the clean-up of those pieces of equipment because *Listeria* cells tend to localize within somatic cells of milk. Cellulose sponge and nylon pads should not be allowed in product contact areas. Cellulose sponges, in particular, tend to be heavily contaminated with microorganisms. Throw-away paper towels should be routinely used. Wherever possible within a plant, movement of air should be from an area of positive pressure, particularly in the pasteurizing and packaging area, outward away from those areas.

Often there is too much traffic coming into an in-house quality control laboratory. It is hazardous because something spilled on the floor can esily be carried into the plant on someone's shoes. Water from a hose can then be splashed across the footprints and the contaminant is one or in equipment. *Listeria* testing should be done by an outside laboratory (8).

Future Direction

Future work remains to be done to delineate other host and organism-sepecific risk factors for invasive disease when intestinal carriage results from foodborne transmission. Although the interaction of *L. monocytogenes* with the cellular immune system has been well characterized in humans and several species of animals, almost nothing is known of possible virulence factors of *Listeria* that mediate attachment to or invasion of the intestinal epithelium.

From a public health view point, the Canadian experience with coleslaw suggests that certain public health measures could aid in the control of both perinatal and adult human listeriosis. Raw manure should not be used for fertilization of soil for vegetable products to be eaten without cooking. Alternatively, if manure is to be used as fertilizer for such crops, it shold undergo some treatment to inactivate pathogens, and there should be appropriate microbial monitoring (82).

Ingestion of raw vegetables, particularly those subject to prolonged cold storage, might be avoided by susceptible individuals unless the sources of foods have been clearly defiend. Health education and more rigorous laboratory investigation are necessary in the future.

Acknowledgement

A contribution from the College of Agricultural and Life Sciences, University of Wisconsin-Madison. One of us (J.B.) was supported by the Korean Government through the Professor's Overseas Research Program while on leave from the Pusan Woman's University, Pusan, Korea.

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(Received August 18, 1989)