

Preventive Medicine

and Screening

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We are all children at heart. When we want something, we want it now and are willing to trust in fate if it means that we can get it now. Many bird owners are like this. They want a bird, so they buy it. They don't necessarily spend the time to research the source of the bird, and often there is little money left over to make sure that their purchase is healthy. Many people buy birds and know very little about them, trusting in the information provided by their friends, the Internet or the pet store. As a result, husbandry-related diseases and infectious disease still remain a critical problem for bird owners and veterinarians.

Preventing the Introduction of Transmissible Diseases

EDUCATION

Preventive medicine is an essential element of avian medicine, aviculture and pet bird ownership. Preventive medicine begins with education. New bird owners should be provided with a basic understanding of nutrition, safe and adequate caging, household hazards, hygiene and bird behavior. Improper nutrition, poor caging, improper sanitation and improper or inadequate socialization can all lead to disease — physical and psychological — that can shorten the bird's life or result in significant lessening of its quality. Similarly, bird owners and potential bird owners must be educated to the risk factors that lead to the introduction of an infectious disease or the acquisition of a bird that is already sick.

The process of educating bird owners and potential bird owners about bird health-related issues works best when it is started before the bird is purchased. It begins at the grass roots level. Aviculturists, pet store owners and their employees have to educate themselves, assure that their stock is healthy and provide accurate information to their clients. Selling a healthy bird and providing the information to keep it healthy will result in a satisfied client and repeat business. Veterinarians also must be proactive. This means educating themselves, other veterinarians, clients and potential clients alike. Speaking to local bird clubs, contributing to newsletters and bird magazines, and being active in local and national avian veterinary organizations are all part of preventive medicine.

ACQUIRING BIRDS

The risk of disease drops dramatically if the person buying a bird researches potential sources of birds prior to purchase. It is reasonable for the buyer to ask a source for references and to request to see the facilities where the bird was raised. Aviculturists who are members of national organizations that promote bird breeding and education of their membership, such as the American Federation of Aviculture, are more likely to have a good preventive medicine plan in place. In the USA, aviculturists can become certified as having the basic elements of a preventive medicine program through the Model Avicultural Program. This or similar certification is a good indication that the aviculturalist is making efforts to produce healthy birds. It also is a positive sign when aviculturists or pet stores have an established relationship with an avian veterinarian. A great contribution to the health of avicultural birds in the USA was the cessation of indiscriminate importation of wild-caught birds. In other countries where the practice of wild bird importation persists, contagious diseases still flourish.

COMBINING DIFFERENT SPECIES OF BIRDS

Risk of disease transmission is increased when birds that originate from different parts of the world are combined. Aviculturists are strongly encouraged to focus on one group of closely related birds rather than raising a wide variety of unrelated species. If many species of birds are to be raised, separating them into different facilities by genus or at least continent of origin may reduce disease problems.

COMPONENTS OF THE PHYSICAL FACILITY³⁰

Any multiple bird facility, including pet stores, should have specifically designated areas including a quarantine area for newly acquired birds, a separate hospital area

for sick birds, the main facility for the breeding birds or pets, a kitchen, and if birds are being hand-raised, a nursery. A clean-up area separate from the kitchen is preferred. Obviously, pet bird owners and small hobbyists may only occasionally need a designated quarantine area, hospital or nursery, whereas large breeding operations may need these facilities all the time. Likewise, the actual physical structure of each component of the aviary will vary enormously. In the home, a bathroom or extra bedroom may serve as a hospital or quarantine area. Large breeding facilities, on the other hand, might have separate buildings for some of these components.

Other elements of aviary design can facilitate or reduce the chances of disease transmission. Outdoor aviaries are practical only in the warmer parts of the country, but they have a number of important advantages. Rain and wind naturally dilute pathogens, and freezing and direct sunlight also can inactivate some pathogens (Chapter 37, Management of Racing Pigeons). On the other hand, birds in outdoor aviaries are more likely to be attacked by raccoons and be exposed to sarcocystis from opossums. Parasites and mosquito-borne diseases also are a risk with outdoor aviaries. The chances of disease transmission can be reduced in indoor collections by making sure there is adequate ventilation and maximal separation of cages. If stocking density is high, especially if cages are stacked on top of each other, then the chances of disease transmission increase dramatically. Pacheco's disease and proventricular dilatation disease (PDD) are examples of diseases that are more likely to occur in an indoor aviary. Aspergillosis is a disease that is more likely to occur in climates with high humidity or indoor collections with poor ventilation.

Movement between these areas should be from the cleanest place to the dirtiest. The kitchen is the cleanest area, followed by the nursery, main collection of birds, hospital and quarantine area. Keeping the traffic flow through the facility to the minimum reduces the risk of disease transfer. The traffic flow can be analyzed by drawing a floor plan of a facility and using a pen to trace movement through it. The more complicated the movement pattern, the more chance for disease spread. It is often the case that a clean area has to be entered several times a day. In large facilities with multiple workers, designating individuals to work in specific areas can solve this problem. When this is not possible, changing clothes or washing up well between areas should be considered. Restricting access to the birds by the public and other bird owners also reduces the risk of disease transmission.

QUARANTINE

Quarantine will be effective only if the basic rules of quarantine are followed. The most important rule of

quarantine is the “all in and all out” rule. One or more birds are brought into quarantine initially and no new birds are allowed into quarantine until the first birds have left. The location of the quarantine facility or area also is important. Just keeping a bird in a separate cage is not good enough. Some degree of distance between the new bird and the previously acquired birds is necessary. A separate building is ideal, but the garage, basement or bedroom also are acceptable.

The proper duration of quarantine is a shifting target that will vary to some degree with each circumstance. The longer the quarantine period, the more likely it is that a disease problem will be recognized before a bird is introduced into the flock. Thirty days is generally the minimum quarantine period recommended for birds, but 45 to 60 days is safer. Some aviculturists who are particularly concerned about PDD may quarantine birds for 6 to 12 months. For the quarantine to be meaningful, it has to apply to all birds entering the collection, even those birds that originated in that facility and are now returning.

Applying quarantine procedures to all birds leaving and returning to the aviary can be onerous, especially for those who show their birds. One solution to this problem is to select birds that will be shown that season and isolate them from the rest of the flock during the show season. At the end of the show season, they can be quarantined for a designated period of time and screened for disease, if necessary, before being reintroduced into the collection.

NEW BIRD EXAMINATIONS

Having every new bird or group of birds acquired examined and possibly screened for disease by an avian veterinarian is a critical element to a preventive medicine program. The extent of testing that might be done with a new bird will vary considerably according to the circumstances. If a bird is going into a home where it will be the only bird, then testing is done to show that the bird is healthy. If the bird is going into a multiple-bird household or aviary, testing is designed not only to make sure that the bird is healthy, but also to determine as best as is possible if it is infected with diseases that could be introduced to a collection.

Preventing Diseases by Common Environmental Pathogens

SANITATION

All environments contain organisms that can potentially

cause disease. These opportunistic organisms, however, cause disease only when they are allowed to reach high concentrations or when other husbandry practices are less than ideal. *Candida albicans*, *Aspergillus* spp., *Pseudomonas* spp. and members of the Enterobacteriaceae are examples of ubiquitous opportunistic pathogens. Diseases caused by these organisms can be greatly minimized with proper hygiene. Whenever possible, food containers should be located so that they are not contaminated with feces. Likewise, water sources should be designed and located to minimize contamination from feces and food dunked in the water. Uneaten perishable foods should be removed from the cage before they have a chance to spoil. Water and food bowls should be cleaned regularly and water sources such as automatic drip systems should be regularly flushed and disinfected. Cages should be designed so they are easily cleaned and fecal and other organic material buildup does not occur.

There are many misconceptions about sanitation and its importance. Many bird owners obsess about it. When considering the degree of sanitation necessary for a pet home or aviary, it should be remembered that birds do not come from a sterile environment and a sterile environment is not the goal. Many bird owners also obsess about transmitting viruses among birds. The best way to keep viruses out is to follow the above guidelines for preventing the introduction of these diseases. If a collection is not infected with viral disease(s), there is no potential for viral transmission within that closed collection.

Much time has been spent discussing which disinfectant is best. This focus on disinfectants ignores the most important part of sanitation, basic cleaning. Organic material must be removed first before any disinfectant can be effective. It is in the organic material that the bacteria can grow, parasite eggs are protected and viruses are at their highest concentration. In aviaries where transmissible diseases are not a problem, cleaning is usually all that is necessary. Studies have shown that one of the best ways to sanitize food and water bowls is to run them through the dishwasher. Syringes and other hand-feeding tools also are readily cleaned and for all practical purposes disinfected in the dishwasher. The dishwasher should be separate from the home kitchen dishwasher. If disinfectants are to be used, they should be used after a surface is cleaned. They work best if they are left in contact with the surface for as long as possible. It is difficult or impossible to disinfect organic material, therefore, it is impossible to disinfect dirt floors and very difficult to disinfect wood surfaces. Disinfectants may be toxic, however, if viral diseases are or have been a problem in an aviary, disinfecting food and water containers and environmental surfaces is indicated. Phenolic

disinfectants and bleach are effective against most viruses and are the only disinfectants that work against viruses that do not have an envelope. Quaternary ammonium and chlorhexidine-based disinfectants are effective only against enveloped viruses.

Testing

Examination and testing of birds has two goals. The first is to make sure the newly acquired bird is healthy and does not have an infectious or non-infectious disease. The second is to make sure the bird is not subclinically infected with a disease that could be transmitted to other birds in the owner's aviary. There are two different types of testing. The first types are non-specific tests that provide general information that suggests a bird is healthy or ill, but do not specifically identify the etiology. The second types of tests are very specific and permit the identification of specific infectious agents. Because we cannot test for all infectious agents, it is common to use both types of tests when evaluating a new bird.

NON-SPECIFIC ASSAYS

The most important diagnostic assays in the broadest manner of speaking are the history, examination of the bird in the cage and the physical exam (see Chapter 6, Maximizing Information from the Physical Examination). However, it is the information derived from this phase of the workup that will lead to the development of a testing plan, and will be important in the interpretation of the results of any diagnostics that may be done.

Common non-specific diagnostic assays that are often included in the new bird exam include the complete blood cell count (CBC), chemistry panel, fecal wet mount and float, and oral and cloacal Gram stains and culture. Plasma electrophoresis and even radiographs are included as part of the new bird exam by some veterinarians. Interpretation of these diagnostic assays is discussed in detail in other chapters and only a few comments will be made about these here.

It has been the experience of the author that the CBC is a very useful tool for screening the new bird. It rarely gives a definitive diagnosis, but if abnormalities in the CBC are found it is a strong indication of an underlying health problem. The plasma electrophoresis also has considerable value, as alterations in this assay are found early in the course of infectious diseases, often before the disease becomes patent.⁶ Fecal wet mounts can reveal *Giardia* spp. infections and are the best way to detect infections with *Macrorhabdus ornithogaster* (megabacterium).¹⁷

The Gram stain and fecal cultures are commonly used

screening tools that have a tremendous potential for abuse. It is generally assumed that parrots should have relatively few bacteria in swabs of their oral cavity and cloaca, and that the predominate bacteria present on these surfaces should be gram-positive rods and cocci. The presence of large numbers of gram-negative bacterial rods, clostridial spores or budding yeasts is considered to be abnormal. It has been the tendency of avian veterinarians to treat birds that have predominately gram-negative flora of these areas. Likewise, when *Pseudomonas* spp. or members of the Enterobacteriaceae such as *E. coli* are cultured, it has been common practice to treat these birds.

Over time, however, it has become clear that this is not the correct approach. The gastrointestinal flora is influenced by many factors. If the bird is not outwardly ill, the presence of the so-called "pathogenic bacteria" is more often a reflection of poor nutrition or other management problems than anything else and does not mean that these organisms are causing this particular bird a problem. In these instances, improving nutrition, hygiene or other management practices will result in the Gram stain becoming normal again without treatment (see Chapter 4, Nutritional Considerations).

The use of aerobic culturing to screen birds also can lead to false impressions. Many of the normal flora found in the bird's gut are anaerobes. When cultures are performed of cloacal swabs and feces, they are generally sent in for aerobic culture. Under these circumstances, only the facultative anaerobes and the aerobic bacteria grow, and the culture becomes skewed toward the smaller numbers of *Pseudomonas* spp. and Enterobacteriaceae that may be present.

The fecal Gram stain should be evaluated in light of the other findings in the individual bird. If the Gram stain is abnormal but the bird is otherwise healthy, management problems may be the underlying cause of the abnormal GI flora. Appropriate corrections to diet and husbandry can then be initiated. After these changes have been implemented for an adequate period (several weeks is commonly employed), a fecal Gram stain should be reexamined. If the bird has diarrhea or is showing other signs of illness and the Gram stain is abnormal, a culture may be indicated. Pending culture results, treatment with appropriate antibiotic, anti-yeast or antifungal therapy should be initiated.

Salmonellosis is a problem that is rarely seen in parrots but is common in other species of birds. Currently, many facilities require that birds be screened for infection with *Salmonella* spp. prior to entry. It is fairly common for otherwise normal birds to have positive fecal cultures. This poses a significant problem, as these birds

Table 21.1 | Diagnostic Assays for Psittacosis⁺

Assay	Sample	Effective in These Species of Birds	Day Positive after Infection	Specifics
Serology*				
Elementary body agglutination	Serum or plasma	Parrots	Approx. 14 days	Negative with successful treatment
Complement fixation	Serum or plasma	All species of birds	Approx. 21 days	May remain positive with successful treatment
Solid phase ELISA	Serum or plasma	Parrots, others?	Unknown	Not available in the USA
PCR*	Whole blood and combined oral and cloacal swab	All species of birds	Oral 5 days, blood 10 days, cloaca 15 days	Rapidly negative after the onset of treatment

+ Commonly affected species include pigeons, doves and parrots (particularly budgerigars & cockatiels).

*A combination of the elementary body agglutination assay and PCR or the complement fixation assay and PCR is more sensitive than either test alone.

cannot be shipped and it is not known if they actually are a health risk to other animals. Little work has been done to determine the success of eliminating intestinal salmonellosis with antimicrobial treatment in exotic birds; it is known that this approach does not work in poultry. Serotyping *Salmonella* isolates may be of some benefit, as certain serotypes are more commonly associated with disease in birds than others. Serology has been an extremely useful tool for the eradication of *S. pullorum* in poultry. Serologic screening for salmonellosis in other species has potential value.¹¹

SPECIFIC ASSAYS

In this day and age, we rely on two important types of diagnostic assays, serologic and polymerase chain reaction (PCR) assays. Serologic assays detect antibodies to specific organisms. PCR assays detect the DNA or RNA of targeted organisms. Serologic assays have the advantage of being generally inexpensive and able to be performed on an easily acquired sample (serum or plasma) that is inexpensively shipped to the laboratory. The disadvantages of serology are that these assays may not become positive until 2 to 3 weeks after infection and may remain positive after the infectious agent is no longer present. Other complicating factors related to serologic assays include non-specific substances in serum that can sometimes cause a virus-neutralizing assay to read positive at high concentrations of serum or plasma, and anti-complementary substances that can invalidate the complement fixation assay. Virus neutralization assays have the disadvantage of requiring that growing cells are always available, and these assays typically take 3 to 5 days to run. Because of the time it takes to set up these assays, they are generally performed only once a week. There are several serological assays that use a secondary antibody that is said to detect all avian immunoglobulins. This type of cross-reactivity is extremely unlikely and this type of assay cannot be recommended.¹⁴

PCR-based assays have the advantages of being extremely sensitive, able to detect pathogens in the early stages of infection, and do not require viable microorganisms to document their presence in the sample. The sensitivity of these assays also is one of their limitations. Contamination

at the sampling site or in the laboratory can give false-positive results. Obtaining a sample that contains the organism and getting it to the laboratory before it degrades also can be a problem for some PCR-based assays. Likewise, inhibitory substances such as antibiotics and the contents of droppings can cause false-negative results.

Not all PCR assays are created equal. Different laboratories offer tests with differing levels of sensitivity. It is important to use a laboratory that has a long history of experience with avian samples.

There are limitations to the sensitivity and specificity of individual infectious agent testing. The practitioner must remember that assays exist for only a select number of the potentially pathologic avian agents. Testing only for specific agents may not yield a diagnosis for an individual sick bird, nor is this testing sufficient to declare an individual bird or a collection free of disease.

PSITTACOSIS

Infection with *Chlamydoiphila psittaci* is common in pet birds. Clinical signs vary from none to a mild respiratory disease to a severe multisystemic, often fatal, disease. Psittacosis is particularly important in avian medicine because it can spread widely before it is recognized and because it is a zoonotic and reportable disease. Clinical signs and traditional diagnostic assays such as hematology, clinical pathology and radiology, while helpful, are generally insufficient to specifically diagnose this disease.

Serology

The elementary body agglutination assay (EBA) is a tried-and-true serologic assay that has been used for the past 10 years. It detects anti-*Chlamydoiphila* IgM. It can detect infected birds within 15 days of infection and generally is positive by the time a bird is showing signs of illness (Table 21.1). Another advantage of this assay is that it becomes negative with successful treatment. It has a few minor limitations. Rarely, a bird may develop signs of disease before the agglutinating antibodies are produced. Also, rarely, a bird with chronic psittacosis may be EBA negative. The EBA works very well for psittacine birds, but may not work in all species, particularly doves

and pigeons. For doves and pigeons, the complement fixation assay (CF) is currently recommended.^{10,20}

The CF test detects anti-*Chlamydoiphila* IgG that is present in blood a few days to a week after anti-*Chlamydoiphila* IgM. Therefore, there is a slight delay between when the EBA and the CF become positive. The CF also may stay positive for an extended period of time after a bird has been successfully treated. To the best of the author's knowledge at this time, only one laboratory offers the CF and EBA[‡]. The CF is a cumbersome test and is not routinely run, so there may be a delay in getting results.

A solid phase enzyme-linked immunoassay (ELISA)[‡] is currently available, although not in the USA. This assay was found to compare favorably to the CF if the serum sample produced a spot as dark or darker than the positive control (J. Grimes, personal communication, 1995). Other serologic assays are offered, but have not been validated by peer-reviewed research.

PCR

PCR testing for psittacosis is another excellent way to identify infected birds. The organism can be detected in swabs of the oral cavity as early as 5 days after infection, in cloacal swabs by 10 days after infection and in the blood by 15 days after infection. The major disadvantage of the PCR is that birds that have been started on treatment before testing may be negative, and cloacal swabs that are heavily contaminated with feces may interfere with this assay. The sensitivity of this assay is improved if both blood and combined oral and cloacal swabs are tested.^{1,8}

No test is 100% sensitive. Therefore, if the greatest degree of sensitivity is sought, the PCR and the EBA and egg inoculation culture or tissue culture could both be performed when screening parrots. PCR can be combined with the CF when screening doves and pigeons.

Chlamydoiphila psittaci infections can occur in almost any species of caged bird. The author recommends testing for this organism in most birds presented for new bird purchase examinations. The author especially recommends testing cockatiels (*Nymphicus hollandicus*) as they can carry this bacterium and not demonstrate clinical signs of disease. The few cases of human infections the author has observed have been acquired from an otherwise healthy pet cockatiel. Pigeons and doves are commonly infected with *C. psittaci* and should be routinely tested.

MYCOBACTERIOSIS

Several mycobacterial species, including *Mycobacterium avium*, *M. genavense*, *M. fortuitum* and *M. tuberculosis*, cause mycobacteriosis in birds. *Mycobacterium avium*

and *M. genavense* cause the majority of avian infections. The time between infection and onset of clinical signs is long, possibly several months. As a result, infected but outwardly healthy birds may be introduced to a collection and infection disseminated widely before it is recognized. Mycobacteriosis is particularly common in captive populations of grey-cheeked parakeets (*Brotogeris pyrrhoptera*), canary-winged parakeets (*B. versicolorus*), *Pionus* spp., canaries (*Serinus canarius*), finches, the red siskin (*Carduelis cuculatta*) and waterfowl. Mycobacterial infections result in a multisystemic but slowly progressive disease that can present with many possible signs. Because signs are rarely specific and infection can remain inapparent for extended periods of time, ancillary diagnostic assays are needed.³³

Detection of the Organism

Many mycobacterial infections colonize the intestinal lamina propria and mycobacteria can be shed in the feces. Acid-fast stains of the feces will reveal the organisms in some cases, but this assay has a very low sensitivity and is of limited value as a screening tool. A PCR assay^{‡‡} is now being offered that can detect mycobacteria in the feces. The major limitation with this assay is that not all birds with avian tuberculosis are actively shedding the organism, or they shed the organism in small numbers or intermittently. A negative result with the PCR assay, therefore, does not rule out the possibility of infection. The technology associated with PCR diagnostics for avian mycobacteriosis is developing rapidly and this assay has significant long-term potential.³³

Serology

Mounting evidence suggests that serology will be an important tool for detecting birds with mycobacteriosis. At the time of this writing, however, serologic assays for mycobacteriosis are still experimental. Successful serologic assays will have to be applicable to all species that are to be tested, and they will have to be able to detect infection with the multiple species of mycobacteria that infect birds. A complement fixation assay was developed to detect mycobacterial antibodies. This assay had the advantage that it did not require species-specific reagents. The complement fixation reaction, however, was very cumbersome and required reagents that had a short shelf life, making it impractical to use. Additionally, culture filtrate was used for this assay and it was necessary to run multiple tests with antigens from each specific serotype of *M. avium* in order to detect all of the infected birds.¹⁸

Indirect ELISAs have been used successfully to detect antimycobacterial antibodies in waterfowl and quail. The indirect ELISA, however, requires a specific secondary

antibody, limiting its usefulness to closely related species. A blocking ELISA has been developed that circumvents the need for a specific secondary antibody. This assay has been tested in canaries, white-winged wood ducks and quail with known or suspected *M. avium* infections. At this point, there has been good correlation between infected birds and birds that are positive with this assay. Protoplasmic antigen from one serotype of *M. avium* was found to cross-react with all other serotypes tested. However, early work suggests that antibodies to other species of mycobacteria can be detected only if their specific antigen is used.³⁴

The immunological response of the host also may complicate the interpretation of serologic assays for mycobacteriosis. The complement fixation assay was used to screen ring-necked turtledoves for infection. The wild-type birds in this collection were all antibody positive, but the majority of the birds that had the white color mutation were seronegative. It is not known if the failure of an antibody response in these birds is limited to this particular color mutation or also may occur in other species and color mutations of birds¹⁸ (see Chapter 28, Implications of Mycobacteria in Clinical Disorders).

MYCOPLASMOSIS

Mycoplasmosis is a common disease of pigeons and poultry, but occurs infrequently in companion birds with the possible exception of cockatiels. The disease in pigeons and companion birds is characterized by conjunctivitis and upper respiratory signs, and less frequently pneumonia. Distention of the infraorbital sinus with fluid or purulent material is common. PCR assays for *Mycoplasma* spp. have been developed and are offered by many diagnostic laboratories. Some of these assays will amplify DNA from all mycoplasmas, so that a *Mycoplasma* sp. infecting a parrot could be detected even if it was not one of the common poultry pathogens. The disadvantage of this assay is that just because mycoplasma is present in a lesion, it is not conclusive proof that it is the cause of disease.

ASPERGILLOSIS

Aspergillosis is an infection of the respiratory system that occurs sporadically in a wide range of birds (see Chapter 29, Implications of Mycoses in Clinical Disorders). Birds from cold and dry climates are highly susceptible to infection. Environments that are conducive to the environmental growth of *Aspergillus* spp. and environments that are poorly ventilated will result in an increased incidence of aspergillosis. Disease can be localized to the upper airways or the syrinx, or it may involve the air sacs and lungs. Respiratory signs are a common feature of this disease, but a bird may not manifest signs until the

disease is advanced. Radiographs, endoscopy and biopsy, cytology and hematology are all valuable tools in the diagnosis of this disease. Even with all these assays, the diagnosis of aspergillosis is often a difficult one.

The diagnosis of aspergillosis has been most extensively studied in humans. Ancillary diagnostic assays used in people include PCR to detect *Aspergillus* DNA from blood, an ELISA to detect *Aspergillus* antigen and an ELISA to detect anti-*Aspergillus* antibody. These studies clearly indicate that even a combination of these three assays will not be adequate to detect many cases of aspergillosis.^{4,5} The problem comes from the fact that most people who contract aspergillosis are immunocompromised. This also may be true in birds. If the infected person's immune system is adequate to contain the disease and the organism is localized in a walled-off granuloma, then these individuals are found to produce antibody. People with generalized disease are generally severely immunocompromised and they do not produce antibody. In these people, *Aspergillus* antigen and DNA are most likely to be found in the blood, but they are not when the lesion is encapsulated. If the pathophysiology of avian aspergillosis resembles that seen in humans, then none of these assays are likely to detect infection in most infected birds. A combination of these assays may be more specific, but false negatives are to be expected.

Serology

Extended efforts have been undertaken to develop a serologic assay for birds infected with *Aspergillus* spp. This work was pioneered at The Minnesota Raptor Center, which currently offers an ELISA assay for the detection of anti-*Aspergillus* antibodies²³. Differing secondary antibodies are used in this assay, depending on the species of bird to be tested. Using well-defined clinical case material, the assay has been validated for use in several species of Falconiformes, but as designed does not work in owls. Immune suppression appears to accompany aspergillosis in raptors. Prior to treatment, many raptors have little or no detectable antibody. Successful treatment results in a subsequent rise followed by a decline in antibody titers. A failure of antibody titers to rise with treatment or an increase in the titers without the expected decrease is considered to be a poor prognostic sign. Thus a medium to high positive antibody titer is highly suggestive of disease. However, negative to low positive results are inconclusive.²⁴ Similar results were found in penguins with aspergillosis.²⁵ Birds with high antibody titers, high beta and gamma globulins, and albumen concentrations above 1.8 g/dl had a favorable prognosis. In contrast, birds with low or undetectable antibody titers and albumin levels below 1.8 g/dl had a poor prognosis.

The accuracy of available serologic and antigen capture assays for the diagnosis of aspergillosis in parrots has been inadequately studied. In one study, a commercially available ELISA for anti-*Aspergillus* antibodies and antigen capture assay^{***} was evaluated in seven birds with confirmed aspergillosis. Of these birds, only one was found to be weakly positive with serology and three birds were positive, two weakly, with the antigen detection assay, suggesting that either parrots in this study did not make anti-*Aspergillus* antibodies and had little circulating antigen, or that these assays were not sensitive.¹⁵ A second study of ten birds found a higher percentage of sero- and antigen-positive birds; however, in neither study were non-infected birds tested, so the specificity of these assays remains to be determined.¹⁶ Currently, the author does not recommend using any of the available *Aspergillus* assays for routine screening of parrots.

PSITTACINE BEAK AND FEATHER DISEASE VIRUS (PBFDV)

PBFDV is a common infection of wild birds in Australia. In the USA and possibly elsewhere, this virus is enzootic in many lovebird collections and also is seen to a lesser degree in budgerigar aviaries. Disease is seen in many species of parrots, including African grey parrots, lovebirds, budgerigars, lorries, lorikeets, eclectus parrots and cockatoos. Infection also occurs in Neotropical parrots, but disease is rare and infections are transient in most cases.

The sequence of the PBFDV's genome varies up to 16% between isolates. This has diagnostic significance, as PCR primers have to be designed in the conserved region of this virus (ORF1) if they are to detect all variants of this virus.^{3,37} Recently, a specific variant of PBFDV has been recognized in collections of lorries in the USA. It also is reported to occur in lovebirds.²⁶ Its sequence and its relationship to previously published sequences of the PBFDV have not been reported. PCR primers derived from the ORF1 also can detect this variant. Primers also have been designed to differentiate it from other PBFDV's. Lorries with this infection may remain viremic for 6 months or more without showing clinical signs. Lorries that develop clinical signs often die, but some will recover.³¹

Serology

Birds that become infected with PBFDV but do not develop disease have high antibody titers. Birds that do develop disease have low antibody titers or no antibody at all. A hemagglutination assay has been developed to detect serum antibodies to PBFDV. Serum antibody has been detected within 1 to 2 weeks of exposure. This assay has been effectively used to study the prevalence of PBFDV infections in wild Australian parrots. Because

PBFDV agglutinates only red blood cells from a few species of cockatoos, this assay is not practical outside of Australia.²³

PCR

Birds become viremic 7 to 14 days after infection with PBFDV. If the birds are unable to mount an appropriate immune response they will remain viremic. If they do mount an appropriate immune response they cease to be viremic. Virus, however, may persist in the feathers and possibly the skin, so that these birds are a potential source of infection until their next molt. PCR is done on heparinized blood.⁷ If multiple birds are to be sampled, care must be taken to prevent contamination between samples. In most circumstances, birds that are PCR positive and have clinical signs of disease will remain positive and are likely to die from their infection. Birds that are positive but are not showing signs of disease should be retested in 3 months. If they are negative at that time they are thought to be cured. Rarely, lorries, lovebirds and occasionally other species of parrots will develop clinical disease, but will then recover and become virus negative.

It has been suggested that it is important to differentiate between the lorry variant of PBFDV and the other variants. The author does not agree with this conclusion. Although the lorry variant may behave somewhat differently than other PBFDV variants, it is still pathogenic, so the significance of a positive test is the same in a lorry or any other parrot species, regardless of the variant.³¹

PBFDV infection in lovebirds (*Agapornis* spp.) may not follow the same patterns as seen in other parrots. PBFDV is widespread in commercial lovebird collections, but disease is rare. It is the author's impression that virus shedding may persist more than 3 months in birds that never show signs of disease.²⁶

AVIAN POLYOMAVIRUS (APV)

APV is a common infection of a wide range of parrots. APV causes morbidity and mortality in nestling budgerigars (*Melopsittacus undulatus*), Indian ring-necked parakeets (*Psittacula krameri*), lovebirds and many parrots. Disease is less common in nestlings of other Old World parrots. Nestling budgerigars in aviaries with enzootic APV become viremic within 7 days of hatch and are serologically positive by 10 days after hatch. If they survive infection, fecal shedding may persist for 6 months or longer, but ceases at some point after the birds become sexually mature. Although they stop shedding virus, infected budgerigars will remain seropositive for life.^{15,16} Nestling parrots of other species become viremic within 2 weeks of infection. They also develop virus-neutralizing antibody at

approximately 14 days postinfection. Antibody titers in most species of nestlings that survive infection are maintained for 10 or more years and possibly for life. Viremia persists for 6 to 8 weeks in most cases. Fecal shedding begins shortly after the onset of viremia, but persists for as long as 12 to 16 weeks.²¹ In rare cases, viremia and fecal virus shedding may persist for more than 10 months.⁷ The duration of viremia and virus shedding in adult birds infected with APV has been studied in only a limited number of birds. However, it appears that viremia and virus shedding occurs only briefly in mature birds or not at all.²¹ Viremia and virus shedding also are significantly impacted by concurrent infections with PBFDV. Birds with co-infections appear to shed APV continuously and may never clear the virus.¹⁶

Serology

An excellent virus-neutralizing (VN) assay has been developed to detect antibodies that neutralize APV. In this assay, virus is first incubated with two-fold dilutions of serum or heparinized plasma. The virus-plasma mixtures are then incubated with chicken embryo fibroblasts, the fibroblasts are washed and the cells are monitored for cytopathic effects (CPE). If CPE do occur at the *highest* concentration of serum, then the bird did not have neutralizing antibody. If virus growth is inhibited and CPE do not occur, then the bird did have neutralizing antibody. In the author's hands, this assay takes 5 days to complete.¹⁵

Use of the APV VN

Parrots infected with APV may begin shedding virus prior to seroconversion and maintain high antibody titers many years after they stop shedding virus. Therefore, the serologic status of a bird is not a good indicator of virus shedding. Sensitive PCR assays should be used in place of serology to detect virus-shedding birds. The APV VN has some limited value in epidemiologic studies and could be used to determine if APV had ever been in a collection. Under these circumstances the immunization status of the birds should be considered. Nestlings do not produce virus-neutralizing antibody to the commercial APV vaccine. Therefore, antibody-positive nestlings have been infected with APV. Adult parrots do develop neutralizing antibody following immunization, but their antibody titers are typically low compared to those seen in birds that survived infection.¹⁹

Use of the APV PCR

Viremia may precede virus shedding and virus shedding continues after the cessation of viremia; therefore, combined oral and cloacal swabs and heparinized blood should be submitted for PCR analysis. If blood is tested alone, many virus-shedding birds will be missed. It

appears that all species of parrots have the potential to become infected with and shed APV, so all birds that are going into an aviary where they might expose nestlings should be tested. Nestlings that survive an outbreak of APV are assumed to be shedding virus. Therefore, testing birds 4 months after the outbreak when virus shedding should have stopped, rather than immediately after the outbreak when virus shedding is expected, best uses the owner's resources.²¹

Testing birds older than 16 weeks that are going into a single-bird household is of questionable value. If they test positive and are not sick, they will shed transiently and stop shedding. If a bird is positive at 16 weeks, it has already been infected and will not generally become clinically ill. It will continue to shed for some time and it should be isolated from other birds.

The author seriously doubts that the veterinarian will be able to detect an infected bird that will subsequently come down with disease as, in his experience, the onset of viremia and the onset of disease occur very close together.

When the value of testing blood was first recognized, it was suggested that PCR of blood detected only fragments of DNA and that a positive did not reflect the true infection status of the bird. It also was suggested that immunized birds that were blood PCR positive were positive because of DNA present in the vaccine. Both these assumptions have been proven to be false. Therefore, if a bird is positive by blood PCR, it is infected with APV.¹⁹ If they test positive and do not have APV disease, they will shed transiently and then stop shedding.

PSITTACID HERPESVIRUSES (PsHVs) OR PACHECO'S DISEASE VIRUSES

PshVs are the causative agent of Pacheco's disease. Pacheco's disease occurs in sporadic outbreaks in newly formed and long-established parrot collections. Losses can range from a single bird up to hundreds of birds. Generally, birds that develop clinical Pacheco's disease die. There are four major genotypes of PshVs. All are capable of causing Pacheco's disease and genotypes 1, 2 and 3 are capable of causing internal papillomas.^{32,35,36} Outbreaks of Pacheco's disease occur when carrier birds expose naïve birds. The dynamics of each outbreak will depend on the genotype of the virus and the species of birds involved. In some collections, Pacheco's disease will not occur, but over time multiple birds will develop papillomas.

Macaws, Amazon parrots and some species of conures are most likely to be carriers of PshVs. Infection prevalence appears to be higher in imported wild-caught birds. Infection also has been recognized in cockatoos

and African grey parrots, and under some circumstances it also may occur in lovebirds and cockatiels. The list of potential carrier species likely will grow as more is learned about these viruses. Any bird that survives an outbreak of Pacheco's disease should be considered infected until shown otherwise. Mounting evidence suggests that parent-to-offspring transmission occurs. The offspring may remain asymptomatic or develop internal papillomatosis, depending on the genotype of the virus. It appears that once a bird is infected they will be infected for life. This includes survivors of Pacheco's disease that were treated with acyclovir.

The key to preventing Pacheco's outbreaks and internal papillomatosis is keeping carrier birds out of the collection or, if they are already in the collection, isolating them from birds that are not infected. Studies to date show that PsHVs in carrier birds are present in significant concentrations in the mucous membranes of the cloaca and oral mucosa. Swabs of these surfaces can be tested by PCR. Virus also may be detected in blood, but concentrations of virus are low in the blood and in one study blood PCR was inconsistently positive, while mucosal swabs were more dependably positive. In rare individuals, birds have been identified that are only blood positive. The biological significance of this is not known; until it is, it is recommended that both blood and combined oral and cloacal swabs be used for PsHV PCR.²²

PCR

Recently discovered sequence data has permitted the development of a single PCR assay that can detect all four genotypes of the PsHV (R. Dahlhausen, personal communication, 2003). Preliminary work with less ideal primer sets suggests that PCR of blood and a combined oral and cloacal swab will detect the majority of birds unapparently infected with PsHVs.

Serology

There are three major serogroups of the PsHV.⁹ Serotype 1 contains genotypes 1 and 4, serotype 2 contains genotype 2 and serotype 3 contains genotype 3.³⁶ It is clear that many birds that are infected with PsHVs are seropositive. It still remains to be determined, however, if all birds infected with PsHVs will demonstrate positive serologic results. Preliminary evidence suggests that antibodies to one serotype inconsistently neutralize viruses of other serotypes.²² Therefore, if serology is to be used to detect PsHV-infected birds, multiple assays using all three viruses or their antigens will have to be run.

PARAMYXOVIRUS 3 (PMV-3)

PMV-3 is a common cause of disease in the Australian grass parakeets (*Neophema* spp.). Clinical signs are vari-

able and include central nervous system disease, respiratory signs, diarrhea and signs of pancreatic insufficiency.^{28,29} In a recent report of an outbreak of PMV-3 in a pet store, the hemagglutination inhibition assay (HI) was found to be a sensitive means of detecting infected birds.¹⁴ Others have tried serologic methods for detecting subclinical infections of PMV-3 in *Neophema* and other species with little success.^{12,29} This discrepancy may be due to the duration of the infection at the time serology is performed. In a recent study with PMV-1, low levels of antibody were detected in African grey parrots. These antibodies were detectable with an experimental ELISA, but not with the HI. This assay is currently under development and may prove useful in the future.¹⁴ In disease outbreaks where PMV-3 is suspected, submission of proper samples for histopathology is currently the most accurate method of confirmation.

Applied Preventive Medicine

TESTING NEWLY ACQUIRED BIRDS

The ultimate decision as to what type of testing should be done for a particular bird will depend on the specific details regarding the source of the bird, species of the bird, the aviary or home into which it is going, the resources of the owner and findings on physical examination. Non-specific assays such as CBC, oral and fecal Gram stain, protein electrophoresis, fecal wet mount and fecal floatation can be applied to all birds. (*Ed. Note: In some practitioners' experience, a negative fecal floatation has not correlated with the absence of intestinal parasites*). Ascarids are commonly expelled from birds, especially those with previous exposure to warm, outdoor environments, following the administration of an appropriate anthelmintic. This occurs in birds with negative fecal floatations, and routine deworming may be advised in these situations (M. Wissman, personal communication, 2002).

Fecal and oral cultures are indicated if abnormalities are found on the Gram stains and birds show other evidence of illness. Chemistry panels are most likely to identify problems in older or unthrifty birds, but can be useful in detecting early disease or establishing baselines for future reference, even in clinically healthy individuals. Radiographs are relatively costly tests that can be used for screening. Generally, however, they are used only when there is some other indication of disease. Currently, the choice of serologic and PCR-based testing is best tailored to the species and background of the bird being examined (**Table 21.2 and Table 21.1**).

Table 21.2 | Diagnostic Tests Used to Screen for Specific Infectious Diseases

Infectious Agent	Assay	Sample for Testing	Species Commonly Infected	Sensitivity	Specificity
Mycobacteria	PCR	Feces	<i>Brotogeris</i> spp., canaries, finches, red siskins, waterfowl	Fair	Good
	Serology	Serum or plasma		Experimental	Experimental
<i>M. ornithogaster</i>	Wet mount	Feces	Budgerigars, finches, cockatiels, parrotlets, lovebirds, lories, other	Fair to poor	Good if many organisms present
PBFDV	PCR	Blood*	Old World parrots	Excellent	Excellent
APV	PCR	Blood and swabs**	Lovebirds, budgerigars, all parrots recently exposed to other birds	Excellent	Excellent
	Serology	Serum or plasma		Proof of previous or current infection	Does not reflect virus-shedding status
PsHV	PCR	Blood and swabs	Macaws, Amazon parrots, conures, others?	Excellent	Excellent
	Serology	Serum or plasma		Unknown	Unknown
PMV-3	HI+	Serum or plasma	<i>Neophema</i> spp., others?	Questionable in chronic infection	Good
	ELISA++	Serum or plasma		Experimental	Experimental

*Heparinized blood

+ HI: Hemoagglutination inhibition

**Combined oral and cloacal swab

++ ELISA: Enzyme-linked immunoassay

There is a saying: “Be careful for what you look for, because you may find it.” This is particularly applicable to testing new birds. It is incumbent upon practitioners to know everything that they can about the tests they are using so that if one does come back positive, it can be properly interpreted. It also is important to correlate test results with the entire clinical picture. If the testing results don’t make sense, then repeat those assays or have them performed by a different laboratory.

PREVENTIVE MEDICINE AND THE VETERINARY HOSPITAL

It is a common practice for veterinarians to board birds. There is no doubt that this is a valuable service to the veterinarian’s clients, but it also poses challenges for the prevention of disease transmission. The greatest risk occurs if birds of uncertain infection status are housed in the same room. If all birds are screened for PBFDV, APV, PsHVs and *Chlamydophila psittaci* before they are allowed to board, then the risk of disease is diminished. There is no test for birds that have the etiologic agent of proventricular dilatation disease, however, so the transmission of this disease cannot be prevented. Other strategies for preventing disease transmission would be to keep birds in isolettes or to house birds separately in different parts of the hospital.

Veterinarians see sick birds and therefore will have birds with infectious diseases in their hospital. A protocol should be developed for every hospital for routine cleaning of the exam, treatment and hospital rooms and caging. Routine PCR testing of swabs of these environments can be used to determine if the cleaning is effective. Boarding birds should be housed separately from hospitalized birds.

PREVENTIVE MEDICINE AND BIRD MARTS

A common way for aviculturists to sell their birds is to bring them to bird marts or bird fairs that are sponsored

by local bird clubs. These marts serve many valuable purposes. They provide an important outlet for the sale of birds and at the same time raise money for the sponsoring organizations. This money is used to help support the bird club and in many cases to fund research and conservation efforts. The bringing together of birds from multiple premises into a confined area and the handling of these birds by the general public, however, results in the ideal opportunity for disease spread, the most common of which is APV.

There are preventive measures that can be taken that will help to mitigate disease transmission at bird marts. The most important is to limit sale of birds to those that are completely weaned. Weaned birds will rarely, if ever, develop APV disease although they are still susceptible to infection. Birds that are taken to a bird mart but not sold should be quarantined away from the rest of the breeder’s birds until they can be sold. A policy of not letting anyone handle birds unless they have bought them also will reduce the spread of disease. Finally, cages made of clear, hard plastic panels can be used to display birds that are for sale. Ideally, these cages would have a fan in the back that draws air out of the cage and a Hepa filter in the front to filter out potential pathogens. Even without these fans, cages made from clear plastic panels are much better than wire cages. If nestlings are allowed at bird marts, then they should be confined to brooders or cages made from this material and taken out to the car or hotel room for feeding. Nestlings that are not sold must go into quarantine after the show.

PREVENTIVE MEDICINE IN THE PET STORE

Pet store owners and managers who intend to sell birds first need to consider what market it is that they wish to reach. Budgerigars, cockatiels, lovebirds, canaries and finches appeal to one type of customer and come with their own significant disease problems. The larger species

of birds appeal to other types of customers. Combining these birds can lead to additional health problems.

It has been a common practice in the USA for individual producers of cockatiels, lovebirds, budgerigars and finches to sell their birds to buyers who combine birds from multiple sources and ship them to other sellers who distribute them to pet stores. This practice maximizes the potential for disease transmission. Cockatiels supplied in this manner have a high incidence of psittacosis. Similarly, lovebirds and budgerigars from these sources are commonly infected with APV, and lovebirds are commonly infected with the PBFDV. When infected birds are mixed with birds from clean collections, disease transmission is likely. When these birds come into a pet store, not only may they be unhealthy, but they also are an important source of infection for other parrots whose retail value may be much higher. The classic example of this is APV outbreaks that occur in nestling macaws, conures and eclectus parrots 2 weeks after they are brought into a pet store. The tendency in these circumstances is to blame the breeder who supplied the nestlings that died, but the problem lies with the budgerigars and lovebirds in the store that are actively shedding virus and that fatally exposed these birds after they entered the store.

It has been the author's experience that pet stores have healthier stock if they establish a relationship with one or more local breeders and buy birds directly from them. If the local breeder can see the possibility of a sustained market, they are more willing to spend money to verify that their flock does not contain the common diseases that can cause so many problems in the pet store. Aviaries that supply birds to stores can be screened for infectious diseases by environmental testing or testing a random selection of birds. The specific types of screening tests should be tailored to the type of birds being purchased, and this protocol is best done with the assistance of an avian veterinarian. Subsequently, if appropriate biosecurity measures are maintained, the pet store owners can feel assured that they are buying clean stock. This requires some initial investment, but this investment is spread out over many birds and is well worth it.

Other management techniques can be used to minimize the risk of disease. First and foremost, a relationship should be established with an avian veterinarian. The veterinarian's role is to provide advice that will help minimize the risk of disease, but at the same time will not result in huge expenditures. A general rule is that any change should increase the pet store owner's profit. If it does not, then another approach should be taken.

The two diseases that can substantially impact the pet store are APV and psittacosis. APV is predominately a dis-

ease of nestling parrots. Lovebirds and budgerigars from many sources may shed this virus. The risk of APV disease can be greatly reduced if stores buy only weaned birds. Alternately, some stores may choose not to sell the smaller species of birds. If nestlings are to be present in the store, all the sources of all birds brought into the store need to be screened for APV. Setting up a separate bird room that the public can look into but may enter only with supervision will help to keep customers from bringing disease into the store. If a customer wants to see a bird, they may be required to put on a clean smock and gloves and possibly even dip their feet in a foot bath before entry into the bird room. If economics require that nestlings that are still being hand-fed be purchased, an alternate approach to keeping them healthy is to raise and wean them in isolation away from the store.

Psittacosis is very common in cockatiels and can occur in any species of parrot. It can cause widespread disease in pet store birds, requires a long treatment period, is a reportable disease in most areas and is transmissible to people. All sources of birds should be tested for this disease.

Quarantine is another element of the preventive medicine that can provide important dividends to the pet store. All birds coming into the store should be isolated for some period of time before they are mixed with other birds in the store. If the incoming birds have been exposed to disease, it is likely that they will begin showing signs during the quarantine period. It has been common practice for some distributors to treat some species of birds with tetracyclines for variable lengths of time before they are sold. This can mask signs of psittacosis but may not cure the birds. Once the birds are off medication signs will often reappear. Money is a factor in any preventive health plan and a careful balance must be established between cost of preventive medicine and its benefits. Careful consideration should be taken so that all preventive measures have a clear economic benefit.

Finally, if preventive measures are undertaken, the public should be made aware of what is being done and birds should be sold as value-added products. For instance, if extensive efforts are undertaken to acquire polyomavirus-free birds, then these birds should be advertised as such. When the consumer sees that one store is concerned about infectious diseases and others do not place similar emphasis on them, the consumer will buy from that store, even if the cost may be somewhat higher.

IMMUNIZATION AND PREVENTIVE MEDICINE

Immunization for poxvirus, paramyxovirus-1 and salmonella can be important elements of disease control in rac-

ing pigeons. Poxvirus immunization also is advised for canaries that are raised outdoors. The current parrot herpesvirus vaccine in the USA is a monovalent vaccine. The exact serotype present in the current vaccine is not known at the time of this writing. It is expected that this vaccine will protect against the serotype from which it is derived. It is not known, however, if this vaccine will protect against other serotypes. In collections of birds where there is a high risk of Pacheco's disease, use of this vaccine may be indicated. A polyvalent vaccine that would protect against infection with the three common serotypes may someday be developed and could potentially protect against Pacheco's disease and internal papillomatosis.

The value of the equine West Nile virus vaccine in birds remains to be proven. The author was not aware of adverse reactions to the vaccine the first year that it was used. However, a hemolytic anemia has been reported in lories that were immunized a year after the first set of immunizations. This problem, however, has not been seen in another collection of birds immunized 2 years in a row. Given that the current information about the

potential value and potential risks of the West Nile virus vaccine is minimal, it should probably be used only as a last resort. Screening in the enclosure of high-risk birds and other mosquito control programs may be the safest ways to prevent disease from the West Nile virus.

A discussion of the avian polyomavirus vaccine is included in Chapter 32, Implications of Viruses in Clinical Disorders. The author believes that management practices are critical to the control of avian polyomavirus, and that there are few circumstances where immunization would be helpful in its control.

Product Mentioned in the Text

a. Immucomb, Biologae Laboratories, Kibbutz Baled, Israel

Resources

- ‡ Texas Veterinary Medical Diagnostic Laboratory, PO Drawer 3040, College Station, TX 77841, 979-845-3414
- ‡‡ Dr. Carlene Emerson, Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-7040
- ‡‡‡ The Raptor Center, 1920 Fitch Ave, Saint Paul, MN 55108, 612-624-4969
- ‡‡‡‡ Avian and Wildlife Laboratory, University of Miami School of Medicine, Miami, FL

References and Suggested Reading

1. Andersen AA: Comparison of pharyngeal, fecal, and cloacal samples for the isolation of *Chlamydia psittaci* from experimentally infected cockatiels and turkeys. *J Vet Diagn Invest* 8:448-450, 1996.
2. Baghian A, et al: Production of a rabbit anti-cockatiel immunoglobulin G and characterization of its cross-reactivities with immunoglobulin G of other psittacine species. *Avian Dis* 43:48-54, 1999.
3. Bassami MR, et al: Genetic diversity of beak and feather disease virus detected in psittacine species in Australia. *Virology* 20:392-400, 2001.
4. Chan C, et al: Detection of antibodies specific to an antigenic cell wall galactomannoprotein for serodiagnosis of *Aspergillus fumigatus* aspergillosis. *J Clin Micro* 40:2041-2045, 2002.
5. Costa C, et al: Real-time PCR coupled with automated DNA extraction and detection of galactomannan antigen in serum by enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis. *J Clin Micro* 40:2224-2227, 2002.
6. Cray C: Plasma protein electrophoresis: An update. *Proc Assoc Avian Vet*, 1997, pp 209-211.
7. Dahlhausen B, Radabaugh CS: Update on psittacine beak and feather disease and avian polyomavirus testing. *Proc Assoc Avian Vet*, 1993, pp 5-7.
8. Dahlhausen B, Radabaugh CS: Detection of *Chlamydia psittaci* infection in pet birds using a molecular-based diagnostic assay. *Proc Assoc Avian Vet*, 1997, pp 191-198.
9. Gravendyck M, et al: Antigenic diversity of psittacine herpesvirus: Cluster analysis of antigenic differences obtained from cross-neutralization test. *Avian Pathol* 25:345-357, 1996.
10. Grimes JE: Evaluation and interpretation of serologic responses in psittacine birds to chlamydiosis and suggested complementary diagnostic procedures. *J Avian Med Surg* 10:75-83, 1996.
11. Grimes JE, Arzmeidi F: *Salmonella typhimurium* agglutinins in exotic bird sera in the USA. *J Vet Diagn Invest* 7:270-274, 1995.
12. Grund C, Grimm F, Kosters J: Serological studies on persistent PMV-1 infection associated with PDD. *Proc Assoc Avian Vet*, 1999, pp 19-23.
13. Ivey ES: Serologic and plasma protein electrophoretic findings in 7 psittacine birds with aspergillosis. *J Avian Med Surg* 14:103-106, 2000.
14. Loudis BG: PMV-3 outbreak: Presentation, diagnosis and management. *Proc Assoc Avian Vet*, 1999, pp 223-227.
15. Phalen DN: Viruses. In Altman RB, et al (eds): *Avian Medicine and Surgery*. Philadelphia, WB Saunders Co, 1997, pp 281-322.
16. Phalen DN: Avian polyomavirus: My thoughts. *Am Fed Avicul Watchbird* 25:28-39, 1998.
17. Phalen DN, Tomaszewski E, Davis A: Investigation into the detection, treatment, and pathogenicity of avian gastric yeast. *Proc Assoc Avian Vet*, 2002, pp. 49-51.
18. Phalen DN, et al: Serologic diagnosis of mycobacteria infections in birds: A preliminary report. *Proc Assoc Avian Vet*, 1995, pp 65-68.
19. Phalen DN, et al: Avian polyomavirus: More pieces to the puzzle. *Proc Assoc Avian Vet*, 1998, pp 151-156.
20. Phalen DN, et al: Diagnosis of *Chlamydia psittaci* infections in cockatiels and Columbiformes. *Proc Assoc Avian Vet*, 1999, pp 13-17.
21. Phalen DN, et al: Viremia, virus shedding, and antibody response during natural avian polyomavirus infection in parrots. *J Am Vet Med Assoc* 217:32-36, 2000.
22. Phalen DN, et al: Diagnosis of parrots infected with Pacheco's disease viruses. *Proc Assoc Avian Vet*, 2001, pp 87-89.
23. Raidal SR, Cross GM: The haemagglutination spectrum of psittacine beak and feather disease virus. *Avian Pathol* 23:621-630, 1994.
24. Redig PT, Orosz S, Cray C: The ELISA as a management guide for aspergillosis in raptors. *Proc Assoc Avian Vet*, 1994, pp 99-104.
25. Reidarson TH, McBain J: Serum protein electrophoresis and *Aspergillus* antibody titers as an aid to diagnosis of aspergillosis in penguins. *Proc Assoc Avian Vet*, 1995, pp 61-64.
26. Ritchie BW, et al: Documentation of a PBFD virus variant in lories. *Proc Assoc Avian Vet*, 2000, pp 263-268.
27. Romagnano A, et al: Aspergillosis testing: Comparison of serological data. *Proc Assoc Avian Vet*, 2002, pp 139-143.
28. Shihmanter EY, et al: Avian paramyxovirus serotype 3 isolated from captive birds in Israel: Clinical signs, pathology and antigenic characterization. *Avian Dis* 42:418-422, 1998.
29. Speer BL: A clinical look at the avian pancreas in health and disease. *Proc Assoc Avian Vet*, 1998, pp 57-64.
30. Speer BL: Avicultural medical practice: The nuts and bolts. *Proc Assoc Avian Vet*, 1998, pp 347-355.
31. Strunk A, et al: Pathobiology and testing recommendations for psittacine circovirus 2 in lories. *Proc Assoc Avian Vet*, 2002, pp 45-47.
32. Styles D, Tomaszewski E, Phalen DN: Papillomas. *J Virol*, submitted 2003.
33. Tell LA, Woods L, Cromie RL: Mycobacteriosis in birds. *Rev Sci Tech Off Int Epiz* 20:180-203, 2001.
34. Tell LA, et al: A multifaceted investigation into the diagnosis of mycobacterial infections in birds. *Proc Assoc Avian Vet*, 2002, pp 61-63.
35. Tomaszewski E, et al: Detection and heterogeneity of herpesviruses causing Pacheco's disease in parrots. *J Clin Micro* 39:533-538, 2001.
36. Tomaszewski E, Kaleta EF, Phalen DN: Molecular phylogeny of the psittacid herpesviruses causing Pacheco's disease: Correlation of genotype with phenotypic expression. *J Virol*, submitted 2003.
37. Ypelar I, et al: A universal polymerase chain reaction for the detection of psittacine beak and feather disease virus. *J Clin Micro* 16:141-8, 1999.

