



Short Communication

Diagnosis and control of an outbreak of leptospirosis in goats with reproductive failure

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ABSTRACT

This study presents a Brazilian goat herd with reproductive failure over 2009–2010, in which there were abortions (22/50; 44%), embryonic resorption (6/50; 12%) and neonatal deaths (2/50; 4%). A diagnosis of leptospirosis was made, based on serology (microscopic agglutination test – MAT), bacterial culture, and polymerase chain reaction (PCR). Antibiotic therapy, specific vaccination protocols and changes in management practices were instigated. One year after the outbreak, diagnostic methods were repeated and reproductive performance re-analysed. Soon after the outbreak, 61/125 (48.8%) of the goats were seropositive for *Leptospira*. Pure isolates of *Leptospira* were not obtained, but *Leptospira* PCR testing was positive in 48/50 (96%) urine samples. After 1 year only 4.2% were seropositive and the occurrence of reproductive problems decreased roughly 10-fold, although five goats (10.4%) remained PCR-positive. A broad-based management approach, including serological and molecular diagnostic methods, vaccination, antibiotic treatment, and alteration of some environmental aspects, were critical to the control of this outbreak, thereby minimising subsequent reproductive failures and economic losses.

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Leptospirosis is prevalent in goats worldwide (Dos Santos et al., 2012) and the chronic form with impaired fertility, neonatal deaths and abortions frequently occurs, leading to important economic losses (Lilenbaum et al., 2008). There is a lack of reports regarding the successful control of leptospirosis outbreaks in small ruminants. Recently, molecular diagnostic methods have been employed for epidemiological and diagnostic purposes in goats and sheep (Silva et al., 2007; Lilenbaum et al., 2008). The present study aimed to document the diagnosis (with serological and molecular tools) and control of an outbreak of leptospirosis in one caprine herd with substantial reproductive failure in Rio de Janeiro, Brazil.

From August to October 2009, several reproductive problems, including 22 abortions during late pregnancy (44% of 50 pregnant animals), occurred in a herd of 125 unvaccinated Saanen goats, confirmed free of brucellosis (AGID and 2-mercaptoethanol) and caprine arthritis–encephalitis (AGID) from Rio de Janeiro, Brazil. The herd reproductive history also included embryonic resorption (6/50; 12%; detected by ultrasonography) and neonatal deaths (2/50; 4%). Necropsy of aborted fetuses showed jaundice, as well as petechiae in the liver and kidneys. The majority (68.2%) of aborted fetuses were macerated. No other clinical signs were

observed besides the reproductive problems. Goats were kept in a stable (intensive breed system) without rodent control.

Over 7–14 days after the abortions, blood samples were collected from all animals. Urine samples were collected from 50 inseminated animals and all samples were transported at room temperature to the laboratory. Urine was chilled and transported to the laboratory for PCR. Blood samples were examined for *Leptospira* antibodies by a microscopic agglutination test (MAT; Lilenbaum et al., 2008). The antigens used were a panel of 24 strains (representing all described serogroups) of live *Leptospira* grown in EMJH. MAT has a sensitivity of 95% and specificity range of 89.0–90.0% (Hernández-Rodríguez et al., 2011).

Samples were seeded for bacteriological culture using a serial dilution technique, incubated at 28–30 °C and examined once weekly for 20 weeks (Lilenbaum et al., 2008). PCR was performed according to Lilenbaum et al. (2008) using primers designed by Stoddard et al. (2009). Despite of the reported high sensitivity and specificity of the assay, each urine sample was tested three times.

Soon after the outbreak, 61/125 (48.8%) serum samples were reactive (titres ≥ 200), mainly to serovar *icterohaemorrhagiae* (65.8%), followed by *hardjo* and *bratislava*, (17.1% each). A large proportion of the reactive sera (31/61; 50.8%) had high titres (≥ 800), whereas 19/61 sera (31.1%) had titres of 400 and 11/61 sera (18.1%) had titres of 200. Although 48/50 goats from which

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Table 1

Diagnostic methods and reproductive problems identified in August 2009 and 1 year later (after broad intervention) in an outbreak of leptospirosis in a goat herd in Rio de Janeiro, Brazil.

	August 2009 n (%)	August 2010 n (%)
<i>Diagnostic methods</i>		
Seropositive	61/125 (48.8%)	5/119 (4.2%)
Culture positive (urine)	0	0
PCR positive (urine)	48/50 (96.0%)	5/48 (10.4%)
<i>Reproductive problems</i>		
Abortions	22/50 (44.0%)	2/48 (4.1%)
Embryonic resorption	6/50 (12.0%)	1/48 (2.1%)
Neonatal death	2/50 (4.0%)	0

urine samples were collected were seropositive, pure isolates of *Leptospira* were not obtained from urine. Nevertheless, PCR was positive in 48/50 (96%) urine samples. The PCR-positive animals had titres of 200, 400, and ≥ 800 (20, 12, and 15 animals, respectively) and only one goat was seronegative. All 22 goats that had aborted tested PCR positive.

All goats were treated with a single dose (25 mg/kg) of dihydrostreptomycin (Ourofino) and vaccinated using an inactivated commercial vaccine against the serovars *bataviae*, *canicola*, *copenhageni*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae*, *pomona*, *pyrogenes*, *tarassovi* e *wolffi* (Leptoven 10, Vencofarma), with a booster dose after 60 days. Environmental measures were also adopted, which mainly consisted of preventing access to flooded areas. Additionally, an extensive rodent control program was implemented, including traps, poisoning, and minimising access to the feed storage room.

In August 2010, serology was performed in 119 goats, and 48 urine samples were collected from inseminated goats for bacteriological culture and PCR. It is noteworthy that six goats were culled after the outbreak and no animals were replaced in the herd. From the 119 serum samples, five (4.2%) were seropositive, four with low titres (200), and one with titre of 400 (all against serovar *icterohaemorrhagiae*). In the year after corrective measures were taken, abortions decreased from 44% to 4.1%, embryonic death from 12% to 2.1%, and neonatal deaths from 4% to 0%. Aborted fetuses did not have signs suggestive of leptospirosis. No leptospire were recovered from the 48 urine samples, although five goats (10.4%) remained positive by PCR (Table 1).

Serovar *icterohaemorrhagiae* was by far the most frequent serovar and was considered the infective agent. Incidental serovars are reported as the most frequent leptospiral agents in goats (Dos Santos et al., 2012; Suepaul et al., 2011), including members of *icterohaemorrhagiae* serogroup (Leon-Vizcaino et al., 1987).

The herd was brucellosis- and CAE-free, which is noteworthy since these are important infectious diseases in small ruminants in the studied area that could most likely contribute to the high level of reproductive failures (Martins et al., 2011). Additionally, based on the high level of seroreactivity and leptospiral carriers (PCR), it was possible to infer that leptospirosis was the cause of the abortions.

The concordance between serology and PCR was very high. This was unusual, since serology is reported to have diagnostic limitations on an individual animal level (Hernández-Rodríguez et al., 2011), and is not reliable for the detection of carriers (Lilenbaum et al., 2008). Discrepancies among methods are particularly

common in chronic or subclinical leptospirosis. In the present study, the high concordance among the tests used was attributed to the collection of samples during the acute phase of the outbreak (Leon-Vizcaino et al., 1987).

A multifaceted management regimen, including vaccination and antibiotic therapy, is ideal for the control of leptospirosis in ruminants (Grooms, 2006), and the reduction in reproductive failure and the bacteriological cure of the infected animals (all except for five that remained PCR-positive) in this study reinforce this principle. Additionally, environmental measures are considered crucial for control of leptospirosis in ruminants (Martins et al., 2010). Importantly, it must be emphasised that the control of leptospirosis must not be limited to the initial treatment of the outbreak, but should be continued for several years.

In conclusion, a broad-based management approach was critical in the diagnosis and control of this outbreak, thereby minimising subsequent reproductive failures and economic losses.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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