

## Clinical variations observed among the main hemoparasitosis caused by *Rhipicephalus sanguineus* in dogs

Variações clínicas observadas entre as principais hemoparasitoses causadas pelo *Rhipicephalus sanguineus* em cães

Variaciones clínicas observadas entre las principales hemoparasitosis causadas por *Rhipicephalus sanguineus* en perros

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### **Abstract**

Hemoparasitosis are diseases of great importance in the practice of a small animal's veterinarian, due to their high number of cases in the clinical routine of these species, and the wide occurrence of their vectors. Related to hemoparasitosis in dogs, the tick *Rhipicephalus sanguineus* has the greatest importance due to parasitizing dogs, perpetuating hemoparasitosis in this host group. The present work describes a literature review about the three main hemoparasitosis in the routine of a veterinarian in Brazil, ehrlichiosis, caused by *Ehrlichia canis*, babesiosis caused by the protozoa *Babesia canis* and *Babesia gibsoni*, and anaplasmosis caused by *Anaplasma platys*, comparing them regarding the differences between clinical signs and laboratory alterations, such as the different types of anemia caused by these conditions that culminate in clinical signs related to them. It also aims to describe the similarity in the diagnostic techniques used and infection treatments, which in all cases is used tetracycline, the most commonly used is doxycycline, and highlight the need for further studies about this topic.

**Keywords:** Anaplasmosis; Babesiosis; Ehrlichiosis; Infectious disease; Tick.

### **Resumo**

As hemoparasitoses são doenças de grande importância na atuação do médico veterinário de pequenos animais, devido a sua elevada casuística na rotina clínica dessas espécies, e da ampla ocorrência de seus vetores. Relacionado

às hemoparasitoses de cães, o carrapato *Rhipicephalus sanguineus* tem a maior importância por parasitar cães, perpetuando hemoparasitoses nesse grupo de hospedeiro. O presente trabalho descreve uma revisão de literatura acerca das três principais hemoparasitoses na rotina do médico veterinário do Brasil, a erliquiose, causada pela *Ehrlichia canis*, a babesiose causada pelos protozoários *Babesia canis* e *Babesia gibsoni* e a anaplasmoze causada pela *Anaplasma platys*, comparando-as, como as diferenças entre os sinais clínicos e alterações laboratoriais, a exemplo dos diferentes tipos de anemias causados por essas afecções que culminam em sinais clínicos relacionados a elas. Visa também descrever a semelhança nas técnicas de diagnóstico empregadas e no tratamento das infecções, que em todos os casos é com a utilização de tetraciclina, mais comumente empregada a doxiciclina e salientar a necessidade de mais estudos sobre esse tema.

**Palavras-chave:** Anaplasmoze; Babesiose; Carrapato; Doença infecciosa; Erliquiose.

### Resumen

Las hemoparasitosis son enfermedades de gran importancia en el papel del veterinario de pequeños animales, debido a su elevado número de casos en la rutina clínica de estas especies, y la amplia ocurrencia de sus vectores. Relacionada con la hemoparasitosis en perros, la garrapata *Rhipicephalus sanguineus* tiene la mayor importancia para parasitar a los perros, perpetuando la hemoparasitosis en este grupo de hospedadores. El presente trabajo describe una revisión de la literatura sobre las tres principales hemoparasitosis en la rutina del veterinario en Brasil, la ehrlichiosis, causada por *Ehrlichia canis*, la babesiosis causada por los protozoos *Babesia canis* y *Babesia gibsoni*, y la anaplasmosis causada por *Anaplasma platys*, comparándolas, tales como las diferencias entre signos clínicos y alteraciones de laboratorio, como los diferentes tipos de anemia provocada por estas condiciones que culminan en signos clínicos relacionados con ellos. También se pretende describir la similitud en las técnicas diagnósticas empleadas y en el tratamiento de las infecciones, que en todos los casos es con el uso de tetraciclina, la más utilizada es la doxiciclina, y resaltar la necesidad de realizar más estudios sobre este tema.

**Palabras clave:** Anaplasmosis; Babesiosis; Erlichiosis; Garrapata; Enfermedad infecciosa.

## 1. Introduction

Hemoparasitosis are among the diseases of great clinical and veterinary importance, especially in dogs, due to their high number of patients in veterinary medical care centers (Witter et al., 2013), they are diseases caused by obligate intracellular parasites, of blood cells. The main diseases in this species are anaplasmosis, caused by the bacterium *Anaplasma platys*; ehrlichiosis, caused by the bacterium *Ehrlichia canis* and babesiosis, caused by the protozoan *Babesia canis vogeli* (Silva et al., 2016). A common factor among these diseases is the fact that they have the same vector, *Rhipicephalus sanguineus*, which transmits the agents through the blood repast (Caponi et al., 2020).

Megid et al. (2016) report that the most common clinical signs and alterations in blood counts in cases of anaplasmosis and ehrlichiosis are anemia, thrombocytopenia, anorexia, pale mucosa, lymphadenomegaly, leukopenia, prostration, and lethargic behavior. In babesiosis, however, it is common to observe lethargy, weight loss, dehydration, hyperthermia, jaundice splenomegaly; in hematological exams: hemolytic anemia, hyperbilirubinemia, thrombocytopenia, metabolic acidosis and azotemia; and in urinalysis: bilirubinuria, hemoglobinuria, and kidney casts (Moraes et al., 2016).

The strict evaluation of clinical signs associated with laboratory tests like complete blood count, biochemical and serology tests, are the most common means of diagnosis (Megid et al., 2016). In cases of *E. canis* and *A. platys*, there is still the possibility of a blood smear of peripheral blood, aiming the direct identification of morulae (Nakaghi et al., 2008). Currently, there is also the possibility of using the Polymerase Chain Reaction (PCR) in the diagnosis of these diseases, including for the detection of specific phases of these diseases' cycle (Witter et al., 2013).

The possibility of coinfection exists since transmission is given by the same vector. This fact is already identified in studies with the presence of multiple hemoparasitosis in dogs, due to the similarity of symptoms, making it difficult to achieve the definitive diagnosis of these infections when concomitant (Leal et al., 2015; Sainz et al., 2015).

Prophylaxis measures for the three diseases are based on vector control, using the technique of spraying the environment with substances composed of amitraz or cypermethrin, and the control on the animal itself, using ectoparasiticides. There is also the recommendation to apply quarantine to animals that arrive in kennels (Megid et al., 2016).

## 2. Ehrlichiosis

Ehrlichiosis is a disease widely diagnosed in dogs in Brazil, presenting as an etiological agent the bacterium *E. canis* (Aguilar et al., 2013). The microorganism is an obligate intracellular organism, affecting cells of the hematopoietic system of dogs, both mature and immature, mainly cells of the monocytic lineage, such as monocytes and lymphocytes. In ticks, it parasitizes intestinal epithelial and salivary glands cells (Silva et al., 2016).

The disease is known as Canine Monocytic Ehrlichiosis (CME) but it can also affect cats. However, the pathogenesis is still unknown in this species, although clinical signs and treatment are quite similar between feline and canine species (Silva et al., 2016; Nagahachi et al., 2014).

### 2.1 Pathophysiology

Ehrlichiosis in dogs can be manifested in three phases: acute, chronic, and subclinical (Chart 1). The incubation period takes between 8 and 20 days. In the acute phase, the agent multiplies inside circulating mononuclear cells, liver, spleen, and lymph nodes, causing lymphadenomegaly and lymphoreticular hyperplasia of the liver and spleen. The transport of these infected cells through the blood distributes the etiologic agent to other organs such as meninges, kidneys, and lungs. Furthermore, they adhere to the endothelium of vessels, causing vasculitis and infection of the subendothelial tissue. Vasculitis causes destruction of peripheral cells (erythrocytes, platelets and endothelial cells) and/or sequestration of these cells, generating thrombocytopenia and leukopenia (Vieira et al., 2012).

In addition to these factors, the clinical signs presented in the acute phase are characterized by hyperthermia, anorexia, weight loss, and asthenia, and there may also be nonspecific signs such as oculonasal discharge, anterior uveitis, epistaxis, depression, polydipsia, lymphadenopathy, dehydration, splenomegaly and diarrhea (Megid et al., 2016; Ettinger & Feldman, 2016).

Biochemical exams in the acute phase detect hyperbilirubinemia and increased alanine aminotransferase (ALT) and alkaline phosphatase (ALP), indicating hepatic impairment (Sainz et al., 2015).

After the acute phase, *E. canis* remains in the host, initiating the subclinical phase of the infection. This is characterized by the absence of clinical signs, but with some possible laboratory alterations in the blood count, such as leukopenia, thrombocytopenia, and anemia. The duration of the subclinical phase can persist for years until the agent is completely eliminated. If this does not occur, the chronic phase begins. In the chronic phase of ehrlichiosis, some animals may develop medullary hypoplasia, resulting in aplastic anemia and severe pancytopenia. There is also the possibility of worsening the condition, when the animal does not have an efficient immune response, in any event of immunosuppression (Sainz et al., 2015).

The chronic disease can cause neurological signs such as ataxia, neuromotor dysfunction, central or peripheral vestibular dysfunction, and localized or generalized hyperesthesia, in addition to cerebellar dysfunction and intense tremors. Other signs that can be observed in this phase are the same as in the acute phase but attenuated. It is not uncommon to find glomerulonephritis and corneal ulcers caused by immune complex deposition. In addition, in the renal parenchyma, this deposition of immune complexes ends up causing elevations in serum creatinine levels, indicating greater nephron involvement. In the cornea, it causes ulcers resulting from vasculitis. When immune complex deposition reaches the joint system, it can lead to arthritis secondary to ehrlichiosis (Megid et al., 2016; Ettinger & Feldman, 2016).

**Chart 1** – Clinicopathological alterations found in the acute, subclinical and chronic clinical phases of canine ehrlichiosis.

ACUTE	SUBCLINICAL	CHRONIC
Hyperthermia, dehydration and diarrhea, polydipsia	Possible absence of clinical signs but with laboratory alterations	Clinical signs of the acute phase, however attenuated.
Anorexia, weight loss and asthenia and depression	Anemia	Ataxia, incoordination, tremors and hyperesthesia
Uveitis, epistaxis, oculonasal discharge, petechiae	Leukopenia	Glomerulonephritis
Lymphadenomegaly and splenomegaly	Thrombocytopenia	Aplastic anemia, monocytosis, lymphocytosis and leukopenia

Fonte: Adaptado de Birchard & Sherding, (2008); Bowman, (2017); Ettinger & Feldman, (2004) e Megid, Paes & Ribeiro, (2016).

## 2.2 Diagnosis

Diagnosis can be given in many ways, and it is not very challenging. The strict evaluation of clinical signs and laboratory tests such as complete blood count, blood smear, biochemical and serology tests, in addition to the clinical history usually reported with the presence of ticks on the animal, are the most common means of diagnosis (Megid et al., 2016). Additionally, there is also the possibility of a peripheral blood smear, aiming at the direct identification of morulae, however, it was reported by Nakaghi et al. (2008) in a study containing data from 30 dogs attended at the UNESP veterinary hospital in Jaboticabal, that the blood smear technique has low specificity and sensitivity, where only 1 of the 30 dogs was possible to directly detect the morulae, presenting an effectiveness result of only 3.3% in this method. It is important noting that Santarém (2003) mentions that in the leukoconcentration extension technique, using buffy coat, there was a significant increase in positive animals, precisely 73.3% of sensitivity. Another possibility is the use of PCR, which provides a precise diagnosis, identifying the specific DNA of the microorganism in peripheral blood leukocytes, as well as detecting specific phases of the disease cycle. Witter et al. (2013) report that a positive PCR characterizes acute or subclinical phases since the second can lead to agent's cycles of parasitemia. On the other hand, a negative and seropositive PCR indicates the chronic phase, considering that the agent is no longer found in the bloodstream, but in the bone marrow, lymph nodes, and spleen. In addition, the study by Nakaghi et al. (2008) showed that the serology techniques aiming to correlate humoral immunology with the phase: Dot-ELISA and Indirect Immunofluorescence Reaction (IIFR), proved to be highly efficient for the detection of the chronic and subclinical phases of the disease, with 70% and 63.3% of positive samples, respectively, and the PCR technique proved to be efficient for detecting the acute phase, with 53.3% of positive samples.

It is also worth noting that no matter what technique is chosen for diagnosis, it is necessary to correlate the clinical signs with the result, and according to the cited authors, the best techniques are serology for the chronic and subclinical phases diagnosis and PCR for chronic phase, using the amplification of the 409 base pair fragment of the *dsb* gene from *Ehrlichia* spp. The blood smear technique, on the other hand, has low efficiency and its use should be replaced by these previously mentioned techniques, when possible (Aguiar et al., 2013; Nakaghi et al., 2008).

Another diagnostic method would be the use of rapid tests, such as the SNAP 4Dx Plus Test, which in the recent study by Liu et al. (2018) using 154 positive samples for *E. canis* confirmed by the aforementioned methods, there was a 97.1% sensitivity with the use of SNAP, proving to be an extremely effective use in the veterinarian clinical routine.

## 2.3 Treatment

The treatment is done with tetracyclines, the most common being doxycycline due to its greater capacity for penetration into tissues as it is the most fat-soluble in this class. It is used at a dose of 10mg per kg/day, orally, for 28 days, and has an effect at any stage of the disease (Ettinger & Feldman, 2016).

### 3. Babesiosis

Canine babesiosis, as well as ehrlichiosis, is also a hemoparasitosis transmitted when there is a blood repast by a contaminated *R. sanguineus*. This one, however, is caused by the protozoan *Babesia* spp. The genus includes obligate intracellular parasites of erythrocytes, being the most common babesiosis in dogs in Brazil, caused by the protozoan *Babesia canis*, which is subdivided into three subspecies: *Babesia canis canis*, *Babesia canis rossi*, and *Babesia canis vogeli*, the last one being predominant in Brazil. Another species of *Babesia* spp. *Babesia gibsoni* can also affect these animals (Megid et al., 2016; Duarte et al., 2011).

Besides blood repast, transmission can also occur by blood transfusion of infected animals, and by any of these forms of infection, after installed in the host, they invade erythrocytes and when they reproduce they destroy them characterizing a regenerative hemolytic anemia a sign by which the disease is recognized (Vieira et al., 2013).

#### 3.1 Pathophysiology

The pathophysiology of babesiosis is closely related to hemolytic anemia, that is, hemolysis caused by the multiplication of this protozoan in red blood cells causes the release of hemoglobin, resulting in hemoglobinuria and bilirubinemia. The indirect bilirubin, released from this occasion, overloads the liver, thus generating hepatic and splenic congestion, hepatosplenomegaly, and jaundice (Ettinger & Feldman, 2016).

The concomitant infection of *B. canis* and *E. canis* in dogs causes a severe normocytic and normochromic anemia, caused by the destruction of mature red cells, the inability of erythropoiesis and a severe and often fatal condition, especially in young dogs (Vieira et al., 2013).

Dogs commonly present an acute condition with anorexia and apathy, diarrhea, fever, hemoglobinuria, mild or severe anemia, jaundice, and there may even be pneumonia. It is important to note that jaundice may not always be present, and it usually lasts from 3 to 10 days. Babesiosis can have a slow recovery that can take more than a month or even lead to death. There is also the possibility of neurological signs, characterized by aggressiveness or extreme apathy, paralysis, ataxia, and imbalance, due to the presence of vasculitis, caused by the adherence of these agents to the blood vessels that supply the nervous system (Ettinger & Feldman, 2016).

The disease can be subdivided into 3 phases: acute, hyperacute and chronic, with the clinical signs of each phase shown in Chart 2.

**Chart 2** – Clinicopathological presentations of canine babesiosis in different hyperacute, acute and chronic phases.

<b>HYPERACUTE</b>	<b>ACUTE</b>	<b>CHRONIC</b>
Metabolic acidosis	Fever, hematuria and jaundice	Intermittent fever
Systemic inflammatory response syndrome	Lethargy and anorexia	Decrease in performance in athlete dogs
Hypoxia	Splenomegaly	Decreased appetite
Shock and venous stasis	Hemolytic anemia	

Fonte: Adaptado de Nelson & Couto (2015).

In laboratory findings, regenerative anemia characterized by macrocytosis is perceptible, referring to a large circulation of immature red blood cells, which may or may not be hypochromia, in addition to hemoglobinuria, hyperbilirubinemia, bilirubinuria, thrombocytopenia, metabolic acidosis, renal cylinders, and azotemia (Moraes et al., 2016).

However, it should be noted that at the beginning of the infection, the anemia found is generally classified as normocytic and normochromic, and of low intensity. As the disease progresses, macrocytosis and hypochromia appear, the

more severe the anemia, the greater the reticulocytosis. Other abnormalities, such as from leukocytes, are inconsistently found: neutrophilia, neutropenia, lymphocytosis, and eosinophilia. There is a possibility that infected adult animals don't show any change in the blood count (Moraes et al., 2016).

The severity of the disease and the involvement of many organs is directly linked to the intensity of hemolysis promoted by parasitemia, the pathogenicity of the strain, and the individual characteristics of the infected dog's organism (Vieira et al., 2013).

### **3.2 Diagnosis**

The most common diagnosis of babesiosis is when the presence of parasitized erythrocytes is demonstrated through a blood smear. The technique consists of collecting peripheral blood and performing the blood smear using Romanowsky stains, such as Giemsa, Wright, Diff-Quick, or Rosenfeld. A big difference between infections with *B. canis* and *B. gibsoni* is that, in the first case, it is commonly identified in pairs and very low parasitemias are usually more common, in addition to this microorganism being larger. In the second case, the parasitemias are usually of 5 to 40% and the protozoan is smaller and usually found alone inside the red cell (Ettinger & Feldman, 2016).

In addition to the correlation of clinical signs, anamnesis, and previous history with ticks, the uses of the ELISA technique and Indirect immunofluorescence in situations of low parasitemia might be useful. These tests even allow the differentiation between animals that have antibodies from previous infections or that are with active disease. There should always be correlated serological tests with clinical signs (Vieira et al., 2013).

It should also be said that molecular biology methods, such as PCR, are much more effective and widely used in epidemiological studies, using the amplification of the BAB1 primer oligonucleotide (5'-GTG AAC CTT ATC ACT TAA AGG-3') which is specific for a region of the 18S rRNA gene of *Babesia* spp (Silva et al., 2012).

### **3.3 Treatment**

The most effective treatment is the one in which there is a tick control, moderating of the immune response, treatment of symptoms and control of *Babesia* spp. The effectiveness of the latter is closely linked to the species of *Babesia* spp. *B. canis* has a better response in symptomatic and control treatment, while *B. gibsoni* is more resistant to treatment in both control and symptomatology (Moraes et al., 2016).

The use of imidocarb dipropionate to combat babesiosis is extremely effective and leads to the complete elimination of the agent, which is inadvisable, as it inhibits the perpetuation of antigenic activity and favors reinfection, being advisable only the treatment with doxycycline, which does not lead to the complete elimination of the agent. Fluid therapy is shown as an ally for animal hydration and assisting in the expansion of vascular volume, reducing toxicity and helping to compensate electrolyte and acid-base imbalances, preventing or reducing metabolic acidosis due to losses like diarrhea and vomiting. In dogs that are severely affected by the disease, it may be necessary to use blood transfusion and administration of bicarbonate in ketoacidosis (Ettinger & Feldman, 2016).

## **4. Anaplasmosis**

Disease caused by a gram-negative bacterium, which belongs to the Rickettsiales order, Anaplasmataceae family, and *Anaplasma* genus, the etiologic agent that infects dogs' platelets causing canine thrombocytic anaplasmosis is *A. platys*. Infection by this organism causes a clinical status called canine cyclic thrombocytopenia (Megid et al., 2016).

Co-infection cases with *E. canis* and *B. canis* are usually more severe than the presentation of anaplasmosis in a solitary infection, which usually has milder clinical signs or even asymptomatic conditions (Aguiar et al., 2008; Leal et al., 2015).

Recently, in Venezuela, a study by Arraga-Alvarado et al. (2014) detected the infection by *A. platys* in two women, showing the parasitism of this agent in humans, but not confirming the existence of disease caused by it. However, both had nonspecific symptoms, such as headache and myalgia after being exposed to *R. sanguineus*. Furthermore, treatment with doxycycline did not alleviate the patients' symptoms.

#### **4.1 Pathophysiology**

As previously mentioned, canine thrombocytic anaplasmosis is responsible for a condition known as canine cyclic thrombocytopenia, in other words, after an incubation period of 8 to 16 days, infection by *A. platys* is characterized by a cyclic platelet parasitemia followed by generalized thrombocytopenia. Anorexia, weight loss, depression, and lethargy may occur as clinical signs, and there might rarely be the presence of hemorrhages when there is severe thrombocytopenia (Ettinger & Feldman, 2016).

#### **4.2 Diagnosis**

For the diagnosis of *A. platys*, as well as the other infections listed here, blood smear techniques with Giemsa staining are commonly used to identify the morulae included in the platelets, in addition to the use of serological tests and molecular biology techniques such as PCR. The latter uses the amplification of genes in two stages, according to Correa *et al.* (2011), the first step is the amplification of the 16S rRNA gene and the second step, the specific amplification for *A. platys*, the oligonucleotide PLATYSF (5' GAT TTT TGT CGT AGC TTG CTA TG 3'). No matter the technique used, it should always be correlated with the clinical history of ectoparasites presence and the clinical signs, which, as mentioned above, are usually mild in solitary *A. platys* infection, but as it is commonly associated with other hemoparasitosis, the concomitant infection by this bacterium must also be considered in cases of *E. canis* and *Babesia* spp. that present more severe symptoms (Nakaghi et al., 2008; Leal et al., 2015).

Another possibility would also be using the SNAP 4Dx Plus Test that in the same study previously mentioned in the ehrlichiosis diagnosis topic, carried out by Liu et al. (2018), the technique was also used to obtain data on its efficacy in the diagnosis of anaplasmosis and the sensitivity found for antibodies against *A. platys* was 83.3%, showing high efficacy and recommendation in the clinical use of this exam.

#### **4.3 Treatment**

The treatment follows the same protocol as the other hemoparasitosis reviewed here: the use of tetracyclines, with doxycycline being more commonly used in addition to symptomatic treatment. However, in infection by *A. platys*, due to its intraplatelet location, there is a limitation in the effectiveness of the complete eradication of this agent in the organism, and it is also important to emphasize that there must be control of ticks in the environment and animal (Ettinger & Feldman, 2016).

## **5. Comparison between hemoparasitoses**

### **5.1 Comparison of clinicopathological findings**

The three diseases have different forms of manifestation in affected animals (MEGID et al., 2016) and as already described, it is not rare the cases in which these diseases appear together, but it is necessary to score the differences in clinical signs and laboratory alterations in individual cases.

The main differences are evident when comparing babesiosis to ehrlichiosis, as shown in Table 1 and Table 2, it is clear that nonspecific signs such as diarrhea, hyperthermia, apathy, dehydration, among others in the group of general clinical signs, are indistinguishable and they can hardly be used as a differential, but when comparing other signs that may be present in ehrlichiosis, especially in the acute phase, such as uveitis, oculonasal discharge, epistaxis and hemorrhagic petechiae, it is possible to see that they do not exist in babesiosis and this can be used as a differential between these two diseases (Vieira et al., 2012) and when comparing laboratory changes, it is feasible that the anemia caused by babesiosis, even initially is normocytic and normochromic, as the disease progresses, the anemia results increasingly in macrocytic and hypochromic (Moraes et al., 2016), an alteration that does not exist in ehrlichiosis, being possible to be guided by this fact as a possible differential. It is noteworthy, however, that the chronic phases of both diseases have many similarities, especially concerning neurological signs, and the veterinarian should be very careful when trying to differentiate these diseases based on this issue (Ettinger & Feldman, 2016).

Regarding anaplasmosis in comparison with other diseases, it is shown in the literature that it is very difficult to perceive this disease alone, it does not have any sign that differs from the others, and its most present condition, thrombocytopenia, is not exclusive to it in the scope of this group of parasites. It is important to say that these diseases are differential diagnoses among them and that other diseases can also be included in this group of differential diagnoses (Ettinger & Feldman, 2016).

### **5.2 Diagnosis comparison**

The diagnosis of these diseases compose common means, mainly in what consists the anamnesis, clinical history of ectoparasites, and also laboratory means, as described here, there is the possibility of performing a blood smear aiming to visualize the parasitic inclusions, having a small difference between each of the diseases due to the predilections that the parasites have for different cell types, *E. canis* and *A. platys* are more easily visualized using the leukoconcentration extension technique, as described by Santarém (2003) because these parasites roost in white cells and platelets respectively and this technique contain more leukocytes and platelets visible under the microscope than the total blood smear, whereas *Babesia* spp. is more easily visualized by the total blood extension technique given that the agent has a predilection for red blood cells, and peripheral blood collection is used as the best choice for this test (Vieira et al., 2013).

However, the blood smear is not the best technique to be used in any of the three cases and that is why it is recommended the use of serology diagnosis techniques such as IIFR and ELISA, and molecular techniques such as PCR, due to their better diagnostic accuracy, but they need to be chosen according to the suspected stage of the disease. In both babesiosis and ehrlichiosis, in situations of low parasitemia, in the chronic phase of the disease it is preferable to use serology and in situations of the acute phase, therefore, high parasitemia, PCR has very high sensitivity (Aguiar et al., 2013; Nakaghi et al., 2008; Vieira et al., 2013).

### **5.3 Treatment comparison**

Regarding the treatment, it felicitates the veterinarian when he realizes that the drug of choice: doxycycline, is the same for all three diseases with the recommended dose of 10 mg/kg, orally, one tablet a day, for 28 days, expecting a clinical recovery within 24h to 72h after starting the treatment. Other drugs from the Tetracycline class can also be used, and even in cases of co-infection, it will be effective in any clinical stage of the disease (Ettinger & Feldman, 2016), with the exception only of infection by *B. gibsoni*, which presents greater resistance to control treatment, and therefore, it is necessary to use the most efficient drug to combat the parasite, imidocarb dipropionate, a hemoparasiticide, with a dose of 5 mg/kg intramuscularly

or subcutaneously and repeat after 15 days, it should be noted that it is a drug with a low therapeutic index, being necessary to observe the animal for at least 30 minutes after application and, if necessary, use atropine (Moraes et al., 2016).

## 6. Conclusion

As the present work aims to clarify the correlations and differences between the three diseases, this topic will be discussed below. As for similarities among the three diseases, we have the same vector, the tick *R. sanguineus*, and the transmission mechanism is through the blood repast for the three diseases. After installed in the body, disregarding co-infections, the first difference is in the cells in which each microorganism parasite, with *E. canis* being a leukocyte parasite, especially monocytes and lymphocytes, whereas *B. canis* is an erythrocyte parasite and *A. platys* a thrombocyte parasite. These factors are closely linked with the clinical signs caused by each parasitemia, especially regarding anemia. It is common that the anemia caused by ehrlichiosis to be normocytic and normochromic, whereas in babesiosis, it initially presents itself as such, but during the disease, it is macrocytic and hypochromic, and in *A. platys* it may not be presented any anemia, depending on the severity of the disease. Already mentioning severity, of the three conditions, the one with the most virulence is ehrlichiosis, but as reported here, in many cases, there is a joint infection of these diseases, making it difficult to perceive the isolated signs, and what comforts, is that the forms of diagnosis and treatment recommended for the three infections are identical, all of them containing a tetracycline combined with symptomatic support.

There are many similarities in clinical signs and laboratory alterations, such as thrombocytopenia, hemoglobinemia, hyperbilirubinemia, making isolated diagnosis difficult in the physical examination, and some laboratory or molecular technique is almost always necessary to identify the presence of which parasite or parasites.

## References

- Aguiar, D. M., Zhang, X., Melo, A. L. T., Pacheco, T. A., Meneses, A. M. C., Zanutto, M. S., Horta, M. C., Santarém, V. A., Camargo, L. M. A., McBride, J. W. & Labruna, M. B. (2013). Genetic diversity of *Ehrlichia canis* in Brazil. *Veterinary Microbiology*, 164(3-4), 315-321.
- Arraga-Alvarado, C. M., Qurollo, B. A., Parra, O. C., Berrueta, M. A., Hegarty, B. C. & Breitschwerdt, E. B. (2014). Molecular evidence of *Anaplasma platys* infection in two women from Venezuela. *American Journal of Tropical Medicine and Hygiene*, 9(16), 1161-1165.
- Birchard, S. J. & Sherding, R. G. (2008). *Manual Saunders: Clínica de Pequenos Animais*. (3rd ed.), Roca.
- Bowman, D. D. (2017). *Georgis Parasitologia Veterinária*. (9th ed.), Elsevier.
- Caponi, A. G., Botelho, L. F. S., Marinho, K. N. S., Sousa, A. K., Carvalho, B. Y. A. & Bonfim, M. E. A. (2020). Incidência de hemoparasitoses em cães da região sul da cidade de Palmas, Tocantins. *PUBVET*, 14(1), 1-4.
- Correa, E. S., Paludo, G. R., Scalon, M. C., Machado, J. A., Lima, A. C. Q., Pinto, A. T. B., Thiebaut, J. T. L. & Albernaz, A.P. (2011). Investigação molecular de *Ehrlichia* spp. e *Anaplasma platys* em felinos domésticos: alterações clínicas, hematológicas e bioquímicas. *Pesquisa Veterinária Brasileira*, 31(10), 899-909.
- Duarte, S. C., Parente, J. A., Pereira, M., Soares, C. M. A. & Linhares, G. F. C. (2011). Phylogenetic characterization of *Babesia canis vogeli* in dogs in the state of Goiás, Brazil. *Revista Brasileira de Parasitologia Veterinária*, 20(4), 274-280.
- Ettinger, S. J. & Feldman, E. C. (2004). *Tratado de Medicina Interna Veterinária: doenças do cão e do gato*. 5ª. ed. Rio de Janeiro: Guanabara Koogan.
- Leal, P. D. S., Moraes, M. I. M. R., Barbosa, L. L. O. & Lopes, C. W. G. (2015). Infecção por hematozoários nos cães domésticos atendidos em serviço de saúde animal, Rio de Janeiro, Brasil. *Revista Brasileira de Medicina Veterinária*, 37(1), 55-62.
- Liu, J., Drexel, J., Andrews, B., Eberts, M. & Breitschwerdt, E. (2018). Comparative Evaluation of 2 In-Clinic Assays for Vector-Borne Disease Testing in Dogs. *Topics in Companion Animal Medicine*, 33(4), 114-118.
- Megid, J., Paes, A. C. & Ribeiro, M. G. (2016). *Doenças infecciosas em animais de produção e de companhia*. Roca.
- Moraes, L. F., Takahira, R. K., Golim, M. A. & Baggio, M. S. (2016). Avaliação das alterações hemostáticas e do risco tromboembólico em cães com AHIM. *Pesquisa Veterinária Brasileira*, 36(5), 405-411.
- Nagahachi, P. Y., Gonçalves, S., Santos, C. R., Pereira, M. A., Lucena H. C. & Menezes, R. C. (2014). Erliquiose felina: relato de caso. *Rev MV&Z*. 12(2), 48.

- Nakaghi, A. C. H., Machado, R. Z., Costa, M. T., André, M. R. & Baldani, C. R. (2008). Canine ehrlichiosis: clinical, hematological, serological and molecular aspects. *Ciência Rural*, 38, (3), 766-770.
- Nelson, R. W. & Couto, C. G. (2015). *Medicina interna de pequenos animais*. (5th ed.), Guanabara Koogan.
- Sainz, Á. Roura, X., Miró, G., Estrada-Peña, A., Kohn, B.; Harrus, S. & Solano-Gallego, L. (2015). Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. *Parasites & Vectors*, 8(75).
- Santarém, V. A. (2003). Achados epidemiológicos, clínicos e hematológicos e comparação de técnicas para diagnóstico de *Ehrlichia canis*. 2003. *Tese (Doutorado em Medicina Veterinária)* – Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu.
- Silva, A. B., Costa, A. P., Sá, J. C., Costa, F. B. Santos, A. C. G. & Guerra, R. M. S. N. C. (2012). Detecção molecular de *Babesia canis vogeli* em cães e em *Rhipicephalus sanguineus* na mesorregião do oeste maranhense, nordeste brasileiro. *Ciência Animal Brasileira*, 13(3), 388-395.
- Silva, V. C. L., Lima, E. R., Dias, M. B. M. C., Fukahori, F. L. P., Rêgo, M. S. A., Junior, J. W. P., Kim, P. C. P., Leitão, R. S. C. S., Mota, R. A. & Carieli, E. P. O. (2016). Parasitological and molecular detection of *Babesia canis vogeli* in dogs of Recife, Pernambuco and evaluation of risk factors associated. *Semina: Ciências Agrárias*, 37(1), 163-172.
- Vieira RFC, et al. Ehrlichiosis in Brazil. *Rev Bras Parasitol Vet*. 2011; 20(1):1-12. <https://doi.org/10.1590/S1984-29612011000100002>
- Vieira, T. S. W. J., Vieira, R. F. C., Nascimento, D. A. G., Tamekuni, K., Toledo, R. S., Chandrashekar, R., Marcondes, M., Biondo, A. W. & Vidotto, O. (2013). Serosurvey of tick-borne pathogens in dogs from urban and rural areas from Parana State, Brazil. *Revista Brasileira de Parasitologia Veterinária*, 22(1), 104-109.
- Witter, R., Vecchi, S. N., Pacheco, T. A., Melo, A. L. T., Borsa, A., Sinkoc, A. L., Mendonça, A. J. & Aguiar, D. M. (2013). Prevalência da erliquiose monocítica canina e anaplasmoose trombocítica. *Semina: Ciências Agrárias*, 34, 3811-3822.