Haematological profiles in canine monocytic ehrlichiosis: a retrospective study of 31 spontaneous cases in Greece

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SUMMARY
This study investigated the haematological parameters in 31 dogs naturally infected with Ehrlichia canis according to clinical signs and serological analyses. Whereas the platelet numeration was depressed in 35.48% dogs, anomalies in erythrocyte numeration and morphology were frequently observed: haemoglobin concentration, haematocrit and erythrocyte numeration were decreased in 25.80%, 19.35% and 19.35% infected animals, respectively, red cell distribution width was enlarged in 80.64% of cases and macrocytosis evidenced by high mean corpuscular volume and confirmed by microscopic examination of blood smear was observed in 90.32% of dogs. In addition, 83.67% of dogs exhibited anisocytosis. A depressed mean corpuscular haemoglobin concentration was obtained in all samples and hypochromia was microscopically confirmed in 93.54% of cases. Monocytopenia and eosinopenia were frequently observed and were eventually coupled to leukopenia (35.48%) and/or neutropenia (25.80%). Variations in haematological profiles may be due to differences in strain virulence and antigenicity, clinical forms of the disease and the eventual occurrence of concomitant infections.

Keywords: canine ehrlichiosis, retrospective study, haematology, anaemia, hypochromia, macrocytosis, monocytopenia, eosinopenia.

RESUME
Cette étude a eu pour but de déterminer la fréquence des anomalies hématologiques chez 31 chiens spontanément infectés par Ehrlichia canis, le diagnostic reposant sur les signes cliniques observés et les analyses sérologiques. Alors que la numération plaquettaire s’est avérée faible chez 35.48% des chiens, des anomalies dans la numération et la morphologie des érythrocytes ont été fréquemment observées : l’hémoglobinémie, la numération érythrocytaire et l’hématocrite ont été abaissées dans respectivement 25.80%, 19.35% et 19.35% des cas, l’étendue de la distribution des globules rouges a été étendue dans 80.64% des cas et une macrocytose révélée par un volume corpusculaire moyen élevé et confirmée par un examen microscopique des frottis sanguins a été observée chez 90.32% des chiens. De plus, 83.67% des chiens ont présenté une anisocytose. Une faible concentration corpusculaire moyenne en hémoglobine a été mise en évidence dans tous les échantillons et l’hypochromie a été confirmée par microscopie dans 93.54% des cas. Une monocytopenie et une eosinopenie ont été fréquemment observées et couplées à une neutropénie (35.48%) et/ou à une neutropénie (25.80%). Les variations dans les profils hématologiques pourraient résulter de la virulence de la souche, de son antigénicité, de la forme clinique de la maladie et de la présence éventuelle d’infections concomitantes.

Mots-clés : Ehrlichiose canine, étude rétrospective, hématologie, anémie, hypochromie, macrocytose, monocytopenie, éosinopenie.

Introduction
Canine monocytic ehrlichiosis is caused by the rickettsia Ehrlichia canis. The disease is transmitted by the brown dog tick Rhipicephalus sanguineus and presents worldwide distribution [2, 9, 15, 20, 21, 22]. Acute, latent and chronic forms of the disease are recognize, d, with the latter usually accompanied by severe haematological and bone marrow dysfunctions [12]. Changes in the immune response to Ehrlichia canis are observed in all forms of infection. In the acute form (approximately 2 months post-infection), serum antibody titer can commonly reach high levels [10, 19]. The clinical picture and haematological parameters have been reported in experimentally [7] and naturally infected dogs [11], as well as in mixed (e.g. ehrlichiosis and babesiosis or ehrlichiosis and leishmaniosis) infections [14].

Thrombocytopenia, anaemia and leukopenia are usually the most common haematological abnormalities in canine monocytic ehrlichiosis. However, these laboratory changes are not specific for ehrlichiosis and may be present in other infectious diseases (e.g. leishmaniosis, bartonellosis etc). The aim of the present study was to present the haematological parameters of 31 dogs with spontaneous monocytic ehrlichiosis in Greece and discuss the results with the ones already known from the veterinary literature.

Material and methods
A total of 31 dogs (22 males and 9 females) naturally infected with Ehrlichia canis were examined from 2002 to 2003. Seventeen were purebred and 14 were stray animals, 1-12 years old. All animals were presented and examined in private small animal veterinary practices. All dogs exhibited...
suggestive symptoms of the disease, mainly depression, lethargy and anorexia, and in several cases petechias and ecchymoses on the skin and/or mucosae, lymphadenopathy and uveitis. The diagnosis of ehrlichiosis was based on suggestive clinical picture and positive serology by indirect immunofluorescence test (IFA). A peripheral blood sample (5 mL) was obtained from each dog for haematological analysis before any treatment administered. Blood samples were analyzed using an haematological analyzer (Abbott CELL-DYN 1700 CS Haematology Analyzer, USA) for total number of platelets (PLT), total number of leukocytes (WBC), absolute number of neutrophils (NEU), lymphocytes (LY), monocytes (MO), eosinophils (EO), basophils (BA), total number of erythrocytes (RBC), haemoglobin concentration (HB), haematocrite (HCT), mean erythrocyte volume (MCV), mean corpuscular haemoglobin content (MCH) and mean haemoglobin concentration in erythrocytes (MCHC) and erythrocyte distribution width (RDW). Additionally, direct blood smears stained with haemacolour stain were microscopically examined for erythrocyte morphology.

Results

The measured haematological parameters are presented in Table I.

The absolute number of platelets was found below 150x10⁹/L in 35.48% of the samples examined, whereas in 58.07% of the cases these cells were found within the usual range (150-500x10⁹/L). In a relatively low percentage of dogs (19.35%) the absolute number of erythrocytes was found below 5.5x10¹²/L and HCT below 37%. Haemoglobin concentration was found below 120 g/L in 25.80% of dogs and the mean haemoglobin content in erythrocytes (MCH) was depressed (< 19 pg) in 32.25% of dogs. In addition, the mean haemoglobin concentration in erythrocytes (MCHC) was low (< 320 g/L) in all examined samples and 90.32% of dogs have exhibited a large mean corpuscular volume (MCV > 77 fL). Hypochromia and macrocytosis were also confirmed by microscopic analysis of blood smears in 93.54% and 90.32% of cases, respectively. Anisocytosis was present in 83.67% of cases. The erythrocyte distribution width was increased (>14.8%) in the majority (80.64%) of samples tested.

The total leukocyte numeration was included in usual ranges in the majority of diseased dogs (58.07%) but 35.48% of them exhibited leucopenia (WBC < 6.0x10⁹/L) and 6.45% exhibited leukocytosis (WBC > 17.0x10⁹/L). The vast majority of animals (93.55%) presented no changes in the absolute number of lymphocytes and basophils, with lymphopenia and basophilia being detected in only 6.45% of animals. Neutropenia (< 3.0x10⁹/L) was observed in 25.80% of samples. Monocytopenia (< 0.1x10⁹/L) and eosinopenia (< 0.1x10⁹/L) were found in 67.74% and 77.41% of samples, respectively.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Usual ranges</th>
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<th>within</th>
<th>above</th>
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<tr>
<td>WBC (10⁹/L)</td>
<td>6.0-17.0</td>
<td>35.48</td>
<td>58.07</td>
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<tr>
<td>NEU (10⁹/L)</td>
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<td>25.80</td>
<td>67.75</td>
<td>6.45</td>
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<tr>
<td>LY (10⁹/L)</td>
<td>1.0-4.0</td>
<td>6.45</td>
<td>93.55</td>
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<td>MO (10⁹/L)</td>
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<td>67.74</td>
<td>29.04</td>
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<tr>
<td>EO (10⁹/L)</td>
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<td>77.41</td>
<td>22.59</td>
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<td>BA (10⁹/L)</td>
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<td>93.55</td>
<td>6.45</td>
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<tr>
<td>RBC (10¹²/L)</td>
<td>5.5-8.5</td>
<td>19.35</td>
<td>80.65</td>
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<td>HB (g/L)</td>
<td>120-180</td>
<td>25.80</td>
<td>71.98</td>
<td>3.22</td>
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<td>HCT (%)</td>
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<td>19.35</td>
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<td>MCV (fL)</td>
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<td>MCH (pg)</td>
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<td>MCHC (g/L)</td>
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<td>0.00</td>
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<td>RDW (%)</td>
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<td>0.00</td>
<td>19.36</td>
<td>80.64</td>
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<td>PLT (10⁹/L)</td>
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<td>58.07</td>
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<th>RBC morphology</th>
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<td>Macrocytosis</td>
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<td>Anisocytosis</td>
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</table>

WBC: white blood cells; NEU: neutrophils; LY: lymphocytes; MO: monocytes; EO: eosinophils; BA: basophils; RBC: red blood cells; HB: haemoglobin; HCT: haematocrite; MCV: mean corpuscular volume; MCH: Mean corpuscular haemoglobin content; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; PLT: platelets.

Table I: Haematological findings in dogs with spontaneous ehrlichiosis in Greece (n = 31).
Discussion

Thrombocytopenia is considered as the most common haematological abnormality in dogs either naturally or experimentally infected with *Ehrlichia canis* [8, 9, 13, 17]. However, in the present study thrombocytopenia was detected in only 35.48% of the samples examined. Similar results have been reported by WOODY and HOSKINS [24], DAGNONE et al. [3] and MACIEIRA et al. [13]. Such discrepancies could be partially explained by the fact that no particular form of the disease was selected for evaluation here and in other studies and dogs included could have acute, latent or chronic form of the disease. Thrombocytopenia may be the result of the decrease of half-life of circulating platelets, endothelium dysfunction and thrombocyte aggregation, increased platelet sequestration in the spleen, and formation of auto-antibodies against platelets [1, 5, 10, 12, 18, 23]. Another possible explanation could be related to variations in virulence of the various strains of *Ehrlichia canis* and antigen heterogeneity [9, 12]. In experimentally *Ehrlichia canis* infected dogs, thrombocytopenia is observed only for 2-3 weeks, after which the number of these cells increases although the antibody titer remained high [12].

Anaemia is also a common clinical pathology abnormality in canine ehrlichiosis [8, 13]. In the current study, decreased haemoglobin concentration, low erythrocyte numerosity and low haematocite were present in 25.80% and 19.5% of dogs respectively. Additionally, macrocytosis, anisocytosis and hypochromia were also observed in the great majority of cases. These observations are different from previous studies in which a normocytic, normochromic and non regenerative anaemia was usually reported [6, 13, 16]. Although these differences can not be easily explained, autoimmune response involved formation of anti-erythrocyte auto-antibodies [5, 7] may participate at least partially to progressive destruction of erythrocytes.

In the current study, leukopenia was observed in about 1/3 of the cases (35.48%). Similar results have been reported by MACIEIRA et al. [13]. In parallel, marked decreases in monocyte and/or eosinophil counts were evidenced in a vast majority of naturally infected dogs (67.74% and 77.41%, respectively) in agreement with previous observations of DUNCAN and PRASSE [4]. It has been postulated that some monocytes infected with *Ehrlichia canis* would adhere to the vascular endothelium, leading to reduction in their peripheral blood numerosity [5]. However, PASA and AZIZOGLU [17] have reported numeration of monocytes and eosinophils within usual ranges in ehrlichiosis diseased dogs. The great majority (93.55%) of infected dogs exhibited absolute number of lymphocytes within usual ranges in the present study whereas lymphopenia was present in only 6.45% of them. By contrast, KUEHN and GAUND [12] observed mainly lymphopenia and PASA and AZIZOGLU [17] have reported occurrence of a slight lymphocytosis.

Variations in haematological profiles in *Ehrlichia canis* infected dogs may be related to differences in the virulence of *Ehrlichia canis* strains, antigen heterogeneity of this bacterial agent and the clinical form of the disease. In addition, further studies are required to determine the influence of concomitant infections with other bacteria or parasites (e.g. *Leishmania infantum, Bartonella* sp., etc) on the observed haematological abnormalities.

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References

10. HARRUS S., WANER, T., WEISS D., BARK H.: Kinetics of the serum antiplatelet antibodies in experimental


