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Epidemiological situation of bovine tuberculosis in the state of Pernambuco, Brazil

Situação epidemiológica da tuberculose bovina no estado de Pernambuco, Brasil

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Abstract

The epidemiological situation of bovine tuberculosis was studied in the state of Pernambuco, Brazil. The state was divided into three regions, and a predetermined number of properties was randomly sampled in each region. In each property, females aged 24 months or older were randomly selected and subjected to the cervical comparative tuberculin test. Altogether, 5,728 animals from 906 properties were tested. In the evaluated properties, an epidemiological questionnaire was applied to identify risk factors related to bovine tuberculosis. The prevalence of infected herds and infected animals and in the state was 2.87% [1.82-4.50] and 0.62% [0.29-1.29], respectively. There was a predominance trend of infected herds in the properties located in the dry areas of Pernambuco, where most dairy properties are found. The risk factors related to the occurrence of positive herds were: presence of 18 or more cows in the herd, milking the cows two or three times a day, and sharing the grazing area. In conclusion, the prevalence of bovine tuberculosis in Pernambuco is low, and the best strategy to be adopted is the implementation of a surveillance system for detection and sanitation of infected herds, preferably by incorporating risk-based surveillance strategies. In addition, the State should implement an effective sanitary program to enable producers to test their animals for bovine tuberculosis before inserting them into the herds, as well as avoid sharing the grazing areas with properties that are not free of bovine tuberculosis.

Key words: Bovine tuberculosis. Brazil. *Mycobacterium bovis*. Pernambuco. Prevalence. Risk factors.

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Resumo

A situação epidemiológica da tuberculose bovina foi estudada no estado de Pernambuco. O estado foi dividido em três regiões e em cada uma delas foi aleatoriamente amostrado um número pré-estabelecido de propriedades. Dentro de cada propriedade, fêmeas com idade igual ou superior a 24 meses foram escolhidas aleatoriamente e submetidas ao teste Tuberculínico Cervical Comparativo. Ao todo foram testados 5.728 animais provenientes de 906 propriedades. Nas propriedades, foi aplicado um questionário epidemiológico para identificar fatores de risco associados à tuberculose bovina. A prevalência de focos no estado foi de 2,87% [1,82; 4,50] e a de animais 0,62% [0,29; 1,29]. Houve tendência de concentração de focos na região do Agreste do estado, caracterizada pelo predomínio de propriedades de leite. Os fatores de risco associados à condição de foco foram ter 18 ou mais vacas no rebanho, ordenhar as vacas 2 ou 3 vezes ao dia e compartilhar pastagem. Concluindo, o estado tem baixa prevalência de tuberculose bovina e a melhor estratégia a ser adotada é a implementação de sistema de vigilância para detecção e saneamento dos focos, de preferência incorporando elementos de vigilância baseada em risco. Além disso, o estado deve realizar uma sólida ação de educação sanitária para que seus produtores passem a testar os animais para tuberculose bovina antes de introduzi-los em seus plantéis e deixem de compartilhar pastagem com propriedades que não sejam livres de tuberculose bovina.

Palavras-chave: Tuberculose bovina. Brasil. Fatores de risco. *Mycobacterium bovis*. Pernambuco, prevalência.

Introduction

Bovine tuberculosis is a zoonosis caused by *Mycobacterium bovis* and causes significant economic losses for meat and milk producers (MODA et al., 1996; COSIVI et al., 1998). For this reason, the Ministry of Agriculture, Livestock, and Supply (Ministério da Agricultura, Pecuária e Abastecimento -MAPA) of Brazil introduced the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis in 2001 (LAGE et al., 2006).

Previous studies have been conducted in 12 Brazilian Federative Units, which hold 75% of the Brazilian herd, to characterize the epidemiological situation of tuberculosis in Brazilian states and implement the best strategies and subsequent management of the actions adopted. The prevalence of infected herds in these units varied between 0.36%, in the Federal District (Brasília), and 9.0%, in São Paulo (BAHIENSE et al., 2016; BARBIERI et al., 2016; DIAS et al., 2016; GALVIS et al., 2016; GUEDES et al., 2016; NÉSPOLI et al., 2016; QUEIROZ et al., 2016; RIBEIRO et al., 2016; ROCHA et al., 2016; SILVA et al., 2016; BAUMGARTEN et al., 2016; VENDRAME et al., 2016).

The meat sector is estimated to grow 2.1% per year in Brazil until 2025 (BRASIL, 2015). For the milk sector, the growth of the Northeast region of Brazil in recent years has been significantly higher than the national average, particularly in the state of Pernambuco (REIS FILHO, 2013).

Pernambuco has a geographic area of 99,000 Km^2 and contains 184 municipalities, which occupy three large regions: Zona da Mata, Agreste, and Sertão. The state has the fourth largest bovine herd of the Northeast of Brazil, with two million heads distributed in approximately 99,000 properties (PERNAMBUCO, 2015).

However, the epidemiological situation of bovine tuberculosis is not well characterized in Pernambuco because the available information is limited to data on carcass condemnation in slaughterhouses, notifications to the official veterinary service of the positive results to tuberculin tests carried out by qualified veterinarians, and a few frequency studies performed in restricted areas of the state, mainly in region of Agreste.

Sobreira Filho et al. (2008) reported that 1.7% of the dairy cattle from 16 farms of the municipality of Gravatá tested was positive to the cervical

comparative tuberculin test. A retrospective study conducted between 2000 and 2009 using data from the bovine clinic of Garanhuns Campus located at the Rural Federal University of Pernambuco (Universidade Federal Rural de Pernambuco) reported 36 cases of bovine tuberculosis (IZAEL et al., 2009). Mendes et al. (2011) examined dairy herds of 15 municipalities and observed that 14% (86/612) of the evaluated animals were positive for the tuberculin test. Milk and blood samples were collected from 401 dairy cattle in 20 properties in the region of Garanhuns, and *M. bovis* was detected by real-time PCR in one milk sample (0.25%) and eight blood samples (2%); the infected animals originated from 6 of the 20 properties sampled (30%) (CEZAR et al., 2016).

Therefore, owing to the scarcity of epidemiological data about bovine tuberculosis in Pernambuco, the aim of this study was to estimate the prevalence of infected herds and animals the risk factors for the disease, aiming at the successful eradication of tuberculosis in the state.

Material and Methods

The study was planned in partnership with MAPA, the Agricultural and Livestock Defense and Inspection Agency of the State of Pernambuco (Agência de Defesa e Fiscalização Agropecuária de Pernambuco-ADAGRO), and the Collaborating Centre in Animal Health, located in the Department of Veterinary Medicine and Animal Husbandry of the University of São Paulo. The fieldwork was conducted by technicians of ADAGRO between February and December 2014.

The state was divided into regions with uniform characteristics to verify possible regional variations. For this purpose, different production systems, handling practices, objectives of exploration, average herd size, marketing system, and the operational and logistical capacity of the official veterinary service of the state were considered for the performance of fieldwork.

A two-stage sampling method was applied to estimate the prevalence of infected herds and animals in the regions and the state. In the first stage, a predetermined number of properties with breeding activity was randomly selected in each region from the register of properties of ADAGRO. In the second stage, a predetermined number of breeding females aged 24 months or older was selected in each property. In properties with herds at different production levels, only the herd with greater economic value or considered the primary objective of the production was studied. A herd was defined as a group of animals kept under the same handling practices, i.e., exposed to the same risk conditions. The selected properties, which for any reason could not be sampled, were replaced using a new drawing. The number of selected properties by region was estimated using the formula for simple random samples (THRUSFIELD, 2007) and the following parameters: estimated prevalence of 20%, level of confidence of 95%, and error of 10%.

For the drawing of the animals in each property, the number of animals to be evaluated was calculated to allow their classification as infected or free from tuberculosis, considering aggregated sensitivity values equal to or higher than 85% and aggregated specificity values equal to or higher than 95%. The individual sensitivity and specificity values adopted were 80.0% and 99.5%, respectively. The calculations were made using Herdacc software version 3.0.

In properties with up to 99 reproducers aged 24 months or older, 20 animals were examined. In properties with 100 animals or more, 40 animals were examined. The animals were randomly selected, and pregnant and parturient cows (from 15 days before delivery to 15 days after delivery) were replaced.

The study animals were subjected to the cervical comparative tuberculin test, which was conducted in compliance with the recommendations of the National Program for the Control and Eradication

of Animal Brucellosis and Tuberculosis (LAGE et al., 2006). The occurrence of suspected animals at the end of the study using this test was prevented by retesting the inconclusive cases using the same diagnostic procedure at a minimum interval of 60 days. All positive animals were euthanized.

For properties with up to 20 tested bovines, one animal should test positive to consider the herd infected with tuberculosis. In the properties in which 40 animals were tested, at least two animals should test positive to consider the herd infected with tuberculosis.

The prevalence of infected herds and animals was evaluated in each region and the entire state. The prevalence of infected herds within each region was also stratified by the type of exploration. The apparent prevalences and their respective confidence intervals were calculated as proposed by Dean et al. (1994). The weighted prevalence of infected herds and animals in the state and the prevalence of animals in each region were calculated (DOHOO et al., 2003). The weighted prevalence of infected herds in the state was calculated by

$$P_1 = \frac{\text{Properties with breeding activity found in the region}}{\text{Properties with breeding activity sampled in the region}}$$

The weight assigned to each animal in the calculation of the prevalence of infected herds in the state was given by

$$P_2 = \frac{\text{Females aged} \geq 24 \text{ months in the property}}{\text{Females aged} \geq 24 \text{ months sampled in the property}} \text{ X}$$

$$\frac{\text{Females aged} \geq 24 \text{ months in the region}}{\text{Females aged} \geq 24 \text{ months sampled in the region}}$$

In the equation above, the first term refers to the weight of each animal in the calculation of the prevalence of infected animals in each region. The calculations were made using the "survey" package of the R Core Team software.

In every property evaluated, a questionnaire was applied to identify the risk factors for infection. The questionnaire was created to assess the exposure to typical risk factors, which have been described in the literature (MARANGON et al., 1998; RAMÍREZ-VILLAESCUSA et al., 2010; SKUCE et al., 2012), and other factors of specific regional interest. The questions addressed the following variables: type of exploration (beef, dairy, or mixed); degree of confinement of the animals (confined, semi-confined, and extensive); number and breeds; use of milking machine and artificial insemination; inclusion of bovines into the herds in the last two years; presence of other animal species in the property (domestic and wildlife); performance of routine tests for bovine tuberculosis; place of slaughter of the breeders; sharing of the grazing area, food and water supply, equipment, and personnel; presence of wetlands; animal feeding with whey; and veterinary care.

The variables were classified in ascending order of risk. These variables were reclassified when necessary. The category with lower risk was considered the basis for the comparison of other categories. The quantitative variables were reclassified in percentages. A preliminary exploratory univariate data analysis was conducted using the χ^2 test or Fisher's exact test to select variables with $p \leq 0.20$ and a subsequent logistic regression analysis was conducted (HOSMER JÚNIOR; LAMESHOW, 1989). The calculations were made using the R Core Team software. All information generated in the field and laboratory work was inserted in a specific database used in the epidemiological analysis.

Results

The State of Pernambuco was divided into three regions (Figure 1) and the summary of census data and the studied samples are presented in Table 1.

The prevalence of infected herds and animals are shown in Table 2, and the prevalence of infected herds by the type of activity is presented in Table 3.

The results of the final logistic regression model for risk factors for bovine tuberculosis in the state of Pernambuco are shown in Table 4.

Figure 1. Map of the state of Pernambuco with the three study regions: 1-Zona da Mata, 2-Agreste, and 3-Sertão. Brazil, 2014.

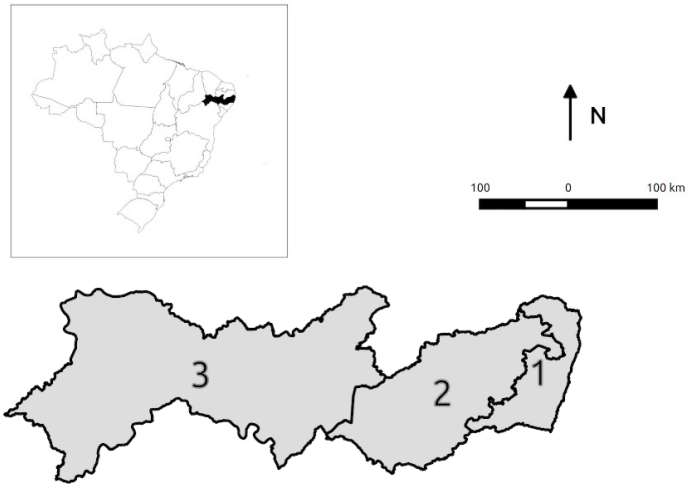

Table 1. Census summary of bovine herds and details of the study sample in different regions of the state of Pernambuco, Brazil, in 2014.

Region	Properties with breeding activity	Sampled properties	Females with aged ≥ 24 months	Sampled females aged ≥ 24 months
1- Zona da Mata	5.518	312	65.522	1.616
2- Agreste	45.381	298	553.384	2.323
3- Sertão	40.056	296	397.222	1.789
Total	90.955	906	1.016.128	5.728

Table 2. Prevalence of infected herds and animals of bovine tuberculosis in the state of Pernambuco, Brazil, in 2014.

Region	Prevalence of infected herds			Prevalence of animals (P)		
	pos/ex*	P%	IC95% (%)	pos/ex*	P%	IC95% (%)
1- Zona da Mata	5/312	1.60	0.67-3.80	8/1616	0.33	0.13-0.86
2- Agreste	13/298	4.36	2.54-7.38	16/2323	0.96	0.47-1.96
3- Sertão	4/296	1.35	0.51-3.55	4/1789	0.18	0.04-0.85
Total	22/906	2.87	1.82-4.50	28/5728	0.62	0.29-1.29

* positive/examined.

Table 3. Prevalence of herds infected with bovine tuberculosis stratified by type of activity in the regions of the State of Pernambuco, Brazil, 2014.

Region	Prevalence of herds infected with tuberculosis (%)					
	Beef		Dairy		Mixed	
	% (positive/ examined)	IC 95% (%)	% (positive/ examined)	IC 95% (%)	% (positive/ examined)	IC 95% (%)
1- Zona da Mata	0 (0/60)	0-0.048*	2.50 (1/40)	0.12-11.72	1.90 (4/210)	0.61-4.53
2- Agreste	0 (0/34)	0-0.082*	6.33 (10/158)	3.26-10.10	2.86 (3/105)	0.73-7.58
3- Sertão	0 (0/61)	0-0.470*	0 (0/45)	0-0.063*	2.15 (4/186)	0.69-5.10

* The calculation was made using the Monte Carlo method and beta distribution.

Table 4. Final model of logistic regression of risk factors for bovine tuberculosis in the State of Pernambuco, Brazil, 2014.

Variable	Odds Ratio	95% CI	P-value
Number of cows ≥ 18*	8.43	1.42-50.18	0.02
Milking of cows 2 or 3 times a day	5.35	2.01-14.19	0.001
Shared grazing	5.12	1.81-14.45	0.002

* percentile 90.

Discussion

The prevalence of herds infected with bovine tuberculosis in the state of Pernambuco was estimated at 2.87% [1.82-4.50] (Table 2), and this rate was statistically similar to that found in the states of Bahia, Rio Grande do Sul, Paraná, Mato Grosso, Mato Grosso do Sul, Rondônia, Goiás, Minas Gerais, and Federal District, lower than that found in the states of Espírito Santo and São Paulo, and higher than that found in the state of Santa Catarina (BAHIENSE et al., 2016; BARBIERI et al., 2016; DIAS et al., 2016; GALVIS et al., 2016; GUEDES et al., 2016; NÉSPOLI et al., 2016; QUEIROZ et al., 2016; RIBEIRO et al., 2016; ROCHA et al., 2016; SILVA et al., 2016; BAUMGARTEN et al., 2016; VENDRAME et al., 2016).

Bovine tuberculosis occurred in every region of the state of Pernambuco and, although the prevalence of infected herds and animals was not significantly different between the state regions, our results strongly suggest the concentration of the disease in the Agreste region (Table 2), which contained a greater number of dairy properties in our sample (Table 3).

No herd infected with bovine tuberculosis was detected in beef cattle, only in dairy or mixed cattle (Table 3).

The risk factors for bovine tuberculosis in the state of Pernambuco were presence of herd of 18 or more cows; milking the animals two or three times a day, and sharing the grazing area (Table 4).

The higher probability of infection in herds from properties with more animals was also verified in the Republic of Ireland (GRIFFIN et al., 1996; CLEGG et al., 2012), United Kingdom (REILLY; COURTENAY, 2007; RAMÍREZ-VILLAESCUSA et al., 2010; BESSELL et al., 2012), New Zealand (PORPHYRE et al., 2008), and Brazil (BAHIENSE et al., 2016; DIAS et al., 2016; GALVIS et al., 2016; SILVA et al., 2016; BAUMGARTEN et al., 2016). Larger herds present greater replacement rates, increasing the risk of inclusion of infected animals. The purchase of animals primarily from suspected farms is the main reason for the transmission of

bovine tuberculosis (REILLY; COURTENAY, 2007; RAMÍREZ-VILLAESCUSA et al., 2010; BESSELL et al., 2012; SKUCE et al., 2012). Therefore, it is reasonable to admit that this variable indicates that the purchase of animals without preliminary testing increases the risk of bovine tuberculosis in a property.

In dairy properties, animals are milked two or three times a day. Dairy herds are more vulnerable to tuberculosis because dairy production involves animal confinement during milking, which increases the risk of transmission (BARLOW et al., 1997), considering that confinement increases the population density and consequently the probability of infectious contacts (HUMBLET et al., 2010; ALVAREZ et al., 2012). Dairy herds also presented greater risk for tuberculosis in New Zealand (PORPHYRE et al., 2008), United Kingdom (RAMÍREZ-VILLAESCUSA et al., 2010; KAROLEMEAS et al., 2011), and Brazil (BAHIENSE et al., 2016; BARBIERI et al., 2016; DIAS et al., 2016; GALVIS et al., 2016; ROCHA et al., 2016; SILVA et al., 2016; BAUMGARTEN et al., 2016). Furthermore, dairy cattle tend to be older than beef cattle and consequently, have a greater probability of exposure to the infectious agent (HUMBLET et al., 2009).

Sharing of the grazing area is a form of indirect contact between the farms because it allows the interaction between animals from different regions and increases the probability of contact between infected and vulnerable animals. The same practice was also identified as a risk factor in the state of São Paulo (DIAS et al., 2016).

Conclusion

The prevalence of bovine tuberculosis in the state of Pernambuco is low, and the best strategy to be adopted is the implementation of a surveillance system for detection and sanitation of infected herds, preferably by incorporating risk-based surveillance strategies. Furthermore, the state should implement an effective sanitary program to allow producers to test their animals for bovine tuberculosis before inserting them into the herds and avoid sharing grazing areas with properties that are not free from bovine tuberculosis.

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