Abstract

Anemia is a relatively common blood disorder but a seemingly underreported and investigated condition in reptiles. Better characterization of anemia in the sick reptile could help determine which available diagnostic tools could be best utilized to determine the cause of low red blood cell counts in these animals. There are, however, a number of limitations that exist when trying to interpret hematologic data for the large number of reptilian species presented to the exotic animal practitioner, including interspecific and intraspecific variation in hematological reference values, limited availability to specific reagents, and a lack of scientific-based studies that focus on anemic conditions in reptiles. Therefore, better documentation and consistency in the scientific-based studies of reptile anemia and rapid dissemination of this information are considered necessary to increase our knowledge in this area of reptile medicine. Collaborative research efforts between practitioners, owners, reptile collection curators, pathologists, immunologists, microbiologists, toxicologists, and laboratory scientists are urgently needed to augment our understanding of anemia and other hematological disorders in reptiles.

Key words: anemia; blood; erythrocytes; hemoglobin; reptiles; treatment

Hematology is the medical science that studies blood in health and disease. As such, the study of hematology has a deep impact on our understanding of multiple diseases. Without a doubt, one of the most useful interpretations of hematology in human and veterinary medicine is the change in the number and morphology of erythrocytes that occur because of pathological conditions. Among these, one of the most commonly observed pathological conditions is anemia.

The word anemia is derived from the Greek word *anæmia*, which means “without (an) blood (aemia).” It can be defined as a reduction in the number of red blood cells (RBC) or erythrocytes per volume of blood, with the subsequent reduction in oxygen delivery to the tissues by hemoglobin (Hb). Ultimately, anemia should be defined as a qualitative or quantitative deficiency in circulating Hb. Anemia can be caused by a primary blood disorder (e.g., aplasia, hypoplasia) or, more frequently, a secondary complication to other diseases. In the latter case, anemia rarely constitutes a diagnosis but is rather a clinical sign of some other pathological condition or illness.

Hb concentration and hematological indexes are invaluable tools to investigate and diagnose reptile hematologic maladies; however, thorough investigation of the anemic patient appears to be rarely conducted in reptile medicine. Identification of anemia in a lizard, snake, crocodile, or chelonian should challenge the veterinary practitioner to perform a comprehensive investigation with the aim to deter-
mine the underlying cause of the condition even in the absence of other clinical signs. Furthermore, early recognition of anemia in a reptile patient during annual or biannual examination helps to identify diseases before they progress into life-threatening disorders and allows the practitioner to implement a specific treatment regimen, thereby helping one establish a prognosis and monitor response to therapy.1-8

More than 400 causes of anemia have been reported in humans, with a smaller, although still significant, number of causes recognized in small and large animals.9,10 It is very likely that there are several causes for anemia in reptiles too, even though these remain unidentified or not clearly defined. Because there are more than 8000 species of reptiles described worldwide, it is unlikely that a detailed knowledge of the erythron and its response to disease for all these species will ever be achieved. However, for the continued development of reptile medicine and hematology, a more exhaustive investigation of the anemic patient, including its causes and associated changes in the erythron, is needed. Exotic animal veterinarians may contribute to this better understanding by reporting systematic investigation and test results from their anemic reptile patients.

The method of blood collection and the use of an appropriate sampling technique are critical steps to the proper interpretation of the reptile hemogram, especially during the investigation of an anemic patient. A basic understanding of reptile anatomy, with emphasis on the topographic anatomy of venipuncture sites, is necessary for collecting blood and reducing the discomfort that the procedure may cause in the patient. Excellent and detailed reviews on these topics have been recently published.4,5,8-14

Blood samples can be collected from multiple locations on a reptile patient. (Figs 1-4).4,5,8,11-14 Choosing the best site for blood collection will be determined by taxonomy, species anatomic charac-

Figure 1. Blood collection from the cervical sinus of a tortoise. (Geochelone sp.) Photo courtesy of Dr. Carolina Torta.

Figure 2. Collecting blood from the subcarapacial vein of an Argentine desert tortoise (Geochelone chilensis). Photo courtesy of Dr. Guillermo Perez Jimeno.

Figure 3. Blood collection from the ventral tail vein of a python (Python sp.). Photo courtesy of Drs. Paula Moreno and Angelica Rojas.

Overview of Blood Collection in Reptiles

The method of blood collection and the use of an appropriate sampling technique are critical steps to the proper interpretation of the reptile hemogram, especially during the investigation of an anemic patient. A basic understanding of reptile anatomy, with emphasis on the topographic anatomy of venipuncture sites, is necessary for collecting blood and reducing the discomfort that the procedure may cause in the patient. Excellent and detailed reviews on these topics have been recently published.4,5,8,11-14

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teristics, body condition, preference and experience of the phlebotomist, size of the patient, and amount of blood required. Selection of an adequate blood sampling site is also essential if a significant volume needs to be collected.4,5,8,11 Peripheral blood vessels should be the first option for reptile blood collection, given that these sites are relatively more accessible and usually adverse to developing secondary complications. The brachial and jugular veins (e.g., lizards, chelonians), ventral tail vein (e.g., lizards, snakes, chelonians, crocodilians), and ventral abdominal vein (e.g., lizards) are readily accessible sites for most species of reptiles.4,5,12,13,15 In ophidians, given that these peripheral sites are not available with the exception of the ventral tail vein, cardiocentesis is usually recommended.4,5,12,16 It is a relatively simple and safe procedure that allows for the collection of a relatively large volume of blood. Another potential venipuncture site in ophidians is the palatine veins.4,5,12,16 In crocodilians, the ventral coccygeal veins and supravertebral vessels are safe and recommended when significant quantities of blood are needed.12,17 In chelonians, the jugular vein is the preferred vessel because there is less risk of collecting lymph-diluted blood as compared with the other peripheral sites.4,5,18 Additional venipuncture sites in chelonians are the postoccipital venous plexus, femoral and coccygeal veins, and the subcarapacial sinus.4,5,12,18

For most reptiles, the total volume of blood collected should not be >1% (in grams) of body weight.3,5,8,11 Usually, a blood volume equivalent to 0.5% to 0.8% of the body weight is enough to provide a diagnostically valuable sample. Ideally, the amount of blood obtained should allow the storage of plasma or serum if additional testing (e.g., serology, plasma protein electrophoresis) is needed at a later date. Large volumes of blood, plasma, and/or serum can be saved at −80°C. In clinically ill patients or those suspected of being severely ill, a smaller volume of blood should be collected (e.g., 0.5 mL is usually enough to run a complete blood count including a plasma biochemistry panel). When investigating the progression of anemia using hematocrit (Ht) percentage and the RBC count, only small volumes of blood should be taken to avoid unnecessary and excessive blood loss in the patient.

Veterinary clinicians must remember that reptiles have very well-developed lymphatic vessels that usually run in close proximity to the main vessels used to collect blood.3,5,12,14,15 As a result, contamination of the blood sample with lymph is a very common complication, especially in chelonians. Lymph contamination causes hemodilution, which can significantly alter the results and diagnostic value of a blood sample.3,5,12,14,15 Recognition of lymph contamination is relatively simple if careful attention is used when collecting the sample. When entering a vessel with the needle, blood will slowly fill the syringe. However, when a lymphatic vessel is entered, a clear, pale yellow or whitish liquid or a change in the color of the previously collected blood (e.g., suddenly becoming more dilute) will be observed in the sample when lymph is being collected instead of blood. While the blood is being collected, careful observation of the syringe during blood draws will help to detect this contamination and consequent change in color.4,5 When lymph contamination is detected, the sample should be discarded and a new one obtained from a different anatomic location.

**Preloading the Anticoagulant in the Syringe**

Anticoagulants are routinely used for holding and storing reptile blood samples. Blood that has been altered with an anticoagulant will allow for the evaluation of blood cells or plasma components from the same sample. Lithium heparin is the recommended anticoagulant for preloading a syringe to collect a blood sample from a reptile.4,5,12,19,20 However, other authors report the use of sodium ethylenediamine tetraacetic acid (EDTA) as a better option for preloading syringes when collecting blood from lizards and snakes.17,21 In chelonians, heparin may cause hemolysis.20 As in birds, the use of EDTA may impair blood cell staining and cause changes in the coloration of erythrocytes and hemolysis.4,5 Nevertheless, blood smears are better prepared when blood is collected without the addition of anticoagulant in the collection syringe. Collecting a

**Figure 4.** Blood collection from the ventral tail vein of a spiny-tailed lizard (*Uromastyx aegyptius*). Photo courtesy Dr. Jaime Samour.
viable blood sample without the use of an anticoagulant may be difficult to achieve in small reptiles, which offer limited blood volumes.

When blood is collected without the use of an anticoagulant, it should be quickly placed in tubes containing anticoagulant to prevent clotting. The use of 0.25 to 0.5 mL Microtainer blood tubes (BD Vacutainer Systems; Beckton, Dickinson and Co., Franklin Lakes, NJ USA) is recommended. Microtainer blood tubes allow for easy and fast mixing of blood. Given that reptile erythrocytes are relatively fragile, it is advisable to remove the needle hub from the syringe when transferring blood to the tube or making the blood film to avoid hemolysis. Once the blood film has been made, they can be stained with the same methods used for birds, including Wright-Giemsa, May-Grünwald-Giemsa, or Diff Quick. Both slide-to-slide and coverslide-to-coverslide techniques are recommended when preparing a blood smear.

The quality of the blood sample is always important for precise examination and diagnosis. Presence of clots, hemolysis, excessive anticoagulant, and contamination with lymph will impede the collection of blood samples and alter diagnostic values. It has been stated that a laboratory test result is only as good as the specimen collected. In another words, extreme caution should be used to prevent sending the laboratory a blood sample for analysis that will confer misleading results. Special precautions should be taken when manipulating reptiles for venipuncture. They can harbor several infectious agents that are zoonotic or potentially zoonotic. Blood samples should be treated as a potential source of blood-borne pathogens for the personnel as well as for other animals. Veterinarians are responsible for enforcing standard biosafety precautions when working with reptiles, especially when collecting blood samples from the diseased patient. Use of gloves, goggles, and/or a mask should always be recommended when working with reptiles that present with clinical signs of disease.

**Hematologic Reference Values**

Although published reference values are available for a number of reptile species, it is important to remember that both intrinsic (e.g., species, gender, age, physiological status, breeding season, hibernation) and extrinsic (e.g., season, temperature, environmental conditions, husbandry, diet, living conditions) factors may cause significant variations in hematological parameters. Thus, when referring to a set of references values, it is important to determine the conditions under which the blood samples were obtained. Understanding and recognizing these possible variations in reptile hematology results will contribute to a judicious interpretation of published reference values and, at the same time, should motivate researchers and clinicians to investigate these differences, obtain references values adjusted to these variations, and demonstrate their clinical significance. Ideally, at least 100 individuals from the same species, age, and sex, housed under similar conditions and fed a diet that is close in nutritional composition, should be used when trying to determine normal blood reference values. Packed cell volume (PCV), RBC counts, and hematological indices vary between species and within individuals, making the use of published reference values of limited value in most cases. Ultimately, comparison of blood reference values in a patient with compiled data from multiple sources that either do not identify the methodology used to collect and test the blood sample or define the population from which the sample is collected is not recommended. In fact, a better approach to evaluating blood work values between sick and healthy patients is to obtain in situ reference values for the individual through investigation of normal blood values for the same patient by collecting and analyzing the samples at least two times in the year. This approach can provide more valuable information regarding what is within the normal range of healthy individuals rather than a single value obtained from a diseased patient. For example, a reptile patient could experience a significant drop in its PCV (e.g., from 45% to 25%), which suggests there is an abnormality, but if the PCV values from the animal were captured by the reference range for that particular species it may be misclassified as being normal. The scenario described above reinforces the need to compare blood reference values obtained from the same patient or from a clearly defined reptile population. In cases of severe anemia when the PCV values fall well below the published reference range, this comparison is not needed.

**Evaluation of the Reptilian Erythron**

Evaluation of the reptilian erythron includes the determination of the PCV or Ht, total RBC (TRBC) count, Hb concentration, and hematological indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Calculation of these indices contributes to effectively measuring the size of erythrocytes and calculating the content of Hb within cells. The detailed examination of these parameters is necessary when diagnosing, investigat-
ing, and characterizing the anemic conditions that affect reptile species.

The TRBC count can be determined by using a manual counter and the Natt Herrick or Unopette techniques. Both methods rely in the dilution of the blood sample and further manual count of cells through the use of a counting chamber. After subsequent adjustment to the dilution factor, the total number of cells/milliliter in blood are calculated. This can be easily performed in a veterinary clinic without the need for expensive equipment. Other methodologies used to obtain a reptile complete blood count include the use of flow cytometry or automatic counters. Although equipment to perform automated complete blood counts on reptile species is expensive and can be purchased, it is not available through most veterinary diagnostic laboratories.

Reptiles usually have a lower number of erythrocytes per volume of blood than mammals or birds. An inverse relationship between size and number of circulating erythrocytes apparently exists. Those species with smaller erythrocytes tend to have a larger number of this cell type in their blood. The PCV or Ht measures the percentage of RBCs in the blood. Reference PCV values in reptiles range from 20% to 45%, whereas some authors report a wider range of values (e.g., 15%-50%).

Hb concentration is measured with a solution of potassium cyanide and potassium ferricyanide. A specific volume of blood is combined with cyanide forming cyanmethemoglobin, which is read using light photometry. Reported Hb concentration values for reptiles range from 5.5 to 12 g/dL. The above-mentioned parameters, together with the hematological indices described below, are necessary to effectively investigate RBC function. Hematological indices allow accurate measurements of erythrocyte size and Hb concentration. When combined, these parameters and indices provide important information that contributes to a better diagnosis, identification of the etiology, and characterization of anemia.

The hematological indices are:

**MCV (fL)** correlates to the average volume of erythrocytes. It can be calculated with the following formula: Ht (%) × 10/RBC count (× 10^{12}/L). MCV values range from 200 to 1200 fl.

**MCH** measures the average weight of Hb in the erythrocyte. The MCH can be calculated as follows: Hb (g/dL) × 10/RBC count (× 10^{12}/L). MCH values range from 6 to 10 g/dL.

**MCHC** expresses the average concentration of Hb in the erythrocyte. It is calculated as: Hb (g/dL) × 100/Hct (%). MCHC values reported for reptiles range from 22% to 41%.

Anemia

Reptile patients with anemia have a decreased oxygen-carrying capacity. This may occur because of a reduction in the number of circulating erythrocytes or a decrease in the concentration of Hb within the erythrocytes. Anemias may be classified as: 1) regenerative, when the bone marrow maintains its erythropoietic function (e.g., hemorrhage, hemolysis) and the patient is capable of producing and releasing newly formed erythrocytes, and 2) nonregenerative, where no new RBCs are formed or released from the hematopoietic sites.

**Regenerative Anemia**

Regenerative anemias are usually caused by hemorrhage and hemolysis. Hemorrhagic anemia in reptiles occurs because of blood loss after trauma and surgery, hemathophagous external parasites, coagulation disorders, gastrointestinal ulcers, and neoplasia. Anemia is a common finding in reptiles with heavy ectoparasite infestations. Ticks from the family...
lies Ixodidae and Argasidae are commonly identified as the underlying cause of anemia in reptile cases in which the arthropods are present (Fig 6).39,40 The introduction of new or recently acquired reptiles in an otherwise well-managed reptiliary could cause outbreaks of tick infestations and anemia in previously healthy individuals. Ophionyssus natricis, the snake mite, is a common cause of anemia in captive snakes. Juvenile animals are especially susceptible to developing anemia from these mite infestations. Leeches may cause significant blood loss in aquatic reptiles and are often a difficult problem to diagnose if the parasites are not observed while attached to their victims.

In chelonians, traumatic wounds caused by fighting, dog bites, and free falling from balconies will result in anemia due to internal or external blood loss (Fig 7). Gastric ulcers or trauma related to the ingestion of a foreign body (e.g., ingesting a fishhook) are other possible causes of internal hemorrhage in reptiles.42 Hemorrhagic conditions are rare in ophidians, although internal hemorrhage caused by the ingestion of rodents exposed to anticoagulant rodenticides may occur in free-ranging snakes.43 Coagulation disorders caused by thrombocytopenia have been described in reptile species and are often the result of decreased production, accelerated use, or destruction of thrombocytes.4,5,8

Hemolytic anemia occurs when erythrocytes are destroyed within the blood vessels (e.g., intravascular hemolysis) or outside the lumen of the vessel (e.g., extravascular hemolysis). Hemolytic anemia can be congenital (e.g., inherited hemolytic anemia) or acquired. An anemia can also be classified based on the mechanism of damage to the plasmatic membrane of the erythrocyte. This can be an immune-mediated response (e.g., antibodies) or the result of an infectious agent, drug, or toxin on the erythrocyte membrane. Inherited hemolytic anemia has not been reported in reptiles to the author’s knowledge.

The most common cause of acquired hemolytic anemia in reptiles is associated with severe infestation by the malarial hemoparasites (Plasmodium).39,40,44,45 Haemoproteus and Sarcocystis are two additional genera of hemoparasites that can also cause anemia in reptiles (Fig 8).4,45 Special considerations should be taken to identify and differentiate these parasites from other nonpathogenic hemoparasites found in reptile blood.

Idiosyncratic drug-induced hemolysis can arise theoretically through the administration of any medication prescribed for reptiles. Drug-induced hemolysis is more likely to occur with drugs well recog-
nized for causing anemic conditions (e.g., nonsteroidal antiinflammatory drugs, antifungal agents, sulfonamide antibiotics). Heavy-metal toxicity, especially lead and zinc toxicosis, can induce a hemolytic anemia, in addition to other gastrointestinal and neurological clinical signs. Lead toxicosis may result from the ingestion of tainted paint, fishing sinkers, spent lead ammunition, or from other environmental sources. Zinc toxicosis has been associated with overdosing zinc supplements, ingestion of pennies coined after 1982, thermometer weights (Fig 9), and zinc-containing ointments. Use of anticoagulants containing calcium EDTA has been reported to cause hemolysis in some species of chelonians. Inappropriate venipuncture techniques, such as excessive negative pressure, prolonged storage, and manipulation of the sample, are common causes of iatrogenic hemolysis.

**Nonregenerative Anemia**

Nonregenerative anemia is the most common manifestation of anemia in reptiles, although it is rarely fully investigated and, as such, rarely reported. In reptiles, the development of a nonregenerative anemia is a slow process, which is likely because of the idiosyncrasy of many chronic diseases and the long half-life of the reptilian erythrocyte.

The most common causes of nonregenerative anemia described in domestic animals and other exotic pets have been identified in reptiles. Neoplasia, systemic infectious diseases, and any moderate to severe chronic degenerative or inflammatory, systemic, or localized disorder that affects the liver, kidney, spleen, or lungs may be accompanied by a nonregenerative anemia typical of chronic diseases.

Infectious diseases should always be considered as one of the top differential diagnoses and are highly prevalent in captive reptiles. Systemic chronic infectious diseases such as mycobacteriosis, mycoplasmosis, chlamydioides, salmonellosis, herpesvirosis, iridovirosis, coccidiomycosis, and aspergillosis are commonly associated with a nonregenerative anemia when disseminated disease is present, or when the liver, kidney, spleen, bone marrow, and/or lungs are seriously compromised.

Renal disorders, including nephritis, nephrosis, amyloidosis, and nephrocalcinosis, have been reported in reptiles. All of the above diseases can cause a nonregenerative form of anemia. Either inflammatory or degenerative disease conditions can adversely affect liver and renal health, ultimately resulting in the development of nonregenerative anemia (Fig 10). Hepatic lipidosis is a common liver disorder in captive lizards, and many infectious agents have been reported as underlying causes of hepatitis. Tumors, toxins, and other liver disorders are rare but can also cause anemia (Fig 11).

Internal hemorrhage caused by the rupture of blood vessels in highly vascularized or ulcerative tumors may cause a regenerative anemia, but most neoplastic conditions are accompanied by nonregenerative forms of anemia. Chronic pneumonia caused by bacterial, viral, or mycotic infections are common in lizards, snakes, and chelonians, and should be investigated in the anemic patient. Gastrointestinal disorders associated with parasitic infestation, chronic diarrhea, and inflammation can result in a nonregenerative anemic condition.

Other causes of nonregenerative anemia observed in birds and other domestic animals may apply to the differential list of the anemic reptile, including hy-
perestrogenism, leukemia, nutritional deficiencies, stomatitis, chronic gastritis, starvation, hypovitaminosis A, urinary calculi, amyloidosis, gout, metabolic bone disease, chronic stress, and hypothyroidism. Chemotherapy for cancer and the use of certain antibiotics (e.g., chloramphenicol) may induce myelosuppression and nonregenerative anemia. Primary causes of bone marrow suppression that have been reported in mammals and birds may also occur in reptiles.

Most nonregenerative anemias will be of the normocytic normochromic type, indicating that hematological indices will be within reference ranges for the species being considered. Hypochromic anemia in reptiles is rare and is classically associated with ferropenic anemia. The development of ferropenic anemia has been associated with excessive iron loss due to sequestration by bacteria, iron-deficient diets, or incomplete iron absorption as seen in malabsorption syndromes and chronic diarrhea. Rarely, a localized infection will manifest with hypochromic anemia. The primary disorders in iron metabolism that are associated with nonregenerative hypochromic anemia in other classes of vertebrates have not been reported in reptiles. Mild anemia with hypochromia is also observed in reptiles coming out of brumation (Fig 12).

Clinical Presentation of Anemia in Reptiles

Clinical signs of anemia can be divided into two main groups: those associated with a reduction in the delivery of oxygen to the tissues and those that are associated with the primary cause of the anemic condition. Only those associated with a reduction in the delivery of oxygen to the tissues are briefly discussed below. A complete description of the clinical signs typically associated with most reptilian diseases that result in secondary anemia is beyond the scope of this review. The reader is encouraged to look at other sources for more details.

Typical clinical signs of anemia in vertebrates are weakness, exercise intolerance, reluctance to walk, tachycardia, and dyspnea. However, reptiles’ physiological idiosyncrasies and captive conditions could contribute to subclinical disease signs that are hard for even the most dedicated keeper, owner, or veterinarian to recognize. Moreover, mild to moderate decreases in the number of circulating RBCs and Hb concentration are not necessarily accompanied by clinical signs. Given their restricted activity, reptiles kept in small enclosures rarely show respiratory distress, even when suffering from the most severe cases of anemia. It is only when the nonregenerative anemia is moderate to severe, and when other clinical signs associated with the primary cause become evident, that the anemic condition will be clinically diagnosed.

Moderate to severe paleness of oral and cloacal mucosa is usually an indication of anemia in a reptile patient. An observant owner should be able to detect this change in the mucosal color. However, this examination parameter is difficult to observe in reptile species with a pigmented oral mucosa. Other common signs of chronic, nonregenerative anemia in reptiles, although nonspecific, are prolonged hibernation or brumation times, anorexia, lethargy, and depression.
Diagnosing Anemia in a Reptile

When compared with nonreptilian species, the principles and approaches used for the diagnosis of anemia in reptiles are the same. Based on the limited knowledge existing for reptiles, anemia rarely appears to constitute a single or primary problem. Often, anemia is just another sign or manifestation of an associated medical condition or disease. Therefore, an exhaustive examination of the whole patient is required to identify the etiology for the underlying disease that may be contributing to the anemic condition.

Most anemic conditions identified in reptiles will have a traumatic, degenerative, inflammatory, neoplastic, toxic, metabolic, nutritional, or infectious etiology.1-5,8,11,13,14,19,36,38 In cases where one of these etiologies is not self-evident, a complete anamnesis, evaluation of the husbandry, thorough physical examination, and judicious use of appropriate complementary diagnostic tests (e.g., fecal tests, radiographs, ultrasound, urinalysis, complete blood count, plasma protein electrophoresis, plasma biochemistry panel, bacterial cultures, fungal cultures, testing using polymerase chain reaction technology, fine-needle aspirates, biopsies, coeliotomy, laparoscopy, blood cultures, lung washes, bronchial washes) can be done to determine the underlying cause of anemia in a reptile patient.2-5,8,67-70

Given the idiosyncratic physiological and behavioral characteristics that tend to mask signs of disease in reptile patients, overt signs of sickness may not be evident for many owners. Without the observed presence of other clinical signs, anemia is easily overlooked in its early stages. Annual or biannual physical examinations and diagnostic testing are recommended for early diagnosis of many diseases in reptiles, including anemia.

A complete investigation of the reptile hemogram is one of the most valuable, simple, and least expensive diagnostic tools available to the practitioner interested in identifying the cause of anemia or disease condition in reptile patients. A complete hematological investigation should include a review of the RBC numbers, morphology and size, and the accompanying white blood cell response.3-5,8

The first specific step needed when investigating the cause of a reptile anemia is to estimate the total RBC count, PCV, and Hb concentration. Unfortunately, reference values for these parameters are only reported for a small percentage of reptiles, and certain parameters, such as the total RBC count, may vary because of intrinsic and extrinsic factors, limiting the value of the reference. Nevertheless, the total RBC counts for most reptiles usually range from 300,000 to 2,500,000 erythrocytes/μL. More specific range values have been provided for lizards (1,000,000 to 1,500,000 erythrocytes/μL), snakes (700,000 to 1,600,000 erythrocytes/μL), and chelonians (300,000 to 500,000 erythrocytes/μL).2-5,8,28-30 It becomes self-evident that for each group, when total RBC counts are well below the lower reference range, the RBC count can be useful in identifying anemia. However, only a limited number of cases will be severe enough to fall under the lower ranges. Mild to moderate physiologic changes may occur with the total RBC count falling within the reported overall reference range, even if anemia is present. Therefore, cautious interpretation of published data and evaluation of other hematological values, indices, and cell morphology is required when evaluating erythrocytic parameters of reptile patients.

The same considerations mentioned for RBC counts may apply to the PCV and Hb concentration. Subsequent calculation of the hematological indices could help to further characterize the anemia. For example, by measuring the average size of the erythrocytes (MCV), it would be possible to determine whether the patient has a microcytic, normocytic, or macrocytic anemia. Additional characterization of the anemia may be achieved through measuring the MCH to determine whether a reptile is hypochromic or normochromic. Overall, anemia in reptiles is usually of the normocytic and normochromic type.1-5,8 Hypochromic anemias are less commonly observed and usually associated with iron deficiency, heavy metal toxicosis, starvation, and malnutrition.4,5 Other types of anemias (e.g., microcytic, macrocytic) have not been widely reported in reptile species.

To determine the etiologic origin of an anemic condition requires the examination of the plasma color, investigation of blood films for RBC morphology and the presence of hemoparasites, and measurement of the reticulocyte count.2-4,5,8,55 The reticulocyte count reflects the ability of the bone marrow to produce new blood cells. Newly produced erythrocytes can be easily identified and counted manually when stained with vital stains (e.g., new methylene blue). Given that reticulocytes are part of the regenerative response to anemia in birds and mammals, they are usually a good quality indicator as to whether the anemic condition is regenerative or nonregenerative in reptiles, and can also provide information about bone marrow function. However, it is important to note that reptiles have a very long erythrocyte half-life (up to 600 days) and a very slow turnover time for these cells.27 For most reptiles, the normal percentage of reticulocytes is less than 2.5%.1,5,8 Therefore, an increase in the number of
reticulocytes may not be present in reptile patients during the early stages of hemolytic or hemorrhagic anemia. Failing to understand the difference between reptiles and other domestic and exotic pet animals can result in the inability to diagnose a nonregenerative cause in the early regenerative anemic patient. In reptiles, it has been reported that it can take up to 2 months to detect an increase in newly formed erythrocytes to rule out a nonregenerative anemia without an obvious cause. An obvious increase in the number of reticulocytes is a clear indicator of regenerative anemia.

When evaluating erythrocyte polychromasia, it is important to recognize that up to 1% of circulating polychromatophilic cells is a normal finding in reptiles. Furthermore, a polychromatic erythrocyte percentage of more than 1% is usually considered a good indicator of RBC regeneration. Conversely, a lack of polychromasia suggests a nonregenerative anemia. Repeated evaluation of more than 500 RBCs may be required to estimate the true status of polychromasia in the reptile patient. Presence of mitotic figures within the erythrocytes, other immature cells, and basophilic stippling of erythrocytes are good indicators, although not exclusive, of regeneration.

When investigating the anemic reptile, practitioners should keep in mind that in certain physiological stages, variations in RBC morphology may occur. Growing reptiles and those undergoing ecdysis may have increased numbers of polychromatic erythrocytes. In brumating reptiles, the number of cells with mitotic figures may be more prevalent. The condition described above should be considered when trying to establish a diagnosis of anemia. On the contrary, anemia is easily masked if blood samples are taken shortly after brumation. The PCV and Hb concentrations will be elevated because of the natural dehydration process that occurs during the time of brumation. A new blood sample should be taken 2 to 4 weeks after the initial test to verify the results, thus taking into account normalization of the brumation-affected blood values if anemia is suspected.

Most regenerative causes of anemia become obvious after completing the anamnesis, physical examination, hematological investigation, and ancillary diagnostic tests. For example, sudden changes in PCV are suggestive of a hemolytic or hemorrhagic disorder. Usually, a sudden drop in PCV is accompanied by a reddish color of the plasma or serum if a hemolytic etiology is present. The plasma color of reptiles is generally a yellow/clear to orange color, but may have a greenish tinge to it. Therefore, a sudden drop in PCV without changes in plasma color is more suggestive of a hemorrhagic problem.

Nevertheless, although a low PCV and reddish coloration of plasma suggest hemolytic anemia, the test should be repeated to rule out technical problems that could result in a misdiagnosis.

Because malaria is a relatively common cause of hemolytic anemia in reptiles, blood films should always be examined for the presence of pathogenic hemoparasites within the RBCs. The presence of malarial organisms will often confirm a diagnosis of a parasite-induced hemolytic anemia. Care should be taken not to assign a parasitic cause of anemia when nonpathogenic hemoparasites are identified (e.g., hemogregarines). Furthermore, it is important to remember that a low prevalence of malarial parasites is not necessarily associated with clinical signs and hemolysis. Other causes of hemolytic anemia in reptiles may include drug-induced toxicity and heavy metal toxicosis, and these should be investigated following the same diagnostic procedures used in small animal and avian medicine.

Diagnosing internal hemorrhage in reptiles can be challenging. In most cases, comprehensive physical examination and judicious use of complementary imaging techniques should be effective enough to identify this type of anemia. Recovery of blood by gentle flushing of the coelomic cavity will be highly supportive of a diagnosis of internal hemorrhage. If these other diagnostic tests are insufficient to confirm active internal hemorrhage, an exploratory coeliotomy or coelioscopy can be performed to confirm the diagnosis.

Secondary poisoning with the newer coumarinic rodenticides is a possible cause of hemorrhage in reptiles fed contaminated rodents. In many cases, melena and epistaxis will be present, and it is rare for internal hemorrhage to be the only overt clinical manifestation. A definitive diagnosis for rodenticide toxicity can be made in reptiles with the same techniques recommended for birds and mammals. Measuring vitamin K–dependent clotting factors is possible in reptiles and may contribute to the diagnosis of a rodenticide poisoning. Investigation of the prothrombin, partial thromboplastin, and coagulation times may also have some diagnostic value if the results are compared with those of unaffected individuals from the same species, although the significance of these tests in reptiles needs further investigation. Primary hemostatic disorders are very rare in reptiles and constitute an area with little scientific investigation.

Nonregenerative causes of anemia present as a completely opposite picture to the regenerative response. The absence of reticulocytes, polychromasia, and mitotic figures is usually a good indicator
of nonregenerative anemia. However, given that a small percentage of reticulocytes (< 1%) and polychromatic erythrocytes (< 2.5%) are usually found in the blood of healthy reptiles, a clinician may not be able to use the absence of these cells as confirmation early in the anemic process. Again, these are general reference values and may not apply to individual cases; therefore, careful interpretation of these parameters is required.

The diagnosis of nonregenerative causes of anemia requires the combination of collecting a complete patient database, thorough physical examination, and the investigation of most common secondary causes of nonregenerative anemia. If a diagnosis cannot be made based on this information, a bone marrow aspirate is required.

**Evaluating the Bone Marrow**

A bone marrow biopsy is required to determine a reptile’s status in a case of nonregenerative anemia. An examination of the cell types from the bone marrow will allow the clinician to determine why the hematopoietic organ is not producing cells in the wake of needed production.4,5,11 Primary failure of erythropoiesis (i.e., the presence of bone marrow aplasia, hypoplasia, or both) is the most common cause of nonregenerative anemia due to bone marrow origin. Possible reasons for a primary failure of bone marrow erythropoiesis include immune-mediated, genetic, radiation, and drug-mediated causes. Infiltration of bone marrow with fat, inflammatory cells, or fibrosis may also result in a reduction in RBC production. A careful anamnesis will help to rule out possible radiation or drug-induced causes for decreased RBC production; however, diagnostics for immune-mediated and genetic causes are not widely available for reptiles. A general lack of basic research limits our ability to diagnose some of these etiologies, and little information about bone marrow interpretation has been published for reptiles.4,5

A bone marrow biopsy can easily be collected from lizards, chelonians, and crocodilians. The preferred biopsy sites are the femur and tibia. The landmarks for inserting the biopsy needle are the trochanteric fossae (e.g., lizards and crocodilians) and tibial crest (e.g., lizards, chelonians, and crocodilians). The technique is conceptually similar to the biopsy technique performed in birds. A spinal needle or a regular hypodermic needle of adequate size and length can be used to collect the sample. When using a regular needle, it is best to use a stylet to avoid blocking the needle channel with bone and/or cartilage. The author uses the stylet that is part of some intravenous catheter sets when performing this procedure.

General anesthesia is highly recommended when performing a bone marrow biopsy, except in the critical patient in which the risk of anesthesia could compromise the patient’s condition. In this last case, the use of a local anesthetic is recommended. Once the area is aseptically prepared, a small incision should be made on the skin and the needle inserted into the bone with continuous and steady pressure and a slight left-right rotational movement. Once the needle is within the medullary cavity, the stylet should be removed and a syringe attached for sample collection. Bone marrow can be collected by aspirating the syringe with a slight but steady negative pressure. No anticoagulant is needed. Once the sample is collected, multiple films can be prepared for evaluating cell morphology and numbers. Samples can be stained with Romanowsky or hematoxylin/eosin stains to evaluate the cells, and Gram’s and/or Ziehl-Neelsen stains to screen for microorganisms. If a bone marrow culture is desired, the hub of the needle can be swabbed and submitted for bacteriology, mycology, or virology.60,67,70

A different approach and technique are recommended in snakes and other reptiles lacking hind and forelimbs. The rib is the preferred site for a bone marrow biopsy in these animals, and collecting the sample requires a surgical approach. This procedure should be done with the patient under general anesthesia. Once the site is aseptically prepared, a section of a rib can be removed and submitted for testing.4,5,8

When evaluating the bone marrow biopsy, it is important to review the myeloid:erythroid relationship, quantitative evaluation of different RBC precursors, morphological abnormalities, presence of inflammatory cells and fatty deposits, and presence of aplasia or hypoplasia. In some cases, hyperplasia of bone marrow RBC precursors is observed and indicates regeneration and normal bone marrow function. However, ineffective erythropoiesis may occur if RBC precursors are observed but not their circulating forms. Seven basic stages of erythrocyte maturation have been reported: rubriblasts, prorubricytes, basophilic rubricytes, early polychromatic rubricytes, late polychromatic rubricytes, polychromatic erythrocytes, and mature erythrocytes.4,5,27,71,72 Relative percentages of these different cell populations in healthy or anemic reptile patients are not available.

When suspected, identification of infectious causes of osteomyelitis should also be investigated. An increased number of inflammatory cells in the biopsy sample may be suggestive of osteomyelitis. Polymerase chain reaction, culture, and specific
stains are necessary to identify fungi as well as various bacteria in the bone marrow. Additional staining with Periodic acid Schiff, Congo red, and Prussian blue are useful to detect amyloid, fat deposits, and iron in the bone marrow.

Therapeutics

Very little information is available regarding treatment of anemia in reptiles; therefore, specific therapeutic recommendations cannot be provided. Anecdotal and clinical evidence suggests that the basic therapeutic principles applied to the anemic nonreptile patient may also be of benefit to the anemic reptile. Detailed information about general husbandry, nutrition, and recommended treatment for diseases such as malaria, lead toxicosis, infectious diseases, kidney and liver disorders, and other common causes of anemia is available in other publications. In the future, reports of clinical experiences and additional scientific research about anemia, its diagnosis, characterization, and specific treatment may enhance the therapeutic options available to the exotic animal practitioner for their anemic reptile patients.

Conclusion

Anemia is a very common presentation in veterinary medicine, but it is underreported in the scientific literature as it relates to reptile species. Therefore, better case documentation, consistency in the investigation of anemia, and rapid dissemination of the available information are needed to increase our knowledge in this area of veterinary medicine. Collaborative research efforts between practitioners, herpetologists, reptile curators, pathologists, immunologists, microbiologists, toxicologists, and clinical pathologists are urgently needed to augment our understanding of anemia in reptiles.

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