

Outbreak of bovine tick-borne disease on a riverside property in the Western Amazonia: Case report*

Surto de tristeza parasitária bovina em propriedade ribeirinha na Amazônia Ocidental: Relato de caso

Guilherme Henrique Reckziegel,** David Prado Bayma,*** Tamyres Izarely Barbosa da Silva,****
Romulo Barros Fernandes,***** Siham Kassab,*** Mariana Dinis Souza,*** Tallison Filipe Lima de Oliveira,***
Heitor Tartari,***** Carlos Alberto do Nascimento Ramos**

Resumo

Objetivou-se relatar um surto de tristeza parasitária bovina (TPB) em propriedade ribeirinha na Amazônia Ocidental. Foi notificado o óbito de 25 bovinos da raça Nelore, em uma propriedade rural às margens do rio Purus, estado do Acre. O produtor observou animais com andar cambaleante, queda na produtividade, perda de peso e evolução ao óbito em aproximadamente 30 dias. Quinze animais do mesmo lote foram selecionados para avaliação clínica e foi procedida a punção da ponta de orelha para pesquisa de hemoparasitos, além da coleta de sangue para avaliação hematológica, bioquímica e molecular. Os principais achados laboratoriais foram anemia, leucocitose, trombocitopenia, hipoproteinemia, elevação da creatina quinase e redução de ureia, creatinina e albumina, além da visualização de formas sugestivas de *Anaplasma* spp. em 13,33% das amostras. Por meio da PCR, foi observado 20% de positividade para *Anaplasma marginale* e 53,33% para *Babesia* sp. As alterações hematológicas e bioquímicas, embora bastante sugestivas, podem sofrer alterações de outros fatores não relacionados à TPB. Por isso, a identificação presuntiva do agente etiológico no sangue ou confirmatória por métodos moleculares é essencial no diagnóstico. A depender da fase da doença, ocorre baixa parasitemia, dificultando a visualização de hemoparasitos em esfregaços sanguíneos. A *Babesia* sp. foi o principal agente do surto de TPB na população avaliada, que, quando associado ao diagnóstico clínico e laboratorial precoce, resulta no direcionamento terapêutico adequado e medidas profiláticas, promovendo uma relação de equilíbrio entre hospedeiro, agente e vetor.

Palavras-chave: Amazônia, anaplasmoze, babesiose, gado, PCR.

Abstract

The objective was to report an outbreak of tick-borne disease (TBD) on riverside property in the Western Amazon. The death of 25 Nelore cattle was reported on a rural property on the banks of the Purus River, state of Acre. The producer observed animals with staggering walking, drop in productivity, weight loss and evolution to death in approximately 30 days. Fifteen animals from the same batch were selected for clinical evaluation and the ear tip was punctured for hemoparasite research, in addition to blood collection for hematological, biochemical and molecular evaluation. The main laboratory findings were leukocytosis, thrombocytopenia, hypoproteinemia, elevated creatine kinase and reduced urea, creatinine and albumin, as well as visualization of forms suggestive of *Anaplasma* spp. in 13.33% of the samples. Through PCR, 20% positivity was observed for *Anaplasma marginale* and 53.33% for *Babesia* sp. Hematological and biochemical changes, although highly suggestive, may suffer changes from other factors not related to TBD. Therefore, the presumptive identification of the etiological agent in the blood or confirmatory by molecular methods is essential in the diagnosis. Depending on the stage of the disease, low parasitemia occurs, making it difficult to see hemoparasites in blood smears. The *Babesia* sp. was the main agent of the outbreak of TBD in the population evaluated, which, when associated with early clinical and laboratory diagnosis, results in adequate therapeutic direction and prophylactic measures, promoting a balance between host, agent and vector.

Keywords: Amazon, anaplasmosis, babesiosis, cattle, PCR.

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**Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal do Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil.

***Unidade de Ensino e Pesquisa em Medicina Veterinária, Universidade Federal do Acre (UFAC), Rio Branco, AC, Brazil.

****Unidade de Ensino e Pesquisa em Medicina Veterinária, Universidade Federal do Acre (UFAC), Rio Branco, AC, Brazil. Correspondence author: tamires.silva@ufac.br.

*****Instituto de Defesa Agropecuária e Florestal (IDAF), Rio Branco, AC, Brazil.

Introduction

According to data from the United States Department of Agriculture (USDA), it was estimated that in 2018 the world cattle herd exceeded the amount of 1 billion bovines. Brazil occupies the second place in this ranking, having just over 232 million bovines, behind only India, with 305 million bovines. Even though it is one of the largest producers of commercial cattle, the country's abundance is still considered low for its potential in this sector. This is due to genetic factors, inefficiency in food, and inadequate reproductive and sanitary management (Oliveira et al., 2015).

As for the sanitary conditions, parasitic diseases are related to important economic losses in the national herd. The bovine tick-borne disease (TBD - Known as "Tristeza Parasitária" in Brazil), which is more prevalent in tropical and subtropical areas, is caused mainly by the agents *Anaplasma marginale*, *Babesia bovis* and *B. bigemina*, transmitted mainly by ticks. The disease has high rates of morbidity and mortality, mainly due to the indiscriminate use of acaricides and hemoparasiticides, creating more resistant populations (Silva et al., 2015; Jirapattharasate et al., 2016).

Epidemiological data on TBD is of great relevance to the advance of cattle farming in Acre, a state located in the Western Brazilian Amazon. In this region, the climatic conditions of high temperature and rainfall favor the spread of the disease through vectors, especially in riverside properties, where the population of hematophagous arthropods is endemic. In addition, there are few studies on the health situation of cattle herds in the region and technical assistance in breeding is precarious, interfering with early treatment, control and prophylaxis of TBD (Kocan et al., 2010; Silva et al., 2015).

The objective of this paper was to report an outbreak of bovine tick-borne disease on a riverside property in the Western Amazon.

Case report

On a property located in the municipality of Manoel Urbano (08° 50' 20" S, 69° 15' 35" W), state of Acre, on the banks of the Purus River, the death of 25 Nelore cattle was notified, males from 10 to 24 months old on average. The producer reported that the animals manifested apathy, staggering, decreased productivity, weight loss, cachexia and progressed to death in approximately 30 days.

As reported by the producer, the symptomatic animals had received treatment with imidocarb dipropionate and ivermectin, in addition to vitamin complexes, however this therapeutic protocol was administered in underdoses and for an insufficient period of time, with no responsiveness of the animals to the therapy, as well as no remission of clinical signs.

In this property, cattle raising was carried out extensively with a stocking rate of approximately 2 bovines per hectare. The pasture was composed of *Brachiaria brizantha* cv. *Xaraes*, which was in abundance, but mineral supplementation was insufficient. There was no well-established protocol for the control of ectoparasites and, when performed, the use of acaricides occurred at intervals of three to six months, in dosages lower than recommended.

After anamnesis, 15 animals that had more evident clinical manifestations were selected from a herd of approximately 115 bovines, all male, from eight to 48 months old. They were

submitted to general clinical evaluation, with the following parameters being verified: body score, heart rate, respiratory rate, rectal temperature (°C), frequency of ruminal movements, palpation of superficial lymph nodes, mucosal color and time capillary perfusion (TCP).

The main signs observed were intense weight loss (Figure 1), apathy, paleness in the mucous membranes or jaundice, dehydration, elevated TCP and rectal temperature, in addition to dyspnea in the most debilitated animals. In relation to the presence of ectoparasites, *Rhipicephalus microplus* were found mainly in the ears and on the animals' dewlap.

Figure 1: Bovine showing weight loss and apathy with hemoparasitosis in the Western Amazon, Brazil



Blood samples were collected by a puncture in the bovine's external jugular vein. One aliquot was placed in sterile plastic tubes with anticoagulant (ethylenediamine tetraacetic acid - EDTA) and the other without anticoagulant, all refrigerated in an isothermal box and transported to the Laboratório de Patologia Clínica of the Unidade de Ensino e Pesquisa em Medicina Veterinária of the Universidade Federal do Acre. Blood smears were also made from peripheral blood obtained by ear tip puncture, which were stored in slide boxes for transportation.

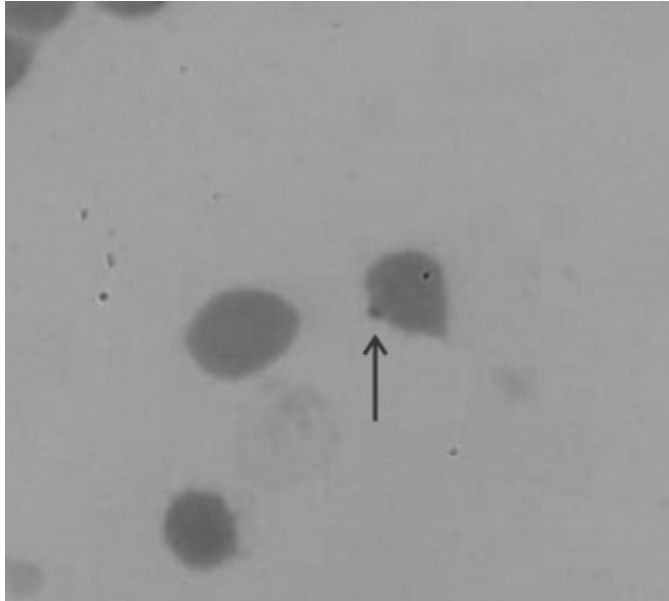
Blood samples in tubes with anticoagulant were processed to obtain erythrogram, leukogram and platelet, as well as the measurement of total plasma proteins. The blood smears of these samples and those from the tip of the ear were stained with rapid panoptic (NewProv®, Rio Branco-AC, Brazil) for differential counting and research of hemoparasites, respectively, under a microscopic at 100X magnification.

Blood samples in tubes without anticoagulant were centrifuged at 5,000 G for serum extraction, which was analyzed using commercial biochemical kits: albumin, urea, creatinine, creatine kinase (CK), alanine aminotransferase (ALT), alkaline phosphatase (AF) and gammaglutamyl transferase (GGT).

In the blood count, it was found that only 6.67% (1/15) of the animals had anemia, while the most common changes corresponded to leukocytosis in 53.3% (8/15), reactive lymphocytes in 33.3% (5/15) and thrombocytopenia in 20% (3/15) of the animals. As for the total protein, 40% (6/15) presented hypoproteinemia.

In the survey of hemoparasites, suggestive forms of *Anaplasma* spp. were seen (Figure 2) in 13.33% (2/15) of the animals. There were no characteristic inclusions of the genus *Babesia* spp.

Figure 2: Erythrocyte inclusions suggestive of *Anaplasma marginale*



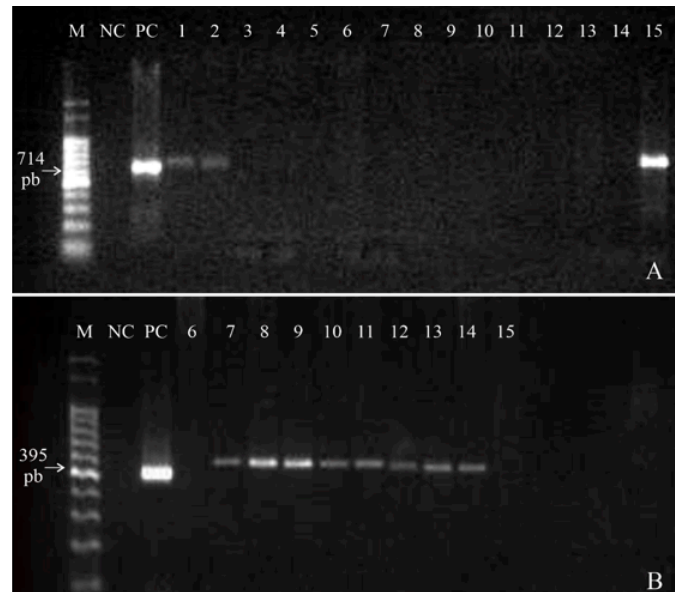
In biochemical assessments, the serum levels of ALT and GGT were within the normal range for the species, while CK was observed to be increased by 100% (15/15) of the animals. The biochemical parameters of urea and creatinine were found below the reference value in 40% (6/15) and 20% (3/15) of cattle, respectively. Albumin had a value lower than the reference for the species in 66.67% (10/15) and higher in 20% (3/15).

For molecular analysis, whole blood was divided into aliquots, kept at -20° C and sent to the Universidade Federal do Mato Grosso do Sul (UFMS). The polymerase chain reaction (PCR) was used, following the methodologies established by Bacanelli et al. (2014) for the identification of *Anaplasma* sp., with Msp5-F / Msp5-R primers, and piroplasmas, with BT-F1, BT-R1 and BT-R2 primers, according to Criado-Fornelio et al. (2003).

As a result, 20% (3/15) of the animals were positive for *Anaplasma* sp. and 53.33% (8/15) for *Babesia* sp (Figure 3). Of these animals, only one obtained a positive result for the presence of nucleic acid from both described agents, while 33.33% (5/15) were concomitantly negative for *Anaplasma* sp. and *Babesia* sp.

In view of the laboratory findings and clinical examination, oxytetracycline (20 mg / kg every 72 hours) was prescribed for 15 days, diminazene diaceturate (4 mg / kg) and 1% ivermectin (200 mcg / kg), both in single administration, in addition to the supply of vitamin B and folic acid. After the indicated therapy, the animals showed gradual recovery. Management changes were also recommended, such as implantation of a pasture rotation system, periodic request for biocarrapaticidogram, establishment of protocols for the control of endo and ectoparasites and mineral supplementation.

Figure 3: Electrophoresis of the nPCR product from bovine blood samples. A) Result for *Anaplasma* sp., using oligonucleotides Msp5-F and Msp5-R. Legend: M = molecular marker, NC = negative control, PC = positive control, 3-14 = negative samples, 1-2 and 15 = positive samples; B) Result for *Babesia* sp., using oligonucleotides BT-F1, BT-R1 and BT-R2. Legend: M = molecular marker, NC = negative control, PC = positive control, 6 and 15 = negative samples, 7-14 = positive samples. Agarose gel 2%, stained with red gel 1%. UFMS, 2019.



Discussion

TBD is a disease of great economic relevance widespread in all regions of Brazil, which leads to the loss of approximately R\$ 17 million / year due to mortality, without considering the losses caused by reduced performance. Among parasitosis diagnosed in cattle, it is the most frequent, with 55.1% of reported cases (Gonçalves, 2000; Brito et al., 2010; Amorim et al., 2014; Oliveira et al., 2015; Paula et al., 2015; Costa et al., 2018).

Study by Brito et al. (2010) confirms that in the South Western Amazon, in the states of Rondônia and Acre, the infection caused by *A. marginale* is high, independent of racial factors, in addition to presenting enzootic stability. In the reported case, *Babesia* sp. demonstrated greater epidemiological importance in the studied population. Infection by these agents can occur in a heterogeneous way, however, their occurrence is directly related to the vectors and the appropriate conditions for their development (Costa et al., 2009; Paula et al., 2015).

At low temperatures, the free life phase of the parasite is hindered, while the equatorial climate, present in the Amazon region, allows the development of vectors, such as *Rhipicephalus microplus*, and the occurrence of outbreaks (Oliveira e Oliveira-Serqueira, 2004; Santos et al., 2009; Piau et al., 2013). As an example, on the property visited, the degree of parasitosis by *R. microplus* was intense in the entire herd.

The lack of instructions for producers on the correct way to control ticks can lead to inefficiency of the product and creation of populations resistant to the acaricides used (Santos et al.,

2009; Carvalho et al., 2014). Thus, it is possible to propose that the indiscriminate use of antiparasitic drugs on the investigated property, through inadequate dosages and frequencies, has contributed to not only an infestation, facilitated by the season, but also to the presence of ticks resistant to the products used.

In addition to the exacerbated presence of ticks in the animals, the clinical manifestation was classic of TBD, which arises as a result of hemolysis and anemia promoted by the agent, resulting in pale mucous membranes, jaundice, anorexia, apathy, dyspnea with compensatory tachycardia and exercise intolerance (Elsify et al., 2015; Jirapatharasate et al., 2016; Ola-Fadunsin et al., 2018), as noted in the case.

Commonly, the fever syndrome in TBD is observed, as a pyrogenic response to the infectious process, which can still be accompanied by severe dehydration, represented by elevated CPT, as occurred in most of the examined cattle. In addition, due to the lack of appetite, there is the development of progressive emaciation, highlighted by the apparent bony protuberances (Feitosa, 2020), as verified in the case.

In line with the clinical examination, the blood count is of great importance for the laboratory diagnosis of TBD, especially in the analysis of the red series and visualization of the hemoparasite through blood smears.

Expressive changes in the erythrogram may be associated with the subclinical phase of the disease, with the compensatory bone marrow response or even with the cyclicity of the parasites in the blood (Brito et al., 2010). Leukocytosis, found in 53.3% of cattle, occurs in response to the ongoing infection in the body, indicating a good prognosis, and the presence of reactive lymphocytes is related to the intense production of antibodies. It is also noted that the total protein concentration is not reliable, since there is an overestimation of the value due to hemoconcentration caused by dehydration of cattle (Thrall et al., 2014).

As for biochemical changes, the increase in CK in all cattle is justified by the poor body condition of the animals, as well as by the administration of intramuscular injections during previous treatment attempts, since the enzyme is indicative of malnutrition and other traumatic muscle injuries. The reduction in creatinine in 20% of the animals, in turn, is proportional to the animal's muscle mass and suggests muscle atrophy due to marked weight loss, corroborating the hypothesis of severe muscle injury (Thrall et al., 2014).

Regarding the low concentrations of albumin and urea identified in a considerable frequency in animals that presented clinical signs, it is known that the animal's inadequate nutritional condition, compatible with weight loss, anorexia and apathy, reflects protein deficiency (Thrall et al., 2014). It is also reported in the literature an increase in ALT and GGT levels due to liver injury in cattle with TBD (Ashuma et al., 2013), which was not verified in this study.

It is observed that the hematological and biochemical changes, although suggestive, may suffer changes from other factors not related to TBD. Therefore, the identification of the etiological

agent in the blood or by molecular methods is essential in the diagnosis. Depending on the stage of the disease, low parasitemia occurs, making it difficult to visualize hemoparasites in blood smears (OIE, 2013).

The presence of artifacts on the slides confuses the precise detection of structures, requiring more sensitive and specific laboratory methods. The detection of specific genomic sequences by means of PCR, although they are more laborious and of high cost, guarantee a high accuracy in the diagnosis (Bacanelli et al., 2014; Ganzinelli et al., 2018), therefore, it was the confirmatory tool used in the outbreak.

Molecular biology data are similar to the study by Almeida et al. (2006) in Rio Grande do Sul, which reported a greater presence of species of *Babesia* sp. compared to *A. marginale*, with a low frequency of mixed infection by both agents. However, in the analysis of bovine blood samples by PCR, described by Brito et al. (2010) in the mesoregions of Rio Branco and Vale do Juruá, positivity for *A. marginale* was higher (92.44% - 208/225) than other hemoparasites. Regarding the PCR results of the present study, it should also be considered that, although the clinical and laboratory findings suggestive of TBD, *Anaplasma* and *Babesia* may present transient parasitemias, according to their genotypic characteristics and the stage of the disease.

For the treatment, the indication of diminazene diaceturate and oxytetracycline in adequate dosages was essential, since they are drugs that promote the potential elimination of hemoparasites, *Babesia* sp. and *A. marginale*, respectively (Alberton et al., 2015). Imidocarb dipropionate is an antiprotozoan that also acts efficiently against intracellular agents, such as *Babesia* sp., but its indiscriminate use by the producer as to the dose and frequency of administration may have limited the remission of the clinical condition, preventing its reuse (Taylor et al., 2017).

According to Alberton et al. (2015), the treatment of TBD should not be restricted to the use of drugs. Thus, the establishment of other complementary measures, such as ectoparasite control, pasture rotation, quality feeding and mineral supplementation (OIE, 2013), were essential to control the reported outbreak. However, these management practices are not always adopted in rural properties in the state of Acre, especially in riverside communities, probably due to the lack of technical assistance in the region.

Conclusion

Bovine tick-borne disease has as its main characteristic a fall in productivity and importantly lethality, resulting in numerous losses. The study of the disease in different regions of the country provides better clarification on its epidemiological aspects. The *Babesia* sp. was the main agent of the outbreak of TBD in the evaluated population, which, when correlated with an early laboratory diagnosis, results in adequate guidance in therapeutic conduct and prophylactic measures, promoting a balance between host, agent and vector.

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