Advanced Diagnostic Approaches and Current Management of Proventricular Dilatation Disease

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Introduction

Proventricular dilatation disease (PDD; Synonyms: proventricular dilatation syndrome, macaw wasting/fading syndrome, neuropathic gastric dilatation of psittaciformes, psittacine encephalomyelitis, myenteric ganglioneuritis, infiltrative splanchnic neuropathy) is a fatal inflammatory disease that affects mainly, but not exclusively, psittacine birds (Order: Psittaciformes). The disease was first recognized in the 1970s in imported macaws (Ara sp.) in Europe and North America (1-7), but has since been reported also from Australia (8,9), the Middle East (10-12), and South America (13). PDD is also present in South Africa (Dr. Emily Lane, personal communication).

Over 70 psittacine species have been reported to be susceptible to PDD (6,14-16, HLS & SC unpublished). These include members of most well-known parrot genera in both the Psittacidae and Cacatuidae families, such as macaws (Ara sp.), the African grey
parrot (*Psittacus erithacus*), cockatoos (*Cacatua* sp.), Amazon parrots (*Amazona* sp.), Conures (e.g. *Aratinga* sp.) Cockatiels (*Nymphicus hollandicus*) and many more (Table 1). While the actual number of susceptible psittacine species is likely much higher, some popular pet species, most notably the budgerigar (*Melopsittacus undulatus*), have not been reported to develop PDD (15,16, HLS unpublished), and may be resistant to the disease.

In addition to *Psittaciformes*, pathological findings identical to those seen in PDD have been reported in a number of captive and free-ranging birds representing at least 5 additional orders. These include Canaries (*Serinus canaria*, Order; *Passeriformes*); a greenfinch (*Carduelis chloris*; Order: *Passeriformes*); a long-wattled umbrella bird (*Cephalopterus penduliger*; Order: *Passeriformes*) Canada geese (*Branta Canadensis*; order: *Anseriformes*), roseate spoonbills (*Ajaja ajaja*; order: *Pelecaniformes*); the peregrine falcon (*Falco peregrinus*; order: *Falconiformes*), toucans (*Ramphastos sp.*; Order: *Piciformes*), and a bearded barbet (*Lybius dubius*; Order: *Piciformes*) (7,17-20).

Based on the occurrence of case-clusters, PDD has been long been considered an infectious disease (5); however, under most circumstances the disease appears to spread slowly within aviaries. Outbreaks affecting dozens of birds during a short time period (e.g. several weeks) have also been described (10,21,22). Crowded indoor aviaries as well as nurseries where parrot chicks are being hand-fed appear to be at the highest risk for PDD outbreaks. While the majority of reported PDD cases are of adult birds (6) birds as young as 5 weeks may be affected (22). Female psittacines have previously been reported to be overrepresented in PDD cases at a ratio of 1:0.6 or more (6,15) while in another study males were over represented at a ratio of 1:0.9 (14). Therefore, it is most
likely that both males and females are equally susceptible to PDD. Very little is known about the occurrence of PDD in wild avian populations. No PDD cases have been reported to date in free-ranging parrots of any continent. PDD is considered the main threat to captive populations of highly endangered psittacine species, such as Spix’s macaw (*Cyanopsitta spixii*), a species that is now extinct in the wild (12).

**Etiology**

PDD has long been suspected to be a viral disease, based on epidemiologic observations, its apparent infectious nature, the typical lesions associated with it, and by ruling out other possible etiologies (5,14,15). Several researchers have attempted to identify the PDD virus using standard virological methods such as culture and electron microscopy (EM). Initially a virus that was recovered from macaws suffering from serositis and that had been tentatively identified as a member of the family *Togoviridae* was suggested to be the candidate etiologic agent of PDD (23,24), but other researchers could not confirm this hypothesis (7,14). Pleomorphic virus-like particles of variable size (30-250nm) have also been described in tissues of affected birds by EM (25). These were suspected to be of the genus avian paramyxovirus (APMV); however, birds affected by PDD have been shown to lack antibodies against APMV of serotypes 1-4, 6, and 7, as well as against avian herpes viruses, polyomavirus, and avian encephalitis virus (5,6). In studies from Germany, APMV-1, closely related to the Hitchner B1 vaccine strain, was isolated from the spinal cords of ~ 20% of PDD patients; however, these isolates showed very low pathogenicity and failed to reproduce the disease in African grey parrots (26,27). Other virus species that have been documented in tissues or excretions of
affected birds include an adeno-like virus, enterovirus, coronavirus and reovirus (6,7,29). These reports were; nevertheless, sporadic.

More consistently, an unidentified enveloped virus of about 80nm in diameter has been demonstrated by EM in feces of affected birds, and a similar virus was isolated from tissues of affected birds using a macaw embryonic cell culture (7,29-31). This virus was initially suspected to be an alphavirus, but further investigation has ruled this possibility out (32). Tissue homogenates from an affected bird that contained this virus were used to inoculate and to successfully reproduce the disease in several psittacine birds (7,31); but despite this success and nearly three decades of PDD research, the identity of the PDD agent remained enigmatic, with some researchers suggesting an autoimmune rather than a viral etiology (15,33).

The major breakthrough in identifying what is now widely believed to be the etiologic agent of PDD only happened very recently, when advanced molecular tools, such as panviral DNA microarrays and high throughput sequencing, were used to test tissues of PDD-positive birds. In 2008 Kistler et al. and Honkavuori et al. independently reported on the recovery of a novel bornavirus from birds with PDD from the US and from Israel (11,34). Based on 16 ABV isolates, 5 distinct genotypes were identified, each sharing only ~ 65% nucleotide sequence identity with previously known members of the *Bornaviridae* family (all originating from mammalian hosts), and ~85% with other ABV genotypes (11).

Bornaviruses are negative-encoded, single-stranded, non-segmented, RNA viruses that are members of the order Mononegavirales. Their placement within a separate family (*Bornaviridae*) was based on several unique characteristics of their genome and
mechanism of replication, most notably the fact that they replicate in the host-cell nucleus rather than in its cytoplasm (35-40). Prior to the discovery of ABV, the single known species within this family was the Borna disease virus (BDV). Borna disease is an encephalitic disease of horses, sheep and occasionally other domesticated mammals. The disease was first described in the early 19th century in Southeast Germany, and has since remained endemic in that area. Many additional species, including the chicken (*Gallus gallus*) are susceptible to BDV infection under experimental conditions, with the outcome ranging from severe encephalomyelitis to persistent asymptomatic infection (37). The pathology seen with BDV is the result of neural invasion by T CD8+ lymphocytes rather than virus-inflicted cellular damage (38).

BDV is an enveloped spherical medium-sized virus, most virions being in the range of 70-130nm (39). The ~ 8,900bp genome encodes 6 major genes, including a nucleoprotein (N), a non structural protein (P10), a regulatory phosphoprotein (P), a matrix protein (M), a membrane-bound glycoprotein (G), and a RNA-dependent RNA polymerase (L) (40). BDV strains show remarkable sequence homogenicity and are all derived from mammalian hosts (11). There is only one report on the recovery of partial BDV RNA sequences from wild avian species (41).

In the short time since the publication of the two pioneering efforts by Kistler et al. (11) and Honkavuori et al. (34), eight additional studies have reported detecting ABV in PDD-positive birds or in birds exposed to PDD cases originating from four continents (20,22,42-47). A sixth ABV genotype has been described (44), and ABV has been recovered from at least 28 psittacine species and one non-psittacine species - a canary (*Serinus canaria*) with typical PDD pathology. Partial sequence analysis has shown the
canary ABV strain to be closely related to ABV5 (20). While most of the recoveries of ABV so far have been from clinically affected birds, a-symptomatic infection and long-term virus shedding have also been identified, and likely play an important role in the epidemiology of PDD (22,43,45,48).

PDD has been successfully reproduced in cockatiels (*Nymphicus hollandicus*) inoculated with brain homogenate containing ABV4, and the presence of an ABV4, nearly identical to that of the inoculum, was demonstrated in various organs of the inoculaes (43). PDD has also been reproduced in cockatiels and Patagonian conures (*Cyanoliseus patagonus*) using cultured ABV, fulfilling Koch’s postulates (47). The distribution of ABV in different tissues and organs of PDD-positive birds has been studied by several researchers, using immuno-histochemical (IHC) staining, western blot, and Quantitative real-time reverse transcription-PCR (20,22,34,42-46). Clear tropism to nervous tissue was demonstrated; however multiple additional tissue types were involved (see below). The route of transmission of ABV is as yet unknown, but is believed to be feco-oral. While our understanding of ABV pathogenesis and epidemiology is still in its infancy, the studies published so far provide convincing evidence, both direct and indirect, that the causative agent of PDD has finally been identified.

**Pathology**

Detailed macroscopic and microscopic pathology in birds with PDD has been described (14-16). Grossly, many birds suffering from PDD can be dehydrated and mildly to severely emaciated with atrophied pectoral muscles especially in birds that had a prolonged history of regurgitation or passing of undigested seeds. Proventriculus may
or may not be dilated in all birds suffering from PDD, but in nearly 70% of the cases, the proventriculus can be distended with seeds and thin-walled (Figure 1). In some cases the proventricular wall may rupture with spillage of food into the coelomic cavity resulting in peritonitis. Duodenum may also be distended and the adrenal glands may be enlarged. In occasional cases heart may have pale area on the epicardium. There may be no significant gross lesions in occasional birds that die suddenly with out any clinical signs of PDD.

Microscopic lesions can be found in various organs involving the gastrointestinal tract, central, peripheral and autonomic nervous systems, heart, and adrenal glands and occasionally in the nerves and ganglia of various visceral organs. It should be pointed out that lesions in various organs may or may not be present consistently in all birds suffering from PDD. In one study cases were selected based on lesions in proventriculus and/or gizzard and compared to other organs. Adrenal gland was the second most frequently affected organ, in 89.3 % of the psittacines followed by intestine (86.5%), heart (79.3 %), Brain/spinal cord (78.8%), esophagus/crop (72.1%), peripheral nerves (71.4%), eye (66.7%) and skin was affected in 25.0% of the psittacines examined (14).

The microscopic lesions consist of infiltration, a few to large number of lymphocytes mixed with few plasma cells in the serosal nerves of the proventriculus and/or gizzard, duodenum and other parts of the intestine (Figure 2). Often in the proventriculus, there is attenuation of glands and fibrosis of the mucosa. In many cases there is infiltration of lymphocytes mixed with a few plasma cells in and around nerves of the muscular tunics most prominent in the gizzard. Similar lesions can also be seen in the serosal and subserosal ganglia and nerves of the crop and esophagus but they tend to be less consistent in these organs. A high percentage of birds can have lesions in the adrenal
glands. These lesions can range from infiltration of a few lymphocytes in the medullary regions to infiltration of large number of lymphocytes mixed with few plasma cells and heterophils (Figure 3). Often the adrenocortical cells will be vacuolated and hypertrophied. The ganglia subjacent to the adrenal gland can also have infiltration of a few to large number of lymphocytes. In the heart there is usually infiltration of similar cells either in the epicardial ganglia and nerves and in and around the subendocardial, myocardial, and subepicardial Purkinje fibers. Brain and spinal cord can have similar lesions characterized by mild to severe perivascular cuffing by lymphocytes scattered throughout the cerebral cortex and cerebellum, brain stem, and spinal cord (Figure 4). Vestibulocochlear ganglia along with nerves and spinal ganglia can also have lymphoplasmacytic infiltration. Similarly, perivascular cuffing by lymphocytes in the peripheral nerves such as sciatic, brachial, and vagus and other nerves can be seen (49). Lesions in the eye when present are characterized by moderate to severe perivascular cuffing in the optic nerves and in the choroid, ciliary body and occasionally in the iris and pecten. Severe retinal lesions and blindness have been reported in a psittacine diagnosed with PDD (50). Lesions in the skin include perivascular infiltration by lymphocytes and plasma cells and occasional necrosis and infiltration of lymphocytes in the *Erector pilae* muscles.

**Immunohistochemistry**

Immunohistochemistry (IHC) has been performed by investigators recently to study the tissue distribution and localization of ABV in psittacines (42-44,46) and a canary (20). Antibodies directed against recombinant ABV N protein, as well as cross-reacting
antibodies against the BDV P protein, have been used as reagents for IHC. ABV N was demonstrated primarily in the nuclei, but also in the cytoplasm, of neurons including Purkinje cells and in glial cells (astrocytes) throughout the brain (43,44). Studies performed using anti BDV P antibodies have demonstrated ABV antigen both in the nucleus and the cytoplasm not only of neural tissues (neurons, glial cells, dendrites, axons of brain, myenteric plexus of proventriculus, conduction fibers of the heart and interstitial nerves in the lung) but also in other cell types including cardiomyocytes, hepatocytes, GI epithelium, and cells in the lamina propria of the intestine (42,44). Similarly ABV antigen has also been demonstrated in both neural as well as extraneural tissues including tubular epithelia of the kidney in a canary (20). In all studies, ABV antigen was found to be widely distributed among host cells, and was not limited only to areas with microscopic lesions (Figure 5).

**Ante-mortem Diagnosis**

*Clinical signs*

The incubation period of PDD appears to be extremely variable. Under experimental conditions, a minimum of 11 days has been reported in one study (31) while in others it was approximately one month (43) or more (47). The maximum time is certainly in the months range, and possibly even years in some cases (31,43). Birds clinically affected by PDD may show symptoms related to malfunction of the digestive tract, neurological signs, or a combination of both (6). Sudden death with no preceding clinical symptoms occurs in some cases.
Birds showing the gastrointestinal (GI) form of PDD often present for marked weight loss, vomiting/regurgitation, and the presence of undigested food (e.g. whole seeds; Figure 6) in their feces (6). Any of the above symptoms, and particularly their coexistence, should alert the clinician to a possible diagnosis of PDD; however, none of them should be considered pathognomonic. Furthermore, the severity of these symptoms will vary among patients, and they may not all be noticeable at the time of presentation. Due to the feather coverage, weight loss often goes unnoticed by the bird’s owner, and passing of undigested food is difficult to detect in birds that are on a pelleted diet.

The range of clinical symptoms possible with the CNS form of PDD is even greater than that seen in the GI form. Signs may be subtle, ranging from a slightly ‘dim’ attitude to profound neurological deficits and/or seizures. Birds may present mildly to severely ataxic, sometime with only one limb noticeably affected. Para-paresis is also quite common, and birds may be sternal at presentation with their legs either rigidly flexed or extended. Torticollis and/or abnormal head movements may be present, and central blindness has recently been described in an African grey parrot with PDD (50). The most severe cases are presented in status epilepticus. As with the GI signs, none of these signs is specific for PDD, and other differential diagnoses should always be considered. It should be noted that mixed GI/neurological PDD cases are not uncommon, and that most birds will have both GI as well as CNS lesions at necropsy, regardless of the clinical form observed antemortem (14).

_Hematology and clinical chemistry_
Birds with PDD often show little or no changes in their blood work (6,51,52). Non-regenerative anemia is the most common hematological change seen with PDD. This finding is similar to what is seen in starving birds, and is likely related to GI malabsorption. Leukocytosis and heterophilia are present in some PDD patients, but are not a consistent finding, and appear to be related to stress and/or to the existence of secondary infections. Likewise, the biochemistry changes seen in PDD patients are mainly those associated with their catabolic state. Total protein and albumin levels are often decreased (52), and mild to moderate plasma elevations of enzymes of muscle origin (lactate dehydrogenase, creatine kinase, and aspartate amino-transferase) may be seen. Other changes are possible, but are not consistent; nevertheless, performing a chemistry panel is important for ruling out other disease conditions, and for assessing the patient’s general health. It is also advisable to test all PDD suspects for blood lead and zinc levels, as the symptoms of heavy metal toxicosis may mimic those of PDD (51,53).

Fecal and crop cytology

There are no fecal or crop cytological findings that are specific for PDD; however, these simple tests should always be performed as part of the diagnostic workup of PDD suspects, because they may help rule in/out other differential diagnoses, or provide important information on changes that are secondary to PDD. It is of particular importance to rule out the presence of avian gastric yeasts (*Macrorhabdus ornithogaster*) and of helminthes, as these can cause GI signs similar to those seen with PDD. Changes of normal GI flora (e.g. increase in gram-negative bacteria, *Clostridium sp.*, and/or *Candida* yeasts) should be interpreted with caution, as they may represent either a
primary or a secondary process. In both cases, however, these will require appropriate therapy.

Diagnostic imaging

Diagnostic imaging techniques, such as survey radiography, contrast radiography, contrast fluoroscopy, and ultrasonography, are useful aids in the diagnosis of PDD, but cannot be used to confirm or rule it out (51,53). The most consistent finding in birds with PDD is a moderately to marked distended proventriculus that contains mainly ingesta and variable amounts of gas. Distention of the proventriculus by gas alone is not typical of PDD. Proventricular diameter has been shown to increase over time in Spix’s macaws with PDD, and has been suggested to be a useful indicator for performing crop biopsy (12). Other GI compartments that may be distended include the crop, ventriculus and small intestine; however, none of these findings is specific for PDD. The degree of distention of the various GI parts will vary among PDD patients, some showing changes only in the intestine or crop. A relatively large proventriculus may be seen in some healthy eclectus parrots, while distention of the proventriculus and crop can be physiological in neonate birds (51,54).

For most PDD cases, survey radiographs are the most cost-effective diagnostic imaging procedure, and provide sufficient information for the assessment of the size of the relevant GI compartments (Figure 7). In cases where findings are equivocal, or when the overall clinical picture does not fit well with PDD, positive contrast studies may be indicated (Figure 8). The technique for performing GI contrast studies in psittacine birds has been previously described (55,56). Following fasting of 4 hours contrast material is
introduced into the crop by gavage. Some authors recommend dosing the patient at 25-50 ml/Kg; however, the use of 10-15 ml/Kg is often sufficient, and will reduce the risk of regurgitation and aspiration. Either barium sulfate or iodine-based contrast media may be used. Barium sulfate generally provides better and longer-lasting positive contrast compared with iodine-based products, but can cause airway irritation if accidentally aspirated, and should be avoided if GI perforation is suspected. The use of barium will also necessitate any GI surgery (e.g. for collecting a crop biopsy) to be delayed until its complete clearance. Contrast studies may be carried out with the patient anesthetized or awake. While the disadvantages of anesthetizing the patient multiple times are obvious (increased anesthetic risk, risk of aspiration, altering GI motility), images obtained from an awake bird (e.g. placed in a cardboard box or on a perch) may not always be sufficiently diagnostic. In many cases, a combination of both options may prove most practical; i.e. the majority of images are obtained with the bird awake, and only once or twice, at well chosen time points, is the bird anesthetized for a short period of time in order to achieve correct positioning.

Contrast studies provide information not only on the size and relative positioning of the GI compartments, but also on the GI transit time. In healthy psittacine birds, barium sulfate should reach the cloaca within 3 hours after administration (55,56), often taking only 90 minutes to do so. The transit time for iodine-based products has not been well documented, but appears to be significantly shorter. In some PDD patients transit time may be markedly prolonged (6,51), while in others it is normal or even shortened (Figure 8). GI transit time may be altered by many pathological as well as physiological conditions, and cannot, therefore, be considered a sensitive or specific indicator of PDD.
Contrast fluoroscopy can also aid the diagnosis of PDD (52). The procedure is similar to that described above. After administration by gavage of 5-10 ml/kg barium sulfate mixed 1:1 with commercial hand-feeding formula, the awake bird is placed in a cardboard box or on a perch and observed intermittently with the fluoroscope until the barium reaches its cloaca. The main advantage of fluoroscopy over standard contrast studies is that it provides real-time views of the GI motility. Knowing the normal motility patterns is obviously necessary for the detection of changes. In the normal psittacine bird boluses of ingesta can be clearly seen leaving the crop and traveling along the thoracic esophagus to the proventriculus. These boluses usually occur at an approximate rate of 1/min and should be unidirectional with no significant amount of barium remaining in the esophagus between boluses. The motility of the proventriculus is less pronounced than that of other GI parts, but every few minutes a relatively large contraction followed by partial emptying into the ventriculus should be seen. Little or no proventricular motility may be present in PDD patients with a grossly distended proventriculus. Most striking of all are the changes seen with PDD in normal ventricular motility. Because of the sequenced contraction of the thick and thin muscle pairs of its wall, a constant ‘washing machine-like’ turning effect is produced, and should be clearly visible in the lateral view of a healthy bird. In PDD patients this pattern may be completely missing, often being replaced by a shallow and irregular flutter of the ventricular wall. The latter finding is the likely cause of failure of the mechanical food grinding action of the ventriculus, leading to the passing of whole seeds in the feces of PDD patients. Peristalsis of the small intestine is bidirectional in psittacine species, with waves traveling down to the cecal remnants and back up to the pylorus. Some PDD patients may show very fast and erratic
peristaltic activity, as well as an increase in duodenal diameter, while in others motility may be slower than usual. As with other imaging techniques, fluoroscopy findings should be regarded suggestive, but not confirmative, for PDD. Unfortunately, this useful technique requires costly equipment, and is, therefore, not readily available to many private practitioners.

**Crop Biopsy**

The gold standard for diagnosing PDD has been, and will likely remain, histological examination. In the live bird this means that at least one appropriately-sized biopsy from a relevant anatomical site must be obtained. Ideally, a biopsy of the serosal surface of the proventriculus and/or ventriculus should be taken, as these sites are the most commonly affected by PDD (14,15,57). However, these procedures are both technically challenging as well as highly invasive compared to the much simpler and less invasive approach to the crop (57).

The sensitivity of crop biopsies for detecting PDD has been a matter of controversy, with the reported prevalence of ganglioneuritis in crops of PDD patients ranging from 22% to 76% (14,15,49,57,58). Proper selection of the biopsy site and preparing multiple biopsy sections has been suggested to increase the sensitivity of crop biopsies (54). The surgical approach to the crop has been previously described (59). In brief, under general anesthesia, the bird is placed in dorsal recumbency and the skin above the crop (i.e. the ventral area of the lower neck) is aseptically prepared. The skin is then incised along the ventral midline or slightly to the left of it and the crop wall is exposed by undermining and retracting the skin laterally. The ventral portion of the crop
is freed from its fascial attachments and lifted gently. Some authors suggest that the cranial portion of the left lateral sac of the crop be preferred as surgical site because this area is less subject to stress and to iatrogenic injury by feeding tubes (57,59). The biopsy should include a prominent blood vessel (Figure 9) as this will increase the chances of obtaining nerve sections (54,57). Stay sutures may be placed cranially and caudally to the biopsy site, which should measure no less than 12mm at its long axis (i.e. along the blood vessel). It is advisable to obtain an elliptical rather than round biopsy (e.g. 12x8 mm) because this enables later identification of the biopsy’s original orientation. A second, smaller piece of about 2x2 mm should be collected in a sterile container and kept frozen for RT-PCR testing (see below). The crop incision is closed in a continuous inverted (e.g. Cushing’s) pattern, using synthetic absorbable suture material, and the skin is closed routinely.

Following fixation for at least 2 hours in 10% buffered formalin, practitioners are encouraged to either section the biopsy themselves or provide the laboratory with specific modulation instructions. The biopsy should be cut perpendicular to its long axis, using a sharp scalpel or razor blade. Special care should be taken not to drag or compress the adventitial side, as it will contain most ganglia. At least five thin slices should be prepared and placed ‘on edge’ in a histological cassette. Under most circumstances this will ensure that at least 10 medium to large nerve sections are represented, while a good biopsy will include > 20 (Figure 10). IHC and RT-PCR for ABV are already offered by some commercial laboratories, and can complement the standard histological examination of crop tissue.
Molecular diagnosis and serology

The recent discovery of ABV and the development of specific molecular and serological assays for its detection, offers new diagnostic tools to the avian veterinarian as well as to aviculturists wishing to clear their flocks from this pathogen. These tools should however, be used cautiously, keeping in mind our limited knowledge of this novel virus, and the inherent limitations of the techniques.

RT-PCR primers for conserved areas of the L, M, and N genes have been designed, and successfully detect at least 40 ABV isolates of 5 distinct genotypes (11,22,42,43). Quantitative real-time PCR, based on primers and probes within the P gene, has also been successfully applied to detect and quantify the presence of ABV in various tissues (34,45). The RT-PCR assays for the highly expressed M and N genes seem to have a similar sensitivity that is somewhat higher than that of the L gene RT-PCR. Based on the limited information available to date, brain, crop, stomachs, and adrenal glands appear to be the most consistent sites for postmortem detection of ABV RNA (34,42-45). Some birds may have ABV RNA present in most major organs and even in the plasma (43,45), while others show a more restricted distribution pattern. It is therefore, important to test several tissue types (brain and stomachs at the very least).

Specimens that may be tested for ABV RNA antemortem, include crop tissue, blood, choanal and cloacal swabs, and feces. Unfortunately, preliminary data shows that ABV-infected birds are not consistently viremic (43,45), shed the virus only intermittently in their saliva/feces, and that crop tissue may test ABV-negative in some PDD patients (43,45). Furthermore, some naturally-infected birds have been reported to shed the virus without obvious clinical signs (43,45). One such cockatiel has had ABV RNA present in
90% of its choanal and cloacal swabs during a period of 110 days (43), and has remained asymptomatic for at least one year thereafter (AYG unpublished). These findings suggest that both false negative as well as false positive results may occur when attempting to determine the PDD status of a bird based on RT-PCR.

Serum from a PDD patient has been shown by Western blot analysis to contain antibodies against an unidentified ABV protein in the bird’s brain. This protein was later identified to be the nucleoprotein (N), one of the two major immunogenic proteins of bornaviruses (the second one being P). The protein from the bird’s brain was extracted and used to test other sera from PDD patients and from control birds with promising results (60). Similarly, Lierz et al. have used Western blot to test sera from symptomatic as well as asymptomatic ABV-positive birds. Recombinant ABV N and P proteins, as well as BDV N and P proteins were used rather than brain extracts, and similar antibody responses were detected, regardless of the birds’ clinical status. The strongest reaction was to the recombinant ABV N protein, showing minimal cross-reactivity with BDV N. Responses to both P proteins were relatively weak and variable. It was concluded that serology could not differentiate between PDD patients and asymptomatic ABV carriers (45). This conclusion is also supported by the findings of another study that used Western blot as well as ELISA to detect anti-ABV antibodies in asymptomatic ABV-positive macaws (48).

Even with the limited data available to date on molecular and serological assays for ABV detection, the advantages and shortcomings of these tests are already apparent. When used for the diagnosis of PDD, false positive as well as false negative results are possible. The tests may detect the ABV status of a bird correctly, but can not be directly
correlated to the patient’s clinical status. The definitive diagnosis of PDD in the single patient will, therefore, continue to be based on histology, with PCR, serology and IHC results as supporting evidence (Figure 11). With that said, the great advantage of these tests is that they offer for the first time practical tools for screening birds for the etiologic agent of PDD. The optimal screening protocol (e.g. serology vs. PCR of several swabs collected serially) is yet to be determined. It is hoped however, that these tests will greatly improve our ability to clear flocks from ABV, and by that to significantly reduce the incidence of PDD.

**Clinical Management of PDD**

PDD is a devastating disease for affected birds, but it is equally devastating for their owner/caretaker. Furthermore, the disease often becomes a flock management problem because many owners of psittacine birds have multiple birds. Transmission between birds in the home environment can be problematic and may lead to sequential illnesses and potentially deaths, which may occur over a period of years. The social implications of a PDD diagnosis can also be devastating. Owners may be shunned from bird club functions or social interaction with other bird owners. Pet sitters often refuse to provide care while the owner is away.

Likewise, the diagnosis of PDD in an avicultural collection can have severe financial and emotional effects on aviculturists. Counseling the owner, as well as establishing a long-term management plan, are important aspects of veterinary care. So the first step in management is client counseling and planning, not only for the affected bird but also other birds in the flock or home.
Initially, the owner may be faced with difficult decisions, choosing between euthanasia and long-term management of affected birds, which may remain infectious. Euthanasia may be the best decision if the bird is critically ill; however, many owners may be reluctant to choose this option. A potential compromise that may allow the client to make a more calculated decision is to treat for 3-4 weeks and re-evaluate for treatment response.

Living with a bird that has a chronic infectious disease that is a risk to other birds, requires a commitment of time as well as limiting birds coming and going from the home. Long term care can entail a significant investment of time and money. The bird could be placed in a rescue center that handles birds with PDD, but the latter will typically request monetary support for long term treatment of the bird. Placement of the bird in a home without other birds for long term management is another option if such a home can be found.

While many birds with clinical PDD can be returned to being clinically normal, effective treatment will typically require months and even years. More research is needed to determine the risk to other birds from those which have been treated but may still be latently infected.

Counseling for owners needs to include the fact that even utilizing current diagnostic methods, the long term consequences of treatment, and risks associated with affected bird post treatment are still unknown. It is also important to understand that the disease can take many forms and can have a very long incubation period. Clinically healthy birds can be infected with ABV and pose a risk of transmission to others.
(43,45,48). The clinician who only diagnoses PDD in the classic emaciated, vomiting, bird that is passing whole seeds, will only be seeing the tip of the iceberg.

The second step in clinical management is assessing the disease status of their other birds which are in contact with the affected bird(s). To be in denial and avoid checking other birds in the home or aviary is placing them at risk. If the PDD is diagnosed before the bird is critically ill, most of them can be helped. Conversely, many birds which are diagnosed by either crop biopsy, ABV-PCR or antibody to ABV may never develop naturally occurring disease (43,45,48, SC unpublished). ABV diagnostics are currently in their infancy. Extensive, long term research is needed to provide the client with a reasonable prognosis, especially when multiple birds or flocks are subject to exposure.

In developing a treatment and control plan, consider each bird individually, but it is also important to evaluate all birds in contact with an affected bird to determine the extent of the problem. Ideally all contact birds should be screened preferably by a combination of Ag and AB tests, and possibly crop biopsy as well. In this way asymptomatically infected birds can be identified, isolated and treated.

Encourage your client to make the commitment not to bring more birds into the home, placing them at risk. Likewise-transferring birds to other owners without disclosure places other birds at risk.

**Treatment considerations**

As an infectious disease that causes inflammation of the central and peripheral nervous system as well as the digestive system, when managing PDD we must think
about preventing transmission of the disease to uninfected individuals, reducing inflammation, aiding digestion and controlling secondary infections, and in many cases, we must do this for a long time. With prolonged therapy and control of secondary infections, birds that are diagnosed early can return to good physical condition. However, their life expectancy cannot be predicted.

Nonsteroidal anti-inflammatory drugs

Initial reports of treatment using the anti-inflammatory drug celicoxib presented the first real hope for birds affected with PDD (61). Nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of structurally diverse compounds used clinically for the treatment of pain and/or inflammation. NSAIDs are believed to exert their analgesic and anti-inflammatory effects through inhibition of the cyclooxygenase (COX) enzymes, which catalyze the conversion of arachidonic acid to the various prostaglandins (61).

Two isoforms of the COX enzyme have been identified in eukaryotic cells, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). The COX-1 protein is constitutively expressed (i.e., it is present under normal conditions and does not need to be induced) and is involved in the maintenance of homeostatic conditions. For example, COX-1 plays a role in blood clotting and elicits a protective role in organs such as the gastrointestinal tract. The COX-2 protein, on the other hand, is inducible and is involved in the immediate early gene response to various stimuli such as cytokines, growth factors and UV light. Older NSAIDs such as, for example, aspirin, ibuprofen and flurbiprofen, inhibit both forms of COX and are referred to as non-selective NSAIDs. Newer NSAIDs
such as, for example, celecoxib and rofecoxib, are selective for COX-2 and are therefore referred to as selective COX-2 inhibitors (61-63).

In addition to their anti-inflammatory properties, NSAID therapy may have other unexpected effects. Chen et al. found that NSAID treatment can suppress the propagation of vesicular stomatitis virus (VSV) in mice. The inhibition of COX antagonized VSV propagation both in vitro and in vivo. In addition, aspirin and celecoxib both prevented the disruption of the blood brain barrier in VSV-infected mice. In vitro experiments showed that the effect of COX inhibition was at least partially mediated by increased production of Nitric Oxide (NO), a molecule known to inhibit VSV replication (64). In another study, Zhu et al demonstrated that COX-2 inhibitors could inhibit the production of human cytomegalovirus in human fibroblast cultures (65). These studies indicate that NSAIDS may have direct antiviral effects.

Celecoxib has been used very successful in treating PDD at the rate of 20 mg/kg bodyweight (BW) once daily if given directly orally (66). However, unless a bird is extremely tame, stress associated with therapeutic protocols must be considered, especially due to the long term nature of therapy. Long term treatment success has been achieved when adding celecoxib to the bird’s food at 40 mg/kg BW once daily (66). A 200 mg capsule of celecoxib may be dissolved in 10 ml of water and used at 0.2 ml/100 grams BW. The drug should be provided on a small amount of food so the chance of consuming adequate amounts is improved. The stability of this suspension has not been studied. Empirically, it is recommended that a new stock be prepared fresh at least once a week, and that it is stored under refrigeration. Many practitioners prefer to have the drug
compounded. In most birds clinical response is slow and gradual and many birds do not show much benefit for at least 2 weeks (66).

Other NASIDs have also been used to treat PDD. Tepoxalin (Zubrin, Schering Plough, Union, NJ, USA), a combined COX-1, COX-2, and 5-lipoxygenase (LOX) inhibitor, was used successfully in treatment of a group of crop biopsy positive bird (66). Through its inhibition of the LOX enzymes, this drug potentially reduces the production of leukotrienes, including leukotriene B4 that may contribute to increased GI tract inflammation. Inhibition of LOX may also reduce the GI effects routinely seen in dogs (and possibly in birds) with COX-1 inhibitors (62).

In a pilot study comparing the effectiveness of celecoxib and tepoxalin (66), 3 treatment groups were compared: 1) Celecoxib 40 mg/kg BW on seed mix (n=9); 2) Tepoxalin 40 mg/kg BW on seed mix (n=8); 3) Tepoxalin 40 mg/kg BW on an extruded rice-based hypo-allergenic diet (n=14). All birds were positive on crop biopsy prior to treatment, and underwent a second crop biopsy after at least 9 months therapy. In group 1, 2 birds still had positive crop biopsies after 9 months treatment, whereas in group 2, 6 birds still had positive crop repeated biopsies. The best results were found in group 3, in which lesions typical of PDD were not found in any of the 14 birds. These results may be attributable to the extruded diet readily absorbing the medication, to its hypo-allergenic nature, to an enhanced efficacy of tepoxalin on this diet, or possibly because the species in group 3 were easier to treat effectively compared with those in groups 1 and 2. Palm cockatoos (Probosciger aterrimus) were found to be particularly difficult to treat effectively, accounting for 7/8 cases of treatment failure. Palm cockatoos and hyacinth macaws (Anodorhyncus hyacinthinus) consume much of their calories
through nuts and seeds, making consistent dosing difficult (66). In these species, some sort of soft or fresh food, such as fruits and vegetables, should be used as a vehicle for administration of the medication.

Meloxicam is another NSAID that is widely used by avian practitioners. Meloxicam is considered COX-2 preferential (not specific) and at higher dosages its COX-2 specificity is diminished (62). However, in the authors’ empirical opinion, the clinical response seen with meloxicam is inferior to that observed with Celecoxib therapy.

The most common side effect of celecoxib and other COX-2 inhibitors is bleeding in the gastro-intestinal tract. The risk may be higher in the first few weeks of therapy. An adult female hybrid macaw with PDD died within 7 days of initiation of celecoxib therapy, exhibiting acute proventricular bleeding (Clubb, unpublished). The feces of birds treated with NSAIDS should be monitored daily. Treatment should be discontinued immediately if melena or fresh blood is detected, and the bird should be evaluated. Fecal cytology, including a Gram’s stain, should be performed to detect *Clostridium* sp. and/or other potential bacterial pathogens.

Some birds seem to develop hypersensitivity to the celecoxib. A mature female hyacinth macaw developed severe pruritus locally on the sides of its face while being treated with celecoxib, which subsided in severity when celecoxib therapy was discontinued (Clubb unpublished).

Most NSAIDS are eliminated by renal clearance and should be used with caution in birds with renal disease. In addition, NSAID-induced renal disease has been
documented in birds (67). It is, therefore, recommended that birds on long-term NSAID therapy be monitored on a regular basis for changes in their chemistry panel.

While the inflammatory lesions in nerves are often reversed in response to NSAID therapy, these drugs are not considered a cure or prophylactic agent for PDD.

*Amantadine hydrochloride*

The prognosis of PDD is especially guarded in patients showing severe central nervous system disorders. Such cases have been poorly responsive to NSAID therapy alone. In one of the authors’ (SC) experience the addition of amantadine hydrochloride (10 mg/kg po SID, or 20 mg/kg SID on food) to the therapeutic protocol resulted in a vast improvement in outcome.

Amantadine was initially used as an antiviral against influenza viruses (62). Its antiviral mechanism of action involves interference with a viral ion channel. Later, it was also found to have an effect in reducing the severity of symptoms of Parkinson’s disease, by antagonizing the N-methyl-D-aspartate receptor and other mechanism that are not yet fully understood (68). Amantadine has many effects on the brain including release of dopamine and norepinephrine. Due to increased viral resistance, amantidine is no longer recommended for influenza treatment or prophylaxis (69), but is still being used to treat various psychological disorders in humans. Common side effects in humans include appetite loss, diarrhea, nausea, lethargy and allergic reactions. Amantadine has been used in combination with celecoxib to treat a large number of PDD patients with only rare adverse reactions, which resolved after cessation of therapy (SC unpublished).
Other drugs used to treat PDD patients

Due to their impaired GI motility, birds with PDD often develop secondary bacterial and fungal GI infections. These should be diagnosed and treated appropriately. Clostridium infections are more common in birds with PDD than in birds with normal intestinal motility and can result in bulky, black, foul smelling feces. Vaccination for clostridium should be considered. A bovine multivalent Clostridium chauvoei/septicum/haemolyticum/novisordellii/perfringens types C and D bacterin-toxoid vaccine (Vision 8; Intervet Inc, Millisboro, DE, USA) administered at 0.25-1 ml intramuscularly or subcutaneously, has been utilized with empirical success. Initially two doses are given two weeks apart with an annual booster (66).

Gas formation and retention in the gastrointestinal tract is a common finding in birds affected with PDD, and can cause discomfort. Gas may be evident radiographically and/or gas bubbles may present in the feces or vomitus. Surfactants (e.g. Infant's Mylicon, Johnson & Johnson, Merck Consumer Pharmaceuticals, Ft. Washington, PA, USA) provide some symptomatic relief. Many birds exhibiting gastrointestinal gas or vomiting respond clinically to combination drug therapy (e.g. clarithromycin, metronidazole, and sucralfate) as if they are infected with helicobacter species, however the presence of helicobacter has not been confirmed in these patients.

Metoclopramide (0.5 mg/kg q12h PO or IM) is an important adjunct therapy to management of severe PDD cases (66). It is beneficial in cases of reduced intestinal motility or intestinal stasis. Treatment is initiated by injection and later continued orally. An adverse reaction to metoclopramide has been reported in a macaw being treated for PDD (69).
Birds with PDD often become anemic and hypo-proteinemic. Supplement of vitamins, especially B Complex vitamins, is helpful.

**Husbandry Considerations**

If possible, birds should be kept outside where sunlight and fresh air will help to dilute/inactivate the virus. It will also enhance the birds well-being. The birds should be spread out as much as possible to reduce the concentration of virus in the environment. Stress should be kept to a minimum. The diet should be easily digested since ventricular and proventricular function is adversely affected by PDD. Liquid diets as well as pelleted diets have been developed specifically for birds with PDD, and juvenile hand feeding formulas can also be used for initial nutritional therapy. Formulated diets are ideal since they are easier to digest than seeds, however extreme caution should be used in converting an ill bird from a seed based diet to a formulated diet. Extruded diets also absorb medication well, enabling long-term stress-free therapy.

Supplementing the diet with vegetables that are high in fiber might be beneficial with early cases of PDD by stimulating intestinal motility. Birds affected by PDD often ingest foreign bodies, especially pieces of wood. These materials may then be passed in vomitus or feces. The bird may be ingesting these materials in an attempt to provide relief from intestinal discomfort. These birds may need toys and cage accessories that cannot be chewed or ingested, and may benefit from high fiber vegetables to fill this need.

Cruciferous vegetables are beneficial sources of raffinose sugars (rich in oligofructusaccarides), which enhance viability of autochinous flora (species of Lactobacillus and Bifidobacterium), thereby inhibiting gram-negative bacteria and
Clostridium. In advanced cases, however, these foods may linger in the intestines and ferment. Periodic supplementation with probiotics may be beneficial.

Due to the inflammatory nature of PDD, supplements that enhance nutrition as well as provide anti-inflammatory effects may augment conventional therapy. Anti-oxidants including oils, specific amino acids, minerals and some natural herbal anti-inflammatory agents may be beneficial. A balance of Omega 3 and Omega 6 fatty acids has been proven to be beneficial in many inflammatory diseases. Salmon oil, flax seed oil, and safflower oil are used as sources of omega 3 and omega 6 fatty acids. Fatty acid supplementation is provided at 50-250 mg/kg body weight of omega 3 fatty acids with an omega 3:omega 6 ratio of 1:2-1:6. If the bird is eating primarily a seed diet, which is naturally high in Omega 6 fatty acids, supplementation with salmon oil and flax seed oil will help to correct the omega 3:omega 6 ratio. Nutritional adjuncts to therapy that may be beneficial in cases with CNS signs include ginko biloba, Vitamin E, alpha lipoic acid, acetyl-L-carnatine and B complex vitamins. There are no studies in the literature on the effect of various diets and/or nutraceuticals on birds with PDD; therefore, all recommendations made above are empirical.

**Monitoring Progress of Therapy**

Response to therapy can be monitored by periodic physical exam, monitoring body condition and weight, repeated radiographs and hematology and plasma biochemistry analysis. Increases in body weight can be misleading as weight gain may be associated with dilation of the proventriculus and intestinal stasis. Monitoring by serial crop biopsies is very useful. On repeated biopsy, the site of previous biopsy should be
avoided, as the presence of old suture material can result in non-specific inflammatory lesions.

If monitoring by radiography the composition of the diet must be considered in evaluation, especially if the bird as eating a primarily seed diet at the time of diagnosis and is converted to a more bulky extruded diet. Birds eating a primarily formulated or extruded diet tend to have a relatively dilated gastrointestinal tract as evident radiographically which can complicate radiographic evaluation (SC unpublished).

**Prevention**

Early epidemiological data on PDD as well as recent studies on ABV (45) suggest that the disease and its causative agent are not equally distributed among flocks. While in some aviaries PDD cases occur on a regular basis, the disease appears to be completely absent from other facilities (AYG unpublished). With this observation in mind, the obvious goals of PDD prevention are: a) to avoid introducing the pathogen into new flocks; and b) to clear it from flocks where it is already present. Until recently; however, these goals were nearly impossible to achieve due to the disease’s long incubation period and the fact that its etiology was unknown. Even facilities that quarantined all new arrivals for extended periods of time, and that performed crop-biopsies on all of their birds, were not completely safe from PDD. Now, with the discovery of ABV and the development of molecular and serological assays for its detection, it is hoped that this situation will change.

Regular monitoring of birds’ body condition and feces (i.e. looking for undigested seeds) are simple ways for detecting clinical PDD cases in flocks where the disease
already exists. While still valid, these simple measures will not detect birds in early stages of PDD or asymptomatic ABV carriers.

ABV RT-PCR and serology are already offered by some commercial laboratories and are expected to become widely available in the near future. At this point, precise recommendations as to the preferred screening protocol can not be made, but when possible both tests should be utilized. Because of intermittent shedding of ABV, it is advisable to submit several serially collected oral/cloacal swabs for RT-PCR. If crop biopsies are collected, they too can be submitted for RT-PCR.

As with other infectious diseases, practicing good hygiene and keeping strict biosafety rules are essential for fighting PDD. Diagnostic necropsies and histopathology should be performed on all birds that die of unknown cause. Overcrowding of aviaries facilitates the spread of PDD, and should be avoided. All new additions should be quarantined and tested (see above), as should be any bird suspected to have clinical signs of the disease. Birds that test ABV-positive must not be allowed into existing flocks, and should be removed from flocks where they already exist. These birds should be placed in a situation where they can not infect other birds. Similar principles may be applied to smaller collections such as multiple-bird households.

Summary

Proventricular dilatation disease (PDD) is a fatal inflammatory disease that affects mainly psittacine birds (Order: Psittaciformes). The disease was first recognized in the 1970’s, but it wasn’t until 2008 that the causative agent of PDD, a novel Borna virus named avian bornavirus (ABV), was discovered. Since its discovery, the number of
publications on ABV has been increasing rapidly, with new information becoming available on an almost monthly basis. RT-PCR and serological and immunohistochemical assays for ABV detection are already commercially available, but the knowledge regarding their optimal application is still lagging behind.

For years, PDD has posed one of the greatest diagnostic as well as therapeutic challenges to the avian veterinarian. It is hoped that the exciting recent progress in PDD research will greatly improve our ability to diagnose, manage and prevent this disease.

Acknowledgements

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Table 1. Psittacine species that have been diagnosed with PDD*

<table>
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<tr>
<th>Genus</th>
<th>Species</th>
<th>Origin</th>
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<td>Nymphicus</td>
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<td>Cacatua</td>
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<td>Anodorhyncus</td>
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* Based on published (6,14-16) and unpublished (SC, HLS) data. A/P = Asian/Pacific; AM = American; AF = African.