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Iron metabolism and oxidative profile of dogs naturally infected by *Ehrlichia canis*: Acute and subclinical disease

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ABSTRACT

The aim of this study was to evaluate the oxidant profile and iron metabolism in serum of dogs infected by *Ehrlichia canis*. Banked sera samples of dogs were divided into two groups: negative control ($n = 17$) and infected by *E. canis* on acute ($n = 24$), and subclinical ($n = 18$) phases of the disease. The eritrogram, leucogram, and platelet counts were evaluate as well as iron, ferritin, and transferrin levels, latent iron binding capacity (LIBC), and transferrin saturation index (TSI) concentration. In addition, the advanced oxidation protein products (AOPP) and ferric reducing ability of plasma (FRAP) in sera were also analyzed. Blood samples were examined for the presence of *E. canis* by PCR techniques. History and clinical signals were recorded for each dog. During the acute phase of the disease, infected animals showed thrombocytopenia and anemia when compared to healthy animals ($P < 0.05$) as a consequence of lower iron levels. Ferritin and transferrin levels were higher in both phases (acute and subclinical) of the disease. The AOPP and FRAP levels increased in infected animals on the acute phase; however, the opposite occurred in the subclinical phase. We concluded that dogs naturally infected by *E. canis* showed changes in the iron metabolism and developed an oxidant status in consequence of disease pathophysiology.

1. Introduction

Canine monocytotropic ehrlichiosis (CME) is an important disease with a worldwide distribution. Although CME has been considered specie-specific, studies have shown that this obligate intracellular gram-negative bacterium *Ehrlichia canis* transmitted by ticks *Rhipicephalus sanguineus* [1,2], may also infect other species besides dogs, including man [3].

CME is considered an endemic disease in Southeastern Brazil, showing acute, subclinical, and chronic phases, which are classified according to clinical signs and clinic-pathological abnormalities [3,4]. The acute phase is easily recognizable due to its clinical manifestation that includes: fever, weight loss, anorexia, bleeding disorders, lymphadenomegaly, anemia thrombocytopenia, and leucopenia [5–8]. On the other hand, the subclinical phase shows significant variable length, extending from months to years [9].

Traditional diagnostic techniques (hematology, cytology, serology, and isolation) are considered valuable tools for CME diagnosis. However, the immune response and the oxidant profile of dogs with CME infection also seem to play a central role in disease pathogenesis, especially when it is observed different clinical

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signs and/or laboratory and pathological findings [7,10].

Previous studies demonstrated that early endosomes containing different species of *Ehrlichia* (morulae) upregulate and accumulate the mammalian transferrin receptor [11], suggesting that *Ehrlichia* spp. has developed strategies for its own iron (Fe) acquisition. Besides other functions, Fe is directly involved with electron transport (cytochromes) and oxygen activation (oxidase and oxygenases), and transportation (hemoglobin and myoglobin) [12], and it is, consequently, important in the mechanism of anemia development. By presenting this information, the aim of this study was to evaluate the hematological parameters, iron metabolism, and oxidative profile in serum samples from dogs naturally infected by *E. canis*.

2. Materials and methods

2.1. Animals

Sera samples were collected from dogs naturally infected by *E. canis*; twenty four (24) on the acute phase of the disease (with clear clinical signs) and eighteen (18) with subclinical disease (asymptomatic). As a control group, sera samples from seventeen (17) healthy dogs were used.

Animal history, clinical signs, complete blood count (CBC), and biochemical profile were analyzed and recorded for each dog. Blood samples from all dogs were tested for the presence of *E. canis*, *Babesia canis*, *Babesia gibsoni,* and *Anaplasma plattys* by nested PCR [13–15].

2.2. Hemogram

Blood was collected and stored in tubes with EDTA for CBC. Blood samples were evaluated using an automated blood cell counter (ABC Vet. Horiba ABX – São Paulo, Brazil) to determine red blood cell (RBC) count, hemoglobin concentration (Hb), total leukocytes and platelets counts. Hematocrit was assessed by using the standard microhematocrit method (Centimicro mod. 1-15-Sigma, Germany). Blood smears were also prepared and stained for microscopic examination.

2.3. Iron metabolism

Iron (Fe) metabolism was assessed through the evaluation of the following variables: serum iron and latent iron-binding capacity (LIBC), through commercial kits (Labtest, Minas Gerais, Brazil) on a semi-automatic analyzer Bio-2000 (Bio Plus Ltda, São Paulo, Brazil). All glassware used was previously soaked into 10% hydrochloric acid for 3 h and rinsed with deionized water (Milli-Q system from Millipore Corporation). Transferrin and ferritin concentrations were assessed using an automated immunoturbidimetry (Labtest, Minas Gerais, Brazil). Additionally, it was estimated the transferrin saturation index (TSI).

2.4. AOPP and FRAP levels

Protein oxidation status was evaluated through the measurement of AOPP concentrations as described by Hanasand et al. [16]. Levels of ferric reducing antioxidant power (FRAP) was measured according to the technique described by Benzie and Strain [17] in sera samples. AOPP and FRAP results were expressed as μmol L^{-1} according to the modified Griess method using the Cobas Mira automated analyzer.

2.5. Statistical analysis

Firstly, the data were subjected to normality test, where we verified a normal distribution. Then, the data were subjected to Tukey test. Values with probability (p) less than 5% were considered statistically different. Data were presented as mean values ± standard deviation.

3. Results

3.1. Clinical signs and hematological parameters

As expected, control group and subclinically *E. canis* infected animals did not show clinical signs of the disease. However, dogs with *E. canis* acute infection showed several clinical signs, such as: apathy, appetite loss and intermittent fever. It is important to emphasize that more than 40% of the dogs with acute infection were also parasitized by ticks. Serology was negative for *B. canis*, *B. gibsoni* and *A. plattys*.

Peripheral blood smears showed hematological changes in animals infected by *E. canis* (Table 1). According to our results, it was possible to observe that animals with the acute phase the CME had a significant decrease ($P < 0.05$) in erythrocyte, hematocrit, hemoglobin and platelet counts when compared to healthy animals. For erythrocyte, hematocrit, hemoglobin and platelet parameters, no significant differences were observed on *E. canis* infected animals with the subclinical disease compared to healthy animals.

Leukocyte, lymphocyte, neutrophil, eosinophil and monocyte counts did not differ during the acute or subclinical phases of the disease when compared to healthy animals. In addition, the bands number, during the acute phase of disease, were higher in infected animals on the acute phase when compared to infected animals on the subclinical phase of CME and to healthy animals.

3.2. Iron metabolism

Iron, ferritin, transferrin, LIBC, and TSI levels are shown in Table 2. *E. canis* infected animals showed a significant increase of ferritin, transferrin, and TSI levels during the acute phase of CME, when compared with healthy animals. Additionally, Fe level significantly decreased during the acute phase of CME compared to healthy animals. Also, ferritin and transferrin levels increased on animals on subclinical disease in comparison to the control group ($P < 0.05$). There were no differences on Fe and TSI levels on infected animals with the subclinical phase of CME. Furthermore, LIBC serum levels did not differ on *E. canis* infected animals.

Ferritin, transferrin and TSI levels were lower in infected animals during subclinical phase, when compared with animals on the acute phase. However, Fe levels were higher in infected animals on subclinical phase than in infected animals on the acute phase of disease.

3.3. AOPP and FRAP levels

AOPP and FRAP results are shown in Table 2. Animals on acute CME presented increased levels of AOPP and FRAP on sera when compared to healthy animals. Similarly, FRAP levels increased in dogs on the subclinical phase when compared to the control group ($P < 0.05$). There were no differences on AOPP levels in infected animals on the subclinical phase of CME. AOPP and FRAP levels were significantly lower in infected animals on the subclinical disease, when compared to dogs on the acute phase of disease.