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Prevalence and risk factors for bovine brucellosis in the state of Alagoas, Brazil

Prevalência e fatores de risco para a brucelose bovina no estado de Alagoas, Brasil

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Highlights

First estimate of prevalence of infected herds and animals for the entire state.

It is the first study to specify brucellosis risk factors for the state.

High quality epidemiological data to guide the decision making.

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Abstract

In order to plan the fight against bovine brucellosis in the state of Alagoas, Brazil, a cross-sectional study was carried out between April and October 2018 to estimate the prevalence of infected herds and animals, as well as the risk factors associated with the disease. Through a two-stage sampling design, blood samples were collected from 3,046 bovine females aged ≥ 24 months from 634 herds, in two different regions of the state. A questionnaire was applied at each farm to assess the association of possible risk factors with the disease. All selected animals were screened by the Rose Bengal test followed by retesting of positives by the Complement Fixation test. For the state, the prevalence of infected herds was estimated at 3.2% [2.1; 4.9] and that of seropositive animals at 0.9% [0.5; 1.4], with no statistical difference between regions. The risk factors identified were pasture rent (OR = 3.11 [1.28; 7.37]) and herd size equal to or greater than 14 females aged ≥ 24 months (OR=4.91 [2.02; 11.66]). It was recommended that the state of Alagoas develop health education action with the beef and dairy cattle production chains, so that producers avoid renting pastures or start practicing it with sanitary care for brucellosis and that they also observe these same concerns when introducing animals in their herds. In addition, the state should consider the convenience of structuring a surveillance system aiming at the eradication of bovine brucellosis, given the low prevalence of infected herds and seropositve animals.
Key words: Bovine brucellosis. Prevalence. Risk factors. Alagoas. Brazil.

Resumo

Para planejar o combate à brucelose bovina no estado de Alagoas, Brasil, entre abril e outubro de 2018 foi realizado um estudo seccional para estimar a prevalência de focos e de animais, além dos fatores de risco associados à doença. Através de amostragem em dois estágios foram coletadas amostras de sangue de 3.046 fêmeas bovinas com idade de ≥ 24 meses de 634 propriedades, oriundas de duas regiões do estado. Um questionário foi aplicado em cada propriedade para avaliar a associação de possíveis fatores de risco com a doença. Os animais selecionados foram submetidos ao diagnóstico sorológico para brucelose (teste Rosa Bengala e Fixação do Complemento, em série). Para o estado, a prevalência de focos foi estimada em 3,2% [2,1; 4,9] e a de animais soropositivos em 0,9% [0,5; 1,4], não havendo diferença estatística entre as regiões. Os fatores de risco identificados foram o aluguel do pasto (OR = 3,11 [1,28; 7,37]) e o tamanho do rebanho igual ou superior a 14 fêmeas com idade ≥ 24 meses (OR=4,91 [2,02; 11,66]). Foi recomendado que o estado de Alagoas desenvolvesse ações de educação sanitária para que os criadores de bovinos evitem o arrendamento de pastagens ou passem a praticá-lo com cuidados sanitários para a brucelose e que também observem esses mesmos cuidados ao introduzir animais em seus rebanhos. Além disso, o estado deve considerar a conveniência de estruturar um sistema de vigilância visando a erradicação da brucelose bovina, tendo em vista a baixa prevalência de focos e de animais soropositivos.
Palavras-chave: Brucelose bovina. Prevalência. Fatores de risco. Alagoas. Brasil.

Introduction

Bovine brucellosis is an anthropozoonosis that also leads to abortion, infertility, and consequently, decreased milk and meat production (Paulin & Ferreira, 2003). The disease is worldwide distributed, even though several countries in Western and Northern Europe, Canada, Japan, Australia, and New Zealand have self-declared free from bovine brucellosis (World Organization for Animal Health [WOAH], 2023). In South America, home to 25% of the world's cattle population and with great expression in the global meat market, the situation is heterogeneous among countries and only Uruguay and Chile are conducting eradication strategies (Ferreira, 2018).

In Brazil, the National Program for the Control and Eradication of Brucellosis and Tuberculosis (Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose [PNCEBT]) was launched in 2001 by the Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento [MAPA]) (Instrução Normativa n. 2, 2001), to reduce the prevalence and incidence of these diseases. As information on the epidemiological situation of diseases in the country was scarce, state studies were conducted with a standardized methodology to generate high quality information to help choose the best strategies and to evaluate the effectiveness of their implementation. To date, the epidemiological situation of bovine brucellosis has been well characterized in 18 states, covering 85% of the Brazilian cattle population, with heterogeneous prevalence between states and, in some cases, within the same state (Alves et al., 2009; Azevedo et al., 2009; Chate et al., 2009; Dias et al., 2009a,b; Gonçalves et al., 2009a,b; Klein-Gunnewiek et al., 2009; Marvulo et al., 2009; Negreiros et al., 2009; Ogata et al., 2009; Poester et al., 2009; Rocha et al., 2009; Sikusawa et al., 2009; V. G. S. O. Silva et al., 2009; Villar et al., 2009; Borba et al., 2013; Almeida et al., 2016; Clementino et al., 2016; Ferreira et al., 2016).

Vaccination of females with bovine brucellosis strains B19 and RB51 was recommended in regions with high and moderate prevalence. Approximately ten years later, the states of Minas Gerais, Rondônia, Mato Grosso, Mato Grosso do Sul, Santa Catarina, São Paulo, Espírito Santo, Rio Grande do Sul, Paraná, and Tocantins conducted a second study to assess the efectiveness of the implemented strategies. Of these, only Minas Gerais, Mato Grosso, Mato Grosso do Sul, Rondônia, and Tocantins found a decreased prevalence of infected herds due to vaccination (Anzai et al., 2016; Barddal et al., 2016; Dias et al., 2016; Inlamea et al., 2016; Leal et al., 2016; Oliveira et al., 2016; Rodrigues et al., 2021; N. S. Silva et al., 2016; Vendrame et al., 2021). The state of Santa Catarina, where vaccination has been banned since 2006 (Portaria SAR nº 17, 2012), also carried out a second study (Baumgarten et al., 2016), but with the aim of confirming the very low prevalence in order to implement eradication strategies safely (Portaria SAR nº 17, 2012).

Epidemiological information on bovine brucellosis in the state of Alagoas is extremely limited, with reports on the occurrence of the desease only at municipal or regional levels (Tenório et al., 2004; K. P. C. Silva et al., 2011; Farias et al., 2019), with the use of different methodologies, which hinder decision-making for the entire state.

Cattle farming is practiced in all 102 municipalities of Alagoas and, therefore, is relevant for its economy (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2018), although the state has only 1.1 million heads, distributed over its 27,843.3 km2, representing 0.5% of the national herd (Instituto Brasileiro de Geografia e Estatística [IBGE], 2019).

Thus, given the lack of accurate information on bovine brucellosis in the state of Alagoas, the objectives of this study was to estimate the prevalence of infectd herds and seropositive animals for bovine brucellosis in the state, and to specify risk factors associated with the disease, aiming at guiding the decision-making process and the implementation of public policies.

Material and Methods

Study design

The study was planned by the Collaborating Center for Animal Health of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo, together with the MAPA and the Agency of Sanitary Defence of Agriculture and Livestock of the state of Alagoas (Agência de Defesa e Inspeção Agropecuária do Estado de Alagoas [ADEAL]), the latter being responsible for the fieldwork, conducted from April to October 2018.

The state of Alagoas was divided into regions based on the predominant production and commercialization systems. For each region, a sample was designed in two stages.

In the first stage, a pre-established number of properties with reproductive activity were randomly drawn, determined by the formula for simple random samples (Thrusfield & Christley, 2018). The parameters used for calculation were a confidence level of 0.95, an estimated prevalence of 0.25, and an error of 0.05. The herd that for some reason could not be sampled was replaced by a new draw.

The concepts of herd sensitivity and specificity were used for the second sample stage (Dohoo et al., 2007), estimating a minimum number of animals to be sampled within each herd to allow their classification as infected or non-infected with brucellosis. The following assumptions were used for the calculations: estimated intra-herd prevalence=0.20, sensitivity of the diagnostic protocol=0.9543, specificity of the diagnostic protocol=0.9993. The serological diagnostic protocol used was serial testing, with screening by the Rose Bengal test (0.962 sensitivity and 0.971 specificity [Davies, 1971]) and retesting of positives by the Complement Fixation test (0.992 sensitivity and 0.975 specificity [MAPA, 2016]). The sensitivity and the specificity of the diagnostic protocol was calculated at the Epitools software (Sergeant, 2018a).

The calculations were performed at the Epitools software (Sergeant, 2018b) to estimate a sample size that would allow herd sensitivity and specificity equal to or greater than 0.89 and 0.99, respectively.

Thus, ten animals were sampled in properties with up to 99 females aged over 24 months, and 15 in those with 100 or more. The females were randomly chosen within the herds, excluding those in the peripartum and post-abortion period.

An epidemiological questionnaire was administered at each sampled herd to identify risk factors associated with the presence of brucellosis. Based on the literature, questions were asked about the type of exploration or production system, type of faring, type and number of milking procedures, use of artificial insemination, herd size, presence of other domestic and wild species, destination of placentas and aborted fetuses, animal purchase and sale, brucellosis vaccination, diagnosis of brucellosis, and indirect contact between herds.

Blood sampling and serological tests

A 10 mL blood sample was collected from the selected animals by puncturing the jugular vein with a sterile disposable needle and stored in a previously identified vacuum tube. The sera were stored in plastic microtubes and kept at -20°C until testing, according to the MAPA protocol: screening by the Rose Bengal test and retesting of positives by the Complement Fixation test (Instrução Normativa n. 10, 2017; Instrução Normativa n. 34, 2017).

Data analysis

Prevalence and respective confidence intervals were calculated according to Dean et al. (1996). Animal and infected herd prevalence values for the state and animal prevalence within the regions were weighted according to Dohoo et al. (2007).

The weight of each property used to calculate the prevalence of outbreaks in the state was given by:

$$P_1 = \frac{\text{Properties with reproductive activity in the region}}{\text{Sampled properties with reproductive activity in the region}}$$

The weight of each animal used to calculate the prevalence of animals in the state was given by:

$$P_2 = \frac{\text{Females} \geq 24 \text{ months in the property}}{\text{Females} \geq 24 \text{ months sampled in the property}} \times \frac{\text{Females} \geq 24 \text{ months in the region}}{\text{Females} \geq 24 \text{ months in the properties sampled in the regions}}$$

In the equation above (P2), the first term refers to the weight of each animal used to calculate the prevalence of animals in each region.

For the analysis of risk factors, chi-square or Fisher's exact test was used first followed by logistic regression, according to Hosmer and Lameshow (1989). The exploratory univariate data analysis was performed and variables were selected with $p \leq 0.20$ for subsequent use in the multivariate analysis using the stepwise forward method with R software (R Core Team [R], 2016). Only the variables with $p \leq 0.05$ remained in the final multivariate regression model.

Results and Discussion

The state of Alagoas was divided into two regions, Zona da Mata and Agreste/Sertão, as shown in Figure 1. Table 1 shows the registration and sample data.

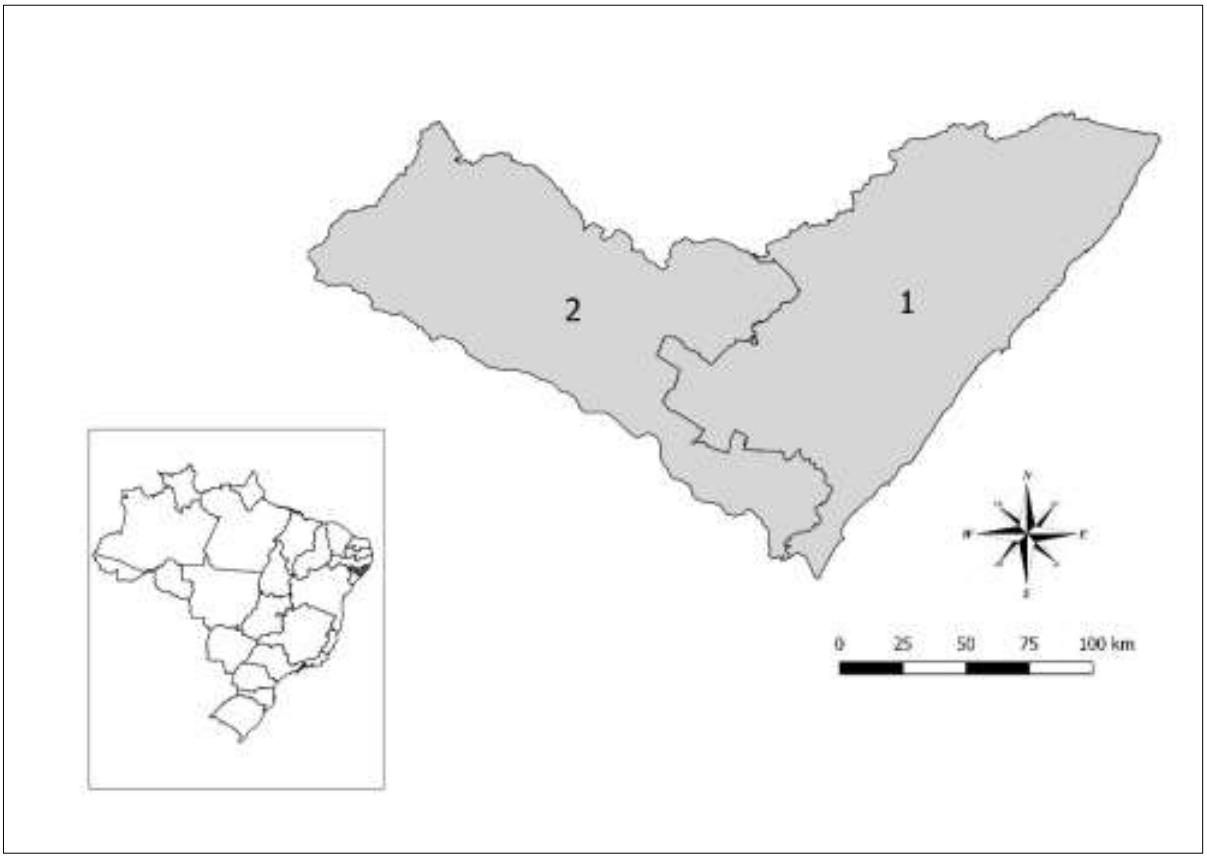

Figure 1. Map of the state of Alagoas divided into Zona da Mata (1) and Agreste/Sertão (2) regions for the study of bovine brucellosis. In detail, the location of the state in Brazil.

Table 1
Registration and sample data from the study of bovine brucellosis in the state of Alagoas, Brazil

region number	number of municipalities	number of herds with reproductive activity	number of females aged ≥ 24 months	number of herds sampled	number of females aged ≥ 24 months sampled
1	64	13.501	244.519	325	1.645
2	38	24.505	217.705	309	1.401
Alagoas	102	38.006	462.224	634	3.046

Source: ADEAL

The estimates of the prevalence of infected herds and seropositive animals for the regions and the state are shown in Table 2. The infected herds prevalence estimates by typology of production unit are organized in the Table 3.

Table 2
Prevalence of infected herds and seropositive bovine animals for brucellosis in the state of Alagoas, Brazil

region	herds				animals			
	tested	positive	prevalence (%)	CI 95% (%)	tested	positive	prevalence (%)	CI 95% (%)
1	325	16	4.9	3.0 - 7.9	1,645	24	1.3	0.7 - 2.1
2	309	7	2.3	1.1 - 4.7	1,401	8	0.5	0.2 - 1.2
Alagoas	634	23	3.2	2.1 - 4.9	3,046	32	0.9	0.5 - 1.4

The results showed no statistical difference between the prevalence of infected herds in the regions (Table 2). Further, within the regions, the disease is homogeneously distributed between beef, milk, and mixed types of herds (Table 3).

Table 3
Prevalence of bovine herds infected with brucellosis in the state of Alagoas, Brazil, by typology of production unit

region	positive/ tested	prevalence (%)	CI 95% (%)	positive/ tested	prevalence (%)	CI 95% (%)	positive/ tested	prevalence (%)	CI 95% (%)
1	8/163	4.9	2.1 - 9.4	4/81	4.9	1.4 - 12.2	4/81	4.9	1.4 - 12.2
2	2/83	2.4	0.3 - 8.4	4/141	2.8	0.8 - 7.1	1/85	1.2	0.03 - 6.4

In Brazil, only the state of Santa Catarina has a lower prevalence of infected herds than Alagoas (Baumgarten et al., 2016). The prevalence of infected herds in Alagoas was similar to that observed for the states of Bahia: 4.2 [3.1; 5.3] (Alves et al., 2009), Minas Gerais: 3.6 [2.8; 4.4] (Oliveira et al., 2016), Pernambuco: 4.5 [3.2; 6.4] (Almeida et al., 2016), Paraíba: 4.6 [3.2; 6.5] (Clementino et al., 2016), Paraná: 4.9 [4.0; 5.9] (Rodrigues et al., 2021), Rio Grande do Sul: 3.5 [2.5; 4.9] (N. S. Silva et al., 2016) and Distrito Federal:2.5 [1.0; 5.1] (Gonçalves et al., 2009b), and lower than the prevalence found in Espírito Santo: 9 [7; 12] (Anzai et al., 2016), Goiás: 16 [15; 20] (Rocha et al., 2009), Mato Grosso: 24 [21; 27] (Barddal et al., 2016), Mato Grosso do Sul: 31 [27; 34] (Leal et al., 2016), Maranhão: 11 [9; 14] (Borba et al., 2013), Sergipe: 13 [9; 16] (V. G. S. O. Silva et al., 2009), Rondônia: 12 [10; 15] (Inlamea et al., 2016), Rio de Janeiro: 15 [13; 18] (Klein-Gunnewiek et al., 2009), São Paulo: 10 [9; 12] (Dias et al., 2016) and Tocantins: 6.4 [4.8; 8.6] (Vendrame et al., 2021).

The prevalence of seropositive animals in both regions was the same (Table 2). The low prevalence of animals verified in the present study differs from those observed by

other authors in previous studies (Tenório et al., 2004; K. P. C. Silva et al., 2011; Farias et al., 2019), probably due to different methods, test protocols, and target populations. Tenório et al. (2004) used a sampling to estimate the prevalence of brucellosis in female dairy herds in five municipalities in region 2 of the present study. Serum samples were obtained from October 2000 to April 2001 and tested with rapid plate agglutination, with 4.14% positive results. K. P. C. Silva et al. (2011) used samples collected from female dairy herds from municipalities located in region 1 of the present study and reported 6.43% positive results with the Rose Bengal test. Farias et al. (2019) analyzed samples from herds located in a municipality in region 2 and found a 2.76% proportion of Rose Bengal test positive results. The methodology used in these studies allowed no confidence interval estimates.

Table 4 shows the final logistic regression model of risk factors for bovine brucellosis in the state of Alagoas.

Table 4
Final multivariate logistic regression model for risk factors for bovine brucellosis in the state of Alagoas

Variable	Odds Ratio	CI 95%	p Value
pasture rental	3.11	1.28 - 7.37	0.01
number of females aged ≥24 months ≥14 (percentile 85)	4.91	2.02 - 11.66	0.0003

Pasture rental is a type of indirect contact between herds and may expose susceptible animals to the infectious agent, since Brucella abortus can survive in the environment for several months in abortion remains, contaminating pastures and water sources (Paulin & Ferreira, 2003; Aune et al., 2012).

Bovine herds with 14 (percentile = 85) or more cows had a significantly greater chance of being infected with brucellosis in Alagoas (Table 4). The association of brucellosis and herd size was already reported by several authors (Nicoletti, 1980; Salman & Meyer, 1984), being verified in the Brazilian states of Mato Grosso do Sul (Chate et al., 2009), São Paulo (Dias et al., 2009b), Rio de Janeiro (Klein-Gunnewiek et al., 2009), Mato Grosso (Negreiros et al., 2009), Tocantins (Ogata et al., 2009) and Sergipe (V. G. S. O. Silva et al., 2009). Some characteristics of larger herds facilitate the transmission of brucellosis, especially due to the increased need for animal replacement (Christie, 1969; Crawford et al., 1990). Thus, this variable indirectly indicates that the more frequent animal replacement practiced in larger herds is the probable cause of greater vulnerability to the disease.

Conclusions

In view of these results, the bovine brucellosis propagation can be reduced in the state of Alagoas through the implementation of health education in the bovine meat and

milk production chains, so that producers avoid renting pastures, or do it according to brucellosis prevention sanitary measures, which should also be implemented when introducing breeding animals into their herds. In addition, the state must consider the convenience of structuring a surveillance system aimed at eradicating bovine brucellosis in view of the low prevalence of infected herds or seropositive animals.

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