First report of an outbreak of ovine septicaemic listeriosis in Saudi Arabia

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Summary
Although a case of human listeriosis has recently been reported in Saudi Arabia, the disease has not been reported in animals to date. The authors describe an outbreak of septicaemic listeriosis in sheep, which occurred during winter. Adult animals and pregnant ewes were principally affected, with a morbidity rate of 7.1% and a mortality rate of 2.4%; no abortions were recorded during the outbreak. Clinical signs included inappetence, weakness, fever, respiratory distress, keratoconjunctivitis and compulsive circling. Listeria monocytogenes was isolated in pure culture from affected sheep. Pathological findings indicated septicaemic listeriosis with encephalitis. Hygienic measures and antibiotics were successful in treating the rest of the flock. Aspects of the outbreak and public health implications are discussed.

Keywords

Introduction
An important infectious disease with world-wide prevalence, listeriosis affects many species of animal as well as humans. In most cases, the disease is caused by a facultative intracellular pathogen, Listeria monocytogenes. The infection may be manifested by three distinct clinical syndromes, namely: encephalitis, septicaemia and abortion (11). Encephalitic listeriosis is more prevalent among ruminants, especially sheep, with an attack rate of approximately 10%-20% and a mortality rate of approximately 5%-10% (4, 5). Involvement of the central nervous system is manifested by unilateral ataxia and meningitis with formation of microabscesses (14). These neuropathological changes account for the common name, ‘circling disease’ of sheep (1). Low et al. reviewed eighty-five cases of ovine listeriosis and reported correlation between isolation of L. monocytogenes from the brain and histological confirmation of listeric encephalitis (11). In an outbreak of septicaemic listeriosis caused by L. monocytogenes, involving 50 young lambs in a flock of 150 sheep, the most significant lesions were necrotic hepatitis and meningoencephalitis (21). Ophthalmitis and corneal opacity have also been described in Listeria-infected cattle and sheep (14).

In general, the disease is more frequent in winter and early spring and has been associated with silage feeding. Listeria monocytogenes was isolated from brains of sheep and goats affected with listerial encephalitis and from silage consumed by the animals (20). A causal relationship between listeriosis and consumption of contaminated silage was reported in sheep,
with a mortality rate of 3.1% in ewes and 1.3% in lambs (12). Listerial meningoencephalitis was diagnosed in early-lambing housed lambs during February and March (6). The frequency of isolation of L. monocytogenes from beef has been found to increase during winter (9).

In the Kingdom of Saudi Arabia, sheep constitute an integral part of the animal population and are raised principally by private owners for meat production. In this paper, the authors report the first outbreak of ovine listeriosis from Saudi Arabia. A case of bacteraemia and meningitis in a male infant, caused by L. monocytogenes, was also recently reported from this country (2).

Materials and methods

Herd history
The outbreak occurred in Al-Khafgi in north-eastern Saudi Arabia, on the coast of the Arabian Gulf. The town lies on a plain with easterly and north-easterly winds. During winter (November to January), an average temperature of 12°C-14°C, a relative humidity (RH) of 65%-70% and an average rainfall of 150 mm-200 mm are recorded in this area. Climatic data for the other seasons is as follows: average temperature of 20°C-22°C in spring, 30°C-32°C in summer, and 28°C-30°C in autumn. Relative humidity ranges between 55% and 65% in spring and autumn, and between 45% and 55% in summer. Rainfall averages 30 mm-50 mm in spring, 10 mm-30 mm in autumn, and no rain falls during the summer.

Between November and December 1999, a flock of 2,100 Naimi sheep in Al-Khafgi showed signs of a disease outbreak. The animals were grazed during the day, kept in pens and provided with grass rolls during the night.

The history of illness included general weakness, respiratory distress and death within one week of the onset of clinical signs. The disease was observed to affect adult animals, mostly pregnant ewes with a morbidity rate of 7.1% and mortality rate of 2.4%. Two representative pregnant ewes were brought to the Veterinary Teaching Hospital, College of Veterinary Medicine, King Faisal University, for medical investigations.

Clinical examination
The two sheep were subjected to thorough clinical examination, including general clinical examination, recording of temperature, pulse rate and respiratory rate.

Pathology
Post-mortem examinations were conducted on the two animals following routine procedures. Tissue samples for histopathology were collected in 10% formol-saline. Paraffin sections were then prepared and stained with haematoxylin and eosin.

Bacteriological investigations
Samples for bacteriological examination were collected from uterine and foetal tissues, conjunctiva, lungs, liver and brain, from two sheep.

The specimens were cultured onto blood agar and MacConkey's agar. Brain tissues were inoculated directly onto McBride's medium or after cold enrichment in brain heart infusion broth at 4°C for 4 weeks. Biochemical tests were performed using a commercial system. The tests used are shown in Table I. Identification of isolates was based on descriptions in monographs (17).

Antimicrobial sensitivity tests of the isolates were performed using twelve antibacterial agents. The results were interpreted as susceptible (S), intermediate (I) or resistant (R), as described in laboratory manuals (3).

Disease management
A total of 99 infected sheep were isolated in separate pens for treatment; 25 were diagnosed as severely-ill and 74 as mildly-ill. A long-acting oxytetracycline (20%) was administered intramuscularly (i.m.) in two doses of 5 ml each. Severely-ill animals received a third dose of 5 ml together with supportive treatment including multivitamins and minerals. All animals were also given an antipyretic (4 ml dipyrone, benzyl alcohol excipient, i.m.). The animals were observed closely for apparent clinical signs and various clinical parameters were recorded, including temperature, pulse and respiratory rate, for treatment follow-up. The owner was advised of the hygienic measures to be adopted to control the outbreak.

Results

Clinical findings
Clinical signs recorded in the affected sheep included anorexia, congested mucous membranes, a rise in temperature
(41°C-42°C), tachycardia, stertorous breathing with productive cough, mucosal nasal discharge, ruminal stasis and hard faeces. The neurological signs included head pressing, compulsive circling, asymmetric jaw closure with dropped ears and nystagmus. Bilateral keratoconjunctivitis was also observed. In the terminal stages, coma and convulsions occurred, followed by death within four days, despite the comprehensive medical approach. Based on clinical and epidemiological features, the herd was suspected to be affected by listeriosis.

**Bacteriological findings**

From the brain tissue of both sheep and the conjunctival specimen and lung of one sheep, an isolate was recovered. After 24 h incubation onto blood agar, smooth round glistening colonies with a narrow zone of β-haemolysis were obtained. Growth was achieved on MacConkey's agar and on McBride's selective medium after direct inoculation or after cold enrichment. Gram-stained smears revealed small, slender Gram-positive rods, which were either single or in pairs and were motile. Confirmatory test results using the commercial Listeria system are displayed in Table I. Based on these results, the isolate was identified as *L. monocytogenes*. No other significant pathogens were recovered from the specimens.

The antimicrobial susceptibility test demonstrated that the isolate was fully susceptible to oxytetracycline, ampicillin, penicillin and erythromycin, intermediately susceptible to gentamicin, flumequine and neomycin and resistant to oxacillin, amoxicillin, nalidixic acid, nitrofurantoin and streptomycin.

**Pathological findings**

Post-mortem examination revealed bilateral keratoconjunctivitis with the presence of localised fibrous tags on the ulcerated cornea. The lungs showed multiple areas of haemorrhage. Marked congestion and turbid meningeal oedema were observed.

Microscopically, acute lymphadenitis was observed, characterised by massive neutrophil infiltration in the subcapsular spaces as well as in the peri- and intrafollicular lymphoid tissue (Fig. 1). The spleen appeared congested with marked haemosiderosis and depletion of white pulp (Fig. 2). Keratoconjunctivitis was observed in both animals. Desquamation of corneal epithelium with inflammatory cell infiltration predominantly of neutrophils, and corneal vascularisation indicative of deep interstitial keratitis were noted (Fig. 3). Conjunctival infiltration with neutrophils and mononuclear cells, and the presence of inflammatory cells in the anterior chamber were also observed (Fig. 4). Brain sections showed marked meningeal congestion, cerebral haemorrhages, perivascular lymphocytic cuffs and liquefactive necrosis with gitter cell aggregation in the subependymal layer of the brain ventricles (Figs 5-7). The liver showed vascular degeneration and lungs were congested with areas of alveolar haemorrhage.

The mildly-ill animals (*n* = 74) started to improve after 5 days of treatment and completely recovered in 10 days, with normal clinical data. After 17 days, some of the severely-ill animals (*n* = 25) started to improve, with some relief of clinical signs. Examination after 4 weeks revealed complete recovery.

**Discussion**

The authors have presented the first report from Saudi Arabia of an outbreak of septicaemic listeriosis in sheep caused by *L. monocytogenes*. Listeriosis is important both economically and from a public health point of view (12, 15). In the present report, the disease was observed to affect only adult sheep, and particularly pregnant animals. However, no abortions were recorded among the infected ewes. The organism was not isolated from the uterus, foetal tissues or placenta. Pregnancy may have acted as a stress factor and predisposed animals to infection. Other studies have reported that encephalitis and abortion rarely occur in the same outbreak, even if the outbreak involves pregnant sheep (14).

*L. monocytogenes* was isolated in the present study from brain tissues, lungs and conjunctiva of the ewes. The organism was isolated readily from brain tissues by plating directly onto blood agar and McBride’s medium or after the cold enrichment technique. The latter has the disadvantage of being a lengthy procedure which can continue for several months and is prone to environmental contamination (7). Hence, in the opinion of the authors, if satisfactory results are obtainable by direct inoculation onto common or selective media, the cold enrichment technique is not necessary. In this study, the recovery rate of *L. monocytogenes* from the brain was 100%. This is in agreement with the findings of other authors (18).

The pathological findings in this study revealed a septicaemic infection and haemorrhages of the internal organs, with a possible haematogenous dissemination. Signs of acute or subacute inflammation were observed. In the brain, meningoencephalitis, perivascular lymphocytic cuffs and micro-granuloma formation were noted. Such lesions are pathognomonic of listerial encephalitis, as described in other investigations of listeriosis in ruminants and humans (8, 10). The organism might have been acquired through food, as *L. monocytogenes* is widespread in the environment (19). Any cuts in the mucous membranes of the buccal cavity may enable the organism to reach the dental terminals of the trigeminal nerve and then the brain. Experimental work in sheep demonstrated that inoculation of *L. monocytogenes* into the teeth pulp cavity produced encephalitis characteristic of listerial encephalitis (1). Bilateral keratoconjunctivitis and opacity were observed, in concordance with other reports of listeriosis (14).

In the present case, ascending ophthalmic infection from the mouth might have occurred during mastication and regurgitation.
Septicaemia is not an uncommon clinical entity in listeriosis, as reported by other studies (11, 21). A high infective dose of the pathogen may counteract the early defence mechanisms of the body, complement bactericidal effect and phagocytosis by macrophages and polymorphonuclear leucocytes. In the present outbreak, this possibly led to dissemination of the infection within the body. Moreover, haemorrhages observed in the internal organs may be the result of toxins released by the organism. Haemolysins secreted by L. monocytogenes have been demonstrated to be important virulence factors correlating with the ability of the bacteria to avoid host defence mechanisms (13).
The ability of *L. monocytogenes* to grow at temperatures as low as 4°C allows the species to multiply and establish infection when many other bacterial species are unable to grow. The present outbreak occurred during winter, when in this part of the country the temperature is relatively low (10°C-21°C), RH is 65%-70% and average rainfall is 150 mm-200 mm; this might have created favourable conditions for contamination of the sheep fodder by the organism. However, the animal feed was not available for bacterial isolation. Many investigators have reported a high incidence of listeriosis during the winter months and in association with silage feeding (6, 9, 12, 20).

Further studies are required in the future to try to isolate *L. monocytogenes* from ruminant foodstuffs and ruminant products such as milk, to monitor the extent of contamination in different seasons. This would outline the basic epidemiological features of the disease in this region.

The hygienic and husbandry measures and the treatment regimen which was followed were successful in bringing the outbreak under control. The isolated strain was highly sensitive to oxytetracyclin which proved to be effective for treatment and prophylaxis. The acute and subacute course of the infection and the prompt intervention assisted in the successful treatment.

The zoonotic nature of listeriosis has been emphasised, and transmission of infection to humans may occur through food originating from carrier animals (16). In a report of bacteraemia and meningitis caused by *L. monocytogenes* in a newborn in Saudi Arabia, it was suggested that the child might have contracted infection from the mother, although maternal blood cultures were negative. Enquiries regarding the possibility that food was the cause of the infection in the mother were not conclusive, but this possibility does exist (2). Further listerial contamination of the food chain may be expected from infected mutton. Studies in this area would be valuable for public health.

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**Premier foyer signalé de listériose septicémique ovine en Arabie saoudite**

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**Résumé**

Malgré la survenue récente d'un cas de listériose humaine en Arabie saoudite, la maladie n'avait pas encore été constatée chez des animaux. Les auteurs décrivent un foyer de listériose septicémique affectant des ovins, qui s'est déclaré pendant l'hiver. Les animaux adultes et les brebis gravides ont été les plus atteints, avec un taux de morbidité de 7,1 % et un taux de mortalité de 2,4 % ; aucun avortement n'a été enregistré pendant l'épidémie. Les signes cliniques étaient les suivants : inappétence, asthénie, hyperthermie, troubles respiratoires, kérato-conjonctivite et marche en cercles de manière impulsive. Des cultures pures de *Listeria monocytogenes* ont été isolées dans des prélèvements provenant d'ovins atteints. Les examens anatomo-pathologiques ont permis de conclure à une listériose septicémique avec encéphalite. L'adoption de mesures d'hygiène et l'administration d'antibiotiques ont été efficaces pour traiter le reste de l'élevage. Les différents aspects de ce foyer et ses conséquences sur la santé publique font l'objet de la discussion.

**Mots-clés**

Arabie saoudite - *Listeria monocytogenes* - Listériose septicémique - Ovins.
Primer informe de un brote de listeriosis septicémica ovina en Arabia Saudí

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Resumen
Aunque se ha descrito recientemente un caso de listeriosis humana en Arabia Saudí, hasta la fecha no se había informado de ningún caso en animales. Los autores describen un brote de listeriosis septicémica en ovejas que se declaró en invierno y afectó sobre todo a animales adultos y hembras grávidas, con una tasa de morbilidad del 7,1% y de mortalidad del 2,4%. Durante el brote no se registró ningún aborto. Los signos clínicos observados fueron pérdida del apetito, debilidad, fiebre, dificultades respiratorias, queratoconjuntivitis y andares compulsivos en círculo. A partir de las ovejas afectadas se aislaron cultivos puros de Listeria monocytogenes. Los datos patológicos corroboraron que se trataba de listeriosis septicémica con encefalitis. La aplicación de medidas de higiene y el tratamiento con antibióticos sirvieron para contener el brote en el resto del rebaño. Los autores abordan aspectos diversos del brote y de sus consecuencias en materia de salud pública.

Palabras clave

References


