REPTILE HEMATOLOGY AND SERUM CHEMISTRY

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Like other exotic animal patients reptiles tend to hide signs of disease. That's why reptile owners typically present their reptiles to veterinarians in an advanced state of disease. This makes an antemortem diagnosis and the initiation of specific therapeutics challenging.

The use of clinical pathology in reptiles is useful, but not as reliable and specific as the results we obtain in traditional small animal medicine. The reasons for this are multifactorial, including the fact that only a limited number of controlled studies have been done with respect to the 7500 plus reptile species that exist. What's more, the studies that have been done are often completed with low numbers of animals compared to studies done with traditional small animals. Seasonal, hormonal, sexual, and dietary influence at the time of blood collection are all factors that likely have an effect on clinical pathology results for reptiles, but have yet to be thoroughly investigated. Also, the nucleated red blood cell of reptiles (as with birds) makes the use of automated systems for white blood cell analysis as used for mammals useless. Therefore, reptile clinicians must use clinical pathology as an adjunct to other clinical information, including a thorough history, physical examination and other diagnostic tools such as radiology, ultrasonography, endoscopy and exploratory surgery.

BLOOD SAMPLING[1, 2, 3]

As a general rule the blood volume for reptiles is thought to be between 5-8% of the total body weight. That means a 100 gm lizard will have 5-8 ml of total blood volume and approximately 10% (0.5-0.8 ml) of that total volume can safely be removed from the patient. Each patient should be evaluated prior to collecting blood, and in some critical patients smaller samples should be drawn until they are more stable. Most commercial laboratories today and in-clinic chemistry machines can easily work these microsamples allowing clinical pathology information on even the smallest of reptile patients.

SNAKES

Common sites for blood collection in snakes include the ventral coccygeal vein of the tail and cardiocentesis. The palatine vein in the oral cavity is not recommended due to haematoma formation and concerns with trauma to the fragile oral mucosa during restraint and collection. All venipuncture sites should be cleaned and aseptically prepared prior to blood collection. Based on the size of the patient a 21-25 gauge needle on a 1-3 cc size syringe is utilized for venipuncture. Anaesthesia is typically not necessary to collect blood from snakes.

Ventral Coccygeal Vein

- Located on the ventral midline of tail
- This sampling site is difficult to collect blood from consistently
- Approach the tail ventrally approximately 1/3 to 1/2 the distance from the vent to the tip
- Use caution to avoid hemipenes in male snakes
- Needle placement is at a 45 degree angle between ventral scales and passed to the point of the vertebra
- May need to move needle along bone to find vein

Cardiocentesis

- Typically by default becomes the preferred site
- Consistent and allows needed volume
- Position snake in dorsal recumbency
- Look for heart beating1/4-1/3 the distance down from the head
- Can be challenging to see heartbeat in larger snakes, can use doppler to isolate
- Once located isolate the heart between index finger and thumb as it can move
- Insert the needle at a 45 degree angle between the ventral scales at the most caudal (distal) point which will result in the needle entering the ventricle
- Aspirate gently and blood will flow into the syringe with each heartbeat
- If no blood is obtained remove the needle and replace with a new needle to retry
- Do not hunt around with the needle as this may lacerate the ventricle or major vessels

LIZARDS

Common sites for blood collection in lizards include the ventral coccygeal vein, jugular vein and abdominal vein. This author prefers to avoid the abdominal vein for routine venipuncture (unless for a cut down and catheterization) as the potential for laceration and free hemorrhage into the coelom are possible. All venipuncture sites should be cleaned and aseptically prepared prior to blood collection. Based on the size of the patient a 21-25 gauge needle on a 1-3 cc size syringe is utilized for venipuncture. Anaesthesia is typically not necessary to collect blood from lizards.

Ventral Coccygeal Vein

- Ttypically the preferred site for collecting blood from lizards
- Vein is located on the ventral midline of the tail between the coccygeal vertebrae
- Preferred site is 1/4 to 1/2 the distance from the vent to the tip of the tail
- In male lizards be distal enough to avoid the hemipenes at the base of the tail

- One technique is similar to what is described for snakes where the needle is directed from ventral to dorsal on the midline toward the coccygeal vertebrae
- As the needle comes in contact with the bone, aspirate gently until blood flows into the syringe
- Another technique is a lateral approach to the same vein at the same distance on the tail, but the needle is placed laterally on the tail where there is a depression between the muscles of the tail and directed medially at a 45 degree angle toward the coccygeal vertebrae
- For this lateral technique the needle will come in contact with bone again (lateral spinous process) where the needle is then guided just ventral to the bone and medial again until blood is noted in the syringe on gentle aspiration

Jugular Vein

- More difficult to routinely find than the ventral coccygeal vein but in larger lizards such as iguanas is useful
- The jugular vein is not visualized without a cut down but is typically located as an imaginary line between the tympanum and the shoulder

CHELONIANS

Turtles and tortoises can be difficult to collect blood samples from due to their ability to pull their bodies into the protective shell. Chelonians may have to be sedated in order to obtain a diagnostic blood sample. Common sites for blood collection in chelonians include the jugular vein, dorsal coccygeal sinus or brachial vein. Both the dorsal coccygeal and the brachial vein are closely associated with lymphatics so contamination of blood samples with lymph may occur. All venipuncture sites should be cleaned and aseptically prepared prior to blood collection. Based on the size of the patient a 21-25 gauge needle on a 1-3 cc size syringe is utilized for venipuncture.

Jugular Vein

- Located at the mid lateral aspect of the neck approximately at the level of the tympanum
- The head must be extended and turned slightly away from the side to be collected
- Pressure may be applied at the base of the neck to allow possible visualization of the jugular vein
- In some smaller chelonians a cut down may be necessary to collect a blood sample

Dorsal Coccygeal Sinus

- Located on the dorsal midline of the tail
- The tail is held and extended out and the needle is inserted on the dorsal midline in a cranial direction
- The needle will contact the vertebrae and with slight aspiration blood will flow into the syringe

Brachial Vein

- This vein works well for larger tortoise species
- The brachial vein is located on the posterior aspect of the elbow
- The needle is passed perpendicular and shallow to the posterior aspect of the elbow and gentle aspiration results in blood collection

PROCESSING BLOOD SAMPLES [1,2,3]

Reptile blood is fragile and should be placed in appropriate collection tubes and processed immediately. Blood samples should be processed in-house when possible or sent to a commercial clinical laboratory that specializes in reptile blood analysis. Experienced laboratory technicians will provide more consistent results and may be able to provide information on cell morphology.

For haematological samples snake and lizard samples can be placed into lithium heparin or ethylenediaminetetraacetic acid (EDTA). Chelonian haematological samples should be placed in lithium heparin only as EDTA has been found to cause red blood cell lysis. If blood smears are being made for white blood cell differentials they should be smeared immediately. Blood collected for plasma chemistry samples should be placed in lithium heparin vials or a sterile clot vial. Plasma samples should be centrifuged immediately to separate the cells from the plasma. Falsely decreased blood glucose values and increased phosphorus and potassium values may occur in plasma samples that have extended exposure to the cell fraction.

COMPLETE BLOOD COUNT (CBC)

Reference ranges for complete blood counts for a variety of reptile species have been published.[2,3] However clinicians must try not to rely on these reference ranges as absolutes for the reasons described earlier. For many species of reptiles there are no published normal values. Clinicians must rely on general trends and obvious changes in the haematological picture of the reptile patient. Serial sampling of the patient or blood sampling from a sibling or cage mate (for comparison) is more valuable than relying on available reference ranges.

The CBC in the reptile patient includes the packed cell volume (PCV) or hematocrit, total white blood cell count (WBC), and a differential white blood cell count.

PCV (Hematocrit)[1,2,3]

- Reptiles have lower hematocrits than traditional small animals (25-35%)
- PCV is used to evaluate general health and hydration of reptile patient
- Hematocrit less than 18-20% would be considered anaemic
- Anaemia in reptiles has been associated with blood loss, chronic infections, malnutrition and exposure to toxins

- In anaemic reptiles the erythron should be evaluated to assess prognosis

WBC[1,2,3]

- Cannot be processed using mammalian based automated cell counters due to nucleated red blood cells
- Blood smears can be made with cover slips or with slides using gentle technique as reptile red blood cells are fragile
- Blood smears can be used to determine the estimated white blood cell count and to provide information on cell morphology
- A variety of manual techniques for performing white blood cell counts on reptiles are possible with two of the most popular methods being the Eosinophil Unopette and Natt Herrick's
- White blood cells that are commonly identified in reptiles include:
- Heterophils - associated with acute inflammation (similar to nuetrophil of mammals)
- Monocytes - chronic inflammatory responses
- Azurophils - thought to be a type of monocyte with unknown function
- Eosinophils, basophils and lymphocytes - have similar functions as seen in mammals
- Thrombocytes - nucleated and appear similar to lymphocytes in morphology except they have an irregular nucleus and irregular cell margins as compared to the acentric nucleus and distinct cell membrane margins of the lymphocytes
- Thrombocytopenia has been associated with bleeding disorders in reptiles

PLASMA CHEMISTRY PROFILES

As with complete blood counts in reptiles, reference ranges for plasma chemistry values have been published for some reptiles.[1,2,3] However these values should be used only as general guidelines and are not as specific as those we rely on for our small animal patients. Serial sampling of the patient or sampling of a sibling or cage mate of the same species may be more valuable. Working with a laboratory that has developed normal values specifically for their lab may be more rewarding.

AST, ALT, ALP

- These enzymes have not been found to be tissue specific as with mammals and birds
- cannot be utilized to evaluate specific damage to organs such as the liver

CPK

- Has been consistently associated with skeletal and cardiac muscle
- The techniques we utilize to collect blood samples from reptiles however may damage muscle during collection which may elevate these values

Glucose

- Values are affected by intrinsic and extrinsic factors
- Hypoglycemia is often associated with inanition and debilitation
- Hyperglycemia can be associated with stress, metabolic disease, neoplasia and pancreatitis
- True diabetes mellitus is rare

Uric Acid

- Rreptiles are uricotelic
- Uric acid is the catabolic end product for nitrogen elimination in most reptiles
- Hyperuricemia can be associated dietary intake, dehydration and renal disease
- Visceral and articular gout may occur in severe cases of hyperuricemia
- Is often **not** sensitive for renal disease

Calcium and Phosphorus

- The primary values used to assess renal disease in lizards
- Not as useful in determining renal disease in chelonians and snakes
- Normal calcium to phosphorus ratio in reptiles is approximately 2:1
- With renal disease an inverse ratio of calcium to phosphorus occurs
- Hypercalcemia may occur physiologically in reproductively active females
- Ionized calcium values useful for determining renal disease and abnormalities of plasma calcium

Plasma Electrolytes

- Abnormalities in plasma electrolytes are often associated with malnutrition and gastrointestinal disease (vomiting and/or diarrhea)
- Laboratory handling error may also affect electrolyte values
- Hypernatremia and hyperchloremia associated with dehydration and excessive dietary supplementation
- Hyponatremia and hypochloremia may be associated with malnutrition
- Hyperkalemia is often associated with renal disease and hypokalemia with inanition related re-feeding syndrome

Plasma Total Protein Values

- Hypoproteinemia is often associated with debilitation and malnutrtion
- Hyperalbuminemia is often associated with dehydration or reproductively active females (vitellogenesis)
- Hyperglobulinemia may be associated with infectious diseases
- Electrophoresis is not well validated in reptiles to date but may be useful looking at trends

Bile Acids[4]

- 3 alpha hydroxyl-bile acids appear to be sensitive for liver disease or damage in reptiles
- May be sensitive to dietary influence so fasting may be important
- More studies are needed to validate bile acids however if high values are found (greater than 60 micromol/L) in anorexic or fasted reptiles they may indicate liver disease

References

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