

Reptile Clinical Pathology

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Abstract: Hematology and chemistry values of the reptile may be influenced by extrinsic and intrinsic factors. Proper processing of the blood sample is imperative to preserve cell morphology and limit sample artifacts. Identifying the abnormal changes in the hemogram and biochemistries associated with anemia, hemoparasites, septicemias and neoplastic disorders will aid in the prognostic and therapeutic decisions.

Introduction

Evaluating the reptile hemogram is challenging. Extrinsic factors (season, temperature, habitat, diet, disease, stress, venipuncture site) and intrinsic factors (species, gender, age, physiologic status) will affect the hemogram numbers, distribution of the leukocytes and the reptile's response to disease. Certain procedures should be adhered to when drawing and processing the blood sample to preserve cell morphology and limit sample artifact. The goal of this paper is to briefly review reptile red blood cell and white blood cell identification, normal cell morphology and terminology. A detailed explanation of abnormal changes seen in the hemogram and biochemistries in response to anemia, hemoparasites, septicemias and neoplasia will be addressed.

Hematology and Chemistries

Blood collection and preparation

Although it is not the scope of this paper to address sites of blood collection and sample preparation, a few important points need to be explained. For best results to preserve cell morphology and decrease sample artifacts, hematologic testing should be performed as soon as possible following blood collection. Prolonged exposure to any anticoagulant will distort cell morphology and increase sample artifacts such as smudge cells. In general, EDTA is not recommended for use in reptiles because it may cause hemolysis, especially in chelonian blood samples. However, EDTA can be used in most of the lizard species without problems. Heparin has the advantage of being used as an anticoagulant for hematology and plasma for blood chemistry evaluation. However, heparinized samples may result in inadequate staining of the white blood cells, affect cell morphology, or stimulate clumping of the white blood cells and thrombocytes.^{1,2}

Regardless of the anticoagulant of choice, blood smears should be made immediately without exposure to anticoagulants. This preserves cell morphology and helps to prevent clumping of the white blood cells and sample artifact. Also important to remember is to air dry the blood smears with a rapid fanning of the slides. Smears allowed to dry too slowly may result in perinuclear ring artifacts. The technique used to make the blood smear is extremely important. Whether the choice is to use two slides, coverslip and slide, or coverslip to coverslip, if the technique is not perfected, lysis of the white blood cells occurs.

Cell identification

Erythrocytes: Reptile erythrocytes are generally larger than avian and mammalian erythrocytes, but smaller than amphibian erythrocytes. The average life span of the reptile erythrocyte in the peripheral blood is 600-800 days depending on the species. The slow metabolic rate of reptiles and age may play a role in the red blood cell life span. In general the packed cell volume (PCV) and the total erythrocyte count increases with age.

The color of the adult cell is orange and contains a nucleus. Less than one percent of the circulating erythrocytes has a polychromatic color to the cytoplasm and is termed polychromatophilic erythrocytes. This reflects the slower turnover rate of reptilian erythrocytes and the long erythrocyte life span. They are similar in size and shape to the mature erythrocytes. These polychromatic erythrocytes reflect the final stage of maturation and are often termed reticulocytes. However a new methylene blue stain is required to verify the aggregate form of the reticulum encircling the erythrocyte nucleus and classifying these as reticulocytes. An increase of reticulocytes suggests a regenerative response to an anemia. Slight anisocytosis and poikilocytosis are considered normal in the peripheral blood. Immature erythrocytes are round to slightly irregular cells with large round nuclei and basophilic cytoplasm. They are occasionally seen in the peripheral blood of reptiles, especially very young reptiles or those going through ecdysis.

Various terms used in the description of erythrocytes are used as a valuable diagnostic tool.¹⁻³ Hypochromasia reflects an erythrocyte with greater than half of the cytoplasm showing pallor. Microcytic erythrocytes reflect older aging cells, while macrocytic erythrocytes are cells recently released from hematopoietic tissue. Atypical erythrocytes, reflected as anisocytosis or poikilocytosis, may normally be seen in the peripheral blood in small numbers. Immature erythrocytes may vary in size, usually round, with a deeply basophilic cytoplasm and nuclear chromatin blue black in color. An important concept to remember is that all reticulocytes are polychromatic, but all polychromatic cells are not reticulocytes. Bone marrow is the primary site for erythropoiesis, granulopoiesis and thrombopoiesis, with the liver and spleen sites of extramedullary erythropoiesis. The thymus is considered the source of lymphoid cells

Lymphocytes: Typically these cells are small with scant cytoplasm, a high nuclear to cytoplasm ratio, and nuclear chromatin clumping. Lymphocytes lack vacuoles but occasionally have azurophilic granules. Small lymphocytes can be confused with reptile thrombocytes. The function of the lymphocyte is similar to mammals and reflects immune stimulation.

Plasma cells: This cell may occasionally be seen in the peripheral blood smear. They are larger than the normal lymphocyte, have a round nucleus that is eccentrically placed with chromatin clumping. The cytoplasm stains a deep blue and a perinuclear halo (Golgi) is present.

Monocytes: A large cell with a round to amoeboid or lobed nucleus, they have less nuclear chromatin clumping as compared to the lymphocytes and the cytoplasm is abundant with a ground glass appearance. Monocytes have phagocytic capabilities and are active with chronic inflammatory conditions. The reptile monocytic cell has been referred to as monocytes, monocytoid azurophils, azurophilic monocytes or azurophils.

Azurophils: An azurophilic leukocyte, the azurophil causes confusion in classification and identification. The granulopoietic origin of the azurophil has not been documented or confirmed. Many researchers believe this cell should be considered a monocyte and not a separate cell type. They may represent an immature form of the monocyte. The azurophil is slightly smaller than the monocyte, has an eccentrically placed round to oval to bilobed nucleus, basophilic cytoplasm and contains small numbers of cytoplasmic azurophilic granules. Similar to the monocyte, this cell has phagocytic capabilities.

Heterophils: The heterophil is functionally equivalent to the mammalian neutrophil. Often the most abundant cell in the peripheral blood, they have a lobed nucleus, primarily rod shaped granules that are bright orange (eosinophilic) and refractive in appearance. They are highly phagocytic with bactericidal activity. Heterophils also participate in viral and parasitic infections.

Eosinophils: Similar in size to heterophils, this cell has a round to oval (lobed in some lizards) slightly eccentric nucleus with primarily round granules. Although their function is not entirely known, these cells may play a role in reflection of parasitic infections or hypersensitivity reactions. In general lizards have lower number of eosinophils versus turtles.

Basophils: This round cell is often smaller compared with the eosinophil. The granules are round and deeply metachromic in color and often obscure the nucleus, which is nonlobed and eccentrically placed. The cytoplasmic granules contain histamine and their function is similar to the mammalian basophil and mast cell. Basophils produce, store and release histamine. The normal basophil percent ranges from 0-40% (desert tortoise) depending on the species.

Thrombocytes: A small nucleated cell, the thrombocyte is the second most numerous cell in the peripheral blood. They are derived from a mononuclear precursor in the bone marrow, have a high nuclear to cytoplasm ratio and may be difficult to distinguish from the lymphocyte. The cytoplasm is colorless and may contain one or more azurophilic granules. The primary function of the thrombocyte is hemostasis. Normal thrombocyte numbers range between 25-350 thrombocytes per 100 leukocytes in the peripheral blood smear.

Clinical points for chemistry evaluation

A detailed description of each biochemical enzyme evaluated in the reptile laboratory profile will not be presented. However, changes or trends of selected enzymes in response to disease will be discussed.

Total protein will increase with dehydration, chronic disease and lipemia (Table 1). Decreases will be seen with starvation, liver dysfunction, kidney disease, gastrointestinal disorders resulting in maldigestion or malabsorption, and parasitism.⁴ Protein electrophoresis for detailed protein evaluation is often recommended, especially when the globulin fractions are elevated.

Physiologic elevations of calcium may be seen with dehydration, hyperalbuminemia, neoplasia and gravid females. Over supplementation of calcium or vitamin D, hyperparathyroidism, and osteolysis may also cause elevations of calcium. Hypocalcaemia is usually the result of a nutritional imbalance. Elevations of phosphorus may normally be present in the young growing reptile. However dietary excess, hypervitaminosis D, and renal disease may cause phosphorus elevations. Uric acid is a primary catabolic product of protein metabolism and tends to be higher in carnivores versus herbivores. Uric acid is often used to evaluate renal disease in the reptile, however 70% of kidney must be damaged for this value to elevate. Elevations may also be seen with gout, nephrocalcinosis and septicemia. An inverted Ca:P ratio is a reliable indication of renal disease.⁴⁻⁶ Elevations of both phosphorus and potassium are often related to hemolysis of the sample, and may not reflect metabolic abnormalities.

Similar to birds, reptile ALT and AST are not tissue specific. AST may be found in high concentrations in the liver and muscle, including the myocardium. Caution is used when evaluating AST in liver disease. Interpretation of reptile electrolytes follows the same guidelines as other animals. Elevations of sodium are seen with dehydration, while low values may reflect gastrointestinal losses. Elevations of potassium may be seen with dietary excess, acidosis or hemolysis of the blood sample. Hypokalemia is seen with gastrointestinal losses or inadequate intake. Elevations of chloride may be seen with dehydration or renal disease.

Table 1. Reptile hematology guide.

Value	Increase	Decrease
PCV	<ol style="list-style-type: none"> 1. Dehydration 2. Abnormal myeloid production 	<ol style="list-style-type: none"> 1. Regenerative anemia, polychromasia 2. Nonregenerative anemia, depression anemia 3. Hemolytic anemia 4. Hemorrhagic anemia
TP	<ol style="list-style-type: none"> 1. Dehydration (elevated alb) 2. Chronic inflammatory disease (elevated globulins) 	<ol style="list-style-type: none"> 1. Metabolic disorder (liver, kidney, GI) 2. Parasites 3. Nutrition
WBC	<ol style="list-style-type: none"> 1. Chronic inflammatory 2. Bacterial infections 3. Parasites 4. Fungal 	<ol style="list-style-type: none"> 1. Viral 2. Severe chronic infection 3. Overwhelming septicemia 4. Toxic chemicals
Thrombocytes	<ol style="list-style-type: none"> 1. Last cell line to become reactive 2. Reactivity with any disease 3. Phagocytic properties 	<ol style="list-style-type: none"> 1. Decrease production 2. Increase peripheral utilization
Heterophils (+/- toxic)	<ol style="list-style-type: none"> 1. Bacterial 2. Chronic inflammatory disease 3. Parasitic infections 4. Stress 5. Neoplasia 	<ol style="list-style-type: none"> 1. Viral infections 2. Overwhelming septicemia
Lymphocytes (+/- reactive)	<ol style="list-style-type: none"> 1. Viral 2. Parasites 3. Wound healing 4. Ecdysis 5. Immune stimulation 	<ol style="list-style-type: none"> 1. Bacterial 2. Immunosuppression 3. Chronic stress 4. Chronic malnutrition
Monocytes/azurophils	<ol style="list-style-type: none"> 1. Chronic bacterial infection 2. Granulomatous disease 3. Parasites 4. Chronic inflammatory disease 	
Eosinophils	<ol style="list-style-type: none"> 1. Parasites 2. Allergic reaction 3. Immune stimulation 	<ol style="list-style-type: none"> 1. Significance not known
Basophils	<ol style="list-style-type: none"> 1. Respiratory tract disease 2. Parasites 	<ol style="list-style-type: none"> 1. Significance not known

Abnormal Clinical Pathology

Evaluating the reptile hemogram is challenging. Extrinsic factors (season, temperature, habitat, diet, disease, stress, venipuncture site) and intrinsic factors (species, gender, age, physiologic status) will affect the hemogram numbers, distribution of the leukocytes and the reptile's response to disease. Low environmental temperatures may suppress and inhibit the immune response of the reptile. Lymphocyte counts in general are lower during the winter months and higher during the summer. Variation of the heterophil numbers is similar to the lymphocyte seasonal variation. Reptiles have the ability to stimulate a marked leukamoid response to an inflammatory disease. This can be confused with an emerging neoplastic disorder, but will resolve itself with treatment of the underlying disease process.

Blood parasites

Blood parasites are common in the reptile species and are often considered an incidental finding. However, some blood parasites have the potential to cause disease and hemolytic anemia.²

Hemoproteus species: is a protozoan parasite found lizards, turtles and snakes, and resembles the Hemoproteus species found in birds. Hemoproteus is transmitted by blood sucking vectors such as midges, mosquitoes and hippoboscids fly. Clinical signs associated with an infection may include anorexia and lethargy. This parasite will cause dehemoglobinization of the infected erythrocyte. The gametocyte may appear as a developing ring or as an elongated crescent-shaped mature gametocyte encircling the erythrocyte nucleus. The granules in the gametocyte contain refractive yellow to brown pigments representing iron pigment deposition. It is also possible to see extraerythrocytic gametocytes in the peripheral blood.

Plasmodium species: is a protozoan parasite that has the potential to cause severe hemolytic anemia and illness in the reptile. The plasmodium life cycle does require the sporogony stage to develop in the mosquito, while the schizogony and gametogony stages develop in the reptile host. This parasite may also distort the erythrocyte and push the nucleus to one end. The presence of schizonts (round to oval inclusions containing deeply staining merozoites) is diagnostic for the identification of Plasmodium sp.

Trypanosome species: is occasionally found in the peripheral blood of reptiles. Transmission is usually a result of a biting insect such as the mosquito, hippoboscids fly, black flies and mites. The leech appears to be the invertebrate host transmitting Trypanosome in the aquatic and semi aquatic reptiles. These large parasites have an undulating membrane, a slender tapering posterior end and a short anterior directed flagellum. They are considered to be an incidental finding and not considered pathogenic.

Microfilaria: is not an uncommon finding in the peripheral blood. For the most part, microfilaria is not considered pathogenic. Reptiles can survive years with this parasite.

Piroplasmids: of reptiles include Sauroplasma (lizards), Serpentoplasma (snakes), Aegyptianella and Babesia. Transmission of this parasite occurs by biting insects or arthropods. The parasite appears as a small round to piriform, nonpigmented signet-ring like vacuole in the erythrocyte cytoplasm.

Lainsonia and Schellackia species: are coccidian parasites, resembling avian Atoxoplasma, and are found in lizards and snakes. This parasite is a round to oval, pale staining, nonpigmented inclusion that deforms the host cell's nucleus (erythrocyte and lymphocyte) into a crescent shape.

Hemogregarine: parasites are the most common hemoparasite affecting reptiles, except for sea turtles and tortoises (considered rare). Hemogregarine, Hepatozoon and Karyolysus species are the three genera commonly found in the reptiles. However classification of this parasite cannot be accomplished based on the gross appearance of the gametocyte within the erythrocyte cytoplasm, so the general term hemogregarine is used when reporting the presence of this parasite. Mites, ticks, mosquitoes and flies transmit this parasite to terrestrial reptiles, while leeches are the primary intermediate host for transmission to aquatic reptiles. The sausage shaped intracytoplasmic gametocyte in the reptile erythrocyte identifies Hemogregarine, and often distorts the erythrocyte nucleus.

Erythrocytes

Erythrocytic values will be affected by species, gender, age and physiologic status of the reptile, season, temperature, habitat, diet, disease, stress, and venipuncture site. Lymphatic contamination of the blood sample draw often occurs in the reptile and will affect the hemogram by decreasing the hematocrit, hemoglobin concentration, total red and white blood cell count. In general, the erythrocytic indices are at their highest prior to hibernation and lowest immediately following hibernation.

Hypochromasia: greater than 2+ may be a result of nutritional deficiencies leading to iron deficiencies, lead toxicity or chronic inflammatory disease

Anisocytosis: greater than 1+ with an increase of polychromasia suggest a regenerative anemia

Poikilocytosis: greater than 1+ is associated with erythrocytic regeneration

(anisocytosis and poikilocytosis may also be found in the post hibernation reptile or those with severe inflammatory disease)

Basophilic stippling suggests a regenerative response, but may also occur in reptiles with iron deficiency or less commonly lead toxicity.

Peri-nuclear rings or clear irregular refractile spaces in the cytoplasm are artifacts in the erythrocyte as a result of the blood smear drying too slowly.^{2,5} Do not confuse these with gametocytes of Hemoproteus or Plasmodium. Round to irregular basophilic inclusions (clumping endoplasmic reticulum) are frequently seen in the erythrocyte cytoplasm and are considered artifact of slide preparation.

Anemia

The normal packed cell volume (PCV) ranges from 15-55%. Values above this range with an elevated total protein suggest dehydration or erythrocytosis (polycythemia) with a normal or low total protein. Values below this range suggest anemia if hemodilution with lymph is not a factor. Anemia is classified according to pathophysiology and termed hemolytic, hemorrhagic or hypoplastic (depression anemia). Polychromasia is usually not graded as it is in the avian species, due to the fact that polychromasia is less than 1% of the total erythrocyte population. The presence of increased polychromatic erythrocytes and reticulocytes is evidence of active erythropoiesis. The presence of rubriblasts indicates a marked erythropoietic response to anemia.

Hemorrhagic anemia: is typically a result of traumatic injuries. Blood sucking parasites and conditions such as coagulopathies or ulcerative lesions should also be considered.

Hemolytic anemia: is often due to systemic or hematogenous bacterial infections (sepsis), toxins (lead, zinc, aflatoxins, petroleum products and certain plants), hemoparasites (plasmodium), or infectious diseases. This usu-

ally presents with a strong response to the anemia with small round erythrocytes (spherocytes) and biliverdinuria due to extravascular hemolysis.

Hypoplastic anemia: is a non-regenerative, normocytic, normochromic anemia. Often termed depression anemia, this is the most common anemia seen in the reptilian species. There is a decrease of erythropoiesis due to chronic inflammation or infection, drug reaction, renal or hepatic disease and neoplasia.

Leukocytes

Leukocyte parameters are affected by many factors and at times may be difficult to properly interpret. As mentioned previously, intrinsic and extrinsic factors affect the reptile's response to disease.

Heterophils become active and toxic with systemic illness. The severity of the systemic illness affects the degree of toxic changes present and the number of immature heterophils released into the peripheral blood. The toxic changes seen in the heterophil include an increase of cytoplasmic basophilia, degranulation, abnormal granulation (rounding), and vacuolization. The immature heterophils consist of bands, metamyelocytes and myelocytes. Significant increases of the heterophils are associated with inflammatory diseases, stress and neoplasia.

Lymphocytes show reactivity by developing a basophilic cytoplasm or forming scalloped edges. Inclusion Body Disease (IBD) viral inclusions can be seen in the lymphocyte and confirmed with H&E stain.

Plasma cells distended with clear to light blue round structures are termed Mott cells. The round structures are termed Russell bodies, which contain immunoglobulins.

Monocytes/ azurophils become larger with exaggerated foamy cytoplasm. Cytoplasmic vacuoles and blebbing may occur with reactivity. A monocytosis suggests chronic or granulomatous inflammation. Monocytes have phagocytic capabilities and often engulf leukocytes and erythrocytes in response to anemia and infectious disease. They also actively engulf bacteria in a sepsis condition.

Eosinophils increase with parasitic infections and stimulation of the immune system.

Basophils increase with parasitic and viral infections, wounds and respiratory disease.

Thrombocytes play an active role in the response to disease. The presence of an increased number of immature thrombocytes is a regenerative response to excessive utilization. A decrease of thrombocytes suggests decrease bone marrow production or excessive peripheral utilization. This may be due to septicemia or disseminated intravascular coagulation (DIC). Activated thrombocytes will appear as aggregated clusters of cells.

Total protein

Most plasma proteins are synthesized in the liver. Exceptions are the immunoglobulins produced by the lymphocytes and plasma cells. Their biological activity is dependent upon their primary and secondary structure (enzymes, hormones, transport and carrier compounds). Hypoproteinemia may be a result of chronic hepatopathies, malabsorption, protein-losing enteropathies, renal disease, blood loss, malignant tumors, starvation, and malnutrition. Hyperproteinemia may result from chronic infectious disease and overproduction of gamma globulins, lymphoproliferative disease or dehydration.

Leukocyte response to disease

Reptiles with a severe inflammatory disease (usually bacterial etiology) develop a marked leukocytosis with the presence of immature heterophils (bands, metaheterophils, myeloheterophils, progranulocytes).^{1-3,5}

Lymphocytosis may occur with wound healing, parasitic infections, viral infections and inflammatory disease. A lymphocytosis may also be present during ecdysis. The presence of reactive lymphocytes and plasma cells reflects stimulation of the immune system. Leukopenia reflects the consumption or decreased production of the peripheral leukocytes. The presence of an absolute heteropenia in conjunction with immature heterophils and toxicity represents overwhelming demand for heterophils in the periphery due to bacterial sepsis or viral disease. This is a degenerative response and carries a grave prognosis. Leukopenia with heteropenia, anemia and thrombocytopenia suggest injury to the bone marrow.

Heteropenia may suggest acute inflammatory disease, degenerative response to disease, stem cell injury or bone marrow toxicity (chemicals, drugs, viral). A heteropenia with no left shift, no anemia and adequate thrombocytes may suggest an acute viral infection or acute marrow toxicity.

Lymphopenia may occur secondary to diseases that cause immunosuppression, chronic malnutrition and chronic stress.

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