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CASE REPORT
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Primary Erythrocytosis in a Bitch - Clinical and Laboratorial Aspects

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ABSTRACT

***Background*:** Primary erythrocytosis is a rare myeloproliferative disorder in dogs and cats characterized by an autonomous proliferation of erythroid precursors in the bone marrow, with low to normal serum erythropoietin concentration, resulting in elevated red blood cell count, hematocrit and hemoglobin concentration. Clinical signs are associated with increased blood volume and viscosity, and may include erythema, hyperemic mucous membranes and neurological signs such as seizures and ataxia. In veterinary medicine, the diagnosis should be made by exclusion of secondary or relative causes, after complementary exams. This report aims to describe a case of primary erythrocytosis in a bitch.

***Case*:** A 4-year-old mixed-breed bitch was referred to the Veterinary Medical Teaching Hospital from UFRGS with 3 convulsive episodes related by the owner. A previous abdominal ultrasonography revealed splenomegaly and the electrocardiogram showed no abnormalities. No alterations were observed at the physical examination. The laboratorial blood tests demonstrated a persistent erythrocytosis, with high hematocrit, hemoglobin and red blood cells count, thrombocytopenia and neutropenia, and total plasmatic protein within the reference interval. The bone marrow cytology revealed reduced cellularity, normal myeloid:erythroid ratio, erythroid hyperplasia, mild myeloid hyperplasia and moderate myelofibrosis. The serum erythropoietin measurement was within the reference range, and the blood gas analysis detected a slight decrease in partial oxygen pressure. Therefore, no evidence of secondary conditions was observed and the diagnosis of primary erythrocytosis could be made.

***Discussion*:** Since there is no definitive method, the diagnosis of primary erythrocytosis could be based on the exclusion of all secondary and relative causes of erythrocytosis. The absence of clinical signs of dehydration and high serum albumin levels were findings that conduced for the exclusion of the relative form of the disturbance. The echocardiography and the abdominal ultrasonography ruled out any cardiopulmonary condition or kidney neoplasm, the most common causes of absolute secondary erythrocytosis. The persistently high hematocrit levels and red blood cell counts are significant for the suspicion of primary erythrocytosis, although thrombocytopenia and neutropenia are not commonly reported. The clinical signs of seizure were correlated with increased blood viscosity and reduced blood flow at the central nervous system. The blood gas analysis discarded the occurrence of systemic hypoxia, and the normal levels of erythropoietin gives higher evidence of the occurrence of an autonomous proliferation of the erythroid precursors within the bone marrow. The bone marrow cytology confirmed erythroid hyperplasia and the reduced cellularity that could be attributed to myelofibrosis. Myelofibrosis was described in humans with polycythemia vera, but there are no reports in veterinary, and this occurrence must be elucidated. An identical mutation in the JAK2 gene was observed in humans with polycythemia vera and dogs with primary erythrocytosis, and occurs in more than 50% of humans with myelofibrosis. Further investigations are necessary for veterinary medicine. In conclusion, the systematic approach of all organic systems and the assessment of complementary exams are necessary for the diagnostic of primary erythrocytosis in dogs. This condition should be considered in the differential diagnosis of any erythrocytosis, considering the guarded prognosis of this hematologic disorder.

Keywords: myeloproliferative disorders, erythropoietin, myelofibrosis.

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INTRODUCTION

Erythrocytosis is a hematologic disorder associated with increase of hematocrit (Hct), hemoglobin (Hb) and red blood cells (RBC) values [8]. The relative form is commonly observed and is associated with hemoconcentration by dehydration or splenic contraction [15]. This secondary condition is easily recognizable and can be normalized after therapeutic procedures [14].

The secondary form occurs after hypoxic stimuli and is commonly related with pulmonary or cardiac diseases. Hypoxia induces the erythropoietin (EPO) production that directly stimulates the erythropoiesis in the bone marrow (BM). However, excessive EPO production is also associated with renal neoplasia, without hypoxia stimulation [19].

Primary erythrocytosis, which occurs with normal or low EPO levels in peripheral blood, is an uncommon myeloproliferative disorder in dogs and cats and is characterized by an autonomous proliferation of erythroid precursors in the BM. The clinical signs include erythema, hyperemic mucous membranes, bleeding episodes, convulsions, ataxia, lethargy, and blindness, all related to the decreased circulatory blood flow and subsequent low tissue perfusion [15]. The diagnosis is confirmed after the exclusion of other erythrocytosis forms, and the treatment consists of phlebotomies, associated or not with chemotherapeutic drugs [18]. The prognosis is guarded, although long-term management has been reported [19].

This report aims to describe a case of primary erythrocytosis in a bitch, emphasizing the diagnosis with clinical and laboratorial findings considering the BM cytology aspects.

CASE

A 4-year-old mixed-breed bitch was referred to the Veterinary Medical Teaching Hospital of the Federal University of Rio Grande do Sul (UFRGS - Porto Alegre, Brazil) with a 4-month convulsive crisis history with interval of 2 to 4 months between each episode. Previous echocardiography excluded cardiac abnormalities. Splenomegaly with smooth borders and normoechogenic parenchyma, and liver with smooth borders and hypoechogenic parenchyma suggestive of congestion, toxemia or hepatopathy were observed at abdominal ultrasonography. At physical examination, the general clinical and neurological parameters were normal.

Alterations in complete blood cell count (CBC) included erythrocytosis (60% Hct, RI 37-55; 21 g/dL Hb, RI 12-18; 9.76 x10^6/µL RBC count, RI 5.5-8.5), and leukopenia (3,900/µL WBC, RI 6,000 - 17,000) due to discrete neutropenia (2,574/µL segmented neutrophils, RI 3,000-11,500) and severe lymphopenia (702/µL, RI 1,000 - 4,800). Severe thrombocytopenia was observed (40x10^3/µL platelets, RI 200-500). Serum biochemistry profile was within the reference interval. Imunochromatographic test for *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, *E. ewingii*, *Anaplasma phagocytophilum*[1] (Snap Combo 4Dx) and conventional PCR for *Rangelia vitalii* were negative and after all investigation procedures the dog was discharged.

Followed-up CBCs (days 7 and 28) revealed persistent erythroid alterations (Table 1). As a treatment, at the day 24, the dog received a granulocyte colony-stimulating factor (G-CSF) analogue[2] [Filgrastin® - 2.5 µg/kg, s.c, SID, for 3 consecutive days]. After this protocol, only a discrete increase of mature neutrophils count was observed (4,028/µL segmented neutrophil, RI 3,000-11,500).

The BM cytology aspirate from the left humerus, under general anesthesia, was performed on day 44. A total of 4 stained[3] smears (Diff Quick Stain, Panótico Rápido) from bone marrow aspirated was observed in light microscopy. Qualitative examination and differential count of nucleated cells under optic microscopic demonstrated hypocellularity, considering the patient's age (50%, RI 25-75) [Figure 1], discrete erythroid hyperplasia in immatures forms (8.98%, RI 1.1-3.3) with moderate dysplasia (Figure 2), discrete myeloid hyperplasia in immature forms (5.35%, RI

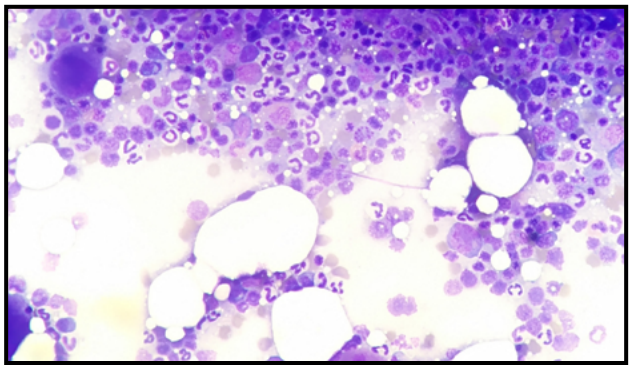

Figure 1. Bone marrow cytology demonstrated a reduced cellularity in a dog with primary erythrocytosis [Diff-Quick stain; 100x].

1.5-3.4), normal megakaryocyte count (14 cells in 10x field, RI 5-15) and moderate myelofibrosis (Figure 3). Iron stores were absent.

A venous blood gas analysis on day 80 demonstrated a mild reduction in blood partial oxygen pressure (PO_2) [43.5 mmHg, RI 47.9-56.3]. The serum EPO was normal (15.6 mU/mL, RI 5-35).

DISCUSSION

Primary erythrocytosis is a myeloproliferative disorder in which erythroid precursors proliferate autonomously, regardless of EPO concentration, resulting in a high number of circulating RBC and consequently higher Hct and Hb values [8]. In veterinary medicine, the asymptomatic chronic progressive course makes the diagnosis more challenging [1] and probably underestimated.

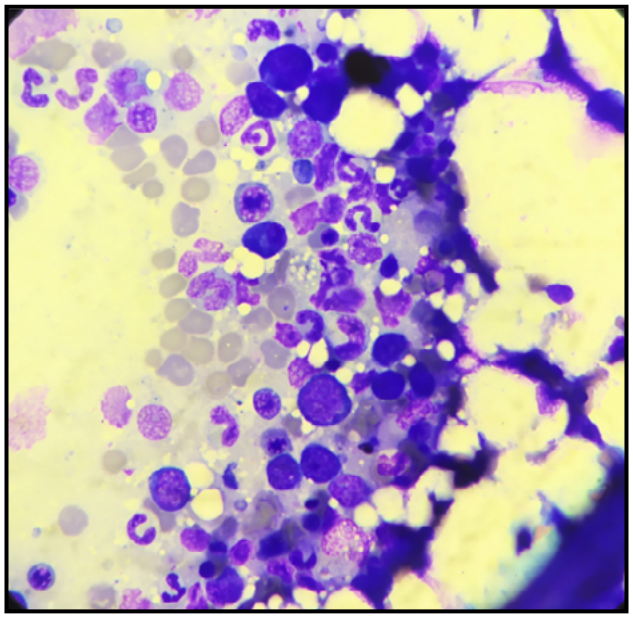

Figure 2. Erythroid hyperplasia in bone marrow cytology; erythroid blasts [Diff-Quick stain; 100x].

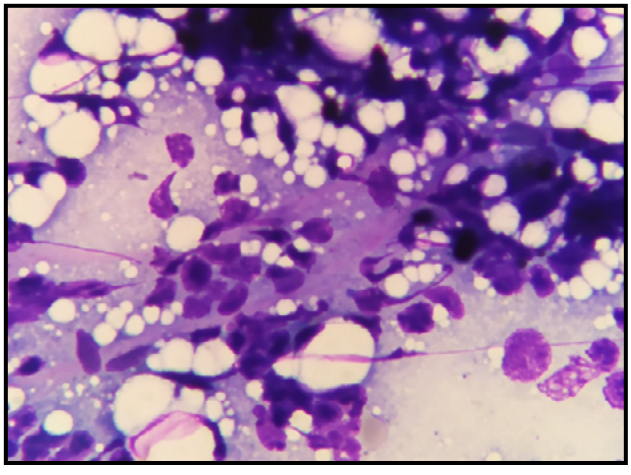

Figure 3. Suggestive myelofibrosis due the presence of stromal cells and collagen [Diff-Quick stain; 100x].

Nonspecific clinical signs are associated with the condition. Hyperemic mucous membranes, polyuria/polydipsia and neurological signs as lethargy, ataxia, blindness and seizures are described in dogs [18]. As a neurological sign, the seizures could be attributed to the hyperviscosity syndrome that leads to a reduction of peripheral oxygen perfusion [6]. Persistent and fatal neurological signals were previously related as a consequence of erythrocytosis hyperviscosity syndrome [12]. Considering the possibility of acute cerebrovascular event in dogs, a CBC must be considered as a screening test for neurological patients.

Regarding the unspecific signals between the different forms of erythrocytosis, the diagnostic approach should consider imaging exams (ultrasonography and echocardiography), to rule out secondary causes of disease. Laboratorial exams beyond CBC must include blood gas analysis, essential to rule out hypoxia and acid-base related etiologies of erythrocytosis. Including all exams and in light of physical examination, the diagnosis of primary erythrocytosis could be made by exclusion as previously described [8,14,18].

Serum EPO concentration must be evaluated although it has a limited application in veterinary medicine. For diagnostic purposes of erythrocytosis, animals with the primary form could have low or normal EPO values [3,14]. EPO levels show variations during the day [3] and its interpretation must be cautious. For dogs and cats with primary erythrocytosis, EPO values ranged from 10.0 to 57.3 mU/mL [3]. In the present case, although the EPO value was within the reference interval, the association between clinical and complementary exams supports the diagnosis of primary erythrocytosis.

Blood gas analysis provides information on tissue perfusion and acid-base balance, being essential in the detection of hypoxia. The PO_2 is an important laboratorial variable to evaluate the tissue perfusion and oxygenation [4]. Animals with primary erythrocytosis could have reduced PO_2 and oxygen saturation (SO_2), explained by blood hyperviscosity [19]. In this case, the discrete reduction of PO_2 could be associated with a venous blood sampling and for the proper assessment of oxygenation, the evaluation of PO_2 must be carried out in arterial blood [11].

Table 1. Complete blood count from a bitch with primary erythrocytosis at day 0 and during the follow-up.

Hematologic variables	Day 0	Day 07	Day 28	Day 44	Day 80	Reference Interval
Hct (%)	60	61	61	62	61	37 - 55
RBC count (106/µL)	9.76	9.51	9.59	9.73	9.62	5.5 - 8.5
Hb (g/dL)	21	21.9	21.9	22.2	22	12 - 18
MCV (/fL)	61.5	64.1	63.6	63.7	63.4	60 - 77
MCHC (%)	35	35.9	35.9	35.8	36	32 - 36
RDW (%)	20.6	20.6	20.9	20.9	20.5	13.6 - 21.7
ABS-RETIC (/µL)	-	-	-	65,550	30,000	60,000 (absent)
WBC Count (/µL)	3,900	4,600	5,300	4,500	4,600	6,000 - 17,000
SEG NEUTR (/µL)	2,574	3,082	4,028	3,420	2,944	3,000 - 11,500
EOS (/µL)	390	414	265	135	414	100 - 1,250
MONO (/µL)	234	230	318	225	276	150 - 1,350
LYMPH (/µL)	702	874	689	720	966	1,000 - 4,800
Platelet (x103/µL)	40	183	95	140	180	200 - 500

HCT hematocrit; RBC red blood cell; MCV mean corpuscular hemoglobin; MCHC mean corpuscular hemoglobin concentration; Hb hemoglobin; ABS-RETIC absolute reticulocyte count; RDW red cell distribution width; WBC white blood cell; SEG NEUT segmented neutrophil; EOS eosinophil; MONO monocytes; LYMPH lymphocytes.

Although uncommon in dogs [6,18], the splenomegaly observed in this case could be related with increased blood viscosity [5,19]. Severe to mild thrombocytopenia [19] is associated with spleen enlargement that result in changes in splenic blood flow and subsequent platelet sequestration, reflecting in low number on the peripheral blood [10].

The leukopenia due to neutropenia and lymphopenia was not correlated with bone marrow findings and could be explained by effect of the disease. At the BM cytology, the normal lymphocytes count meant that the sequestration of these cells in lymphoid organs or peripheral consumption could be occurring. Related to granulocytic lineage, the reduced release of neutrophils from BM can be as result from conditions in which their precursors are present in normal or increased numbers, but the release of the mature form in the blood is reduced, as commonly seen in secondary myelodysplastic syndromes (MDS) [9]. Normal BM values in both lineages suggest that the lymphopenia and neutropenia are ineffective and related with a chronic course of MDS.

Other effect of MDS in the BM was the absent effect after granulopoiesis stimulant use at day 28. The use of G-SCF was not associated with an increase of mature neutrophils counts on follow-up CBCs, suggesting the inability of the BM to respond to stimuli. Synthetic G-CSF is widely used in neutropenic dogs and cats. The use of synthetic G-CSF is indicated to promote the proliferation and maturation of granulocytic precursors and release of mature neutrophils to the circulation [13]. As consequence, a marked neutrophilia should be observed in peripheral blood one to two hours after administration for one to three days [7].

In erythrocytosis cases, the BM cytology could be recommended when hematological abnormalities cannot be explained [2]. Although many authors do not recommend the BM cytology to diagnose and differentiate between primary and secondary erythrocytosis, some clinical and laboratorial aspects have been highlighted [19]. In this case, the persistent bicytopenia (neutropenia and lymphopenia) was the decisive factor to perform the BM cytology.

BM cellularity is evaluated comparing the nucleated cells and fat ratio, although a BM biopsy is more sensitive to determine the hypo or hypercellularity [16]. Elderly animals have a naturally reduced

cellularity, but in young animals reduced cellularity is associated with several BM disorders, as myelofibrosis [18]. Other findings that support myelofibrosis in this case were the moderate to marked presence of fibroblasts, reticular stromal cells and excess collagen or reticulin fibers. A report of 3 dogs with concomitant myelofibrosis and MDS has been previously published, but the author could not determine if this condition is an expected finding in all MDS affected animals [20]. In humans with polycythemia vera, transformation to myelofibrosis or acute leukemia occurs in 10 to 15% of cases, but there are still no reports of these changes in animals [6]. To this date, there are no reports of dogs with primary erythrocytosis and concomitant myelofibrosis.

The moderate erythroid dysplasia (dyserythropoiesis) observed is associated with accelerated production of RBC in the bone marrow. This finding has been described in a dog with primary erythrocytosis with secondary myelodysplasia [20]. Dyserythropoiesis is a common finding observed in MDS [21] as a primary erythrocytosis [20], a type of MDS. At the same way, the slight dysgranulopoiesis observed can also be related to the occurrence of MDS [21].

Lack of iron (Fe) stores, as an absence of stainable hemosiderin in the BM cytology, can be associated with a higher demand of Fe due to the increased erythropoiesis. This finding was previously reported in humans with polycythemia vera and in dogs with primary erythrocytosis [9]. In dogs, the absence of Fe stores in the BM is particularly associated with deficiency of this mineral and could be expected in this case due the intense RBC production in the BM.

As in humans with polycythemia vera, dogs with primary erythrocytosis showed an identical mutation in the JAK2 gene [1]. This same mutation occurs in more than 50% of human patients with myelofibrosis and has a central role in the pathogenesis of myeloproliferative neoplasms [17]. There is a need for further investigations about the occurrence of the JAK2 mutation and its relationship with myelofibrosis in dogs. The detection of this mutation is a key marker in the World Health Organization classification of myeloproliferative neoplasms and this tool is likely to be useful in veterinary medicine [1]. A limitation of this report was the inability to evaluate the JAK2 mutation.

In conclusion, this is the first report of concurrent primary erythrocytosis and myelofibrosis in dogs. Even that it is an uncommon diagnosis in veterinary medicine, a BM cytology with complementary stains and cytogenetic tests must be used to investigate the myelofibrosis in primary erythrocytosis. This process will likely aid clinicians in decision making regarding treatment and prognostics expectations.

MANUFACTURERS

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***Declaration of interest*.** The authors declare no conflicts of interest. The authors alone are responsible for the content of the paper.

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