

SCIENTIFIC COMMUNICATION

GRANULOMATOUS MENINGOMYELOENCEPHALITIS IN A CALF CAUSED BY *MYCOBACTERIUM BOVIS**

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ABSTRACT

Bovine tuberculosis is a chronic disease caused by *Mycobacterium bovis*, characterized by respiratory disorders, emaciation and lymphadenomegaly. Neurological signs associated with granulomatous lesions in the central nervous system are rarely seen. In Brazil, the National Program for Control and Eradication of bovine tuberculosis has begun in 2001, and notification of the disease is compulsory. A case of tuberculous meningomyeloencephalitis is reported in a dairy calf less than one year old presenting neurological signs (irritability, aggressiveness, weakness, dizziness and recumbency) which progressed to death in three days. Brain, spinal cord, and lung tissue samples were examined histopathologically. The spinal cord showed granulomatous meningomyelitis with Langhans giant cells in the meninges and perivascular mononuclear cuffing in the white and gray matter. There were a few granulomas in the cerebral cortex with a central area of necrosis surrounded by mononuclear infiltrate. Mononuclear perivascular cuffing, satellitosis, hyperemia, and severe granulomatous meningitis with Langhans giant cells were observed in the neuropile. Samples of brain were inoculated into Petragani and Stonebrink media. *M. bovis* was isolated and identified by PCR with JB21-JB22 primers. Considering the 2,888 samples of bovine CNS sent for surveillance of neurological syndrome and negative for Rabies, this was the only case of tuberculous meningomyeloencephalitis (0,035%) diagnosed from 2004 to 2008 by the Laboratory of Anatomopathology of Instituto Biológico. Although tuberculous meningomyeloencephalitis is not so frequent, it's important to diagnose *M. bovis* as a causative agent of bovine neurological disorders. In accordance with Brazilian regulations, sanitary measures in the infected herd (test-and-slaughter) should be adopted.

KEY WORDS: Granulomatous meningomyeloencephalitis, tuberculosis, bovine, neurological syndrome, Brazil.

RESUMO

MENINGOMIELOENCEFALITE GRANULOMATOSA EM UM BEZERRO, CAUSADA POR *MYCOBACTERIUM BOVIS*. A tuberculose bovina é uma doença crônica causada por *Mycobacterium bovis*, caracterizada por distúrbios respiratórios, emaciação e linfadenomegalia. Em raros casos, sinais neurológicos associados com lesões granulomatosas no sistema nervoso central são observados. O Brasil iniciou o Programa Nacional para Controle e Erradicação dessa doença em 2001, tornando a notificação compulsória. Um caso de meningomielite tuberculosa é relatado em uma bezerra leiteira com um ano de idade, que apresentou sinais neurológicos (irritabilidade e agressividade, fraqueza e tontura, decúbito) que progrediram para morte em três dias. A medula espinhal apresentou meningomielite granulomatosa, com células gigantes tipo Langhans nas meninges e manguito perivascular mononuclear na substância branca e cinzenta. Havia alguns granulomas no córtex cerebral, com área central de necrose circundada por infiltrado mononuclear. No neurópilo observou-se manguito perivascular mononuclear, satelitose, hiperemia e severa meningite granulomatosa com células gigantes tipo Langhans. Amostras de cérebro foram inoculadas nos meios de cultivo Petragani e Stonebrink e *M. bovis* foi isolado e identificado por PCR com primers JB21-JB22. Considerando as 2.888 amostras de SNC bovino enviadas para a vigilância epidemiológica de síndrome neurológica e negativas para raiva, este foi o único caso

*Todos os procedimentos foram realizados conforme os Princípios Éticos de Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA) e aprovado pela Comissão de Ética em Experimentação Animal do Instituto Biológico.

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(0,035%) de meningomieloencefalite tuberculosa diagnosticado pelo Laboratório de Anatomia Patológica do Instituto Biológico no período de 2004 a 2008. Embora a meningomieloencefalite tuberculosa não seja tão freqüente, é importante incluir a infecção por *M. bovis* no diagnóstico diferencial de lesões do sistema nervoso central de bovinos. De acordo com a legislação brasileira, medidas sanitárias no rebanho infectado (teste e abate) devem ser adotadas.

PALAVRAS-CHAVE: Meningomieloencefalite granulomatosa, tuberculose, bovinos, síndrome neurológica, Brasil.

Bovine tuberculosis is a chronic disease caused by *Mycobacterium bovis*. The majority of cattle are asymptomatic, but in herds with a high rate of infection, some can present respiratory disorders, emaciation, lymphadenomegaly, and reduced milk and meat production (RIET-CORREA; GARCIA, 2007). Young cattle most frequently become infected by ingestion of infected milk, whereas lung infection by inhalation is most common in adults (McGAVIN; ZACHARY, 2007). Neurological signs, associated with granulomatous meningoencephalitis or other lesions in the central nervous system (CNS), are uncommon in *M. bovis* infection. Neurological lesions are categorized into four symptomatic groups: meningitis (the most common), tubercles in the brain or the spinal cord, compression of the spinal cord by tuberculous spondylitis, and neuritis caused by lesions in other organs (BARROS et al., 2006).

Sporadic cases of tuberculosis in the CNS of cattle have been reported in Brazil. CORREA et al. (1980) reported in São Paulo State a case of disseminated tuberculosis in a Holstein cow with nervous symptoms. Necropsy revealed caseated granulomas in the CNS and on the pleura and peritoneum. Microscopy showed calcified granulomas in the meninges and cerebral neuropile as well as perivascular cuffing with mononuclear cells. *Mycobacterium* was revealed by the Ziehl-Neelsen and auramine fluorecence methods in smears of neural tissue lesions.

GUEDES et al. (1997) reported in Rio Grande do Sul State a case of granulomatous meningitis in a crossbred cow, in which tuberculous lesions replaced most of the parenchyma. Microscopy revealed typical granulomatous inflammation of the cerebral meninges with lymphocytes, epithelioid cell, and Langhans giant cell infiltration. Ziehl-Neelsen stain demonstrated acid-fast bacilli within the cytoplasm of epithelioid and giant cells.

In the same state, three cases of tuberculosis in the CNS with a neurological syndrome associated to visceral tuberculosis were also found by DRIEMEIER et al. (1999). Macroscopically, the brains of all animals showed meningeal thickening with a turbid aspect at the base of the brain, cerebellum, and brain stem, with some areas of mineralization at the cut surface. Ziehl-Neelsen stain revealed acid-fast bacilli mainly at the focus of caseated necrosis. Grocott staining for detection of fungi was negative.

A retrospective study performed over 36-year period in southern Brazil (SANCHES et al., 2000), data from 6,021 necropsies of cattle revealed that 552 (9.2%) animals presented neurological clinical signs, 305 (66.9%) showed inflammatory lesions, and tuberculosis was diagnosed in only three cases (0.98% of 305 cases). The lesions were in the meninges or in the brain parenchyma, with a caseous calcified aspect, the presence of Langhans giant cells, and acid-fast bacilli.

In Paraíba State, northeast Brazil, BANDARRA et al. (2007) reported two cases of bovine tuberculosis in the CNS. One of the animals presented weight loss, enlargement of lymph nodes, and a positive tuberculin skin test. The second presented incoordination, weakness of hind limbs, and bladder incontinency and was negative to the tuberculin skin test. In both cases, necropsy revealed meningeal thickening with granular mass, multiple caseated nodules in the lungs, and lymph node enlargement with caseated necrosis. Microscopy showed diffuse granulomatous meningitis and, meningitis and multifocal granulomatous encephalomyelitis were seen in the second animal. Ziehl-Neelsen stain revealed acid-fast bacilli in the cytoplasm of epithelioid and giant cells and positive staining for *Mycobacterium* sp. by immunohistochemistry.

The Brazilian Ministry of Agriculture introduced the National Surveillance System for Transmissible Spongiform Encephalopathies, which standardized procedures and provided technical information for the diagnosis of neurological syndromes in cattle over two years old (BRASIL, 2009a). Currently, diagnosis is made by authorized laboratories in Brazil providing epidemiological information and giving support to agricultural authorities in establishing strategic measures against bovine neurological disorders (BARROS et al., 2006; BRASIL, 2009a). From January 2004 to May 2007, the Instituto Mineiro Agropecuário at Minas Gerais State, Brazil examined CNS samples from 751 bovines over two years old reporting microscopic lesions of tuberculosis in only two cases, although 78.0% of the cattle had presented a neurological syndrome (OLIVEIRA et al., 2007). From January 2004 to June 2007, the Laboratorio de Anatomia Patológica of the Instituto Biológico in São Paulo State, Brazil, examined for the surveillance of rabies, other encephalitis and bovine spongiform encephalopathy, 1,666 CNS samples from

bovines and histological alterations were found in 378 animals (22.7%), but none of them suggesting tuberculosis (DEL FAVA et al., 2007).

Case report

The present case of tuberculous meningoencephalitis was diagnosed in a female dairy calf less than one year old from a herd in Goiás State, Brazil. The veterinarian's herd who performed the necropsy reported that the animal presented neurological signs (irritability, aggressiveness, weakness, dizziness and recumbency), which progressed to death in three days. At necropsy, white nodules were found in the liver, lungs, and diaphragm. Small fragments of CNS (spinal cord and brain), lungs and liver were sent for laboratory analysis. Direct immunofluorescence assay and intracerebral inoculation into mice were negative for Rabies. Samples of brain, spinal cord, and lung tissue were preserved in 10% formalin for histology (hematoxylin and eosin). Granulomatous meningomyelitis was observed in the spinal cord with Langhans giant cells in meninges (Fig. 1) and perivascular mononuclear cuffing in the white and gray matter. The cerebral cortex contained multifocal granulomas with central areas of necrosis surrounded by mononuclear infiltrate (Fig. 2). The neuropile showed mononuclear perivascular cuffing, satellitosis and hyperemia, and severe granulomatous meningitis with Langhans giant cells. Lung contained numerous caseated granulomas with mild calcification and central necrosis surrounded by epithelioid macrophages, lymphocytes, plasma cells, and Langhans giant cells, and fibrosis at the periphery. Due to the granulomatous inflammatory pattern observed in histopathological analysis (HE), it was performed differential staining for fungic infection (Grocott-Gomori and Schiff Periodic Acid) and tuberculosis

(Ziehl-Neelsen). Some acid-fast bacilli were observed within the cytoplasm of Langhans giant cells.

Refrigerated samples of lung and brain were decontaminated by the Petroff method and inoculated into Petragani and Stonebrink media, and acid-fast bacilli were isolated (KANTOR, 1988). The isolate was identified as *M. bovis* by PCR using a pair of primers, JB21-JB22, which amplified a 500 bp DNA sequence (RODRIGUEZ et al., 1995).

Considering the 2,888 samples of bovine CNS sent for surveillance of neurological syndrome and negative for rabies, no matter the age of the animals, this was the only case of tuberculous meningoencephalitis (0.035%) diagnosed from 2004 to 2008 by the Laboratorio de Anatomia Patológica of the Instituto Biológico. It is demonstrated that, for the diagnosis of tuberculosis, the macroscopic and histopathological analysis with differential staining for acid-fast bacilli, associated with isolation and molecular identification of the bacteria have to be done.

Considering the symptomatic groups described by BARROS et al. (2006), this case can be classified as a granulomatous meningoencephalitis, due the presence of meningitis and tubercles in the brain and spinal cord, what explains the neurological signs presented in the animal. A complete study of the pathogeny of the disease, with a more detailed description of anatomical parts of the CNS involved in this process was not possible to be done because only small fragments of spinal cord and cerebral cortex were sent to the laboratory. It's necessary to alert veterinarians that, for the differential diagnosis of neurological syndrome, it's necessary to collect for laboratorial analysis the spinal cord, brain stem, cerebellum and cerebral hemispheres, and this procedure is well described and can be accessed in the National Program of Herbivorous Rabies Control and other Encephalopathies (BRASIL, 2009a).

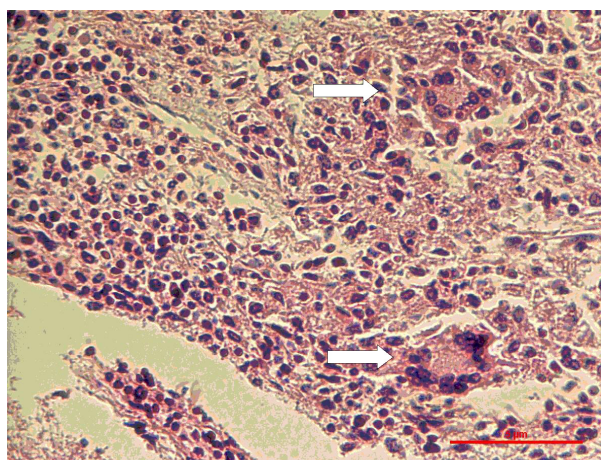


Fig. 1 - Granulomatous meningomyelitis with Langhans giant cells (arrows) in a calf brain. Hematoxylin and eosin staining.

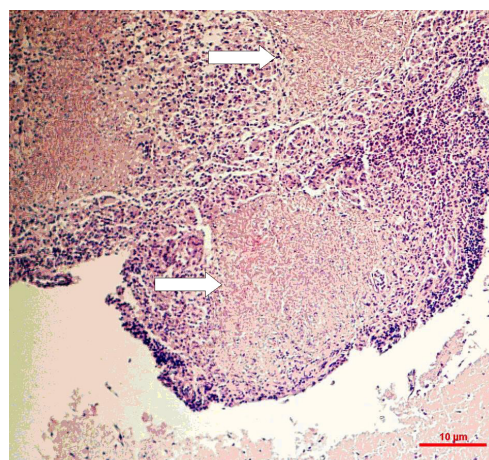


Fig. 2 - Granulomas with a central area of necrosis (arrows) in the cerebral neuropile of a calf brain. Hematoxylin and eosin staining.

Although sporadic cases of tuberculosis in the CNS of cattle had been reported in Brazil (CORREA et al., 1980; GUEDES et al., 1997; DRIEMEIER et al., 1999; SANCHES et al., 2000); BANDARRA et al., 2007; OLIVEIRA et al., 2007) it's important to diagnose *M. bovis* as a causative agent of bovine neurological disorders. According to Brazilian regulations, sanitary measures in a tuberculosis infected herd (test-and-slaughter) should be adopted (BRASIL, 2009b).

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