Over the last 10 years, there have been significant advances in avian medicine. Diagnostic testing for poultry has been an area of active research due to the economic impact of this industry. More recently, though, exotic birds owned by families and fanciers, like cockatiels, pigeons, and parakeets, as well as endangered species held by zoos, such as flamingos, hawks, and even the kagu (Figure 1), have been the focus of more and more research. This research is paying dividends, as new molecular tests are now available to rule out many of the significant diseases we see in all our avian patients.

The DCPAH has always been a leader in avian diagnostics, with two board-certified avian pathologists, one specializing in poultry and the other in wildlife, as well as numerous ACVP board-certified pathologists with significant zoo, wildlife, and exotic avian experience. Avian gross necropsies, histopathology, bacteriology, toxicology, nutrition, and mycology are routine procedures in the laboratory.

By: Dalen W. Agnew, DVM, PhD, Dipl ACVP; Roger Maes, DVM, PhD; Annabel Wise, DVM, PhD; Steve Bolin, DVM, PhD; Scott Fitzgerald, DVM, PhD, Dipl ACVP, Dipl ACVP; Matti Kiupel, DVM, PhD, Dipl ACVP

Advanced Diagnostics for Exotic Pet and Zoo Birds

In addition, however, advanced molecular techniques, such as polymerase chain reaction (PCR) tests, are also available for many viruses, bacteria, and fungi. Fresh or frozen tissues are usually preferred, but for many PCR tests, even formalin fixed samples or archived paraffin-embedded samples can be used. Swabs, tissue samples, or blood samples can also be used from live animals. Some of the most important viruses that can now be routinely detected include influenza, psittacine beak and feather disease, and Newcastle disease, and West Nile virus among many others (see Table 1). In addition, efforts are currently under way to validate a test for Boravirus, the suspected viral cause of proventricular dilatation syndrome in psittacines (Figure 2 shows the thin-walled and non-functional proventriculus). This test should be available by the fall.

Important avian bacteria and fungi that can now be detected via routine culture, PCR, or both include Aspergillus spp., Salmonella spp., and Chlamydophila. Tests are also available for Cryptosporidium spp., Trichomonas spp., Sarcocystis spp., and other pathogenic protozoa (see Table 2). And while this is an extensive list, efforts continue and new tests are continually in development to take advantage of the latest scientific information in serving our clients.

Besides disease diagnostics, the DCPAH also has the capability to determine the sex of many avian species using a test called sexing by tRNA amplification (STRA) or sexing by DNA amplification (SDA). This test can be used for many species of birds, including psittacines, pigeons, and parakeets. The test works by amplifying a specific region of DNA that is unique to males and females. The results are typically available within 24 hours.

Table 1: Avian viruses for which tests are available at the DCPAH:

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>Test Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza (including avian and swine strains)</td>
<td>PCR</td>
</tr>
<tr>
<td>Herpesviruses (e.g., Pacheco’s disease)</td>
<td>PCR</td>
</tr>
<tr>
<td>Circoviruses (e.g., psittacine beak and feather disease and pigeon circovirus)</td>
<td>PCR</td>
</tr>
<tr>
<td>Paramyxoviruses (e.g., Newcastle disease and pigeon paramyxovirus)</td>
<td>PCR</td>
</tr>
<tr>
<td>Polyomavirus (Budgerigar fledgling disease)</td>
<td>PCR</td>
</tr>
<tr>
<td>Eastern and Western equine encephalomyelitis viruses</td>
<td>PCR</td>
</tr>
<tr>
<td>West nile virus</td>
<td>PCR</td>
</tr>
<tr>
<td>Poxvirus</td>
<td>PCR</td>
</tr>
</tbody>
</table>

Table 2: Bacteria, fungi, and protozoa for which tests are available at DCPAH:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Test Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella spp. (including pullorum and gallinarum)</td>
<td>PCR</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>PCR</td>
</tr>
<tr>
<td>Mycobacterium spp. (avium, genevense, bovis, and tuberculosis)</td>
<td>PCR</td>
</tr>
<tr>
<td>Chlamydophila</td>
<td>PCR</td>
</tr>
<tr>
<td>Megabacteria (gastric yeast)</td>
<td>PCR</td>
</tr>
<tr>
<td>Cryptosporidium spp.</td>
<td>PCR</td>
</tr>
<tr>
<td>Trichomonas spp.</td>
<td>PCR</td>
</tr>
<tr>
<td>Sarcocystis spp.</td>
<td>PCR</td>
</tr>
</tbody>
</table>

Figure 1: Kagu from the island of New Caledonia.

Figure 2: Proventriculus of an African grey parrot with proventricular dilatation syndrome.
Transmission Electron Microscopy

By: Matti Kiupel, DVM, PhD, Dipl ACVP; Dalen W. Agnew, DVM, PhD, Dipl ACVP

Visceral larval migrations, larval neurodegenerative disorders, and larval neoplastic cells are the preferred service. Ultrastructural examination is a highly useful tool to elucidate a number of different disease mechanisms:

1. Detection and characterization of unknown viral particles. As an example, Figure 1 shows an ultrastructural image of porcine circoviral particles forming characteristic intracytoplasmic inclusions in macrophages in a pig with postweaning multisystemic wasting syndrome.

2. Identification of parasites, in particular protozoal organisms in tissue sections. As an example, Figure 2 shows Sarcocystis neurona in a skeletal muscle cell in the tongue of a 4-month-old filly. The finding of mature, intact schizonts in the brain and sarcocysts in the tongue further elucidates the life cycle of this parasite, strongly suggesting horses as natural intermediate hosts.

3. Identification and characterization of neoplastic cells.

4. Characterization of degenerative processes, in particular neurodegenerative disorders.


Samples should be submitted formalin fixed. Preferably we would like to get a 3 cm in diameter, well fixed tissue sample, together with a detailed report of the clinical presentation and gross and microscopic lesions. We will cut submitted sections (Figure 3), paraffin embed one-half of the tissue to produce an H&E slide and use this slide to identify the best location for further ultrastructural examination (Figure 4) by comparing the microscopic tissue half with the mirror image of the remaining formalin fixed tissue. For each submission, we will then cut 4 trapezoid 2.3 mm tissue samples (Figure 5) to produce plastic embedded “thick” sections (Figure 6). We also accept the submission of samples prepared in a similar manner by the client for processing. For each case, 2 “thin” sections will be produced and examined by our team of highly experienced pathologists. The cost of an electron microscopy submission, including photomicrographs and interpretation, is $300.

Avian Blood Cells: The ABCs of Avian CBCs

By: DCPAH Clinical Pathologists

A complete blood count (CBC) can be a useful diagnostic tool to evaluate avian patients. However, nucleated erythrocytes and thrombocytes make automated cell concentrations unreliable. A blood smear must be used in conjunction with hemocytometer cell counts to determine total and differential leukocyte concentrations. Avian cells can be challenging to differentiate. In most avian species, heterophils and eosinophils contain red cytoplasmic granules, but the cells can be differentiated based upon granule staining intensity and shape (often round in eosinophils and fusiform in heterophils). Small lymphocytes can be confused with thrombocytes, but lymphocytes have bluer cytoplasm and less dense nuclei. Thrombocytes are often vacuolated and may clump. Large lymphocytes may be confused with monocytes, but monocytes have more cytoplasm and often irregularly shaped nuclei. All avian CBCs at the DCPAH include a pathologist’s review of the blood smear. Submission of anticoagulated blood and unstained, freshly-made blood smears is recommended.

Common Intoxications in Exotic Species

By: Wilson Rumbeiha, BVM, PhD, DABVT, DABT, Clinical Toxicologist

Like other animals, exotic species (including wildlife kept in zoos) and exotic pets are vulnerable to poisoning from a variety of toxicants. Everything is a poison depending on the dose. Domesticated exotic pets, particularly birds, reptiles and fish are highly vulnerable to insecticides used around homes. Among the more toxic ones are organophosphorus and carbamate pesticides. Exotic birds and fish are also very susceptible to mycotoxins, particularly aflatoxins. whereas dogs and cats are affected by diets contaminated with > 60 ppb, fish and exotic birds are affected at aflatoxin concentrations as low as 10 ppb. During evolution, exotics have not had much encounter with pesticides and mycotoxins. As such, they do not have enzyme systems capable of detoxifying even small amounts of these compounds. Indoor birds are also commonly affected by lead and zinc present in household items. Because of their elaborate breathing apparatus, pet birds are very vulnerable to toxic inhalants such as Teflon fumes (from burning Teflon-coated cooking pans). Birds have also succumbed to vapors in homes generated from house freshening products or even burning cooking oils. Zoo animals have their share of toxicants that commonly affect them. High on the list is zinc poisoning from pennies. Zinc causes hemolysis in animals that ingest pennies and other zinc-containing foreign objects. A common diagnosis in zoo birds, particularly birds of prey, is anticoagulant poisoning, commonly brodifacoum, which is a potent second generation anticoagulant compound. This compound is capable of causing relay toxicosis (i.e., birds of prey feeding on rodents that have been poisoned by this compound within zoo premises). Some bird species suffer from iron accumulation disease, which manifests itself as liver failure. Poisonous plants are also reported to affect animals in zoos. Finally, llamas and alpacas are sensitive to copper poisoning. Feed meant for these species should be formulated not to exceed 15 ppm copper, with a 6:1 ratio balance with molybdenum. In these species, copper toxicosis is manifest as acute liver injury. For aquarium species, water quality should be monitored. Fish have died following exposure to moderately elevated copper in water.
Ferret Disease Diagnostics

By: Matti Kniupel, DVM, PhD, Dipl ACVP; Roger Maes, DVM, PhD; Annabel Wise, DVM, PhD

Ferrets are one of the most common pet animals in the United States. According to the California State Bird and Mammal Conservation Program, more than 800,000 ferrets were kept as pets in 1996. Ferrets belong to the family of Mustelidae and as such have a unique physiology and may develop a number of ferret-specific diseases that require expertise to properly diagnose. Such expertise in ferret medicine and diagnostics as well as research into the etiology, pathogenesis and treatment of a number of important and emerging ferret diseases, has a longstanding history at Michigan State University (for more details please go to www.ferrethealth.msu.edu). Our infectious disease research has been focusing primarily on enteric diseases of ferrets, in particular ferret coronaviruses, ferret rotaviruses, enteric coccidiosis, and Tyzzer’s disease. We have also been on the forefront of research into prognostication and carcinogenesis of adenocortical neoplasms. Our research has resulted in numerous specific diagnostic tests that are now offered to our clients as routine services and thereby directly benefit pet ferrets. The DCPAH is the only laboratory that currently offers PCR for both ferret enteric coronavirus (the cause of epizootic catarhal enteritis) and ferret systemic coronavirus (the cause of FIP-like disease). (Figure 1) Additionally, PCRs for ferret rotavirus groups A and C and a PCR for Eimeria furonis were developed at DCPAH. For a complete list of ferret-specific tests for infectious diseases, see Table 1.

In regard to the diagnosis of neoplastic diseases, the DCPAH is the only laboratory to offer an extensive range of immunohistochemical stains that have been used in ferrets to diagnose and prognosticate neoplastic diseases (for a complete list please see our webpage: immunohistochemistry). We offer a complete array of markers for endocrine neoplasms and established transcription factor GATA-4 as a prognostic tool for predicting the risk of adenocortical metastases and for discriminating potentially aggressive tumors from more benign variants. Only recently, we were able to show that increased cytochrome b5 expression accounts in part for the preferential production of adrenal androgens and estrogen by adenocortical neoplasms and can serve as a marker of androgen synthetic potential in these tumors. Please visit our web site for more detailed information or call for information about the various areas of our diagnostic services.

Insectivorous amphibians are at great risk of vitamin A deficiency because their insect prey do not store vitamin A in their bodies. Therefore, monitoring the vitamin A status in colonies of toads and frogs is warranted. It is, however, challenging because few reference values are available. Over the past few months, we have accumulated a number of serum vitamin A values from toads and toads from multiple submissions from several zoos and wildlife organizations. We have no information about the diets of these animals, but the data do provide us with some insight into the interpretation of values. (Figure 1) The most frequently occurring values are very low, near the detection limit of the assay. These are clearly deficient. More challenging to interpret, however, is the substantial portion of values in a low but measurable range. These values are deficient for domestic mammals, but might they represent normal serum vitamin A concentrations for amphibians?

Adequate serum vitamin A concentrations in most domestic mammals range between 200 and 500 ng/mL. Of particular interest in the amphibian data is that approximately 15% of the serum values are above 200 ng/mL, thus into the range considered adequate for mammals. Most of these 15% were from one location, suggesting that dietary management at that location may have been responsible for the higher vitamin A status. This relatively small but significant proportion of amphibian vitamin A values that fall into the range of normal mammals suggests that adequate serum vitamin A concentrations in amphibians may be similar to adequate values observed in mammals. Furthermore, these data suggest that vitamin A deficiency is widespread in captive amphibian colonies and that monitoring vitamin A status of these colonies may be useful in assessing the sufficiency of dietary strategies.

Table 1: Infectious Disease Diagnostics

<table>
<thead>
<tr>
<th>Test Description</th>
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<tbody>
<tr>
<td>- PCRs specific for ferret enteric coronavirus (cause of ECE) and ferret systemic coronavirus (cause of FIP-like diseases), immunohistochemistry and serology for ferret coronavirus</td>
</tr>
<tr>
<td>- PCR and in-situ hybridization for Aleutian disease</td>
</tr>
<tr>
<td>- PCR for group A and C rotaviruses</td>
</tr>
<tr>
<td>- PCR, immunohistochemistry and serology for canine distemper</td>
</tr>
<tr>
<td>- PCR and immunohistochemistry for influenza</td>
</tr>
<tr>
<td>- PCR for enteric coccidiosis (Eimeria furonis)</td>
</tr>
<tr>
<td>- PCR for Tyzzer’s disease (Clostridium piliforme)</td>
</tr>
<tr>
<td>- Immunohistochemistry for proliferative colitis (Lawsonia intracellularis)</td>
</tr>
<tr>
<td>- Serology and fluorescent antibody testing on tissues and urine for Leptospira</td>
</tr>
<tr>
<td>- Bacterial culture including mycobacterial organisms</td>
</tr>
<tr>
<td>- Parasitology for ecto- and endoparasites</td>
</tr>
</tbody>
</table>

Monitoring Vitamin A Status in Captive Amphibians

By: Thomas H. Herdt, DVM, MS, Dip ACVN

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Figure 1: The cause of FIP-like disease.

Figure 1: Distribution of serum vitamin A concentrations among 97 samples from frogs and toads in 4 separate colonies.
Reporting Options Available

DCPAH clients may select from several options for receiving laboratory reports:

1) Web-based reporting through WebView, our online results program. WebView is designed to provide users with quick access to DCPAH test results using the internet. Online reports are posted to the web within a few hours after they are released by the individual laboratories. To use the online system, it will be necessary to register for a DCPAH Web ID and Password. This will allow the user to access the system from anywhere in the world.

2) A FAX-transmitted report

3) An e-mail-transmitted report

4) A printed copy sent through the U.S. Postal Service. (NOTE: Due to recent postage increases, DCPAH encourages clients to use FAX, web-based, and email resulting options. Use of these options is faster and helps DCPAH keep test prices reasonable.)

5) Combination of two formats, FAX and e-mail, or mail and e-mail, etc. (Web-based reporting is available to all clients, regardless of result reporting preferences.)

FAX transmissions can be sent as soon as results are released or scheduled for any one of the following time periods:

- 8 a.m.-Noon
- Noon-5 p.m.
- 5 p.m.-Midnight
- Midnight-8 a.m.

Contact the DCPAH Business Office at 517.353.3045 to set up your preferred report distribution method. Established clients can set up WebView for online access to report results, or modify their report distribution method at our website: www.animalhealth.msu.edu.