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Review on Public Importance and Diagnostic Method of Listeria Monocytogenes, Ethiopia

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Abstract: Listeriosis is a disease in humans and an animal caused by Listeria monocytogenes and is one of the most important emerging bacterial zoonotic diseases worldwide. Among the Listeria species, Listeria monocytogenes causes listeriosis in humans and animals and has the highest case fatality rate among foodborne diseases. It is one of the major microorganisms responsible for food-borne illness. The main sources of infection are reservoir hosts, contaminated food of animal origin, dairy products, fish and fish products, vegetables and the environment. The immunocompromised people, elderly, newborns and pregnant women are the most susceptible groups to listeriosis. Listeria monocytogenes could be a gram-positive, rod-shaped, facultatively anaerobic, non-spore-forming, microscopic bacterium with a low G+C concentration. It can withstand and tolerate a wide range of pH, temperature, and salt. Consumption of contaminated food and ready-to-eat foods is the chief source of infection for humans. Listeria is identified in suspected samples using isolation and identification, biochemical, serological, and molecular methods. Studies show that *L. monocytogenes* becomes resistant to some types of antibiotic therapy. The effects of listeriosis on social health and economic importance have been not well documented in our country. As a result, this review's objective is to inform the public about the importance of the diseases, a diagnostic tool, and a summary of the data on food-borne listeriosis in meals containing animal products. Good cleanliness and secure handling during manufacturing, distribution, storage, and transport are necessary for preventive actions against diseases.

Keywords: Food of Animal Origin, Human, Listeriosis, *L. monocytogenes*, Zoonotic

1. Introduction

Listeriosis, also called silage disease, circling diseases, and meningoencephalitis, is caused by *Listeria monocytogenes*. It is an infectious and fatal disease of animals, birds, fish, crustaceans, and humans where septicemia and encephalitis are predominantly observed [1, 2]. Listeria monocytogenes has been identified as a major pathogenic microorganism that causes foodborne infections [3]. Listeriosis outbreaks might be sporadic or epidemic in nature [4]. Infection in animals is usually subclinical, but severe forms can also occur [1]. Some of the clinical indications are abortion, stillbirth, septicemia, perinatal infections, gastroenteritis, and meningoencephalitis [5, 6]. The organism has an intracellular life cycle that may pass from cell to cell without a release from the cell. This ability explains its potential to cross the placental and blood brain barrier, explaining its pathogenesis and clinical signs [5]. In recent years, *L. monocytogenes* has grown to be a major opportunistic human foodborne pathogen amongst meals and medical traces of bacteria [7, 8]. Primary isolation is enhanced under microaerobic conditions. It is a saprophyte which will survive for long periods in soil, water, feed, and animal feces, making it easily detectable within the environment [9] and animals are infected through the feeding of contaminated water, pasture, or silage.[6, 10].

According to some studies, food becomes contaminated during production, collection, transportation, storage, and processing, as well as from contaminated soil or water [11, 12]. The quantity of contaminating organisms will vary depending upon the route of contamination [13]. Infection in humans can occur through direct contact with infected animals, infectious material such as aborted fetuses [14], and consumption of contaminated unpasteurized milk and by-products, raw vegetables, certain processed foods such as soft cheeses, hot dogs, and deli meats and fish and fish products

[15, 16]. Listeriosis is comparatively rare but it is a significant disease, with an estimated hospitalization rate that exceeds 90% and a mortality rate of approximately 15-30% [4].

Aged people, newborns, immunocompromised people, and pregnant women are at high risk of contracting a listeria infection. Pregnant women are ten times more susceptible to listeria infection than other healthy adults are, and older people are even vulnerable [17]. The occurrence and prevalence of the organism in foodborne diseases in Africa is hardly reported [18]. However, in Ethiopia, there is limited data regarding the prevalence of *L. monocytogenes* in animal-origin foods [19].

The importance of *L. monocytogenes* cannot be underemphasized because it may cause huge economic losses in livestock industries and cause food poisoning. Good quality control strategies along with adequate prevention measures are suggested to effectively prevent and control this food-borne illness in health, agricultural, and environmental systems [20]. In developing countries, there have been very few or no reports on *L. monocytogenes*. This might be true because no one has given it due attention or been unaware of its occurrence [21]. Listeria monocytogenes is the main human pathogen among Listeria spp. and clinical strains of bacteria [22]. It is essential to possess knowledge concerning the characteristics, environmental factors, and interactions of virulence with host susceptibilities. Therefore, the objective of this review is to assess the public importance of its diagnostic methods and to compile available data.

2. Literature Review

2.1. General Characteristics of Listeria Monocytogenes

Listeria monocytogenes has the capacity to vary from an ecological saprophyte to a possible pathogen [4, 23]. Because of somatic (O) and flagellar (H) antigens, seventeen (17) recognized species belong to the Listeria genus., namely *L. monocytogenes, Listeria seeligeri, Listeria ivanovii, Listeria welshimeri, Listeria innocua, Listeria grayi, Listeria marthii, Listeria fleischmannii, Listeria aquatica, Listeria floridensis, Listeria grandensis, Listeria newyorkensis, Listeria cornellensis, Listeria rocourtiae, Listeria weihenstephanensis, Listeria riparian, and Listeria booriae. Among those species, L. monocytogenes and L. ivanovii* are considered pathogenic [24].

L. monocytogenes is highly pathogenic to humans and animals [25]. Ruminants, warm-blooded animals, and humans are, in rare cases, infected by L. ivanovii [21, 26]. There are 13 *L. monocytogenes* serotypes recognized, including 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and seven [27]. The annual endemic ranges from two to fifteen cases per million people [29], because serovars 1/2a, 1/2b, 1/2c, and 4b account for more than 98.6% of all human infections [28]. Based on the Multiplex PCR assay, L. monocytogenes strains could even be categorized into four distinct serogroups, IIa (serovars 1/2a, 1/2c, 3a, and 3c), IIb (1/2b, 3b, 4b, 4d, and 4e), IIc (1/2c and 3c), and IVb (4b, 4d, and 4e) by targeting four marker genes [30]. Lineages II and I represent the majority of isolates involved in human clinical cases, whereas lineages III and IV strains are found frequently among humans and are more common among animals [30, 31].

2.2. Morphology and Growth Characteristics

Listeria spp. is non-spore-forming, facultative anaerobic, catalase-positive and oxidase-negative organisms and intracellular bacterium with small gram-positive rods (0.5-4 m in diameter and 0.5-2 m in length). *They are motile at 25°C, showing tumbling motility, but are non-motile at 37°C.* Listeria are motile with peritrichous flagella at 37°C, but not or only slightly motile [1]. The taxonomic characteristics are those of the bacillus family, listeriaceae, and supermolecule. Listeria species grow as saprobes in soil, sewage, silage, animal manure, litter, and bird droppings. They are facultative anaerobes that grow best under oxygen and higher carbon dioxide concentrations. L. monocytogenes grows best at 30-37°C, but it can survive and multiply at temperatures ranging from 0 to 45°C, making it more resistant to disinfectants and sanitizing agents [1, 27].

Listeria monocytogenes grows at 40c (the cold enrichment method for isolation of the organism from brain tissue but not from placental or fetal tissues) [32]. Laboratory media supports growth, preferably in nutrient broth supplemented with up to 10% (w/v) NaCl [32]. L. monocytogenes grows in an alkaline and neutral pH range of 4 to 9.6, which promotes multiplication and necessitates a minimum water activity of 0.92 [33]. Listeria specious is both a mesophlic and psychotropic organism [27]. It has greater heat tolerance than other non-spore-forming bacteria. However, short-time high-temperature pasteurization is effective for killing listeria [28, 34]. Listeria spp. grows well on an outsized sort of non-selective laboratory media, hence culture from normally sterile sites like blood or liquid body substance don't require special media. Special selective media are required for specimens like feces, vaginal secretions, food, and environmental samples [35].

It can form biofilms on solid surfaces, including those in food-processing facilities, and adheres to a variety of surfaces, which could be a source of contamination [27, 36]. The infective dose of *Listeria monocytogenes* is unknown and may vary with the strain and susceptibility of the host. Once introduced into the food processing industry, it can survive and remain for an extended period under adverse conditions within the food industry, and it is positive for the Christe Atkins Munch Peterson test (CAMP) reaction on sheep blood medium (5% v/v) with Staphylococcus aureus but not with Rhodoccocus equi [27, 37].

3. Epidemiology

3.1. Global Distribution

Listeria affects a list of species within the Animalia, including sheep, camels, goats, buffalo, cattle, horses,

canines, rodents, birds, wild animals, pigs, and humans. Sheep are severely suffering from *L. monocytogenes* [37]. Listeria spp. can be isolated from cow and goat milk as well as animal meat [38, 39]. Animals are usually asymptomatic carriers of *L. monocytogenes*. In wildlife animals, outbreaks of diseases are typically sporadic and associated with exposure to high burdens of the bacterium over a short period. Otherwise, clinical signs may arise secondary to immunocompromised individuals, predisposing diseases, and excessive environmental stress [40].

Listeriosis is an emerging disease with the main community public health concerns worldwide. The outbreak of the disease is associated with food contamination and thus many risks of morbidity and mortality [41]. Earlier, it was known predominantly in most European and Western countries and many gastroenteritis cases were reported, mainly from the USA due to L. monocytogenes [42].

In the alliance, in 2013, 1,743 cases were reported with 15.6% mortality [43]. In most EU members as well as the USA, Australia, and Canada, the listeriosis rate was around 0.3 cases per 100,000 people [44]. In Iceland, this rate was 1.3, in Sweden, it was 0.6, in Denmark, it was 1.6, and in Norway, it was 1.0. This could be due to a high intake of smoked fish [45]. In New Zealand, the listeriosis rate was 0.6 cases per 100,000 individuals [46]. There has been no appropriate consideration of its incidence in Africa, and there have been few reports on L. monocytogenes [47]. On the other hand, there is a range of knowledge about the occurrence of L. monocytogenes in numerous samples. As an example, in Nigeria, the prevalence rate of L. monocytogenes in food of animal origin is 88%, chicken at 64.7%, and fish at 33.7% [18].

Table 1. *Some records of global Listeria outbreaks between 2014 and 2017.*

Country	Types of foods	Source
USA	Cream, soft milk and cheese	[48]
South Africa	Various food products	[49]
USA	Raw milk chocolate milk product	[50]
USA	Soft cheese	[44]
EU/EEU	Various food product	[51]
USA	Cheese products	[52]

CDC: Centre for Disease Control, NICD: National Institute for Communicable Diseases, ECDC: European Centre for Disease Prevention and Control, USA: United States of America, EU: European Union, EEA: European Economic Area.

Table 2. *Study on Prevalence of L. monocytogenes from food of animal origin in Africa.*

Authors	Country	Foodstuffs	Overall prevalence
[53]	Algeria	Ready-to-eat dairy and meat foods (227 samples)	2.6% *L. monocytogenes*
[53]	Ethiopia	Retail meat and dairy products (240 samples)	4.1% *L. monocytogenes*
[54]	Morocco	426 samples: (a) raw meat (n = 112), meat products (n = 240), poultry (n = 74)	2.4% *L. monocytogenes*
[55]	Egypt	Street vended Ready-to-eat food (576 samples)	14% *L. monocytogenes*
[56]	Botswana	Food samples from supermarkets and street vendors (1324 samples)	4.3% *L. monocytogenes*
[57]	South Africa	Meat and meat products (2017 samples)	14.7% *L. monocytogenes*

Source: Jooste, P., Jordan, K., Leong, D. & Alvarez-Ordóñez, A., 2016, Listeria monocytogenes in food: Control by monitoring the food-processing environment. *African Journal of Microbiology Research 10 (1), 1–14.*

3.2. Prevalence Study of Listeria Monocytogenes in Ethiopia

In Ethiopia, *L. monocytogenes* has been reported since 2004 with a p*revalence* rate of 5.1% and Listeria spp. 32.6% [47]. Detection of L. monocytogenes was in minced beef at 1.6%, ice cream at 19.6%, fish at 2.3%, pork at 7.5%, and chicken at 1.9%, and the serotype identified belongs to ½b, 4b, and 4c. A meta-analysis by [58] shows that among the seven species of Listeria spp. reported in Ethiopia, raw beef meat has the highest prevalence rate (62%), followed by desserts (43%). Similarly, [59] meta-analysis shows 27 original studies from 7828 meat samples, 4% of *L. monocytogenes* were recorded.

The contamination of the meat and vegetables is due to the poor hygienic environment at the point of slaughter, processing, and promotion. Traditionally, people consume uncooked beef meat and raw animal food products throughout the state, which aggravates the overall public health associated with listeria species [58].

According to [60] report, human Listeriosis in Tigray Regional states the prevalence rate amongst pregnant women 12/141 (8.5%) were recorded. With relevance the socio-demographic character, a high prevalence rate was seen within the cohort of 20–24 years 18.6% (8/43), rural dwellers 10% (3/30), homemakers 11.4% (10/88), and school 9.6% (5/52) reported. Similarly, among women attending Jimma University center, *L. monocytogenes* was isolated in eight (5.56%) of 144. The isolation rate was comparatively higher among women with a record of fetal failure (9.7%) and women with preterm deliverance 6.25% [61]. In Ethiopia, few reports on listeriosis in restricted areas and no well-organized epidemiological surveillance systems at the national level [34]. Conversely, nowadays there are various reports on Listeriosis from diverse samples from different areas summarized in table 3 below.

Table 3. *Shows Prevalence rate of L. monocytogenes and L. spp in samples of animal source, Ethiopia.*

Study area	No. of samples	Types of Samples	Prevalence of *L. monocytogenes*	Prevalence of L. spp	Reference
Central highlands of Ethiopia	343	raw milk	7 (2.04%)	65 (18.9%)	[19]
	65	Pasteurized	13 (20%)	26 (40%)	
	20	Yoghurt	1 (5%)	1 (5%)	
	15	Cheese	4 (26.7%)	9 (60%)	
Total	443		25 (5.6%)	126 (28.4%)	
A. A (different sub city)	100	Cottage cheese	1 (1%)	4 (4%)	[62]
	76	Raw beef	2 (2.6%)	39 (51.3%)	
	100	Raw Milk	13 (13%)	22 (22%)	
	115	Liquid whole egg	5 (4.3%)	37 (32.2%)	
Total	391		21 (5.4%)	102 (26.1%)	
Gonder town	60	Raw meat	4 (6.6%)	31 (51.6%)	[63]
	25	Minced beef	3 (12%)	6 (24%)	
	50	Fish meat	3 (6%)	13 (26%)	
	24	Pizza	2 (8.3%)	7 (29.1%)	
	50	Past. Milk	0	0	
	50	Raw milk	2 (4%)	14 (25%)	
	40	Cottage cheese	0	5 (12.5%)	
	20	Ice cream	3 (15%)	9 (45%)	
	65	Cream Cakes	7 (10.7%)	11 (16.9%)	
	384		24 (6.5%)	96 (25%)	
A. A (7 sub city and surrounding)		Raw meat	4 (6.8%)	(62.7%)	[38]
		Raw milk	2 (3.4%)	8.5%	
		Cottage cheese	1 (1.7%)	6.8%	
		Cream cake	3 (5.10%)	20%	
Total	240		4.1%	27.5%	
Debre –Birhan	384	Raw milk dairy	33 (8.59%)	78 (20.3%)	[64]
	23	Raw milk Vendors	3 (13.04%)	7 (30.4%)	
Total	407		35 (8.8%)	85 (20.88%)	
Municipal Abattoir and butcher shops in Addis Ababa	384	Swab abattoir	8 (2.1%)		[65]
	384	Butcher shops	21 (5.5%)		
	105	equipment	7 (6.7%)		
Total	873		36 (4.1%)		
Ambo and Holeta, west Shoa.	150	Abattoirs	33%	39 (26%)	[66]
	150	Butchers	1.6%	44 (29.3%)	
	150	restaurants	1.6%	45 (30%)	
Total	450		20 (4.4%)	128 (28.4%)	
From Asella Dairy Union and Ada Primary Dairy Cooperative, Ethiopia	106	bulk milk samples	1.2%	41.7%	[67]
Bishoftu, Dukem towns, and centeral Ethiopia	80	Raw beef	9 (11.2%)	40 (11.2%)	[68]
	80	Raw Milk	7 (8.7%)	27 (8.7%)	
	25	Pasteu. Milk	-	2 (8%)	
	40	Cottage Che.	2 (5%)	21 (52.5%)	
	30	Yogurt	1 (3.3%)	6 (20%)	
	25	Ice Cream	-	4 (16%)	
	30	Chicken	1 (3.3%)	4 (16%)	
	30	Fish	-	6 (20%)	
				112 (32.9%)	
Total	340		5.8%		

3.3. Source of Infection and Mode of Transmission

The major source of infection was animal feces, Human feces, Farm surry, sewerage sludge, farm water troughs, Surface water, and plants animal feed and the walls [30].

Listeria spp. are isolated from various animals' origin like goats, cattle, pigs, sheep, chicken, quail, partridge, ostrich, and buffaloes [39] and isolated from ice creams [5], Seafood [69], and mushroom production [70]. Infected animals can also serve as a source of infection from their urine, feces, aborted fetuses, uterine discharges and the milk [7]. The most common route of human infection is through ingestion of contaminated foods like, unpasteurized dairy products, raw meat, and meat products, soft cheese, contaminated vegetables and, fish and fish products, [12, 71]. Calves may acquire infection from contamination of cow teat, ingestion of milk containing the organism from carrier's animals [30]. The natural habitat of Listeria is might be decaying plant matter, as saprophytes, in soils, water, vegetables, effluents, plants, [72] Some reports indicated that one out of 5 percent of healthy humans are a transporter of this pathogen [73]. The Listeria monocytogenes may spread to the fetus using transplacental passing through the maternal bloodstream or mounting from a colonized genital tract [34].

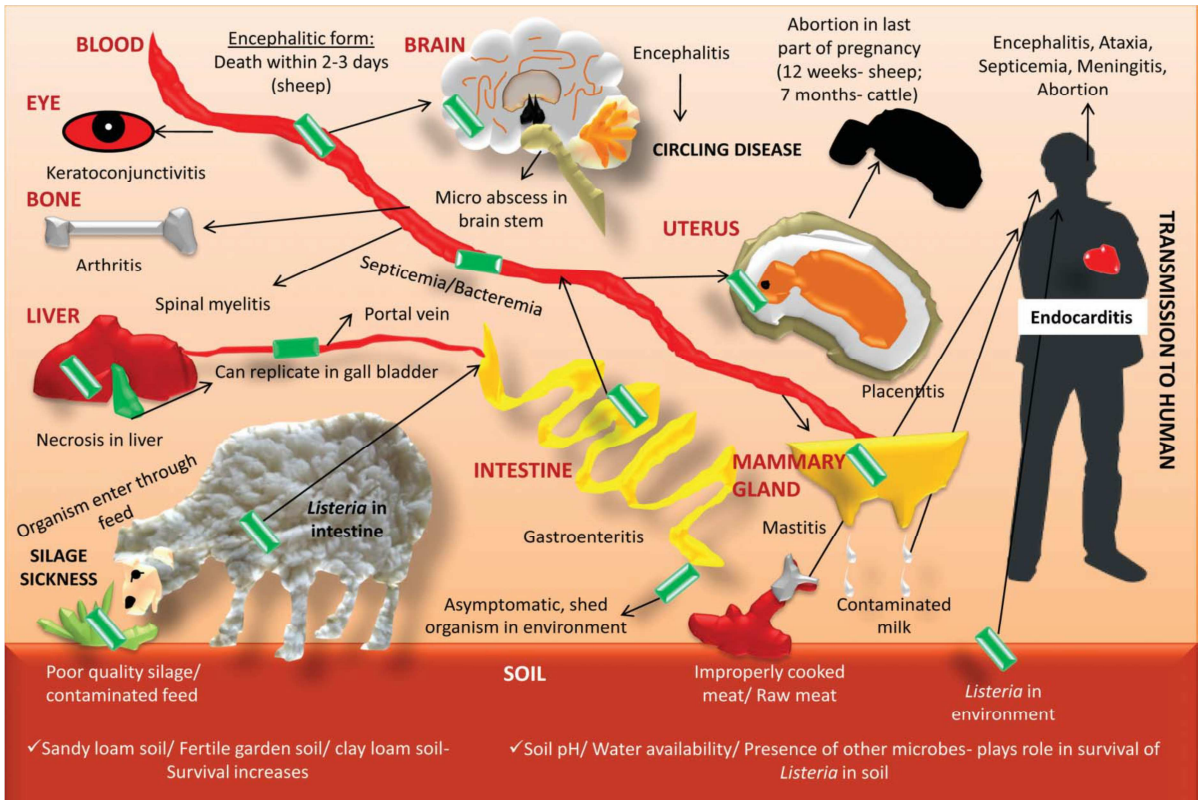

Figure 1. *Transmission and clinical signs of Listeriosis in animals and humans. Source: K. Dhama et al. (2015).*

Host Range

Listeria monocytogenes infects a wide range of animals, including cattle, goats, cats, rabbits, pigs, sheep, deer, dogs, guinea pigs, horses, foxes, fish, crustaceans, and birds, and is a zoonotic bacterial pathogen to humans [74]. The foremost clinical listeriosis cases happen in ruminants and pigs infrequently develop the disease, but birds are subclinical transporters of the organism [1].

Host Risk Factor: Concerning sensitivity, virtually all wild and domestic animals are susceptible to infection. [75]. Listeriosis in livestock can be sporadic or enzootic, with an inactive character and no affinity to spread within the outbreak. According to [38], the morbidity rate ranges from 5-15%, rarely 20-25%, and mortality varies depending on clinical form, being 90% higher in the nervous type and much lower in the abortive type [76]. In pregnancy, the disease is capable of causing premature delivery, stillbirth, and miscarriage as alternatives to severe health problems for infants [34].

Management risk Factors: poor nutritional status, changes in weather, transport, long periods of flooding, unhygienic conditions, early pregnancy, and parturition stresses, poor access to pasture and feed supplies, overcrowding are most typically reducing host response [77]. In the immunocompromised phase and the presence of continual disorder, the infectivity and severity of listeria are high [78].

Pathogenic risk factor: The pathogenic risk factor of infection increases due to an enormous multiplication of *L. monocytogenes* within the feed or environment. In food hygiene, the important aspect is the ability of the bacteria to stay alive in an exceedingly wide range of temperatures. It can form biofilm on firm surfaces in food processing facilities and is challenging to sanitizing agents and disinfectants. Once established in the processing of food and vegetation, it can stay alive and continue for a protracted period in an unfavorable environment [27]. Biofilm can act as a possible source of contagion [79]. The organism also processes diverse cellular contents to escape the protective means of its host. Listeria is a facultative intracellular pathogen that infects cells and intestinal cells through directed endocytosis. Bacterial Superoxide Dismutase (SOD) protects against the bactericidal action of the respiratory burst of the phagocyte. Additionally, listeriolysin O disrupts lysosomal membranes, allowing the organism to grow within the cytoplasm [80].

Reservoirs: Listeria species are dispersed widely within the natural environment, including soil, water, and decaying vegetation. Animal reservoirs are domestic and wild mammals. Humans may act as reservoirs, mostly abattoir personnel and laboratory employees exposed to *L. monocytogenes* culture [77].

Pathogenesis and Virulence: Internalin, a surface protein, aids in adhesion and internalization to host cell receptors. Macrophages uptake Listeria, the bacterium primarily localized within the vacuole. Listeria secretes listeriolysin O proteins, which break down the vacuole barrier and enable the bacteria to escape into the cytoplasm. Once liberated from the vacuole into the cytoplasm of the host cell, *L. monocytogenes* can replicate. The invasive form of Listeriosis has the flexibility to multiply between host cells

and cross the placental and blood-brain barriers [81]. It colonizes host cells, survives within the stomach environment and enters the intestine through the intestinal epithelial cells. In the digestive region and macrophages; the acid tolerance of the organism is the foremost vital cause for boosting the virulence when the pathogen encounters acidic circumstances [82].

Listeria possesses unique virulence factors to attack the host, escape immune cells, and can multiply extracellular and intracellular. It causes damage to parenchymal cells and the hepatocytes of the liver. After enter of host cells, antigen might be taken up by non-phagocytes and phagocyte cells and might deliver antigen to mutually endogenous and exogenous antigen processing. Listeria passes through different stages in the host cell, from the harbor and dominant invasion, lysis of vacuoles, adhesion, multiplication, and evasion of the host resistance mechanisms, and cell-to-cell multiplying [83].

Clinical Signs: Among livestock, the disease severely affects sheep, and the clinical signs show encephalitis, (circling movement) through nervus and brainstem dysfunction, with abortion within the last trimester of three months. Septicaemia, placentitis, and gastroenteritis might be observed [1]. Adult sheep ages four to eight months show the encephalitic form, and young sheep ages five to eight months may develop the septicemic type. Sheep show clinical signs like incoordination, head deviation with tilting of the absolute, propelling themselves ahead until they get a solid or wall/entry, one-sided facial paralysis, saliva drooling, loss of ear observed [84].

Death may occur within three days due to respiratory failure. Cattle and goats show signs of the diseases [1]. In cattle, the disease path is elongated and may have several periods. In cattle, keratoconjunctivitis and unilateral uveitis were reported [85]. Buffaloes are in peril of listeriosis, a genital tract infection that is familiar [86]. In camels, the cerebral kind of listeriosis also occurs [87]. Young birds are more prone to high mortality; maybe 40% show persistent infection. In adult birds, the septicemic form and irregular meningoencephalitis cause unexpected death. However, in poultry, paralysis of the limbs, torticollis, tremors, incoordination, and depression signs may occur. Moist litter's damp/moisture environment, wintry and immunosuppressive conditions are predisposing factors [88].

3.4. Listeria Monocytogenes in Animal Farm

On the farm, *L. monocytogenes* is found in the soil as a natural inhabitant but in moderately low numbers [89]. It can survive in farming soil and even grow. In agricultural soils, the prevalence rate ranges from 8.7% to 51.4%, but in non-agricultural soils, the prevalence rate is between 15.2% and 43.2% [90, 91]. Some reports suggest that it is the chief reservoir of *L. monocytogenes*. Dairy and meat-producing animals are exposed to this pathogen during the interface with the natural environment. Polluted dust is well known to carry this pathogen. The animal can get the infection through the air or by ingesting contaminated feed [92].

Silages are a normal feed used to help save animals during forage scarcity since the seasonal changes within the year [93]. Inadequately prepared silage can be foot-dragging in the event of *L. monocytogenes* resulting in infected animals [94]. Animal feces contaminated with silages and shed by the organisms serve as vehicles for infection and reintroduction into the environment [95]. Asymptomatic infected animals may transmit this pathogen to people through food [96]. Poor farming practices, natural water and wastewater sources near farming communities that harbor large amounts of pathogens and could be sources of animal contamination are also sources of L. monocytogenes on farms [97].

Since *L. monocytogenes* exists within the environment, control actions must begin from the farm stage until the meat and animal by-products reach the consumers. Control of the pathogen includes all activities during harvesting and management practices. It may reduce the likelihood of pathogens being present in animals and meat products [98]. This method will reduce the number of sources of infection, as well as the entry and spread of contaminants into the animal. Successful biosecurity and the most precious animal welfare to cut back pathogen levels in animals incorporate diet treatment [99]. Pathogens are reduced in the environment by proper handling of animal feed and animal management practices, clean water, and waste disposal [100]. Conversely, it is challenging farmers to manage pathogens at the farm stage because of an absence of awareness, income, and money [89].

3.5. Listeriosis in Humans

Listeriosis has occurred as a severe invasive or non-invasive form. The non-invasive forms are fever and gastroenteritis. Infections in humans can be invasive or non-invasive [97, 101]. The severe invasive forms are meningitis, septicemia, conjunctivitis, endocarditic, and flu-like infection [102].

Human cases were first reported in 1929 [103], and perinatal cases in 1936 [104]. Nowadays, it is believed to be a food-borne disease with a significant public health concern and a death rate of 20–30% [105]. Human infection is through the ingestion of contaminated food, which could cause severe and life-threatening infection. The disease is associated with newborns, pregnant women, aged people, and immunocompromised people. In humans, the disease causes meningoencephalitis, severe sepsis, uterine infection, and sometimes death [106]. Patients with diabetes, colitis, asthma, and inflammatory bowel diseases are at greater risk. In healthy humans, it can cause febrile gastroenteritis [32]. In general, food borne Listeriosis has three clinical signs: meningitis, abortion, and septicemia [107].

3.6. Laboratory Diagnosis

For presumptive diagnosis, the previous history of the disease, pathological lesions, feeding habits, grazing pasture, and observation of clinical signs are supportive. Confirmatory diagnoses are made after isolation and

detection of the bacterium [88]. The conventional methods based on international regulatory purposes comprise "the U.S. Food and Drug Administration (FDA) method, the ISO11290 Standards, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) method, the Association of Official Analytical Chemists (AOAC) official method, and thus the French Standards"[1]. Samples were collected based on clinical appearance: lesions within the kidneys, liver, and spleen in the case of septicemic form; liquid substances, pons, and medulla in the case of encephalitic form; and placenta (cotyledons), fetal abomasal contents, and uterine discharges in abortion cases [35].

For Listeria spp., isolation and identification of suspected samples are cultured directly on selective agar and aerobically incubated at 37°C for twenty-four to 48 hours. Commercially available indicator media like Listeria Oxford, which is a selective agar, are intended chiefly for the isolation of Listeria from human food [87]. The classical methods for spotting Listeria species are microbiological culture-based isolation and PCR-based testing, which are used to characterize serotypes further [1].

Direct Microscopy: Detection of gram-positive cocci from liver or cotyledons lesions might reveal the organism. For presumptive diagnosis of neural listeriosis, brain tissue fixed with (10% formalin) for histopathology examination reveals a micro abscess within the brain stem with perivascular cuffing [101]. A confirmatory diagnosis is held by isolating the pathogen from suitable specimens [31]. Different conventional methods used for the isolation and identification of Listeria spp. from food and clinical samples have gained acceptance for international regulatory purposes. The methods differ by their criteria of choosing primary, secondary, and selective inhibitory compounds in relevance to the food types [108].

“The International Standard ISO-11290 and USDA methods” use a two-step enrichment in Fraser broth, but the Association Française de Normalization (AFNOR) uses the single-step broth method. The AFNOR method requires lower incubation time and yields results in 2 days "as opposed to the 4-5 days required [109]. In 1989, OXA (Oxford agar), was developed, which is a selective medium and thus the most extensively used. Most literature suggested using OXA agar and either one of the following media: MOX (Modified Oxford Agar), PALCAM (Polymixin Acriflavine, Lithium chloride, Ceftazidime, Aesculine Mannitol), and LPM (Lithium Chloride Phenyl ethanol Moxacalatam medium)," utilizing ferric iron and esculine [110].

For brain tissue, culturing a cold-enrichment method is used. Small pieces of the spinal cord and medulla become homogenized, and a tenth suspension is ready during a nutrient broth. The broth is sub-cultured once weekly for up to 12 weeks and incubated in the refrigerator at 4°C. On agar culture, small white colonies with smooth margins appear in 24 hours and are grayish-white. *L. monocytogenes*, predominantly, shows the feature "tumbling motility’ after 2–4 hours of broth culture and is positive for catalase test [68]. Listeria species have the power to hydrolyze esculine incorporated in media. All Listeria species utilize B-D-Glycosidase and cleaved esculine (esculinase) via the blackening of the medium. Antimicrobial agents, like Acriflavine, cyclohexamide, and nalidixic acid), suppress non-listeria organisms [111].

Rapid detection methods include polymerase chain reaction (PCR), fluorogenic tests, monoclonal antibodies, and supermolecule hybridization tests. Agars like ALOA, BCM, and CHROM agar are rapid and alternate new chromogenic differential selective media. It could be used instead of one in each of the selective agars [112]. According to the FDA, chromogenic kits are recommended and validated for detection of 104 CFU/ml enrichment culture within 4-6 hours. Rapid chromogenic tests have the advantages of identifying particular virulence factors, phosphatidylinositol-specific phospolipase and an account of the organism within the food samples [113].

3.7. Biochemical Test

After isolation of a pure culture of Listeria, a biochemical test was performed for identification and confirmation. The common media for the biochemical tests include carbohydrate fermentation tests; rhamnose, D-xylose, mannitol; and hemolysis on blood agar. In general, *L. monocytogenes* is unreactive for oxidase and indole tests, ferments glucose and lactose with acid production, but no gas produced. Listeria monocytogenes is a positive reaction to catalase, Voges Proskauer, and methyl red, and on blood agar Beta, hemolysis forms as a clear zone. *The Christe Atkins Munch Peterson (CAMP) test for S. aures is positive for L. monocytogenes but negative for Rhodoccocus equi [114]*.

3.8. Serological Test

The serodiagnosis test method is beneficial for the detection and serotyping of *L. monocytogenes*. Antibody detection methods include the growth inhibition test, complement fixation (CFT), antibody precipitation, and enzyme-linked immunosorbent assay (ELISA). Conversely, when the conventional antigens used in serological tests reveal significant false positive results due to cross-reaction, in septicemic abortion cases, anti-Heamolysin (LLO) antibodies of Listeriosis were detected using an ELISA test [1, 115].

4. Molecular Method

Molecular methods are sensitive, specific, and accurate for detecting *L. monocytogenes* DNA from avirulent Listeria species. The molecular tests commonly used to detect Listeria spp. are conventional PCR, Real-Time PCR, and DNA hybridization. Real-time PCR is a sensitive test that can be used to detect and quantify L. monocytogenes in food [116]. Isolates from animal origin are mainly virulent, typically confirmed by animal inoculation. In rabbits or guinea pigs, a drop of broth culture is inoculated into the conjunctiva. After 24 to 36 hours, rabbits or guinea pigs

show purulent keratoconjunctivitis as seen in the case of *L. monocytogenes. However, other Listeria spp. is not affected.* Mice are in peril from L. *monocytogenes* and L. ivanovii. Mice inoculated intraperitoneally die within five days because of the necrotic liver [35].

5. Treatment, Control and Prevention

Treatment: Antibiotic therapy has been used for a long time for the treatment of listeriosis in livestock and humans. Treatment of neurological or chronic forms in goats and sheep has little value soon after the clinical signs. Human listeriosis treatment is difficult because L. monocytogenes can attack a wide range of cell types [116]. Moreover, the treatment of human listeriosis is usually unsuccessful for a long time, which makes the treatment period vary in line with the extent of the infection. However, antibiotics have been used to treat human listeriosis successfully for a while [117]. Listeria is in peril of most antibiotics, but isolates from the environment, food, animals, and human sources are known to be proof against commonly used antibiotics. In humans, common antibiotics used are gentamicin, penicillin, cotrimoxazole, ofloxacin, cephalosporin, fosfomycin, fluoroquinolones, and erythromycin [40, 118, 119].

The inherent resistance of *L. monocytogenes* against these antibiotics is because of the dearth of the low affinity of enzymes catalyzing the last step of plasma membrane synthesis [110]. Chemotherapy with antibiotics is the sole access method for the treatment of listeriosis in medical and veterinary. It is critical to use antibiotic drugs while utilizing and monitoring the vulnerability [120]. Nevertheless, *L. monocytogenes* growth towards resistance has been accelerating [121].

Control and Prevention: The control of Listeria in foods relies on the Hazard Analysis Critical Control Point (HACCP) approach to establish effective critical control points within the strategy. Listeria contamination in processed foods can be significantly reduced by the careful design and layout of processing equipment, as well as the concept of thorough cleaning regimes of the processing environment. Because of its ubiquitous nature, it is virtually impossible to eliminate the pathogen from many food products. To reduce food contamination, effective Listeriosis prevention necessitates sanitation of food contact surfaces [122]. Pregnant women and immunocompromised people who are mainly in peril of these diseases should take extra precautions with these food types. It is important to note that sanitation in food establishments, proper hygiene, cooking of meat and fish, pasteurization of dairy products, and health education of high-risk groups, mode of transmission, and defense will help to cut back the incidence of Listeriosis [123].

6. Conclusion and Recommendations

Listeria monocytogenes, the chief explanation for listeriosis, is one of the important emerging food-borne bacterial zoonotic pathogens of worldwide significance. Listeria spp. exists everywhere as an opportunistic and major food-borne pathogen. L. monocytogenes has been isolated from different foods of animal origin like meat, milk, cheese, other dairy products, ready-to-eat foods and vegetables. It primarily affects infants, pregnant women, and the elderly and immunocompromised people [21]. Humans become infected through the consumption of contaminated food and ready-to-eat foods are the chief sources of infection [1]. Control measures for a decent prevention and control program of this emerging food-borne pathogen. Furthermore, the sources of infection and modes of transmission should be recognized. Additionally, addressing communication, a risk perception and consumer practice to the final public is mandatory. As a result, standard food hygiene, processing, farming, transportation, storage, and marketing of foods are the best ways to reduce the prevalence of Listeriosis in humans and animals.

Based on conclusion, recommendations are as follow:

1) Disease Surveillance and epidemiological investigation for prevention and control.
2) Food Surveillance: are at the import, retail levels, and wholesale to create sure food safety.
3) Health Promotion: health education to the trade and members of the public for food safety measures against listeriosis targeted at the high risk groups.
4) Safe storage of animal feeding and good animal husbandry management practice.

Conflict of Interest

The authors declare that they have no competing interests.

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