

RESEARCH ARTICLE

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Clinicopathological findings in dogs naturally infected dogs with Babesia

EGON ANDONI*, DHIMITËR RAPTË, REZART POSTOLI, ELENICA DIMCO, JETA ABESHI

Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania

Abstract:

The aim of the study was to describe the clinicopathological findings in dogs naturally infected with *Babesia*. During the period of February 2011-February 2012 twenty-nine cases of *Babesia Canis* were diagnosed in the clinic of Small Animal at the Veterinary Faculty of Tirana. The animal was considered infected when pear shaped babesia was observed inside infected erythrocytes in Wright-Giemsa stained blood smears. The animal stay for one week in our clinic and during this period we evaluated the canine babesiosis cases by means of clinical history, physical examination, hematological and biochemical tests. Based in the clinical sing of babesia the animal was divided in two groups with uncomplicated and complicate babesia. The duration of clinical signs ranged from 1 to 5 days prior to the arrival at the clinic and all the animals were positive with *B. c. canis* infections. The main clinical signs were dehydration and anemia in (79%), apathy (74%), anorexia or decrease appetite (70%) and fever (68%). The anemia was present in the dogs and classified severe (13%) as mild (45%), and moderate (52%) of all the cases. In conclusion, the main clinicopathological findings were a mild to moderate hemolytic anemia and mild to severe thrombocytopenia.

Keywords: Clinicopathological; Canine babesiosis; Tirana

1. Introduction

Canine babesiosis is a protozoal tick-borne disease with worldwide distribution caused by different species of *Babesia*. The geographical distribution of the causative agent and the occurrence of babesiosis dependent on the presence of hard tick species of the Ixodus family. At the same time it is strongly related with the seasons, with most cases are found during the Spring and Autumn. Based in morphological size of trophozoites canine *Babesia* is divided in two groups, small *Babesia* were trophozoites size range from 1.0 to 2.5 μm and large *Babesia*, were trophozoites range from 2.5 to 5.0 μm . On the basis of molecular phylogeny and vector specificity large piroplasms are classified into three sub-species: *B. canis canis*, *B. canis rossi* and *B. canis vogeli*. Three are the distinct species of small piroplasms to cause the disease in dogs *Babesia gibsoni*, *Babesia conradae* and *Babesia microti*-like piroplasm named *Theileria annae*. The small canine piroplasm with the greatest geographical distribution is *B. gibsoni*. *Babesia canis* was first described by Piana and Galli-Valerio (1895) and the pathogenicity of this organism is intermediate. *B. canis canis* infection is distributed in Europe and in Asia. The three subspecies demonstrate tremendous variations in clinical signs, geographical distribution and vector specificity [2]. *B. c. rossi* infection transmitted by *Haemaphysalis leachi* is found in South Africa [1], and is the most virulent subspecies causing hemolytic

anemia and an inflammatory response. *B. c. canis* infection transmitted by *Dermacentor reticulatus* and *B. c. vogeli* infection transmitted by *Rhipicephalus sanguineus*. Clinical cases of large form canine babesiosis have been reported mainly in Italy and in Central-North Europe such as Holland, Germany, Poland, France, and other countries from Balkan areas. Babesiosis may have a hyperacute, an acute, or a chronic course and is capable of causing a wide range of clinical signs such as fever, lethargy, anorexia, jaundice, anemia and thrombocytopenia [1]. The anemia in the begging is mild, normocytic and normochromic, then becomes macrocytic, hypochromic, and regenerative with the progresses of the disease. Thrombocytopenia is common in canine babesiosis, independent of the subspecies involved. Leukocyte abnormalities may include leucocytosis, leucopenia, neutrophilia or neutropenia, lymphocytosis, and eosinophilia [9]. Biochemical profile abnormalities in *B. canis* infection are related with the severity of the disease and the hypoxia degree. The serum activity of Asparate aminotransferase (ALT) and alanine aminotransferase increase at canine babesiosis. Also hyperbilirubinemia, hypoalbuminemia, electrolyte and acid-base abnormaliies are common finding in babesiosis. Hematological abnormalities found in canine babesiosis are anemia and thrombocytopenia. Thrombocytopenia is common in all animals infected with babesiosis and independ of the subspecies involved. Some authors classified babesiosis into

uncomplicated and complicated forms. The various clinical abnormalities appearing in complicated babesiosis such as: cerebral babesiosis acute renal failure, acute pancreatitis, cardiac changes, icterus, caugulopathy and acute respiratory distress syndrome. This clinical manifestation of caused by *B. canis* is highly variable and are not easy to explained by the hemolytic disease process. Some authors have proposed that although the clinical manifestations are diverse, the mechanism promoting them is probably uniform [10]. Pathogenesis involves immunologic factors, increased lipid peroxidation and activation of the coagulation cascade. Soluble parasite proteases activate the kallikrein system and induce the fibrinogen-like protein (FLP) formation. At the same time tissue hypoxia is a common feature in canine babesiosis and is probably one of the major causes for the release of cytokines and other inflammatory mediators. Also lactic acid generation from tissue hypoxia is considered the main reason for metabolic acidosis that develops in animal with babesiosis. The main goal of this work is to provide additional information about the pathogenicity of *B. canis* in blood samples from dogs naturally infected at Tirana region. At the same time we won't to describe the clinical signs and clinicopathological findings of these dogs based in clinical history and physical examination, hematological and biochemical tests.

1. Materials and methods.

Twelve cases of *Babesia Canis* were diagnosed in the clinic of Small Animal at the Veterinary Faculty of Tirana during the period of February 2011-February 2012. Babesia was diagnosed by microscopically examination of blood smear from peripheral blood and also we determineted the species of the parasite which cause the infection. The animals were considered infected when intraerythrocyte pear-shape merozoites were found inside parasites erythrocytes. In each infected animals were observed daily for clinical signs by veterinary clinical examination. They undergo the physical examination and data results were obtained for each case. We were focused in the color of the mucous membranes of mouth and eyelid, dehydration, apathy, anorexia, fever, decreased appetite or absence, spleen size, epistaxis, and abdominal pain. The level of parasitemia was ranked using the scale + (1-10 erythrocytes parasited per blood smear), ++ (11-50 erythrocytes parasited per blood smear), +++ (>50 erythrocytes parasited per blood smear). At the same time blood pressure was measured using the Doppler

method using Doppler Detector (Model 811-B) in all dogs, complete blood count (CBC) was performed by an automatic hematologic analyzer (Mindray BC-2800Vet). The erythrocyte count included: Hemoglobin (Hb), Red Blood Cell (RBC), Platelet, Packet Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC). The level of anemia was classified as described elsewhere (Weiss and Tvedted, 2004a). Full biochemistry analysis included Blood Urea Nitrogen (BUN), Creatinine, Total protein, bilirubine, Asparate amino Transferase (AST) and Alanine amino Transferase(ALT). These values were measured by automated clinical chemistry analyzer (EMP -168 Vet) using standard kits.

3. Results and Discussion

3.1 Clinical signs

The duration of clinical signs ranged from 1 to 5 days prior to the arrival at the clinic (estimation by the owners). The mean of 29 patients is 4.56 ± 2.43 with median age is 4 years old. The minimal age is 0.9 years old and maximal age 9.3 years old and it come under normal spread based on the Kolmogorov-Smirnov test for normal distribution =0.8. The mean temperature of 29 patients $39.47 \pm 0.64^{\circ}\text{C}$ with median temperature 39.2°C . The minimal temperature is 38.7°C and maximal temperature is 41°C .The clinical signs were as follows: 5–10% dehydration (26/29, 89.65%), apathy (17/29; 58.62%), anorexia (11/29; 37.93%), decreased appetite (3/29; 10.3%), fever (16/29; 55.1%), icterus (8/29; 27.5%), petechiae (3/29; 10.3%) and abdominal pain (12/29; 41%).

Table 1. Statistics summary of age and temperature in cases with babesia.

	Mean	SD	Mediana	Min	Max
Age	4.566	2.437	4.000	0.900	9.300
Temp	39.479	0.6494\	39.200	38.700	41.000

3.2 Abdominal pain

We notice that 12 (41%) have abdominal pain and 17 (59%) don't have abdominal pain without important statistic change, $p=0.4$

3.3 Parasitemia

The level of parasitemia was different between dogs. In the best part of than (16/29 55.17%)

presented the low level of parasites (+), (10/29 34.48) shows medium amount of parasites (++) and (3/29 10.3%) shows high level of parasites (+++).

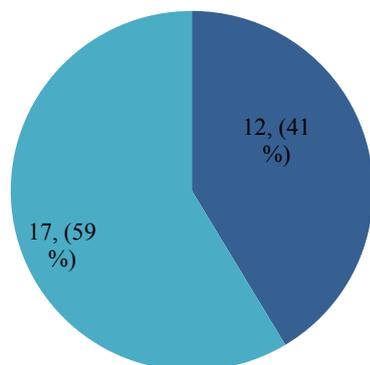


Figure 1. Percentage of the patients with abdominal pain

3.4 Haematocrit

The haematocrit value of the animals was determined daily and was expressed as the PCV of a sample of heparinised blood. The anemia was considered when hematocrit was lower than 37%. The

severity of anemia was classified as described elsewhere (Furlanello et al., 2005). A single dose of imidocarb dipropionate (Imizol) injection was given intramuscular to all the dogs to eliminate the *Babesia* infection.

3.5 Anemia

Anemia was present in 23/29 (79.31%) and further classified as moderate 14/23 (60.8%) mild in 7/23 (30.4%), and severe 2/23 (8.69%). In all cases, the anemia was normocytic and normochromic.

3.6 Biochemical abnormalities

The value of Alanine amino Transferase (ALT) was within normal range mean \pm SD (71.94 \pm 40.51), Aspartate amino Transferase (AST), mean \pm SD (37.73 \pm 13.14) and Bilirubine mean \pm SD (0.75 \pm 0.3). Azotemia was present in seven dogs (7/29 24.1%) of all the cases. Urea was elevated with normal creatinine in eight dogs (8/29 27.5%) of all the cases.

Table 2. Hematological and biochemical parameters in 29 dogs with babesiosis

	Mean	Mediana	SD	Minimal value	Maximal value
RBC($10^6/\mu\text{l}$)	3.62	3.8	1.28	1.5	6.9
HCT (%)	21.66	19	7.99	10.2	38
MCV(fL)	58.1	58	6.57	48	73
MCHC(g/dl)	38.28	38	5.92	27.5	50.5
PLT($10^6/\mu\text{l}$)	148.9	149	41.55	55	260
ALT(U/I)	71.94	58	40.51	16	190
AST(U/I)	37.73	35	13.14	18.5	78
UREA(mg/dl)	22.17	25	9.32	1.5	32.7
CREA(mg/dl)	1.53	1.4	0.59	0.5	3.1
BILT(mg/dl)	0.75	0.7	0.3	0.2	1.5
PT(g/dl)	6.3	5.8	1.74	2.3	10.6

4. Conclusions

Canine babesiosis is considered worldwide a cause of hemolytic anemia [1]. The classical clinical presentation of canine babesiosis is a febrile and hemolytic syndrome with apparent anemia and hemoglobinuria. However, the severity of this illness is very variable depending mainly on the species of *Babesia* involved. The temperature in our study was (39,45 °C), which shows a moderated elevation. The values is in concordance with others studies, and it is well now that sub-species *Babesia canis canis*, which

is less virulent from the forms caused by *Babesia canis rossii* sub-specie with are the most virulent strains [3]. Temperature is one of the most common sings in canine babesiosis. In general it has a moderate increase and doesn't need a specific treatment. A study made from some authors [7], shows that temperature is a parameter with the concentration of parasite antigens in blood plasma. Usually the temperature values return within normal values 2-3 days after infection. The body temperature may increase from both antigens released from infected erythrocytes and from soluble parasite antigen .The mean of the blood pressure was 117.5 \pm 32.5 mmHg.

These results show a clear tendency of hypotension in animal's severe ill with complicated babesiosis. Animals infected with the highest levels of parasitemia induced to levels that are life threatening [8], and blood pressure was low for the second consecutive day. In the others cases blood pressure was restored to normal levels within one day after treatment. Similar blood pressure changes have been observed in clinical canine babesiosis [4] and were suspected in experimental *B. canis* infection [7]. However the mechanisms which induce the canine babesiosis remain unclear, but it is suspected that it is a combination of the factors which include vasodilatation and the increase of permeability of blood vessels. There are contradictory indications regarding the level of abdominal pain in animals infected with babesia. In 41% of cases we observe abdominal pain. In a recent study from [5], in Hungary this level was 13%, while in a study from in Spain these level was 66.35%. Anyway in our study the level of abdominal pain was higher in animals infected with complicate babesiosis. This abdominal pain may be inflicted from splenomegaly, acute pancreatitis [6] and alternation in gastro-intestinal system together with azotemia. The level of anemia range from the easy to severe forms. The level of anemia is many variable in infections caused from babesia [1]. These changes may be caused from the different stages in the diagnosis of the disease. In some cases the disease was identify in the early stages preventing the intravascular hemolize and other complications. In others cases the diagnosis of infected animals was done advance stage of the disease with values of hematocrite was very low and the prognoses was suspect. The majority of the dogs showed hemolytic anemia and only four dog's shows sing of non-hemolytic anemia. Hemodilution and erythrocyte sequestration play a role in phenomenon instead of erythrocyte lysis. There was no correlation between the severity of the disease and the degree of anemia. Possible explanation of the lack of correlation between the degree of anemia and the severity of the disease are that many of the pathogenic process are a result of host immune response and not from the destruction of the erythrocyte from parasite. Anyway in some cases babesiosis may be associate with other hematozoa, viral and bacterial disease, which may influence in the clinical condition of the animal. Biochemicals findings include elevate value of Alanine amino Transferase (ALT), Asparate amino Transferase (AST), and hyperbilirubinemia. As regards the functional work of the kidneys we were

based in the determination of the values of Creatinine and BUN. The increase value of creatinine and BUN may be caused from different factors. Some time these values may increase as a result of dehydration caused from anorexia or hyperthermia [1; 5]. The elevated serum urea and the increase value of creatinine shows azotemia, which could reflect decreased renal blood flow, possibly because of decreased blood pressure or hypovoalemia. In other cases patients shows elevated value of serum urea without a corresponding increase in the serum creatinine. The phenomenon has been attributed to catabolism of lysed erythrocytes, resulting in an increased ammonia load on the liver and consequent increased urea production. The increase value only of the BUN cannot be used to assess renal insufficiency in dogs with babesiosis. The elevated creatinine level is a trust indicator of renal failure, and studies has shows that elevated creatinine is associate with the increase risk of death in canine babesiosis. The main clinicopathological findings was thrombocytopenia that range from mild to severe and a moderade or mild hemolytic anemia.

5. References

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