ANALYSIS OF HAEMATOLOGICAL ABNORMALITIES OBSERVED IN DOGS INFECTED BY A LARGE BABESIA

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Abstract

The aim of presented study was a statistical analysis of haematological abnormalities in cases of canine babesiosis, especially according to age and breed of animals. Data submitted to analysis consisted of 350 haematological results from dogs that were diagnosed as Babesia positive, based on May-Grünwald-Giemsa stained blood smears. Haematology tests (erythrocyte, leukocyte, and platelet parameters) were performed on automated analyser. Haematological parameters that showed abnormalities were submitted to the statistical analysis with the group division based on animals’ age and breed. It was found that the most significant haematological abnormality in the course of babesiosis is thrombocytopoenia, less severe abnormalities included anaemia, leukopenias (both neutropoenia and lymphopenia), moreover bi- or pancytopoenia were noted more seldom. Additionally, significant differences of PCV value between the group of young animals and both groups of adult and old animals were observed, and also in case of WBC value significant difference between German shepherds and mongrels were noted.

Key words: dog, Babesia canis, babesiosis, blood disorders.

Canine babesiosis is a protozoal tick-borne disease with worldwide distribution and global significance (7, 8). The disease is caused by different Babesia species, historically divided into large form designated as Babesia canis and small form named Babesia gibsoni. Molecular analysis has identified three distinct subspecies of B. canis: Babesia canis rossi, Babesia canis canis, and Babesia canis vogeli, which are morphologically identical (6, 10). The distribution and incidence of canine babesiosis in Europe was summed in the paper by Trotz-Williams and Trees (12). Though, different Babesia species have been described in Europe, in Poland, only clinical cases of B. c. canis infection in dogs have been reported so far.

The mechanisms of the pathogenesis of babesiosis result from host immune response to the organism rather than from a direct destruction of the erythrocytes by the parasite. Haemolytic anaemia and hypotensive shock syndrome induced by inflammatory mediators are two basic disease disorders. Haemolytic anaemia is a prominent feature of babesiosis in dogs. Direct red blood cell damage, intravascular haemolysis, and extravascular haemolysis are thought to occur (2).

B. c. canis infection is transmitted by Dermacentor reticulatus (14) and is capable of causing a wide range of clinical signs. The clinical signs of babesiosis can range from subclinical infections to hyperacute fatal disease. Clinical signs of canine babesiosis may involve anaemia, thrombocytopenia, lethargy, anorexia, splenomegaly, haemoglobinuria, bilirubinuria, fever, and jaundice. The most common haematological abnormalities found in Babesia canis infection are anaemia and thrombocytopenia. The anaemia is initially mild, normocytic, and normochromic, then becomes macrocytic, hypocromic, and regenerative as the disease progresses.

There are not many studies from Europe concerning the haematological abnormalities during the course of large Babesia infection in dogs, and these existing are represented by small sample size (5, 11). Several papers (1, 9) were published in Poland that coped with some aspects of laboratory abnormalities during the course of babesiosis, but there is only one study dealing with the problem (13) that involves respectively large sample size (248 dogs), however it concerns mainly the descriptive statistics of haematological abnormalities. This study intended to present more thorough analysis of the haematological data, obtained from dogs with Babesia infection, on the basis of blood smears, with statistical analysis between groups of different age and breeds.

Material and Methods

Data submitted to analysis consisted of 350 haematological results originating from dogs of different age, breed, and sex that were referred to the Faculty’s
Small Animal Clinic with the clinical signs corresponding to large Babesia infection. Blood smears stained by the May-Grünwald-Giemsa technique were found as Babesia positive. Haematology tests were performed on automated analyser (Diatron®, Abacus). The following data were taken into analysis: erythrocyte parameters - red blood cell count (RBC), haemoglobin concentration (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and red blood cell distribution width (RDW); leukocyte parameters - white blood cell (WBC), granulocyte, monocyte, and lymphocyte counts; platelet parameters - platelet count (PLT) and mean platelet volume (MPV).

Babesia positive haematological values were compared to the reference values (Table 1). The study of the degree of abnormalities in haematological values was based on the deviations from standard values. To evaluate the degree of anaemia, the classification of Couto (4) was adopted where PCV below 18% indicates severe anaemia, PCV between 18% and 29% - moderate anaemia, and PCV between 30% and 36% - mild anaemia. For the evaluation of thrombocytopenia, classification proposed by Furlanello et al. (5) was used, where severe thrombocytopenia is considered when PLT values are below 25,000, moderate between 25,000 and 49,000, mild between 50,000 and 99,000, and slight between 100,000 and 192,000. Additionally, based on haematological results, an analysis of bicythopoenia and pancythopoenia occurrence was made.

In further analysis, haematological parameters that showed the most degree of abnormalities were submitted to the statistical analysis with the group division based on animals’ age (46 young dogs from 0 to 1 year, 130 adult dogs from 1 to 7 years, and 105 old dogs above 7 years; only dogs with available age data were included in these analyses), and breed (42 German shepherds vs 44 mongrels). In a statistical analysis, an SPSS software package was employed. Age and breed groups were compared with the Student’s t-test.

**Results**

**Erythrocyte parameters.** In 157 dogs out of 350 (45%), RBC count was below the minimal reference value. The haemoglobin concentration was lower than minimal standard value in 119 (34.6%) dogs. The PCV values below the reference minimum were found in 175 (50%) dogs. According to the anaemia classification by Couto, eight (2.3%) dogs were categorised to a group with severe anaemia, 50 (14.3%) dogs to moderate anaemia, and 117 (33.4%) dogs to mild anaemia. The MCV was predominantly in a range of reference values; only in 14 (4%) dogs it was slightly below the values. The MCHC concentration was above the maximum standard value in 140 (40%) dogs. The RBC distribution width was slightly increased in 76 (21.7%) dogs.

**Leukocyte parameters.** In 203 (58%) dogs, the WBC count decreased beneath the minimum standard value, while in seven (2%) dogs leukocyte count was above maximum reference value. Neutropoenia was observed in 98 (28%) dogs and neutrophilia in 14 (4%) dogs. Lymphopenia was noted in 126 (36%) dogs and lymphocytosis in seven (2%) dogs. Monocyte count was below the reference value in 63 (18%) cases and one result was above the maximum reference value.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Haematological parameters of the dogs included in a study</th>
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<tr>
<td></td>
<td>Haematological parameters</td>
</tr>
<tr>
<td>RBC (10^{12}/L)</td>
<td>5.5</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>12</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>37</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>60</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>32</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>11.9</td>
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<tr>
<td>WBC (10^{9}/L)</td>
<td>6.0</td>
</tr>
<tr>
<td>granulocytes (10^{9}/L)</td>
<td>3.0</td>
</tr>
<tr>
<td>monocytes (10^{9}/L)</td>
<td>0.15</td>
</tr>
<tr>
<td>lymphocytes (10^{9}/L)</td>
<td>1.0</td>
</tr>
<tr>
<td>PLT (10^{9}/L)</td>
<td>200</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>5.8</td>
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</tbody>
</table>
Platelet parameters. With respect to platelet count in 342 (97.7%) dogs, the number was below the reference value. According to the scale proposed by Furlanello et al. (5), slight thrombocytopenia was observed in 12 (3.5%) dogs, mild in 45 (12.8%), moderate in 90 (25.7%), and severe in 191 (54.6%). Mean platelet volume was increased in 20% of dogs.

Bicytopoenia and pancytopoenia analysis. The pancytopoenia was observed in 89 (25.7%) of the studied dogs. Bicytopoenia due to anaemia and thrombocytopenia was observed in 65 (19%) dogs and due to leukopenia and thrombocytopenia in 109 (31%) dogs. No cases of bicytopoenia due to anaemia and leukopenia were observed. Altogether bicytopoenia and pancytopoenia were observed in 263 (75%) dogs.

Statistical analysis. The haematological parameters that showed the greatest abnormalities, and were submitted to the statistical analysis in age and breed groups, included PCV, WBC count, and PLT count.

Statistically significant (P≤0.001) differences were noted only in PCV values between the group of young dogs (28.31 ±8.72), and both groups of adult (37.20 ±6.85) and old dogs (37.89 ±6.91). We also noted statistically significant (P≤0.05) difference in PLT count between the groups of adult and old dogs. However, after withdrawing one result from analysis (from the old dogs’ group), which was extremely high above the maximum reference value (1,943 million), the observed significance disappeared.

In case of breed group analysis, statistically significant (P≤0.001) difference in WBC count was found between German shepherds (8.62 ±3.80) and mongrels (5.96 ±2.61).

Discussion

In dogs, diagnosis of babesiosis is based on examination of stained blood smear (e.g. by May-Grünwald-Giemsa method), when the presence of parasites, with morphology characteristic for Babesia canis is revealed in erythrocytes. In most cases, the decision of undertaking the laboratory examination is supported by the evidence of characteristic clinical signs of anaemia, anorexia, lethargy, fever, and jaundice. With respect to different incubation period for babesiosis, determining the moment of invasion is extremely difficult in clinical cases. In practice, when determining the moment of invasion is extremely difficult in clinical cases, we can only base on the estimating the onset of clinical signs, that usually coincides with the dog’s health worsening to such an extent that owner decides to report his animal to veterinarian.

In the textbooks dealing with small animal pathology, main haematological features during the course of canine babesiosis are described as regenerative, normochromic, and normocytic anaemia and thrombocytopenia. Babesia infection is also listed among the causes of bi- and pancytopoenia. In the paper of Bourdoiseau (3), that reviews several studies on canine babesiosis in France, thrombocytopenia and lymphopenia were found as the first haematological changes with lymphopenia tending to fade during the course of the disease. In a thorough study of Furlanello et al. (5) on 23 Babesia infected dogs, haematological findings revealed anaemia in 17 (74%) dogs with the degree of mild in six cases (26%), moderate in 10 (43%) cases, and severe in one (4%) case. In our study, anaemia based on PCV was noted in half of the animals, which is a much smaller number than in the study of Italian authors. The percentage of anaemia degree from our study is very close in case of mild (33.4%) and severe (2.3%), but differs for moderate anaemia (14.3%). In a study of Zygner et al. (13), anaemia was demonstrated in 31.4% of dogs, based solely on PCV. However, in this study, the reference minimum value was lower than in our study (35 instead of 37). With this lower value taken into consideration during the analysis of our data, the distance between results presented in this paper and in paper by Zygner et al. (13) is even closer, reaching a value of 40% instead of 50%. The results of both studies seem to be distinct from those of the Italian study. Nevertheless, from our study, where results for RBC, PCV, and haemoglobin concentration were below reference values, in case of 45%, 50%, and 34.6% of the data respectively, it is evident that in less than half of the dogs with babesiosis, peripheral blood smears show no signs of erythrocyte abnormalities, although anaemia is one of the main features referred to the disease. According to Furlanello et al. (5), it is possible that in some cases anaemia is masked by dehydration. We also observed that MCHC was above the maximum standard value in 140 (40%) dogs. This is often met in a course of babesiosis since high MCHC values are resulting from haemolysis. The value of RBC distribution width was slightly increased in 76 (21.7%) dogs but in most of those dogs (57), the value of the rise was just 1%. RDW value is often increased in regenerative anaemia because reticulocytes and young RBCs are larger than mature erythrocytes so it could be considered as a slight sign of regeneration in Babesia infected dogs. In the cited Italian study, erythrocyte regeneration was observed only in three dogs (based on reticulocyte count).

The most differences between the studies can be observed in white blood cells values. In studies by Furlanello et al. (5), Zygner et al. (13) and in the present study neutropoenia was observed in 73.9%, 36.3% and 28%, respectively. Lymphopenia was observed in 65.2%, 7.2% and 36%, respectively. As it seems that leukocyte counts is dependent on more various factors than just Babesia infection, influencing the immunological cellular response, these values are the most inconsistent and the less reliable in terms of characterising the organism’s reaction in canine babesiosis.

The most consistent and severe change in blood picture observed in the course of canine babesiosis is thrombocytopenia. In studies by Furlanello et al. (5), Zygner et al. (13), and in our investigations, thrombocytopenia occurred in all dogs, 99.5% and
97.7% of dogs, respectively. Moreover, the level of thrombocytopoenia estimated as a severe (PLT values below 25,000) was found in two latter studies as 13% and even 54.6%, respectively. High MPV values observed in 20% of the dogs included in our study are a sign of increased haemopoiesis, indicating the regeneration processes. Noteworthy is that the number of dogs meeting the haematological abnormalities, at least in two populations of blood cells, reached 75% of the studied population.

Statistical analysis of age and breed groups, which included such parameters as PCV, WBC, and PLT, revealed statistically significant differences only in the case of PCV values between the group of young animals and both groups of adult and old animals (P≤0.001), and in a case of WBC value, the difference (P≤0.001) between German shepherds and mongrels. In our opinion, it is hard to attribute any direct connection to the disease course in case of the difference in WBC values between the analysed breed groups, despite the high statistical power, when we take into consideration the observations aforementioned in the discussion of WBC values in canine babesiosis. In case of PCV, it has to be remembered that although it seems statistically significant this difference possibly has its basis in physiology, since normal PCV value in young animals can be as low as 25% instead of minimal value (37%) for adult dogs.

In conclusion, it can be noted that the most significant haematological abnormality in the course of babesiosis is thrombocytopoenia, when surprisingly laboratory signs of anaemia are not met in more than half of the infected dogs. Additionally, our analysis showed, that it is difficult to observe any significant differences in the occurrence of haematological abnormalities that can be correlated to age or breed.

References