

CYTOLOGY

Blood Smear Review: How to Identify and Interpret Neutrophil Abnormalities

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Blood smear evaluation remains a critical part of a hematologic evaluation of veterinary patients, complementing the results of a CBC by enabling clinicians to identify morphologic abnormalities that may not be detected by an in-house benchtop analyzer.¹⁻³ This article focuses on the identification of abnormalities of peripheral blood neutrophils that may facilitate the early detection of inflammation, monitor response to treatment, and inform long-term prognosis (**BOXES 1 AND 2**). In some instances, neutrophils may contain certain infectious agents or other abnormal inclusions, and the ability to recognize these abnormalities may lead the clinician to an immediate diagnosis.

NEUTROPHIL MATURATION AND NORMAL MORPHOLOGY

Neutrophils are the first immune cells to respond to inflammation and infection and are derived from myeloid (granulocytic) precursors in the bone marrow. Granulocytic precursors exhibit pyramidal maturation progressing from the earliest precursors, myeloblasts, to progranulocytes; myelocytes; metamyelocytes; band cells; and, lastly, mature, segmented neutrophils. The stages can be distinguished by their nuclear and cytoplasmic features.

■ **Myeloblast (FIGURE 1A):** Myeloblasts have high nuclear-to-cytoplasmic ratios with round to oval, eccentric nuclei containing finely stippled chromatin with 1 to multiple prominent nucleoli. They have

Abstract

Blood smear evaluation is a necessary diagnostic tool for assessing and recognizing neutrophil abnormalities. Properly interpreting morphologic abnormalities and identifying infectious agents can lead to improved patient care by enabling the clinician to recognize the early onset of inflammation and/or disease; monitor treatment efficacy; and, in some instances, provide an immediate diagnosis. Automated hematology analyzers aid in the detection of abnormal leukocyte counts; however, manual blood smear evaluation is recommended because analyzers may not accurately classify neutrophils with certain morphologic abnormalities (e.g., left shift). In addition, accurately interpreting abnormalities of neutrophil concentration provides valuable diagnostic information and facilitates the inclusion and exclusion of a wide variety of differential diagnoses.



Take-Home Points

- The predominant leukocyte in most domestic mammals is the neutrophil, and its normal cell morphology differs between species.
- Manual blood smear evaluation is recommended to confirm an automated leukocyte count and differential reported by an analyzer.
- Abnormalities of neutrophil morphology can be the first sign of disease and may be seen before clinical signs and/or other alterations of a CBC.
- The ability to interpret neutrophil abnormalities is a diagnostic skill that can provide valuable insight into patient health, response to treatment, and prognosis.

small amounts of medium basophilic cytoplasm that often display prominent paranuclear clearing (Golgi zone).

- **Progranulocyte (or promyelocyte) (FIGURE 1B):** Progranulocytes have round to oval, eccentric nuclei containing coarsely stippled chromatin and lightly basophilic cytoplasm that contains few fine, magenta-colored primary granules. Primary granules are the first granules seen as myeloblasts transition to progranulocytes. They contain many antimicrobial compounds and are myeloperoxidase positive.
- **Myelocyte (FIGURE 1C):** Myelocytes have round to oval, often eccentric nuclei containing coarse or ropy chromatin and have paler blue cytoplasm than progranulocytes.
- **Metamyelocyte (FIGURE 1D):** Metamyelocytes have reniform nuclei with coarse chromatin and clear to variably basophilic cytoplasm that, depending on the degree of immaturity, contains neutral-staining secondary granules. Secondary granules are smaller and less dense than primary granules and are specific to the type of granulocyte present (e.g., neutrophils have neutral-staining granules, eosinophils have pink

granules). These granules contain compounds involved with the formation of reactive oxygen species and other proteins that aid in neutrophil migration.

- **Band cell (FIGURE 1E):** Bands have nuclei with no lobulations and virtually parallel sides of which no area has a diameter less than two-thirds the diameter of any other area, often resembling a horseshoe with more coarse, paler-staining chromatin.⁸ The cytoplasm resembles that of a mature neutrophil. Bands are rarely seen in the blood of healthy animals.
- **Segmented (mature) neutrophil (FIGURE 2A):** Mature neutrophils have a segmented nucleus containing 3 to 5 separate lobes; condensed, dark purple chromatin; and colorless to pale blue cytoplasm that contains neutral (i.e., nonstaining) secondary granules and measure approximately 10 to 14 μm in diameter (twice the size of an erythrocyte). Female animals may have a few neutrophils containing a Barr body (FIGURE 2B), which appears as a small, teardrop-shaped protrusion of the nucleus and represents the inactivated X chromosome.⁶ In cats, low numbers of neutrophils may contain a few Döhle bodies that appear as small, round to ovoid, blue

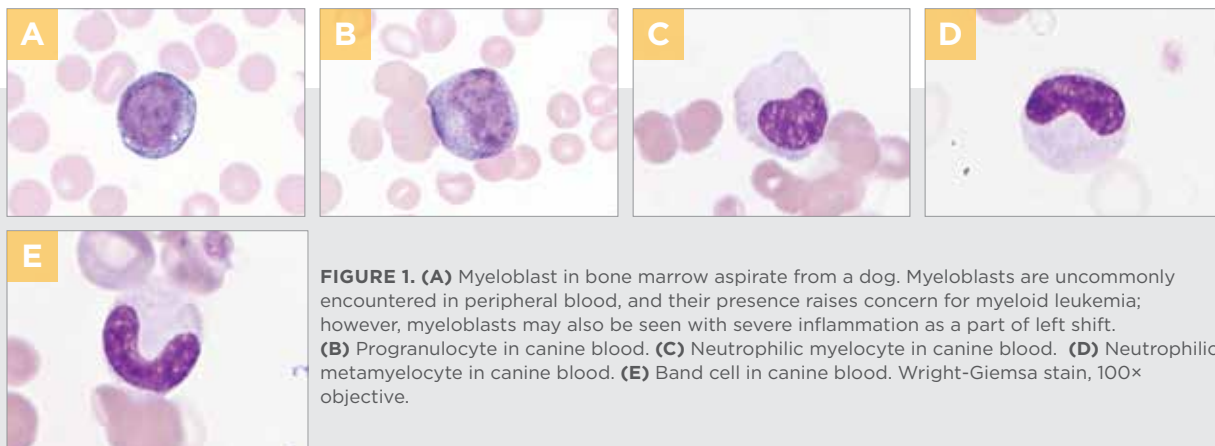


FIGURE 1. (A) Myeloblast in bone marrow aspirate from a dog. Myeloblasts are uncommonly encountered in peripheral blood, and their presence raises concern for myeloid leukemia; however, myeloblasts may also be seen with severe inflammation as a part of left shift. **(B)** Progranulocyte in canine blood. **(C)** Neutrophilic myelocyte in canine blood. **(D)** Neutrophilic metamyelocyte in canine blood. **(E)** Band cell in canine blood. Wright-Giemsa stain, 100 $\times$ objective.

cytoplasmic inclusions and represent aggregates of rough endoplasmic reticulum (**FIGURE 2C**). Certain cat breeds (e.g., Birman, Siamese, Himalayan) may have neutrophils containing fine, red to pink cytoplasmic granules that are often discovered incidentally on blood smear examination and are not an indication of illness.^{8,9}

ABNORMAL NEUTROPHIL MORPHOLOGY

Nuclear Abnormalities

Left Shifting

The presence of increased numbers of band neutrophils and earlier precursors is called a *left shift*, which is a response to inflammation and increased peripheral demand. Left shift should be orderly and pyramidal with higher numbers of more mature precursors than less mature precursors. When the number of immature neutrophils is greater than the number of mature segmented neutrophils, a *degenerative left shift* is present, which may be seen with severe inflammation and may confer a guarded clinical prognosis.^{5,10}

BOX 1 Morphologic Abnormalities of Neutrophils Detected by Manual Blood Smear Evaluation

Nuclear abnormalities

- Left shift
- Pelger-Huët anomaly
- Hypersegmentation
- Pyknosis and karyorrhexis
- Karyolysis

Cytoplasmic abnormalities

- Toxic change
- Granulation
 - Breed-specific granulation (Birman, Siamese, and Himalayan cats)
 - Chédiak-Higashi syndrome (Persian cats; very rare)
- Miscellaneous inclusions
 - Hemosiderin/iron (sideroleukocytes)
 - Lipofuscin-like
- Infectious agents
 - *Anaplasma phagocytophilum*, *Ehrlichia ewingii* (mostly dogs)
 - *Hepatozoon canis*, *Hepatozoon americanum* (dogs)

Pelger-Huët Anomaly

Pelger-Huët anomaly (PHA) is a congenital morphologic abnormality that has been documented in dogs, cats, rabbits, and horses.¹¹⁻¹⁴ PHA is suspected to result from a mutation in the gene that encodes the lamin B receptor (a protein involved with nuclear envelope structure and function).¹¹⁻¹⁴ Neutrophils of animals with PHA contain condensed chromatin and

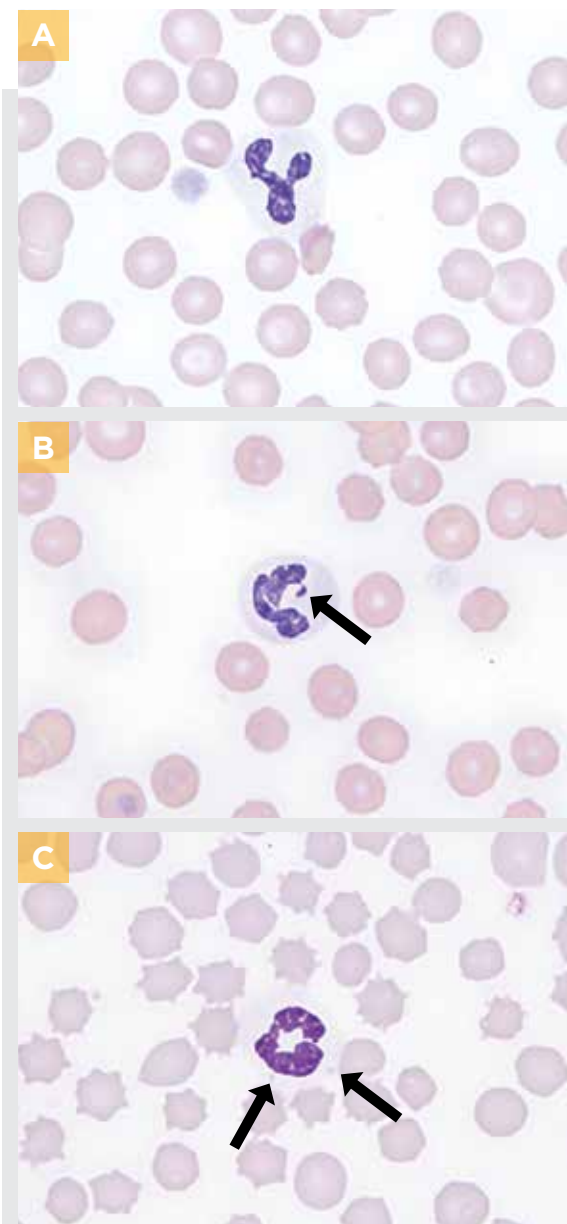


FIGURE 2. (A) Normal neutrophil in canine blood. (B) Normal neutrophil in canine blood. Note the small teardrop-shaped Barr body (**arrow**) that can be present in females. (C) Normal neutrophil in feline blood. Note the 2 small Döhle body inclusions (**arrows**). Wright-Giemsa stain, 100× objective.



cytoplasmic features similar to a typical mature neutrophil; however, their nuclei appear hypolobulated (round, oval, or reniform-shaped) (FIGURE 3). The nuclei of affected granulocytes and monocytes fail to undergo segmentation but retain normal function. PHA is often incidentally discovered during blood smear evaluation, typically after suspected abnormalities are flagged by an analyzer (e.g., suspected bands) because affected cells can resemble bands. PHA can often be distinguished from left shift because many cells will be affected and their cytoplasm will lack typical features of immature cells (e.g., cytoplasmic basophilia, primary granules).^{2,4,8} An acquired form, called pseudo-PHA, has been documented in association with myeloid leukemias, severe infections, and administration of chemotherapy.^{8,15} In contrast to congenital PHA, pseudo-PHA is transient and generally only a subset of neutrophils appear hyposegmented.⁴

Hypersegmentation

Hypersegmentation occurs as a part of cell aging after neutrophils remain in peripheral blood for an extended period. In most domestic species, a neutrophil is considered hypersegmented if the nucleus consists of 5 or more distinct segments (FIGURE 4A). Hypersegmentation may be seen in patients with chronic inflammation, excess endogenous or exogenous glucocorticoids, myeloid leukemias, and certain congenital conditions.^{4,8,16} Botryoid neutrophils are neutrophils with hypersegmented nuclei that are radially arranged and exhibit a distinct “pinwheel” appearance; they are often associated with canine heatstroke (FIGURE 4B).¹⁷

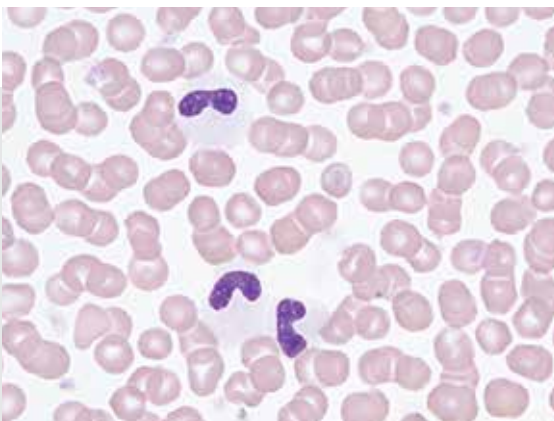


FIGURE 3. Neutrophils in the blood of a dog with Pelger-Huët anomaly. Wright-Giemsa stain, 100× objective.

BOX 2 Differentials for Changes in Peripheral Neutrophil Concentrations

Neutrophilia

- Stress neutrophilia (corticosteroid-mediated)^a
 - Transient shift of neutrophils from marginating pool to circulating pool
 - Usually mild, rarely exceeds 2× upper reference range
 - Often associated with concurrent monocytosis (dogs), lymphopenia, and eosinopenia
 - Left shift and/or toxic change not expected
- Physiologic neutrophilia (epinephrine-mediated)^b
 - Fear or excitement leading to splenic contraction and transient neutrophilia
 - Usually mild, rarely exceeds 2× upper reference range
 - Often associated with concurrent lymphocytosis
 - Left shift and/or toxic change not expected
- Inflammation (acute or chronic)
 - Severity is variable depending on the nature and duration of the inciting cause
 - May have concurrent monocytosis and lymphocytosis
 - Supported by presence of left shift or toxic change

Neutropenia

- Inflammation (acute or chronic)^a
 - Severe inflammation that results in depletion of bone marrow storage pool or insufficient time for granulopoiesis
 - Supported by presence of left shift or toxic change
 - Decreased production (bone marrow dysfunction)
 - Decreased granulopoiesis secondary to myelofibrosis, myelonecrosis, myelophthisis, certain infectious agents (e.g., parvovirus, feline leukemia virus, *Toxoplasma gondii*, *Ehrlichia canis*), or toxins (e.g., estrogen, chemotherapy)^{4,5}
 - Persistent, often associated with other cytopenias (e.g., anemia, thrombocytopenia)
- Destruction (immune-mediated destruction of neutrophils and their precursors)
 - Uncommon in dogs and cats
 - Persistent, often with left shift, and responsive to immunosuppressive therapy^{4,6,7}

^amost common

^bless common in dogs

Cytoplasmic Abnormalities

Toxic Change

Toxic change is one of the most common morphologic abnormalities of neutrophils in the peripheral blood and suggests accelerated granulopoiesis resulting from systemic inflammation.^{2,4} Neutrophils are classified as toxic if they exhibit certain cytoplasmic features (e.g., increased cytoplasmic basophilia, vacuolated cytoplasm, Döhle bodies) (**FIGURE 5**). Toxic change grading is often subjectively based on the features and severity, which can vary among laboratories. Toxic changes are often most severe in animals with sepsis, endotoxemia, and/or tissue necrosis.² The morphology of neutrophils with severe toxic changes may be similar to that of monocytes, making differentiation difficult and limiting the accuracy of a leukocyte differential. *Pseudotoxic change* represents an in vitro change secondary to prolonged storage (Döhle body-like inclusions have been noted as soon as 4 hours after blood collection).¹⁸

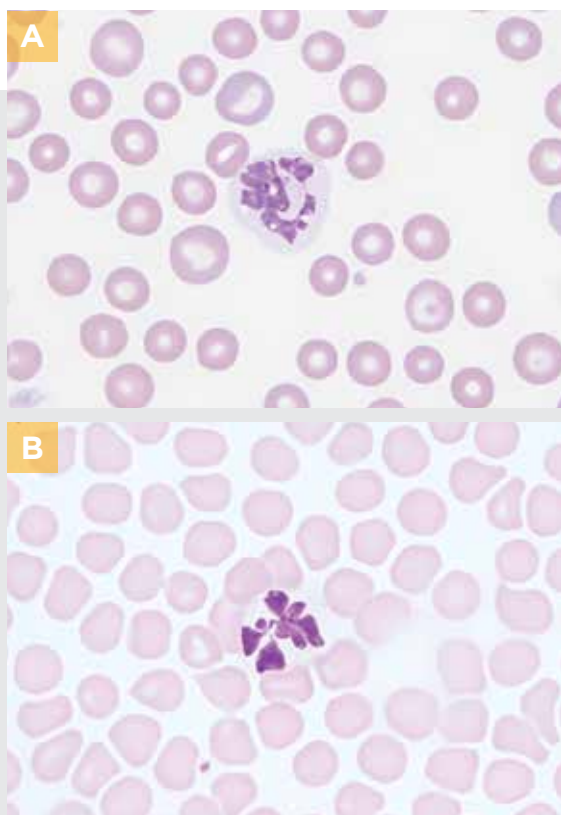


FIGURE 4. (A) Hypersegmented neutrophil in the blood of a dog with a history of long-term prednisone administration. **(B)** Botryoid neutrophil in the blood of a dog with heatstroke. Wright-Giemsa stain, 100× objective.

Sideroleukocytes

Sideroleukocytes are neutrophils that contain hemosiderin inclusions within their cytoplasm that appear as coarse, dark blue–black granules (**FIGURE 6**).⁴ The inclusions may be seen in animals with hemolytic anemia and can be confirmed only through special cytochemical staining for iron (e.g., Prussian blue stain).⁴

Lipofuscin-like Inclusions

Lipofuscin-like inclusions, also referred to as critical green inclusions, are fine to coarse, dark blue–green granules within the cytoplasm of neutrophils and monocytes (**FIGURE 7**).¹⁹ In dogs and cats, the inclusions are suspected to represent lipofuscin pigment and are often associated with severe hepatocellular injury and higher risk for death.²⁰

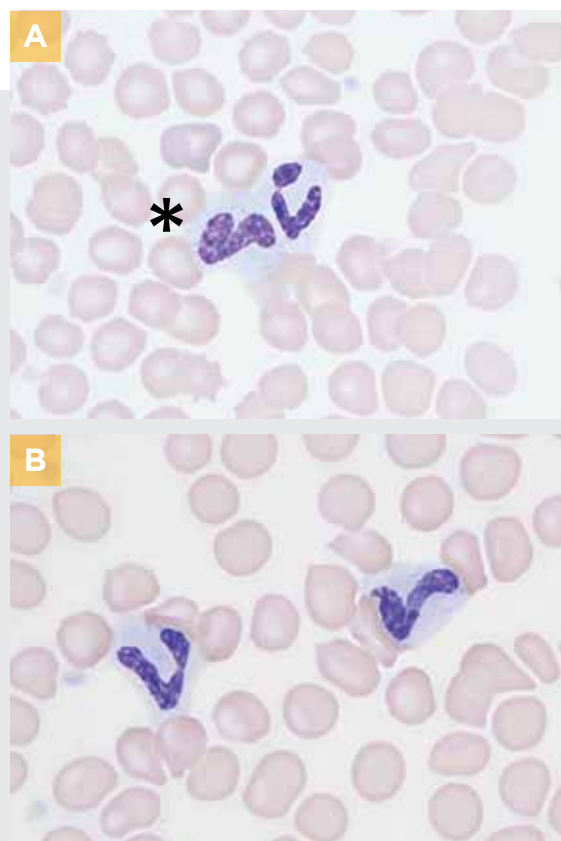


FIGURE 5. (A) Toxic neutrophil (asterisk) adjacent to normal neutrophil in canine blood. **(B)** Toxic neutrophils in feline blood. Wright-Giemsa stain, 100× objective.



Infectious Organisms

Certain species of *Rickettsia* (e.g., *Ehrlichia ewingii*, *Anaplasma phagocytophilum*) may be seen within the cytoplasm of neutrophils of dogs during the acute phase of infection. The morulae of these species appear similar and cannot be distinguished via cytology alone, necessitating the use of PCR or serology for further differentiation (**FIGURE 8**). Affected patients often have characteristic hematologic abnormalities (e.g., thrombocytopenia, anemia, leukopenia), which can contribute to the clinical suspicion of tick-borne diseases.² Gamonts of *Hepatozoon americanum* protozoa may occasionally be seen within the cytoplasm of neutrophils and monocytes of dogs (**FIGURE 9**).^{2,8} Because these intracellular parasites are often found in low numbers in the peripheral blood, a buffy coat preparation may be needed for visualization.² Dogs

with hepatozoonosis may also have an extreme neutrophilia, often with a left shift.^{2,4} Infrequently, phagocytized intracellular bacteria may be seen in the peripheral blood, which indicates bacteremia.

ABNORMAL NEUTROPHIL CONCENTRATIONS

Abnormal neutrophil concentrations are best determined by evaluating the absolute neutrophil counts reported by a hematology analyzer and confirming the automated findings via manual blood smear evaluation.² Differentials for abnormalities, their general mechanisms, and defining characteristics are summarized in **BOX 2**. Detailed descriptions of the

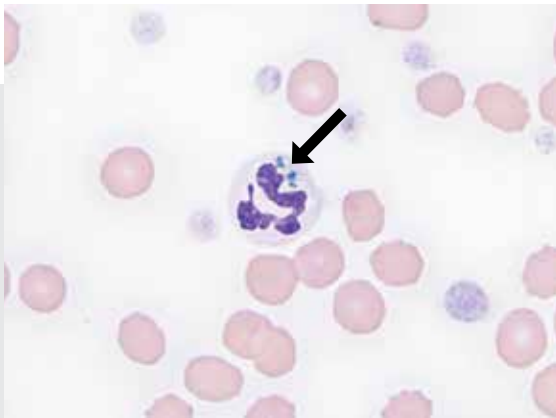


FIGURE 6. Sideroleukocyte containing coarse blue-green hemosiderin inclusions (**arrow**) in canine blood. Wright-Giemsa stain, 100× objective.

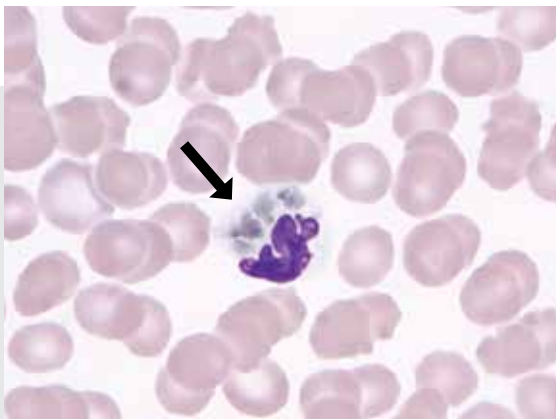


FIGURE 7. Neutrophil containing coarse green-brown lipofuscin-like inclusions (**arrow**) in the blood of a dog with acute hepatocellular necrosis. Wright-Giemsa stain, 100× objective.

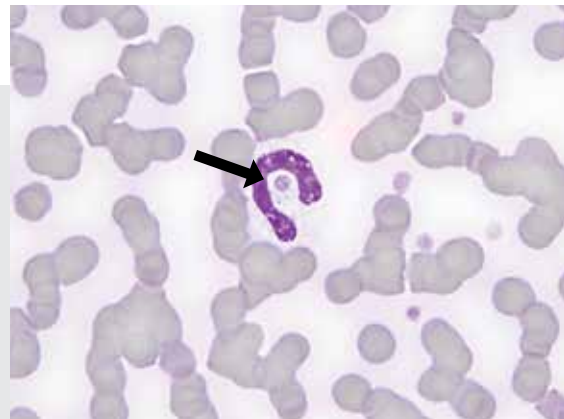


FIGURE 8. Neutrophil containing a single morula (**arrow**) in equine blood and round to irregular dark blue coccobacilli ~0.5 µm in diameter. PCR confirmed the diagnosis of anaplasmosis (*Anaplasma phagocytophilum*). Wright-Giemsa stain, 100× objective.



FIGURE 9. Toxic neutrophil containing a nonstaining *Hepatozoon americanum* gamont (**arrow**) in canine buffy coat that appears as negatively staining and light blue cigar-shaped inclusions that contain a small purple nucleus. Wright-Giemsa stain, 100× objective.

mechanisms of these abnormalities and less common differentials can be found elsewhere.^{2,4,8,9}

SUMMARY

Neutrophil abnormalities are common among veterinary patients. In addition to an automated CBC, blood smear evaluation is encouraged to recognize

atypical morphology and confirm an analyzer's findings. Ultimately, the ability to interpret neutrophil abnormalities is a useful diagnostic skill that can provide further valuable insight into patient health, response to treatment, and prognosis. **TVP**

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References

- Schlemmer SN, Garner B. Blood smear review: a step-by-step guide to normal findings for cats and dogs. *Today's Vet Pract.* 2024;15(1):38-47.
- Zabolotzky SM, Walker DB. Peripheral blood smears. In: Valenciano AC, Cowell RL, eds. *Cowell and Tyler's Diagnostic Cytology and Hematology of the Dog and Cat*. 5th ed. Mosby; 2020:438-467.
- Harvey JW. Hematology procedures. In: Harvey JW, ed. *Veterinary Hematology*. 1st ed. W.B. Saunders; 2012:11-32.
- Stockham SL, Scott MA. Leukocytes. In: Stockham SL, Scott MA, eds. *Fundamentals of Veterinary Clinical Pathology*. 2nd ed. Blackwell; 2008:53-106.
- Burton AG, Harris LA, Owens SD, Jandrey KE. Degenerative left shift as a prognostic tool in cats. *J Vet Intern Med.* 2014;28(3):912-917. doi:10.1111/jvim.12338
- Karni RJ, Wangh LJ, Sanchez JA. Nonrandom location and orientation of the inactive X chromosome in human neutrophil nuclei. *Chromosoma.* 2001;110(4):267-274. doi:10.1007/s004120100145
- Devine L, Armstrong PJ, Whittmore JC, et al. Presumed primary immune-mediated neutropenia in 35 dogs: a retrospective study. *J Small Anim Pract.* 2017;58(6):307-313. doi:10.1111/jsap.12636
- Harvey JW. Evaluation of leukocytic disorders. In: Harvey JW, ed. *Veterinary Hematology*. 1st ed. W.B. Saunders; 2012:122-176.
- Burton AG. Leukocytes. In: Burton AG, ed. *Clinical Atlas of Small Animal Cytology and Hematology*. 2nd ed. Wiley Blackwell; 2024:484-488.
- Burton AG, Harris LA, Owens SD, Jandrey KE. The prognostic utility of degenerative left shifts in dogs. *J Vet Intern Med.* 2013;27(6):1517-1522. doi:10.1111/jvim.12208
- Comazzi S, Aresu L, Weiss DJ. Neutrophil function disorders. In: Brooks MB, Harr KE, Seelig KJ, Wardrop KJ, Weiss DJ, eds. *Schalm's Veterinary Hematology*. 7th ed. John Wiley & Sons; 2022:347-353.
- Deshuillers P, Raskin R, Messick J. Pelger-Huët anomaly in a cat. *Vet Clin Pathol.* 2014;43(3):337-341. doi:10.1111/vcp.12176
- Gill AF, Gaunt S, Sirninger J. Congenital Pelger-Huët anomaly in a horse. *Vet Clin Pathol.* 2006;35(1):460-462. doi:10.1111/j.1939-165X.2006.tb00165.x
- Vale AM, Tomaz LR, Sousa RS, Soto-Blanco B. Pelger-Huët anomaly in two related mixed-breed dogs. *J Vet Diagn Invest.* 2011;23(4):863-865. doi:10.1177/1040638711407891
- Feldman BF. The Pelger-Huët anomaly of granulocytic leucocytes in the canine. *Vet Clin Pathol.* 1975;4(2):15-19. doi:10.1111/j.1939-165x.1975.tb00044.x
- Fyfe JC, Giger U, Hall CA, et al. Inherited selective intestinal cobalamin malabsorption and cobalamin deficiency in dogs. *Pediatr Res.* 1991;29(1):24-31. doi:10.1203/00006450-199101000-00006
- Mastorilli C, Welles EG, Hux B, Christopherson PW. Botryoid nuclei in the peripheral blood of a dog with heatstroke. *Vet Clin Pathol.* 2013;42(2):145-149. doi:10.1111/vcp.12041
- Bau-Gaudreault L, Grimes CN. Effect of time and storage on toxic or pseudo-toxic change in canine neutrophils. *Vet Clin Pathol.* 2019;48(3):400-405. doi:10.1111/vcp.12755
- Hodgson TO, Ruskova A, Shugg CJ, McCallum VJ, Morison IM. Green neutrophil and monocyte inclusions - time to acknowledge and report. *Br J Haematol.* 2015;170(2):229-235. doi:10.1111/bjh.13434
- Sebastian KN, Lucidi CA, Scott MA. Characterization of blue-green blood leukocyte inclusions and accompanying clinical, hematologic, and serum biochemical changes in dogs. *Vet Clin Pathol.* 2024;53(2):168-178. doi:10.1111/vcp.13348



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