An Outbreak of Ovine Listeriosis in Qassim Region, Saudi Arabia: Epidemiological, Clinical and Treatment Outcomes

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ABSTRACT

This study was carried out to describe the epidemiological, clinical and treatment outcomes of an outbreak of listeriosis in a sheep flock at Qassim Region, central of Saudi Arabia during January 2020. The flock consisted of 700 sheep of different age and sex. The overall morbidity rate was 57.14%, while the mortality rate was 50% and the case fatality rate was 87.5%. The morbidity rate was statistically lower ($P=0.0001$) in sheep having age less than 6 months (16.66%) than those over 6 months (74.48%). On the contrary, the case fatality rate was not statistically different ($P=0.9$) in young sheep (97.14%) compared to adult animals (86.57%). The clinical signs observed were acute deaths, nervous manifestation in the form of staggers, ataxia, head tilt and circling. Initial diagnosis was made on the basis of the typical clinical signs, while confirmatory diagnosis was made on the basis of bacteriological examination. Treatment using penicillin as antibiotic in addition to flunixin meglumine as anti-inflammatory gave good results in case of early intervention whereas late-stage treatment gave bad prognosis. So, early intervention is the key to treatment success.

Key words: Listeriosis, Sheep, Outbreak, Treatment.

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INTRODUCTION

Listeriosis is a highly fatal infectious disease caused by different *Listeria* (L.) species, but mainly by *L. monocytogenes*, affecting a lot of mammals, including different animal species as cattle, sheep, horses, dogs and humans (Fentahun and Fresebehat 2012). *L. monocytogenes* is a Gram-positive intracellular bacterium causing fatal meningoencephalitis in humans and ruminants (Pfister et al. 2002). It is considered as a significant food borne pathogen present in the surrounding environment. Moreover, ruminant animals are mainly the natural hosts for the microorganism where, these species can disseminate the microorganism through milk and meat (Shamloo et al. 2019; Rodriguez et al. 2021; Zhao et al. 2021).

Sheep is the most affected animal species representing clinical features in the form of septicemic, nervous, abortive, mastitis and gastroenteritis (Smith, 2001; Brugere-Picoux 2008; Limmahakhun and Chayakulkeeree 2013; OIE 2014). The disease has a worldwide distribution, particularly in temperate climates and favorable

environmental conditions for growth of the microorganism such as bad quality silage (McLauchlin 1987; Fenlon 1988, 1999).

Ingestion of bad quality silage is considered the main route of infection particularly common in ruminants (Low and Donachie 1997). The microorganism can affect many organs as intestine especially in young animals and monogastric animals, reproductive systems and nervous system. So, the disease manifest itself in three forms namely, septicemia, abortion and encephalitis (Nash et al. 1995). Transmission of listeriosis from animals to humans has been associated either with ingestion of fecal contaminated food (Blenden et al. 1987; Pearson and Marth 1990) or directly from infected ruminants to man (Blenden et al. 1987). The economic losses of listeriosis are high, not only due to the losses in animal production but also due to the public health concern (Blenden et al. 1987; Pearson and Marth 1990).

Listeria monocytogenes, which is a foodborne bacterium is a major agent responsible for infectious diseases in humans and animals. In human, listeriosis occurs as sporadic cases, which is epidemiologically linked with

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consumption of contaminated food products. The signs of listeriosis in humans ranged from mild self-limiting disease to severe systemic infections in susceptible people (Ranjbar and Halaji 2018). Milk and dairy products from infected animals may result in life threatening diseases in a large population of people (Shamloo et al. 2019).

There is a little literature concerning listeria infection in Saudi Arabia in farm animals, so far, only two reports have been documented. The first was an outbreak in a sheep flock in the eastern region (Al-Dughaym et al. 2001) and the second was a case of cerebral listeriosis in she camel in the eastern region as well (Al-Swailem et al. 2010). So, this report is the third and it is about an outbreak occurred in a new area, Qassim Region, central region of Saudi Arabia. This study was carried out to describe the epidemiological, clinical and treatment outcomes of an outbreak of listeriosis in a sheep flock.

MATERIALS AND METHODS

Ethical Approval

Animal Ethical Committee, Scientific Research Deanship, the University of Qassim, Saudi Arabia approved this study.

Animals and Sample Collection

This investigation was done in winter (January 2020), on sheep flock consisted of 700 sheep of different age and sex belonging to a private farm at Qassim Region, central of Saudi Arabia. Animals were let to graze during the day and kept in pens and provided with fodder rolls (barley, grass, alfa alfa) during the night.

Epidemiological and Clinical Investigation

All sheep in this study were carefully examined clinically (Rosenberger 1979). Morbidity, mortality and case fatality rates were estimated epidemiologically according to the method described by Martin et al. (1987).

Specimens for Bacteriological Examination

Specimens of brain and liver tissues were aseptically collected from several clinical cases in sterile containers and immediately sent for laboratory investigation.

Bacteriological Examination

Bacterial isolation was attempted from the sterile tissue samples obtained from hind brain and liver tissues. Direct smears were stained by Gram's staining method. Specimens from the brain and liver were cultured on blood agar, MacConkey agar and brain heart infusion broth and agar. Samples were incubated at 37°C for 48h. Blood agar and brain heart infusion broth were also inoculated and incubated at 4°C for six weeks with weekly examination of growth and subculture of broth on solid medium and incubation at 37°C. The colonial characters of *L. monocytogenes* were observed on blood agar and MacConkey agar. Further characterization of *L. monocytogenes* was done according to catalase test, umbrella motility in semi-solid medium, tumbling motility in 3h broth culture at 25°C by hanging drop technique and CAMP test (McKellar 1994).

Therapeutic Intervention

Diseased sheep was treated using penicillin-streptomycin (Pen & Strep, NorBrook) administered by deep intramuscular once daily for 7 consecutive days at doses of 8mg procaine penicillin and 10mg dihydrostreptomycin sulphate per kg bodyweight achieved by administering 1ml/25kg BW. Flunixin meglumine (Finadyne, Schering-Plough) as anti-inflammatory agent was administered at a dose of 2.2mg/kg body weight achieved by administration of 2ml/45kg BW. Sulphadiazine Sodium-trimethoprim (Joprim, Jordan Veterinary and Agriculture Medical Industrial Company) was administered at a dose of 1gm/15kg BW, daily for 5 days to healthy in-contact animals.

Statistical Analysis

The obtained data were analyzed by Chi-Square and *t* test using the SPSS for Windows (2009) statistical software program and a P value of less than 0.05 was considered significant.

RESULTS

Out of the examined (700) sheep, 400 animals (57%) showed nervous manifestation representing a morbidity rate 57.14%. Out of these 400 sheep, 350 were died representing mortality rate of 50% and case fatality rate of 87.5% (Fig. 1).

The incidence of the disease in this study was lower in young sheep less than 6 months than those over 6 months. The morbidity and mortality rates in young sheep were 16.66% (35/210) and 16.19% (34/210) compared to 74.48% (365/490) and 64.48% (316/490) respectively in animals over 6 months. On contrary, the case fatality rate was 97.14% (34/35) in young sheep compared to 86.57% (316/365) in adult ones (Fig. 1).

In the early stages of the disease, the sick animals were moving aimlessly with deviated neck. With progression of disease, animals showed unilateral cranial nerve deficits and started moving in circles. Severe depression, ataxia and circling were observed in all cases. Blindness was observed in 100 cases without any eye lesions. Abortion was occurred in 5 out of 80 pregnant ewes. Fever (41-42°C) was observed only at the early stages of the disease, whereas in the late stages, the temperature was normal. Before death, the infected animals became recumbent, unable to eat and paddling of the forelimbs. Deaths were occurred within 10 days from the disease onset.

Microscopical examination of Gram-stained smears, prepared from solid medium, revealed Gram-positive short rods, coccobacilli or coccoid forms (Fig. 2). After 48 hours of incubation at 37°C on sheep blood agar, greyish white small dew drops like colonies with narrow slight clearing zones of β -hemolysis were observed. It was catalase-positive and cells were motile at 25°C.

In this study, the results of the treatment revealed that, early intervention using penicillin (administered by deep intramuscular daily once for 7 consecutive days) in addition to Flunixin meglumine as anti-inflammatory agent gave good results. However, administration of the drug in late stages of the disease had no effects. Sulphadiazine Sodium-trimethoprim offered to all healthy sheep as a method of control gave good efficacy in lowering the occurrence of new cases.

Fig. 1: Morbidity, mortality and case fatality rates during the outbreak.

Fig. 2: Gram-stained smears showing Gram-positive short rods, coccobacilli or coccoid forms.

DISCUSSION

Listeriosis is one of the most important infectious diseases of most animal species with zoonotic importance worldwide; it has considerable economic significance as it is mainly a foodborne pathogen (Barman et al. 2020). Listeriosis is an infection characterized by high case fatality rate. All of domestic animals can attract the disease, but cattle and sheep are the most commonly affected (Cooper and Walker, 1998). In this study, the morbidity rate was 57.14%, the mortality rate was 50% and the fatality rate was 87.5%. Variations in these rates were recorded in the previous outbreaks, where Vandegraaff et al. (1981) recorded morbidity rate ranged from 0.2 to 8.0%, and 100% fatality rate. Nash et al. (1995) reported that 3.1 and 1.3% of ewes and lambs were died from listeriosis subsequently. Al-Dughaym et al. (2001) found that the disease was observed in adult animals, mostly pregnant ewes with a morbidity rate of 7.1% and mortality rate of 2.4%. George (2002) mentioned that the incidence rate might reach 9%, but rarely over 2%. These variations may be attributed to the differences in the animal susceptibility in different areas.

The high mortality rate that was observed in this outbreak may be due to the occurrence of the infection for the first time in this area in highly susceptible animals, free from any source of previous immunity due to no vaccination or previous exposure. Also, the misdiagnosis of the infection as coenurosis by the owners and using of anti-parasitic drug instead of antibiotics aggravated the problem. The morbidity and mortality rates in sheep less than 6 months (16.66 and 16.19%) were statistically lower ($P=0.0001$) than those in sheep more than 6 months 74.48 and 64.48%. This may be referred to the feeding of young animals on milk mainly. On contrary, the case fatality rate was not statistically different ($P=0.9$) in young sheep (97.14%) than that in adult ones (86.57%). Similar observation was recorded by Nash et al. (1995) and Kumar et al. (2007). The high case fatality rates in late treated sheep may be attributed to the irreversible injuries and limited repair capacity for the brain (Niederkorn 2006).

L. monocytogenes is major widespread zoonoses, especially in domestic animals. It is found commonly in the soils, decaying vegetation, and as fecal flora of some mammals (Schuchat et al. 1991; Bille et al. 2003). About 5% of healthy adult animals carry the microorganism in their feces (Schlech et al. 1983; Schuchat et al. 1991), these healthy carriers were documented in different species of animals (Low and Donachie 1997). In particular, subclinically mastitic animals due to *L. monocytogenes* can excrete the microorganism in their milk (Fthenakis et al. 1998; Wagner et al. 2000).

The outbreak in this study was occurred in winter season. Similar observations were reported previously by Nash et al. (1995), Al-Dughaym et al. (2001) and Radostits et al. (2007) who reported that the occurrence of listeriosis outbreaks was mainly during winter and within 2 weeks after the introduction of silage. This may be referred to the wide scale of temperature (4 to 37°C), with the fastest growth rates occurring at 30 to 37°C. Several factors including abrupt changes in food, extremely cold weather, transportation and lactation lower the host immunity and increase the animal susceptibility to infection. Feeding of sheep on bad quality silage had an immunosuppressive effect and make animal more reliable to infection (Brugere-Picoux 2008).

The clinical signs of listeriosis may be septicemic, nervous or abortive according to the tissue affected (Scott 2013). In the present study, the clinical signs observed in infected sheep were in the form of nervous manifestation. Deaths were occurred within 10 days from the disease onset. Similar clinical signs were also recorded previously (Low and Linklater 1985; Smith and Sherman 1994; Rebhun 1995; Low and Donachie 1997; Braun et al. 2002; Mahajan et al. 2020). The observed nervous signs may be attributed to the proliferation of *L. monocytogenes* within the subarachnoid and ventricular space which produced a severe host inflammatory response resulting in bacterial lysis in the CSF and release of bacterial components which stimulate the release of neuro-inflammatory molecules and other pro-inflammatory host proteins (Yadav et al. 2009). Barlow and McGorum (1985) postulated that the organism reached the brain by way of circulating blood or branches of the trigeminal nerve that terminated in the oral cavity, nasal cavity or conjunctiva. Moreover, as a result of the facial and tongue paralysis, undigested food was observed inside the animal mouth (Kumar et al. 2007).

The microscopic examination of Gram-stained smears, prepared from solid medium, demonstrated Gram-positive short rods, coccobacilli or coccoid forms. The suspected colonies were identified on the bases of being β -hemolytic, catalase-positive, and cells were motile at 25°C.

Confirmatory diagnosis in this study was mainly based on the bacteriological examination in animal specimens. This procedure is the most accurate confirmatory tool for diagnosis to which other methods are compared (Quinn et al. 1999). Gram-stained smears revealed Gram-positive short rods, coccobacilli or coccoid forms. The suspected colonies were identified on the bases of being β -hemolytic, catalase-positive, and cells were motile at 25°C. Similar observation was recorded previously by Kumar et al. (2007).

In this outbreak, infected sheep were not admitted to silage feeding indicating that infection by listeriosis in sheep can occur without feeding on silage. Similar observation was recorded by Kumar et al. (2007) who concluded that ovine listeriosis outbreaks can be happened without the association with the feeding on silage. This may be explained by the wide occurrence of *L. monocytogenes* in the environment other than silage such as soil, water, sewage, wild animal feces and the fecal matter of some normal animals. Under stress factors, *L. monocytogenes* can convert to pathogenic type causing clinical disease (Sauders and Wiedmann 2007; Brugere-Picoux 2008).

In this study, the results of the treatment revealed that, early intervention using penicillin in addition to flunixin meglumine as anti-inflammatory agent gave good results. However, administration of these drugs in late stages of the disease gave no effects. Similar observation was reported by Radostits et al. (2007) who recorded that listeriosis had a per-acute course causing rapid deaths within 2-4 days and treatment is often not effective.

Low and Donachie (1997) reported that antibiotics such as penicillin or tetracycline can be used successfully in the treatment of listeriosis. Hof (2003) found all commonly used antibiotics, except cephalosporins were active against *L. monocytogenes* *in vitro* and amoxicillin and gentamicin combination was the best choice. In the present study, Sulphadiazine Sodium-trimethoprim was offered to all healthy sheep in drinking water as a method of control and gave good efficacy in lowering the occurrence of new cases. Hawkins et al. (1984) found that the use of trimethoprim-sulfamethoxazole combination was superior to either drug alone.

Conclusion

It is concluded that, ovine listeriosis in Saudi Arabia could pose a significant public health concern where meat, milk or meat products from sheep are widely used by the people and so listeriosis in Saudi Arabia must take a great attention. Also, early intervention using penicillin is the key to treatment success. Moreover, Sulphadiazine Sodium-trimethoprim administration orally to healthy in-contact sheep during the outbreak as a method of control gave good efficacy in lowering the occurrence of new cases.

Author's Contribution

All authors contributed equally to this study.

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