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

Prevalência e fatores de risco para brucelose bovina no Estado de Pernambuco, Brasil

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Abstract

This study was conducted to characterize the epidemiology of bovine brucellosis in the state of Pernambuco, Brazil. The state was divided into three regions, and in each region, approximately 300 properties were randomly sampled. From these selected properties, a pre-established number of animals were randomly selected and blood serum samples were obtained. A total of 3,901 animals were selected from 900 properties. For each selected property, an epidemiological questionnaire was administered to assess the type of farming, the animal husbandry practices and the sanitary practices that could be associated with the presence of brucellosis infection. The testing protocol consisted of screening the samples with a buffered acidified plate antigen test and retesting the positive samples with a complement fixation test (CF). One positive animal was enough to define an infected herd. The prevalence rates of infected herds and animals in the state were 4.5% [3.2; 6.4%] and 1.4% [0.7; 2.7%], respectively. By region, the prevalence rates of infected herds and animals, respectively, were as follows: Zona da Mata, 3.3% [1.8; 6.1%] and 1.7% [0.5; 3.0%]; Agreste, 7.4% [4.9; 10.9%] and 1.9% [0.8; 3.0%]; and Sertão, 1.3% [0.5; 3.5%] and 0.7% [0.0; 1.6%]. Flooded pastures (OR = 2.86 [1.37; 6.42]) and the presence of 13 or more females in the herd (3rd quartile) (OR = 2.65 [1.19; 5.89]) were identified as risk factors. The existence of veterinary care emerged as a protective factor against bovine brucellosis in the state of Pernambuco (OR = 0.24 [0.10; 0.58]).

Key words: Bovine. Brazil. Brucellosis. Prevalence. Risk factors. Pernambuco. Brazil.

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Resumo

Realizou-se um estudo para caracterizar a situação epidemiológica da brucelose bovina no Estado de Pernambuco. O estado foi dividido em três circuitos pecuários e em cada um foram amostradas aleatoriamente cerca de 300 propriedades e, dentro dessas, foi escolhido, de forma aleatória, um número pré-estabelecido de animais, dos quais foi obtida uma amostra soro sanguíneo. No total, foram amostrados 3.901 animais, provenientes de 900 propriedades. Em cada propriedade amostrada foi aplicado um questionário epidemiológico para verificar o tipo de exploração e as práticas zootécnicas e sanitárias que poderiam estar associadas ao risco de infecção pela doença. O protocolo de testes utilizado foi o da triagem com o teste do antígeno acidificado tamponado (AAT) e o reteste dos positivos com o teste de Fixação de Complemento (FC). O rebanho foi considerado positivo, se pelo menos um animal foi reagente às duas provas sorológicas. As prevalências de focos e de animais infectados do Estado foram de 4,5% [3,2; 6,4%] e 1,4% [0,7; 2,7%], respectivamente. Os resultados para os circuitos pecuários da prevalência de focos e de animais infectados foram respectivamente: Zona da Mata, 3,3% [1,8; 6,1%] e 1,7% [0,5; 3,0%]; Agreste, 7,4% [4,9; 10,9%] e 1,9% [0,8; 3,0%] e Sertão, 1,3% [0,5; 3,5%] e 0,7% [0,0; 1,6%]. Os fatores de risco (*odds ratio*, OR) associado à condição de foco foram: presença de pastos alagados (OR = 2,86 [1,37; 6,42]) e a presença de 13 ou mais fêmeas no rebanho (terceiro quartil) (OR = 2,65 [1,19; 5,89]). A existência de assistência veterinária na propriedade foi considerado um fator de proteção contra a brucelose bovina no Estado de Pernambuco (OR = 0,24 [0,10; 0,58]).

Palavras-chave: Bovino. Brasil. Brucelose. Prevalência. Fatores de risco. Pernambuco. Brasil.

Introduction

Brucellosis, an infectious disease caused by bacteria of the genus *Brucella*, is endemic in many countries. It results in significant economic losses in production systems and has serious implications for animal and public health, given its zoonotic character (PAULIN; FERREIRA NETO, 2003).

Brazil has never had a well structured program to combat brucellosis until 2001, when MAPA launched the National Program for control and eradication of brucellosis and tuberculosis (PNCEBT), harmonized with those strategies recommended by the OIE, based on vaccination of calves with the B19 and certification of free herds through a routine of serological tests.

Along with the establishment of the PNCEBT, epidemiological surveys were conducted in 17 Brazilian States (84% of bovine population) using farms with reproductive activity as primary sample units. The prevalence of brucellosis infected herds varied from 0.32% in Santa Catarina state to 41.6% in Mato Grosso do Sul state (ALVES et al., 2009; AZEVEDO et al., 2009; BORBA et al., 2013; CHATE et al., 2009; DIAS et al., 2009a, 2009b; GONÇALVES et al., 2009a, 2009b; KLEIN-

GUNNEWIEK et al., 2009; MARVULO et al., 2009; NEGREIROS et al., 2009; OGATA et al., 2009; ROCHA et al., 2009; SIKUSAWA et al., 2009; SILVA et al., 2009; VILLAR et al., 2009; CLEMENTINO et al., 2016). The states of Santa Catarina, São Paulo, Minas Gerais, Rondônia, Mato Grosso, Mato Grosso do Sul, Espírito Santo and Rio Grande do Sul carried out the second study on brucellosis prevalence. Of these, only in Mato Grosso, Mato Grosso do Sul, Minas Gerais and Rondônia a decreasing prevalence of infected herds was observed as a consequence of vaccination program (BARDDAL et al., 2016; BAUMGARTEN et al., 2016; DIAS et al., 2016a; ANZAI et al., 2016; INLAMEA et al., 2016; LEAL FILHO et al., 2016; OLIVEIRA et al., 2016; SILVA et al., 2016b).

The state of Pernambuco (PE) is located in the eastern central area of northeastern Brazil. It includes 184 municipalities and the Archipelago of Fernando de Noronha, covering a total of 98,146.31 km². There are several major rivers in the state, including the São Francisco, the Capibaribe, the Una, the Ipojuca and the Pajeú.

Cattle were introduced to Pernambuco in 1534 by Duarte Coelho Pereira, the donee of the Captaincy

of Pernambuco, who brought nearly a hundred head of cattle from Cape Verde, Africa during his tenure. The animals were imported to feed the families of the lords of mills, for transportation, to power the mills that were not powered by water and to supply leather, handicrafts, tobacco wrappers and other products (PRADO, 1987).

Currently, the bovine herd includes 2,403,912 cattle and 7,433 buffaloes distributed over 110,319 properties. Small herds prevail, with an average of 20.3 animals per property, and there is a slight predominance of mixed-type exploitation and semi-intensive farming. According to the Brazilian Institute of Geography and Statistics (IBGE), Pernambuco has 1.16% of the cattle in the country and accounts for 2.53% of the total bovine milk produced in Brazil (IBGE, 2006).

The Official Veterinary Service of the State of Pernambuco (ADAGRO) performs animal-related sanitary functions in the state. ADAGRO consists of a central headquarters, 11 regional units (RUs), 47 local veterinary units (LVUs) and 96 offices for community assistance (OCAs). Veterinary services are provided by 142 professionals distributed among the existing RUs, LVUs and OCAs.

For cultural reasons, the cattle industry in the state has an intense movement of animals, mainly for the participation of animals in traditional fairs and festivals. Each year, there are approximately 2500 of these events, becoming the control of infectious diseases very challenging.

In 1975, the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) performed the first large study of bovine brucellosis in the country. The state of Pernambuco was included in this study; the prevalence of infected herd was 4.75%, and 1.5% of the state's animals were seropositive. The serological test used was the rapid plate serum agglutination (BRASIL, 1977). Although the state is composed of three climatically very distinct regions (Zona da Mata, Agreste and Sertão), the prevalence of infected herds and of seropositive

animals were estimated for the entire state only, without dividing it into these regions to check for possible heterogeneities. Since then, no official statewide studies have been conducted to determine the prevalence of bovine brucellosis.

One serological survey of bovine brucellosis conducted in isolation in Pernambuco assessed 600 samples of female sera from 16 dairy cattle from various cities in the Agreste region of the state and detected anti-*Brucella abortus* antibodies in 4.2% of the females in five herds (TENÓRIO et al., 2005). Another study carried out in the city of Correntes in the Agreste region reported a prevalence rate of 6.8% (74/1089) among cows (TENÓRIO et al., 2008).

Considering the long time elapsed since the last study of brucellosis in the state (1975), the massive movement of cattle and the lack of information about heterogeneities between regions, this study aims to estimate the prevalence of infected herds and seropositive animals, and individualize the risk factors for the introduction of the disease, providing high quality epidemiological information for implementation and management of PNCEBT in the Pernambuco state.

Material and Methods

The present study was planned by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA), the Collaborator Centre in Animal Health of the School of Veterinary Medicine of the University of São Paulo (FMVZ-USP) and the Animal Health Service of the State of Pernambuco (Agência de Defesa e Fiscalização Agropecuária de Pernambuco – ADAGRO). The field work was performed by ADAGRO staff from July 2008 and April 2009, after being trained to standardize the procedures.

Based on the geographic divisions used by the IBGE, the state was divided into three regions: Zona da Mata, Agreste and Sertão.

The Zona da Mata region is the most important in the state, both from a demographic and an economic point of view. The climate is tropical: hot and humid with an average temperature of 24°C and annual rainfall that varies between 800 mm and more than 2,000 mm. The hydrographic network is perennial and copious, and the vegetation is characteristic of an Atlantic forest. The Agreste region is a transitional area between Zona da Mata and Sertão; the climate ranges from the tropical climate of Zona da Mata to the semi-arid climate of Sertão, and the average rainfall is between 800 and 1,000 mm. Of the total cattle herd in the state, 56% is concentrated in the Agreste region, which is the main dairy region of Pernambuco. In the Sertão region, which is located entirely in the semi-arid region of the state, the climate is hot and dry, with high temperatures and a small amount of rain that is poorly distributed through the year. Bovine husbandry is semi-extensive and combined with mixed breeding, highlighting the extensive breeding of goats and sheep (PERNAMBUCO, 2012).

Within each of these regions, a pre-established number of properties with reproductive activity (primary sampling units) were randomly selected.

In farms with more than one herd, if the animals were subjected to the same handling practices (i.e., exposed to the same risk of exposure to infection), the herd of greater economic importance was selected. The primary sampling unit was selected at random, based on the registration of farms with bovine reproductive activity. Each selected property that could not be visited (for various reasons) was replaced by another property selected at random. The number of properties selected in each region was estimated using the formula for simple random samples (THRUSFIELD, 2007). The following parameters were used for the calculation: a confidence level of 0.95, an estimated prevalence of 0.25, and an error of 0.05.

Within each selected property, a pre-established number of cows older than 24 months (secondary

sampling units) were randomly selected. The sampling strategy for the secondary units aimed to estimate the minimum number of animals to examine within each property to enable the classification of the property as a brucellosis in infected herd location or a non-infected herd location. The concept of aggregated sensitivity and specificity (DOHOO et al., 2003) was used to accomplish this goal. For the calculations, 95% sensitivity and 99.5% specificity were used for the testing protocol (FLETCHER et al., 1998), and the estimated prevalence was 20%. Herdacc version 3 (Ausvet: Canberra, ACT, Australia) was used for this process. The sample size that brought the sensitivity and specificity of the measurements to 90% or greater was used. Thus, at the properties with up to 99 females aged 24 months or older, 10 animals were sampled; at the properties with 100 or more females aged 24 months or older, 15 animals were sampled. The females in the peripartum period (i.e., approximately 15 days before and after delivery) were excluded from the sample. The choice of females within the properties was random and systematic.

The sero-diagnostic protocol consisted of a screening with a buffered acidified antigen test (Rosa Bengala) and a retest of the positive samples with a complement fixation test (CF), following the recommendations of the PNCEBT (LAGE, 2006). Blood samples were collected by a jugular vein puncture with a disposable sterile needle into a vacuum tube that was previously identified. The blood was centrifuged, and the sera were stored at -20°C in polypropylene microtubes until analysis. The serological tests were performed at the National Laboratory of Agriculture – LANAGRO) in Recife.

The property was considered positive if at least one seropositive animal was detected.

The sample design allowed us to determine the prevalence of brucellosis infected herds and seropositive adult females (≥ 24 months) in the state of Pernambuco and in each region. The calculations of the prevalence and the confidence

intervals were performed as recommended by Dean and colleagues (DEAN et al., 1994). The estimates of the prevalence of infected herds and seropositive animals in the state and within the regions were weighted (DOHOO et al., 2003).

In the calculation of the prevalence of infected herds in the state of Pernambuco, the weight for each farm was determined by the following equation:

$$W_f = \frac{\text{farms in the region}}{\text{sampled farms in the region}}$$

In the calculation of the prevalence of seropositive animals in the state, the weight of each animal was determined by the following equation:

$$W_{la} = \frac{(\text{females} \geq 2 \text{ years in the farm}) / (\text{sampled females} \geq 2 \text{ years in the farm}) * (\text{females} \geq 2 \text{ years in the region}) / (\text{females} \geq 2 \text{ years in the sampled farms of the region})}{1}$$

In the above formula, the first term refers to the weight of each animal in the calculation of the prevalence of seropositive animals within each region.

The prevalence estimates and the 95% confidence intervals were calculated using the software *Epi Info* 6.0 (DEAN et al., 1994) and SPSS version 9.0. The prevalence rates were compared with the confidence intervals. The prevalences were compared by the proportion comparison test, using SPSS 9.0 software.

In each sampled farm, a questionnaire was applied in order to generate data about its managing practices. All information generated in the field and in the laboratory was inserted in a database.

In this cross sectional study, risk factors such as production system (meat, milk, mixed), raising system (extensive, any degree of confinement), artificial insemination, cattle breeds, number of cows above two years of age, total herd size, presence of other domesticated species, presence of wild species, destination of the placenta and aborted fetuses, animal trade, vaccination against brucellosis, slaughter in the farm, pasture sharing, indirect contact between farms, flooded pastures, breeding paddock and veterinary assistance were assessed.

These variables were organized in an increasing risk scale. When necessary, a recategorization was made. The least risk category was always considered as baseline for comparisons with the other categories. Quantitative variables were categorized using quartiles as cut points.

An exploratory univariate analysis using chi-square (χ^2) or Fischer exact test was made with all variables, considering the whole State. Those below significance level of 0.20 were selected to a multivariate analysis using logistic regression, according to Hosmer and Lameshow (1989). All calculations were made in SPSS 9.0 and EpiInfo 7.0 computer softwares.

In order to check if the variables were correlated, a Spearman correlation test was performed. If two variables were correlated, the one that was less associated with the dependent variable was excluded from the multivariate analysis.

Results

Pernambuco state was divided into three regions based on the characteristics of the livestock production (Figure 1). Table 1 shows the state census data and the sample data.

Figure 1. Map of the state of Pernambuco showing the division of regions: Zona da Mata (East), Agreste (Central) and Sertão (West) and the spatial distribution of positive and negative farms for bovine brucellosis. 2009.

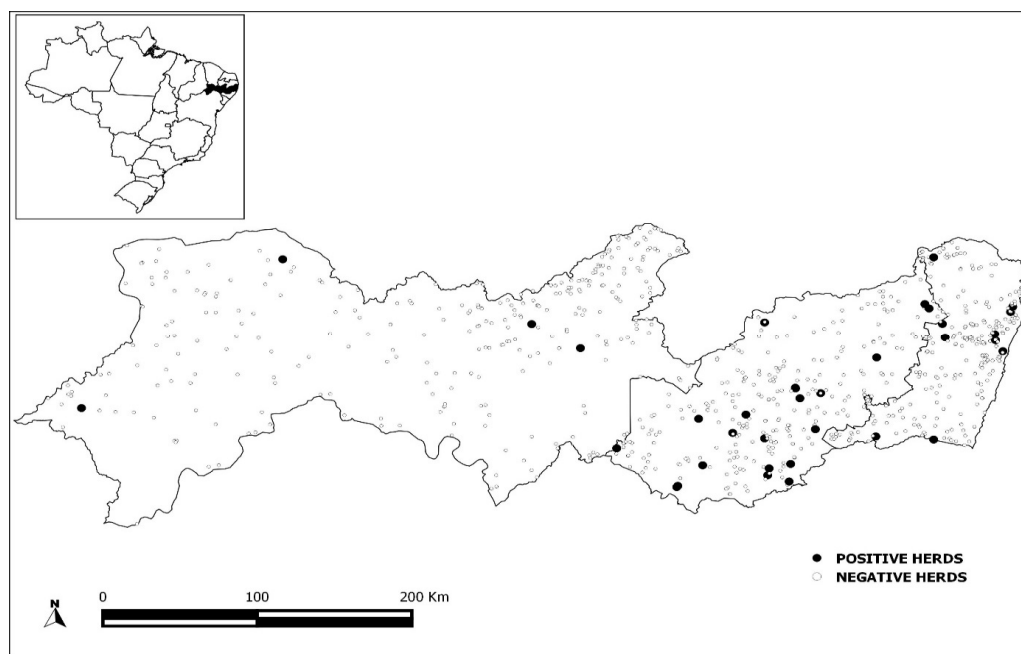


Table 1. Census data for the bovine population in the state of Pernambuco, Brazil. 2008.

Region	Farms with reproductive activity	Sampled farms	Bovine population	Females ≥ 24 months	Sampled females ≥ 24 months
Zona da Mata	9,330	301	193,012	58,562	1,024
Agreste	52,680	299	1,182,163	417,204	1,550
Sertão	42,509	300	746,737	311,665	1,327
Total	104,519	900	2,121,912	787,431	3,901

The Zona da Mata region had the lowest cattle density (9% of the cattle in the state), distributed mostly on the small farms of agrarian reform settlers. The predominant type of farming was mixed, and the rearing system was semi-intensive. The Agreste region had mixed-farming herds, semi-intensive rearing, approximately 56% of the bovine herd in the state and the largest number of rural properties, with an average of 22 bovines per property. Agreste is the main milk-producing region. In the Sertão region, the herd was mixed with semi-extensive rearing. The herds were small, with an average of 17.6 animals, and the handling practices were simple, with no automation. The Sertão region has 35% of the herd in the state.

The census data used as the basis for the calculation of the sample and the prevalence rates were the most recent data available at the time of the fieldwork in 2008.

Tables 2 and 3 show the prevalence of infected herds by region and the prevalence of infected herds stratified by the type of farming in the regions. Figure 1 shows the spatial distribution of the positive properties (infected herds) and the properties sampled by region. Table 4 shows the prevalence of seropositive animals. Table 5 presents the results of the univariate analyses, and Table 6 presents the final logistic regression model.

Table 2. Prevalence of brucellosis infected farms (P) in the state of Pernambuco, Brazil, by region.

Region	Farms			
	Sampled	Positive	P (%)	CI 95% (%)
Zona da Mata	301	10	3.3	[1.8; 6.1]
Agreste	299	22	7.4	[4.9; 10.9]
Sertão	300	4	1.3	[0.5; 3.5]
Total	900	36	4.5	[3.2; 6.4]

Table 3. Prevalence (P) of infected herds of bovine brucellosis stratified by the type of farming system in the regions in the state of Pernambuco, Brazil.

Region	Beef		Dairy		Mixed	
	P (%)	CI 95% (%)	P (%)	CI 95% (%)	P (%)	CI 95% (%)
Zona da Mata	1.5	[0.0; 7.9]	11.5	[4.3; 23.4]	1.7	[0.3; 5.0]
Agreste	0	[0.0; 16.1]	5.2	[2.1; 10.4]	10.7	[6.1; 17.0]
Sertão	3.4	[0.1; 17.8]	1.1	[0.0; 6.1]	1.1	[0.4; 4.1]

Table 4. Prevalence of cows seropositive for brucellosis in the state of Pernambuco, Brazil, by region.

Region	Animals			
	Sampled	Positive	Prevalence (%)	CI 95% (%)
Zona da Mata	1,024	19	1.7	[0.5; 3.0]
Agreste	1,550	28	1.9	[0.8; 3.0]
Sertão	1,327	5	0.67	[0.0; 1.6]
Total	3,901	52	1.4	[0.7; 2.7]

Table 5. Variables with $p \geq 0.20$ in the univariate analysis of the risk factors for bovine brucellosis in the state of Pernambuco, Brazil.

Variable	Exposed/Cases	Exposed/Controls	p value
number of cows ≥ 13 (third quartile)	21/36	187/864	<0.001
number of lactating animals ≥ 5 (third quartile)	25/36	184/864	<0.001
Having veterinary assistance	12/36	76/864	<0.001
Presence of a calving paddock	12/36	99/864	0.001
Presence of flooded pastures	14/36	151/864	0.001
Occurrence of abortions in the last two years	7/36	42/864	0.002
Fate of abortions			
Bury, throw away and burn	8/36	285/864	0.002
Feed to pigs or dogs	9/36	70/864	
Do nothing	19/36	509/864	
Introduction of breeders	24/36	379/864	0.007
Use of artificial insemination	3/36	8/864	0.008
Pasture renting	7/36	98/864	0.138

Table 6. Final multivariate logistic regression model for risk factors of bovine brucellosis in the state of Pernambuco, Brazil.

Variable	Odds ratio	95% CI	p value
Presence of flooded pastures	2.86	[1.27; 6.42]	0.011
number of cows ≥ 13	2.65	[1.19; 5.89]	0.017
Having veterinary assistance	0.24	[0.10; 0.58]	0.001

$r^2 = 13.6\%$.

Discussion

In the state of Pernambuco, the estimated prevalence of brucellosis infected herds was 4.5% [3.2; 6.4%]. Similar results were reported in Bahia (4.2% [3.1; 5.3%], (ALVES et al., 2009) and Paraná (4.0% [3.2; 4.8%], (DIAS et al., 2009a). In Pernambuco, a higher frequency of infected herds was observed in the Agreste region compared with Zona da Mata or Sertão (Table 2). This result is most likely due to the production system in Agreste, which has the largest bovine herd (Table 1), larger farms (the median number of cows in Agreste was 8 compared with 6 in Sertão and 3 in Zona da Mata) and greater movement of animals (PERNAMBUCO, 2012). The climatic conditions in Sertão, which are predominantly arid, combined with the low density of bovines on subsistence properties, hamper the transmission of brucellosis.

The prevalence of infected herds stratified by the type of farming was homogeneous (Table 3), with no statistically significant differences. The prevalence of brucellosis infected herds was higher in dairy-farming herds (11.5%) in Zona da Mata, in mixed-farming herds (10.7%) in the Agreste region and in beef-farming herds in Sertão. There was no statistically significant difference in beef and dairy farming between the three regions. However, in mixed herds, the prevalence was much higher in the Agreste region than in Zona da Mata and Sertão, most likely because of the concentration of mixed-type herds in the region.

The prevalence of brucellosis-positive animals in the state of Pernambuco was 1.4% [0.7; 2.7%] (Table 4). Zona da Mata and Agreste had the highest

prevalence rates: 1.7% [0.5; 3.0%] and 1.9% [0.8; 3.0%], respectively. Sertão had the lowest prevalence (0.7%) [0.0; 1.6%].

Considering the national study from the 70s, when the prevalence of infected herds was 4.75% and the prevalence of seropositive animals was 1.5% (BRASIL, 1977), these results show that the epidemiology of brucellosis has not changed over the past 30 years, despite the massive movement of animals in the state, suggesting that the disease reached equilibrium in the state. It should be noted that the methodology used in the previous study was different from that used in the present study, both in relation to the testing protocol and the sample design.

The prevalence results showed that the state can be divided into two regions: one covering Zona da Mata and Agreste (Tables 2 and 4), where the prevalence is higher, and Sertão, where the prevalence is very low. The prevalence of seropositive animals in regions with a higher prevalence may be reduced with a well-structured vaccination program. In Sertão, which had a low prevalence, vaccination would not noticeably improve the conditions. Thus, the best approach would be to implement eradication strategies, including the prohibition of vaccination and the implementation of a surveillance system for the detection of residual infected herds and testing them to eliminate the disease, as described by Paulin and Ferreira Neto (2003).

The final logistic regression model indicated that the presence of flooded pastures and having a herd of females with ≥ 13 animals were risk factors for bovine brucellosis, whereas having veterinary care was a protective factor (Table 6).

The presence of flooded pastures was also confirmed as a risk factor for bovine brucellosis in the state of Bahia, Brazil (ALVES et al., 2009). The survival of *Brucella* spp. increases in a humid environment (FAO/WHO, 1986). The longer they are able to survive in the environment, the greater the chance that they will infect a new susceptible animal. Thus, flooded areas and flooded pastures on a property can facilitate the spread of the disease. Zona da Mata is the region of the state with the highest rainfall and humid areas for several months of the year. The Agreste region has a semi-arid climate, but the annual average rainfall ranges between 800 and 1000 mm. The wet forests and the presence of humid areas may favor the persistence of *Brucella* spp. in the soil.

Herds with 13 or more cows had a higher risk of brucellosis infected herds. The association between larger herds and the presence of brucellosis has already been observed in Brazil in the states of Mato Grosso do Sul, São Paulo, Rio de Janeiro, Mato Grosso, Tocantins and Sergipe (CHATE et al., 2009; DIAS et al., 2009b; KLEIN-GUNNEWIEK et al., 2009; NEGREIROS et al., 2009; OGATA et al., 2009; SILVA et al., 2009). In larger herds, there are no individual differences in terms of susceptibility to disease, but some factors may influence disease dynamics independently or in combination (e.g., the more frequent replacement of animals and the greater number of problems related to sanitary control) (CRAWFORD et al., 1990). Christie (1969) found that the increase in the herd size increased the probability of brucellosis and the persistence of the infection, increasing the prevalence of the disease and the difficulty eradicating it. Kellar et al. (1976) found that properties with infected herds acquired replacement animals more frequently than the properties that were free of disease.

However, the main problem is not simply the introduction of bovines into the herd but introducing them without testing for brucellosis and/or without knowledge about the sanitary conditions of the herd of origin.

The fact that veterinary assistance has emerged as a protective factor means that professionals are correctly advising their clients about control and prevention.

The results of this study will guide the government of Pernambuco state in planning and optimizing the fight against brucellosis and in periodically assessing the effectiveness of the measures taken.

Conclusions

The prevalence results showed that the state can be divided into two regions: one covering Zona da Mata and Agreste (Tables 2 and 4), where the prevalence is higher, and Sertão, where the prevalence is very low. The prevalence of seropositive animals in Zona da Mata and Agreste may be reduced with a well-structured vaccination program, using B19 and RB 51 strains (AMAKU et al., 2009; SOUZA et al., 2016). In Sertão, which had a low prevalence, the best approach would be to implement eradication strategies. Moreover, an efficient animal health education program to orientate farmers to test replacement animals for brucellosis prior to introduction in their herds and avoid calving in flooded areas should also be implemented.

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