

## The first recorded epidemic of leptospirosis in sheep in Egypt

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### 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 imply 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, on the Cairo–Alexandria road, near Alexandria.

## **Materials and methods**

In December 2012 (wet season), a sheep farm in Northern Egypt on the Cairo–Alexandria road, 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 leptospire 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  $\times 100$  magnification. Titres were calculated as the highest serum dilution that agglutinated at least 50% of the 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, United States [USA]) was prepared and autoclaved, then EMJH enrichment (BD, USA) and 5-fluorouracil (200  $\mu\text{g}/\text{ml}$ ; 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 transmitting leptospires 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 leptospires does not eliminate the possibility that the animal is infected, it merely indicates that the animal was not excreting detectable numbers of leptospires 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.

## **Conflicts of interest**

Nothing to declare.

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**Table I**  
**Titres of two serovars of *Leptospira* spp. in acute and convalescent serum samples 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
45				