
Anemia description in *Babesia* spp. infected dogs

Răzvan MĂLĂNCUȘ, Geta PAVEL, Mihai CONDREA

Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine
"Ion Ionescu de la Brad" Iași, 8 Mihail Sadoveanu Alley
E-mail: razvanmalancus@uaiasi.ro

Abstract

Babesiosis is a tick-borne malaria-like illness caused by species of the intraerythrocytic protozoan Babesia. Infection in dogs may occur by tick transmission, direct transmission via blood transfer from dog bites, blood transfusions or transplacental transmission. The most common mode of transmission is by tick bite, as the Babesia parasite uses the tick as a reservoir. The study was undertaken between 2015-2017 in Physiology and Pathophysiology laboratory, Faculty of Veterinary Medicine Iasi on 21 dogs of different breeds and age. Babesia infected dogs represented 4,3% of the total number of investigated blood samples. Due to Babesia spp. affinity for erythrocytes, anemia is the most commonly diagnosed disorder in babesiosis, being observed in 11 patients (52,4%). Direct action of parasites on erythrocytes by producing toxins or indirectly by stimulating an autoimmune response leads to destruction of red blood cells in large numbers according to the degree of parasitemia. The average value of erythrocytes, hemoglobin and hematocrit are inversely proportional to the expressed parasitemia by the studied individuals. As previous studies have shown before, in Babesia spp. infected dogs, anemia is accompanied by monocytosis. The increase in monocyte counts correlates to the leukocyte proliferation found in babesiosis. Monocytosis certifies the chronic evolution of the disease and the autoimmune character induced by the development of the parasitic stages.

Key words: *Babesia* spp., dogs, anemia

Introduction

The primary function of the red blood cells is to transport oxygen to tissues. Anemia is defined as a significant deficit in the mass of circulating red blood cells. As a result, the capacity of the blood to deliver oxygen is compromised. The presence of anemia can be documented by measurement of either the concentration of hemoglobin in the blood or the hematocrit, which is the ratio of the volume of red blood cells to the total volume of a blood sample. A patient is anemic if the hemoglobin or hematocrit value is more than two standard deviations below normal. The lower limits of normal vary with the age of the individual and, in adults, with gender. Occasionally, the documentation of anemia is confounded by a concurrent alteration in the plasma volume. For example, if a patient with a low mass of circulating red blood cells is also hypovolemic, owing to a concurrent loss of plasma volume from dehydration, the blood hemoglobin and hematocrit levels will be falsely elevated and may even be in the normal range. Another case is represented by acute hemorrhage, in which there is concomitant loss of both red blood cells and plasma.⁴

Babesiosis is a tick-borne malaria-like illness caused by species of the intraerythrocytic protozoan *Babesia*. Infection in dogs may occur by tick transmission, direct transmission via blood transfer from dog bites, blood transfusions or transplacental transmission. The most common mode of transmission is by tick bite, as the *Babesia* parasite uses the tick as a reservoir.²

In babesiosis, the parasite of the *Babesia* genus, *Babesiidae* family, sets in the parasitized organism erythrocytes in variable number (1-4 parasites), putting on different shapes and forms, depending on the species. The parasite has several species that can be found in dogs, like *Babesia canis*, *Babesia vogeli* or *Babesia gibsoni*.²

The effect of Babesia spp. over the red blood cells is their destruction, causing hemolytic anemia. Animal contamination is achieved by transcutaneous inoculation during the feeding of

infected ticks that inserts the parasites together with saliva. The inoculated parasites initially penetrate red blood cells, they multiply, secret metabolic toxins that causes the lysis of red blood cells, so anemia occurs. Due to these phenomena the entire functioning of the body is disturbed developing liver and kidney disorders, nervous, respiratory and cardiac disease.³

Material and methods

The study was conducted at the Faculty of Veterinary Medicine Iasi, over a two-year period, the research being performed on a total of 21 dogs, of different breeds and ages, all these subjects being affected by babesiosis. For each of these cases blood sample collection has been performed using EDTA as anticoagulant.

The investigated hematological parameters have been represented by the number of erythrocytes, hemoglobin, hematocrit, derived erythrocyte constants (mean corpuscular volume - MCV, mean corpuscular hemoglobin - MCH and mean corpuscular hemoglobin concentration - MCHC), reticulocyte count, ESR, both platelets and leukocytes count. The determination of red blood cells parameters have been made by conventional methods.⁵

Determination of the red series main parameters (number of erythrocytes, hemoglobin, hematocrit) can provide relevant data on the existence of anemia, which is common in babesiosis due to destruction of large numbers of red blood cells. However, the persistence of anemia in animals in convalescence may be maintained by the presence of erythrocyte self antibodies and immune complexes, erythrocytes lysis being induced by complement.

The observation of *Babesia spp.* infestation degree has been made by reading the May Grumwald Giemsa stained blood smears and determining of leucocytes formula, the hematological examination allowing to appreciate blood parameters changes, disturbances accompanying *Babesia spp.* infestation. It must be considered that *Babesia spp.* may not always be identified in the blood smear. It is considered that they are visible on the first day after inoculation, and then they disappear until day 10. From day 11 to day 21 after inoculation *Babesia spp.* can be observed in erythrocytes, their presence being directly proportional to the degree of parasitemia.⁴

Assessment of hematologic changes allows to ascertain between different types of anemia, focusing on the infestation development and allowing to appreciate the parasitemia degree. Thus, the determination of leukocyte formula ascertains the evolutionary parasitic forms located in the intermediate hosts blood, these data corroborated with other hematological parameters helping to establish a correct diagnosis that allows precise identification of the starting point of infestation.³

The obtained data has been tabulated in contingency tables, statistical appreciation being achieved by using the SPSS Statistics 18 statistical software and Fisher's exact test which illustrates the association between two different categories of investigated parameters

Results and discussions

The conducted study has investigated and allowed to diagnose the types of anemia developed by the *Babesia spp.* infected subjects. The main changes induced by babesiosis had repercussions on the red series regarding the number of erythrocytes, quantity of hemoglobin, hematocrit and derived erythrocyte constants.

Out of 596 laboratory samples examined over the two-year period, 494 represented blood samples. From those, 21 patients have been identified with babesiosis, representing 4,3% of the investigated blood samples. The most *Babesia spp.* infected dogs were identified in 2016 (13 patients) while in 2015 and 2017 babesiosis was observed in 5, respectively 3 dogs (fig. 1). Although a peak was recorded in 2016, the babesia infected patients represented 3,9% of the total investigated patients, while in 2015 represented 4,5% and in 2017, almost 3% (2,8%).

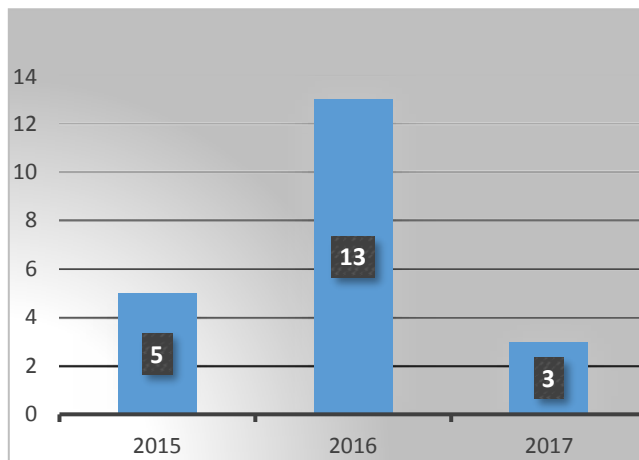


Fig. 1. Babesiosis cases in dogs between 2015-2017

Because of *Babesia spp.* affinity for erythrocytes, anemia is the most commonly diagnosed disorder in babesiosis. The direct action on erythrocytes by producing toxins or indirect action by stimulating an autoimmune response leads to mass destruction of the red blood cells depending on the parasitemia degree. Thus, the reduction of the red blood cell counts, hemoglobin and hematocrit are directly proportional to the expressed parasitemia by the studied subjects.³

Patients with mild or moderate anemia are often asymptomatic. Many note breathlessness and/or fatigue only upon strenuous exercise. In severe anemia, dyspnea and fatigue are common complaints. These symptoms reflect limitations in the earlier-mentioned compensations for the tissue hypoxia imposed by a low red blood cell mass. Physical findings also depend on the severity of the anemia. Pallor reflects a compensatory shunting of blood away from the skin to ensure adequate flow to vital organs. Those with severe anemia may have tachycardia at rest, owing to a compensatory increase in basal cardiac output.² The hyperdynamic circulation in such patients often gives rise to a systolic “flow” murmur that is transmitted into the neck. In patients with lesser degrees of anemia, the heart rate is normal at rest but, on exercise, increases more than normally. Anemic patients may have many other informative physical findings that depend on specific underlying pathophysiology. For example, those with hemolytic anemia often have splenomegaly, owing to trapping of defective or damaged red blood cells in the spleen, and jaundice, reflecting increased plasma bilirubin levels due to rapid destruction of red blood cells.⁴

The characterization of the anemia tried to assess the following factors: presence of reticulocytosis, dimension of the red blood cells, presence of poikilocytosis and modification in hemoglobin content of the red blood cells in investigated patients.

The anemias can be divided into three broad categories: decreased red cell production, increased red cell destruction, and blood loss. Often, the patient’s history and physical examination provide information as to which process is going on. For example, the presence of blood loss is usually apparent from the history. Physical findings such as jaundice and splenomegaly suggest hemolysis.⁵ Among the available laboratory tests, the reticulocyte count is the simplest and most reliable way to distinguish among the three major categories of anemia. This laboratory test is a measurement of the fraction of young red cells in the blood (<2.5 days old). In patients with impaired red cell production, the reticulocyte count will be inappropriately low. Despite elevated levels of plasma erythropoietin, the bone marrow is unable to respond to produce adequate numbers of new red cells. In contrast, the reticulocyte count is generally elevated in both hemolytic anemia and in acute blood loss.⁶

We recorded 13 cases (61,9%) that presented reticulocytosis, with the most pronounced values of 78,5%, respectively 610.000 reticulocytes/mm³ being observed in a patient with severe anemia due to *Babesia spp.* infestation associated to antiparasitic treatment (fig. 2).

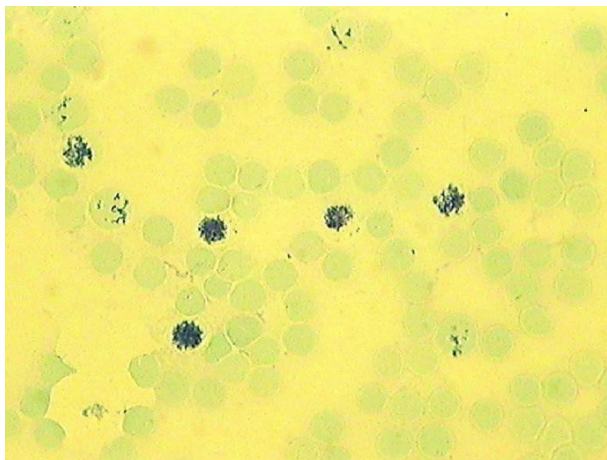


Fig. 2. Increased number of reticulocytes in a *Babesia spp.* infested dog

A wide range of structural, metabolic, immunologic, and mechanical defects can result in premature destruction of circulating red cells. However, irrespective of etiology, uncomplicated hemolytic anemias have a number of features in common. The capacity for efficient erythropoiesis is preserved, and indeed, in response to hypoxia-induced erythropoietin production, red cell production is often markedly increased, an adaptive response that is reflected by an elevation of the reticulocyte count. Usually, destruction of red blood cells is accompanied by a stimulation of erythropoiesis and release of immature red cell precursors in the blood stream, one of the adaptive mechanisms being represented by the merge or skipping of some precursor stages of development and their early release from the bone marrow. Anemia accompanied by reticulocytosis of 5% or greater strongly suggests the presence of hemolysis. However, elevated reticulocyte counts can also be seen in nutritional anemias during the first two weeks of replacement therapy with iron, cobalamin (vitamin B12), or folic acid. Acute hypoxia can also cause a transient elevation of the reticulocyte count. Finally, infiltrative bone marrow disorders such as metastatic neoplasms can also induce a modest sustained elevation of the reticulocyte count due to early release.

Regarding the changes observed in erythrocyte derived constants, 81,0% (17 cases) of investigated dogs presented either decreased MCV, MCH or MCHC, the association being considered statistically significant in dogs with *Babesia spp.* hemolytic anemia, with $p < 0,02$. Changes in volume of the red blood cell were noticed in 19 patients, the association between the presence of anisocytosis and anemia in dogs being very statistically significant, with $p < 0,001$. Changes of MCV and MCHC were recorded in 7, respectively 5 dogs, with no statistical association with manifested anemia.

A statistically significant association ($p < 0,02$) was noticed between increased erythrocyte sedimentation rate (ESR) and severe infestation with *Babesia spp.*, with 90,9% of the severely affected dogs (10 out of 11 dogs) presenting high values for ESR.

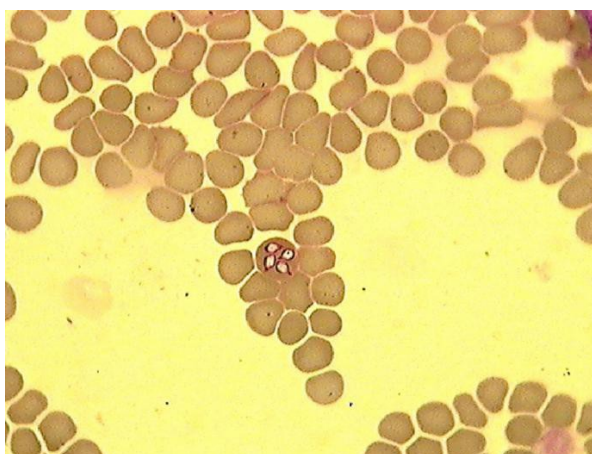


Fig. 3. Massive infestation with *Babesia canis* inside a red blood cell

Although increased ESR has been observed in 14 cases, there is no association between the destruction of red blood cells in moderately infested dogs and the high rate of sedimentation for the red blood cells.

Changes in the shape of the red blood cells, usually associated with anemia, can only be recorded by reading a stained blood film. Microscopic examination of a carefully spread and well-stained blood smear is an important part of the evaluation of any unexplained anemia, but it is particularly informative in identifying the cause of hemolysis.¹ Out of 21 cases, 19 dogs, representing 90,5% of the patients, had poikilocytosis, characterized by the presence of schizocytes (fragments of red blood cells), echinocytes, keratocytes, drepanocytes (sickle cell) or dacriocytes (tear shaped cell) (fig. 4).

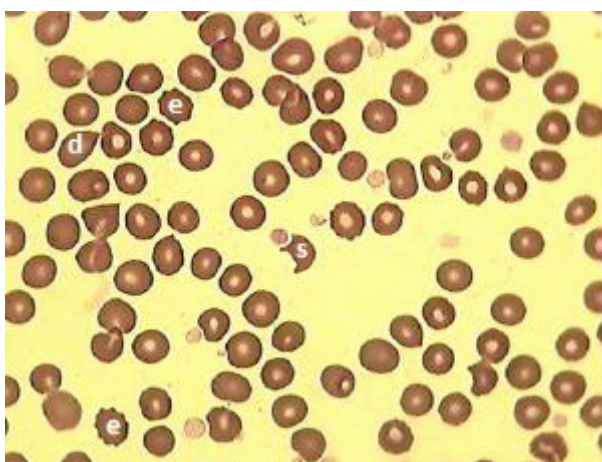


Fig. 4. Poikilocytosis - variety of shapes between red blood cells:
s - schizocyte; e - echinocyte; d - dacriocyte

The changes in shape are considered to be the result of parasite action against the red blood cells and also increased fragility of red cells membrane.

Conclusions

The undertaken study allowed to be drawn some relevant conclusions about the evolution

of anemia in *Babesia spp.* infested animals:

1. *Babesia spp.* infested dogs over a two-year period, between 2015-2017, represented 4,3% of the total number of investigated patients;
2. Thirteen cases (61,9%) presented reticulocytosis, with the most pronounced values of 78,5%, respectively 610.000 reticulocytes/mm³ being observed in a patient with severe anemia due to *Babesia spp.* infestation associated to antiparasitic treatment;
3. There was identified a very statistically significant association between the presence of anisocytosis and anemia in dogs with babesiosis, with $p < 0,001$;
4. A statistically significant association ($p < 0,02$) was noticed between increased erythrocyte sedimentation rate (ESR) and severe infestation with *Babesia spp.*, with 90,9% of the severely affected dogs (10 out of 11 dogs) presenting high values for ESR

References

1. Hossain M.A., Yamato O., Yamasaki M., Maede Y., 2003 – Clinical and haematological studies on experimentally induced chronic babesiosis in splenectomized dogs, *Bangl. J. Vet. Med.*, 1:53-56;
2. Irwin P.J., 2010 – Canine babesiosis, *Vet Clin Small Anim*, Elsevier, 40, 1141-1156;
3. Pavel Geta, Mălăncuș R.N., 2013 - Correlation between hematological parameters and babesia spp. infestation in animals, *Buletinul USAMVCN CN nr. 70(1-2)/2013/USAMVCN-STA 1(1-2)/2013*;
4. Schoeman J.P., 2009 – Canine babesiosis, *Onderstepoort Journal of Veterinary Research*, 76:59-66;
5. Zdravko Zvorc, Renata Baric Rafaj, Kules J., Vladimir Mrljak, 2010 – Erythrocyte and platelet indices in babesiosis of dogs, *Veterinarski Arhiv* 80(2), 259-267.
6. Wellman M.L., Radin Judith, 2004 - Bone marrow evaluation in dogs and cats, The Gloyd Group, Wilmington, Delaware.