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Mahendra Pal
Narayan Consultancy on Veterinary Public Health and Microbiology, Anand, Gujarat, India

Margo Yonas Shuramo
Dagam District Agricultural Office, North Shoa, Oromia, Ethiopia

Fikru Shiferawu
Dagam District Agricultural Office, North Shoa, Oromia, Ethiopia

Bhupendra C Parmar
Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Anand, Gujarat India

Correspondence
Mahendra Pal
Narayan Consultancy on Veterinary Public Health and Microbiology, Anand, Gujarat, India

Listeriosis: An emerging foodborne disease of public health concern

Mahendra Pal, Margo Yonas Shuramo, Fikru Shiferawu and Bhupendra C Parmar

Abstract
Globally, foodborne diseases are estimated to cause over 600 million illnesses and 420,000 deaths each year. Listeriosis is a major foodborne bacterial zoonotic disease that is reported many countries of the world. The disease is caused by the ingestion of *Listeria* contaminated foods. Almost all infections in humans are caused by *Listeria monocytogenes* and sometimes *Listeria ivanovii* may be implicated in the disease. Elderly persons, infants, pregnant women, and immunocompromised individuals are highly susceptible to infection. The bacterium has ability to multiply at refrigeration temperatures. Cross contamination can occur in food industries. Various types of foods including the raw milk, butter, soft cheese, ice cream, meat and others are implicated in the outbreaks of foodborne listeriosis. *Listeria monocytogenes* has zoonotic potential due to their ability of transmission from animals to humans. Disease is categorized into two forms, namely severe invasive listeriosis and non-invasive febrile gastroenteritis. The organism contains important virulence factors. The definitive diagnosis of listeriosis was based on the isolation and identification of the bacteria in samples. The drug sensitivity pattern of bacteria poses major public health problems since antibiotics have been used since long time. The poor laboratory facilities pose a challenge in early diagnosis in developing countries. The best preventive strategy is to avoid the consumption of contaminated raw foods, especially of animal origin.

Keywords: Epidemiology, listeriosis, listeria monocytogenes public health importance, zoonotic pathogen

Introduction
Foodborne diseases are significant cause of morbidity as well as mortality in both sexes and in all age groups. Foodborne diseases are attributed to cause over 600 million illnesses and over 420,000 deaths every year worldwide. There are multiple aetiologies of foodborne diseases, which are reported from the developing and developed countries of the world (Pal, 2005; Pal, 2007) [29, 30]. Listeriosis is an important emerging food borne disease that primarily causes problems in pregnant, new-borns, elderly and persons with a weakened immune system. However, the healthy people are often asymptomatic or presents with mild gastrointestinal symptoms (Pal *et al*., 2017) [33].

Listeria species are widely distributed in various environments due to its adaptability to various harsh conditions. The bacterium has been isolated from the soil, plants, silage, and water, particularly from food processing environment and especially in refrigerated premises, despite them being routinely cleaned and disinfected (Danion *et al*., 2017) [9]. Foods, in particular ready-to-eat foodstuffs, including meat, fish, milk, dairy products, fruits, and vegetables, represent the major vehicle for sporadic cases and outbreaks of listeriosis (Buchanan *et al*., 2016) [5]. *Listeria monocytogenes* occurs widely in food processing environments, and can survive for long periods in foods, in processing plants, in households, or in the environment, particularly at refrigeration or frozen storage temperatures. Environmental monitoring programs assess the effectiveness of cleaning and sanitation as well as identify hard-to-clean areas in the facility (Abdollahzadeh *et al*., 2016) [2].

Human listeriosis cases have been reported throughout the world (Lomonaco *et al*., 2015; Najjar *et al*., 2015) [21, 25]. *Listeria monocytogenes*, *Listeria ivanovii*, and very few *Listeria innocua* strains show infection cause capacity. The individuals those are immunocompromised, pregnant, new born and elders are highly susceptible to the disease (Duru *et al*., 2020) [13]. It is able to breach the placental barrier and the blood-brain barrier gives the organism an ability to cause fatal infection, neonatal illness, and invasive central nervous system disease (Pal *et al*., 2017; Pal, 2020) [33, 31]. *Listeria monocytogenes* has also

been associated with gastro- enteric manifestations with fever (Robert *et al*., 2018) [37]. *Listeri*a has ability to survive, grow and multiply at low temperatures, survive high osmotic condition and ability to attach to materials are create conditions for the virulence and pathogenesis (Holch *et al*., 2013) [18]. *Listeria monocytogenes* can be differentiated from the other biochemical testes like xylose, rhamnose, and type of hemolysis on blood agar (OIE, 2018) [27]. Proper hygiene and sanitation programs are important for control of pathogen in a food processing and handling practices (González-Rivas *et al*., 2018) [16]. The objectives of this paper is to delineate the epidemiology and public health importance of foodborne listeriosis.

Etiology

Listeriosis is caused by members of the genus *Listeria*, which has now 17 species (*Listeria ivanovii, Listeria grayi, Listeria rocourtiae, Listeria fleischmannii, Listeria newyorkensis, Listeria weihenstephanensis, Listeria floridensis, Listeria aquatica, Listeria thailandensis, Listeria cornellensis, Listeria riparia, Listeria booriae, Listeria Goaensis* and *Listeria Grandensis)* (Weller *et al*., 2015) [41].

Listeria is a small (0.5-2 µm x 0.5 µm), Gram-positive bacillus, facultative aerobic, oxidase negative isolated or arranged in small chains (Vera *et al*., 2013). [39] Strains of *Listeria monocytogenes* are always D-xylose negative and produce lecithinase. They are generally β-haemolytic and L-rhamnose positive. *Listeria* spp. also has the ability to tolerate salt conditions (NaCl) up to 20% (weight/volume (Holch *et al*., 2013) [18].

Listeria monocytogenes 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 (Wieczorek *et al*., 2012) [42]. Low temperatures induce enzymes such as RNA helicase which improves *Listeria monocytogenes'* activity and replication at low temperatures. This mechanism enables the ability to propel itself and catch onto enterocytes early in infection, but eventually losing the flagella the bacteria is longer exposed to higher temperatures. The ability to produce biofilms enhances *Listeria monocytogenes* ability to survive harsh environments. It can grow at pH 4.5-9.6 with an optimum around 7 PH (OIE, 2014) [26]. *Listeria monocytogenes* has been divided into 13 serotypes (those are 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7) on the basis of somatic and flagellar antigens. Serotypes 1/2a, 1/2b and 4b are commonly reported in humans, and serotypes 1/2a and 4b in animals (Datta *et al*., 2013) [9].

Pathogenesis

The organism contains important virulence factors, such as haemolysin, listeriolysin O, fibronectin-binding protein, Act A protein, two phospholipases, metalloprotease, Vip protein, a bile exclusion system and a bile salt hydrolase that are necessary for adhesion, intracellular multiplication and pathogenicity, and are primarily regulated by PrfA protein (Jeyaletchumi *et al*., 2012; Osanai *et al*., 2013; Vera *et al*., 2013; OIE, 2018) [39, 28, 20].

Upon ingestion through contaminated food, *Listeria monocytogenes* survives different exposures like acidity environment, bile salts, and enzymes secreted from the host defense system (Jeyaletchumi *et al*., 2012). [20] Having survived this stage, *Listeria monocytogenes* adheres to and enters phagocytic cells (Carvalho *et al*., 2014) [7]. The ability of *Listeria monocytogenes* to survive within cells contributes to its pathogenicity (Wilson *et al*., 2018) [43] [42]. After the *Listeria monocytogenes* is internalised by the macrophages it escapes by helps of listeriolysin O and phosphatidylinositol-specific phospholipase (plcA). By help of actin-based movement, the organism passes from one cell to another cells without re-exposure to host extracellular immune surveillance (Osanai *et al*., 2013) [28].

Epidemiology

Listeria are distributed worldwide affects a many species in the Kingdom Animalia, including sheep, goat, cattle, buffalo, horse, pig, camel, canine, rodent, wild animals, birds and also humans. Small ruminants, especially sheep, are mostly affected (Pal,2007; OIE, 2018). *Listeria* has been isolated from the milk, cheese, meat of goats, sheep, cattle, pig, chicken, quail, partridge, ostrich and buffaloes (Rahimi *et al*., 2012; Derra *et al*., 2013; Pal and Awel,2014; Mulu and Pal,2016) [35, 32, 12, 24,], fish and fishery, ice creams, vegetables, other ready-to-eat foods, seafoods (Ahmed *et al*., 2013) [3] and mushroom production facility (Viswanath *et al*., 2013) [40].

Listeria species are mainly acquired by ingestion, but they can also enter the body by other routes including inhalation or inoculation into broken skin or the eye (Pal,2007). Animals and humans can shed *Listeria* in milk and/or vaginal secretions with or without clinical signs, and sick individuals may excrete the organisms in other secretions and excretions including nasal discharges and urine. The fetus is also infected in utero, and some neonates may acquire *Listeria* from the vagina at time of birth. Listeriosis has been transmitted between human new-borns in the hospital via direct contact or on fomites, although person-to-person transmission seems to be absent or insignificant (Mateus *et al*., 2013) [22].

Disease in humans

The incubation period of disease is 4 to 21 days (Pal, 2007). The invasive forms of diseases include septicaemia, meningitis, and the infection of the uterus. Bacteria cause abortions, meningitis, septicaemia, and febrile gastroenteritis characterised by fever and diarrhoea (Mateus *et al*., 2013; Matle *et al*., 2019) [23].

The forms of listeriosis depends on the immune status, and age of the individual, and also virulence factors, dose and mode of transmission (Poimenidou *et al*., 2018). [34] Among *Listeria* species *Listeria monocytogenes* is infective to all human population groups as it has a propensity to cause especially severe problems in pregnant women, neonates, the elderly and immune suppressed individuals (Sintayehu, 2017) [38]. It is important to mention that 10–100 million colony forming units (CFU) in healthy hosts and only 0.1–10 million CFU can cause diseases in people at high risk (Dele, 2016) [11].

Public health importance

Listeriosis is a foodborne bacterial disease that can cause relatively rare, but often lethal infections. The disease is severe in the children, pregnant, elderly and immunocompromised subjects. In under developed country, indirect transmission occur simply by consumption of food

products from diseased animals (Hiwot *et al*., 2016)[17].

Listeria mostly contaminates and become sources of infection when consuming raw foods of both plant and animal origin and unhygienic treated processed foods. The organism was isolated from milk, milk products, vegetables, animal's meat and smoked seafood (Pal and Awel, 2014; Dele, 2016; Mulu and Pal, 2016) [32, 11, 24]. The storage, distributions, and handling of food, changes in the eating habits of people, particularly towards convenience and ready-to-eat foods (Zeinali *et al*., 2015) [44] and increase in the number of people considered to be at high risk for the disease (elderly, pregnant women, new born, immune compromised) are main reasons of transmission and distribution of diseases (Ricci *et al*., 2018) [36]. In this context, Pal and Awel (2014) [32] discussed the public health significance of *L. monocytogenes* in milk and milk products. Unpasteurized milk and several milk products like ice cream, butter, and soft cheese may act as vehicle of *L. monocytogenes*; and the ingestion of contaminated products can result in infection. A comprehensive and systematic study conducted in an abattoir at Addis Ababa, Ethiopia indicated the presence of *L. monocytogenes* in abattoir, butcher's shops and equipment like knives and meat cutting tables (Mulu and Pal, 2016) [24]. These investigators also studied the susceptibility of *L. monocytogenes* isolates to different antimicrobials and found that multidrug resistance of *L. monocytogenes* with two or more antibiotics. The outbreaks of listeriosis due to the consumption of cheese and meats have been described by Dele (2016) [11].

Population at risk

Pregnant women are at a great risk of acquiring the infection. Around 20% of pregnancies affected by listeriosis do not give birth at time. Therefore, abortion or stillbirth, and meningitis, neonatal pneumonia, sepsis is major challenge (Charleen *et al*., 2017) [8]. People with AIDS are at least 300 times more likely to get ill than those with a normally functioning immune system. People suffering with disease like cancers, liver disease and diabetes all confer a moderate risk of infection (Ricci *et al*., 2018) [36].

The new-born babies and the elderly with age greater than 65 years are at a greater risk of acquiring the infection. The immune systems of those young are very immature and also older age immunes are very weak for defence of bacteria and extremely susceptible to these types of infections. *Listeria* can cause life-threatening infection in the neonate, including bacteremia and pneumonia, and are a common cause of neonatal bacterial meningitis (Abdi *et al*., 2016) [1].

However, in some cases, a *listeria* infection can lead to life-threatening complications to those have generalized blood infection and Inflammation of the membranes and fluid surrounding the brain (meningitis) (Zi-Wei *et al*., 2020) [45]. Diabetes mellitus, cardiovascular disease, neoplastic disease, or haemodialysis failure are important factors in the pathogenesis of listeriosis. A patient without coexisting disease has an approximately 10.7% mortality rate versus a patient with several co-morbidities including diabetes and heart disease who has a mortality rate is near 24% (Cao, 2018) [6].

Diagnostic techniques

The diagnosis of listeriosis can be established by isolation of the bacteria from a variety of clinical specimens, such as blood, cerebrospinal fluid, brain tissue, spleen liver, abomasal fluid meconium, faeces, vomitus, foods (milk, meat and their products) or animal feed. Suitable samples from septicemic cases include liver, spleen and blood (Pal *et al*., 2017; FDA, 2019) [33, 15].

For the isolation and identification of *Listeria* species, following steps recommended by the International Standardization Organization (ISO) 11290 method was important. Identification of *Listeria* species included culture methods, in light of specific enrichment followed by the characterization dependent on morphology of its colonies, fermentation of sugar and its haemolytic properties. Isolation of *Listeria monocytogenes* includes antibody-based tests, ELISA, culture-based methods and immune-capture techniques (Itumeleng *et al*., 2020) [19]. The other species that can cause hemolysis can be differentiated from *Listeria monocytogenes* by uses of CAMP (Atul *et al*., 2020) [4].

Molecular tools such as PCR, multiplex PCR and real-time PCR employing virulence-associated genes such as Internalin A (inlA), inlB and inl C for specific detection of *Listeria* monocytogenes whereas Internalin D (inl D) for detection of *Listeria* ivanovii have been found rapid, specific, reproducible and reliable. PCR is as a reliable and effective strategy for isolations and identifications of *Listeria* species and, differentiation of *Listeria monocytogenes*. Multiplex PCR permits the synchronous identification of more than one pathogen in a same test sample, for example, *Listeria* and *Salmonella*; only pathogenic living *Listeria* cells can cause the disease so testing *Listeria monocytogenes* and other *Listeria* species and even differentiation of serovars 1/2a and 4b from other serovars of *monocytogenes* by targeting 2 virulence genes at a onetime. This approach is very attractive for food analysis, due to reduction in reagents, labour costs and testing time (Atul *et al*., 2020) [4].

The API *Listeria* system is one of the rapid diagnostic methods, which takes 24 hours to identify *Listeria* species without the need for additional tests. Immunofluorescence is effective for rapidly identifying *Listeria monocytogenes* in smears from animals dead or aborted from listeriosis and from milk, meat, and other sources (Itumeleng *et al*., 2020) [19].

Treatment

Treatment of listeriosis may be a difficult task because *Listeria* can invade virtually all cell types. However, the recovery depends on early diagnosis and prompt, aggressive antibiotic treatment. The most commonly used for treatments of listeriosis are penicillin, steptomycine (the drug of choice), ceftiofur, erythromycin, and trimethoprim/ sulphonamide. Resistance to tetracycline, streptomycin, erythromycin, rifampin, and kanamycin has been reported from worldwide. Supportive therapy, including fluids and electrolytes, is required for animals and human having diarrhea, difficulty of eating and drinking. Heating ready to eat food at good temperature can also kill bacteria (Abdi *et al*., 2016) [1]. Attempts should be made to develop the chemotherapeutic agent that is safe, effective, low cost and does not develop resistance.

Prevention and control

Early detection and reporting of a listeriosis outbreak are important in preventing the spread from continuing. In order to prevent formation of bacteria in animals feed the good handling and preservation of silage, decrease chance of

contamination of animals feed with soil or faeces and ensuring optimal conditions that do not favour for multiplication of bacteria by assuring that the pH falls below 5.0 (Abdi *et al*., 2016)[1].

After the food is contaminated by *Listeria*, the refrigerator temperature may not be effective because of the ability of the bacteria to survive at low temperature. Hence, application of HACCP approach and the establishment of effective critical control points in the food processing plant are important (Abdi *et al*., 2016) [1]. The people who are at risk should avoid taking raw milk, soft cheeses, refrigerated ready-to-eat foods without effective heat treatment to an internal temperature of 73.9 °C (165 °F) or raw animal's products (Eyasu *et al*., 2015) [14].

Conclusion and Recommendations

Listeriosis, primarily caused by *L.monocytogenes*, is an emerging foodborne bacterial zoonosis of global public health importance. The disease can occur in sporadic as well as in epidemic form. *Listeria monocytogenes* is an important pathogen of food safety concern as it can survive at refrigerator temperature. The primary source of transmission is by ingestion of contaminated food. The immunocompromised patients, new born, pregnant and old individuals are highly susceptible to L. *monocytogenes* infection. Therefore, one health program was best approach for control and prevention of disease. Depend on the conclusion above the below recommendations are forwarded:

- Identifies all sectors of the food chain to implement good hygienic practices (GHP) and good manufacturing practices (GMP) as well as to implement a food safety management system based on the HACCP.
- Identify the virulence factors, major vehicle, and survival strategies of organism in environment.
- Emphasis should be given to study the molecular epidemiology of foodborne listeriosis.

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Author's contribution

All the authors contributed equally. They read the final version, and approved it for the publication.

Conflict of interest

The authors declare that they do not have conflict of interest.

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