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A serological study on *Brucella abortus*, caprine arthritis–encephalitis virus and *Leptospira* in dairy goats in Rio de Janeiro, Brazil

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Abstract

In spite of the large number of goats found in several developing tropical countries, milk production remains unsatisfactory. The occurrence of infectious diseases, such as leptospirosis, brucellosis and caprine arthritis–encephalitis (CAE) may in part be responsible for sub-optimal production. In this study, 1000 serum samples were tested for leptospirosis, 953 for brucellosis and 562 for CAE. All tested flocks presented at least one seroreactive animal for leptospirosis and for CAE. Reactivity to leptospirosis was 11.1%, and serovar *hardjo* was the most frequently found. Anti-*B. abortus* agglutinins were found in 0.5% of the samples presented and 14.1% were seroreactive to CAE. Leptospirosis was considered to represent the major infectious problem in the studied goat flocks. The occurrence of infectious diseases in the tested flocks may represent an important factor contributing to the decreased productivity of the animals. These findings may be similar to those observed in other developing countries and require further study to define the relationship between seropositivity and reduced production.

Keywords: Leptospirosis; Brucellosis; CAE; Goats; Reproduction

1. Introduction

Dairy goat breeding is an increasing important economic activity in Brazil (Cordeiro, 1998). According to Devendra (1990), approximately 95% of all goats world-wide are located in developing countries, yielding some 76% of total goat milk production. Despite a population of approximately 12 million goats, Brazil ranks as only 18th in terms of the amount of goat milk produced (FAO, 2004), mainly due to the low milk productivity per goat. Amongst other factors, infectious diseases such as leptospirosis, brucellosis or caprine arthritis-encephalitis (CAE) may contribute to this problem, leading to impaired milk production.

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 output, occurs more frequently, leading to important economic losses (Faine et al., 2000). In several states of Brazil, the disease has been reported since the 1960s (Favero et al., 2002).

Although the most common agent of caprine brucellosis, *Brucella melitensis*, has never been isolated in Brazil, infection in goats due to *Brucella abortus* occurs sporadically and may represent a source of economic losses and a public health hazard (Alton et al., 1988; Poester et al., 2002). As in cattle, the disease is characterised by late abortion, stillbirths, decreased fertility and low milk

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production. It has been sporadically reported in several Brazilian states (Alves et al., 1997). Diagnosis follows official recommendations for bovine brucellosis control, and is mainly based on serological tests (Office International des Epizooties, 2004).

Caprine arthritis-encephalitis (CAE) is a debilitating and progressive disease caused by a lentivirus (CAEV) belonging to the *Retroviridae* family. Its clinical manifestations can include chronic synovitis and arthritis, demyelinising encephalitis, chronic interstitial pneumonia and indurative mastitis, which greatly reduces milk production (Callado et al., 2001). In Brazil, the disease has been reported since the 1980s (Hötzel et al., 1993), but few serological surveys have been conducted, and breeders have only begun to be more concerned about it in recent years. Although diagnosis is mainly performed by such serological tests as enzyme-linked immunosorbent assay (ELISA) and agarose gel immunodiffusion (AGID), a clinical examination based on measuring the difference between the carpo-metacarpal articulation and the metacarpal diameter has been proposed (Monicat, 1987), termed the "Clinical Index" (CI).

The purpose of this report was to gather the results of studies previously conducted by our group, aiming to estimate the seroprevalence of three main infectious diseases in goat flocks in Rio de Janeiro. This information should contribute to the understanding of these diseases and to assist in decision-making about controls, with the goal of increasing the productivity of the animals.

2. Material and methods

2.1. Sampling procedures

Rio de Janeiro has approximately 13,500 goats distributed in 277 flocks. The goats are predominantly Saanen and Toggenburg breeds and are all kept under intensive care for milk production. Sample size assumed an estimated average prevalence according to previous studies conducted in the same region, with an allowable error of 5% and a confidence interval of 95% according to recommendations (Centro Panamericano de Zoonosis, 1979). A minimum of 948 samples for brucellosis and leptospirosis and of 541 samples for CAEV were deemed necessary. During 2003 and 2004, we tested 1000 serum samples from adult animals for leptospirosis, 953 for brucellosis and 562 for CAE, from 48, 46 and 27 flocks, respectively.

Goat flocks were located in several regions of the state of Rio de Janeiro and comprised 60–350 adult animals. Flocks were chosen according to geographical area in order to represent the whole state. In each area, flocks were chosen randomly and samples were also collected at random from about 20% of the herd (minimum of 20 samples per herd). At the time of sample collection, no information on the history of each goat was provided to avoid sampling bias.

Blood was collected in Vacutainer tubes from the jugular vein of each goat. Blood 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.

2.2. Laboratory assay procedures

Leptospirosis was diagnosed using a microscopic agglutination test (MAT), as recommended by OIE (Faine et al., 2000; Office International des Epizooties, 2004). Samples were screened at a 1:100 dilution using a battery of live antigen strains of *Leptospira interrogans* serovars *australis* (Ballico), *bataviae* (Swart), *bratislava* (Jez bratislava), *canicola* (Hond Utrech IV), *grippotyphosa* (Moskva V), *icterohaemorrhagiae* (RGA), *pomona* (Pomona), *pyrogenes* (Salinem) and *wolffi* (3705), and *Leptospira borgpetersenii* serovars *ballum* (Mus 127), *hardjo* (Hardjobovis), *sejroe* (M 84) and *tarassovi* (Perepelicin).

All strains were grown in liquid medium – EMJH for 7–10 days. Samples with agglutinating activity at a 1:100 dilution were subsequently titrated against reacting antigens using serial two-fold dilutions of serum until the highest titre was obtained in order to identify the infective serovar.

For the detection of anti-*B. abortus* agglutinins, the specific 2-mercaptoethanol test (2-ME) was performed following international standards (Alton et al., 1988; Office International des Epizooties, 2004). Antigen was elaborated using strain 1119/3 of heat-inactivated *B. abortus* and interpretation of results followed a ruling of the Brazilian Department of Agriculture (Brasil, 2004). Briefly, 2-ME was considered reactive when complete agglutination was obtained for sera dilutions higher or equal to 1/25. CAEV antibodies were detected using a commercially available agarose gel immunodiffusion (AGID) kit (AGID-CAEV P_{28}, Institute Pourquier – sensitivity 79.7%, specificity 99%).

Tests were carried out in Petri dishes in agarose gels containing seven equidistant holes of the same size (one central and six peripheral). The positive control serum and the recombinant p28 antigen samples were loaded into these wells, and the plates incubated in a humid chamber at 25 °C. Results were read after 96 h. Samples were considered as reactive when a precipitation line appeared between the wells containing the serum and the antigen. The test is, however, only qualitative and so antibody titres were not measured. On the same day as blood collection, the Clinical Index (CI), as described by Monicat (1987), was measured. Forty-eight goats from the same herd randomly chosen were measured, based on the difference between the carpo-metacarpal articulation and the metacarpal diameter, resulting positive when this difference was >7.0 cm.

3. Results

All of the 48 flocks tested for leptospirosis presented at least one seroreactive animal. This was also observed among the flocks tested for CAE, whereas only 3/46 flocks tested for brucellosis presented seroreactive animals (Table 1).

Table 1
Reactive goat sera for brucellosis, leptospirosis and CAE in Rio de Janeiro, Brazil

Flock	No. of samples	Leptospirosis	Brucellosis	CAE
1	21	2	–	2
2	22	2	–	NT
3	20	2	–	1
4	20	2	–	NT
5	20	3	–	2
6	20	2	–	NT
7	22	2	–	2
8	20	4	–	2
9	20	2	2	NT
10	20	2	–	NT
11	20	1	–	NT
12	22	2	–	6
13	20	3	–	1
14	20	2	–	NT
15	20	4	–	NT
16	20	2	–	3
17	20	3	–	2
18	22	2	–	1
19	21	1	–	NT
20	20	4	–	5
21	20	2	2	NT
22	24	4	NT	NT
23	20	3	–	NT
24	20	2	–	2
25	20	2	–	NT
26	20	4	1	NT
27	20	3	–	NT
28	26	4	–	3
29	24	4	–	4
30	20	3	–	3
31	20	2	–	2
32	20	2	–	2
33	22	4	–	NT
34	20	2	–	6
35	20	1	–	4
36	24	2	–	NT
37	23	2	NT	2
38	20	3	–	4
39	20	2	–	NT
40	20	4	–	NT
41	22	3	–	2
42	20	2	–	5
43	20	2	–	4
44	24	4	–	NT
45	20	3	–	6
46	20	3	–	2
47	20	2	–	1
48	21	2	–	NT
Total	1000	111	5	79

NT, not tested.

One hundred and eleven animals were positive for leptospirosis, with an average flock frequency of reactivity of 11.1%, (CI 8.8–13.3%; $P = 0.05$). Serovar *hardjo* was most frequently found in 80 (72.1% of the seroreactive) animals, followed by *wolffi*, *bratislava* and *grippotyphosa*, with 24 (21.6%), five (4.5%) and two (1.8%) reactions, respectively (Table 2). Reactivity was similar in all regions of Rio de Janeiro. Acute cases of clinical leptospirosis, as jaundice, fever or anorexia were not observed.

Table 2
Distribution of goat serum samples seroreactive in the Leptospirosis Microscopic Agglutination Test

Serovar	Titre		Total	% Total
	100	200		
hardjo	68	12	80	72.1
wolffi	24	0	24	21.6
bratislava	5	0	5	4.5
grippotyphosa	2	0	2	1.8
Total	99	12	111	100.0

Of the 953 serum samples tested for anti-*B. abortus* agglutinins, only five (0.5%) were reactive to the 2-ME test indicating a sporadic occurrence of the disease in goat flocks from Rio de Janeiro. All seroreactive animals had a history of nursing with cow milk, but testing and detection of the presumably infected cows was not possible.

In the case of CAE virus (CAEV), 79/562 (14.1%) of samples tested by AGID were seroreactive. The disease seems to be widespread in Rio de Janeiro, since all of the tested flocks presented at least one seroreactive animal. Of the 48 animals in which the CI was identified, 22 were reactive to AGID and 26 presented negative results at the serological test. Of the 22 seroreactive goats, only three presented a CI > 7.0 cm, whereas from the 26 seronegative animals, four presented a CI > 7.0 cm. The sensitivity of the CI in relation to AGID was 13.6% and the specificity 84.6%. Concordance between the tests was poor ($k = 0.057$).

4. Discussion

Although seroreactivity to an organism does not translate into verification that the animal was clinically affected by that organism, the infectious diseases leptospirosis and CAEV seem to be widespread amongst goat flocks from Rio de Janeiro and probably represents an important factor that contributes to the decreased productivity of these animals.

Reactivity to leptospirosis is very high and alarming. Although goats are considered less susceptible to this infection (Leon-Vizcaino et al., 1987), some reports in Brazil demonstrated seroreactivity ranging from 24% to 76% (Cunha et al., 1999). Nevertheless, when only intensively bred animals are considered, rates decrease significantly, ranging from 2.4% to 14.2% (Favero et al., 2002), which agree with the results of the present study conducted in intensively bred animals.

Earlier we demonstrated in cattle that the influence of herd management has a significant impact on the overall seroprevalence of leptospirosis (Lilenbaum and Souza, 2003). We suggest that the adoption of adequate husbandry practices observed in the intensive breeding system may have allowed the reduction of seroreactivity rates when compared to those regions where goats are bred extensively.

Serovar *hardjo* is strongly related antigenically to serovar *wolffi* and cross-reactions may occur (Faine et al., 2000). Therefore, the total amount of seroreactive animals to *hardjo* could be as high as 104 (80 + 24), or 93.7% of the observed reactions. *Hardjo* is a strain adapted to cattle (Faine et al., 2000) and the most frequently described serovar in cattle both worldwide and also in Rio de Janeiro (Lilenbaum and Santos, 1996). Goats do not appear to act as primary reservoirs of *Leptospira* (Faine et al., 2000) and so their infection depends to a great extent on the possibilities of contact with these microorganisms in their natural environment. Since the role of Leptospira in the impaired fertility and milk production in cattle is well recognized (Faine et al., 2000), it is suggested that *hardjo* infection observed may be one of the most important reasons of the reduced productivity of Brazilian goat flocks.

In relation to brucellosis, the seroreactivity observed in the present study agrees with reports from other authors in Brazil (0–2.0%; Poester et al., 2002). Although seroreactivity to *B. melitensis* in Brazil has not been demonstrated in goats, *B. abortus* has not been eradicated from bovine herds, and can infect small ruminants and cause reproductive disease (Alton et al., 1988). Infection by *B. abortus* in goats has been reported in several countries, mainly in developing areas (Leal-Klevezas et al., 2000). Kabagambe et al. (2001) reported that even mixed infections by both *B. abortus* and *B. melitensis* may occur, while Ocholi et al. (2004) recently reported the recovery of *B. abortus* from livestock, including goats, in Nigeria.

The seroreactivity rates to CAEV were extremely high in all of our studied herds. Nevertheless, the overall reactivity of 14.1% is significantly lower than the 21.1% reported previously in the same region by Cunha and Nascimento (1995). This difference reflects the concern of some veterinarians and breeders regarding the disease and its control. Although few herds effectively undergo a control programme based on test-and-culling, an increasing interest has been observed in acquiring tested animals and preventing the uptake of infected colostrum by newborn animals.

The use of the CI for the diagnosis of CAE, even as a complementary method, was considered inadequate. In spite of its low cost, the occurrence of 4/26 (15.4%) of seronegative animals with a CI > 7.0 cm, and 19/22 (86.4%) seroreactive animals with a CI < 7.0 cm revealed a low specificity, as well as a very low sensitivity of the test. We cannot therefore recommend the clinical diagnosis of CAE using the CI.

In the present study, seropositivity to *B. abortus* was shown to occur only sporadically, which agrees with other reports (Poester et al., 2002). We conclude that brucellosis caused by *B. abortus* does not represent a major cause of decreased performance of goat milk production in Brazil. However, other infectious diseases such as CAE and leptospirosis, which are much more frequent and may pose important economic hazards, are not being adequately considered for official control programmes. We believe that those infections need more attention and a more intensive diagnostic and control programme is desirable for increasing goat milk production.

Leptospirosis caused by serovar *hardjo* infection occurs worldwide and is accepted as a major cause of reproductive problems, leading to impaired milking production (Faine et al., 2000). The anti-*Leptospira* seroreactivity observed in the present study indicates that leptospirosis represents a major infectious problem in goat flocks in Rio de Janeiro. Surprisingly, in spite of its importance and prevalence, no official control measures are carried out in Brazil or in other developing countries in order to increase the effectiveness of diagnosis and reduce its occurrence, and the disease remains underdiagnosed in several regions of the developing world, and especially in tropical regions.

We believe that the seroprevalence noted in this study may be similar to that observed in many other regions of Brazil, and possibly elsewhere in the developing world. The relationship between production and seropositivity for each of the organisms should be studied in detail. If economically justified, routine monitoring and the establishment of official control programmes for diseases such as leptospirosis may well result in an increase in the productivity and health of goat herds in affected regions.

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