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Epidemiologic characterization of bovine tuberculosis in the state of Espírito Santo, Brazil

Situação epidemiológica da tuberculose bovina no Estado do Espírito Santo, Brasil

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Abstract

A cross-sectional study was carried out between January 2012 and May 2014 to investigate the status of bovine tuberculosis in the state of Espírito Santo. The state was divided into two regions, and in each of them, 300 farms with reproductive activity were randomly selected and considered as the primary sampling units. In the selected farms, a fixed number of female bovines aged over 2 years were randomly selected to undergo a comparative cervical tuberculin test; an epidemiologic questionnaire was also applied. In the state of Espírito Santo, the apparent prevalence of tuberculosis-positive farms was 7.6% (95% confidence interval [CI] = 5.7-9.9). Prevalence at the herd level varied from 4.6% (95% CI = 2.6-7.3) in region 1 to 11.1% (95% CI = 7.7-15.3) in region 2. The apparent prevalence of tuberculosis-positive animals was 0.7% (95% CI = 0.3-1.1) in the state, and the prevalence varied from 0.3% (95% CI = 0.2-0.6) in region 1 to 1.2% (95% CI = 0.3-2.9) in region 2. The risk factors associated with tuberculosis prevalence in Espírito Santo were the number of adult females ≥ 10 (odds ratio [OR] = 2.40; 95% CI = 1.17-5.31) and milking type (milking machine/milking parlor) (OR = 2.88; 95% CI = 1.36-5.86]). The state of Espírito Santo should set up a surveillance system to detect and control bovine tuberculosis, taking into account the importance of dairy farms and animal trade in the state.
Key words: Bovine. Brazil. Espírito Santo. Prevalence. Risk fator. Tuberculosis.

Resumo

Para estimar a prevalência e os fatores de risco da tuberculose bovina no Estado do Espírito Santo (Brasil), foi realizado um estudo transversal entre janeiro de 2012 e maio de 2014. O estado foi dividido em duas regiões, e em cada uma foram amostradas aleatoriamente 300 fazendas com atividade reprodutiva, consideradas unidades primárias de amostragem. Nas propriedades selecionadas um número fixo de fêmeas bovinas acima de 2 anos de idade foram aleatoriamente selecionadas para realização do teste

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cervical comparativo; também foi aplicado um questionário epidemiológico. No Estado do Espírito Santo a prevalência aparente de fazendas positivas para tuberculose foi de 7,6% (intervalo de confiança 95% [IC 95%] = 5,7-9,9). A prevalência de rebanhos positivos variou de 4,6% (IC 95% = 2,6-7,3) na região 1 a 11,1% (IC 95% = 7,7-15,3) na região 2. A prevalência aparente de animais positivos para tuberculose foi de 0,7% (IC 95% = 0,3-1,1) no estado, variando de 0,3% (IC 95% = 0,2-0,6) na região 1 a 1,2% (IC 95% = 0,3-2,9) na região 2. Os fatores de risco associados com a infecção por tuberculose no Espírito Santo foram: número de fêmeas adultas ≥ 10 (odds ratio [OR] = 2,40; IC 95% = 1,17-5,31) e tipo de ordenha (ordenhadeira mecânica/sala de ordenha) (OR = 2,88; IC 95% = 1,36-5,86). O estado do Espírito Santo deve implementar um sistema de vigilância para detectar e controlar a tuberculose bovina, levando em consideração a importância de propriedades leiteiras e comércio animal na epidemiologia da doença no estado.
Palavras-chave: Bovina. Brasil. Espírito Santo. Fator de risco. Prevalência. Tuberculose.

Introduction

Bovine tuberculosis (bTB) is a chronic zoonotic disease caused by *Mycobacterium bovis*, which in addition to cattle, also infects small ruminants, humans, and other domestic and wild animals. The bacterium initiates a chronic, granulomatous, caseous-necrotising, inflammatory process in many organs depending on its route of entry. The main route of entry is inhalation (causing primary lesions in the lungs and accessory lymph nodes), but it can also be ingested (causing primary lesions in the mesenteric lymph nodes) (DOMINGO et al., 2014). Apart from the risk to public health, the disease causes economic losses to the cattle industry (ZINSSTAG et al., 2006). Usually, bTB is endemic and widespread in large territories connected by commercial relations, especially animal trade (GILBERT et al., 2005).

In 2001, the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) launched the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (PNCEBT), with an aim to reduce the negative impacts of bovine brucellosis and tuberculosis on human health, as well as to promote the competitiveness of the national livestock industry (LAGE et al., 2006). The control measures recommended by the PNCEBT included movement control of reproducing animals and certification of tuberculosis-free farms (LAGE et al., 2006). This includes diagnostic tests and veterinary services, which are paid for by the farmers. The official veterinary service is only responsible for auditing the entire system.

Recent studies carried out in 12 Brazilian states, which hold 74% of the Brazilian cattle population, showed that the prevalence of tuberculosis-infected herds ranged from 0.36% in the Federal District to 9.0% in São Paulo (BAHIENSE et al., 2016; BARBIERI et al., 2016; DIAS et al., 2016; GUEDES et al., 2016; LIMA et al., 2016; NÉSPOLI et al., 2016; QUEIROZ et al., 2016; RIBEIRO et al., 2016; ROCHA et al., 2016; SILVA et al., 2016; VELOSO et al., 2016; VENDRAME et al., 2016). The state of Espírito Santo has a herd of 2,216,144 heads, of which 43.9% is concentrated in the north of the state-a region in which beef cattle farms are predominant. Dairy farming is practiced in the southern region of the state – a region which has a more traditional organization, with a predominance of small farms (BRASIL, 2015).

To date, no survey has tried to accurately characterize bTB in the entire territory of the state of Espírito Santo. Lavagnoli et al. (2010) collated data on routine tests for tuberculosis conducted throughout Espírito Santo between January 2005 and June 2007, and found a bTB prevalence of 0.54% at the individual animal level. However, that study used non-random sampling and could have yielded biased results. From January 2009 to December 2012, the percentage of bovines that had to be slaughtered under the provisions of State Inspection because of the presence of macroscopic

lesions suspected of being induced by bTB was 0.23%. Among the 1260 suspected bTB lesions detected during the abattoir inspection, 1026 (81.4%) were in bovines from the southern region of the state, which has a predominantly dairy and dual-purpose livestock, and which regularly sends discarded animals for slaughter (PAOLI, 2013).

The present study aimed to estimate bTB prevalence and risk factors in the state of Espírito Santo in order to better inform the management of the animal health policy. As stated by Skuce et al. (2012), "Risk factors will vary across regions due to factors such as differing farm structures, farm management practices, local TB control, and the relative importance of specific risk factors within individual areas." Therefore, local epidemiological studies like the one reported here are extremely important to understand tuberculosis dynamics, spread, and maintenance within a region, and, consequently, to better inform decision makers and control agencies on how to deal with this important disease.

Materials and Methods

Sample design

The present study was designed by the MAPA; the Collaborator Centre in Animal Health, School of Veterinary Medicine, University of São Paulo (FMVZ-USP); and the Animal Health Service of the State of Espírito Santo (Instituto de Defesa Agropecuária e Florestal do Espírito Santo-IDAF). The field work was performed by IDAF staff from January 2012 to May 2014, after being trained to standardize the procedures.

In order to characterize regional differences in epidemiological parameters of bTB, the state of Espírito Santo was divided into two regions, taking into account the livestock production systems, management practice, herd size, and trade systems according to Azevedo et al. (2009). This division also considered the operational and logistic capacity of the IDAF to perform the field work based around its 40 regional offices. A map of the livestock regions was plotted using the software ArcGIS 10.0.

In each region, a cross-sectional study was performed to estimate the herd- and animal-level prevalence of bTB by using a two-stage sampling method. In the first stage, a predetermined number of farms with reproductive activity were randomly selected (primary sampling units). In the second stage, a predetermined number of female bovines aged over 2 years were randomly selected (secondary sampling units).

In farms with more than one individual herd, we chose the biggest, more economically important one, in which the animals were submitted to the same management procedures, i.e., animals exposed to identical risk factors. The choice of the primary sampling units was based on the official farm registry database. If a selected farm could not be visited by IDAF staff, a new one was randomly selected to replace it. The number of selected farms per livestock region was estimated by the simple random sample formula proposed by Thrusfield (2007) and Noordhuizen et al. (2001):

$$N = \frac{Z_{\alpha}^{2} * P * (1 - P)}{d^{2}}$$

where N denotes the sample size, Z_{α} is the normal distribution value for the 95% confidence level, P is the expected prevalence (20%), and d is the absolute error (5%).

The choice of the secondary sampling units aimed at the appropriate classification of a farm as positive or negative. To achieve this, we used the herd sensitivity and specificity concept (DOHOO et al., 2003). The values for sensitivity and specificity for the test protocol were 80% and 99.5%, respectively (FLETCHER et al., 1998), and the expected prevalence was considered to be 20%. The calculations were made using Herdacc version 3, and the selected sample size was the one that allowed herd sensitivity and specificity above 90%.

Thus, in farms with 99 or less females aged over 2 years, 20 animals were sampled, and in farms with more than 99 females aged over 2 years, 40 animals were sampled. If the selected herd was smaller than the required sample size, all animals were sampled. The sampling method for the selection of females was systematic.

If up to 20 animals in a herd were tested, and if there was at least one bTB-positive animal, the herd was classified as an infected herd. If 40 animals in a herd were tested, at least two animals had to test positive for the herd to be classified as an infected herd.

Test protocol

The comparative cervical tuberculin test was used according to the guidelines of the Brazilian Brucellosis and Tuberculosis Control and Eradication National Program (LAGE et al., 2006). Tested animals were separated from the herd for 72 h, when the results were being analyzed. Animals with inconclusive results were retested 60 days after the first test. If the second test result was inconclusive or positive, the animal was considered positive according to the guidelines of the national program (LAGE et al., 2006).

Prevalence estimation

The sample design allowed us to estimate the herd- and animal-level prevalence of bTB in Espírito Santo and in the livestock regions as well. The apparent prevalence and the respective confidence intervals (CIs) were estimated according to Lumley (2004). All calculations were weighted (DOHOO et al., 2003). The weight of each farm (W_f) in the calculation of the positive herd prevalence in the whole state was derived as follows:

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

The weight of each female bovine aged over 2 years (W_a) in the calculation of animal prevalence in the whole state was derived as follows:

$$W_a = \frac{females \geq 2\ years\ in\ the\ farm}{sampled\ females \geq 2\ years\ in\ the\ farm} \times \frac{females \geq 2\ years\ in\ the\ region}{females \geq 2\ years\ in\ the\ sampled\ farms\ of\ the\ region}$$

In the above expression, the first term refers to the weight of each sampled animal in the farm and the second term refers to the weight of each farm in the livestock region. The prevalence estimates and respective 95% CIs were calculated using R (R DEVELOPMENT CORE TEAM, 2012) along with the "survey" package (LUMLEY, 2004).

Risk factor analysis

In each sampled farm, a questionnaire was applied in order to generate data about its management practices. All information generated was archived in a database.

In this cross-sectional study, risk factors such as production system (meat, milk, or mixed), raising system (extensive or any degree of confinement), cattle breeds, number of cows, total herd size, presence of other domesticated species, presence of wild animals, animal trade, routine of tuberculosis testing, slaughter in the farm, pasture sharing, feeding calves with whey, indirect contact between farms, flooded pastures, and veterinary assistance were assessed according to the literature (MARANGON et al., 1998; SKUCE et al., 2012). These variables were organized on an increasing risk scale. When necessary, a recategorization was performed. The least-risk category was always considered the baseline for comparisons with the other categories. Quantitative variables were categorized using the median as cutoff.

An exploratory univariate analysis was performed using the chi-square (χ^2) or Fisher exact

test with all variables, taking into account all of the data obtained from the entire state. Variables with a significance level of less than 0.20 were selected for use in a multivariate analysis using logistic regression, performed according to Hosmer and Lemeshow (2000). All calculations were conducted using R (R DEVELOPMENT CORE TEAM, 2012).

Results

The state of Espírito Santo was divided into two livestock regions (Figure 1), according to Azevedo et al. (AZEVEDO et al., 2009). The apparent prevalence of bTB-positive herds in the state was estimated to be 7.6% (95% CI = 5.7-9.9), ranging from 4.6% (95% CI = 2.6-7.3) in region 1 to 11.1% (95% CI = 7.7-15.3) in region 2. The results showed that the prevalence of bTB-positive farms was significantly different between the two regions (Table 1).

Figure 1. Map of the state of Espírito Santo showing the division of the two livestock regions, 2013.

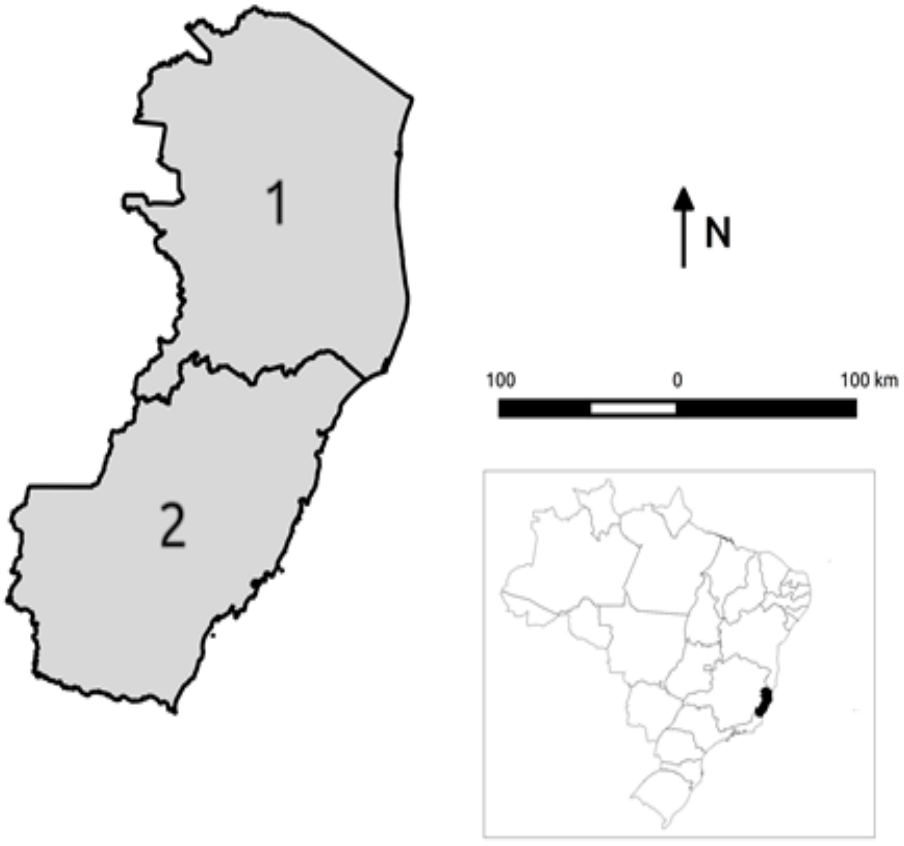

Table 1. Apparent prevalence of bovine tuberculosis at the herd level in the state of Espírito Santo, 2013.

Region	Farms with reproductive activities	Sampled farms	Positive farms	Prevalence (%)	95% CI (%)
1	17,496	349	16	4.6	2.6-7.3
2	15,100	288	32	11.1	7.7-15.3
Total	32,596	637	48	7.6	5.7-9.9

Moreover, no significant differences in the herd-level apparent prevalence were observed between farm enterprises (beef, mixed, and dairy) and for each of the farm enterprises between the regions (Table 2). The apparent prevalence of bTB-positive females aged over 2 years in the state of Espírito Santo was estimated to be 0.7% (95% CI = 0.3-1.1), ranging from 0.3% (95% CI = 0.2-0.6) in region 1 to 1.2% (95% CI = 0.3-2.9) in region 2 (Table 3).

Table 2. Apparent prevalence of bovine tuberculosis at the herd level, categorized by farm enterprise type, in the state of Espírito Santo, 2013.

Region	Beef cattle			Dairy cattle			Mixed cattle		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
1	0/38	0.0	0.0-7.4*	12/201	6.0	3.1-10.2	3/106	2.8	0.6-8.1
2	2/30	6.7	0.8-22.1	27/195	13.9	9.3-19.5	3/62	4.8	1.0-13.5

*Estimated with the Beta distribution, according to Vose (2008).

Table 3. Apparent prevalence of tuberculosis-positive female bovines aged over 2 years in the state of Espírito Santo, 2013.

Region	Females > 2 years of age	Sampled females > 2 years of age	Positive animals	Prevalence (%)	95% CI (%)
1	660,512	4,589	20	0.3	0.2-0.6
2	383,789	3,110	52	1.2	0.3-2.9
Total	1,044,301	7,699	72	0.7	0.3-1.1

The results of the univariate analysis, showing risk factors with $p < 0.20$ are presented in Table 4, and the results of the multivariate analysis are presented in Table 5. The final multivariate model indicated two risk factors: number of adult females ≥ 10 (odds ratio [OR] = 2.4; 95% CI = 1.17-5.31) and milking type (having a milking parlor or portable milking machine; OR = 2.88; 95% CI = 1.36-5.86).

Table 4. Univariate analysis of the risk factors ($p \leq 0.20$) for bovine tuberculosis in the state of Espírito Santo, 2013.

Variable	Proportion of infected herds	%	*p*
Milking type			0.001
No milking/Manual milking	31/547	5.7	
Milking machine/Milking parlor	13/71	18.3	
Number of females > 2 years			0.004
1-10*	12/285	4.2	
≥10	36/352	10.2	
Number of milkings per day			0.004
No milking	5/95	5.3	
1	27/445	6.1	
2-3	15/89	16.9	
Milk refrigeration			0.011
No	19/366	5.2	
Yes	26/240	10.8	
Farm enterprise			0.011
Beef	2/68	2.9	
Dairy	39/396	9.8	
Mixed	6/168	3.6	
Raising system			0.015
Extensive	37/559	6.6	
Any degree of confinement	11/71	15.5	
Acquisition of breeding animals**			0.021
No	22/386	5.7	
Yes	26/240	10.8	
Veterinary assistance			0.033
No	27/439	6.2	
Yes	21/187	11.2	
Breed			0.046
Beef	1/48	2.1	
European dairy	3/26	11.5	
European beef	3/10	30	
Mixed-breed	38/513	7.4	
Others	2/21	9.5	
Acquisition of animals**			0.073
No	19/328	5.8	
Yes	29/301	9.6	
Bulk tank			0.14
Owned	15/102	14.7	
Shared	11/138	8	
Presence of wild animals			0.18
Yes	30/338	8.9	
No	18/299	6	
Equipment, feedstock, or personal sharing			0.183
Yes	3/83	3.6	
No	45/554	8.1	

*Median. **During the previous 12 months.

Table 5. Final multivariate model of the risk factors for bovine tuberculosis in the state of Espírito Santo, 2013.

Variable	Odds ratio	95% CI	*p*
Number of females > 2 years of age			
1-9 (reference category)	-	-	-
≥10	2.40	1.17 – 5.31	0.022
Milking type			
No milking/Manual (reference category)	-	-	-
Milking machine/Milking parlor	2.88	1.36 – 5.86	0.004

r^2 = 6.9%.

Discussion

The prevalence of bovine herds infected with bTB in Espírito Santo was estimated to be 7.6% (95% CI = 5.7-9.9) (Table 2), which was statistically equal to the prevalence in the state of São Paulo and higher than that in the states of Bahia, Pernambuco, Paraná, Santa Catarina, Rio Grande do Sul, Mato Grosso do Sul, Mato Grosso, Rondônia, Goiás, Minas Gerais, and the Federal District (BAHIENSE et al., 2016; BARBIERI et al., 2016; DIAS et al., 2016; GUEDES et al., 2016; LIMA et al., 2016; NÉSPOLI et al., 2016; QUEIROZ et al., 2016; RIBEIRO et al., 2016; ROCHA et al., 2016; SILVA et al., 2016; VELOSO et al., 2016; VENDRAME et al., 2016). Moreover, in the state of Espírito Santo, the prevalence of bTB-infected herds was higher in dairy farms in both livestock regions, but it was significantly higher in region 2, which has more dairy cattle than does region 1 (Tables 1 and 2) (LAVAGNOLI et al., 2010). The prevalence of bTB-positive animals was also higher in region 2 than in region 1, but the confidence intervals for these proportions overlapped greatly (Table 3).

Among the different farm enterprises, dairy farms have been reported previously as risk factors for bTB (ALVAREZ et al., 2012; GRISI-FILHO et al., 2011; KAROLEMEAS et al., 2011; PORPHYRE et al., 2008; RAMÍREZ-VILLAESCUSA et al., 2010). This relationship may be attributed to the diary cattle's longer life expectancy and higher production stress, as well as to their gathering in large numbers during milking (HUMBLET et al., 2009). We also found this relationship (Table 4), but the variable did not make it to the final logistic model (Table 5), mainly because of its association with another more important variable – milking type. This latter variable is interesting, since it indicates two risk factors. First, a beef farm typically does not milk its cows, and thus its herds are in the baseline category of this variable. Second, dairy farms with milking machines or milking parlors have better technological infrastructure, and as such are more likely to have a higher animal density than its counterparts; this increases the risk of bTB transmission and maintenance in a farm, as previously identified in Belgium (HUMBLET et al., 2010). Moreover, enterprises with milking machines or milking parlors have shown a higher probability of purchasing breeding animals in the past 12 months (Table 6). Animal purchase has been found to be a risk factor in previous studies (BESSELL et al., 2012; CARRIQUE-MAS et al., 2008; CLEGG et al., 2012; OLOYA et al., 2007; RAMÍREZ-VILLAESCUSA et al., 2010; REILLY; COURTENAY, 2007; SKUCE et al., 2012; TSCHOPP et al., 2009) therefore, this variable is probably increasing the statistical significance of the milking type variable even further.

Table 6. Proportion of farms that purchased breeding animals in the past 12 months, according to its milking type, in the state of Espírito Santo, 2013.

Purchase of breeding animals	No milking/Manual milking	Milking machine/ Milking parlor	Total
No	64%	41%	62%
Yes	36%	59%	38%

The number of adult females in a herd was also found as a risk factor in the final model (Table 5). The relationship between herd size and bTB infection has already been found in several studies (BESSELL et al., 2012; CLEGG et al., 2012; GREEN; CORNELL, 2005; GRIFFIN et al., 1996; PORPHYRE et al., 2008; RAMÍREZ-VILLAESCUSA et al., 2010; REILLY; COURTENAY, 2007; SKUCE et al., 2012). The two main mechanisms underlying this finding are the increased risk induced by purchasing animals, since bigger herds are typically more likely to purchase animals (CIPULLO et al., 2016), and the greater persistence of the disease (BROOKS-POLLOCK; KEELING, 2009).

Conclusion

The southern region of Espírito Santo has a higher bTB prevalence at the herd level (11%) probably because of its higher concentration of dairy farms, whereas bTB prevalence in the northern region is moderate (5%). Herd size and milking type (having a milking machine or milking parlor) were found to be risk factors for bTB infection in herds. The latter probably indicates the influence of other variables, such as enterprise type (dairy), animal density, and animal introduction. On the basis of these findings, we recommend that the abovementioned risk factors should be incorporated into the design of risk-based surveillance and control programs.

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