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ORIGINAL RESEARCH

# Leptospirosis as the most frequent infectious disease impairing productivity in small ruminants in Rio de Janeiro, Brazil

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**Abstract** Despite the importance of small ruminants breeding in developing countries, milk/meat productivity remains unsatisfactory. Infectious diseases, such as leptospirosis, brucellosis, and small ruminant lentiviruses (SRLVs), contribute to this scenario. The objective of the present study was to determine the role of each of these diseases in the productivity of small ruminants breeding in Rio de Janeiro, Brazil. In goats, 343 samples were tested for leptospirosis, 560 for *Brucella abortus*, and 506 for caprine arthritis-encephalitis (CAE), whereas in sheep, 308 samples were tested for leptospirosis, 319 for *B. abortus*, 374 for *Brucella ovis*, and 278 for Maedi-Visna (MV). Regarding leptospirosis, 25.9% of goats and 47.4% sheep were seroreactive, with serovar Hardjo the most prevalent in both species. Anti-*B. abortus* agglutinins were found in 0.7% of all samples, exclusively in goats. In relation to SRLVs, 8.6% of goats and 3.2% of sheep samples were positive for CAE and MV, respectively. Leptospirosis was the major infectious problem in the small ruminants sampled and may contribute to impaired productivity of these animals.

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## Introduction

Small ruminants (sheep and goats) are ubiquitous, with important contributions to the subsistence, economic, and social livelihoods of many humans, particularly in developing countries (Kosgey and Okeyo 2007). According to FAO (2010), approximately 95.7% of all goats and 63.3% of all ewes worldwide are located in developing countries and represent more than 70% of total animal production. Among the various factors that may lead to low productivity in tropical countries, infectious diseases may be very prevalent, impairing milk and meat production (Lilenbaum et al. 2007; Leitner et al. 2010). From those, the most commonly reported are Leptospirosis, Brucellosis (caused by either *Brucella abortus* or *Brucella ovis*), Caprine arthritis-encephalitis (CAE), and Maedi-Visna (MV) (Reina et al. 2009; Bekele et al. 2011; Suepaul et al. 2011). This scenario is barely the same in several developing countries.

Although goats and sheep have been reported as less susceptible to leptospirosis than other domestic farm animal species, e.g. cattle (Leon-Vizcaino et al. 1987), there are recent reports documenting reproductive failure by this agent (Lilenbaum et al. 2009). Leptospirosis in small ruminants may present in an acute form, with increased body temperature, anorexia, depression, jaundice, and anemic or hemorrhagic syndromes (Adler and de la Peña Moctezuma 2010). Nevertheless, the chronic form, with impaired fertility, neonatal deaths, abortions, and decreased milk output, occurs more frequently, causing substantial economic losses (Lilenbaum et al. 2009).

Although the most common agent of caprine and ovine brucellosis are *Brucella melitensis* (never isolated in Brazil) and *B. ovis*, respectively, infection in small ruminants due to *B. abortus* occurs sporadically and may represent a source of economic losses and a public health hazard (Poester et al. 2002). As in cattle, *B. abortus* infection is characterized by late abortion, stillbirths, decreased fertility, and low milk production. Diagnosis follows official recommendations for bovine brucellosis control and is usually based on serological tests, mainly the acidified antigen test (AAT) and 2-mercaptoethanol test (2-ME) (OIE 2008).

Brucellosis caused by *B. ovis* is a non-zoonotic infection characterized by a clinical or subclinical disease in sheep, with genital lesions in rams and placentitis and abortions in ewes. Infected ewes may excrete *B. ovis* in vaginal discharges and milk, so that ewe-to-ram and lactating ewe-to-lamb transmission could be means of spreading the infection (López et al. 2006). The disease has been described to cause considerable economic losses in many countries in Latin America, as Argentina (Samartino 2002) and Brazil (Poester et al. 2002; Alves et al. 2010), as well as in Europe (Spicić et al. 2010). The most widely used diagnostic techniques are serological tests such as complement fixation, agar gel immunodiffusion (AGID), and indirect ELISAs (OIE 2008).

Infections due to small ruminant lentiviruses (SRLVs) cause MV (sheep) and CAE (goats). These retroviruses are widespread and may cause a persistent, lifelong infection, resulting in subclinical disease in one or more organs, including articular joints, brain, lung, and mammary gland, or pleuropneumonia (de Andrés et al. 2005). The viruses are transmitted primarily via colostrum, although transmission via aerosol, horizontal contact, and sexual activity can also occur (Leitner et al. 2010). Clinical disease is rarely observed before the age of 2 years, except in the case of goat kids with CAE, where encephalitis can become evident within a few months after infection (de Andrés et al. 2005). The most convenient way to diagnose SRLVs infections is to perform serology. A variety of laboratory techniques are available, including AGID and indirect ELISA (OIE 2008).

In 2007, our group (Lilenbaum et al. 2007) gathered some information about infectious diseases in small ruminants from Rio de Janeiro, Brazil. In that retrospective study, although sampling was not statistically determined, leptospirosis was considered the most frequent infection in those species. Therefore, we now designed a prospective study, conducted in a representative sampling and with all the tests conducted in the same flocks at the same time. The objective of the present study was to determine the role of leptospirosis, brucellosis—either by *B. abortus* or *B. ovis*—CAE, and MV in the productivity of small ruminants breeding in Rio de Janeiro, Brazil with regard to other tropical countries.

## Material and methods

### Sampling procedures

This was a prospective study. Rio de Janeiro state in Brazil has approximately 30,000 goats and 50,000 sheep distributed in 297 herds and 176 flocks, respectively (IBGE 2008). From those, 96 goat dairy herds (10,500 goats) and 72 sheep meat flocks (32,000 sheep) represent approximately 80% of the total milk/meat production and were selected for the study group. Sampling assumed an estimated average prevalence according to previous studies conducted, when possible, in the same region with a margin of error of 5% and a confidence interval of 95%. For leptospirosis, expected prevalence was 20.9% for goats and 13.7% for sheep (Lilenbaum et al. 2009), for CAE, 35% (Bandeira et al. 2009), while for Maedi-Visna, 8.2% (Oliveira et al. 2006), for *B. ovis*, an expected prevalence of 7.5% (Alves et al. 2010) was used. Nevertheless, it was not possible to find local references regarding the expected prevalence for *B. abortus*, and it was considered as unknown. It is noteworthy to observe that for all infections, sampling was bigger than the minimum required. Samples were collected from April 2009 to November 2010. Herds were randomly selected within each of the six regions studied in Rio de Janeiro state, and only herds with at least 20 animals were selected to the study. None of the herds/flocks reported historic of outbreaks or perceptible losses due to diseases. From each flock, approximately 20% of adult animals were randomly sampled. For goats, 343 samples were tested for leptospirosis, 560 for *B. abortus*, and 506 for CAE, representing 19 herds. Whereas for sheep, 308 samples were tested for leptospirosis, 319 for *B. abortus*, 374 for *B. ovis*, and 278 for MV, representing 16 flocks. Blood was collected in Vacutainer® tubes from the vena jugularis of each animal, chilled, and transported to the laboratory where they were centrifuged (1,000×*g* for 10 min). Serum was stored in 1.5-mL Eppendorf® tubes at −20°C for batch testing.

### Laboratory assay procedures

*Leptospirosis* A microscopic agglutination test (MAT) was employed, as recommended (OIE 2008; Adler and de la Peña Moctezuma 2010). Samples were screened at a 1:100 dilution using a panel 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 at 28–30°C), free of contamination or auto-agglutination. All samples with agglutinating activity at a 1:100 dilution were considered positive and subsequently titrated against reacting antigens, using serial two-fold dilutions of serum. The endpoint was the highest tube in which 50% agglutination was recorded and measured by comparison with a control suspension. The highest titer reached was used to identify the infective serovar (Adler and de la Peña Moctezuma 2010).

*Brucellosis* For the detection of anti-*B. abortus* agglutinins, a screening test was performed with AAT, and a 2-ME was performed as a confirmatory assay, following international standards (OIE 2008). Antigen was elaborated using strain 1119/3 of heat-inactivated *B. abortus* (TECPAR, Curitiba, PR, Brazil), and interpretation of results followed the Brazilian Department of Agriculture guidelines (Brasil 2004). Serological diagnosis of *B. ovis* infection was carried out using the AGID with lipopolysaccharides and protein antigens from *B. ovis*, strain Reo 198 (TECPAR, Curitiba, PR, Brazil) and according to recommendations (OIE 2008).

*Lentiviruses* SRLV antibodies (both CAE and MV) were detected using a commercially available AGID kit (AGID-CAEV P28, Biovetech, Brazil), according to the manufacturer's instructions.

### Statistics

The results of seroprevalence for each herd and each disease were analyzed by the $\chi^2$ test (Chi-square) using GraphPad InStat version 3.05 (GraphPad Software Inc., San Diego, CA). Analysis showing a confidence interval above 95% ($P<0.05$) was considered significant.

## Results

For leptospirosis, 25.9% and 47.4% of goats and sheep, respectively, were seroreactive (Table 1). Serovar Hardjo was the most frequent in both species (approximately 50% of reactive goats and sheep). Agglutinins against other serovars, as Icterohaemorrhagiae, Grippotyphosa, and Pomona were also detected (Fig. 1). Seroreactivity for leptospirosis was more prevalent ($P<0.0001$) than for other infectious diseases, as brucellosis or SRLVs.

Of the 560 goats tested for anti-*B. abortus* agglutinins, only four (0.7%) were reactive to the AAT test, but none of them was confirmed by the 2-ME test, whereas no sheep were seroreactive against this agent (*B. abortus*). In contrast, 23 of 374 (6.1%) sheep presented anti-*B. ovis* agglutinins (Table 1).

**Table 1** Serological survey for anti-*Leptospira* sp., anti-*B. ovis*, anti-*B. abortus*, anti-CAEV, and anti-MVV antibodies in small ruminants from Rio de Janeiro, Brazil (Apr 2009–Nov 2010)

| Species | Disease | No. samples tested | Reactive |
|---|---|---|---|
| Goats | Leptospirosis | 343 | 89[a] (25.9%) |
| | *B. abortus* | 560 | 4[b] (0.7%) |
| | CAE | 506 | 43 (8.6%) |
| Sheep | Leptospirosis | 308 | 146[a] (47.4%) |
| | *B. abortus* | 319 | 0 (0.0%) |
| | *B. ovis* | 374 | 23 (6.1%) |
| | MV | 278 | 9 (3.2%) |

[a] Significant difference ($P<0.0001$) in relation to other infections

[b] Reactive to the ATT test, but not confirmed by the 2-ME test

Regarding the diagnosis of SRLV (CAEV and MV), 8.6% of goats were CAEV-positive, whereas 3.2% of sheep samples were MVV-positive (Table 1).

Twelve goats presented mixed infection, showing seroreactivity against leptospirosis and CAEV simultaneously, and one goat presented seroreactivity against the three studied pathogens. In sheep, eight animals presented seroreactivity against leptospirosis and ovine brucellosis at the same time, and two animals to ovine brucellosis and MVV.

## Discussion

This was a prospective study, conducted in a well-determined and representative population. Therefore, we believe that these results represent an advance in representativeness and reliability of those previously described (Lilenbaum et al. 2007). Despite some minor differences with regard to prevalence rates, the major results previously described were confirmed in the present study, and leptospirosis undoubtedly represents the major infectious disease that impairs reproduction in small ruminants in Rio de Janeiro, Brazil.

Although seroreactivity to an agent does not necessarily mean that the animal was clinically affected by that pathogen, we inferred that the infectious diseases leptospirosis, ovine brucellosis, and SRLVs (mainly CAE) were widespread among those small ruminants. Furthermore, we inferred that these pathogens contributed to the decreased productivity of these animals. This scenario does not differ from that observed in other countries, as Argentina (Samartino 2002), Turkey (Ozdemir and Erol 2002; Yilmaz et al. 2002), Croatia (Spicić et al. 2010), Central America (Desvars et al. 2011; Suepaul et al. 2011), or Tajikistan (Jackson et al. 2007), what reinforces the worldwide (mainly developing countries) validity of the presented results. Reactivity to leptospirosis was alarmingly high, particularly in sheep (47.4%). Furthermore, it was significantly more prevalent than other diseases, as brucel-

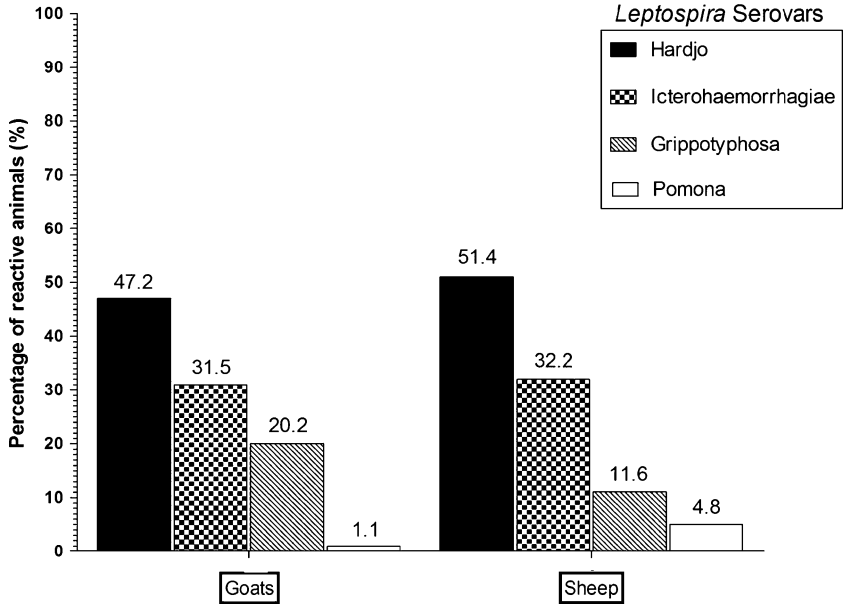


**Fig. 1** Serovar distribution of anti-*Leptospira* sp. antibodies in MAT in 89 goats and 146 sheep from Rio de Janeiro, Brazil

losis or SRLVs. Although those findings agree with those reported to other regions of Brazil (Melo et al. 2010), they are higher than those reported in a recent report from Brazil (Seixas et al. 2011) and other tropical regions, as 4.3% (sheep) and 9.3% (goats) in Barbados and 6.4% (goats), 5.0% (sheep), and 3.3% (goats) in Trinidad, both in Central America (Desvars et al. 2011; Suepaul et al. 2011) or in Andaman Island (India), where 16.4% of goats were seroreactive (Sunder et al. 2005), and even in non-tropical countries, as Poland—12% in goats and 4.5% in sheep (Krawczyk 2005) or Turkey—8% of seroprevalence in sheep (Ozdemir and Erol 2002).

Seroreactivity to Hardjo in this study reached 48.8% of the observed reactions. Hardjo is a strain adapted to cattle (Adler and de la Peña Moctezuma 2010) and the most frequently described serovar in cattle and small ruminants worldwide, including Brazil (Lilenbaum et al. 2007). Although goats and sheep were reported to not act as primary reservoirs of *Leptospira* sp. (Leon-Vizcaino et al. 1987), there was more recent evidence that they are renal and reproductive tract carriers (Silva et al. 2007; Lilenbaum et al. 2009). It is well established that leptospirosis in goats determines important reproductive problems, abortion being the most visible symptom of the syndrome (Leon-Vizcaino et al. 1987; Lilenbaum et al. 2009; Adler and de la Peña Moctezuma 2010). Consequently, due to the reduction of the reproductive performance of the herd, the total milk, as well as the meat production, tends to decrease, representing an important hazard to the farmers.

The low prevalence of seroreactivity for brucellosis in the present study was not unexpected. Although seroreactivity to *B. melitensis* in Brazil has not been reported in goats, *B. abortus* has not yet been eradicated from cattle and can occasionally infect small ruminants (Poester et al. 2002). The same scenario can be observed in other countries, where *B. abortus* has been reported in cattle (Rahman et al. 2006) as well as in small ruminants (Samartino 2002; Sunder et al. 2005; Jackson et al. 2007; Bekele et al. 2011). Furthermore, mixed infections by both *B. abortus* and *B. melitensis* have already been reported (McDermott and Arimi 2002).

Serology for *B. ovis* was done only in sheep, since this agent had low affinity for other hosts (López et al. 2006). The results agree with seroprevalence of anti-*B. ovis* agglutinins in Brazil (Lima et al. 2007; Alves et al. 2010) as well as in other countries, e.g., 2.0% in rams in Croatia (Spicić et al. 2010).

Although seroreactivity to CAE was similar to that recently reported in Brazil (Bandeira et al. 2009), it was lower than that previously reported in the same region by our group (Lilenbaum et al. 2007), what may reflect the impact of control programs which have been adopted in the last years. With regard to MV, prevalence was similar to other regions in Brazil (Lombardi et al. 2009). Although SRLVs represent a concern in small ruminant exploration (Synge and Ritchie 2010), particularly in developing countries (Ploumi et al. 2001; Yilmaz et al. 2002), a minor effect on flock performance parameters has been described (Leitner et al. 2010). The observed reduction in seroreactivity is an interesting finding and demonstrates that control programs may rapidly reduce its occurrence in affected areas.

Based on the present results, we inferred that among the infectious diseases, leptospirosis is the most frequent and potentially the major infection impairing productivity in small ruminants in Rio de Janeiro, Brazil and possibly in other developing countries Therefore, the adoption of adequate programs to reduce the occurrence of infectious diseases,

mainly leptospirosis, is mandatory for the increase of milk/meat production in small ruminants in tropical areas.

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