Acta Scientiae Veterinariae, 2023. 51(Suppl 1): 905.

CASE REPORT
Pub. 905

ISSN 1679-9216

Cerebral Babesiosis: Transplacental Infection by *Babesia bovis* in a Calf

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ABSTRACT

***Background*:** Cerebral babesiosis is a significant cause of mortality in calves, as it can be transmitted through the placenta during gestation and presents with hemolytic and/or neurological symptoms. However, the role of this agent as a cause of abortion is still poorly understood. In endemic areas, the disease primarily affects cattle between the ages of 1 and 12 months and occasionally neonates. This study aimed to describe the epidemiological, clinical, anatomopathological, molecular, and differential diagnostic aspects of a naturally infected newborn Nelore calf.

***Case*:** A newborn Nelore calf from Mato Grosso do Sul was discovered dead 1 h after birth and was subjected to necropsy, cytopathological and histopathological examinations by the Pathological Anatomy Laboratory (LAP) at the Federal University of Mato Grosso do Sul (UFMS), as well as complementary *in situ* hybridization (ISH) and polymerase chain reaction (PCR) tests. Hemoparasite research yielded negative results in all cows within the herd, including the mother of the necropsied calf, which were all clinically healthy. The clinical and hematological examination results of the cows were normal. At necropsy, the calf appeared thin, with a moderately jaundiced carcass and multiple petechiae on the pleural and pericardial surfaces. The lung was inflated until the opening of the thoracic cavity and floated when immersed in formalin, indicating that the calf was born alive and breathing. The spleen and liver were moderately enlarged with rounded edges, and the liver appeared slightly orange. The gray matter of the brain was significantly cherry red. Histologically, the central nervous system capillaries were slightly congested, with numerous punctiform and basophilic structures observed in the erythrocytes obliterating these vessels, either alone or in pairs, measuring 1 to 2 μm in diameter, which were morphologically consistent with *Babesia bovis*. In the cytological evaluation of the imprint slides of the brain cortex, blood capillaries filled with erythrocytes parasitized by solitary or paired punctiform basophilic structures, approximately 1 μm in diameter, morphologically compatible with *B. bovis*, were detected. Positive labeling for *Babesia* spp. was observed in the ISH examination, while the PCR identified *B. bovis* and *B. bigemina* in were identified in the brain fragments. The macroscopic findings, including pale or icteric mucous membranes, yellow and enlarged liver, and splenomegaly observed in cases of anaplasmosis, may be confused with those of cerebral babesiosis when the latter does not present with hemoglobinuria. Brain congestion indicates *B. bovis* infection but does not rule out co-infection with *Anaplasma marginale* in this case, although this rickettsia was not identified in the blood smear.

***Discussion*:** The diagnosis of *Babesia bovis* infection was based on epidemiological and histological data, as well as the identification of the protozoan in spleen and brain smears and through PCR and ISH exams, which are highly sensitive and can aid in the diagnosis of *B. bovis* in cases of perinatal deaths. *In situ* hybridization is effective in cases where the material has autolysis, as the technique allows the genetic material of the agent to be associated with the lesion, even with tissue alterations caused by fixation in formalin. The findings of this study highlight the importance of considering this disease as a differential diagnosis among those that cause abortions or neonatal losses in cattle and emphasize the importance of conducting anatomopathological exams for definitive diagnosis.

Keywords: cerebral babesiosis, *Babesia bovis*, pathology, transplacental transmission, intrauterine infection, *in situ* hybridization.

DOI: 10.22456/1679-9216.132031

Received: 16 June 2023 Accepted: 30 September 2023 Published: 21 October 2023

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INTRODUCTION

Cerebral babesiosis, caused by the protozoan *Babesia bovis*, is a significant cause of death in cattle, presenting with hemolytic and neurological symptoms [5,8,30]. The protozoan is transmitted by the tick *Rhipicephalus microplus*, which is distributed in tropical and subtropical areas, making babesiosis a condition of enzootic stability in these regions [25,26,28].

The occurrence of the disease and deaths caused by it are influenced by various epidemiological factors, including the transmission capacity of the tick population, as well as the susceptibility of cattle related to their immunological status, breed, and age of affected animals [10,25,30]. The decline in colostral antibody levels and the high burden of tick infestation, leading to the phase of greatest susceptibility to infection, can result in the development of the clinical manifestation of the disease, with high rates of morbidity and mortality [10].

Considering that babesiosis is a disease that has been causing significant neonatal losses and abortions in cattle [1,14,15,20], it is important to consider this disease as a differential diagnosis for babesiosis caused by *B. bigemina* and anaplasmosis [3,4,9,12], as well as for bovine infectious rhinotracheitis (IBR), bovine viral diarrhea (BVDV), leptospirosis, neosporosis, and brucellosis [19]. However, reports of neonatal deaths and transplacental infections caused by *Babesia* sp. are rare [30].

This study aimed to describe a case of bovine neonatal death caused by *Babesia bovis* in the state of Mato Grosso do Sul, covering the main epidemiological, clinical, pathological, and molecular aspects, as well as the differential diagnoses for this disease.

CASE

A newly born Nellore calf underwent necropsy and histopathological examination by the team at the Pathological Anatomy Laboratory (LAP) of the Federal University of Santa Maria (UFMS). Epidemiological and clinical data of the affected herd were obtained through an interview with the veterinarian responsible for the herd during visits to the property by the LAP-UFMS team where the case occurred. During the visits, all cows were subjected to body temperature measurement, and blood samples were collected by jugular vein puncture, which was stored in tubes containing EDTA anticoagulant for direct investigation of hemoparasites and a complete blood count.

In April 2019, a male Nelore calf was born on a rural property in Maracaju (21º 36' 52" S; 55º 10' 06" W), Mato Grosso do Sul state, from a healthy cow and was found dead 1 h after birth. The property where the case occurred consisted of 18 dairy cows raised extensively on pasture under tropical climate conditions. During a visit to the property, it was found that the cows had an intense infestation of *R. microplus* ticks during the last months of gestation.

During the necropsy, fragments from various organs were collected, fixed in a 10% formalin solution, routinely processed for hematoxylin and eosin (HE)[1] staining, and examined under light microscopy. Additional procedures were carried out to investigate hemoparasites, such as spleen imprints and cerebral cortex smears stained with Panótico Rápido®[1].

Brain fragments were collected, refrigerated, and sent for PCR tests. Paraffin-embedded brain blocks were sent for *in situ* hybridization (ISH). PCR used the Cybi/Cbbr[2] primers specific for *B. bigemina* and Cybo/Cbbr[2] primers specific for *B. bovis* [6]. *In situ* hybridization procedures followed the methodology described in 2020 by Hülskötter *et al*. [16]. The previously used riboprobe (probe for ribosomal RNA) detected *Babesia microti* in experimentally infected chickens. The primers for *B. microti* 16S rRNA similar gene were BM16SFW 5′-CAT GTCTTAGTA TAA GCT TTT ATA CAG CGA AA-3′ and BM16SRW 5′-AAC GCT CGG AAG CGA GAT TAA TGA CAA GGC AG-3′. As negative controls for the technique, chicken tissues infected with a probe in the same direction were processed.

RESULTS

The results of the hemoparasite research were negative in all cows in the herd, including the necropsied calf's mother, that was heavily parasitized by ticks. The clinical and hematological examination results of the cows did not show abnormalities. At necropsy, the calf was thin, with a moderately icteric carcass and multiple petechiae on the pleural and pericardial surfaces. The lung was armed to the opening of the thoracic cavity and floated when immersed in formaldehyde, indicating that there was breathing and, therefore, the calf was born alive. There was no colostrum in the abomasum, indicating that no colostrum had been fed. The spleen and liver were diffusely and moderately enlarged with rounded edges. The liver was slightly orange. The brain's gray matter was markedly cherry red (Figure 1).

Histologically, the capillaries of the central nervous system were diffusely and mildly congested; numerous pinpoint and basophilic structures measuring 1-2 µm in diameter, morphologically compatible with *B. bovis*, were observed in the red blood cells that obliterated these vessels. Cytological evaluation of the imprint slides of the cerebral cortex showed blood capillaries filled with erythrocytes parasitized by solitary or paired basophilic pinpoint structures, approximately 1 µm in diameter, morphologically compatible with *B. bovis* (Figure 2A). ISH examination showed positive labeling for *Babesia* spp. (Figure 2B). The PCR analysis identified *B. bovis* and *B. bigemina* in the brain fragments.

DISCUSSION

The diagnosis of *Babesia bovis* infection as the cause of death in this calf was based on epidemiological data, macroscopic and histological findings, and the identification of the protozoan in spleen and brain imprints, as well as through PCR and ISH. Similar findings have been described in aborted fetuses, stillborns and neonates resulting from transplacental *B. bovis* infection in cattle [14,21,24].

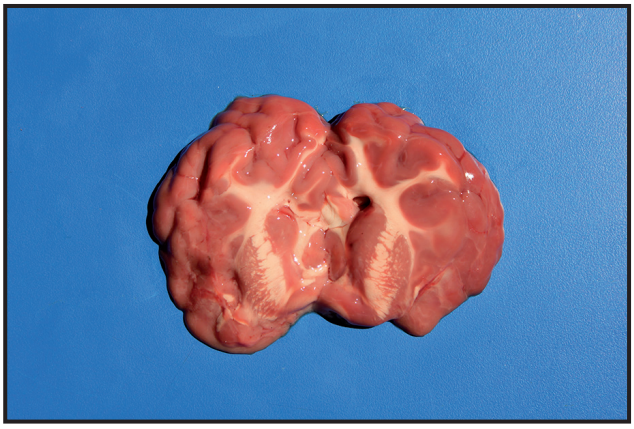

Figure 1. Macroscopic changes in a calf infected *in utero* by *Babesia bovis*. Transverse section of the frontal portion of the brain. The gray matter is diffusely cherry red.

Previous reports of abortions or neonatal deaths caused by *B. bovis* have shown that mothers were asymptomatic at the time of parturition [9,14,29], similar to the present case where all cows in the herd were clinically healthy, and *Babesia* spp. was not detected in blood smears, including that of the cow that gave birth to the necropsied calf. This occurs because adult animals living in areas of enzootic stability have sufficient immunity and normally do not become ill, and tests such as blood smears or complete blood counts do not detect minimum levels of infection [9].

The pathogenesis of transplacental infection by *B. bovis* is not well understood. However, some authors suggest that the transmission capacity may be affected by the intrinsic characteristics of the parasite, such as size, shape, and strain, and of the host, such as damage to blood vessels in the placental membrane and immunity [9]. Another hypothesis is that synepitheliochorial placentation in ruminants allows fetal infection by pathogenic agents but limits the transfer of larger molecules, mainly antibodies, from mother to fetus [14,28,29]. Although it does not show clinical signs of the disease and no hemoparasites were found, the calf's mother was heavily parasitized by ticks. It is possible that the cow had the presence of the protozoan at basal levels and transmitted it to the calf through the

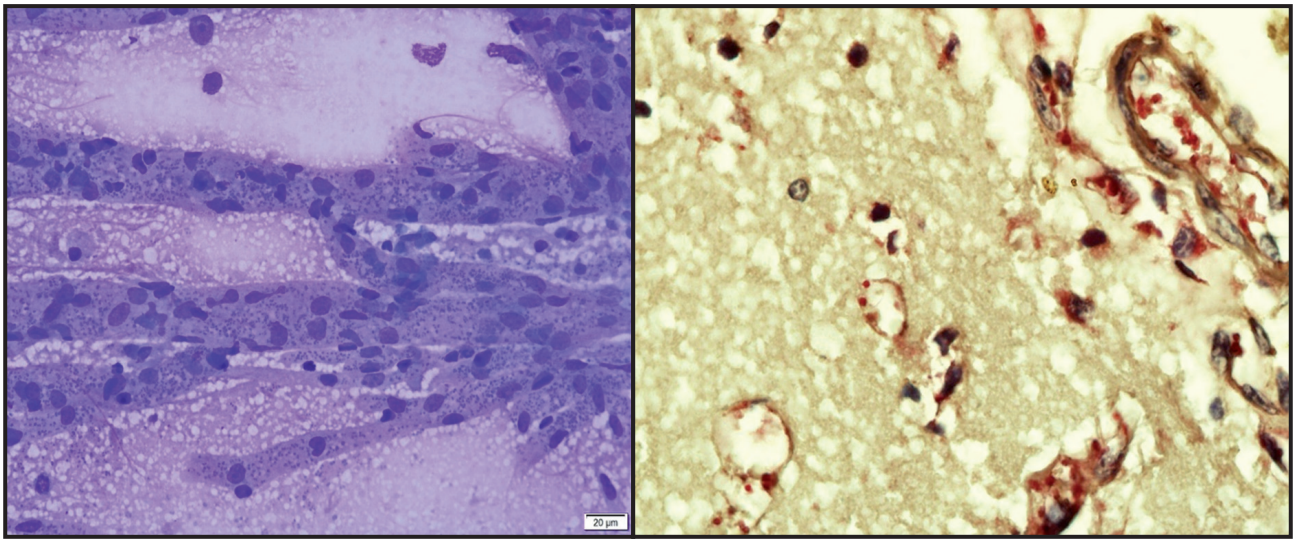

Figure 2. Cytological and molecular findings in a calf infected *in utero* by *Babesia bovis*. A- Cytology of the cerebral cortex smear. Blood capillaries are filled with erythrocytes containing basophilic structures measuring approximately 1-2 µm in diameter, compatible with *Babesia bovis* [Panotic Rapid; 10x]. B- Brain. Positive staining for *Babesia* sp. inside erythrocytes [*In situ* hybridization; 40x].

placenta, this happens in chronically infected cows living in areas of enzootic stability, as observed in some studies [17].

Tick infestation in pregnant cows may be a result of immunosuppression that occurs 2 to 3 weeks before calving. [18]. In one study, a greater demand for oxygen by the fetus was observed in the last 3rd of pregnancy, which may be one of the determinants of fetal death in cases of lack of blood supply [23]. Therefore, it is possible that the reduction in blood supply could lead to fetal anoxia and its consequent death.

Besides babesiosis, other diseases may cause abortions in cattle, including anaplasmosis, leptospirosis, and neosporosis [2,7,9,11,13,14,22]. In anaplasmosis, the macroscopic findings, including pale or icteric mucous membranes, yellow and enlarged liver, and splenomegaly in anaplasmosis, can be confused with those of cerebral babesiosis when it does not present hemoglobinuria [9]. In our case, brain congestion demonstrates infection by *B. bovis* but does not exclude co-infection by *Anaplasma marginale*. However, this rickettsia was not identified in the blood smear. Infection by *Neospora caninum* is an important cause of abortion in cattle, and post-mortem macroscopic findings include degenerative to inflammatory lesions of fetal tissues. However, in this case no macroscopic changes were observed.

Both PCR and ISH confirmed the presence of *B. bovis* in the necropsied calf; however, its high sensitivity should be interpreted cautiously. As babesiosis is confirmed by visualizing the protozoan in red blood cells, the use of molecular techniques (PCR and ISH) to identify the pathogen associated with necropsy and histological findings facilitates the confirmation of the diagnosis, even in cases where the smear is not performed or the parasite is not visualized [6,16]. In cases of autolysis, these techniques are also useful, as ISH mainly associates the genetic material of the agent with the lesion. The fact that *B. bigemina* was identified in the brain tissue does not indicate that this agent was responsible for the death of the calf, it only points to the presence of infection. Macroscopic and microscopic findings demonstrated the characteristic brain lesions caused by *B. bovis* would not be observed in *B. bigemina* [26].

This case demonstrates the need to include cerebral babesiosis in the list of differential diagnoses of diseases that cause neonatal deaths in cattle. Therefore, the importance of conducting necropsy exams, smears of fragments of the cerebral cortex, and histopathological exams to reach a conclusive diagnosis is highlighted. Identifying the agent through PCR and ISH proved to be useful tools for the conclusion of the diagnosis.

MANUFACTURERS

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Declaration of interest. The authors declare no conflicts of interest. Only the authors are responsible for the content and writing of this article.

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