Haematological diagnosis of anemia in dogs and cats

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Abstract

Anemia is part of the erythrocytic system pathology and is characterized by a decrease in hemoglobin and in number of red blood cells in circulating blood, which is a common disorder both in animals and in humans. This study proposes to identify types of anemia according to morphological and etiopathogenetic criteria in 26 patients. The diagnosis of anemia in dogs and cats was based on anamnestic data, clinical and paraclinical examinations. By quantitative haematological determinations and blood smear examination, there were identified 13 cases of normocytic normochromic anemias, 4 of macrocytic hyperchromic anemias and 9 of microcytic hypochromic anemias. Depending on the number of immature erythrocytes circulating in the blood, were identified 7 cases of hyperregenerative anemias, 6 hyporegenerative, 10 generative and one normoregenerative anemias, 2 of these cases remaining unclassified. Regarding the etiopathogenesis of anemias, were identified 11 cases of parasitic hemolytic anemias, 4 cases of autoimmune hemolytic anemias, one case of infectious hemolytic anemia, 2 cases of posthemorrhagic anemias and 8 hemolytic anemia associated with unknown causes. The results obtained indicate 92.3% of peripheral hemolytic anemias and 7.7% of anemias caused by excessive red blood cell loss.

Key words: *anemia, pets, hematology*

Introduction

Anemia is part of the erythrocytic system pathology and is characterized by a decrease in hemoglobin and in number of red blood cells in circulating blood, which is a common disorder both in animals and in humans. Anemia appears as a result of changes of one or more factors involved in erythrogenesis: marrow, "building materials" of erythrocytes, catalytic or stimulatory factors. Sometimes, although the production of erythrocytes is normal, it can appear destruction or loss of erythrocytes due to other globular or extraglobular causes.

Depending on the morphological criterion, anemia is classified into three types: macrocytic, normocytic, microcytic hypochromic. Macrocytic anemias, characterized by an increased mean cell volume (MCV), hemoglobin (HEM), and reduced red blood cells are less common in animals but may be a transient response to haemorrhage, hemolysis, etc., when macrocytosis is consequence of releasing in the blood of immature erythrocytes, larger than adult ones. Normocytic anemias, where MCV and MCHC are normal , can be commonly caused by hemolysis or bone marrow depression, following inflammatory disorders. Microcytic hypochromic anemias, characterized by small size of erythrocyte, decrease erythocyte number, hemoglobin and HEM, is a frequent deficiency in iron and other anti-anemic microelements (Nicolae Avram, 1999). Normochromic anemia can also be added to this classification, hemoglobin being in normal limits.

Depending on the number of reticulocytes, anemia can be classified as: regenerative, when the bone marrow can respond to anemia and produce new erythrocytes in the blood; hyperregenerative, when reticulocytes are above normal; aregenerative, characterized by absence of immature erythrocytes in anemias; hyporegenerative due to deficient erythropoiesis.

From etiopathogenetic classification, depending on the response of the marrow and the circulating blood, there are:

- central anemia caused by hypofunction of bone marrow, characterized by blocking red cell precursors. Usually are included protein-vitamin-mineral anemias and toxic anemias;

- hemolytic anemia with peripheral origin, caused by excessive lysis of red blood cells and premature destruction. The etiology of these anemias are some endogenous (autoimmune mechanisms) or exogenous factors.

- anemia caused by excessive loss of red blood cells in haemorrhages due to injuries or other causes (Nicolae Avram, 1999). Hemorrhage can be internal or external. Hemorrhage into joints and the abdominal cavity are examples of internal hemorrhage. Hemorrhage from lacerations, loss from the gastrointestinal or urinary tract, or external or internal parasites are examples of external hemorrhage (Maxey L. Wellman).

Size changes (anisocytosis) are characterized by present of red elements in different sizes: megalocyte (12-15 μm), macrocyte (8-12 μm), microcyte (4-6 μm), schizocytes (2-3 μm). Anisocytosis involves abnormal red blood cell regeneration.

Shape changes (poikilocytosis) refer to the detection in smears of different shapes of erythrocytes such as ovalocytes, drepanocyte, rocket, drop, etc. Also, may also appear nucleated erythrocytes, Howell-Jolly corpuscles (indicates exaggerated regeneration), Heinz corpuscles (indicates serious anemias).

Color changes (anisochromia) of RBC are depending on the content and the quality of hemoglobin. RBC with insufficient hemoglobin (hypochromia) appears with a clear central area colored only on the periphery or pale. Hyperchromia is an intense and uniform coloring of the erythrocytes, possibly with a dark hue in center. In some pathological conditions, hemoglobin affinity for acidic is replaced by a more or less pronounced basophilia. Polychromatophilic or basophilic coloration of erythrocytes are aspects found in anemias , indicate a red cell regeneration.

Nucleated RBCs (NRBCs) can indicate active regeneration but are also seen with splenic dysfunction, shock, heavy metal toxicity and bone marrow disorders. The presence of polychromasia, anisocytosis and NRBCs on blood smears may indicate regeneration (Séverine Tasker, 2006).

Although kidney failure and some infections (flea infestation, FeLV infection and hemobartonellosis) are likely the most common causes of anemia, there are many other differential diagnoses to consider, such as bleeding disorders, toxicity, metabolic disturbances, hereditary defects, and immune-mediated hemolytic anemias. It is therefore crucial to carefully assess the feline patient by history taking, physical exam and routine laboratory tests in order to determine the cause and offer the most appropriate treatment (Urs Giger, 2016).

Material and methods

Investigations were conducted on 26 cats and dogs of various breeds and ages that were presented during the March 30, 2016 to March 21, 2017 in the Medical Clinic of Faculty of Veterinary Science from Iași and in private Veterinary Clinic from Iași.

Diagnosis of anemia in animals is based on anamnetic data, clinical and paraclinical examinations. In clinical examination, some common disorders occur in all anemia: pale skin, membranes; red, brown or black urine; hepatomegaly with increased sensitivity, splenomegaly; tachycardia, polypnea or dyspnoea at rest, or at low effort. Paraclinic examinations require haematological determinations and examination of blood smears. Hematologic examination is essential in diagnosis. A low number of erythrocytes, hemoglobin and hematocrit values are the main parameters to diagnose anemia.

Initial diagnostics in an anemic patient should focus on identifying the cause of anemia. A diagnosis of anemia secondary to an underlying immunemediated pathogenesis is based on evidence of accelerated red blood cell (RBC) destruction (Andrew Mackin, Todd Archer, 2014).

The investigation stages to diagnose anemia are:

-determination of hemoglobin (Hb) and hematocrit (Ht) as the main parameters, accompanied by count of red blood cells (E);

-determination of erythrocyte constants: MCV, HEM, MCHC, to specify the morphological type of anemia;

-examination of the blood smear for determining the morphology of the red blood cells: size, color, shape, young elements (Nicolae Avram, 1999).

In order to establish the diagnosis of anemia were used classical methods of haematological analysis such as the hemocitometric method for counting erythrocytes, Sahli colorimetric method for hemoglobin dosing, the microhematocrit method for hematocrit determination, mathematical methods for derived erythrocyte constants (MCV, HEM, MCHC), specific staining techniques with brilliant cresyl blue for reticulocytes, May-Grünwald-Giemsa (MGG) or Diff-Quick (DQ) for erythrocytes.

Hematocrit is the percentage expression of globular blood volume in relation to total blood volume (in other words, as percent of the total blood volume is erythrocytes, since the volume occupied by the other elements is negligible). Determining hematocrit with microhematocrit method uses heparinated capillary tubes. The end of the tube is closed to the flame and then centrifuged at a special centrifuge. To reading a hematocrit it is used a special reading device (fig. 1).

Fig. 1. Janetzky centrifuge (right) and reader for microhematocrit determination (FMV Laboratory Iasi)

Determination of erythrocytes by haemocytometric method uses: the counting chamber, also called hemocytometer (Bürker-Türk, Thoma, Neubauer), Potain pipette for erythrocytes, Hayem dilution fluid and microscope (fig. 2).

Fig. 2. Materials for the haemocytometric method

The Sahli colorimetric method uses: the Sahli hemoglobinometer, hydrochloric acid and distilled water. The Sahli hemoglobinometer contain a comparator and a capillary pipette.

Fig. 3. Sahli hemoglobinometer

The mean cell volume (MCV) is the volume of isolated erythrocyte. It is measured in μ^3 and calculated with formula: VEM = Ht x 10 / E.

Medium erythrocyte hemoglobin (HEM) is the average hemoglobin content of an erythrocyte. It is measured in picograms and calculated with formula: HEM = Hb x 10 / E.

The mean cell haemoglobin concentration (MCHC) is the average hemoglobin concentration in the blood. It is expressed as a percentage or in g / dl red blood mass and is calculated with formula: MCHC = Hb x 100 / Ht (Geta Pavel, Răzvan Mălancuş, 2015).

Results and discussions

By quantitative haematological tests and blood smear examination, were identified 13 (50%) normocytic normochromic anemias, in 5 cats and 8 dogs, 4 (15.4%) macrocytic hyperchromic anemias in 2 cats and 2 dogs and 9 (34.6%) microcytic hypochromic anemias, in 2 cats and 7 dogs.

Depending on the number of immature erythrocytes circulating in the blood, were identified 7 cases of hyperregenerative anemias, 6 hyporegenerative, 10 generative and one normoregenerative anemias, 2 of these cases remaining unclassified. Reticulocytes are erythrocytes with vital grains; the granulocyte substance (identical to the basophilic polychromatic substance) appears colored blue on a pink background, being placed in different positions: sometimes at the edge of the cell in granules, sometimes in the center, or can even fill the whole cell. Increased reticulocyte counts occur in the red cell regeneration phase (adapted from I. Adamesteanu, A. Nicolau, H. Bârză, 1966). Regeneration is evidenced by anisocytosis, polychromatic macrocytes, large numbers of reticulocytes.

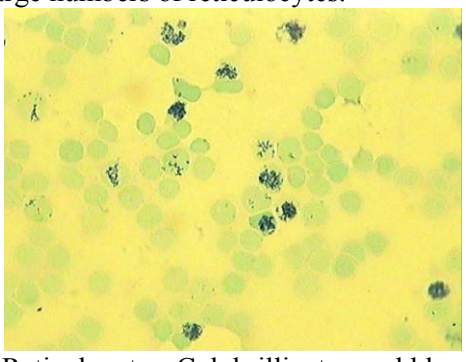

Fig. 4 Reticulocytes, Col. brilliant cresyl blue x1000

Regarding the etiopathogenesis of anemias, were identified 11 cases of parasitic hemolytic anemias, 4 cases of autoimmune hemolytic anemias, one case of infectious hemolytic anemia, 2 cases of posthemorrhagic anemias and 8 hemolytic anemia associated with unknown causes. The results obtained indicate 92.3% of peripheral hemolytic anemias and 7.7% of anemias caused by excessive red blood cell loss. Parasitic haemolytic anemias were caused by Mycoplasma hemofelis in 3 cats and by Babesia gibsoni in 9 dogs. In a study by Shalm (1975) it was found that Mycoplasma hemofelis disease is rare, affecting both sexes but with a higher frequency in males. Infectious feline anemia can affect all ages of animals, most of which are described in cats aged 1-3 years. Based on the findings of the previous study, in the present study only one cases from 3 cases of feline infection were identified. In the blood smear were seen changes of erythrocytes such as: anisocytosis, Jolly bodies, schizocytes, echinocytosis. The analysis of the blood smear in cases with the Babesia gibsoni parasite revealed: intraerythrocytic babesies (fig. 5A), echinocytes (fig. 5B), schizocytes (fig. 5C), Jolly bodies (fig. 5D), target cells, polychromatophiles, young nucleated erythrocytes indicating splenic hypofunction and excessive regeneration, anisocytosis.

The four cases of autoimmune hemolytic anemia (AHAI) were found only in Bichon and American Bulldog dogs. These occurred after post-transfusion reactions, when the donor's incompatible erythrocytes are hemolyzed by the recipient's pre-existing antibodies. Haematological changes characteristic of the disease are the presence of spherocytes, polychromatophilic macrocytes, agglutination and anisocytosis. Recent studies have indicated that all dog breeds are prone to this type of anemia, but predominantly Cocker spaniels, English Springer spaniel, Poodle and English Sheepdogs (Andrew Mackin, 2014). Also, AHAI is more common in dogs than in cats, but recent studies (Husbands, 2002, Kohn, 2006) show that this is also common in cats. IMHA is primarily a disease of middle-aged to older dogs. It may occur at any age but is rare in dogs younger than 1 year (Michael J. Day, 2012).

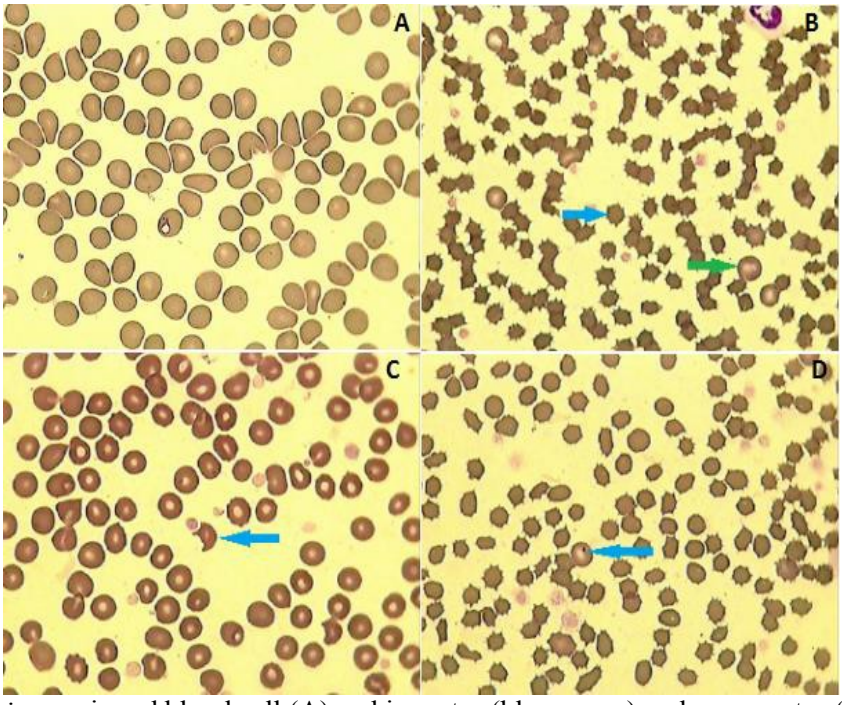

Fig. 5. *Babesia spp.* in red blood cell (A); echinocytes (blue arrow) and macrocytes (green arrow) (B); horn-shaped schizocyte (C), intraerythrocytic Jolly bodies, Col. MGGx1000

Anemias caused by excessive red blood cell loss due to external haemorrhages were found in a dog and was microcytic, hypochromic, normoregenerative anemia (MCV, HEM under normal range), and a cat with a macrocytic, hyperchromic, aregenerative anemia (MCV, HEM over normal limits).

Conclusions

Dysfunction of the red blood cell line, anemia is a common disease in animals. Red blood cells, also known as erythrocytes are important for the transport of oxygen from lungs to all organs in the body. Reducing the number of erythrocytes below the normal limit, the low amount of hemoglobin in anemia has different causes and is associated with many diseases that can be treated more effectively when symptoms are discovered in short time. The diagnosis of anemia in dogs and cats can be based on anamnestic data, clinical and paraclinical examinations. An important role are quantitative haematological tests and blood smear examination. Analysis of haematological determinations leads to a morphological diagnosis, and the analysis of blood smear orientates to an ethiological diagnosis as nucleated cells are indicators of splenic hypofunction in babes and excessive regeneration, spherocytes are characteristic in autoimmune diseases, target cells idicate diseases with hepatic origin, polychromatophiles regeneration, schizocytes and echinocytosis a pathological orientation of the blood vessels.

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