Listeriosis in Large Ruminants: A Review

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Abstract: Listeriosis is a sporadic bacterial disease caused by different species under genus Listeria: Among those Listeria monocytogenes have the only and most pathogenic importance for large ruminants where by other have no impact in large ruminants. Listeriosis is more prevalent in temperate climate (cold and winter). Listeria monocytogens is highly multiply in poor quality silage, with a PH greater than 5.5. Listeria is a food borne pathogen that is transmitted by ingestion, inhalation or direct contact with infected animal. Among those ways, ingestion of contaminated silage is the primary modes of transmission. Host management as well as pathogen has the risk factors for the disease. The clinical manifestation of listeriosis in large ruminants includes neonatal septicemia, encephalitis and abortion in late term of pregnancy. Under such condition there are different diseases which have as same as clinical sign with listeriosis. Those are rabies, ketosis, hypomagnesaemia and polioencephalomalacia. In most case Listeria can be diagnosed either by laboratory like direct microscopy, culture, animal inoculation or necropsy findings. The most recommended drug for the disease listeriosis includes penicillin G, aminoglycoside, trimethoprim, sulfamethazole and tetracycline and the best control measure for the disease is vaccination and discontinued of eating poor quality silage.

Key words: Listeria monocytogenes · Listeriosis · Large ruminants

INTRODUCTION

Listeriosis is a sporadic bacterial disease that affects a wide range animals including mammals, birds, fish and crustaceans, although the most clinical listeriosis occurs in ruminants; pigs rarely develop disease and birds are generally subclinical carriers of the organism [1]. Most infection in animal is subclinical, but listeriosis can occur either sporadically or in epidemic form. In addition to the economic impact of listeriosis in animals, there is a link between animals and their role as a source of infection for humans primarly from consumption of contaminated animal products. Infection can be as a result of direct contact with infected animals especially during calving or lambing [2]. However, these infections are very rare. The relative importance of the zoonotic transmission of the disease to humans is not clear and contamination from the food processing environment is apparently more relevant to public health [3].

The clinical manifestations of listeriosis in large ruminants include encephalitis, septicemia and abortion. The septicemic form is relatively uncommon and generally, but not invariably, occurs in the neonate. It is marked by depression, weakness, inappetance, pyrexia, emaciation and diarrhea in some cases [4]. The encephalitic form sometimes referred to as ‘circling disease’ because of a tendency to circle in one direction, it is the most common manifestation of the disease in ruminants. The signs include depression, anorexia, head pressing or turning of the head to one side and unilateral facial paralysis [5]. Abortion is common in late term after 7 month in cattle and 12 week in sheep [3].

The post-mortem finding and histopathology in ruminants depends on clinical manifestation. In the encephalitic form, the CSF may be cloudy and the meningeal vessels congested. Gross pathological lesions of the brain are rare. In the septicemic form, multiple foci of necrosis in the liver and, less frequently the spleen; aborted foeti of ruminants show very little gross lesions, but autolysis may be present if the fetus was retained before being expelled [6].

The evidence indicates that listeriosis in animal predominantly associated with stored forage, silage and with the environment as the main route of entry, after oral
ingestion, in the case of septicemic or abortive listeriosis. The incubation period can be as short as one day. The incubation period for encephalitic form is usually 2-3 weeks and the course of the disease is usually short in sheep and goats; 1-4 days although it can be more practiced in cattle [7].

In general, listeriosis is an infectious diseases that affect many species; the genus composed of six recognized species, those are Listeria innocua, Listeria ivanovii, Listeria grayi, Listeria monocytogenes, Listeria seeligeri and Listeria welshimeri, of which three of them have more pathogenic. Among those L. monocytogenes has the most significant and zoonotic diseases [5]. Therefore the main objective of this seminar paper was to review imperative points on listeriosis in large ruminants.

**Listeriosis in Large Ruminants:** Listeriosis in ruminants may present as encephalitis, abortion and septicemia. Usually only one form of disease occurs in a group of affected animals. Septicemia, often encountered in new born animals although, L. monocytogenes is widely distributed in the environment and can replicate in the surface layers of poor quality silage with PH values above 5.5. In such circumstances, listerial number may reach 10^7 CFU per Kg of silage. In good quality silage, multiplication of the organism is inhibited by the acid produced by fermentation. Susceptibility to infection with L. monocytogenes has been attributed to decreased cell-mediated immunity associated with advanced pregnancy [8].

**Etiological Agents:** Listeria are aerobic or facultatively anaerobic, non-sporulating gram positive coccobacilli that grows at 4°C-45°C and typically have tumbling motility when cultured at 20°C-25°C. Characteristics that help to distinguish L. monocytogenes from other Listeria species include the formation of a narrow zone of β-hemolysis on sheep blood agar and the production of acid from glucose, maltose, L-rhamnose and α-methyl-D-mannoside but not from D-xylose. Determination of the serotype of L. monocytogenes is based on somatic (O) and flagellar (H) antigens [11].

**Morphology and Growth Characteristics:** Listeria is gram positive coccobacilli that have a tendency to occur in short chains of 3-5 organisms. In stained preparations they often assume a typical diphtheroid palisade arrangement, a properly that was responsible for their previous incorrect classification with the Corynebacteria. L. monocytogenes is 0.4-0.5µm by 0.5-2.0µm in size. In cultures incubated for 3-6 hours at 37°C, the bacillary form predominates, but thereafter the prevalent form is coccolid. In cultures 3-5 days old long filamentous structure 6-20µm or more in length often occur especially in rough strains. At temperature of 20-25°C, L. monocytogenes is actively motile by means of four peritrichous flagella, but at 37°C only one polar flagellum is formed. The motility of Listeria is useful in their differentiation from Erysiplothrix and the Corynebacteria [12].

**Epidemiology**

**Occurrence**

**Geographical:** although L. monocytogenes is sporadic in occurrence and worldwide in its distribution the clinical disease in animals mainly in the northern and southern latitudes and is much less common in tropical and subtropical than in temperate climates. The disease is important in North America, Europe, United Kingdom, New Zealand and Australia [4]. Seasonally in the northern hemisphere, listeriosis has a distinct seasonal occurrence, probably associated with seasonal feeding of silage, with the highest prevalence in the months of December but seasonal occurrence is not a features of Australia [8].

regarding the host listeriosis is primarily a disease of ruminants and the major disease associated with L. monocytogenes are encephalitis and abortion; rarely producing syndromes of septicemia, spinalmyelitis, uveitis, gastroenteritis and mastitis [13].

**Source of Infection:** The organism is common in the environments and infection is not limited to agricultural animals. L. monocytogenes is ubiquitous in the environment and can be commonly isolated from animal feces, animal feeds and the walls, floors, drains, etc. most feed hays, grains and formulated feed have the potential to contain L. monocytogenes but, with most, low levels of available water restrict its multiplication [4].
In ruminants, *L. monocytogenes* can be isolated from the feces and nasal secretions of an animal. *L. monocytogenes* is commonly present in silage, but it does not multiply to any significant extent in effectively preserved silage which is characterized by anaerobic silage, high density, a high concentration of organic acids and a PH below 5.5. It may be present in silage which is poorly fermented. The risk for contamination of silage with Listeria is higher when it contains soil. Moist preserved feeds other than grass silage are at risk for Listeria growth. Infected animal serve as a source of infection from their urine, feces, aborted fetus, uterine discharges and in milk [1].

**Transmission:** Animals are exposed by ingestion, inhalation, or direct contact with the Listeria bacterium. The organism has been found in water, birds, urine, aborted fetuses, uterine discharge and milk. Food-borne transmission is the most common mode of transmission and Listeria is considered a food-borne pathogen for large ruminant. In large ruminant, Listeria is most frequently transmitted by contaminated silage. It is most often found in silage that is poorly fermented with a PH =5.6, in moldy silage, or in silage spoiled with soil [15]. In some case, the disease results from infection of the terminals of the trigeminal nerve consequent to abrasions of the buccal mucosa from feed or from infection of tooth cavities [14].

**Risk Factors:** Even if *L. monocytogenes* occur everywhere, only a small proportion of animals develop clinical disease. A number of predisposing factors have been observed as a risk factor for disease. These include factor that cause a lowering of the host animal’s resistance and factors that increase the infection pressure of the organisms. These risk factors related with host management includes: poor nutritional status, sudden changes of weather to very cold and wet, late pregnancy and parturition stress, transport, long periods of flooding with resulting poor access to pasture and overcrowding and insanitary conditions with poor access to feed supplies The factor that increases the infection pressure largely involves a massive multiplication of *L. monocytogenes* in the feed or environment. The feeding of grass or corn silage as a major risk factor for the occurrence of *Listeriosis* has been recognized for many decades. The increase in use of silage for feed may be the reason for the apparent increase in the prevalence of the disease in recent years [4]. Introduction of virulent strains to the flock may also occur via carrier animal and this may carry a heavy population of the bacteria and can contaminate feed or pastures for silage. The organism persists for as long as 16.5 months in cattle feces, up to 207 days on dry straw and for more than 2 years in dry soil and feces. It is resistant to temperature of -20°C for 2 years and is still viable after repeated freezing and thawing [1].

**Pathogenesis**

**Mechanism of Pathogenesis:** Exposure to Listeria occurs via oral route. Entry into intestinal epithelial cells or M cells is mediated by internalin, a surface protein and its interaction with host cell receptors. After passage through the intestinal barrier, Listeria can be observed in phagocytic cells within the lamina propria. Further dissemination occurs via the blood stream. Listeria can be internalized by phagocytic cells or by non-phagocytic cells through induced phagocytosis. After internalization, it escapes from phagosome, becomes associated with actins filaments in the cytoplasm and propels itself to the cell’s plasma membrane protrusions and thus avoids host defense mechanism [15].

An alternative proposed route of entry has been through damaged mucosal surfaces, inhalation, or by conjunctival contamination. It then invades the CNS by travelling along the trigeminal nerve sheath. This route of infection usually results in meningoencephalitis. However, there has been no evidence for tropism of the organism to neural tissue and it is unknown whether the organism can use nerves other the trigeminal nerve to access the brain. The incubation period of *L. monocytogenes* infection is usually 2-6 weeks [16].

**Clinical Signs:** The clinical outcomes of listeriosis depend on pathogenic strains and immune status of the host. There are three major forms of clinical signs. These are septicemia, encephalitis and abortion [5].

**Septicemia:** Acute septicemia due to *L. monocytogenes* is not common in adult ruminants but does occur in new born calves. There is no sign suggestive of nervous system involvement, the syndrome being a general one comprising depression, weakness, emaciation, pyrexia and diarrhea in some cases, with hepatic necrosis and gastroenteritis at necropsy. A better defined but less common syndrome has been described in calves 3-7 days old. Corneal opacity is accompanied by dyspnea, nystagmus and mid opisthotones. Death follows in about 12 hours [4].
Encephalitis: The neural (encephalitic) form of listeriosis occurs sporadically, affecting a single animal in a herd. It is referred to as “circling disease” due to the effects of encephalitis and meningitis [16]. In cattle, the signs range from sub-acute to chronic and include depression, anorexia and tendency to circle in one direction, head pressing or turning of the head to one side, unilateral facial paralysis and bilateral keratoconjunctivitis [17].

Abortion: Abortion is usually late term after 7 months in cattle. The fetus may be macerated or delivered weak. Systemic signs are rare in the cow unless the fetus is retained and triggers a fatal septicemia [13]. Although abortion or still birth occurs sporadically and the rate is up to 100%; it is uncommon to find the encephalitic form and abortions occurring in a single outbreak [6]. Usually abortion has been observed soon after the commencement of silage feeding but does not always have this association [4].

Diagnosis: Listeria monocytogenes is present in soil and water. It can also found in intestinal tract of an animal. Listeriosis usually occurs in calves and pregnant cow and immunocompromised cattle. Although meningitis and bacteremia are the most common manifestations, the organism can also cause spontaneous abortions [18]. The disease can be tentatively diagnosed based on clinical signs and its confirmation is achieved by isolating the pathogen from the specimens [19].

Laboratory Diagnosis

Direct Microscopy: A direct smear of infected tissue may reveal numerous gram positive rods in septicemia and abortions; however, only few numbers of organisms are observed in encephalitic form [20]. Histological examination of fixed 10% formalin brain tissue can often give a presumptive diagnosis of neural Listeriosis [19].

Culture: Appropriate specimens include CSF, blood and amniotic fluid. Listeria is facultatively anaerobic and readily grows on routine laboratory media such as BAP, chocolate (CHOC) and CNA agars. Its small, gray colonies are surrounded by a narrow zone of β-hemolysis. At times it may be necessary to remove a colony in order to observe the hemolysis [18].

Isolation: Sample are plated on sheep blood agar and incubated at 35°C in 10% CO2. Isolation of L. monocytogenes from brain tissue may be enhanced by pour plate methods. After the initial isolation attempts, remaining tissue is stored at 4°C for “cold enrichment”. Such tissue is sub cultured weekly for up to 12 weeks. Cold enrichment is not necessary for isolation from listerial abortions or septicemias [3]. For samples where contamination is likely, enrichment and the use of selective media (lithium chloride phylethanol-moxalactam medium, oxford medium, or PALCAM Listeria selective medium) are advisable [5].

Identification: This small, gram positive, non-spore forming rod may appear coccobacillary or coccoid. Listeria monocytogenes is catalase and bile-esculin positive. Motility tests are important in Listeria identification [3]. Broth motility; two broth tubes are inoculated with the test organism and incubated for several hours. One tube is incubated at 35°C and another is hold at room temperature (25°C). When examined microscopically in a wet mount preparation, L. monocytogenes exhibits tumbling, end-over-end (“head-over-heals”) motility at 25°C and little motility at 35°C. Whereas semisolid agar motility; this test is performed by stabbing the test organisms once in to a tube of semisolid agar and then incubating the tube overnight at 25°C. L. monocytogenes produces an umbrella-like growth [18].

Animal Inoculation: In pure culture are placed in to the conjunctiva of the rabbit or guinea pig, or swabbad on the everted lid, a sever keratoconjunctivitis develops within 24 hours, followed by opacity of the cornea [8]. Anton test is positive test consist of the production of a purulent conjunctivitis in 24-26 hours after installation of a small amount of broth culture in to the conjunctival sac of guinea pig or rabbits [21].

Necropsy Findings: Typically there are no distinct gross changes associated with Listeria encephalitis. Histological examination of CNS tissue is necessary to demonstrate the micro-abscesses that are characteristics of the disease. Those are present in the brain stem in listerial encephalitis and in the cervical and/or lumbar spinal cord in outbreak of spinomyelitis. Gram staining of paraffin-embedded tissue may permit confirmation of the diagnosis in cases for which suitable culture material is unavailable [4]. Visceral (septicemic) lesions occur as multiple foci of necrosis in the liver, spleen and myocardium in the septicemic form and in aborted fetus, aborted fetuses are usually edematous and autolyzed, with very large number of bacteria visible microscopically in a variety of tissues [8].
Differential Diagnosis: Listeriosis can be differentiated from different diseases based on their clinical signs [11]. Disease affecting nervous system can be: ketosis, is a common disease of cattle typically occur in dairy cows in the early lactation and characterized by partial anorexia, depression, nervous dysfunction, abnormal licking, incoordination, abnormal gait and aggression are followed by marked ketonuria [4]. Polioencephalomalacia, is an important neurologic disease that have seen sporadically or as a herd outbreaks. The disease is characterized by blindness, recumbency, tonic-colic seizures, hypermetric gait and coma [1]. Rabies: it often occurs in a number of animals at one time due to the case with which a number of cattle bitten by a dog. The main features are drooling saliva, aggressiveness, incoordination, recumbency and paralysis. Gid, even if the disease occurrence is rare in cattle, there is similar localizing sign with listeriosis, but the clinical course is longer [14]. Hypomagnesaemia (grass tetany, grass staggers), it is a disease that show septicemic sign and confused with listeriosis, but the high rectal temperature and absence of true hyperaesthesia in listeriosis should be enough to distinguish the disease [13].

Zoonotic Implication: In humans, listeriosis may occur as a sporadic disease or as a food-borne outbreak to produce septicemic disease, meningoencephalitis, abortion and infection in other organs. Sporadic disease may involve healthy humans of any age but the disease usually occurs in the very young, very old and people who are otherwise immunocompromised [22]. The similarity of the disease spectrum in humans and animals and the occurrence of food-borne outbreak have led to concern that the disease could be zoonosis. These are a potential for zoonotic transmission. It would appear that the majority of human exposures to the organism and the risk for disease, result from contamination of foods during processing the particular ability of the organism to grow at refrigerator temperature [4].

Prevention and Control: The treatment of choice of listeriosis is intravenous administration of either ampicillin or penicillin, often in combination with an aminoglycoside for synergy. Trimethoprim-sulfamethazole is bactericidal against Listeria monocytogenes and has been used successfully in the treatment of patients with penicillin allergy. Listeria monocytogenes susceptible in vitro to penicillin G, ampicillin, erythromycin, trimethoprim-sulfamethoxazole, chloramphenicol, rifampin, tetracycline, aminoglycosides and impanel. However, Chloramphenicol and rifampin may antagonize the bactericidal effect of penicillin because Listeria monocytogenes is not sensitive to cephalosporin [11]. Intravenous administration of chlorotetracycline (10mg/kg body weight per day for 5 day) is reasonably effective in meningoencephalitis of cattle. Penicillin at dosage of 44,000 IU/kg BW given IM daily for 7 days and in many case for 10-14 days can also be used. The recovery rates depend largely on the time that treatment is started after the onset of clinical signs. If sever course of events in an outbreak is that the first case dies but subsequent cases are detected sufficiently early for treatment [4].

Although the occurrence of the disease is low occasional epidemics may occurs. These associated with high rate of environmental contamination. Poor quality silage should not be fed to pregnant ruminants and also silage feeding should be discontinued if an outbreak of listeriosis is confirmed. If a calf has listeriosis, fed Pasteurized colostrum’s milk or milk substitute [5]. Vaccination with killed vaccines, which do not induce an effective cell-mediated response, is not protective because L. monocytogenes is an intracellular pathogen. Live, attenuated vaccines, which are available in some countries, are reported to reduce the prevalence of listeriosis [8].

Treatment of maternal bacteremia during pregnancy can prevent neonatal infection. Antibiotic therapy for the new born can limit sequel, although the widely disseminated disease characteristics of granulomatosis is frequently fatal regardless of treatment. Early onset of disease carries a higher mortality risk than late onset infection and immunocompromised hosts have a worse prognosis than do otherwise healthy adults with listeriosis [11].

CONCLUSION

Listeriosis is an intracellular, sporadic bacterial disease found as contaminants of environment and different materials. It can also be isolated from feces of apparently healthy animals. Listeria monocytogenes is a food borne pathogen and they can multiply at higher stage in poorly stored silage and rotting vegetation in which these are aerobic condition and a PH is higher than 5.4 and in some case the disease result from infection of the terminals of the trigeminal nerve. The organism can survive at a refrigerator temperature and it can tolerate pasteurization treatment if the heat applied is insufficient. Finally, Listeria monocytogenes are prevalent during
winter season and the clinical sign show encephalitis, septicemic form and abortion. Therefore, based on the above conclusions the following recommendations are forwarded: Food borne listeriosis should be prevented by proper application of heat treatment and by maintenance of satisfactory hygienic condition. Effectively preserved silage should be used in order to reduce the occurrence of listeriosis and silage that is obviously decayed should be avoided from the environment. Susceptible animals should not be exposed to wet, cool and unhygienic environment. Aborting cow should be isolated from their offspring in order to reduce further infection of dam. Surveillance systems should be established for listeriosis investigation to reveal the existing situation of the disease in Ethiopia.

REFERENCES