

Diagnosing causes of anemia

History, physical examination and a minimum database set the stage for diagnosing underlying conditions in anemic Pets.

Be it a 6-week-old puppy with hookworm infection, a kitten with feline leukemia or a geriatric Pet with renal disease, anemia is a common finding for veterinarians. Anemia itself is not a disease; rather, it is a laboratory finding indicative of a disease process. Because many conditions can cause anemia and anemia can affect Pets of any age or species, developing an effective diagnostic and treatment plan can be frustrating. No single treatment plan applies to all cases of anemia. But, if you think of it as a clinical sign and develop a thorough diagnostic approach, you are more likely to successfully discover the primary disease process and deliver appropriate therapy.

Anemia is an absolute decrease in the number of circulating red blood cells (RBCs). It can be identified by a low hematocrit, hemoglobin concentration or RBC count. However, remember that hemodilution caused by parenteral fluid administration, low sample volume in an EDTA tube or improper withdrawal of blood from an intravenous fluid line may cause the same laboratory findings, but they do not always

constitute true anemia.

A hematocrit of less than 37 percent in dogs or less than 25 percent in cats is considered significant and needs to be evaluated. If a Pet shows a decrease in hematocrit from its baseline over time, this may also be considered a significant finding, even if the Pet is still within the normal reference values for the population. Reference ranges are designed to include approximately 95 percent of healthy Pets. This means that up to one in 20 Pets can fall below the normal range and still be physiologically normal. However, since it is impossible to know ahead of time which Pets normally fall outside of the reference range, it is desirable to follow a diagnostic protocol for all Pets that fall below the normal reference range to rule out the various causes of anemia, many of which can be life threatening.

The diagnostic approach to anemia must be systematic and thorough. Start with a thorough history, complete physical examination and minimum database. Additional diagnostics (*e.g.*, radiography, ultrasonography, endoscopy, systolic blood pressure measurement, bone marrow aspirate or biopsy, testing of iron levels and coagulation



By Jeffrey Mills,
DVM

Contributing Author

Table 1: Causes of Anemia

- Acute trauma or blood loss
- Chronic infection or disease
- Flea infestation
- Hookworm infection
- Blood parasite infection
- Chronic renal failure
- Immune-mediated hemolytic anemia
- Nutritional anemia
- FeLV/FIV infection (cats)
- Aplastic anemia
- Neoplasia
- Disseminated intravascular coagulation
- Endocrine or hormonal imbalances
- Bone marrow disorders
- Toxin exposure

profiling) may be required depending on the results of your initial testing. As always, you may need to provide emergency or supportive care before obtaining diagnostic samples and pursuing a complete diagnosis.

The number of conditions that can cause anemia are as daunting as the number of diagnostic tests needed to elucidate the primary disease process.¹ Some of the more common diagnoses associated with anemia are shown in *Table 1*.

In this article, we will provide suggestions with regard to a physical examination, thorough history and minimum database that includes microscopic examination of a blood smear. We will also discuss when a bone marrow biopsy or aspirate is indicated. *Blood transfusions in anemic Pets* (page 38) discusses the basics of anemia treatment.

History and signalment

As with most disease processes, the first step in discovering the cause of anemia is to take an accurate and thorough history. The purpose of taking a history is to deter-

mine what clinical signs the Pet is having (*e.g.*, weakness, vomiting, diarrhea, anorexia), what risk factors the Pet may have been exposed to (*e.g.*, hit by car, rodenticide ingestion, acetaminophen ingestion, travel) and the Pet's overall health (*e.g.*, vaccine status, family history, presence of chronic diseases). The most likely causes of the anemia will vary with the Pet's age, species and environment.

Remember that the body can compensate for a slowly progressing anemia, making the clinical signs of chronic anemia more difficult for the owners to detect. Pets with chronic anemia may present to the veterinarian with nonspecific signs, such as decreased activity, decreased appetite or weight loss. Conversely, Pets with acute anemia have had no time to compensate and will commonly have a history of sudden collapse or respiratory distress. On the extreme end of the scale, a rapid loss of 50 percent or more of the blood volume can result in shock and death.

The signalment can also provide some clues. Juvenile patients are more likely to be anemic due to internal or external parasite infestation. Stray kittens and cats or those with an unknown background are more likely to test positive for feline leukemia virus (FeLV) or feline immunodeficiency virus (FIV). Certain breeds are predisposed to congenital anemia, such as nonspherocytic hemolytic anemia (*e.g.*, Poodle, Beagle), phosphofructokinase (PFK) deficiency (*e.g.*, Cocker Spaniel, English Springer Spaniel), pyruvate kinase (PK) deficiency (*e.g.*, Abyssinian, American Eskimo, Basenji, Beagle, Chihuahua, Cairn Terrier, Dachshund, Poodle, Pug, Somali, West Highland White Terrier) or stomatocytosis (*e.g.*, Alaskan Malamute, Miniature Schnauzer).² Middle-aged to geriatric dogs that

present with acute collapse should be evaluated for internal bleeding due to a ruptured splenic tumor, such as hemangiosarcoma. This is especially true for large breeds.

Physical exam findings

A complete physical examination may reveal findings that could reflect the cause of the anemia. Clinical findings can be related to the primary cause, such as small kidneys on palpation associated with chronic renal disease, or they can be related to the lack of blood volume itself, as in the case of pale mucous membranes. Toxins such as lead may cause central nervous system signs or seizures. Pets with neoplasia may present with findings such as cachexia, lymphadenopathy or a palpable abdominal mass. Again, remember that the common sign of increased respiratory effort may not be present if the Pet has had a long standing anemia; in contrast, the Pet that acutely develops anemia will tend to have an increased respiratory effort to compensate for hypoxia at the cellular level.

In all cases, it is crucial to be sure that the clinical signs are related to anemia in the first place. If a Pet presents with pale mucous membranes, it is important to differentiate anemia from hypoperfusion. On a complete blood count, many Pets with hypoperfusion will have a normal hematocrit.

Clinical laboratory findings

Appropriate diagnostic testing will complement a thorough history and complete physical exam. Every anemic Pet requires a CBC with manual differential done by microscopic evaluation of a blood smear, internal organ function screen, electrolyte determination, urinalysis, fecal exam for intestinal parasites, skin evaluation for external parasites, heartworm testing and

testing for FeLV and FIV in cats and *Rickettsia* infections in dogs. Other tests (e.g., thyroid, cortisol and sex steroid levels, radiographs, ultrasound) may be indicated based on the history, signalment, physical exam and initial testing results.

Minimum database

Complete blood count. The CBC can be used to help verify the presence of anemia and classify it. Correctly classifying the anemia as regenerative vs. nonregenerative will help identify the primary disease process. A low hematocrit is the hallmark of anemia; however, it is important to note that a mildly anemic patient may present with a low-normal hematocrit in the face of dehydration and that a normal patient may have a low hematocrit in the face of hemodilution caused by fluid therapy. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) are also useful in classifying anemias (see Classification of anemias, page 29). Aplastic anemia is a condition that affects the entire bone marrow and is also characterized by neutropenia, lymphopenia and thrombocytopenia.

Internal organ function screen and electrolytes. Hemoglobinemia (noted as a hemolyzed serum sample) and hyperbilirubinemia can be seen in cases of hemolysis, which is commonly caused by infectious diseases (e.g., bacteria, *Rickettsia*, blood parasites), immune-mediated mechanisms, hematopoietic neoplasia, hemangiosarcoma and drug or toxin ingestion.

A low albumin and low total protein concentration may indicate blood loss. If no external bleeding is evident, check the stool for hematochezia or melena and examine both the chest and abdomen for free fluid via radiographs, ultrasound and/or centesis.

Pets with chronic renal disease will likely have an elevated blood urea nitrogen (BUN), creatinine and phosphorus level. The results of a urinalysis will also aid in the definitive diagnosis of renal disease.

Pets with a portosystemic shunt may have elevated liver enzymes and dilute urine; however, a few may have normal liver values and require a bile acid assay to reveal an abnormality in liver function.

Nearly all endocrinopathies can cause anemia of chronic disease. Some clues that may be evident in the internal organ function screen include elevated cholesterol with hypothyroidism, electrolyte imbalances with hypoadrenocorticism and elevated ALT with hyperthyroidism.

Urinalysis. Hemoglobinuria and bilirubinuria can be seen in cases of hemolysis, and bilirubinuria can also be seen with liver disease. Pets with renal failure will have a low specific gravity even in the face of azotemia. Pets with coagulation defects or trauma may have RBCs in the urine sediment.

Saline agglutination test. This test can be used to differentiate autoagglutination from rouleaux formation. A positive agglutination test is associated with immune-mediated anemia, whereas rouleaux formation is more likely to be an artifact or associated with inflammatory or neoplastic diseases. If RBC clumping remains on the slide after a drop of saline has been added, then the test is positive and the cells are exhibiting true autoagglutination. If the clumping dissipates, then the blood was most likely exhibiting rouleaux. A positive saline agglutination test is pathognomonic for immune-mediated hemolytic anemia.

Imaging. Radiography and/or ultrasonography can be useful to detect changes in organ size or shape and to rule out the presence of free fluid in the chest or

abdomen. Intra-abdominal or thoracic bleeding, a ruptured spleen, hemorrhaging hemangiosarcoma and perforated gastrointestinal ulcerations can all cause anemia from internal blood loss. Cancer and organ failure can cause anemia of chronic disease or directly impact the bone marrow's ability to produce new RBCs.

Classification of anemias

Anemia can be classified according to the RBC size and hemoglobin concentration and according to the bone marrow's response (reticulocyte count). This can help narrow the differential diagnosis list and focus your search for the cause of an anemia. Anemias are classed in terms of three main categories:

1. Normocytic, microcytic or macrocytic. A normal, low or high MCV indicates normocytic, microcytic or macrocytic RBCs, respectively.

2. Normochromic or hypochromic. A normal MCHC indicates normochromic RBCs, and a low MCHC indicates hypochromic RBCs. Hyperchromatism does not exist as there is no way to load an RBC with excessive hemoglobin. If the MCHC is high, it indicates free hemoglobin in the serum which might occur with hemolysis or Oxyglobin (Biopure) treatment.

Knowing the MCV and MCHC can help narrow the diagnosis because many causes of anemia fall into categories based on these classifications. For example, puppies with a portosystemic shunt tend to have a microcytic anemia, and anemia of chronic disease tends to be microcytic and hypochromic; immune-mediated hemolytic anemia, on the other hand, tends to be macrocytic and hypochromic.

3. Regenerative or nonregenerative. The degree of RBC regeneration can be

determined from a reticulocyte count. Reticulocytes are immature RBCs that are anuclear but contain residual RNA and mitochondria that absorb vital stains (*e.g.*, methylene blue) in a reticular pattern.⁵

Reticulocytes will appear as polychromatic RBCs on Wright's stained preparations and can be found in circulation 48 to 72 hours following the onset of anemia, reaching peak levels in about seven days.⁵ Early in the disease process, an anemia may falsely appear to be nonregenerative due to this lag time.

An absolute reticulocyte count of $>80,000/\mu\text{L}$ in dogs and $>60,000/\mu\text{L}$ in cats is consistent with a regenerative response. Corrected reticulocyte percentages of >1 percent in dogs and >0.4 percent in cats are consistent with a regenerative response. A regenerative response indicates that the cause of the anemia doesn't involve the bone marrow (*i.e.*, hemorrhage or hemolysis). On the other hand, a nonregenerative anemia indicates either that there has been insufficient time for a bone marrow response, that the bone marrow itself is diseased or that the underlying disease process is suppressing the bone marrow (*i.e.*, anemia of chronic disease). Reticulocyte response will be higher in cases of hemolysis than in cases of external hemorrhage because the iron released from lysed RBCs is available for immediate use by the bone marrow. See the diagnostic algorithm in *Figure 1* (page 32) for the equation to calculate a corrected reticulocyte count.

A quicker but less specific index to distinguish regenerative from nonregenerative anemia is to look for macrocytosis and hypochromasia on the CBC and/or polychromasia on the differential. Regenerative anemia will tend to be macrocytic (high MCV) and hypochromic (low MCHC)

due to the bone marrow's increased production of reticulocytes. Nonregenerative anemias tend not to be macrocytic or polychromatic. Polychromasia, a variation in the color among RBCs, will appear with regeneration because the residual RNA in the reticulocyte will absorb more blue stain. Reticulocytes also tend to be larger than mature RBCs, but they have the same amount of hemoglobin. Thus, most regenerative anemias will be macrocytic and hypochromic and will exhibit polychromasia.

A useful list of rule-outs based on RBC size and hemoglobin concentration and regenerative status is beyond the scope of this article but can be found in any clinical pathology or internal medicine text.⁴

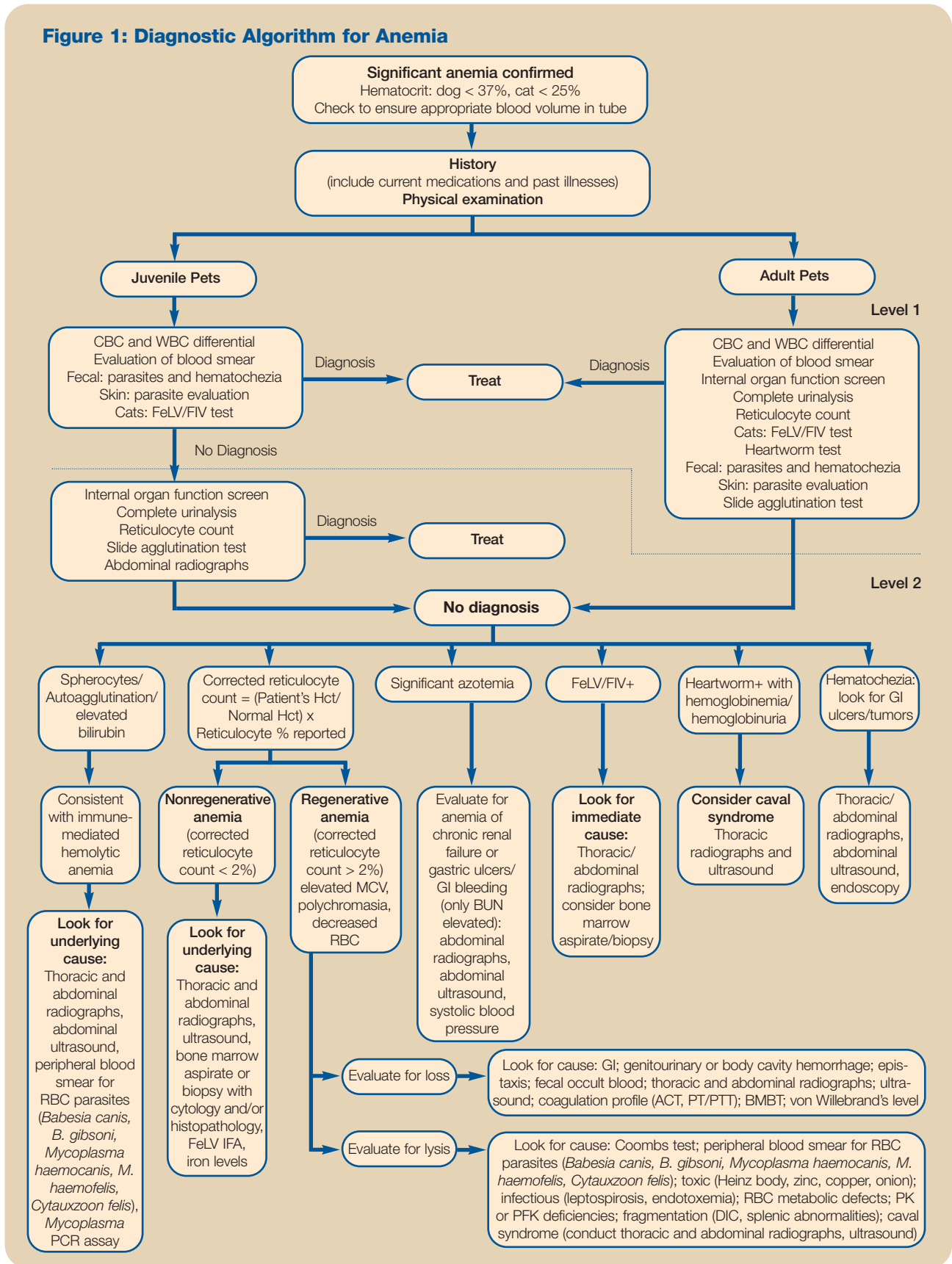
Blood smear evaluation

The ability to accurately evaluate a blood smear is often critical in reaching an accurate diagnosis of the cause of anemia. The procedures for the proper preparation and evaluation of a blood smear have been well described in the literature.⁵ You should become comfortable with evaluating the size and morphology of the RBCs and the white blood cells, as well as the size and number of the platelets present.

Autoagglutination and blood cell parasites can also be detected. Blood smear evaluation is important to verify the CBC results and to help determine the adequacy of the bone marrow's response. The white blood cells should also be evaluated because the presence of band neutrophils indicates an inflammatory process that may be the cause of the anemia.

The monolayer should be examined under oil immersion when you are counting cells and evaluating cell morphology. Anemic patients will usually have thin films overall. In general, the presence of

Figure 1: Diagnostic Algorithm for Anemia



polychromasia, nucleated RBCs, Howell-Jolly bodies and basophilic stippling indicates a regenerative anemia. See *Figure 2* (page 36) for common abnormal blood smear findings.

Other diagnostic testing

While working through the diagnostic algorithm, you will often need to perform tests beyond the minimum database to find the primary disease process causing the anemia.

While a positive saline agglutination test is pathognomonic for immune-mediated hemolytic anemia, a negative result does not rule it out. In those cases, a Coombs test may be needed. A positive Coombs test is found in about 60 percent of Pets with immune-mediated hemolytic anemia. This test detects antibodies and complement on the surface of canine RBCs. It does not help you determine whether the disease process is primary immune-mediated hemolytic anemia or it is secondary to a drug reaction.

Coagulation testing may be appropriate if the cause of anemia is narrowed down to blood loss with no obvious cause. Antinuclear antibody titers and/or a lupus erythematosus cell test may be appropriate if systemic lupus erythematosus is suspected. Serum lead measurements are indicated in Pets with nucleated RBCs. Leptospirosis titers may be indicated because leptospirosis can cause bone marrow suppression. Other tests may be needed depending on the individual case.

Bone marrow evaluation

In cases of anemia, bone marrow evaluation is indicated when a nonregenerative anemia has been confirmed despite an adequate time for a normal bone marrow

response. Other indications for bone marrow evaluation include unexplained thrombocytopenia, neutropenia, pancytopenia and morphologic abnormalities, such as neoplastic cells or nucleated RBCs noted on a microscopic blood smear examination. Further investigation of the bone marrow is also warranted if you suspect a hematopoietic neoplasia or other bone marrow disease. In general, a regenerative anemia is associated with a functional marrow; therefore, evaluation is not usually indicated in these cases.

There are two main ways to evaluate the bone marrow: a bone marrow aspirate or bone marrow core biopsy. Bone marrow aspirates are typically collected by aspirating through an 18-gauge needle or special bone marrow needles with stylets. Aspirates do not allow evaluation of the bone marrow architecture but are useful for evaluating the morphology of individual bone marrow cells. A core biopsy is needed for evaluation of the bone marrow cells in relation to each other and the surrounding stroma. In many cases, a core biopsy provides better diagnostic information. A core biopsy is typically performed with a 16- or 18-gauge bone marrow biopsy needle. The techniques involved in collecting bone marrow samples are outside of the scope of this article but are well documented elsewhere.⁶

Discussion

Anemia is a clinical state associated with an extremely broad range of causes, from trauma to parasites to any number of clinical conditions. Thus, a thorough history and physical examination are vital to providing useful suggestions for potential diagnostic paths. Attention to the minimum database results is likewise crucial to

Figure 2: Abnormalities Found in Blood Smear Evaluation

Acanthocytes

Acanthocytes, also called spur cells, should not be confused with echinocytes. Acanthocytes have two to 10 finger-like blunt projections that are not evenly spaced.⁵ They are associated with altered lipid metabolism such as neoplastic and non-neoplastic conditions of the liver, spleen and kidney but most commonly liver disease and hemangiosarcoma.⁵



Basophilic stippling

Basophilic stippling is the presence of basophilic dots within the RBCs resulting from aggregated ribosomes. It is associated with regenerative anemia or lead poisoning.



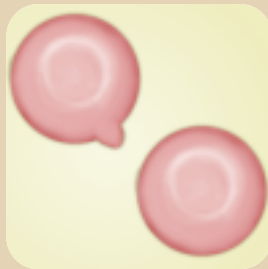
Echinocytes

Echinocytes, also called burr cells, have short projections that are evenly spaced. They can be caused by crenation (*i.e.*, artifact due to slow drying or small sample volume relative to the amount of EDTA) but are also associated with kidney disease, chemotherapeutic agents, lymphoma, pyruvate kinase deficiency and rattlesnake envenomation.



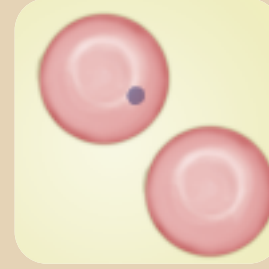
Heinz bodies

Heinz bodies are clumps of denatured hemoglobin that appear as small circular projections off the RBC membrane. The projections are often paler than the rest of the cell. They will stain dark blue with new methylene blue. Their presence indicates oxidative damage. In cats, they are associated with hyperthyroidism, lymphoma and diabetes mellitus. In dogs and cats, they are associated with acetaminophen toxicity, benzocaine toxicity, garlic toxicity, onion toxicity, propofol administration, phenothiazine, propylene glycol, zinc toxicity and others.



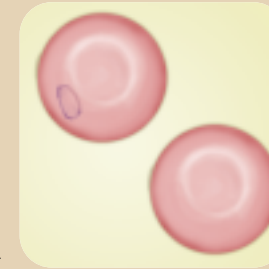
Howell-Jolly bodies

These are basophilic inclusions resulting from nuclear remnants. They are associated with regenerative anemia or hyposplenism.



Parasites

Parasites that may be seen on feline RBCs include *Mycoplasma haemofelis* (previously *Haemobartonella felis*) and *Cytauxzoon felis*. Parasites that may be seen on canine RBCs include *Mycoplasma haemocanis* (previously *Haemobartonella canis*), *Babesia gibsoni* (depicted here) and *Babesia canis*. Heartworm microfilaria can be seen in the feathered edge of the blood smear.



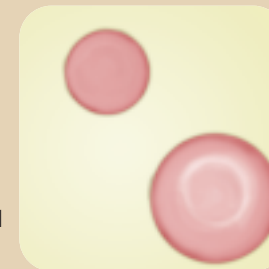
Schistocytes

Schistocytes are fragmented RBCs. They indicate disseminated intravascular coagulation (DIC), hemangiosarcoma, vasculitis, heartworm disease, hyposplenism, liver disease or any other disease with an associated microangiopathy.



Spherocytes

Spherocytes are smaller and darker red than normal RBCs. They are easily recognized in canine blood smears, but normal feline erythrocytes tend to be small and have less central pallor normally, so they can be hard to identify in the cat. These cells form when macrophages remove part of the cell membrane due to the presence of complement or antibodies. The hemoglobin remains in the cell at the same original concentration; thus, the cell stains darker. Spherocytes are highly suggestive of immune-mediated hemolytic anemia but can be seen in a variety of conditions (*e.g.*, Heinz body anemia, zinc toxicity, vasculitis, post-blood transfusion, mononuclear phagocytic neoplasms and DIC).



reaching an appropriate diagnosis; classification of the anemia as regenerative or nonregenerative; macrocytic, normocytic or microcytic; and hypochromic or normochromic provides you with important diagnostic rule-outs that can lead you to the underlying cause of the anemia. Once the anemia is correctly classified and all additional clinical signs are documented, comprehensive diagnostic and pathology textbooks can aid greatly in this diagnosis.

Once a diagnosis is reached, treatment of the anemia's underlying cause can begin, and you can improve the Pet's overall health and quality of life. Acute treatment of the anemia itself is covered in *Blood transfusions in anemic Pets* on page 38. 🏥

References

1. Latimer KS, Mahaffey EA, Prasse KW. *Duncan and*

Prasse's veterinary laboratory medicine: Clinical pathology. 4th ed. Ames: Iowa State Press, 2003:193-214.

2. Couto G. Anemia. In: Nelson RW, Couto G. *Small animal internal medicine*. 3rd ed. St. Louis: Mosby; 2003:1152-1169.

3. Latimer KS, Mahaffey EA, Prasse KW. *Duncan and Prasse's veterinary laboratory medicine: Clinical pathology*. 4th ed. Ames: Iowa State Press, 2003:16-18.

4. Latimer KS, Mahaffey EA, Prasse KW. *Duncan and Prasse's veterinary laboratory medicine: Clinical pathology*. 4th ed. Ames: Iowa State Press, 2003:28.

5. Metzger FL, Rebar A. Three-minute peripheral blood-film evaluation: The erythron and thrombon. *Vet Med* 2004;1026-1034.

6. Fossum TW, ed. *Small animal surgery*. 3rd ed. St. Louis: Mosby, 2007:1341-1342.

Jeffrey Mills, DVM, graduated from Purdue University School of Veterinary Medicine in 1998. He joined Banfield in 2004 and is currently a charter owner in Southwest Louisville. He is also associate editor for the *Journal of Exotic Pet Medicine* and is involved in box turtle and opossum rehabilitation. He lives with his wife, Vashti; three children, Nick, Kirsten and Lilly; five cats; three dogs; three doves; a lovebird and a hairless rat.