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Classification of anemia as to the medullary response through reticulocyte count in dogs attended to in the Veterinary Hospital from Federal University of Mato Grosso, *Campus Sinop*.

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Abstract. Anemia is defined as the presence of erythrocytes, hemoglobin concentration and/or hematocrit below the reference values, leading to death if not treated. It is manifested clinically in the presence of pale mucosae, dyspnea, exercise intolerance and increased heart rate due to reduced tissue oxygenation. The aim of this study was to classify the anemia according to the medullary response in regenerative with weak, moderate or intense release and non-regenerative with null response, correlating with its possible causes by reticulocyte count. Thus, 50 blood samples from anemic dogs treated at the Veterinary Hospital of the Universidade Federal de Mato Grosso, Sinop Campus, were evaluated. After collection, blood smears were prepared for reticulocyte counting using supravital staining. Of the 50 animals evaluated, 54% (27/50) showed non-regenerative anemia with null response (<60,000 cells/ μ L), 36% (18/50) showed regeneration with weak release (60,000 to 150,000 cells/ μ L) and 10% (5/50) presented moderate to intense regeneration (>150,000 cells/ μ L). In conclusion, in this study was observed a predominance of non-regenerative anemia and a lower incidence of responsive anemia; the medullary response was more evident in hemoparasitosis anemia and non-regenerative anemia occurred mainly in animals with renal insufficiency, possibly due to insufficient production of erythropoietin.

Keywords: erythropoietic activity, regeneration, red blood cell count.

Introduction

Anemia is a reduction in the number of red blood cells resulting in a decrease in oxygen in the tissues (Thrall, 2015). There are three main mechanisms that favor its development: increased erythrocyte destruction rate, decreased production of new erythrocytes and acute or chronic hemorrhages (Jonker & Van Hensbroek, 2014).

Three parameters are used to classify anemia: the size and hemoglobin content of the erythrocyte, erythroid response of the bone marrow (regenerative or non-regenerative) and the pathogenic mechanisms, that is, the etiology of the problem (Thrall, 2015).

In order to reverse anemia, effective erythropoiesis is necessary. For this, it is important to stimulate the production of erythrocytes in the bone marrow, the ability of hematopoietic precursor cells to respond to the stimulus and the nutrients necessary for the production of new cells (Lane & Vieth, 2014). The acceleration of erythropoiesis aims to increase circulating hemoglobin and, consequently, the

transport of oxygen to tissues (Failace, 2009; Hoffbrand, 2013).

A practical strategy in the presence of an anemic animal is to evaluate the reticulocyte count, which is also indicative of bone marrow response. Reticulocyte counting is an effective method in the diagnosis, classification and monitoring of anemic patients (Costa et al., 2008).

Reticulocytes are young anucleated erythrocytes, but without loss of their structures, being considered immature erythrocytes that contain some organelles such as: RNA, mitochondria and ribosomes (Cowgill et al., 2003). After about two days in the bone marrow, reticulocytes are released into the peripheral blood to become the mature erythrocyte (Riley et al., 2001).

The term reticulocytes refers to the microscopic characteristics that these cells obtain when subjected to supravital dyes, such as the new methylene blue and the bright cresyl blue (Piva et al., 2010). To enumerate reticulocytes, staining is applied to erythrocytes, thus causing the

aggregation of residual organelles, resulting in a microscopically visible granular material. (Weiser, 2015)

Reticulocytes are the same polychromatophilic cells seen in panoptic-stained blood smears. The evaluation of these cells is suggestive for the response of the bone marrow to anemia. However, the appearance of these cells is more subjective and it is difficult to quantify the corresponding cell count in the smear stained for reticulocytes (Thrall, 2015).

Thrall (2015) reported that the main causes of regenerative anemia are hemorrhage and hemolysis (intravascular or extravascular). Non-regenerative anemias are mainly caused by aplastic pancytopenia due to the use of drugs, chemicals, toxins, estrogen and infectious agents. In addition, in relation to non-regenerative anemia, erythrocyte hypoplasia may come from extrinsic disorders of the bone marrow, including inflammatory diseases, chronic renal failure, associated with endocrine disease and, less commonly, anemia associated with nutritional deficiencies.

This study aimed to evaluate the classification of anemia, regarding the spinal response in regenerative with weak release, moderate to intense and non-regenerative with null response correlating with its possible causes, by means of the reticulocyte count in anemic dogs treated at the Veterinary Hospital of the Federal University Mato Grosso, Campus Sinop.

Methods

Fifty samples of whole blood from dogs were collected, regardless of gender, race and age. Blood collection was performed from peripheral veins (jugular, cephalic and saphenous) using sterile syringes containing EDTA (ethylene diaminetetraacetic acid) with a capacity of 3 mL and 25 x 7 mm needles. The anemic animals in the study were seen at the Medical Clinic, Surgical Clinic and Small Animal Reproduction admitted to the Veterinary Hospital. All analyzes were performed at the Clinical Pathology Laboratory. Right after the collections, the samples were processed and analyzed immediately. The cut-off point for inclusion in this study was determined by the hematocrit value (Ht) below 30%, obtained using the hematological analyzer (Mindray model BC-2800 VET). After completing the blood count, the sample preparation process for reticulocyte counting began. For animals with Ht between 20 and 30%, 50 μ L of bright cresil blue in 150 μ L of blood, those with Ht below 20%, 50 μ L of bright cresil blue in 200 μ L of blood (Santos, 1999). The samples were kept in a water bath at 37 $^{\circ}$ C for 25 minutes for subsequent preparation of the blood smear and counting of 1,000 erythrocytes by manual technique under optical microscopy. The absolute value was obtained by multiplying the number of erythrocytes by the percentage of reticulocytes (Weiser, 2015).

According to Pereira et al. (2008) and Weiser (2015), anemia is classified as responsive in dogs when the absolute reticulocyte count shows results above 60,000 cells / μ L.

Results and discussion

Considering the classification of Tvedten (2010) for the degree of anemia, of the 50 animals evaluated, 38 had moderate anemia (20 to 30% Ht) and 12 severe anemia (Ht <20%). Still, 54% (27/50) presented non-regenerative anemia with null response (<60,000 cells / μ L), 12 animals with suspected infectious agent, 14 with renal failure and one animal with suspected inflammatory disease; 36% (18/50) presented regeneration conditions with weak release (60,000 to 150,000 cells / μ L), nine due to infectious agents, one due to neoplasia, eight due to trauma and 10% (5/50) to moderate to intense regeneration. (> 150,000 cells / μ L), three animals per infectious agent, one for neoplasia and one for trauma, according to the adapted classification described by Stockham & Scott (2002).

In relation to the 38 moderately anemic animals, 55% were not regenerating or with little regeneration. Gonçalves et al. (2014) reported that the absence of reticulocytosis in mild to moderate anemia does not result in sufficient hypoxia to stimulate increased reticulocyte release, because the more intense the hypoxia, the greater the signs of regeneration as occurs in cases of blood loss (mainly acute) and erythrocyte destruction.

However, the other 45% of these 38 animals showed bone marrow regeneration with moderate to intense release. Although the anemia in these animals is not so severe, reticulocytosis was more evident, probably due to the cause, such as hemolysis (intra or extravascular), the most common extravascular occurring, in addition to hemostatic disorders that trigger hemorrhage or chronic blood loss due to gastrointestinal parasitism as a source of occult bleeding. Corroborating, Latimer et al. (2003) reported that the rupture of red cells or the loss of these to the internal or external environment will cause a greater acceleration of erythropoiesis with consequent release of reticulocytes.

Regarding animals with more severe anemia (12 dogs), moderate to severe regeneration predominated in seven animals. It is important to note that in these cases, the early release of immature erythrocytes into the bloodstream is a normal response of the bone marrow due to the lost erythrocytes, as a result of infectious agents such as *Babesia* spp., *Anaplasma* spp. *Mycoplasma* spp. inducing greater synthesis of erythropoietin by the kidneys, caused by hypoxia detected by justaglomerular cells (Gonçalves et al. 2014), .

Feldman et al. (2000) reported that each infectious agent can cause anemia through different mechanisms, from a responsive one, as it occurs in giardiasis, hookworms and isosporoses, to an unresponsive one, such as erlichiosis.

Anemia with little or no bone marrow response was seen in five dogs with severe anemia. In these situations, it should be considered that, despite the greater severity and the hypoxia is more pronounced, there are cases in which the stimulus for erythropoiesis is ineffective, such as in the cases of renal failure, resulting in moderate to severe regenerative anemia due to lack of erythropoietin production. The degree of azotemia shows the severity of renal failure, which is correlated with the severity of anemia and the production of reticulocytes (Thrall et al., 2015). The kidneys do not have the capacity to adapt when they are insufficient, as it is not possible to form new nephrons in adult life, however these organs are capable of performing contralateral hypertrophy. To change kidney function, more than 75% of its cells must be functional (Camargo et al., 2006). However, although there are situations in which the red cell population is well below the reference values, the production of reticulocytes will be inadequate due to the lesser stimulus for erythropoietin production due to insufficient kidney.

Non-regenerative anemias can also have other causes, mainly due to aplastic pancytopenia due to the use of antineoplastic drugs, toxins and estrogen, in addition to infectious agents such as immunosuppressive viruses. Bone marrow is sensitive to most antineoplastic drugs because it has a high mitotic index and proliferative fraction, as reported by Lanore & Delprat (2004). The mechanism of estrogen-induced myelotoxicity is not fully understood, but studies have confirmed the existence of an erythropoiesis inhibitory factor, the production of which is induced by estrogen, synthesized by stromal thymus cells. In this way, excessive exogenous administration, in repeated doses or in doses recommended for animals with idiosyncratic sensitivity in ovarian or estrogen-producing testicular tumors, induce toxicity (Sontas et al., 2009). Even though the prognosis is reserved, some dogs with aplastic pancytopenia recover, as described by Brazzell & Weiss (2006).

Also in relation to these non-responsive anemias, erythrocyte hypoplasia can result in extrinsic bone marrow disorders, including inflammatory diseases, chronic renal failure, associated with endocrine disease and, less commonly, anemia associated with nutritional deficiencies (Thrall, 2015). Even in anemia, there may still be no bone marrow response. One of the causes is believed to be due to insufficient time to release immature cells into the circulation, since the period for detecting hypoxia and stimulating erythropoiesis for reticulocyte production is approximately 72 hours and not necessarily that the animal has a bone marrow dysfunction.

Anemia results in tissue hypoxia and generally shows clinical signs such as pale mucous membranes, lethargy, intolerance to physical exercise and changes in heart and respiratory rates (Thrall, 2015). Clinical findings can be associated

with laboratory tests to aid diagnosis, and it is the role of the veterinarian to determine the causes of the problem for subsequent treatment and to indicate appropriate preventive measures (Mills, 2012).

The possible diagnoses of the causes of anemia collected in this report were classified into infectious agents (distemper, gastrointestinal endoparasitosis, hemoparasitosis, leishmaniasis and parvovirus), inflammatory processes (pyometra), neoplasms (TVT) and trauma (being run over, fractures of the femur, radio and ulna, in addition to fights with other animals). However, even with laboratory tests, blood smear analysis, in addition to imaging tests, such as ultrasound and x-ray, the importance of other complementary tests to confirm the diagnosis of infectious diseases cannot be ruled out, and it is necessary to perform PCR, RIFI, ELISA and other possible serological tests (Soares et al., 2006; Carlos et al., 2007).

Conclusion

Even though hemogram findings contribute to the identification of anemias (nucleated erythrocytes, anisocytosis, polychromasia, Howell-Jolly bodies and variation of the Red Cell Distribution Width), one of the main ways to assess erythropoiesis and spinal response is the quantification of reticulocytes.

There was a predominance of non-regenerative type anemias. Regeneration was more evident in anemia caused by infectious agents resulting from hemoparasitosis, followed by anemia caused by trauma. Non-regenerative anemias occurred mainly in animals with renal failure, where this count was reduced.

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