CHAPTER
32

Implications of Viruses in Clinical Disorders

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The past 15 years have seen remarkable growth in the understanding of the viral diseases of companion and aviculture birds. Molecular-based and traditional investigative and diagnostic tools allowed scientists to discover and understand the biology of many of the viruses that cause the common diseases seen in these birds. This information resulted in the development of management strategies that, when implemented, mitigate or completely eliminate the risk of several viral diseases. Unfortunately, we still lack critical information on a number of viral diseases and diseases thought to be caused by viruses. Additionally, not all bird owners are aware that they should apply what has been learned, and many are unwilling to do so. Therefore, viral diseases are still a significant threat to captive populations of birds.

Diagnostic Assays Used to Detect Viral Infections

SEROLOGY IN THE LIVE BIRD

Historically, serologic assays were used to screen large groups of animals to determine if a disease agent was present in the flock or herd in question. If a significant number of animals tested positive, then there was sufficient proof that the infectious agent was present and management changes were made accordingly. We ask much more from serology in pet bird and aviary medicine. We ask that each assay applied to a single sample tell us if the bird is or is not currently infected with whatever agent we are interested in; unfortunately, this is often not possible. Birds in the early stages of infection may not have had time to develop antibody. Assays that detect immunoglobulin M (IgM) will not become

positive for 7 to 14 days after infection, while assays that detect immunoglobulin Y (IgY) may take an additional 7 days before they are positive. The other major limitation of serology is that many birds remain seropositive after they are no longer infected with the virus and, without proper knowledge, a practitioner or bird owner could be misled into believing that a serologically positive bird was actively shedding virus.

Sadly, it must be noted that serologic and other diagnostic assays for avian infectious diseases have been and are still being offered commercially that are meaningless or their meaning is not known. For a diagnostic assay for any infectious disease to be valid, it must be tested rigorously in controlled infection trials or by the compilation and careful analysis of clinical data obtained from naturally infected animals. The accuracy of assays that have not been presented and preferably published in a peer-reviewed journal is suspect until proven otherwise.

Enzyme-linked Immunoassay (ELISA)

The ELISA detects antibodies from test plasma that react with viral antigens. To do this, the assay depends on a specific secondary antibody that can recognize the antibody of the bird being tested. If a single species is being tested and a secondary antibody to that species is available, the ELISA is an excellent assay. Cross-reactivity between secondary antibodies made to the antibody of one species of bird and the antibodies of other birds, however, will vary. Therefore, an ELISA using anti-Amazon parrot antibody may work for all species of Amazons and with careful controls may be applied to all species of parrots. It is, however, not likely to work in divergent species, such as passerines or ducks.

Hemagglutination Inhibition Assay (HI)

Several avian viruses, including the psittacine beak and feather disease virus, avian influenza and the paramyxoviruses, when added to red blood cells of the appropriate species will cause them to agglutinate. If the viruses are mixed with diluted serum containing antibodies to that virus, hemagglutination may be inhibited. HI can then be used to detect and quantitate circulating antibody. This assay is highly sensitive; however, non-specific hemagglutins and hemagglutin inhibitors occur in the serum of birds, complicating this assay. At times this assay may prove cumbersome, as some viruses will agglutinate the cells of some species and not others. If the necessary species are rare, this assay becomes impractical. Evidence suggests that the HI may not detect antibody in birds chronically infected with paramyxovirus 3.

Virus Neutralization Assay (VN)

The VN is a very sensitive and specific assay and can detect both IgM and IgY as long as they are neutralizing. In this assay, serum or plasma is diluted and each dilution is incubated with a specific concentration of live virus. The virus-serum mixture is then incubated with cells that are susceptible to infection. The cells are monitored for several days. If antibody is present in the serum and neutralizes the virus, cytopathic effects (CPE) to the cells are prevented. If the serum does not contain antibody, CPE occurs at all dilutions of serum. The antibody titer is defined as the reciprocal of the highest serum dilution that results in a 50% or 100% reduction in CPE.

The biggest disadvantages of the VN are that it requires that live cells be available and the assay itself takes 3 to 5 days. As a result, most laboratories will do this assay only once a week, and the turnaround time may be as long as 2 weeks.

POLYMERASE CHAIN REACTION (PCR) IN THE LIVE BIRD

PCR has been the single most important advancement in the detection of subclinical virus infection of birds. PCR detects viruses by amplifying a portion of the viral DNA, or viral RNA converted to DNA, to detectable levels. Blood, oral and cloacal swabs, tissue swabs and even environmental swabs can be used in this assay. Which samples need to be examined for each virus will depend on the virus and the stage of infection at the time of sampling. Development of a PCR assay requires knowledge of the DNA or RNA sequence of the virus to be detected. It also is necessary to know the variations in the sequences of the specific viruses. If there is considerable genetic variability but little biological variation within a virus, it may be critical to develop an assay that can detect all of the viruses. On the other hand, if significant biological variation is correlated with genetic differences, it may be important to develop multiple PCR assays that can differentiate among the genetic variants.

PCR assays are highly sensitive, but not all PCR assays are equally sensitive. When screening birds for avian polyomavirus, psittacid herpesviruses and the psittacine beak and feather disease virus, it is important to use PCR assays that have the highest level of sensitivity. The most sensitive assays typically use a nested or semi-nested PCR reaction that produces labeled amplification products that can be detected with an automated system. The sensitivity of the PCR assay also can be a disadvantage. Contamination of the sample at the time of collection or at the laboratory can result in false-positive results. It takes contamination with only one infected cell to cause a sample to become positive. Contamination is much more likely to occur when multiple birds are sampled. This technology is rapidly advancing, and it is certain

that the ability to screen for many more diseases soon will be available. Likewise, the cost and convenience of these assays will greatly improve.

POSTMORTEM DIAGNOSTICS FOR VIRAL INFECTIONS

Many viruses leave a characteristic histologic pattern of disease in their victims. Therefore, if the whole bird or a complete set of fixed tissues is submitted to the pathologist, a diagnosis can often be made based on the presence of specific histologic lesions, such as patterns of necrosis, the inflammatory response and the presence of viral inclusion bodies. When inclusions cannot be found or the inclusions are not specific, macerated fresh tissue and even formalin-fixed tissue can be examined for virus particles using electron microscopy. Virus isolation sometimes is necessary to detect specific viruses. Viruses can be isolated in embryonated chicken eggs and in primary cell cultures. Virus isolation often requires multiple blind passages before the virus is detected, and the whole process may take one to several weeks. Not all viruses grow readily in eggs or cell culture, so a negative finding does not conclusively rule out the possibility of a viral infection.

Molecular-based diagnostic assays have greatly improved the pathologist's ability to detect infectious agents in necropsy specimens. Fresh tissues from birds that die with viral infections typically contain high concentrations of virus. This virus is readily detected by PCR, if an assay for that virus is available. Formalin-fixed tissues also can be examined for virus DNA. However, formalin degrades DNA into small pieces; therefore, it is best to screen tissues that have been fixed for only a short time or have been fixed and then imbedded in paraffin within 2 or 3 days. Selecting PCR primers that amplify a short segment of the viral DNA also will increase the chance of detecting the viral DNA in formalin-fixed tissues. In situ hybridization and in situ PCR are techniques where the viral DNA actually can be detected in thin sections of formalin-fixed tissues. These assays have only limited availability and have a reduced sensitivity as compared to PCR of fresh tissue; however, they have important applications under some circumstances.

DNA Viruses

CIRCOVIRUS: PSITTACINE BEAK AND FEATHER DISEASE VIRUS (PBFDV)

Applied Biology

PBFDV is a non-enveloped DNA virus. Its single-stranded genome appears to code for seven proteins. Multiple variants of this virus have been identified, and the DNA sequence of these variants differs up to 11% compared to the originally sequenced variants. Studies in Australia have not shown a host specificity for any one of these variants or an indication that one variant is more pathogenic than others.[4] Work in the USA has identified a variant that is commonly found in lories and lorikeets.[35] The biology of this virus in lories may differ somewhat from other PBFDV variants in other species.[42] Genetic variation in these viruses has significant implications for testing. In order to detect all PBFDV variants, PCR assays must be designed to detect conserved areas of these viruses that are identical in all of them. Alternately, multiple assays that are variant-specific must be used.[35,53]

Infected birds shed virus in feather and skin dander, feces and crop secretions. Transmission has been postulated to result from inhalation of the virus, ingestion of the virus or possibly by movement of the virus across the bursal follicular epithelium. Vertical transmission also has been postulated; however, the overall role that vertical transmission plays in the dissemination of beak and feather infection remains uncertain. Naturally occurring disease predominates in juvenile and young adult birds. Whether birds become persistently infected and develop disease depends on the age and species of the bird exposed and possibly the specific variant of the infecting virus.

Virus replication occurs in a wide range of tissues, including the thymus, bursa of Fabricius, crop, esophagus, intestine, skin and feathers. Virus also has been identified in circulating leukocytes. Feather dysplasia results from virus-induced necrosis and disruption of the epidermal collar, intermediate basal epidermis and feather pulp, and thrombosis and hemorrhage within the feather pulp. Damage to the germinal epithelium of the beak is similar, resulting in the observed gross changes. Necrosis of the bursa, thymus and possibly circulating leukocytes results in varying degrees of immune suppression. Diseases caused by opportunistic pathogens are common in PBFDV-infected birds.[36]

Clinical disease may develop within 2 to 4 weeks in exposed nestling parrots, but prolonged incubation periods of months and possibly even years are more likely when young adult birds are infected. Virus can be detected in the blood before clinical signs are observed. In one report, virus could be detected in an experimentally infected bird 2 days after infection. The onset of viremia may be longer in naturally infected birds.

Clinical Presentation

Species Distribution

Psittacine beak and feather disease (PBFD) occurs in a wide range of wild and captive parrots, particularly the

cockatoos, eclectus parrots (*Eclectus roratus*), budgerigar (*Melopsittacus undulatus*) and lories and lorikeets from Australia, the Pacific Islands and Southeast Asia. African parrots, including the African grey parrot (*Psittacus erithacus*) and lovebirds (*Agapornis* spp.), also are highly susceptible to infection and disease, and infection has been found in the wild African parrots. Infection in Neotropical parrots in captivity occurs at a low to moderate rate, but disease is rare. A small number of macaws and Amazon parrots and a single pionus parrot have been reported with PBFDV.[24] PBFDV infections in wild Neotropical parrots are not documented.

Signs in Cockatoos

PBFDV causes chronic progressive disease in birds older than 8 to 10 months. The large majority of birds with the chronic form of PBFDV first develop lesions between 6 months and 3 years of age. The first signs of PBFDV are subtle. A lack of powder on the beak may be the first indication that powder down feathers are diseased. Some birds will present with a history of a delayed molt. Close inspection of these birds will generally reveal at least a few dysplastic feathers. Both down and contour feathers are affected, but the disease may predominate in one or the other. Initially, diseased feathers are widely scattered and are associated with the pattern of molt. As the disease advances, all feather tracts will become involved, generally in a somewhat symmetrical fashion (Fig 32.1). In advanced cases, only down feathers, a few scattered contour feathers or no feathers at all may remain.

Affected feathers show varying degrees of dysplasia. Hyperkeratosis of the feather sheath is common, resulting in sheath thickening and retention. Growing feathers are short and may be pinched off either at their proximal ends or near their base (clubbing). Thinning of the rachis and recent and previous hemorrhage within the feather shaft is common. In some feathers there is so much disruption of feather growth that the sheath contains only a disorganized mass of keratin. Mildly affected feathers may show bowing, have transverse dystrophic lines and fracture at any location along their length.

Beak lesions are common in the sulphur-crested (*Cacatua galerita*), Major Mitchell's (*C. leadbeateri*), Moluccan (*C. moluccensis*) and umbrella cockatoos (*C. alba*), little corella (*C. sanguinea*) and galahs (*Eolophus roseicapillus*). They are less frequent or entirely absent in other species. These lesions may occur at any stage of the disease but are seen most commonly in birds with advanced disease. Early changes in the beak are the result of hyperkeratosis of its superficial layer. These changes cause beak elongation and overgrowth. Longitudinal fissures develop subsequently. In the terminal stages of the disease, the distal portion of the beak will fracture, leaving underlying necrotic debris and bone. Necrosis of the palatine mucosa causes it to separate from the beak (Fig 32.2). The resulting space fills with caseous material. Beneath the caseous material is bone. These lesions are painful and birds may become partially or completely anorectic. Secondary infections of the beak and oral cavity are common. A pathologic process similar to the one occurring in the beak also may affect the nails of the feet. These lesions, however, generally are not a significant manifestation of PBFD.

If the beak lesions are not severe, birds can live with the PBFD for many years. However, the vast majority of these birds die, either from their primary lesions or from secondary infectious diseases within 6 to 12 months after onset of the signs. Mounting evidence suggests that birds with PBFD have significant alterations in their immune function. As a result, opportunistic pathogens, eg, yeasts and other fungi, both gram-positive and gram-negative bacteria, cryptosporidia and avian polyomavirus, are common complications and often terminal manifestations of PBFD. A survey of cockatoos with PBFD showed that most have high concentrations of avian polyomavirus in their skin and would be expected to be continuously shedding this virus.

Acute PBFD in nestling cockatoos may begin with nonspecific signs such as depression and regurgitation. Feather lesions develop rapidly and are extensive. These lesions may be identical to those seen in the chronic form of PBFD, or more often, annular constricting bands near the base of the feather develop simultaneously in numerous feathers (Fig 32.3). These feathers break off easily and may bleed. They also tend to be loose in the follicles and are easily pulled out. An understated feature of this disease is the discomfort of the nestling. The damaged feathers are painful and these birds do not want to be handled. Like the chronic form of PBFD, an early sign of infection is reduced powder on the beak. This last sign is not specific because young cockatoos do not always groom themselves as intensively as the adults and will routinely have less powder on their beaks. Advanced beak lesions rarely have time to develop, as the acute disease is often rapidly fatal. As with the older birds, the rate of disease progression varies. Infection studies suggest that rapidly fatal disease is likely to occur in umbrella and sulphur-crested cockatoos, whereas a more chronic form of the disease can be expected in galahs.

PBFD was rampant in wild-caught cockatoos imported into the USA and Europe prior to 1992. Importation has ceased or is dramatically diminished; as a result PBFD in cockatoos has become rare. Circumstances are entirely different in Australia, where the disease is common in the wild and infected wild-caught birds sold as pets.

Bob Dahlhausen

Fig 32.1 | A cockatoo with generalized feather dysplasia characteristic of psittacine beak and feather disease.

Bob Dahlhausen

Fig 32.2 | Necrosis of the junction of the rhinotheca and the oral mucosa in a cockatoo with advanced psittacine beak and feather disease.

Bob Dahlhausen

Fig 32.3 | A nestling Moluccan cockatoo with the acute form of psittacine beak and feather disease. All growing feathers are involved. Many are seen to have hemorrhage within their shafts.

Signs in African Grey Parrots

Acute PBFD also occurs in juvenile African grey parrots. In experimentally infected birds, non-specific systemic signs preceded feather lesions. Dystrophic feathers identical to those seen in cockatoos also occur in African grey parrots. Additionally, newly formed contour feathers that would normally be gray will sometimes be red. Red coloration of contour feathers, however, is not specific for PBFD. Not all African grey parrots with PBFD have demonstrable feather lesions. A rapidly fatal form of PBFD was described in 7-week-old to 9-month-old African grey parrots. Birds typically presented with an acute onset of crop status, regurgitation and weakness. Feather loss was present in 3 of 14 birds. Total white blood cell counts fewer than 1000 cells/μl were common. An acute, often massive, liver necrosis also was a common finding, although changes in serum chemistry findings did not consistently correlate with the degree of liver disease. Most of these birds died or were euthanized within 2 weeks of presentation.[36,37]

Signs in Lovebirds

PBFDV infection is extremely common in lovebirds. Up to 40% of the lovebird samples submitted to one laboratory for genetic probing were positive. A survey of commercial lovebird producers in Texas found that 60% of the facilities sampled had PBFDV in their collections. Many, possibly most, PBFDV infections in lovebirds do not result in clinical disease. The dynamics of PBFDV infection in lovebirds have not been studied extensively, but it appears that asymptomatically infected lovebirds are only transiently infected. When disease does occur in lovebirds, it is most common in young adult birds. These birds appear unthrifty, they may shed feathers and not regrow them, or they may have a delayed molt. Dystrophic feathers may predominate, be scattered or absent entirely. Some of these birds survive for many months or years, and some will recover and may eliminate the virus. It has been reported that the lory variant of PBFDV may be common in lovebirds.

Encephalitozoon hellem is a common infection in lovebirds and a potential zoonotic disease. The prevalence of *E. hellem* shedding is significantly higher in lovebirds infected with PBFDV.[3]

Signs in Budgerigars

PBFDV infection is enzootic in some budgerigar breeding facilities, but it is not as widely disseminated as it is in the lovebird. Most affected birds are fledglings. In the author's experience, diffuse feather changes similar to those seen in cockatoos are uncommon. Instead, many of these birds have normal feathering except for the complete absence of primary and secondary wing feathers (Fig 32.4). The owners refer to these birds as runners or creepers. These lesions are not specific for PBFD, and identical feather abnormalities are caused by avian polyomavirus infections. PBFDV and polyomavirus infections in budgerigars also can occur concurrently.

Signs in Eclectus Parrots

PBFD in the eclectus is very similar to that in the lovebird. Dystrophic feathers may or may not be present, but

Fig 32.4 | A budgerigar with "French molt." There is incomplete development of the primary wing feathers and the tail feathers. These lesions are the result of either psittacine beak and feather disease, avian polyomavirus or a concurrent infection with both.

feather quality of clinically affected birds is poor. Many of these parrots are unthrifty and are plagued with other infectious diseases. Fatal polyomavirus infections in adult eclectus parrots have been correlated with concurrent PBFDV infections.

Signs in Lories

PBFD appears to be relatively common in free-ranging Australian rainbow lorikeets (*Trichoglossus haematodus*). Fledgling lorikeets with typical dysplastic feather lesions are found walking on the ground. Histology of these birds reveals characteristic bursal lesions. Approximately one-third of these birds die before their first molt, another third have persistent feather abnormalities, but the remaining birds go on to molt and develop normal feathers (L. Filippich, personal communication, 1997). A similar disease has been described in lory and lorikeet collections in the USA. Some of these birds never develop signs, while others develop transient feather disease and still others develop persistent feather lesions and other manifestations of PBFD.[35]

Signs in Neotropical Parrots

PBFDV infection in New World parrots has been documented in 3 to 5% of samples submitted for PBFDV screening in one laboratory. Disease in these birds, however, is extremely rare. Clinically affected birds have feather lesions essentially identical to those of cockatoos. Also like the cockatoos, disease has been seen in both adult and nestling birds. Resolution of the clinical signs has been documented in some of these birds.

DIAGNOSIS

When typical clinical signs are observed, they strongly support the diagnosis of PBFD. PBFDV infection can be confirmed in birds with clinical disease, those with non-specific signs and inapparently infected birds using a PCR assay of heparinized blood. In capable hands, this is a highly sensitive and specific assay. Birds with clinical signs of PBFD that are positive by PCR have a guarded prognosis. With supportive care, some will live many years with the disease. Uncommonly, clinical signs will resolve in some birds and they will subsequently become virus-negative. Birds infected with the lory variant may be more likely to survive infection. Histopathologic examination of biopsies from PBFD birds also can be used to confirm the clinical diagnosis. Clinically normal birds with a positive test result represent birds in the early stages of infection or birds with a transient subclinical infection or a sample that was contaminated at the time of collection. Clinically normal birds with positive test results should be retested in 90 days. Lories infected with the lory variant have remained positive for over 6 months without showing signs of disease. It is critical to remember that all positive birds are actively shedding virus whether they are showing signs or not. Also, birds that no longer have virus in the blood may continue to shed virus in their feather and skin dander until their next molt.

Before the PCR-based assay was developed, diagnosis of PBFD was made by histopathologic examination of plucked growing feathers or biopsies of feathers and feather follicles. Characteristic changes in the growing feather and its follicle and the presence of virus-induced intranuclear and intra-cytoplasmic inclusion bodies (basophilic globule cells) are considered diagnostic. Similar inclusion bodies are irregularly found in other tissues. In the African grey and eclectus parrots, feather lesions may not be present. In birds without feather lesions, the clinician often does not suspect PBFD. As inclusion bodies may be found only in the bursa, submission of a complete set of tissues is necessary for an accurate diagnosis.[36]

CONTROL

With the advent of a sensitive and specific diagnostic assay for PBFDV, control of this disease has been greatly simplified. All new birds should be tested for the virus at the time of purchase. Alternately, testing can be delayed a month to permit a recently exposed bird time to become viremic. The most conservative method would be to test initially after purchase and repeat the test in 30 days. Testing of new birds is of no value unless all other birds in the aviary also are tested. Although expensive, testing all birds in valuable collections of at-risk species can avert future catastrophic loses. Birds with a positive test result should be immediately removed from the aviary, as they are sources of massive amounts of virus.

The cost of testing individual birds can make it difficult to persuade pet store owners and private individuals to

test all lovebirds and budgerigars. If private owners are unwilling to test these birds but have other birds at risk at home, they should be discouraged from keeping them. Rather than test individual birds coming into a collection, pet stores can require that their sources swab their aviary or holding areas for PBFDV before birds are purchased from them. PBFDV is believed to be highly resistant to commonly used disinfectants; therefore, aggressive cleaning is necessary to eliminate it from a contaminated environment. Following cleaning, PCR of environmental swabs can be used to determine if a facility has been adequately cleaned. Routine environmental testing in veterinary hospitals is recommended. A vaccine for PBFDV is not available at the time of this writing.

CIRCOVIRUS INFECTION IN THE CANARY

A disease with high morbidity and mortality has been reported in nestling canaries (*Serinus canarius*). Affected birds have a distended abdomen and an enlarged gall bladder. Exudate in the air sacs also is reported. Canary fanciers refer to the disease as "black spot," as the enlarged gall bladder can be observed through the nestlings' skin. Lesions characteristic of circovirus infections in other birds are present in these canaries, as are the characteristic intranuclear and cytoplasmic inclusion bodies. Diagnosis is most readily made in birds 10 to 20 days old. The partial DNA sequence of a novel circovirus — named the canary circovirus — was amplified from a flock of canaries experiencing a high degree of mortality. Gross lesions were confined to petechial hemorrhages in two of four birds examined. Microscopic examination of the tissues was not done. This sequence information will permit more extensive studies of this virus in the future.[46]

CIRCOVIRUS INFECTIONS IN COLUMBIFORMES

A circovirus infection also has been documented in pigeons. Unlike the disease seen in psittacine birds, it is not usually associated with abnormal feathering. Signs are rarely specific, and birds generally have other diseases as well. Chlamydophila, mycoplasma, adenovirus and herpesvirus infections and systemic bacterial infections have all been described in pigeons with circovirus infection. It is possible that this virus is immunosuppressive and weakens the pigeon's immune system to a point that other diseases develop[47] (see Chapter 13, Integument).

Diagnosis is made by finding basophilic globule cells in tissue sections. A complete set of tissues, including the bursa in the young bird, may be necessary to detect this virus by histopathology. This virus has been sequenced and a PCR assay capable of detecting the virus in tissue has been developed. Using this assay, it was shown that the Columbid circovirus, as it is now called, is widespread in European racing pigeons. It is highly likely that it is present in flocks of feral and domestic pigeons worldwide. Like PBFDV, Columbid circovirus also can be detected in the blood of live birds.[14]

AVIAN POLYOMAVIRUS (APV)

Applied Biology

APV is widespread and can be found in most countries of the world where psittacine birds are raised. It is a nonenveloped DNA virus that codes for six proteins. Some degree of genetic variation has been identified in APV but it is relatively small, and it is assumed that all APV variants have the same host range.[29] The route of natural infection has never been experimentally verified, but infection has been induced through the respiratory route. Given the rapid spread of this virus at bird sales and in the nursery, natural infection through the respiratory system is likely. In the budgerigar, the virus replicates in a wide range of tissues, including growing feathers, skin, liver, spleen, renal tubular epithelium, heart and cerebellum. Clinical signs and necropsy findings are largely associated with tissue distribution of virus replication. Disease in budgerigars is confined to nestlings. Not all budgerigars die with APV infection, and surviving birds shed virus in skin and feather dander and in droppings.[24]

Most non-budgerigar parrots are assumed to be susceptible to APV infection. Disease, however, typically is limited to nestling parrots. Macaws, conures and eclectus parrots are over-represented, although diagnosis of this disease has occurred in most psittacine species (Fig 32.5). Birds become viremic sometime between 1 week and 10 days after infection. Disease, if it is going to occur, develops 10 to 14 days after exposure. It is characterized by generalized hemorrhage, moderate to massive hepatic necrosis, and an immune-complex glomerulopathy. Characteristic karyomegalic changes and intranuclear inclusion bodies are typically found in macrophages and other antigen processing cells, including the mesangial cells of the glomerulus. The vast majority of birds with these lesions die. Adult birds and nestlings that are infected but do not develop disease will remain viremic for a variable period of time and shed virus in their droppings, and possibly in feather dander and skin, before becoming virus-negative. Most infected birds clear the virus after several weeks to several months and, although they maintain a persistent antibody titer, are not thought to be persistently infected.[30]

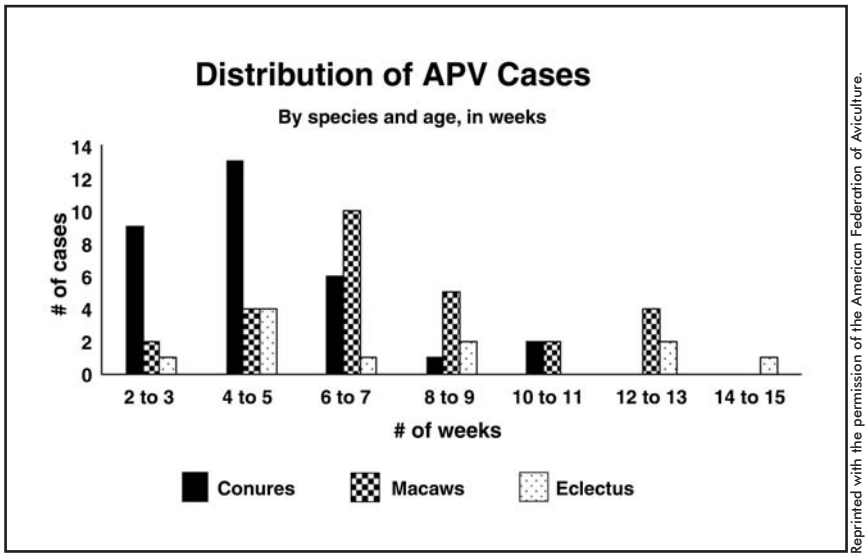

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Fig 32.5 | Age distribution of nestling macaws, conures, and eclectus parrots with avian polyomavirus disease, from the literature and birds submitted to the Schubot Exotic Birds Health Center.[2, 5]

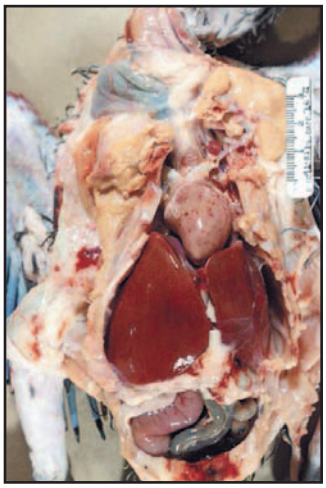

Fig 32.6 | A nestling macaw with avian polyomavirus disease. Note the extensive petechial and ecchymotic hemorrhage and pale musculature.

Clinical Presentations

APV in Budgerigars

Budgerigar breeders first detect this problem in their flocks when there is a sudden increase in the number of dead nestlings in the nest boxes. The nestling mortality rate often is high and may approach 100% when the virus is first introduced to an aviary. If there is no intervention in subsequent breeding seasons, mortality rates will decline but production will always remain depressed. The signs of APV disease in budgerigar nestlings are somewhat variable. Most often, the young birds experience an abbreviated course of disease. At death, birds are found to be stunted and have abnormal feather development, skin discoloration, abdominal distension, ascites, hepatomegaly with localized areas of necrosis and scattered areas of hemorrhage. In some outbreaks, the virus attacks the cerebellum and these birds will have head tremors. Death predominates in birds that are 10 to 20 days old.[25]

Not every budgerigar nestling infected with APV dies. Some never show signs. Other nestlings will fail to develop their primary and secondary wing feathers and tail feathers (see Fig 32.4). These birds have been referred to as runners or creepers, and this form of the disease has been described as French molt. PBFDV or a combination of APV and PBFDV can cause identical lesions. It is possible that one or more additional diseases may cause similar feather disease. Not all budgerigars appear to be equally susceptible to infection and disease. In one study in the United States, English budgerigars were rarely found to be infected with APV, although they were housed with other birds shedding the virus.

APV in Non-budgerigar Parrots

Non-budgerigar parrots are susceptible to APV. Some are highly susceptible to disease, while others rarely, if ever, develop disease. APV disease in these birds occurs at different ages in different birds. In conures, deaths typically occur in birds less than 6 weeks of age. Deaths in macaws and eclectus parrots occur in birds 14 weeks and younger (see Fig 32.5). Most, possibly all, of the nestlings lost are being hand-fed. Infected nestlings appear healthy, show very few premonitory signs and then die suddenly. When signs do occur, they precede death by only a few hours. Observant owners may notice delayed crop emptying, weakness and a generalized pallor or bruising under the skin in the preceding hours before death. Yellow discoloration of the urates is another rare observation. Occasionally, complete blood count and serum chemistry tests can be performed prior to death. Increases in the liver enzyme aspartate aminotransferase are expected. Near death, birds have a marked thrombocytopenia. Birds that die are typically in excellent body condition. Additional findings commonly include generalized pallor with subcutaneous and subserosal hemorrhage and enlargement of the spleen and liver (Fig 32.6). Less commonly, ascites and pericardial effusion may be present.

APV in Lovebirds

APV disease in lovebirds is distinct enough to merit special attention. As in the budgerigar, this disease occurs in nestling birds, and inclusion bodies can be found in multiple organs. Unlike the budgerigar, birds up to 1 year of age also can be affected. This unusual age susceptibility has not been fully explained. However, in at least some of these older birds, concurrent infection with PBFDV also is occurring and may permit APV disease in a bird that would otherwise be resistant to it.

APV in Nestling Cockatoos

A unique presentation of APV disease occurs in nestling cockatoos. These birds present at an age of 4 to 8 weeks with a history of difficulty breathing. Physical examination reveals a severely dyspneic bird that is underweight and may be stunted in its growth. These birds have all the appearances of a bird that has aspirated food. Most of these birds die. Necropsy reveals heavy, wet lungs that may not float in formalin. Histologically, there is a severe generalized interstitial pneumonia with huge numbers of inclusion bodies in what are believed to be type II pneumonocytes. Preliminary sequence data suggests that this form of APV disease is caused by a specific APV variant. This variant is still capable of causing the classic form of APV disease in other susceptible species.[32]

Post-APV Edema and Ascites Syndrome

Some birds that have APV disease survive. An unknown percentage of these birds go on to develop ascites and generalized edema. They still appear bright and alert and may continue to eat and empty their crops, but they are edematous and have a fluid-filled peritoneum. The fluid is a transudate or modified transudate and does not contain inflammatory cells. These birds do not improve and either die or are euthanized. Histologic lesions include a sclerosis of the glomeruli and regenerative lesions in the liver. It is suspected that the edema and ascites syndrome is secondary to hypoproteinemia, either from a failure of albumen production in the liver or a loss of protein from the kidney. Viral inclusion bodies are rare or absent in these birds. This disease very closely resembles the viral serositis lesions described in nestling parrots with eastern equine encephalitis and may be mistaken for it. These birds are still loaded with APV, and PCR of blood or cloacal swabs in the live bird or blood or a liver swab in the dead bird will be strongly positive.[32]

APV in Adult Parrots

APV readily infects adult parrots. Most infections, probably greater than 99.9% of them, are completely asymptomatic. These birds become infected, shed virus for a few weeks or do not shed virus at all, and do not show signs of illness. APV disease, however, has been documented in adult birds. Disease in these birds resembles that seen in the nestling. An atypical form of a progressive virus encephalopathy also has been reported in two cockatoos. Why rare adult birds or groups of birds develop disease is not known in all cases. In many cases, however, adult birds that die with APV disease have concurrent PBFDV infections. PBFDV is believed to be immunosuppressive, allowing APV to cause disease in a bird that would normally be refractory to infection. APV disease does uncommonly occur in adult parrots that do not have concurrent PBFDV infections. In the author's experience, these deaths typically occur in pet stores, suggesting that stress may play a role in the pathogenesis of disease.

Diagnosis

Testing Inapparently Infected Birds

The goal of testing is to detect inapparently infected birds that are shedding virus and to keep them from exposing other birds. Budgerigars, when infected as nestlings, shed virus for 6 or more months. Larger species of parrots, when infected as nestlings, become viremic for 4 to 8 weeks and shed virus from 6 to 16 weeks. Viremia and virus shedding have rarely been detected for as long as 10 months. Birds infected with PBFDV and APV may shed virus continuously. To detect virus-shedding birds, both blood and a combined oral and cloacal swab are examined by PCR. Testing blood alone is not recommended, as viremia ceases before virus shedding ends. Limited studies have been done on the duration of virus shedding in adult birds. However, it appears that after exposure, viremia and virus shedding are absent or greatly abbreviated as compared to nestlings.[25,28]

All birds infected with APV develop a detectable virus-neutralizing (VN) antibody titer within 2 to 3 weeks. The presence of antibody has no bearing on virus shedding, as antibody-positive birds continue to shed virus for many weeks. Also, once a bird develops antibody, most will maintain a high antibody titer many years after they have stopped shedding virus. Even though virus shedding cannot be predicted by serology, serology still has some value in the control of APV. Serology can be used to screen young budgerigars and lovebirds coming out of an aviary or returning from the show circuit. If they are coming from an aviary and are seropositive, they have been recently infected with APV and are most likely shedding virus. If birds that have been on the show circuit are seronegative after a 2-week quarantine, they are not infected with APV.

Postmortem Diagnosis of APV Disease

Gross and microscopic lesions seen in birds that die with APV infection are characteristic. Spleen, liver, lung and kidney are essential tissues to provide to the pathologist. These birds are viremic at the time of death, and swabs of any tissue will be positive by PCR. Immunofluorescent staining of impression smears and in situ hybridization also can be used to confirm infection if other histologic findings are inconclusive.[36]

Prevention and Control of APV[25]

The key to prevention of APV disease is to make sure that birds that are shedding virus are not introduced into an aviary or that materials that might be contaminated with APV are not brought into the aviary. Testing can be an important part of a prevention plan. Excellent

PCR assays have been developed to detect infection in the live bird. Serology can be used to determine if a bird has been infected in the past but does not adequately predict the virus-shedding status of the bird.

Control in Budgerigar Aviaries

If a prevention program for APV infection is to be instituted in a budgerigar aviary, the first step is to make sure that it is not already there. The virus is readily detected by PCR in the environment of aviaries where the infection is enzootic. Alternately, blood and combined oral and cloacal swabs can be used to test nestlings and young adult birds. A virus neutralization assay can be used to detect antibodies to APV. Most birds in an aviary with enzootic APV will be seropositive.

Exhaustive efforts are required to keep APV out of a budgerigar collection. The movement of birds on and off the property must be carefully restricted. All new birds coming onto the property should be seronegative or PCR-negative. Alternatively, environmental swabs of their aviary of origin can be tested by PCR. If the aviary is a commercial aviary, dealers, feed salespeople, delivery trucks and other bird breeders should be banned from the aviary entirely. Young birds taken to the bird dealer and rejected should not be returned to the aviary. Food should be purchased directly from the feed mill so that it is never in contact with other birds. If the aviary is primarily breeding show budgerigars, then all birds going to the show should be quarantined until the end of the show season and tested by serology or PCR before they are returned to the breeding colony.

Budgerigars are not the only birds that can bring APV into a collection. Lovebirds and possibly cockatiels (*Nymphicus hollandicus*) also can be sources. Devastating outbreaks have occurred in budgerigar operations when lovebirds have been introduced into previously closed budgerigar colonies.

Elimination of APV from a budgerigar collection is challenging, but not impossible. The first critical step is to stop breeding. The infection cycle is perpetuated by the constant presence of infected nestlings, fledglings and young adult birds. These birds shed virus for up to 6 months or more after infection, seeding the environment with virus. Chicks are then exposed immediately upon hatch and the cycle continues. Once breeding is stopped, all birds that have not been used for breeding should be removed from the property. Adult birds should be moved to a temporary environment and the aviary totally disinfected. Nest boxes can be cleaned and painted but are better off destroyed and replaced with new boxes. All wood surfaces should either be discarded or cleaned and painted over. After a 6-month hiatus, the adult breeding stock can be returned to the clean aviary and set up for breeding again.

Prevention and Control in Lovebird Collections

The sad state of the matter is that both PBFDV and APV are enzootic in many lovebird aviaries. Oddly, disease in these birds is often rare. Shedding, however, occurs in young lovebirds and may be continuous in birds that are concurrently infected with PBFDV. Breeders who wish to have a lovebird collection that does not have APV should first test their birds for infection. Again, serology using the virus neutralization assay or PCR of swabs from the environment or blood and combined oral and cloacal swabs of individual nestlings and fledglings will readily detect virus. To prevent the introduction of APV to a lovebird aviary, a representative number of each lot of incoming birds is tested by serology or PCR. Alternately, environmental swabs are used to verify that the aviary from which the birds originate is free of APV.

Prevention and Control in Aviaries Breeding Non-budgerigar Parrots

Outbreaks of APV do not occur in adult breeding birds, they occur exclusively in nurseries. Outbreaks occur when birds with inapparent infections, generally nestlings, are introduced to the nursery. If the following rules are followed, APV outbreaks in the nursery are extremely unlikely. Breeders of the larger species of parrots should not breed cockatiels, lovebirds or budgerigars. If they must breed these species, they must test them thoroughly to make sure they are not infected with APV. The breeder should raise only the chicks that they produce. If they must raise chicks from other sources, these birds must be quarantined and tested by PCR before they are brought into the nursery. Extensive and repeated environmental testing of the aviary of origin may be substituted for individual bird testing. Any bird that leaves the nursery and is in direct or even indirect contact with other birds must not be allowed back into the nursery. Adult birds coming into the aviary also should be quarantined and tested for APV. Ideally, people taking care of the nursery would not take care of the adult birds. Often, this is not possible. In these situations, cleaning up and changing clothes before working with the nestlings is recommended.

APV outbreaks in the nursery are devastating. In most cases, once APV is introduced to a nursery it spreads rapidly, so that by the time the first case is recognized most of the nestlings are already infected. This concept is important for two reasons. First, vaccination at this point will do no good. Second, testing during the outbreak will prove only that the virus is widely disseminated. To save money, the aviculturalist should be encouraged to reserve testing to determine when shedding has stopped and the chicks can be sold.

Little can be done to keep exposed chicks from becom-

ing infected with APV in most nurseries. However, efforts should be made to improve hygiene, decrease density of birds and use individual syringes for hand-feeding individual chicks. The most important element in the control of APV outbreaks is to stop bringing babies into the nursery. Chicks can be left in the nest to be raised by the parents or pulled and sent to another facility to be raised. It remains unclear why, but parent-raised chicks (excepting lovebirds and budgerigars) are not reported to develop APV disease. Surviving chicks will shed virus for 8 to 14 weeks, rarely as long as 16 weeks. The older the chick at the time of exposure, the shorter the period of virus shedding. Chicks should be negative by PCR of blood and a combined oral and cloacal swab before they are sold. PCR of the oral and cloacal swab is critical, as viremia ceases before shedding stops.

After the outbreak has stopped, a close inspection of the aviary must be done. Birds that might be shedding virus need to be identified and tested or eliminated from the aviary. Extensive cleaning and disinfection of the nursery also will have to be done. In aviaries where the underlying source of disease has been eliminated, subsequent breeding seasons can be free of the disease.

Preventing APV Disease in the Pet Store

The pet store is one of the most common places where APV outbreaks occur. Most pet stores get their birds from multiple sources. They sell budgerigars, lovebirds and cockatiels, the three species that are most likely to be shedding virus, and many stores will acquire susceptible species when they are still nestlings. To avoid disease, pet stores can use several strategies. The easiest and best method for preventing APV disease in the pet store is to buy only weaned nestlings. These birds will be old enough that, if infected with APV, they will not develop disease. If unweaned nestlings are to be purchased, they should be raised outside of the store until weaned. If nestlings must be in the store, they should be separated from all other birds, and have a person designated to take care of only them and no other birds. The public should not be allowed to handle these birds. Stores that sell high-value nestling parrots should consider limiting their bird sales to these birds only and not selling lovebirds, budgerigars and cockatiels. Establishing long-term relationships with breeders also can reduce the risk of disease transmission. Breeders supplying pet stores should be encouraged to develop a preventive medicine program to develop and maintain APV-free flocks.

Immunization

A commercial APV vaccine[a] is available in the USA. Its value as a tool in the prevention of APV disease is controversial. The author's research has raised several questions about this vaccine and its ability to protect against APV infection. The vaccine is to be given to nestlings that are 4 weeks of age or older and is thought to provide protection to nestlings 2 weeks after the second vaccine, or by the age of 8 weeks. From the dynamics of the disease, however, most birds cannot be immunized early enough in life to be protected. An additional concern is that VN antibody was not detected in nestlings immunized with this vaccine, and immunization of adult birds resulted only in low antibody titers.[28] Clinical trials that claim to show that the vaccine stopped outbreaks of APV disease in nurseries did not study control flocks where the vaccine was not used. If new nestlings were not added to a nursery experiencing an outbreak, deaths would stop on their own within 2 to 4 weeks of the first death.[34] Claims that immunizing already infected birds will result in a shortening of the duration of virus shedding are not documented.

APV is, by and large, a completely preventable disease through appropriate management strategies and selective testing. As a result, the author stresses these avenues of control and does not recommend immunization.

APV Infection and Disease in Non-psittacine Birds

One or more APV strains can infect non-psittacine birds. Several species of passerines have been documented to have classical APV disease. In the author's experience, flocks of Gouldian finches (*Chloebia gouldiae*) are perhaps at greatest risk. Again in the author's experience, mortality is limited to nestling and young adult finches during one breeding season but is not seen again in the following year. Surviving birds have moderate levels of antibody that will neutralize a lovebird-derived APV. APV DNA was detected in the tissues of one finch with PCR primers derived from the psittacine APV sequence, suggesting that this bird was infected with a psittacine variant. However, other studies suggest that another significantly different virus also may infect passerines.

There is a single published report of a rhamphastid dying with an APV disease. The bird was a green aracaris (*Pteroglossus viridis*). The virus sequenced from this bird was found to be nearly identical to those derived from psittacine birds. It was speculated that the original source was a cockatoo. In this study, in-contact birds, including zebra finches (*Poephila guttata*), a kookaburra (*Dacelo novaguineae*) and a Lady Ross turaco (*Musophaga rossae*), became seropositive but did not develop disease.[19]

APV Infection in Free-ranging Birds

There is strong evidence that APV infection occurs in wild birds on multiple continents. A high prevalence of anti-APV antibody was found in free-ranging greater

sulphur-crested cockatoos in Australia.[33] APV disease has not been reported to occur in wild Australian birds, but a disease with characteristic APV lesions was induced in a cockatoo infected with a preparation of PBFD virus derived from the feathers of a wild bird, suggesting that APV was present in these tissues and was copurified with the PBFD virus.[33] Recently, APVs were identified in five buzzards (*Buteo buteo*) and a falcon (*Falco tinnunculus*) in Europe. Genetically, the sequence of the falcon virus was nearly identical to other APV variants of psittacine origin and the virus in the buzzard amplified with PCR primers derived from the sequence of the original APV isolated from a budgerigar. Because of autolysis in the buzzards, the histologic lesions associated with this disease could not be characterized.[17]

Preliminary evidence that APV may occur in wild birds in North America also exists. A house sparrow (*Passer domesticus*) was found to have a glomerulopathy with characteristic APV-like inclusions within mesangial cells and PAS-positive deposits within the mesangium and glomerular capillaries.[24]

Goose Hemorrhagic Polyomavirus

A genetically distinct polyomavirus with limited homology to the avian polyomavirus has been implicated as the cause of the hemorrhagic nephritis and enteritis of geese in Europe. Little is known about the importance of this virus in waterfowl, but it may be widespread, as the histologic lesions that it is reported to cause are not specific.[13]

PAPILLOMAVIRUSES

Diseases caused by papillomaviruses in birds have been described only in wild European finches and imported African grey parrots. The African grey parrots had papilliferous plaques of the commissures of the beak, eyelids and face that became more extensive over the course of the year the birds were monitored. Lesions in European finches predominate on the legs and feet; lesions of the face are rare. These lesions should be differentiated from those caused by poxviruses.[43]

ADENOVIRUSES

Adenoviruses in Companion Birds

Adenovirus infections and disease in companion birds are rare. They have been associated with hepatitis, acute necrotizing pancreatitis, conjunctivitis and a multisystemic disease in lovebirds. However, recent reports of these diseases have been lacking. Adenovirus-associated encephalitis also is a rare disease that has not been recently reported. Characteristic basophilic intranuclear inclusion bodies are infrequently seen in renal tubular epithelial cells in parrots that die with other diseases. These lesions are most common in lovebirds and budgerigars.[21,36]

A fatal adenovirus infection causing hepatitis is described in the nestlings of Senegal parrots (*Poicephalus senegalus*) and related species. The disease occurs sporadically within aviaries. In one collection, the disease occurred in 3 out of 4 years in offspring from a single pair of Senegal parrots. Affected nestlings typically present acutely ill or are found dead. Grossly, the liver is discolored red-black, and scattered yellow-gray areas may be present. Multifocal hepatic necrosis and the presence of large, darkly basophilic intranuclear inclusion bodies within hepatocytes characterize this disease.[36]

The author has seen adenovirus infections in several mixed flocks of finches. Typically, clinical signs are not observed. Concurrent diseases, such as candidiasis and atoxoplasmosis, were common. Poor hygiene and high stocking density may have played roles in these deaths.

Pigeon Adenovirus

Adenoviruses in pigeons cause two distinctly different diseases. The first occurs in pigeons less than 1 year old and may be associated with the onset of the racing season. This virus replicates predominately in the intestinal epithelium, causing villus atrophy. Many birds will develop disease. Signs are those of acute severe enteritis, diarrhea and vomiting. Severely affected birds die, but many uncomplicated infections resolve within 1 week. A common complication of this adenovirus infection is an *Escherichia coli* overgrowth of the intestinal tract. These birds have persistent diarrhea, lose condition and will die if not aggressively treated. *E. coli* overgrowth of the intestine also can result in septicemia and sudden death. Mild to moderate hepatic necrosis may occur in some infections and contribute to the clinical signs and duration of the disease.

A second adenovirus causes massive hepatic necrosis. All ages of birds are susceptible. Disease, however, is sporadic in a flock and spreads slowly. Signs of infection include vomiting and biliverdin-stained urates; however, most birds die before signs are recognized. Birds showing signs die within 24 to 48 hours.

Diagnosis for both adenovirus infections can be made only at necropsy. Treating for dehydration and secondary bacterial infections can mitigate mortality in birds with the enteric adenovirus infection.[8,52]

PSITTACID HERPESVIRUSES (PsHVs)

Applied Biology

PsHVs are alpha herpesviruses that are the causative

agent of Pacheco's disease (PD) and internal papillomatosis of parrots (IP). The PsHV1 virus contains three major serotypes. Two additional serotypes (serotypes 4 and 5) are described, but they are each represented by only a single virus isolate. It is unclear if 5th serotype is a PsHV1 or an entirely different herpesvirus. There are four major genotypes of PsHV1. The viruses in genotypes 1 and 4 comprise serotype 1, the viruses in genotype 2 comprise serotype 2 and the viruses in genotype 3 comprise serotype 3. The single serotype 4 isolate is a genotype 4, but appears to have evolved into a unique serotype.[49] A new herpesvirus, PsHV2, has been discovered. This virus has been identified in mucosal tissues from Congo African grey parrots and a single blue and gold macaw. Most birds were not showing signs of disease, however this virus was found in a mucosal papilloma in one African grey parrot and cutaneous papilloma from another.

The study of the complexity of these viruses and the correlation between genotype and pathotype is still in its infancy, but patterns are beginning to unfold. Current sequence data has allowed the development of PCR primers that can detect all of the viruses discovered to date. This discovery allowed investigators to determine that the PsHVs that cause PD persist in the mucous membranes of the oral cavity and cloaca and can be inconsistently detected in the blood.[31,49,50]

Based on these data, the following epizootiologic picture is proposed. Transmission between birds occurs when a naïve bird is exposed to the oral secretions, droppings or vomitus of a persistently infected bird. The route of infection can be by ingestion or contact with conjunctival or respiratory mucous membranes. The outcome of infection will depend on the genotype of the virus and the species of bird exposed. Genotypes 1, 2 and 3 are highly pathogenic to Amazon parrots. In Europe, genotype 4 PsHV also kills Amazon parrots, but this virus is not found to cause PD in Amazon parrots in the USA.[49] In contrast, genotype 4 is the most common cause of PD in macaws and conures. Cockatiels, cockatoos and other Pacific species of birds are relatively resistant to PD, but when they do develop disease, any of the four genotypes may be responsible. African grey parrots are susceptible to genotypes 2, 3 and 4. Genotype 1 has not been found in African grey parrots with PD, but the number of African greys tested to date is small, so this should be considered only a preliminary finding.

Birds that become infected with PsHVs and either do not develop PD or do develop PD but are treated and survive will become persistently infected and will remain persistently infected for life. There is a possibility that some subclinical infections may result in a cure, but this remains to be proven. There is no evidence that persistently infected birds will subsequently develop PD. However, some persistently infected birds will develop IP. Which birds will develop IP may depend on the species of the bird, the genotype of the virus and as yet undetermined factors.

Most persistently infected birds are readily detected by PCR analysis of blood and combined oral and cloacal swabs. These birds will be consistently positive with repeated samplings.[31] Even though PsHVs are continuously present in the mucosa of persistently infected birds, field data suggest that actual virus shedding or the degree of virus shedding may fluctuate over time. Species persistently infected with PsHVs include the macaws, Amazon parrots, some of the *Aratinga* conures and the Patagonian conure (*Cyanoliseus patagonus*). Increasing evidence also suggests that cockatiels, lovebirds, cockatoos and possibly other species may be persistently infected with one or more PsHVs. Wild-caught birds that have passed through a quarantine station, parent-raised chicks of wild-caught birds and birds that have survived an outbreak of PD are at highest risk for persistent infection.

The incubation period for PD typically ranges from 5 to 14 days. Virus replication occurs in a number of organs, and birds are viremic. Inclusion bodies are most often found in the liver and spleen and to a lesser extent the crop, small intestine and pancreas. Necrosis of the infected cells, particularly hepatocytes, accounts for the clinical signs.[36]

Clinical Presentation

PD occurs almost exclusively in psittacine birds. Disease is most common in avicultural collections, quarantined birds and pet stores. The most common clinical presentation is a dead bird that died with little or no advanced evidence that it was ill. PD occurs most frequently in mixed collections of parrots that contain Amazons, macaws and conures, particularly Patagonian and *Aratinga* conures. The onset of the breeding season or recent changes in the aviary may predispose to virus shedding and PD outbreaks. Clinical signs may precede death in macaws and less frequently in other species. Signs are non-specific and include lethargy, depression and anorexia. Profuse sulfur-colored (biliverdin-stained) urates are another non-specific but consistently reported sign. Regurgitation, bloody diarrhea and terminal central nervous system signs are infrequently reported. Duration of clinical signs ranges from a few minutes to a few days. Only a few birds are known to survive infection once clinical signs develop. Elevation in the serum aspartate amino transferase concentrations and a marked leukopenia are reported in these birds. Radiographically, hepatomegaly, splenomegaly and renal enlargement also are

documented. The number of affected birds can vary from a single isolated case to hundreds.[24]

To the author's knowledge, the only documented naturally occurring case of Pacheco's disease in a non-psittacine bird occurred in a keel-billed toucan (*Ramphastos sulfuratus*). Lesions in this bird and a second keel-billed toucan experimentally infected with Pacheco's virus were characteristic of the psittacine infection. In another toucan (species not reported), a disease resembling Pacheco's disease was described. Herpesvirus virions were identified in the tissues of this bird; however, fluorescent antibody-staining of the tissues with a Pacheco's virus-specific antibody was negative.

Diagnosis

Diagnosis in the live bird is difficult and rarely made. History, signs and laboratory findings are strongly suggestive of PD but are not specific. These birds are strongly positive on PCR of combined oral and cloacal swabs and blood but usually die before the samples can be analyzed. In the author's experience, once a bird is confirmed to have disease and owners know what to look for, they will detect the early stages of the disease in birds, often in time to save them with treatment.[24, 36]

Most birds that die are well muscled and may have recently ingested food. Common gross lesions include hepatomegaly, splenomegaly, renal swelling and serosal and epicardial hemorrhage. The affected liver may be uniformly pale yellow, resembling the appearance of a diffuse lipidosis (Fig 32.7), have a diffuse mottling, or have scattered, irregularly shaped, discolored foci. In many birds, liver lesions are not observed grossly. Less commonly, submucosal hemorrhage of the intestines with or without intraluminal blood also may be present. Because of the acute nature of this disease, gross lesions may be entirely absent in some birds.

Histologically, hepatic necrosis is present in the vast majority of the cases. Varying degrees of splenic lymphoid hyperplasia and necrosis, pancreatitis and enteritis also occur. Eosinophilic and, less frequently, basophilic intranuclear inclusion bodies are found in the liver on the margins of the necrotic areas and in bile duct epithelium. Inclusions will sometimes be present in the spleen, intestinal epithelium, crop and pancreas. Although these lesions are characteristic for PD, the diagnosis can be verified by PCR of tissue swabs, staining impression smears with specific fluorescently labeled anti-Pacheco's virus antibody and in situ hybridization.

Treatment

Mortality in Pacheco's virus outbreaks can be minimized by prophylactic use of acyclovir[b]. In the author's experience, mortality stops within 24 hours after the initiation of flock treatment. Treatment options include administration of acyclovir in the drinking water (1 mg/ml) and food (400 mg/quart of seed) simultaneously or by gavage (80 mg/kg q 8 h). A higher oral dose of 330 mg/kg q 12 h also has been recommended. The necessary length of treatment is not known. The author treats flocks for 7 days and birds with signs of disease for 2 weeks.[23]

Preventing virus spread is another important aspect of bringing a Pacheco's disease outbreak under control. Traffic through the aviary should be minimized and hygiene improved. Additionally, barriers between cages can be erected, or cages can be moved farther apart. Intensive cleaning efforts may result in the increased aerosolization and further dissemination of the virus. Immunization in the face of an outbreak is of questionable benefit, as protective antibody titers would not be expected for 2 weeks after vaccination.

Prevention and Control

Control measures fall into three categories: savvy management practices, testing and immunization. Given that some conure species have repeatedly been implicated in the outbreak of this disease, these birds should not be kept in a mixed collection. General concepts, such as a closed aviary, proper quarantine procedures and acquisition of birds from reputable sources, will help to minimize the likelihood of Pacheco's virus being introduced to an aviary. Adequate spacing between cages and limiting human traffic in the aviary also are important preventive measures. Outbreaks are less likely to occur in outdoor aviaries.

Testing is becoming an increasingly practical means of preventing the introduction of PsHVs into a collection. Persistently infected birds are readily detected by PCR of blood and combined oral and cloacal swabs. Birds that are at highest risk for being persistently infected are those that have survived PD outbreaks and wild-caught parrots and chicks raised by wild-caught parrots. Macaws, Amazon parrots, Patagonian conures and *Aratinga* spp. conures commonly are demonstrated to be infected persistently.

A single Pacheco's disease virus vaccine[c] is currently being marketed in the USA. The serotype of the virus in this vaccine and its ability to protect against all serotypes of PsHVs are not known. Immunizing parrots in mixed collections of high-risk birds may be beneficial.

Internal Papillomatosis of Parrots (IP)

Clinical Manifestations

IP is a disease that primarily affects macaws, Amazon parrots, hawk-headed parrots and, less commonly,

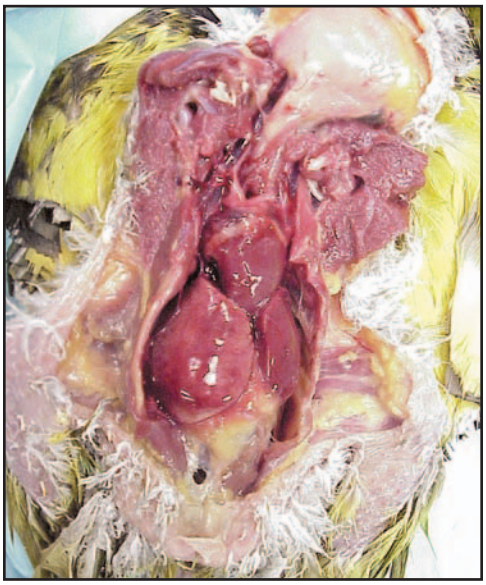

Fig 32.7 | An eclectus parrot with Pacheco's disease. The liver has a diffuse yellow mottling that is caused by extensive hepatic necrosis. Although many cases do not present with this lesion, the presence of this lesion should alert the practitioner or pathologist to the possibility that they are dealing with Pacheco's disease.

Fig 32.8 | Extensive papillomatous changes to the cloacal mucosa of an Amazon parrot.

conures. Most lesions are confined to the oral and cloacal mucosa, although lesions also may be found in the conjunctiva, nasal lacrimal duct, bursa, esophagus, crop, proventriculus and ventriculus. Cloacal lesions are the most common manifestation of IP in Amazon parrots and generally are present in the macaw as well. Oral papillomas are common in macaws. Of the macaws, the green-winged macaw (*Ara chloroptera*) is prone to develop the most widely disseminated form of IP. In these birds, lesions generally are present in both the cloaca and oral cavity and may extend into the esophagus, crop and even the proventriculus and ventriculus. Less frequently, blue and gold (*Ara ararauna*) and scarlet macaws (*Ara macao*) and, uncommonly, an Amazon parrot will develop this diffuse form of IP.[23]

Owners usually first recognize that their bird has IP when they see blood in the bottom of the cage from an ulcerated cloacal papilloma or when the papilloma prolapses through the cloaca (Fig 32.8). Oral lesions may be extensive but rarely result in clinical signs. Papillomatous lesions are rarely static; they wax and wane and may disappear entirely. Often, the only indication that IP is present is a slight roughening of the cloacal mucosa or a thickening of the choanal edges and blunting of the choanal papillae. If the lesions do not spontaneously resolve, each time they recur they generally are more severe. The lesions in some birds will be consistently present. Birds with IP may live for many years and even be reproductively successful. The general life expectancy of these birds, however, is diminished as compared to other birds without this disease. The chronic irritation associated with cloacal lesions and repeated surgeries to remove the lesions may result in cloacal strictures. Birds with diffuse lesions of their upper digestive system often develop a wasting disease that may resemble proventricular dilatation disease.

A small to moderate percent of birds with IP will go on to develop bile duct or pancreatic duct carcinomas. Signs of bile duct carcinomas are not specific. Birds typically lose body condition, appear unthrifty and may have an overgrown beak. Elevated gamma glutamyl transferase levels have been reported in birds with advanced lesions. Bile duct carcinomas are readily demonstrated with ultrasonography and appear as hyperechoic round to irregular masses. Infection with PsHV genotype 3 may predispose to the development of bile duct carcinomas.

Treatment

Birds with cloacal papillomas are often in pain, so it has been common practice to remove all or part of these lesions. Cryotherapy, electrocautery, chemical cautery, laser surgery and sharp dissection have all been used. In the experience of the author, removing part of a lesion will often cause the remainder to regress; these lesions generally return, however. To minimize the risk of stricture formation, surgery is limited to a small portion of the cloaca or not done at all. Carboplatin has been used in a few birds to treat bile duct carcinomas (B. Speer, personal communication, 2001) (see Chapter 35, Surgical Resolution of Soft Tissue Disorders).

Prevention

Mounting evidence strongly suggests the viruses that cause PD are the same viruses that cause IP.[18,27,43,44,48] Depending on the genotype of the viruses and the species involved, however, these viruses can disseminate through a collection without causing Pacheco's disease. The first clue that the virus is there is when birds begin to develop papillomas. Transmission can occur from parent to offspring and between cages of birds. Transmission is much more likely to occur if cages are in close proximity and birds are housed indoors. A meticulous physical examination of birds before entry into a collection coupled with a PCR assay for PsHVs will detect infected birds.

Treating birds with anti-herpesvirus drugs will not cure them of infection and does not appear to impact the course of IP. These viruses presumably persist in a non-replicating form and are not susceptible to these drugs.

Cutaneous Plaques and Papilliferous Lesions of the Foot

Herpesvirus virions are documented in proliferative lesions on the feet of macaws and cockatoos. These lesions show some species-specific variations. Lesions in cockatoos tend to be papilliferous, while those of macaws are raised, depigmented plaques. These lesions regress if treated topically with acyclovir. The sequence of a herpesvirus from a plaque from the foot of a macaw was found to closely resemble that of the PsHVs that cause PD and IP.[48]

MISCELLANEOUS HERPESVIRUS INFECTIONS IN COMPANION BIRDS

An infection resembling a herpesvirus infection is described in Gouldian, melba (*Pytilia melba*) and purple grenadier (*Uraeginthus ianthinogaster*) finches. Clinical signs include weight loss, anorexia, dyspnea, severe conjunctivitis and, less commonly, a head tilt. Mortality ranges from 25 to 100%. Gross and microscopic lesions are found in the conjunctiva, trachea and air sacs.[40]

A herpesvirus has been isolated from English budgerigars in Europe. Its presence has been correlated with reduced hatchability and is believed to be transmitted vertically.

A virus believed to be a mutation of the infectious laryngotracheitis virus of chickens has been observed to cause a severe upper respiratory and tracheal disease in Amazon parrots and Bourke's parakeet (*Neophema bourkii*). The duration of this disease is variable, but clinical signs have been reported to last up to 9 months. This disease is reported in the USA, but if it occurs in the USA, it is rare. Additionally, tracheitis occurs uncommonly in some birds with Pacheco's disease, and this could be mistaken for Amazon tracheitis.

DUCK VIRUS ENTERITIS (DVE)

This herpesvirus has caused massive die-offs of wild ducks on several occasions in the USA. In Texas, the disease is seen on a smaller scale in the early summer on small community or golf course ponds. Generally, these ponds are densely populated with semi-domestic ducks. A common factor in most of the outbreaks is the presence of Muscovy ducks on the pond. There is a significant amount of controversy surrounding this virus and what to do when an outbreak occurs. In the past, attempts were made to euthanize all ducks on the pond in an effort to eliminate the carrier birds. This approach to control is rarely practical and almost certainly will offend some members of the local community. Additionally, genetic-based tests now support the idea that this virus is widespread in wild waterfowl. At this point, the best method of control is to limit the density of ducks maintained on ponds and, if possible, prevent the introduction of Muscovy ducks onto these ponds.[38]

PIGEON HERPESVIRUS (PHV)

PHV has a worldwide distribution and is a particular problem in racing pigeons, as birds are exposed when closely housed with birds from other lofts prior to each race. When the virus is first introduced to the loft, many birds will be affected. Signs include depression, reluctance to move, protrusion of the third eyelid, conjunctivitis, vomiting, rhinitis, dyspnea, anorexia, weight loss and loose green droppings. Characteristic gray to yellow oral and pharyngeal diphtheritic plaques mark this disease. Birds that survive infection appear to remain infected for life. Virus shedding increases during the breeding season, resulting in infection of squabs. Infected squabs become carriers but disease does not occur, possibly because of passive transfer of antibody.[8,23] Egg transmission of PHV does not occur, and fostering eggs from infected flocks under hens that are not infected may break the infection cycle.[23]

POXVIRUSES

Applied Biology

There are many poxviruses. Each poxvirus has its own host range, a range that may include one or several species of birds. Examples of poxviruses with a limited host range include the canary poxvirus, which affects only canaries and canaries hybridized with other species, and the parrot pox that appears to be confined to South American parrots. A mynah poxvirus appears to affect only mynahs, but mynahs may be susceptible to the starling poxvirus.[24,51] In contrast, poxviruses brought into

Hawaii in non-native species have had a devastating impact on native Hawaiian forest birds. Fowl pox can cause disease in a number of gallinaceous birds and appears to be the cause of pox lesions seen in ostrich chicks in the USA.[39]

Poxviruses require an injury to enter the body. Mosquitoes are the most common vectors for poxviruses, allowing the virus to enter the body through a bite wound. When wild-caught nestling blue-fronted Amazon parrots were held in quarantine, incompletely sanitized hand-feeding utensils were believed to spread the virus from one bird to the next. Generally, canary outbreaks occur in birds that are housed outdoors, but conspecific aggression and cannibalism also may result in rapid dissemination if latently infected birds are present in the flock. Rarely, aerosolized virus in feces or feather dander may directly infect respiratory epithelium.

Clinical Presentation

The practitioner is most likely to see this disease in canaries and chickens housed outdoors and in free-ranging birds and young domestic pigeons. Historically, disease was seen in nestling blue-fronted Amazon parrots held in quarantine. This problem is not seen in captive-raised birds and, because the importation of these birds has essentially ceased, this manifestation of disease is no longer seen in the USA.

Three forms of disease are recognized. The so-called dry pox is the most common disease manifestation. In this form of the disease, lesions are most commonly seen around the face (especially the eyelid and commissures of the mouth), on the feet and under the wings (Fig 32.9). Lesions are raised, smooth to nodular, and may ulcerate. Lesions may be small and clinically insignificant to extensive, deforming lids and even the beak. Extreme cases result in lesions that may appear neoplastic. Secondary superficial bacterial and fungal infections occur in ulcerated lesions. Conjunctivitis and keratitis are common when there is extensive lid involvement. Extensive lesions also may impair vision, resulting in the birds not being able to find food. The lesions develop rapidly over the course of several days, but take up to 6 weeks to regress. When they do begin to regress, they regress rapidly.

Wet or mucosal pox is a second manifestation of some poxvirus infections. It is seen in canaries, lovebirds, mynahs and imported blue-fronted Amazon parrots. The disease in canaries is characterized by a unilateral or bilateral blepharitis, chemosis and conjunctivitis. Typically, there will be considerable ocular discharge and the eyelids will swell shut. Diphtheritic lesions of the oral cavity and trachea develop subsequently. These lesions cause the birds to stop eating, and secondary infections of these lesions are common. Extensive oral lesions and tracheal lesions may obstruct airflow and cause asphyxiation. Mortality in canary aviaries is often high. If treated aggressively, some of these birds will survive but may have persistent ocular lesions. Wet pox sometimes may accompany dry pox.

Systemic pox occurs in canaries as an acute onset disease. Chemosis, depression, anorexia and dyspnea characterize this disease. Birds die within a few days. If they survive, they will develop cutaneous lesions. Necropsy findings include air sacculitis and pneumonia.

Diagnosis

The gross appearance of the lesions in the appropriate species is highly suggestive of the disease. Biopsies of mucous membranes and cutaneous masses will reveal the classic large eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies) (Fig 32.10). Impression smears of these lesions also may reveal these inclusion bodies. Because the lesions often ulcerate, inflammatory cells, bacteria and yeasts are likely to be present in scrapings and impression smears.

Treatment

The poxviruses themselves cannot be treated. In the mild form of the disease, treatment is generally not necessary. Severe lesions may cause the birds to stop eating. In these cases, supportive care (tube-feeding and fluids) is indicated. Ulcerated lesions may become infected and antibiotic therapy is indicated in these birds. Vitamin A supplementation also is suggested to be therapeutic. Surgical removal of pox lesions will only cause scarring and should not be attempted.

Prevention and Control

Poxviruses are transmitted by insect bites or by inoculation into abrasions on the skin or mucous membranes. Raising birds indoors or screening the aviary best controls infections in canaries. A canary pox vaccine[d] is available. Immunization of pigeons with the pigeon pox vaccine also is an important means of control. Racing pigeons are immunized[e] no less than 6 weeks before the racing season begins, as the vaccine is live and will cause the birds to show some signs of illness.

RNA Viruses

PARAMYXOVIRUS 1 (PMV-1) EXOTIC NEWCASTLE DISEASE VIRUS

Applied Biology

Nine serogroups of avian paramyxoviruses are recognized.

Fig 32.9 | The dry form of pox in a chicken.

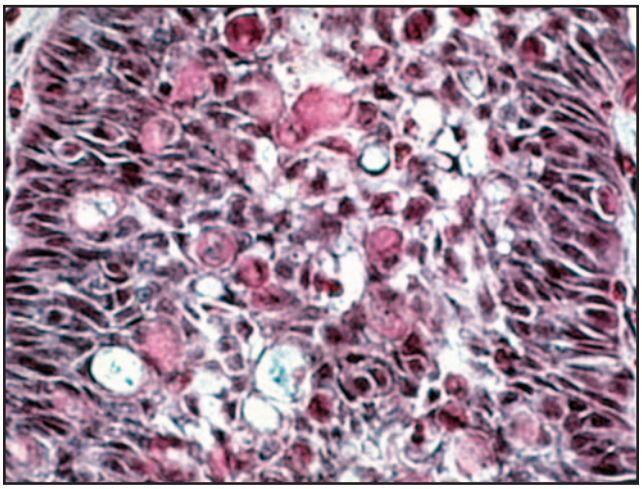

Fig 32.10 | Hematoxylin and eosin-stained section of an avian pox lesion. The round eosinophilic intracytoplasmic inclusions are characteristic of those produced by poxviruses.

Within each paramyxovirus serogroup there may be many strains. PMV-1 or the Newcastle disease virus strains are defined immunologically, genetically and by their pathogenicity to chicken embryos or chicks. Velogenic viruses are highly pathogenic to chickens. These viruses can be divided into those that cause predominately hemorrhagic lesions of the digestive tract — viscerotrophic velogenic Newcastle disease virus (VVND) — and those that cause predominately respiratory and central nervous system lesions — neurotropic velogenic Newcastle disease virus (NVND). Less pathogenic forms that primarily cause disease in young chickens are called mesogenic pathotypes, and those that cause little or no disease in the chicken are called lentogenic pathotypes. The virulence of PMV-1 virus for chickens does not consistently reflect the virulence of the virus in other species, as VVND may cause only mild signs in companion birds, and mesogenic viruses have caused devastating outbreaks of disease in wild birds. The two most important PMV-1 viruses that the practitioner confronts are the highly pathogenic VVND strain known as exotic Newcastle disease virus (END) and pigeon paramyxovirus.[1,24]

EXOTIC NEWCASTLE DISEASE VIRUS

END is a highly virulent virus that has a devastating impact on poultry worldwide. It has been excluded from many nations in the world by strict laws governing the movement of birds. In the USA, all birds entering the country are required to go through a 30-day government-monitored quarantine. Random birds and birds that die in quarantine are tested for END. Illegal movements of parrots across the Mexican border have been responsible for limited outbreaks of END in the USA in the past. The 2003 outbreak occurring in California, Nevada, Arizona and Texas is speculated to have resulted from illegal movements of fighting cocks from Mexico. Infected chickens shed virus through their respiratory system and feces. Inhalation of the virus may be an important means of transmission when birds are in direct contact. Movement of the virus between flocks results from the movement of infected birds and the movement of the virus on contaminated vehicles and other equipment, clothes and feed sacks. END can colonize the conjunctiva of the human eye where it can persist for at least 48 hours, but it is not known if this plays a role in END dissemination. Vertical transmission of virus is not believed to play an important role in END dissemination.

Clinical Presentation and Diagnosis

END in Poultry

END is as likely to appear in small, privately owned collections of chickens as it is in large poultry operations. Therefore, it is entirely possible that private practitioners will be presented with chickens with this reportable disease.

The first indication of END in a flock of chickens may be the sudden onset of mortality with few antemortem signs. Signs are generally non-specific and may involve the gastrointestinal tract, respiratory system, central nervous system or a combination of these systems. Sneezing, coughing, nasal discharge and dyspnea, swelling around the eyes and the head, green diarrhea, depression, weakness, muscle fasciculations, torticollis, paralysis and sudden death are all listed as signs associated with the 2003 outbreak in the southwestern USA. Birds with these signs should be immediately reported to local regulatory veterinarians. Necropsies of these birds are not performed in the clinic but at official diagnostic facilities.

Gross lesions also can be extremely variable. However, lesions that are highly suggestive of END include hemor-

rhagic lesions of the conjunctiva, esophagus, proventriculus, small intestines, ceca and cloaca. Tracheal hemorrhage may be a significant lesion in birds infected with certain strains. Birds with CNS signs may not have gross lesions.

END in Parrots

END has entered the USA on several occasions in smuggled parrots. Although the entry was along the USA-Mexico border, often the disease was not recognized until these parrots had made it to northern states. Outbreaks have typically been associated with nestling yellow-naped (*Amazona ochrocephala auropalliata*) and double yellow-headed Amazon parrots (*A. ochrocephala ochrocephala*). Signs are not specific and include depression, anorexia, weight loss and diarrhea. Respiratory signs may or may not be present. Ataxia, torticollis, opisthotonus, head bobbing, chorea and paralysis may occur in birds surviving the acute form of the disease. The development of neurologic signs in a sick bird should alert the veterinarian that he or she may be dealing with END. Recovery can occur, and recovered birds may shed virus for months to years. If this disease is suspected, a regulatory veterinarian should be immediately contacted.

Prevention and Protection

The first line of defense against END is controlling the movement of birds into a country. When this fails and the disease infects poultry, the situation is catastrophic. Under these circumstances, efforts are made to isolate the virus to a specific geographic area by stopping the movement of birds. Then a door-to-door campaign is undertaken to identify and slaughter all flocks with the disease. An extensive public education effort is necessary to keep this disease from spreading. In affected areas, breeders of companion birds are significantly impacted. Bird shows and sales are banned, and movement of birds out of the quarantined areas is prohibited.

Veterinarians in the quarantined areas and in areas where the disease has the potential of spreading will be asked by aviculturists what they can do to protect their flocks. The most important means of preventing END is to maintain a closed flock. This means that no birds are introduced to the aviary until the outbreak is under control. If owners are outside of the quarantine area and must bring in new birds, these birds are isolated in a separate facility for 30 days before being introduced into a flock. A veterinarian should examine all birds showing any sign of disease. A closed colony also means restricted access to the public. In quarantine areas, visitors who own birds or might have been around birds should be completely barred from the facility. Footbaths should be placed at the entranceway to the aviary, and every effort should be made to make sure that anything brought onto the premises, including food, has not been exposed to other birds.

There is no desire on the part of regulatory veterinarians to unnecessarily kill companion birds. Therefore, in the USA, consideration is given to the specific circumstances of the home or the aviary before action is taken. For maximum protection, birds are housed indoors. If this is not possible, they should be housed in a way that keeps out all wild birds and rodents. A fence preventing loose neighborhood birds from entering the facility is critical. It is highly recommended that all exotic bird owners not keep poultry, or if they do, that the poultry be confined to a cage and isolated the same way the companion birds are isolated. Food and water dishes are covered so the droppings of wild birds cannot contaminate them. An effective biosecurity protocol as described is critical. If END is found in close proximity to an aviary, the aviary is likely to be quarantined. Owners will then be required to follow specific quarantine measures, including having their birds swabbed twice at a 15-day interval. If END is found in a flock, the birds will be euthanized. For more detailed information see the California Animal Health and Food Safety Services Web site at www.cdfa.ca.gov. Immunization of companion birds with Newcastle disease vaccines intended for poultry is not recommended. It also should be noted that immunized poultry still may contract END, and their vaccination status will not impact the outcome of the flock if they are exposed.

PMV-1 IN PIGEONS (PPMV-1)

Applied Biology

Pigeon paramyxovirus-1 (PPMV-1) was first recognized in the early 1980s and has since disseminated throughout the world. It is found in feral pigeon populations and is a significant problem in racing pigeon flocks. Evolution of this virus has resulted in changes in its virulence and clinical manifestations. PPMV-1 is not restricted to pigeons and has been identified in feral Eurasian collared doves (*Streptopelia decaocto*).[45] The strain of virus identified in these doves, however, appears to be adapted to them and varies somewhat from that found in pigeons.[8,24,45]

Clinical Presentation and Diagnosis

PPMV-1 may present in two ways. In the first, neurologic signs predominate. Ataxia and torticollis are the most common signs. The second presentation is polyuria with or without neurologic signs. In both forms of the disease, many birds in a loft will show signs and mortality can be high. Affected birds should be submitted for necropsy to verify the infection.

Prevention

PPMV-1 is disseminated by contact with other pigeons. It is particularly common for it to be introduced to a loft when pigeons are raced, as the birds are in close contact with birds from other flocks prior to the race. Immunization with a PPMV-1 vaccine[f] at least 6 weeks prior to the racing season is recommended on a yearly basis.

OTHER PARAMYXOVIRUSES

Paramyxovirus 2 (PMV-2)

PMV-2 rarely causes disease in companion birds. It has been associated with contact with wild passerine birds, particularly finches, and should be considered a differential for birds that are showing respiratory signs.

Paramyxovirus 3 (PMV-3)

PMV-3 has been recognized in multiple nations around the world, but its basic biology is poorly understood. It appears to cause subclinical infections in some birds and it is these birds that are responsible for the spread of infection. Disease is reported most frequently in *Neophema* species, lovebirds, cockatiels and Amazon parrots, although recently the disease also has been seen in African grey parrots. Signs of infection may be non-specific and precede death by 24 to 48 hours. Birds with a longer duration of signs will develop CNS signs resembling those seen with END. In some cases, respiratory signs also may occur. Chronic infections in *Neophema* species often result in chronic pancreatitis. These birds have voluminous stools that contain undigested starch and fat. PMV-3 infections also are reported in finches with signs of diarrhea, dysphagia, conjunctivitis and dyspnea.[22,24]

Antemortem diagnostic assays have not been consistently effective in identifying birds with disease and asymptomatically infected birds. Traditionally, birds infected with PMVs will produce antibodies that will inhibit virus-induced agglutination of red blood cells. This may be true in some parrots with acute PMV-3, but may not be true in chronic infections. Current efforts to develop an ELISA assay to detect chronically infected birds may help to resolve this issue. Molecular-based technology also may prove to be beneficial in the diagnosis of these infections.

AVIAN INFLUENZA

Avian influenza is a rare disease of companion birds. It is reported to cause non-specific signs as well as signs related to the central nervous system. It is readily recovered from swabs of the cloaca and trachea.

EASTERN EQUINE ENCEPHALITIS (EEE)

The importance of EEE in parrots is unclear. EEE was implicated in a disease of 7- to 12-week-old macaws. The birds showed varied signs from sudden death to decreased appetite with abdominal distention. Grossly, serositis with extensive abdominal effusion was noted. Histologically, hepatic disease, interstitial pneumonia and lymphocytic proventriculitis were consistent findings. This disease has been termed polyserositis. It is the author's experience that this disease is extremely rare. However, edema and ascites occur with some degree of frequency following APV infections, and this disease and its associated lesions may be mistaken for polyserositis.

WEST NILE VIRUS (WNV)

Applied Biology

WNV is a flavivirus that is a member of the Japanese encephalitis virus complex. WNV can be divided into two lineages. Lineage 1 has a wide distribution. It has been isolated from Africa, Europe, the Middle East, India, Australia and the USA. Lineage 2 is confined to Africa. Recently, outbreaks of WNV have occurred in France, Romania, Italy, Russia and Israel. The outbreak in Israel was atypical because the virus had an apparent increased virulence for humans and birds. A strain of WNV essentially identical to the strain found in Israel was first identified in New York City in 1999. By the time of this writing, it has spread widely and has been identified in the majority of states in the USA and in several provinces in Canada. It is expected that it will continue to expand its range into Central and South America in the coming years.[15,20]

Birds are the primary vertebrate host for WNV. Mosquitoes, particularly the members of the genus *Culex*, are the insect vectors. It has been postulated that hippoboscid flies also may be vectors of WNV, but this has not been experimentally tested (M. Taylor, personal communication, 2002). After ingesting blood from a viremic bird, the virus is amplified in the digestive tract and salivary gland of the mosquito. Bird infection occurs when an infected mosquito bites a bird. After a bird is infected, it remains viremic from 4 to 7 days. The magnitude of the viremia depends on the species infected. Crows, magpies, house sparrows (*Passer domesticus*) and other passerines appear to develop the highest concentrations of virus in the blood and have the longest duration of viremia. WNV may persist in the skin after the cessation of viremia, allowing mosquito infection for an as-yet-undetermined period of time after infection.

Preliminary studies suggest that consumption of animals infected with WNV and even ingestion of infected mos-

quitoes will result in infection in some species of birds. Close contact also can result in viral dissemination between birds. Virus can be found in the oral cavity and cloaca of infected birds for 9 or more days after infection, and it is a reasonable hypothesis to assume that it is present in saliva and droppings. WNV persists in the tissues of some species that survive for as long as 14 days after infection. Experiments have not been done to show if it persists longer than this.[16]

Clinical Presentation

WNV can infect and is believed to have caused death in a wide range of species. Birds particularly susceptible to disease caused by WNV include crows, blue jays (*Cyanocitta cristata*), magpies, accipiters, red-tailed hawks (*Buteo jamaicensis*) and several species of northern owls. Ruffed grouse (*Bonasa umbellus*), gulls, house sparrows, robins (*Turdus migratorius*) and mourning doves (*Zenaida macroura*) make a significant number of reported cases, but reported disease in these species is less than 10% of that reported for crows. Naturally and experimentally infected geese appear to be sensitive to disease from WNV. Chickens, turkeys and at least some species of psittacine birds appear to be relatively refractory to disease.

WNV infection has a seasonal distribution in temperate climates. The first cases are seen in the spring and then continue through the summer. A short course of lethargy followed by death may be the only signs seen. Other birds, however, develop signs of central nervous system disease, including ataxia, tremors, weakness, seizures and abnormal head postures prior to death. Anisocoria and impaired vision also were noted in some birds. Observations by practitioners suggest that some birds may show mild signs of illness and then recover.[41]

Diagnosis

Necropsy findings suggestive of WNV disease include intraosseous hemorrhage of the calvaria and hemorrhage of the meninges, mucosa and serosa of the gastrointestinal tract. Splenomegaly is minimal to marked. Focal, linear, or diffuse myocardial pallor also may be present. Microscopic lesions of the brain, heart, pancreas, intestines and spleen are highly suggestive of WNV disease. Infection can be confirmed by isolating the virus from oral and cloacal swabs, brain, heart, kidney, liver, lung and spleen or PCR of these tissues.

Neutralizing virus antibody is detected in the majority of birds within 14 days of infection. Plaque reduction assays and hemagglutination inhibition assays are used to detect antibodies to WNV.[15,16,41]

Prevention

WNV has created panic in the general bird-owning population in the USA and has been of great concern to wildlife and zoo veterinarians. Many zoo veterinarians have elected to use the West Nile virus vaccine[g] for horses to vaccinate birds. The equine dose is 1 ml. Experimental immunization using a 0.5-ml dose given twice 2 weeks apart did not induce an antibody response in cockatiels. A full 1.0-ml dose given three times, 3 weeks apart, did induce an antibody titer in some but not all birds immunized at the Houston Zoo (J. Flanagan, personal communication, 2003). Adverse reactions to the vaccine were not noted. A hemolytic anemia, however, has been reported in lories immunized 1 year after their first set of immunizations the year before. Similar problems have not been noted at the Houston Zoo in birds that have been immunized 2 years in a row (J. Flanagan, personal communication, 2003). Given that it is not known how protective this vaccine is for birds and that at least one institution has seen adverse effects that may be linked to immunization with it, use of this vaccine should be considered a last resort when other mosquito control options are not available. An immunization schedule that has been used has been to give a 1-ml dose of the vaccine every 3 weeks for three immunizations. The vaccine can be divided and given in multiple sites in smaller birds.

Disease in psittacine birds is rare; therefore, immunization of psittacine birds is not recommended at this time. Concerned pet owners should keep their birds indoors in the warmer months of the year.

REOVIRUS

A reovirus was found to be one of several pathogens causing a complex of diseases in recently imported African grey parrots, but also was seen in several other species including cockatoos.[6] Because imported wild birds are rarely seen in practice today in the USA, this disease has essentially disappeared. Wild-caught African grey parrots are, however, still imported into Europe, and this disease continues to be a problem there.

Clinical Presentation

The signs of disease have varied to some degree with the specific outbreak. Signs in outbreaks seen in the USA included depression, weakness, weight loss, edema of the legs and head, and paralysis. Anemia, leukopenia and elevated liver enzymes also are reported. More recent outbreaks in African grey parrots imported into Italy showed respiratory signs, including coughing, nasal discharge and increased lung sounds. The disease had a prolonged course and affected birds died. Australian king parrots also were affected with this disease, but these birds died suddenly.

Natural reovirus infections are often complicated by concurrent infections with multiple other infectious agents, including bacteria, *Aspergillus* spp. and other viruses. Each of these pathogens contributes to the clinical picture of disease.

Diagnosis

Diagnosis in the live bird is probably impossible. However, this disease should be suspected in recently imported wild-caught African grey parrots and birds that are in contact with them. Gross lesions include enlargement of the liver and the kidney, with focal depressed discoloration of the capsular and cut surfaces of the liver. Serosal hemorrhages, enteritis and renal enlargement occur less commonly. Lesions from other pathogens also may be present. Histologic lesions are not specific, and virus isolation is necessary to confirm reovirus infection.

Control

There is no means of control for this disease other than to stop importing wild-caught African grey parrots. Isolation of imported African grey parrots from other imported species may prevent some losses.

RETROVIRUSES

Retroviruses are important causes of disease in waterfowl and gallinaceous birds. Documentation of retrovirus diseases in companion birds is lacking.

Diseases Thought to be Caused by Viruses

PSITTACINE PROVENTRICULAR DILATATION DISEASE (PDD)

Applied Biology

PDD is a disease of unknown etiology, but circumstantial evidence suggests that one or more viruses cause it. Numerous clinical reports document the spread of PDD through a collection after the introduction of birds that subsequently develop disease. Inoculation of tissue homogenates derived from a bird with PDD induced a histologically identical disease in experimentally infected birds. Viruses and virus particles have been identified in a number of birds with PDD. Several viruses have been identified in birds with PDD or in flocks where PDD was a problem. These include eastern equine encephalitis, enterovirus, coronavirus, reovirus, avian paramyxovirus 1 and paramyxovirus 3.[9,10,12] The potential role of paramyxoviruses as the cause of PDD has been strengthened by the development of lesions identical to those seen in PDD in a flock of *Neophema* spp.. They were experiencing an outbreak of PMV-3 infection: these findings were strengthened by the discovery of antibodies to PMV-1 in birds with PDD, and isolation of a low virulent strain of PMV-1 from the spinal cord of 6 of 32 parrots with PDD.[11,12,22] It is hoped that the slow but steady progress made by investigators working with this disease will soon lead to the discovery of its etiologic agent.

Clinical manifestations of the disease are the result of a lymphoplasmacytic inflammation of the nerves of the gastrointestinal tract and brain, spinal cord and peripheral nerves.[5] Lesions also may be found in the heart and adrenal gland. Lesions are rarely diffuse and are variable in their severity. As a result, clinical signs of disease vary from case to case.

Clinical Presentation and Diagnosis

PDD occurs most frequently in African grey parrots, macaws, Amazon parrots, cockatoos and conures, but it is possible that all parrot species are susceptible.[24] It also may occur in non-psittacine birds, as a disease with similar lesions has been observed in Canada geese, a red-tailed hawk and flamingos. There is no sex predilection for PDD. The median age of onset of PDD is 3 to 4 years, but birds as young as 10 weeks and as old as 17 years have been documented with lesions consistent with PDD. Domestically raised and imported birds are equally susceptible to disease. The incubation period for this disease is not known but may be long, as birds isolated from contact with other birds for up to 2 years still have developed this disease.

The number of birds affected by PDD in a collection, the rapidity with which it spreads through a collection and the clinical signs of disease can vary significantly. Birds with the most common form of the disease present with what the owner considers to be an acute onset of disease. There may be a history of regurgitation, anorexia and the presence of undigested seeds in the droppings. Physical examination, however, reveals an emaciated bird. Often the ventriculus can be palpated in the coelomic cavity caudal to the edge of the sternum. Radiographically, the proventriculus is often massively dilated, filling the left side of the coelomic cavity (Figs 32.11, 32.12). Typically, it develops a "J" shape causing the ventriculus to be displaced to the right and ventrally (Fig 32.13). Ultrasound will demonstrate a widely distended proventriculus and ventriculus. Muscle contractions typically are weak, and there is a failure of the junction between the proventriculus and ventriculus to close. Contrast studies show distention of the proventriculus and ventriculus and often the proximal duodenum. Transit time of the contrast material is markedly reduced. Various permutations of this disease may occur, and dilation of the crop, ventriculus, proventriculus or

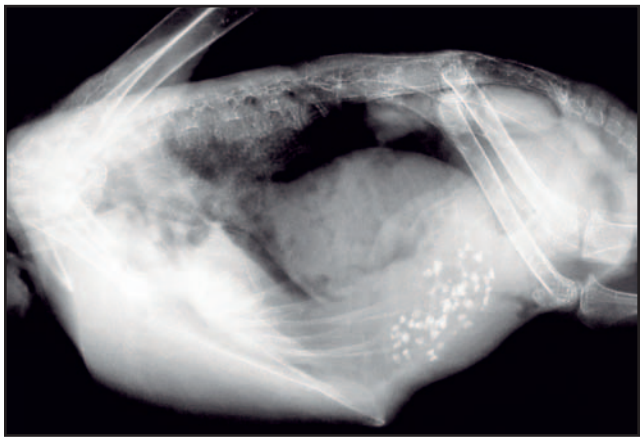

Fig 32.11 | Lateral radiograph of an eclectus parrot reveals proventricular dilatation disease (PDD). Note the massively distended proventriculus.

Fig 32.12 | In this ventral dorsal radiograph of the eclectus parrot in Fig 32.11, the hugely distended proventriculus fills the left lateral coelomic cavity.

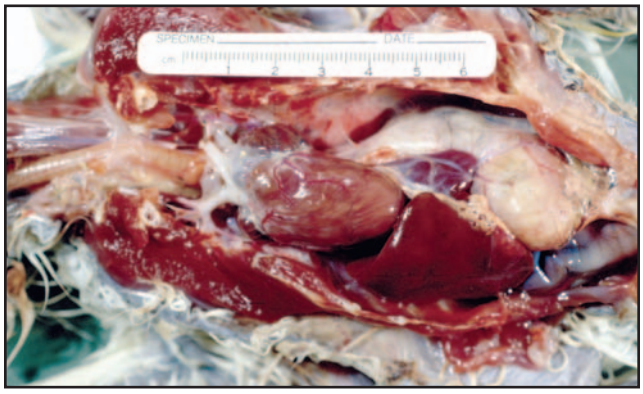

Fig 32.13 | In the gross necropsy of a eclectus with PDD in Figs 32.11 and 32.12, note the massively distended proventriculus that extends laterally beyond the edge of the left liver lobe. The ventriculus is displaced to the right and ventrally.

duodenum may be seen alone or in combination.

CNS signs may be absent, occur in combination with the gastrointestinal signs, or be the only presenting signs. CNS signs may have an acute onset or be very slowly progressive. They may reflect disease of the brain or the spinal cord, and recent evidence suggests that they also may reflect lesions of the lower motor neurons. Ataxia that may be slowly progressive, proprioceptive deficits, paresis and less commonly paralysis, head tremors and rarely seizures have been reported in birds with PDD.

Clinical pathologic findings are not specific but typically reflect the fact that these birds are starving. High uric acid levels were seen in one of the author's cases, because the bird's neurologic disease prevented it from reaching the water bowl and drinking. Overgrowth of the digestive tract with yeast and gram-negative bacteria occurs as the result of gastrointestinal stasis. Initial attempts at diagnosing this disease by serology and electron microscopy of the feces have subsequently been discontinued.

Demonstrating characteristic lesions in a crop biopsy can make a definitive diagnosis of this disease. Not all birds with PDD have crop lesions, so failure to find lesions in a biopsy does not rule out the disease. It is suggested that biopsying the right cranial ventral aspect of the crop, while making sure that it contains a blood vessel, will increase the probability of a lesion being found.[10] Biopsy of the proventriculus is not indicated, as the proventriculus will already be diseased and is likely to dehisce. A partial thickness biopsy of the ventriculus to get a section of the splanchnic nerves is safe but is rarely done.

The progression of PDD through a flock cannot be predicted. Typically, once it is recognized in the flock, additional birds will develop disease in the following months to years. However, there are times when only an individual bird is affected. Uncommonly, there are outbreaks where multiple birds develop disease within a very short period of time.

As common as this disease is, there are other diseases that can cause nearly identical signs.[2] Gastrointestinal signs identical to those seen in PDD can be caused by neoplasia of the intestines, intestinal foreign bodies and even massive worm burdens that cause intestinal obstruction. Emptying of the proventriculus and ventriculus appears to be inhibited as long as the intestinal tract is distended. As a result, proventricular dilatation occurs in cases of lower bowel obstruction. Inflammatory disease and neoplastic diseases of the ventriculus and proventriculus also can cause gastrointestinal stasis. Another common cause of gastrointestinal stasis is heavy metal poisoning. Heavy metal poisoning is commonly associated with central nervous system signs as well. A chronic wasting disease caused by internal papillomatosis also resembles PDD.

PDD should be considered as a differential in any bird

with CNS disease. Traumatic injuries, heavy metal poisoning, neoplasia, viral, bacterial and fungal infections of the CNS, nutritional deficiencies and hydrocephalus are additional diseases that can cause similar signs.

Treatment

Celecoxib[h], a COX-2 inhibitor, has been advocated as a treatment for PDD.[7] Controlled trials with this drug for the treatment of PDD have not been done, but careful clinical reporting suggests that celecoxib does cause regression of the signs of PDD, and birds that otherwise would have died are still alive up to 2 years after treatment. Successful treatment appears to be more likely if PDD is diagnosed before the bird is extremely debilitated. The recommended treatment protocol is to make a suspension of celecoxib in lactulose and to administer 10 mg/kg orally once a day for a minimum of 6 weeks or until the signs resolve completely. Although lactulose was the first agent used to suspend this drug, others may work just as affectively. Anecdotal information suggests that other COX-2 inhibitors also may be effective.

Additional supportive care will need to be supplied to these birds in addition to celecoxib treatment. Birds that are dehydrated should be given fluids. Liquid diets sometimes will pass through the digestive system while solid diets will not. Secondary yeast and bacterial infections also should be treated.

Prevention and Control

Until the etiologic agent of this disease is identified and an appropriate test for that agent is developed, preventive measures will depend on conservative management practices. A detailed history of the source of all new birds and long quarantine periods (>6 months) will reduce the risk of introducing this disease. Historical findings suggest that this disease is more likely to spread in indoor collections. Keeping breeding birds outdoors when possible and maximizing hygiene, ventilation and cage separation in indoor aviaries may reduce the risk of PDD transmission.

The diagnosis of PDD in a collection can be a devastating blow to the aviculturalist. Remember, however, that sooner or later most large collections of birds that have been assembled from multiple sources will have a case of PDD. Isolating birds in contact with birds that have had PDD may prevent dissemination. Incubator-hatching eggs and hand-raising these chicks in isolation also may break the infection cycle, although this has not been proven scientifically.

SEASONAL MORTALITY IN GREAT-BILLED PARROTS

A disease of unknown etiology is killing great-billed parrots (*Tanygnathus megalorhynchos*) in the USA.[26] A survey of great-billed parrot owners suggests that this disease has killed approximately 50% of the great-billed parrots housed in the southern USA during the period of 1995 to 2000. The disease occurs predominately in Gulf Coast States from July to October. Other species that have had similar signs of this disease include the mealy Amazon parrot (*Amazona farinosa*) and lories. The disease occurs typically in outbreaks, with multiple birds developing signs over a period of 1 to 4 weeks. Both hand-fed nestling parrots and adult parrots are susceptible. Birds housed outside are primarily affected, but disease also has occurred in indoor birds.

Clinical signs of this disease are dramatic. Gastrointestinal motility ceases, so birds stop eating. They may have diarrhea or no droppings at all. Even liquid diets do not pass through the digestive tract. Affected birds rapidly become dehydrated. Birds die within 1 to 3 days, despite aggressive therapy. One case did survive after 2 weeks of treatment, during which time the bird lost nearly 50% of its body weight before its digestive tract began to function again. Intussusception of the intestines occurs in a significant number of cases, resulting in bowel strangulation and necrosis. Blood counts and clinical chemistries show a moderate drop in the total white blood cell count, concentration of the blood from dehydration, an imbalance of electrolytes, elevation in muscle enzymes and an elevation in uric acid, indicating severe dehydration or primary kidney disease or a combination of both.

Consistent specific necropsy findings do not occur. Given the seasonality of this disease, an insect-borne virus has been suspected to cause this disease. However, repeated virus isolation attempts have been unsuccessful. Investigators are currently examining the possibility that a clostridium toxin may be causing this disease.

EPIZOOTIC RESPIRATORY NEOPLASIA OF COCKATIELS

Rapidly growing tumors of the air sacs and lungs occur in cockatiels. Multiple birds in a collection may be affected over a period of several years. The most common clinical sign is the sudden onset of severe dyspnea, although observant owners may see the signs develop over the course of several days. By the time the birds are severely dyspneic, restraint for physical examination or radiographs can be life threatening. If the bird can be radiographed, masses will be seen either in the lung or lungs or in an air sac. These are highly invasive tumors and will penetrate through adjacent vertebrae, compressing the spinal cord. When this occurs, birds present

with a progressive or sudden onset of paresis or paralysis of the legs. Attempts to treat these tumors have not been reported.[24]

These tumors are light tan to yellow. A single pulmonary or air sac mass may be present, but multiple masses are more common. Often they are expanding into the thoracic inlet, resulting in compression of the interclavicular air sac and the trachea. Some of these tumors contain a considerable amount of well-differentiated fat tissue; they may outwardly appear as lipomas. The appearance of the nuclei of the neoplastic cells resembles the nuclear changes seen in cells infected with APV. A history of APV disease has been documented in some of these aviaries, but APV DNA has not been identified in these tumors.

Products Mentioned in the Text

a. Psittimune APV Avian Polyomavirus Vaccine, Biomune, Lenexa, KS, USA 913-894-0230, www.BiomuneCompany.com
b. Zovirax, Burroughs Wellcome Co, Phoenix, AZ, USA 8572-9349
c. Psittimune PDV Pacheco's Disease Vaccine, Biomune, Lenexa, KS, USA 913-894-0230, www.BiomuneCompany.com
d. Poximune Canary Pox Vaccine, Biomune, Lenexa, KS, USA 913-894-0230, www.BiomuneCompany.com
e. Pigeon Pox vaccine, Maine Biological Laboratories, Waterville, ME, USA, 207-873-3989, www.mainebiolab.com
f. Paramyxovirus 1 Vaccine, Maine Biological Laboratory, Waterville ME, USA, 207-873-3989, www.mainebiolab.com
g. West Nile Virus Vaccine, Fort Dodge Laboratories, Fort Dodge, IA, USA, 800-477-1365, www.wyeth.com/divisions/fort_dodge.asp
h. Celebrex, Pharmacia, Pfizer, www.celebrex.com

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