# Prevalence and risk factors for bovine tuberculosis in the State of Mato Grosso do Sul, Brazil

# Prevalência e fatores de risco para tuberculose bovina no estado de Mato Grosso do Sul, Brasil

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## Abstract

The objective of this study was to describe the epidemiological situation of bovine tuberculosis in Mato Grosso do Sul. The State was divided into three regions, Pantanal, Planalto Norte, and Planalto Sul. In each region, properties were randomly chosen and a pre-established number of animals were also randomly selected; these animals were submitted to the intradermal comparative cervical tuberculin diagnostic test. In total, 17,121 animals from 938 properties were tested. In cases of inconclusive results, the animals were retested with the same diagnostic procedure within a minimum interval of 60 days. An epidemiological questionnaire was administered in the properties sampled to investigate possible risk factors for the disease. In the State, the prevalence of infected herds was 1.3% (95% confidence interval [CI]: 0.72–2.37%) and that of infected animals was 0.035% (95% CI: 0.017–0.069%). Point estimates indicated a higher concentration of infected herds (2.61%; 95% CI: 1.31–5.15%) and infected animals (0.132%; 95% CI: 0.055–0.315%) in the Planalto Sul region, which has a predominance of dairy properties. The prevalence of herds infected with bovine tuberculosis was found to be associated with the production of milk, with technification in the mode of production represented by the use of mechanical milking. Thus, it is recommended that the State adopt strategies for the eradication of bovine tuberculosis, with the structuring of the surveillance system for detection and mitigation of infected herds, incorporating risk-based surveillance elements.

**Key words**: Bovine tuberculosis. Brazil. Mato Grosso do Sul. Prevalence. Risk factors.

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## Resumo

O objetivo do presente estudo foi descrever a situação epidemiológica da tuberculose bovina no Mato Grosso do Sul. O estado foi dividido em três regiões, Pantanal, Planalto Norte e Planalto Sul. Em cada região, propriedades foram sorteadas aleatoriamente e, dentro dessas, selecionou-se também de forma aleatória um número pré-estabelecido de animais, os quais foram submetidos ao teste Tuberculínico Cervical Comparativo. No total, foram testados 17.121 animais, provenientes de 938 propriedades. Os animais que resultaram inconclusivos foram retestados com o mesmo procedimento diagnóstico em intervalo mínimo de 60 dias. Nas propriedades amostradas foi aplicado um questionário epidemiológico para averiguar possíveis fatores de risco para a enfermidade. No estado, a prevalência de focos foi de 1,3% [0,72; 2,37%] e a de animais 0,035% [0,017; 0,069%]. As estimativas pontuais indicaram uma maior concentração de focos (2,61% [1,31; 5,15%]) e de animais (0,132% [0,055; 0,315%]) na região Planalto Sul, que tem predomínio de propriedades leiteiras. Verificou-se que a condição do foco de tuberculose bovina está associada à produção de leite, com sofisticação no modo de produção representado pela utilização de ordenha mecânica. Assim, recomenda-se que o estado adote estratégias de erradicação, com a estruturação de sistema de vigilância para detecção e saneamento de focos, incorporando elementos de vigilância baseada em risco.

**Palavras-chave**: Brasil. Fatores de risco. Mato Grosso do Sul. Prevalência. Tuberculose bovina.

## Introduction

Mato Grosso do Sul is the sixth largest Federative Unit of Brazil, with an area of 357,000 km$^2$, geographically divided into four mesoregions and 11 microregions, containing 79 municipalities, with a resident population of approximately 2.4 million with urbanization of 86%.

Agribusiness is one of the major economic activities in the State. Currently, Mato Grosso do Sul holds the fourth largest bovine herd in Brazil, with approximately 21 million head. It is one of the largest State producers of beef in the country, in addition to having important dairy basins near large centers.

According to the Ministry of Development, Industry and Foreign Trade (MDIC), in 2014, the State exported, preferentially to Asia and the European Union, approximately US$1 billion in boneless meat and frozen beef, US$102 million in fresh or chilled meat, and US$170 million in offal and leather, generating approximately 280 million reais in taxes on movement of goods and services (ICMS).

Regarding animal health, Mato Grosso do Sul still faces major challenges, including the fight against tuberculosis and brucellosis. In 2001, the Ministry of Agriculture, Livestock and Supply (MAPA) launched the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (PNCEBT) (LAGE et al., 2006), with methodologies harmonized with those recommended by the World Organization for Animal Health (OIE). These methodologies are based on vaccination against brucellosis, certification of properties through routine indirect tests, and surveillance systems for areas in the eradication phase.

In order to generate high-quality data to permit selection of the optimal strategies and management of eradication processes, following implementation of the PNCEBT, the Collaborating Center in Animal Health of the MAPA, based in the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo, along with the MAPA and the official veterinary services of the States, have conducted studies on the epidemiological situations of these two infections.

Studies conducted on bovine tuberculosis in 12 Federative Units, which account for 66% of the Brazilian herd, showed prevalence of infected herds ranging from 0.36% in the Distrito Federal to 9.0% in São Paulo (BAHIENSE et al., 2016; BARBIERI

et al., 2016; DIAS et al., 2016; GALVIS 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; BAUMGARTEN et al., 2016; VENDRAME et al., 2016).

Some studies regarding tuberculosis in the State of Mato Grosso do Sul have been published in the literature. Schenk and Schenk (1982) reported a prevalence of 0.2% of suggestive lesions in slaughterhouses from 1974 to 1979. From tuberculous lesions collected from cattle in the State, Jorge (2001) isolated mycobacteria belonging to the *Mycobacterium tuberculosis* complex and Araújo et al. (2005) obtained isolates identified as *M. bovis*. Cazola et al. (2015), working with tissues of animals with positive tuberculin reactions collected from various regions of the State, identified seven isolates as *M. bovis*, belonging to spoligotypes SB0121, SB1145, and SB0881. Azambuja (2010) found that 12% (24/200) of bovines tested positive for the intradermal comparative cervical tuberculin diagnostic test in the milk basin of Caarapó, a municipality located in the center of the Southern Plateau region of the State. In contrast, Piva Filho (2013), using the same diagnostic test, found a rate of positive-testing animals of 0.26% in the municipality of Parnaíba (MS).

Thus, noting that bovine tuberculosis is rampant in the State of Mato Grosso do Sul and its epidemiological situation is not adequately known, the present study aimed to estimate the prevalence of infected herds and infected animals, exploring the possibility of regional differences, and to identify the risk factors associated with the disease.

## Materials and Methods

The preparations for the study were carried out in collaboration with the MAPA, the State Agency for Animal and Plant Health Protection (IAGRO-MS) and the Collaborating Center in Animal Health, located in the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo. The fieldwork was performed by veterinarians of IAGRO-MS from April to December 2009.

In order to verify the existence of possible regional differences, the State was divided in regions with homogeneous characteristics, taking into account production systems, management practices, purposes of production, average sizes of herds, and marketing systems, as well as the operational capacity and logistics of the official veterinary service of the State to conduct field activities, as described by Negreiros et al. (2009).

A two-stage sampling was performed to allow estimation of the prevalence of infected herds and infected animals in the regions and in the State. In the first stage, within each region, a pre-established number of properties with reproductive activity were randomly selected from the property registry of IAGRO-MS. In the second stage, within each property, a pre-established number of reproductive cows with ages equal to or greater than 24 months were randomly selected. On properties where herds were separated, only the predominant one, that with the highest economic value or considered as the main objective of production, was the target of the study. This herd was defined as a group of animals maintained under the same management practices, i.e., exposed to equal risk conditions. Properties that had been selected randomly, but that for any reason could not be sampled, were replaced using a new round of randomization. The number of selected properties per region was estimated by the formula for a simple random sample (THRUSFIELD, 2007), according to the following parameters: estimated prevalence of 20%, 95% confidence level, and 5% error.

For the randomization of animals within each property, the number of bovines to be examined with the goal of classifying them as either an infected herd or free of tuberculosis was calculated, considering aggregate sensitivity values equal to or higher than 85% and specificity aggregate

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equal to or higher than 95%. The individual values of sensitivity and specificity adopted were 80% and 99.5%, respectively. The calculations were performed with the aid of the Herdacc program version 3.0.

On properties with up to 99 breeding cows, with ages equal to or greater than 24 months, 20 animals were examined. On properties with 100 or more cows, 40 were examined. These animals were always randomly selected from the sample and those within 15 days prior and subsequent to delivery were replaced.

The animals of the sample were submitted to the intradermal comparative cervical tuberculin diagnostic test conducted in accordance with the Technical Manual of the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (LAGE et al., 2006). In order to avoid the maintenance of suspect animals at the end of the study, those with inconclusive results were retested with the same diagnostic procedure within a minimum interval of 60 days. All animals testing positive were euthanized.

For the properties with up to 20 tested animals, only one positive result was required to classify them as a herd infected with tuberculosis. For the properties with 40 tested animals, at least two positive results were required in order to classify them as an infected herd.

For each region and for the entire State, the prevalence of herds infected by bovine tuberculosis was obtained. The prevalence of infected herds within each region was also stratified by type of production. The calculations of apparent prevalence and the respective confidence intervals were performed as recommended by Dean et al. (1994). The calculations of prevalence of infected herds in the State, as well as prevalence of positive animals within the regions were weighted (DOHOO et al., 2003). The weight of each property in calculating prevalence of infected herds in the State was given by:

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

The weight of each animal in calculating prevalence of infected animals in the State was given by:

$$P_2 = \frac{\text{Cows} \geq 24 \text{ months on the property}}{\text{Cows} \geq 24 \text{ months sampled on the property}} \times \frac{\text{Cows} \geq 24 \text{ months in the region}}{\text{Cows} \geq 24 \text{ months sampled in the region}}$$

In the expression above, the first term refers to the weight of each animal in the calculation of prevalence of infected animals within the regions. Calculations were performed using the SPSS program, version 20.

In all the properties selected for this study, a questionnaire was also administered to individualize the risk factors associated with the condition of an infected herd. The questionnaire was formulated to examine the existence of exposure to classic risk factors, already described in the literature (MARANGON et al., 1998; RAMÍREZ-VILLAESCUSA et al., 2010; SCKUCE et al., 2012), as well as others of particular regional interest. The questions addressed the following variables: type of production (beef, dairy, or mixed); degree of agglomeration of animals (confined, semi-confined, and extended); number and breed of the animals; use of mechanical milking and artificial insemination; form of milk delivery; introduction of cattle; presence of other animal species in the property (domestic and wild); existence of border with forests; routine testing for bovine tuberculosis; place of slaughter of breeding animals; sharing of pastures, waterholes/drinkers; inputs, equipment, and staff; presence of flooded areas; cattle feed with whey; and existence of veterinary assistance.

The variables were displayed on an increasing risk scale. When necessary, reclassification of these variables was performed. The category of lower risk was considered as a basis for comparison of other categories. The quantitative variables were reclassified into quartiles. A first exploratory data analysis was performed (univariate analysis) to select those variables with p ≤ 0.20 with the $\chi^2$ or Fisher's exact test, and these variables were subsequently subjected to logistic regression, as recommended by Hosmer and Lemeshow (1989). Calculations were performed using the SPSS program, version 20. All the data generated by the fieldwork were inserted in a specific database, used in epidemiological analyses.

## Results

The State was divided into three regions (Figure 1). Region 1 refers to the Pantanal, where the main type of production is extensive breeding. Regions 2 and 3 are located in the Planalto and are renowned for their production of beef, but they also have significant dairy production. In the Planalto Norte, the activities of cattle breeding, rebreeding, and fattening predominate, while fattening and finishing predominate in the Planalto Sul.

**Figure 1.** Map of the State of Mato Grosso do Sul with division into regions: Pantanal (1), Planalto Sul (2), and Planalto Norte (3). Detailed location of the State in Brazil. 2009.
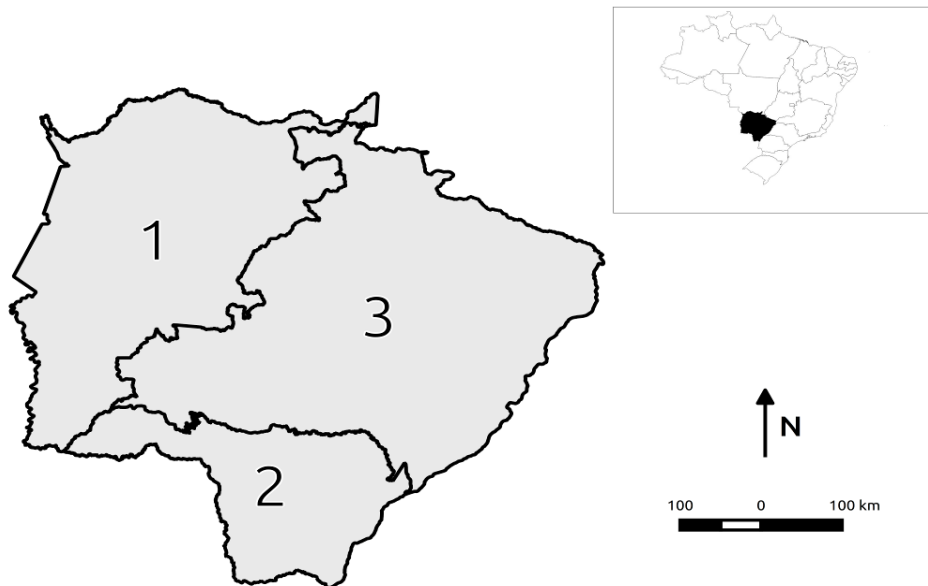


Table 1 presents a summary of census data and the studied sample. Table 2 shows the prevalence of herds infected with bovine tuberculosis in the regions and in the State. The prevalence values of infected herds per type of farming are presented in Table 3 and the prevalence values of cows testing positive to the tuberculin test are presented in Table 4.

**Table 1.** Summary of bovine herd data and census details of the studied sample, according to the regions of the State of Mato Grosso do Sul, Brazil. 2009.

| Region | Number of Municipalities | Properties with reproductive activity | Sampled properties | Cows with ages ≥ 24 months | Cows with ages ≥ 24 months sampled |
|---|---|---|---|---|---|
| 1 - Pantanal | 9 | 7,543 | 305 | 2,130,725 | 6,343 |
| 2 -Planalto Sul | 31 | 25,242 | 306 | 1,455,919 | 4,345 |
| 3- Planalto Norte | 38 | 40,494 | 327 | 4,488,153 | 6,433 |
| Mato Grosso do Sul | 78 | 73,279 | 938 | 8,074,797 | 17,121 |

**Source:** IAGRO-MS, 2009.

**Table 2.** Prevalence of herds infected with bovine tuberculosis for the regions and for the State of Mato Grosso do Sul, Brazil. 2009.

| Region | Properties | | Prevalence (%) | 95% CI (%) |
|---|---|---|---|---|
| | Examined | Positive | | |
| 1 - Pantanal | 305 | 2 | 0.66 | [0.16–2.60] |
| 2 - Planalto Sul | 306 | 8 | 2.61 | [1.31–5.15] |
| 3- Planalto Norte | 327 | 2 | 0.61 | [0.15–2.42] |
| Mato Grosso do Sul | 938 | 12 | 1.30 | [0.72–2.37] |

**Table 3.** Prevalence of herds infected with bovine tuberculosis stratified by type of farming in regions of the State of Mato Grosso do Sul, Brazil. 2009.

| Region | Prevalence of herds infected with tuberculosis (P) (%) | | | | | |
|---|---|---|---|---|---|---|
| | Beef | | Dairy | | Mixed | |
| | P (%) (positive/ examined) | 95% CI (%) | P (%) (positive/ examined) | 95% CI (%) | P (%) (positive/ examined) | 95% CI (%) |
| 1 - Pantanal | 1.0 (2/191) | [0.17–3.42] | 0.0 (0/86) | [0.0–0.34] * | 0.0 (0/26) | [0.0–0.10]* |
| 2- Planalto Sul | 1.0 (1/100) | [0.05–4.83] | 4.4 (7/158) | [1.96–8.56] | 0.0 (0/48) | [0.0–0.06]* |
| 3 - Planalto Norte | 0.0 (0/146) | [0.0–0.02]* | 1.6 (2/124) | [0.27–5.23] | 0.0 (0/55) | [0.0–0.05]* |

* Calculation using the method of Monte Carlo and distribution beta. CI: confidence interval.

**Table 4.** Prevalence of cows positive for tuberculosis for the region and for the State of Mato Grosso do Sul, Brazil. 2009.

| Region | Cows | | Prevalence (%) | 95% CI (%) |
|---|---|---|---|---|
| | Examined | Positive | | |
| 1 - Pantanal | 6,343 | 2 | 0.005 | [0.001; 0.031] |
| 2 - Planalto Sul | 4,345 | 12 | 0.132 | [0.055; 0.315] |
| 3- Planalto Norte | 6,433 | 5 | 0.016 | [0.005; 0.051] |
| Mato Grosso do Sul | 17,121 | 19 | 0.035 | [0.017; 0.069] |

Table 5 shows the final model of logistic regression for the risk factors for bovine tuberculosis in the State of Mato Grosso do Sul.

**Table 5.** Final logistic regression model showing the risk factors for bovine tuberculosis in the State of Mato Grosso do Sul, 2009.

| Variable | Odds ratio | 95% CI (%) |
|---|---|---|
| Dairy enterprise | 3.87 | [1.00–14.80] |
| Mechanical milking | 4.86 | [1.23–19.28] |

## Discussion

Herds infected with bovine tuberculosis were detected in all regions of the State of Mato Grosso do Sul, and the prevalence of infected herds in the State was 1.3% (95% confidence interval [CI]: 0.72–2.37%) (Table 2). This prevalence of infected herds is equal to those observed in the states of Bahia, Paraná, Rio Grande do Sul, Mato Grosso, Rondônia, Goiás, Pernambuco, and Distrito Federal. It exceeds the prevalence of infected herds in the State of Santa Catarina and is lower than those observed in São Paulo, Minas Gerais, and Espírito Santo (BAHIENSE et al., 2016; BARBIERI et al., 2016; DIAS et al., 2016; GALVIS 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; BAUMGARTEN et al., 2016; VENDRAME et al., 2016).

Point estimates indicated a higher concentration of infected herds in the Planalto Sul region, albeit without statistical significance (Table 2). Table 3 shows that this is the only region with a predominance of dairy farms, while in the other two -Pantanal and Planalto Norte -beef farming predominates. In fact, the point prevalence estimates of infected herds are higher in the dairy properties, which lie in the Planalto Sul and Planalto Norte regions (Table 3).

The association between bovine tuberculosis and dairy activity was observed by several international authors (PEREZ et al., 2002; PORPHYRE et al., 2008; RAMÍREZ-VILLAESCUSA et al., 2010; ZENDEJAS-MARTÍNEZ et al., 2007, 2008), and this may be attributed to factors inherent to this type of farming, described as predisposing to the disease, such as high-density (CLEAVELAND et al., 2007; HUMBLET et al., 2009) and longer productive cycles (REGASSA et al., 2010; BIFFA et al., 2011).

The prevalence of infected animals in the State was 0.035% (95% CI: 0.017–0.069), and there was a higher number of cows testing positive to tuberculin in the Planalto Sul region compared to the other two regions (Table 4). The same analysis, performed previously for the prevalence of infected herds, may be applied to the prevalence in animals.

According to studies conducted to date in the country, Mato Grosso do Sul has a low prevalence of bovine tuberculosis. The adoption of strategies for the eradication of the disease with the structuring of a surveillance system for detection and mitigation of infected herds is recommended.

Table 5 shows that the risk factors for bovine tuberculosis in the State are related to dairy farms with some degree of sophistication in the production system, represented by the use of mechanical milking.

Dairy herds are more vulnerable to tuberculosis, because milk production features the agglomeration of animals on the occasion of milking, which increases the risk of transmission (BARLOW et al., 1997) since it raises the population density and consequently the probability of infectious contacts (HUMBLET et al., 2010; ALVAREZ et al., 2012). Dairy herds were also found to have a higher risk of tuberculosis in New Zealand (PORPHYRE et al., 2008) and the United Kingdom (RAMÍREZ-VILLAESCUSA et al., 2010; KAROLEMEAS et al., 2011). In Brazil, this association was previously detected in the states of Bahia, Goiás, Mato

Grosso, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina, and São Paulo (BAHIENSE et al., 2016; ROCHA et al., 2016; NÉSPOLI et al., 2016; BARBIERI et al., 2016; SILVA et al., 2016; QUEIROZ et al., 2016; BAUMGARTEN et al., 2016; DIAS et al., 2016). Moreover, animals from dairy herds tend to be older than animals from beef herds, therefore having a higher likelihood of exposure to the infectious agent (HUMBLET et al., 2009).

In addition, the sophistication of the dairy properties, using fully automatic mechanical milking in milking rooms or partially automatic mechanical milking, increased the risk of bovine tuberculosis. The stress of high milk production was associated with an increased risk of bovine tuberculosis (GRIFFIN et al., 1996).

## Conclusion

The State has a low prevalence of bovine tuberculosis and the best strategy to be adopted is the implementation of a surveillance system to detect and mitigate infected herds, preferably incorporating elements of risk-based surveillance.

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