DIAGNOSIS OF INFECTIOUS DISEASES IN REPTILES
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Introduction
Reptiles, like many wild animals, mask their illnesses well, which can make it difficult to assess their health. Laboratory testing therefore plays an especially important role in these animals. Diagnosing infectious agents in reptiles is also challenging, due to the rapid changes and growing availability of testing in recent years. In many cases, little information is available on which tests on what samples are ideal in specific instances. Knowing what samples are best to test, and what tests may be helpful in specific situations, from acutely diseased animals to quarantine situations is important for patient care as well as for optimizing costs and planning treatments.

Tests available for diagnosing infectious agents can be divided into two general categories – methods for direct detection of the infectious agent, and tests for the detection of an immune response to the infection. The second category is generally limited to antibody detection in reptiles.

Understanding what questions a specific test can answer and what the clinical implications of the results are is a central aspect of infectious disease diagnostics. In some cases, e.g. for questions of herd health or chronic problems in a collection, the general presence of a pathogen may be of primary interest. In an individual animal, the current presence of an infectious agent in that patient, and its correlation with disease may be the most important question.

In cases in which specific disease processes are being examined, it is also crucial to consider additional diagnostics, e.g. clinical examination, cytology, and histopathology in addition to direct detection of an infectious agent or immunological testing. The presence of a single infectious agent should always be interpreted in conjunction with clinical and other findings.

Sampling

Before submitting samples to a laboratory for diagnostic testing, it is always a good idea to contact the laboratory to discuss diagnostic methods and procedures. The success of laboratory testing will depend a great deal on the samples that are submitted. Important factors include the choice of samples, timing, and method of collection and handling of samples.

Immunological testing

Immunological testing methods used in reptile medicine detect antibodies against a given pathogen. Detection of antibodies provides evidence that an animal has been exposed to a pathogen, but does not say anything about the current presence of the pathogen in the animal. Repeat testing over time and
detection of changes can provide more information. The samples necessary for this type of testing are generally easily determined, as antibodies are usually found in the blood.

Methods used for serological testing in reptiles include neutralization testing, mostly for antibodies against viruses, e.g. herpesviruses in tortoises. In these tests, a virus is grown in cell culture, in the presence of plasma (or serum) from the patient. If antibodies are present in the plasma, they will bind to the virus and prevent infection of the cells. ELISAs are also used for serological testing. This involves coating a well with antigen, adding serum from the patient, and detecting antibodies that bind to the antigen using a secondary antibody that detects the patient antibody. The diversity of reptile species limits the use of this system in reptile medicine due to the need for specific secondary antibodies. ELISAs have been described e.g. for the detection of antibodies against mycoplasma in tortoises. Hemagglutination inhibition (HI) is another serologic test method used in reptile medicine, mostly for the detection of antibodies against ferlaviruses. For pathogens that bind to red blood cells and cause them to clump together, antibodies in plasma from a patient can prevent this reaction, which can be used for antibody detection and quantification.

When using immunodiagnostic methods, it is important to realize that these methods detect a host reaction to a pathogen. Knowledge of the biology of the pathogen is needed to interpret the importance of antibody detection. Antigenetic diversity and cross-reactivity should also be considered. In some cases, a group of pathogens may have significant antigenetic diversity, and not all strains will cross react, e.g. herpesviruses in tortoises or ferlaviruses. In other cases, some antibodies may cross react with related pathogens that have different clinical implications.

**Direct pathogen detection methods**

Direct detection methods look for the presence of a pathogen in a sample. Choice of samples for direct pathogen detection is more complicated than for serological testing, since the pathogen must be present in the sample. Choosing a specific sample therefore requires some knowledge of the biology of the infectious agent. In general, it is important to remember that a negative test does not mean that the pathogen was not present elsewhere in the reptile.

Isolation of a pathogen in the laboratory is an important tool in diagnostics as well as in research, as it is helpful in studying multiple aspects of specific agents. Culture methods are used mostly for bacteria and fungi. Virus isolation requires cell lines and specific conditions in which the virus can replicate, is time consuming and expensive. Virus isolation is not commonly used as a diagnostic test, although it has been described for a number of viruses of reptiles.

Detection of a portion of the genome of a pathogen by polymerase chain reaction (PCR) is the most commonly used test for detection of a wide variety of pathogens in reptiles. PCR uses a set of primers that bind to specific parts of the genome and a polymerase to synthesize double stranded DNA of a specific length (defined by the binding sites of the primers) that matches the template, resulting in an exponential amplification of these copies of the DNA. For RNA, the first step is a reverse transcription
(RT) to convert it to DNA. Evaluation of the results of the PCR can be done by several methods. The size of the amplicon can be determined by gel electrophoresis, but additional testing is generally necessary in order to determine the specificity of the reaction. Sequencing of the product provides definitive information on the identity of the product and can also provide information on the specific pathogen detected, and its relationship to other described, related agents. Real-time and quantitative PCRs use a labeled DNA probe corresponding to part of the target sequence to increase specificity, detect the product, and, in some cases, to provide information on the amount of template in the original sample. The probe does this by binding to the target sequence and producing a color signal during the amplification process. SYBR green real-time PCRs use a dye that detects double stranded DNA, rather than a probe with a specific sequence, and are therefore less specific and should be interpreted with care. One factor to consider when using PCRs for the detection of pathogens in reptiles is the variability of the pathogens of interest. This is particularly true for viruses, especially RNA viruses. The variability of viruses can mean that primers that can detect specific viruses will not work with other, related viruses. For this reason, many of the PCRs used in reptile virology have a low specificity in order to allow the detection of a range of related viruses. This, on the other hand, means that it is particularly important to verify the results by other means (e.g. sequencing).

In recent years, new sequencing methods, known as next generation sequencing (NGS) have been increasingly used in the detection, identification, and characterization of reptile pathogens. This has led to a huge increase in the availability of tests for specific viruses. So far, this technology is not used for normal clinical diagnostics, but as technology advances and prices for this type of testing go down, it is likely to become a clinically relevant method for pathogen detection.

**Interpretation of Results**

The use of best practices for laboratory testing for infectious diseases is important, and must be followed by a careful evaluation of the results obtained. Once testing methods have been chosen, interpretation of results should take the history, clinical signs, and adjunct testing into account. It is also important to remember that detection of a single potential pathogen does not rule out the presence of other factors in a disease process and multiple infections are common in captive reptiles.