Clinical description of transplacental anaplasmosis and the importance of congenital transmission in the epidemiology of cattle tick fever

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Case Report

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Abstract

Anaplasmosis, caused by *Anaplasma marginale*, is a limiting factor in calves breeding, causing considerable economic losses due to the high prevalence in cattle herds. Transmission of the agent can occur biologically, through ticks; mechanically, via the bite of blood-sucking flies, or by iatrogenic and congenital routes. The aim of the present study was to report a calf clinical case of congenital anaplasmosis. Clinical examinations, blood smears, and blood collection were performed for hematological and biochemical tests and molecular diagnosis. Regarding the clinical examination, the mucous membranes were altered, as well as respiratory movements and heartbeats. Blood smears showed structures suggestive of *A. marginale*. In the hematological analysis, the animal presented hypochromic macrocytic anemia and moderate anisocytosis, confirmed in blood smear. The leukogram revealed intense leukocytosis due to neutrophilia, lymphopenia, and monocytosis. The biochemical profile showed aspartate aminotransferase (AST) below the reference values for the species; azotemia; gamma glutamyltransferase (GGT) extremely higher than the maximum limit for the species; and globulins and alkaline phosphatase slightly above the reference values for the species. The PCR confirmed the infection by the presence of *A. marginale* DNA. The occurrence of transplacental transmission of anaplasmosis is mainly associated with asymptomatic cases. Symptomatic cases, such as the present study, are rarely reported in the literature. Transplacental transmission of *A. marginale* is variable on farms, but it can cause significant losses, since infected calves become the source of rickettsia for the herd. In addition, although clinical cases are rare, anaplasmosis should be included in the list of differential diagnosis of anemia in newborn calves.

Introduction

Cattle Tick Fever (**TF**) is a complex of diseases caused mainly by protozoa and rickettsiae, *Babesia* spp*.* and *Anaplasma* spp., respectively (De Vos, 1992). In Brazil, the main species involved are *Babesia bovis*, *B. bigemina* and *Anaplasma marginale* (Kessler & Schenk, 1998). The TF is responsible for serious economic losses in livestock, resulting from high mortality rates, decreased milk production, abortions in the acute phase of the disease, delay in weight gain, and additional expenses with medications (Vidal, 2000).

Anaplasmosis, caused by *A. marginale*, has been considered a limiting factor in the rearing of calves in endemic areas (M.F.B. Ribeiro et al., 1983). The clinical signs observed consist of anemia, jaundice, dyspnea, tachycardia, fever, fatigue, sialorrhea, diarrhea, anorexia, weight loss, and abortion (Alderink, F.J., Dietrick, 1983; Barbet, 1995; Miodrag Ristic, 1981). Transmission of *A. marginale* can occur biologically by ticks (Aguirre et al., 1994), mechanically, by blood-sucking flies, iatrogenically (Hawkins et al., 1982) or congenitally (M.F.B. Ribeiro et al., 1995). Congenital transmission can result in abortion or birth of infected calves (Aubry & Geale, 2011; Trueman & McLennan, 1987), possibly contributing to the epidemiology of bovine anaplasmosis (Kocan et al., 2003; Potgieter & van Rensburg, 1987).

Although the majority of calves infected by the transplacental route are asymptomatic (Añez-Rojas et al., 2010), part of them may show clinical signs of TF, suggesting its role as a cause of undiagnosed neonatal death (Costa et al., 2016). From an epidemiological point of view, asymptomatic calves can become important maintainers of *Anaplasma* spp. in the herd and the frequency of these animals on the farms is not certain (Aubry & Geale, 2011). In the light of the clinic, the diagnosis of anaplasmosis must be made in order to carry out adequate treatment of the disease (Kocan et al., 2010).

The aim of the present study was to report a clinical case of transplacental transmission of bovine anaplasmosis.

Case Description

A male Holstein calf, born in May 2017 on a dairy farm located in Minas Gerais, Brazil, was referred, with five days old, to the Ruminant Clinic of the Veterinary Hospital of Universidade Federal de Minas Gerais presenting jaundiced mucous membranes, and extreme apathy. During the days of hospitalization, clinical examinations, blood collections were performed to obtain smears, hematological and biochemical analysis and molecular diagnosis.

Results

The calf mucosae were initially intensely jaundiced, and after the third day of hospitalization, they were pale. Heartbeat and breathing movements remained fast throughout all daily clinical examinations. In blood smears, structures suggestive of *Anaplasma* spp. were observed, as shown in Figure 1.

The data acquired with the measurement of temperature and rickettsemia during the days of hospitalization are correlated in Figure 2.

On the first day of hospitalization, umbilical infection was observed and, from the third day onwards, the animal presented diarrhea. On the eighth day, the diarrhea became intense, evolving to a dehydrating condition, with retraction of eyeball and high capillary perfusion. Therefore, an intervention was carried out with the administration of glycophysiological serum by oral, intravenous and peritoneal routes, in addition to the administration of anti-inflammatory. The animal did not recover and died after 15 days of life.

In the hematological analysis, the animal presented hypochromic macrocytic anemia and moderate anisocytosis, confirmed in blood smear. The leukogram revealed intense leukocytosis due to neutrophilia, lymphopenia, and monocytosis. Although it was observed the presence of platelet microaggregates, the platelet account was within the normal range for the specie. The biochemical profile revealed aspartate aminotransferase (AST) below the reference values for the species; azotemia; gamma glutamyltransferase (GGT) extremely higher than the maximum limit for the species; and globulins and alkaline phosphatase slightly above the reference values for the species (Table 1).

DNA extraction from the blood of animal was performed with the Wizard® Promega kit, following the commercial protocol and the nested PCR using the MSP45 / MSP43 primer pairs (1st reaction) (de la Fuente et al., 2008) and AnapF / AnapR (2nd reaction) (Silveira et al., 2012). The reaction products were for MSP1a 800 base pairs (bp) and for MSP4 294 bp as seen in Figure 3.

Discussion

The incubation period, the time elapsed between the exposure of the animal to the infectious agent and the appearance of the first clinical signs, of anaplasmosis can vary between two and five weeks (Kocan et al., 2010). In this context, considering the age of the hospitalized animal (5 days of life), the clinical signs suggestive of anaplasmosis, such as anemia, jaundice and apathy, and high rickettsemia, it is concluded that the infection occurred through the transplacental route. In addition, congenital transmission was also confirmed by the presence of *A. marginale* DNA in the blood of the calf collected on the first day of hospitalization.

There are few reports on the occurrence of natural congenital infection of bovine anaplasmosis. Transplacental transmission has been linked to the occurrence of the acute form in the dam during pregnancy, especially at the end of pregnancy (Salaberria & Pino, 1988). However, other studies suggest that transplacental transmission can occur in chronic carrier cows (Grau et al., 2013). The mechanisms of this transmission are unclear, although it probably occurs through an active extra-erythrocyte phase of the microorganism, since the erythrocytes do not pass through the bovine placenta. Once reaching the fetal circulation, *A. marginale* can cause infection, establishing an intra- erythrocyte phase (Fowler & Swift, 1975; Zaugg, 1985).

Considering the clinical examination of the calf, it was noticed that the mucosae of the calf were initially jaundiced and, later, pale. There was also a compensatory increase of heartbeat and breathing movements, as a result of anemia and dehydration, in order to maintain the oxygen transport capacity from the lungs to the tissues (Marques, 2003).

According to Kocan et al. (2010), at the beginning of anaplasmosis, the rectal temperature can rise to 41ºC, remain elevated for a certain time and decrease to normal. The rectal temperature remained unchanged during the period of the calf hospitalization, and it is possible that, in the days prior to hospitalization, the animal presented hyperthermia.

The calf presented hypochromic macrocytic anemia in the erythrogram which suggests that the anemia was regenerative. Anemia can be explained by the invasion of erythrocytes by *A. marginale*. Rickettsia invades erythrocytes and initiates replication cycles, removal of cells infected by the mononuclear phagocytic system, and subsequent invasion of new erythrocytes, causing a reduction in the number of circulating red blood cells (Aubry & Geale, 2011). This extravascular hemolysis causes a reduction in the concentration of oxygen in the blood, which leads to an increase in the production of erythropoietin by the kidneys. Erythropoietin stimulates erythropoiesis through the bone marrow. Thus, the bone marrow releases immature cells and/or erythrocytes produced with fewer cell divisions. Extravascular hemolysis

also leads to the degradation of hemoglobin into heme and globin. Globin, as well as heme iron, is recycled by the body. The rest of the heme is oxidized to biliverdin which is converted to unconjugated bilirubin that is metabolized in the liver to conjugated bilirubin to be excreted. However, extravascular hemolysis was intense in the calf, so that its liver was unable to conjugate all bilirubin, causing its accumulation in the tissues and consequent jaundice (Kaneko et al., 2008; Santos & Alessi, 2011).

The leukogram showed leukocytosis due to neutrophilia, lymphocytosis, and monocytosis. Possibly leukocytosis occurred due to a sum of factors. Among which can be mentioned the stress of collection with consequent release of adrenaline and increase in leukocytes. In addition, because it is a newborn, another justification for the leukocytes to be increased would be in reflex to calving (Kaneko et al., 2008; Rosenberger, 1993). During calving there is a peak of glucocorticoids, both in the cow and in the calf, causing leukocytosis that can extend until the seventh day of calf life (Rosenberger, 1993). Umbilical infection was another possible cause of the change in leukocytes (Fecteau et al., 2009). Finally, leukocytosis can still be justified by the chronic infection associated with *A. marginale*. It is expected that cattle show a predominance of lymphocyte elements in the leukogram, which was not observed in this calf. The absence of young neutrophils indicates that the acute inflammatory state had already passed at the time of collection. In addition, benign lymphocytosis and monocytosis are findings commonly associated with chronic rickettsemia in this species (Kaneko et al., 2008; Rosenberger, 1993). Such observation suggests that the condition was already chronic in this calf, with the infection starting in the intrauterine period. Although the immune system of fetus is immature, it is capable of producing an immune response (Chase et al., 2008).

As for the biochemical examination, it is observed azotemia that possibly occurred as a result of an immune-mediated glomerulonephritis. The persistent infection by *A. marginale* may have led to the formation of soluble immune complexes and antibodies that, when in excess, can be deposited in the glomeruli, stimulating the fixation of the complement and consequent glomerular injury (Santos & Alessi, 2011). However, since the kidneys were still responsive to hypoxia and producing erythropoietin, the calf was considered to be in acute renal failure. In addition, the clinical picture of dehydration may have contributed to azotemia.

The proteinogram indicates that the calf acquired excellent passive immunity, as the serum protein was elevated due to the increase in globulins. The substantially high value of the GGT enzyme corroborates with this result, indicating that the calf ingested maternal colostrum, which is rich in this enzyme (Godden et al., 2019). However, the increase in globulins may also have occurred in response to infection by *A. marginale*. Three-months-old fetuses already have an active immune system (Chase et al., 2008). Considering that the calf was infected by *A. marginale* via transplacental, the stimulus for antibody production was already occurring since the fetal period. Grau et al., (2013), when performing indirect fluorescent antibody testing of samples from 30 beef cows and their respective calves, observed that all cows in the experiment were positive for antibodies specific to *A. marginale* from the beginning to the end of the experiment. Three calves (10%) were seropositive when tested before ingesting colostrum, indicating that they were producing antibodies anti *A. marginale* already as fetuses. Three days after

birth, when the test was repeated, 100% of the newborn calves were positive, indicating that they had ingested maternal colostrum with antibodies anti *A. marginale*.

The AST enzyme was below the reference value for the species. Neonate calves have low enzyme activity, which can take days or weeks to approach the reference values for adult cattle (Rosenberger, 1993). Alkaline phosphatase was above the reference value for the species. Contrary to what happens with other enzymes, alkaline phosphatase is high in neonates and is expected due to bone growth and decays as growth stabilizes (Kaneko et al., 2008; Rosenberger, 1993).

Serial blood smears indicate that the calf had a spontaneous clinical cure for anaplasmosis. Rickettsemia decreased from 4% to less than 0.5% in three days of hospitalization, zeroing on the seventh day, even though no treatment for anaplasmosis was instituted. Young animals have colostral antibodies (Corrier & Guzman, 1977), serum and fetal hemoglobin, which partially impair the multiplication of the agent in the blood and determine a greater erythropoietic activity of the bone marrow (M. Ristic, 1960).

Even with the rapid reduction in rickettsemia, the calf died. The calf probably died as a result of the weakness caused by anemia. Anemia possibly facilitated infection with enteropathogens, leading to profuse diarrhea, severe dehydration and death. According to Ribeiro & Passos (2002), death from anaplasmosis is usually associated with the severity of anemia.

Due to the fact that anaplasmosis is endemic in the region and the ease of complementary exams confirming this disease, the diagnosis of anaplasmosis by transplacental transmission was quickly reached. However, the following differential diagnosis for anemia and/or jaundice in newborn calves must be considered: iron deficiency; blood loss in the peripartum, for example due to umbilical injuries; leptospirosis; bacillary hemoglobinuria/hemorrhagic jejunum; coccidiosis; neonatal isoerythrolysis; among others (Divers, 2005).

It is believed that the transplacental transmission of *A. marginale* is not rare, however, as many calves are asymptomatic, the problem is possibly underdiagnosed. Grau et al. (2013) detected *A. marginale* by PCR in 10.5% of the newborn calves tested. A study conducted by Silva et al. (2014) revealed the presence of DNA from *A. marginale* in 41% (9/22) in blood samples from newborn calves, suggesting a high prevalence of transplacental transmission in the analyzed herd. Even with the animals being asymptomatic, the author points out that this form of transmission proved to be epidemiologically important for the maintenance of the agent in the herd through different generations.

However, there is a dearth of studies on transplacental transmission in herds. Costa et al. (2016), in a study evaluating the transplacental transmission of TF agents, states that when there is an imbalance in enzootic stability - parasites, vectors and host, this form of transmission can become common, constituting an important mechanism for the dissemination of agents in herds.

Conclusion

Congenital anaplasmosis represents damage to herds. The asymptomatic individual may be epidemiologically important, as it is also a source of transmission of *A. marginale*, leading to losses in property. When symptomatic, it can lead calves to death, and should be included in the list of differential diagnosis of anemia in newborn calves for timely treatment. It is important that surveys on newborn calves are carried out in order to have knowledge of the dynamics of transplacental transmission and its relevance in bovine anaplasmosis.

Declarations

Declaration of Competing Interest

The authors have no conflict of interest.

Ethical approval

The article reports a clinical case presented at the Escola de Veterinária of Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. All protocols followed were in accordance with the guidelines from the standard textbooks in Veterinary Medicine.

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Tables

Table 1. Results of the complete blood account, leukogram, platelet and biochemistry of the calf.

Complete blood count			
Physical aspect of the plasma:	Intensely jaundiced		
Erythrogram			
Test	**Result**		**Reference values***
Globular volume (%)	26		22-33
Hemoglobin (g/dL)	8.8		8.5-12.2
Red blood cells(x 10^6 cells/µL)	3.66		5.1-7.6
VCM (fL)	71.03		38-50
CHCM (g/dL)	33.84		36-39
HCM (g/dL)	24.04		14-18
Fibrinogen	200		200-700
Slide observation:	Moderate anisocytosis		
Leukogram			
Test	**Result**		**Reference values***
Total leukocytes	25,500		4,900-12,000
Differential leukometry	**Relative (%)**	**Absolute (cells / µL)**	**Reference values***
Myelocytes	0	0	Rare
Metamyelocytes	0	0	Rare
Band Neutrophils	0	0	Rare
Segmented Neutrophilis	49	12,495	1,800-6,300
Eosinophils	3	765	0-900
Basophils	0	0	Rare
Lymphocytes	35	8,925	1,600-5,600
Monocytes	13	3,315	0-800
Slide observation:	-		
Plaquetogram			
Test	**Result**		**Reference values***
Platelets (x 10 ^ 3/µL)	523,000		200,000-650,000
Slide observation:	Presence of platelet microaggregates		

Examination date:	29/05/2017
Biochemist	

Test	Result	Reference values*
Alanine aminotransferase (U/L)	27.36	11–40
Aspartate aminotransferase (U/L)	53.6	78–132
Creatinine (mg/dL)	4.46	1.0–2.0
Glutamyltransferase range (U/L)	507.68	6.1–17.4
Glucose (mg/dL)	57	45–75
Total proteins (g/dL)	7.6	5.7–8.1
Albumin (g/dL)	2.78	2.1–3.6
Globulins (g dL)	4.82	3.6-4.5
Urea (mg dL)	189.92	6.0–27
Alkaline phosphatase (U/L)	221.05	0–200
*(Constable et al., 2017)		

Figures

Figures 1-3 were not provided with this version of the manuscript.