Systemic bovine tuberculosis: a case report

Tuberculose bovina sistêmica: um relato de caso

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Abstract

Gross and histopathological lesions associated with *Mycobacterium bovis* in an ox are described. Differential diagnoses of frequently occurring gross lesions in cattle that could be easily confused with bovine tuberculosis are discussed, and a comparative analysis of *M. bovis* induced tuberculosis lesions in wildlife is made.
Key words: Tuberculosis, Bovine, *Mycobacterium bovis*, Pathology.

Resumo

Alterações macroscópicas e histopatológicas associadas a *Mycobacterium bovis* são descritas em um bovino. O diagnóstico diferencial de lesões macroscópicas mais freqüentemente encontradas em bovinos que podem ser confundidas com a tuberculose bovina é discutida. Uma análise comparativa de lesões induzidas por *M. bovis* em animais silvestres foi realizada.
Palavras chave: Tuberculose, Bovino, *Mycobacterium bovis*, Patologia.

Introduction

Tuberculosis (TB) is a highly progressive granulomatous infectious disease caused by the rod-shaped, gram-positive, acid-fast bacteria from the genus *Mycobacterium* (JONES, HUNT; KING, 1997). Bovine tuberculosis is more frequently induced by *Mycobacterium bovis* (TIMONEY et al., 1992; JONES; HUNT; KING, 1997; RADOSTITS et al., 2002). Bovines are considered the primary natural host of *M. bovis*, but horses, swine, cats, and non-human primates are highly susceptible to develop the disease (TIMONEY et al., 1992; JONES; HUNT; KING; 1997). Sheep, goats, and dogs are relatively resistant to infections by *M. bovis* (TIMONEY et al., 1992). Zoonotic tuberculosis caused by *M. bovis* is sporadic in Brazil (COSIVI et al., 1998), but a recent Medline search did not reveal any reference of *M. bovis* infection in Brazilian captive or free-ranging wildlife. However, several free-ranging wildlife are considered reservoirs of *M. bovis* in the United Kingdom (DELAHAY; CHEESEMAN; CIFTON-HADLEY, 2001), New Zealand (COSIVI et al., 1998), North America (BRUNING-FANN et al., 2001), South Africa (KEET; KRIEK; MICHEL, 2000), Ireland, and Australia (RADOSTITS et al., 2002).

Gross bovine TB lesions are more frequently observed in the bronchial, mediastinal, submaxillary, and retropharyngeal lymph nodes than in the lung or liver (TIMONEY et al., 1992). Disseminated

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granulomatous TB lesions have also been described in the pleural and peritoneal surfaces of bovines (TIMONEY et al., 1992; JONES; HUNT; KING, 1997), and there are reported cases of TB-associated meningitis (JONES; HUNT; KING, 1997). TB-like lesions have been observed in the skin of cattle (RADOSTITS et al., 2002), but these have not been directly related to *M. bovis* (JONES; HUNT; KING, 1997). Typical TB lesions are characterized by various different sized, superficial or deeply located, firm or hard tubercles bulging from mucous or serous surfaces (SMITH, 1990; JONES; HUNT; KING, 1997). Sectioned surface of these tubercles reveals a yellowish, caseous, solid and dry necrotic center that is normally calcified (JONES; HUNT; KING, 1997).

Microscopically, tubercles are granulomatous lesions characterized by a collection of epithelioid and giant cells surrounded by a layer of fibroblasts and lymphocytes, having the center of the granuloma necrotic and calcified (JONES; HUNT; KING, 1997). Acid-fast (red) bacteria identified by the Ziehl-Neelsen staining technique within typical tubercles are sufficient to establish a diagnosis for tuberculosis (TIMONEY et al., 1992; JONES; HUNT; KING, 1997).

The present report describes the gross and histopathological findings of systemic tuberculosis in an ox that was submitted for routine necropsy, and offers differential diagnosis for other common granulomatous lesions in cattle. The importance of this paper is to demonstrate that even though TB is easily diagnosed in advanced cases, practicing large animal veterinarians could inadvertently confuse this disease with other granulomatous alterations.

Case report

The animal in question was a 3-year-old mixed breed, male bovine that originated from a rural village located approximately 50 km from the city of Maringá, Paraná, Southern Brazil. According to the referring veterinarian, the animal presented firm, bulging, submandibular and cervical masses of several months duration and was receiving large spectrum antibiotic therapy. Repeated antibiotic therapy was unsuccessful. A differential diagnosis of bovine lymphosarcoma was then suspected because of the enlarged lymph nodes and the animal was remitted to the Hospital Veterinário, Centro Universitário de Maringá, Maringá, PR. Further therapeutic measures were not implemented. The animal was sacrificed and submitted to the Department of Veterinary Pathology for routine necropsy and histopathological evaluations. Affected tissues were fixed in 10% formalin solution and routinely processed for histopathological evaluation. Selected formalin-fixed-paraffin-embedded (FFPE) tissue samples were submitted to the Gram and the Ziehl-Neelsen staining techniques to identify gram positive/negative and acid fast intracellular bacteria, respectively (TIMONEY et al., 1992; JONES; HUNT; KING, 1997).

Results

The animal presented for necropsy was slightly emaciated. There were several, large (varying from 8 to10 cm diameter), firm, bulging, multi-lobulated, and intact mass (submandibular lymph node) located on each jaw (Figure 1) and between the branches of the mandible bone. These huge masses were well fixed to subcutaneous tissue without any contact with the underlying bone. When perforated, a yellowish, odorless liquid to granular secretion was observed in one of the nodular compartments. This mass consisted of various separated nodular formations, varying from 4 to 8 cm in diameter; these forms were united by connective tissue. Sectioned surface showed various tubercles formed by a yellowish, dry caseous center that was lined by a thick surrounding layer of connective tissue (Figure 2).

Retropharyngeal, cervical, and thoracic lymph nodes were markedly enlarged and demonstrated gross alterations similar to that observed in the submandibular lymph node. Mesenteric, hepatic, and pulmonary lymph nodes also demonstrated this gross pattern. Small tubercles (0.5 to 3 cm in diameter)

were observed within the pulmonary and hepatic parenchyma, these being more noticeable in the liver than the lung.

Figure 1– Bovine tuberculosis. Note severe enlargement of submandibular lymph nodes.

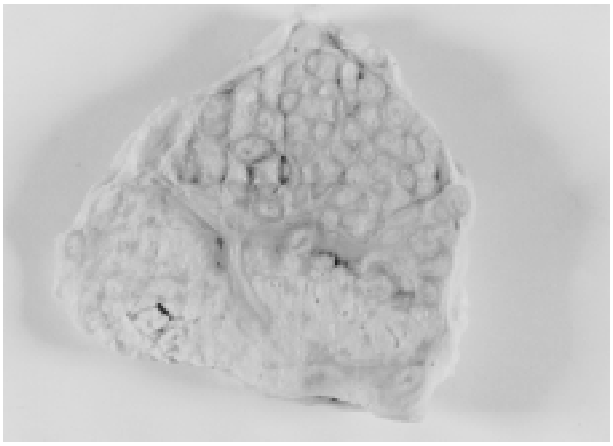

Figure 2 – Bovine tuberculosis. Sectioned surface of submandibular lymph node (after fixation in formalin solution). There are various different sized tubercles surrounded by fibrous connective tissue.

Microscopic evaluation of all affected lymph nodes revealed a similar pattern with little individual variation, being characterized by various tubercles that substituted the normal lymphoid tissue. The center of these tubercles revealed caseous necrosis with varying degrees of calcification. A layer of inflammatory cells, consisting of lymphocytes, macrophages, epithelioid cells, and Langhans's giant cells, surrounded this necrotic area. These inflammatory cells were surrounded by an extensive layer of fibrous connective tissue (Figure 3). Various gram-positive and acid fast positive bacteria were identified within these necrotic centers by the gram and Ziehl-Neelsen staining techniques respectively, realized in FFPE tissues. Tubercles were also observed within the hepatic and pulmonary parenchyma resulting in distortion of the normal hepatic and pulmonary architecture by invading tubercles.

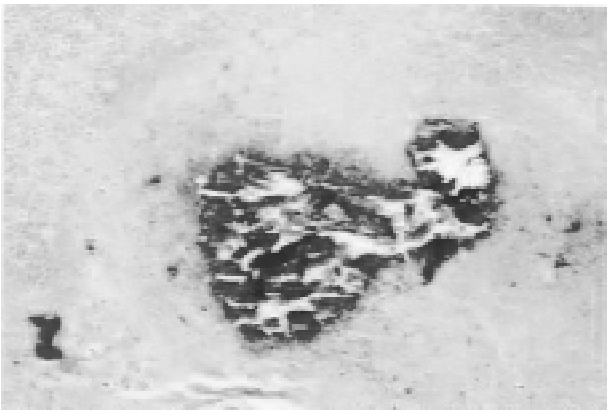

Figure 3 – Bovine tuberculosis. Note calcified necrotic centre of tubercles surrounded by mononuclear inflammatory infiltrate and connective tissue (HE x40) in bovine tuberculosis.

Discussion

The gross and histopathological alterations associated with the results of the histotechnical techniques, realized with FFPE tissues described, in this article are characteristic of tuberculosis induced by *Mycobacterium bovis* (SMITH, 1990; TIMONEY et al., 1992; JONES; HUNT; KING, 1997; RADOSTITS et al., 2002). Severe diffused lymphadenopathy associated with *M. bovis* tubercles, as was observed in this case, correlates with the lymphatic macrophage-associated dissemination characteristic of this organism (TIMONEY *et al*., 1992; JONES; HUNT; KING, 1997). This animal also demonstrated hepatic and pulmonary associated *M. bovis* lesions. Extra-lymphoid hepatic and pulmonary TB lesions have been associated with the intracellular multiplication of *M. bovis* in the circulatory system after initial entry via the efferent lymphatic and thoracic ducts (TIMONEY et al., 1992).

Severe diffused lymphadenopathy occurs in bovine actinobacillosis and lymphosarcoma (TIMONEY et al., 1992; JONES; HUNT; KING, 1997). The location of the granulomatous reaction observed in the submandibular lymph node described in the present report could de easily confused with actinomycosis (SMITH, 1990). These lesions although microscopically distinct, could be inadvertently confused with gross bovine TB lymphoid-associated granulomatous lesions (SMITH, 1990) therefore routine biopsy evaluation by histopathology associated with histotechnical staining techniques, immunohistochemistry, or molecular diagnosis (Polymerase Chain Reaction) of the suspected lesion is of extreme importance to determine a definite diagnosis.

Actinobacillosis of cattle (wooden tongue) is caused by *Actinobacillus lignieresii*, a gram-negative bacterium commensal to the oral cavity and pharynx of ruminants (JONES; HUNT; KING, 1997; RYCROFT; GARSIDE, 2000). *A. lignieresii* granulomatous-associated lesions are restricted to soft subcutaneous tissues of the head, neck, the gums, cheeks, and the tongue (JONES; HUNT; KING, 1997In the present report TB granulomas were observed in the head and neck. Gross evaluation of lesions induced by *A. lignieresii* reveals a pus-filled center that resembles sulfur granules (TIMONEY *et al*., 1992), different to the lesions observed in this animal. Microscopically, these lesions are granulomatous reactions demarcated by epithelioid and giant cells with a purulent central area (JONES; HUNT; KING, 1997)Frequently, characteristic club-shaped immune-mediated structures are observed in the center of these purulent areas (JONES; HUNT; KING, 1997; RYCROFT; GARSIDE, 2000), these structures were not observed in the present case.

Bovine actinomycosis (lumpy jaw) is caused by *Actinomyces bovis*, a non-acid fast, gram-positive bacterium that resides in the oral cavity and intestinal tracts of bovines (TIMONEY et al., 1992; JONES; HUNT; KING, 1997). This disease frequently produces marked granulomatous enlargement of the mandible or the maxillary bone that is observed microscopically as purulent centers surrounded by extensive proliferation of fibrous connective tissue (JONES; HUNT; KING, 1997). A remarkable microscopic feature of *A. bovis* is the forming of basophilic irregularly shaped rosettes surrounded by a layer of radially arranged eosinophylic projections (known as the Splendore-Hoeppli reaction), considered an immunoreactive host-induced response to the invading organism. (TIMONEY et al., 1992; JONES; HUNT; KING, 1997). This reaction does not occur in bovine tuberculosis (JONES; HUNT; KING, 1997), and was not observed in the present case.

Enzootic bovine lymphoma is a malignant tumor caused by the bovine leukemia virus that has marked tropism for lymphoid and other tissues (JONES; HUNT; KING, 1997), frequently resulting in severe diffused lymphadenopathy. The remarkably enlarged lymph nodes observed in bovine lymphoma, and as described in the present report, could be confused with the granulomatous lesions seen in bovine tuberculosis (SMITH, 1990; RADOSTITS *et al*., 2002). Grossly, affected tissues are firm, white-gray and homogenous at the sectioned surface that microscopically represents widespread proliferation of neoplastic lymphocytes substituting normal lymphoid tissue (JONES; HUNT; KING, 1997). Consequently, even though bovine lymphosarcoma may be confused with TB lesions grossly, these two diseases demonstrate distinct histopathological patterns. A more precise diagnosis of enzootic bovine lymphoma could be obtained by the detection of virus antibody using the agar-gel immunodiffusion test or by molecular diagnosis (TIMONEY et al., 1992; RADOSTITS et al., 2002).

Although cases of bovine TB have not been registered in free-ranging and/or captive wildlife in Brazil, this does not necessarily means that these species have not been previously infected. In Europe the extremely large number of wildlife species that are considered reservoirs of *M. bovis* posses a threat to farm animals and humans (DELAHAY;

CHEESEMAN; CIFTON-HADLEY, 2001). Tuberculosis lesions induced by *M. bovis* in free-ranging and captive wildlife are considered grossly different from those observed in cattle (KEET; KRIEK; MICHEL, 2000; BRUNING-FANN *et al*., 2001), which may partially be responsible for the absence of related reports in Brazilian wildlife. Granulomatous panopthalmitis, choroiditis, uveitis, conjunctivitis, and cystic cortical dilation of lymph nodes were the principal *M. bovis* induced lesions described in free-ranging lions in South Africa (KEET; KRIEK; MICHEL, 2000). These lesions are not commonly associated with bovine tuberculosis and would probably be overlooked by the consulting veterinarian. In another report of *M. bovis* tuberculosis in wildlife from Michigan, United States (BRUNING-FANN *et al*., 2001), only 1% (3/294) of all wildlife demonstrated gross lesions that raised a clinical suspect of tuberculosis. Reports from the United States (BRUNING-FANN et al., 2001) and South Africa (KEET; KRIEK; MICHEL, 2000), have indicated that diagnoses of tuberculosis in wildlife were only made after histopathological evaluation, bacteriological culture, or Polymerase Chain Reaction analysis of tissues collected from affected animals. Therefore, all cases of granulomatous lesions in ruminants and free-ranging and/or captive wildlife in Brazil should be thoroughly evaluated to determine the underlined etiological agent, implement recognized control and/or eradication procedures, thereby restricting the dissemination of bovine tuberculosis in Brazil.

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