

The first recorded epidemic of leptospirosis in sheep in Egypt

M.E. Hatem & A. Samir*

Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, P.O. Box 12211, Cairo, Egypt
*Corresponding author: ahmed.samir@cu.edu.eg; ahmedsamir121@hotmail.com

Summary

An outbreak of leptospirosis in sheep is recorded as the first epidemic of ovine leptospirosis in Egypt. The sick animals showed typical symptoms such as fever, jaundice, red urine and abortion, with a fatal course. Acute and convalescent sera were tested for demonstration of seroconversion using the microscopic agglutination test to confirm infection. Long-acting tetracycline was successfully used for treatment.

Keywords

Egypt – Epidemic – Leptospirosis – Ovine – Record – Sheep.

Introduction

Leptospirosis is a re-emerging zoonosis caused by *Leptospira* spp. The natural reservoirs and carriers of leptospires are domestic and wild animals (1). The disease is serious, with public health significance, and is considered a hazard for occupations that involve close contact with animals, such as agricultural and dairy farmers, sewer workers, veterinarians and fishing industry workers (2). Serological evidence of leptospirosis in sheep has been reported by many authors in India (3). In Brazil, the epidemiology of leptospirosis in sheep is complex and the bacterium can infect a large number of animals in the herd (4). The disease may become endemic for a small number of serovars more adapted to sheep. In Egypt, several recent studies have confirmed the re-emergence of leptospirosis in humans (2, 5, 6) but few (1, 7, 8) have reported on the surveillance of leptospirosis in animals. One report in farm animals over 20 years ago found that 4.2% of sheep were seropositive for *Leptospira* spp. (8), whereas in a recent study (1) none of the collected ovine sera was seroreactive. In this paper, a recent outbreak of leptospirosis is documented on a sheep farm in Northern Egypt.

Materials and methods

In December 2012 (wet season), a sheep farm in Northern Egypt on the Cairo–Alexandria road near Alexandria, with

1,500 ewes and 500 suckling lambs, had 45 cases (2.3%) of leptospiral infection. The animals suffered from fever, were off their feed, and showed icteric symptoms and red urine.

Blood samples were collected from the 45 cases during the first week of clinical signs (acute phase), and sera were used in the microscopic agglutination test (MAT). Some of the sick animals had received medication before the blood samples were collected. A further 20 blood samples were taken as controls from animals in the same flock that showed no clinical signs. Veterinarians at the farm started treatment at the onset of clinical signs with a full dose of Pen & Strep (Norbrook, Ireland), a combination of procaine penicillin (dose: 8 mg/kg bodyweight) and dihydrostreptomycin sulphate (dose: 10 mg/kg bodyweight), and then changed the medication to the long-acting tetracycline Alamycin 300 (Norbrook, Ireland), dose: 20 mg/kg bodyweight.

The MAT was performed according to Cole *et al.* (9), using 12 serovars of the genus *Leptospira* previously isolated in Egypt as antigens (1, 2, 5, 10). The serovars used were Alexi, Australis, Bataviae, Bratislava, Canicola, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogenes and Wolfii. Briefly, live suspensions of leptospires representing the 12 serovars were added to serially diluted serum specimens in 96-well microtitre plates and incubated at an ambient temperature for 1.5 h. Agglutination was examined using dark-field microscopy at 100 × magnification. Titres were calculated as the highest serum dilution that agglutinated at least 50% of the

Table I
Titres of two serovars of *Leptospira* spp. in acute and convalescent serum samples ($n = 45$) showing seroconversion (rising titres)

No. of serum specimens	Acute sera		Convalescent sera	
	Icterohaemorrhagiae	Pomona	Icterohaemorrhagiae	Pomona
8	1:400	Nil	1:6,400	Nil
15	1:800	Nil	1:6,400	Nil
10	1:800	Nil	1:12,800	Nil
2	1:800	1:200	1:3,200	1:800
6	1:800	1:200	1:6,400	1:1,600
4	1:800	1:400	1:6,400	1:3,200

leptospire for each serovar used. Follow-up serum samples (convalescent stage) were obtained two weeks later and tested for seroconversion.

Isolation of leptospire was attempted by inoculating each sample of body fluids (blood, milk, urine) and uterine discharges into three or four tubes of Ellinghausen McCullough Johnson Harris (EMJH) liquid medium. The EMJH base (BD, USA) was prepared and autoclaved, then EMJH enrichment (BD, USA) and 5-fluorouracil (200 µg/ml; Sigma Chemical Co., St. Louis, MO) were added aseptically (1). All cultures were incubated at 30°C for up to 12 weeks and examined weekly by dark-field microscopy.

Results

The MAT results (Table I) showed that all the sera from the acute cases were reactive with the Icterohaemorrhagiae serovar (titres 1:400 to 1:800) and some also showed reactivity with the Pomona serovar (titres 1:200 to 1:400). Sera from the convalescent cases reacted with the same two serovars: Icterohaemorrhagiae (titres 1:1,600 to 1:12,800) and Pomona (titres 1:800 to 1:6,400). The 20 control sera showed no seroreactivity. The isolation of leptospire from blood, urine, milk and uterine discharges in the affected cases was unsuccessful (12 weeks post-culture).

Discussion

An outbreak of leptospirosis in sheep was recorded for the first time in Northern Egypt. The animals were suffering from typical symptoms and infection was confirmed by the demonstration of seroconversion using MAT. Inspection at the affected farm identified brown rats (*Rattus norvegicus*) in the feed store area (silage store); these animals are a possible source of infection, as confirmed in a recent study in the Delta region in Egypt (1), where brown rats have been captured, bled and found to be an important source

for transmitting leptospire to humans and other animal species.

Although treatment with the full dose of Pen & Strep was initiated at the onset of clinical signs, the disease was not controlled and new clinical cases appeared (a total of 45 cases, among which four ewes aborted); in total, 26 of the 1,500 ewes died (1.7%). The medication strategy was changed to long-acting tetracycline, which helped in the recovery of the sick animals and in the prevention of new cases. Tetracycline is recommended (2) as an anti-leptospiral drug; however, it is unclear why the penicillin/streptomycin combination failed, as it is considered an effective treatment (6).

Isolation of the pathogen was unsuccessful (12 weeks post-inoculation), although some of the animals had started treatment before sample collection and this might have affected the isolation process. Some culture tubes showed variable rates of contamination despite the addition of a selective supplement to the media and this may be due to the nature of the samples: urine, milk and uterine discharges after abortions. Failure to recover leptospire does not eliminate the possibility that the animal is infected, it merely indicates that the animal was not excreting detectable numbers of leptospire at the time of testing (11).

Although clinical leptospirosis is uncommon in sheep, both sporadic and epidemic disease can occur (12). This report is of an outbreak of leptospirosis among sheep in Egypt in which the animals showed typical clinical presentation. The diagnosis was confirmed in the laboratory using the gold standard serological test (MAT), which has been proven to detect rising titres (seroconversion) of antibodies in serum samples from clinical cases. The epidemic disappeared after appropriate treatment was initiated.

Première épidémie de leptospirose ovine enregistrée en Égypte

M.E. Hatem & A. Samir

Résumé

Un épisode de leptospirose affectant les moutons a été signalé en Égypte. Il s'agit de la première épidémie de leptospirose ovine enregistrée dans ce pays. Les animaux atteints présentaient les signes caractéristiques de la maladie, à savoir de la fièvre, une jaunisse, une hématurie et des avortements, suivis de la mort. Des sérums prélevés en phase aiguë et convalescente ont été soumis à un test d'agglutination microscopique afin de confirmer l'infection par la mise en évidence d'anticorps. La tétracycline à action prolongée a été administrée avec succès pour traiter les animaux atteints.

Mots-clés

Égypte – Épidémie – Leptospirose – Mouton – Notification – Ovin.



Primera epidemia registrada en Egipto de leptospirosis en ovejas

M.E. Hatem & A. Samir

Resumen

Un brote de leptospirosis que afectó a las ovejas constituye la primera epidemia de leptospirosis ovina registrada en Egipto. Los animales enfermos exhibían síntomas típicos de la infección, como fiebre, ictericia, hematuria y aborto, con evolución mortal. Se analizó el suero de animales en fase de infección aguda y animales convalecientes para evidenciar una posible conversión sérica, utilizando la prueba de aglutinación microscópica para confirmar la infección. Se administró con resultados positivos un tratamiento a base de tetraciclina de acción prolongada.

Palabras clave

Egipto – Epidemia – Leptospirosis – Oveja – Ovino – Registro.



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