

MILIARY TUBERCULOSIS IN CATTLE IN SERGIPE STATE, BRAZIL

(*Tuberculose miliar em bovino no estado de Sergipe*)

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RESUMO

O objetivo deste trabalho é relatar o primeiro caso de tuberculose miliar em bovino no estado de Sergipe, seus achados clínicos e patológicos. Um bovino, fêmea, com histórico de que há dez dias apresentava emagrecimento progressivo e no oitavo dia de evolução clínica começou a apresentar incoordenação e anorexia. No exame clínico do sistema nervoso foi observado desvio lateral da cabeça, déficit proprioceptivo com dismetria, ataxia, redução do tônus lingual, flacidez de mandíbula, ausência do reflexo de deglutição e compressão da cabeça contra objeto. Macroscopicamente foram observados múltiplos nódulos nos pulmões, diafragma, linfonodos mediastínicos, glândula mamária, encéfalo e medula espinhal, amarelados, de tamanhos variados e consistência firme que, ao corte, apresentavam conteúdo amarelado pastoso com aspecto arenoso. O coração continha abundante líquido serosanguinolento no saco pericárdico, com presença de fibrina livre no líquido e aderida ao epicárdio. Histologicamente, as lesões nodulares caracterizavam-se pela formação de lesão granulomatosa caracterizada por uma área central de necrose, rodeada por macrófagos epitelióides, poucos linfócitos e abundantes células gigantes multinucleadas do tipo Langhans, exibindo áreas multifocais de calcificação caracterizando o quadro de tuberculose miliar, confirmado com a coloração de Ziehl Neelsen. A tuberculose miliar deve ser considerada no diagnóstico diferencial de enfermidades que acometem o sistema nervoso em bovinos oriundos de rebanhos com elevados índices de animais positivos para tuberculose, reforçando a importância do inquérito a respeito da prevalência desta enfermidade.

Palavras-chave: *Mycobacterium bovis*, sinais clínicos, encéfalo.

ABSTRACT

The objective of this work is to report the first case of miliary tuberculosis in cattle in the state of Sergipe, Brazil, with its clinical and pathological findings. A female bovine with a ten-day history had progressive weight loss and on the eighth day of clinical evolution showed incoordination and anorexia. The clinical examination of the nervous system indicated lateral deviation of the head, proprioceptive deficit with dysmetria, ataxia, reduced lingual tone, flaccidity of the jaw, absence of the swallowing reflex and compression of the head against an object. After euthanasia, multiple nodules were observed in the lungs, diaphragm, mediastinal lymph nodes, mammary glands, brain and spinal cord. Yellowish of varying sizes and firm consistency were observed at the macroscopic level and when cut, had a pasty yellowish content with a sandy aspect. The heart contained abundant serosanguinous fluid in the pericardial sac, with the presence of free fibrin in the liquid and adhered to the epicardium. In the histological analysis, the nodular lesions were characterized by the formation of granulomatous lesions with a distinctive central area of necrosis, surrounded by epithelioid macrophages, few lymphocytes and abundant giant multinucleated cells of the Langhans type, showing multifocal areas of calcification characterizing miliary tuberculosis, confirmed with the Ziehl Neelsen stain. Miliary tuberculosis must be considered in the differential diagnosis of diseases that affect the nervous system in cattle from herds with high levels of animals positive for tuberculosis, reinforcing the importance of the investigation regarding the prevalence of this disease.

Key words: *Mycobacterium bovis*, clinical signs, brain.

INTRODUCTION

Bovine tuberculosis is a chronic infectious disease with zoonotic potential, caused by the *Mycobacterium bovis*, a bacterium that belongs to the family Mycobacteriaceae, genus *Mycobacterium*, where bacilli are short and anaerobic, not flagellated, not encapsulated, immobile and that have a granular aspect when stained (ANDREAZZA *et al.*, 2015).

It is currently estimated that approximately 50 million cattle are infected worldwide, causing economic losses of approximately 3 billion dollars per year (SWEETLINE ANNE *et al.*, 2017). The disease causes losses in the production processes of cattle, sheep, goats, camels and pigs (ANDREAZZA *et al.*, 2015; GHEBREMARIAM *et al.*, 2018).

Tuberculosis is characterized by granular nodules that are called tubercles which mainly affect the lungs and mediastinal lymph nodes in their most common form, but when it spreads and takes on the miliary form, tuberculosis can affect organs such as the liver, spleen, lung, pleura, peritoneum, intestines, lymph nodes of the head and thorax (SILVA *et al.*, 2016).

Usually the infection occurs through the respiratory or oral route and the most common clinical signs are cough and progressive weight loss, due to pulmonary impairment. However, different clinical signs can be observed when it affects other organs, in these cases the dissemination usually occurs via hematogen and the tuberculosis is characterized by the presence of chronic granulomatous inflammation with the presence of caseous necrosis, and alcohol-resistant bacilli in the various affected organs (SILVA *et al.*, 2016; SILVEIRA *et al.*, 2018).

Calcification, caseification or abscesses may occur after the appearance of granulomatous lesions. With the course of the disease, which initially affects the ganglionic system, different types of lesions can be caused depending on the type of organ affected and the grade (type) of immune resistance of the individual (CANAL *et al.*, 2017).

Tuberculosis can be easily transmitted from animals to humans, either through direct contact with the affected animals, or through the consumption of milk or dairy products produced with unpasteurized milk. When it takes the miliary form, tuberculosis can be confused with other diseases by presenting different symptoms, which makes it difficult to accurately diagnose it. These symptoms are related to the organs affected by the tuberculosis and can vary from gastrointestinal to neurological disorders. This work aims to describe a case of miliary bovine tuberculosis, with its clinical presentation and pathological findings.

PATIENT CARE

The animal was examined at the Veterinary Hospital Dr. Vicente Borelli, from the Faculdade Pio Décimo in Aracaju, SE, Brazil, a mixed breed female animal, approximately two years old, raised in an extensive regime, from the municipality Itaporanga d'Ajuda, SE. The property was in Sapé village (10° 59'01.9 "S 37° 22'43.0" W, altitude 12 meters). The owner reported that the animal had been experiencing progressive weight loss, apathy, capricious appetite evolving to anorexia for approximately 10 days. It had difficulty getting up when in sternal decubitus and the condition would evolve if it remained in decubitus for long periods.

In the general physical examination the animal presented mild dehydration, with mild enophthalmos, difficulty in getting up and dry stools in small quantities. The mammary glands

were hardened and the left posterior hoof was swollen. At the end of the clinical examination the animal showed drowsiness, uncoordinated gait and pressured the head against obstacles, showing that the nervous system was also affected. In the specific clinical examination of the nervous system, the alterations observed were lateral deviation of the head, proprioceptive deficit with dysmetria (Fig. 01A), uncoordinated walking (Fig. 01B), reduction of tongue tonus, flaccidity of the jaw and absence of the swallowing reflex.

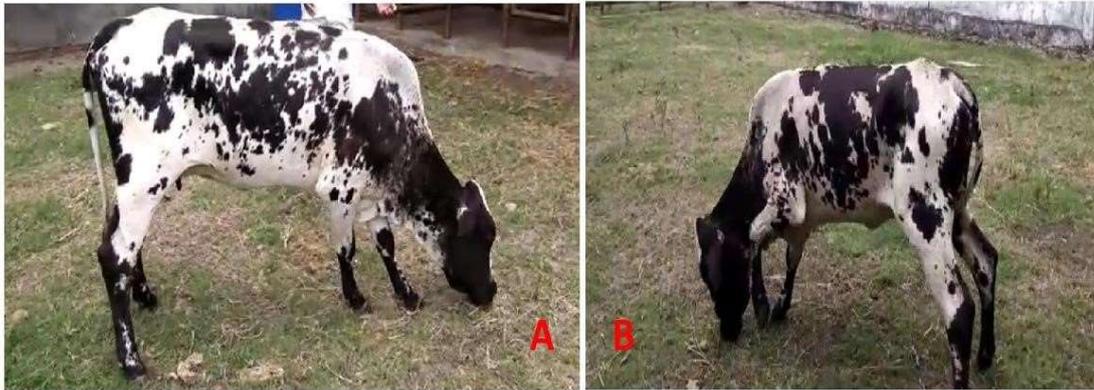


Figure 01: Female bovine, showing dysmetria with increased support base of the pelvic limbs and crossing of the thoracic limbs, characterizing proprioceptive deficit (1A) with consequent mobility difficulties (1B).

The presumptive diagnosis was rabies, considering that it was observed signs of diffuse lesion in the clinical evaluation of the nervous system and polysephalomalacia being one of the main diseases that differential diagnosis must be performed. A treatment with vitamin B1 (10mg/kg/TID) and dexamethasone (0.2mg/kg/SID) for three days was initiated, during which time the animal remained under observation for evaluation of the clinical picture. As supportive treatment, oral fluid therapy of approximately 10 liters of hydroelectrolytic solution was applied. After the 72-hour period, as the clinical signs of effects on nervous system became more accentuated and the animal started to present circling, greater motor incoordination, hypermetria, a wider support base, in addition to frequent falls, it became evident that the treatment was not effective. Consequently, it was decided to euthanize the animal based upon the evolution of the clinical picture and the lack of therapeutic response.

During the clinical examination and before euthanasia, the animal was also presenting decreased visual acuity, jaw relaxation, absence of corneal reflex, absence of sensitive reflex on the face, loss of auditory reflex, absence of swallowing reflex, absence of lingual tone, reflex of decreased flexion and withdrawal characterizing clinical alteration of a diffuse lesion in the central nervous system.

Euthanasia was performed after sedation of the animal with Xylazine (0.1mg/kg/IV) and subsequent intrathecal application of 60 mL of lidocaine hydrochloride.

At necropsy, a moderate amount of bloody fluid was observed in the thoracic cavity and it was identified multiple yellowish nodules of varying sizes and firm consistency, which had a pasty yellowish content with a sandy aspect on the periphery of the nodules when cut, at the lungs. The mediastinal lymph nodes were enlarged with the presence of multifocal nodular areas with a caseous aspect. Such nodulations were also observed in the diaphragm. The heart

had abundant serosanguinous fluid in the pericardial sac with the presence of free fibrin filaments in the fluid. A large amount of fibrinous material and yellowish granular multifocal areas were attached to the epicardium.

It was also noted ruminal adhesion to the diaphragm during the necropsy, as well as the presence of nodular structures on the wall of the rumen. The spleen of the animal had a wrinkled capsule and the presence of nodules, as well as adhered material similar to fibrin deposited on its surface. The liver had a yellowish surface with an accentuated lobular pattern and the kidneys had a yellowish surface.

During the evaluation of the mammary glands, extensive areas of yellowish friable material were observed, with sandy areas compressing and intermingling the breast tissue (Fig. 02).

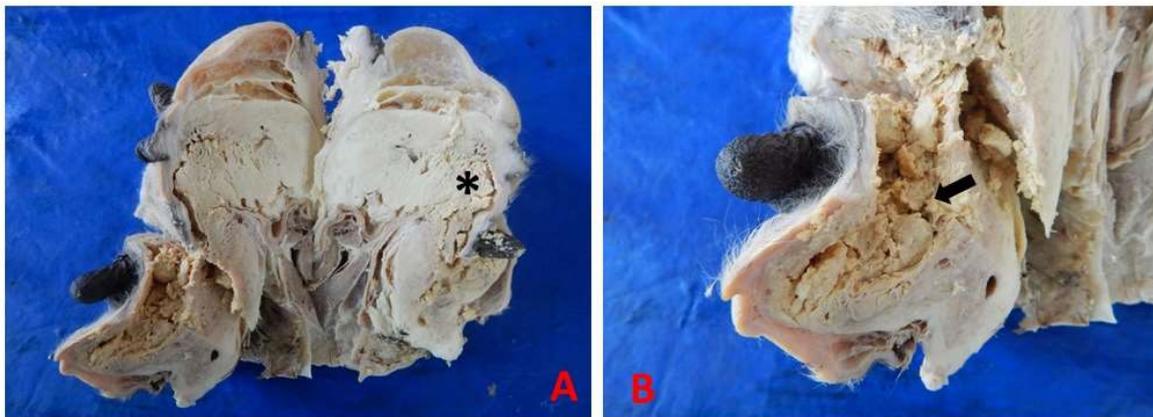


Figure 02: Mammary glands showing extensive areas of yellowish friable material (2nd) with sandy areas compressing and interminglings the breast tissue (*). Destruction of he glandular parenchyma in the region of the glands cisterna (arrow).

The brain and spinal cord had a large number of small caseous nodules adhered to the leptomeninges, similar to those described in the diaphragm but of smaller size (Fig. 03), and the meninges were congested.



Figure 03: Cross-section of the brain showing a large amount of granulomas adhered to the surface of the brain, filling the brain turns and longitudinal fissure of the brain (*).

Material was collected for microbiological examination with sterile swab and fragments for histopathological analysis which were stored in a container containing 10% formaldehyde. The Histological analysis showed that the formation of granulomatous lesions at the nodular lesions characterized by a central area of necrosis surrounded by epithelioid macrophages, few lymphocytes and abundant giant multinucleated cells of the Langhans type showing multifocal areas of calcification. In the lungs, the nodules were multifocal to coalescent, compressing to the adjacent alveoli, where moderate congestion and slight thickening of the alveolar septa was observed (Fig. 04). The affected lymph nodes exhibited loss of the cortical spinal cord and the nodular lesions contained abundant necrosis and calcification. The lesions were less pronounced in the liver and kidneys. The brain and spinal cord shared the same type of injury. The Ziehl Neelsen technique was applied to stain the brain and lungs, both had granulomas and were positive.

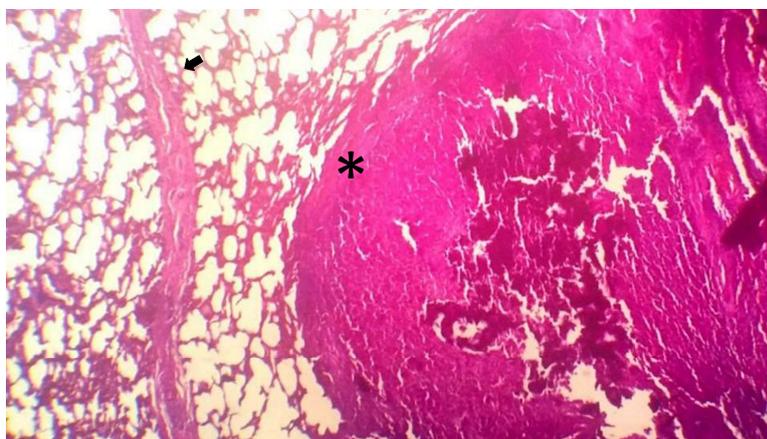


Figure 04: Histological section of the lungs showing the multifocal to coalescent nodules, compressing to the adjacent alveoli (*), where moderate congestion and slight thickening of the alveolar septa was observed (arrow).

RESULTS AND DISCUSSION

The clinical signs exhibited by the animal, associated with necropsy and histopathological findings, allow us to affirm that the reported case is of miliary tuberculosis. For (FREITAS *et al.*, 2001), although tuberculosis can manifest itself in several clinical forms which are dependent on the route of infection and subsequent response, the most common form of manifestation affects the lungs and the mediastinal and bronchial lymph nodes, which may or may not be associated with a respiratory infection.

The description of the etiopathogenesis of tuberculosis and the clinical forms of that tuberculosis can present itself in humans relates to the miliary form of the hematogenic spread of the bacillus, which leads to the formation of very small and diffuse granulomatous lesions which affects not only the lungs but several other organs (CAMPOS, 2006) as shown in this report. Miliary tuberculosis is considered uncommon in animals. This information is reinforced by Freitas *et al.* (2001) in his study of 1735 buffaloes slaughtered in the state of Pará, Brazil, where this type of tuberculosis occurred in only five animals. Such small occurrences allows us

to characterize the occurrence as rare in this species, and this is the first known reported case in the state of Sergipe.

Before the clinical analysis, the animal in this report had a history of progressive weight loss, uncoordinated walking, reluctance to exercise, yet did not have a cough. However, these symptoms suggests a chronic disease and the symptom of uncoordinated walking could indicate the involvement of the nervous system. Those symptoms are similar to those described by (OLIVEIRA *et al.*, 2012), where the authors reports an animal with chronic cough in the city of Viçosa/MG, Brazil.

Symptoms such as cachexia and lymph node hyperplasia followed by lymphadenitis and dyspnoea are common to animals that present a clinical picture of the disease, these symptoms were evidenced in this report and are emphasized by the Ministry of Agriculture of Brazil when describing animals with tuberculosis (GALIZA *et al.*, 2010).

In cattle with miliary tuberculosis, when the nervous system is affected clinical signs may appear such as locomotor disorders, circling, in addition to the changes produced by the disease in the brain, in the spinal cord and in the meninges. When present in the brain, animals have insecure walking, rigid movements, chronic seizures, mandibular trismus, a general loss of sensation and paralysis. In the spinal cord, the rigidity of the posterior train and of the tail is also observed, with consequent paraplegia and the animal may also have urinary incontinence. On the other hand, in meningeal tuberculosis the animal is easily excited, has exaltation of reflexes, anxiety and loss of consciousness (SILVEIRA *et al.*, 2018). In the present report, the clinical manifestation of the disease are consistent with the description above.

The description of the diseases that affect the nervous system of cattle in the Northeastern semiarid, of Brazil, made by (MELO *et al.*, 2012) states that, once the brain, brainstem and spinal cord are affected by tuberculosis, the animals may present blindness, pressure the head against objects, walk in circles, trismus or mandibular relaxation, lingual paralysis, dragging of the clamps and decreased sensitivity of the face. All of these clinical signs were observed in this case study, with the exception of mandibular trismus. It led to the conclusion that these were evidences due to the large amount of granulomas present in the central nervous system.

Since rabies is a disease that also causes diffuse nervous symptoms and has a diversified clinical presentation, in addition to presenting high frequency in Brazilian herds, it must be considered whenever animals present symptoms in the nervous system. The main disease to be considered in the differential diagnosis of rabies is botulism due to the similarity of its clinical signs (GALIZA *et al.*, 2010). Taking into account cattle herds with a high incidence of tuberculosis and the reported data, we can consider miliary tuberculosis as a differential diagnosis of diseases of the nervous system, even in sporadic cases.

In addition to the nervous symptoms, the mammary glands of the animal were also affected, which according to (RADOSTITS *et al.*, 2002) it is a form that can be considered rare and occurs in less than 1% of diagnosed cases. Therefore, the authors state that when the mammary gland is affected, it indicates a spread of the agent throughout the whole animal's body (FREITAS *et al.*, 2001). In a study with 239 parts of buffalo affected by tuberculosis, obtained in slaughterhouses in the state of Pará, Brazil, only nine animals had affected mammary glands, three of which had the glandular parenchyma affected and six with the retro

mammary lymph node affected, which corresponds to 1.3% and 2.5%, respectively, when compared to 60.3% of involvement of the respiratory system in the same study.

In miliary tuberculosis, it is common to find several outbreaks of chronic inflammatory reaction of the granulomatous type, with areas of caseous necrosis with central calcification, this lesion is usually surrounded by a halo of epithelioid cells and giant cells. On the other hand, it was also observed macrophages, lymphocytes at the periphery of the lesion and proliferation of fibrous tissue encapsulating it (OLIVEIRA *et al.*, 2012). Similar injuries are described in a study carried out with tuberculosis affecting dairy goats in the state of Pernambuco, Brazil (COELHO *et al.*, 2008). This pattern of lesions was also shown in this case study when the liver, kidneys, brain, spinal cord, and lymph nodes were examined.

In addition to the lesions described above, multifocal coalescent nodules compressing to the adjacent alveoli where moderate congestion and slight thickening of the alveolar septa were observed at the lungs. These findings in the lungs are consistent with the IV stage granulomas described by (SOUZA *et al.*, 2013), where histomorphological analysis characterize the granulomatous lesions suggestive of ganglion and pulmonary tuberculosis in cattle slaughtered in the northwest region of the state of São Paulo, Brazil. Lungs injuries due to Tuberculosis are the most common and the most reported, as well as described (RUGGIERO *et al.*, 2007).

The Ziehl-Neelsen staining technique is based on the ability of mycobacteria to retain fuscine after being stained and not discolored by the action of acid-alcohol, being considered by (COELHO *et al.*, 2008) as a method traditionally used for the diagnosis of mycobacteriosis due to its simplicity and speed of execution. Mycobacteria stained by this technique appear as thin, slightly curved, isolated or even pairs, stained in reds with a blue background. For this reason they are called acid-resistant bacilli (BRASIL, 2008).

This was the technique used to confirm the diagnosis of tuberculosis in this report with similar results by using samples of the brain and lungs.

CONCLUSIONS

In view of the clinical and pathological findings, it can be concluded that tuberculosis, when in miliary form, can manifest itself in several organs and thereby induce diffuse symptoms.

Lesions in the mammary glands, even though reported as rare in the literature, can occur and cause great impairment of this structure.

Miliary tuberculosis must be considered in the differential diagnosis of diseases that affect the nervous system in cattle from herds with high levels of animals positive for tuberculosis, reinforcing the importance of the investigation regarding the prevalence of this disease.

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