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# HEMATOLOGICAL CHANGES IN DIFFERENT CLINICAL FORMS AS A COMPLEMENTARY DIAGNOSTIC TOOL IN CANINE LEISHMANIASIS[1]

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## ABSTRACT

The aim of this study was to evaluate the alterations in hematology parameters in relations to the different clinical forms in seropositive dogs to *Leishmania infantum (L. infantum)*. Thirty seropositive dogs for *Canine leishmaniasis* (CanL) confirmed with an indirect fluorescent antibody test (IFAT), according to the clinical signs were allocated to three equal groups: asymptomatic (AD), oligosymptomatic (OD) and symptomatic dogs (SD). Blood samples were collected for hematology parameters analysis in all groups. A significant anemic profile was observed in the SD that correlates to the presence of specific symptoms in SD. Reduced granulocytes count and percentage in the SD and increased number of lymphocytes in the OD indicates on the complexity of immune response in the different clinical forms of CanL. The hematological changes can be considered a useful complementary tool for further evaluation of disease progression and prognosis in different clinical forms of CanL.

**Keywords:** Leishmaniasis, clinical form, hematology, dogs.

[1] original scientific paper

## INTRODUCTION

Leishmaniasis is a life-threatening vector-borne disease, caused by obligate intracellular protozoan parasites of the genus *Leishmania*. *Leishmania infantum* (syn. *Leishmania chagasi*) has been identified as the causative agent of *Canine Leishmaniosis* (CanL) and is considered a leading factor of visceral leishmaniosis (VL) (Gramiccia, Gradoni, 2005). Domestic dogs are major reservoirs for urban and periurban settings, playing a key role in parasite transmission (Ciaramella,et al., 1997). The disease is transmitted by the bite of blood-suckling sandflies (*Flebotomus* and *Lutzomyia genus*). Possible transmissions via vertical, venereal and transfusional routes were also reported (Svobodova et al., 2017). The parasite has a biphasic life cycle that includes two hosts, the mammalian host and the insect vector. In mammal, the promastigote form is deposited by the vectors bite into the skin and been phagocytized by the host cells of the reticuloendothelial system predominantly macrophages. Here, promastigote form evolves into the amastigote form, leading to macrophage destruction (Gharbi et al., 2015; Bates et al., 2007). Invading the mononuclear phagocytic system in many organs (bone marrow, liver, lymph node and spleen), parasite activates the host defense components of both, innate and adaptive immunity. Susceptibility is related to high parasite load and dissemination, leading to polyclonal B cell proliferation and plasma cell differentiation with high antibody production (Giunchetti et al., 2019). Parasite load, pathological progression and immune response are in a direct relationship with clinical manifestations. Since the parasite can load various organs, the disease can be presented with broad clinic pathological manifestations including the various clinical signs, hematological and histological changes. The diversity of clinical signs includes the general, nonspecific spectrum, cutaneous signs which are the most frequent, ocular manifestations and signs related with the severe disease progression like chronic renal failure (Solano-Gallego et al., 2011). Two clinical staging has been proposed with a good level of agreement between them (LeishVet system and Canine Leishmaniasis Working group (CLWG)) (Proverbio et al., 2016). In previous study dogs were grouped into three groups, according to clinical forms of CanL,: asymptomatic dogs (AD), oligosymptomatic (OD), and symptomatic dogs (SD) (Manciantiet al., 1988). Although this categorization according to some authors has limited value (Solano-Gallego et al., 2011), in the recent review was described as a favored approach of clinical form categorization and correlation with immunopathological features of CanL (Giunchetti et al., 2019). Despite the fact that the diagnosis of CanL requires the serological or

molecular parasite confirmation, some authors found very useful performing the routine hematological test investigating the hematology alterations caused by the *L. infantum* infections (Freitas et al., 2012; Ulchar et al., 2015; Torrecilha at al., 2016). The most frequent hematological findings were alterations in erythrogram status consistent with anemia, low red blood cell count (RBC), low hematocrit values (HCT) and low hemoglobin values (HGB). Inconsistency has been reported regarding findings on the relation between anemia and the severity of the disease (Ribeiroat al., 2013). Leukocytosis with neutrophilia and eosinopenia has been described as a typical alteration in white blood cell count (Freitas et al., 2012; Torrecilha at al., 2016). Data about additional hematological parameters and the relation with other cell count values are very few and changes are considered insignificant (Ulchar at al., 2015). Regarding the abovementioned this study is yet another attempt to identify the usefulness of the hematological aspect in evaluating the clinical status in dogs naturally infected by *L.infantum*.

## MATERIALS AND METHODS

Thirty dogs (n=30) of different breeds were enrolled in this study. The criteria for the inclusion of the subject were: adult dogs (no younger than 2 years, to exclude the subject with possibility of immature immune system) and seropositive dogs for CanL confirmed with an indirect fluorescent antibody test (IFAT) performed according to the World Organization for Animal Health (OIE) manual (≥1:40 dilutions) (OIE, M., 2000).

All included dogs were brought in to the clinic for evaluation of the eventual presence of clinical signs and sampling for additional hematology investigations. According to the presence or absence of clinical signs, the infected dogs were categorized as: asymptomatic dogs (n=10) (AD - with no external clinical signs,), oligosymptomatic dogs (n=10) (OD - no typical signs for leishmaniosis infection: lymphadenopathy, lethargy, fever, anorexia, n=10) and symptomatic dogs (n=10) (SD - dogs with typical clinical signs for CanL: fever, lymphadenopathy, weight loss, emaciation, hepatosplenomegaly, conjunctivitis, keratitis, onychogryphosis, cutaneous lesions) (Mancianti et al., 1988).

For hematology analysis, blood samples were collected once from *Vena cephalica antebrachii externa* using tubes containing anticoagulant ethylendiamineteraacetic acid (EDTA).The analysis was performed by the automated blood analyzer Humacount 30ST – veterinary mode. The analysis involved: red blood cell count (RBC), white blood cell count (WBC), lymphocytes count (LYM), lymphocyte percentage (LYM%), monocytes

count (MON), monocyte percentage (MON%), granulocytes count (GRA), granulocyte percentage (GRA%), hematocrit (HCT), hemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) count. The results were compared to the reference values for canine species according to Moritz at al. (2004). Parameters considered consistent to anemia: red blood cell count (RBC), hematocrit values (HCT), hemoglobin values (HGB) and additional parameters which allow categorization of anemia (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). Comparisons between groups were made using the analysis of variance (ANOVA) followed by Tukey's test. Possible association between laboratory findings and different groups were analyzed using Fisher's Exact Test. Spearman correlation method was performed for the analysis of the correlation between clinical form and laboratory parameters. Statistical analyses were performed by using SPSS software (SPSS for Windows, version 20, SPSS Inc, Chicago, IL, USA). Significance level was set at 95% and p value less than 0.05 was considered significant.

## RESULTS

The results of the hematology parameters evaluation are presented in Table 1. The most alterations from the reference range were observed in SD. We found reduced mean value of number of RBC cells and reduced mean value in number and percentage of GRA, but increased percentage of LYM in SD compared to reference values. Significantly decreased mean value of HCT and HGB, have been observed in SD. Increased MCHC concentration was observed in OD, but the differences compared to other clinical forms were not significant. Significant differences were observed between the OD and AD in the MCH values. Differences observed between the three clinical forms in mean value of PLT were significant, although there was no alteration from the reference values. Significantly increased frequencies of hematology alterations consistent with anemia were observed in SD compared to the other two groups of dogs ($p<0.05$, Fisher's Exact Test) (Figure 1). Statistical analysis indicated significant correlation between clinical forms and alterations consistent to anemia ($p < 0.05$, Spearman) (Table 2). Negative correlation was observed between RBC and MCV in SD (Table 3).

**Table 1**: Hematological parameters in dogs naturally infected and seropositive on *L. infantum* with different clinical forms.

| Parameters | Clinical forms | | | |
|---|---|---|---|---|
| | Reference interval | Symptomatic dog (SD) n=10 | Oligosymptomatic dog (OD) n=10 | Asymptomatic dog (AD) n=10 |
| **WBC** | 5.84-20.26 ($\times 10^9$/L) | 8,79 ± 6,66 | 12,70 ± 3,02 | 14,42 ± 4,03 |
| **LYM** | 2.04-4.66 ($\times 10^9$/L) | 4,12±2,60 | **4,93±1,24** | 4,46±1,44 |
| **MON** | 0.24-2.04 ($\times 10^9$/L) | 1,70 ±1,05 | 2,01 ±1,01 | 1,11± 0,54 |
| **GRA** | 4.38–10.34($\times 10^9$/L) | **3,24 ±3,20**[a] | 7,59±3,16[b] | 6,83±3,09[b] |
| **LYM%** | 18.97-41.28 (%) | **50,86±10,01**[a] | 37,62±8,92[b] | 38,46±11,32[b] |
| **GRA%** | 47.57-83.66(%) | **28,73 ±14,41**[a] | 63,21 ±20,33[b] | 55,80 ±15,50[b] |
| **RBC** | 5.68-9.08 ($\times 10^{12}$/L) | **4,55±1,12**[a] | 5,76±1,42[b] | 6,22± 0,79[b] |
| **PLT** | 173.05-486.50 ($\times 10^9$/L) | 422,10±245,54[a] | 263,30±121,76[b] | 222,4±124,50[c] |
| **HGB** | 137–203(g/L) | **109,70 ±27,10**[a] | 147,60 ±31,08[b] | 139,20 ±17,95[b] |
| **HCT** | 42–62 (%) | **33,48 ± 8,98**[a] | 44,62 ± 7,78[b] | 43,90 ± 6,39[b] |
| **MCV** | 62.7-74.56 (fL) | 68,70 ± 5,69 | 68,60 ± 8,54 | 69,60 ± 6,34 |
| **MCH** | 20.46-24.81 (pg) | 22,35 ±1,15 | 23,25 ±1,97[a] | 22,56 ±1,66[b] |
| **MCHC** | 316–343 (g/L) | 333,70±29,66 | **351,60±39,13** | 319,40±17,14 |

Means ± standard deviation in bold are above or below the reference interval; $p<0.05$ indicates a significant difference among clinical forms; [a,b,c] differences between the values involving different letters on the same row is found to be statistically significant.

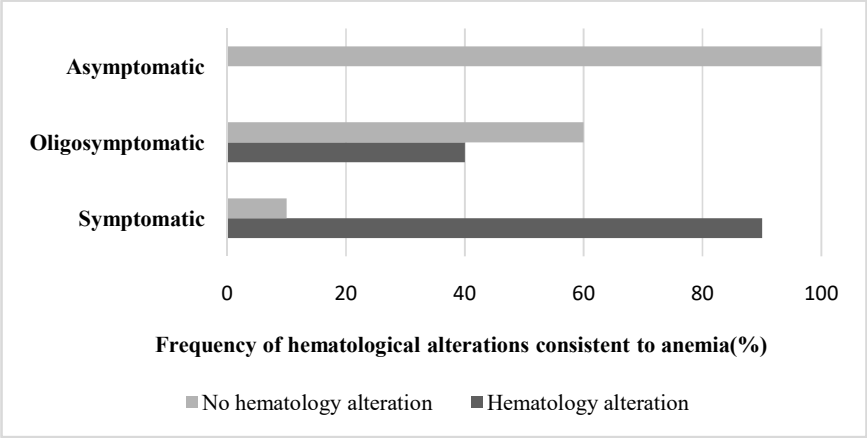


**Figure 1:** Frequency of hematological alteration consistent to anemia (%) (Reduced RBC, HCT and HGB)

**Table 2:** Correlation between clinical forms and hematology alterations consistent with anemia in dogs naturally infected and seropositive on *L. infantum*

| | WBC | RBC | HGB | HCT | PLT |
|---|---|---|---|---|---|
| **Clinical forms** | r = 0.328<br>p = 0.076 | r = 0.539<br>p = 0.002 * | r = 0.386<br>p = 0.034* | r = 0.396<br>p = 0.03* | r = 0.347<br>p = 0.059 |

$p < 0.05$ indicates a significant correlation between hematology parameters and clinical forms (SD, OD, AD)

**Table 3:** Correlation between some hematology parameters in dogs with overt specific clinical symptoms of *L. infantum* infection

| | RBC | | HGB |
|---|---|---|---|
| MCV | r = - 0.662<br>p = 0.72 | MCH | r = 0.177<br>p = 0.34 |

r indicates on correlation between parameters related to anemia and hemoglobin concentration.
$p < 0.05$ indicates a significant correlation between parameters related to anemia and hemoglobin concentration

## DISCUSSION

In this study, data provided during the evaluation of hematology parameters in dogs infected with *L. infantum*, pointed to alterations which are mostly

observed in severe clinical cases with overt specific clinical symptoms. Hematology parameter alterations were prominent in dogs with the most severe clinical form, which is in accordance with the findings that the cytological alterations and the parasite load increase as soon as the dogs show clinical signs (De Abreu, et al., 2011). Regarding the clinical manifestations, dogs were categorized into three groups according to the categorizations used by other authors (Mancianti, et al., 1988; Ribeiro, at al., 2013). Dogs were seropositive to *L. infantum*, but not all manifested specific symptoms for CanL. Asymptomatic dogs may be considered as exposed or infected but clinically normal or dogs with signs associated with other diseases with no clinicopathological abnormalities related to CanL. The other two categories were based on manifestation of clinical symptoms. Dogs with symptoms nonspecific to CanL were referred as OD and dogs with symptoms specific to CanL and clinicopathological abnormalities suggestive of leishmaniasis, referred to as SD (Proverbio, et al., 2016). The SD had exaggerated manifestation of common signs established as the most indicative to CanL (Solano-Gallego, at al., 2009). The categorization of seropositive dogs, as it is recommended by other authors (Ribeiro, et al., 2018), was to enable a more accurate approach in evaluation of hematology profile in dogs within a different clinical stage.

Anemia is the most common finding in CanL (Temizel, et al., 2011; Ribeiro, at al 2013) and also it was the most significant in SD in this study. Low RBC, compared to the reference level, was accompanied by a low level of HGB and a low percentage of HCT in SD. These values were also significantly lower compared to the other two groups of dogs. A significant positive correlation has been found between hematology parameter alterations consistent with anemia and clinical form, which is in accordance with findings to other authors (Ribeiro, et al., 2013). According to most studies, more than 50% of patients were presented with normocytic/normochromic and nonregenerative anemia (Ribeiro, et al., 2013;Ribeiro, et al., 2018). But some authors, in dogs with severe clinical manifestations, described normocytic hypochromic anemia presented by decreased MCHC paired with the normal level of MCV which is indicative for iron deficiency (Torrecilha, et al., 2016). Pavel at al., (2017) also reported normochromic regenerative type anemia since reticulocyte count was significantly above the reference level. Compared to reference range in MCV and MCHC level in SD, no significant alterations were observed, indicating normocytic and normochromic anemia. In this study, no reticulocyte count and no bone marrow evaluation could be performed in order to determine whether dogs have regenerative or nonregenerative anemia. However, increased MCV in anemic dogs and in humans is thought

to be associated with the regenerative type of anemia (Paltrinieri, at al., 2016). Data analysis reveals negative, yet no significant, association between MCV and RBC in SD, meaning that lowering the number of RBC is followed by a slightly increased level of MCV. This means that the bone marrow compensates RBC requirements into circulation, releasing young not fully matured RBC into peripheral circulation which is indicative of regenerative anemia. Thus, the anemia in symptomatic dogs could be considered poorly regenerative since no significant increase of MCV was observed. To elucidate the genesis of anemia, the levels of HCT and HGB concentration have also been considered. Hematocrit and HGB were below the reference level and significantly low in SD compared to the other two groups and corroborate with previous studies (Freitas, at al., 2012; de Carvalho Nicolato, at al., 2013). Reduced RBC count associated to reduce level of HGB due to impaired erythropoiesis, could be the main reason for low HCT and the lowest level of MCH (Reis, at al., 2009). Some authors have suggested that despite the hemodilution due to decreased erythropoiesis and/or increased hemolysis, polydipsia could also contribute to the low HCT (Ulchar, at al., 2015). Low WBC count, but still within the reference range and no significant changes, were also observed in the SD. These results are in agreement with previous studies (Freitas et al., 2012; Ulchar, at al., 2015).The GRA were significantly decreased in the SD. Significantly higher GRA, especially neutrophils and eosinophils, were observed in different segments of intestinal tissue, lymph nodes (Silva, et al., 2018), skin (Reis, et al., 2009), liver (Giunchetti, at al., 2008) and spleen (Barrouin-Melo, at al., 2006) in naturally or experimentally infected dogs with *L. infantum*. These studies suggest on an increased demand of granulocytes as an inflammatory response in multiple organs affected by parasitism. Despite that neutrophils are the most abundant leucocytes and the first cells to be recruited to the site of infection (Giunchetti,et al., 2008), some authors have reported decreased neutrophils and eosinophils counts due to increased migration and trapping in affected tissues (Alvar, at al., 2004). In addition, possible depletion of myeloid lineage affected by an intense bone marrow parasitism, may also contribute to decrease GRA count in dogs within SD. Lymphocytes were above reference interval in the OD, although these alterations were not significant compared to the other groups. These results corroborate with findings that in hosts bearing mild disease, immune response becomes regulated by the increased number of circulating T lymphocytes (both CD4(+) and CD8(+) T cells) creating a microenvironment efficient to remove the parasites (Reis, at al., 2006). Lymphocytosis in bone marrow, but not in peripheral blood, reported by de Carvalho Nicolato at al., (2013) is explained as a compensatory response that provides lymphocytes to target

organs affected by the parasite, which is reflected by the peripheral blood lymphopenia observed in advanced stages of CanL. These findings may explain the increased percentage of lymphocyte but not in their total count in peripheral blood in the SD. Mild to moderate thrombocytopenia have been reported to be frequent in CanL and is due to possible peripheral consumption of circulating PLT (Paltrinieri, at al., 2016). Moreover, decreased production due to the depressed bone marrow activity and dysmyelopoiesis may be the additional cause of low PLT in peripheral blood.

## CONCLUSION

The results in this study points out the association of hematology alterations and the clinical form in seropositive dogs. Anemic profile with low level of RBC, HCT and HGB were the most prominent hematological changes in dogs with clear specific clinical symptoms, indicating on the severity and progression of the disease. The WBC reflects the differences in immune response in different clinical forms in CanL and is not always relevant indicator for disease progression. Further analysis can be conducted to evaluate the follow up of the hematology parameter changes in seropositive dogs with nonspecific clinical manifestation or the ones with no clinical symptomatology suggestive to CanL. Asymptomatic dogs are of great importance for long term monitoring since these groups are considered the most important in disease spreading. Overall, the hematology analysis could be a useful clinical tool in the evaluation of disease progression and prognosis.

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