PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 45:e07538, 2025
DOI: 10.1590/1678-5150-PVB-7538

Original Article
Livestock Diseases

Epidemiological and pathological dynamics of tuberculosis in Jersey cattle in southeastern Rio Grande do Sul[1]

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ABSTRACT.- Alberti T.S., Marcolongo-Pereira C., Venancio F.R., Scheid H.V., Souza C.G., Martins K.R., Schild A.L. & Sallis E.S.V. 2025. **Epidemiological and pathological dynamics of a tuberculosis outbreak in young Jersey calves in southeastern Rio Grande do Sul**. *Pesquisa Veterinária Brasileira 45:e07538, 2025.* Faculdade de Veterinária, Universidade de Cruz Alta de Cruz Alta, Rodovia Municipal Jacob Della Mea Km 5,6, Parada Benito, Cruz Alta, RS 98020-290, Brazil. E-mail: taina_alberti@yahoo.com

Bovine tuberculosis (TB), caused primarily by *Mycobacterium bovis*, is a zoonotic infectious disease with significant economic impacts on the milk production chain due to the reduction in zootechnical indices. This study aimed to investigate and describe the epidemiological and pathological characteristics of tuberculosis in a Jersey cattle property in southeastern Rio Grande do Sul, focusing on transmission routes and clinical and histopathological findings. The outbreak, affecting 15% of the herd, suggested potential transmission routes, including human-mediated transmission, facility contamination, wild animal vectors, and undetected infection in founder animals. Both adult and young animals, including calves that were only a few days old, were affected, suggesting aerogenous and congenital transmission, respectively. Pathological examination of the affected calves showed granulomatous lesions, primarily in the respiratory tract, significant necrosis, and abundant acid-fast bacilli. These findings highlight the need for vigilant diagnostic practices and effective management strategies to control bovine tuberculosis, particularly in endemic regions. This study underscores the importance of considering tuberculosis as a differential diagnosis in cases of weight loss and respiratory symptoms in animals, including young animals.

INDEX TERMS: Cattle disease, granuloma, bovine tuberculosis, calves, zoonoses.

RESUMO.- [**Dinâmica epidemiológica e patológica de um surto de tuberculose em bezerros jovens da raça Jersey no sudeste do Rio Grande do Sul.**] A tuberculose bovina (TB), causada principalmente pelo *Mycobacterium bovis*, é uma doença infecciosa zoonótica que provoca impactos econômicos significativos na cadeia produtiva do leite devido à redução dos índices zootécnicos. Este estudo teve como objetivo investigar e descrever as características epidemiológicas e patológicas da tuberculose em uma propriedade de bovinos Jersey no sudeste do Rio Grande do Sul, com foco nas vias de transmissão e nos achados clínicos e histopatológicos. O surto, que afetou 15% do rebanho, sugeriu potenciais vias de transmissão, incluindo transmissão mediada por humanos, contaminação das instalações, vetores de animais selvagens e infecção não detectada em animais fundadores. Tanto animais adultos quanto jovens, incluindo bezerros com apenas alguns dias de idade, foram afetados, sugerindo transmissão aerógena e congênita respectivamente. O exame anatomopatológico dos bezerros afetados mostrou lesões granulomatosas principalmente no trato respiratório, necrose significativa e abundantes bacilos álcool-ácido resistentes. Estas conclusões destacam a necessidade de práticas de diagnóstico vigilantes e estratégias de gestão eficazes para controlar a tuberculose bovina, especialmente em regiões

[1] Received on September 2, 2024.
Accepted for publication on October 26, 2024.

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endêmicas. O estudo ressalta a importância de considerar a tuberculose como diagnóstico diferencial em casos de perda de peso e sintomas respiratórios em animais, incluindo os jovens.

TERMOS DE INDEXAÇÃO: Doenças de bovinos, granuloma, tuberculose bovina, bezerros, zoonoses.

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by acid-fast bacteria (AFB) belonging to the *Mycobacterium tuberculosis* complex. In cattle, it is primarily caused by *Mycobacterium bovis*, which is a highly pathogenic and potentially zoonotic species (Riet-Correa & Garcia 2007). Transmission mainly occurs via the airborne route through the inhalation of aerosols contaminated with bacteria. It can also occur via the oral route and, less frequently, via genital, cutaneous, and congenital routes (Ozyigit et al. 2007, Moral et al. 2018).

Owing to its chronic nature, TB is considered a disease of middle-aged to elderly animals. However, in cases of congenital transmission, clinical signs are observed within the first few days of life (Ozyigit et al. 2007, Alberti et al. 2020). Although rare, congenital transmission is highly relevant in areas endemic to bovine tuberculosis (Moral et al. 2018). Young animals can also become infected in the first days of life by inhalation of aerosols from adult cattle with pulmonary tuberculosis (Mekonnen et al. 2020).

Therefore, the objective of this study was to investigate and describe the epidemiological and pathological characteristics of tuberculosis in Jersey cattle in southeastern Rio Grande do Sul. This includes exploring the potential transmission routes, with a focus on aerogenous and congenital pathways, and analyzing the clinical and histopathological findings associated with the disease in young animals within an endemic region.

MATERIALS AND METHODS

Ethical approval. This study was not submitted to the Ethics Committee on the Use of Animals (CEUA) of the "Universidade Federal de Pelotas" (UFPel), because the cases studied were sick or dead cattle from laboratory routine and the legislation only provides for the use of live animals for experimentation.

An outbreak of infectious diseases, including 25 lactating cows and two calves, was detected in 53 Jersey dairy cattle herds. Two years ago, a veterinarian was called to treat a cow with eye injury and recommended testing the herd for tuberculosis and brucellosis. Tests revealed the presence of tuberculosis. Subsequently, a series of *post mortem* tuberculosis tests were performed.

Five animals died, including three calves. Two of these were Jersey calves, aged two and three months, and were sent for necropsy to the "Laboratório Regional de Diagnóstico" (Regional Diagnostic Laboratory), "Faculdade de Veterinária" (Faculty of Veterinary Medicine), "Universidade Federal de Pelotas" (LRD-FV-UFPel). During necropsy, organ fragments from the abdominal and thoracic cavities, as well as from the brain, were collected and placed in 10% buffered formalin. Lung and lymph node samples were collected and sent under refrigeration to the "Laboratório Veterinário de Diagnóstico Molecular" (Veterinary Molecular Biology Laboratory – LaBMol-Vet) for molecular analysis using polymerase chain reaction (PCR). Organs from the adult cows were sent to the laboratory for analysis.

After 48 hours, the formalin-fixed samples were sectioned, embedded in paraffin, cut into 3 µm thick sections, and stained using hematoxylin and eosin (HE), Ziehl-Neelsen (ZN), Masson's trichrome (MT), and Von Kossa (VK) techniques. Selected sections from the lungs and lymph nodes were stained using immunohistochemistry (IHC). Histological sections were placed on silanized DAKO slides and placed in an oven. Subsequently, the slides were deparaffinized and antigenically recovered using the PT LINK DAKO system in the Envision Flex DAKO solution for 25 min at 97 °C. The antibodies used were CD3 (polyclonal rabbit) and CD68 (Kp-1). Primary antibodies were incubated in the Autostainer Link 48 DAKO/Agilent device for 24 and 26 min. The detection system employed was Envision DAKO, with diaminobenzidine (DAB) as the substrate. A palatine tonsil section was used as a positive control. After developing with DAB, the slides were dehydrated, cleared, and counterstained with Harris hematoxylin. They were then mounted and examined under an optical microscope and classified as positive or negative based on the staining intensity.

DNA extraction for *Mycobacterium bovis.* Identification after colonies suggestive of *M. bovis* were grown, they were collected using an inoculation loop and resuspended in an Eppendorf tube containing 200 µL tris-EDTA. The bacterial suspension was inactivated by heating in a thermoblock at 85 °C for 30 min. The sample was then centrifuged at 13,000 × g for two minutes. The precipitate was discarded, and the supernatant containing mycobacterial DNA was transferred to new tubes and stored at -20 °C for further analysis.

PCR identification. For the detection of DNA from *Mycobacterium* spp. in the *M. tuberculosis* complex, PCR was performed as described by Wilton & Cousins (1992). The reaction mixture consisted of enzyme buffer (2.5 µL), 1.5 µL of dNTP (2.5 mM), 1.25 µL of 50 mM $MgCl_2$, 0.5 µL of each primer at 10 pmol (MYCGEN-F: 5'-AGAGTTGATCCTGGCTCAG-3' and MYCGEN-R: 5'-TGCACACAGGCCACAAGGGA-3'), 0.25 µL of Taq Polymerase (5 U/µL) (Ludwig Biotecnologia, Porto Alegre, Brazil), 2 µL of DNA, and 16.5 µL of DNase- and RNase-free water, for a total volume of 25 µL. A 2720 Thermal Cycler (Applied Biosystems, MA, USA) was used. The thermocycling conditions were 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 3 min, extension at 72 °C for 3 min, and final extension at 72 °C for 7 min. A sample of *M. tuberculosis* DNA was used as a positive control, whereas 2 µL of endonuclease-free water served as a negative control. Results from the samples, including positive and negative controls and a 100 bp ladder (Ludwig Biotecnologia, RS, Brazil), were visualized and interpreted using 1.5% agarose gel electrophoresis stained with 0.5µg/mL ethidium bromide.

RESULTS

Chronic diseases occurred from late August 2022 to early March 2023, with a morbidity rate of 15%, affecting 20 animals. The affected animals exhibited progressive weight loss and apathy, with some presenting with enlarged lymph nodes. Additionally, the lungs and lymph nodes of two animals (cows and calves) were sent to the LRD with a suspected diagnosis of tuberculosis. All other animals tested negative for tuberculosis in the allergic test, but showed lesions upon sanitary slaughter. The dairy farm is in Capão do Leão, Rio Grande do Sul.

The two necropsied animals had a history of progressive weight loss, and one of them presented with diarrhea. Neither of the animals was tested, and both were euthanized because of their clinical conditions and the history of their mothers testing positive in the tuberculin skin test.

During necropsy, one animal (Bovine 1) exhibited areas of consolidation in the cranioventral regions of the lung lobes (Fig.1) with yellowish, caseous nodules. The lymph nodes of this animal showed punctate, yellowish areas. The other animal (Bovine 2) presented with ascites, hydrothorax, hydropericardium, and pulmonary edema, but no significant macroscopic lesions were found in the lungs and lymph nodes.

Histopathological evaluation of the Bovine 1's lung revealed multifocal to coalescent areas of caseous necrosis and marked bronchointerstitial pneumonia, characterized by an intense inflammatory infiltration of neutrophils, macrophages, epithelioid cells, and lymphocytes (Fig.2). Rare Langhans-type giant cells are also observed. The lymph nodes of this animal exhibited multifocal to coalescent areas of caseous necrosis, lymphoid rarefaction, and a loss of normal architecture (Fig.3). In Bovine 2, intense multifocal bronchointerstitial pneumonia with areas of atelectasis and an inflammatory infiltrate composed of macrophages, neutrophils, and lymphocytes was observed. The lymph nodes of this animal showed lymphoid depletion, medullary edema, and predominance of epithelioid cells in the germinal centers.

In both cases, ZN staining revealed many acid-fast bacilli amidst necrosis and within the phagocytic cells (Fig.4). In lung and lymph node sections from Bovine 1, which were subjected to VK and MT staining, discrete areas of dystrophic calcification were evident amidst caseous necrosis and immature, disorganized fibroplasia near the lesion (Fig.5 and 6). In Bovine 2, no labeling of collagen or calcium fibers was observed.

Molecular analysis confirmed the presence of *Mycobacterium bovis* in samples from both animals. Immunohistochemical evaluation of Calf 1 showed positive immunostaining for CD3 in the lymph nodes and lungs, and negative immunostaining for CD68 (Fig.7). In Calf 2, no staining for CD3 or CD68 was observed in lung lesions. However, in the lymph nodes of this animal, positive staining was observed in the cortical region for CD3, and negative staining for CD68 (Fig.8).

Fig.1. Tuberculosis in young Jersey calves. The lung exhibited cranioventral consolidation and yellowish nodules on its pleural surface.

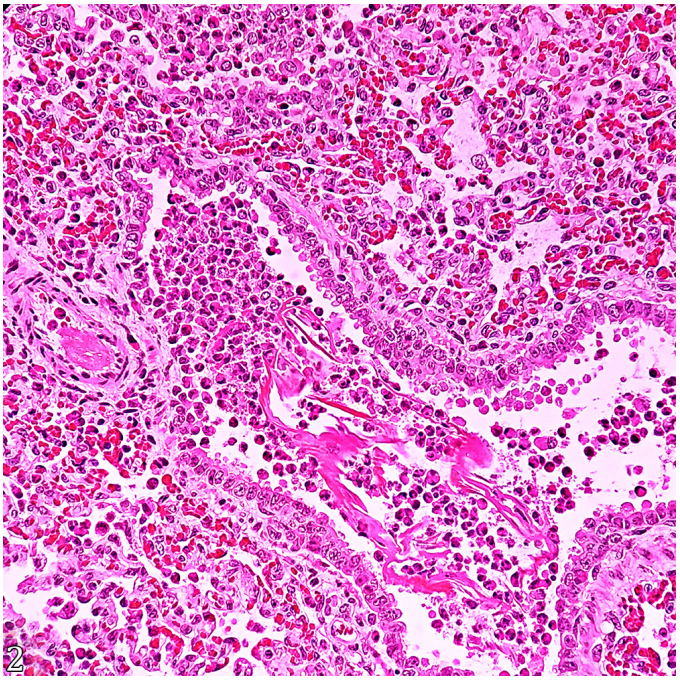

Fig.2. Tuberculosis in young Jersey calves. Bovine 1 lung tissue showing marked bronchointerstitial pneumonia, characterized by an intense inflammatory infiltrate of neutrophils, macrophages, epithelioid cells, and lymphocytes. HE, obj.40x.

DISCUSSION

Bovine tuberculosis is a zoonotic infectious disease that negatively affects the milk production chain, causing economic losses due to the gradual reduction of zootechnical indices (Boland et al. 2010). This impact was evident in this outbreak, where 15%

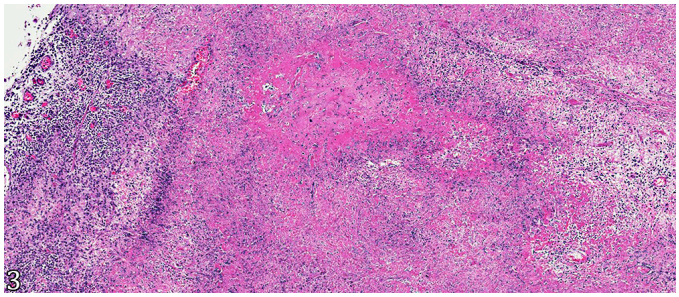

Fig.3. Tuberculosis in young Jersey calves. The lung tissue of Bovine 2 showed a focally extensive area of consolidation owing to an inflammatory infiltrate predominantly consisting of neutrophils. HE, obj.4x.

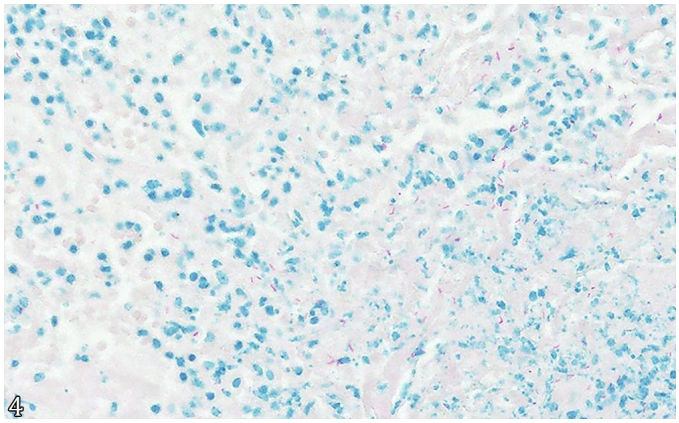

Fig.4. Tuberculosis in young Jersey calves. Bovine 1 lung tissue showing acid-fast bacilli amid necrosis and within phagocytic cells. Ziehl-Neelsen special staining, obj.40x.

of the herds tested positive for tuberculosis. Such outbreaks underscore the vulnerability of livestock products to sanitary barriers imposed by international markets (Michel et al. 2010).

Currently, there is no effective vaccine for bovine tuberculosis (OIE 2013). Disease control and eradication programs in most countries, including Brazil, depend on traditional diagnostic methods such as the intradermal test (Álvarez et al. 2012). In Brazil, the "Programa Nacional de Controle e Erradicação da Brucelose e da Tuberculose Animal" (National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis – PNCEBT) uses this method to detect delayed hypersensitivity reactions (DTH). This reaction occurs after inoculation with a purified protein derivative (PPD), leading to a local inflammatory response in infected animals before the presence of the bacillus (Xin et al. 2013).

In this outbreak, four potential routes of *Mycobacterium bovis* transmission to the herd were considered: unintentional transmission by humans, facility contamination, introduction through wild animal vectors, and undetected infection in one of the founder animals. While bovine tuberculosis is typically observed in adult animals owing to its chronic nature (Alberti et al. 2020), our study documented cases in both adult and very young animals, including those only a few days old. Although transmission in these young animals may be linked to tuberculosis lesions in the genitourinary tract or placenta of infected mothers (Moral et al. 2018), the data did not allow us to conclusively identify the primary transmission route.

The inability to definitively determine the primary transmission route highlights the complexity of tuberculosis transmission dynamics in endemic regions. Evidence suggests multiple possible pathways, but without definitive proof, it remains unclear which route was predominant in this outbreak.

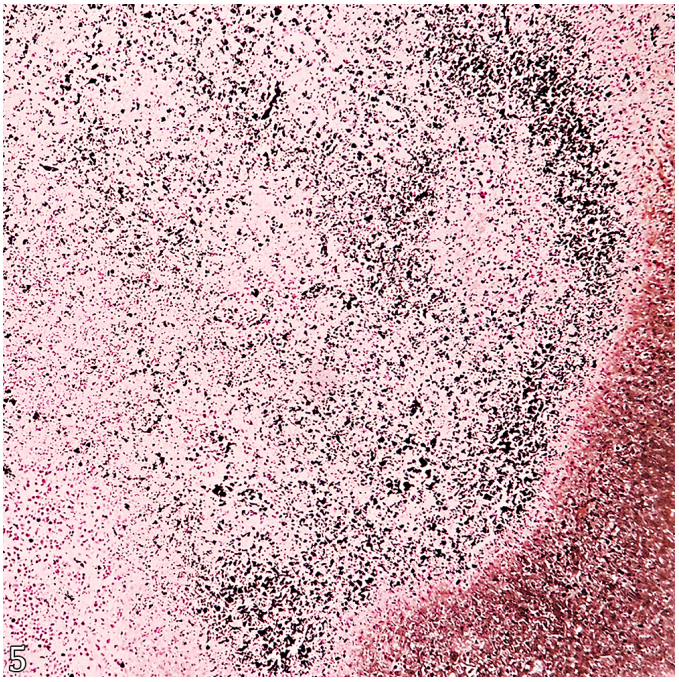

Fig.5. Tuberculosis in young Jersey calves. Bovine 1 lymph node showing dystrophic calcification amid caseous necrosis. Von Kossa staining, obj.10x.

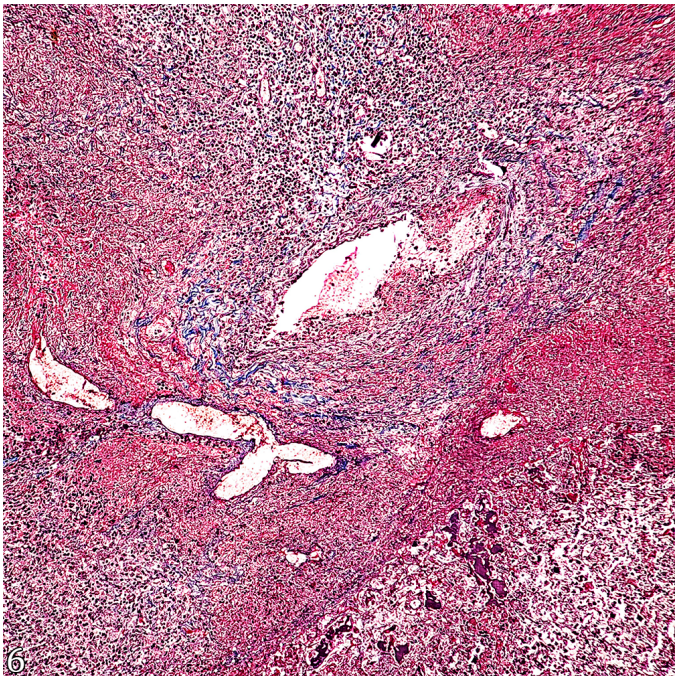

Fig.6. Tuberculosis in young Jersey calves. Bovine 1 lung showing an area of immature fibrosis. Masson's trichrome special staining, obj.10x.

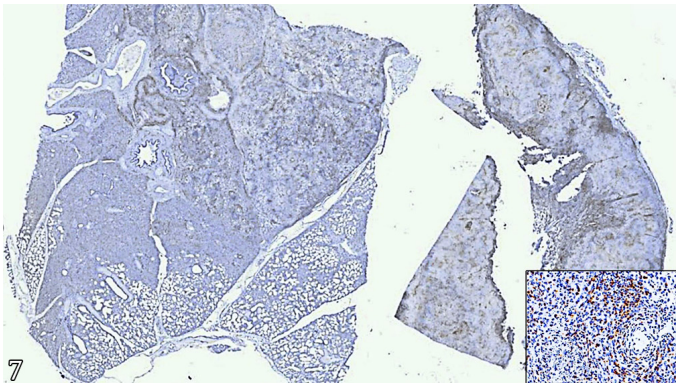

Fig.7. Tuberculosis in young Jersey calves. Bovine 1 showed positive immunolabeling for CD3 in the lungs and on the periphery of the necrotic areas in the lymph nodes. Inset: evidence of lung immunolabeling. IHC (CD3), submicroscopic view.

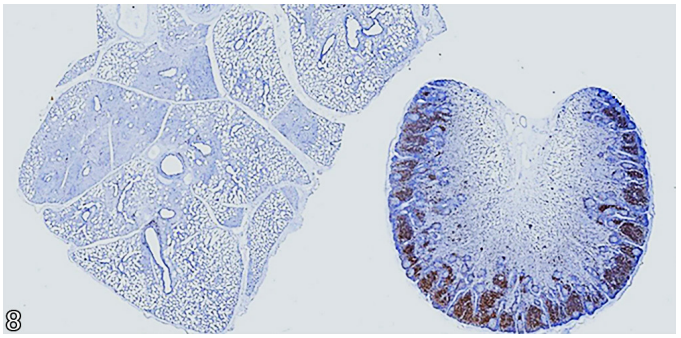

Fig.8. Tuberculosis in young Jersey calves. Bovine 2 showed negative immunolabeling for CD3 in the lungs and positive immunolabeling in the cortical lymph nodes. IHC (CD3), submicroscopic view.

This uncertainty underscores the need for further research and more advanced diagnostic tools to better understand *M. bovis* transmission in similar settings.

The dynamics of the disease within the herd were observed through progressive spread and development of clinical and pathological signs over time. Initially, infection was detected in adult cows, characterized by gradual weight loss and enlarged lymph nodes. As the outbreak progressed, it became evident that the disease was not confined to adult animals; young calves, including neonates, also began to exhibit signs of tuberculosis, indicating multiple routes of transmission.

Clinically, the disease is characterized by progressive development of granulomatous lesions in various organs, with the lungs, head, and mediastinal lymph nodes being the most affected (Carrisoza-Urbina et al. 2019, 2023). The histopathological findings of this study, particularly the presence of disorganized granulomas with significant necrosis in young calves, suggest a deficient immune response, allowing substantial bacterial multiplication. T lymphocytes (CD3) were detected in the granulomas of one of the calves but only in areas with extensive necrosis, indicating that these cells play a role in disease progression when the body's adaptive immune response becomes active (Beytut 2011, Andreazza et al. 2015). The presence of these cells in the context of severe lesions suggests a delayed but critical immune response that attempts to contain infection.

The atypical presentation of tuberculosis in young calves, characterized by disorganized granulomas and a high bacterial load, aligns with the literature on immune responses in young animals (Carrisoza-Urbina et al. 2019, 2023). These findings emphasize the importance of early detection and intervention, particularly in herds where tuberculosis is endemic.

CONCLUSION

While this study explored potential transmission routes of bovine tuberculosis, it also underscored the inherent challenges in definitively identifying the primary route, thereby reflecting the complexity of the dynamics of the disease. Clinical and histopathological findings, especially in young calves, offer valuable insights into disease progression and the associated immune response. These findings emphasize the critical need for vigilant monitoring and implementation of effective control strategies to manage and prevent similar outbreaks in the future.

Acknowledgments.- This study was financed in part by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES), Brasil, by "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq) and by "Fundação de Amparo à Pesquisa e Inovação do Espírito Santo" (FAPES).

Conflict of interest statement.- The authors declare that they have no conflicts of interest.

Credit author statement.- Tainá S. Alberti and Clairton M. Pereira – Data analysis and article writing. Fabiano R. Venâncio and Caroline G. Sousa – Performing necropsies and processing samples. Haide V. Scheid – Performing immunohistochemistry. Kauê R. Martins – Performing the PCR technique. Ana Lucia Schild and Eliza Simone V. Sallis – Article review.

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