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Tuberculosis Lesions Associated with the Production and Epidemiological Profiles of Cattle with Positive Intradermal Test

Lesões de Tuberculose Associadas aos Perfis Produtivo e Epidemiológico de Bovinos Positivos ao Teste Intradérmico

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Abstract

Bovine tuberculosis (BT) is an infectious disease caused by *Mycobacterium bovis*, characterized by nodular and granulomatous lesions in organs, lymph nodes and carcasses, leading to significant economic and sanitary losses in cattle production. The comparative cervical test (CCT) is the main method recommended by the World Organization for Animal Health (WOAH) for diagnosis. This study aimed to correlate the presence of characteristic BT lesions in the sanitary slaughter of 257 bovines from nine cities in Northern Paraná State (2021-2022) with the productive (management system and aptitude) and epidemiological (sex, age and origin) characteristics of the animals. Only 28% (71/257) of the CCT-positive animals had lesions, and 91% (64/71) of these were females over 36 months old. The correlations between the presence of lesions and age and sex were significant, indicating a higher prevalence of the disease in older females. Lesions were mainly in the head (97.1%), lung (69.1%), lung lymph nodes (69.1%), liver (50.7%), intestines (31.0%) and spleen (29.6%). There is no direct correlation between the animals' location and lesions' presence. Still, the distribution of these

injuries suggests possible differences in the mechanisms of infection, the strains of *M. bovis* or the stages of the disease.

Keywords: Age. Aptitude. Sex. Management System. Origin.

Resumo

A tuberculose bovina (TB) é uma doença infecciosa causada pela *Mycobacterium bovis*, caracterizada por lesões nodulares e granulomatosas em órgãos, linfonodos e carcaça. levando a perdas econômicas e sanitárias significativas na produção de gado. O teste cervical comparativo (TCC) é o principal método recomendado pela Organização Mundial de Sanidade Animal (OMSA) para diagnóstico. Este estudo analisou dados de abate sanitário de 257 bovinos oriundos de nove municípios do norte do Paraná (2021-2022), correlacionando características produtivas (sistema de manejo e aptidão) e epidemiológicas (sexo, idade e origem) com a presença de lesões características de TB. Apenas 28% (71/257) dos animais positivos no TCC apresentaram lesões, sendo que 91% (64/71) eram fêmeas com mais de 36 meses. As relações entre a presença de lesões com idade e sexo foram significativas, indicando maior prevalência da doença em fêmeas mais velhas. As lesões estavam principalmente na cabeça (97,1%), pulmão (69,1%), linfonodos pulmonares (69,1%), fígado (50,7%), intestinos (31,0%) e baço (29,6%). Não houve correlação direta entre localidade das amostras e lesões, mas a distribuição das lesões sugere possíveis diferenças nos mecanismos de infecção, cepas de *M. bovis* ou estágios da doença.

Palavras-chave: Aptidão. Idade. Sexo. Sistema de Manejo. Origem.

1 Introduction

Bovine tuberculosis (BT) is a progressive, chronic infectious disease caused by *Mycobacterium bovis*. It is characterized by the presence of nodular and granulomatous lesions in various bovine tissues, especially in the lung (De Souza *et al*., 2016; Ferreira *et al*., 2020). This disease is one of the main causes of cattle carcasses condemned in slaughterhouses, causing significant economic losses (Garozi *et al*., 2022; Funke; Dalto; Kindlein, 2023), which represents a major problem for Brazil, one of the world's leading producers and exporters of beef. At the last census, the country had around 202 million heads of cattle, with 42.3 million heads slaughtered in the year (ABIEC, 2025).

The prevalence of BT in different regions of Brazil varies due to differences in socio-economic conditions, territorial extension, herds, production characteristics and vaccination rates (MAPA, 2024). Between 2017 and 2021, the South and Southeast regions were responsible for 82% of national BT condemnations in slaughterhouses under federal inspection and the State of Parana ranked fourth in prevalence of the disease in the country during the same period (Funke; Dalto; Kindlein, 2023). In addition to the high incidence in cattle, BT is also a major concern because of its zoonotic nature and the fact it can be transmitted to humans and other species of animals, such as other ruminants, pigs, birds and wild animals (Broughan *et al*., 2013; Kanipe; Palmer, 2020).

In the month of February 2025 only, the Paraná Agricultural Defense Agency recorded 22 new hotspots and 39 new cases of BT in 20 municipalities in the State (ADAPAR, 2025). In addition, the proximity to São Paulo, one of the states with the highest number of outbreaks of the disease in the country, reinforces the need for intensive surveillance and continuous monitoring to contain the

spread of BT to reduce the observed rates (MAPA, 2024).

The most used diagnostic method to verify the presence of BT in cattle herds is the intradermal tuberculin test, which evaluates the cellular immune response. Screening tests include the simple cervical test (SCT), which involves applying tuberculin (a specific antigen for *M. bovis*) to the animal's cervical region, and the caudal fold test (CFT), which is very similar to the SCT, but the tuberculin is inoculated at the base of the tail, at the junction between the hairy and glabrous skin (caudal fold) (BRASIL, 2017).

The comparative cervical test (CCT) can be used as a confirmatory method in animals with positive or inconclusive results in the other tests. CCT-positive animals are sent for sanitary slaughter where, through post-mortem examinations, it is possible to verify the incidence of macroscopic lesions suggestive of BT such as granulomas (caseous or calcified) in the head and chest lymph nodes, lungs, liver, spleen and serous surfaces of the body cavities (MAPA, 2023).

It is important to note that the macroscopic lesions characteristic of the disease may be absent, since the intradermal test has low specificity and consequently false positive results. In addition, these lesions may also be absent in the early stages of infection or in cases of an efficient host immune response. The use of complementary and differential tests for a definitive diagnosis, such as PCR, is fundamental, since granulomatous inflammatory processes caused by actinobacillosis, actinomycosis and other chronic bacterial infections promote similar morphological characteristics (De Souza *et al*., 2016; Santos *et al.*, 2024).

Although there is a consensus in the literature and legislation on carcass lesions associated with BT, there are still a few studies that relate the productive and epidemiological characteristics of the animals to the lesions suggestive of the disease identified in post-mortem examinations. The aim of this study was to correlate production characteristics, such as aptitude and management system, and epidemiological characteristics, including sex, age and place of origin of CCT-positive animals, with the characteristic BT lesions observed at slaughter.

2 Material and Methods

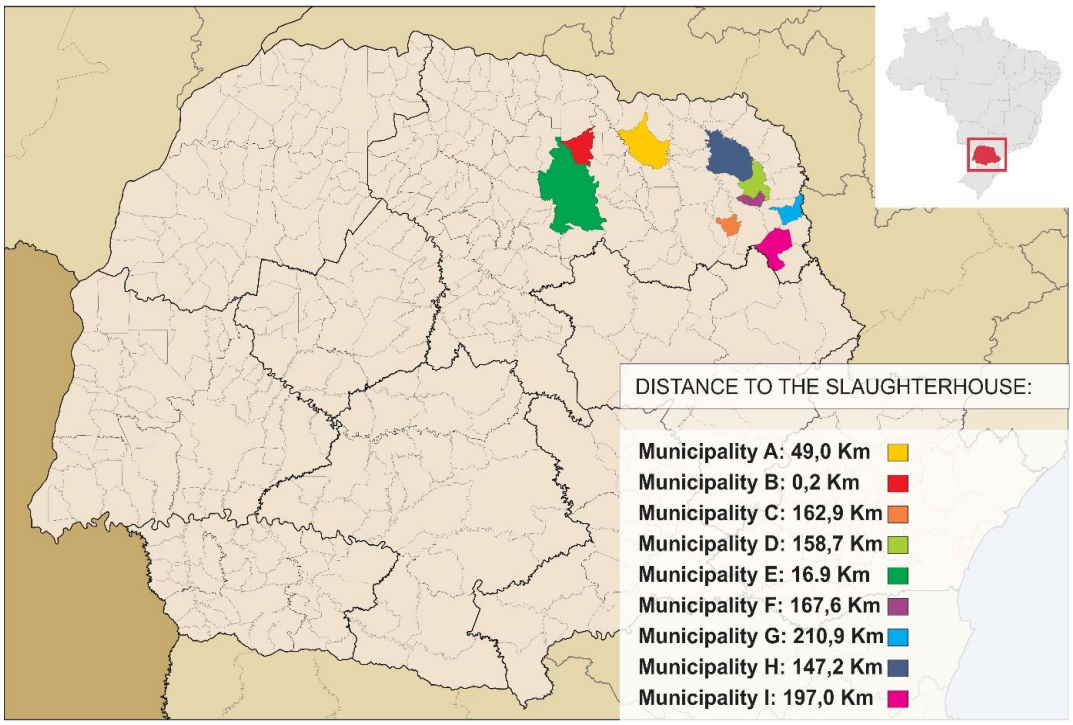
The study was carried out in a slaughterhouse under the state inspection service (SIE), located in a city in the North of the State of Paraná, Brazil (23º16'09" S and 51º02'53" O). The slaughterhouse was selected because it is a reference for sanitary slaughter in the region, due to the implementation of detailed self-control measures and procedures for this type of slaughter. Its capacity is 170 cattle/day and 160 pigs/day, which corresponds to approximately 4,500 animals/month.

Between March 2021 and September 2022, 257 BT-positive animals were identified at the CCT and sent for sanitary slaughter. During the post-mortem inspection, carcass, parenchyma, organs and

lymph nodes were examined, following the guidelines established by current legislation, to macroscopically identify characteristic BT lesions, both nodular and granulomatous (BRASIL, 2020). In this study, the lungs (lung parenchyma and pleura), lung chain lymph nodes (mediastinal, bronchial and ventral), head (muscles and parotid, submandibular and retropharyngeal lymph nodes), heart, intestine (mesenteric lymph nodes), kidneys, spleen, liver (liver parenchyma and lymph nodes) and carcass (muscles and lymph nodes) were evaluated.

The animals evaluated in this study included males and females of varying ages, from both beef and dairy farming (aptitude) and raised in different management systems (intensive, semi-intensive and extensive). The nine municipalities from which the animals originated were located at an average of 131 ± 79 km from the slaughterhouse, as shown in Figure 1.

Figure 1 – Origin of the animals that tested positive for tuberculosis in the comparative cervical test and were sent for sanitary slaughter in the North of the State of Paraná, and the respective distances to the slaughterhouse (2021-2022)



Source: the authors.

A mixed factorial design $2^4 \times 3^1$ (Gunst; Mason, 2009) was used to assess the influence of place of origin, management system (intensive, semi-intensive and extensive), aptitude (beef or dairy), sex (male or female) and age (< 36 months and ≥ 36 months) on the occurrence of lesions characteristic of BT identified in the sanitary slaughter of CCT-positive animals. The variables were parameterized to facilitate the interpretation of the results and to guarantee the normal distribution of the data.

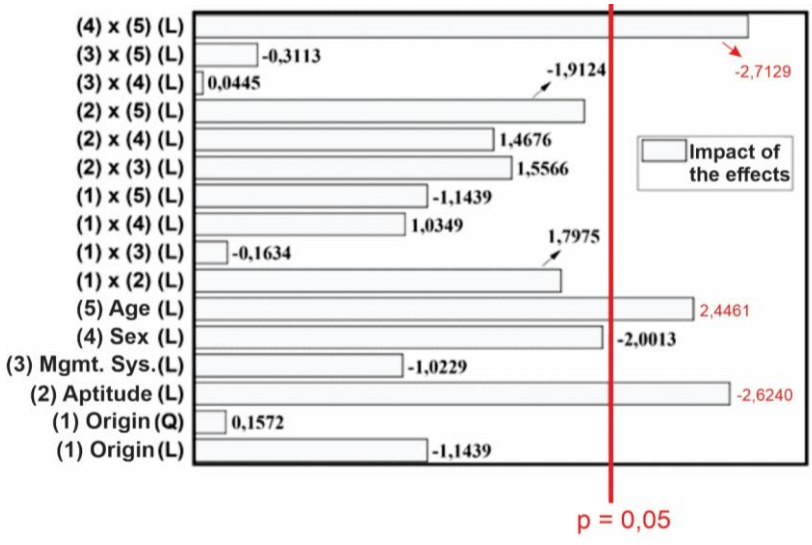
The results were evaluated through Analysis of Variance (ANOVA) and Pareto diagrams

(Karuppusami; Grandhinathan, 2006; Sawyer, 2009) for the five variables (origin, management system, aptitude, sex, age), using Statistica (STATSOFT, v. 10.0), with a significance level of 95% ($p < 0.05$). The significant variables were those with $p<0.05$ in the ANOVA table and $p>0.05$ in the Pareto diagram.

3 Results and Discussion

Among the CCT-positive cattle, only 28% (71/257) showed macroscopic lesions, while 72% (186/257), despite being CCT-positive, did not show lesions characteristic of BT. These data are possibly associated with the age of the animals evaluated, and consequently the length of exposure to the etiological agent (Hamed *et al*., 2021; Zhu *et al.,* 2023), since BT lesions were only observed in cattle ≥ 36 months of age ($p<0.05$; Table 2) ($p>0.05$; Figure 2). When the sampling population was restricted to cattle aged ≥ 36 months (n= 180), the relationship between a positive CCT and the presence of macroscopic lesions suggestive of BT increased to 39%. It is important to note that the percentage of macroscopic lesions observed in positive samples in the CCT can vary, especially when young cattle are being evaluated. In addition, factors such as the dose of tuberculin used, the experience of the professional responsible for application and interpretation, the post-infection interval, the peripartum period and the nutritional condition of the animals can influence the sensitivity of the test (De Kantor; Ritacco, 2006; Souza *et al*., 2014).

Figure 2 – Pareto diagram of the productive (aptitude and management system) and epidemiological (sex, age and origin) variables of cattle that tested positive in the comparative cervical test, were sent for sanitary slaughter and showed lesions characteristic of bovine tuberculosis in lymph nodes and tissues, between March 2021 and September 2022



The values shown in black refer to effects that are not significant at 95% ($p>0.05$), while the values in red indicate effects that are significant at 95% ($p<0.05$). The variables are: (1) = origin; (2) = aptitude; (3) = management system; (4) = sex; (5) = age; (L) = linear relationship; (Q) = quadratic relationship (to the second power).
Source: the authors.

When the relationship between age and sex of cattle with characteristic BT lesions was evaluated jointly (Table 1), a positive effect was observed for age and a negative effect for sex, indicating a greater occurrence of lesions in lymph nodes and tissues in samples from females (in absolute quantities) and in cattle ≥36 months of age ($p>0.05$; Figure 2). When analyzing the sex and age variables individually, only the latter was associated with the macroscopic lesions characteristic of BT observed in the post-mortem examination ($p<0.05$; Table 2; $p>0.05$; Figure 2). Therefore, the interpretation of sex and age data should be carried out in an integrated manner, suggesting that BT tends to be higher in cattle populations made up of females and older animals.

Table 1 – Productive characteristics (aptitude and management system), epidemiological characteristics (sex and age) and presence of bovine tuberculosis lesions in cattle that tested positive in the comparative cervical test and were sent for sanitary slaughter between 2021 and 2022 in the northern region of the State of Paraná (Brazil)

Productive and Epidemiological Characteristics						**Tuberculosis Lesions at Post-Mortem**
Origin (municipalities A to I)	**Sex***		**Age (months)**	**Aptitude**	**Management system****	**Absence/Presence and Sites of Lesions (n)*****
A	7	0	≥ 36	Beef	E	Absence
B	1	0	≥ 36	Milk	I	Absence
C	21	0	≥ 36	Milk	SI	Absence
D	6	0	<36	Milk	SI	Absence
E	47 35	7 1	<36 ≥ 36	Beef Milk	E E and SI	Absence Presence (35) in Lun, H, He, LCL, Int, Spl, Carc e Li
F	1 47	0 5	<36 ≥ 36	Milk Milk	SI SI	Absence Presence (22) in Lun, H, Li
G	14	0	≥ 36	Milk	SI	Absence
H	03 17 17 10	0 0 0 0	≥ 36 <36 ≥ 36 ≥ 36	Milk Milk Milk Milk	SI SI SI E	Absence Absence Presence (8) in Lun, H, He Presence (6) in Lun, H, He
	10	0	≥ 36	Milk	SI	Absence
I	8	0	≥ 36	Beef	SI	Absence

*Female (244)/ Male (13) ** E=extensive; I=intensive; SI=semi-intensive **Lun= lungs (including lung parenchyma and pleura); LCL= Lung chain lymph nodes (mediastinal, bronchial and ventral); H= heart; He= head (muscles and parotid, submandibular and retropharyngeal lymph nodes); Int= intestine (including mesenteric lymph nodes); Spl= spleen; Li= liver (including liver parenchyma and lymph nodes); Carc= carcass (muscles and associated lymph nodes).
Source: the authors.

In general, cows sent for sanitary slaughter come from dairy farming and remain in the herd for longer before commercial slaughter. This prolonged exposure period can increase the risk of contact

with areas contaminated by *M. bovis* (Grisi-Filho *et al*., 2011). This is reinforced by the fact that, in this study, the type of livestock farming was a significant variable in the occurrence of lesions characteristic of BT ($p<0.05$, Table 2; $p>0.05$ Figure 2), with a greater number of animals from dairy farming being sent for sanitary slaughter and showing lesions on post-mortem inspection (87.3%, 65/71) (Table 1). In addition, the data from this study also indicated that, despite the significantly higher number of positive females in the CCT (n=244) compared to males (n=13), it was possible to identify more macroscopic lesions at post-mortem inspection in male cattle (46.0%; 6/13) than in females (23.0%; 56/244).

Table 2 - Analysis of variance (ANOVA) between the productive characteristics (aptitude and management system), epidemiological characteristics (sex, age and origin) and characteristic lesions of bovine tuberculosis in lymph nodes and tissues of cattle positive to the comparative cervical test, sent for sanitary slaughter between 2021 and 2022

Variable	SQ	F	*p*
(1) Origin	13,78	1,31	0,26
(2) Aptitude	72,52	6,89	0,013
(3) Management system	11,02	1,05	0,31
(4) Sex	45,19	4,01	0,054
(5) Age	63,02	5,98	0,020
(1) x (2)	34,03	3,23	0,082
(1) x (3)	0,28	0,027	0,87
(1) x (4)	11,28	1,07	0,31
(1) x (5)	13,78	1,31	0,26
(2) x (3)	25,52	2,42	0,13
(2) x (4)	22,69	2,15	0,15
(2) x (5)	38,52	3,66	0,065
(3) x (4)	0,021	0,002	0,96
(3) x (5)	1,02	0,097	0,76
(4) x (5)	77,52	7,36	0,011

(1) = origin; (2) = aptitude; (3) = management system; (4) = sex; (5) = age. Data from quadratic relationships were omitted as they were not significant at the 95% confidence level. SQ = Sum of squares. F= SQ between groups / SQ within groups. p= p-value <0.05.
Source: the authors.

Although it was not possible to establish a direct relationship between the origin of the cattle and the number of samples showing BT lesions ($p>0.05$; Table 2), it was observed that in only three (E, F and H) of the nine municipalities evaluated were the evidence of the disease was confirmed. In the animals from location E, which has a larger land area and was more densely occupied, post-mortem inspection revealed typical BT lesions in all the locations inspected (n=35), except for the

kidneys. At location F, the characteristic BT lesions were observed in the head, lymph nodes of the pulmonary chain and liver (n=22), and at location H, the cattle showed lesions of BT in the lungs, head and heart (n=14) (Table 1), thus indicating different routes of entry for the pathogen and stages of the disease at the time of slaughter (Andreazza *et al*., 2015; Domingo *et al*., 2014).

Thus, BT lesions were present in the head (parotid, submandibular and retropharyngeal muscles and lymph nodes) in 97.1% of cases, followed by the lungs (lung parenchyma and pleura - 69.1%), lung chain lymph nodes (mediastinal, bronchial and ventral - 69.1%), liver (liver parenchyma and lymph nodes - 50.7%), intestines (mesenteric lymph nodes - 31%%), carcass (muscles and lymph nodes - 35.2%) and spleen (29.6%).

Inhalation is the most common route of infection for BT and can cause lesions in the nasopharynx and lower respiratory tract, including the lungs and associated lymph nodes. In contrast, ingestion of *M. bovis* from contaminated pasture, feed or water generally causes lesions in the mesenteric lymph nodes and few or no visible lesions in the intestinal wall (Domingo *et al.*, 2014).

4 Conclusion

The CCT-positive bovines with lesions suggestive of BT on post-mortem examination are ≥ 36 months old, predominantly females, and mostly dairy cattle. Although males have a low occurrence of lesions characteristic of BT, they are identified in a higher percentage when compared to females.

It is not possible to correlate the origin of the cattle with the number of lesions found. However, based on the locations with characteristic BT lesions, we can infer the possibility of different contamination mechanisms or different stages of the disease between the origins of the cattle.

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