Listeria monocytogenes Monographic Study

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Abstract

Listeria monocytogenes is a ubiquitous bacteria with a remarkable resistance in discordant condition which produce listeriosis, an infectious disease that affects multiple domestic and wild animals' species, but also humans.

Receptive to listeriosis are the majority of domestic or wild mammals and birds, in the last years being registered an increase of receptivity in humans.

The concept of listeriosis in human pathology, a disease caused by eating or drinking contaminated food and water, appeared for the first time in 1981, during an outbreak in Canada with seven cases in adults and 34 cases of maternal-fetal listeriosis. The alimentary origin of human listeriosis can be easily explained if considered some general characteristics of the bacteria. Thus, resistance in various conditions, especially at lower temperatures, justifies its dissemination and food contamination, particularly when is conserved by refrigeration.

Also, *L. monocytogenes* has a significant presence in alimentary products. Some studies showed that 4% of the milk products, 29% of the meat products, 5% of the vegetable products and 26% of the products obtained from fishes and shell fishes are positive for *L. monocytogenes*, which allows us to say that battle against these bacteria is a war against microbial contamination.

Keywords: bacteria, epidemiology, incidence, *Listeria monocytogenes*

1. Introduction

Listeria monocytogenes is a Gram-positive bacteria, asporogenous, catalase positive, small size, with round extremities, first described in 1926 in Cambridge, United Kingdom [20]. Is a psychrophilic bacteria recognized as a pathogen of great importance of food. It is accepted that listeriosis in humans is a disease that is transmitted mainly through food. The bacterium is widespread in different environments and was isolated from soil, from animals, seafood, milk, and meat products [2,3,4].

Human listeriosis can take various forms, including encephalitis, meningitis, abortion and septicemia. But by 1980, human listeriosis remains a hidden disease that has attracted limited attention, although it often evolves with high morbidity and mortality.

From early to mid 1980s was found a total increase of listeriosis in both humans and animals in Europe and North America. Many episodes of listeriosis have occurred because of consumption especially of milk products, which were the primary source of contamination with *L. monocytogenes.*

Listeria species are widespread in the environment and are transmitted to animals, the outcome of these infections being forwarded in the environment through feces, blood, and milk. Under these circumstances, foodstuffs of animal origin are exposed in a considerable extent to contamination that can occur during processing, transport and storage [5,6].

Even though milk and milk products have been classified as primarily responsible in cases of listeriosis due to consumption of foods, studies have shown that these microorganisms can contaminate meat and meat products.

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The series of outbreaks of the 1980s showed that *L. monocytogenes* causes a very serious invasion and often life-threatening disease, constituting an economic burden for both public health services and food industry. Recent estimations ranked listeriosis as the second and fourth most common cause of death due to food toxiinfection in the United States, England, and Wales. This disease has been estimated to be responsible for 14-15% of all deaths due to food poisoning in both studies. A strict control of food quality, but also a public education is needed to reduce the future occurrence of such episodes and sporadic cases of listeriosis [7,8].

2. History of listeriosis

Listeriosis was first described in 1920 as an infectious disease of rodents and other small animals. In 1926, Murraz et al. described a septic disease in rabbits, which included a peripheral monocytosis so that the microorganism was called *Bacterium monocytogenes*. Listeriosis was first described in humans in 1936, when it was isolated in three children with bacteremia and/or meningitis. Later, the genus name was changed to *Listerella* followed by *Listeria*, in honor of Lord Lister. Although monocytosis is not a typical finding in human infections, species *monocytogenes* remained, and the microorganism is now known as *Listeria monocytogenes* [8].

The bacteria has been recognized as causative agents of diseases in humans during World War II, when were described numerous cases of meningitis. With the introduction of chemotherapeutic agents, between 1950 and 1960 listeriosis was more frequently recognized as a significant infection among individuals with major deficits of the immune system. Since then, numerous factors, including increasing use of immunosuppressive drugs (e.g. corticosteroids), organ transplantation and renal dialysis, and increased life expectancy of the population caused listeriosis to become increasingly important zoonose.

In the last two decades, two major events have brought the infection with *L. monocytogenes* to the attention of clinicians. First, the epidemy caused by HIV virus and secondly the recognition of food-borne listeriosis episodes caused by pre-packaged meat products, sausages, soft cheeses, fine, salads and other foods [9]. Today, infection with *L. monocytogenes* is recognized as an important cause of CNS infections, including meningitis and encephalitis, perinatal infections, gastroenteritis and a variety of other less common clinical diseases.

In our country, the first strains of *L. monocytogenes* were isolated by Volintir, in 1951, from the brains of goats with signs of rabies and from one pig in an outbreak of swine fever. Subsequently, during 1956-1958, it was described the first cases of listeriosis in sheep, cattle, pigs, buffalo, trout, duck and the bacteria was isolated from the water and the frog [10].

3. Description and taxonomy

Listeria genus comprises a group of Gram-positive rod-shaped bacteria, classified taxonomically in seven species: *L. monocytogenes*, *L. murrayi*, *L. seeligeri*, *L. welshimeri*, *L. innocua*, *L. gray*, and *L. ivanovii*. Since *L. monocytogenes* is a facultative intracellular pathogen met the human and animals, *L. ivanovii* (formerly known as *L. monocytogenes* serotype 5), infect mainly ruminants (eg. sheep and cattle). The other five species are saprophytic without apparent properties of pathogenity [4].

Based on serological reactions, *Listeria* species were divided into more than 15 distinct serotypes, *L. monocytogenes* serotypes having 1/2a, 1/2B, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, and 7, *L. ivanovii* having serotype 5, and other species of *Listeria* serotypes 6a and 6b and some serotypes shared with *L. monocytogenes*. Using genetic identification methods, *L. monocytogenes* was separated into three lines: Line I consists of serotypes 1/2B, 3b, 4b, 4d, and 4e, line II is composed of serotypes 1/2a, 1/2c, 3a, and 3c, and line III of serotypes 4a and 4c. In addition, the line III can be divided into subgroups IIIA, IIIB, and IIIC [11,12,13].

Species of the *Listeria* Genus have biochemical, morphological, and molecular similarities and occupy similar environment. The bacteria grow well on blood agar and produces a narrow zone of β hemolysis due haemolisin and listeriolisin O. *L. monocytogenes* possesses temperature dependent willing polar flagella responsible for motility at 25°C. Another unique feature of *L. monocytogenes* is its ability to survive at wide range of temperatures, including in the refrigeration temperatures (4 to 10°C). As the bacteria growth at the temperature of refrigeration

more than most organizations, this feature can be used in isolation of *L. monocytogenes* in mixed cultures, the so-called cold enrichment technique. Selective media can also be used to isolate the microorganism and has proven to be superior to the cold enrichment [4,14,15].

4. Epidemiology

Infection with *L. monocytogenes* is a widespread zoonose, affecting mainly cattle, sheep, and goat herds. Bacteria are ubiquitous in the environment and are usually present in animal faeces, soil, decomposing vegetation, plants and water courses. Once an area is contaminated with microorganisms, they are difficult to eradicate, due to their high degree of survival. *L. monocytogenes* is able to live in the biofilm and to survive in severe environmental conditions, including a wide range of temperatures (-1.5 to 50°C) and wide limits of pH (from 4.3 to 9.6) [15].

Bacteria were also identified in the faeces of 5% of healthy human subjects, where are considered transient colonizers of the gastrointestinal tract. A study of patients' families with listeriosis showed gastrointestinal porting rate of 21%, although there was no significant difference in occurrence of symptoms between carriers and non-carriers [8].

Rate of gastrointestinal colonization in healthy adults and the fact that 15% to 70% of food products are contaminated with *L. monocytogenes* suggests that people frequently ingest bacteria. However, listeriosis is a relatively uncommon infection in humans, implying that host factors related to inoculums size are important in the emergence and evolution of disease.

The most numerous cases of listeriosis occur in urban areas without a clear association with animals, suggesting that food serves as a major vector of infection. Potentially contaminated foods include raw vegetables, unpasteurized milk, poultry, meat products from gourmet foods category, soft cheeses, and many other foods. Although marine foods have received little attention on the potential for contamination with *L. monocytogenes*, the disease has been linked to consumption of shrimp, crab meat, smoked rainbow salmon, and lobster [7,16].

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In 2000, the Centers for Disease Control and Prevention (CDC) reported approximately 650 cases of listeriosis. However, because listeriosis isn't a notifiable disease in the U.S., this number is an underestimate of disease incidence. CDC has coordinated a study of active surveillance in 1986, estimating the annual infection rate of 0.07 to 100,000 people, with approximately 1850 cases and 425 deaths per year in the US. Rate of infection is higher among adults aged over 60 years (1.4 per 100,000 people) and infants less than one month age (10 per 100,000 people). The increased incidence of listeriosis in extreme age groups is probably due to the decline or immaturity of the immune system [20,21].

Pregnant women represents one third of cases and their risk of infection is 17 times higher than the risk of other categories. In two episodes of food infections, 50% to 87% of all affected adults that weren't pregnant women had a poor medical condition. A study conducted by the CDC on sporadic cases of listeriosis has shown that 69% of cases of disease in patients who are not pregnant involved humans with a poor medical condition. Immunosuppressive state, which reduces cellular immunity, increases the risk of listeriosis [14,26].

Especially because of prevention efforts in the food industry, the incidence of listeriosis decreased by 44% from 1989 to 1993. These strategies include a zero-tolerance policy for ready meat, poultry and dairy products.

5. Incidence and pathogenity

This listeriosis has been confirmed in various animal species, including humans, and often has been isolated from the uterus (during pregnancy), central nervous system and blood circulation.

In humans, subclinical infections occur frequently and are difficult diagnoses having regard to the widespread distribution of *Listeria* spp. Almost all human cases of listeriosis are due to *L. monocytogenes*, being described rare cases of

infections caused by other species such as *Listeria ivanovii* and *L. seeligeri*.

Factors that increase or decrease the risk of listeriosis are associated with consumption of foods contaminated with *L. monocytogenes*. Infection is most often diagnosed in immunosuppressed individuals, elderly, pregnant women, unborn and newborn children. In these individuals, listeriosis can take various clinical symptoms, the most common being septicemia, meningitis and encephalitis, with a mortality rate of approximately 20-30% [24,25,26].

The incidence of infection increases with age, so that the average age of infection in adults is approximately 55 years. Men are more commonly affected than women aged over 40 years, because women are infected especially during periods in which they are pregnant. Human listeriosis is a seasonal pattern, with a peak in the late summer and early autumn. In contrast, listeriosis in animals has a seasonal peak in spring [4].

L. monocytogenes is also a major pathogen of listeriosis in animals, although approximately 10% of septicemia in sheep has been reported following infection with *L. ivanovii*. In domestic animals (especially sheep and goats), listeriosis generally develop signs of encephalitis, abortion, and septicemia inducing considerable economic loss. A large number of animals (mammals, birds, fish, and invertebrates) are known to be asymptomatic carriers of germs, eliminating *Listeria* spp in faeces, and therefore constitute an important reservoir of germs. Thus, *Listeria* sp. was often identified in food and living environment of the animals. For example 42-87% of straw samples collected from cattle farms in Austria contained *Listeria monocytogenes*. Regarding sensitivity, virtually all wild or domestic animals are susceptible to infection caused by *Listeria* [27].

In sheep, listeriosis was first reported in New Zealand by Gill in 1931 in the form of encephalitis, subsequently being reported in other countries. In Romania were diagnosed first by Volintir in 1954 and then studied by many researchers [28]. Among domestic animals, sheep are most susceptible, and within species, lambs and pregnant ewes are most sensitive. Listeriosis in sheep may develop sporadically or enzootic, having a stationary character, without tendency to disseminate in the outbreak. Morbidity may vary between 5-15%, sometimes 20-25% and mortality varies according to clinical form, being very high in nervous form (over 90%) and much lower in the abortive form [28].

In cattle, listeriosis was first reported by Mathews in 1928 and in Romania, Rosca (1972) describes the first listerial abortion in cattle. According to the symptoms, three forms can be differentiated: sepsis, nervous and listerial abortion [10].

In pigs, listeriosis was first described in Russia (Slabospickij, 1938), then in the USA (Bister et al., 1940), and in some European countries (De Blieck et al., 1948). In Romania, *Listeria monocytogenes* was isolated from pigs for the first time in 1951 (Volintir et al.), later disease in pigs being described as a morbid entity by Gluhovschi et al., 1958, Rosca et al., 1961, Marica et al., 1963 etc. Pigs' listeriosis has much greater incidence than that indicated in some statistics, morphoclinical manifestations of the disease being quite devoid of specificity [29].

In horses, infection was seen and described, among others, by Schiladze (1953) in Russia.

In dogs there were few cases of listeriosis. It is estimated that rats are the most important vectors of the disease; dogs became sick in natural conditions and after healing spread microorganism in the environment. The disease generally has a short evolution, from several hours to several days [10].

In rabbits, the infection was described in 1926 (Murray). The disease occurs usually with reduction of green food and the emergence of cold. Rabbits' listeriosis is, in most cases, a septicemia; death is often sudden, boosted by factors stressful and particularly bad maintenance conditions [29].

In fur animals, the disease has a low contagiousness, being described in polar and silver foxes, mink, nutria, and chinchilla. Listeriosis in fur animals is mainly a septicemia, the youth nutrias under 2 months and foxes to the age of 4 months, constituting main targets of the infection [29].

Avian listeriosis is a sporadic infection, more common in autumn and winter, seen in chickens, geese, canaries, etc. Birds are considered as vectors of human and animal infections. Among the factors favoring of infection, Guarda (1989) mentions the presence of infection with Marek's disease virus and *Salmonella*, the first being recognized as immunosuppressive agents, and the last as blocking agents of the mononuclear phagocyte system [29]. The disease was first reported in chickens by Seastone, in 1935.

Subsequently, infection was diagnosed in canaries, wild cocks, blue eagles, goslings and ducks [28].

In food industry (milk processing factories), *L. monocytogenes* was isolated from raw milk tanks. Although proper pasteurization is a safe method for *L. monocytogenes* destruction, the risks may occur when milk or milk products are contaminated during technology flow or if unpasteurized milk is used.

Luppi et al. [30], conducted a bacteriological study in Italy following which identified 13 strains of *Listeria* (nine of *L. monocytogenes* and four of *L. innocua*) in 113 samples of meat (11.5%) and four strains (two of *L. monocytogenes* and two of *L. innocua*) in 75 samples frozen meat (5.3%). In England, MacGowan et al. [31], following measurements on samples of meat from different animal species, isolated *L. monocytogenes* in these proportions: in chickens' products (21/32 or 65.6%), beef (9 / 26 or 34.6%), lamb (8/20 or 40%), pig (9/32 or 28.1%) and sausages (8/23 or 34.7%).

Also, cut vegetables and fruits are an ideal environment, rich in nutrients, for *Listeria* growth and development. After examining a total of 855 samples (425 samples of cabbage, 205 of water, and 225 samples of fungus) from four farms in Texas in 1999-2000, Prazak et al. [32] have isolated 26 strains of *L. monocytogenes* (3%)

Ready-products offer an excellent environment for development of *L. monocytogenes*. Thus, Van Coillie et al. [34], following studies regarding prevalence of *L. monocytogenes* in 252 ready-prepared foods in Belgium, isolated bacteria in 23.4% (34) of samples, minced and smoked meat being heavily contaminated.

Although many initiatives have been undertaken to decrease the incidence of listeriosis in the world, recent reports have shown a dramatic increase in its incidence in Germany, England and Wales. More than 60% of all actions taken in the United States between 1996 and 2000 had as main objective the identification of contamination with *L. monocytogenes*. This underlines the fact that *L. monocytogenes* is not only important for public health, but also has a socio-economic importance upon the production of food and on food businesses worldwide, including those involved in international trade [34].

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