IMPORTANCE OF HAEMATOLOGICAL CHANGES IN DIAGNOSING CANINE BABESIOSIS

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Abstract. Over the past ten years, canine babesiosis spread widely in Lithuania. In this retrospective study during 2003–2012, blood of 300 dogs with babesiosis was investigated at the Veterinary Academy of Lithuanian University of Health Sciences (LUHS) and Dr. L. Kriaučeliūnas Small Animal Clinic. Morphological changes (RBC, HCT, Hb, PLT, WBC, leukogram and core shift) of blood were tested searching for regularities, which may be enough to reasonably suspect that the dog is suffering from babesiosis. Blood tests were carried out in 300 dogs: 186 dogs with haematological abnormalities and 114 dogs with normal haematological findings. This study shows that babesiosis can be characterized by marked thrombocytopenia (30.90±4.97 x 10^9/l, P<0.05), neutrophilic leukogram profile change to lymphocytic-plasmocytic and monocytosis (16.20±1.30 %, P<0.05). Also there is often a tendency to anemia and leukopenia. Haemolysis of erythrocytes often cause renal failure and azotemia develops (UREA 29.32±3.75 mmol/l, P<0.05). Complication such as renal failure is of particular importance in predicting epicrisis. Less common babesiosis is accompanied by liver failure.

Keywords: dogs, babesiosis, diagnostics, haematological investigation.

Introduction

Over the last decade, canine babesiosis has spread widely among the dogs in Lithuania. Disease cases have been recorded in all territory of the Republic, but the highest incidence of canine babesiosis has been observed in the western and central parts of Lithuania. The tick *Ixodes ricinus*, which transmits *Babesia* spp. protozoa, is found high in the mountains and near the Northern pole. There is an opinion that one of the factors affecting the spread of babesiosis is climate change (Trapp et al., 2006; Hartelt et al., 2007; Irwin, 2007; Skotarczak, 2008; Bashir et al., 2009; Irwin, 2009; Kirtz et al., 2012).

Clinical signs of babesiosis are determined by disease form which can vary from subclinical to severe, and often causes death because of dysfunction in multiple organs. Dog becomes lethargic, loses appetite, is unwilling to move and just lies on the ground in the first days of illness, body temperature rises to 40-41 ^{0}C and lasts for 2–3 days, tremor of peripheral muscles develops. Mucous membranes and skin becomes pale or yellowish, urine colour darkens from brownish to brown or attains a red colour as the result of haemolysis. Splenomegaly develops: enlarged spleen can rupture and excessive bleeding into abdominal cavity leads to the lethal outcome. Tachycardia and increased respiratory rate are common signs, which are caused by pulmonary oedema. Secondary GI tract signs develop as a result of kidney and liver insufficiency and animals start to vomit, sometimes they have diarrhea or conversely – coprostasis develops. Irreversible CNS changes are possible due to whole body intoxication: animals develop coordination impairment, paresis or paralysis (Leisewitz et al., 2001; Lobetti et al., 2002; Evers et al., 2003; Duh et al., 2004; Harikrishnan et al., 2005; Zambelli, Leisewitz, 2009; Lobetti, 2010).

Young, old, weak, animals with poor body condition and animals kept in bad circumstances are affected more severely. Pregnant bitches can abort due to babesiosis (Bastos et al., 2004; Keller et al., 2004; Nel et al., 2004; Sobczyk et al., 2005; Niwetpathomwat et al., 2006; Ayoob et al., 2010; Cardoso et al., 2010).

When first clinical signs appear, protozoa become visible in erythrocytes during blood smear test, taken from peripheral blood. At the beginning of illness, only single *Babesia* spp. in blood smear are found, but when they are starting to multiply extensively, the count of protozoa in blood rises significantly. The main test currently used to diagnose babesiosis in Lithuania is peripheral blood smear microscopy. Protozoa found in erythrocytes are thought to be *Babesia canis canis*, but accurate species differentiation is often difficult using microscopy, or sometimes impossible (Földvari et al., 2005; Garcia de Sá et al., 2006; Hartelt et al., 2007; Irwin, 2009; Kirtz et al., 2012).

Most often owners are trying to describe the clinical appearance of their animals using less informative symptoms, witch often accompanies most diseases. Only few owners are able to describe symptoms, which could be pathognostic initially to babesiosis, and let the veterinarian to apply purposely one or more tests. Due to this reason, veterinarians must render various tests to estimate overall status of the animal, differentiate between various pathologic processes, determine clinical diagnosis and prescribe treatment. Complete blood count is one of the most used tests. Numerous changes and variations in morphological parameters have been observed in cases of babesiosis (Niwetpathomwat et al., 2006; Hartelt et al., 2007; Jefferies et al., 2007; Joubert et al., 2007; Köster, 2009; Ayoob et al., 2010; Kirtz et al., 2012). These

changes are quite specific and with reference to this veterinarians can suspect babesiosis, and diagnosis can be confirmed or denied after peripheral blood smear test.

Aims of the study – to assess the changes of haematological parameters in a large number (large population) of dogs with babesiosis (red blood cells (RBC), hematocrit (HCT), hemoglobin (Hgb), platelets (PLT), leukocytes (WBC), leukogram and core shift, leukocytic pattern). Also to assess the specificity of these parameters which could lead to further purposeful use of diagnostic procedures for diagnosing and differentiating parasitosis.

Materials and methods

In 2003–2012, blood of 300 dogs with babesiosis was investigated at the Veterinary Academy of Lithuanian University of Health Sciences (LUHS) and Dr. L. Kriaučeliūnas Small Animal Clinic. Two canine groups were created. In the 1st group, there were dogs with visible clinical signs of babesiosis and changes in haematological parameters while the 2nd group was composed of individuals without typical signs of babesiosis and with none or nonspecific changes in haematology. Haematological parameters from *Babesia* spp. positive dogs were compared with the results of healthy dogs. Also creatinine (CREA), urea (UREA), alanin aminotransferase (ALT), aspartat aminotransferase (AST), bilirubin (BIL) in serum using "Reflotron Manual (Roche, Germany)" were measured for dogs if haematological abnormalities in complete blood count (CBC) were noticed. Haematological parameters (RBC, HCT, packed cell volume (PCV), Hgb, PLT, and WBC) were measured using IDEXX LaserCyte (IDEXX laboratories, USA) device, peripheral blood smears and core shift in the leucogram were assessed using Nikon Eclipse E200 (Nikon Corporation, Japan) binocular optic microscope using 10x magnification oculars and 100x magnifying immersion oil objective-lens. The results were summarised and their statistic means were calculated using „Microsoft Excel 2003" and „Graph Prism™. Version 2.0" programs. Arithmetic averages of results (X), bias of averages (S_x), p value on differences of averages (p), correlation coefficients (r) were also calculated. The difference of arithmetic averages was reliable, when $p<0.05$ (comparisons made between I and II groups with healthy dogs).

Results

It was found that the average RBC was 5,37±0,23 x 10^{12}/l ($p<0,05$). The lowest value was found to be 1.59 x 10^{12}/l, the highest – 7.74 x 10^{12}/l and the most common measurement was 5,20 x 10^{12}/l after analysing 186 dogs affected by *Babesia* spp. which had haematological abnormalities (1st group) (Table 1).

The mean HCT value was 36.71±1,6 % ($p<0,05$). The lowest measured value was 11.20 %, the highest 49.70 %, and the most common one was 47.0 % (Table 1).

The mean measured Hgb value was 126.30±4,70 g/l ($p<0,.5$). The lowest measured Hgb value was 58.0 g/l, highest 162.0 g/l and the most common one 100.0 g/l (Table 1).

PLT on average was 30.90±4.97 x 10^9/l ($p<0.05$). The lowest measured PLT amount was 1.0 x 10^9/l, the highest 114.0 x 10^9/l and the most common 16,0 x 10^9/l (Table 1).

The average value of leukocytes was 10.97±1.60 x 10^9/l ($p>0.05$). The lowest leukocyte amount was 2.86 x 10^9/l, the highest 63.0 x 10^9/l. The average amount of neutrophils was 57.31±2.62 % ($p<0.05$, in comparison with healthy dogs), lymphocytes 22.76±1.76 % ($p>0.05$), monocytes 16.20±1.30 % ($p<0.05$, in comparison with healthy dogs) (Table 1).

A weak negative correlation was found between hematocrit and monocytes (r = -0.37663), a weak negative correlation between platelets and monocytes (r = -0.45927) and an average positive correlation between platelets and neutrophils (r = 0.578325).

No haematological abnormalities were found in the 2nd (114 dogs) compared with the reference range. In this group, the mean RBC was 6.37±0.20 x 10^{12}/l. The lowest RBC amount was 5.60 x 10^{12}/l and the highest 8.70 x 10^{12}/l, while the most common measured value was 6.50 x 10^{12}/l (Table 2).

The mean HCT value was 47.78±1.04 %. The lowest measured HCT value was 39.40 %, the highest 56.90 % and the most common 44.70 % (Table 2).

The mean measured hemoglobin value was 158.90±3.40 g/l. The lowest HCT value was 127.0 g/l, the highest 194.0 g/l and the most common 169.0 g/l (Table 2).

In the 2nd group of animals without haematological abnormalities yet suffering from babesiosis, platelets count on average was 460.80±130.50 x 10^9/l. The lowest measured PLT value was 187.0 x 10^9/l, the highest value 513.0 x 10^9/l and the most common measured value was 293.0 x 10^9/l (Table 2).

The amount of leukocytes in the 2nd group dogs was measured on average 12.28±0.65 x 10^9/l. The lowest leukocyte value was 6.16 x 10^9/l, the highest 16.89 x 10^9/l. The average of neutrophils was 65.30±2.41 %, lymphocytes 20.05±1.87 %, and monocytes 9.36±0.76 % ($p<0,05$) (Table 2).

A weak negative correlation between HCT and PLT count (r = -0.32269), a weak negative correlation between HCT and monocytes (r = -0.3394), a weak positive correlation among PLT and monocytes (r = 0.224052) and a weak negative correlation between Hgb and PLT (r = -0.36448) were noticed in the 2nd group dogs.

Diagnosis was confirmed after *Babesia* spp. protozoa were found in peripheral blood smear using microscopic examination.

The blood biochemistry was checked in dogs with haematological changes in complete blood count. CREA in blood plasma on average was 141..40±13.54 µmol/l ($p<0.05$) (Table 3). The lowest CREA value was 39.60 µmol/l, the highest 884.0 µmol/l and the most common measurement was 44.20 µmol/l.

UREA in blood plasma was estimated on average 29.32±3,75 mmol/l ($p<0.05$) (Table 3). The lowest estimated UREA value was 4.74 mmol/l, the highest 50.0 mmol/l and the most common measurement was 50.0 mmol/l.

Table 1. **Blood results of dogs with haematological abnormalities clinical signs of babesiosis**

Parameter	Measured value	
	Dogs with babesiosis	Healthy dogs
RBC,·10^{12}/l	5.37±0.23*	6.87±0.10
HCT, %	36.71±1.60*	48.07±0.67
Hgb, g/l	126.30±4.70*	161.90±1.96
PLT,·10^{9}/l	30.90±4.97*	322.70±13.05
LEU,·10^{9}/l	10.97±1.60	11.46±0.42
Leukocyte forms		
Neutrophils, %	57.31±2.62*	68.96±1.53
Lymphocytes, %	22.76±1.76	19.90±1.15
Monocytes, %	16.20±1.30*	7.43±0.41
Note: * – p<0.05		

Table 2. **Blood results of dogs without haematological abnormalities and clinical signs of babesiosis**

Parameter	Measured value	
	Dogs with babesiosis	Healthy dogs
RBC,·10^{12}/l	6.37±0.20	6.87±0.10
HCT, %	47.78±1.04	48.07±0.67
Hgb, g/l	158.90±3.40	161.90±1.96
PLT,·10^{9}/l	460.80±130.50	322.70±13.05
LEU,·10^{9}/l	12.28±0.65	11.46±0.42
Leukocyte forms		
Neutrophils, %	65.30±2.41	68.96±1.53
Lymphocytes, %	20.05±1.87	19.90±1.15
Monocytes, %	9.36±0.76*	7.43±0.41
Note: * – p<0.05		

ALT value in blood plasma on average was 69.92±6.65 V/l (p<0.05) (Table 3). The lowest estimated value of this enzyme was 24.0 V/l, the highest 273.0 V/l and the most common measurement was 36.0 V/l.

AST value in blood plasma on average was 172.10±28.47 V/l (p<0.05) (Table 3). The lowest estimated value of this enzyme was 24.0 V/l, the highest 622.0 V/l and the most common measurement was 500.0 V/l.

Table 3. **Biochemical profile values of dogs with haematological abnormalities and clinical signs of babesiosis**

Parameter	Measured value	
	Dogs with babesiosis	Healthy dogs
CREA,·µmol/l	141.40±13.54*	72.00±1.84
UREA, mmol/l	29.32±3.75*	6.80±0.52
ALT, V/l	69.92±6.65*	44.35±2.02
AST, V/l	172.10±28.47*	39.32±3.55
BIL, µmol/l	49.03±8.43*	7.88±0.38
Note: * – p<0.05		

Total bilirubin in blood plasma was estimated on average 49.03±8.43 µmol/l (p<0.05) (Table 3). The lowest BIL value was 5.60 µmol/l, the highest 176.0 µmol/l and the most common measurement was 8.55 µmol/l.

Discussion

Complete blood count analysis is one of the most frequently used method, which enables veterinarian to assess the condition of sick animal. It also helps to diagnose and differentiate one pathological process from other, and prescribe treatment (Harikrishnan et al., 2005; Köster, 2009; Cardoso et al., 2010; Lobetti, 2010). It was observed that most frequently (up to 65% cases) babesiosis is diagnosed in April and May, when weather temperature rises to 9–14 ^{0}C while the second peak, up to 19% cases, occurs in August–October months, when weather temperature stays around 8–18 ^{0}C (Nel et al., 2004; Skotarczak, 2008; Cardoso et al., 2010; Kirtz et al., 2012). Moreover, it has been observed that certain typical haematological abnormalities accompany babesiosis and allow veterinarians to purposely use additional diagnostic procedures in parasitosis differentiation among other diseases with similar symptoms. Other authors also confirm this observation (Bastos et al., 2004; Duh et al., 2004; Joubert et al., 2007; Kirtz et al., 2012).

Protozoa are found in erythrocytes in peripheral blood smear after microscopic examination. Only single *Babesia* spp. are visible when clinical signs are mild, however they start to multiply rapidly and the count of protozoa increases significantly. Hemolysis of erythrocytes increases and hypocromic microcytic anemia develops when the disease progresses (Duh et al., 2004; Irwin, 2007; Jefferies et al., 2007; Joubert et al., 2007; Köster et al., 2009). It is not always possible to detect for sure the parasites in erythrocytes because sometimes veterinarians lack experience in making blood smears. However, it is often possible to identify haematological abnormalities in complete blood count. (Kirtz et al., 2012).

Blood results of 300 babesiosis dogs with babesiosis revealed that most of the dogs (1st group, 62 % cases) had tendency to anemia. However, anemia can be present in various diseases including babesiosis. A detailed CBC analysis of dogs with babesiosis demonstrated that 40 % of affected individuals did not have changes in CBC.

62 % of affected dogs (186 cases, 1st group) had changes in complete blood count: mild decrease in RBC (on average 5.37±0.23 x 10^{12}/l, i.e.by 23.3 % less when compared with the reference range 5.50–8.50 x 10^{12}/l, p<0.05, and in HCT (on average 36.71±1.60 %, i.e. by 9.29 % less when compared with the reference range 37.0–55.0 %,. p<0.05. Hgb values were at the limit of reference range (the estimated average 126.30±4.70 g/l, p<0.05; the reference range 120.0–180.0 g/l (Aiello, 1998). All these changes allow suspecting emergent anemia. A quite severe trombocytopaenia is one of the pathognostic signs of babesiosis (the average values were measured to be 30.90±4.97 x 10^{9}/l (by 90.03 % less when compared with the reference range), p<0.05 (the reference range 120.0–500.0 x 10^{9}/l (Aiello, 1998)).

Thirty eight percent of dogs (114 cases, 2nd group) had no haematological changes. However, there is a tendency of decreasing hematocrit and increasing amount of monocytes in this group (Table 2). It was observed that there were no haematological abnormalities in dogs, which were brought to the clinic with mild symptoms, which are not specific to babesiosis.

The literary data point out that leucopenia is often accompanied by babesiosis. Leucogram profile changes to lymphocytic-plasmocytic profile and monocytosis are often observed and sometimes can be marked (Evers et al., 2003; Duh et al., 2004; Hartelt et al., 2007; Jefferies et al., 2007; Kirtz et al., 2012).

Leukopenia, sometimes quite severe, was found in dogs affected by *Babesia* spp. Although leucocytes on average were measured to be 10.97±1.60 x 10^9/l ($p>0.05$; the reference range 6.0–17.0 x 10^9/l), neutrophils 57.31±2.62 % ($p<0.05$; the reference range 43.0–71.0 %), lymphocytes 22.76±1.76 % ($p>0.05$; the reference range 12.0–40.0 %), and monocytes 16.20±1.30 % ($p<0.05$; the reference range 3.0–10.0 % (Aiello, 1998)).

Hemolysis of erythrocytes is a common cause of renal insufficiency, which leads to azotemia and increasing creatinine and urea values in blood plasma (Niwetpathomwat et al., 2006; Joubert et al., 2007; Cardoso et al., 2010). Creatinine levels on average were estimated to be 141.40±13.54 µmol/l ($p<0.05$; the reference range 44.2–159.0 µmol/l), urea 29.32±3.75 mmol/l ($p<0.05$; the reference range 3.33–8.99 mmol/l) in dogs affected by babesiosis and having concurrent haematological abnormalities (Aiello, 1998).

Noncojugated and total bilirubin levels increase in blood plasma due to intensive hemolysis (Keller et al., 2004; Köster et al., 2009). The total bilirubin values in blood plasma of dogs with babesiosis and CBC on average was 49.03±8.43 µmol/l ($p<0.05$; the reference range can be ≤8.55 µmol/l (Aiello, 1998)).

Less commonly, babesiosis is accompanied by liver failure. ALT and AST are increasing, while urea and albumin concentrations in blood plasma decrease (Leisewitz et al., 2001; Niwetpathomwat et al., 2006; Joubert et al., 2007). In dogs which were suffering from babesiosis and had haematological abnormalities alanin aminotransferase was estimated on average to be 69.92±6.65 V/l ($p<0.05$; the reference range 8.20–57.30 V/l) and aspartat aminotransferase 172.10±28.47 V/l ($p<0.05$; the reference range 8.90–48.50 V/l (Aiello, 1998)).

Conclusions

1. Mild anemia was established (RBC 5.37±0.23 x 10^{12}/l, $p<0.05$; HCT 36.71±1.60 %, $p<0.05$) and marked trombocytopenia (PLT 30.90±4.97 x 10^9/l, $p<0.05$) in dogs with overt symptoms of babesiosis (62 % cases, group I).

2. Neutrophilic leukogram profile change to lymphocytic-plasmocytic with predominant monocytosis (16.20±1.30 %, $p<0.05$) were noticed as a haematological abnormalities accompanying babesiosis. Neutropenia also was observed (57.31±2.62 %, $p<0.05$).

3. Blood results within physiological ranges were observed in 38 % of affected dogs (group II) which had no typical clinical signs of babesiosis. However they all had a tendency for monocytosis (9.36±0.76 %, $p<0.05$).

4. Thrombocytopenia, monocytosis, tendency to anemia and leukopenia, also weather temperature within the range of 8–18 °C are the main indexes that justify the necessity of blood smear microscopic analysis.

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