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Risk factors associated with leptospirosis in dairy goats under tropical conditions in Brazil

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Abstract

Serum samples from 248 adult dairy goats from 13 flocks with lowered fertility farmed in the Rio de Janeiro region of Brazil were examined for *Leptospira* antibodies by MAT with 24 serovars, cut off 100. A questionnaire was completed for each herd. Antibodies were detected in 20.8% of these goats, mainly to serovar Hardjo. Risk factors associated with seroprevalence to leptospirosis were the frequency of professional veterinary supervision (OR = 2.35), climate (OR = 2.63) and grazing for more than 2 h a day. Flock factors as size, type of milking and offering of food supplementation, as well as the location and topography, the type of animal housing or the presence of silos did not significantly affect seroprevalence. We suggest that a successful control program for goat leptospirosis should include a complete investigation of herd management practices, which could influence in the occurrence of the infection.
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Dairy goat breeding is an increasing economic activity in Brazil (Cordeiro, 1998). In spite of its large number of animals, of approximately 12 million, Brazil ranks as only 18th in terms of the amount of goat milk produced (FAO, 2004), mainly due to the low per goat milk productivity. Amongst other factors, infectious diseases such as leptospirosis may contribute to this problem, leading to impaired milk production.

Goats are known to be less susceptible to leptospirosis than other domestic species, as cattle (Leon-Vizcaino et al., 1987). Leptospirosis in goats may present in an acute form, with an increase in body temperature, anorexia, depression, jaundice, and anaemic or haemorrhagic syndromes (Faine et al., 2000). Nevertheless, the chronic form with impaired fertility, neonatal deaths, abortions and decreased milk production occurs more frequently, leading to important economic losses (Cunha et al., 1999; Lilenbaum et al., 2007). In several states of Brazil, the disease has been reported since the 1960s (Favero et al., 2002). In Rio de Janeiro state, leptospirosis is considered to be one of the major reproductive infectious diseases. In a recent study, 11.1% of the tested animals showed to be seroreactive, predominantly due to serovar Hardjo (Lilenbaum et al., 2007).

In cattle, leptospiral infection has been classified into two major groups: one consisting of strains adapted to and carried by cattle, such as Hardjo, which are independent of region or rainfall; and a second that consists of incidental infections caused by strains carried by other domestic and free-living animals, which are dependents

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on environmental factors and farm management practices (Ellis, 1984). It has been suggested that the second group could be relatively more important in tropical countries than in other regions. It is also widely accepted that this classification is valid for other species, e.g. sheep and goats.

Under tropical conditions, it has been demonstrated that herd management practices may affect the overall seroprevalence of the disease and the distribution of serovars in cattle (Lilenbaum and Santos, 1996). Factors such as herd size, replacement policy and herd type – beef or dairy – appear to be important contributors (Alonso-Andicoberry et al., 2001). In Brazil, previous studies (Lilenbaum and Santos, 1996; Lilenbaum and Souza, 2003) confirmed that herd management has a significant impact not only on the overall seroprevalence of leptospirosis, but also on the serovar distribution, while the geographic distribution of the farms had no significant influence. The aim of this study was to identify specific factors that may be associated to seroreactivity to caprine leptospirosis under tropical conditions.

Rio de Janeiro state has approximately 13,500 goats distributed in 277 flocks. The goats are predominantly Saanen and Toggenburg breeds. Sample size calculations assumed an estimated average prevalence according to previous studies conducted in the same region, an allowable error of 5% and a confidence interval of 95%, according to recommendations (Centro Panamericano de Zoonosis, 1979). Therefore, a minimum of 218 samples should be collected.

Thirteen flocks located in several regions of the state of Rio de Janeiro, in order to significantly represent the whole state, were studied. Flocks comprised 60–350 adult animals. In each area, flocks were chosen randomly and samples were also collected at random from about 20% of the flock. Besides location, the existence of a reliable identification system of the goats and the absence of a vaccination program against leptospirosis were the only inclusion criteria. At the time of sample collection, no information on the history of each goat was provided to avoid sampling bias.

Although it was not an inclusion criterion, all flocks presented a certain level of lowered fertility. The most frequent reproductive problems identified were estrous repetition and low conception rates (all flocks), premature calving (eight flocks) and sporadic abortion (eight flocks). The goats had not been vaccinated for leptospirosis and had been previously tested for brucellosis with negative results. A 48-point questionnaire was completed for each flock. Questions were related to management and sanitary conditions, climate, housing and frequency of veterinary attendance.

Serum samples were obtained from 248 adult (1–5 years old) goats. Blood was collected in Vacutainer tubes from the jugular vein of each goat. Samples were chilled and transported to the laboratory where they were centrifuged at 1000*g* for 10 min. Serum was stored in 1.5 mL Eppendorf tubes and stored at −20 °C for batch testing.

Samples were examined for *Leptospira* antibodies by the microscopic agglutination test (MAT), using live antigens grown in liquid medium (EMJH) free of contamination or self-agglutination. A complete panel of 24 strains of live *Leptospira* were used as antigens *L. biflexa* serovars Andamana and Patoc; *L. interrogans* serovars Australis, Autumnalis, Bataviae, Bratislava, Canicola, Copenhageni, Icterohaemorrhagiae, Pomona, Sentot and Wolffi; *L. borgpetersenii* serovars Castellonis, Hardjo, Hebdomadis, Javanica, Pyrogenes, Tarassovi and Whitcombi; *L. kirschneri* serovars Butembo, Cynopteri and Grippotyphosa; *L. noguchi* serovar Panama and *L. santarosai* serovar Shermani. Samples that showed agglutinating activity at 1:50 dilution had the antibody titres further tested by twofold serial dilutions. Samples were considered as reactive when agglutination was evident on a 1:100 dilution. The antigen with the highest titre was considered to be the infective serovar (Faine et al., 2000).

Data was analyzed using the statistics software package SPSS version 8.0 (Statistical Package for the Social Science, SPSS Inc., Chicago, 1998). First bivariate analysis was used to test for simple associations between each independent variable. The *t*-test was used for continuous variables and the chi-square test was used for categorical variables. Identification of a risk factor required confidence level of 95% ($p < 0.05$) as well as a biologically plausible association between the factor and seroreactivity to leptospirosis. The variable was then selected for further analysis in step 2, a logistic regression (Frankena and Graat, 1997; Hosmer and Lemeshow, 1989; Lemeshow and Hosmer, 1984).

The logistic model used to estimate the odds ratio, describe the effects of presence of veterinary assistance and system of production on serological results was: $\mathrm{LOGIT}(Y_{ij}) = b0 + b1(i) + b2(j)$, where Y_{ij} is the outcome variable (serological results –0 = negative and 1 = positive), "*i*" is veterinary assistance ($i = 1$ (frequently), 2 (absence or sporadic)) and "*j*" represents the system of production ($j = 1$ (intensive), 2 (semi-intensive or extensive).

A total of 52 positive reactions were observed, corresponding to 20.9% of the samples. The most prevalent reactions, which could not be attributed to cross reactions, were to serovars Hardjo (36.5%), Shermani (30.8%), Icterohaemorrhagiae (9.6%), Grippotyphosa (9.6%), Autumnalis (5.8%), Castellonis (3.8%) and Bratislava (3.8%), according to Table 1. The rate of seroreactivity ranged from 10.5% to 46.4% of the examined group. Goats are not a maintenance population for Hardjo; the infection occurs due to cattle contact, since the bovine is the maintenance for this serovar (Schollum and Blackmore, 1981). The rate prevalence of 20.9% and predominance of Hardjo were not unexpected for this population and are consistent with recent reports of seroprevalence of leptospirosis in Brazil, either on goats (Favero et al., 2002) or in bovines (Lilenbaum and Santos, 1996). It is higher than the 11.1% that was recently reported in the same region (Lilenbaum et al., 2007). Nevertheless, instead of a large epidemiological survey, the present study examined flocks with

Table 1
Prevalence of *Leptospira* serovars in dairy goats from Rio de Janeiro, Brazil

Serovar	No. of samples	Reactives (%)	Total (%)
Hardjo	19	36.5	7.7
Shermani	16	30.8	6.4
Grippotyphosa	5	9.6	2.0
Icterohaemorrhagiae	5	9.6	2.0
Autumnalis	3	5.8	1.2
Castellonis	2	3.8	0.8
Bratislava	2	3.8	0.8
Sub-total	52	100	20.9
Non-reactives	196	–	79.1
Total	248	–	–

different levels of impaired fertility, what increased the seroreactivity rate, although not altering the serovar distribution.

Environmental factors, such as location and topography, as well as the type of animal housing or the presence of silos did not significantly affect seroprevalence. This finding indicates that no flock is condemned to be infected with *Leptospira* as a direct consequence of geographic location, and that control of leptospirosis does not necessarily need high investments in stables or silos. This finding may be particularly important in developing countries where goat breeding is beginning to develop, since it demonstrates that the use of adequate management practices, rather than high big funds apports, is crucial for the sanity of the flock. Breed data were not analyzed since the majority of the flocks were composed by Saanen animals. Findings are very similar to those observed in cattle, since it has been demonstrated (Lilenbaum and Santos, 1996; Lilenbaum and Souza, 2003) that in dairy cow breeding location of the herd does not significantly interfere, while management system has a significant impact both in the seroprevalence and in the distribution of the serovars.

Nevertheless, climate was significantly associated as a risk factor to seroreactivity to leptospirosis. Animals from flocks in tropical climate have 2.63 (odd ratio) more chances to be seroreactive than animals from flocks in temperate climate, which can be found in the mountain region of the state. Heat stress has been clearly demonstrated as an important factor associated to welfare and reproductive performance of goats in tropics (Devendra and Burns, 1983). Since Saanen is a goat breed from Swiss origin, this finding probably reflects the better adaptation of those animals to this climate. This finding can also be due to the rain precipitation, an environmental factor that has already been associated to goat leptospirosis in a tropical region of Northeast Brazil (Alves et al., 1996) and is well recognized as an important climatic factor associated to leptospirosis (Faine et al., 2000).

Surprisingly some herd factors as herd size, type of milking (milking machine or manual) and offering of food supplementation did not significantly affect leptospirosis seroprevalence. Herd size was reported to be not associated to bovine leptospirosis in Spain (Alonso-Andicoberry et al., 2001), but seems to be an important risk factor in sows from Viet Nam (Boqvist et al., 2002).

A clear association was demonstrated by chi-square test between the occurrence of abortions and seroreactivity to leptospirosis in the studied goats. It is not an unexpected finding. It is well established that leptospirosis in goats determines important reproductive problems, being abortion the most visible symptom of the syndrome (Faine et al., 2000). In spite of this clear association, abortion was not included in the logistic regression analysis since it was considered more as a consequence of the leptospiric infection rather than an associate risk factor itself.

Another possible factor associated to the occurrence of seroreactivity to leptospirosis was the grazing for more than 2 h a day. Although logistical regression analysis showed this aspect to be not significant, the chi-square test demonstrated a clear association ($p < 0.01$) between grazing and reactivity to serological tests. Animals that are allowed to graze showed mean seroreactivity rates of 28.0%, while for animals confined to stables the rate was 12.9%. It is important to consider that leptospirosis in goats depends to a great extent on the possibilities of contact with leptospires in the environment. Thus, animals that graze present a higher risk of environmental infection. This finding agrees to that observed for cattle in Spain (Alonso-Andicoberry et al., 2001), where grazing was also observed to be strongly associated to the leptospiric infection.

An important risk factor significantly associated to seroprevalence to leptospirosis was the frequency of professional veterinary supervision (OR = 2.35). Herds under frequent veterinary supervision or visited at least every two weeks showed mean seroreactivity rates of 12.4%. On the other hand, herds visited infrequently by a veterinarian and those without any veterinary assistance showed mean seroreactivity rate of 25.8%. Interestingly, less than 15% of the veterinarians involved in goat breeding were concerned and warned about leptospirosis in the flock. This means that, even when the veterinarian is not directly involved in a leptospirosis control program, his presence is responsible for sanitary and management practices that indirectly improve the conditions of the animals, resulting in an overall decrease in the incidence of parasitic and infectious diseases, including leptospirosis. This is a very important finding and had been previously demonstrated for cattle breeding in the same region (Lilenbaum and Souza, 2003).

In conclusion, this study demonstrates that, under tropical conditions, a successful leptospirosis control program, in addition to vaccinations and chemotherapy, should include an investigation of flock management practices to try to identify factors that are likely to influence the prevalence of the disease. Substantial investment in stables is not necessary, but the use of adequate management practices, as well as the presence of veterinary assistance, is mandatory for the sanity of the flock.

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