INTRODUCTION

A process-driven approach to disease diagnosis is most efficient at identifying existing and emerging diseases. Physical examination should direct the diagnostic approach and no single plan will work in each situation. Each plan should involve specific or non-specific diagnostics that evaluate a clinical problem or differential. Non-specific diagnostics include hematology, plasma biochemistries, protein electrophoresis, culture, virus isolation, and histopathology. Specific diagnostics include PCR, ELISA, and immunohistochemistry.

PATHOGEN DETECTION

PCR/qPCR

Polymerase chain reaction utilizes technology that amplifies a segment of the DNA of the target, in most cases part of the pathogen genome. Typically, conventional PCR amplifies a segment of DNA >200 bp, while qPCR amplifies 50-120 bp. Conventional PCR and SYBR green qPCR require sequencing to determine if the pathogen is truly present. If a positive result is reported without sequencing, then the therapeutic plan should be approached cautiously. TaqMan qPCR utilizes an additional step with a probe that allows a result to be determined without sequencing. Always ask the lab what type of PCR they are using to better inform your clinical decisions.

Oral and cloacal swabs often lead to the most rewarding diagnostic results for common upper respiratory pathogens and GI pathogens of chelonians. Oral swabbing needs to include both the respiratory epithelium accessed from the choanae on the roof of the oral cavity as well as the oral epithelium itself. Sampling can occur using many types of swabs, but sterile cotton-tipped applicators are the most common. Synthetic swabs, such as nylon flocked swabs produce superior samples for culture, virus isolation, and molecular diagnostics and may improve the ability to detect a pathogen. Blood may be submitted for pathogens that are transported in blood.

Positive results do not indicate that the pathogen is causing disease, nor that the pathogen is even alive in the sample. It only tells you if the segment of DNA is present, it is possible that in oral swabs the animals had eaten something with the pathogen DNA and the patient isn’t even infected.

Culture/Virus Isolation

Isolating the pathogen allows the clinician to determine that a live pathogen was recovered from a patient. It still does not inform about infection or disease. However, there are several test factors that influence results obtained. Some bacterial pathogens are incredibly difficult to culture (i.e Mycoplasma) and thus a negative results is not a truly negative animals. While most bacterial infections are only 70% sensitive (only 7 out of 10 true positives will test positive). Many bacterial agents require specialized media or conditions, always check with the lab to determine how these can influence your results.

Virus isolation is mainly used to identify noel viruses or cultivate a virus for transmission studies. A diagnosis of viral infection is better obtained clinically with histopathology and qPCR. Furthermore, virus
isolation requires specialized media and some pathogens are difficult to grow (herpesviruses). This is a fairly specialized test and only a few labs in the country can do reptile samples. Call your lab to determine if this is available before sending.

HOST RESPONSE

Reptile Clinical Pathology

A single complete blood count result in a reptile is rarely helpful in identifying disease, but serial samples or comparisons among similarly grouped animals may detect significant changes. A single sample in certain cases can help direct therapy in cases of non-regenerative anemia, hypoglycemia, hypoproteinemia, or increased PCV/TS.

Several studies have reported reference ranges for free-ranging reptiles,1,2,6-13,18 but these studies have also demonstrated the effect of season, age class, sex, venipuncture site, and disease status can have on the hemogram.16,17

In the literature, studies in chelonia make up a large percentage of the variations reported. For example, packed cell volume (PCV) was found to be higher in desert tortoises Gopherus agassizzi,11 radiated tortoises Astrochelys radiata held outdoors in the US, and yellow-margined box turtles Cuora flavomarginata during drier or warmer months, respectively.18 Total white blood cell counts (WBC) in both the yellow-margined box turtle and Asian yellow pond turtles Mauremys mutica have also varied by season making comparisons between individuals in different season difficult.10 Males were observed with higher PCV in New Guinea snapping turtles Elseya novaeguineae5 and desert tortoises,9 total WBC concentrations in yellow-headed temple turtles Heosemys annandalii,8 and eosinophils in the yellow-margined box turtle, while females had greater monocytes concentrations in the yellow-margined box turtles. Red-eared sliders Trachemys scripta elegans with experimental ranavirus infections were observed with very few changes, but decreases in total solids were observed in the last sample before they died.4 Inclusions in the white blood cells has been observed in free-ranging eastern box turtles associated with ranaviral infection and might direct further diagnostic investigations.3

In addition to chelonian species, environmental, gender, and seasonal variations have been well documented across many reptile orders, including but not limited to bearded dragons Pogona vitticeps, green iguanas, Indian cobras Naja Naja, Nile monitors Varanus niloticus, Argentine tegus Salvator merianae, and rock lizards Psammophilus blandfordanus.

Thus with all of the demographic and physiological differences between individuals, interpretation of the hemogram should be done with caution and rarely can a decision on therapy or prognosis can be made on this test alone, except for extremes of reference ranges.

Protein Electrophoresis

The acute phase response (APR) is a biological reaction to trauma or infection in the body to control damage induced by invading pathogens, mediate tissue damage, and promote a rapid return to hemostasis. The primary organ mediating this response is the liver, resulting in synthesis of acute phase proteins (APP). The acute phase proteins include 3 major components: serum amyloid A (SAA), fibrinogen (Fib), and albumin (Alb). In most vertebrates, SAA is a major positive APP which rises and falls
rapidly, Fib is a major positive APP which is somewhat slower to increase and can stay elevated for several weeks, and Alb is a major negative APP. Evaluation of these in combination may provide an opportunity to assess both the active inflammatory status and a more chronic inflammatory status of an affected patient.

Changes in APP are a very non-specific indicator of inflammatory response. However, they are a crucial part of diagnostic evaluation in species where more classic signs of inflammation are not present. Evaluating the nature of an inflammatory state in reptiles can also be difficult. In a recent article, the point is made that decreased, increased or normal WBC counts can be present during infection in turtles. Instead, one of the authors recommends using a relatively non-specific method of evaluating APP, protein electrophoresis (EPH), in conjunction with the leukogram as a more robust (though in most species currently non-validated) means of evaluating inflammation.17

In a study on 324 eastern box turtles Terrapene carolina carolina, blood samples were obtained for 3 years at three sites in Illinois and one site in Tennessee, USA. Significant differences were observed with total protein (sex, season, state, Illinois location), albumin (age class, season, state, Illinois location), a-1 globulins (sex, season, Illinois location), a-2 globulins (sex, season, state, Illinois location), b globulins (age class, sex, season, state, Illinois location), c globulins (sex, season, state, Illinois location), and hemoglobin binding protein (age class, sex, state, Illinois location). This study allowed for establishment of references intervals in the eastern box turtle and emphasized differences that occurred based on age, sex, season, and location.

At this time, it is safe to conclude that as more studies investigate acute phase protein ranges across different reptile orders, its clinical utility shows promise. However, as results may be influenced by environmental, gender, and seasonal factors, conclusive interpretations will remain challenging.

Serology
The diagnosis of specific infectious diseases can be complicated and careful consideration of the benefits and limitations of each test is warranted. Serologic assays determine exposure to a particular pathogen, but rely on a healthy robust immune system to produce cellular or humoral responses. Certain diseases elicit a cell-mediated response, and thus testing for antibodies is of no clinical value. But moreover, the responses to many diseases in reptiles are completely unknown.

Herpesvirus diagnosis in many species of tortoises has been reported based on results of an ELISA.14 However, this requires the use of species specific antibodies or interpretation of the results based on the assay being validated in other species.

At present, the hemagglutination inhibition (HI) assay is the sole commercially available serologic method available to detect exposure to ophidian paramyxovirus (OPMV) in snakes. A study of 26 eastern massasaugas Sistrurus catenatus catenatus was performed and blood was sampled to determine their OPMV status. All snakes were tested for antibodies by using HI assays against the green tree python (GTP), San Lucan rattlesnake (SLR), and Aruba Island rattlesnake (AIR) isolates. Twenty-five snakes were tested for antibodies against the western diamondback rattlesnake (WDR) isolate. All samples tested against the GTP and SLR were positive (26/26), whereas 56% (14/25) of the WDR assays were positive, and none (0/26) of the AIR assays yielded a positive result. There was 100% agreement between the GTP and SLR assays, and complete disagreement between the SLR and AIR, as well as the
GTP and AIR assays. The results demonstrate that current HI assays are not reliable in the eastern massasauga, and likely other species.

REFERENCES


