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Epidemiological characterization and risk factors associated with *Brucella ovis* infection in sheep from the states of Rio Grande do Norte, Paraíba, and Sergipe[1]

Caracterização epidemiológica e fatores de risco associados à infecção por *Brucella ovis* em ovinos dos estados do Rio Grande do Norte, Paraíba e Sergipe

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Highlights:
Prevalence of 5.37% (70/1304) for ovine brucellosis in the three northeastern states.
Higher positivity of adults compared to youth (p <0.001-*odds ratio* = 3.41).
Females are significantly more infected than males. (p <0.01-*odds ratio* = 0.41).
Mothers sheep more infected than young animals (p <0.001 - *odds ratio* = 3.64).
Lack of worker training is an associated risk factor (*odds ratio* = 3.68).

Abstract

This study aimed to determine the prevalence of ovine brucellosis in the states of Rio Grande do Norte, Paraíba, and Sergipe. A seroepidemiological survey was carried out in 121 properties under 23 municipalities between 2011 and 2012. The 1,034 serological samples collected from the sheep were subjected to the agar gel immunodiffusion (AGID) test using kits produced by the Paraná Institute of Technology (TECPAR). Anti-*Brucella ovis* antibodies were observed in 5.37% (70/1,304) of the animals evaluated, and the difference in the incidence of brucellosis between the adults and young animals of both sexes, and between the males and females was statistically significant ($p < 0.05$). The prevalence of brucellosis in Rio Grande do Norte, Paraíba, and was Sergipe 7.66% (36/470), 5.40% (13/241) and 3.54% (21/593), respectively. Of the municipalities visited, 91.30% (21/23) harbored

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herds with positive serology, and 42.15% (51/121) of the properties had at least one seropositive animal. The lack of training among the workers ($p \leq 0.05$) (*odds ratio* = 3.68; 95% CI = 1.08-12.60; p = 0.038) was identified as a risk factor. The results obtained in this study indicate that the sheep from Rio Grande do Norte, Paraíba, and Sergipe are infected by *B. ovis*, thus necessitating the adoption of proper sanitary measures for preventing the spread of the disease in the herds.
Key words: Ovine brucellosis. Epidemiology. AGID. Semi-arid. Ovine culture.

Resumo

O estudo teve como objetivo determinar a prevalência da Brucelose Ovina nos Estados do Rio Grande do Norte, Paraíba e Sergipe. Foi realizado o levantamento soroepidemiológico em 121 propriedades de 23 municípios durante os anos de 2011 e 2012. As 1.034 amostras sorológicas de ovinos foram submetidas à técnica de Imunodifusão em Gel de Ágar (IDGA), utilizando o kit produzido pelo Instituto de Tecnologia do Paraná (TECPAR). Anticorpos anti-*Brucella ovis* foram observados em 5,37% (70/1.304) dos animais testados, havendo diferença estatística significativa ($p < 0,05$) para adultos e jovens de ambos os sexos, e entre machos e fêmeas. A prevalência encontrada no Estado do Rio Grande do Norte, Paraíba e Sergipe foi de 7,66% (36/470), 5,40% (13/241) e 3,54% (21/593), respectivamente. Dos municípios visitados, 91,30% (21/23) eram compostos por rebanhos com sorologia positiva e 42,15% (51/121) das propriedades possuíam pelo menos um animal soropositivo. A falta de capacitação de trabalhadores ($p \leq 0,05$) (*odds ratio* = 3,68; IC 95% = 1,08-12,60; p = 0, 038) foi identificada como fator de risco. Os resultados obtidos neste trabalho indicam que a infecção por *B. ovis* encontra-se presente nos ovinos dos estados do Rio Grande do Norte, Paraíba e Sergipe, sendo necessária a adoção de medidas sanitárias para evitar a propagação da doença nos rebanhos.
Palavras-chave: Brucelose ovina. Epidemiologia. IDGA. Semiárido. Ovinocultura.

Introduction

Brucellosis in sheep is an infectious and chronic disease caused by *Brucella ovis*, and is responsible for causing epididymal lesions in sheep, placentitis, abortion in mothers, and high lamb mortality (World Organization for Animal Health [OIE], 2015).

Ovine brucellosis was first reported in Brazil in the state of Rio Grande do Sul by Ramos et al. 1966. Later, Blobel, Fernandes, Mies, Ramos and Trein (1972), also reported incidences of ovine brucellosis in Rio Grande do Sul, and isolated the agent from sheep with clinical epididymal lesions.

Serological studies have demonstrated that *B. ovis* infections in Brazil are distributed in herds. The prevalence of ovine brucellosis ranges from 0.72% to 16.25% in the Northeastern regions of Brazil (Coleto et al., 2003; Souza et al., 2012).

The disease is predominantly diagnosed by serological tests. For ensuring safer results, it is desirable to associate the results of the serological tests with the history of the herd, the clinical picture, the origin of the animals, and incidences of the disease in the nearby regions.

The most commonly serological tests used for the serological diagnosis of *B. ovis* infections are Complement Fixation Reaction (CFR), Agarose Gel Immunodiffusion (AGID), and Enzyme-Linked Immunosorbent Assay (ELISA) (Xavier et al., 2011; França et al., 2014; F. S. F. Alves et al., 2017). The tests can be associated with bacteriological tests and examination with Polymerase Chain Reaction (PCR) in order to increase the diagnostic efficiency (Xavier et al., 2011).

AGID is a test with favorable cost benefit, since it has a good sensitivity and specificity of 96.4% and 100%, respectively, low cost, and is easy to interpret (Myers & Siniuk, 1970; Marín et al., 1989).

Considering the importance of sheep exploration in Northeastern Brazil and the impact of brucellosis

in sheep breeding, this study aimed to investigate the epidemiological aspects and risk factors associated with the disease in the states of Paraíba, Rio Grande do Norte, and Sergipe.

Material and Methods

This study was conducted in the states of Rio Grande do Norte, Paraíba, and Sergipe, which together represent 8.8% of the total Brazilian herds (Instituto Brasileiro de Geografia e Estatística [IBGE], 2017). The mesoregions and municipalities of the three states were selected according to the animal density, organizational structure of the herds, and support from institutional structures.

A total of 470 serological samples were collected in May 2011 from the sheep harbored in 47 properties under seven municipalities of Rio Grande do Norte. A total of 241 samples were collected from 24 herds harbored under eight municipalities of Paraíba, and were subsequently analyzed in July 2011. A total of 593 samples were collected in February 2012 from the sheep harbored in 50 properties under eight municipalities of Sergipe.

The municipalities visited in Rio Grande do Norte included the municipalities of Apodi, Caraúbas, Mossoró (West Potiguar mesoregion), Afonso Bezerra, Angicos, Lages, and Pedro Avelino (Central Potiguar mesoregion). The municipalities visited in Paraíba included the municipalities of Cacimba de Areia, Pombal, Passagem, Quixaba (Sertão Paraibano mesoregion), São João do Cariri, Sumé, Monteiro, and Prata (Borborema mesoregion). The municipalities visited in Sergipe included the municipalities of Nossa Senhora da Glória, Canindé de São Francisco, Poço Redondo, Gararu (Sertão Sergipano mesoregion), Poço Verde, Simão Dias, Lagarto, and Tobias Barreto (Agreste Sergipano mesoregion).

The minimum number of samples was statistically calculated according to the methodology provided by Thrusfield (2007). An expected minimum prevalence of 8%, a sample error of 30%, and a confidence level of 95% (z = 1.96) were considered for the statistical analyses. Ten to twelve serological samples were tested from each of the properties visited.

For data collection, a questionnaire containing questions pertaining to the general status, sanitary conditions, food, and reproductive management of the herds was created. The questionnaire was answered *in loco* by a group of properly trained and leveled technicians and fellows of Embrapa Goats and Sheep. This information allowed mapping the health profiles of the herds, and analysis of the possible risk factors associated with the incidence of ovine brucellosis in the herds.

Univariate and multivariate analyses of the risk factors related to disease incidence were performed with SPSS version 20.0 for Windows OS. During univariate analysis, each independent variable was crossed with the dependent variable. The variables that had values of $p \leq 0.2$ in the Chi-squared test (Zar, 1999) were selected and subjected to multivariate analysis. Logistic regression was used (Hosmer & Lemeshow, 2000) to define a model that best identified the risk factors. The final fit of the model was assessed with the Hosmer and Lemeshow test of goodness of fit, in which a $p \geq 0.05$ indicated that the model is well-fitted to the data.

Blood samples were collected by venipuncture of the jugular vein using a vacuum tube. Following blood collection, the samples were properly identified, stored under refrigeration, and taken to Embrapa Goats and Sheep in Sobral, in Ceará, where they were stored at -20°C till experimentation. The samples from Rio Grande do Norte and Paraíba were analyzed in October 2011, while the serological samples collected from the herds in Sergipe were analyzed in May 2012.

Serological testing was performed by AGID for detecting anti-*B. ovis* antibodies using the commercial diagnostic kit for *B. ovis*, and a soluble antigen comprising proteins and lipopolysaccharides

obtained from the *B. ovis* Reo 198 strain, produced by the Paraná Institute of Technology (TECPAR). The test was performed according to the manufacturer's instructions.

Results

The results indicated that the overall prevalence of ovine brucellosis in the three Northeastern states was 5.37% (70/1304) and 42.15% (51/121) of the properties harbored at least one animal seropositive for *B. ovis*. It was also found that the animals in Rio Grande do Norte were more seropositive for *B. ovis* compared to the animals in Sergipe ($p < 0.05$) (Table 1).

The total number of seropositive animals in the three states studied herein was categorized according to the age, sex, and animal group, and depicted in Table 2. A higher number of adult animals (7.08%; 60/847) were found to be seropositive for *B. ovis* ($p < 0.001$ - *odds ratio* = 3.41; 95% CI = 1.73 - 6.72) in comparison to the young animals (2.19%; 10/457). This may be attributed to the fact that adults are more likely to come in contact with the sources of infection.

Table 1
Prevalence of ovine brucellosis per animal and per herd in the states of Rio Grande do Norte, Paraíba, and Sergipe (2019)

State	Prevalence per animal		Prevalence per herd	
	Positive	Negative	Positive	Negative
	N (%)	N (%)	N (%)	N (%)
Rio Grande do Norte	36 (7.66)[a]	434 (92.34)	25 (53.20)[a]	22 (46.80)
Paraíba	13 (5.40)[ab]	228 (94.60)	9 (37.50)[a]	15 (62.50)
Sergipe	21 (3.54)[b]	572 (96.46)	17 (34.0)[a]	33 (66.00)
Total	70 (5.37)	1.234 (94.63)	51 (42.15)	70 (57.85)

[a]The different letters in the same column indicate significant differences as calculated by the Chi-squared test ($p < 0.05$).

Table 2
Categorization of the incidence of ovine brucellosis by sex, age, animal category, and breed in the states of Rio Grande do Norte, Paraíba, and Sergipe (2019)

Classification by	Animal group	Result of AGID test		Total
		Positive	Negative	
		N (%)	N (%)	
Age	Adult (over 12 months)	60 (7.08)[a]	787 (92.92)	847
	Young (between 6 and 12 months)	10 (2.19)[b]	447 (97.81)	457
Sex	Male	15 (2.96)[a]	492 (97.04)	507
	Female	55 (6.90)[b]	742 (93.10)	797
Category	Mother	52 (7.54)[a]	638 (92.46)	690
	Father	8 (5.10)[ab]	149 (94.90)	157
	Young	10 (2.19)[b]	447 (97.81)	457

[a]The different letters in the same column indicate significant differences as calculated by the Chi-squared test ($p < 0.05$).

There was a significant difference in the seropositivity for *B. ovis* with respect to the gender of the animals ($p < 0.01$ - *odds ratio* = 0.41; 95% CI = 0.23 - 0.74), and while 6.90% (55/797) of the female animals were seropositive for *B. ovis*, only 2.96% (15/507) of the male animals had positive serology (Table 2).

In this study, 2.19% of the seropositive samples were from young animals. However, the mother animals represented the group with the highest number of seropositive animals (7.54%). Of the three categories evaluated, the mothers and young animals were statistically different from each other ($p < 0.001$ - *odds ratio* = 3.64; 95% CI = 1.83 - 7.25) (Table 2).

It was observed that 43.75% (42/96) of the properties harbored seropositive animals that were used in an extensive/semi-extensive breeding system. The other 36.00% (9/25) of the properties used the animals in an intensive/semi-intensive sheep breeding system (Table 3).

The variables for multiple analysis were selected by analyzing the risk factors, with the lack of training among the workers being an associated risk factor (*odds ratio* = 3.68; 95% CI = 1.08 - 12.60) (Table 4). A total of 62.81% (76/121) of the farmers reported they were not qualified for performing the activity, against 37.19% (45/121) of the farmers who reported being somewhat qualified.

Table 3
Variables associated with the incidence of *B. ovis* infections in properties harboring sheep, obtained by univariate analysis, in the states of Rio Grande do Norte, Paraíba, and Sergipe (2019)

Variables	Total No. of Properties	No. of Properties with seropositive sheep	*Odds ratio*	95% CI	p-Value
	N (%)	N (%)			
Animal identification					
No	92 (76.03)	38 (41.30%)	0.866	0.373-2.009	0.738*
Yes	29 (23.97)	13 (44.83%)			
Presence of management center (cradle/sty)					
No	9 (7.44)	3 (33.33%)	0.667	0.159-2.801	0.732**
Yes	112 (92.56)	48 (42.86%)			
Cleaning of facilities					
No	5 (4.13)	3 (60.00%)	2.125	0.342-13.207	0.649**
Yes	116 (95.87)	48 (41.38%)			
Breeding purpose					
Beef	102 (84.30)	43 (42.16%)	1.002	0.3717-2.702	0.997*
Mixed	19 (15.70)	8 (42.10%)			
Continuation					
Breeding system					
Intensive/ Semi-intensive	25 (20.66)	9 (36.00%)	1.383	0.556-3.438	0.485*
Extensive/ Semi-extensive	96 (79.34)	42 (43.75%)			
Production animals					
Sheep	16 (13.22)	6 (37.5%)			
Sheep and goats	18 (14.88)	4 (22.22%)	*	*	0.208*
Sheep and cattle	29 (23.97)	12 (41.38%)			
Sheep, goats, and cattle	58 (47.93)	29 (50.00%)			

continue

continuation

Breed					
Non-exotic	117 (96.70)	49 (41.88%)	1.388	0.189-10.194	1.000**
Exotic	4 (3.30)	2 (50.00%)			
Trained workers					
No	76 (62.81)	36 (47.37%)	1.800	8.37-3.872	0.131*
Yes	45 (37.19)	15 (33.33%)			
Acquisition of animals from known and neighboring herds					
No	21 (17.36)	12 (57.14%)	0.480	0.185-1.244	0.126*
Yes	100 (82.64)	39 (39.00%)			
Acquisition of animals from fairs, unknown herds, or exhibitions					
No	71 (58.68)	33 (47.48%)	0.648	0.308-1.361	0.250*
Yes	50 (41.32)	18 (36.00%)			
Fathers from the herd					
No	93 (76.86)	40 (43.01%)	1.166	0.492-2.763	0.726*
Yes	28 (23.14)	11 (39.29%)			
Mothers from the herd					
No	16 (13.22)	4 (25.00%)	0.411	0.124-1.359	0.136*
Yes	105 (86.78)	47 (44.76%)			
Separation of animals by age					
No	111 (91.74)	47 (42.34%)	1.102	0.294-4.124	1.000**
Yes	10 (8.26)	4 (40.00%)			
Separation of females before calving					
No	47 (38.84)	17 (36.17%)	0.667	0.315-1.412	0.289*
Yes	74 (61.16)	34 (45.95%)			
Care is taken when including new animals					
No	96 (79.34)	39 (40.63%)	0.741	0.306-1.794	0.506*
Yes	25 (20.66)	12 (48.00%)			
Animal vaccination					
No	51 (42.15)	26 (50.98%)	1.872	0.898-3.905	0.093*
Yes	70 (57.85)	25 (35.71%)			
Observation of deworming practices					
No	5 (4.13)	3 (60.00%)	2.125	0.342-13.207	0.649**
Yes	116 (95.87)	48 (41.38%)			
Observation of reproductive practices					
Controlled Natural Breeding	32 (26.45)	17 (53.13%)	0.545	0.241-1.233	0.143*
Uncontrolled Natural Breeding	89 (73.55)	34 (38.20%)			
Promotion of breeding season					
No	89 (73.55)	34 (38.20%)	0.545	0.241-1.233	0.143*
Yes	32 (26.45)	17 (53.13%)			
Criteria for the first mating of females					
No	89 (73.55)	39 (43.82%)	0.769	0.336-1.769	0.535*
Yes	32 (26.45)	12 (37.50%)			

continue

continuation

Adoption of neonatal care					
No	45 (37.19)	16 (35.56%)	0.646	0.303-1.381	0.258*
Yes	76 (62.81)	35 (46.05%)			
Navel treatment					
No	46 (38.02)	16 (34.78%)	0.610	0.286-1.300	0.199*
Yes	75 (61.98)	35 (46.67%)			
Mortality rate					
0 to 10	57 (47.11)	26 (45.61%)			
11 to 40	51 (42.15)	21 (41.18%)	*	*	0.711*
41 to 60	8 (6.61)	3 (37.50%)			
Over 60	5 (4.13)	1 (20.00%)			
Death before weaning					
No	50 (41.32)	17 (34.00%)	1.784	0.844-3.768	0.128*
Yes	71 (58.68)	34 (47.89%)			
Supplementation					
No	42 (34.71)	15 (35.71%)	0.664	0.307-1.435	0.296*
Yes	79 (65.29)	36 (45.57%)			
Supply of mineral salt					
No	21 (17.36)	9 (42.86%)	1.036	0.400-2.681	0.942*
Yes	100 (82.64)	42 (42.00%)			
Native pasture - Caatinga					
No	11 (9.09)	6 (54.55%)	1.733	0.499-6.027	0.383*
Yes	110 (90.91)	45 (40.91%)			

* Variables selected by the Chi-squared test ($p \leq 0.20$).
** Variables selected by Fisher's exact test ($p \leq 0.20$).

Table 4
Variables associated with the incidence of *B. ovis* infection in the sheep from the states of Rio Grande do Norte, Paraíba, and Sergipe, as estimated by logistic regression

Risk factors	*Odds ratio*	95% CI		p-Value
Lack of training among workers	3.68	1.08	12.60	0.038

Discussion

The prevalence of ovine brucellosis determined in this study was consistent with the results of the study by J. B. A. Silva, Feijó, Teixeira and Silva (2003), who reported that 34.48% (100/290) of the animals in Rio Grande do Norte are seropositive for *B. ovis*, using the AGID test. Using anti-*B. ovis* antibodies, Azevedo et al. (2004) reported that 11.30% (13/115) of the 115 sheep tested from Rio Grande do Norte are seropositive for *B. ovis*. Clementino, Alves, Azevedo, Paulin and Madeiros (2007) observed that 5.62% (28/498) of the sheep from Paraíba are seropositive for *B. ovis*, and found that 8.83% (25/283) of the properties harbor at least one seropositive animal. In the study by Santos et al. (2013), 1,134 woolless sheep from Paraíba were evaluated for *B. ovis* infections, which revealed that 20.39% (21/103) of the properties in Paraíba contain seropositive animals, and 5.20% (59/1,134) of the animals are seropositive for *B. ovis*. Mendonça et al. (2017) reported that 4.40% (41/932) of the sheep from the 46.30% (25/54) properties evaluated in Sergipe are seropositive for *B. ovis*. Souza et al. (2012) reported a prevalence of 0.72%

seropositivity among the animals in Bahia, while J. R. A. Alves et al. (2017) reported a prevalence of 5.88% (7/119) seropositivity in the sheep sold at the animal fairs of Sertão of Pernambuco. In Teresina, state of Piauí, 4.6% of the samples were reported to be seropositive for *B. ovis*, following testing with anti-*B. ovis* antibodies (G. A. Silva et al., 2017).

The results of previous studies corroborate the observations of the present study. However, they differ from the present study with respect to the strategies used for selecting the mesoregions, municipalities, properties, and samples collected. Studies conducted by Batista (2012) on the prevalence of *B. ovis* infections in the sheep from the states of Ceará and Piauí, reported a 8.21% (70/852) seropositivity. By analyzing the serological samples of sheep from Piauí using the AGID test, Costa et al. (2012) observed that 17.8% (16/90) of the animals were seropositive for *B. ovis* and 12.9% (4/31) of the properties harbored seropositive animals.

Marinho and Mathias (1996) did not find any seropositive animals in the State of São Paulo using the AGID test. The differences in the prevalence of *B. ovis* could be associated with the breeding systems used and the specific characteristics of each of the regions in the country. This agrees with the study by Salaberry, Paulin, Santana, Castro and Lima-Ribeiro (2011), who did not find any seropositive sheep (0/334) in the municipality of Uberlândia in the state of Minas Gerais.

Analysis of the number of seropositive animals categorized by age, sex, and animal group in the three states covered by this study, reveal that a higher number of adult sheep (7.08%; 60/847; $p < 0.05$) were seropositive for *B. ovis*, compared to the young animals (2.19%; 10/457). This may be due to the fact that the adult animals are more likely to come in contact with the sources of infection. The presence of seropositive young animals aged less than one year may be partly due to the transmission of the infectious agent through the milk of the infected mother sheep (Clementino et al., 2007).

There was a significant difference in seropositivity with respect to the gender of the animals ($p < 0.05$), since 6.90% (55/797) of the females were found to be seropositive for *B. ovis*, while only 2.96% (15/507) of the male animals showed positive serology. This could be attributed to the fact that the infected sheep released the bacteria through vaginal secretions, placenta, aborted fetus, and milk (Libal & Kirkbride, 1983; Homse, Casaro, & Campero, 1995; Estein, 1999; Baigún, Conigliaro, & Luna, 2000), thus contaminating the environment and promoting the dissemination of the microorganism in the herd, since oral and nasal mucous membranes and wounded skin serve as entry points for *B. ovis* (Plant, Eamens, & Seaman, 1986; Alton, Jones, Angus, & Verger, 1988; Bulgin, 1990). These factors could also be responsible for the incidence of ovine brucellosis in the young animals.

Similar results were reported by Pinheiro et al. (2009), who observed that 1.43% (1/70) of the males were seropositive for *B. ovis*, while 3.34% (17/509) of the female animals in the State of Alagoas were seropositive for the bacteria.

However, studies by J. B. A. Silva et al. (2003) and Azevedo et al. (2004) in the state of Rio Grande do Norte, by Batista (2012) in Ceará and Piauí, by N. S. Silva et al. (2009) in the state of Bahia, and by Manhezzo, Conceição and Castro (2015) in Mato Grosso reported no difference ($p \geq 0.05$) in the incidence of ovine brucellosis between the sexes. However, it is important to note that the exchange, loan, and sale of breeding sheep among sheep owners is a common practice in Northeastern Brazil. Thus, the infected sheep play a crucial role in spreading the disease in the herds (Paolicchi et al., 1999).

In this study, 2.19% of the young animals were found to be seropositive for *B. ovis*. However, the category with the highest number of seropositive animals comprised the mother sheep (7.54%). Of the three categories evaluated, the mothers and the young animals were statistically different from each other ($p < 0.05$).

Females play an important role in spreading the disease in the herd, as they release the bacteria via their vaginal secretions, thus contaminating the males. The exposure of the young animals to the bacteria is therefore lesser than the adults owing to their limited sexual experience, and the exposure of the older animals is also limited by their decreased sexual activity (Ficapal, Jordana, Blasco, & Moriyón, 1998).

Rizzo et al. (2009) analyzed 22 males and 182 females with a history of reproductive disorders in São Paulo using the AGID test and found 4 (1.96%) females to be seropositive for *B. ovis*.

It was observed that 43.75% (42/96) of the properties that harbored seropositive animals used an extensive/semi-extensive breeding system. The other 36.00% (9/25) of the properties used an intensive/semi-intensive system for sheep breeding (Table 3).

Magalhães and Gil-Turnes (1996) observed a higher prevalence of *B. ovis* in the animals that were kept in huts (intensive) in comparison to those that remained in the fields (extensive), which explains the results with a higher concentration of animals. These results were also corroborated by the results of the study by J. R. A. Alves et al. (2017), wherein intensive breeding was observed to be a risk factor (*odds ratio* = 11.5; p = 0.005) for the incidence of *B. ovis* infections in the sheep from the state of Sertão of Pernambuco.

Pinheiro et al. (2009) found a higher incidence of seropositivity in the animals that were harbored in the extensive systems of Alagoas, while Clementino et al. (2007) observed no significant differences in the seropositivity for *B. ovis* between animals harbored in extensive systems and those harbored in the intensive/semi-intensive systems of Paraíba.

According to Souza et al. (2012), extensive systems predominate in the microregion of Juazeiro da Bahia, where the prevalence of brucellosis is considered to be low (0.72%). The authors further discuss that the type of system cannot be analyzed in isolation, although it can be considered to be a predisposing factor. This demonstrates the importance of associating the property information with the presence of seropositive animals identified by serological tests, in identifying the primary points of disease entry and studying the permanence in the herd.

The variables for multiple analysis were selected by analyzing the risk factors, which revealed that the lack of training among the workers is an associated risk factor (*odds ratio* = 3.68; 95% CI = 1.08-12.60). It was observed that 62.81% (76/121) of the proprietors affirmed that they had not received any training for performing the activity, while 37.19% (45/121) confirmed that they had received some form of training.

The climatic conditions of the region, particularly during the rainy season, can make it difficult for the workers to clean the shelves, pens, and pastures, thus contributing to the survival of *B. ovis* and increasing the number of seropositive animals (Martins et al., 2013).

Although no differences ($p \geq 0.05$) were observed among the properties harboring sheep with or without ovine brucellosis, cleaning the facilities is a basic measure recommended for combating the dissemination of diseases. According to Santos et al. (2013), the properties in which the facilities were cleaned on an annual basis had 50% seropositivity, with an *odds ratio* of 7.13. On the other hand, the incidence of seropositivity in the properties that promoted daily and/or monthly cleaning was 17.1%.

Another important factor in the dissemination of diseases, especially ovine brucellosis, is animal traffic, which is frequent in the Northeastern region, where the entry of sheep with unknown health status is favored. The acquisition, loan, consortium, and/or exchange of animals in this region are important factors in the eco-epidemiology of ovine brucellosis, especially among father animals. However, no statistically significant differences were observed in the parameters evaluated in this study, with respect

to the origin and acquisition of animals. The practice of selling animals without ensuring proper sanitary control may favor the spread of *B. ovis* among the herds in the region (Clementino et al., 2007).

Santos et al. (2013) observed that animal acquisition is a potential risk factor for disease incidence, since 27.6% of the properties that acquired animals showed seropositivity, in comparison to the 12.8% seropositivity observed in the properties that did not acquire animals. These studies emphasize the significance of intensifying the inspection of animals participating in events such as fairs and exhibitions, among others, and of the animals being brought from regions reported to harbor ovine brucellosis or have unknown health status, although the results of this study do not indicate any differences in disease incidence with respect to animal acquisition ($p \geq 0.05$).

Ficapal et al. (1998) observed a higher seropositivity in imported breeds in comparison to that observed in the local animal breeds. However, no differences were observed in this study ($p \geq 0.05$) with respect to the animal breeds, probably due to the small number of imported breeds considered herein.

Conclusions

Ovine brucellosis is prevalent in the states of Rio Grande do Norte, Paraíba, and Sergipe, and the lack of training among the sheep breeding workers in Northeastern Brazil is an important risk factor associated with the occurrence of the disease. The state of Rio Grande do Norte has the highest prevalence of seropositivity for *B. ovis*, followed by the states of Paraíba and Sergipe. It is therefore suggested that the official institutions should implement proper sanitary measures, promote awareness about ovine brucellosis among sheep producers, and arrange programs for training the technicians, with the aim of controlling and eradicating the disease in the Northeastern states of Brazil.

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