Avian Bornaviral Ganglioneuritis in Clinical Practice

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Abstract: Avian Borna virus (ABV) has been shown to be a causative agent of Proventricular Dilatation Disease in birds. The avian Bornaviradae represent a genetically diverse group of viruses that are widely distributed in captive and wild populations around the world. They are widely distributed in tissues of affected birds and generally produce a non-suppurative ganglioneuritis in the gastro-intestinal tract and central nervous system. Diseased birds may or may not demonstrate ABV ribonucleic acid on ante-mortem tests or test positive on serological assays. Alternatively, clinically normal individuals may test positive on molecular and serological assays. The route of transmission in natural infections has not been thoroughly ascertained. Management of affected birds is beneficial and currently centered at reducing neurological inflammation, managing secondary complications, and providing nutritional support.

Key words: Avian Borna virus, Proventricular dilatation disease, PDD, ganglioneuritis, COX-2

Introduction

Neuropathic gastric dilatation of psittacine birds was initially reported as a wasting disease in macaws imported into North America and Europe from Bolivia in the late 1970s. [1,2,3,4,5,6,7,8] Originally limited to macaw species (*Ara* spp.), the disease was later identified in other parrot species as well. One of the first reports was by Ridgeway and Gallerstein in 1983 followed by the report of impaction, dilatation, and degeneration of the proventriculus in 16 large psittacine birds by Clark in 1984.[1,6] Originally called Macaw Wasting Disease, the disease has also been referred to as Macaw Fading Syndrome, Myenteric Ganglioneuritis, Infiltrative Splachnic Neuropathy, Neuropathic Gastric Dilatation, and Proventricular Dilatation Disease (PDD). The disease may be more appropriately termed Avian Myenteric Ganglioneuritis, Non-suppurative Ganglioneuritis, or Avian Auto-immune Ganglioneuritis.[9] These articulate the disease process better and remove the focus on the proventriculus.

PDD has been described in over 80 species of psittacine and non-psittacine birds worldwide, both captive and in the wild.[10,11] African grey parrots (*Psittacus erithacus*), macaws (*Ara* spp.), Amazon parrots (*Amazona* spp.), and cockatoos (*Cacatua* spp.) are the most common psittacine species affected.[10] The disease is minimally represented in Quaker parrots (*Myiopsitta monachus*) and Lovebird species (*Agapornis*).[9] Lesions suggestive of PDD have also been described in canaries (*Serinus canaria)*, the greenfinch (*Carduelis chloris)*, long-wattled umbrella bird (*Cephalopterus penduliger),* a bearded barbet *(Lybius dubius),* Canada geese (*Branta canadensis*), toucans (*Rhamphastidae*), honey creepers(*Drepanidinae*), roseate spoonbill (*Platalea ajaja*) and a peregrine falcon (*Falco peregrines*).[12,13,14,15] PDD is a progressive neurologic disease with a high case fatality rate once clinical signs are present.[16] The disease presents a serious threat to captive propagation and conservation efforts for endangered psittacines such as the Spix's macaw (*Cyanopsitta spixii*).

PDD

Clinical Signs

Clinical signs are variable and depend upon the host species involved, severity of disease, distribution of lesions and affected organ system involved. They vary from case to case. They may present in the central, peripheral, and/or autonomic nervous system. Although neurogenic in nature, signs are generally classified as gastrointestinal (GI) or central nervous system in character. Birds may exhibit only gastrointestinal or neurologic signs or a combination of both. GI tract signs reflect pathology of the terminal ganglia of the vagus nerve (cranial nerve X). The vagus, also referred to as the pneumogastric nerve, is the Autonomic Nervous System's parasympathetic control of the heart and digestive tract. It regulates homeostatic function of the proximal GI tract, pancreatic endocrine and exocrine function, hepatic glucose production, and heart rate. Gastrointestinal signs reflect varying degrees of dysfunction and neurogenic atrophy including delayed crop emptying and impaired GI tract transit, regurgitation, anorexia, dilatation and sometimes impaction of the upper GI tract. Pyloric motility is disturbed with severe alteration of gastric emptying and stasis.

Affected birds have impaired ability to digest and absorb dietary nutrients leading to loss of body weight, passage of undigested food material in the feces and diarrhea. The vagus nerve is also a major constituent of the inflammatory reflex, a neural reflex that controls innate immune responses and inflammation during GI tract pathogen invasion and tissue injury.[17,18,19] Impairment of this reflex and reduced vagal stimulation of gastric acids impairs the natural resistance to bacterial overgrowth and leads to alterations in the intestinal microbiome. Overgrowth of pathogenic organisms such as Clostridial spp. and fungal organisms often occur in affected birds. This classic form of the disease is most often observed in new world psittacines.

Central nervous system lesions commonly involve the Cerebrum or Cerebellum. Cerebral perivascular cuffing and Glial cell injury can cause seizures in affected birds. Optic lobe lesions lead to a cortical blindness which may be reversed with effective treatment. Disruption of the Perkinje, Glial, and Granule cell layers of the Cerebellum produce disorders in fine movement and equilibrium evidenced as ataxia, proprioceptive deficits, intention tremors, incoordination, dysarthria (vocalization abnormalities), motor deficits and reduced cognitive ability. Old world psittacines often exhibit these central nervous system signs although concurrent, non-clinical lesions in the GI tract are usually present.

Inflammation and myelin degeneration of the dorsal nerve roots, white matter, and associated ganglia have been identified at all levels of the spinal cord in PDD affected birds.[20] Thoracolumbar lesions were the most common and severe. The dorsal root ganglia contain cell bodies of sensory neurons that bring information from the periphery to the spinal cord. PDD associated peripheral neuritis has been implicated as a cause of feather picking and self-mutilation in affected birds.[20]

Myocarditis as a component of PDD has been previously reported.[21] Lesions are more frequent and severe in the right side of the heart which may reflect the higher density of nervous tissues in this area. Additionally, parasympathetic innervation of the heart is partially controlled by the right vagus branch which innervates the sinoatrial node. Dilatation of the right ventricle of the heart of affected birds has previously been described. Arrhythmias and alterations in blood pressure may also be observed in affected birds. Cardiac lesions can result in acute death in otherwise clinically normal birds.

A survey of pet birds with clinical signs of PDD found 66% of birds exhibited central nervous system signs, 22% GI tract signs, 9% feather picking and mutilation, and 9% acute death.[22]

Pathology

Characteristic histological lesions of PDD involve lymphocytic, plasmacytic inflammatory infiltrates of nervous tissues, axonal swelling, myelin degeneration, and perivascular mononuclear cell infiltrates of blood vessels and connective tissue surrounding affected nerves.[20] Lesions often present in ganglia of the GI tract (ganglioneuritis), central nervous system (encephalitis, myelitis), peripheral nerves including the sciatic, brachial, and vagal nerves (neuritis), and retina (retinitis).

Abnormalities may be observed at all levels of the spinal cord but are especially severe in the thoracolumbar portion. They include vacuolation and spongiosus of the white matter, axonal swelling with myelin degeneration, perivascular infiltrates in the grey and white matter and associated ganglia and dorsal nerve roots, and gliosis. Lesions may also be observed in the heart which demonstrates focal to diffuse areas of myocardial necrosis associated with infiltration of mononuclear cells. The intracardiac nerve plexus of the right side of the heart is often more severely affected.[20] Lymphocytes may be scattered diffusely throughout the adrenal medulla or localized in clusters adjacent to cortical tissue.
The disease process of PDD involves inflammation of the nerve ganglia which exposes normally sequestered ganglioside proteins to the host immune system. Studies by Rossi et.al. have shown that the release of G 50 proteins from nerve ganglia produce pathologic lesions similar to that noted in the autoimmune neuropathy of Guillian Barré syndrome. [24,25,26,27] Disease occurs when the host immune response is directed to these exposed proteins causing corresponding neurological dysfunction.

Diagnosis
PDD should be considered in the differential diagnosis of any bird exhibiting neurological and/or gastrointestinal signs. A presumptive diagnosis can be made based upon the history, clinical signs, and radiographic evaluation of the digestive tract. Definitive diagnosis can be confirmed by demonstrating the characteristic histopathologic lesions in tissues from affected birds.

Plain and contrast radiography, and fluoroscopic evaluation of gastrointestinal motility, have been used for tentative ante mortem diagnosis. Radiology and ultrasonography often reveal various degrees of enlargement, thinning and/or impaction of the ingluvies, proventriculus, ventriculus, and proximal duodenum . The proventriculus is often severely dilated, filling the left side of the coelomic cavity. It often appears as a "J" shape causing the ventriculus to be displaced to the right and ventrally.[28] Dilatation and thinning of the wall of these organs may lead to impaction and rupture. Contrast studies often demonstrate prolonged transit times throughout the gastrointestinal tract.[29]

Clinical pathologic findings in PDD are inconsistent and generally reflect the state of malnourishment, dehydration, and secondary infections that may occur with this disease. Serum chemistry profiles and complete blood counts are often normal although a relative and absolute heterophilia, hypoproteinemia, anemia, gram-negative and Clostridial bacterial enteritis have been reported.[28] Gastrointestinal stasis predisposes to overgrowth of the digestive tract with yeast and gram-negative bacteria.

Ante-mortem diagnosis of PDD can be confirmed by identification of the characteristic myenteric ganglioneuritis in biopsy samples of the crop, ventriculus, and adrenal gland.[10] Crop biopsy specimens should include a visible blood vessel/nerve complex. While reported to be an effective method of ante mortem diagnosis with an accuracy of 76%, it has been reported that only about 76% of PDD affected birds have crop lesions.[10, 30] In practicality, crop biopsies are reported to be indicative of PDD in only about 30 to 35% of cases.[10,31]

Post-mortem examination often reveals emaciation and the presence of a distended, impacted proventriculus and ventriculus. Thinning of the wall of these organs occurs and rupture of these organs may be present. Histological examination of a wide range of tissues should be conducted on birds suspected of succumbing to PDD. Tissues submitted should include the ingluvies, proventriculus, ventriculus, duodenum, adrenal gland, heart, spleen, and brain.

Differential Diagnoses
Tumors or papillomas of the crop, proventriculus, ventriculus, and intestines, ingestion of foreign bodies, megabacteriosis, and parasitism can cause gastrointestinal signs identical to those seen in PDD.[10] Emptying of the proventriculus and ventriculus appears to be inhibited as long as the intestinal tract is distended. Inflammatory disease and neoplastic diseases of the ventriculus and proventriculus can also cause gastrointestinal stasis. Heavy metal poisoning is commonly associated with central nervous system signs but can cause gastrointestinal stasis as well. Internal papillomatosis may result in a chronic wasting disease that 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 appear similar.[10]

Avian Bornavirus (ABV)

ABV
An infectious etiology for PDD has long been suspected based on its apparent spread through aviary collections. Transmission electron microscopy studies in the 1990s provided the first evidence of a viral etiology demonstrating inclusion bodies and enveloped virus-like particles in the myenteric plexus, celiac ganglia and fresh feces from affected birds.[32] Attempts to isolate an infectious agent were unsuccessful at the time.
In 2008, two independent research groups identified a novel virus in tissues from PDD-affected birds and named it avian bornavirus (ABV).[33, 34] It was proposed as the causative agent of PDD. Subsequent studies have confirmed the association between ABV infection and PDD.[38,39,40,41,42] Disease development was reproduced by parenteral inoculation of psittacine birds, such as cockatiels (*Nymphicus hollandicus*) or Patagonian conures (Cyanoliseus patagonus), with genotypes ABV-2 and ABV-4. However, ABV has also been found in healthy birds which may remain apparently disease free for years.[35,36,38,39]

Bornaviruses are enveloped, non-segmented single-stranded negative sense RNA viruses. They are members of the family *Bornaviridae* in the order *Mononegavirales*. Other families within the order include the Filoviridae (West Nile virus), Rhabdoviridae (rabies virus), and the Paramyxoviridae. Currently 15 ABV genotypes have been identified. The recent reclassification of Avian Bornaviruses is Psittaciform Bornavirus 1 (PaBV- 1,2 ,3 4,7), Passeriform Bornavirus 1 (CnBV-1,2,3, canary) and (MuBV-1, munia finch), Waterbird Bornavirus 1 (ABBV-1, aquatic bird bornavirus 1), Passeriform Bornavirus 2 (EsBV-1,estrildid finch bornavirus 1), and tentative, unclassified bornaviruses of Avian Bornavirus MALL (ABV-MALL), Parrot Bornavirus (5 PaBV-5), Parrot Bornavirus 6 (PaBV-6), and parrot bornavirus (8 PaBV-8).[43] PaBV 2 and 4 are the predominate genotypes in psittacine birds.

The genetic variability of ABV is much greater than that observed in Borna Disease Virus. There is a 91 to 100% shared nucleotide identity within a genotype and only 68 to 85% between genotypes. Different genotypes seem to cause different disease in different species and individuals but the relationship between genotype, species of bird, and observed clinical disease is obscure at this time. Infection with one genotype does not appear to be protective against another. Simultaneous infection with two genotypes can occur and may result in severely worse clinical disease.

ABV is widely distributed in the body of infected birds.[44] It reproduces in a non-cytopathic manner in the host nucleus and persists due to mechanisms that evade the host immune system.[45] As a result, ABV infections are considered chronic and life-long. It is there for unlikely that anti-viral or vaccine therapy will effectively eliminate the viral infected state.

Infection Rate
ABV is widely distributed in both captive and wild avian populations. Approximately 15- 40% of normal healthy birds are positive for the presence of ABV. Lierz detected ABV RNA in 27 (45.8%) of 59 healthy appearing pet birds.[46] Thirty five of 77 (45%) healthy birds from an aviary with a past history of PDD were found positive for ABV-specific serum antibodies.[47] A survey of laboratory samples submitted for other testing revealed approximately 34% (271/791) avian samples tested positive from across the USA.[48]
Almost all collections of psittacine birds will contain individuals infected with ABV. A large-scale survey revealed that ABV infection is widespread among captive psittacines in Europe, with 23% of 1,442 birds considered infected.[36] Similarly, a high infection rate was observed in captive canaries in Germany[49] (Rubbenstroth et al., 2013), as well as in certain populations of wild waterfowl in North America.[49,50]

While a significant percentage of the avian population is infected with ABV, the frequency of clinical borna viral disease is much lower. The majority of ABV positive birds do not exhibit clinical disease. When it does occur, disease presents on a continuum of intensity with many birds exhibiting only mild disease. The clinically "wasted" bird represents the more severe form of untreated, chronic disease.

Transmission

The epidemiology of BDV is currently not well understood. ABV is shed in the urine and feces of infected birds. The urofaecal-oral route is assumed to be important for horizontal transmission but other routes cannot be excluded. Transmission via the respiratory tract and vertically through the egg have also been discussed but supporting experimental evidence is lacking at this time. Piepenbring et al. did report successful ABV transmission to one cockatiel placed in contact with a group of cockatiels that were experimentally infected with ABV-4.[38] Most other studies indicate that horizontal transmission of ABV by direct contact is inefficient in immunocompetent fully fledged birds.[51] Cockatiels (*Nymphicus hollandicus*) were infected with ABV-4 by oral and intranasal application. Clinical signs of disease were not observed in any of the birds during the 174 day observation period. At the end of the study, pathohistological and immunohistochemical examination revealed neither lesions typical for PDD nor ABV specific antigen in any of the birds.[52] Kistler et. al. reported a dramatic PDD outbreak during which 13 unweaned chicks of various psittacine species died.[54] These observations suggest that ABV transmission may be much more efficient in unweaned nestlings with immaturely developed immune systems compared to older individuals.

Experimental studies have shown that ABV infections can be induced experimentally when the virus is injected intramuscularly, intravenously, and intracranially in birds. Gancz et al. (2009) were the first to demonstrate that PDD could be transmitted to healthy birds by the use of infected-brain tissue. They inoculated cockatiels by multiple routes with a brain homogenate from either an ABV4-positive bird or from a PDD-/ABV-control bird. The birds inoculated with healthy control bird homogenate remained healthy, whereas all three birds inoculated with brain homogenate from ABV-infected birds developed both gross and microscopic lesions typical of PDD.[39] Gray et al. isolated ABV in cultured DEF. After six passages, these infected cells were injected intramuscularly into two Patagonian conures (Cyanoliseus patagonis). Clinical signs of PDD developed within 66 days post-infection in both challenged birds. The presence of typical PDD was demonstrated on necropsy and histopathology. RT-PCR demonstrated the presence of the inoculated strain in the brains of the challenged birds.[40] Piepenbring et al. inoculated 18 cockatiels by both the intracerebral and intravenous routes with an isolate of ABV4 cultured for six passages in a quail cell line (CEC-32). All challenged birds became persistently infected but the clinical disease patterns that developed varied among individuals. Five birds developed clinical signs of PDD, while on necropsy 7 of the 18 had a dilated proventriculus. All infected birds did, however, show mononuclear cell infiltrates characteristic of PDD in a wide range of organs.[38] Strong suggestive evidence for vertical transmission of ABV from the hen to the egg has been reported in psittacines and canaries. Several studies found eggs from infected hens to be ABV-positive by PCR assays.[51,55,56,57] Final proof of vertical transmission provided by the detection of productive infection in embryos or chicks reared under isolated conditions is lacking at this time.

The incubation period of PDD is unknown. Clinical observations suggest that this could be as short as several weeks to as long as many years.[8,28] In general, transmission is believed to require long term, close contact among birds. Unlike other RNA viruses that are more stable (West Nile Virus), endonuclease enzymes in the environment tend to rapidly degrade the avian bornavirus. Soap and dilute bleach appear to be effective in disinfecting enclosures and items that come in contact with ABV-positive individuals.

ABV Diagnostics

PCR

The diagnosis of PDD has traditionally been based on the identification of histological lesions in biopsy or necropsy tissues. With the discovery of the association of ABV with PDD, diagnostic testing has focused on reverse transcription polymerase chain reaction (RT-PCR) for the detection of ABV ribonucleic acid (RNA) in affected birds. Ante mortem detection of viral infection in the naturally or experimentally infected bird is however challenging. Urine, feces, and cloacal swabs are samples reportedly most likely to contain virus however a whole blood sample in combination with choanal/cloacal swabs may provide the greater sensitivity. Fecal swabs are less desirable as RNAases and other degrading or inhibiting agents may rapidly degrade the viral RNA in these samples. Cloacal sampling, however, likely underestimates the prevalence of ABV infection. Intermittent urofecal shedding of ABV is described in both experimentally and naturally-infected parrots which can lead to a false negative test result.[38,58,59] One research study recommended the use of feather calami for ABV RNA testing.[60] The consensus of opinion among most other researchers at the 2014 ABV Research Forum was that this was not an appropriate sample for accurate ABV testing.[9] It should be noted that there is no consistent or standardized method of testing among laboratories offering this service. Reported rates of ABV positive tests among several university and commercial laboratories range from 3-33%. With only about 68 to 85% sequence identity among the known ABV genotypes, some assays may not be able to detect all ABV genotypes. Primers have been designed to target the nucleocapsid (N) gene, matrix (M) gene, phosphoprotein (P) gene, and polymerase (L) gene. Assays designed for detection of the M and immunodominant N gene sequences appear to have a similar high sensitivity.[35] Those for the L and P genes are generally less accurate. Both gel-based RT-PCR and real-time RT-PCR have been successfully used to detect ABV RNA. Real-time PCR assays appear to be the more sensitive of the two techniques.

Serology

Serologic assays have been used to detect ABV exposed or infected birds. The indirect fluorescent antibody (IFA)assay is preferred by many investigators, particularly in Europe. Researchers in the USA tend to use the Western Blot assay.[61,62] Both appear to be appear to be sensitive and specific assays. They cannot however differentiate between diseased birds and healthy carriers. Not all birds shedding viral RNA are antibody positive.[47] The discrepancy between viral shedding, presence of antibodies, and clinical disease is now well recognized in parrots infected with ABV.[38,62,63] Viral shedding and presence of antibodies coincided in only one-fifth of the samples in a large study of captive psittacines.[36] In another study of free-ranging psittacines, 50% of ABV RNA positive birds did not show antibodies against ABV by IFA.[11] Many apparently healthy birds may however be seronegative while, at the same time, shedding ABV in their feces. In a large-scale study conducted in captive psittacine birds in Europe, 17% of the birds had detectable antibodies.[36] Widespread persistent asymptomatic infection with less common clinical disease is a consistent feature of bornavirus infections, and may be attributed to the lack of cytopathic effect of bornaviruses and their ability to escape recognition by the innate immune system.

Serologic testing for antiganglioside antibodies appears to more accurately detect clinically affected birds. Antiganglioside autoantibodies are utilized as markers of immune-mediated disease and are triggered by a variety of pathogens. High antiganglioside antibody levels were detected in 15.5% of 650 avian serum samples and 98% of symptomatic and histologically positive PDD birds.[65] Further studies to help determine the sensitivity and specificity of antiganglioside autoantibodies in the diagnosis of PDD are needed.

PDD Pathogenesis

Infection studies with ABV have satisfied Koch's postulates and shown that it is a cause of PDD in birds. Some researchers are of the opinion that it is the sole cause of PDD in birds.[66] While ABV is a causative agent of PDD, the relationship of ABV in the pathogenesis of this disease remains unclear. Although the virus is non-cytopathic it can induce inflammation and a selective loss of glial cells and neurons. This cell loss appears secondary to T cell cytotoxicity. The presence of CD8+ T cells parallels the onset of neurologic dysfunction. Rossi et.al. have demonstrated immunocomplex deposition around affected nerve ganglia associated with high positive expression of complement fractions by immunofluorescence in birds with clinical PDD.[65] They suggest that PDD is induced by an autoimmune mechanism similar to the pathogenesis of Guillain Barrè Syndrome where gangliosides act as major antigens. To confirm this theory, they challenged 6 cockatiels (*Nymphicus hollandicus*) intraperitoneally (IP) or orally with purified avian gangliosides. One month post infection, 100% of I.P. inoculated and 33% of orally challenged parrots developed neurological and GI tract signs compatible with PDD. Four of the birds demonstrated the classic lymphoplasmacytic

ganglioneuritis in crop biopsies and all showed histopathological changes compatible with PDD.[67] These findings warrant further investigation into the mechanisms of autoimmune reactions against nerve ganglioside proteins and their possible involvement in the pathogensis of PDD.

Treatment

PDD has historically been a fatal disease of psittacine birds with mortality approaching near 100%. Even with appropriate supportive care, most birds succumb to progressive debilitation, starvation, secondary infections, or CNS disturbances. The ganglioneuritis and encephalomyelitis associated with PDD is inflammatory in nature, the chronicity of which, contributes to the progressive, debilitating nature of the disease. The rationale for a therapeutic approach was that diminishing this reaction was expected to lead to clinical improvement and possible resolution of clinical signs in affected birds. In 2002, we demonstrated the resolution of clinical PDD by Cyclooxygenase 2 (COX-2) inhition.[68] Since this time, clinicians have recognized the early signs of avian ganglioneuritis and the variable ways it can presents in a clinical setting. The use of preferential and selective COX-2 inhibitors has improved and extended the quality of life of affected birds.

Gastrointestinal prokinetic agents such as Cisipride (Propulsid, Janssen Pharmaceutica Inc. Titusville, NJ,USA) and metoclopramide (Reglan, Schwarz Pharma, Seymour, IN,USA) are helpful in improving transit in birds with GI tract involvement, especially early in the course of therapy. Appropriate antibacterial and antifungal therapy should be instituted to control overgrowth of intestinal anerobes, yeasts, and Macrorhadus also in these individuals. GI tract disease results in an altered intestinal microbiome. Probiotics and prebiotics (Sivoy, Rome, Italy) may prove helpful in restoring a normal intestinal environment. Omega fatty acids are also helpful in reducing inflammation and clinically beneficial in affected birds.[70] Semi-elemental diets such as Emeraid Omnivore and Carnivore (Lafeber Emeraid LLC, Cornell, IL, USA) require minimal digestion and provide a readily absorbable source of essential nutrients and Omega fatty acids). The liver is often exposed to bacterial showers from the abnormal intestinal environment in affected birds. Herbal supplements like Milk Thistle (Milk Thistle, Low alcohol, Gaia herbs, Brevard, NC, USA) and ginger (Ginger Root, Certified Organic, Gaia herbs, Brevard, NC, USA) are helpful in reducing inflammation, preserving hepatic function, and improving GI tract transit.
Gabapentin (Neurontin, Pfizer, New York, NY, USA) is increasingly being used in birds to help treat self-mutilation and to control neurogenic pain. It is a useful adjunct in managing birds with avian ganglioneuritis. Clinical signs in affected birds tend to increase with the onset of breeding activity. Reduction of the stress of elevated hormonal activity through the use Leuprolide acetate(Lupron Depot, AbbVie, Chicago, IL, USA) or Deslorelin implants (Suprelorin, Virbac Animal Health, Fort Worth, TX, USA) is very beneficial.

Mammalian Borna Disease virus shows a high degree of sensitivity to Ribivirin (Virazole, Valeant Pharmaceuticals International,Bridgewater,NJ,USA) , a ribonucleic analog that stops viral RNA synthesis. To date, the use of this and other antiviral agents in the treatment of avian bornaviral disease has not been rewarding or adequately researched.

Immunomodulation therapy through the use of *Mycobacterium bovis* extracts to redirected trafficking of activated antigen-specific CD4+T cells to local inflammatory sites has been shown to modulate the initiation and progression of a Th1-mediated peripheral and CNS autoimmune disease. This researched group found significant improvement in clinically diseased birds when this approach was combined with selective anti-COX-2 therapy.[69]

With patience, perseverance, prolonged therapy, reduced stress, and attention to correction of secondary problems, quality and longevity of life in many ABV clinically affected birds can be significantly improved. Implementation of appropriate therapeutic plans, early in the course of clinical disease, are most productive. Therapies may be minimally effective in severely affected individuals.

Formulary

Celecoxib (Celebrex, Pfizer Inc.,Mission,KS, USA), initially 30-40 mg/kg divided BID PO, Long term 15-30 mg/kg BID PO.
60-80 mg/kg divided BID PO for central nervous system involvement.

Robenacoxib (Onsior, Novartis Animal Health,North Ryde, NSW) Robenacoxib, 2-10 mg/kg IM weekly, and subsequently monthly[69]

Meloxicam (Metacam, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO USA). a preferential COX-2 inhibitor, not selective.).0.5 mg/kg IM, PO once daily; Oral form is formulated for canine species and may have reduced bioavailability in avian species; once clinical trial has shown it use exacerbates signs of clinical disease in ABV infected cocaktiels (*Nymphicus hollandicus*).[71]

Gabapentin (Pfizer) 10-25mg/kg BID PO up to 50 mg/kg BID PO in self -mutilating birds (Cathy Johnson-Delaney , written communication, 2015)

References

1. Clark FD. Proventricular dilatation syndrome in large psittacine birds. *Avian Dis*. 1984 Jul-Sep;28(3):813-5.
2. Gerlach H. Uber das Sog. “Macaw Wasting Syndrome.” Vortrag, 4. Arbeitstagung der Zootierarzte im deutschsprachigen Raum. Munster. 24-25 Nov. 1984.
3. Graham DL. Infiltrative splachnic neuropathy, a component of the “wasting macaw” complex. *Proc. Int. Conf. on Avian Med*., Toronto. P 275.1984
4. Hughes PE. The pathology of myenteric ganglioneuritis, psittacine encephalomyelitis, proventricular dilatation of psittacines, and macaw wasting syndrome. *Proc 33rd West Poult Dis Conf.* 1984;85–87.
5. Phalen DN. An outbreak of psittacine proventricular dilatation syndrome (PPDS) in a private collection of birds and an atypical form of PPDS in a nanday conure. *Proc Annu Conf Assoc Avian Vet.* 1986;27–34.
6. Ridgway RA, Gallerstein GA. Proventricular dilatation in psittacines. *Proc. 4th Ann. Meet. Assoc. Avian Vet.* San Diego. pp. 228-230. 1983.
7. Woerpel, RW, Rosskopf WJ. Clinical and pathological features of macaw wasting disease (proventricular dilatation syndrome). *Proc. 33rd Western Poultry Dis. Conf*., Davis, Calif. pp. 89-90. 1984.
8. Woerpel, RW, Rosskopf WJ. Proventricular dilatation and wasting syndrome: myenteric ganglioneuritis and encephalomyelitis of psittacines; an update. *Proc. Int. Conf. on Avian Med*., Toronto. pp 25-28.1984.
9. Avian Bornavirus Research Forum. Assoc. of Avian Vet. Annual Conference, 2014, New Orleans, LA.
10. Schmidt RE, Reivilo DR, Phalen DN. In: *Pathology of Pet and Aviary Birds*. Iowa State Press, Iowa, 2003:47–55.

11. EnCana's-Nagel N, Enderlein D, Piepenbring A, et al. Avian Bornavirus in Free-Ranging Psittacine Birds, Brazil. *Emerg Infect Dis*. 2014; 20: 12:2103-2106

12. Daoust PY, Julian RJ, Yason CV, Artsob H. Proventricular impaction associated with nonsuppurative encephalomyelitis and ganglioneuritis in two Canada geese. *J Wildl Dis*. 1991 Jul;27(3):513-7

13. Gregory CR, Ritchie BW, Latimer KS, et al. Progress in understanding proventricular dilatation disease. *Proc Annu Conf Assoc Avian Vet.* 2000;269–275

14. Perpiñán D, Fernández-Bellon H, López C, Ramis A. Lymphoplasmacytic myenteric, subepicardial, and pulmonary ganglioneuritis in four nonpsittacine birds. *J Avian Med Surg*. 2007 Sep;21(3):210-4.

15. Shivaprasad HL. Proventricular dilatation disease in a peregrine falcon (*Falco peregrinus*). *Proc Annu Conf Assoc Avian Vet* 2005.

16. Weissenock H, Bakonyi T, Sekulin K, et al. Avian coronaviruses in psittacine birds from Europe and Australia with proventricular dilatation disease. *Emerg Infect Dis*. 2009; 15:1453-1459.

17. Pavlov VA, Tracey KJ. The vagus nerve and the inflammatory reflex linking immunity and metabolism. *Nat Rev Endocrinol.* 2012;8 :743-754.

18. Andersson U, Tracey KJ. Reflex principles of immunological homeostasis. *Annu. Rev. Immunol*.2011; 30:313–335

19. Tracey KJ. The inflammatory reflex. *Nature*. 2002; 420:853–859

20. Berhane Y, Smith D, Newman S, et al. Peripheral neuritis in psittacine birds with proventricular dilatation disease. *Avian Pathol.* 2001;30:563–570

21. Cazayoux Vice C.A. Myocarditis as a component of psittacine proventricular dilatation syndrome in a Patagonian conure. *Avian Dis*. 1992;36(4):1117-9.

22. Philadelpho NA, Rubbenstroth D, Guimara MB, et.al. Survey of bornaviruses in pet psittacines in Brazil reveals a novel parrot bornavirus. *Vet. Micro.* 2014;174:584-590.

23 Rossi G, Crosta L, Ceccherelli R, Pesaro S. New evidence in PDD pathogenesis: can ganglioside sensitization satisfy Koch's postulates? Proc Eur Assoc Avian Vet. 2009;155.

24 Pesaro S, Crosta L, Bertoni P, et.al. Anti-ganglioside antibodies production as a theory of PDD pathogenesis. Proc Eur Assoc Avian Vet. 2009;89.

25. Rossi G, Ceccherelli R, Crosta L, et al. Anti-ganglioside specific auto-antibodies in ganglia of PDD-affected parrots. Proc 11th Eur AAV Conf, 1st Sci ECZM Meeting, Madrid. 2011;177–178

26. Rossi G, Enderlein D, Herzog S, et al. Comparison of anti-ganglioside antibodies and anti-ABV antibodies in psittacines. Proc 11th Eur AAV Conf, 1st Sci ECZM Meeting, Madrid. 2011;187–189.

27. Rossi G, Crosta L, Pesaro S. Parrot proventricular dilation disease. Vet Rec. 2008;163:310

28. Phalen DN. Implications of viruses in clinical disorders. In: Harrison GJ and Lightfoot TL, eds. *Clinical Avian Medicine*. Spix Publishing, Inc. 2006.

29. Gerlach S. Macaw wasting disease-a 4 year study on clinical cases, history, epizootiology analysis of species, diagnosis and differential diagnosis, microbiological and virological results. *Proc. Annu Conf Eur Chapt Assoc Avian Vet.* 1991:273-281.

30. Gregory CR, Latimer KS, Campagnoli RP, Ritchie BW. Histologic evaluation of the crop for diagnosis of proventricular dilatation syndrome in psittacine birds. *J Vet Diagn Invest*. 1996 Jan;8(1):76-80.

31. Bond MW, Downs D, Wolf S. Screening for Psittacine Proventricular Dilatation Syndrome. Proc Annu Conf Assoc Avian Vet. 1983;92–97. Nashville, TN

32. Gough RE, Drury SE, Harcourt-Brown NH, Higgins RJ. Virus-like particles associated with macaw wasting disease. *Vet Rec.* 1996;139:24.

33. Kistler AL, Gancz A, Clubb S, Skewes-Cox P, et al. Recovery of divergent avian bornaviruses from cases of proventricular dilatation disease: identification of a candidate etiologic agent. *Virol. J.* 2008;5:88.

34. Honkavuori KS, Shivaprasad HL, Williams BL, , et.al. Novel borna virus in psittacine birds with proventricular dilatation disease. *Emerg. Infect. Dis.* 2008;14:1883-1886.

35. Lierz M, Hafez HM, Honkavuori KS, et al. Anatomical distribution of avian bornavirus in parrots, its occurrence in clinically healthy birds and ABV antibody detection. *Avian Pathol*. 2009;38:491–6

36. Heffels-Redmann U, Enderlein D, Herzog S, et al. Occurrence of avian bornavirus infection in captive psittacines in various European countries and its association with proventricular dilatation disease. *Avian Pathol.* 2011;40:419–26.

37. Weissenböck H, Bakonyi T, Sekulin K, et al. Avian bornaviruses in psittacine birds from Europe and Australia with proventricular dilatation disease. *Emerg Infect Dis.* 2009;15:1453–9.

38. Piepenbring AK, Enderlein D, Herzog S, et al. Pathogenesis of avian bornavirus in experimentally infected cockatiels. *Emerg Infect Dis*. 2012;18:234–41.

39. Gancz AY, Kistler AL, Greninger AL, et al. Experimental induction of proventricular dilatation disease in cockatiels (Nymphicus hollandicus) inoculated with brain homogenates containing avian bornavirus 4. *Virol J* 2009;6:11.

40 Gray P, Hoppes S, Suchodolski P, et al. Use of avian bornavirus isolates to induce proventricular dilatation disease in conures. *Emerg Infec Dis* 2010;16(3):473–9.

41 Mirhosseini N, Gray PL, Hoppes S, Tizard I, et al. Proventricular dilatation disease in cockatiels (Nymphicus hollandicus) after infection with a genotype 2 avian bornavirus. *J Avian Med Surg*. 2011;25(3):199-204.

42 Payne S, Shivaprasad HL, Mirhosseinia N, et al. Unusual and severe lesions of proventricular dilatation disease in cockatiels (Nymphicus hollandicus) acting as healthy carriers of avian bornavirus (ABV) and subsequently infected with a virulent strain of ABV. *Avian Pathology.* 2011;40(1):15-22.

43. Kuhn JH, Dürrwald R, Bào Y, et al. Taxonomic reorganization of the family Bornaviridae. *Arch Virol*. 2015;160:621–632.

44. Rinder M, Ackermann A, Kempf H, et al. Broad tissue and cell tropism of avian bornavirus in parrots with proventricular dilatation disease. *J Virol* 2009;83:5401–5407.

45. Reuter A, Ackermann A, Kothlow S, et al. Avian bornaviruses escape recognition by the innate immune system. 2010. *Viruses* 2010;2:927-938

46. Lierz M. Avian Borna virus research at the free University of Berlin. Presented at the Research Forum of the 10th European AAV Conf.Antwerp, Belgium, 2009.

47. Herzog S, Enderlein D, Heffels-Redmann U, et al. Indirect immunofluorescence assay for intra vitam diagnostic of avian Borna virus infection in psittacine birds. *J Clin Microbiol.* 2010;48(6): 2282–2284

48. Dahlhausen RD, Orosz SE. Avian Borna Virus Infection Rates in Domestic Psittacine Birds. In: Proc Association of Avian Veterinarians, San Diego, CA.2010.

49. Rubbenstroth D, Rinder M, Stein M, et.al. Avian bornaviruses are widely distributed in canary birds (Serinus canaria f. domestica). *Vet Microbiol* 2013;165:287–295.

50. Payne S, Covaleda L, Jianhua G, et.al. Detection and characterization of a distinct bornavirus lineage from healthy Canada geese (Branta canadensis). *J Virol*. 2011;85(22):12053-12056.

51. Rubbenstroth D, Brosinski K, Rinder M, et al. No contact transmission of avian bornavirus in experimentally infected cockatiels (*Nymphicus hollandicus*) and domestic canaries (*Serinus canaria forma domestica*). *Vet Microbiol* 2014;172 (2014):146–156.

52. Heckmann J, Enderlein D, Piepenbring A, et al. Investigation on Different Routes of Transmission of Avian Bornavirus in Cockatiels (*Nymphicus hollandicus*). 2015, Proceedings ICARE Conference, Paris, FR.

53. Lierz M, Piepenbring A, Herden C, et.al. Vertical transmission of avian bornavirus in psittacines. *D Emerg Infect Dis.* 2011;17(12):2390-2391

54. Kistler AL, Smith JM, Greninger AL, et.al. Analysis of naturally occurring avian bornavirus infection and transmission during an outbreak of proventricular dilatation disease among captive psittacine birds. *J. Virol*. 2010;84:2176–2179.

55. Kerski A, de Kloet AH, de Kloet SR. Vertical transmission of avian bornavirus in Psittaciformes: avian bornavirus RNA and anti-avian bornavirus antibodies in eggs embryos, and hatchlings obtained from infected sun conures (*Aratinga solstitialis*). *Avian Dis.* 2012;56:471–478.

56. Lierz M, Piepenbring A, Herden C, et.al. Vertical transmission of avian bornavirus in psittacines. *Emerg. Infect. Dis*. 2011;17:2390–2391.

57. Monaco E, Hoppes S, Guo J, Tizard I. The detection of avian bornavirus within psittacine eggs. *J. Avian Med. Surg.* 2012;26:144–148.

58. Raghav R, Taylor M, Delay J, et.al. Avian bornavirus is present in many tissues of psittacine birds with histopathologic evidence of proventricular dilatation disease. *Journal of Veterinary Diagnostic Investigation* 2010;22:495–508.

59. Heatley JJ, Villalobos AR. Avian bornavirus in the urine of infected birds. *Vet Med Res Rep* 2012;3:19–23

60. de Kloet AH, Kerski A, de Kloet SR. Diagnosis of Avian bornavirus infection in psittaciformes by serum antibody detection and reverse transcription polymerase chain reaction assay using feather calami. *Journal of Veterinary Diagnostic Investigation*. 2011;23(3): 421–429

61. Lierz M, Piepenbring A, Heffels-Redmann U, et.al. Experimental Infection of Cockatiels with Different Avian Bornavirus Genotype. *Proc Annu Conf Assoc Avian Vet* 2012 Louisville, KY.

62. Villanueva I, Gray P, Mirhosseini N, Payne S. The diagnosis of proventricular dilatation disease: Use of a Western blot assay to detect antibodies against avian Borna virus. *Veterinary Microbiology* 2010;143:196–201.

63. Heffels-Redmann U, Enderlein D, Herzog S, et.al. Follow-Up Investigations on Different Courses of Natural Avian Bornavirus Infections. in Psittacines. *Avian Diseases*. 2012; 56:153–159.

64. Rossi G, Ceccherelli R, Crosta L, et al. Anti-ganglioside specific auto-antibodies in ganglia of PDD-affected parrots. *Proc 11th Eur AAV Conf*, *1st Sci ECZM Meeting*, Madrid, Spain. 2011;177–178.

65. Pesaro S, Crosta L, Bertoni P, et al. Anti-ganglioside antibodies production as a theory of PDD pathogenesis. *Proc Eur Assoc Avian Vet*. Antwerp, Belgium.2009;89.

66. Payne SL, Delnatte P, Guo J, et.al. Birds and bornaviruses. *Animal Health Research Reviews* 2012;13(2):145–156

67. Rossi G, Crosta L, Ceccherelli R, Pesaro S. New evidence in PDD pathogenesis: can ganglioside sensitization satisfy Koch's postulates? *Proc Eur Assoc Avian Vet*. Antwerp, Belgium.2009;155.

68. Dahlhausen B, Aldred S, Colaizzi E. Resolution of clinical proventricular dilatation disease by Cyclooxygenase 2 inhibition. *Proc Annu Conf Assoc Avian Vet.* 2002;9–12.

69. Rossi G, DVM, Crosta L, Ceccherelli R, DVM, PhD, Stefano Pesaro S, PDD: Our Point of View after 7 Years of Research. *Proc Annu Conf Assoc Avian Vet.* Louisville, KY. 2009.

70. Clubb SL: Clinical management of psittacine birds affected with proventricular dilatation disease. *Proc Annu Conf Assoc Avian Vet.* San Antonio, TX, 2006

71. Hoppes S, Heatley J, Guo J, et al. Meloxicam treatment in cockatiels (*Nymphicus hollandicus*) infected with avian bornavirus. *J Exotic Pet Med* 2013;22:275–279.