

Clinical and Hematological Changes in Dogs with Canine Monocytic Ehrlichiosis Attended in a Private Clinic in São Bernardo do Campo, São Paulo

Pasqualetti VB, Gandolfi AM, dos Santos Pessini PG and da Silva EB*

Laboratório de Análises Clínicas/Parasitologia do Centro Universitário FMABC, FMABC, Brazil

***Corresponding author:** da Silva EB, Laboratório de Análises Clínicas-Parasitologia do Centro Universitário Saúde ABC. Avenue Lauro Gomes, 2000. Santo André, São Paulo, Brazil, Tel: +5511955505397, E-mail: emerson.silva@fmabc.br

Citation: Pasqualetti VB, Gandolfi AM, dos Santos Pessini PG, da Silva EB (2021) Clinical and Hematological Changes in Dogs with Canine Monocytic Ehrlichiosis Attended in a Private Clinic in São Bernardo do Campo, São Paulo. J Vet Ani Res 4: 101

Abstract

The Ehrlichiosis Monocytic Canina (EMC) is a character disease zoonotic and has as main vector red dog tick (*Rhipicephalus sanguineus*). The disease has several clinical and laboratory presentations depending on the stage it is in. Animals commonly have fever, anorexia, apathy, pale mucous membranes, and petechiae. The most frequent laboratory alterations include mild to moderate anemia, thrombocytopenia, neutropenia or lymphopenia, hypoalbuminemia, among others. Diagnostic tests to confirm the disease can be through serology, molecular tests or the presence of morulae in a blood smear. In this study, the medical records of 23 dogs suspected of having CME treated at a private clinic in São Bernardo do Campo, São Paulo, were evaluated, observing the clinical-hematological and serological changes. The main symptom reported was apathy, followed by fever and the presence of ixodidiosis. The main alteration found on the CBC was thrombocytopenia, followed by anemia and lymphopenia. Morula was found in only one patient and only 13 were positive for serology.

Keywords: Canine Monocytic Ehrlichiosis (EMC); Thrombocytopenia; Anemia; Dogs

Introduction

The Ehrlichiosis canine disease is not a zoonotic caused by a gram-negative obligate intracellular and presenting the main vectors ticks (Rickihisa, 1991).

Ehrlichia canis is a gram-negative pleomorphic coccoid bacterium that resides in the host cell cytoplasm: monocytes, lymphocytes and macrophages. The vertebrate hosts for *E. canis* are members of the *Canidae* family such as coyotes, foxes, jackals and domestic dogs. Its transmission occurs through the vector *Rhipicephalus sanguineus*, the brown tick of the dog. During feeding, ticks inoculate salivary secretion contaminated with ehrlichias inside the feeding site in the host. All stages of the vector can transmit the disease (larva, nymph and adult) [1].

Dogs can be affected by various agents of the *Anaplasmatecea* family, such as *Ehrlichia canis*, *Ehrlichia ewingii*, *Ehrlichia chaffeensis*, *Anaplasma plays* and *Anaplasma phagocitophilum*. The most widely studied species is *E. canis*, which causes a multisystem disease, canine monocytotropic ehrlichiosis (EMC) that manifests in acute, subclinical and chronic forms. It is a potentially fatal disease if left untreated, transmitted by the bite of the dog's brown tick, *Rhipicephalus sanguineus* [1].

In the acute phase, the microorganism enters the mononuclear cells and replicates, spreading to other organs of the host; it can last from two to four weeks (Breitscherdt, 2004). In this phase, clinical signs such as petechiae, ecchymosis, vacuities, neurological and muscular, ocular signs can occur. Common laboratory findings at this stage are thrombocytopenia, mild anemia and leukopenia [1].

The changes that we normally find in the blood count of patients with CME are anemia, leukopenia, thrombocytopenia and pancytopenia and are directly related to the animal's symptoms [2]. According to the author, EMC is the main cause of thrombocytopenia in dogs, being a very important change that leads to the suspicion of the disease.

The most common clinical signs are depression, lethargy, anorexia, weight loss, fever, lymphadenomegaly, splenomegaly and bleeding. Hemorrhage in EMC does not always occur due to the drop in the number of platelets, but due to the vasculitis caused by the disease and the splenic sequestration [2].

The *E. canis* occurs in many countries with temperate climates, tropical and subtropical world, coinciding with the prevalence of its vector, *R. sanguineus* [3].

Material and Methods

Information was collected from the medical records of 23 dogs treated at the Clínica e Laboratório Veterinário, in São Bernardo do Campo, São Paulo, after requesting free and informed consent (TCLE) to the owners, observing changes in laboratory tests and confirmatory tests performed. Complete blood count was performed in all described patients. Animals with clinical suspicion of EMC were included in the study, presenting the signs and symptoms of anorexia, depression and fever, and occasionally petechiae, ecchymosis and epistaxis. Anticoagulants were collected using venipuncture, preferably in the cephalic vein, as they were collected in everything containing anticoagulant EDTA K2 and everything with separating gel. Confirmatory tests were performed as serology (Enzyme-Linked Immunosorbent Assay – ELISA and Immunochromatography) and Polymerase Chain Reaction (PCR). The animals tested were suspected of having the disease and even those with a negative result were treated as Ehrlichiosis Monocytic Canina, with the improvement of the condition.

Results

Of the 23 dogs suspected of having CME evaluated in this study, there was no significant racial predilection, with a greater number of mixed-breed dogs (SRD). There was a slight predilection for the male gender and the most frequent age group was middle age (3 to 6 years), as shown in Table 1 below.

Feature	n	%
Sex		
Male	15	65.2
Female	8	34.8
Age		
<6 months	1	4.3
7 - 12 months	2	8.6
12 years	3	13.0
3 to 6 years	13	56.5
> 7 years	4	17.3
Breed		
Chihuahua	1	4.3
Golden Retriever	1	4.3
Lhasa apso	3	13.0
Pequines	1	4.3
Pinscher	1	4.3
Poodle	1	4.3
Shih tzu	5	21.8
SRD	9	39.2
Yorkshire	1	4.3

Table 1: Sample characterization

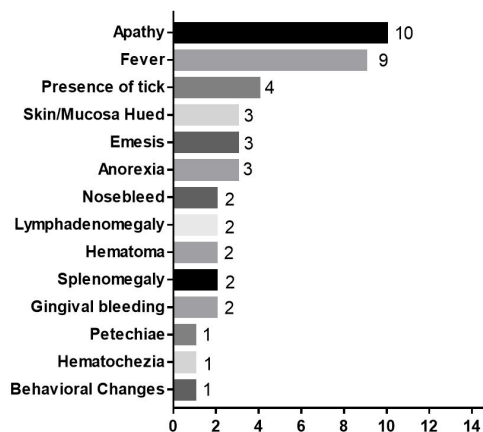


Figure 1: Main clinical signs of the 23 dogs with EMC seen at Clínica e Laboratório Veterinário Da Villa, in São Bernardo do Campo, São Paulo

The most common clinical sign described in the medical records was apathy, followed by fever. Anorexia, emesis, and pale mucous membranes have also been described in some animals. Signs of bleeding such as gingival bleeding, petechiae, bruises and hematochezia have been described in some patients. More rare clinical signs included lymphadenomegaly, splenomegaly and behavioral changes (Figure 1).

Of the hemograms evaluated, all had thrombocytopenia. Anemia and lymphopenia were found to be the second most frequent changes. 3 animals showed leukopenia and neutrophilia. No animal presented thrombocytosis. Serological tests such as ELISA and Immunochromatography were performed, showing some positive results, as shown in the Table 2 below. 10 patients were positive for ELISA and 3 for the immunochromatographic method.

Parameter	n	%
Anemia	6	26.0
Leukocytosis	1	4.3
Leukopenia	3	13.0
Neutrophilia	3	13.0
Neutropenia	2	8.7
Lymphocytosis	1	4.3
Lymphopenia	6	26.1
Thrombocytosis	0	0.0
Thrombocytopenia	23	100.0
ELISA (+)	10	43.4
Immunochromatographic (+)	3	13.0

Table 2: Main hematological and serological changes of the 23 dogs with EMC treated at Clinica e Laboratório Veterinário Da Villa, in São Bernardo do Campo, São Paulo

Discussion

The results observed in this work show that all animals had platelet count below 200 thousand/ μL , this being the main alteration observed, 18 patients presented platelet count below 100 thousand, showing that the platelet count found in EMC was, in its great majority, below this value.

Platelet values below 30.000 were found in 5 patients, none of whom had active mucosal bleeding, only petechiae or bruises. Patients who had mucosal bleeding had platelet counts of 68 and 172 thousand/ μL , which creates the hypothesis that hemorrhage is not only related to the number of platelets but to other factors such as vasculitis, platelet function and clotting factors that may be affected by the disease [1-3]. (2008) [4] describe platelet counts with values less than 200.000/ μL found in 60.0% (15/25) of the animals. This finding is cited in many studies in the literature that indicate the importance of this hematological variable for the presumptive diagnosis of the disease in endemic areas.

Morphologically, it was observed in this work the presence of macroblocks in 4 patients. In the work of [4], the presence of platelet anisocytosis was also observed in 64.0% (16/25) of the animals and macro-platelets in 16.0% (4/25), proving that, despite the tendency to thrombocytopenia, there is active thrombocytopenia [4].

Thrombocytopenia in the acute phase can occur due to an increase in the consumption of platelets by the inflamed vascular endothelium, an increase in splenic sequestration and immune-mediated destruction, or by a decrease in the median life of platelets. It has been proposed that *E. canis* infection in dogs alters the immune system, resulting in an excess of anti-platelet antibodies, which would be a major cause of thrombocytopenia although other non-immunologically mediated mechanisms may also be involved [3,5,6].

Only 6 patients had hematocrit below the reference value followed. 3 patients presented hematocrit with values below 30% and 3 patients presented this between 30 and 39%. In the study by [4], anemia was confirmed in 15 of the 25 (60%) positive animals by PCR and, of these, 73.33% (11/15) presented anemia of the normocytic and normochromic type, which is commonly found in dogs with ehrlichiosis and described in the literature [1-3].

The erythrocyte morphology was altered in 12 patients in this study, with mild to moderate anisocytosis and polychromasia. The cause of the observed non-regenerative anemia is believed to be multifactorial, such as anemia of acute inflammation, a consequence of a hemolytic process, or decreased erythropoiesis.

In the present study, only 1 patient found an *E. canis* morula in the blood smear. [7] also found only a cytoplasmic inclusion of *E. canis* by analyzing samples from 30 patients. In the research carried out by [4], in smears of peripheral blood and leukocyte popes, inclusions suggestive of elementary corpuscles and morulae of *E. canis* and in the cytoplasm of mononuclear cells were found in 5.33% (4/75) of the animals. Of these, 75% (3/4) were positive by PCR, which can be justified by the subjectivity of the parasitological technique.

The total leukocyte value showed a moderate variation, the most leukopenic with 5,100 mm³ leukocytes and the patient with the highest leukocytosis did not exceed 15,100 mm³. In total, 3 patients had leukopenia, while 1 had leukocytosis. [7] mentioned that he found leukocytosis in 4 patients (4/30) while 8 (8/30) had pancytopenia. This last alteration mentioned was found, in this study, in only 1 patient.

Neutrophilia was found in 3 patients, while neutropenia in only 2. Neutrophil morphology was found to be altered in 7 patients, 4 of whom had fine toxic cytoplasmic granulations and 3 had nuclear hypersegmentation. Mild lymphocytosis was present in 1 patient while lymphopenia was present in 6 patients. 4 patients showed changes in lymphocyte morphology, which were reactive. According to [3], about 50% of dogs have lymphopenia and eosinopenia. Animals receiving corticosteroid therapy may experience lymphopenia, eosinopenia, as well as neutrophilia due to the use of these drugs. The lymphocytic atypia observed in animals infected naturally or experimentally was justified by the intense immune response observed in canine ehrlichiosis.

In the study by [8], the mean values of the number of leukocytes, although in the normal range (14,915), presented a very wide range of variation (minimum 5,300 and maximum 40,100). This variation is probably due to individual factors such as race, age, sex, pregnancy, lactation, nutrition or medication, factors inherent to the pathogenicity of the infectious agent, the stage of infection or the presence of co-infections.

The most frequent clinical sign observed was apathy, followed by fever, anorexia / hyporexia, lymph node enlargement, emesis, among others. 2 animals had mucosal bleeding, while 3 had petechiae or bruises on the skin.

In the study by Silva (2010) [9] the clinical signs of the 24 animals mono-infected with *E. canis*, the main findings were: lymphadenopathy, 50% (12/24); some type of dermatological alteration, 45.83% (11/24); apathy, 37.5% (9/24) presence or previous history of tick infestation, 37.5% (9/24) and pale mucous membranes, 33.33%.

Ocular changes, mainly related to inflammatory reaction and deposition of immune complexes causing uveitis are relatively common. Neurological conditions are also described as ataxia and seizures due to inflammation or bleeding in meninges [1].

In the most affected breeds, we found the SRD (9/23) in greater quantity, followed by the breeds Shih Tzu (5/23), Lhasa apso (3/23) and others (6/23). The work did not show a significant racial predilection, the hypothesis created by the presence of more SRD dogs would be because since they are present in most of the general complaints in the casuistry of this Veterinary Clinic. The same applies to sex and age. More males were found than infected females (15 males to 8 females). The age group was varied, with the youngest being 3 months old and the oldest being 9 years old. The highest frequency appeared in adult dogs, aged 3 to 6 years (13/23).

In the study by [5] variables such as sex, race and age had little influence on the prognosis of patients, except for the German shepherd breed, which was more susceptible to the disease, with a higher rate of infection and death.

An important observation in another study by [10], it was that 7-16 days after the beginning of the treatment of infected dogs the platelet value returned to the reference value, however, the DNA of *E. canis* was still found in splenic samples, showing that the disease was still present, emphasizing the importance of monitoring by specific tests and not just by blood count.

EMC can present variations in the severity of the disease related to the diversity found in different samples found around the world, and in cases of concomitant diseases or co-infection with other pathogens transmitted by the same tick vector [1].

In recent years, case reports of co-infection with multiple organisms that cause "tick disease" in dogs and humans have been published. However, the consequences of co-infection have not been well established, compared to infection with a single organism. According to [8], the combined infection between *A. platys* and *E. canis* is common, which also suggests that infection with *E. canis* contributes to the pathogenesis of *A. platys*.

The presence of clinical symptoms, hematological changes and positive results in serology and / or PCR constitute a more reliable diagnostic pattern in EMC [11].

Conclusion

It was concluded, with this work, that EMC is a disease that leads to varied hematological clinical changes, with fever, apathy and anorexia as the main symptoms and thrombocytopenia, anemia and lymphopenia as the main changes in blood count. Such hematological changes suggest the presence of the disease, as well as the presence of *Ehrlichia canis* morulae in blood smears. The changes found are generally related to the severity of the disease and the stage at which it is found, and animals with pancytopenia at blood count are animals that are usually in the chronic phase and that present more severe symptoms. Despite the hematological changes are suggestive of EMC, it is always necessary to perform confirmatory tests for diagnosis and molecular tests and serology.

Reference

1. Dagnone ASE, Costa MT (2015) *Ehrlichia canis* (Erlíquiose Monocitotrófica Canina – Emc) In: Dagnone, A. S. E Costa, M. T. Doenças Infeciosas De Rotina Em Cães E Gatos No Brasil. São Paulo: Medvep 2015: 177-83.
2. Baker DC (2012) Diagnosis Of Disorders Of Hemostasis. In: Thrall, M. A. Et Al. Veterinary Hematology and Clinical Chemistry. New Jersey: Wiley-Blackwell: 185-205.
3. Almosny NRP (2002) Hemoparasitoses Em Pequenos Animais Domésticos E Como Zoonoses. Rio De Janeiro: L. F. Livros De Veterinária 2002: 13-56.

4. Meneses, Iris Daniela Santos De Et Al (2008) Perfil Clínico-Laboratorial Da Erliquiose Monocítica Canina Em Cães De Salvador E Região Metropolitana, Bahia. *Revista Brasileira De Saúde E Produção Animal* 9: 4.
5. Harrus, Shimon, (1997) Canine Monocytic Ehrlichiosis: A Retrospective Study Of 100 Cases, And An Epidemiological Investigation Of Prognostic Indicators For The Disease. *Veterinary Record*, V. 141: 14: 360-63.
6. Mylonakis, Mathios E, Theodorou, Konstantina N (2017) Canine Monocytic Ehrlichiosis: An Update on Diagnosis And Treatment. *Acta Veterinaria* 67: 299-317.
7. Nakaghi, Andréa Cristina Higa et al. (2008) Canine Ehrlichiosis: Clinical, Hematological, Serological And Molecular Aspects. *Ciência Rural*, 38: 766-70.
8. Santos, Vania Gomes Dos (2008) Clinical Aspects And Laboratories From Cinema, Ehrlichiosis And Borreliosis In Caves (Canis Familiaris, Linnaeus, 1758) Naturally Infected. Thesis De Mestrado. Universidade Federal Rural Do Rio De Janeiro.
9. Silva, Lucilene Dos Santos (2010) Erliquiose E Anaplasose Canina Em Teresina, Piauí. Tese De Mestrado. Universidade Federal Do Piauí.
10. Harrus, Shimon (2004) Comparison of Simultaneous Splenic Sample Pcr With Blood Sample Pcr For Diagnosis and Treatment Of Experimental *Ehrlichia canis* Infection. *Antimicrobial Agents and Chemotherapy* 48: 4488-90.
11. Manoel, Camila Santos (2010) Clinical Alterations, Hematologists and Surgeons Infectious Diseases by *Ehrlichia canis*. Thesis De Doutorado. University of São Paulo.