


# Serological survey of leptospirosis, brucellosis, and lentivirus in herds of small ruminants in Recôncavo Baiano, Bahia, Brazil

## *Inquérito sorológico de leptospirose, brucelose e lentivirose em rebanhos de pequenos ruminantes no Recôncavo Baiano, Bahia, Brasil*

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### ABSTRACT

A serological survey was carried out to assess the frequency of leptospirosis, small ruminants lentivirus (SRLV), and brucellosis in small ruminant herds in the Recôncavo Baiano, State of Bahia, Brazil, from February to December 2017. In four goat herds, 125 animals were tested for SRLV and leptospirosis, while in five sheep herds, 378 animals were tested for leptospirosis, brucellosis, and SRLV. Regarding leptospirosis, MAT detected 80% of goats and 15.34% of sheep seroreactive. Reactivity was most frequent to serogroups Autumnalis and Grippotyphosa in goats and sheep, respectively. Regarding SRLV, 8.8% of goats and 0.79% of sheep were reactive. Search for anti-*B. ovis* antibodies revealed 0.52% reactivity. In sheep, three animals showed simultaneous seroreactivity for SRLV and leptospirosis, while one animal had a serological response for brucellosis and leptospirosis. In goats, simultaneous seroreactivity for SRLV and leptospirosis was observed in only one animal. Leptospirosis was the most frequent of the three infectious diseases in investigated herds.

**Keywords:** Diagnosis. Reproductive changes. *Leptospira* spp. Caprine arthritis-encephalitis. Maedi-Visna. *Brucella ovis*.

### RESUMO

Foi realizado um inquérito sorológico para avaliar a frequência de ocorrência de leptospirose, lentivirose de pequenos ruminantes (LVPR) e brucelose em rebanhos de pequenos ruminantes no Recôncavo Baiano, estado da Bahia, Brasil, no período de fevereiro a dezembro de 2017. Em quatro rebanhos de caprinos, foram testados 125 animais para LVPR e leptospirose, enquanto em cinco rebanhos de ovinos, foram testados 378 animais para leptospirose, brucelose e LVPR. Em relação à leptospirose, 80% das cabras e 15,34% das ovelhas foram sororreativas. Os sorogrupos de *Leptospira* spp. predominantes foram Autumnalis e Grippotyphosa para caprinos e ovinos, respectivamente. Em relação as LVPR, 8,8% dos caprinos e 0,79% dos ovinos foram reativos. Adicionalmente, a pesquisa de anticorpos Anti-*B. ovis* revelou 0,52% de ovinos reativos. Em ovinos, três animais apresentaram sororreatividade simultânea para LVPR e leptospirose, enquanto um animal teve resposta sorológica para brucelose e leptospirose. Em caprinos, sororreatividade simultânea para LVPR e leptospirose foi observada em apenas um animal. A leptospirose foi a doença infecciosa mais frequente nos rebanhos investigados.

**Palavras-chave:** Diagnóstico. Alterações reprodutivas. *Leptospira* spp. Artrite encefalite caprina. Maedi-Visna. *Brucella ovis*.

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## Introduction

Animal diseases that cross the species barrier cause high rates of death worldwide, heavily impacting low- and middle-income countries that practice small ruminant breeding (Torres-Velez et al., 2019). It is estimated that over 20% of animal production worldwide is hampered by infectious diseases, causing large economic losses (World Organisation for Animal Health, 2019). In Brazil, the annual production of goat and sheep meat was estimated at 172 thousand tons (US \$153 million—0.02% of GDP) and 22 thousand tons (US \$6 million—0.0008% of GDP) for goat milk (Instituto Brasileiro de Geografia e Estatística, 2017). Northeast is the region with the highest growth in this sector, with 7.6 million goats and 9 million sheep (Instituto Brasileiro de Geografia e Estatística, 2017). The state of Bahia concentrates the largest goat (2.960.443) and sheep (3.763.732) numbers, highlighting the importance of small ruminant breeding as a socioeconomic activity for the local scenario (Instituto Brasileiro de Geografia e Estatística, 2017; Magalhães et al., 2018). Additionally, this activity poses one of the main sources of food and income for producers (Ribeiro & de Alencar, 2018).

The most-reported infectious diseases in goats and sheep are brucellosis, caprine arthritis-encephalitis (CAE), maedi-visna (MV), and leptospirosis, the latter was the most impactful in studies performed in goats and sheep, from April to November 2010 in Rio de Janeiro (Martins et al., 2012) and sheep of Minas Gerais, in 2007 (Dorneles et al., 2020). In Paraíba, search for anti-*B. ovis* antibodies in sheep revealed seroreactivity in the herds investigated (Santos et al., 2013). CAE (Lima et al., 2013) and MV (Martinez et al., 2011) were also reported in small ruminants in the micro-

region of Juazeiro, in the state of Bahia. Leptospirosis is a zoonotic disease caused by pathogenic leptospire, in which humans are occasional hosts of an epidemiological cycle involving wild and domestic animals (Costa et al., 2015). Most infected livestock present the chronic form of leptospirosis with impaired fertility, abortion, stillbirth, and decreased milk and meat production with consequent extensive economic losses (Ellis, 2015).

*Brucella ovis* is the causative agent of a sexually transmitted infectious disease in domestic sheep. It is characterized by genital lesions and epididymitis in rams, placentitis, and abortions (rare) in ewes, and neonatal mortality in lambs (Elderbrook et al., 2019). Infected rams are responsible for introducing the disease into a herd. Brucellosis has already been described as one of the main causes of reproductive impairment and economic losses (Dorneles et al., 2020).

Small ruminants are also commonly infected by lentiviruses, which belong to the *Retroviridae* family, genus *Lentivirus*, represented by two phylogenetic groups whose prototypes are the virus of Maedi-Visna (MV) and caprine arthritis-encephalitis (CAE) (Junkuszew et al., 2016). The primary clinical outcomes of lentivirus are arthritic, mammary, neurological, and pulmonary manifestations, the latter more frequently observed in sheep (World Organisation for Animal Health, 2018).

Considering the growth of breeding activities and the importance of epidemiologic data about infectious diseases in small ruminants, the objective of the study was to assess the occurrence of leptospirosis, SRLV, and brucellosis in sheep and goat to determine which infectious disease had the highest frequency in the Recôncavo Baiano, Northeastern of Brazil.

## Materials and Methods

### Number of samples and laboratory test

The study was conducted in different herds recruited as convenience samples located in the region of the Recôncavo, in the state of Bahia, between February and December 2017. The animals included in this study belonged to five distinct herds. Four herds were composed of both species in a total of 290 sheep and 125 goats, whilst one herd consisted of 88 sheep. The animals were between 10 months and five years of age. The farms were under semi-intensive (2/5) and intensive (3/5) management systems. The herds were characterized by intense acquisition and transit of animals. The production was focused on animals of the Boer breed for meat production and dairy goat herds of the Saanen and

Anglo-Nubiana breeds. The sheep herds sampled were of the Santa Inês breed with meat production aptitude.

Sera from 125 goats were tested for leptospirosis and SRLV, while sera from 378 sheep were tested for leptospirosis, *B. ovis*, and SRLV. For the detection of anti-*Leptospira* spp. antibodies, microscopic agglutination test (MAT) was performed, as recommended (World Organisation for Animal Health, 2018). The MAT panel was composed of 24 serovars representing 21 serogroups maintained in liquid medium (EMJH) through subculture (at 28-30°C) in the collection of the Bacterial Laboratory of the Federal University of Bahia (LABAC-UFBA) (Table 1). Sera with titers  $\geq 100$  were considered reactive and endpoint titers were determined starting from an initial dilution of 1:100.

For the detection of anti-*Brucella ovis* antibodies, the Agar Gel Immunodiffusion method (AGID) was performed, using a commercial kit (TECPAR, Curitiba, PR, Brazil). Serological diagnosis for both lentiviruses (CAE and MV) was performed using AGID, with a commercial kit (BIOVETECH, Recife, PE, Brazil). The commercial test of AGID does not distinguish infection between CAE and MV. Therefore, we inferred the diagnosis as SRLV seroreactive animals. All procedures were performed according to the manufacturer's recommendations.

## Statistical analysis

Seropositivity of each disease was expressed based on descriptive analysis, using absolute and relative distributions. The confidence interval (CI) of 95% with an acceptable error of 5% was considered significant. The proportion CI was analyzed using Epi Info™ 7.2.3.1.

## Results

Of the 125 goats evaluated for leptospirosis, 100 (80%) were seroreactive (Table 2). Autumnalis (37%), followed by Grippytyphosa (28%), and Sejroe (23%) were the most frequent serogroups. Titers of 100 were predominant (54%, 54/100), followed by 200 (41%, 41/100) (Table 3). Of the 378 sheep samples tested, 58 (15.34%) were reactive (Table 2). Serogroups Grippytyphosa (39.66%) and Tarassovi (27.58%) were predominant. In sheep, titers of 100 were the most frequent (75.86%, 44/58) (Table 4).

The serological survey for anti-SRLV antibodies showed 8.8% and 0.79% of seroreactivity for goats and sheep, respectively (Table 2). Of the 378 samples tested for the presence of anti-*B. ovis* antibodies, only two (0.52%) were reactive (Table 2).

In sheep, three animals were simultaneously seroreactive for SRLV and leptospirosis (all with titers of 100 for Grippytyphosa), while one animal demonstrated concomitant serological response for brucellosis and leptospirosis (titer of

Table 1 - Serovars used as antigen to perform the microscopic agglutination test (MAT)

Specie	Serogroup	Serovar	Strain
<i>L. interrogans</i>	Australis	Bratislava	Jez Bratislava
<i>L. interrogans</i>	Autumnalis	Autumnalis	Akiyami A
<i>L. borgpetersenii</i>	Ballum	Castellonis	Castellon 3
<i>L. interrogans</i>	Bataviae	Bataviae	Van Tienen
<i>L. interrogans</i>	Canicola	Canicola	Hond Utrecht IV
<i>L. weilii</i>	Celledoni	Celledoni	Celledoni
<i>L. kirschneri</i>	Cynopteri	Cynopteri	3522 C
<i>L. interrogans</i>	Djasiman	Djasiman	Djasiman
<i>L. kirschneri</i>	Grippytyphosa	Grippytyphosa	Moskva V
<i>L. interrogans</i>	Hebdomadis	Hebdomadis	Hebdomadis
<i>L. interrogans</i>	Icterohaemorrhagiae	Copenhageni	M20
<i>L. interrogans</i>	Icterohaemorrhagiae	Copenhageni	L1 130
<i>L. interrogans</i>	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
<i>L. borgpetersenii</i>	Javanica	Javanica	Poi
<i>L. noguchii</i>	Louisiana	Louisiana	LUC1945
<i>L. borgpetersenii</i>	Mini	ND	2008/01925
<i>L. noguchii</i>	Panama	Panama	CZ 214 K
<i>L. interrogans</i>	Pomona	Pomona	Pomona
<i>L. interrogans</i>	Pyrogenes	Pyrogenes	Salinem
<i>L. interrogans</i>	Sejroe	Hardjo	Hardjoprajitino
<i>L. borgpetersenii</i>	Sejroë	Sejroë	M 84
<i>L. biflexa</i>	Semaranga	Patoc	Patoc 1
<i>L. santarosai</i>	Shermani	Shermani	1342 K
<i>L. borgpetersenii</i>	Tarassovi	Tarassovi	Mitis Johnson

Table 2 - Serological survey for anti-*Leptospira* spp., Anti- SRLV, and anti-*B. ovis* antibodies in small ruminants in the Recôncavo region, Bahia, Brazil, from February to December 2017

Species	Disease	No. samples tested	Reactive (%)
Goats	Leptospirosis	125	100 <sup>a</sup> (80.0%)
	SRLV	125	11 (8.8%)
Sheep	Leptospirosis	378	58 <sup>a</sup> (15.34%)
	<i>B. ovis</i>	378	2 (0.52%)
	SRLV	378	3 (0.79%)

<sup>a</sup> Significant difference with other infections

SRLV = small ruminants lentiviruses

Table 3 - Distribution of serogroups reactive by microscopic agglutination test (MAT) performed seroreactive goats from Recôncavo, in the state of Bahia, Brazil, from February to December 2017

Serogroup	Titers				Total N (%)
	100	200	400	≥ 800	
Pyrogenes	0	0	0	1	1 (1.0)
Hebdomadis	4	0	0	0	4 (4.0)
Grippotyphosa	24	3	1	0	28 (28.0)
Sejroe	23	0	0	0	23 (23.0)
Australis	0	1	0	0	1 (1.0)
Celledoni	0	1	0	0	1 (1.0)
Tarassovi	2	0	2	0	4 (4.0)
Autumnalis	0	36	1	0	37 (37.0)
Sejroe	1	0	0	0	1 (1.0)
Total	54	41	4	1	100 (100.0)

Table 4 - Distribution of serogroups reactive in microscopic agglutination test (MAT) performed seroreactive sheep from Recôncavo, in the state of Bahia, Brazil, from February to December 2017

Serogroup	Titers				Total N (%)
	100	200	400	≥ 800	
Pyrogenes	0	1	1	0	2 (3.45)
Grippotyphosa	21	1	1	0	23 (39.66)
Australis	1	1	0	0	2 (3.45)
Celledoni	4	1	0	0	5 (8.62)
Tarassovi	15	1	0	0	16 (27.58)
Autumnalis	0	4	1	0	5 (8.62)
Sejroe	2	1	0	0	3 (5.17)
Shermani	1	0	1	0	2 (3.45)
Total	44	10	4	0	58 (100.0)

400 for Autumnalis). In goats, simultaneous seroreactivity to SRLV and leptospirosis was observed in only one animal (titer of 200 for Autumnalis). It must be emphasized that leptospirosis was the common disease in all animals that presented simultaneous seroreactivity.

## Discussion

Small ruminants can act as reservoir hosts for different infectious agents with relevant economic losses, representing a growing concern to public health (Dorjee et al., 2008;

Martins & Lilenbaum, 2014). Here was demonstrated the exposure of the studied herds to *Leptospira* spp. through the high detection of anti-*Leptospira* spp. antibodies in goats and sheep. Additionally, it is inferred that leptospirosis is the main reproduction disease in small ruminants in the studied herds. Similar findings were observed in other studies performed in southeastern Brazil, in the state of Rio de Janeiro (Martins et al., 2012; Lilenbaum et al., 2007). This suggests possible sanitary management failures in the herds, denoting the importance of implementing diagnostic, prophylactic, and control measures in the region.

Other serological surveys in sheep and goats from Rio de Janeiro (Martins et al., 2012), Minas Gerais (Santos et al., 2012), and the semiarid region of the state of Paraíba (Higino et al., 2013) revealed the occurrence of reactions to serogroups Autumnalis, Grippotyphosa, Sejroe, Celledoni, and Tarassovi, corroborating our findings. In the present study reactions to serogroups Autumnalis and Grippotyphosa were the most frequent in goats and sheep, respectively, corroborating with other investigations carried out in the National Park of Serra das Confusões, state Piauí and rural areas of the state of Pernambuco (Santos et al., 2017), as well as the Sertão mesoregion of the state of Paraíba. All are located in northeastern Brazil (Silva et al., 2019).

In the herds studied, it was observed serological reactions to a variety of serogroups. Therefore, it is believed that contact with wild animals and rodents, as well as the presence of cattle on the properties, contributed to the occurrence of the disease since serological reactions have been evidenced for serovars usually maintained by more than one species (Araújo Neto et al., 2010; Soares et al., 2020). The serogroups Autumnalis and Grippotyphosa were previously isolated from wild animals, especially rodents found in rural areas, suggesting their role as reservoirs and possible transmitters of leptospirosis (Agudelo-Flórez et al., 2009). The occurrence of seroreactivity to these serogroups demonstrates the possibility of rodent participation in leptospiral transmission, emphasizing the need for control of synanthropic populations (Santos et al., 2017).

The occurrence of serological reactions to serovars Hardjo and Wolffi, representatives of the Sejroe serogroup, could be attributed to the close contact between sheep and cattle. The members of the Sejroe serogroup are maintained by cattle and usually transmitted from them to other species, mainly small ruminants (Adler & De La Peña Moctezuma, 2010; Pinna et al., 2018). Studies suggest that small ruminants have been establishing the status of gradual adaptation to other serovars, including Hardjo, acting as carriers of leptospires and shedding the agent in the environment for long periods (Dorjee et al., 2008; Lilenbaum et al., 2009).

The climatic conditions of the studied region may also contribute to the high prevalence of leptospirosis. Small ruminants located in tropical climates are almost three times more likely to be seroreactive than those from temperate ones (Lilenbaum et al., 2008). Although the present findings were similar to other studies conducted in Brazil, the leptospiral seroreactivity found in the examined herds was higher than those reported in other tropical countries: 4.7% (sheep) and 7.9% (goats) in Thailand (Suwancharoen et al., 2013), 5% (sheep) and 3.3% (goats) in Trinidad (Suepaul et al., 2011) and 8.47% (goats) in Tanzania (Assenga et al., 2015).

In tropical regions, the presence of larger herds and the high number of serovars isolated increase the chances of exposure to the agent. (Faine et al., 2000). Therefore, tropics pose as ideal scenarios for the maintenance and spread of leptospirosis: elevated rainfall rates together with a variety of wild, synanthropic, and domestic reservoirs may contribute to the continuous transmission of the bacteria (Pappas et al., 2008; Almeida et al., 2019). The occurrence of leptospirosis represents an important factor affecting herd performance. Consequently, milk and meat production tend to decrease, leading to important financial difficulties for farmers (Martins & Lilenbaum, 2014).

SRLV and *Brucella* spp. infections cause debilitating illnesses with induced inflammatory pathology of several organs, leading to the progressive emaciation of the animal (Junkuszew et al., 2016; Larruskain & Jugo, 2013). These agents mainly infect polymorphonuclear cells and monocytic-phagocytic lineage (Larruskain & Jugo, 2013; Li et al., 2014). Therefore, it is known that the animals presenting concomitant diseases may have drawbacks in their humoral response, with a consequent predisposition to develop the most severe form of leptospirosis (Chen et al., 2020).

Studies report the occurrence of CAE and MV in various states of Brazil, with seroreactivity ranging from 0.29% to 17.6% (Bandeira et al., 2009; Sousa et al., 2019; Dorneles et al., 2020). The low seroreactivity to SRLV detected in the present study corroborates other studies performed in the northeastern region of Brazil. Lima et al., (2013) found 0.29% reactivity in goats from the Baixo Médio São Francisco region, Bahia. In the state of Pernambuco, 1.89% of goats' and 0.26% of sheep's samples were reactive (Melo et al., 2016). Regarding brucellosis, the presence of anti-*B. ovis* antibodies have a variable presentation in different states of the country, such as 7.5% in Paraíba (Alves et al., 2010), 6.1% in Rio de Janeiro (Martins et al., 2012), and 24.04% in Minas Gerais (Dorneles et al., 2020). The seroreactivity to brucellosis was extremely low in all herds studied, corroborating with other reports from Bahia

(Souza et al., 2012) and Rio de Janeiro (Lilenbaum et al., 2007). The occurrence of these diseases is of great concern in small ruminants due to the consequent decrease in milk and meat production, reduced fertility in rams, lower conception rates in ewes, abortion, and decreasing numbers of healthy lambs' birth (Elderbrook et al., 2019; Martins et al., 2012).

The variable frequency between distinct regions can be a consequence of different management systems, geographical location, demographic concentration, animal transit, host susceptibility, the interaction between species, and characteristics of the etiologic agent (Higino et al., 2013). The herds studied were characterized by intense acquisition and transit of animals. Therefore, the occurrence of these pathogens should be expected, since intensive management promotes higher contact between animals, favoring the spread and transmission of infectious agents (Junkuszew et al., 2016; Campos et al., 2017; Santos et al., 2017).

The lower seroreactivity for brucellosis and SRLV observed in this study demonstrates that control and sanitary programs may reduce the occurrence of these diseases in the affected areas, corroborating the report of Martins et al. (2012). In contrast, leptospirosis is a neglected disease within the governmental health program. The high occurrence of leptospirosis has a relevant impact on public health since sheep may carry the bacteria in the kidneys for long periods (Almeida et al., 2019). This extended shedding via urine constitutes a zoonotic risk for handlers and owners. Therefore, to reduce exposure and infections by leptospires, more efforts towards health education, disease control, and inspection are needed.

The development of these activities is affected by the inefficient sanitary management practices for leptospirosis control. In small ruminant breeding negligence towards leptospirosis controls and detection increases the incidence of the disease, determining the low productivity of the sector (Campos et al., 2017; Salaberry et al., 2011). However, as observed in the present study and as reported in the herds studied by Martins et al. (2012), the frequency of CAE, MV and Brucellosis was reduced due to the implementation of animal health and control programs. The low seroreactivity for these diseases is an interesting finding and suggests advantages in the inclusion of leptospirosis within an official program, emphasizing the importance of implementation of sanitary management applied to the control and the prevention of infectious agents in small ruminant herds.

## Conclusion

The serological investigation of leptospirosis, small ruminants lentivirus (SRLV), and brucellosis

in small ruminants from herds of Recôncavo region Bahia State, Brazil showed that leptospirosis was the most frequent of them. It is proposed that the establishment of leptospirosis control programs with identification of infected animals, combined with treatment, prophylaxis, health management, and requirement of health status documents for animal transit may be applied for reducing the economic and productive losses in these herds.

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