Hematological evaluation of dogs naturally infected by *Leishmania (Leishmania) chagasi* submitted to treatment with meglumine antimoniate

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Abstract

The present research was carried out aiming to assess the hematological response of dogs with visceral leishmaniasis submitted to treatment. For this, seven animals naturally infected by *Leishmania sp.* were submitted to a treatment with 75 mg/kg meglumine antimoniate subcutaneously, 12-12h / 3 weeks. In all animals, a complete blood count and bone marrow aspiration biopsy were carried out for a descriptive evaluation at up to seven moments: before the treatment, 30, 60, 90, 120, 150 and 180 days after the start of the treatment. Before the beginning of the experiment hematological alterations were observed in four of the seven dogs (57.1%), among them, nonregenerative anemia, lymphopenia, lymphocytosis and monocytosis. During the course of the experiment the occurrence of leukocytoses, such as left shift neutrophilia and eosinophilia, were observed in some of the animals. Before the beginning of the treatment (M1), the occurrence of erythrocytic hypoplasia was detected by bone marrow cytology in two of the dogs (28.6%). This was reversed through an increase in the amount of erythroid progenitor cells after the administration of meglumine antimoniate. Thus, it can be concluded that the treatment led to normalization of the hematological alterations and recovery of the bone marrow.

Key words:
Dogs.
Leishmania sp.
Complete blood count.

Introduction

Visceral leishmaniasis is a disease caused by a protozoan belonging to the order *Kinetoplastida*, family *Trypanosomatidae* and genus *Leishmania*.[1,2,3] In the Americas the etiological agent is *Leishmania (Leishmania) chagasi*.[1,2,4] The infection usually causes a chronic systemic disease. However, depending on the properties of the parasite and on the immune competence of the host, the evolution may be acute and severe, leading the animal to death in a few weeks.[5,6] The clinical manifestations of the disease in dogs and humans are similar and include fever for long periods, anemia, progressive loss of weight and cachexia in its final stage.[7] In the lymphoid organs, the proliferation of B lymphocytes, plasmocytes, histiocytes and macrophages may result in generalized lymphadenomegaly and hepatosplenomegaly.[5,8,9,10]

Dermatological alterations are very frequent in animals with visceral leishmaniasis and they may occur in the absence of other symptoms.[10] The buildup of immune complexes in the kidneys occasionally results in proliferative glomerulonephritis and, in many cases, in interstitial nephritis, which can lead to kidney insufficiency.[3,8,9,11,12,13,14,15] *Leishmania* also multiply within macrophages in the liver, producing an active chronic hepatitis and, occasionally, hepatomegaly, vomiting, polyuria, polydipsia, anorexia and weight loss.[3,8,11,13,16] Some animals present chronic diarrhea and melena due to the presence of ulcerations in the gastric and

intestinal mucosa. Enteritis may be a result of direct parasitic damage [11] or a consequence of kidney insufficiency[8,9,17]. It is possible to observe animals with locomotor, respiratory, cardiac, ophthalmic and neurologic alterations.[11]

Dogs with leishmaniasis may present hemorrhagic diatheses such as hematuria, petechia, suffusions, and mainly, epistaxis. Besides the occurrence of ulcerations in the nasal cavity, other causes for the occurrence of hemorrhages include vasculitis, uremia, splenic sequestration of platelets and, occasionally, thrombocytopenia due to aplasia or bone marrow hypoplasia.[2,11,18,19,20]

Pentavalent antimonials, particularly meglumine antimoniate, are the medications of choice in the treatment of human visceral leishmaniasis, and have been used as treatment protocol for dogs.[21] The mechanism of action of these medications have not been completely established, but it is known that they act on the amastigote forms of the parasite, blocking their metabolism by inhibiting the glycolitic activity and the oxidation pathway of fatty acids, being, thus, considered leishmanicidal.[1,21] Pentavalent antimonials cause reduction of the symptoms and, in some cases, even clinical cure of the dogs.[22,23,24,25,26]

The occurrence of blood alterations in dogs with visceral leishmaniasis is very important and frequent. The erythrogram shows anemia, generally normocytic normochromic nonregenerative in 57 to 94.2% of the animals.[2,8,9,27] Anemia in visceral leishmaniasis results from several causes, among them splenic sequestration of red blood cells, decrease in bone marrow production, chronic renal failure, blood loss, hemolysis and immune-mediated mechanisms.[2,9,27,28,29]

With regards to the alterations present in the leukocyte series, some authors report normal total leukocyte count [8], others describe the presence of leukocytosis associated to neutrophilia [30], while others report the occurrence of leucopenia [10,31]. There are discrepancies in the literature concerning the occurrence of lymphocytosis or lymphopenia in dogs with visceral leishmaniasis. Bourdoiseau et al. [32] carried out a study comparing the lymphocytic abnormalities of seropositive dogs with a mild clinical picture, with a severe clinical picture and seronegative dogs and concluded that the seropositive animals with a good clinical status presented lymphocytosis, whereas those with a severe clinical picture presented lymphopenia. On the other hand, Ikeda et al.[27], in a retrospective study on the hematological alterations of 191 dogs naturally infected by *Leishmania (L.) chagasi,* observed that lymphocytosis as well as lymphopenia occurred in animals regardless of the severity of their clinical pictures. Furthermore, the observation of monocytosis is common [27,33,34], and it can be accompanied by the presence of large activated monocytes [27].

Animals with visceral leishmaniasis may present normal platelet counts or thrombocytopenia.[2,10,12] The possible causes for thrombocytopenia are the formation of self-antibodies, splenic sequestration and bone marrow suppression.[8,10,35]

Despite the scarce literature concerning bone marrow cytology of dogs with visceral leishmaniasis, the occurrence of hyperplasia of precursors of neutrophilic granulocytes has been reported, thus enhancing the myeloid:erythroid relation (M:E). An increase in the population of monocytes and macrophages and the elevation in the number of plasmocytes and Mott cells also occur, which may indicate antigenic stimulation associated to infection.[30,33,34]

Hence, the present study aimed to verify the hematological response of dogs with visceral leishmaniasis submitted to a treatment with meglumine antimoniate.

Material and Method

Seven pet dogs naturally infected by *Leishmania sp.*, four of them male and three females, of varied breeds and with ages ranging from seven to sixty months, were used. The diagnosis of the disease was

reached by the identification of amastigote forms of *Leishmania sp.*in the cytological examination of lymph node and bone marrow aspirates and confirmed by enzyme immune assay (ELISA). The serum samples collected from these dogs were screened to rule out the possibility of co-infection with *Ehrlichia canis* e *Babesia canis*. The indirect immunofluorescent antibody test was performed to detect antibodies to *B. canis*, following the protocol described by Machado[36], and an enzyme immunoassay test (SNAP*3Dx*-IDDEX Laboratories) for the detection of *E. canis* antibodies. Before the beginning of the experiment the animals were dewormed with a combination of praziquantel, pyrantel palmoate and febantel. During the whole experimental period the dogs were fed with balanced commercial dog food (Selection Special Croc Evolution® - Royal Canin), were given water *ad libitum*, wore deltamethrin antiparasitic collar (Scalibor® - Intervet Production S.A.), and were kept in a screened kennel to avoid reinfection.

The dogs were submitted to a treatment with 75 mg/kg meglumine antimoniate (Glucantime® - Aventis Pharma Ltda) subcutaneously, 12-12h / 3 weeks. A complete blood count (erythrogram, leukogram and qualitative platelet count) and a descriptive myelogram were carried out at seven moments: M1- before the beginning of the treatment; M2- 30 days; M3- 60 days; M4- 90 days; M5- 120 days; M6- 150 days and M7- 180 days after the beginning of the treatment.

The total cell count was carried out by an automated blood cell counter (Celm – CC510). Hemoglobin determination was carried out by the hemiglobincyanide method (CELM E – 205D spectrophotometry) and packed cell volume by the microhematocrit method (SIGMA 1-13 microhematocrit centrifuge). The calculation of hematimetric indices was carried out according to Jain [37]. The differential count of 100 leukocytes and the morphological and qualitative evaluation of the platelets were processed in smears stained with quick hematological stain, according to Jain's recommendations and criteria. [38] The bone marrow was collected with an aspiration biopsy needle (Monoject®), through a puncture on in the iliac crest of the animals. Immediately after puncturing five smears were prepared for each animal. The slides were stained with hematological stain (Panótico Rápido® – Laborclin – Curitiba, PR) and observed under an optical microscope with 100 times magnification for descriptive evaluation. In cell differentiation, Harvey's classification was used. [39] In compliance with the recommendations of the Brazilian Ministry of Health, after the 180 days of follow-up, the dogs were submitted to euthanasia with 15 mg/kg intravenous sodium pentobarbital (Hypnol 3% - Fontoveter – Itapira, SP), followed by an ampule of 10 mL potassium chloride (Cloreto de potássio a 19,1% - Darrow – Rio de Janeiro, RJ).

Results, Discussion and Conclusion

The haemathological analysis of the seven dogs area summarized in table 1. Four animals (57.1%) presented anemia, verified by the decrease in the number of erythrocytes, hematocrit and hemoglobin, before and 30 days after the beginning of the treatment (M1 e M2). M.C.V. and M.C.H.C. indices were within the normal range described by Jain[37] at all moments, characterizing an anemia of the normocytic normochromic type, which corroborates the descriptions of Abranches et al.[31], Ciaramella et al.[12], Koutinas et al. [9] and Ikeda et al.[27], who reported that this type of anemia is the most frequent in the cases of visceral leishmaniasis. Another reason for anemia was ruled out by means of serological methods and by cytological examination of bone marrow smears. In general, the values of the erythrocytic series increased after the treatment, with the disappearance of the anemic picture between sixty and ninety days after the beginning of the treatment (M3 e M4). However, one dog (14.3%) presented anemia again 120 and 180 days after the

Table 1 - Haematological analysis before the beginning of the treatment (M1), 30 days (M2), 60 days (M3), 90 days (M4), 120 days (M5), 150 days (M6) and 180 days (M7) after the beginning of the treatment. Araçatuba- SP, 2008

	Haematological alterations						
Dog	M1	M2	M3	M4	M5	M6	M7
1	Anaemia and lymphopenia	anaemia	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	monocytosis
2	Anemia, leukopenia and lymphopenia	Anaemia and leukocytosis due to left shift neutrophilia	normal haemathological evaluation	normal haemathological evaluation	anaemia	normal haemathological evaluation	anaemia
3	normal haemathological evaluation	eosinophilia	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation
4	Anaemia and lymphopenia	Anaemia, leukocytosis due to left shift neutrophilia and eosinophilia	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation
5	Anaemia, leucopenia and lymphopenia	Anaemia, leukocytosis due to left shift neutrophilia and eosinophilia	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation
6	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation
7	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation	normal haemathological evaluation

beginning of the treatment. At 120 days the identification of the etiology was not possible, as the animal did not present any clinical alteration. At 180 days, however, amastigote forms of the parasite were detected through lymph node and bone marrow aspiration biopsy and liver and spleen imprint, which explains the anemic picture as a result of the multiplication of the parasite in several organs, including in the bone marrow.

Before the beginning of the treatment erythrocytic hypoplasia was observed in the bone marrow cytology of two of the dogs (28.6%), which agrees with the findings of Anosa and Idowu[30], Yamaguchi et al.[34] and Buracco et al.[33], who reported that animals with visceral leishmaniasis presented bone marrow hypoplasia, and also with the reports of Kontos and Koutinas[8], Koutinas et al.[9], Ciaramella and Corona [2] and Ikeda et al.[27], who affirmed that the anemia in dogs with visceral leishmaniasis is nonregenerative. After the administration of meglumine antimoniate, all of the anemic animals presented a regenerative erythrocytic response, evidenced by an increase in the number of erythroid progenitor cells in the bone marrow, and by the presence of polychromatophilia, polychromatic rubricytes and metarubricytes in the peripheral blood.

Of the seven dogs, two (28.6%) presented leukopenia, whereas the other five (71.4%) had normal leukocyte count in the first evaluation. Such observations disagree with Abranches et al.[31], who observed leukopenia in 100% of the dogs studied, and corroborate the reports of Kontos and Koutinas[8] who affirmed that the occurrence of alterations in the number of leukocytes in dogs with visceral leishmaniasis is rare. Thirty days after the beginning of the treatment three animals (42.9%) presented leukocytosis due to left shift neutrophilia, associated to the presence of crepitant rale in lung fields, indicating pneumonia, which was confirmed by radiographic examination. In humans, pentavalent medications may provoke disturbances in several organs, among them, the lungs.[40] Nonetheless, such observations have not been described so far in veterinary medicine literature. Two dogs (28.6%) presented eosinophilia associated to leukocytosis due to neutrophilia, probably resulting from the pulmonary picture. Besides these, another animal presented eosinophilia 30 days after the beginning of

the treatment (M2), which was associated to an allergic reaction to a deltamethrin collar. The occurrence of eosinophilia is common in alterations of tissues containing a great amount of mastocytes, such as the skin and the lungs.[41]

During the experiment four dogs (57.1%) presented lymphopenia that could not be related to the severity of the clinical picture, since other animals with a similar clinical picture had lymphocyte count within the normal limits. Although all animals presented monocyte count within the normal limits in the beginning of the experiment, one animal presented monocytosis 180 days after the beginning of the treatment (M7), when amastigote forms of the parasite were evidenced again by the lymph node and bone marrow aspiration biopsies. When the first blood count was performed, one of the dogs (14.3%) presented a normal leukogram, but with the presence of activated monocytes.

Before the beginning of the experiment two animals (28.6%) presented granulocytic hypoplasia in the bone marrow cytology, associated to leukopenia in the blood count, and in the course of the experiment, three dogs presented granulocytic hyperplasia, with leukocytosis in the blood count. An increase in the number of macrophages and monocytes shown by bone marrow cytology was not observed in any of the animals, which disagrees with the findings of Anosa and Idowu[30], Yamaguchi et al.[34] and Buracco et al.[33], who stated that an increase in the number of monocytes and macrophages is very frequent in dogs with visceral leishmaniasis. In the course of the experiment one of the alterations observed through the myelogram was the occurrence of plasmocytosis in two dogs, probably due to the antigenic stimulation provoked by the infection.[30,33,34] No animal presented alterations in the values of fibrinogen determination or in the qualitative platelet count, which disagrees with the reports of Slappendel and Ferrer[10], who verified the occurrence of thrombocytopenia in 50% of the animals studied.

Thus, it can be concluded that the treatment promoted normalization of the hematological alterations and recovery of the bone marrow. Nevertheless, as the treatment was not able to promote parasitological healing, in some of the animals the hematological abnormalities reappeared.

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Avaliação hematológica de cães naturalmente infectados por *Leishmania* (*Leishmania*) *chagasi* submetidos a tratamento com antimoniato de meglumina

Resumo

A presente pesquisa foi realizada com o objetivo de avaliar a resposta hematológica de cães com leishmaniose visceral submetidos a tratamento. Para tanto, sete animais naturalmente infectados por *Leishmania sp*. foram submetidos a um tratamento com 75 mg/kg de antimoniato de meglumina por via subcutânea, 12-12 h /3 semanas. Em todos os animais, uma contagem hematológica completa e punção biópsia aspirativa de medula óssea foi realizada para uma avaliação descritiva em até sete momentos: antes do tratamento, 30, 60, 90, 120, 150 e 180 dias após o início do tratamento. Antes do início do experimento foram observadas alterações hematológicas em quatro dos sete cães (57,1%), entre eles, anemia não regenerativa, linfopenia, linfocitose e monocitose. Durante o curso do experimento a ocorrência de leucocitose, como neutrofilia com desvio à esquerda e eosinofilia,

Palavras-chave:
Cães.
Leishmania sp.
Hemograma.

foram observadas em alguns dos animais. Antes do início do tratamento (M1), a ocorrência de hipoplasia da série eritrocítica foi detectada pela citologia de medula óssea em dois animais (28,6%). Isto foi revertido por um aumento na quantidade de células eritróides progenitoras após a administração de antimoniato de meglumina. Desta forma, pode-se concluir que o tratamento promoveu a normalização das alterações hematológicas e recuperação da medula óssea.

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