# Distribution of lesions in cattle affected by bovine tuberculosis in the State of Minas Gerais from 2004-2008

*Distribuição das lesões e caracterização dos animais acometidos pela tuberculose bovina no estado de Minas Gerais, 2004-2008*

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## Abstract

The distribution of lesions and the characteristics of cattle affected by bovine tuberculosis (TB) in the state of Minas Gerais (MG) were evaluated using data from samples sent to the official laboratory of the Brazilian Ministry of Agriculture for bacteriological diagnosis. It was conducted a descriptive study using 1,081 samples with lesions suggestive of TB that were sent for bacteriological diagnosis of *Mycobacterium bovis*. The respiratory system had the highest injury frequency (53.01%), followed by the carcass (20.23%), the abdominal cavity (16.52%), the tongue (8.26%) and the breast (1.98%). The pulmonary lymph nodes (19.7%), mediastinal lymph nodes (12.8%) and lung parenchyma (12.0%) were the main tissues from which *M. bovis* was isolated with great regularity. This study revealed the existence of a higher percentage of female (63.8%) dairy cattle (40.1%) from two and a half to seven years of age (85.2%) among the affected

animals. The results of this study will help to subsidize the National Program for Control and Eradication Tuberculosis in Brazil, which aims to collect samples for bacterial isolation and molecular diagnosis.

**Keywords:** Bovine tuberculosis. Diagnosis. Lesions. Animals affected.

## *Resumo*

*A distribuição das lesões e a caracterização dos animais acometidos por Tuberculose Bovina (TB) no estado de Minas Gerais, foram avaliadas por meio dos dados relativos às amostras enviadas para o diagnóstico bacteriológico no Laboratório Nacional Agropecuário (LANAGRO) em Pedro Leopoldo (MG), de 2004 a 2008. Foi feito um estudo descritivo a partir de 1081 amostras com lesões sugestivas de TB enviadas para o diagnóstico bacteriológico de Mycobacterium bovis. O aparelho respiratório apresentou a maior frequência de lesões (53,01%), seguida da carcaça (20,23%), cavidade abdominal (16,52%), conjunto cabeça-língua (8,26%) e mama (1,98%). Os linfonodos pulmonares (19,7%), mediastínicos (12,8%) e o parênquima pulmonar (12,0%) foram os principais tecidos onde foi isolado o M. bovis com maior regularidade. Evidenciou-se a existência de um maior percentual de fêmeas (63,8%) de raças leiteiras (40,1%), com idade acima de dois anos e meio até sete anos (85,2%) entre os animais acometidos.*

***Palavras-chave:*** *Tuberculose. Bovino. Lesões. Animais acometidos. Diagnóstico bacteriológico.*

## Introduction

Bovine tuberculosis (TB) is a globally distributed zoonotic disease caused by *Mycobacterium bovis* and origins numerous economic and social losses associated with the activity of dairy and beef cattle (SILVA et al., 2013).

Official reports indicated a prevalence of infected animals of 1.3% in Brazil between 1989 and 1998 (BRASIL, 2006). The overall prevalence in slaughtered animals in the state of Minas Gerais, which is the main milk-producing state in Brazil, was 0.7%, with temporal and spatial variation between 0% and 8.7% (BRASIL, 2006). It was attributed the large spatial and temporal variation observed in the prevalence of tuberculosis in relation to the technical and material conditions of each slaughterhouse, to cattle origin and to categories such as age, sex and zootechnical breeding system.

TB is been characterized by the formation of granulomatous lesions that are nodular in appearance and called tubers (FLAMAND et al., 1994). In cattle, these lesions are observed more frequently in the bronchial and mediastinal lymph nodes, which may be the only infected tissues. However, the lungs, the liver, the spleen, the surfaces of body cavities and other anatomic sites may also be infected (OIE, 2010). It is important to understand the distribution of these lesions in bovine tissues. The microbiological diagnosis of TB is arduous and time-consuming (i.e., this process can last up to three months), so the samples must be chosen carefully to achieve a better efficiency in laboratory diagnosis (MEDEIROS et al., 2010). Sample selection must be performed carefully, even if the laboratory chooses to use faster methods, such as PCR (FIGUEIREDO et al., 2010; ARAÚJO et al., 2014).

Within this context, the aim of this study was to evaluate the distribution of the lesions and the characteristics of cattle affected by TB using samples that were collected in the Brazilian state of Minas Gerais and sent to the official laboratory (Lanagro/MG) of the Brazilian Ministry of Agriculture, Livestock and Supply for bacteriological diagnosis between 2004 and 2008.

## Materials and methods

In this epidemiological survey, data were collected using information from forms that were sent with each sample that required *Mycobacterium* isolation. This information was processed using

methods for the descriptive analysis of variables, as described by Sampaio et al. (2007).

A total of 1,081 tissue fragments with gross lesions suggestive of TB were collected from 792 animals, including males and females of various ages, in slaughterhouses from the State of Minas Gerais. These lesions had nodular features, and were predominantly hemorrhagic, of varying sizes and shapes, caseous or calcified, and purulent or not purulent. This material was obtained during the sanitary inspection of carcasses in a slaughterhouse or during the performance of autopsies on rural properties and was primarily composed of fragments of lung, pre-scapular, pre-pectoral and mediastinal lymph nodes and hepatic parenchyma. The samples were placed in wide-mouth bottles labeled with the identification of the animal and tissue, the origin and the date of collection; the bottles were sealed and wrapped in a plastic bag. The specimens used for diagnosis were sent in a polystyrene box with ice, as recommended by the Technical Manual of the National Program of Control and Eradication of Brucellosis and Tuberculosis (BRASIL, 2006). The material was kept frozen in the laboratory until processing.

For each sample sent for bacteriological examination, the following procedures were performed: thawing to room temperature; macroscopic evaluation of lesions; maceration with sterile distilled water and sand; decontamination with 6% sulfuric acid ($H_2SO_4$); washes with phosphate buffer, pH 7.0; inoculum preparation for smear slides stained using the Ziehl Neelsen stain; and inoculation in Lowenstein-Jensen and Stonebrink-Lesslie media. The culture media were incubated at 37 °C for up to 10 weeks. Each isolated bacterium was characterized using biochemical tests to confirm the isolation of *M. bovis*. The employed procedures were based on previous published methodologies (KENT; KUBICA, 1985).

All activities involving the isolation and biochemical identification of *M. bovis* were performed in the Laboratory of Diagnosis of Bacterial Diseases of Lanagro/MG in the period from 2004 to 2008.

## Results and discussion

Of the 1,081 samples, 871 samples were suitable for bacteriological diagnosis. Of these samples, *M. bovis* was isolated from 574 (65.9%) samples. The remaining 210 samples were found to be unfit for diagnosis due to the high rate of contamination.

Table 1 shows the distribution of the TB lesions in anatomic specimens sent to the laboratory for bacteriological diagnosis. The respiratory tissues had the highest injury frequency (53.01%), followed by the carcass (20.23%), the abdominal cavity (16.52%), the tongue (8.26 %) and the breast (1.98%). In the respiratory system, the macroscopic changes were distributed in the following order: lung lymph nodes, mediastinal lymph nodes and lung parenchyma. In the carcass, the lesions were more frequent in the scapular lymph nodes. In the abdominal cavity, the lesions were prevalent in the hepatic parenchyma, the hepatic lymph nodes and the intestinal lymph nodes. In the head, most lesions were present in the sublingual and retropharyngeal lymph nodes. In the udder, the lesions were present most frequently in the mammary lymph nodes.

The lesions were present more frequently in the respiratory tract (53.01%). This result reinforces previous publications that demonstrated that the respiratory pathway is the most important site of infection in cattle (CORNER et al., 1990; FREITAS; GUERRA; PANETTA, 2001; GRISI FILHO et al., 2011; ROGERS; DONALD; SCHULTZ, 1980; SOUSA et al., 2003; WHIPPLE; BOLIN; MILLER, 1996). The carcass was the area with the second highest distribution of lesions, with an occurrence rate of 20.23%. This result differed from that reported by Freitas, Guerra and Panetta (2001), who collected samples from slaughtered buffalos and found that the carcass was the third most common site of lesions, with an occurrence rate of 9.6%. Grisi Filho et al. (2011) also reported different results, as those authors observed that the carcass and liver were the fourth most common localization of tuberculous granulomas (11.86%). The abdominal cavity was the third most common site of granulomas in our study (16.52%), but this site had a frequency of 6.3% and occupied the fourth position (FREITAS; GUERRA; PANETTA, 2001) or a frequency of 39.13% and occupied the second position (GRISI FILHO et al., 2011) in other studies.

The head occupied was the fourth most common site of lesions among the five regions analyzed, with a frequency of 8.26%. This result differs from the results reported in other studies, which found that the head region was the second common site

**Table 1** - Distribution of gross lesions caused by bovine tuberculosis in anatomic specimens sent to an official laboratory of the Brazilian Ministry of Agriculture from 2004 to 2008.

| Local | Nº | % |
|---|---|---|
| Respiratory tract | 304 | 53.01 |
| Pulmonary lymph nodes [1] | 113 | 19.65 |
| Mediastinal lymph nodes | 73 | 12.8 |
| Pulmonary parenchyma | 69 | 11.97 |
| Tracheobronchial lymph node | 19 | 3.3 |
| Esophageal lymph node | 12 | 2.15 |
| Apical lymph node | 9 | 1.65 |
| Diaphragm (fragment) | 6 | 1.07 |
| Pleura | 2 | 0.41 |
| Carcass | 116 | 20.23 |
| Pre-scapular lymph node | 43 | 7.43 |
| Pre-pectoral lymph node | 27 | 4.62 |
| Pre-crural lymph node | 14 | 2.48 |
| Iliac lymph node | 14 | 2.39 |
| Sciatic lymph node | 11 | 1.9 |
| Popliteal lymph node | 8 | 1.32 |
| Inguinal lymph node | 0 | 0.08 |
| Abdominal Cavity | 95 | 16.52 |
| Hepatic parenchyma (liver) | 40 | 6.94 |
| Intestinal lymph [2] | 21 | 3.72 |
| Hepatic lymph node | 20 | 3.47 |
| Intestines (fragments) | 7 | 1.24 |
| Mesenteric lymph node | 5 | 0.91 |
| Spleen | 1 | 0.17 |
| Suprarenal lymph node | 0 | 0.08 |
| Head-tongue set | 47 | 8.26 |
| Retropharyngeal lymph node | 29 | 5.12 |
| Sublingual lymph node | 8 | 1.4 |
| Parotid lymph node | 6 | 0.99 |
| Sublingual gland | 3 | 0.5 |
| Lymph node | 1 | 0.17 |
| Mandibular lymph node | 0 | 0.08 |
| Udder | 11 | 1.98 |
| Mammary lymph node | 9 | 1.57 |
| Retromammary lymph node | 2 | 0.41 |
| TOTAL | 574 | 100 |

Legend: [1] Lung lymph lodes e [2] Intestinal lymph nodes: nominated in accordance with the findings in the original database.

Source: Research data.

of bovine tuberculosis lesions (CORNER et al., 1990; FREITAS; GUERRA; PANETTA, 2001; GRISI et al., 2011; ROGERS; DONALD; SCHULTZ, 1980; SOUSA et al., 2003; WHIPPLE; BOLIN; MILLER, 1996). These differences are likely linked to individual immune responses. According to Neill, Bryson and Pollock (2001), the bovine immune response that occurs during infection with *M. bovis* is extremely complex and dynamic, involving a variety of cellular events that result in clinical and pathological characteristics that are markedly different among individual animals.

Finally, the udder and teat region exhibited the lowest percentage of lesions (1.98%), corroborating the findings of other studies (FREITAS et al., 2001; GRISI FILHO et al., 2011). Even if udder lesions are uncommon, they are important for both public health and disease transmission to young animals. The ingestion of contaminated raw milk is one of the principal routes of human infection with *M. bovis*, and young animals that receive milk from cows with mammary gland tuberculosis are at risk of infection (ABRAHÃO; NOGUEIRA; MALUCELLI, 2005; MODA et al., 1996).

The lesions observed in the lymph nodes were found in the following frequencies: 12.8% in the mediastinal lymph nodes, 7.4% in the pre-scapular lymph nodes, 0.9% in the mesenteric lymph nodes and 1.9% in the sciatic lymph nodes (Table 1). These findings were similar to those observed in buffalos (FREITAS; GUERRA; PANETTA, 2001), which had the following frequencies: 10% in the mediastinal lymph nodes, 2.5% in the pre-scapular lymph nodes, 0.8% in the mesenteric lymph nodes, and 1.3% in the sciatic lymph nodes. A study conducted with samples collected from beef cattle in a slaughterhouse (SOUSA et al., 2003) observed frequencies of 42.9% for the mediastinal lymph nodes, 14.5% for the pre-scapular lymph nodes, 5.9% in the mesenteric lymph nodes and 3.7% in the ischiatic lymph nodes. In part, these differences may have been observed due to the diversity of lymph node sizes and the presence of caseous masses or discrete lesions, which could have influenced the identification of lesions during sanitary inspection.

The frequency of animals with lesions according to gender was 63.8% (505/792) in females and 31.7% (251/792) in males. Only 4.5% (36/792) of the samples failed to specify gender. These results were similar to those reported by Grisi Filho et al.

(2011), with frequencies 58.89% in females and 37.94% in males.

A higher incidence of animals with a positive bacteriological diagnosis was observed in animals aged between 2.5 years and 4.5 years (72.2%). The age group between 4.5 years and 7 years was the second most frequent, with a distribution of 13%. Animals aged up to 2.5 years comprised the third most frequent group (8.8%). Therefore, the disease was concentrated among animals aged from 2.5 to 7 years (85.2%).

Mixed animals provided 37.3% of the samples. Among the dairy breeds, Jersey (16.2%), Netherlands (12.2%), Girolando (6.7%) and Brown Swiss (5.0%) were those that most contributed most to the material sent for laboratory analysis (approximately 40% of the samples).

## Conclusion

In this study, gender, age and race profiles had a significant impact on the results, likely due to the characteristics of the herds in the State of Minas Gerais, where there is a vocation for dairy cattle.

This study is the first study performed using samples sent directly to the official laboratory of the Brazilian Ministry of Agriculture. According to the findings of this study, the incidence of TB lesions was higher in the respiratory tract, confirming that this route is the most important route of infection in cattle. Most of the animals were female dairy breeds two and a half years to seven years old. The results of this study will help subsidize the National Program for Control and Eradication of Brucellosis and Tuberculosis (PNCEBT) instituted by the Ministry of Agriculture, Livestock and Supply (MAPA) by providing data related the profiles of cattle affected by tuberculosis in Brazil.

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