



Management of Common Avian Infectious Diseases

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Most infectious diseases are the result of a complex interaction between all of the microbes present in a host and the ability of the host to defend itself against primary and secondary pathogens. By developing a thorough understanding of available diagnostic tests, one can frequently determine which portion of a disease process, if any, may be associated with a particular infectious agent. When correctly interpreted, the results from these tests can then guide management decisions for the individual patient, or for the associated population of animals. Improper interpretation of test results can lead to detrimental actions. Frequently overlooked in the management of infectious disease is that all animals are a member of a population. This population may be as vast as a large aviary, or rather transient (such as a boarding facility, bird show or veterinary clinic). While treatments or other attempts to prevent infectious diseases are usually focused on the individual, it is prudent to consider that proper or improper management of the individual patient has its most dramatic effects on the population. For example, when used correctly, a diagnostic test that suggests or confirms the presence of *Chlamydophila spp* in a bird with upper respiratory disease should lead to treatment of the individual patient, quarantine of the affected individual and monitoring, or possibly treatment, of other birds at risk in the same population. By comparison, if one were to incorrectly use a single positive IgG-based antibody titer to diagnose and initiate treatment of chlamydiosis in a clinically stable apparently healthy bird, then one might incorrectly expose the bird to unnecessary antimicrobial drugs. For the individual patient, the unnecessary use of an antimicrobial drug will have unwanted effects on organ systems (even the safest of drugs have side effects) and will, at least temporarily, alter the normal flora of microbes in the host. Unnecessary use of antimicrobial drugs can result in the development of microbes that are resistant to the particular drug, resulting in long-term detrimental effects for the individual and population to which it belongs.

When managing infectious disease, it is important to remember that the interaction between microbes and a host and the detection of this interaction using diagnostic tests is a biologic event with inherent variability. The clinician must manage these infectious agents based on events that are likely to occur in most hosts and accept that there will always be some exceptions to the rule. For example, the rule for human immunodeficiency virus (HIV) is that an infected individual will die; the exception is that humans who lack CCR5 receptors are not susceptible to disease. Most dogs and cats infected with rabies virus usually develop a progressive neurologic disease that is invariably fatal. The exception is that some infected dogs and cats develop chronic subclinical infections and may recover. As a rule, non-budgerigar psittacine birds infected with avian polyomavirus will either die or will develop an appropriate immune response and clear the virus, within weeks to months. The exception is that an occasional non-budgerigar psittacine bird will remain infected for a prolonged time. Consider the



impact on individuals and the population they represent if management of HIV in humans, rabies virus in dogs and cats, or avian polyomavirus in psittacine birds were based on the exception to the rule rather than the rule.

Historically, four general methods have been used to prevent infectious diseases in animal populations. These methods include: 1) isolation of susceptible or naive animals, 2) diagnostic testing followed by isolation or removal of test-positive animals, 3) genetic selection of a disease-resistant population of animals, and 4) vaccination to prevent disease and restrict an organism's amplification within a host or population of hosts.

Because of the difficulties in controlling infectious organisms by isolation or diagnostic testing, vaccination has played an integral role in disease prevention and control in many animals, including birds. The goal of any vaccine is to stimulate immunity to a specific pathogenic organism via controlled antigen exposure of the host. While this goal usually is achieved to high efficacy, no vaccine protects all vaccinates from infection. However, the few animals that are vaccinated and fail to develop immunity are still afforded some protection by being surrounded by a population of animals that have been vaccinated successfully. This is one basic goal of "herd" or "flock" immunity from a vaccination program.

It should be noted that the effectiveness of a vaccination program depends on the quality of management practices that directly affect the health and immune status of each animal in a population. Vaccines would be expected to be of decreased efficacy for infectious disease control in flocks with risk factors such as poor hygiene, low quality diets, overcrowding, accumulation of waste, vermin infestations, stagnant air, low quality light and inappropriate use of antibiotics.

There have been numerous discussions and publications that debate the use of vaccines in companion animals. It should be noted that these discussions concern the frequency of vaccination and not the importance of primary immunization in reducing the risk or impact of preventable infectious diseases.

VACCINES

Vaccines have reduced the incidence of (or eradicated in specific populations) some of the most devastating infectious diseases found in humans and other animals. There are three main types of vaccines that are used to prevent viral diseases: attenuated-live (also called modified live), inactivated (also called killed) or synthetic (also called recombinant or subunit). In the laboratory, attenuated-live vaccines are produced by growing a strain of an organism that is altered so that it infects an animal but does not cause severe disease. The animal's immunologic response is able to fight off the weakened strain of organism contained in the attenuated-live virus vaccine. The vaccinated animal is then protected against future infections by the virulent organism. Inactivated vaccines are produced by using chemicals to destroy the infectivity of an organism present in a solution. Synthetic vaccines are produced by laboratory manipulations to separate the specific portion of the organism that is responsible for inducing an immunogenic response, and concentrating this portion of the organism in a



vaccine. An ideal vaccine would be highly immunogenic and stimulate strong sustained immunity in the absence of adverse side effects. To date, these goals have been unfortunately mutually exclusive.

Attenuated-live vaccines

The primary advantage of attenuated-live vaccines is their ability to stimulate a stronger, more complete, natural immune response than that induced by an inactivated vaccine. They are also generally less expensive than killed vaccines because comparatively low concentrations of a attenuated organism can be used in the vaccine. The superior immune response occurs because the vaccinates are actually being infected by an organism that is able to replicate. Most attenuated-live vaccines are produced by growing an organism repeatedly *in vitro* until mutants are identified that are less virulent than the wild-type organism. Many of the attenuated-live vaccines are easy to administer because they can be given orally, in eye drops or by inhalation of a mist. Attenuated-live vaccines administered by these natural routes of exposure are designed to stimulate a local immune response in the mucosa of the respiratory or gastrointestinal tract and thus improve a vaccinate's ability to prevent infectious agents from entering the body through these routes. By replicating in the vaccinate, a single dose of an attenuated-live virus vaccine commonly produces a strong and long-lasting immunologic reaction.

There are advantages and disadvantages to attenuated-live virus vaccines. The function of an attenuated-live virus vaccine is to produce an infection that will stimulate the immune system without producing disease. Because the organism present in the vaccine is infectious, there is a possibility the vaccine strain of organism can revert to a virulent form causing a severe (rather than mild) infection. Additionally, attenuated-live vaccines may be virulent in animals that are immunosuppressed, or they may be contaminated with other undetected infectious agents.

When given to an animal species other than the one they were developed to protect, attenuated-live vaccines may induce severe disease.

Inactivated vaccines

Inactivated vaccines are produced by taking an infectious organism and destroying its ability to replicate without changing the organism's immunogenicity. These vaccines are not likely to induce a disease as long as the infectious organism has been inactivated. Attenuated-live vaccines are highly immunogenic. By comparison, inactivated vaccines are poorly immunogenic. To improve their immunogenicity, inactivated vaccines generally contain large quantities of an organism (frequently over 1 million per ml), most contain adjuvants to enhance a vaccinate's immune response and these vaccines must be administered multiple times, exposing the vaccinate to a multitude of foreign proteins with each injection. Inactivated vaccines are used in place of attenuated-live vaccines because of their stability and safety.

Synthetic vaccines

The inactivated or attenuated-live vaccines currently in use will soon be replaced with more effective, safer vaccines that contain (subunit), or induce the production of (recombinant and plasmid-mediated) only the specific proteins of a microbe that stimulate an immune response. These vaccines are safer



than traditional vaccines because they contain fewer extraneous proteins (subunit) and do not contain the genome of an organism necessary for replication (subunit, plasmid-mediated). The USDA has classified recombinant vaccines into three groups: type I recombinant (subunit) vaccines; type II recombinant (gene-deleted) vaccines; and type III recombinant (vectored) vaccines.

Subunit vaccines are produced by inserting nucleic acid that codes for a gene or epitope into an expression system (usually yeast or bacteria), growing the expression vector organism so that the desired protein is produced, and then harvesting and purifying the desired protein. This purified protein, frequently without an adjuvant, can then be used as a vaccine. Subunit vaccines are free of extraneous proteins or other contaminating infectious agents. Thus, vaccinates are only exposed to high concentrations of the protein or proteins that are necessary to stimulate a protective immune response and adjuvants are usually not necessary. Because the immunogenic proteins are recovered and purified from a stable expression vector, subunit vaccines are a highly consistent product. When necessary, several immunogenic proteins from the same organism can be combined in a single injection to broaden the immunologic response or subunit proteins from several different organisms can be incorporated into the same vaccine. Unfortunately, like inactivated vaccines, subunit vaccines primarily stimulate the humoral portion of the immune system.

Type II recombinant (gene-deleted) vaccines are similar in concept to attenuated-live vaccines. Attenuated-live vaccines are generally produced by isolating avirulent strains of an organism from a population of hosts or by repeatedly replicating the organism in the laboratory until random mutations create an organism with reduced virulence. Gene-deleted vaccines are produced by intentionally removing the genes from an organism that are responsible for virulence or pathogenicity while leaving the genes intact that code for replication and protective proteins. These changes make it less likely for an organism to induce disease while simulating a natural infection that stimulates protective immunity. However, because most of the genome of an organism remains intact and the gene-deleted organism replicates within the host, these vaccines could have safety concerns similar to those with attenuated-live vaccines.

Type III recombinant (vectored) vaccines are produced by determining the nucleic acid sequence that codes for an immunogenic protein, or epitope from that protein, and then inserting this nucleic acid sequence into a vector (currently nonpathogenic or gene-deleted bacteria or viruses) that when introduced to a vaccinate stimulates the expression of the protein coded by the inserted nucleic acid. Because these vaccines simulate a natural infection, they are generally more efficacious than inactivated vaccines and induce a long-lasting immunity. Yet, they are far safer than attenuated-live vaccines because they contain only nucleic acid sequences that code for specific proteins, they do not induce organism-associated disease, will not revert to a virulent form and are not shed into the environment. Data suggests that some recombinant vaccines may be able to stimulate protective immunity in young animals despite the presence of maternal antibodies, which typically interfere with other vaccines.



Plasmid-mediated vaccines

Plasmid-mediated vaccines, also known as nucleic acid or naked DNA vaccines, have the greatest potential for revolutionizing vaccinology. Until the early 1990s, it was theorized that foreign nucleic acid that was introduced into an animal would be quickly destroyed by nucleases in a host. A study in mice demonstrated that nucleic acid sequences could be injected into muscle and, instead of being destroyed, the protein coded by the nucleic acid would be produced by the muscle cells. If the injected nucleic acid codes for a protein that protects against a virus, bacteria, parasite or neoplasm then it would be a highly specific and comparatively safe vaccine. Plasmid-mediated vaccines are produced by inserting the nucleic acid sequence that codes for a protective protein or individual epitope into a plasmid (a circularized extrachromosomal DNA sequence used by bacteria to share genetic information). When this plasmid, containing selected nucleic acid sequence, is injected into a vaccinate, the plasmid is taken inside a cell. The cell then directs production of the proteins coded by the inserted nucleic acid sequence (similar to a natural viral infection). The proteins that are produced from this plasmid are recognized as foreign by the host cell and an immune response is generated against the protein. Thus, plasmid-mediated vaccines simulate a natural infection, stimulating a strong immune response while exposing the vaccinate to only the proteins produced by the inserted nucleic acid. These vaccines can be administered in saline and do not require an adjuvant eliminating problems associated with vaccine site reactions. When combined, these attributes make plasmid-mediated vaccines candidates for the safest and most efficacious of all current vaccine technologies.

DIAGNOSTIC TESTING

When a vaccine is not available to help reduce the spread of an infectious agent, one is forced to attempt control using other methods including pathogen detection. When using a diagnostic test to detect the presence of an organism (ie, culture), a portion of an organism (ie, antigen capture ELISA or nucleic acid amplification and detection-PCR), or a host response to an organism (antibody assay), it is important for one to remember that a positive test does not mean that a host is diseased, and with some tests (PCR particularly) may not even indicate that a host is or has been infected. A host exposed to an infectious agent can remain uninfected. For example, a bird that is vaccinated for avian polyomavirus but swallows a virus could pass detectable levels of virus in its excrement even though it was not infected. Also the host can be infected but remain subclinical (this is the most common event with the majority of infectious agents) or can be infected and develop disease. In most cases, considering clinical signs, defining gross lesions, finding abnormalities in measurable blood parameters (CBC, chemistry profile) or detecting specific histologic changes remain necessary to differentiate exposure from infection and infection from disease. For example, using PCR to detect a target segment of PBFD virus nucleic acid in the blood of a bird with or without feather dystrophy does not confirm psittacine beak and feather disease. A diagnosis of psittacine beak and feather disease requires demonstration of characteristic microscopic changes in conjunction with the etiologic agent in affected tissues.

When choosing a diagnostic assay, it is best for the clinician to determine specifically the question for which an answer is desired and then use the test that best answers that question. For example, if



one is trying to determine if a bird has been recently infected with *Chlamydophila spp*, then the best test to choose would be a serologic assay that detects anti-chlamydial IgM. To determine if a bird with upper respiratory disease was actively shedding chlamydia, a test that detects the presence of *Chlamydophila spp* (ie, culture, cytology) or a portion of the chlamydial organism (ie, antigen ELISA or PCR) in a secretion or excretion would be best. To determine if a bird had been infected with *Chlamydophila spp* in its immunologically detectable past, choose a serologic assay that detects anti-chlamydial IgG. To establish the *Chlamydophila spp* status of a bird, choose a combination of tests that will determine if the bird is shedding infectious organisms (ie, culture), has portions of the infectious organisms in its body (ie, antigen capture ELISA or PCR), or has been infected in its immunologic detectable past (serologic assays for IgM and IgG). If a bird were infected with *C. pneumonia* (the dominate isolate in humans) rather than *C. psittaci* (presumed to be the dominate isolate in psittacines), an assay designed to differentiate between these two closely related organisms is necessary.

Irrespective of the type of diagnostic test, proper sample collection, handling and shipping are important for good and accurate test results. The better the quality of samples that are sent to a laboratory, the more likely the laboratory can provide clinically relevant information. Incorrectly collected or submitted samples are generally useless. Tests requiring whole blood should be collected in the correct anticoagulant with the correct blood to anticoagulant ratio (20 units per ml of blood for heparin and 1-2 mg EDTA per ml). For serologic assays, serum should be separated from the clotted blood as soon as possible to prevent deteriorating RBCs from contaminating the serum. Sterile, sealable culturettes, not cotton-tipped applicators, should be used for collecting samples for culture, cytology or PCR. The type of culturette chosen will vary based on the type of culture submitted (ie, aerobic or anaerobic, viral, bacterial, etc). Culturettes containing a liquid transport media, not gel, should be used for collecting samples for PCR. Swabs of the oral or choanal areas should be slightly to moderately moist following sample collection. Swabs intended to detect the presence of an organism in feces should be covered with readily visible quantities of excrement following sample collection. A cloacal swab is most likely to detect what is present within a host compared to a fresh fecal sample that is more likely to detect what is present within a host as well as what is present on and around the sampled.

Accuracy of Diagnostic Tests

The value of any diagnostic test is based on its sensitivity (ability to correctly find small quantities of target) and specificity (ability to differentiate between two related targets). The ideal diagnostic test would provide maximum sensitivity (no false negatives), maximum specificity (no false positives) and rapid results. Because biologic interactions between organisms represent an infinite number of variables that can affect test results, this ideal is difficult, if not currently impossible, to achieve. The higher the sensitivity and specificity of a test, the more reliable it is as a diagnostic aid. No diagnostic test is 100% accurate. In general, as a test increases in sensitivity (reduced numbers or false negatives) there is a decrease in specificity (increased number of false positives). As a test increases in specificity (reduced numbers of false positives) there is a decrease in sensitivity



(increased number of false negatives). Even the best PCR assays we have developed for detection of pathogens of importance in companion birds are subject to this statistical variability. Commonly used diagnostic tests are biologic assays. Samples are collected by veterinary professionals who are subject to some error. Assays performed by laboratory personnel are subject to some error and are being evaluated to determine the presence, or past presence of a biologic organism (the potential pathogen) that in some fashion has interacted with another biologic organism (the host). Thus, scientific data that describes 100% sensitivity and specificity usually is the result of invalid controls or small sample size. As the number of tested samples is increased for any biologic assay, the sensitivity and specificity of the test will inherently become less than 100%.

Each type of test will have an associated level of sensitivity and specificity (Table 1). For example, for a virus to be detectable by electron microscopy, there must be at least 1 million viral particles per milliliter of sample. This test has a low sensitivity. However, electron microscopy is relatively straightforward in differentiating between the physical characteristics of a herpesvirus and an adenovirus. Thus, electron microscopy has a high specificity in some instances. DNA probe-based tests that are designed to detect a target strand of an organism's nucleic acid can be designed to be extremely specific (detect only PBFV virus) or less specific (detect any conserved sequence of a related circovirus). PCR technology, which is designed to amplify the number of strands of target nucleic acid in a sample, can be used to produce a test with a theoretical sensitivity of one strand of target nucleic acid per sample volume. When combined, PCR technology and organism-specific DNA probes can be used to create tests that detect a target segment of nucleic acid from a single organism (highly sensitive) and can differentiate between two closely related organisms (highly specific).

Types of Diagnostic Tests

There are two general types of tests for managing infectious diseases: direct tests that demonstrate the presence of an organism (ie, culture, cytology/microscopy) or a portion of the organism (ie, antigen capture ELISA or PCR), and indirect tests that demonstrate that a host's immune system has responded to an infection by the production of antibodies or a particular type of lymphocyte (Table 2). Each type of test provides specific data and varies in sensitivity and specificity.



Tests that directly demonstrate the presence of an organism, or a portion of an organism, are best used to confirm or refute a suspected infection in an individual patient that is experiencing clinical problems. Tests that indirectly demonstrate that an infection has occurred by the detection of antibodies are best for establishing the incidence or prevalence of an organism within a group of birds. For example, if a bird is having clinical signs that are consistent with those caused by Pacheco's disease virus, then a test that directly demonstrates the presence of the avian herpesvirus that causes this disease is going to provide the most diagnostic information. On the other hand, to determine whether any bird in an aviary has recently been infected with Eastern equine encephalomyelitis virus, then a test that demonstrates the presence of antibodies to this virus would be best. The types of direct and indirect tests that are commonly used in diagnosing infectious diseases are listed in Table 2.

Antibody tests

The detection of organism-specific antibodies requires that the host survive an infection, the immune response of the host produces a detectable quantity of antibodies and that the antibody titer remains high for a sufficient period of time for detection. The types of tests that can be used to demonstrate antibodies to an organism are listed in Table 2.

All antibody detection assays share basic characteristics that influence their diagnostic value:

- An IgG-based antibody test indicates whether or not detectable concentrations of this class of antibodies is present in a host's serum. A single positive sample indicates that the host has responded to an infection in its immunologically detectable past, not that the host is currently infected (exception is an organism that usually causes a persistent type infection). Demonstrating a 4-fold or greater increase in antibody titer in at least two serum samples collected 2 to 4 weeks apart is necessary to confirm an active infection. Because antibody levels may decline over time (typically months to years) to undetectable concentrations and some hosts mount a poor immune response, a negative antibody test does not mean a host has not been infected.
- The detection of IgM against a particular organism in a single test is usually indicative of a recent infection (days to weeks) but does not indicate if the host is currently infected (exception is an organism that usually, but not always, causes a persistent type infection).
- In some cases, an antibody titer may not be detectable for several weeks after infection. If a serum sample for a single antibody test were collected prior to serologic conversion, the resulting low titer would suggest that the host had not been exposed to an organism when in fact it was actively infected.
- Detection of antibodies in a single serum sample alone is of diagnostic (in some cases, prognostic) value only when an infection is usually, but not always, associated with a persistent or life-altering infection (ie, HIV, herpesviruses).
- Most serologic assays are not designed to differentiate between antibodies produced in response to a natural infection and antibodies produced in response to vaccination.
- Some antibodies generated against certain organisms will cross-react with the antibodies produced against other organisms causing a false positive result.



- A bird's immune system must be properly functioning to produce detectable levels of antibodies against an infectious organism.

ELISA

An enzyme-linked immunosorbent assay (ELISA) can be designed so that it detects antigen or antibody. For example, an antigen-capture ELISA could be designed to detect the presence of proteins from avian polyomavirus in the feces of a bird; an antibody ELISA could be designed to detect the presence of avian polyomavirus antibodies in the serum of a bird. An ELISA designed to detect antibodies to avian polyomavirus cannot be used to detect the virus; the ELISA designed to detect viral proteins in fecal samples cannot be used to detect antibodies. Correctly designed, antibody ELISAs can be sensitive to 0.0005 ug of protein/ml.(ref) For antibody detection, this sensitivity is bested only by virus neutralization and bactericidal activity tests (Table 1). The disadvantage to ELISA for use in Psittaciformes is this test requires a secondary antibody that binds to psittacine immunoglobulin. Unfortunately, polyclonal anti-psittacine Ig antibodies have not reliably detected heterologous immunoglobulin from other psittacine species. The development of an anti-psittacine Ig monoclonal antibody that appears to detect the immunoglobulin from most genera of Psittaciformes may increase the availability of ELISA, particularly assays intended for in-clinic use. (Ritchie, et al, Unpublished Data)

Fluorescent antibody assays

There are two types of commonly used fluorescent antibody assays; direct and indirect. Direct fluorescent antibody assays test for the presence of an organism in tissues using anti-organism antibodies. Indirect fluorescent antibody assays test for the presence of antibodies in serum by reacting the test sera with tissues that contain the target organism, usually infected cell culture, or for the presence of an organism in tissues. Indirect fluorescent antibody assays are considered moderately sensitive (they can detect as little as 0.1 ug of protein/ml) but like ELISA they require a secondary antibody.

Virus neutralization/Bactericidal activity

Most antibody assays detect only the presence of a specific immunoglobulin and they provide no information on the biologic activity of the antibodies. By comparison, virus neutralization and bactericidal activity assays indicate the biologic activity of the antibodies. These assays are among the most sensitive and specific of all antibody tests (Table 1), but they are less commonly used than other assays because they are time and labor intensive and not all infectious organisms elicit neutralizing antibodies.

Culture

Except for inducing an experimental infection, culture is the only definitive method to demonstrate that an infectious form of an organism is present in the excretions, secretions or tissues of a host. Anaerobic culture of oral, choanal and fecal samples seems to have increased in popularity as a screening assay in companion birds. It is important to understand that the presence of an organism in



a bird's secretions or excretions does not indicate disease. Additionally, colonization of ingesta in the lumen of the gastrointestinal tract, or of a mucosal surface, by a particular organism does not indicate that this organism is, or will be, associated with disease.

Multiple variants of many bacteria, some of which can be pathogenic and others of which are non-pathogenic, may be cultured from a host and the pathogenicity of these variants is not usually distinguishable without extensive biochemical testing. Those who use culture to the exclusion of fecal cytology should be aware that anaerobes can be associated with disease and are often difficult to culture even using strict anaerobic conditions, and some microbial organisms that are readily visible by fecal cytology may be restricted from growing in culture based on the chosen media or through inhibitory agents secreted by other microbes.

Cell culture for the isolation of viruses is available through many state or university laboratories but is not routinely used as a diagnostic technique in companion and aviary birds. When compared to other virus detection methods, cell culture procedures are inefficient, labor-intensive, expensive and may require weeks to months to complete. Additionally, many of the viruses that infect companion and aviary birds are difficult to recover in cell culture and the failure to recover a virus from a bird with a disease of viral origin does not indicate the bird is virus-free. As is the case with bacteria, the isolation of a virus from a host does not necessarily mean that the recovered virus is the cause of a disease process.

Electron microscopy

Electron microscopy can be used to demonstrate the presence of organisms (ie, viruses, chlamydia, rickettsia, etc) that are difficult or currently impossible to see by light microscopy in excretions, secretions or tissues. Because electron microscopy is relatively insensitive, when an organism is detected it is assumed that it is present in concentrations of at least 1 million organisms per milliliter of sample. Many viruses that replicate in cells of the digestive tract cause asymptomatic infections. Thus, it is common to see virus particles by electron microscopy in the feces of normal-appearing birds.

Nucleic acid amplification and detection (PCR and DNA probes)

Organism-specific DNA probes can be used to detect a target strand of nucleic acid that is extracted from a sample and attached to a membrane or it can be used to detect organism-specific nucleic acid in a section of formalin-fixed tissue that has been processed for histologic evaluation. The process of using nucleic acid probes to detect organism-specific nucleic acid sequences in tissues is called in situ hybridization.

In situ hybridization using organism-specific nucleic acid probes is particularly valuable in diagnosing an infection when an organism is present in small numbers or produces lesions that microscopically resemble those induced by other viruses. For example, the intranuclear inclusion bodies caused by polyomavirus can appear morphologically similar to the intranuclear inclusion



bodies caused by PBFV virus, adenovirus or herpesvirus. In situ hybridization using viral-specific DNA probes can quickly and correctly determine which of these viruses may have produced the inclusion bodies. Organism-specific antibodies can also be used to determine if the proteins of an organism are present in an affected tissue. However, DNA probe-based assays are generally more sensitive and specific than antibody staining techniques for the detection of an organism in tissues.

Use of DNA probes to detect the presence of an organism's nucleic acid in infected tissues, where high numbers of the organism are usually present, is fairly straight forward. In contrast, detection of an organism in excretions or secretions where numbers of the organism may be limited requires additional processing. To increase the likelihood of finding an organism in a diagnostic specimen (improved sensitivity), a sample to be tested is often subjected to a group of reactions that will amplify (increase) the number of DNA molecules in the sample that originated from the target organism. Increasing the number of target molecules in a sample is the purpose of the polymerase chain reaction (PCR) step of many nucleic acid detection tests. Theoretically, amplification procedures can use one copy of a nucleic acid sequence from an organism to produce 1,000,000 replicates of the same sequence. It is obviously easier to detect 1,000,000 copies of the target nucleic acid sequence than it is to detect 1 copy of the target nucleic acid sequence.

The primary advantage of PCR is its extreme sensitivity in detecting the presence of small quantities of target nucleic acid. The major disadvantage of PCR is its extreme sensitivity in detecting the presence of small quantities of contaminating or irrelevant target nucleic acid, which may or may not have originated from an organism within a host and may or may not have been recovered from a viable organism. When interpreted correctly, PCR technology provides indispensable information. When interpreted incorrectly, PCR technology can be extremely detrimental. Any sample collected for PCR should be treated at least as carefully as a sample collected for culture.

It is of interest that clinicians frequently discuss the recovery of certain bacteria from the oral, choanal or cloacal areas of birds as contaminants, but it is rare to hear clinicians discuss the detection of a target segment of nucleic acid in samples from the oral, choanal or cloacal areas as having been caused by sample contamination, even though PCR is magnitudes more sensitive and thus more prone to contamination error than culture. For example, if a clinician were testing a bird to determine if PBFV virus nucleic acid was present in the blood, and the blood sample was collected from a toenail, then a positive result might indicate that viral nucleic acid was present in the blood, or it could indicate that the bird's toenail was contaminated with viral nucleic acid that was introduced into the sample tube during the collection process. Because of large surface irregularities, washing the bird's nail before collection may not reduce the potential for contaminating the sample. Use of feathers for the identification of a bird's sex by PCR is similarly problematic. Female birds are heterozygous. If nucleic acid from the W chromosome, which is present in every cell in a female bird, were to contaminate the feather of a male and then the contaminated male's feather were processed for sex identification, the bird could be incorrectly identified as a female.



PCR assays detect only a target segment of an organism's nucleic acid; they do not routinely differentiate between nucleic acid that originated from a viable or non-viable organism. Therefore, interpretation of a positive result, particularly in a patient that is asymptomatic, is limited to saying that the target segment of nucleic acid was detected in the tested sample, not that the patient is or has been infected. In most cases, a positive PCR result in a patient that is asymptomatic should be interpreted as a reflection of the organisms to which the host has been exposed in its immediate environment. Retesting of asymptomatic birds that are positive by PCR for common pathogens like PBFD virus, avian polyomavirus or chlamydia, is necessary because most PCR-based tests do not differentiate between viable and non-viable nucleic acid.

When interpreting PCR-based results, consider that non-viable nucleic acid can persist in a host for months following exposure, many hosts are exposed to an organism but do not become infected and most hosts that are infected remain subclinical. A study of PCR results from cattle experimentally infected with bluetongue virus provides example of the care that must be exercised in interpreting the biologic importance of a positive PCR result in a clinically normal animal. In experimentally infected cattle, infectious bluetongue virus could be detected in the blood for 2 to 8 weeks following inoculation, while target nucleic acid sequences, described as being of "little biologic significance", could be detected in the blood for up to 5 months as the cattle's immune system was clearing the virus. Thus, stating that PCR detected a target segment of bluetongue viral nucleic acid in the blood of a cow would be correct; stating that PCR detected bluetongue virus in the blood would be incorrect.

PCR based tests are biologic assays that are designed to detect a biologic sample. As such, both the target nucleic acid within a test sample and the test reagents themselves are subject to interference by biologic or chemical reactions that affect nucleic acid or proteins (enzymes used in the reaction). Substances that are known to interfere with PCR include hematin, formalin, DMSO, NaCl, KCl > 50mM, phosphate buffered saline, melanin, excess EDTA, excess heparin, detergents and glove powder. A clinician commented during a talk at a national meeting that he failed to amplify avian polyomavirus from a commercially produced inactivated vaccine. This clinician failed to include controls that would have shown that the B-propiolactone used to inactivate the virus interferes with the PCR reaction as does the aluminum hydroxide used as an adjuvant. (Ritchie, et al, In preparation)

AVIAN POLYOMAVIRUS (APV)

From its initial description in the early 1980's, avian polyomavirus infections have caused frustration to aviculturists and veterinarians who, until 1995, had no vaccine available for reducing the spread of this virus. Epizootiologic data suggest that avian polyomavirus is a leading cause of mortality in young psittacine birds (≤ 150 days old), with a reported mortality rate of 10% to 93% in at-risk neonates. Acute infections are characterized by death following a 12-to 48-hour period of clinical changes that may include depression, anorexia, weight loss, delayed crop emptying, regurgitation, diarrhea and subcutaneous hemorrhage. In addition to chicks, adult psittacine birds are readily susceptible to infection, can become ill, and some may



die.

Virus exposure through direct contact with clinically or subclinically infected birds, or through contact with virus contaminated environments, is considered important in the transmission of the environmentally stable polyomavirus. Polyomavirus epornitics have been linked to: 1) inadequate quarantine procedures, 2) virus contaminated nest boxes, 3) virus contaminated incubators, 4) shipment of unvaccinated or incompletely vaccinated birds to brokers or pet retailers, 5) mixing unvaccinated birds from numerous locations and 6) exposing unvaccinated flock residents or neonates to infected birds or a contaminated environment and returning them to the aviary without quarantine.

Until the avian polyomavirus vaccine^b was registered by the USDA, control of polyomavirus epornitics was problematic because of the prevalence of virus activity in psittacine birds and the inherent difficulties in reducing potential exposure to this environmentally stable virus by maintaining closed aviaries, practicing extraordinary hygiene and attempting to detect and isolate transiently-infected birds. Techniques developed at the University of Georgia College of Veterinary Medicine to facilitate this latter task include assays to detect anti-polyomavirus antibodies and a DNA probe test to detect polyomavirus nucleic acid.^a Both types of assays have inherent limitations. Detection of anti-polyomavirus antibodies in a single serum sample merely indicates a previous, and typically transient, infection. Polyomavirus-specific DNA probes^a can be used to detect viral nucleic acid in cloacal swabs, fresh tissues (blood, liver, spleen, etc.), or in environmental samples collected from areas (hospital, nursery, incubators, etc.) that may have been contaminated with the virus. Birds that are DNA probe negative and seronegative could be susceptible to infection.

The strategies for using the avian polyomavirus vaccine are similar to those used to control infectious diseases of other companion animals, like parvovirus in dogs. By vaccinating the adults, the population of birds at-risk for infection is substantially decreased, and the likelihood of a progressive cycle of transmission among the mature birds is reduced. This in turn lessens the chances that the adult population will serve as a source of virus exposure for the neonates. If virus activity in the breeding aviary is reduced through vaccination, then careless avicultural practices (i.e., no quarantine procedures, bringing birds from other aviaries into the nursery, allowing visitors with direct or indirect contact with birds access to the nursery) become the only route by which the virus enters the aviary.

Breeding birds should be vaccinated twice with a two-week interval between vaccinations. It is best to vaccinate breeding birds during the non-breeding season. Recommendations for vaccinating neonates are based on the fact that the older a chick is when it is vaccinated, the more likely it's immune system will respond. It is best to wait until a chick is greater than 35 days of age to initiate the vaccination process; these vaccinates should receive a booster vaccination 2 to 3 weeks later. If



an outbreak is occurring, young birds can be safely vaccinated beginning at 10 to 20 days of age. These birds should receive two additional boosters with a 2- to 3-week interval between doses. In any case, a bird should receive the last vaccination at least 2 weeks prior to leaving the aviary.

Data from experimental and field settings suggest that the inactivated avian polyomavirus vaccine is safe and efficacious in psittacine birds that vary in age, species, and immunologic status. Additionally, vaccination has been shown to be effective and valuable in helping to control polyomavirus epornitics and has not been associated with clinically recognizable adverse reactions even when used in the face of an outbreak.

An expected field efficacy has been established by vaccinating flocks during an outbreak. In 9 flocks, the cumulative mortality rate in at-risk chicks prior to and during the vaccination process was 422 of 1,474 (29%). After the original epornitics were controlled, the cumulative mortality rate in chicks which were vaccinated (all the chicks which died were incompletely vaccinated) and then potentially exposed to polyomavirus was 21 of 2,081 (1%).

While it is not recommended to vaccinate a flock during breeding season, vaccination can be used to help stop an epornitic even while birds are breeding. When vaccinating during an outbreak, it is important that the veterinary staff and aviary personnel exercise extraordinary care to prevent handling and injection procedures from serving as methods of virus transmission from bird to bird. While it is recommended that neonates be at least 35 to 40 days of age before being vaccinated, chicks from flocks experiencing an outbreak can be vaccinated from 10 to 20 days of age.

PACHECO'S DISEASE VIRUS (PDV)

The usual clinical history associated with virulent strains of PDV is for a normal appearing bird to be found dead in its enclosure. If present, clinical signs may include depression; anorexia; diarrhea (which may contain blood); regurgitation; biliverdinuria; sinusitis; central nervous system signs; polydypsia and polyuria; and conjunctivitis. As they manifest the final stages of the disease prior to death, many affected birds may develop neurologic signs in the form of tremors, opisthotonos and violent seizures. Birds exposed to virulent strains of PDV usually develop clinical signs or die within 3 to 10 days after being exposed to the virus. Most psittacine birds affected by PDV die within several hours to 2 days of showing clinical signs, but actual exposure to the virus probably occurred several days earlier. The incubation period, or progression of disease, may vary with different viral strains.

Virus spread depends on many factors - the hygiene in the aviary, the species of exposed birds, the distance between enclosures, the strain of the virus and the condition of the flock. Crowding, poor air circulation, accumulation of excrement and stacking of enclosures increase the likelihood of PDV transmission from infected to susceptible birds. Psittacine birds with clinical signs of Pacheco's disease shed large concentrations of virus in their feces and in



pharyngeal secretions, particularly in the last few days of life.

It is safest to assume that any bird which recovers from a PDV infection is latently infected. Birds that recover from PDV infections may develop low, but detectable, levels of virus-neutralizing antibodies.^a Precipitating antibodies have been shown to develop early during an infection but decrease in abundance within months. A detectable increase in antibodies may occur in some latently infected birds when they are actively shedding the virus. However, a decrease in the antibody level with no detectable increase in viral shedding also has been demonstrated to occur in latently infected birds.

Most birds are infected with PDV after they ingest contaminated excrement. Thus, aviary hygiene is critical in preventing PDV outbreaks. Because clinically affected birds shed large quantities of virus in their feces and respiratory secretions, any bird suspected of having Pacheco's disease should be maintained in strict isolation. There are at least three problems which make control of Pacheco's disease virus through testing impractical: 1. What will one do with serologically positive birds? 2. False negative antibody results are common thus, latently infected birds are frequently not detected. 3. After testing, one has chosen serologically negative birds which would be considered highly susceptible to infection.

Based on the protective nature of experimental vaccines, several inactivated PDV vaccines containing an oil emulsion adjuvant became commercially available in the late 1980s. These vaccines provided good protection against disease, but the oil adjuvant was associated with muscle necrosis in a small number of vaccinates, particularly cockatoos. The vaccine responsible for most of the reported reactions is no longer available. Side effects are minimal when the currently available vaccine is administered subcutaneously and not intramuscularly.

When a Pacheco's disease outbreak is occurring, it is important to treat with acyclovir to provide some immediate resistance to disease and to initiate a vaccination program to provide longer term protection from disease. When vaccinating during an outbreak, the attending clinician must exercise extreme caution to prevent aviary personnel, or the vaccination procedure itself, from spreading the virus from bird to bird.

PSITTACINE BEAK AND FEATHER DISEASE VIRUS (PSITTACINE CIRCOVIRUS - PSCV)

Psittacine beak and feather disease (Pbfd) was first described in various species of cockatoos in the early 1970s. The disease initially was characterized by symmetric feather dystrophy and loss, development of beak deformities and eventual death. In the mid 1980s, it was demonstrated that Pbfd was caused by a previously undescribed virus that represents one of the prototype viruses for the Circoviridae family of viruses. Research data collected from the past 12 years has suggested that the protein structure and genome of the PsCVaffecting psittacine birds (a virus we are now calling PsCV 1) was relatively conserved. However, a variant of psittacine circovirus (a virus we are calling PsCV 2)



has been documented as the cause of feather dystrophy in a group of lories. Sequence analysis confirms that PsCV 2 has sufficient nucleic acid differences that it is not detected using the proprietary nucleic acid primers^a to PsCV 1 developed by the Psittacine Disease Research Group at the UGA College of Veterinary Medicine.

This variant was detected when 9 blood and feather samples from lories with dystrophic feathers were examined. DNA in situ hybridization with a virus specific probe demonstrated that cells associated with affected feathers from all of these lories contained circoviral nucleic acid. Using proprietary PsCV 1 specific primers, PsCV 1 nucleic acid was not detected in the blood of these lories. By comparison, 16 budgerigars with dystrophic feathers confirmed by in situ hybridization to be associated with PsCV were also blood positive for PsCV 1 nucleic acid.

Most birds infected with PsCV 1 develop a transient infection that can be detected by demonstrating target segments of nucleic acid in the blood. The immune response clears the virus in these subclinically infected birds with no detectable affect on the host. The clinical changes that occur in birds that do not mount an effective immune response against PsCV 1 can be peracute, acute or chronic.

In general, PsCV 1 associated disease in Old World psittacine birds is considered progressive and fatal. By comparison, some New World psittacine birds with established PsCV 1 associated disease have been shown to recover. The documentation of PsCV 2 would be academic were it not for the fact that the microscopic lesions in affected lories were less severe than those seen with PsCV 1 and several of the affected lories apparently recovered. It is of interest that two porcine circoviruses have been defined; porcine circovirus 1 (PCV 1) and porcine circovirus 2 (PCV 2). Porcine circovirus 1 is considered of low to no pathogenicity, while PCV 2 causes substantial disease in affected pigs.

Management of birds with dystrophic feathers in which PsCV nucleic acid is detected has been based on data collected by the Psittacine Disease Research Group using proprietary PsCV 1 sequences. Because PsCV 2 appears to behave differently in some birds than PsCV 1, clinicians must be extremely careful to determine whether a DNA probe-based test is documenting PsCV 1, PsCV 2 or some other target sequence that cross-reacts with conserved circovirus sequences. Use of less specific primers (designed to amplify target sequence that may not be specific to PsCV 1) to screen for viral DNA can result in improper recommendations for patients.

If a bird infected with PsCV 2 has a greater chance of recovery compared to those infected with PsCV 1, then euthanasia of a bird with feather abnormalities associated with PsCV 2 would be a disservice to the individual patient and its species. It should be stressed that the management of the individual patient is different from that of the flock and diseased birds must be completely separated from others (see Figure 1). Birds that are able to recover from PsCV 2 associated disease may transfer the factors responsible for their recovery to their chicks, which would be a decided genetic advantage.



Figure 1. Diagnostic flow chart for Psittacine Circovirus

Bird Has Normal Feathers

Test blood for PsCV nucleic acid using DNA probe-based assay

- A positive test in a bird with no feather abnormalities indicates that the bird has been exposed to PsCV and that viral nucleic acid is present in the blood. This bird must be retested in 90 days. If the bird is still positive when retested at 90 days, this indicates that the bird is either subclinically infected or that the bird is being repeatedly exposed to the virus. Subclinically infected birds can develop feather lesions at some future date. If the bird is negative when retested, this indicates that the bird was transiently infected and that the bird's immune system was able to clear the viral nucleic acid from the blood. Birds with normal feathers that have cleared an infection should be considered resistant to PBFD. Most birds that are exposed to the PsCV will have viral nucleic acid present in their blood for a brief period.

- A negative test indicates that the target segment of PsCV nucleic acid was not detected in the blood.

Bird Has Abnormally Developing Feathers

Submit affected feathers for histologic examination and blood for PsCV 1 nucleic acid detection using DNA probe-based assay

- A positive DNA probe test on the blood of a bird with characteristic inclusion bodies in cells of affected feathers suggests that the bird has an active PsCV 1 infection.

- If the feather biopsy contains characteristic inclusion bodies but the blood DNA probe test is negative for PsCV 1 nucleic acid, then the blood sample should be retested using a less specific circovirus DNA assay.^a Birds that are found to be infected with variants of PsCV other than PsCV 1 should be isolated, not euthanized, and monitored closely for the development of normal pin feathers that would suggest recovery. Birds that are recovering from psittacine beak and feather disease will be blood negative for nucleic acid for months before all of the affected feathers (the cells of which will retain PsCV until molted) are replaced during the molting process with new uninfected feathers. As long as dystrophic feathers, or their associated dust, are present the bird should be considered infectious.

- It should be noted that some PsCV -infected psittacines of South American descent have spontaneously recovered from the disease.

Management of a positive bird:

If a bird **with feather abnormalities** from a breeding aviary is found to be positive for PsCV 1, PsCV 2 or any variant of circovirus, the bird should be removed from the area as quickly as possible. Virus-infected birds with feather abnormalities shed large concentrations of virus in their feather dust which can be easily carried to other birds by the wind or on clothes, skin or hair. All areas, supplies and equipment that could be contaminated with feather dust should be repeatedly cleaned and disinfected.

PROVENTRICULAR DILATATION DISEASE

Proventricular dilatation disease (PDD) is used to describe an inflammatory response characterized by the accumulation of lymphocytes and plasma cells in the nervous system, especially the nerves that



supply the muscles in the proventriculus and other digestive organs including crop, ventriculus and small intestine. The most common clinical signs of PDD include depression, weight loss (with or without decreased appetite), constant or intermittent regurgitation, and/or passage of undigested food in the feces indicating a malabsorptive or maldigestive disorder. Central nervous system signs associated with PDD, which may occur in addition to or independent of gastrointestinal signs, may include ataxia, abnormal head movements, seizures and proprioceptive or motor deficits.

This disease was first discussed in the late 1970's in birds imported into the United States and Germany. Subsequently, an epornitic of this disease has been occurring in psittacine birds in North America and Europe, probably as a result of the widespread importation and shipment of birds to satisfy the demands of the pet trade. In the order Psittaciformes, PDD has been reported in more than 50 species. Suggestive lesions also have been reported in toucans, honey-creepers, canaries, weaver finches, two free-ranging Canada Geese, and captive and free-ranging roseate spoonbills. (Dr. Robert Schmidt, personal communication) The description of PDD in multiple families of birds would suggest that its cause is not restricted to a particular host. It is expected that additional non-psittacine birds will be diagnosed with this disease as improved diagnostic tests are used to accurately detect affected individuals. There is no reference to spontaneous disease in free-ranging psittacine birds; however, there is every reason to assume that these birds would be susceptible. Given the severe nature of PDD and its apparent ability to affect a wide range of bird species, the importation of psittacine birds or their eggs into any region with indigenous Psittaciformes must be considered extremely risky.

Etiology of PDD

Since its initial description in the late 1970's, multiple etiologies have been proposed for the lymphoplasmacytic ganglioneuritis described as PDD. Adenovirus-like particles were demonstrated within intranuclear inclusion bodies in one affected bird. Paramyxovirus-like viral particles were demonstrated within inclusion bodies located in the neural cells of the spinal cord and in visceral nerve ganglia of another bird. Similar inclusion bodies have been described in the nerves of pigeons with paramyxovirus infections. Birds with PDD have been shown to lack detectable levels of antibodies to paramyxovirus (serotypes 1, 2, 3, 4, 6 and 7), Pacheco's disease virus (an avian herpesvirus), avian polyomavirus and avian encephalitis virus. An eastern equine encephalomyelitis (EEE) virus was recovered from neonates with abdominal distention from an aviary with a history of PDD. The disease in these neonates was termed avian viral serositis. This finding was used to suggest that PDD may be caused by EEE virus, even though EEE virus occurs primarily in the eastern portion of the United States, and PDD has been shown to occur throughout the United States, Canada and Europe. Experimental and epizootiologic findings suggests that EEE virus is not the cause of PDD.

A paramyxovirus related to Hitchner B1 was recovered from a bird with PDD. Antibodies to this virus could be detected using an ELISA but antibodies were not detected using standard hemagglutination-inhibition assays used for paramyxoviruses. Experimentally infected African grey parrots either died soon after or inoculation or seroconverted and shed virus with



morphologic characteristics suggestive of paramyxovirus in their excrement.

Using electron microscopy, viruses with morphologic characteristics suggestive of paramyxovirus, enterovirus, coronavirus and reovirus have been detected in tissues, secretions or excretions from birds that have been histologically diagnosed with PDD.

Experimental Induction of PDD

The lymphoplasmacytic ganglioneuritis that characterizes PDD can be experimentally induced by exposing susceptible adult psittacine birds to a tissue homogenate derived from affected birds which contains an enveloped virus approximately 80 nm in diameter. A virus with similar ultrastructural characteristics was recovered from the excrement of a macaw in Europe using macaw embryos to produce a primary cell culture.

Clinical changes in experimentally infected birds varied from acute onset of a combination of central nervous system and gastrointestinal signs, followed by death within 11 days of inoculation, to induction of only gastrointestinal signs that were first noted 3 months after inoculation. Virus particles, ultrastructurally similar to those of the virus in the experimental inoculum, were recovered from the tissues and/or excrement of the experimentally infected birds. None of the contact controls used in this study developed clinical signs of PDD, suggesting that the suspect PDD virus is not readily transmissible or requires a specific route of inoculation that was not favored by the experimental conditions. Alternatively, it could be speculated that the unaffected contact controls were already immune to infection. However, all birds, both those that received the virus containing inoculum and the birds which received the control inoculum were derived from the same initial group of research birds. All of the experimentally infected birds were susceptible and none of the contact control birds developed disease. These findings suggest that a lack of successful transfer of the virus from an infected to an uninfected bird was more likely than some specific immunity in all of the contact control birds.

Diagnosing PDD

Clinical laboratory findings in PDD-affected birds are inconsistent. Survey and contrast radiographs are useful for demonstrating gastric dysfunction in suspect birds. Distention of the proventriculus and increased transit time of barium are common findings in chronically affected birds. The proventriculus of neonates is normally dilated, a condition which should not be misinterpreted as PDD. Ultrasonic examination may be used to demonstrate dilatation and impaction of the proventriculus. Endoscopic examination may show impaction, ulceration and dilatation of the proventriculus. Fluoroscopy has been used to demonstrate reduced gastric motility which can be an indication of PDD.

A presumptive diagnosis of PDD often is based on historical information, clinical signs, and radiographic evidence of proventricular dilatation or dysfunction. However, the presence of characteristic histologic lesions in nervous tissues is necessary for a definitive diagnosis. In most cases, a post-mortem diagnosis is rendered when a complete set of tissues (including proventriculus, ventriculus and brain) are examined microscopically. In some suspect patients, it is possible to obtain a



diagnosis before death by submitting a biopsy of the crop. In one study, histologic evaluation of a crop biopsy correctly diagnosed PDD in (68%) of positive birds and in another study the sensitivity of crop biopsy was 76%. Thus, a positive crop biopsy in a bird with suggestive clinical changes is of diagnostic value, but a negative crop biopsy in a bird with suggestive clinical changes does not rule out PDD. To increase the likelihood of histologic detection of PDD-associated lesions, practitioners should obtain full-thickness crop biopsies containing **at least one large blood vessel** and its associated ganglia. Evaluating step sections of the biopsy sample may also increase the likelihood of detecting segmental lesions.

The presence of lymphoplasmacytic ganglioneuritis and variable clinical signs (GI only, CNS only or GI and CNS) have led several researchers to propose that PDD might be caused by more than one etiologic agent. Our experimental transmission studies have demonstrated that the same virus containing inoculum can cause varying clinical changes even within birds of the same species. Additionally, a morphologically similar virus has been recovered from the tissues or excrement of birds that were diagnosed by crop biopsy with PDD, even though some of these naturally affected birds had predominately CNS signs, some had gastrointestinal signs and some had both. It should be cautioned that PDD should not be diagnosed based on clinical changes or gross lesions, particularly in birds with predominately neurological signs. Paramyxovirus-3 has been isolated from several birds with neurologic signs suspected to be associated with PDD, and serology was effective in documenting these infections (Dr Judy St Leger, personal communication). We suggest that tissues (including the pancreas when paramyxovirus is suspected) from birds that die following a progressive neurologic disease be submitted for virus isolation (available through many state diagnostic laboratories or the Infectious Diseases Laboratory at the UGA College of Veterinary Medicine).

Treatment and Prevention

Proventricular dilatation disease can occur in any aviary despite excellent hygiene, valid quarantine procedures and the absence of new additions to the flock. In some aviaries, numerous cases of PDD will occur simultaneously. In others, several affected birds may die, and the problem seemingly resolves, only to reappear 1 to 2 years later. In other cases, a single bird in a breeding pair may die, with no subsequent losses in the aviary even 4 to 5 years later. It is common for many birds exposed directly or indirectly to an affected bird to remain asymptomatic.

Mates, offspring or siblings of birds that are diagnosed microscopically with PDD should be considered at extra risk of developing the disease; however, they should not be euthanized. Many of the birds that are directly exposed to those with PDD never develop the disease. Until appropriate preventative measures can be developed, it would be prudent to place exposed birds in isolation.

Provided with an easily digested high energy diet, a stress-free environment and treatment for secondary bacterial or fungal infections, affected companion birds can survive for months or years. Any bird with the disease that is being treated should be placed in strict isolation with no direct or indirect contact with other birds. Some birds with clinical changes suggestive of PDD have been reported to recover when provided supportive care. However, a positive diagnosis of



this disease requires the demonstration of microscopic lesions in the nerves and none of the reported recoveries have been in birds confirmed to have PDD. Birds with PDD may stabilize or improve when treated with antiinflammatory agents.

PRODUCTS MENTIONED IN THE TEXT AND REFERENCES

a. Several laboratories offer antigen and antibody tests for chlamydia, Circovirus (formerly PBFD virus), polyomavirus and Pacheco's disease virus including ANTECH, IDEXX, Infectious Diseases Laboratory, University of Georgia, Athens, GA 30602, (706-542-8092) and the Avian/Wildlife Laboratory, University of Miami, Miami, FL 33101 (800-232-1056).

b. Avian Polyomavirus Vaccine, Biomune, Lenexa, KS 913-894-0230.

References available by sending a stamped self-addressed envelope to the author.