LIVER ENZYME ACTIVITY IN DOGS INFECTED WITH BABESIA CANIS

WOJCIECH ZYGNER¹, OLGA GÓJSKA-ZYGNER², EWA DLUGOSZ¹, AND HALINA WĘDRYCHOWICZ¹³

¹Division of Parasitology and Parasitic Diseases, Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, 02-786 Warsaw, Poland wojciechzygner@yahoo.pl
²Center of Small Animal Health Clinic Multiwit, 00-753 Warsaw, Poland
³W. Stefański Institute of Parasitology, 00-818 Warsaw, Poland

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Abstract

The influence of anaemia on alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities in dogs infected with B. canis was investigated. Samples of blood and serum from 230 infected dogs were divided into two groups: A (with anaemia) and B (without anaemia). The differences in the activities of the enzymes between both groups were not statistically significant. These results suggest that mild anaemia, as the only factor, has no influence on ALT, AST, or ALP activity in canine babesiosis. However, this study certainly cannot exclude the possibility that a more severe anaemia can have a major effect on the liver.

Key words: dogs, Babesia canis, babesiosis, hypoxia, aminotransferases, liver injury.

Material and Methods

Two hundred and thirty samples of whole blood and serum from 129 male and 101 female dogs, infected with large Babesia, were collected. All infected dogs were presented to the Center of Small Animal Health Clinic Multiwit with clinical signs of babesiosis including lethargy, anorexia or decreased appetite, fever, pale mucous membranes, dehydration, vomiting, and brown discoloured urine (due to haemoglobinuria). The blood samples were collected before treatment within 1–3 d of disease duration. The first day when the dog was lethargic was considered as the first day of the disease. Exclusion criteria were as follows: any drug therapy in the preceding 4 weeks (including dogs misdiagnosed with babesiosis), known concurrent disease or infection, history of travelling abroad in the preceding one year, and inability to clearly identify anaemia (haematocrit within reference interval and haemoglobin concentration below normal values, and detection of dehydration in clinical examination of non-anaemic dogs causing possible masking of anaemia by dehydration). The inclusion criteria were: diagnosis of babesiosis and collection of blood samples for analysis before treatment.

EDTA was used as an anticoagulant. Serum was obtained by the centrifugation of blood samples.
without an anticoagulant. ALT, AST, and ALP activities were determined by a clinical chemistry analyser (XL 640, Erba Mannheim, Germany). The diagnosis was based on the detection of Babesia organisms in blood smears stained with Giemsa. The level of parasitaemia was evaluated as it was described in the previous study by Jacobson et al. (15). Parasitaemia was expressed as the percentage of parasitised red blood cells.

All blood samples were tested using PCR in order to detect B. canis DNA. The DNA was extracted from the blood using the Blood Mini kit (A&A Biotechnology, Poland) according to the manufacturer’s instructions. The efficiency of the DNA isolation was confirmed by electrophoresis in a 1.5% agarose gel. PCR was performed according to Sobczyk et al. (21) with the primers BcW (5'-CAT CTA AAG AGG GCA GCA GG-3') and BcW-B (5'-TTA ATG GAA ACG TCC TTG GC-3') used to amplify the 18S rDNA gene fragment of B. canis. The expected product was about 500 bp. As a positive control, the DNA lysate from the blood of a dog infected with B. canis was used. The infection in this dog was confirmed by the sequencing of the PCR product, which was found to be 100% identical to a fragment of the B. canis 18S ribosomal DNA gene under accession No. AY321119 in the GenBank® database. The size of the PCR product was analysed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide. The PCR products with the expected amplicon size were isolated from the agarose gel using the Gel-Out kit (A&A Biotechnology). Next, 20 out of 230 PCR products were randomly selected for sequencing in order to verify the PCR test results. The sequencing reaction was carried out on an AbiPrism® Genetic Analyzer using the GeneScan® Analysis Software computer programme. Obtained sequences were compared to the sequence data available in the GenBank® using the BLAST programme (http://www.ncbi.nlm.nih.gov/BLAST/).

The red blood cell (RBC) count (normal values, 5.5–8.0 10^6/L), haematocrit (Hct; normal values 0.37–0.55 L/L), concentration of haemoglobin (Hb; normal values 7.45–11.17 mmol/L), mean corpuscular haemoglobin concentration (MCHC; normal values 19.8–22.3 mmol/L), and mean corpuscular volume (MCV; normal values 60–77 fL) were calculated in blood samples. These parameters were assessed with an automatic haematologic analyser (DiaTron®, Abacus). Dogs with both haematocrit and Hb concentration between these groups were assessed with the Shapiro-Wilk’s test showed that the distribution of enzyme activity values was normal. The activity of ALT within the reference values (3–50 IU/L) was found in 35 (39%) serum samples in group A and in 42 (30%) samples in group B. An increased ALT activity was detected in remaining serum samples of both groups (61% and 70%, respectively). Two-fold or greater increase in ALT activity was observed in 18 (20%) samples from group A and in 34 (24%) samples from group B. The difference in the ALT activity between both groups was not statistically significant (P=0.0623; Table 2).

The activity of AST within the reference values (1–37 IU/L) was present in seven (7.8%) serum samples in group A and in eight (5.7%) samples in group B. An increased AST activity was observed in the rest of the samples. Two-fold or greater increase of AST activity was detected in 50 (55%) samples in group A and in 94 (67%) samples in group B. The difference in the AST activity between both groups was not statistically significant (P=0.526; Table 2).

The activity of ALP within the reference values (20–155 IU/L) was detected in 61 (67.8%) serum samples in group A and in 94 (67.1%) samples in group B. An increased ALP activity was present in the rest of the samples. Two-fold or greater increase of ALP activity was detected in seven (97.8%) samples from group A and in nine (6.4%) samples from group B. The difference in the ALP activity between these groups was not statistically significant (P=0.951; Table 2).

**Results**

The dogs examined in this study belonged to various breeds. The mean age of the dogs was 4 years and 6 months (the youngest dog was 10 months of age and the oldest was 11-year-old). Lethargy, anorexia, or decreased appetite were present in all dogs. Ninety (39.1%) dogs had anaemia (group A), and 140 (60.9%) dogs had no anaemia (group B). A hundred and sixty-six (72.2%) dogs had fever (67 in group A and 99 in group B). Haemoglobinuria indicating intravascular haemolysis was detected in 16 (17.8%) dogs of group A and in three (2.1%) dogs of group B.

The expected PCR product (500 bp) was detected in all 230 blood samples. The 20 selected product sequences showed 100% similarity to the 18S rDNA partial sequence of B. canis isolated from dogs in Poland.

The values of the erythrocyte parameters are presented in Table 1. The mean level of parasitaemia in groups A and B amounted to 0.13% ±0.04% and 0.12% ±0.04%, respectively. The differences in RBC count, Hct and Hb concentration between groups A and B were statistically significant. However, the differences in MCV and MCHC between these groups were not statistically significant. The obtained results allowed dividing the blood and serum samples into two groups: group A (anaemic dogs), group B (non-anaemic dogs). The results were analysed using the Statistica 8.0 programme. Shapiro-Wilk’s test was used for the estimation of normality in ALT, AST, and ALP activity distribution in groups A and B. Wald-Wolfowitz Runs test was used to compare the activity of the ALT, AST, and ALP in the groups A and B, and to compare values of the erythrocyte parameters in these groups. The value of P<0.05 was considered significant. Results are depicted as median and 25-75 percentiles.
Table 1
Statistical values of the erythrocyte parameters in groups A and B

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Group</th>
<th>RBC</th>
<th>Hct</th>
<th>Hb</th>
<th>MCV</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_{med}$</td>
<td>A</td>
<td>4.56</td>
<td>0.295</td>
<td>6.3</td>
<td>66</td>
<td>21.35</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6.395</td>
<td>0.425</td>
<td>9.1</td>
<td>67</td>
<td>21.6</td>
</tr>
<tr>
<td>$\mu$</td>
<td>A</td>
<td>4.202</td>
<td>0.274</td>
<td>5.92</td>
<td>66.0</td>
<td>21.50</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6.457</td>
<td>0.427</td>
<td>9.22</td>
<td>66.9</td>
<td>21.67</td>
</tr>
<tr>
<td>S.D.</td>
<td>A</td>
<td>0.989</td>
<td>0.063</td>
<td>1.38</td>
<td>3.6</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.668</td>
<td>0.044</td>
<td>0.92</td>
<td>3.1</td>
<td>1.75</td>
</tr>
</tbody>
</table>

Reference range: 5.5–8.0, 0.37–0.55, 7.45–11.17, 60–77, 19.8–22.3

Table 2
Comparison of medians of ALT, AST, and ALP activities (IU/L) in groups A and B

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Group</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_{med}$</td>
<td>A</td>
<td>54.5</td>
<td>85.5</td>
<td>122.5</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>62.5</td>
<td>101.5</td>
<td>120</td>
</tr>
<tr>
<td>25%-75%</td>
<td>A</td>
<td>43–91</td>
<td>50–120</td>
<td>84–180</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>48–99</td>
<td>61.5–149.5</td>
<td>90–176</td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>A</td>
<td>20–725</td>
<td>20–424</td>
<td>29–2295</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>20–502</td>
<td>25–498</td>
<td>37–763</td>
</tr>
</tbody>
</table>

Reference range: 3–50, 1–37, 20–155

ALT – alanine aminotransferase (IU/L), AST – aspartate aminotransferase (IU/L), ALP – alkaline phosphatase (IU/L), $M_{med}$ – median, 25%-75% – 25th and 75th percentile, Min.-Max. – values of minimum and maximum, $p$ – a value of $P$ calculated for Wald-Wolfowitz Runs test, * - statistically significant differences, A – anaemic dogs, B – non-anaemic dogs.

Discussion

The detection of *B. canis* DNA in all examined samples is not surprising because *B. canis* is the only piroplasm species detected so far in dogs in Poland (1, 26, 28). The occurrence of *B. canis* and the absence of the other *Babesia* species infecting dogs result from the fact that among the tick species transmitting these pathogens, only *Dermacentor reticulatus* tick has been detected in Poland (27, 30).

The prevalence of anaemia in the examined blood samples is similar to the results of the previous studies from Poland (11, 25, 31) but differs from the results from Italy and Spain, where anaemia was detected in 74%–93.1% and in 11.1% of dogs infected with *B. canis*, respectively (12, 20, 22). The difference in the prevalence of anaemic dogs infected with *B. canis* probably results from the different virulence of the parasite strains and/or the duration of the infection.

The lack of significant differences in MCV and MCHC values between groups A and B, and observed means and medians of MCV and MCHC within reference intervals in both groups, show that there was no marked difference in the RBC count regarding the relative number of immature erythrocytes in the blood. This is shown by the lack of macrocytosis (increase in MCV) and hypochromasia (decrease in MCHC), which are typical for regenerative response after erythrocyte destruction (6). The anaemia observed in this study was normocytic and normochromic. This kind of anaemia is typical at the start of haemolysis and appears to be non-regenerative (lack of immature erythrocytes) during the first 2–3 d of haemolysis (6). In canine babesiosis anaemia becomes regenerative (macrocytic and hypochromic) after a few days of infection (23). Thus, the values of MCV and MCHC observed in this study within reference intervals indicate short duration (2–3 d) of the disease. This was in agreement with the information given by the owners of the dogs.
According to Allred (3) anaemia correlates with parasitaemia in Babesia infected hosts. Moreover, Böhm et al. (5) showed that high parasitaemia was significantly associated with mortality among dogs infected with B. rossi in South Africa. However, the level of parasitaemia detected in this study, was not different between groups A and B. This result is similar to the results of the previous studies in Europe, which demonstrated no correlation between parasitaemia and severity of anaemia (9, 12). Jacobson (14) suggested that anaemia and parasitaemia might correlate in experimental infections.

The prevalence of elevated aminotransferase and ALP activities in the examined serum samples is similar to the results of previous studies from Poland and Italy (12, 29). An increase in the enzyme activity above reference values in the examined dogs suggests liver injury. Both ALT and AST are present in high concentrations in hepatocytes. Therefore, their leakage into the circulation is observed when hepatocytes or their membranes are damaged (4, 13). In this study, AST activity increased in a larger number of serum samples than ALT activity. This probably resulted from the fact that AST is also detected in other tissues, such as kidneys and RBC, which are also insulted during the course of canine babesiosis (12, 23). However, if haemolysis was a significant cause of increased AST activity in serum of these dogs, there should be a higher increase in AST activity in group A. Nevertheless, no statistical difference in AST activity between both groups was found. Moreover, the median of AST activity was higher in group B. The observed higher median of AST activity in group B may result from the fact that this enzyme is also detected in the kidneys, muscles, and pancreas (24). In group B, a 10-fold or higher increase in AST activity was observed in seven dogs, whereas, in group A only in one. It seems probable that the kidneys, muscles, or pancreas could be also the origin of AST in these dogs. Yet, the influence of anaemia on these organs and tissues was not assessed in this study. It is also probable that other undetected diseases or opportunistic infections could additionally influence the increase in AST activity. Thus, this result shows that there is no influence of haemolytic anaemia, or at least mild anaemia, on AST activity in dogs infected with B. canis. ALT is a more specific marker of hepatocellular injury in dogs (4, 8). Rajamohan et al. (18) showed that ALT mRNA is also present in canine heart, fat, brain, skeletal muscles, and kidneys. However, in that study only ALT1 isoform expression among two isoforms of this enzyme was investigated and relative expression of ALT1 was noted as follows: heart > liver > fat ~ brain ~ skeletal muscles > kidneys. The authors of this publication suppose that the increase in ALT activity in dogs infected with B. canis resulted mainly from hepatocellular injury. Yet, anaemia had no influence on liver damage.

ALT does not leak from the hepatocyte with increased membrane permeability but the increase in this enzyme activity is caused by the induction by drugs or hormones. ALP derives from the bile ducts. Cholestasis in canine babesiosis, probably caused by hepatomegaly, could be one of the reasons of the marked increase in ALP activity. This precedes the development of hyperbilirubinemia (4). The lack of a significant difference in ALP activity between groups A and B shows that anaemia had no influence on ALP activity. The results of this study differ from the results of previous investigations in which ALT, lactate dehydrogenase, and ALP activities were compared in anaemic and non-anaemic dogs infected with Babesia rossi (19). The authors observed relatively higher liver enzyme activity in anaemic dogs. However, means of ALT and ALP activities were within reference values both in anaemic and non-anaemic dogs. In this study, the authors observed an increase in median ALT and AST activities above reference values in both groups. Medians of ALP activity were similar and within normal values in both groups, and statistical analysis showed no differences between these groups. The differences between results of this study and results obtained by Reyers et al. (19) probably result from the fact that blood samples were taken from dogs infected with different Babesia species. Moreover, Reyers et al. (19) did not exclude the fact that destructed erythrocytes might be the source of ALT and LDH in anaemic dogs.

The authors of this study investigated the influence of anaemia on ALT, AST, and ALP activities. Anaemia may be one of the reasons of hypoxia and hypoxic liver injury observed during the course of canine babesiosis. Yet, the results of this study did not show correlations between anaemia and increased aminotransferase and ALP activities. However, the possibility that more severe anaemia can effect the liver cannot be certainly excluded. Therefore, further investigations dealing with hepatopathy in canine babesiosis are necessary. Probably, TNFα, like in cases of bovine babesiosis and human falciparum malaria, may play the main role in liver damage causing hypotension and oxidative damage (7, 14, 17).

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References