

Forma heterozigota da anomalia de Pelger-Huët em cão

A Heterozygote Form of Pelger-Huët Anomaly in Dog

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ABSTRACT

Background: The Pelger-Huët anomaly is a congenital alteration in segmented neutrophil, eosinophil and basophil morphology consisting of nuclear hypo-segmentation. It presents in two ways: the homozygote (very rare) presenting granulocytes with rounded nuclei and dense chromatin, where nuclei with more than two lobes are not found; and in the heterozygote form which presents leukocytes with bi-lobulated nuclei, yet leukocytes that seem immature can also be found. Diagnosis is important for preventing WBC interpretation errors. The objective of this study is to report a case of the heterozygote form of Pelger-Huët anomaly in an Australian Shepherd bit.

Case: A 6-year-old Australian Shepherd bitch, weighing 28 kg, was received to perform tartar cleaning at the Veterinary Hospital (HV) of the Universidade Estadual de Maringá (UEM), in Umuarama in Paraná State. In the anamnesis, the owner reported that the animal was healthy, vaccinated, and had not made use of medications recently. Upon physical examination, the animal was overweight, with adequate hydration, alert consciousness level, no exo-parasites present, rectal temperature of 39.3°C (37.5 to 39.2), heart rate of 96 beats per min (60 to 120 beats per min), breathing (18 to 36 movements per min), a two second capillary perfusion time, strong and regular pulse, hyperemic oral mucosa, and otherwise, a normal-colored and popliteal reactive left lymph. Semiological evaluation presented no alteration. Due to the tartar cleaning procedure, a hemogram was requested. Blood smear analysis from the first blood workup showed a large increase in the number of hypo-segmented leukocytes, with a pattern of mature chromatin, in the form of bands and meta-myelocytes, characterizing a deviation to the left, all of which did not correspond to the patient's clinical presentation. In addition, other cells in the granulocytic lineage, such as eosinophils, also presented hypo-segmentation. Since the dog was healthy, the Pelger-Huët anomaly was suspected. To confirm, the hemogram was repeated at 12 days and a bone marrow collection was performed; the same pattern of morphological alterations in leukocytes was observed both in the peripheral blood and the bone marrow, compatible with the Pelger-Huët anomaly.

Discussion: In order to arrive at an APH diagnosis, hypo-segmentation of granulocytes in the blood smear must be found and compared with the clinical results that the animal presents; associating any use of medication, serious infections, myeloid leukemia, and metastatic tumors in the bone marrow that might result in acquired hypo-segmentation; also known as pseudo Pelger-Huët anomaly. It is also necessary to evaluate family members for confirmation. Diagnosis is based on persistent nuclear hypo-segmentation of granulocytes, a clinically healthy animal, absence of medication use, and analysis of the bone marrow. It was not possible to determine a hereditary origin; for not having access to the animal's family, but earlier studies have shown that the incidence of this anomaly is high in the Australian Shepherd race. Generally, the Pelger-Huët anomaly is a hematological finding that must be differentiated from other forms of hypo-segmentation. Because of its hereditary origin, it is appropriate that the owner limits the animal's reproductive interactions to avoid the risk of offspring inheriting the homozygote, which leads to death. The clinical pathologist should be aware of the patient's history to diagnose and differentiate true from pseudo Pelger-Huët anomaly. Direct blood smear microscopy is essential, because automated hematological analysis alone will not detect such changes.

Keywords: Pelger-Huët Anomaly, WBC, nuclear hypo-segmentation, Australian Shepherd.

INTRODUCTION

The Pelger-Huët anomaly (APH) was described for the first time in humans in 1928, by the Dutch doctor Karl Pelger, who observed two patients with morphological leukocyte changes consisting of nuclear hypo-segmentation, and a pattern of mature, dense, and coarse chromatin. Subsequently, the pediatrician G. J. Huët described this anomaly as being a genetic alteration [3]. APH has now been reported sporadically in animals, such as rabbits, cats, horses, and dogs [1,6,12].

APH is a morphological alteration of the nucleus of segmented neutrophils, eosinophils and basophils presenting nuclear hypo-segmentation, in the form of bands, with individual smooth, round or oval lobes [12].

APH presents in homozygous and heterozygous forms. The homozygous form is very rare, and presents all granulocyte nuclei as rounded with dense chromatin; nuclei with more than two lobes are not found. Animals with the homozygote may die in the womb, at birth, or shortly afterwards [2,6]. In the heterozygote form, the leukocytes present bi-lobulated nuclei in up to 93% of neutrophils, but mature leukocytes can also be found appearing in their younger forms having bands and as meta-myelocytes [1,6].

APH is a benign anomaly that alters neither leukocyte half-life nor function [1,10]. To avoid unnecessary therapeutic action, diagnosis is important for preventing WBC interpretation errors, such as left offset, and misidentification of inflammatory processes.

The objective of this study is to report a heterozygote form APH case in a bitch of Australian Shepherd breed.

CASE

A 6-year-old Australian Shepherd bitch, weighing 28 kg, was received to perform tartar cleaning at the Veterinary Hospital (HV) of the Universidade Estadual de Maringá (UEM), in Umuarama in Paraná State, Brazil. In the anamnesis, the owner reported that the animal was healthy, vaccinated, and had not made use of medications recently. The animal's deworming routine was not up to date.

The animal lived in the backyard on the floor and in the grass, with access to the street in supervised day trips. Its food was commercial adult ration exclusively. Upon physical examination, the animal was overweight, with adequate hydration, alert conscious-

ness level, no exo-parasites present, rectal temperature of 39.3°C (37.5 to 39.2), heart rate of 96 beats per min (60 to 120 beats per min), breathing (18 to 36 movements per min), a 2 s capillary perfusion time, strong and regular pulse, hyperemic oral mucosa, and otherwise, a normal-colored and popliteal reactive left lymph [4]. The semiological system evaluation presented no alterations.

Due to the dental prophylaxis procedure, a hemogram examination was requested. Four mL of blood were collected by puncturing the jugular vein and packaged in a tube containing an aqueous solution of ethylenediaminetetraacetic tripotassium acid (EDTA-K3) at 10%. The sample was properly identified and sent to the Pathology Laboratory of the HV, Veterinary Clinic at UEM.

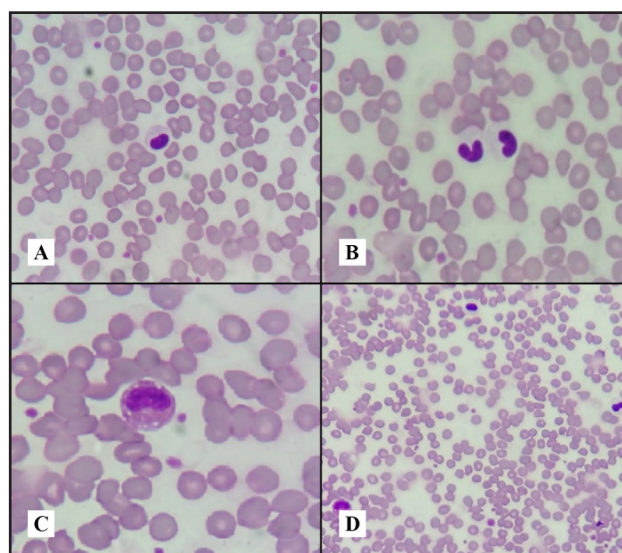


Figure 1. Blood smear from the bitch with Pelger-Huët Anomaly (different fields). A- There is neutrophil hypo-segmentation in meta-myelocytic form. B- It is observed in the rod form. C- There is an eosinophil without lobes [100x]. D- Presents hypo-segmentation in neutrophils and an eosinophil [40x].

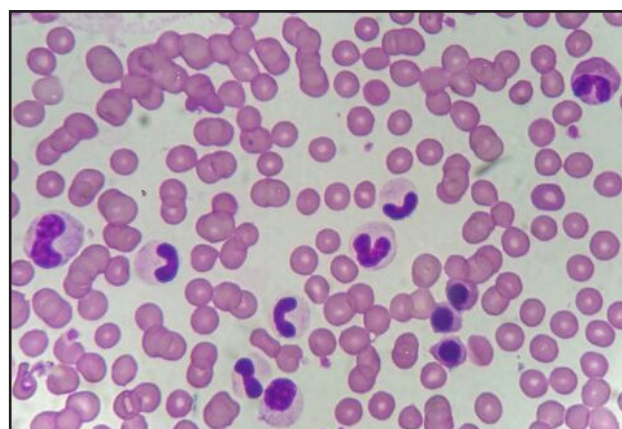


Figure 2. Bone marrow smears evidencing neutrophil hypo-segmentation, it is also possible to observe a group of immature erythroid cells.

Table 1. Results of the first WBC held on 17 March 2017, at the Veterinary Clinic Pathology Laboratory (HV - UEM).

WBC	Results				Reference Values	
	1 st count		2 st count		Percentage	Absolute
	%	µL	%	µL		
Total leukocytes	7,400				100	6,000 - 17,000/µL
Segmented neutrophils	10	740	74	5,476	55 - 80%	3,000 - 11,500/µL
Bands	35	2,590	0	0	0 - 1%	0 - 300 µ/L
Meta-mielocytes	28	2,072	0	0	0%	0 µ/L
Lymphocytes	18	1,332	19	1,406	13 - 40%	1,000 - 4,800/µL
Monocytes	5	370	4	296	1 - 6%	150 - 1,350/µL
Eosinophil	4	296	3	222	1 - 9%	100 - 1,250/µL
Basophil	0	0	0	0	0 - 1%	Raro

Table 2. Result of the second WBC held on 29 March 2017, at the Veterinary Clinic Pathology Laboratory (HV - UEM).

WBC	Results				Reference Values	
	1 st count		2 st count		Percentage	Absolute
	%	µL	%	µL		
Total leukocytes	6,400				100	6,000 - 17,000/µL
Segmented neutrophils	0	0	78	4,992	55 - 80%	3,000 - 11,500/µL
Bands	47	3,008	0	0	0 - 1%	0 - 300 µ/L
Meta-myelocytes	31	1,984	0	0	0%	0 µ/L
Lymphocytes	18	1,152	18	1,152	13 - 40%	1,000 - 4,800/µL
Monocytes	0	0	0	0	1 - 6%	150 - 1,350/µL
Eosinophil	4	256	4	256	1 - 9%	100 - 1,250/µL
Basophil	0	0	0	0	0 - 1%	Raro

In the automated laboratory, erythrogram, thrombogram and total leukocyte count were conducted on Hemacounter 60 (Vet) (Hemogram®)¹ equipment; and in order to obtain the differential leukocyte count and qualitative analysis of blood cells, a blood smear stained with Romanowsky stain (Panoptic fast®)² type was prepared.

To confirm the anomaly, a new hemogram and bone marrow collection were carried out after 12 days; with the animal anesthetized moments before the dental prophylaxis procedure. The bone marrow collection was performed by means of puncture and aspiration from the sternum following the (modified) method proposed by Stewart *et al.* [9]. We collected 10 mL of bone marrow by means of a single puncture in the sternal flow region. Subsequently 15 microscope slides using the peaks, stained with quick Panoptic fast®² were performed.

DISCUSSION

Analysis of the first blood smear workup showed a large increase in the number of hypo-segmented leukocytes, with a pattern of mature chromatin, in the form of bands and meta-myelocytes, presenting a deviation to the left, which did not correspond with the patient's clinical presentation. In addition, other cells in the granulocytic lineage such as eosinophils also presented hypo-segmentation. A new blood smear from the same blood sample confirmed the result. As the patient did not present any clinical alterations; the Pelger-Huët anomaly was suspected. The results of the first WBC are shown in Table 1.

For confirmation of this suspicion, a new hemogram was performed 12 days afterwards, shortly before the tartar cleaning procedure. The same pattern of morphological changes in granulocytic leukocytes, compatible with the Pelger-Huët anomaly was found. To correct the values in the WBC a new differential count was

performed, counting hypo-segmented neutrophils with mature chromatin patterns as segmented neutrophils, the results can be found in Table 2.

To arrive at an APH diagnosis, one must find evident granulocyte hypo-segmentation in the blood smear, such as meta-myelocytes and bands; and then compare with the animal's clinical presentation. The use of drugs, such as sulfonamides and colchicine, serious infections, myeloid leukemias, and metastatic tumors in the bone marrow may also result in acquired hypo-segmentation, also called pseudo Pelger-Huët anomaly. Considering the hereditary origin of the anomaly, searching family members for confirmation should also be considered [1,5,8].

Pseudo APH cells tend to have asymmetric nuclei lobes and cytoplasm with large numbers of toxic granules, vacuoles, and Dohle corpuscles. The nuclei of congenital APH cells have up to two round symmetrical lobes and few cytoplasmic granulations (Figure 1) [7]. However, several authors [8,10,11] claim that hereditary and acquired anomaly cells are morphologically indistinguishable.

In the marrow slide analyses it was possible to observe the same morphological pattern of peripheral neutrophils; and no segmented neutrophils were found, indicating true APH (Figure 2).

In this case the persistence of granulocyte hypo-segmentation in both blood smears, associated

with the healthy state of the animal, exclusion of the previously mentioned diseases, non-exposure to medicines, and the bone marrow analysis confirmed the finding. For not having access to either the parents or siblings of the animal, it was not possible to determine a hereditary origin. Latimer, Campagnoli & Danilenko [6] in a prospective study conducted with 892 Australian Shepherd breed dogs, found that 87 (9.8%) of the animals had APH, this determines that the race has a high incidence of this anomaly, without predilection for age or sex.

Generally, APH is a hematological finding that must be differentiated from other forms of hypo-segmentation. Being inherited and possessing a fatal homozygote form, owners should be instructed to not allow the animal to bear offspring.

APH is a rarely occurring hematological finding that should be diagnosed to avoid erroneous interpretations of the WBC. The clinical pathologist should be aware of the patient's history to differentiate pseudo Pelger-Huët anomaly from true.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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