# Interpreting non-domesticated animal blood profiles – part three: reptiles

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**VICKI BALDREY and IAN ASHPOLE** discuss, in the penultimate part of their series on blood sampling, approaches to dealing with blood profiling in exotic species

**EXOTIC pets are being encountered with increasing frequency in general practice and it is important clinicians are aware of some of the peculiarities they may present**.

This, the third in a series of four articles, discusses the interpretation of blood results from some of the more commonly encountered reptilian species. Please refer back to the first article in this series (*VT* 42.39), "Obtaining a sample", for further guidance regarding the collection of blood samples from reptiles. Part two can be found in *VT* 42.42.

Due to the "blind" sampling techniques used in many cases, contamination of blood samples with lymphatic fluid is not uncommon; some parameters, such as glucose, calcium, urea and enzymes, are comparable with that of plasma or serum – however, total protein and potassium values may be artificially low in a contaminated sample and haematological parameters will also be lowered. It is worth noting reptilian plasma is usually colourless, although it may appear slightly yellow, orange or even green, due to the presence of carotenoid pigments (especially in herbivores) or biliverdin.

## Reference ranges

Interpreting reptilian blood results is challenging due to the broad range of species, across many different families, and the lack of confirmed "normal" ranges in many cases. Reptiles pose another challenge, as they are so heavily influenced by external factors, such as environmental temperature and humidity, which must also be considered when evaluating a set of blood results.

Serial blood profiles and monitoring changes over time in the same individual may have greater value than standalone profiles in some cases.

That said, Tables 1 and 2 provide a general set of reference ranges for haematological and biochemical parameters in selected reptile species. Due to the lack of published ranges, some values are not given.

## Haematology

As with avian specimens, both erythrocytes and thrombocytes are nucleated – therefore, manual haematology counts are required.

A large amount of information can be gained from just a packed-cell volume (PCV) and examination of a blood smear; morphological changes to blood cells can be just as informative as quantitative values. Rapid Romanowsky stains, such as Diff-Quik, are commonly used to prepare blood films.

The aetiologies of anaemia in reptiles are similar to those in avian and mammalian species, with anaemia classified as haemorrhagic, haemolytic or depression anaemia. Haemorrhagic anaemia may be secondary to trauma, gastrointestinal bleeding, ulcerative lesions or parasitic infestations. Haemolytic anaemia may be seen with toxicosis, and depression anaemia is commonly secondary to chronic inflammatory or infectious disease or neoplasia.

Due to the much longer lifespan of their erythrocytes (up to 800 days in some species) and slow regenerative response, anaemia may take longer to develop in reptiles, with an associated longer recovery period. An elevated PCV is commonly encountered in dehydrated reptiles – however, it should be borne in mind that the PCV may be normal in a dehydrated reptile with concurrent anaemia. Rehydration may be necessary prior to blood sampling in some cases ( Figure 1 ).

As with birds, the main granulocytic cell in reptiles is the heterophil, which has a similar function. Heterophils may increase in number and show “toxic” changes (such as abnormal granulation and vacuolation) in the face of inflammatory or infectious processes. Stress may also cause a heterophilia.

Heteropaenia may be observed in response to severe, overwhelming inflammation or infection, and also during hibernation of chelonian species. Heterophils show seasonal variation, with the highest numbers generally seen during the summer months.

Reptilian lymphocytes have similar functions to those of birds and mammals, with elevated counts occurring in response to chronic inflammation and infection, and also during wound healing and skin shedding (ecdysis).

It should, however, be remembered that, as these animals are ectothermic, the reptilian immune system is significantly influenced by environmental factors, especially temperature.

A seasonal variation in lymphocyte numbers is also seen. Lymphopaenia is a common finding in reptiles and is often associated with malnutrition, stress and poor husbandry, leading to immunosuppression, as commonly occurs with Mediterranean tortoises post-hibernation.

Eosinophils are found in high numbers in certain reptiles, such as some chelonian species, with seasonal variation also occurring in this cell line. It is thought they may play a role in parasitic infections and stimulation of the immune system in preparation for, and during, hibernation. Basophils are found in varying concentrations in different reptiles and increased numbers have been associated with parasitic and viral infections in chelonians. Seasonal variation in this cell line is minimal.

Reptilian monocytes have a similar role to those in birds and mammals and may be increased in response to more chronic processes, such as granulomatous inflammation. Some confusion exists regarding the term “azurophil” in reptiles; these cells are described more commonly in snakes and some lizards. In snakes, these cells are thought to function similarly to neutrophils, but are more like monocytes in other species; in both cases, azurophils are involved with the phagocytic and granulomatous response to microbial infection. The confusion appears to be one of nomenclature and revolves around the staining characteristics of the so-called azurophilic granules within these cells. Where confusion exists, azurophils should be counted separately in snakes but alongside monocytes in other species.

The function of the reptilian thrombocyte is similar to the mammalian platelet, including roles in haemostasis and wound healing. Elevated numbers may also be associated with severe inflammatory disease or sepsis.

## Proteins

As with birds, serum or plasma protein electrophoresis provides the most accurate measurement of albumin concentration and allows globulins to be separated into alpha, beta and gamma fractions, although specific reference ranges for globulin fractions have not yet been documented in reptiles. Increased total protein and albumin values may indicate dehydration, while hyperglobulinaemia is often associated with chronic inflammatory processes.

It is important to note that female reptiles show a significant increase in total protein concentration during periods of reproductive activity and folliculogenesis ( Figure 2 ). This biochemical change is often accompanied by an increase in the total calcium value, which may reach two to four times the “normal” level.

Hypoproteinaemia may be seen in cases of chronic malnutrition, or as a result of proteinlosing enteropathy (such as gastrointestinal parasitism) or nephropathy, and secondary to severe haemorrhage.

## Calcium and phosphorous

While total calcium values may have diagnostic value, such as the significantly elevated concentration commonly seen in reproductively active females, the physiologically active, ionised calcium component is generally considered more informative.

Low ionised calcium values are seen commonly in sick reptiles and may manifest as neurological signs, including muscle tremors, paresis and seizures.

Many herbivorous diets are low in calcium and high in phosphorous, which may lead to secondary nutritional hyperparathyroidism, with fibrous osteodystrophy and pathologic long bone fractures, if calcium and/ or vitamin D3 supplementation and exposure to UVB radiation are not provided. Changes to phosphorus concentration in association with renal disease are discussed below.

## Renal parameters

Assessment of renal function is more complicated in reptiles than it is in domestic mammals, with both urea and creatinine considered to be poor indicators of renal function. Uric acid is the main end product of protein metabolism in terrestrial reptiles, and accounts for 80 to 90 per cent of the nitrogenous waste produced. Aquatic species are an exception, where ammonia and urea are the predominant products excreted.

Hyperuricaemia with a value more than double the upper normal limit is usually suggestive of renal disease, although the actual value does not appear to correlate reliably with the severity of renal damage. Raised uric acid values may also reflect recent protein ingestion, particularly in carnivorous species, where uric acid concentration reaches a peak approximately 24 hours post-feeding. Gastrointestinal haemorrhage should also be considered. Hyperuricaemia may be associated with articular or visceral gout (although biochemical findings are inconsistent), which may be either primary, or secondary to renal failure, chronic dehydration or following excessive animal protein consumption by herbivorous reptiles.

Hyperphosphataemia is a more consistent finding associated with renal failure in reptiles, usually resulting in inversion of the calcium:phosphorus ratio. Haemolysis of the sample and excessive dietary phosphorus content must be ruled out.

Urea may be a useful marker of hydration status in some chelonians, with raised concentrations commonly seen in dehydrated specimens, particularly following hibernation. Chronic dehydration has been associated with urolithiasis in chelonians ( Figure 3 ).

## Liver parameters

Liver disease, such as hepatic lipidosis, is relatively common in exotic practice, but antemortem diagnosis of liver disease in reptiles can be challenging and often requires visual assessment of the liver, combined with histopathological examination ( Figures 4 and 5 ).

Aspartate aminotransferase (AST) is found in skeletal muscle and kidney, but also in high concentration within hepatic cells and is, therefore, the most commonly used parameter to evaluate hepatocellular damage. This enzyme should be assessed in conjunction with creatine kinase (CK) concentration to help differentiate hepatocellular damage from muscle breakdown. A raised AST value with a normal CK value is suggestive of some degree of hepatic damage, while a raised CK with a normal AST value would be considered due to muscle cell damage alone, as may occur secondary to restraint for blood sampling or due to intramuscular administration of some drugs. Elevations to both parameters are more difficult to interpret and indicate tissue damage, with or without hepatic insult.

Alanine aminotransferase (ALT) is not considered organ-specific in reptiles and is, therefore, not commonly used. Lactate dehydrogenase may be released in cases of hepatocellular damage, but has widespread tissue distribution and, therefore, lacks specificity. Artefactual elevations to this enzyme are also common secondary to haemolysis. Alkaline phosphatase is widely distributed throughout the body and, therefore, also lacks specificity. Elevations to this enzyme have been documented in association with osteoblastic and reproductive activity and in juveniles. As in birds, gammaglutamyltransferase (GGT) may be a more liver-specific enzyme. However, tissue levels are very low, reducing the sensitivity of this test.

No liver function tests have been validated in reptiles at this point and reptile bile acid values can be unreliable and misleading in many cases.

## Glucose

Glucose values in reptiles are subject to marked physiological variation, depending on the species, nutritional status and environmental conditions.

Hyperglycaemia in reptiles may occur due to stress or an underlying systemic problem. Although diabetes mellitus has been documented in some reptile species, such as the green iguana (*Iguana iguana*) and various chelonians, it is considered extremely uncommon and a thorough investigation into other possible causes of the hyperglycaemia should be performed before making that diagnosis.

Hypoglycaemia is usually associated with prolonged anorexia, severe hepatobiliary disease or sepsis, and can lead to clinical signs, including tremors, loss of the righting reflex and dilated, non-responsive pupils.

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