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Key Words

- Anemia
- Erythrocyte morphology
- Erythrocytosis
- Poikilocytes
- Red blood cells

The interpretation of erythrogram in dog and cat

> Abstract

Erythrogram is part of the complete blood count and includes the number of erythrocytes, hemoglobin concentration, hematocrit, erythrocyte indices (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration), red cell distribution width, the number and percentage of reticulocytes and erythrocyte morphology. Appropriate blood sampling and handling are essential for the validity of the erythrogram interpretation. Erythrogram major abnormalities are anemia, erythrocytosis/polycythemia, erythrocyte morphologic abnormalities and erythrocyte inclusions. Anemia can be either regenerative or nonregenerative. Regenerative anemia is divided into blood loss anemia and hemolytic anemia. Common causes of blood loss anemia are traumatic injuries and hemostatic disorders, while hemolytic anemia can be immune-mediated, microangiopathic, associated with Heinzbody formation or due to genetic defects of red blood cells. Nonregenerative anemia includes anemia of chronic disease, anemia of chronic renal failure, aplastic anemia and nutritional anemia. Erythrocytosis/polycythemia can be relative (hemoconcentration) or absolute, which is further divided into primary (polycythemia vera) and secondary. The appearance of abnormal erythrocytes can be either an artifact or associated with certain disorders. The erythrocyte inclusions are either of non-infectious origin, such as Howell-Jolly and Heinz bodies or related to infections, such as *Babesia spp.* and hemotropic *Mycoplasma spp.*, the observation of which into the erythrocytes sets the definitive diagnosis of the relevant diseases.

> Introduction

Erythrogram is part of the complete blood count (CBC) and includes red blood cells (RBC) count, hemoglobin concentration, hematocrit, red blood cell indices [mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)], red cell distribution width (RDW), reticulocytes percentage and count and the evaluation of erythrocyte morphology on stained blood smears (Table 1). Appropriate blood collection, sample handling and storage are essential for the reliability of the results. Overnight fasting should precede blood collection in order to prevent postprandial lipemia, which can interfere with the measurement of plasma hemoglobin concentration.¹ The blood should be collected into EDTA tubes and filled up to the defined limit in order to avoid blood clotting (overfilled tubes) or decrease of the hematocrit (underfilled tubes).² Avoidance of iatrogenic blood hemolysis, due to poor blood collection technique or sample handling, is critical because it can interfere with various erythrocyte parameters. The CBC and preparation of blood smears should be completed as soon as possible, preferably within 2-3 hours after blood collection. However, blood in EDTA tubes can be safely stored in the refrigerator for 24 hours.^{1,3} Refrigerated blood samples should be kept at room temperature and be gently inverted before analysis. It should be noted that inappropriate drying, fixation and staining can lead to low-quality

**Table 1.** Erythrogram reference intervals. Modified from Moritz, 2004³⁷

Analytes	Canine reference intervals	Feline reference intervals
Red blood cells (10 ¹² /L)	5.68-9.08	5.92-11.16
Haemoglobin (g/dL)	13.8-20.4	8.2-15.3
Haematocrit (%)	42-62	24-46
MCV (fL)	62.7-74.6	37.0-55.0
MCH (pg)	20.5-24.8	11.3-17.2
MCHC (g/dL)	31.6-34.4	26.2-35.9
RDW (%)	12.0-13.2	13.8-21.1
Reticulocytes (%)	0.1-1.5	0.1-1.2
Reticulocytes (10 ⁹ /L)	10.9-111.0	3.7-94.1

stained blood smears. The most commonly used stains are the Romanowsky-type (for routine morphologic evaluation of blood cells), new methylene blue (for the determination of reticulocytes number and presence of Heinz bodies) and Prussian blue (for the verification of iron-containing inclusions in blood cells).

> Anemia

Anemia is defined as decreased hemoglobin concentration, which is typically associated with a decreased number of circulating RBCs and low hematocrit.¹ The above mentioned pathologic state, also named absolute anemia, should be differentiated from relative anemia, which is the result of hemodilution.⁴ Anemia is classified by bone marrow responsiveness (regenerative, nonregenerative), erythrocyte indices (microcytic, normocytic, macrocytic and hypochromic, normochromic) and pathophysiology (anemia due to a decreased red blood cell life span and anemia caused by decreased erythrocyte production).^{4,5} However, the ultimate goal is the classification of anemia according to the etiology.

> Regenerative anemia

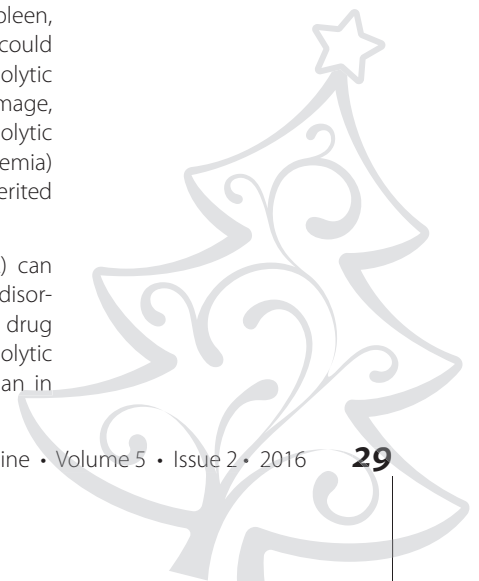
Regenerative anemia is characterized by reticulocytosis, which is the best marker for accelerated erythropoiesis, and hence by anisocytosis (increased RDW), macrocytosis (increased MCV), hypochromasia (decreased MCHC) and polychromasia (highly indicative of regeneration).^{6,7} Regenerative anemia is divided into two major categories: blood loss anemia (BLA) and hemolytic anemia.

BLA is characterized by moderate regeneration, although it may be nonregenerative in early stages and poorly regenerative or even nonregenerative in late stages. In the first 2-4 days, blood loss anemia is nonregenerative, normocytic and normochromic, because reticulocytosis is observed 2-4 days after the onset of the hemorrhage.^{6,8} In the next stage,

anemia becomes regenerative, macrocytic and hypochromic and remains as such, unless iron deficiency anemia is developed secondary to chronic hemorrhage. The most common causes of BLA are traumatic injuries (including those surgically-induced) and hemostatic disorders, which occur as a result of severe thrombocytopenia, anticoagulant intoxication, functional defects of platelets (uremia, severe liver disease, specific drug administration, etc.) or inherited disorders (such as hemophilia A, B and von Willebrand's disease).⁹ Neoplasms, especially hemangiomas and hemangiosarcomas in the spleen, liver and lungs, as well as those of the gastrointestinal or urinary tract are strongly associated with chronic blood loss.⁹ Parasitic infections with ectoparasites (e.g. fleas, lice and ticks) or endoparasites (e.g. *Ancylostoma spp*, *Uncinaria spp* and coccidia), especially in young and small dog breeds, gastrointestinal ulcers, foreign bodies (particularly in the gastrointestinal tract), and urinary calculi are also reported as causes of BLA.⁹

Hemolytic anemia is a condition in which the rate of erythrocyte destruction is higher than the erythrocyte production in the bone marrow. It is the regenerative anemia type with the greatest response.^{6,8} However, in some cases, such as bone marrow damage or production of antibodies against RBC precursors in bone marrow, the regeneration is poor to none.⁹ Hemolysis can be intravascular (within the blood vessels or heart) or extravascular (destruction of erythrocytes in the macrophages of the spleen, liver and bone marrow). Hemolytic anemia could be classified into four main categories. Hemolytic anemia due to RBCs immune-mediated damage, mechanical damage (microangiopathic hemolytic anemia), oxidative damage (Heinz body anemia) and because of a heterogeneous group of inherited defects.

Immune-mediated hemolytic anemia (IMHA) can be primary or secondary, due to immune disorders, infectious and neoplastic diseases or drug administration. Primary (autoimmune hemolytic anemia) is much more common in dogs than in





cats.⁴ Infectious agents can cause hemolytic anemia directly by attacking erythrocytes or indirectly by the effect that their products have on erythrocytes. IMHA may be seen in cases of leishmaniosis, ehrlichiosis, dirofilariasis, hemotropic mycoplasmosis and babesiosis.^{6,10} Immune-mediated hemolysis has been observed in lymphoproliferative diseases, such as lymphomas and leukemias.⁶ Drugs that are commonly used in clinical practice and potentially cause IMHA are antibiotics (penicillins, cephalosporins, trimethoprim-sulfonamide), non-steroidal anti-inflammatory drugs (paracetamol), antiarrhythmics, etc.^{4,10} Immune-mediated hemolysis may also be seen in transfusion reactions, vaccine administration and neonatal isoerythrolysis.¹¹

The mechanical injury of RBCs (microangiopathic hemolytic anemia) is the result of certain pathologic conditions and diseases, such as vasculitis, disseminated intravascular coagulation (DIC) (anemia due to DIC may also be hemorrhagic due to multiple hemorrhages), hemangiosarcoma, dirofilariasis and cardiac, hepatic or splenic disease.^{4,6,10}

Heinz body anemia is a result of a variety of factors, which may be easily identified from the history of the animal patient. Specific drug administration (e.g. paracetamol, benzocaine), as well as the consumption of onion, garlic and their products, are associated with this type of hemolytic anemia.^{4,12,13} Heinz body anemia can emerge in the progress of some endocrine diseases, such as diabetes mellitus and hyperthyroidism, as well as in neoplastic diseases such as lymphomas.^{10,12,14} Furthermore, chemicals (e.g. heavy metals, propylene glycol, phenolics) are also associated with the onset of Heinz body anemia, while hemolytic anemia which accompanies hypophosphatemia may occasionally be related to Heinz body formation.^{4,6,12}

Hemolytic anemia due to inherited genetic defects of RBCs are rarely encountered in clinical practice and include erythrocyte membrane defects (e.g. hereditary elliptocytosis, hereditary stomatocytosis), erythrocyte enzyme abnormalities (e.g. phosphofructokinase deficiency, pyruvate kinase deficiency, feline porphyria, methemoglobin reductase deficiency) and other pathologic conditions, such as familiar non-spherocytic hemolytic anemia (as reported in Beagle and Poodle), in which there is no evidence of disorders in morphology or in the enzymes of erythrocytes.^{4,15-18}

> Non-regenerative anemia

Non-regenerative anemia is the most common type of anemia in clinical practice.¹⁹ This anemia may arise either because of abnormalities of the stem cells, their modulators and the bone marrow environment or because of disorders in the later-stage regulators of erythroid proliferation, including eryth-

ropoietin, cytokines and iron.¹⁹ As the average life span of erythrocytes is 100-120 days in dogs and 70-78 days in cats, the development of anemia, which is only caused by decreased erythropoiesis, might take several weeks or even months.¹⁹ Reticulocytes are either not seen in great numbers or not seen at all in circulation; hence, non-regenerative anemia is characterized as normocytic and normochromic.^{9,20} Exceptions include nutritional anemias, anemias associated with myeloproliferative disorders, and certain cases with anemia of chronic disease.⁹ Non-regenerative anemia can be classified into five major categories: i) anemia of chronic disease (ACD), ii) erythropoietin-related anemia (ERA), iii) anemia due to bone marrow disorders, iv) nutritional deficiency anemia and v) anemia due to acute and peracute blood loss or hemolysis (first 48 to 96 hours).

ACD (also called anemia of inflammation) is the most frequently encountered anemia in small animals.¹⁹ The production of cytokines during inflammation results in the decrease of iron availability, erythropoietic response and RBCs life span.¹⁹ Typically, laboratory evaluation reveals mild to moderate normocytic and normochromic anemia.¹⁸ Moreover, anemia is accompanied by decreased levels of serum iron and serum transferrin and normal or increased serum ferritin concentration.^{19,20} ACD can be a result of both infectious (bacterial, viral, protozoal, fungal) and non-infectious (immunological, neoplastic) diseases.⁴ Regarding anemia due to neoplasia, it should be mentioned that this can also be a consequence of hemorrhage, hemolysis, myelophthisis, myelofibrosis and myelodysplasia.^{19,21}

ERA has as its main pathogenic mechanism the suppression of erythropoietin and can be caused either by chronic renal disease or by specific endocrine disorders. Anemia of chronic renal disease (or chronic renal failure), which refers to end-stage renal disease or renal failure, is mainly a result of erythropoietin deficiency and uremia.^{19,22} This type of anemia is mild to moderate, normocytic, normochromic, with normal iron distribution, unless there is evidence of inflammation.¹⁹ ERA can be developed secondary to endocrine disorders, such as hypothyroidism, hypoadrenocorticism and pituitary dwarfism.¹⁹ The anemia due to hypothyroidism or hypoadrenocorticism is usually mild, normocytic and normochromic.²³

Non-regenerative anemia can be the result of the following bone marrow disorders: bone marrow or erythroid aplasia/hypoplasia [known as aplastic anemia and pure red cell aplasia (PRCA), respectively], myelophthisis, myelodysplasia and myelofibrosis. All these disorders, apart from pure red cell aplasia, affect two or even all three cell lineages in the bone marrow; thus, leukopenia and/or thrombocytopenia are also present along with anemia. Pure red cell aplasia (PRCA) leads to a severe, normocytic, normochromic anemia, with absolute



reticulocytopenia and the virtual absence of bone marrow erythroid precursor cells.²⁴ PRCA can be immune-mediated (it is considered a type of non-regenerative IMHA), related to viral infections [canine parvovirus, feline leukemia virus (FeLV) infection] or rarely congenital.²⁴⁻²⁶ Aplastic anemia (also called aplastic pancytopenia) is characterized by bicytopenia or pancytopenia and replacement of normal bone marrow elements by adipose tissue.²⁷ Infectious diseases, such as ehrlichiosis, parvovirus, leishmaniasis, histoplasmosis in dog and FeLV, feline immunodeficiency virus (FIV) infections and panleukopenia in cat, frequently cause aplasia of bone marrow.²⁷ Hyperestrogenism, either of endogenous origin (e.g. Sertoli cell neoplasia) or of exogenous-iatrogenic origin caused by an overdose of estrogens, is a causative factor for both dogs and cats, although dogs are much more susceptible to high levels of estrogens.^{19,27} Transient aplastic anemia is reported in radiation syndrome and after the administration of specific drugs, such as chemotherapeutics (azathioprine, cyclophosphamide, etc.), phenylbutazone, sulfadiazine, griseofulvin, chloramphenicol and fenbendazole.^{18,19,27,28} If an etiologic diagnosis cannot be established, aplastic anemia is defined as idiopathic.²⁹ Myelophthistic anemia develops secondary to a space-occupying lesion in the bone marrow where non-marrow elements, such as neoplastic, inflammatory and stromal cells, crowd out the normal hematopoietic precursors of bone marrow.¹⁹ The laboratory evaluation shows a mild to severe, normocytic, normochromic anemia, reticulocytopenia and leukoerythroblastic reaction.¹⁹ Diseases associated with myelophthistic anemia are hematopoietic or metastatic neoplasms and granulomatous inflammatory diseases.^{18,20} Myelodysplastic syndromes (MDS) are a heterogeneous group of hematopoietic stem cell disorders characterized by cytologic dysplasia in the blood and bone marrow and by various combinations of anemia, neutropenia and thrombocytopenia.¹⁹ Myelodysplasia is seen in both dogs and cats, but is more common in cats with FeLV infection.²⁰ In dogs, myelodysplasia can be genetically-based or secondary to drug administration.¹⁹ All MDS are associated with non-regenerative, normocytic and normochromic anemia.³⁰ Myelofibrosis is the replacement of normal elements of bone marrow from fibrous tissue produced by fibroblasts.²⁰ Myelofibrosis may lead to another similar condition, called osteosclerosis or myelosclerosis, in which instead of fibrous tissue, osseous tissue is produced by osteoblasts.^{20,31} Both syndromes are rare and have been observed in FeLV infection in cats, in dogs with congenital pyruvate kinase deficiency, in carcinomas and in cases without any underlying condition (idiopathic).²⁰ Most patients with these syndromes are severely anemic and present leukoerythroblastic reaction.¹⁹

Nutritional deficiency anemia is caused by a prolonged lack of minerals, vitamins and proteins essential, for the production of erythrocytes.¹⁸ By far

the most frequent anemia of this group is iron deficiency anemia (IDA). Iron is required for the synthesis of hemoglobin and therefore iron deficiency can cause anemia, which is classified as non-regenerative, even though mild to moderate regeneration is often observed.³² Although dietary deficiency is the major cause of IDA in humans, in small animals it is rare and almost always concerns puppies and kittens that are exclusively fed on milk.³² IDA is caused mainly by external hemorrhage and also by insufficient intestinal absorption of iron.³² Regarding RBC indices, acute IDA is normocytic and normochromic and only after the lapse of several weeks or months does it turn into microcytic and hypochromic.^{9,32} At this point, it should be noted that microcytosis is normally seen in some dog breeds, such as Akita and Sharpei.²⁰ Apart from iron, copper deficiency can also lead to anemia, which, in contrast to IDA, is normocytic and normochromic.^{18,32} Vitamin deficiencies, usually observed in animals suffering from gastrointestinal or pancreatic diseases, constitute rare causes of anemia in dogs and cats. Vitamin B₁₂ and folic acid deficiency cause macrocytic or normocytic, normochromic anemia, which can be called megaloblastic.^{9,18} Niacin deficiency also causes macrocytic and normochromic anemia.⁵ Pyridoxine (vitamin B₆) and riboflavin (vitamin B₂) deficiencies are very rare and constitute etiologic factors of microcytic and hypochromic anemia.⁹

> Erythrocytosis and polycythemia

Erythrocytosis is defined as an increase in hemoglobin concentration, RBC count and hematocrit above the established reference intervals.³³ The terms erythrocytosis and polycythemia are often used to describe the same pathologic condition, although erythrocytosis implies an increase in the number of erythrocytes and polycythemia an increase in the number of all three cell lines.³⁴ Erythrocytosis is much more frequent in veterinary medicine than polycythemia.³⁴ It is important to know that certain breeds of dogs, such as Greyhounds, Afghan hounds, Salukis and Whippets, have higher hematocrit values (usually low 60s).⁴ Based on the pathogenesis, erythrocytosis is classified into relative or absolute and furthermore, absolute erythrocytosis is divided into primary (polycythemia vera) or secondary.³³

In relative erythrocytosis, the RBC mass is within the normal reference range, but hematocrit is increased because of the decreased plasma volume (hemocentration).³³ Relative erythrocytosis is mild to moderate and is the most common type of erythrocytosis in dogs and cats.³³ Dehydration is the major causative factor of this pathologic state.³⁴ Splenic contraction due to excitement, anxiety or exercise can cause physiologic erythrocytosis, which is mild to moderate, transient, concerns mainly dogs and may mask concurrent anemia.⁴ In cases where

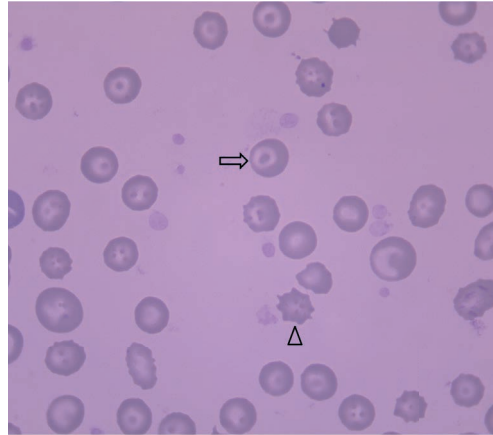


Figure 1. Blood smear from a dog. A codocyte (arrow) and an echinocyte (arrowhead) are observed (Giemsa, 100x objective).

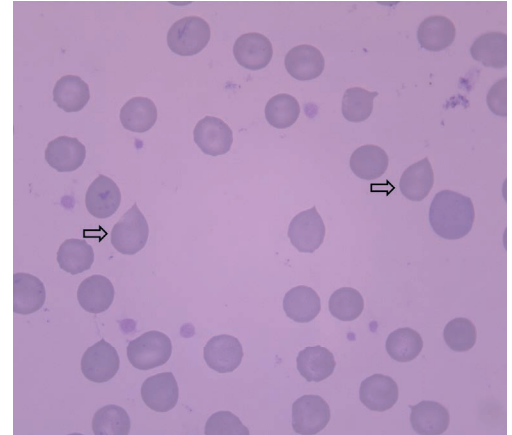


Figure 2. Blood smear from a dog. Two dacryocytes (arrow) are witnessed (Giemsa, 100x objective).

splenic contraction is suspected, a new blood sample should be collected with the minimum of stress.³⁴

Absolute erythrocytosis is defined as an increase in total RBC mass.³³ As mentioned above, it is divided into primary (polycythemia vera) or secondary. Polycythemia vera is a chronic myeloproliferative disorder characterized by the autonomous clonal proliferation of hematopoietic progenitor cells.³³ Secondary erythrocytosis results from increased production of erythropoietin, which is typically seen in animals with congenital heart abnormalities

(e.g. tetralogy of Fallot, patent ductus arteriosus), chronic severe pulmonary disease, in animals living at high altitudes or those that are physically trained (working dogs).^{4,33,34} Furthermore, renal inflammatory, neoplastic or other diseases (e.g. amyloidosis) can cause inappropriate secondary erythrocytosis directly, by secreting erythropoietin, or indirectly, through local hypoxia.^{33,34} Finally, endocrinopathies, such as hyperadrenocorticism and hyperthyroidism are associated with mild erythrocytosis, due to the hormonal stimulation of erythropoiesis.³³

> Erythrocyte morphology

In a blood smear stained with a Romanowsky-type stain, four features of RBCs are evaluated: the color, size, shape and, if present, the inclusions. The color (normochromia, hypochromia) and the size (microcytosis, normocytosis, macrocytosis) can be evaluated both quantitatively (RBC indices) and qualitatively (blood smear). Considering the above, only the shape and the inclusions of RBCs will be discussed in this section, as information regarding the connection between the color and size of RBCs with specific hematologic disorders has been previously provided, based on RBC indices.

Pathophysiologically, poikilocyte formation is linked to a variety of changes primarily or secondarily affecting the erythrocytes. Modifications in RBC membrane lipids or proteins are related to the formation of acanthocytes, codocytes (Figure 1), elliptocytes (also called ovalocytes) and echinocytes (although, the latter have a very complex pathogenesis) (Figure 1).^{35,36} On the other hand, the presence of schistocytes, keratocytes

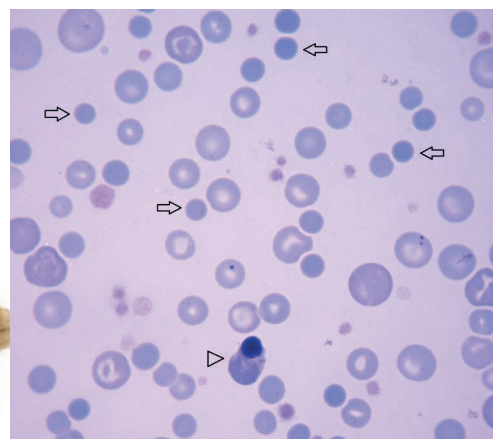


Figure 3. Blood smear from a dog with IMHA. Polychromasia and anisocytosis are obvious. Many spherocytes (arrow) and one metarubricyte (arrowhead) are observed (Giemsa, 100x objective).

**Table 2.** Common etiology of poikilocytes

Poikilocyte	Associated diseases
Acanthocytes	Disseminated intravascular coagulation, hemangiosarcoma, liver disease, glomerulonephritis
Codocytes (target cells)	Regenerative anemia, iron deficiency anemia, congenital dyserythropoiesis
Dacryocytes	Myelofibrosis, hypersplenism, glomerulonephritis
Eccentricocytes (erythrocyte hemighosts)	Etiology of Heinz body anemia
Echinocytes (crenated erythrocytes)	Uremia, glomerulonephritis, snake envenomation, various neoplasms
Elliptocytes (ovalocytes)	Various bone marrow disorders, hepatic lipidosis, portosystemic shunts, glomerulonephritis
Keratocytes	Iron deficiency anemia, myelodysplastic syndrome, liver disease
Schistocytes	Disseminated intravascular coagulation, hemangiosarcoma, vasculitis, heart failure, caval syndrome of dirofilariasis, glomerulonephritis, myelofibrosis
Spherocytes	Immune-mediated hemolytic anemia, erythrocyte parasites, snake envenomation, bee sting, zinc toxicity
Stomatocytes	Hereditary

and dacryocytes (Figure 2) in peripheral blood is owed to mechanical damage of RBCs.^{35,36} Furthermore, erythrocytes oxidative damage and their fragmentation due to phagocytosis leads to the formation of eccentricocytes and spherocytes (Figure 3), respectively, whereas stomatocytes have a hereditary etiopathogenesis.^{35,36} The presence of echinocytes, dacryocytes and stomatocytes on the blood smear is commonly considered an artifact of inappropriate sample storage or preparation.^{35,36} The identification of spherocytes is difficult in cat, because the RBC central pallor is not prominent, while eccentricocytes are more easily recognized on new methylene blue (NMB) stained preparations.^{35,36} Finally, it should be mentioned that the concurrent presence of schistocytes, keratocytes and acanthocytes is indicative of lymphoma, hemangiosarcoma, DIC, hepatic cirrhosis, glomerulonephritis or pancreatitis.^{35,36} Additionally, in the case of snake bites, either echinocytes or spherocytes are produced, depending on the time course and dose of venom.^{35,36} Further information concerning the etiology of the formation of the various poikilocytes is provided in Table 2.^{4,35,36}

Nucleated RBCs (Figure 3), concurrently with reticulocytes, are seen most frequently in patients with regenerative anemia (Table 3). Spleen or bone marrow diseases, such as hematopoietic neoplasms, myelofibrosis and MDS, are associated with an increased number of circulating nucleated RBCs including less mature erythrocyte precursors, such as rubriblasts and prorubricytes, while lead toxicity is related to metarubricytosis, which is usually not accompanied by anemia.^{35,36}

Howell-Jolly bodies are nuclear remnants which are observed in the erythrocyte cytoplasm and appear as round, clearly basophilic inclusions which vary

in size. They are normally removed by the spleen, therefore low numbers may be seen in healthy cats, because of the unique structure of their spleen. Regenerative anemia, hypofunctioning spleen, splenectomy and glucocorticoids administration are the major causes of increased Howell-Jolly bodies.^{35,36}

Heinz bodies are aggregates of oxidized, denatured hemoglobin which appear as small, red to pale pink, with Romanowsky-type stains, projections in erythrocyte surface. On NMB blood smears, they are stained bluish-green and are visualized much easier (Figure 4). In healthy cats, Heinz bodies may

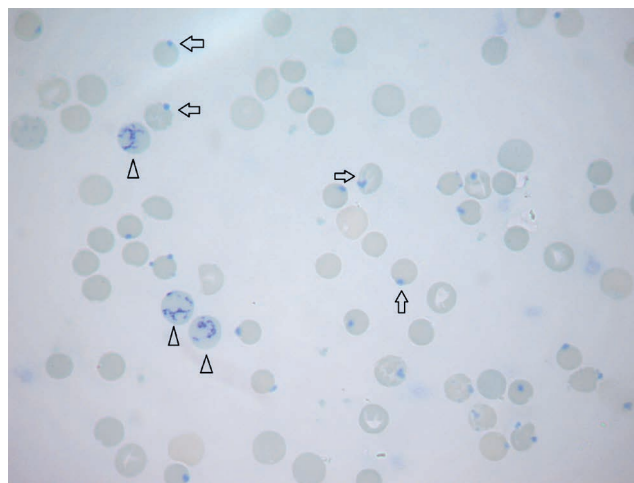


Figure 4. Blood smear from a dog with Heinz body anemia. Many Heinz bodies (arrow) are noticed, as well as three reticulocytes (arrowhead) (New methylene blue, 63x objective).



Table 3. Degree of erythroid regeneration in anemia. Modified from Weiss D and Tvedten H, 2004⁵

Degree of bone marrow stimulation	Percentage of reticulocytes		Reticulocytes count (/μL)		
	Dogs	Cats (aggregate reticulocytes)	Dogs	Cats (aggregate reticulocytes)	Cats (punctate reticulocytes)
Normal	1	0-0.4	60,000	<15,000	<200,000
Mild	1-4	0.5-2	150,000	50,000	500,000
Moderate	5-20	3-4	300,000	100,000	1,000,000
Marked	21-50	5+	>500,000	>200,000	1,500,000

be observed in more than 5% of erythrocytes because of the decreased splenic removal and the susceptibility of cats to the denaturation of hemoglobin by endogenous oxidants.³⁵ The etiology of Heinz bodies formation has been described thoroughly in Heinz body anemia.

Basophilic stippling is the formation of blue-staining punctate inclusions (in Romanowsky-stained blood smears) in erythrocytes, which represent aggregates of ribosomes and polyribosomes. Basophilic stippling must be differentiated from siderotic granules, which are usually seen adhered in clusters. In small animals, it occasionally occurs in regenerative anemia, whereas when observed in the absence of severe anemia, lead toxicity becomes a great possibility.^{35,36}

Siderotic inclusions (also known as Pappenheimer bodies) are iron-containing inclusions which, on Romanowsky-stained blood smears, appear as basophilic granules located in clusters near the periphery of the erythrocyte. Prussian blue staining is necessary for the confirmation of these inclusions. Erythrocytes or reticulocytes with siderotic inclusions are called siderocytes,

they are rarely if ever seen on normal blood smears and are associated with myeloproliferative diseases, hemolytic anemias, dyserythropoiesis and lead toxicity.^{35,36} Nucleated erythrocytes with siderotic inclusions are called sideroblasts and are linked to MDS, myeloproliferative disorders or inflammation.^{35,36}

The most common erythrocyte inclusions of infectious origin are the intracellular protozoan *Babesia* spp., epicellular mycoplasma organisms, *Mycoplasma haemofelis* and *Mycoplasma haemocanis*, as well as distemper virus inclusions. *Babesia* spp. are oval to pear-shaped (usually) inclusions, which have a colorless to light blue cytoplasm and red to purple nucleus in Romanowsky-stained blood smears.³⁵ *Babesia canis* is large and thus easily observed (Figure 5). On the other hand, *Babesia felis* and *Babesia gibsoni* are small and it is difficult to visualize them on blood smears. Hemotropic *Mycoplasma* spp. typically appear as cocci and occasionally as rings or rods, which are all stained in the shades of blue with Romanowsky-type stains.³⁵ *Mycoplasma haemofelis* is seen individualized or in short chains,

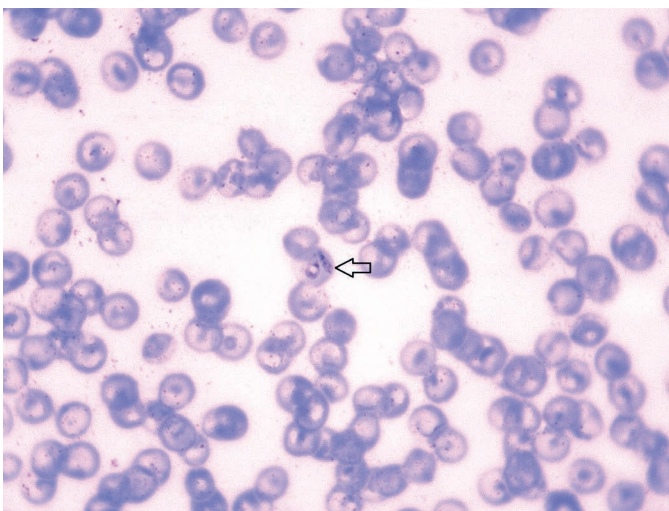


Figure 5. Blood smear from a dog with babesiosis. Two pear-shaped merozoites of *Babesia canis* (arrow) in a red blood cell are observed (Giemsa, 100x objective).

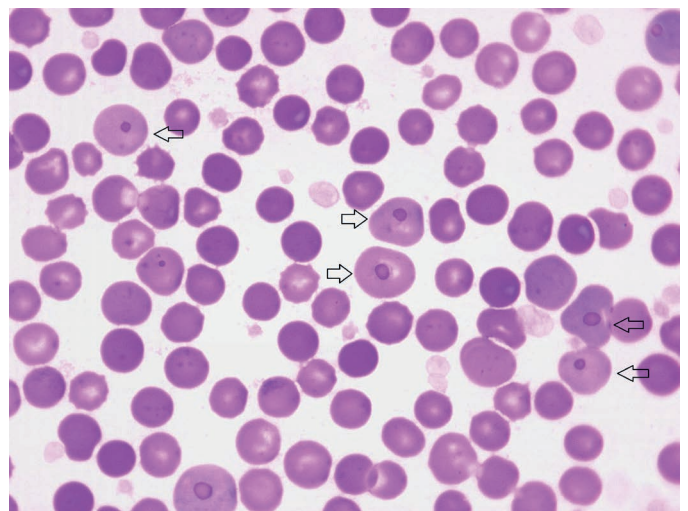


Figure 6. Blood smear from a dog with distemper. Many virus inclusions (arrow) are seen in red blood cells. Diff-Quik, 100x objective.



in contrast to *Mycoplasma haemocanis* that is seen in chains, forming various patterns.³⁵ Distemper viral inclusions in dogs are blue-grey inclusions, variable in size and shape, formed within the erythrocyte precursors; hence, they most often

occur in polychromatophilic cells, although they can be observed in erythrocytes.³⁵ For reasons as yet unknown, the fact remains that distemper inclusions are easier to recognize with Diff-Quik staining (Figure 6).³⁵

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