ISSN 2675-6617

Epidemiological, clinical and hematological aspects of bovine anaplasmosis: a retrospective hospital-based study (2012-2021)

[*Aspectos epidemiológicos, clínicos e hematológicos da anaplasmose bovina: um estudo retrospectivo (2012-2021) de base hospitalar*]

"Scientific Article/Artigo Científico"

Amanda Reges **Guedes**[1*], Rodolfo José Cavalcanti **Souto**[2], Gliére Silmara Leite **Soares**[1,3], Ângela Imperiano da **Conceição**[1], Jobson Filipe de Paula **Cajueiro**[2], Maria Isabel de **Souza**[1,2], José Augusto Bastos **Afonso**[2], Carla Lopes de **Mendonça**[2]

[1]Programa de Pós-Graduação em Medicina Veterinária, Universidade Federal Rural de Pernambuco, Recife-PE, Brasil.
[2]Clínica de Bovinos de Garanhuns, Universidade Federal Rural de Pernambuco, Garanhuns-PE, Brasil.
[3]Universidade Federal do Agreste de Pernambuco, Garanhuns-PE, Brasil.
[*]Corresponding author/Autora para correspondência: E-mail: amandaguedes00@gmail.com

Abstract
The aim was to carry out a retrospective study of bovine anaplasmosis diagnosed at the Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE) from 2012 to 2021. A descriptive analysis was performed of the epidemiological, clinical, and hematological data of 230 clinical records of cattle of different age groups. The disease represented 79.9% of the hemoparasites diagnosed at the Institution. Females aged ≥ 24 months of taurine lineage and their crosses, reared under intensive and semi-intensive systems, were the most affected. A higher occurrence of the disease was observed in the months of August to December. The most frequently observed clinical findings were apathy, decreased appetite, icteric mucous membranes, and tachycardia. Spontaneous abortion occurred in 12.8% of pregnant cows. Hematological findings revealed regenerative anemia, and the severity of the anemia was associated with a greater intensity of bone marrow response. Antimicrobials (oxytetracycline LA and enrofloxacin) were effective in reducing parasitemia, which, together with blood transfusion, were essential for the patient's clinical improvement. Clinical resolution with hospital discharge occurred in 82.6% of patients, with a mean hospital stay of eight days. Anaplasmosis was the most frequent hemoparasitosis in CBG/UFRPE. It is important to provide guidance measures for producers in the region, especially at the end of the rainy season before the dry season, so that the diagnosis and disease treatment are carried out as early as possible, allowing a faster recovery and return of the animals to production, and thus minimizing the economic impact to the dairy basin of the state of Pernambuco.

Keywords: *Anaplasma marginale*; clinical records; epidemiology; hemoparasitosis; regenerative anemia.

Resumo
Objetivou-se realizar um estudo retrospectivo da anaplasmose bovina diagnosticada na Clínica de Bovinos de Garanhuns/Universidade Federal Rural de Pernambuco (CBG/UFRPE) no período de 2012 a 2021. Realizou-se análise descritiva dos dados epidemiológicos, clínicos e hematológicos de 230 prontuários clínicos de bovinos de diferentes faixas etárias. A enfermidade representou 79,9% das hemoparasitoses diagnosticadas na Instituição. As fêmeas com idade ≥ 24 meses de linhagem europeia e suas cruzas, criadas sob os sistemas intensivo e semi-intensivo foram as mais acometidas. Observou-se maior ocorrência da enfermidade nos meses de agosto a dezembro. Os achados clínicos observados em maior frequência foram apatia, comprometimento do apetite, mucosas ictéricas e taquicardia. O aborto ocorreu em 12,8% das vacas gestantes. Os achados hematológicos revelaram anemia regenerativa, cuja maior intensidade da resposta medular esteve associada à gravidade da anemia. Os antimicrobianos (oxitetraciclina LA e enrofloxacino) foram eficazes na redução da parasitemia, que aliado à transfusão sanguínea, foram fundamentais para a melhora clínica do paciente. A resolução clínica com desfecho de alta hospitalar ocorreu em 82,6% dos pacientes com período de internação médio de oito dias. A anaplasmose foi a hemoparasitose de maior ocorrência na CBG/UFRPE, sendo relevante

Recebido 04 de março de 2023. Aceito 24 de julho de 2023.
DOI: https://doi.org/10.26605/medvet-v17n3-5682

a adoção de medidas orientativas aos produtores da região, principalmente no final do período chuvoso para o período seco, a fim de que o diagnóstico e tratamento da enfermidade sejam realizados o mais precocemente, possibilitando a recuperação mais rápida e retorno dos animais à produção, minimizando o impacto econômico que acarreta a bacia leiteira do estado de Pernambuco.

Palavras-chave: *Anaplasma marginale*; anemia regenerativa; epidemiologia; hemoparasitose; prontuários clínicos.

Introduction

Anaplasmosis is considered an important disease in cattle, that causes hemolytic anemia, production losses, spontaneous abortion, and death, generating significant economic losses to livestock, and being considered endemic in countries with tropical and subtropical climates (Kocan et al., 2010; Ferreira et al., 2022).

The disease agent, *Anaplasma marginale*, is an obligate intracellular Gram-negative bacterium. Transmission is carried out by ticks, *Rhipicephalus microplus*, by hematophagous fly bites, and by iatrogenic routes. Studies have shown that biological transmission by ticks is more efficient than mechanical transmission, but in herds where arthropods are absent, flies act as the main form of dissemination (Kocan et al., 2010). Transplacental transmission can also occur (Brito et al., 2019). Taurine cattle are more susceptible than zebu cattle, as well as demonstrating greater resistance to the vector tick (Costa et al., 2011). Several factors influence the dynamics of the infection, including the virulence of the strain, the amount of inoculum, the population and transmission capacity of the vectors, and the susceptibility of the animals (Kocan et al., 2010).

Cattle of all age groups are susceptible to anaplasmosis, however, there is a higher prevalence in older animals, which may result in more severe clinical cases (Costa et al., 2011; Atif, 2015). The movement of animals from tick-free regions to endemic regions constitutes a risk factor for the development of the infection (Atif, 2015).

The intensity of the clinical signs can vary and progress with marked pallor of the mucous membranes associated with jaundice, fever, tachycardia, tachypnea, decreased appetite, reduced lactation, and spontaneous abortion in pregnant cows (Constable et al., 2017). The main hematological alterations are verified in the erythrogram (Lima et al., 2019), in which regenerative anemia resulting from extravascular hemolysis is found, and some indicators of bone marrow response can be observed in the blood smear (Jain, 1993; Tvedten, 2010).

Considering the relevance of the socioeconomic role of cattle farming for the economy of Pernambuco, mainly for the Southern Agreste Microregion, combined with the impact that bovine anaplasmosis represents on the herd of the state's dairy basin, the current study aimed to carry out a retrospective hospital-based study of clinical cases of anaplasmosis diagnosed in the period from 2012 to 2021, highlighting the main epidemiological, clinical, hematological, and therapeutic aspects of the disease.

Material and Methods

The retrospective study of clinical cases of anaplasmosis was carried out from the descriptive analysis of the medical records of the Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE) referring to the period from January 2012 to December 2021, totaling 10 years. Only hospital care data were included in this study, excluding field clinical care carried out on the farms.

As recommended, all animals underwent clinical (Dirksen et al., 1993) and hematological examinations (Tvedten and Weiss, 2000) at the time of hospital care. Information was recorded (anamnesis, physical and laboratory examinations) in the clinical record, following the same reporting protocol, established by the Institution.

Initially, the screening of clinical cases was carried out by consulting the register book, to identify the cases of anaplasmosis diagnosed between January 2012 and December 2021. Subsequently, the clinical records were accessed for the collection of information and a database was created using Microsoft Excel® software, version 2019. In total, 230 clinical cases of bovines diagnosed with anaplasmosis were analyzed, using the presence of the agent *A. marginale* in the blood smear as an inclusion criterion in all cases (WOAH 2018), associated with clinical findings and the detection of anemia (hematocrit ≤ 24%) with different degrees (Jain, 1993; Tvedten and Weiss, 2000). All cases of anaplasmosis that co-occurred with other concomitant diseases were excluded from this study.

With respect to the epidemiological data, information was recorded regarding origin (municipality and state), sex, breed, age group (≤ 2 months; > 2 to 6 months; > 6 to 24 months; > 24 months), type of exploitation, presence of ticks/flies, and rearing system. For the seasonality study, the period from September to March was

considered the dry period, with an average monthly rainfall below 75 mm, and the period from April to August as the rainy period, with above 75 mm. This criterion was defined based on the assessment of rainfall over the 10 years studied, using the National Institute of Meteorology (INMET) database.

Regarding clinical data, information was obtained regarding appetite, mucosa color, rectal temperature, degree of dehydration, respiratory rate, heart rate, and the occurrence of spontaneous abortions. As concerns to hematological parameters, information was collected of the complete blood count, plasma protein and fibrinogen, and the peripheral blood smear analysis, the latter also having the objective of evaluating the parasitemia (Kessler and Schenk, 1998) and characterizing the bone marrow response. In order to assess the degree of severity of the anemia, the classification based on the hematocrit value was adopted as mild (between 20% and 24%), moderate (between 14% and 19%), severe (between 10% and 13 %), and very severe (values below 10%) according to Tvedten and Weiss (2000). With respect to the therapy performed at the institution, information was obtained regarding commonly used antimicrobials, long-acting oxytetracycline - LA (20mg/kg- IM) and enrofloxacin (7.5 mg/kg-SC), and the use of hematopoiesis promoters (Hemolitan® and Polivin B_{12} oral), as well as whether a blood transfusion was carried out (Reichmann and Dearo, 2001). A blood transfusion is indicated in cases in which moderate, severe, or very severe anemia is observed, associated with the severity of the clinical condition. Ruminal fluid was used with the aim of improving the digestive dynamics in animals with motility impairment.

For the analysis of epidemiological, clinical, and laboratory information, a descriptive statistical model was used, and the distribution of data was observed through relative and absolute frequencies in the case of categorical variables. For numerical variables, the distribution took place through measures of central tendency (median) (Sampaio, 2007).

Results

During the studied period, a total of 6275 cattle were hospitalized and, of these, 439 (7.0%) were diagnosed with hemoparasitosis [*A. marginale, Babesia* spp. and Bovine Parasitic Sadness (BPS) Complex]. *A. marginale* was responsible for infecting 351 animals, which represented 79.9% of clinical cases. Of the cases of anaplasmosis diagnosed in the period (n=351), 230 clinical cases were selected for this study, excluding all cases of anaplasmosis that co-occurred with other intercurrent infections.

Epidemiological data – A growing number of clinical cases of anaplasmosis was observed over the 10 years studied, particularly from 2017 onwards, with a significant increase in the years 2020 and 2021. It is noteworthy that in the year 2021 the number of hospitalized cattle at CBG/UFRPE also increased, however, the number of cases of the disease in relation to the number hospitalized cattle per year was also higher, showing a magnitude in percentage points of almost eight times when compared to the first year studied (Figure 1).

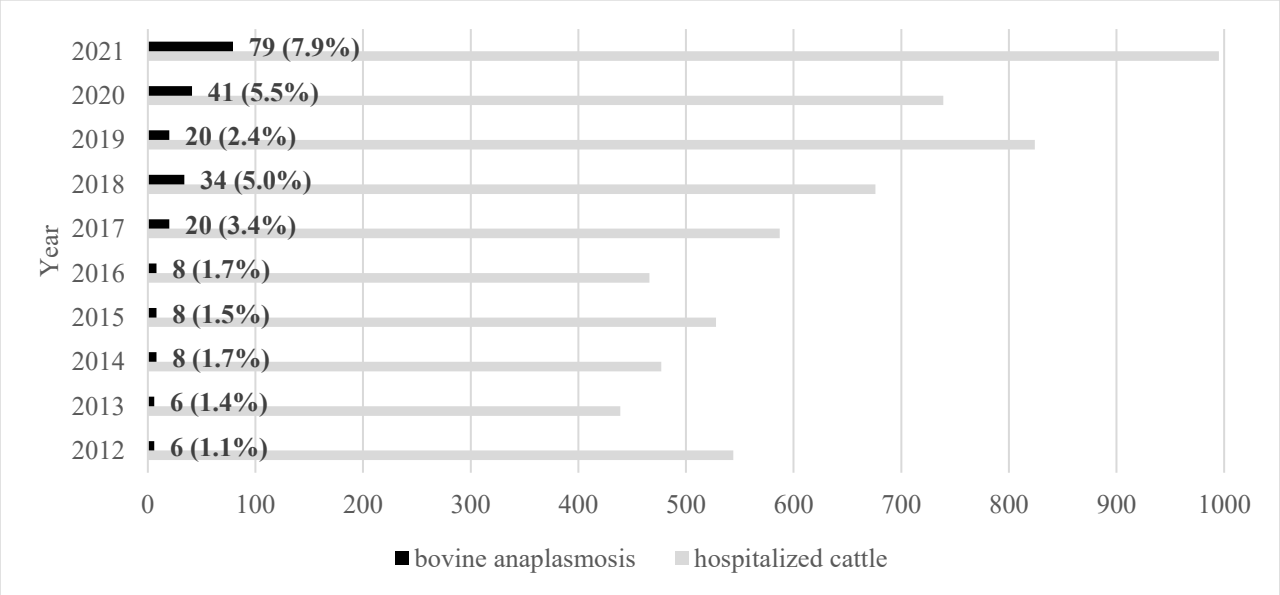

Figure 1. Absolute (n) and relative (%) frequency of diagnosed cases of anaplasmosis in relation to the number of cattle hospitalized at Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE) in the period from 2012 to 2021.

Considering the origin of the animals, 97.8% were from the state of Pernambuco (225/230), from 24 municipalities, mainly the Southern Agreste Microregion (Figure 2), where the dairy basin of the state is concentrated. The other animals (5/230) came from three municipalities from the state of Alagoas. With respect to the property rearing system, 86.5% adopted the intensive and semi-intensive system. Holstein, Girolando, and mixed breed animals were the most affected with the disease (90.9%), when compared to other breeds (Brown Swiss, Jersey, Gir, and Nelore).

A higher occurrence of the disease was observed in adult cattle (≥ 24 months) (n=143), when compared to calves aged less than six months (n=32). However, the percentage of deaths was higher in calves aged less than two months (50%) (Figure 3), highlighting the death of a calf with only two days of age.

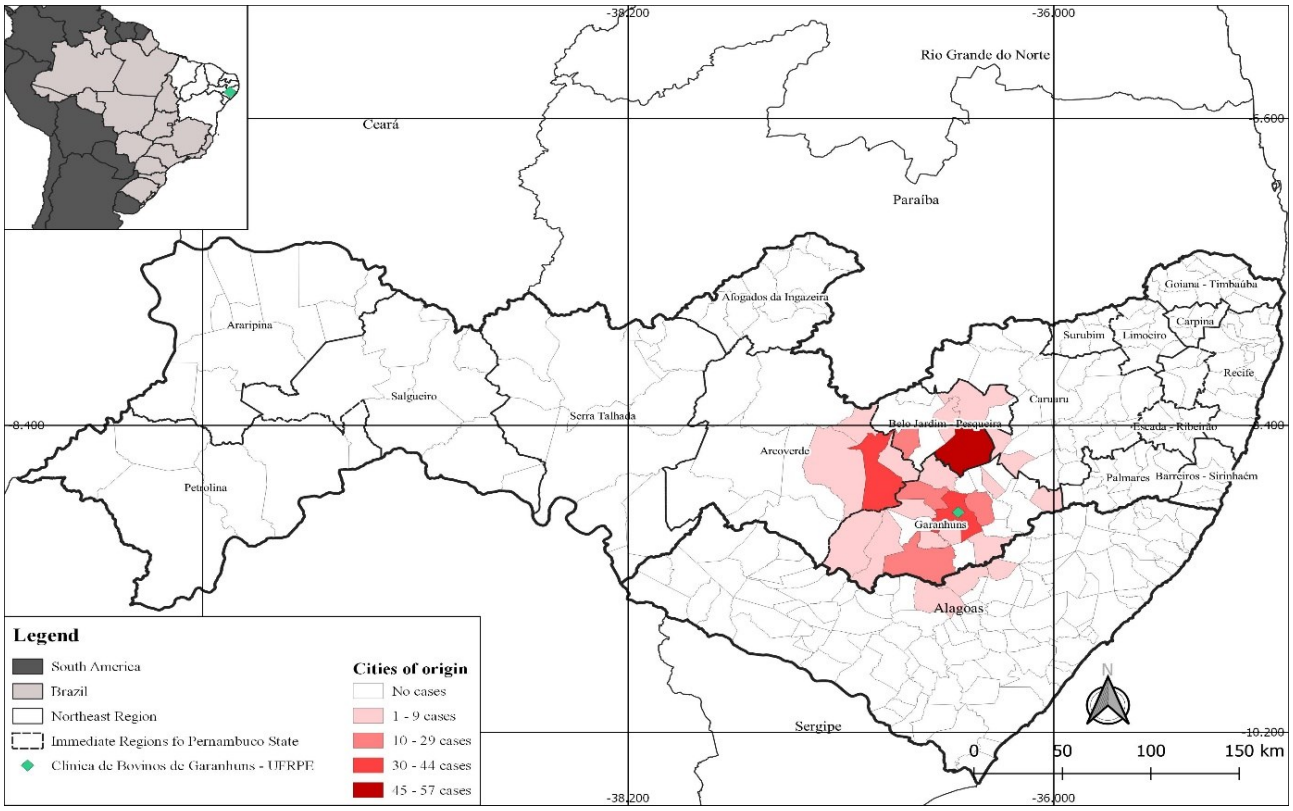

Figure 2. Spatial distribution of the municipalities of origin of cattle diagnosed with anaplasmosis at Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE), in the period from 2012 to 2021.

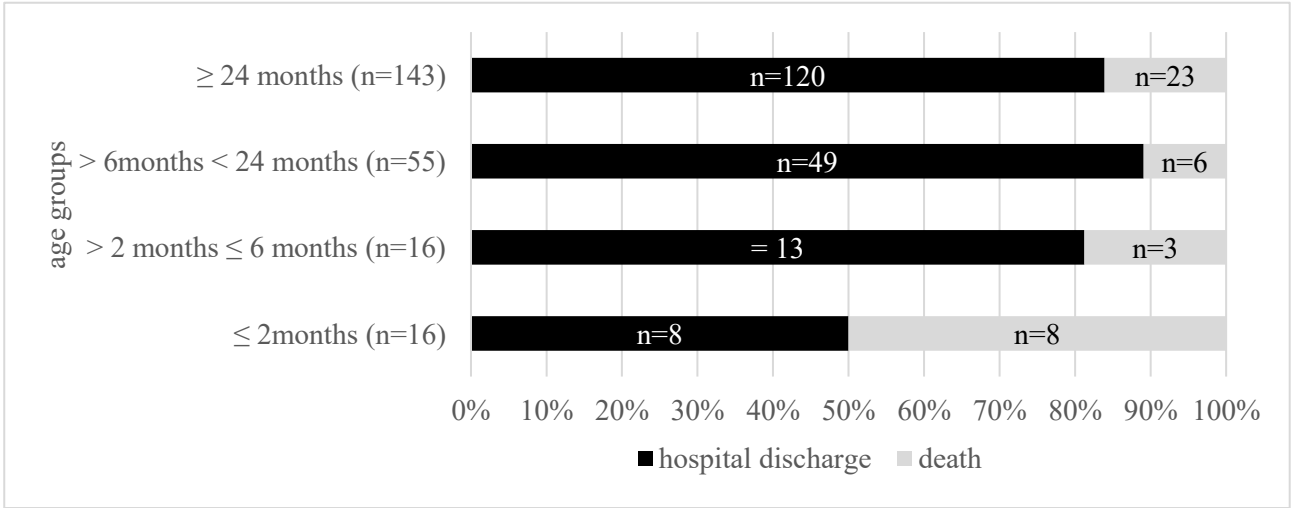

Figure 3. Relative (%) and absolute (n) frequency of clinical resolution of cattle with anaplasmosis diagnosed at Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE) from 2012 to 2021 in different age groups (n=230).

Of the animals aged ≥ 24 months affected with anaplasmosis, 115 were females and of these 67% (77/115) were lactating and 34.0% (39/115) were pregnant. The presence of the vector (ticks and/or flies) was observed on 56.5% of the animals.

With regard to seasonality, the highest occurrence of cases over the course of the ten years of the study was observed in the months of August to December (Figure 4), the phase in which the transition from the rainy period ("winter") to the dry period ("summer") begins in the region.

Clinical findings – Table 1 presents the relative and absolute frequency of the main clinical findings, highlighting apathy, impaired appetite, pale/jaundiced mucous membranes, and tachycardia. Considering cases of spontaneous abortion of pregnant females with anaplasmosis, 12.8% (5/39) aborted at different stages of pregnancy. Clinical resolution with hospital discharge occurred in 82.6% (190/230) of clinical cases, with a mean hospital stay of approximately eight days.

Hematological findings – Table 2 presents the hematological variables. Regarding the degree of anemia, 41.3% (95/230) of the cattle had severe anemia, 32.6% (75/230) very severe, 20.9% (48/230) moderate, and 5.2% (12/230) mild. In the evaluation of the blood smear, moderate to high rickettsemia was verified in the large majority of the animals. The regenerative response of the bone marrow was evidenced in 94.8% (218/230) of the cases, with Howell-Jolly bodies, anisocytosis/ macrocytosis, polychromasia, nucleated red blood cells, and basophilic stippling being observed in the blood smear. It was observed that the severity of anemia was associated with greater intensity of bone marrow response (Table 3). Leukogram values revealed leukocytosis by lymphocytosis accompanied by neutrophilia and a mild regenerative left shift (Table 2).

Drug therapy was administered to 93.0% of the cattle (214/230), and the remainder died even before any therapeutic intervention due to the acute clinical framework of the disease or the owner's interest in treating them on the farm. Among the drugs used, oxytetracycline LA was administered in 80.4% of the animals (172/214), enrofloxacin in 12.6% (27/214), and both in 7% (15/214). In the latter situation, it became necessary to change the pharmacological basis when one of the drugs was not effective in reducing rickettsemia. As for the clinical resolution, 86.6% (149/172), 96.3% (26/27), and 80.0% (12/15) of the animals treated with oxytetracycline, enrofloxacin, and both, respectively, were discharged from the hospital (Figure 5). The protocol therapy using oxytetracycline LA in three doses was satisfactory, requiring four doses to be administered in 7.55% (13/172) of the cases in which the parasitemia was persistent. A blood transfusion was indicated in 85.9% (164/191) of cattle with greater clinical and hematological impairment (VG ≤ 15%), resulting in clinical recovery in 82.3% (135/164) of cases (Figure 6). It should be noted that of these 164 animals, 32 (19.5%) required an additional transfusion.

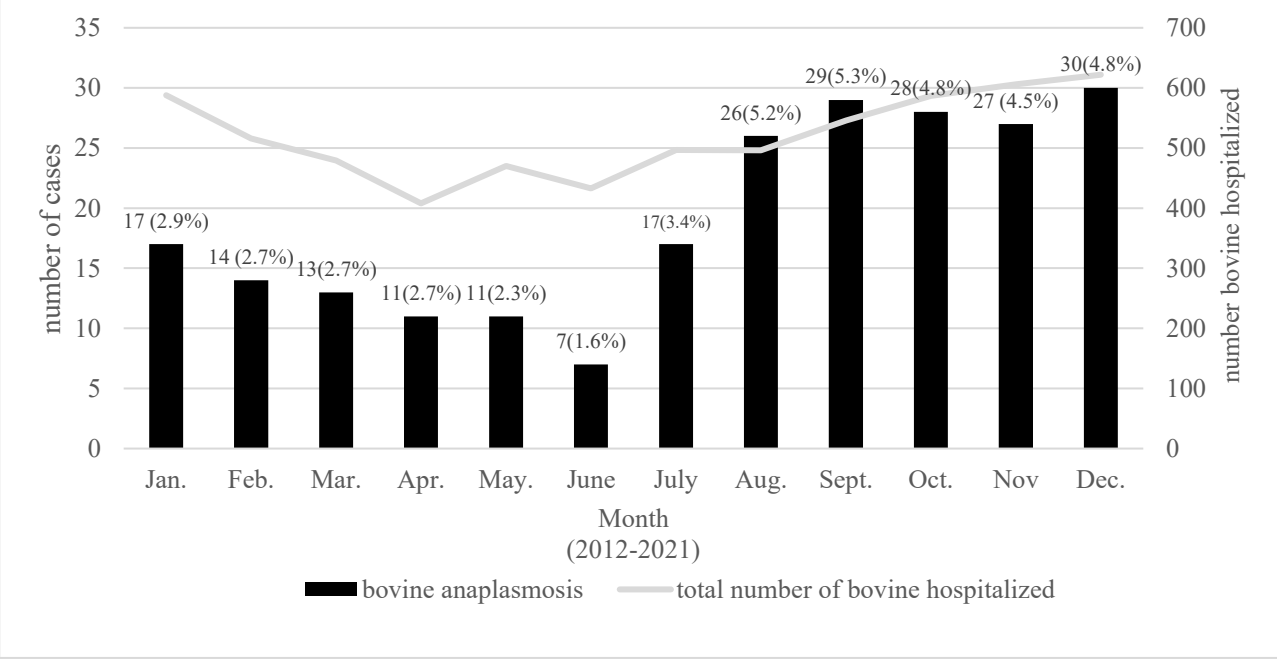

Figure 4. Relative (%) and absolute (n) frequency of clinical cases of bovine anaplasmosis at Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE), in relation to the number of cattle diagnosed in the respective months of the year in the period from 2012 to 2021.

Table 1. Relative (%) and absolute (n) frequency of the main clinical findings of cattle diagnosed with anaplasmosis at Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE) in the period from 2012 to 2021.

Clinical findings	N	Variables	Number of animals	
			Absolute value (n)	Relative value (%)
Appetite	218	Absent	92	42.20
		Reduced	40	18.34
		Present	86	39.44
Rectal temperature	230	≥ 39°C	111	48.3
		< 39°C	119	51.7
Degree of dehydration	223	No change	55	24.66
		Mild (6%)	122	54.70
		Moderate (8%)	40	17.93
		Severe (10%)	6	2.69
Mucosa color	230	No change	69	30.00
		Jaundice	106	46.08
		Pale	50	21.73
		Porcelain white	5	2.17
Respiratory frequency (breaths/min)	229	Bradypnea (< 24)	20	8.73
		No change (24-36)	106	46.28
		Tachypnea (> 36)	103	44.97
Heart rate (beats/min)	230	Bradycardia (< 60)	1	0.43
		No change (60-80)	27	11.78
		Tachycardia (> 80)	202	87.82
Spontaneous abortion	39	No	34	87.2
		Yes	5	12.8

Table 2. Median and interquartile range of hematological variables of cattle diagnosed with anaplasmosis (n=230) at Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE) in the period from 2012 to 2021.

Variables	Q_1[a]	Median	Q_3[b]	Reference value[c]
Erythrocytes (x10^6 /µL)	1.6	2.1	3.2	5.0-10.0
Hematocrit (%)	9	11	15	24-46
Hemoglobin (g/dL)	2.9	3.6	4.9	8-15
MCV (ƑL)	44	53.6	62	40-60
MCHC (%)	29	31.5	34	30-36
Total leukocytes (/µL)	10075	14350	19000	4000-12000
Neutrophil (band) (/µL)	0	141	318	0-120
Neutrophil (segmented) (/µL)	3088	5236	7275	600-4000
Lymphocyte (/µL)	5456	8125	8125	2500-7500
Monocyte (/µL)	0	118	226	25-840
Eosinophil (/µL)	0	0	320	0-2400
Basophil (/µL)	0	0	0	0-200
Plasma Protein (g/dL)	6.8	7.3	7.8	7.0-8.5
Fibrinogen (mg/dL)	400	600	700	300-700

[a] first quartile; [b] third quartile; [c] Kramer (2010)

Discussion

Bovine anaplasmosis was the most frequent hemoparasitosis diagnosed at CBG/UFRPE in the 10-year period that comprised the study. Gonçalves et al. (2011) and Almeida et al. (2006) in retrospective studies in the states of São Paulo and Rio Grande do Sul, identified lower occurrences of the disease, of 31.5% and 29.4%, respectively. The high occurrence is probably due to the endemic nature of the disease in some the northeastern region, where the climatic characteristics allow the constant occurrence of ticks due to the higher moisture content in the region (Furlong et al., 2005; Ferreira et al., 2022).

The lower occurrence of cases from 2012 to 2016 may have been influenced by the intense drought that affected the state of Pernambuco in 2012. Marengo et al. (2016) reported that although the impact of the dry period resulting from the 2012 drought diminished in subsequent years, the *El Niño* event in 2015 caused a rainfall deficit. The increasing number of cases from 2017 may be associated with the greater volume of rainfall compared to previous years, which, together with mild temperatures, favored the tick cycle, consequently the transmission of the disease, combined with the possibility of a long period without contact with the tick compromised the immune response (Costa et al., 2011).

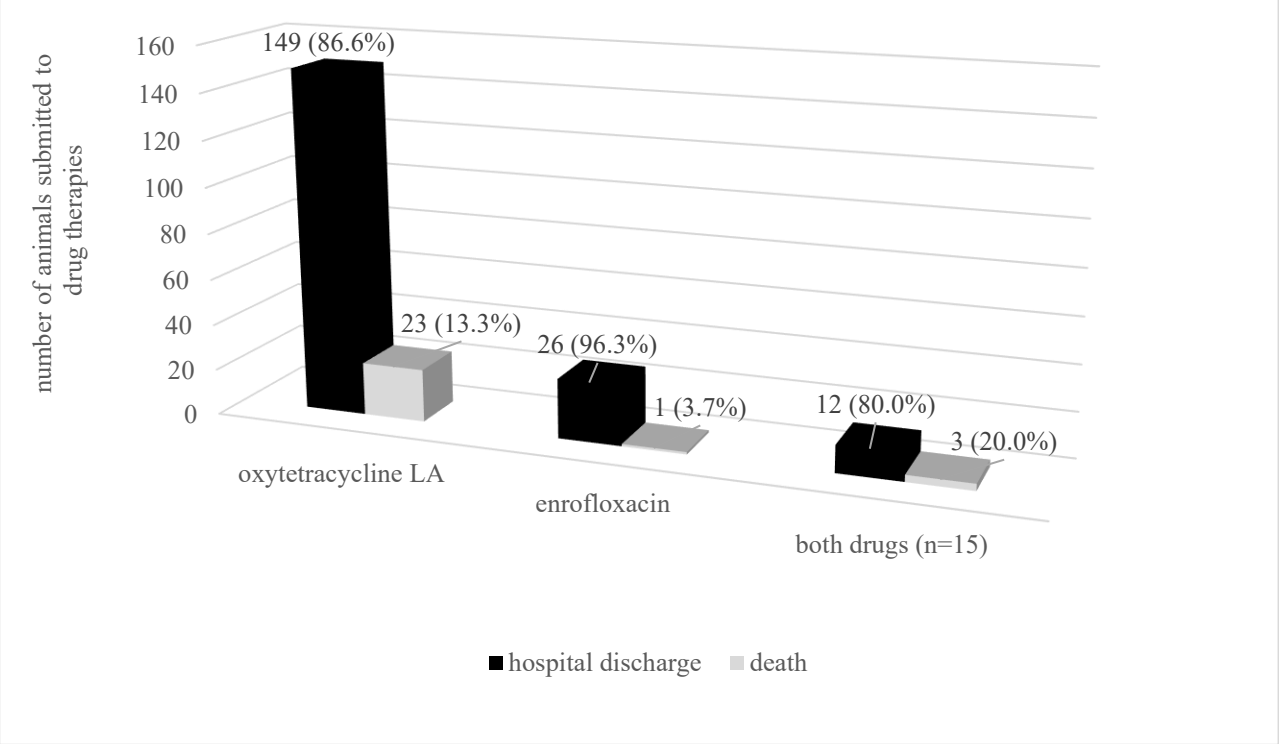

Figure 5. Relative (%) and absolute (n) frequency of clinical resolution of cattle with anaplasmosis diagnosed at Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE) in the period from 2012 to 2021 submitted to different drug therapies (n=214).

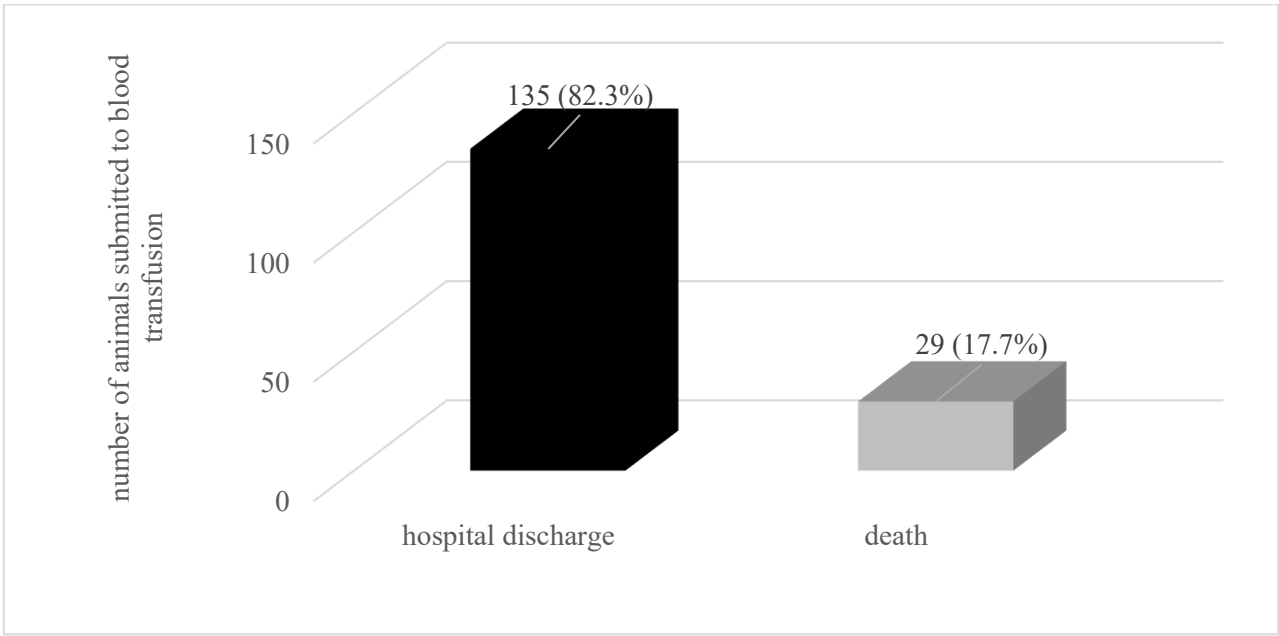

Figure 6. Relative (%) and absolute (n) frequency of clinical resolution of cattle with anaplasmosis diagnosed at Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE) in the period from 2012 to 2021 submitted to blood transfusion (n=164).

Table 3. Relative (%) and absolute (n) frequency of some findings, indicators of bone marrow response, observed in blood smears of cattle with different degrees of anemia (n=230) diagnosed with anaplasmosis at Bovine Clinic of Garanhuns, Federal Rural University of Pernambuco (CBG/UFRPE) in the period from 2012 to 2021.

Erythrocyte findings	Degree of anemia			
	Mild (n=12)	Moderate (n=48)	Severe (n=95)	Very Severe (n=75)
Howell-Jolly bodies	66.7% (8/12)	89.6% (43/48)	91.6% (87/95)	98.7% (74/75)
Anisocytosis/Macrocytosis	33.3% (4/12)	70.8% (34/48)	95.8% (91/95)	97.3% (73/75)
Polychromasia	76.71% (2/12)	50% (24/48)	88.4% (84/95)	92.0% (69/75)
Basophilic stippling	16.7% (2/12)	33.3% (16/48)	73.7% (70/95)	78.7% (59/75)
Nucleated red blood cells (erythroblasts/metarubricytes)	16.7% (2/12)	43.7% (21/48)	78.9% (75/95)	84% (63/75)

Cattle of the Holstein, Girolando, and mixed breeds, predominant in the region, were the most affected, confirming that the European lineage and its crosses are more sensitive to ticks and, consequently, to anaplasmosis (Costa et al., 2011).

Cattle aged over two years were the most affected in relation to other age groups, similar to what was reported by Santos et al. (2017) and Constable et al. (2017). The greater susceptibility of adults is commonly related to compromised immunity, due to poor prior contact with the agent, poor nutritional status, stress, and pregnancy, which may result in more severe clinical cases (Costa et al., 2011; Santos et al., 2017). The practice of selling cattle during the dry season in semi-arid regions to areas with a greater supply of food and rainfall, which is common in the region (Santos et al., 2017; Silva de Jesus, 2019), may have contributed to the number of infected adult cattle.

In this study, mortality in calves less than two months old was higher than in adult cattle, contradicting the literature, which highlights that mortality is higher in older animals. (Hungerford and Smith, 1997). Management of colostrum feeding can result in failed transfer of passive immunity, leading to higher calf mortality (Rebhum, 1995).

The predominance of affected females can be explained by the characteristics of the region, whose main economic activity is focused on milk production, confirming the economic impact of the disease, where 67% of females aged ≥ 24 months were in the lactation phase and 30.5% were pregnant. The immunosuppression observed during pregnancy is one of the reasons that explain the greater susceptibility to infections and reinfection processes, which favor the vertical transmission of the agent (Brito et al., 2019). In this study, a two-day-old calf died, suggesting transplacental transmission was responsible for this outcome. Silvestre et al. (2016) verified transplacental transmission in 10% of calves born to chronically infected cows.

The percentage of 12.8% of cows that were pregnant aborted at between three and eight months of pregnancy. There are few data available on spontaneous abortion resulting from anaplasmosis in herds, supposedly due to failure in observation associated with lack of zootechnical control and difficulty in determining the etiology. Abortion is reported as one of the main reproductive consequences in cows with anaplasmosis (Constable et al. 2017), highlighting the impairment of the disease on herd productivity. For Henker et al. (2020), anaplasmosis should be considered as a differential diagnosis in cases of pregnancy loss and neonatal death in cattle, also highlighting its importance in transplacental infections.

Bovine anaplasmosis showed a seasonal influence, with a greater number of cases in the months of August to December, which corresponds in the region to the transition from the rainy period ("winter") to the dry period ("summer"). This can be explained by the favorable conditions at this time of year for the transmission of the agent by the vectors, due to the temperature and humidity at this time, which favor the acceleration of the biological cycle and increase in the tick population compared to previous months (Furlong et al., 2005). Similarly, Costa et al. (2011), also in the Northeast

region, observed a concentration of bovine parasitic sadness outbreaks at the end of the rainy season and the beginning of the dry season.

The clinical findings of the disease were similar to those described in the literature (Constable et al., 2017). Appetite impairment was present in most of the animals, and should be taken into account in the early identification of the disease, as it is possibly the primary finding that is noticeable to the farmer. It is noteworthy that the color of the mucous membranes did not always reflect the degree of anemia, as sometimes pink mucous membranes were observed in anemic animals, and hematocrit testing is recommended whenever possible. These findings corroborate Coelho (2007) who reported a low correlation between the color of the mucous membranes and the hematocrit value, indicating the failure of colors mucous of evaluation to identify anemia.

The absence of fever in some animals may be related to the fact that some of them were medicated on the farm with drugs commonly used in the treatment of anaplasmosis. Treatment with these drugs reduces rickettsemia, consequently decreasing the levels of antigenic stimulus, necessary for the synthesis of endogenous pyrogens that result in fever (Garcia-Zapata and Souza Júnior, 2006). In the current study, most animals presented a physiological to mild degree of dehydration, in agreement with values within the normal range of plasma protein (Jain, 1993).

The erythrogram, despite demonstrating severe anemia, revealed normocytic normochromic anemia. According to Jain (1993), the analysis of the bovine blood smear assumes importance in the characterization of regenerative anemia, indicative of the response of the bone marrow, when compared to the hematimetric indices. Some findings, such as polychromasia, macrocytosis, basophilic stippling, Howell-Jolly bodies, and nucleated red blood cells are observed with more severe anemia. These findings confirm that the evaluation of the blood smear is fundamental in the classification of hemolytic anemias, such as anaplasmosis, in species such as cattle, without consistent reticulocyte responses (Tvedten, 2010). Swenson and Jacobs (1986) and Alvim et al. (2019) also reported a regenerative response to anemia in two Angus cows and a Jersey heifer, respectively, diagnosed with anaplasmosis.

Lymphocytic leukocytosis accompanied by neutrophilia was also reported by Singh et al. (2014), although the leukogram varies in animals with anaplasmosis (Ferreira et al., 2013; Alvim et al., 2019). Hyperfibrinogenemia, even to a lesser extent when compared to other acute phase proteins, as reported by Nazifi et al. (2012) in cases of bovine anaplasmosis, was not observed in the vast majority of animals (89%) in this study, with plasma fibrinogen remaining within normal values (Jain, 1993), probably due to the lower sensitivity of this acute phase protein in inflammatory processes in cattle infected with this agent (Nazifi et al., 2012).

The therapeutic approach used in most cases in the current study was oxytetracycline LA, adopting the protocol of three doses with an interval of 72 hours (Wilkinson, 2005) however, it is worth mentioning that enrofloxacin for the treatment of anaplasmosis started to be used in institution from 2015. This therapeutic protocol was satisfactory, resulting in the discharge from hospital of 87.4% of the treated animals. This response was probably due to the number of doses and the concentration administered, as reported by Facury-Filho et al. (2012) who emphasized that the success of the treatment depends on the time and concentration of the drug, which must be above the minimum inhibitory concentration (MIC). These same authors found that the use of enrofloxacin proved to be more effective in terms of reducing the parasitemia and recovering the hematocrit, when compared to oxytetracycline. Kaartinen et al. (1997) attributed this faster enrofloxacin response to the high plasma concentration achieved in a short period of time.

In 6.5% of the animals, it was necessary to change the pharmacological basis, as it was not efficient in reducing parasitemia, as also occurred in 7.5% of the cattle in which oxytetracycline LA was used, which required the administration of four doses. These responses are probably of concern due to the greater virulence and/or resistance of strains that do not respond to the recommended treatment as expected. Shahbazi et al. (2021) recently identified resistance genes (otrA and otrB) to oxytetracycline in strains of *Anaplasma* spp.

A blood transfusion was essential as supportive therapy, resulting in high recovery rates for patients. This is an emergency measure that is recommended in cases of hemolytic disease, even if the transfused red blood cells have a short half-life of 2-4 days (Basile and Barca Júnior, 2000; Reichmann and Dearo, 2001). In the current study, blood transfusions were performed in a crucial period, in which the drugs had not yet reached the MIC, with the continuation of agent multiplication and consequent hemolysis occurring (Facury-Filho et al., 2012).

The performance of another blood transfusion, necessary in 19.5% of cattle with severe to very severe anemia, was probably due to the volume of blood administered not being sufficient to compensate for the anemia, which may

have been aggravated by the poor clinical condition of the patient. The hematopoiesis promoters employed, as recommended by the manufacturers, were used daily to aid in hematocrit recovery, providing an adequate supply of vitamins and minerals, as also reported by Coelho (2007), as well as the administration of rumen fluid, which added to the supportive therapy.

The average period of hospitalization of the animals with anaplasmosis was eight days, regardless of clinical resolution. Gonçalves et al. (2011) found that cattle diagnosed with BPS were hospitalized for an average of four days. The greater number of days of hospitalization may be associated with more intense clinical conditions, since most of the animals had severe to very severe anemia, which requires a longer period for recovery of hematocrit and clinical condition, and these criteria are adopted together with a negative blood smear parasite for determination of hospital discharge.

Conclusion

- Anaplasmosis was the most frequent hemoparasitosis diagnosed at CBG/UFRPE between the years 2012 to 2021, affecting mainly cows in the production stage;

- Among the main clinical findings, apathy, impaired appetite, icteric mucosal coloration, and tachycardia stand out;

- The evaluation of blood smears in cattle with anaplasmosis is essential in the characterization of regenerative anemia, indicative of bone marrow response, marked by the presence of erythrocyte indicators of active erythropoiesis in the peripheral blood, when the degree of anemia is more severe;

- Blood transfusion is vital as a supportive therapy associated with drug therapy for the satisfactory resolution of severe cases of anemia from anaplasmosis;

- The adoption of guiding measures for farmers in the region, especially at the end of the rainy season before the dry season, are relevant so that the diagnosis and treatment of anaplasmosis are carried out as early as possible, allowing the animal to recover more quickly, and therefore return to production, thus minimizing the economic impact that this disease causes to the dairy basin in the state of Pernambuco.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgements

To the Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE) for granting a Scholarship (Process IBPG1012-5.05/21). We would like to thank the technical staff and residents of the Clínica de Bovinos de Garanhuns/UFRPE for their care and discretion in recording information in the clinical records, making this work possible.

References

Almeida, M.B.D.; Tortelli, F.P.; Riet-Correa, B.; Ferreira, J.L.M.; Soares, M.P.; Farias, N.A.; Riet-Correa, F.; Schild, A.L. Tristeza parasitária bovina na região sul do Rio Grande do Sul: estudo retrospectivo de 1978-2005. **Pesquisa Veterinária Brasileira**, 26(4): 237-242, 2006.

Alvim, G.R.; Costa, L.G.; Deschk, M.; Silva-Neto, A.F.; Araujo, R.F.; Franciscato, C. Basophilic stippling and reactive lymphocytosis in bovine anaplasmosis – A case report. **Revista Brasileira de Higiene e Sanidade Animal**, 13(1): 98-105, 2019.

Atif, F.A. *Anaplasma marginale* and *Anaplasma phagocytophilum*: *Rickettsiales* pathogens of veterinary and public health significance. **Parasitology Research**, 114(11): 3941-3957, 2015.

Basile, J.R.; Barca Júnior, F.A. Os grupos sanguíneos e a transfusão de sangue nos bovinos. **Revista Ciências Biológicas e da Saúde**, 2(1): 161-170, 2000.

Brito, L.G.; Barbieri, F.S.; Ferreira, T.A.A.; Carneiro, D.S.; Amaral, T.M.; Figueiró, M.R.; Oliveira, M.C.S. Transmissão congênita de *Babesia bovis* e *Anaplasma marginale* na epidemiologia da tristeza parasitária bovina. **Embrapa Amazônia Oriental, Circular Técnica**, 48: 1-18, 2019.

Coelho, L.C.T. **Anaplasmose bovina: parâmetros clínicos e de patologia clínica em bezerros infectados experimentalmente**. Dissertação (Mestrado em Medicina Veterinária) – Universidade Federal de Minas Gerais, Belo Horizonte, 2007. 65p.

Constable, P.D.; Hinchcliff, K.W.; Done, S.H.; Grünberg, W. **Veterinary Medicine: a textbook of the diseases of cattle, horses, sheep, pigs, and goats.** 11th ed. Missouri: Saunders Elsevier, 2017. 1091p.

Costa, V.M.M.; Rodrigues, A.L.; Medeiros, J.M.A.; Labruna, M.B.; Simões, S.V.D.; Riet-Correa, F. Tristeza parasitária bovina no Sertão da Paraíba. **Pesquisa Veterinária Brasileira**, 31(3): 239-243, 2011.

Dirksen, G.; Gründer, H. D.; Stöber, M. **Rosenberger: exame clínico dos bovinos**. 3rd

ed. Rio de Janeiro: Guanabara Koogan, 1993. 448p.

Facury-Filho, E.J.; Carvalho, A.Ú.; Ferreira, P.M.; Moura, M.F.; Apolinário, B.C.; Santos, L.P.H.; Ribeiro, M.F.B. Effectiveness of enrofloxacin for the treatment of experimentally-induced bovine anaplasmosis. **Revista Brasileira de Parasitologia Veterinária**, 21(1): 32-36, 2012.

Ferreira, M.R.A.; Arantes, T.Q.; Silva, M.D.; Dias, M.; Moreira, C.N. Influências de infecções por *Anaplasma marginale* no leucograma e no metabolismo oxidativo dos neutrófilos em bezerros da raça Girolando. **Enciclopédia Biosfera**, 9(17): 1744-1752, 2013.

Ferreira, G.C.M.; Canozzi, M.E.A.; Peripolli, V.; Moura, G.P.; Sánchez, J.; Martins, C.E.N. Prevalence of bovine *Babesia* spp., *Anaplasma marginale*, and their co-infections in Latin America: systematic review-meta-analysis. **Ticks and Tick-borne Diseases**, 13(4): 101967, 2022.

Furlong, J.; Martins, J.R.S.; Prata, M.C.A. **Carrapato: problemas e soluções**. 1st ed. Juiz de Fora: Embrapa Gado de Leite, 2005. 65p.

Garcia-Zapata, M.T.A.; Souza Júnior, E.S. Aspectos fisiopatológicos da febre nas doenças infecto-parasitárias. **Universitas: Ciências da Saúde**, 4(1): 111-117, 2006.

Gonçalves, R.C.; Silva, A.A.; Ferreira, D.O.L.; Chiacchio, S.B.; Lopes, R.S., Borges, A.S.; Amorim, R.M. Tristeza Parasitária em bovinos na região de Botucatu–SP: estudo retrospectivo de 1986–2007. **Semina: Ciências Agrárias**, 32(1): 307-312, 2011.

Henker, L.C.; Lorenzett, M.P.; Fagundes-Moreira, R.; Dalto, A.G.C., Sonne, L.; Driemeier, D.; Soares, J.F.; Pavarini, S.P. Bovine abortion, stillbirth and neonatal death associated with *Babesia bovis* and *Anaplasma* sp. infections in southern Brazil. **Ticks and Tick-borne Diseases**, 11(4): 101443, 2020.

Hungerford, L.L., Smith, R.D. Variations in seroprevalence and host factors for bovine anaplasmosis in Illinois. **Veterinary Research Community**, 21(1): 9-18, 1997.

Jain, N.C. **Essentials of veterinary hematology**. 1st ed. Philadelphia: Lea & Febger, 1993. 420p.

Kaartinen, L.; Pyorala, S.; Moilanen, M.; Raisanen, S. Pharmacokinetics of enrofloxacin in newborn and one-week-old calves. **Journal of Veterinary Pharmacology and Therapeutics**, 20(6): 479-482, 1997.

Kramer, J. W. Normal hematology of cattle, sheep, and goats. In: Feldman, B.F.F. Zinkl, J.G. Jain, N.C. **Schalm's veterinary hematology**. 5th ed. Philadelphia: Williams & Wilkins, 2000. p.1075-1074.

Kessler, R.H.; Schenk, M.A.M. **Carrapato, tristeza parasitária e tripanossomose dos bovinos**. Campo Grande: EMBRAPA, 1998. 157p.

Kocan, K.M.; De La Fuente, J.; Blouin, E.F.; Coetzee, J.F.; Ewing, S.A. The natural history of *Anaplasma marginale*. **Veterinary Parasitology**, 167(2): 95-107, 2010.

Lima, D.H.S.; Vinhote, W.M.S.; Ubiali, D.G.; Soares, P.C.; Cordeiro, M.D.; Silva, J.B.; Fonseca, A.H.; Barbosa, J.D. Experimental infection by *Anaplasma marginale* in buffaloes and cattle: clinical, hematological, molecular and pathological aspects. **Pesquisa Veterinária Brasileira**, 39(9): 700-709, 2019.

Marengo, J.A.; Cunha, A.P.; Alves, L.M. A seca de 2012-15 no semiárido do Nordeste do Brasil no contexto histórico. **Revista Climanálise**, 3(1): 49-54, 2016.

Nazifi, S.; Razavi, S.M.; Kaviani, F.; Rakhshandehroo, E. Acute phase response in cattle infected with *Anaplasma marginale*. **Veterinary Microbiology**, 155(2-4), 267-271, 2012.

WOAH. World Organization for Animal Health. Bovine anaplasmosis. In: **Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**. 2018. p. 999-1013.

Reichmann, P.; Dearo, A.C.O. Blood and blood component transfusion in large animals. **Semina Ciências Agrárias**, 22(2): 223-228, 2001.

Rebhun, W.C. **Diseases of dairy cattle**. Baltimore: Williams & Wilkins, 1995. 530p.

Sampaio, I.B.M. **Estatística aplicada à experimentação animal**. 3rd ed. Belo Horizonte: FEP-MVZ Editora, 2007. 265p.

Santos, G.B.; Gomes, I.M.; Silveira, J.A.; Pires, L.C.; Azevedo, S.S.; Antonelli, A.C.; Horta, M.C. Tristeza Parasitária em bovinos do semiárido pernambucano. **Pesquisa Veterinária Brasileira**, 37(1): 1-7, 2017.

Shahbazi, P.; Gharajalar, S.N.; Mohebbi, K.; Taeb, J.; Farhang, H.H.; Nikvand, A.A.; Norouzi, R. First survey on the presence and distribution of oxytetracycline-resistance genes in *Anaplasma* species. **Acta Parasitologica**, 66: 501-507, 2021.

Silva de Jesus, R. **Surtos de anaplasmose bovina na Bahia: aspectos clínicos, laboratoriais e anatomopatológicos**. Monografia (Graduação em Medicina Veterinária) – Centro de Ciências Agrárias, Ambientais e Biológicas da Universidade Federal do Recôncavo da Bahia, Cruz das Almas, 2019. 54p.

Silvestre, B.T.; Silveira, J.A.G; Meneses, R.M.; Facury-Filho, E.J.; Carvalho, A.U.; Ribeiro,

M.F.B. Identification of a vertically transmitted strain from *Anaplasma marginale* (UFMG3): molecular and phylogenetic characterization, and evaluation of virulence. **Ticks and Tick-borne Diseases**, 7(1): 80-84, 2016.

Singh, N.D.; Banga, H.S.; Gadhave, P.D.; Chougule, A.A.; Goyal, A.; Brar, R. S. Clinico-pathological alterations in a case of bovine (Anaplasmosis). **Indian Journal of Animal Research**, 48(3): 295-297, 2014.

Swenson, C.; Jacobs, R. Spherocytosis associated with anaplasmosis in two cows. **Journal of the American Veterinary Medical Association**, 188(9): 1061-1063, 1986.

Tvedten, H.; Weiss, D.J. Laboratory and clinical diagnosis of anemia. In: Feldman, B.F.F. Zinkl, J.G. Jain, N.C. **Schalm's veterinary hematology**. 4th ed. Philadelphia: Williams & Wilkins, 2000. p.143-150.

Tvedten, H. Laboratory and clinical diagnosis of anemia. In: Weiss, D.W.; Wardrop, K.J. **Schalm's veterinary hematology**. 6th ed. Ames: Wiley-Blackwell, 2010. p.152-161.

Wilkinson, R. **Bovine anaplasmosis**. 2005. Available at: <https://www.vet.k-state.edu/docs/vhc/farm/ag-practice-updates/Bovine_Anaplasmosis.pdf.> Accessed on: 07 nov. 2023.