

Diagnostic Hematology of Reptiles

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KEYWORDS

• Blood cell morphology • Hematology • Reptiles • Hemogram

Microscopic evaluation of the peripheral blood film is a powerful diagnostic tool and an essential part of the complete blood count in human and veterinary medicine. Blood cell counts and morphology vary greatly among the more than 8000 species of reptiles described, even among species within the same genus. In addition, many intrinsic and extrinsic factors complicate the evaluation of hematologic data in reptiles; thus, published reference intervals provide only a baseline for interpretation, and veterinarians need to be aware of these factors to accurately interpret and correlate hematologic and clinical findings in the reptile patient. Reptiles have become increasingly popular as pets and are frequently found in settings such as zoos and wildlife parks. Wild reptile populations often are subjects of health assessment studies and investigations of naturally occurring disease. For example, the recently discovered novel siadenovirus of Sulawesi tortoises (genus: *Siadenovirus*; species: *Sulawesi tortoise AdV1*) was associated with severe systemic disease; bone marrow myeloid necrosis was observed in 20 of 33 tortoises, and intranuclear inclusions were observed in myeloid and stromal cells of hematopoietic tissue in 19 of 20 tortoises. Several hematologic abnormalities also were observed, including anemia, leukopenia or leukocytosis, heteropenia or heterophilia, lymphopenia or lymphocytosis, and monocytosis, all of which are nonspecific indicators of a chronic inflammatory response.¹ This is just one example of how systemic disease can manifest in the hemogram of reptiles, alerting the veterinarian to the need for further (molecular) diagnostics, if clinically warranted.

Routine hematologic evaluation of reptiles includes determination of packed cell volume (PCV), hemoglobin (Hb) concentration, red blood cell (RBC) count, RBC indices, total white blood cell (WBC) count, leukocyte differential counts, and assessment of blood cell morphology. In small reptile patients, when only a limited amount of

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blood can safely be withdrawn, a properly prepared blood film has priority, because microscopic evaluation alone can provide clinically relevant diagnostic information. Morphologic changes in peripheral blood cells can indicate specific disease processes, help to establish a list of differential diagnoses, and help monitor the health status of a patient during the course of disease or response to therapy. Annual or biannual health checks with blood analysis can help establish baseline values within individuals, which can be valuable for detecting hematologic abnormalities that develop with disease later in life. Because of their unique physiology and behavior, many chronic disease states in reptiles are not detected until in the advanced stages.²

This article describes the normal morphologic and functional features of reptilian blood cells and discusses the manifestation of physiologic and pathologic changes in the reptilian hemogram. The morphology of reptilian blood cells is based on their staining characteristics with Romanowsky-type stains. There are significant differences in the physiology of reptiles compared with common domestic animals; hematologic evaluation starts with blood sample collection, sample-handling techniques, and laboratory procedures, details of which are well documented elsewhere in the literature.^{3,4}

One of the most challenging aspects of diagnostic hematology of reptiles is the accuracy of cell counts. Because reptiles have nucleated RBCs, manual methods must be used to quantify leukocytes. The Natt-Herrick method for obtaining total WBC counts has multiple sources of errors, including inadequate mixing or dilution of blood and stains, incorrect charging of the hemocytometer chamber, and errors in differentiating leukocytes from thrombocytes (which also are nucleated in reptiles). Therefore, total leukocyte estimates with designated formulas are useful during blood film evaluation to verify the manual counts. Erroneous manual counts can lead to misinterpretation of the leukogram, with potentially serious effect on the individual or study population. Manual counts are necessary, however, for determining absolute leukocyte counts (the concentration of cells per microliter of blood), which should be used (rather than percentages) for accurate interpretation of the leukogram. Cautious and consistent use of sampling techniques, specimen handling, and laboratory methods provide the most reliable laboratory results. With these aspects in mind, the validity of abnormalities observed in the hemogram must be interpreted in relation to the clinical presentation of the individual reptile.

Despite the availability of peer-reviewed information and recent advances in reptile medicine, there is an abundance of misinformation and speculations in the literature. The information in this article is based on the authors' collective experience with extensive clinical case material from the Zoological Medicine Service and department of Aquatic Animal Health at the College of Veterinary Medicine, University of Florida, as well as through multiple collaborative institutions. All of the information cited from textbooks and conference proceedings has been confirmed as accurate to the best of the authors' abilities.

BLOOD CELL MORPHOLOGY AND FUNCTION IN REPTILES

Erythrocytes

Erythrocytes of reptiles are similar in microscopic and ultrastructural morphology to those of other nonmammalian vertebrates. Reptilian, avian, amphibian, and fish erythrocytes are nucleated and therefore larger than their mammalian counterparts. When stained with Romanowsky-type stains, the mature erythrocyte of reptiles is elliptical and has abundant orange-pink cytoplasm. The nucleus is centrally positioned, is irregular to elliptical, and has condensed, deeply basophilic chromatin (**Fig. 1**).

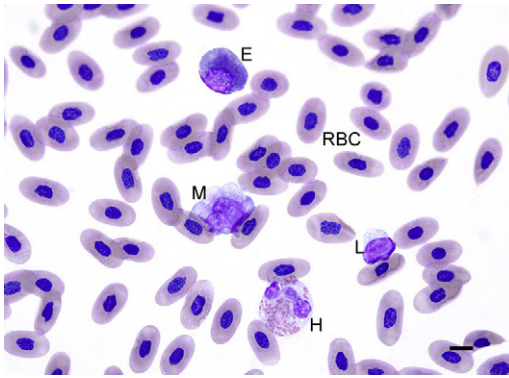


Fig. 1. Peripheral blood from a clinically healthy green iguana (*Iguana iguana*). E, green eosinophil; H, bilobed heterophil; L, lymphocyte; M, monocyte; RBC, mature erythrocytes. Wright-Giemsa, bar = 10 μm .

Erythroplastids (anucleated erythrocytes) are also occasionally observed in healthy reptiles (<0.5%), mostly snakes; they do not seem to have any clinical significance.^{5–8} Aged erythrocytes with small round pyknotic nuclei can be identified in the circulation in low numbers in healthy reptiles. A low number of teardrop-shaped or fusiform erythrocytes may be observed in healthy reptiles and must be differentiated from erythrocytes deformed during the smearing process (personal observation of authors).⁹

The PCV of most clinically healthy reptiles ranges from 20% to 40%, lower than that of mammals and birds and indicating less oxygen-carrying capacity.^{9–12} Hb concentration is similarly lower (5.5–12 g/dL).² Mean cell volume (MCV) is higher than that of mammals and varies with species. Because of the inverse relationship between erythrocyte number and size, species with higher MCV, such as turtles and snakes, have lower RBC counts than lizards, which have a lower MCV and higher RBC count.^{10,13} The average erythrocyte lifespan ranges from 600 to 800 days in reptiles. This extremely slow turnover of erythrocytes (relative to human erythrocytes, which have a 120-day lifespan) is thought to be associated with the slow metabolic rate of reptiles.^{9,10,13,14}

It is common to find a low percentage (<1%) of polychromatophils in the blood of healthy reptiles, particularly in young animals or animals in ecdysis (periodic skin shedding that can be complete [snakes and some lizards] or partial [chelonians and other species]).³ Unlike polychromatophils in mammals, those in reptiles are smaller than mature erythrocytes and gradually enlarge (rather than decrease in size) during maturation. Reptilian polychromatophils are also rounder and more basophilic and have larger round, oval, or irregular nuclei than mature erythrocytes, with higher nuclear to cytoplasmic (N:C) ratios (**Fig. 2**). The nuclei of immature erythrocytes contain areas of less-densely packed euchromatin, indicating active Hb production. Earlier stages of immature erythrocytes also may be seen in reptile blood, in particular rubricytes, which have darker basophilic cytoplasm, larger round to oval nuclei, and coarser chromatin than polychromatophils. Rubricytes resemble lymphocytes and must be correctly identified during the leukocyte differential count (see **Fig. 2**). Mitotic erythroid precursors also are occasionally observed in the peripheral blood of healthy reptiles, but are more frequently observed in patients with active erythroid regeneration (see **Fig. 2**).

Reticulocyte stains such as new methylene blue can be used to quantify immature erythrocytes, in which residual RNA precipitates to form a distinct ring of basophilic aggregates surrounding the nucleus. Most healthy reptiles have less than 5%

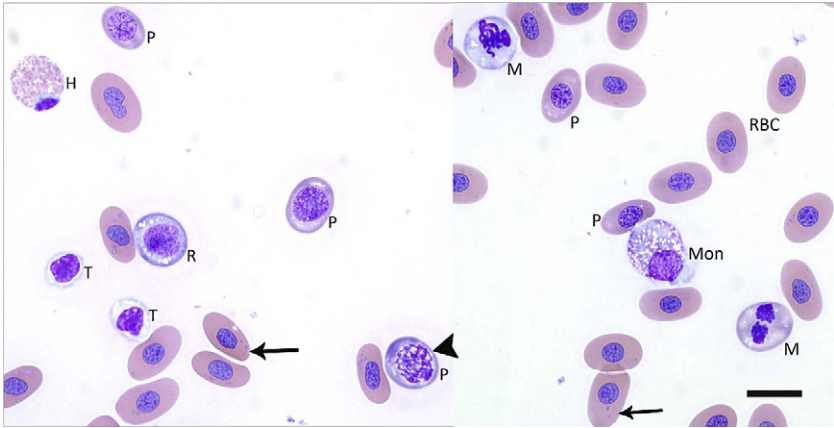


Fig. 2. Peripheral blood from a green sea turtle (*Chelonia mydas*) with anemia (PCV = 12%) and evidence of erythroid regeneration. Mature erythrocytes (RBC) with mild basophilic stippling (arrows). Polychromatophil undergoing mitosis (arrowhead). H, heterophil; M, mitotic figures in erythroid cell line; Mon, reactive monocyte; P, polychromatophils; R, rubriblast; T, thrombocytes. Wright-Giemsa, bar = 10 μ m.

reticulocytes.¹⁵ Absolute reticulocyte counts are not routinely evaluated. Assessment of the degree of polychromasia and quantitation of immature erythroid precursors are critical in determining whether an anemia is regenerative.

A low number of small punctate, basophilic inclusions and/or clear, distinct vacuoles are frequently observed in erythrocytes from healthy Chelonians (turtles and tortoises) and other reptile species.^{3,9} These inclusions have been identified by electron microscopy as degenerated organelles; their clinical significance is unknown, but they must be differentiated from drying artifacts.¹⁶ Similar single basophilic irregular inclusions have been identified ultrastructurally as aggregates of endoplasmic reticulum in erythrocytes of Eastern water dragons.¹⁷ Symmetric, pale; and square, rectangular, or hexagonal inclusions resembling Hb crystals are frequently identified in erythrocytes of healthy iguanas (Fig. 3); the cause and clinical significance are unknown.^{18,19}

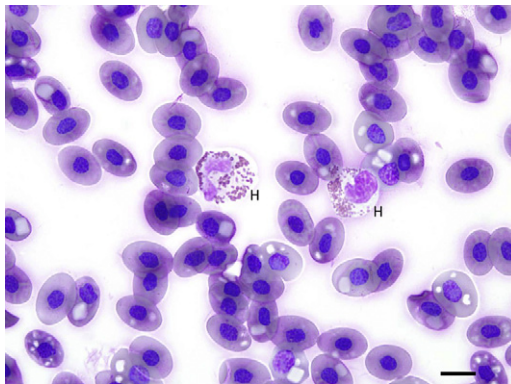


Fig. 3. Peripheral blood from a clinically healthy green iguana (*Iguana iguana*). Erythrocytes contain variably sized, pale rectangular to square cytoplasmic inclusions of unknown origin. H, bilobed heterophils. Wright-Giemsa, bar = 10 μ m.

Leukocytes

Reptilian leukocytes can be classified as granulocytes (heterophils, eosinophils, basophils) and mononuclear cells (lymphocytes, monocytes, azurophils). Leukocytes vary greatly in number and morphology of granules, cytochemical staining patterns, and relative concentration in the peripheral blood depending on species and genera.²⁰ In general, heterophils (named as such because of their prominent bright pink-orange cytoplasmic granules) are the equivalent of mammalian neutrophils, whereas monocytes and lymphocytes of reptiles have similar morphology and function as those of mammals, birds, and fish. Azurophils are unique to reptile species.

Heterophils

Reptilian heterophils are large (10–23 μm) round cells with clear cytoplasm filled with bright pink-orange granules.^{10,21} Crocodylians (alligators and crocodiles) and Chelonians have distinct fusiform granules, whereas Squamata (lizards and snakes) have angular, pleomorphic, and densely packed granules (see **Figs. 1–3**).^{3,22} Heterophil nuclei are eccentric and vary from round to oval (in most snakes, Chelonians, and Crocodylians) to bi- or multilobed (in lizards) (see **Figs. 1–3**).^{21,22}

Heterophils in most species of reptiles compose 30% to 45% of leukocytes in the peripheral blood^{9,10,13,23}; in chelonian and crocodylian species, they account for more than 50%.^{16,22,24,25} Based on cytochemical and ultrastructural studies, heterophils appear similar to mammalian neutrophils, likely functioning to phagocytose bacteria and foreign material. They play a significant role in innate immunity in response to various inflammatory stimuli.^{10,13,16,22,24,26} Toxic heterophils can be observed in reptiles with bacterial infections, severe inflammation, or necrosis; the degree of toxicity reflects the severity of disease. Morphologic findings in mild toxicity include cytoplasmic basophilia and degranulation; severe toxicity is characterized by cytoplasmic vacuolation, aberrant (pleomorphic) cytoplasmic granules, and excessive nuclear lobulation (**Figs. 4 and 5**).^{3,4} As in mammals, quantitative and qualitative assessment of toxicity is important as a prognostic indicator.^{3,4,27} Degranulation without basophilia can be an artifact of inappropriate sample handling, prolonged

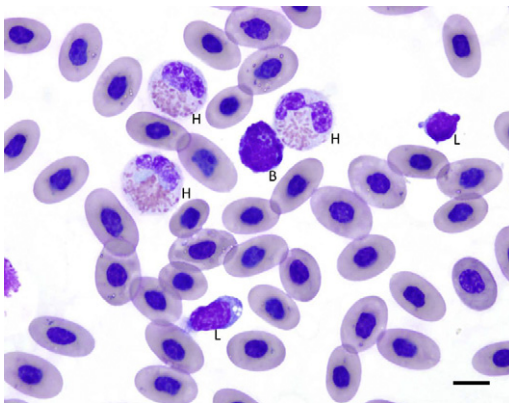


Fig. 4. Peripheral blood from a Chinese dragon (*Physignathus cocincinus*) with multiple subcutaneous abscesses and heterophilia. Heterophils (H) are mildly toxic (degranulation and cytoplasmic basophilia). Erythrocytes are mature and contain small, pale basophilic inclusions consistent with degenerate organelles. B, basophil; L, small lymphocytes. Wright-Giemsa, bar = 10 μm .

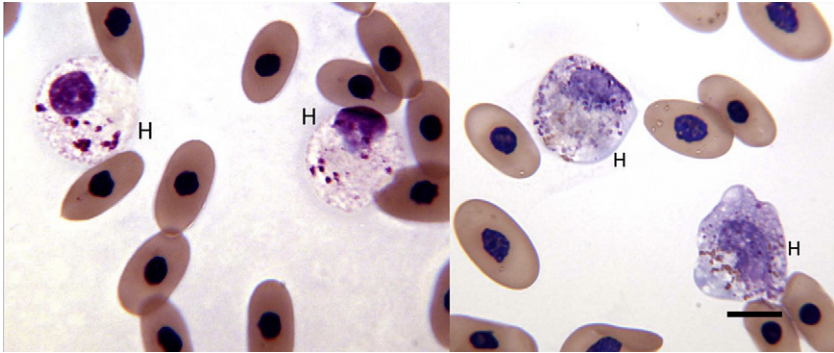


Fig. 5. Peripheral blood from (left) an American crocodile (*Crocodylus acutus*) and (right) a spectacled caiman (*Caiman crocodylus*). Heterophils (H) are severely toxic, with degranulation, indistinct cytoplasmic vacuolation, and abnormal granules. The caiman heterophils also have increased cytoplasmic basophilia and immature nuclei. Wright-Giemsa, bar = 10 μm .

storage, or inappropriate fixation of the blood film.^{3,20,28} As in mammals, the presence of immature heterophils (left shift) is generally associated with inflammation. Compared with mature heterophils, immature heterophils have larger, occasionally pleomorphic nuclei; higher N:C ratios; and increased cytoplasmic basophilia and can contain a low number of small, dark purple primary granules.⁴

Eosinophils

Eosinophil morphology in reptiles is similar to that of mammals. Eosinophils vary from 9 to 20 μm in diameter both between and within species. Eosinophils have a clear cytoplasm and round pink granules. Nuclei are central or eccentric and round, oval, elongated, or bilobed (**Fig. 6**).^{3,22} Eosinophils are absent in most snake species but have been identified in king cobras (*Ophiophagus hannah*).^{20,28,29} Eosinophil granules in iguanas, tegus, and rainbow lizards uniquely stain pale blue-green and are referred to as green eosinophils (see **Fig. 1**).³⁻⁵ The authors have observed immature eosinophils in blood from a box turtle, based on the presence of dark blue primary granules

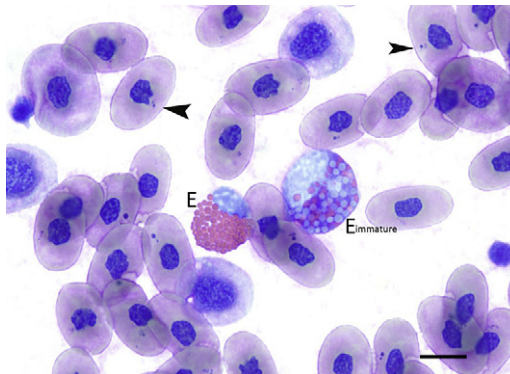


Fig. 6. Peripheral blood from a clinically healthy flowerback box turtle (*Cuora galbinifrons*). A mature eosinophil (E) and an immature eosinophil (E_{immature}). A few of the mature erythrocytes contain small, basophilic inclusions consistent with degenerate organelles (arrowheads). P, polychromatophils. Wright-Giemsa, bar = 10 μm .

admixed with the bright eosinophilic secondary granules (see **Fig. 6**) and by their larger and more pleomorphic nuclei.

Eosinophils compose 7% to 20% of leukocytes in healthy reptiles, with lower percentages in lizards and higher percentages in turtles. Although eosinophil function in reptiles has not been well studied, abnormally high eosinophil numbers have been associated with parasitic infections (eg, protozoa, helminths) and other types of antigenic stimulation.^{3,30} Lower eosinophil percentages in free-ranging nesting leatherback turtles compared with loggerhead and green sea turtles were attributed to differences in diet and parasite load. Only a few helminth species have been found in leatherbacks, and because they mainly prefer jellyfish, the omnivorous loggerheads and green turtles are frequently infected with spirorchids and other parasites.^{31,32} Eosinophils from infected snapping turtles have been reported to be able to phagocytize immune complexes,³⁰ and eosinophils from a healthy young American alligator had phagocytic and microbicidal capacity against *Staphylococcus aureus*.²⁴

Basophils

Basophils in reptiles are usually small cells (7–12 μm) but may reach 20 μm in some species. As in other species, basophils contain numerous small, round, dark purple (metachromatic) granules that frequently obscure the round nucleus (see **Fig. 4**).^{9,22} Basophils with pale purple cytoplasm and clear, distinct vacuoles rather than granules can result from degranulation or lack of metachromatic staining (**Fig. 7**). Basophils of reptiles may degranulate during blood collection, delayed sample processing, or slide preparation. A lack of metachromatic staining of basophils and mast cells has been associated with the use of aqueous stains on blood films and cytologic preparations.³³

The percentage of basophils varies greatly among reptile species.³⁴ Healthy turtles and tortoises have up to 40% basophils,^{10,16} whereas healthy freshwater turtles (eg, Northern red-bellied cooters) have up to 65% basophils.^{10,35–41} The percentage of basophils is reported to increase with certain hemoparasitic (eg, hemogregarines and trypanosomes) and viral (eg, iridovirus) infections.¹⁰ The function of basophils in reptiles is not well understood. Basophils of snapping turtles express surface immunoglobulin and release histamine.^{10,35,42}

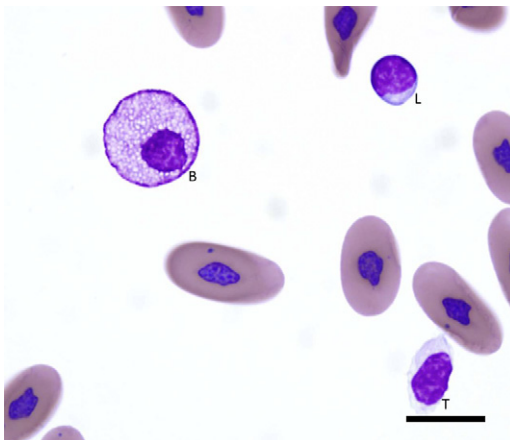


Fig. 7. Peripheral blood from a clinically healthy American alligator (*Alligator mississippiensis*). B, degranulated basophil; L, small lymphocyte; T, thrombocyte. Wright-Giemsa, bar = 10 μm .

Lymphocytes

Reptilian lymphocytes are similar in their morphology to those of mammals and birds and vary in size from 5 to 15 μm (see **Figs. 1, 4 and 7**).¹⁰ It can be challenging to differentiate small lymphocytes from thrombocytes when performing a total WBC count using a hemocytometer or during blood film evaluation (see **Fig. 7**). Large lymphocytes, reactive lymphocytes, and lymphoblasts may be observed occasionally, especially in disease conditions that cause immune stimulation. Plasmacytoid lymphocytes and granular lymphocytes can also be observed during immune stimulation. Plasma cells are rarely observed in the peripheral blood of reptiles with inflammatory or infectious diseases.⁹ Similar to the lymphocytes of birds and mammals, reptilian lymphocytes are categorized as B cells and T cells with corresponding functions, including immunoglobulin production and cell-mediated immune responses, respectively.¹⁰

In most reptile species, lymphocytes are the predominant leukocyte and compose up to 80% of leukocytes.^{10,20,34,43–45} Causes of lymphocytosis include inflammation or infection, wound healing, parasitism (eg, anisakiasis, spirorchidiasis, hematozoa), and viral diseases.³ Lymphopenia can be associated with malnutrition and excess endogenous and exogenous corticosteroids.³

Monocytes

Monocytes in reptiles are variable in size (8–25 μm) and shape (round or oval) and have distinct cytoplasmic borders and abundant pale blue-gray cytoplasm. Nuclei are round, oval, reniform, or multilobed and have smooth to slightly clumped chromatin (see **Fig. 1**).¹⁰ Reactive monocytes can contain cytoplasmic vacuoles (see **Fig. 2**).

Monocytes usually compose 0% to 10% of leukocytes^{10,13}; however, some reptile species have up to 20% monocytes.⁴⁶ Monocytes develop into macrophages after leaving the peripheral blood to enter into tissues. They are essential for granuloma and giant cell formation, a common response to microbial infections in reptiles.⁴⁷ The percentage of monocytes increases during chronic antigenic stimulation, chronic inflammation, and bacterial or parasitic diseases.⁴⁸

Unique to reptiles, circulating monocytes and macrophages that contain melanin pigment (melanomacrophages), nucleoproteinaceous debris, or lipid vacuoles (**Fig. 8**) can be observed, all of which must be differentiated from intracellular organisms.⁴⁷ Erythrophagocytic macrophages can also be found in the peripheral blood. Potential causes include delayed sample processing and immune-mediated, infectious, or neoplastic disease.²⁷ The authors observed marked erythrophagia in an emerald tree boa that had a positive blood culture for *Corynebacterium* sp (**Fig. 9**); the erythrophagocytic macrophages disappeared shortly after the initiation of antimicrobial therapy (Stacy NI, DrMedVet, Alleman AR, DMV, PhD, unpublished data, 2009). Siderophagocytes and erythrophagocytes without anemia were identified in blood films of a corn snake 20 to 79 days after ovariosalpingectomy.⁴⁹

Azurophils

The azurophil is unique to reptile species. Azurophils are commonly observed in squamates and crocodylians, and occasionally in tortoises and turtles, and are morphologically (and possibly functionally) similar to both granulocytes and monocytes.^{5,20,22,28} Azurophils are round cells with distinct cytoplasmic borders and pale blue-gray cytoplasm that contains numerous dustlike azurophilic to purple granules and sometimes a few clear, punctuate vacuoles. Nuclei are usually round or oval, eccentric, and have clumped chromatin (**Fig. 10**).³ Immature azurophils have higher N:C ratios and more pleomorphic nuclei. Cytochemically, azurophils in snakes are similar to mammalian

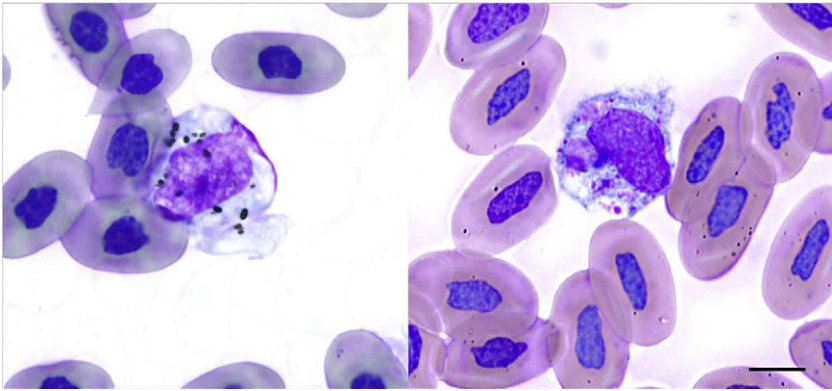


Fig. 8. Macrophages in peripheral blood. (Left) Melanomacrophage in a clinically healthy loggerhead sea turtle (*Caretta caretta*). (Right) Macrophage with intracytoplasmic nucleoproteinaceous debris in a common boa constrictor (*Boa constrictor imperator*). Macrophages are occasionally observed in the blood of clinically normal reptiles. Wright-Giemsa, bar = 10 μ m.

neutrophils (positive for benzidine peroxidase, sudan black B (SBB), and periodic acid-Schiff), whereas azurophils of lizards are similar to mammalian monocytes (positive for acid phosphatase, negative for benzidine peroxidase and SBB).^{15,18,20,22} Therefore, the authors recommend counting azurophils separately in snakes, but grouping them with monocytes in other reptile species. Azurophils are the second most common leukocyte type in snakes and may normally represent up to 35% of circulating leukocytes in some species.^{20,29,45} Increased numbers are frequently

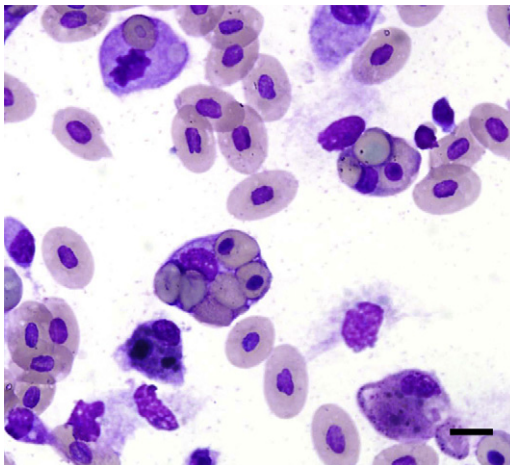


Fig. 9. Peripheral blood from an emerald tree boa (*Corallus caninus*) with positive blood culture for *Corynebacterium* sp. Several monocytes (macrophages) contain phagocytized erythrocytes and greenish black hemosiderin pigment. Cell in the upper left appears mitotic. Wright-Giemsa, bar = 10 μ m.

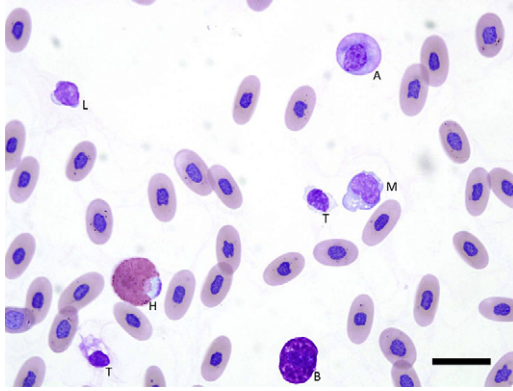


Fig. 10. Peripheral blood from a blood python (*Python brongersmai*) with chronic constipation. A, azurophil; B, basophil; H, heterophil; L, small lymphocyte; M, mildly vacuolated monocyte; T, thrombocytes, and mature erythrocytes. Wright-Giemsa, bar = 10 μm .

associated with inflammatory and infectious (ie, bacterial) diseases, particularly in acute stages.⁵⁰ Azurophils in reptile species other than snakes are found in low percentages, and increased numbers are considered to occur more frequently in chronic disease states, similar to monocytes.

Thrombocytes

Unlike mammalian platelets, which are cytoplasmic fragments of megakaryocytes,²⁷ thrombocytes of reptiles, birds, amphibians, and fish are nucleated and represent a distinct cell line that most likely originates from the thromboplast in hematopoietic tissue, hence their name. Morphologic features of thrombocytes are similar to those of small lymphocytes, and their differentiation may be challenging. Thrombocytes are ellipsoid to oval, are approximately 8 to 16 \times 5 to 9 μm , and have distinct cytoplasmic borders and scant, clear cytoplasm that may contain a few fine, dustlike, pink granules. Nuclei are round to oval, central, and have dense, dark chromatin (see **Figs. 2, 7, and 10**).^{4,10} During blood collection and/or blood film preparation, thrombocytes often become activated or rupture. Activated thrombocytes often clump and can have pseudopods or contain a few cytoplasmic vacuoles (see **Fig. 2**).⁴ When thrombocytes are ruptured, they appear as free nuclei with smooth chromatin. Blood samples of reptiles are usually collected in lithium-heparin, which often causes thrombocytes and possibly leukocytes to clump.⁵ Thrombocyte clumps can be helpful in identifying thrombocyte morphology of a particular species and aid in differentiating them from lymphocytes. Compared with lymphocytes, thrombocytes are slightly smaller; are round, oval, or elliptic; and have distinct cytoplasmic borders and central round or oval nuclei with denser, darker chromatin.

Thrombocytes function similar to mammalian platelets, including involvement in hemostasis and wound healing.¹⁰ Thrombocytes may also have phagocytic capabilities.⁵¹ Activated thrombocytes can phagocytize bacteria, nucleoproteinaceous debris, erythrocytes, hemosiderin, and melanin (personal observation of authors).⁹ Immature thrombocytes are larger than mature cells and have higher N:C ratios and slightly basophilic cytoplasm.

Because thrombocytes frequently clump in heparinized blood samples, hemocytometer counts and blood film estimates can vary greatly and cannot be considered accurate. Thrombocyte numbers can be subjectively assessed by the examiner as

normal, decreased, or increased. When thrombocytopenia is observed, difficult or slow blood withdrawal, delay in sample processing, clotted samples, and laboratory error need to be ruled out. As in thrombocytopenic mammals, there are numerous differentials for thrombocytopenia in reptiles.

INTRINSIC AND EXTRINSIC FACTORS AFFECTING THE HEMOGRAM OF REPTILES

Age, sex, environment, and diet can dramatically affect the reptile hemogram with regard to both cell morphology and cell concentration in the peripheral blood.

Age

Captive adult mugger crocodiles had higher RBC counts and significantly lower percentages of lymphocytes compared with juveniles and subadults.⁵² Other described age-related hemogram changes include higher lymphocyte percentages and lower heterophil percentages in juvenile loggerhead turtles between the ages 1 month to 3 years, compared with adult turtles.⁵³

Sex

Hb and PCV values in captive New Guinea snapping turtles and in free-ranging desert tortoises were significantly higher in males compared with females.^{25,39} However, PCVs in free-ranging juvenile green sea turtles, African pancake tortoises, and Gopher tortoises did not differ significantly based on sex.^{54–56} Both gravid and nongravid female captive green iguanas had higher PCV and mean corpuscular hemoglobin concentration (MCHC) values than did males.¹⁸ Male free-ranging radiated tortoises had higher RBC counts and PCVs than females,⁵⁷ similar to free-ranging desert tortoises, in which significantly higher RBC mass was documented in males than in females throughout the year.²⁵

Higher heterophil counts were observed in adult male captive mugger crocodiles than in adult females.⁵² Females reportedly have higher percentages of lymphocytes than males of the same species and age, under identical environmental conditions.^{10,13,37}

Ambient Environment and Season

Several components of the hemogram can be significantly affected by seasonal variation in temperature and other environmental factors and by hibernation status. Seasonal effects are multifactorial and can be influenced by rainfall, food availability, and temperature extremes.²⁵ Thus, it is difficult to apply broad patterns of changes across species, and any inferences drawn should be limited to a particular species and geographic area.

Reptiles have been reported to have higher RBC counts posthibernation (spring) than prehibernation (fall).^{9,10,13,21} Free-ranging radiated tortoises had higher RBC counts and PCVs in summer than in winter (the hibernation period).⁵⁷ Captive South American rattlesnakes had significantly higher RBC count, PCV, Hb level, MCV, mean corpuscular hemoglobin (MCH), and MCHC and lower total WBC and thrombocyte counts in winter than in summer.⁴⁵ In contrast, a long-term health assessment study of alligator snapping turtles in Georgia and Florida revealed higher PCVs and basophil percentages in summer than in spring.⁵⁸ Gopher tortoises had lower total WBC counts and monocyte percentages in spring than in fall.⁵⁶ Higher heterophil counts¹³ and fewer eosinophils^{9,10,13,59} were observed in summer months than in hibernation periods. Lymphocyte percentages reportedly are lower in animals during ecdysis and winter than during summer months.^{10,11,13,25} Monocyte numbers are not

significantly affected by seasonal factors,^{10,13} although high percentages of monocytes were reported in hibernating desert tortoises and dystocic chameleons.^{25,60} Compared with other leukocytes, seasonal variation in basophil concentration is mild, with fewer basophils in desert tortoises during hibernation and higher numbers during active periods.^{21,25} The percentage of basophils is rather affected by age and geographic region.³⁴

In one study involving a large number of free-ranging desert tortoises, hibernating tortoises had lower lymphocyte and basophil percentages and higher monocyte and azurophil percentages than nonhibernating animals.²⁵ However, there were no significant seasonal, geographic, or sexual differences in total WBC and heterophil counts. In a group of 31 captive viperid snakes, no differences were observed in pre- and posthibernation samples in PCV or total and differential WBC counts.⁶¹

Captive Versus Wild Reptiles

Differences in hemogram results from healthy captive reptiles compared with wild-caught reptiles of the same species have been attributed to ectoparasites and hemoparasites in free-ranging animals and stress and husbandry in captive animals. Higher RBC and lymphocyte counts and lower heterophil and azurophil counts were reported in captive-bred king cobras than in wild-caught king cobras.²⁹ Similarly, estimated total WBC counts were higher and percentage of heterophils was lower in captive bog turtles compared with wild bog turtles.³⁷

Contamination of Blood Samples with Lymph

Many venipuncture sites in reptiles are in close proximity to lymph vessels such that hematologic (and biochemical) values can vary significantly depending on the collection site and potential dilution of the blood sample with extravascular fluid, lymph, or both.⁶² Lymph contamination resulted in a significantly lower PCV and Hb concentration and a significantly higher lymphocyte count in samples from the dorsal coccygeal vein, subcarapacial venipuncture site, or postoccipital venous plexus of chelonian species.^{62–64} When a blood sample from a reptile has a low PCV without evidence of erythroid regeneration and a high number of small lymphocytes, contamination with lymph should be suspected and another sample from a different site should be collected.

DIAGNOSIS AND CAUSES OF ANEMIA IN REPTILES

In addition to an increase in polychromasia and earlier erythroid precursors, erythrocyte morphologic findings associated with regenerative anemia in reptiles include basophilic stippling, binucleation, increased anisocytosis and anisokaryosis, and an increased number of mitotic figures. However, the nuclear changes also can be observed in erythrocytes of reptiles with severe inflammatory disease, malnutrition, or starvation or posthibernation, all of which usually are associated with nonregenerative anemia.^{5,65} Posthibernating reptiles can have a marked regenerative erythroid response with basophilic stippling.⁶⁵ Basophilic stippling also can be observed in reptiles with lead toxicosis.³ An increased number of fusiform or teardrop-shaped erythrocytes has been associated with septicemia or chronic infectious disease (personal observation of authors).⁹ RBC indices may help to characterize the erythroid response to disease, similar to their use in mammals.² A regenerative response in reptiles is typically associated with a decrease in MCV and MCHC.

Given the long life span of erythrocytes in reptiles, the duration and degree of anemia needs to be considered when evaluating the individual patient. Anemic reptiles

with evidence of erythroid regeneration generally have a better prognosis than patients having no or a mild regenerative response. Anemia of chronic disease associated with decreased erythrocyte production (nonregenerative anemia) develops slowly and has been described as the most frequent type of anemia in reptile patients.^{2,66} Commonly reported causes include systemic infectious disease; chronic degenerative or inflammatory diseases of the liver, kidney, spleen, or lungs; gastrointestinal disease; inappropriate husbandry; starvation; and hematopoietic neoplasia.^{2,3,66} Most stranded, debilitated loggerhead turtles have nonregenerative anemia, which probably is multifactorial in origin.³¹

Erythrocytes from reptiles with iron-deficiency anemia often appear hypochromic in blood films and MCH and MCHC are lower. Causes for iron deficiency in reptiles include chronic inflammatory disease, iron-deficient diets, and malabsorption due to gastrointestinal disease.^{2,3} Causes of hemorrhagic anemia in reptiles include trauma, ectoparasite infections (eg, ticks, mites, leeches), coagulopathies, gastrointestinal ulceration, and neoplasia.^{2,3,66} Hemolysis can be associated with bacterial and parasitic infections, such as heavy *Plasmodium* sp infection, drugs, or toxins such as lead and zinc.²

DIAGNOSIS AND CAUSES OF INFLAMMATION IN REPTILES

Heterophilia is frequently associated with inflammatory conditions, including infectious diseases (bacterial, parasitic), tissue injury, and necrosis. Other causes include neoplasia, gravidity, excess exogenous or endogenous glucocorticoids, and, rarely, granulocytic leukemia.^{3,13,67} Acute, overwhelming infections in reptiles may result in heteropenia with a left shift and toxicity.⁴ Severe heteropenia has been associated with fenbendazole administration in Hermann tortoises.⁶⁸

In snakes, increased numbers of azurophils, with or without a left shift, are frequently associated with inflammatory or infectious (ie, bacterial) diseases, particularly in the acute stages.⁵⁰ As with monocytes, increased azurophil percentages in reptile species other than snakes are considered to occur more frequently in chronic disease states.

Inflammation in reptiles often results in granuloma formation, depending on the underlying cause of the lesion.^{22,47} Although heterophils are among the first inflammatory cells involved in inflammatory reactions of reptiles, granulomas form within days, with densely packed necrotic heterophils in the center and monocytes, macrophages, and multinucleated giant cells at the periphery.^{47,69} The presence of lymphocytes and plasma cells may indicate chronicity of the lesion. The reptilian inflammatory response is modulated by a variety of intrinsic and extrinsic factors, with temperature, season, and hormonal effects among the most extensively investigated.^{47,69} The efficacy and duration of the inflammatory response in ectothermic reptiles can be influenced by ambient temperatures, with higher temperatures stimulating the host response and resulting in earlier resolution of inflammatory lesions.⁴⁷ These tissue reactions are typically reflected in the peripheral blood by heterophilia with or without a toxic left shift, monocytosis, and azurophilia. The main cause of leukocytosis in reptiles is infectious disease.^{47,66}

A hallmark of bacterial infection is the presence of a toxic left shift, together with heterophilia or heteropenia. Bacteremia rarely is diagnosed microscopically by observation of intracytoplasmic bacteria within leukocytes in peripheral blood smears. Case reports of bacteremia include the description of a spirilliform bacterium in the peripheral blood and bone marrow of a rhinoceros iguana, *Chlamydia* sp inclusions in peripheral monocytes of flap-necked chameleons, and *Chlamydophila* inclusions in

peripheral monocytes of emerald tree boas with pneumonia (as observed by the authors and confirmed by PCR).^{4,70,71} When bacterial infection is suspected, further diagnostic testing is indicated (eg, blood culture or molecular diagnostics). Serial hemogram evaluations can help to monitor the progress of disease and response to treatment and to establish a prognosis.

VIRAL INFECTIONS IN THE PERIPHERAL BLOOD

Some viral infections of reptiles may be diagnosed by observing characteristic cytoplasmic viral inclusions in blood cells. Viral inclusions in erythrocytes must be differentiated from Hb crystals, drying artifacts, and degenerated organelles. Viral inclusions in leukocytes must be differentiated from phagocytized cellular debris, hemosiderin, and melanin granules.

Inclusion body disease (IBD) of boas and pythons can result in mild to marked lymphocytosis and characteristic intracytoplasmic inclusions in lymphocytes (rarely in thrombocytes and basophils).⁴ The cause of IBD is still unknown; a retrovirus has been suspected to be the causative agent but has yet to be confirmed by future research.⁷² In Romanowsky-stained blood films, IBD inclusions are smooth, homogenous, pale, basophilic structures that often fill the cytoplasm and can displace the nucleus (Fig. 11). All body systems are affected by IBD, but inclusions can mostly be found in the neurons and glial cells of the central nervous system, epithelial cells of the mucosa of the alimentary tract, hepatocytes, renal tubular epithelial cells, and pancreas.⁷² Identification of IBD inclusions in peripheral blood (buffy coat preparations are recommended) can help to confirm a clinical suspicion and establish an ante-mortem diagnosis. If inclusions are absent in peripheral blood from an animal with suspected IBD, histopathologic examination of biopsies of the liver, stomach, or esophageal tonsils is indicated to make a diagnosis.⁷³

Iridoviral inclusions have been reported in blood cells of snakes, lizards, and turtles.^{74–77} Iridoviral infections, formerly termed pirhemocytosis, were correctly identified by using transmission electron microscopy to demonstrate viral particles

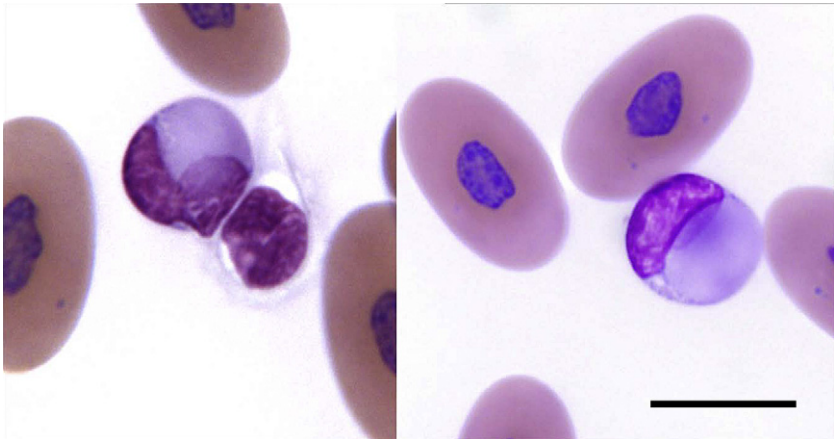


Fig. 11. Peripheral blood from (left) a rainbow boa (*Epicrates senchria senchria*) and (right) a common boa constrictor (*Boa constrictor imperator*) with inclusion body disease. Lymphocytes contain homogenous basophilic inclusions that displace the nucleus. A partially lysed thrombocyte is also seen in the image on the left. Wright-Giemsa, bar = 10 μ m.

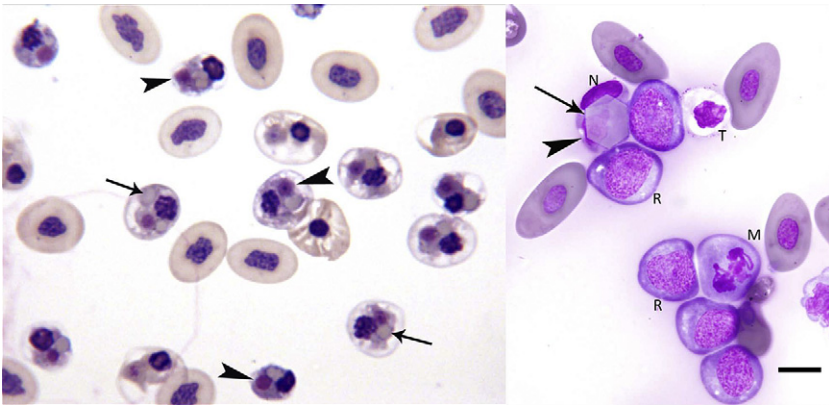


Fig. 12. Peripheral blood from a peninsula ribbon snake (*Thamnophis sauritus sackerii*) (left) and terciopelo (*Bothrops asper*) (right) with SEV infections. The erythrocytes contain crystalline inclusions (arrows) and granular eosinophilic viral inclusions (arrowheads) characteristic of SEV. Nucleus (N) of an erythroid precursor that contains a viral inclusion. M, mitotic figure; R, rubricyte; T, thrombocyte. Wright-Giemsa, bar = 10 μm .

consistent with *Iridoviridae*.^{76,78} Iridoviral inclusions are seen in a variety of target cells, and their morphology varies in different reptile species. Infections have been reported as pathogens in reptiles, but inclusions in circulating erythrocytes have been noted without any apparent adverse effects. In lizards, the virus is termed lizard erythrocyte virus (LEV); inclusions appear in the cytoplasm of erythrocytes as small, punctuate to oval, dark pink amorphous structures, sometimes associated with rectangular albuminoid vacuoles.⁷⁶ Natural LEV infections have not been associated with clinical disease,⁷⁶ whereas experimental infections can induce systemic disease.⁷⁹ In snakes, the virus is termed snake erythrocyte virus (SEV) and inclusions are of 2 types. One type of inclusion is viral in origin and appears as punctuate aggregates of granular pink to dark purple material. The other type of inclusion is pale orange to pink, round to hexagonal, and crystalline and is thought to be composed of cellular and viral byproducts of lipids and proteins (Fig. 12).^{77,80} SEV infection often is associated with severe anemia.^{77,78,80} Iridoviral inclusions (frog virus 3; genus *Ranavirus*) have also been identified in monocytes, azurophils, and heterophils of an eastern box turtle. These cytoplasmic inclusions were 3 to 7 μm in diameter, round to oval, pink, and granular; this viral infection can also cause systemic illness.⁷⁵

Poxviral inclusions were first described in a blood smear from a flap-necked chameleon as pleomorphic, basophilic to purple inclusions within monocytes.⁷¹ Poxvirus infection has been reported in crocodylians, tegu lizards, and tortoises and can cause generalized skin disease with pustular lesions or benign skin tumors.⁸¹

HEMOPARASITES

Most hemoparasites of reptiles are nonpathogenic; they are observed often in the blood of healthy, wild-caught animals. Pathogenic hemoparasites are associated with hemolytic anemia and other clinical disease, particularly when stress is a factor. This section briefly describes the morphology of the most common hemoparasites in reptiles. Detailed information can be found in a recent textbook.⁸²

The term hemogregarine is used to describe a variety of morphologically similar organisms from 4 different genera. They can be found in most reptile species and

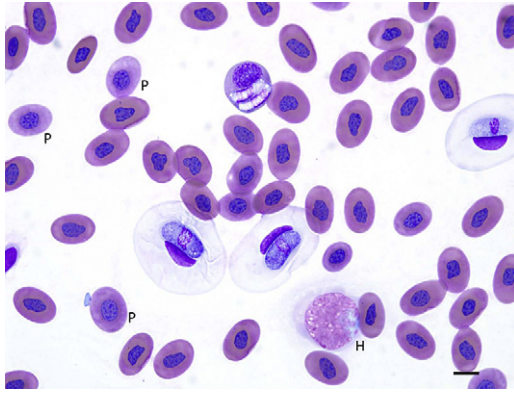


Fig. 13. Peripheral blood from an eastern indigo snake (*Drymarchon corais couperi*) with *Hepatozoon* sp infection. Gametocytes can be seen in 3 highly swollen erythrocytes and 1 rubricyte. H, heterophil; P, polychromatophils. Wright-Giemsa, bar = 10 μ m.

cannot be differentiated based on morphology alone.⁸² Hemogregarine gametocytes are readily identified within the cytoplasm of erythrocytes of infected animals. They are oblong organisms with a pale basophilic cytoplasm and central round to oval nuclei with dark purple chromatin. The organism may displace or wrap itself around the nucleus of the host cell (**Fig. 13**). Hemogregarines are generally considered nonpathogenic but have the ability to provoke a significant inflammatory response in unnatural or aberrant host species.^{29,83,84}

More than 90 species and subspecies of *Plasmodium* have been described in reptiles.⁸² Gametocytes of *Plasmodium* are morphologically similar to those of hemogregarines, with the difference that most malarial parasites typically contain refractile, golden-brown pigment granules (hemozoin). In addition, meronts and trophozoites (small, signet-ring structures) may also be identified in the peripheral blood of infected animals. Most *Plasmodium* spp are nonpathogenic in reptiles, but cases of mild to severe anemia have been reported.^{9,85}

Trypanosomes of reptiles are morphologically similar to those infecting mammals and birds. They are extracellular, flagellate protozoa with a kinetoplast and an undulating membrane. Trypanosome infections have been reported in many reptile species; they generally result in lifelong subclinical infections and rarely cause clinical disease.^{9,85,86}

Microfilarial infections have been described in many reptile species.⁸⁵ Although generally considered subclinical and an incidental finding, heavy infestations may result in clinical disease.^{85,87} Filarid worms are readily identified in blood films of infected animals.

HEMATOPOIETIC NEOPLASIA

As with other chronic diseases, hematopoietic neoplasms are not usually detected in reptiles until an advanced stage of disease has developed.² Diagnosis and differentiation of hematopoietic neoplasia in reptiles is based on the leukocyte differential and morphology (eg, atypical blast cells),^{88,89} bone marrow evaluation, and cytochemical, immunocytochemical, or immunohistochemical staining.^{89,90} Lymphoid malignancies with or without leukemia are among the most commonly described hematopoietic neoplasms in reptiles, particularly in snakes and lizards (**Fig. 14**), and also have

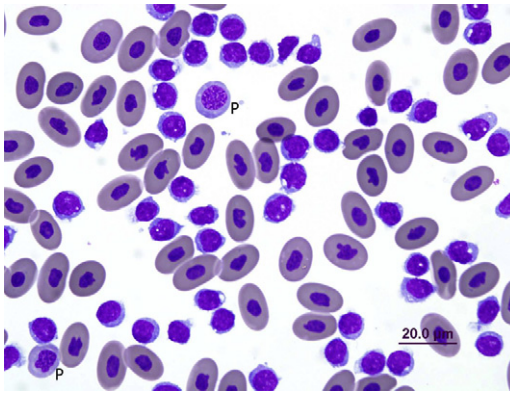


Fig. 14. Peripheral blood from an Asian cobra (*Naja naja kaouthia*) with marked leukocytosis (388,000/ μ L) diagnosed as a chronic lymphocytic leukemia. Neoplastic lymphocytes (L), polychromatophils (P). Lymphocytes were identified as T cell in origin by using immunocytochemistry. Wright-Giemsa, $\times 100$ objective.

been rarely reported in chelonians and crocodylians.^{88,91–94} Reported cases of lymphoid malignancies are sporadic, but a high incidence of multicentric lymphoma was documented in a colony of Egyptian spiny-tailed lizards.⁹⁵ Other hematopoietic neoplasms reported in reptile species include myelogenous leukemia,^{90,96} chronic monocytic leukemia,⁴⁸ other myeloproliferative disorders,^{91,97} and leukemia of undetermined origin in a desert spiny lizard.⁹⁸

SUMMARY

There have been significant advancements in the understanding of reptile hematology in recent years. Much work has been done to identify blood cell types and function in many species of reptiles using cytochemical and ultrastructural methods. Baseline data and reference intervals have been established for many species, and many of the infectious, environmental, and neoplastic processes affecting the hemogram of reptiles have been documented. However, given the vast number of species of reptiles and the increasing recognition of new disease processes using molecular techniques, continued investigations are needed in the future, especially evidence-based studies of disease and associated hematologic abnormalities.

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