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Original Research Article

EMERGENCE OF CLINICALLY SUSPECTED LISTERIOSIS IN GOATS: A SPORADIC OUTBREAK IN JAFFNA DISTRICT, SRI LANKA

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SUMMARY: Listeriosis is an infectious disease affecting ruminants and a serious foodborne illness in humans. Index cases with neurological signs suspected of Listeriosis were reported from ten Veterinary ranges of Jaffna District in August 2021. Field investigation with 937 animals from 262 farms revealed the characteristic clinical manifestations of the disease. The brain samples of dead animals were subjected to microbiological and histopathological analysis and confirmed as Listeriosis. Antibiotic sensitivity test was performed using disc diffusion method and amoxicillin, tetracycline, enrofloxacin and trimethoprim/ sulfamethoxazole were found to be sensitive to the isolates of suspected *Listeria monocytogenes.*The outbreak affected the goats regardless of breed and sex with a high incidence in goats of 1-3 years of age. Encephalitic form of Listeriosis was primarily observed with a high case fatality rate (77.8%). The mortality rate was high (84%) in adult goats and a low recovery rate (22.22%) was recorded in the overall population despite the routine therapeutic interventions. However, preventive measures adapted to control the outbreak within and outside the district were a success. This is the first report of the emergence of a Listeriosis outbreak in Jaffna District and it highlights the need to initiate preventive strategies for the future occurrence of Listeriosis as it is a possible threat to public health.

KEYWORDS: Listeriosis, goat diseases, encephalitic form, Jaffna district

INTRODUCTION

Listeriosis is a fatal disease affecting both animals and humans. It is caused by *Listeria monocytogenes,* a Gram-positive,facultative anaerobic coccobacillus. The organism is ubiquitous in nature as it can survive in different environments such as soil, water, sewage and decaying plant materials (Vazquez-Boland *et al*., 2001). It is a virulent foodborne pathogen causing serious illness in humans, especially inimmunocompromised individuals. The transmission of *L.monocytogenes* to humans occurs through direct contact with infected animals and consumption of contaminated foods of animal origin such as unpasteurized milk, cheese, cold meats and smoked sea food. The virulence of the pathogen is attributed to its ability to survive intracellularly which allows the pathogen to invade a wide range of host cells (Rogalla and Bomar, 2023. Although thirteen different serotypes of *L. monocytogenes* have been identified, only three (1/2a, 1/2b, 4a) are responsible for human Listeriosis (Chen *et al*., 2016).

Notwithstanding that Listeriosis has a wide host range, it is recognized as the most lethal disease for small ruminants. The transmission takes place via ingestion of food and water contaminated with faeces, saliva, nasal secretions, and aborted materials from infected animals (Dhama *et al*., 2015). Furthermore, the consumption of poorly fermented silage and contaminated commercial foods (Brugere-Picoux, 2008) is also a major source of infection. The infection can occur through the conjunctiva, small wounds in lips, oral and nasal mucosae (Braun*et al*., 2002), through carrier animals either by venereal or by faeces (Borku *et al*, 2006; Wesley *et al*., 2002). Vertical transmission is considered another potential source of infection (Braun*et al*., 2002).

Listeriosis can be manifested in three distinct forms in ruminants; septicaemia in young animals, encephalitis in adult animals and intrauterine infections leading to late-term abortion in pregnant animals (Headley *et al*., 2013).Moreover, keratoconjunctivitis, iritis and mastitis are uncommon manifestations in ruminants (Addis *et al*., 2019; Gaya *et al*., 1996; Nightingale *et al*., 2004). Among these syndromes, encephalitic form is the most common manifestation in goats and sheep (Headley *et al*., 2013). Encephalitic

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Listeriosis which leads to vestibular ataxia and unilateral cranial nerve deficits is manifested by unilateral facial paralysis, circling movements and drooling of saliva (Nightingale *et al*., 2004). Since encephalitis is more acute in nature and causes death within 4 to 48 hours of disease onset, its mortality rates are higher than those associated with other syndromes (Kumar *et al.*, 2007; Singh Parihar, 2004).

Listeriosis is a threat to both public health and food safety. In addition, the disease inflicts significant economic losses on livestock farmers. Listeria outbreaks in animals have been reported from various regions of the world at different times and there are reported cases from New Zealand, Serbia, Germany, Eastern Europe, Brazil, Turkey, South Africa, Canada, Switzerland and the Netherlands (Wesley *et al*., 2002; Kalender *et al*., 2003; Headley *et al*., 2013). The changes in the distribution of susceptible hosts, poor quality silage production and environmental factors may predispose the occurrence of the disease in the reported countries and the cases have been encountered in cattle, goats, sheep and pigs. The incidences of animal Listeriosis have also been reported from Asian countries at varying periods including different parts from the Indian sub-continent (Assam, Uttar Pradesh, Andhra Pradesh), Kuwait, Japan, Korea and Azerbaijan (Kim *et al*., 2023; Dhama *et al*., 2015; Khalimbekov, 1955). Several major outbreaks were reported in many countries as discussed above (Barman *et al*., 2020; Braun *et al*., 2002). This study focused on describing the epidemiology, clinical manifestations, laboratory diagnosis, management and control measures of the disease in goats with Listeriosis in Jaffna district, Sri Lanka.

MATERIALS AND METHODS

Outbreak location

Jaffna is one of the 25 administrative districts of Sri Lanka, consisting of fifteen veterinary ranges with a total goat population of 61,025 in 2021 (Annual Report 2021, Department of Animal Production and Health, Northern Province). Goat rearing has been popular in the Jaffna district for many decades and significantly contributes to the livelihood of the rural community. The Goat population in Jaffna district represents 43.71% of the provincial population and 23.29% of the national herd (Annual Report 2021, Department of Animal Production and Health, Northern Province and Department of Census and Statistics). Saanen, Jamunapari, Boer, local breeds and their crosses are the predominant breeds reared in Jaffna mostly under the semi-intensive management system (Annual Report 2021, Department of Animal Production and Health, Northern Province).

Affected population

This report describes the investigation of an outbreak among 937goats belonging to 262 farms with a total sample population of 2358 between August and October2021 in ten veterinary ranges of Jaffna District: Jaffna, Thellipalai, Uduvil, Pointpedro, Karaveddy, Chavakacheri, Nallur, Sandilipay, Vaddukodai and Kopay(Figure 1). The goats presented with nervous signs were included in this investigation. The affected farms were visited and detailed information on history and management practices adopted by farmers were recorded using a questionnaire.All the farmers provided their informed consent to be included in the study.

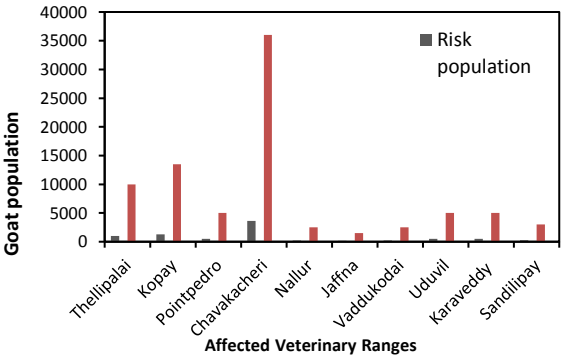

Figure 1. The total and risk goat population of different affected Veterinary range of Jaffna District, Sri Lanka in 2021

Case history

There had been no incidence of similar disease occurrence before in the study area and the first case was reported in August 2021. Each farm under survey consisted of 4-10 animals and the flocks were housed in common stables irrespective of age groups. All the affected animals were allowed for free grazing and fed with leaves, grass, concentrates, poonac and rice bran in the evening. Silage feeding was observed in some farms, especially for cattle which were reared together with goats. All age groups of both sexes were affected.

Case presentation

Upon clinical examination, the affected goats appeared to show the following clinical signs: Pyrexia (104.2±0.35°F), ocular discharge, salivation, nasal discharge, facial paralysis, lateral protrusion of tongue, tilted head, anorexia, depression, drooping of lips and ears, droopy lower eyelid (paralysed side), circling of head, impacted food particles in muzzle, convulsions, ataxia, incoordination, recumbency (later stage) and death within 1-3 days (Figure 2).

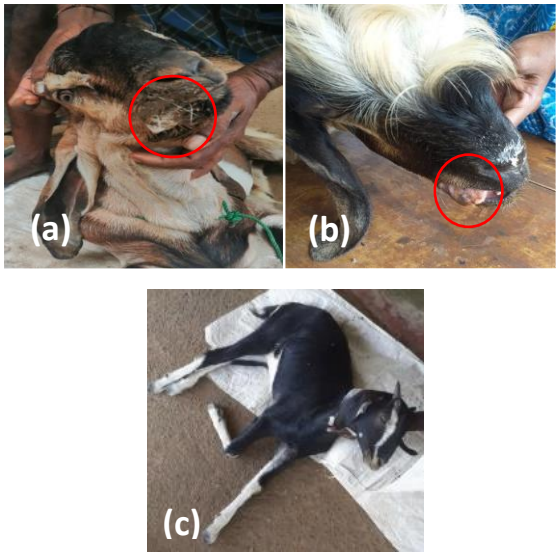

Figure 2. The most characteristic clinical manifestations of Listeriosis in affected goats (Encephalitic Listeriosis): (a) Profuse salivation with impacted food in the mouth due to cranial nerve paralysis in an acutely affected goat (b) Unilateral facial paralysis with droopy lip and protrusion of tongue due to cranial nerve deficit (c) Terminal recumbency and paddling movements exhibited by a cross-bred goat with the progression of the disease. The discussed features of (a) and (b) are encircled in each image.(a,b.c labels not visible)

Differential diagnosis

Listeriosis
Caprine arthritis encephalitis

Laboratory investigations

Sample collection

Nasal swabs (n=20), blood and milk samples (n=30) from the sick animals and blood samples (serum) from recovered live animals (n=05) were collected and analysed at the Veterinary Investigation Centre, Jaffna and were sent to the Veterinary Research Institute (VRI), Gannoruwa.

Necropsy examinations were performed on five dead animals at the site of an outbreak in a routine manner. Sections of the brain, spinal cord, heart, liver, lungs, spleen, kidney and intestine were collected, kept in ice, fixed in 10% formalin and analysed at the VIC. Then the samples were dispatched to VRI for confirmation.

Bacteriological testing

Bacteriological isolation was attempted from nasal swabs, milk and brain samples by culturing aerobically in different agar media including Mueller Hinton agar, nutrient agar and Mac Conkey agar. The aforementioned media cannot be used for the primary isolation of Listeria. However, because of the unavailability of blood agar and any Listeria selective agar in Veterinary Investigation Centres, during the time of the outbreak, and due to the prevailing transportation restrictions due to the COVID-19 pandemic, these media were used to culture the samples as an alternative method.Positive cultures were subjected to Gram staining and biochemical tests including catalase, oxidase, indole, urease citrate and methyl red.Further confirmation was done by necropsy findings and histopathological findings.

Virological testing

The serum samples were processed at Animal Virus Laboratory, Polgolla using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (IDEXX CAEV/ MVV total Ab test Kit). Whole-virus antigen preparations were used for the detection of antibodies against the Caprine Arthritis Encephalitis Virus (CAEV)with the use of positive and negative controls. The sera were tested in 1:2 dilutions at room temperature according to the manufacturer's instructions.

Antimicrobial susceptibility testing

Antibiotic sensitivity test (ABST) was performed at VIC, Jaffna and Veterinary Research Institute, Gannoruwa using the disc diffusion method (manuals of clinical laboratory standard institute). Amoxicillin(25µg), tetracycline (30µg), enrofloxacin(10µg),trimethoprim sulfa(25µg),gentamicin (10µg), ampicillin (10µg) and streptomycin (25µg) discs were used.

Statistical analysis

The descriptive analysis was performed using Excel 2016 software. Student's t-test was performed using Minitab 18 software to determine the influence of age (kids < 1year, adults>1year), sex (male, female) and breed (Saanen, Jamunapari, Boer, Cross breeds) on the occurrence of the disease. Also, case fatality, mortality, morbidity, prevalence and recovery rates were calculated using Microsoft Excel 2016.

RESULTS

Necropsy findings

Post-mortem examination of five goats revealed moderate to severe, multifocal, sub-acute, suppurative encephalitis (4/5 cases),moderate to severe, diffuse pulmonary congestion and haemorrhage with sub-acute, suppurative pneumonia (3/5 cases) and moderate,multifocal,

sub-acute, hepatic centrilobular necrosis(2/5 cases) (Figure 3).

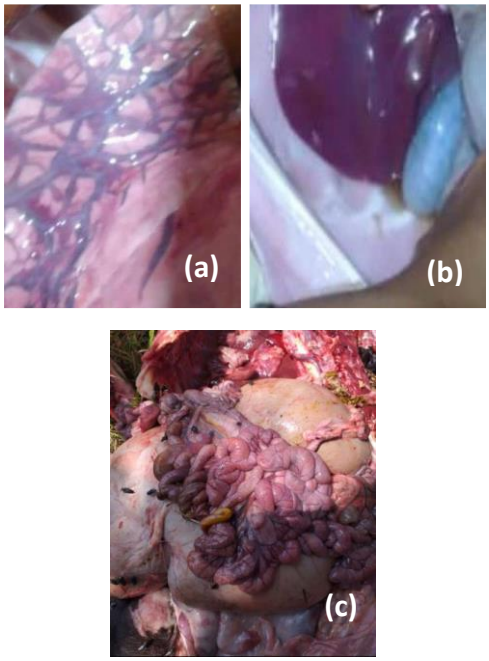

Figure 3. Pathological characteristics of Listeria infection in affected goats at necropsy: (a) Severe pulmonary congestion and necrosis with the evident signs of suppurative pneumonia and haemorrhage (b) Multiple, disseminated necrotic foci in the liver surface in a goat infected with septicaemicListeriosis. (c) Gastroenteritis with consistent haemorrhage in the mucosal surface of the abomsaum and diffuse congestion in the intestines (a,b,c not visible).

Table 1. Biochemical and cultural properties of Listeria monocytogenes isolated from the culture

Biochemical & Cultural properties		**Reaction of *Listeria monocytogenes***
Gram stain		Positive
Cell morphology		Short non spore forming rods
Culture	Mueller Hinton	Small, greyish glistening colonies with smooth surface with 1-2 mm in diameter
	Nutrient agar	Bluish smooth, small, round colonies with 1-2 mm in diameter (minimal growth)
	Mac Conkey	No growth
Catalase		Positive
Oxidase		Negative
Indole		Negative
Urease		Negative
Methyl red		Positive

Moderate to severe, multi-focal perivascular cuffing with mononuclear cells was recorded in the brain section of all cases. Multi-focal areas of degeneration with infiltration of neutrophils, lymphocytes and macrophages (60% of the samples), degenerated neutrophils and macrophages suggesting micro-abscesses (40%of the samples), presence of polymorphonuclear leukocytes (40%of the samples), marked diffuse congestion in meninges (60%of the samples) and locally extensive area of haemorrhage in gray matter of cerebrum (20%of the samples) were the other findings observed in the brain tissue of the dead animals. In a few samples (20%), the white matter of the spinal cord consisted of polymorphonuclear leukocytes with mild diffuse vacuolation. Further, all lung samples showed moderate to severe oedema and haemorrhage with multi focal infiltration of neutrophils in bronchioles and alveoli. The liver samples of the vast majority of dead animals had multi focal centrilobular necrosis with diffuse congestion. Diffuse lymphatic depletion with congestion in spleen was observed in two cases. Multi-focal tubular necrosis and haemorrhage were found in the renal surface and parenchyma of nearly half of the samples.The histological findings were mostly suppurative in nature and the cases were highly suggestive of bacterial encephalitis.

Microbiological findings

Small, greyish glistening colonies with smooth surfaces measuring 1-2 mm in diameter were observed on Mueller Hinton Agar. A minimal growth of bluish smooth, small, round colonies with 1-2 mm in diameter was detected on nutrient agar. There was no growth on Mac Conkey agar. Microscopically organisms were Gram positive and short rods in morphology (Figure 4). All the isolates were positive for catalase and methyl red and negative for oxidase, indole, urease and citrate (Table 1).

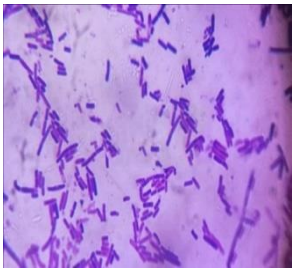

Figure 4. *Listeria monocytogenes* as Gram-positive short rods from the culture in Mueller Hinton Agar

Among the submitted samples, bacterial isolation (suspected *Listeria monocytogenes*) was made from the brain tissues of the affected goats. Therefore, the disease was diagnosed as Listeriosis on the basis of typical clinical signs, conventional bacteriological tests, necropsy findings and characteristic histopathological features.

Virology

Virology results clearly suggested that the antibodies for Caprine arthritis encephalitis Virus (CAEV) were negative in the serum samples of the affected goats and thus, Caprine arthritis encephalitis was ruled out.

ABST results

ABST results revealed that the suspected *Listeria monocytogenes* isolates were sensitive to amoxicillin, tetracycline, enrofloxacin and trimethoprim/ sulfamethoxazole. Isolates were intermediately sensitive to gentamicin and ampicillin while the isolates were resistant to streptomycin.

Treatment and Management

The affected animals were treated with one of the antibiotics that were sensitive according to ABST as follows (Figure 5): amoxicillin (8% of the cases), tetracycline (12% of the cases), enrofloxacin (26% of the cases),trimethoprim sulfa (18% of the cases) and the combination of amoxicillin and enrofloxacin (36% of the cases). The antibiotics were administered intramuscularly at the dose rate of 20 mg/kg (amoxicillin,tetracycline), 15 mg/kg (trimethoprim sulfa) and 5mg/kg (enrofloxacin). Additionally, all the animals were treated with the anti-inflammatory agent (ketoprofen – 2.2mg/kg), antihistamines (chlorphenamine maleate – 0.5 mg/kg) and supportive therapy(multivitamin-6ml/animal) intramuscularly for 3-5 days.The severely affected animals were provided with fluid therapy (Rintose, Normal Saline) intravenously in addition to the above treatment protocol.Persistent ataxia, torticollis, kerato-conjunctivitis and abortion were some of the complications encountered during the investigation (Figure 6).

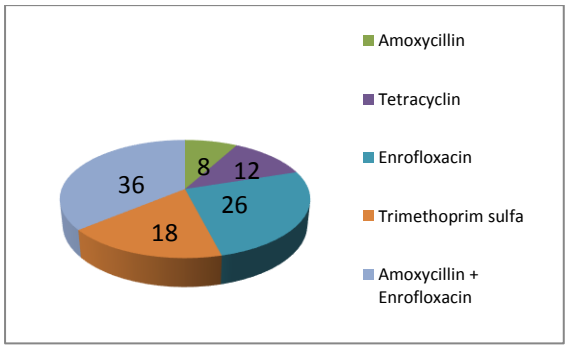

Figure 5. Different antibiotics used to treat the affected animals based on the ABST (The percentage of each antibiotic used is given in the figure). The combined therapy of antibiotics with amoxycillin and enrofloxacin was more frequently used compared to the usage of individual antibiotics (Series 1 overlapped)

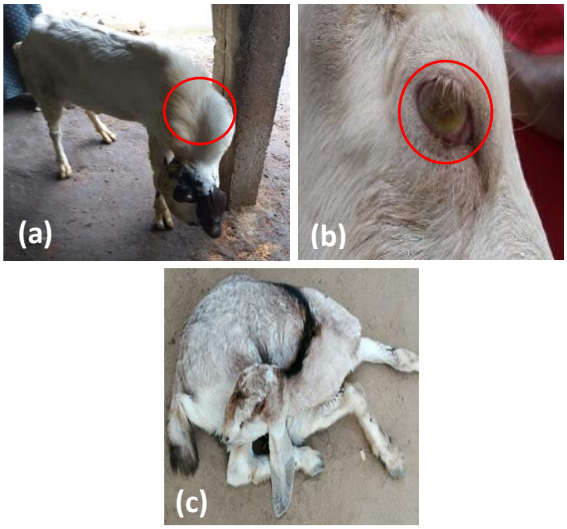

Figure 6. Different complications encountered in the outbreak in affected goats treated at the terminal stage of the disease: (a) Torticollis of a goat having involuntary, circling movement due to focal lesions of the brain stem (twisted neck is encircled in the picture) (b) Unilateral keratoconjunctivitis in a severely affected goat characterized by blepharospasm and lacrimation with hyperaemic palpebral conjunctivae (the affected eye is encircled) (c) Ataxia in a kid manifested initially as in-coordination followed by tetraparesis resulted in lateral recumbency(a,b,c not visible)

Epidemiology

Among the three forms of Listeriosis (septicaemic, encephalitic and reproductive), encephalitic Listeriosis was predominantly observed during this outbreak with the prevalence rate of 39.7%. Morbidity, mortality and case fatality rates of the incidence were 1.54, 1.2 and 77.8%, respectively. Despite the efforts made to save the sick animals, only 22.22% recovery rate was achieved. A high mortality rate was recorded in goats above one year compared to goats below one year ($p<0.05$). The highest number of cases was reported in 1-3 years. There is no statistical significance on breed and sex on the occurrence of disease ($p>0.05$).

Outbreak Control

The disease was tentatively diagnosed as Listeriosis with the typical clinical manifestations together with the laboratory findings and treatment was initiated for the index cases. Further spread of the disease was controlled by the isolation of susceptible animals in the herd from the infected, movement restriction of animals, animal products and manure. Free grazing of animals in the affected ranges was controlled. Guidance was given on the proper disposal of carcasses, excreta and left over feed. Proper disinfection processes were carried out

on farm premises, feed bunks, mineral feeders and floor. The surfaces of farm premises were thoroughly cleaned using soapy water followed by chemical sanitizer: diluted chlorine bleach solution (5-6g/lit). Moreover, awareness was made via media (newspapers, notices) on prevention and zoonotic aspects of the disease. The outbreak was effectively controlled both within and outside the study district with immediate implementation of aforementioned preventive measures.

DISCUSSION

The outbreak of Listeriosis was not documented before in the Jaffna district and the in the country. This outbreak occurred during the North East monsoon.

This was a severe outbreak indicated by a high case fatality rate which was in line with a previous report by Younis *et al*., (2010). Despite the low mortality in the entire population, significantly high mortality rate was observed in goats above one year of age. However, this finding was not in agreement with previous report by Borku *et al*., (2006) where high mortality in kids (83.3%) was observed in an outbreak in Turkey. The above differences in case fatality and mortality rates are attributed to age factor, the form of Listeriosis observed and the responsiveness to treatment (Radostits *et al*., 2007). Moreover, relatively significant prevalence rate (nearly 40%) denotes increased risk of Listeriosis for the population.

Despite the disease affecting all goats irrespective of sex and breed, there was a high incidence among goats aged between 1-3 years. According to the literature, young animals are more prone to develop septicaemic form, while adult animals develop encephalitic form of Listeriosis (Hamidi *et al*., 2020).

Encephalitic Listeriosis was predominantly observed wherein few cases were manifested together with either septicaemic form or reproductive form. The septicaemic form was clearly indicated by the necropsy findings in liver (multiple necrotic foci) and spleen and few of the cases encountered with abortion in pregnant does were suggestive of reproductive Listeriosis.Encephalitic form was commonly reported in several outbreaks from various countries (Barman *et al*., 2020; Borku *et al*., 2006; Younis *et al*., 2010). The clinical presentation with encephalitic Listeriosis was similarly documented in many other studies (Braun *et al*., 2002; Hamidi *et al*., 2020; Kumar *et al*., 2007) which could facilitate on a rapid clinical diagnosis of the disease.
The variations in clinical manifestations of the disease are attributed to the site affected in nervous system (medulla, pons, cerebellum, cerebrum or anterior spinal cord) and impairment of cranial nerves (Brugere-Picoux. 2008; Younis *et al*., 2010).
The histopathological lesions observed in this study are in conformity to previous observations by Hamidi *et al*., (2020) and Kumar *et al*., (2007) where marked perivascular cuffing comprising of mononuclear cells, polymorphonuclear leukocytes forming abscesses and congested meninges with infiltration of mono nuclear cells were evidenced. The results were also consistent with the findings reported by Younis *et al*., (2010).

According to the literature, procaine penicillin, tetracycline, erythromycin, trimethoprim/ sulfamethoxazole are generally recommended for the treatment of Listeriosis(Hamidi *et al*., 2020; Lianou *et al*., 2022). Among the aforementioned antibiotics, most were shown sensitive for ABST conducted for isolates from the current outbreak and were used for the treatment. Although the combination of ampicillin and gentamycin were indicated as the treatment of choice for Listeriosis in a few studies and were proved to be effective (Braun *et al*., 2002; Christ-Donald *et al*., 2020), our study finds intermediate sensitivity to the above two antibiotics. Despite streptomycin showed sensitivity to *L. monocytogenes* in a previous report by Hamidi *et al*., (2020), it was found resistant in this study.

Intravenous fluid therapy with sodium chloride and sodium lactate was aimed to correct metabolic acidosis and dehydration. Listeriosis causes diminished ruminal activity and movement which is manifested by impaired defecation in the affected animals, and this subsequently leads to reduced production of Vitamin B1 by ruminal microbes. Therefore, the treatment strategy also includes the administration of supportive which consists of Vitamin B1.

The animals which were treated at the terminal stage after the disease progression had poor prognosis denoted by the low recovery rate (22.2%) and the survivors from this group also developed complications. However, the affected goats that received regular, rigorous and prolonged treatment from early onset of disease survived with minimal complications. This finding agrees with Braun *et al*., 2002 where poor clinical outcome was seen in severely affected animals. Therefore, both the selection of antibiotics and the commencement of treatment during the acute stage of the disease appear crucial for a successful clinical outcome.

Zoonotic potential of Listeriosis and its highly contagious nature that can cause high case fatality and poor recovery highlights the need to initiate

eminent control measures. Vaccination using attenuated or killed vaccine is practiced in some countries to reduce the annual incidence of the disease (Brugere-Picoux, 2008). Even though the disease is highly contagious, prophylactic vaccination couldn't be carried out due to the unavailability of listeria vaccines in Sri Lanka and only the conventional control measures were adapted.

Poor quality silage, feed stuffs, contaminated pastures with faeces of wild and farm animals, contaminated feed (grains and cereals) and water with the access of migratory/wild birds, rodents or with their carcasses can be the sources of *Listeria* (Rodriguez *et al*., 2021). The newly introduced silage production and consumption in dairy sector or recently used feed stuffs or increased rearing of pet birds and the changes in the environmental and climatic conditions could be the suspected causes of this outbreak. However the prevention of future occurrences requires the investigation on the definite cause of the outbreak which is crucial to prevent the unwanted zoonotic infection via milk, milk products or direct contact. Under reporting of cases due to the prevailed Corona pandemic during the study period and lack of laboratory facilities for confirmatory diagnosis are the limitations of this study. Further, research is required to identify the root cause of the outbreak of caprine Listeriosis in Jaffna District.

CONCLUSION

The emergence of Listeriosis in Jaffna district was documented for the first time with this outbreak which inflicted significant losses to dairy goat husbandry. Since there is lack of vaccination for Listeriosis in Sri Lanka, prevention highly depends on the control measures: timely diagnosis and attentive treatment, movement restriction, proper disposal and disinfection of contaminated materials and surfaces and conducting awareness programs.

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