INTERPRETING DIAGNOSTIC TESTS FOR EXOTIC PETS

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Introduction

Exotic pets have evolved to mask their illness to avoid predation. Although this method of self-preservation is valuable to these animals in the wild, it can make disease diagnosis for captive animals a challenge for the veterinarian. Historically, diagnostic methods used to characterize the health status of exotic species were based on domestic species assays. Unfortunately, some of these diagnostic assays may not have been sensitive or specific enough to characterize avian or reptilian disease processes. Veterinarians have also been limited by the amount of research specifically focused on the characterizing the health status of these animals. Fortunately, over the past two decades there has been a movement in the scientific community to develop diagnostic assays specifically tailored at evaluating the health status of these patients. The purpose of this presentation is to introduce veterinarians to the diagnostic assays that are currently available for exotic pet patients and discuss the advantages and disadvantages associated with these assays.

When considering diagnostic tests it is always important to consider the test characteristics (e.g., sensitivity and specificity). Yes, this is one of those things we should have learned in epidemiology but may have forgotten (or slept through). By having an understanding of what the sensitivity and specificity are for a test, it is possible to draw the most appropriate conclusion about a test result. For example, what would you think of a positive test result from a test with a high sensitivity and low specificity? Most would likely give it little thought and presume they have a diagnosis; however, the low specificity implies that the test has an increased likelihood of false positives. If the test is measuring a contagious infectious disease, it is possible that an animal without disease may be classified as positive when it isn't. Drawing the wrong conclusion could result in an animal being euthanized. Because of the potential for misclassification, it is important for veterinarians to consider the test characteristics when interpreting a test result. All tests have a sensitivity and specificity. Unfortunately, they +may not all be well defined. In cases where they are not, it is important to use information available from other similar tests to assist with test interpretation.

Physical exam as a diagnostic test

The physical examination is an important diagnostic test that is not generally considered as such. When performing a physical examination it is essential that it be done in a thorough and consistent manner. Performing an incomplete examination can result in mis-classification of potential disease problems and misdirecting the clinician when making plans for additional tests. The sensitivity and specificity of the physical examination should improve with the veterinarian's experience, although some veterinarian's examinations become less complete over time. Because the physical examination serves as the primary driver behind directing the development of differential disease lists and diagnostic tests, veterinarians should focus on their patients during this most important examination.

Clinical pathology: interpreting blood results

Hematology and plasma biochemistry testing remain important diagnostic tests in exotic pet medicine, as these diagnostics can provide insight into the physiologic and immunologic status of a patient. Veterinarians often rely on "normal" values to interpret clinical pathology results; however, with so many different species of exotic animals it is not possible to obtain these comparisons. The use of "normals" to describe the values used for comparison is not exactly correct either. The author prefers to describe these parameters as reference ranges. To truly be "normals" the data should follow a Gaussian distribution and be evaluated for outliers. In most references this is never evaluated. Veterinarians should avoid blindly accepting "flags" or "low/high" results from a laboratory. Instead, veterinarians need to learn to interpret the blood results based on their patient's clinical presentation and validated references that consider the physiologic fluctuations that can be found in exotic pets.

Diagnostic Imaging

Diagnostic imaging is an underutilized tool in exotic pet medicine, especially the more advanced forms (e.g., ultrasound, computed tomography, and magnetic resonance imaging). Survey radiographs can be used as an important screening tool, and are often invaluable for ruling out disease processes. However, there are limitations regarding their capacity to rule in disease, with some exceptions (e.g., foreign body). Again, as with the physical examination, survey radiographs are subject to misclassification. Most misclassification is associated with inexperience, although the time committed to analyzing the images can also affect the interpretation. Developing background knowledge of the radiographic anatomy of the exotic species being presented to your practice is important for having success with diagnostic imaging.

Microbiologic Culture

Historically, microbiological culture has been used to detect bacterial organisms from various tissues and secretions. Although culture has been considered the gold standard for detecting microbes, the test characteristics for this assay are generally unknown. In general, culture is considered to have a moderate sensitivity and high specificity. There are a number of parameters that can affect the reliability of culture, including method of specimen collection, quantity or type of sample collected, temporal or seasonal variation in shedding, and method of culture. An additional limitation of microbiologic culture is the time required to confirm a diagnosis, which may exceed 48 h to isolate an organism and 96 h to confirm the organism with biochemical tests. The delay imposed by culture techniques may defer initiation of appropriate antimicrobial therapy and control procedures. Culture of certain microbes can be difficult, if not impossible, based on our current knowledge of growth requirements. The inability to confirm the presence of a fastidious organism can influence the management of a clinical case. It is not uncommon for veterinarians to receive culture results for a sample that are negative or have multiple organisms isolated. Again, because of the inherent weaknesses with this diagnostic test, interpretation of the results is sometimes more of an art than science.

Serologic and Molecular Based Assays

Hemagglutination inhibition assays are used as screening tests for a variety of different pathogenic organisms; however, these assays are susceptible to cross-reactions with related organisms. This can make it more difficult to characterize a specific virus within a group of viruses. An example of this is with paramyxovirus in snakes. While commonly used to screen snakes in the USA, the HI assay has been found to be subject to misclassification based on the source of the virus used to run the assay. Caution should be used when

interpreting the result of these types of assays. To limit the potential for misclassification, parallel testing strategies (i.e., using multiple types of diagnostics to prove or disprove positive status) should be used.

The impact of infectious diseases on exotic pet health has created a need for rapid and accurate detection methods for pathogens from both animal and environmental samples. Enzyme-linked immunosorbent assays combine a specific anti-immunoglobulin with an enzyme to detect a specific microbial antigen or antibody. Benefits of the procedure include speed and low labor requirements compared to culture. The sensitivity of the ELISA is generally considered to be higher than the sensitivity for culture. Because the ELISA detects antigen or antibody, it is not necessary for a sample to contain live pathogen. ELISA generally requires fewer overall organisms for detection than culture.

Polymerase chain reaction, once considered only a research technique, is being used both in the clinical diagnosis of disease and for epidemiological investigations. Polymerase chain reaction assay is an enzyme-mediated process used to replicate DNA from an organism with specific oligonucleotide primers that are complimentary to specific nucleotide sequences of the subject organism. Because the PCR technique can be used to create logarithmic copies of microbial DNA from a limited amount of sample, the technique can identify organisms in clinical or environmental samples at levels too low to detect with culture. Several techniques can be used to detect microbial DNA with PCR, including specific PCR, broad-range PCR, multiplexing PCR, nested PCR, and reverse-transcriptase PCR. The PCR assay amplifies DNA with a thermostable DNA polymerase in combination with a buffer, magnesium, deoxyribonucleoside triphosphates, and oligonucleotide primers. The primers anneal to complimentary regions on the coding and noncoding strand of DNA. The DNA polymerase attaches to DNA primer complexes and extends the DNA. The copy made in the first cycle serves as a template for further amplification. Multiple cycles at various temperatures are repeated and the process of disrupting the double-stranded DNA, annealing the primers to the DNA, and extending the DNA produces a logarithmic increase in the template. There are a number of different techniques used to confirm the presence of target DNA, including gel electrophoresis, DNA sequencing, oligonucleotide probes, and restriction fragment length polymorphism.

The PCR assay is considered to be more sensitive than culture because it can amplify DNA from a single organism or part of an organism under suitable conditions. Specificity of the PCR assay can also be very high depending on the primers used. PCR assay may be prone to false positive reactions if processing of the samples is not performed under controlled conditions. To prevent amplification of contaminant DNA, processing should be conducted in separate pre-and post-PCR rooms. A number of biological inhibitors affect the results of a PCR assay, including blood, blood culture media, urine, sputum, and vitreous humor. Sample processing can also influence the PCR assay results, yielding false negatives if the DNA extraction technique does not release microbial DNA, or if the quantity of DNA available for the reaction is low.

A variety of serologic and molecular assays are available to test for the exposure or presence of a range of exotic pet pathogens, including the paramyxovirus, herpesvirus, *Aspergillus* sp., *Chlamydophila psittaci*, and *Mycoplasma* sp, among others. To ensure that the results associated with a particular assay are correct, it is important for the veterinarian to understand the methods used to perform the assay and its specific test characteristics.

Post-mortem testing

Necropsy is generally regarded as the gold standard for disease diagnosis in veterinary medicine. However, this diagnostic method is also subject to mis-classification. Sample collection during the necropsy may be subject to sampling bias. Macroscopic lesions are generally sampled without error, whereas microscopic lesions may

be overlooked. The number of samples collected at the necropsy can also affect the outcome. The author has observed two sampling methods among pathologists, those that collect a series of samples from a single organ and those that collect only one sample from an organ. Microscopic interpretation of the histologic samples may also be subject to mis-classification. One pathologist's interpretation of a histologic lesion may be vastly different from another pathologist. Although necropsy provides important insight into the disease processes in a wildlife patient, veterinarians should consider that it is subject to mis-classification.

Conclusions

Recent advances in exotic pet medicine have generated new diagnostic assays to assist the veterinarian with disease diagnosis. Veterinarians should become familiar with the advantages and disadvantages associated with specific diagnostic assays to improve their understanding of these testing methods. It is important that we remain vigilant and continue searching for new methods to detect pathogens that have not yet been characterized.

Figure 1. Estimating test sensitivity and specificity for an assay.

	Disease	
	Positive	**Negative**
Assay 1	a	b
Assay 2	c	d

$$Sensitivity = \frac{a}{a+c}$$

$$Specificity = \frac{d}{b+d}$$