FULL ARTICLE

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A likely case of Theileriosis in cattle in Colombia. First report

Um provável caso de Theileriose em bovinos na Colômbia. Primeiro relato

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ABSTRACT

Bovine theileriosis is a tick-borne disease caused by protozoa of the genus *Theileria* and may affect different ruminant species, mainly cattle among domesticated species. The disease occurs mainly in tropical and subtropical regions where climate and environmental conditions favor the development of their vectors. The clinical signs vary according to the infecting species. This study aimed to characterize clinically and present the first report of Theileriosis in Holstein and Simmental cows in Colombia. Two 22-month-old pregnant heifers were referred to the Large Animal Clinic at the National University of Colombia with a history of depression, pigmenturia, and dark feces. The main clinical signs on clinical examination were recumbency, severe dehydration (10-12%), icteric mucous membranes, and weak pulse. The blood cell count revealed severe anemia, hypoproteinemia, and lymphopenia; microscopic alterations in red cells, such as structures compatible with *Theileria* spp., were observed in lymphocytes and red cells. Increased levels of AST, GGT, urea, chloride, and sodium were observed, and the blood gases revealed metabolic acidosis; ventricular tachycardia was observed in one heifer. The anemia and the morphological alterations could be due to the *Theileria* in the erythrocytes, which increases their elimination rate along with oxidative changes or immune-mediated mechanisms. The observed lymphopenia could be attributed to the microorganisms within the cells that favor cell destruction. An increase in the hepatic enzyme levels might have resulted from anemia and dehydration that causes poor blood perfusion of the liver. The observed metabolic acidosis and hyperlactatemia were associated with inadequate tissue perfusion. In conclusion, this is a likely outbreak of Theileriosis in Holstein and Simmental heifers in Colombia.

Keywords: Cattle. *Theileria*. Anemia. *Rhipicephalus* (*Boophilus) microplus*. Acidosis.

RESUMO

A teileriose bovina é uma doença transmitida por carrapatos causada por protozoários do gênero *Theileria*, podendo acometer diferentes espécies de ruminantes, sendo principalmente bovinos entre as espécies domesticadas. A doença ocorre principalmente em regiões tropicais e subtropicais do mundo onde as condições climáticas e ambientais favorecem o desenvolvimento de seus vetores. Os sinais clínicos variam de acordo com a espécie infectante. O objetivo deste estudo foi caracterizar clinicamente e apresentar o primeiro relato de Theileriose em vacas Holandesas e Simental na Colômbia. Duas novilhas gestantes de 22 meses de idade foram encaminhadas à Clínica de Grandes Animais da Universidade Nacional da Colômbia com histórico de depressão, pigmentúria e fezes escuras. Ao exame clínico, os principais sinais clínicos foram decúbito, desidratação grave (10-12%), mucosas ictéricas, pulso fraco. O hemograma revelou anemia grave, hipoproteinemia e linfopenia; alterações microscópicas nas hemácias como estruturas compatíveis com *Theileria* spp. foram observadas em linfócitos e hemácias. Observou-se elevação dos níveis de AST, GGT, uréia, cloreto e sódio e a gasometria revelou acidose metabólica; taquicardia ventricular foi observada em uma novilha. A anemia e as alterações morfológicas podem ser devidas à presença de *Theileria* nos eritrócitos que aumenta sua taxa de eliminação juntamente com alterações oxidativas ou mecanismos imunomediados. A linfopenia observada poderia ser atribuída à presença de microrganismos no interior das células que favorecem a destruição celular. O aumento nos níveis de enzimas hepáticas pode ter sido resultado de anemia, desidratação que causa má perfusão sanguínea do fígado. A acidose metabólica observada e a hiperlactatemia estiveram associadas à perfusão tissular inadequada. Em conclusão, este é um surto muito provável de Theileriose em novilhas Holandesas e Simental na Colômbia.

Palavras-chave: Gado. *Theileria*. Anemia. *Rhipicephalus* (*Boophilus) microplus.* Acidose.

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Introduction

Bovine theileriosis is a tick-borne disease caused by protozoa of the genus *Theileria* that belongs to the infraphylum Apicomplexa. This infraphylum includes the protozoa with an apical complex that allows the parasite to enter the host cell (Perera et al., 2013; Ruggiero et al., 2015; Ganaie et al., 2019). Ticks of the genera *Amblyomma* spp., *Hyalomma* spp., *Riphicephalus* spp., and *Haemaphysalis* spp. transmit these protozoa. (Bishop et al., 2004; Perera et al., 2013; Sitotaw et al., 2014; Gul et al., 2015; Islam et al., 2017; El-Dakhly et al., 2018; Agina et al., 2020). *Theileria* spp. may affect wild and domestic ruminant species such as cattle, sheep, goats, and buffaloes. However, cattle are the mainly affected species among domestic ones. *Theileria annulata* (*T. annulata*) and *Theileria parva* (*T. parva*) are the species more commonly associated with severe clinical pictures in cattle (Perera et al., 2013; Abdela & Bekele, 2016a; Islam et al., 2011, 2017; Dandasena et al., 2018; Agina et al., 2020); among the genus *Theileria*, the ones belonging to the orientalis group (*T. buffeli/ T. orientalis/ T. sergenti),* with 11 genotypes identified up to now, have been reported as causative agent of the diseases called bovine anemia associated to *Theileria* or Oriental theileriosis (Islam et al., 2011; Spickler, 2019; Agina et al., 2020). Other species, such as *T. mutans*, *T. tarurotragi*, and *T. velife*, have also been reported to affect cattle. These *Theileria* species cause very mild cases, and the animals can be asymptomatic (Perera et al., 2013; Spickler, 2019; Agina et al., 2020).

Theileria spp. is obligated intracellular parasites that infect bovine leucocytes (lymphocytes, monocytes/macrophages) and erythrocytes (Abdela & Bekele, 2016a; Islam et al., 2017; Khan et al., 2017; Ganaie et al., 2019) developing a life lasting infection (Izzo et al., 2010). It has been determined that this disease occurs mainly in tropical and subtropical regions of the world where climate and environmental conditions favor the development of their vectors (Islam et al., 2011; Perera et al., 2013; Sitotaw et al., 2014; Abdela & Bekele, 2016a; Ganaie et al., 2019; Agina et al., 2020).

The clinical signs vary according to the infecting species, the host susceptibility, and the inoculated parasite load (Perera et al., 2013). Clinical signs include weakness, anorexia, fever, anemia, lethargy, jaundice, tachycardia, tachypnea, increased size of lymphatic nodes, conjunctival petechiae, dyspnea, nasal secretion, decreased milk production, loss of body condition, abortion and death (Perera et al., 2014; Temiz et al., 2014; Gul et al., 2015; Abdela & Bekele, 2016a; El-Dakhly et al., 2018; Ganaie et al., 2019; Spickler, 2019; Agina et al., 2020). In cases caused by *T. orientalis*, the animals can be asymptomatic or present mainly clinical signs related to the severity of the hemolysis and subsequent anemia (Perera et al., 2013; Abdela & Bekele, 2016a; Ganaie et al., 2019). The infected animals may remain usually asymptomatic throughout life but may develop the disease in stressful conditions (Izzo et al., 2010; Watts et al., 2016). Due to these different manifestations, theileriosis is an infection that causes severe economic losses due to high morbidity and mortality rates, decreased milk yield, treatment costs, and possible culling of unproductive animals (Gul et al., 2015; Ganaie et al., 2019).

The hematological findings in theileriosis cases include a decrease in red blood cell count, hemoglobin levels, packed cell volume (PCV), and the mean corpuscular hemoglobin (MCH), but an increase in mean corpuscular volume (MCV) and reticulocyte count may occur (Ganguly et al., 2015; Ayadi et al., 2017; Khan et al., 2017; Kim et al., 2017; Somu et al., 2017; Charaya et al., 2021); regarding the white blood cell count, it is variable. It has been reported from leukopenia to leukocytosis, neutrophilia, and neutropenia, as well as neutrophilia with mild left shift, lymphocytosis, monocytosis, and hypoproteinemia, and all these alterations are thought due to the evolution of the clinical disease (Cöl & Uslu, 2006; Temiz et al., 2014; Khan et al., 2017; Somu et al., 2017). These findings are very similar to the ones observed in buffaloes and include a marked decrease in red blood cell count, PCV, hemoglobin, neutropenia, lymphopenia, eosinopenia, and monocytopenia along with thrombocytosis (Osman & Al-Gaabary, 2007; El-Deeb & Iacob, 2012).

Clinical pathology tests reveal hyperbilirubinemia, hypergammaglobulinemia, Creatinine kinase (CK), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) increases; with a marked decrease in blood triglycerides, albumin, globulin, glucose, calcium, magnesium, and phosphate (Izzo et al., 2010; Islam et al., 2011). Acid-base disorders are not commonly reported in these cases (Perera et al., 2013, 2014). However, a study showed a compensated metabolic acidosis that progressed to a non-compensated acidosis in severely anemic cattle infected with *Theileria* (Temiz et al., 2014).

Diagnosis is usually made by evaluating blood smears to determine the presence of piroplasm in erythrocytes and leukocytes, also using the alterations of hematological parameters, serological tests such as enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT); there are also molecular tests such Polymerase Chain Reaction (PCR), Quantitative Real-time Polymerase Chain Reaction (qPCR), nested PCR that allows determining the species or subspecies of *Theileria*. The use of loop-mediated isothermal amplification (LAMP) allows the identification of genomic material, and it is a test that can be used for diagnosis in field conditions (Radostits et al., 2007; Islam et al., 2011; Gul et al., 2015; Dandasena et al., 2018; El-Dakhly et al., 2018; Ganaie et al., 2019; Agina et al., 2020).

The objective of this study was to characterize clinically and report for the first time the presence of theileriosis in Colombia.

Materials and Methods

Two 22-month-old pregnant Holstein and Simmental heifers that weighed 380 Kg were referred to the Large Animal Clinic at the National University of Colombia with a history of depression, pigmenturia, and dark feces. They were treated with oxytetracycline, diminazene, vitamin B, and flunixin meglumine. The heifers came from a farm with cattle of various breeds on pasture supplemented with concentrate and corn silage. Twenty days before admission, a Simmental adult cow and a heifer with similar clinical signs were treated with oxytetracycline/imidocarb and died within two days despite treatment. Hematological evaluation requested by the referring veterinarian of other Zebu cattle showed PCV values between 20-22%, and the blood smears revealed the presence of Babesia spp. in 30% of the heifers and 50% of the female calves. Fecal exams revealed a low intestinal parasite burden, and no diarrhea was observed.

On clinical examination, the heifers were in recumbency, lethargic, unable to get up, with a low body condition score (2/5), sunken eyeballs (15 mm), severe dehydration (10-12%), pale and icteric mucous membranes, weak pulse, increased capillary refill time (5"), tachycardia (86 and 160 BPM), tachypnea (16 and 52 breaths per minute), hypothermia (36.7 °C), ruminal atony, and in one animal, pigmenturia, increased paralumbar lymph nodes were observed.

Rectal palpation of both patients indicated a lack of feces and a collapsed rumen. *Rhipicephalus* (*Boophilus*) *microplus* ticks were also observed in both patients (Figure 1).

Venous blood was collected for cell blood count (CBC), serum biochemistry, and venous blood gases. Urine was sent for urinalysis, and an electrocardiogram (ECG) was done on one heifer due to an arrhythmia detected on auscultation.

Results

CBC showed severe anemia in both animals (PCV: 9 and 10%), hypoproteinemia, and lymphopenia in one heifer (Table 1).

In both cases, microscopic structures compatible with *Theileria* spp. were observed in high numbers in lymphocytes and red cells (Figure 2A and B), and neither were *Babesia* spp. nor *Anaplasma marginale* were observed.

Serum chemistry tests showed increased urea, AST, Cl^- and lactate levels in both heifers as well as a decrease in K^+, ionized Ca^{++}, Glucose, and tCO_2; only one heifer showed increased GGT and Na^+ (Table 2).

On venous blood gases and electrolyte evaluation, metabolic acidosis, hyperchloremia, hyperlactatemia, and an increased base deficit were observed (Table 3). From a physicochemical approach to acid-base, it had a SID acidosis. Urinalysis revealed aciduria, hemoglobinuria, and proteinuria in one patient. ECG on the Holstein heifer indicated ventricular tachycardia (Figure 3).

Figure 1 – Tick of the *Rhipicephalus* (*Boophilus*) *microplus* was observed in both patients.

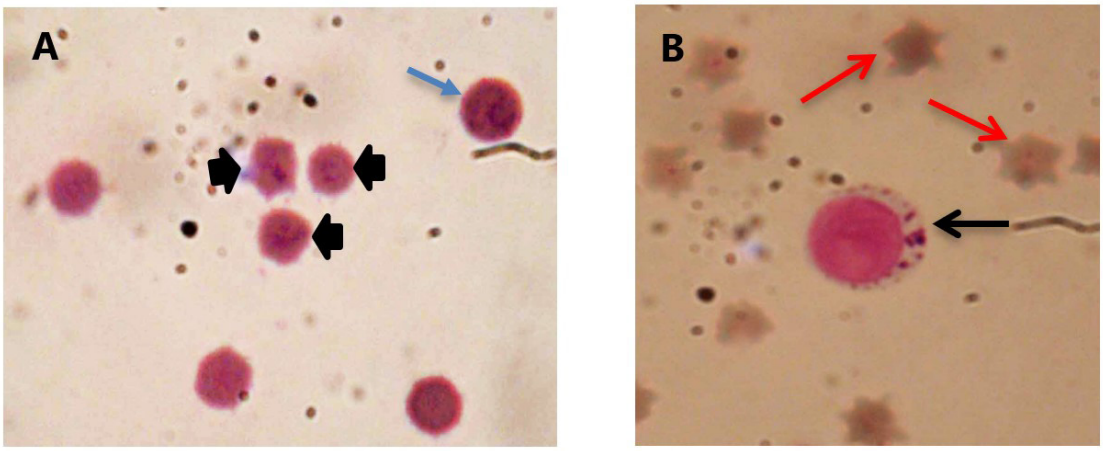

Figure 2 – (A) Merozoites (Piroplasm) in red Blood cells (arrowhead), spherocytes, and basophilic stippling (blue arrow); (B) Schizonts (Koch bodies) in a leukocyte (black arrow) and echinocytes (red arrows).

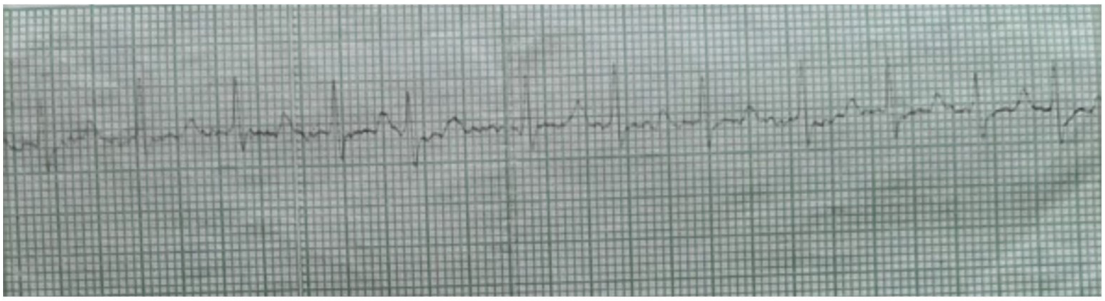

Figure 3 – Ventricular tachycardia observed on the electrocardiogram.

Table 1 – Results of Blood Cell Count in both heifers

Cell Blood Counts			
	Heifer 1	**Heifer 2**	**Reference Values (Sharkey & Burton 2019; Sharkey & Heinrich, 2019)**
PCV (%)	10	9	25.3– 32.3
TPP (g/dL)	5.1	6.4	6.7 – 7.46
Fibrinogen (mg/dL)	200	500	100 – 700
WBC (cel/µL)	7280	8900	6590 – 14375
NØ (cel/µL)	2912 (40%)	3293 (37%)	2230 – 4350
LØ (cel/µL)	4295 (59%)	5607 (63%)	5200 – 7482
EØ (cel/µL)	73 (1%)	0	0 – 240

Table 2 – Serum Chemistry tests in both heifers

Serum Chemistry Tests			
	Heifer 1	**Heifer 2**	**Reference Values (Garzón-Audor et al., 2020, Staempfli & Oliver-Espinosa, 2019)**
Urea (mmol/L)	**9.5**	**13.7**	2.3 – 8
Creatinine (µmol/L)	82	89	80 -168
AST (u/L)	**357**	**497**	53 – 138
GGT (u/L)	33	**44**	17 – 33
Na (mmol/L)	139	147	136 – 144
K (mmol/L)	**3.1**	**3.4**	3.6 – 4.9
Ca (mmol/L)	**1.02**	**1.21**	2.1 – 2.7
TCO_2 (mmol/L)	**16.4**	**8.5**	33 – 34
Cl^- (mmol/L)	**111**	**119**	99 – 107
Lactate (mmol/L)	**14.51**	**17.62**	0.56 – 1.39

Table 3 – Blood Gases in both heifers

Blood Gases			
	Heifer 1	**Heifer 2**	**Reference Values (Castañeda-Salazar et al., 2002; Garzón-Audor et al., 2020)**
pH	7.2	7.2	7.33 – 7.42
pCO_2 (mm Hg)	34.7	20.1	37.14 – 50.98
pO_2 (mm Hg)	15.9	20.4	30 – 37
HCO_3 (mEq/L)	15.3	7.9	21.66 – 30.34
SID (mmol/L)	25.33	19.7	38 – 44
BD (mmol/L)	– 11	– 20.2	-2.07 – 5.93

Both cases were treated with 7.5% hypertonic saline solution, isotonic saline to treat dehydration, and 5% $NaHCO_3$ solution to correct acidosis. Blood transfusions were performed to treat hemolytic anemia. Calcium borogluconate was administered to correct hypocalcemia, and flunixin meglumine was given as an anti-inflammatory medication.

A combination of oxytetracycline and diminazene was administered several times to treat the theileriosis, given that no buparvaquone is available in Colombia. The ventricular tachycardia was corrected using IV lidocaine, and vitamin B complex was used as hematinic. To treat the *Rhipicephalus* (*Boophilus) microplus* tick infestation, ivermectin was given, and ruminal transfaunation was performed to stimulate appetite.

After these therapies, hematological, serum and acid-base values returned to physiological ranges. The two heifers were discharged from the clinic 29 days after admission.

Discussion

There have not been previous reports of the presence of *Theileria* spp. in Colombian cattle. However, it is essential to remark that given the country's geographical location in the tropics, this is an area with a high prevalence of potential vectors (ticks) and hemoparasitic diseases (Vecino et al., 2010; Abdela & Bekele, 2016a; Ganaie et al., 2019; Acevedo-Gutiérrez et al., 2020; Agina et al., 2020). Along with these epidemiological risk factors, the clinical findings and the demonstration of the piroplasm within red cells and leukocytes of the evaluated patients indicated the theileriosis, and it is also highly suggestive of its presence in the farm of origin.

The clinical findings observed in these cattle such as lethargy, low body condition, weakness, icterus, anorexia, pigmenturia, severe dehydration, anemia, hypothermia, increased paralumbar lymph nodes have been reported in different studies (Islam et al., 2011; Perera et al., 2013, 2014; Temiz et al., 2014; Abdela & Bekele, 2016a, 2016b; Ganaie et al., 2019; Agina et al., 2020; Ma et al., 2020; Aziz et al., 2022). None of the subjects showed body temperature increase (fever). This could be attributed to the long-case evolution, previous treatments that may have had some beneficial effects, the species that caused the infection, the severity of the anemia, the hypovolemia, and the impending hypovolemic shock (Abdela & Bekele, 2016a, 2016b). The alterations of the cardiac rhythm, such as sinus tachycardia observed in ruminants, including buffaloes, have been suggested to be due to either fever, pain, anemia, and electrolyte disturbances or their combinations (Hansapour et al., 2008; Fartashvand et al., 2013; Gupta et al., 2018). Other clinical alterations observed in theileriosis that include ruminal atony, atrial fibrillation, atrioventricular blockage, and sinus arrhythmia have been thought to be due to anemia, electrolyte, and acid-base imbalances (Hansapour et al., 2008; Fartashvand et al., 2013; Mehmet et al., 2014). Sunken eyeballs, weak pulse, and increased capillary refill time have also been reported in cases of *T. orientalis* and are associated with the degree of anemia, dehydration, fever, and hypovolemic shock as was observed in these animals (McFadden et al., 2011; Watts et al., 2016). These clinical findings are prevalent in cases of anaplasmosis and babesiosis in cattle as it has been shown that hemolytic anemia, decrease in the adequate circulatory volume, electrolyte and acid-base imbalances are shown to be responsible for the physiological and pathological alterations observed in diseases caused by *Anaplasma marginale and Babesia bigemina* in cattle (Castañeda-Salazar et al., 2002; Sherlock et al., 2003; Garzón-Audor et al., 2020).

Severe anemia was observed in both cases. The cause of anemia in theileriosis has been attributed to tick infestation, red cell oxidative lesions, increased red cell fragility, metabolic products from the parasite that alters erythropoiesis, and increased erythrophagocytosis by macrophages in the reticuloendothelial system (Ananda et al., 2009; Khan et al., 2011, 2017; Gul et al., 2015; Ayadi et al., 2017; Charaya et al., 2021); however, the leading cause of the anemia is hemolysis that has been associated with the presence of merozoites in the red cells and it has been suggested to be due to the proteases present in the merozoites as occurs some other hemoparasites (Shimizu et al., 1990; Hagiwara et al., 1995). However, Ayadi et al. (2017) and Agina et al. (2020) suggest that anemia results from the extensive destruction of hematopoietic cells in the bone marrow. The anemic process results in alterations of red cell morphology with appearance of echinocytes, spherocytes and basophilic stippling (Khan et al., 2011; Gul et al., 2015); these researchers also detected acanthocytes, schistocytes, keratocytes and Howell-Jolly bodies in cattle infected with *T. buffeli*, which were thought to be caused by the alteration brought about by the intravascular hemolysis (Stockham et al., 2000; Gul et al., 2015). However, Khan et al. (2011) proposed that the morphological alterations of red cells are mainly caused by the presence of *Theileria* in the erythrocytes, oxidative changes, and immune-mediated processes.

The findings on the leukocyte numbers observed in these patients are relatively different from cases reported that indicated mild leukopenia and neutropenia along with lymphocytosis and monocytosis (Izzo et al., 2010; Ganguly et al., 2015; Khan et al., 2017; Patial et al., 2021; Selim et al., 2022). However, the lymphopenia observed in one of the animals could be thought to be due to the destruction of infected lymphocytes by the reticuloendothelial system or due to hematopoiesis alteration caused by the metabolites from the *Theileria* as has been proposed by Selim et al. (2022). This lymphocyte decrease has also been reported in studies in bovines (Omer et al., 2002, 2003; Charaya et al., 2021) and buffaloes (Osman & Al-Gaabary, 2007; El-Deeb & Younis, 2009; El-Deeb & Iacob, 2012). On the other hand, hypoproteinemia was observed in the two cases. This alteration has also been reported to take place and is thought to be generated by anorexia, extravascular leakage, and hepatic damage that cause a decrease in

protein synthesis (Charaya et al., 2021; Stockham et al., 2000; Singh et al., 2001; Omer et al., 2003; Cöl & Uslu, 2007; Somu et al., 2017). The fibrinogen levels observed in these cases were within normal parameters, in partial accordance with a study that indicates that the fibrinogen can be normal or increased in infections by *T. buffeli* (Stockham et al., 2000). However, others have observed increased fibrinogen due to the acute inflammatory process (Cöl & Uslu, 2006).

The increase in AST and GGT are indicative of hepatic damage. This damage could be caused by inadequate perfusion because of the anemia, dehydration, degenerative changes, and the parasite itself (Omer et al., 2003; Cöl & Uslu, 2007; Saber et al., 2008; Charaya et al., 2021). Inadequate renal perfusion due to the anemia and decreased adequate circulating volume occur in theileriosis, as observed in these patients (Singh et al., 2001; Omer et al., 2003; Cöl & Uslu, 2007). The mild hyperchloremia could be due to the fluids administered at the farm before being referred; however, Stockham et al. (2000) observed an increased lactate and normal chloride and urea.

Calcium levels in these animals were determined to be decreased. This finding has been previously reported in theileriosis, and it has been attributed mainly to a decrease in feed consumption, alteration in intestinal absorption, or renal damage (Singh et al., 2001; Omer et al., 2003; Cöl & Uslu, 2007; Hansapour et al., 2008; Khan et al., 2011; Ganguly et al., 2015; Somu et al., 2017). The hypokalemia observed could have been the result of anorexia and diarrhea, as has been observed in *T. annulata* infections. Other authors have observed hyperkalemia (Hansapour et al., 2008; Saber et al., 2008). The slight hypernatremia observed could also be explained by the previous use of IV solutions and due to dehydration (Saber et al., 2008). However, Hansapour et al. (2008), Temiz et al. (2014) and Somu et al. (2017) observed decreased blood sodium levels.

Regarding the acid-base balance, the animals had metabolic acidosis with hyperlactatemia, indicating an acid gain and reflected by a decrease in bicarbonate or total CO_2 (TCO_2) and an increment in the anion gap as reported by Mehmet et al. (2014). However, Temiz et al. (2014) detected a very mild metabolic acidosis without an increase in the anion gap in severely anemic patients with Theileriosis. Acidosis and hyperlactatemia could have occurred due to inadequate tissue perfusion and lactate formation. Metabolic acidosis has been reported in cases of anaplasmosis, that is a disease with a hallmark of intravascular hemolytic anemia as occurs in theileriosis cases by *T. sergenti* (Castañeda Salazar et al., 2002; Sherlock et al., 2003; Oliver-Espinosa & Castañeda-Salazar, 2022).

Conclusion

In conclusion, this is a likely outbreak of Theileriosis in Holstein and Simmental heifers. It is the first report of this exotic disease in Colombia, and given the clinical features, it may have been caused by *T. orientalis*.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

Ethics statement

The study was conducted on clinical cases referred to the Large Animal Clinic at the National University of Colombia, so ethical approval was unnecessary.

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