PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 45:e07740, 2025
DOI: 10.1590/1678-5150-PVB-7740

Original Article
Livestock Diseases

Epidemiological characterization and risk factors associated with brucellosis in sheep in the municipality of Rio Branco, Acre[1]

Mario C.S. Araújo[2]*, Heloisa S. Maia[2], Lorena Y.C. Tomaya[2], Leonardo A.K. Melchior[2], Renato M. Peixoto[3], Patrícia G. Souza[4], Pâmela A. Lima[5], Rafael R. Nicolino[5], Renato L. Santos[5], Tamyres I.B. Silva[2]

ABSTRACT.- Araújo MCS, Maia HS, Tomaya LYC, Melchior LAK, Peixoto RM, Souza PG, Lima PA, Nicolino RR, Santos RL, Silva TIB. **Epidemiological characterization and risk factors associated with brucellosis in sheep in the municipality of Rio Branco, Acre**. *Pesquisa Veterinária Brasileira 45:e07740, 2025*. Universidade Federal do Acre, Rodovia BR-364 Km 04, Distrito Industrial, Rio Branco, AC 69920-900, Brazil. E-mail: mcesar171971@gmail.com

Brucellosis in sheep is a chronic infectious disease with a worldwide distribution, primarily caused by the bacterium *Brucella ovis*. The disease is characterized by the development of epididymitis and sperm granulomas, which result in reproductive disorders and are responsible for significant health problems and economic losses in sheep farming. This cross-sectional sampling study, the first of its kind in Western Amazonia, aimed to characterize the seroprevalence of brucellosis in sheep from rural properties in the municipality of Rio Branco, Acre, and to analyze the risk factors associated with the infection. Forty-eight sheep farms in the municipality were selected, and 511 blood samples were collected from animals of both sexes, starting at six months of age. Serological samples were tested using an indirect enzyme-linked immunosorbent assay (iELISA) to detect anti-*Brucella ovis* antibodies (IgG) with the crude *B. ovis* extract antigen and, in a complementary manner, with the purified recombinant periplasmic protein BP26r. Epidemiological questionnaires were applied during the visit to the farms. Of the farms with sampled sheep, 33.4% (16/48) had at least one seropositive animal, and 5.5% (28/511) of the sheep tested were considered seropositive. Among the risk factors analyzed by multiple logistic regression, the "segregation of sick animals" (odds ratio = 3.67; $p = 0.043$) showed epidemiological and statistical relevance for the control of *B. ovis* infection. The results obtained in this study indicate that *B. ovis* infection occurs in sheep from Acre, in the Western Amazon, requiring the implementation of control and prophylaxis measures, especially health education, to prevent or minimize the spread of the disease in herds in Acre.

INDEX TERMS: *Brucella ovis*, ELISAi, risk factors, sheep farming, seroprevalence.

RESUMO.- [**Caracterização epidemiológica e fatores de risco associados à brucelose em ovinos no município de Rio Branco, Acre.**] A brucelose nos ovinos é uma doença infectocontagiosa, de evolução crônica, com distribuição cosmopolita, causada primordialmente pela bactéria *Brucella ovis*. A enfermidade é caracterizada pelo desenvolvimento de epididimite e granulomas espermáticos que resultam em distúrbios reprodutivos, sendo responsável por relevantes problemas sanitários e vultosos prejuízos econômicos para a ovinocultura. Este estudo transversal por amostragem, inédito na Amazônia Ocidental, teve como objetivo caracterizar a soroprevalência da brucelose em ovinos de propriedades rurais no município de Rio Branco, estado do Acre, e análise dos fatores de risco associados à infecção. Foram selecionadas 48 propriedades com criação de ovinos do município e colhidas 511 amostras de sangue de animais de ambos os sexos, a partir de seis meses de idade. As amostras sorológicas

[1] Received on August 1, 2025.
Accepted for publication on September 2, 2025.

[2] Universidade Federal do Acre (UFAC), Rodovia BR-364 Km 04, Distrito Industrial, Rio Branco, AC 69920-900, Brazil. *Corresponding author: mcesar171971@gmail.com

[3] Universidade Estadual do Ceará (UECE), Rodovia BR-020, Bezerra de Sousa, Tauá, CE 63660-000, Brazil.

[4] Laboratório Federal de Defesa Agropecuária, Av. Rômulo Joviano s/n, Olaria, Pedro Leopoldo, MG 33250-220, Brazil.

[5] Universidade Federal de Minas Gerais (UFMG), Av. Pres. Antônio Carlos 6627, Pampulha, Belo Horizonte, MG 31270-901, Brazil.

foram testadas pelo ensaio imunoenzimático indireto (ELISAi) para detecção de anticorpos (IgG) anti-*Brucella ovis* com o antígeno extrato bruto de *B. ovis* e, de modo complementar, com a proteína periplasmática recombinante purificada BP26r. Durante a visita às propriedades foram aplicados questionários epidemiológicos. Das propriedades rurais com ovinos amostrados, 33,4% (16/48) apresentaram pelo menos um animal sororreagente e 5,5% (28/511) dos ovinos testados foram considerados soropositivos. Dentre os fatores de riscos analisados por regressão logística múltipla, a "segregação de animais doentes" (odds ratio = 3,67; p = 0,043) apresentou relevância epidemiológica e estatística para o controle da infecção por *B. ovis*. Os resultados obtidos neste estudo indicam que ocorre a infecção por *B. ovis* nos ovinos do Acre, Amazônia Ocidental, requerendo a implantação de medidas de controle e profilaxia, notadamente de educação em saúde, que impeçam ou minimizem a difusão da doença nos rebanhos acreanos.

TERMOS DE INDEXAÇÃO: *Brucella ovis*, ELISAi, fatores de risco, ovinocultura, soroprevalência.

INTRODUCTION

Ovine brucellosis is a chronic, infectious, and contagious disease with worldwide distribution, exerting significant socioeconomic and sanitary impacts on sheep farming (Xavier et al. 2009). *Brucella melitensis* and *Brucella ovis* are the main etiological agents of brucellosis in small ruminants (Rossetti et al. 2022). However, *B. melitensis* is considered exotic in Brazil (Rostami et al. 2023).

Infection by *B. ovis* is one of the most important causes of reproductive disorders in sheep, as well as trade restrictions on animal-derived products (meat, milk, and dairy products), economic losses for industries due to the condemnation of by-products, and costs associated with control, eradication, and research programs (Jardim et al. 2006, Sobrinho et al. 2010).

Serological tests play a key role as screening tools for determining animal exposure to *B. ovis* (Ridler 2014). Commonly used techniques include complement fixation (CF), agar gel immunodiffusion (AGID), and indirect enzyme-linked immunosorbent assay (iELISA), which employ soluble surface antigens obtained from the *B. ovis* REO 198 strain (OIE 2018).

The iELISA is a sensitive test for the diagnosis of ovine brucellosis; however, its results and sensitivity are directly influenced by the antigen employed (Rahaley et al. 1983). Antigens may include crude pathogen extracts and specific microbial structures, such as LPS, proteins, and peptides (Muñoz et al. 2005, Gan & Patel 2013). Crude extracts of *Brucella* spp. have been frequently described as antigens in serological assays in different hosts (Barrouin-Melo et al. 2007, Oliveira et al. 2011, Corrente et al. 2015, Tabasi et al. 2019).

Given the lack of official epidemiological data on the prevalence of ovine brucellosis in municipalities of Acre and the Western Amazon, and considering the disease's relevance to the production system, this study may contribute to identifying the epidemiological scenario of ovine brucellosis in the region. Furthermore, it is expected to propose alternatives to support the implementation of control and prophylactic strategies within the sanitary programs of official Animal Health Defense agencies, as well as to enhance awareness among sheep farmers in the municipality of Rio Branco, Acre State.

This study aimed to investigate the epidemiological status of *B. ovis* infection in sheep in the municipality of Rio Branco, Acre, and to characterize the associated risk factors.

MATERIALS AND METHODS

Ethical approval. The study was approved by the Animal Use Ethics Committee of the "Universidade Federal do Acre" (CEUA/UFAC) under protocol no. 27/2022.

Study area. The research was conducted in the municipality of Rio Branco, which is part of the Baixo Acre Regional District in the state of Acre, located in the Western Amazon and the northern region of Brazil (Fig. 1).

Sampling. For the sampling design, the primary units (sheep farms) were selected for convenience based on information from the official registry of the "Instituto de Defesa Agropecuária e Florestal do estado do Acre" (Institute of Agricultural and Forestry Defense of the State of Acre) (IDAF-AC 2021). To calculate the sample size of sheep to be randomly selected per flock (n), an unknown expected prevalence of 50% (p = 0.5) was assumed, in order to maximize the sample size, with a 95% confidence level (p = 0.95), a maximum error margin of 5%, and a total population of 11,936 sheep (IDAF-AC 2021). The minimum sample size estimated for this study was 371 animals (Thrusfield & Christley 2018). The criteria established for selecting animals included diverse zootechnical and breed patterns: an apparently healthy condition, both sexes, reproductive age of six months or older (Brasil 2005), potential for commercialization, and rearing under different production systems.

To determine the number of animals to be sampled on the selected farms, the following criteria were applied: 10 males and females were sampled when the flock comprised up to 99 animals, and 15 males and females were sampled when the flock comprised 100 or more animals. In cases where the flock had fewer than 10 animals of reproductive age, all were included. These parameters were based on regulations concerning bovine brucellosis (Brasil 2002). In total, 511 blood samples were collected from sheep belonging to 48 farms in the municipality of Rio Branco, Acre (Fig. 2).

Sample collection and storage. During visits to the selected farms, the rural producer was initially asked to sign the Informed Consent Form. Sheep were then physically restrained, and blood samples were collected via puncture of the external jugular vein using 10 mL vacuum collection tubes without anticoagulant, following local antisepsis with 2% iodine alcohol. The biological material was properly labeled and transported in an insulated cooler to the "Laboratório de Doenças Infecciosas dos Animais" (Animal Infectious Diseases Laboratory - LADIA) at the UFAC. Collected blood samples were centrifuged at 3,500 × g for 10 minutes to obtain serum, which was then transferred to 2 mL microtubes and stored at -20 °C until laboratory diagnostic processing.

Serological tests. For the detection of anti-*Brucella ovis* antibodies, the indirect enzyme-linked immunosorbent assay (iELISA) was performed following the adapted protocol described by Barrouin-Melo et al. (2007) at the "Laboratório de Patologia Molecular"(Molecular Pathology Laboratory) of the "Escola de Veterinária" (School of Veterinary Medicine), "Universidade Federal de Minas Gerais" (UFMG). Samples were exposed to antigens extracted from *Brucella ovis* periplasmic lipopolysaccharides (crude extract) and, complementarily, to the purified recombinant protein antigen of *Brucella* spp. (BP26r), at a 1:250 dilution in bovine serum albumin and disodium phosphate buffer solution (0.05 M, pH 9.6) in the wells of microtiter plates pre-coated with controls and reagents. Plate readings were conducted using a microplate reader at a wavelength

of 492 nm. The ELISA cut-off for defining the positive and negative seroreactive samples was calculated as the sum of the mean of the negative control values plus two times the standard deviation of the negative control ($\bar{x}$ neg. + 2.0 SD). Additionally, serological tests for smooth LPS were performed using the Rose Bengal Test (RBT) (*Brucella abortus*) on all samples at the "Laboratório Federal de Defesa Agropecuária" (Federal Laboratory of Agricultural Defense - LFDA), Bacterial Diseases Section, Pedro Leopoldo/MG. Farms with at least one seropositive animal were considered positive.

Epidemiological questionnaire. To characterize risk factors, an epidemiological questionnaire was administered, comprising information on the sheep production systems of the selected farms (Table 1). The questionnaire included management type, technological level, applied sanitary practices, and other relevant characteristics, totaling 45 closed-ended questions.

Statistical analysis. The responses from the epidemiological questionnaires were tabulated in an Excel spreadsheet for subsequent statistical analysis. Risk factor analysis was conducted in two stages:

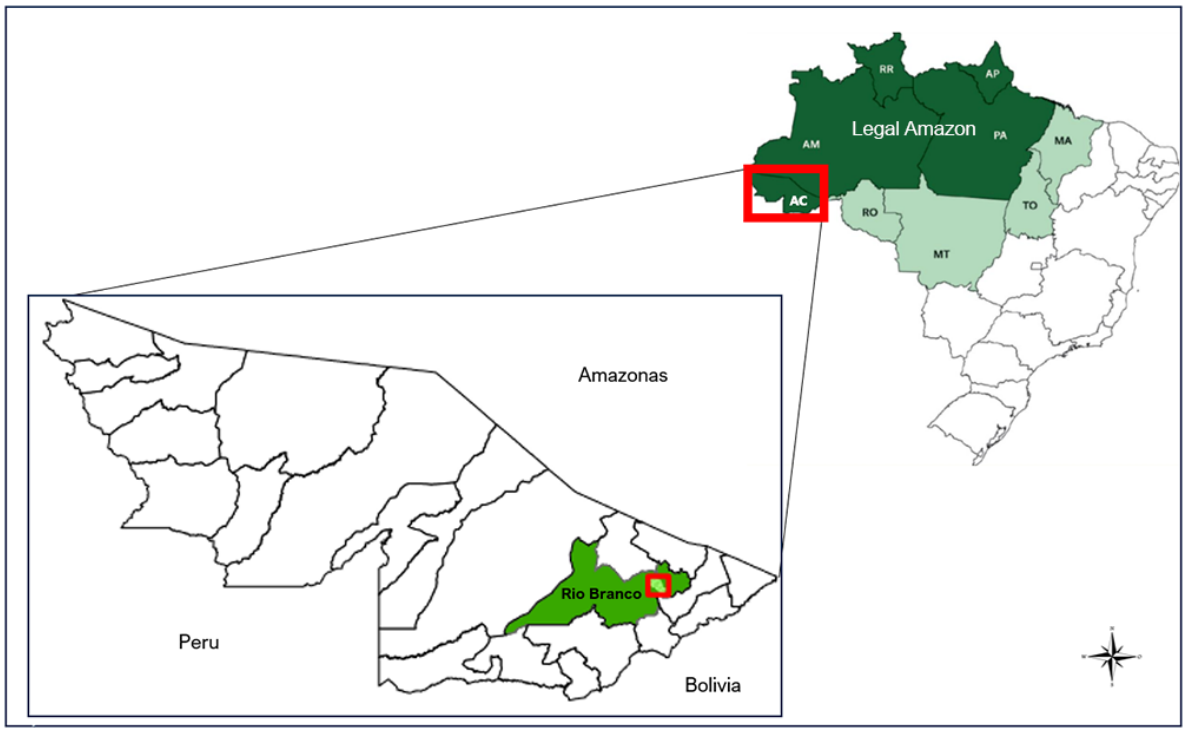

Fig. 1. Illustration of the map of Acre State, highlighting the municipality of Rio Branco.

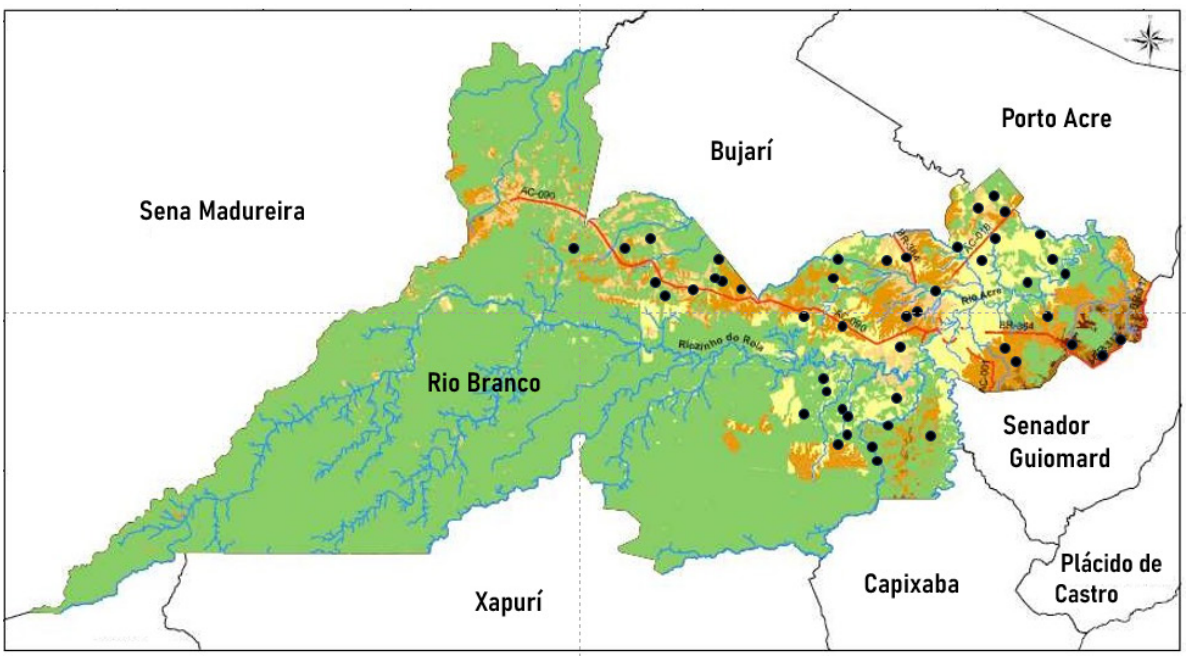

Fig. 2. Illustration of the map of the municipality of Rio Branco, highlighting the location of the selected livestock farms.

Table 1. Bivariate analysis of risk factors associated with *Brucella ovis* seropositivity in sheep from livestock farms in the municipality of Rio Branco, Acre, Brazil

Variables	Total number of farms N (%)	Number of farms with seropositive sheep N (%)	OR (95% CI)	*p*-value
Breed origin				
Mixed	20 (41.67%)	5 (25.00%)	1.94 (0.57 – 7.36)	0.300*
Native	28 (58.33%)	11 (39.29%)		
Contact with wildlife species				
No	33 (68.75%)	10 (30.30%)	1.53 (0.42 – 5.42)	0.519*
Yes	15 (31.25%)	6 (40.00%)		
Commercialization				
Animal trade	10 (20.83%)	4 (40.00%)	-	0.322**
Product trade	1 (2.08%)	1 (100.00%)		
Does not commercialize	37 (77.08%)	11 (29.73%)		
Origin of breeding males from the same herd				
No	5 (10.42%)	2 (40.00%)	0.72 (0.11 – 5.97)	1.000**
Yes	43 (89.58%)	14 (32.56%)		
Has facilities for handling animals				
Sheep pen	33 (68.75%)	11 (33.33%)	1.00 (0.26 – 3.59)	1.000*
Does not have	15 (31.25%)	5 (33.33%)		
Feed supplementation				
No	4 (8.33%)	4 (100.00%)	-	0.009**
Yes	44 (91.67%)	12 (27.27%)		
Fed with bovine milk serum				
No	25 (52.08%)	10 (40.00%)	0.53 (0.15 – 1.78)	0.307*
Yes	23 (47.92%)	6 (26.09%)		
Rents pastures				
No	45 (93.75%)	16 (35.56%)	-	0.541**
Yes	3 (6.25%)	0 (0.00%)		
Shared pastures with other farms				
No	45 (93.75%)	14 (31.11%)	4.43 (0.39 – 110.97)	0.254**
Yes	3 (6.25%)	2 (66.67%)		
Shares water sources/drinking troughs				
No	47 (97.92%)	16 (34.04%)	-	1.000**
Yes	1 (2.08%)	0 (0.00%)		
There are swampy areas				
No	35 (72.92%)	12 (34.29%)	0.85 (0.20 – 3.23)	1.000**
Yes	13 (27.08%)	4 (30.77%)		
Separates pregnant females				
No	41 (85.42%)	13 (31.71%)	1.62 (0.28–8.39)	0.672**
Yes	7 (14.58%)	3 (42.86%)		
Separates sick animals				
No	28 (58.33%)	6 (21.43%)	3.67 (1.07 – 13.62)	0.038*
Yes	20 (41.67%)	10 (50.00%)		
Acquisition of animals				
Livestock event	2 (4.17%)	0 (0.00%)		1.000**
Known herd	41 (85.42%)	14 (34.15%)	-	
Unknown herd	5 (10.42%)	2 (40.00%)		
Implement preventive measures upon animal introduction				
No	40 (83.33%)	12 (30.00%)	2.33 (0.48 – 11.44)	0.413**
Yes	8 (16.67%)	4 (50.00%)		
Performs deworming				
No	5 (10.42%)	0 (0.00%)	-	0.154**
Yes	43 (89.58%)	16 (37.21%)		
Implements a vaccination program				

Variables	Total number of farms N (%)	Number of farms with seropositive sheep N (%)	OR (95% CI)	*p*-value
No	44 (91.67%)	15 (34.09%)	0.64 (0.03 – 5.54)	1.000**
Yes	4 (8.33%)	1 (25.00%)		
Performs diagnostic tests or examinations				
No	46 (95.83%)	15 (32.61%)	2.07 (0.08 – 54.72)	1.000**
Yes	2 (4.17%)	1 (50.00%)		
Veterinary assistance				
No	40 (83.33%)	11 (27.50%)	4.39 (0.92 – 24.53)	0.100**
Yes	8 (16.67%)	5 (62.50%)		
Recent introduction of breeding males				
No	41 (85.42%)	13 (31.71%)	1.62 (0.28–8.39)	0.672**
Yes	7 (14.58%)	3 (42.86%)		
Occurrence of abortion				
No	39 (81.25%)	11 (28.21%)	3.18 (0.72 – 15.09)	0.138**
Yes	9 (18.75%)	5 (55.56%)		
Disposition of aborted fetus				
Buried	11 (22.92%)	5 (45.45%)	0.51 (0.13 – 2.09)	0.468**
None	37 (77.08%)	11 (29.73%)		
Provide neonatal care				
No	4 (8.33%)	0 (0.00%)	-	0.286**
Yes	44 (91.67%)	16 (36.36%)		
Mortality rate				
< 10%	45 (93.75%)	15 (33.33%)	1.00 (0.04 – 11.27)	1.000**
>10%	3 (6.25%)	1 (33.33%)		
Animal slaughter				
On the farm	42 (87.50%)	14 (33.33%)	1.00 (0.13 – 5.80)	1.000*
Does not slaughter	6 (12.50%)	2 (33.33%)		
Manure disposal				
Accumulation in the open	21 (43.75%)	6 (28.57%)	1.47 (0.44 – 5.24)	0.537*
Used as fertilizer	27 (56.25%)	10 (37.04%)		

OR = Odds ratio, 95% CI = confidence interval; *Pearson's chi-square test, **Fisher's exact test.

bivariate and multivariate analysis. In the bivariate analysis, using R software for Windows (R Core Team 2024), each independent variable was cross-tabulated with the dependent variable (serological status at the flock level). Variables with a *p*-value ≤ 0.20 in the Chi-square or Fisher's exact test were selected for the multivariate analysis, which employed multiple logistic regression (Santos et al. 2013, Machado et al. 2015). Logistic regression was used to define the model that best identifies risk factors associated with serological status at the flock level. A significance level of 5% was adopted in the multivariate logistic regression. To assess collinearity among all selected independent variables, the variance inflation factor (VIF) criterion was applied, with a value below 5 indicating independence (Kim 2019).

RESULTS AND DISCUSSION

Among the sampled farms, 33.4% (16/48) had sheep that tested seropositive for *Brucella ovis* by iELISA. Regarding the total number of animals tested, 5.5% (28/511) of the sheep tested seropositive using the crude *B. ovis* extract antigen. These results indicate that *B. ovis* is likely present in the studied municipality, as the sampled animals showed serological evidence of infection.

A high proportion of farms with seropositive sheep was observed, despite a lower seroprevalence among the individual animals on these farms. Santos et al. (2013), in the state of Paraíba, reported a similar situation when testing 1,134 hairless sheep, finding 20.39% (21/103) of farms and 5.20% (59/1,134) of animals to be positive. From an epidemiological perspective, these findings suggest that the infection in the studied area may be endemic. In regions where the disease has been recently introduced, high prevalences of 20% to 60% are observed, whereas endemic regions tend to exhibit lower prevalences (Ficapal et al. 1998).

The scatter plot in Figure 3 shows points representing the mean optical density (OD) values from the replicates and the established iELISA cut-off using the crude *B. ovis* extract antigen. The 28 points above the dashed cut-off line (cut-off = 1.0207) were considered seropositive, while the 493 points below the dashed line (cut-off = 1.0207) were considered seronegative.

Regarding the sex of the tested animals, a higher proportion of females (6.0%, 24/403) were seropositive for *B. ovis* compared to males (3.7%, 4/108). Using the binomial test with a 5% significance level, this difference between seropositive males and females was statistically significant ($p = 0.00018$). Similar results were reported by Lima et al. (2020), who observed

Table 2. Final multivariate logistic regression model for risk factors associated with ovine brucellosis at the herd level in the municipality of Rio Branco, Acre, Brazil

Variables	OR	95% CI	*p*-value	EC	SE
Separation of sick animals	3.67	1.07 – 13.62	0.043	1.29	0.64

OR = Odds ratio, CI = confidence interval, EC = estimated coefficient, SE = standard error of the estimate.

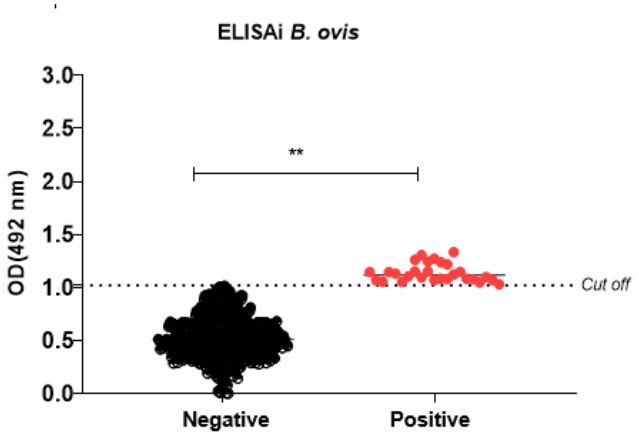

Fig. 3. Scatter plot of optical density (OD) values by indirect enzyme-linked immunosorbent assay (iELISA) using the crude extract antigen of *Brucella ovis*.

that 6.90% (55/797) of females were seropositive for *B. ovis*, whereas only 2.96% (15/507) of males tested positive.

This may be attributed to the fact that sexually mature females are considered more susceptible to the disease (Martins et al. 2013). Adult females can act as disseminators of the infection within the flock, primarily transmitting it to males during mating by shedding the agent in vaginal secretions (Plant et al. 1986). Infected ewes release the bacterium through vaginal secretions, placenta, aborted fetuses, and milk (Libal & Kirkbride 1983, Homse et al. 1995, Estein 1999, Baigún et al. 2000), contaminating the environment and promoting the spread of the microorganism within the flock, since the oral and nasal mucosae and damaged skin serve as entry points for *B. ovis* (Plant et al. 1986, Alton et al. 1988, Bulgin 1990). The presence and persistence of these tissues and secretions in the facilities facilitate *B. ovis* infection among flock sheep, particularly females, as males are generally housed separately (Clementino et al. 2007).

Complementary to the study, the sheep serological samples were also tested by iELISA using the purified recombinant protein antigen BP26r. An increase in seropositivity (20.74%, 106/511) was observed in animals tested with the BP26r antigen compared to the crude *B. ovis* extract. This may suggest the occurrence of false positives, likely because BP26r is an outer membrane protein of *Brucella* spp., with 100% identity to *Brucella abortus*, *Brucella melitensis*, *Brucella suis* and *B. ovis* (Kim et al. 2013, França et al. 2014).

Considering the iELISA using the crude extract antigen of *B. ovis* as a reference test, a sensitivity of only 50% and a specificity of 81% were observed in the iELISA results with the purified recombinant protein antigen – BP26r. Thus, given the serological results obtained with the use of both antigens, only the prevalence data derived from the crude total extract antigen of *B. ovis* were considered for the present epidemiological study in the municipality of Rio Branco, Acre. It is also worth noting that all samples tested with the RBT for the detection of anti-smooth LPS antibodies yielded negative results.

All selected livestock establishments were characterized as traditional rural holdings, with sheep of all age groups present, kept without segregation and not individually identified. The production system was extensive, oriented towards meat production; feeding was based primarily on pasture; reproduction occurred through natural mating; and the animals were raised under rustic conditions, without the implementation of any technical improvements or technological innovations in the production system. Furthermore, no training or updating of procedures was provided to the workers involved in the enterprise.

Table 1 presents the results of the bivariate analysis of the association between the investigated risk factor variables in the rural properties and the seropositivity of sheep for *B. ovis* by iELISA, considering associations statistically significant when $p \leq 0.200$.

In the bivariate analysis, which identifies whether there is an association between the risk factor and infection in animals, the variables "feed supplementation" ($p = 0.009$), "segregation of sick animals" ($p = 0.038$), "veterinary assistance" ($p = 0.096$), "occurrence of abortion" ($p = 0.138$), and "deworming" ($p = 0.154$) were statistically significant at the 20% probability level.

The variables selected in the bivariate analysis were subjected to multivariate logistic regression analysis. The existence of collinearity among the independent variables was assessed, with variance inflation factor values ranging from 1.00 to 1.19, indicating no association among them.

The risk factor "segregation of sick animals" (Table 2) was considered epidemiologically and statistically significant in the multivariate analysis. In general, when dealing with brucellosis, good sanitary management practices (Rossetti et al. 2022) in sheep farming are essential to prevent the dissemination of the infectious agent in small ruminant herds.

The control of *B. ovis* can be based on the identification, isolation, and elimination of seropositive animals. As preventive and control measures, the isolated rearing of young and adult rams is recommended (Embrapa Caprinos e Ovinos 2020), with the separation of sexually active males, in addition to reproductive examination and the gradual elimination of animals presenting palpable testicular lesions or sperm abnormalities (Traldi 2006).

CONCLUSIONS

In the present study, based on serological evidence, we indicate the occurrence of *Brucella ovis* infection in sheep from the municipality of Rio Branco, Acre State, Brazil. The iELISA diagnostic method proved to be sensitive for identifying

sheep with anti-*B. ovis* antibodies. Additionally, the negative serological tests for smooth LPS suggest the maintenance of the exotic status of *Brucella melitensis* in the country.

According to the analyzed risk factors, it is recommended to segregate and gradually cull animals presenting suggestive clinical signs, sperm abnormalities, and/or a laboratory diagnosis of *B. ovis* infection. Other sanitary measures are also relevant and should be encouraged.

As this is the first study conducted in the Western Amazon, complementary approaches are required to achieve a better understanding of the epidemiology of *B. ovis* and the infection profile in the Amazon region, aiming at strategic planning and the implementation of control and prophylactic measures.

Acknowledgments.- The authors thank the undergraduate Veterinary Medicine students of the "Universidade Federal do Acre" for their technical support during field activities.

Conflict of interest statement.- The authors declare no conflicts of interest.

Credit author statement.- Mario C.S. Araújo was responsible for the study conception. All authors contributed to the study design and the initial drafting of the manuscript. Mario C.S. Araújo, Tamyres I.B. Silva, Heloisa S. Maia, Patrícia G. Souza, Pâmela A. Lima, and Renato L. Santos developed the field and/or laboratory methodology. Data analysis was performed by Lorena Y.C. Tomaya, Leonardo A.K. Melchior, and Rafael R. Nicolino. Tamyres I.B. Silva and Renato L. Santos carried out the manuscript revision.

Data availability statement.- The authors report that the study data are available to the scientific community and can be requested by contacting the corresponding author.

REFERENCES

Alton GG, Jones LM, Angus RD, Verger JM. Techniques for the Brucellosis Laboratory. Paris: Institut National de la Recherche Agronomique; 1988.

Baigún R, Conigliaro AS, Luna F. Aislamiento de *Brucella ovis* y control de reaccionantes serológicos en epididimitis ovina. Vet Argentina 2000;17:103-107.

Barrouin-Melo SM, Poester FP, Ribeiro MB, Alcântara AC, Aguiar PHP, Nascimento IL, Schaer RE, Nascimento RM, Freire SM. Diagnosis of canine brucellosis by ELISA using an antigen obtained from wild *Brucella canis*. Res Vet Sci 2007; https://doi.org/10.1016/j.rvsc.2007.02.006

Brasil. Inquérito Soroepidemiológico da Brucelose - Manual de Procedimentos. Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal. Brasília: Ministério da Agricultura, Pecuária e Abastecimento (MAPA); 2002.

Brasil. Instrução Normativa n° 20, de 15 de agosto de 2005. Procedimentos para operacionalização do cadastro sanitário de estabelecimentos de criação de caprinos e ovinos. Secretaria de Defesa Agropecuária (SDA), Ministério da Agricultura, Pecuária e Abastecimento (MAPA), Diário Oficial da União, Brasília; 2005. Accessed on Jul 23, 2024. https://www.gov.br/agricultura/pt-br/assuntos/sanidade-animal-e-vegetal/saude-animal/qualidade-dos-servicos-veterinarios/arquivos/pnsco/in_20_2005_cadastr_est_criacao_caprinos_ovinos.pdf/view

Bulgin MS. *Brucella ovis* excretion in semen of seronegative clinically normal breeding rams. J Am Vet Med Assoc 1990; https://doi.org/10.2460/javma.1990.196.02.313

Clementino IJ, Alves CJ, Azevedo SS, Paulin LM, Medeiros KA. Inquérito soro-epidemiológico e fatores de risco associados à infecção por Brucella ovis em carneiros deslanados do semi-árido da Paraíba. Pesq Vet Bras 2007; https://doi.org/10.1590/S0100-736X2007000400002

Corrente M, Desario C, Parisi A, Grandolfo E, Scaltrito D, Vesco G, Calao V, Buonavoglia D. Serological diagnosis of bovine brucellosis using B. melitensis strain B115. J Microbiol Methods 2015; https://doi.org/10.1016/j.mimet.2015.10.012

Embrapa Caprinos e Ovinos. Brucelose ovina. Sobral: Centro de Inteligência e Mercado de Caprinos e Ovinos (CIM), Empresa Brasileira de Pesquisa Agropecuária (Embrapa); 2020. Accessed on Dec 27, 2023. https://www.embrapa.br/cim-inteligencia-e-mercado-de-caprinos-e-ovinos/zoossanitario-brucelose

Estein SM. Aspectos inmunológicos en el diagnóstico y control de la epidemitis contagiosa del carnero por *Brucella ovis*. Arch Med Vet 1999; https://doi.org/10.4067/S0301-732X1999000100001

Ficapal A, Jordana J, Blasco JM, Moriyón I. Diagnosis and epidemiology of Brucella ovis infection in rams. Small Rumin Res 1998; https://doi.org/10.1016/S0921-4488(97)00108-9

França SA, Mol JPS, Costa EA, Silva APC, Xavier MN, Tsolis RM, Reis JKP, Paixão TA, Santos RL. Indirect ELISA for diagnosis of Brucella ovis infection in rams. Arq Bras Med Vet Zootec 2014; https://doi.org/10.1590/1678-6767

Gan SD, Patel KR. Enzyme immunoassay and enzyme-linked immunosorbent assay. J Investig Dermatol 2013; https://doi.org/10.1038/jid.2013.287

Homse AC, Casaro AP, Campero CM. Infertilidad en ovejas por *Brucella ovis*. Vet Argent 1995; https://www.researchgate.net/publication/277956434_Infertilidad_en_ovejas_por_Brucella_ovis

IDAF-AC. Cadastro oficial de explorações de ovinos. Instituto de Defesa Agropecuária e Florestal do Estado do Acre; 2021. Accessed on Jan 18, 2024. https://idaf.ac.gov.br

Jardim GC, Pires PP, Mathias LA, Ribeiro OC, Kuchembuck MRG. Diagnóstico sorológico da brucelose bovina em animais adultos vacinados com dose reduzida da cepa 19 de *Brucella abortus*. Pesq Vet Bras 2006; https://doi.org/10.1590/S0100-736X2006000300009

Kim D, Park J, Kim SJ, Soh Y-M, Kim HM, Oh B-H, Song J-J. *Brucella* immunogenic BP26 forms a channel-like structure. J Mol Biol 2013; https://doi.org/10.1016/j.jmb.2013.01.015

Kim JH. Multicollinearity and misleading statistical results. Korean J Anesthesiol 2019; https://doi.org/10.4097/kja.19087

Libal MC, Kirkbride CA. *Brucella ovis*-induced abortion in ewes. J Am Vet Med Assoc 1983; https://doi.org/10.2460/javma.1983.183.05.553

Lima AMC, Alves FSF, Alves SM, Santos VWS, Andrioli A, Eloy AMX, Farias DA, Paula NRO, Pinheiro RR. Epidemiological characterization and risk factors associated with *Brucella ovis* infection in sheep from the states of Rio Grande do Norte, Paraíba, and Sergipe. Semina, Ciências Agrárias 2020; https://doi.org/10.5433/1679-0359.2020v41n2p531

Machado G, Santos DV, Kohek I, Steinc MC, Heina HE, Poeta AS, Vidor ACM, Corbellini LG. Seroprevalence of *Brucella ovis* in rams and associated flock level risk factors in the state of Rio Grande do Sul, Brazil. Prev Vet Med 2015; https://doi.org/10.1016/j.prevetmed.2015.05.009

Martins NEX, Almeida JDM, Silva MG, Sousa MG, Mathias LA, Almeida KS. Prevalência de anticorpos anti-*Brucella ovis* e anti-*Brucella abortus* em ovinos do município de Colinas, Tocantins, Brasil. Rev Patol Trop 2013; https://doi.org/10.5216/rpt.v42i2.25525

Muñoz PM, Marín CM, Monreal D, González D, Garin-Bastuji B, Díaz R, Mainar-Jaime RC, Moriyón I, Blasco JM. Efficacy of several serological tests and antigens for diagnosis of bovine brucellosis in the presence of false-positive serological results due to *Yersinia enterocolitica* O:9. Clin Diagn Lab Immunol 2005; https://doi.org/10.1128%2FCDLI.12.1.141-151.2005

OIE. Ovine epididymitis (*Brucella ovis*). v.2, Chapter 3.8.7. Manual of Diagnostic Test and Vaccines for Terrestrial Animals, World Organisation for Animal Health; 2018. Accessed on May 19, 2024. https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.08.07_OVINE_EPID.pdf

Oliveira MZD, Vale V, Keid L, Freire SM, Meyer R, Portela RW, Barrouin-Melo SM. Validation of an ELISA method for the serological diagnosis of

canine brucellosis due to *Brucella canis*. Res Vet Sci 2011; https://doi.org/10.1016/j.rvsc.2010.07.004

Plant JW, Eamens GJ, Seaman JT. Serological, bacteriological and pathological changes in rams following different routes of exposure to *Brucella ovis*. Aust Vet J 1986;63(12):409-412. PMid:3800795

R Core Team. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing; 2024. Accessed on Apr 4, 2024. https://www.R-project.org/

Rahaley RS, Dennis SM, Smeltzer MS. Comparison of the enzyme-linked immunosorbent assay and complement fixation test for detecting *Brucella ovis* antibodies in sheep. Vet Rec 1983;113(20):467-470. PMid:6359661

Ridler AL, Smith SL, West DM. Seroconversion and semen shedding in rams experimentally infected with *Brucella ovis*. N Z Vet J 2014; https://doi.org/10.1080/00480169.2013.836697

Rossetti CA, Maurizio E, Rossi UA. Comparative review of brucellosis in small domestic ruminants. Front Vet Sci 2022; https://doi.org/10.3389/fvets.2022.887671

Rostami S, Rashidian E, Jaydari A, Rahimi H. Investigation of the proportion of *Brucella abortus* and *Brucella melitensis* in sheep and goat milk. Med Vet Int 2023; https://doi.org/10.1155/2023/6751152

Santos FA, Higino SSS, Azevedo SS, Costa DF, Farias AEM, Alves FAL, Paulin LM, Alves CJ. Caracterização epidemiológica e fatores de risco associados à infecção por *Brucella ovis* em ovinos deslanados do semiárido paraibano. Pesq Vet Bras 2013; https://doi.org/10.1590/S0100-736X2013000400008

Sobrinho PAM, Ramos TRR, Fernandes CHC, Campos AC, Costa LM, Castro RS. Prevalência e fatores associados à infecção por lentivírus de pequenos ruminantes em caprinos no Estado do Tocantins. Ciênc Anim Bras 2010; https://doi.org/10.5216/cab.v11i1.3957

Tabasi M, Eybpoosh S, Bouzari S. Development of an indirect ELISA based on whole cell *Brucella abortus* S99 lysates for detection of IgM anti-*Brucella* antibodies in human serum. Comp Immunol Microbiol Infect Dis 2019; https://doi.org/10.1016/j.cimid.2019.01.007

Thrusfield M, Christley R. Veterinary Epidemiology. 2018; https://doi.org/10.1002/9781118280249

Traldi A. Enfermidades de caprinos e ovinos - formas de controle e erradicação. 2006. Accessed on Jan 12, 2024. http://www.montesaltos.com/ENFERMIDADES_DE_CAPRINOS_E_OVINOS.pdf

Xavier MN, Costa ÉA, Paixão TA, Santos RL. The genus *Brucella* and clinical manifestations of brucellosis. Ciência Rural 2009; https://doi.org/10.1590/S0103-84782009005000167