# CURRENT RESEARCH AND ADVANCES IN AVIAN BORNAVIRUS AND PROVENTRICULAR DILATATION DISEASE (AVIAN BORNAVIRAL GANGLIONEURITIS) FOR VETERINARY PROFESSIONALS 2016

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*(Terms in italics are defined in the glossary beginning on p. 26.)*

## ABSTRACT

The Avian Bornavirus is an immune-mediated disease and is responsible for what is known as Proventricular Dilatation Disease, more recently termed Avian Bornaviral Ganglioneuritis. It suppresses *apoptosis*, causing life-long, persistent symptoms. As many as one-third of the birds in collections are currently testing positive for Avian Bornaviral Disease, and the number is as high as 70% in high-stress conditions. Although captive psittacines are the most impacted, other species are testing positive for ABV as well. ABV is not the only cause of PDD signs; two other viruses have been proven to cause these same symptoms, and the number could be much higher. ABV is not a zoonotic disease, so humans and other mammals cannot contract this virus. Avian Bornaviral Disease is transmitted both horizontally and vertically. Treatment protocols and proper care are extending the lives of many birds infected with the Avian Bornavirus. Without treatment, it is always fatal.

## INTRODUCTION

Much has been written about the Avian Bornavirus (ABV) and Proventricular Dilatation Disease (PDD) in the past, and a significant amount of it is now out-of-date. Even data just three or four years old have been replaced with newer, more technologically supported facts. In an attempt to clarify the nature of this disease and distinguish it from other viruses which can cause the same symptoms, Drs. R. Dahlhausen and S. Orosz have chosen to refer to PDD as **Avian Bornaviral Ganglioneuritis (ABG),** which better describes the disease and "removes the focus on the proventriculus."[4] In this paper, the term, **Avian Bornaviral Ganglioneuritis (ABG/PDD)** will be used to denote the disease which causes the specific ganglioneuritis symptoms brought on by Avian Bornaviral infection.

> "This is complex disease. ABV has been shown to be A cause of PDD. But most birds that test positive for the virus are clinically normal. Also, there are birds with PDD disease that test negative for the virus. Disease involves an immune response that targets the nerve ganglia. It is theorized that other agents, such as viruses and bacteria, can sensitize the immune system, and through molecular mimicry, cause the host's immune system to react with self- (nerve ganglia) producing disease that is indistinguishable from PDD. PDD is not an appropriate term for the disease as there are forms that only show disease in the central nervous system; i.e., there is no proventricular dilatation present. Avian ganglioneuritis is a more appropriate term."
>
> R. Dahlhausen

## WORLDWIDE DISTRIBUTION OF AVIAN BORNAVIRAL GANGLIONEURITIS (PDD)

The disease originally known as "Macaw Wasting Disease" was initially reported in the late 1970's in the U.S. and Europe, and its country of origin was Bolivia.[4] The term, "Proventricular Dilatation Disease" was given to the disease in a 1983 report describing "impaction, dilatation, and degeneration of the *proventriculus*."[4] Originally thought to affect only macaws, as of 2009, verified cases had been reported in as many as 80 psittacine and non-psittacine species, both captive and wild.[4] Since then, the Avian Borna Virus has been proven as the virus responsible for ABG/PDD, and it has been detected in birds

around the world largely due to the expansive bird trade of the 1970's and 1980's.Now it is found not only in America and Europe, but in most countries. Asia, Africa, and S. America are seeing growing numbers of ABV-positive birds. This disease "now presents a serious threat to both captive propagation and conservation efforts for endangered psittacines such as the Spix macaw."[4]

## DEFINING AVIAN BORNAVIRAL GANGLIONEURITIS (PDD)

The Avian Bornavirus (ABV) is an *enveloped*, negative-stranded, RNA-virus *genome*. Avian Bornaviral Ganglioneuritis (PDD) is a fatal, inflammatory wasting disease affecting mostly birds in the psittacine family (Order *Psittaciformes*).[1] It is caused by the Avian Bornavirus and is a disorder in which "inflammation of the central, peripheral, and autonomic nervous systems is associated with gastrointestinal (GI) dysfunction and neurologic signs."[2] In *clinical* terms, ABG/PDD is described as a "non-*suppurative*, lymphocytic-plasmacytic ganglioneuritis of the nerve plexi of the crop, proventriculus, *ventriculus* or gizzard (depending on the species), and duodenum."[23, 2]

**Figure 1: A macaw suffering from the effects of the Avian Bornavirus (ABG/PDD). Note the poor condition of the beak and feathers and depressed stance. (Courtesy R. Dahlhausen)**


## ETIOLOGY AND PATHOGENESIS OF ABG/PDD

In the 1970's, when the huge influx of captive birds from other countries began showing clinical signs of ABG/PDD, researchers began searching for the etiology and pathogenesis of this disease but for decades were unable to identify it. [2] Researchers, suspecting a viral cause for the signs of avian ganglioneuritis, noted that the disease could be transferred to naïve birds through inoculation with tissue homogenates or fecal material from affected birds.[2] Histopathological lesions associated with ABG/PDD also suggested a viral etiology."[2]

In 2008, “inclusion bodies and enveloped particles similar to viruses in the *myenteric plexus* and celiac ganglion of infected birds” were discovered using transmission electron microscopy studies. [20] Dr. Amy Kistler et al. and Dr. Kirsi.S Honkavuori et al., independent researchers, provided confirmation that the etiological agent and pathogenesis of ABG/PDD are found in the Bornavirus. “The use of the Virus Chip, a DNA microarray containing representation of all viral taxonomy, enabled tissue to be screened for the presence of most known viral pathogens.”[2] They are credited with discovering a novel, enveloped, negative-stranded, RNA virus genome and designating it the “Avian Bornavirus.”[1,20] The isolated Borna Disease Virus (BDV) genome revealed a high degree of sequence divergence from all prior Bornavirus isolates.”[2] With this, Kistler’s group conclusively establishing the connection between the BDV and ABG/PDD.[2]

ABV and ABG/PDD had previously been regarded as carrying a high-mortality but low-infection risk. As of a few years ago, it has been shown to occur much more frequently but involve a “low incidence of clinical disease and a much lower incidence of severe disease.” [15] But now, even this concept is changing. Although the number of ABV-positive, clinically ill birds is growing, new treatment protocols have moved this disease to a more chronic state and one which often responds to treatment. Nevertheless, an infected bird will ever eliminate the viral infection.[2]

**Related Studies on the Etiology and Pathogenesis of PDD May Be Found in Appendix-A, p. 27**

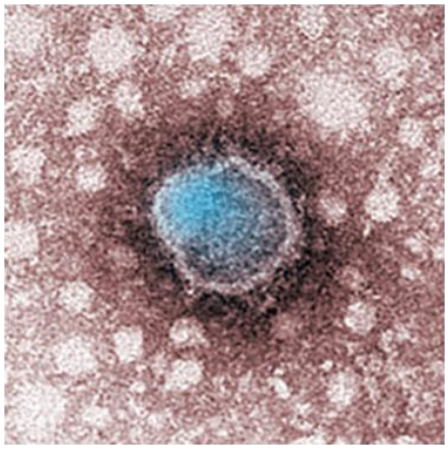


**Figure 2: The Avian Bornavirus (courtesy R. Dahlhausen)** 

## THE PARENT DISEASE: BORNA DISEASE VIRUS

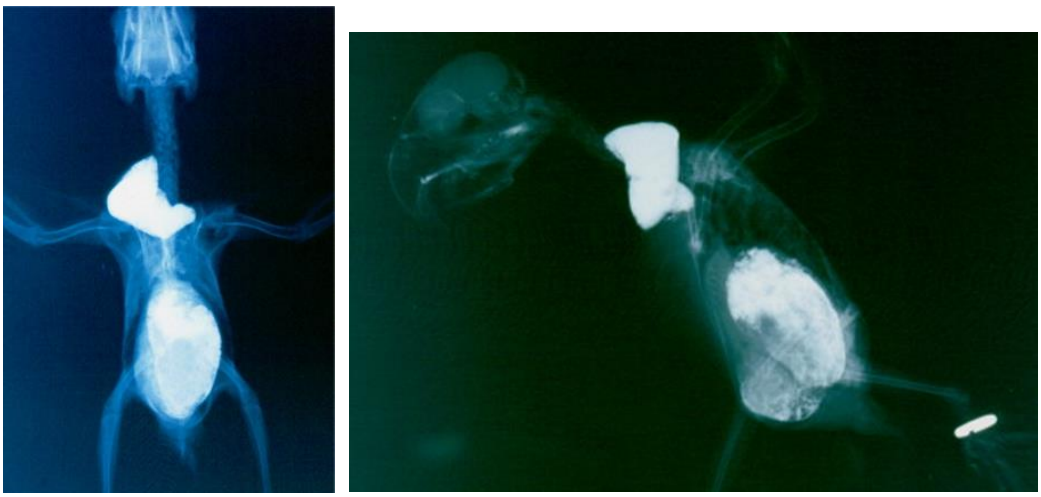
The Borna Disease Virus (BDV) was originally discovered in cavalry horses in Borna, Saxony, Germany in 1885. It has since that time become known as a “neurological disease a wide range of animal species and even in humans.” [2, 4]

Most viruses spread by cell-to-cell contact, first invading and destroying the host cell, then moving on to infect more cells.[20] The Borna Disease Virus, however, uses the nuclear compartment of the host cells, in which infectious BDV ribonucleoprotiens are present, for transcription and replication.“ [20] The Bornavirus, through *non-cytopathogenicity*, does not destroy the cell; therefore, the infected cells suffer very little damage.[3] Because the cell is not destroyed, the virus is able to avoid being recognized by the host’s immune system.[2,3] “This is a detection-evasion strategy which allows the molecules to go unrecognized by the cytosolic RNA sensor that triggers the host’s innate immune responses.” [20]

Since the virus is plentiful in the infected cells, it must suppress *apoptosis*.[20] "Programmed cell death appears to be reduced by the presence of a BDV viral accessory protein (protein X)." [3, 20] "This leads to the continual, persistent, life-long CNS infections." [2] This infection process is most certainly the same for ABV.[2,3]

The effects of Bornaviral Disease are many. "Manifestations range from fatal meningoencephalitis to subtle behavioral alterations."[2] The animal may even be infected over a long period of time and never exhibit any signs.[3] "The virus can remain in the brain, blood, and other body cells for an indeterminate amount of time," possibly for the lifetime of the individual. [2]

ABV belongs to the order *Mononegavirales* and has been serologically sequenced. Testing has shown it to be genetically "related to the mammalian BDV with which it exhibits approximately 65% *homology*."[3] Serological testing has also confirmed the link between ABV and BVD.
But the Avian Bornavirus is different from the mammalian form of Bornavirus—it does not grow in mammalian cell lines. Therefore, it is not thought to infect humans or animals. [2]



**Figures 3 and 4: Ventrodorsal and lateral radiographs showing barium in a distended crop with poor motility and enlarged proventriculus and ventriculus. (Courtesy R Dahlhausen)**


Researchers have confirmed that BDV RNA virus is responsible for "persistent infections of the Central Nervous System (CNS)." [4,2] BDV attacks are limited to the nervous system; however, ABV affects multiple organs: the brain, GI tract, liver, kidneys, heart, and lungs, as well as in the peripheral blood vessels.[3] Because BVD is extremely neurotropic, it attacks the central, peripheral and autonomic nervous systems. There is a wide range of signs from the infection; in some birds, only slight changes in behavior are noted, but in others, the birds suffer severe neurological disease which results in fatal infections. [4, 2] The animal may even be infected over a long period of time and never exhibit any signs.[2]

## INCREASING INFECTION RATES

Practitioners have been reporting high numbers of birds testing positive for ABV. Even single pet birds housed in the same environment for many years have been diagnosed with the disease.[2] In one study

of both “suspect and non-suspect birds,” over 5000 cases of ABG/PDD-diagnosed birds had been confirmed to have both ABV *antibodies* and Bornavirus RNA. [4]

## Studies

In an experiment using blood sample testing on healthy-appearing pets, Michael Lierz et al. detected ABV RNA in 27 out of 59 (45.8%) birds tested. In another study, he discovered that 35 of 77 (45%) birds who had previously dealt with ABG/PDD tested positive for ABV-specific serum antibodies. [3]

In a 2010, molecular samples from 791 individual psittacine birds from across the U.S. were submitted to the Veterinary Molecular Diagnostics Laboratory (VMD, Milford, Ohio). In this experiment, “Heparinized whole blood and cloacal swab samples were tested for the presence of ABV-specific RNA using an in-house developed assay. Primers targeted a conserved region of the L gene of the ABV genome. ABV-specific RNA was detected in cloacal swabs by qPCR (real-time PCR). Individuals testing positive for ABV-specific RNA on either blood sample, cloacal swab, or both were considered positive.” [3]

The study revealed an “overall detection rate of 34.3% (271/791) for ABV-specific RNA” in the samples taken.[3] The species with the highest number of positive results were cockatoos, Amazons, eclectus, African greys, and macaws. Those with the lowest number of positive results were cockatiels, budgerigars, pionus and conures. [3]

In a later study, VMD lab tested 100+ psittacines living in a stressful shelter situation; 52% of this population tested positive for ABV-specific RNA. [3] And in a screening of birds in another stressful shelter situation in 2012, 71% of the birds tested positive.[3]

While the number of birds testing positive for ABV is growing, birds seen with clinical signs is much lower. Most birds do not exhibit signs, and those that do show only mild signs. The severe form of the disease resulting in death is a result of on-going, untreated disease.

## THE AVIAN BORNAVIRUS AND ITS GENOTYPES

A genotype refers to the genetic constitution of an individual or a group or class of organisms having the same genetic constitution. Viruses are capable of developing subgroups, called genotypes—related viruses within a genus or genetic subgroup. (The number after ABV [*e.g.*, ABV-4, ABV-6], is the number relegated to the individual genotype in order of discovery.) “The family *Bornaviridae* is no exception, consisting of more viral genotypes (or viral species) than had been previously assumed.”[6] Bornaviruses are natural pathogens of several avian species. “Recent research has shown that the Avian Bornavirus represents a diverse viral group of at least 15 distinct ABV genetic subgroups.”[2, 4] If the bird is not tested for each specific genotype, the test can result in a false negative. Genotypes 2 and 4 are the ones mostly commonly found in psittacines and therefore are the ones for which most veterinarians test, although sometimes the clinician must expand the testing to other genotypes if his tests results are negative yet he still suspects ABV. (Dahlhausen, personal communication)

Recognition of the virus is dependent upon matching the tests to the specific genogroups, and each genogroup is dominated by a single, distinct protein. A virus can have many sub-groups, depending on the proteins to which they are attached. The number after ABV (e.g., ABV-4, ABV-6), is the number relegated to the individual genotype in order of discovery. Since each genotype is dependent on a particular protein, if the sample were tested for a specific protein and none other, it is possible that the bird could be ABV-positive even if the test results came back negative. A bird can display

signs, but if the results do not match any existing genotype, the results can be negative. The bird may still have ABV; if so, the researcher has discovered a new genotype.[17]

To prove the existence of multiple genotypes, researchers conducted a study involving naturally infected birds. Those which were treated and recovered did so in about 4 weeks; however, even after recovery, they continued to display lesions in the GI tract and have ABV in their tissues. Surviving, asymptomatic birds tested positive for ABV. This verified the existence of multiple strains of the virus.[17]

## DETECTION OF AVIAN BORNAVIRAL DISEASE AND ABG/PDD

Despite the high incidence of viral infection in psittacines, only a small percentage actually exhibit clinical signs and serious disease. Clinical signs vary—all the way from infrequent episodes of mild signs to sudden and severe illness.[12] A bird may experience just some of the symptoms, not necessarily all. Some birds become symptomatic years or decades after becoming ABV-infected, and some never show signs at all but may continue to shed the virus and thus infect other birds.[4] Healthy birds can experience *viremia* due to ABV without becoming clinically ill. [2] In fact, most of the ABV-positive birds never display clinical disease.[15]

Clinicians encounter some challenges when testing for Avian Bornaviral infections. Reasons for this are:

- *Lymphoplasmacytic ganglioneuritis*, pathological lesions, or a similar disease state may be caused by another infectious agent. [1,2] Two other viruses have been proven to cause the same ganglioneuritis symptoms as ABV.
- Recognition of the virus is dependent upon matching the tests to the specific genogroups, and each genogroup is dominated by a single, distinct protein.
- The virus may have many sub-groups, depending on the proteins to which they are attached. Since each genotype is dependent on a particular protein, if the sample were tested for one specific protein and none other, it is possible that the bird could be ABV-positive even if the test results came back negative.
- Some psittacine species are more vulnerable to specific genotypes than others.
- The various ABV genotypes may each produce distinctive and specific clinical symptoms.
- "New mutations may occur in the viral genome that could affect the assay, making it difficult to detect all subtypes." [4]
- A bird may be ABV-infected and display signs, but if the results do not match any known genotype, the results can be negative.
- "Some reported primer sequences used in ABV RT-PCR testing have been shown to also detect paramyxovirus-1, a related virus." [2]
- Some reported primer sequences used in ABV RT-PCR testing do not detect all identified ABV genotypes. [2]
- "While the virus can be detected in cloacal swabs from both clinically and non-clinically infected birds, cloacal swab samples are not consistently positive in affected birds, possibly due to the high level of RNA enzymes in fecal material that can destroy viral RNA" and inconsistent shedding of the virus.
- Other destructive factors may be present, like bacteria, enzymes and other contaminants found in the feces. [4] This results in a high number of false negatives.[4]
- "The low replication rate of BDV and low number of infected blood cells may limit BDV-RNA detection in all cases." [2]

Some genotypes produce certain symptoms, and others produce different symptoms. This may explain why some birds develop certain symptoms and others do not, and why some birds, when exposed, do not contract the virus at all.

(Any relationship between genotype and species infected and/or clinical disease observed remains to be elucidated. [Dahlhausen, personal communication]).

The bird's own system can offer conflicting test results:

- There are multiple ABV antigens, depending on the type of protein involved, and if the bird isn't tested for the specific antigen affecting it, a false negative could result.
- The bird may not be shedding the virus at the time of testing; cloacal swabs are less sensitive because of the intermittent shedding of the virus in some birds.
- The bird might not be shedding the virus at all because its intestinal tract has not been affected, or the infection might be transient. The result is an inflammatory process that has become latent, as in an autoimmune disease.[1]
- If a crop biopsy is performed, there is a chance that the sample will not contain the infected ganglions; therefore, it will come back as negative when the bird is actually positive.

## SPECIES AFFECTED BY ABV DISEASE

### Psittacines

For many years, the Avian Bornavirus has spread uncurbed throughout the avian population. Until recently, there had been no means of detection or control in existence.[3] Psittacines appear to be the most affected by the Avian Bornavirus; therefore, almost all of the studies have concentrated on the captive psittacine population. Most psittacine species have been tested, and the species with the highest number of positive results were cockatoos, Amazons, eclectus, African greys, and macaws. Quakers and Lovebirds have been minimally represented.[4] Those with the lowest number were cockatiels, budgerigars, pionus and conures. [3]

### Free-ranging Psittacine Species

Investigations of ABV infection in wild psittacines had not been presented until recently.

### A Study

In research performed by Dirk Enderlein et al. in 2011, more than 80 free-ranging, clinically healthy psittacines were used in the ABV tests. The birds were located in Brazil and belonged to seven different species.[7]

ABV RNA was discovered using RT-PCR and qPCR. IHC was also used to "detect ABV antigen in paraffin-embedded organ samples."[7] A fourth test, immunofluorescence assay, was involved in the discovery of ABV-specific antibodies. The results revealed that:

- ✓ 33% of the birds tested demonstrated ABV infection by positive qPCR results. ABV genotype-4 was confirmed by the sequencing of PCR products of the positive samples.
- ✓ More than 50% of the birds presented specific anti-ABV antibodies but tested negative when they were subjected to PCR testing.[7]

The possibility exists that ABV genotypes other than ABV-2 and 4, or as-yet-unknown genotypes, or "cross reactivity with related agents" were involved. [7]

**Non-psittacine species affected**

Avian species other than psittacines are vulnerable to ABV. Researchers from Texas A&M University studied evidence of ABV in non-psittacine species, specifically waterfowl. Their study involved the testing of Canada Geese, and it resulted in the discovery of a new genotype. [2] This Bornavirus genotype contained characteristics that bore more similarity to the mammalian Bornavirus than to Avian Bornavirus. In 2011, Payne, et al, using the rt-PCR assay, "screened brain tissue from water birds for the presence of the ABV-M gene."[13] They tested Mute Swans, Snow Geese, Ross' Geese, and Greater White-fronted Geese. The ABV was detected in all but the White-fronted Geese. Of the ducks tested, 11% tested positive for the ABV, and 13% of gulls tested positive. The virus also caused neurological symptoms of ABG/PDD in the form of acute *encephalitis* in geese and swans. A novel ABV genotype was discovered in a Bald Eagle *(Haliaeetus leucocephalus)* as well, suggesting that raptors could be vulnerable to ABV through predation. [13, 16]

Testing of Canada geese *(Branta canadensis)* resulted in the discovery of two new genotypes, ABV-CG (Canada goose) and ABV-4. Using the RT-PCR assay, they "screened for the presence of the ABV-M gene in the brain tissue." [16] The positive percentage rates of detection were:

- Mute swans *(Cygnus olor):* 23%
- Snow geese *(Chen caerulescens):* 18%
- Ross' geese *(Chen rossii):* 10%
- Greater White-fronted geese *(Anser albifrons):* 0%
- Ducks (*Anas* and *Aythya* species): 11%
- Gulls (*Larus* species and *Leucophaeus atricilla*): 13%

Canaries are another of the non-psittacine species which have been found to host ABV. The canaries in Dr. Monica Rinder's studies contracted ABV by natural means. One canary showed signs of apathy just three days before it succumbed to the virus.[13] Death was caused by both encephalitis and non-suppurative ganglioneuritis of the proventriculus. The lesions found in it were similar to the lesions found in the psittacine birds with ABV. Both neural and *extraneural* tissues contained Bornaviral antigens.[6]

A second canary's illness was chronic and "included prolonged depression, CNS symptoms, and visual impairment with c*horioretinitis* (ocular inflammation)*."* [15] Analysis of the viral sequence suggested a different, yet similar, genotype from the ones found in psittacines.[15]

Other species affected are the greenfinch, long-wattled umbrella bird, the bearded barbet, toucans, honey creepers, reseate spoonbill, and a peregrine falcon. "The disease presents a serious threat to captive propagation and conservation efforts for endangered psittacines such as the Spix macaw."[4]

## PERIODS OF VULNERABILITY

Birds may be more vulnerable to viral attack during some periods in their life cycles than others.

**Susceptibility to infection in nestlings**

Jean Smith and Amy Kistler et al., studying various species of recently hatched psittacines during a sudden surge of ABG/PDD in the captive flock, tested an Umbrella Cockatoo *(Cacatua alba),* a Moluccan Cockatoo *(Cacatua molaccensis)*, a Scarlet Macaw *(Ara macao)*, and several Jenday conures *(Aratinga jandaya)*. They determined that 'unweaned birds were more vulnerable to the ABV-2 infections than the adults. The estimated incubation period was between two and four weeks." [6]

**Susceptibility to infection during breeding seasons**
The other highly susceptible period for contracting ABV-ABG/PPD is the breeding season; the disease cycles from active to dormant states, and the stresses of this increased hormonal state depress the immune system, allowing symptoms to flare up. Clinicians frequently see upsurges in new cases and relapses in ABV and ABG/PDD patients during this time.[2] One clinician has treated a patient who had experienced severe relapses of gastrointestinal symptoms during four consecutive breeding seasons.[2]

**Incubation Periods, Triggers, and Relapses**

The Avian Bornavirus' ordinarily long incubation period is shorter for the immunocompromised or very young.[16] It can range from several weeks to many years. [2] In a recent, acute outbreak of ABG/PDD in an avian nursery which houses and hand-feeds multiple species of psittacines, researchers, using controlled infectivity, noted that symptoms appeared in as few as 20 to 31 days.[2]

In some naturally occurring flocks, ABG/PDD is described as a "sporadic disease;" the bird may experience bouts with the symptoms for months, even years, after diagnosis. [2] Even single, pet birds, housed in the same environment for many years, have been diagnosed with the disease.

The clinician should not automatically assume a bird is afflicted with ABG/PDD simply because it tests positive for antibodies against ABV. It might take years before an ABV-positive bird develops signs of ABG/PDD, and some never become symptomatic. It is not known if or when the bird will become symptomatic or what will trigger the onset of symptoms; however, stress is a major factor.[6]

ABV disease is "defined by the absence or presence of *antigenemia*." [2] R. Dahlhausen attributes the development of clinical disease to the following predisposing factors:

- Genetics, age, the host species involved, and the developmental competency or compromised condition of the host's immune system.[2]
- Stress due to malnutrition, concurrent disease, reproductive activity, and improper husbandry. Stress is generally accepted as the primary trigger in the activation or recurrence of ABG/PDD, and it will accelerate the spread of the virus.[2]

Each of the above factors will change the way the bird reacts to the virus, and these signs differ from case to case. Clinical signs vary and are dependent upon the "host species involved, severity of disease, distribution of lesions and affected organ systems involved." [4]

## CLINICAL SIGNS OF ABV INFECTIONS

Although the Avian Bornaviral Ganglioneuritis signs are "primarily neurogenic in nature, they are classified as gastrointestinal (GI) or Central Nervous System (CNS) in character."[4] Birds may exhibit only the GI or the Central Nervous System (CNS) signs, but some experience both, and the virus affects both, even if they are not initially obvious. The GI signs indicate pathology of the terminal ganglia of the vagus nerve (Cranial Nerve X), also referred to as the *pneumogastric* nerve.[4]

A survey of birds affected by Avian Bornavirus and displaying clinical signs of Avian Bornaviral ganglioneuritis (PDD) revealed that 66% of birds exhibited CNS signs, 22% experienced GI tract signs, 9% displayed feather picking and mutilation, and 9% suffered acute death.[4]

**The Gastrointestinal Signs of Avian Bornaviral Disease**

ABG/PDD is a non-suppurative inflammation or infection of the networks of nervous tissues due to the invasion of infected lymphocytes and plasmacytes into the organs. When the virus attacks the gastrointestinal system's nerves, inflammation results. [18] It is "an immunologic attack of infiltrating immune cells on the autonomous (involuntary) nervous system of the upper digestive tract."[18] Affected birds are unable to digest and absorb their dietary nutrients properly.

When this invasion of the nerve clusters by lymphocytes and plasmacytes reaches the GI system's nerves, "symptomatic, persistent infections often result in intestinal colic and ganglioneuritis in the upper intestinal tract."[2, 23] S. Orosz discovered that the GI symptoms developed from the "disruption of innervation to one of the cranial nerves of the brainstem—the vagus nerve." [15] The vagus nerve (Cranial Nerve X) in the brain "supplies the first part of the intestinal tract—from the crop, proventriculus and ventriculus to the duodenum and the heart. When the vagus nerve malfunctions, there is a disruption of the normal GI motility and a thinning of the wall of the GI tract." [15] This can result in the rupture of the proventriculus and immediate, painful death. Finding the infected nerve ganglions confirms that the individual is positive for ABV disease.[15] Other signs include varying degrees of abdominal enlargement, muscular and neurogenic atrophy, weakness, crop stasis, impaired GI tract transit, and *polyuria.*

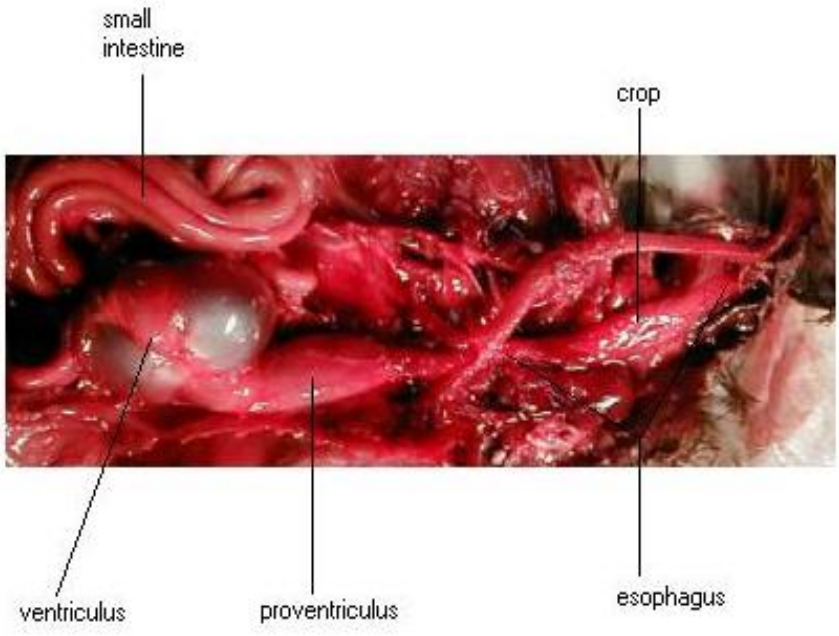



**Figure 5: A normal intestinal tract (courtesy www.LookforDiagnosis.com.)**

The most frequently seen clinical GI signs include depression, anorexia and weight loss (due to poor motility), loss of body condition, regurgitation, passage of undigested food in the feces, poor absorption, *polyphagia*, and *inappetence*. Crop biopsy and radiographs revealed dilation of the proventriculus.[2] *Histopathology* slides show a *lymphocytic, plasmacytic ganglioneuritis*. [15] When this invasion of the nerve clusters by lymph and plasma cells attacks the GI system's nerves, symptomatic, "persistent infections often result in intestinal colic and ganglioneuritis in the upper intestinal tract."[2, 23] Unless treated, it is always fatal.

In addition, clinicians frequently observe secondary bacterial and fungal infections of the GI tract which must be addressed immediately.[21] The vagus nerve also controls the "inflammatory reflex, a neural reflex that controls immune responses and inflammation during GI-tract pathogen invasion and tissue

injury."[4] When this reflex is impaired, natural resistance to bacterial overgrowth takes place; this leads to alterations in the intestinal microbiome and overgrowth of pathogenic organisms such as Clostridium perfringens and fungal organisms. [4] Many of the affected birds develop C. perfringens and enteritis due to sluggish GI transit and should be treated appropriately if that sign is present. (Dahlhausen, personal communication)

**Figure 6: Gross lesions in a blue and gold macaw. Normally, the proventriculus would not be larger than the liver. Organ displacement is due to the enlarged proventriculus (in blue at top) and ventriculus (in grey, below the proventriculus.) (Courtesy Dr. Louise Bauck and Beautyofbirds.com)**

**Figure 7: Necropsy photo of a crop containing undigested foods. Whole seeds can be seen in this picture. (Courtesy R. Dahlhausen)**

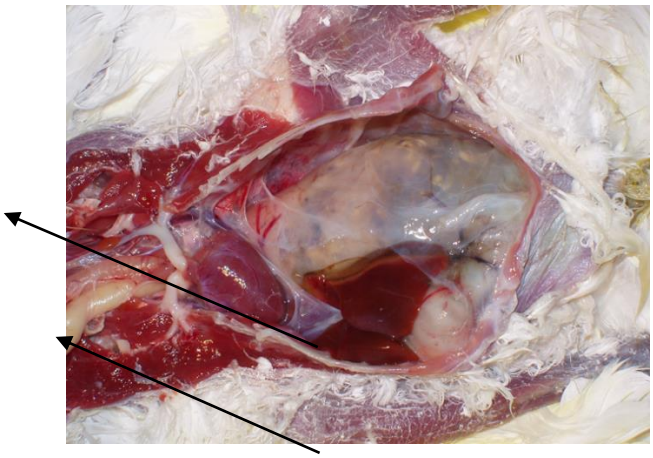


**Figure 8: Necropsy photo of an enlarged proventriculus (top arrow) and ventriculus.**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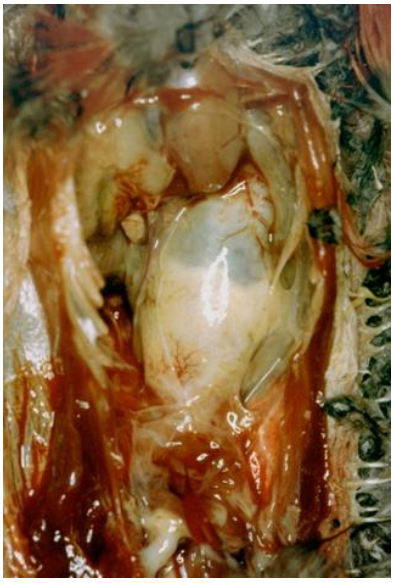

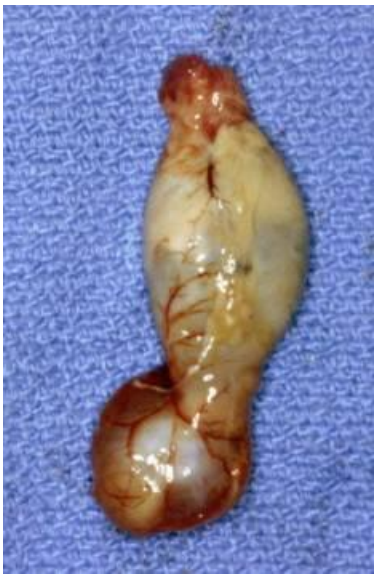

**Figures 9 and 10: Necropsy photos showing the displacement of organs caused by dilated proventriculus and ventriculus. The large, pale, center organ (the proventriculus) and the smaller yellowish extension of it (the ventriculus) are displacing the lungs, kidneys, heart, and liver. The second picture is the proventriculus and ventriculus from the same necropsy. (Courtesy R. Dahlhausen)** 

**Figure 11: A necropsy photo displaying the wasting effect of the disease in a cockatoo. (Courtesy R. Dahlhausen)** 

## The Neurological Signs of Avian Bornaviral Ganglioneuritis (PDD)

Some individuals only display neurological signs of ABV/PDD. However, when ABV assaults the Central Nervous System, it assaults all the nerves, so the bird will still eventually experience damage to the GI tract. These signs simply might not be obvious early in the disease process.

With neurological signs, inflammation leads to non-supperative *encephalomyelitis*.[23] As a *neurotropic* virus, ABV preferentially infects nerve cells, causing inflammation of the nerve ganglions and exposing ganglioside proteins.[4] The immune response to these causes nerve dystrophy which leads to clinical disease (Dahlhausen, personal communication).

The neurological symptoms are controlled by the area of the brain which was affected by the invasion of the lymphocytes. Since the lymphocytes determine whether or not the tissue contains the virus, finding lymphocytic ganglioneuritis confirms that the individual is positive for the Avian Bornavirus. [15]

CNS lesions are usually found in the cerebrum or cerebellum. Disruptions of the cell layers within the cerebellum produce disorders in fine-muscle movement and equilibrium. The affected bird displays "lack of coordination, *ataxia*, intention tremors, progressive paresis, paralysis, head tremors, seizures, and motor and *proprioceptive* deficits."[4] Neurological signs also include feather plucking and self-mutilation, difficulty balancing, moaning or crying due to digestive discomfort, aggressive behavior, reduced cognitive ability, dysarthria (vocalization abnormalities) and intermittent head-shaking. [4, 2] *Hyperesthesia*, *hyperalgesia*, and *allodynia* have also been noted. Old World species often exhibit the CNS signs and many times experience concurrent, non-clinical lesions in the GI tract as well.[4] The nervous system signs are more difficult to control and more rapidly fatal than GI signs. (Dahlhausen, personal communication)

Feather-picking and self-mutilation have been associated with peripheral neuritis. "Inflammation and myelin degeneration of the dorsal nerve roots, white matter, and associated ganglia have been identified on all levels of the spinal cord in ABG/PDD affected birds."[4]

## Self-mutilation and Feather Destruction

**Figure 12: Neurological signs of self-mutilation. (Courtesy R. Dahlhausen)**


## A Study

In 2011, A. Fluck et al., in Giessen, Germany, set up experiments to "establish a correlation between the ABV viral infections and other neurological signs, including feather plucking." [7]
Researchers collected crop and cloacal swabs from all the birds in the study, then subjected the samples to reverse transcription PCR (RT-PCR) testing in search of the presence of ABV RNA. They also examined tissue samples for the presence of specific anti-ABV antibodies by immunofluorescence assay.[8]

The test yielded the following results:

- 42.5% of the birds tested positive for ABV infection in one of the test systems.
- 24% of the birds in the control group tested positive in at least one of the ABV tests, compared to 54.1% of the feather-plucking birds and 68.4% of the neurologically diseased birds. [8]

The ABV antibody titers were highest in birds with neurological signs. The feather-plucking birds displayed the highest levels of antibodies, and those birds in the control group which were ABV-positive had very low titers. At the same time, highest viral loads were found in the group of neurologically diseased birds.

The results unmistakably substantiated a correlation between an ABV infection and neurological signs. They also confirms the correlation between an ABV infection and feather plucking. [8]

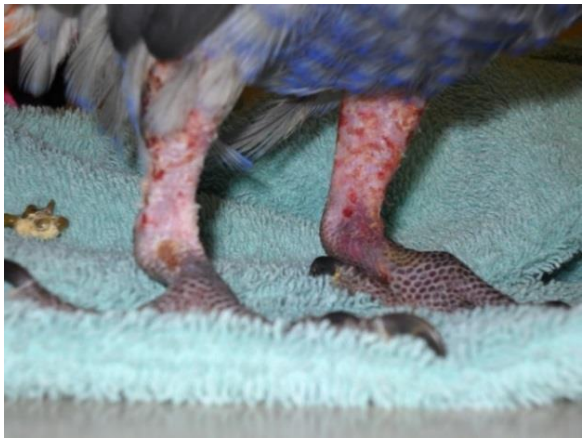

**Figure 13: Signs of self-mutilation in an African Grey (Courtesy R. Dahlhausen)**


**Figures 14-22**


**These video clips demonstrate a cockatoo experiencing nervous system signs: tremors, ataxia, and agitation. Double click on each to open. (Courtesy R. Dahlhausen)** 

## Other organs affected

The ABV attacks a wide variety of tissues in the body, not only the GI tract and nervous system. The viral RNA has been discovered in the liver, kidney, adrenal glands, heart, and lungs.

## Cardiac Impairment (Autonomic Nervous System)

Myocarditis has been reported as a component of ABG/PDD. In studies, "Viral antigen ABV has been identified in the *epithelial* cells within the *myocytes* in ABV-induced cardiac lesions." [2] ABV is known to attack the myocardium (the heart muscle), and clinicians have discovered "lesions in extraneural tissues, such as smooth or heart muscle fibers." [23] Clinicians have found lesions in the "conduction pathways of the heart, causing acute (sudden) death in otherwise normal-appearing individuals." [18, 2] In studies, "Viral antigen ABV has been identified in the cells which generate the electrical impulses that control the heart rate." [2]

Lesions are "more frequent and severe in the right side of the heart, possibly due to the higher density of nervous tissues in that area."[4] Also, "parasympathetic innervation of the heart is partially controlled by the right vagus branch which innervates the *sinoatrial node*. Enlargement of the right ventricle of the heart of affected birds has also been described." [4] Arrhythmias and alterations in blood pressure have been noted in affected birds. Cardiac lesions are known to result in acute death in birds that appear clinically normal. [4]

## Ocular Damage

The Avian Bornavirus is also responsible for disorders of the eyes, leading to optic lobe lesions and blindness.[4] Rudiger Korbel and Monika Rinder, using both ophthalmoscopy and digital imaging with digital scanning ophthalmoscopy, discovered that the birds which were naturally infected with ABV, and were clinically ill with ABG/PDD, experienced lesions within the *fundus* of the eye (retina) and decreased visual acuity. Birds which were unaffected by ABV and ABG/PDD showed no lesions or changes in acuity. [11] Cortical blindness is also found in ABV birds. In this case, the eye is functioning properly, but the nerves between the eye and brain have become infected. In most cases of cortical blindness, the eyes respond to correct treatment.[2, 4]

The pathogenesis of ABV is the result of an *auto-immune reaction*.[6, 17] The immune system's failure to recognize and destroy an infectious agent results in fatal disease." [17] Auto-immune disease is a result of the breakdown of "self-tolerance of the immune system."[17] Different phases of an individual's life alter the immune system. These differing demands on the immune system change the system's responses and lead to "permanent alterations and adaptations throughout life." [17]

Studies showed that birds need not come in direct contact with the virus to develop ABG/PDD symptoms. By inoculating six healthy birds with lipids from the peripheral nervous systems of ABV-affected parrots, Rossi and Pessaro proved that naïve birds can develop characteristic ganglioneuritis, even though there was no virus involved; their immune systems attacked the newly introduced *gangliosides* despite lack of infection. The leakage of ganglioside proteins from the damaged nerves causes an auto-immune reaction, and the resulting inflammation produces the symptoms of the disease.[15] These are regarded as the definitive indicators of the presence of ABV. [6] The virus is triggered by an infection which leads to the production of specific antibodies. These antibodies bind to gangliosides; this leads to *immune-mediated* damage to peripheral nerves and results in clinical symptoms. This concept is centered on the correlation between ABG/PDD and an auto-immune response.[17]

Advanced technology is assisting scientists. Studies involving the use of an electron microscope showed that *macrophages* attack nerves that had been infected with the virus, thus causing a "leakage of protein-ganglioside proteins from these damaged nerves."[12, 23] An auto-immune reaction follows; the macrophages are turning on the body, attacking the body's own nerves. It is this reaction and the inflammation due to the immune system's attack that leads to the onset of clinical symptoms of ABG/PDD.[12]

**The body's auto-immune defense:**

- The virus attacks the nerve cell.
- The nerve cell ingests the virus.
- The immune system fails to recognize it and so does not attack it. It is now an auto-immune disorder resulting in infection.
- Lymphocytes and plasmacytes respond.
- The lymphocytes produce anti-ganglioside antibodies which triggers a *cytokine* storm.
- The leakage of the proteins from the immune complexes initiates inflammation and symptoms.
- The inflammation brings about more lymphocytes, but these are also infected. This becomes a recurring cycle.

## GANGLIOSIDES AND ANTI-GANGLIOSIDE ANTIBODIES

According to Susan Orosz, "The lymphocytes go into the brain because of the leakage of the ganglioside proteins. This sets up an autoimmune reaction. It's not the virus that brings about the symptoms; it's the leakage of the proteins from these immune complexes that initiates them." [15] Therefore, if there are anti-ganglioside antibodies in the blood, the clinician is able to substantiate the diagnosis of Avian Bornaviral Ganglioneuritis (PDD) even before the individual exhibits clinical signs. They are regarded as the definitive indicators of the presence of ABG/PDD. [4, 6]

**For Further Reference on Gangliosides and Anti-Ganglioside Antibodies, See Appendix B, p. 28.**

## *CYTOKINES* AND CYTOKINE STORMS

**For Reference on Cytokines and Cytokine Storms, See Appendix C, p. 29.**

## TRANSMISSION

### Direct transmission via contact

Transmission and epidemiology of the Avian Bornavirus is not completely understood. Researchers know that the virus is shed in the urine and feces of infected birds; therefore, it is assumed that the oral/fecal route is important for horizontal (bird-to-bird) transmission, but other routes must be considered also.

In a 2009 study, Dr. Monika Rinder et al. detected ABV in numerous organs and tissues. Since the "viral nucleic acids were found in the fecal and cloacal swabs of infected birds," Rinder concluded that the virus is transmitted by the fecal, oral, and nasal routes. [6] Birds must come in close contact with the saliva and fecal matter to become infected.[2, 4]

It has been theorized, but not proven, that the virus can become aerosolized when the infected individual regurgitates or defecates and the matter dries. This theory states that even if this material is cleaned up immediately, infection can result if another individual eats food or breathes air near the site of the vomitus or droppings. [12]

ABV transmission from bird to bird is not always clearly understood. Although some clinicians consider the virus to be highly contagious at all times, others believe it is more likely to spread in homes and aviaries in which good hygiene is not practiced. This is a "chronic disease; asymptomatic, long-term survival does not mean that the bird has been cured. The bird will continue to carry and shed the virus even after symptoms cease, and the virus can be detected many weeks after infection." [2]

Since the affected bird is a potential source of transmission to other birds, some clinicians believe it is essential that the affected bird be isolated from others in the flock.[2, 21] Others believe that isolation is necessary in a shelter or breeding situation, but in a home, careful hygiene and distance are usually sufficient to prevent infection. For the most part, horizontal transmission is thought to "require long-term, close contact among birds."[4] While other RNA viruses are more stable in the environment, ABV "enzymes tend to degrade rapidly."[4] Cleaning with soap and dilute bleach is generally sufficient to "disinfect enclosures and any items that come in contact with the ABV-positive individual." [4]

**Vertical transmission, from the hen to the egg, is now considered to be the primary cause of ABV transmission.**

### Vertical transmission

Early studies had shown the difficulty in proving that the Avian Bornavirus is passed from hen to egg through vertical transmission. While this form of transmission was suspected, it was not proven until recently. It is now considered to the primary cause of Avian Bornaviral transmission in birds. Many of the earlier studies gave strong evidence for the passing of the virus through the egg; however, these studies were insufficient to support the theory with absolute certainty.

**For Reference on These Early Studies, See Appendix D, P. 30.**

Previously considered of little risk in avian collections, the Avian Bornavirus is now considered a major threat to both avicultural and companion birds. As many as 71% of some populations are testing positive for ABV; “the high incidence of asymptomatic or latent ABV infections makes managing both types of collections quite complex.” [2]

Some clinicians believe it is important to rule out other diseases before they screen for ABV infection. However, it is prudent to test for ABV at the same time rather than wait for other test results to come in; this way treatment can begin sooner. Screening allows the clinician to discover the ABV RNA, or serum antibodies, that fight infection by attacking foreign substances, even before the bird demonstrates clinical signs. [6]

In the past, screening presented a problem, even in verified ABV cases, due to conflicting test results. But today’s molecular diagnostic tests have removed that difficulty. Screening is vital for the “management of subclinical infections” and for breeding purposes.[1] Buyers need to have confidence that their new birds are healthy, and aviary owners need to know that any bird entering or leaving has not tested positive for ABV.[1]

**Figures 23, 24: Necropsy photos show the dramatically reduced pectoral mass in a macaw and another bird. Wasting occurs when the proventriculus dilates as the disease progresses. The proventricular wall thins due to the altered mixing pattern of food; the food is not digested or absorbed properly. This, combined with reduced absorption of nutrients, causes the bird to use the muscle mass for its energy source. (Courtesy R. Dahlhausen and S. Orosz)** 

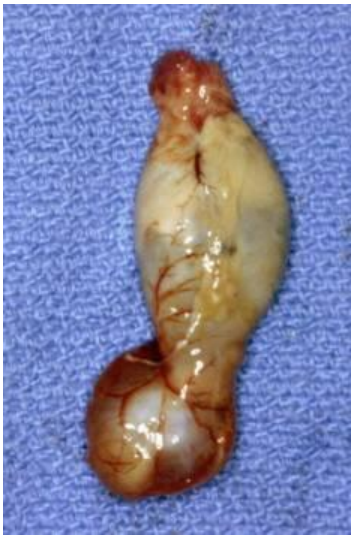

**Figure 25: The enlarged proventriculus is the top organ; the ventriculus is below.**


## DIAGNOSTICS

Until just a few years ago, a "presumptive ABV-ABG/PDD diagnosis was a systematic investigation based on serology tests, signs, cytological evidence, and radiography or CT-scan to view the dilated proventriculus." [2, 6] As a result, clinicians were often unsure of their diagnoses.

Many clinicians wish to explore the differential diagnoses for the ganglioneuritis symptoms the bird is experiencing before diagnosing it as Avian Bornaviral Disease. There are other diseases or conditions which can cause the same gastrointestinal signs as ABV/PDD. These are:

- "Tumors or papillomas of the crop, proventriculus, ventriculus, and intestines. The presence of internal papillomas may lead to a chronic wasting signs similar to ABG/PDD.
- Ingestion of foreign bodies
- Megabacteriosis
- Parasitism
- Inflammatory disease and neoplastic diseases, causing gastrointestinal stasis.
- Heavy metal poisoning."[4]

As long as the intestinal tract is distended, the proventriculus and ventriculus will not become fully emptied, and motility will be inhibited. Gastrointestinal stasis will result.[4]

Other conditions are responsible for CNS and GI signs similar to those seen in ABG/PDD. Some of these also cause distention of the organs in the digestive tract. [4] Other diseases that produce similar CNS signs in birds are:

- Traumatic injuries
- Heavy metal poisoning
- Neoplasia
- Viral, bacterial, and fungal infections of the CNS
- Nutritional deficiencies
- Hydrocephalus.[4]

ABV/PDD should be considered as a possible differential in any bird with CNS and GI signs. Once a presumptive diagnosis is made based on history, clinical signs and radiographs of the digestive tract, it may be confirmed by the discovery of histopathologic lesions in the bird's tissues and molecular diagnostic testing.

## TESTING

Practitioners rely on various tests to give them a conclusive diagnosis of Avian Bornaviral Disease. Serology tests, radiology, and biopsies were (and still are) the standard diagnostic tests available.

Crop biopsies are useful in locating viral infiltrates in the neural tissues, and radiographs will show the distension of the proventriculus, ventriculus and other GI organs.[6] However, one drawback to both radiography and crop biopsy is that they must be performed when the bird is displaying advanced clinical symptoms. In addition, crop biopsy is invasive and can be dangerous; it often results in false negatives and false positives due to inconsistent data. [6]

### Standard Testing Protocols

- **Radiographs and CT-Scans**: "Contrast radiography is used to diagnose the dilatation of the proventriculus and the duodenum descendens" and to determine "extended transit times of ingestion. It may also show spontaneous ruptures of the dilated proventriculus, with ingesta filling the caudal air sac group." [20]
- **Crop Biopsy**: A section of crop and a prominent blood vessel are removed and examined. If the sample contains pathological lesions consistent with ABV, the test results will be positive for ABV, even if the bird is asymptomatic. The presence of lymphoplasmacytic ganglioneuritis in crop tissue is required for a positive result. [1]
- **ELISA** (Enzyme-linked Immunosorbent Assay): This is a serology test which looks for immunological exposure to specific ABV antigens. ELISA is a primary binding test used to detect either antigens (proteins) or antibodies in the blood. After blood is collected from the *brachial vein* under anesthesia, pathologists search for the presence of characteristic lymphoplasmacytic ganglioneuritis found in ABV. [1]
- **Molecular Diagnostics:** These are genetic DNA tests which will detect shedding of the Avian Bornavirus.[1] Direct rt-PCR detects the presence of ABV-specific RNA. [1]
- **Anti-ganglioside antibody test**. This is a non-invasive serology test which detects the presence and increase of anti-ganglioside antibodies in the blood of parrots. [21] It is the most accurate test of all (Dahlhausen, personal communication).
- **The Western Blot assay** is capable of identifying the N-Protein which is "associated with ABV infection in the brains of affected birds. [2] This assay has detected antibodies against the Avian Bornavirus. [6] However, "recommendations regarding the application of this assay to the ante-mortem diagnosis of ABV in pet birds have yet to be determined." [2]

## PREVIOUS TESTING METHODS

Before the introduction of PCR testing, researchers had struggled with inconsistent, unreliable testing methods and results. These tests continue to be used by some clinicians, thus making diagnosis difficult. The following study demonstrates the difficulty of using these tests.

### A Study

Studies have demonstrated that the outcomes of the standard methods of testing can be ambiguous. In 2009, Dr. Susan Clubb and Dr. Siwo de Kloet compared the results of the three standard tests used to confirm the presence of ABV in birds. They used four aviaries with a combined number of 137

birds for the testing, and each of the birds had these three tests performed: Crop biopsy, ELISA, and rt-PCR.[1]

The findings were:
- 2% tested positive in all three tests
- .7% tested positive on crop biopsy and PCR
- 12.4% tested positive only on crop biopsy
- 2.8% tested positive on ELISA and PCR
- 5% tested positive only on ELISA
- 4.3 tested positive only on PCR[1]

These results were quite unexpected. The three tests did not correlate well, further complicating the diagnostic process. [1] The researchers proffered the following possible explanations:

- It is possible that another agent might be causing lymphoplasmacytic ganglioneuritis.[1]
- Tissue samples might not be "directly tested by PCR or *immunohistochemistry* for the presence of ABV."[1]
- "ABV has been found in clinically healthy birds." [1]
- "RT-PCR assays for ABV from cloacal swabs are often less sensitive due to the intermittent shedding of ABV or poor handling procedures."[1] They would therefore be less reliable. [1]
- "The relationship between lymphoplasmacytic ganglioneuritis found on crop biopsy and the classic clinical form of ABV-ABGPDD" has not been sufficiently investigated."[1]
- There are multiple ABV antigens, depending on the type of protein involved, and if the bird isn't tested for the specific antigen affecting it, a false negative could result. [1]
- The bird may not be shedding the virus at the time of testing—or not at all—because the intestinal tract has not been affected, or the infection might be transient. The result is an inflammatory process that has become latent, as in an autoimmune disease.[1]
- "Some assays may not be able to detect all ABV genotypes since only about 68 to 85% sequence identity exists among the known ABV genotypes."[4]

## MOLECULAR DIAGNOSTICS

Although the clinician may tentatively diagnose Avian Bornaviral disease based on traditional methods, he can verify the presence of the disease by locating the characteristic signs of ganglioneuritis in the crop biopsy. However, false-negative crop biopsies occur at least one-fourth of the time due to the "variable distribution of lesions in birds."[2] Biopsies must include infected ganglia; if these aren't present, the test will come back negative. The bird may still be positive for ABV even he is not displaying signs of nerve damage and the tests results come back negative.[2] With the standard tests, only necropsy findings are definitive, providing proof of nerve destruction in the proventriculus, ventriculus and brain. [2]

The field of Molecular Diagnostic Veterinary Pathology specializes in the diagnosis of diseases through examination of molecules within organs, tissues or body fluids. Due to its sensitivity and specificity in detecting nucleic acid targets, PCR is one of the most important tools in molecular diagnostics.[2]

Identification of the ABV genome has allowed researchers to use PCR genetic analysis in order to identify the virus in tissue samples. This allows practitioners to "detect a broad range of ABV

genotypic variants which can be used for a wide spectrum of clinical samples and necropsy materials."[6]

A positive ABV test is one that traditionally has been based on finding histological lesions in biopsy or necropsy tissues. Recently, however, the use of Reverse-Transcription Polymerase Chain Reaction (RT-PCR) has allowed clinicians to detect ABV ribonucleic acid (RNA) in affected birds. "Both gel-based RT-PCR and real-time RT-PCR have been successfully used to detect ABV RNA; however, real-time PCR assays appear to be the more sensitive of the two techniques."[4]

The standard tests continue to be performed, but they are now ancillary to the more sophisticated genetic testing. (Dahlhausen, personal communication) Today, identification of ganglioneuritis in the lymphocytes and plasma cells (*lymphoplasmacytic ganglioneuritis*) provides a definitive diagnosis of ABV." [2, 4]

## DRUG TESTING

In 2010, Gray et al. conducted a trial to evaluate two anti-viral drugs on the market—amantadine and ribavirin—for use in the treatment of Avian Bornaviral Ganglioneuritis (PDD). She tested three, in-vivo Congo African Grey parrots *(Psittacus erithacus erithacus)* naturally infected with ABV. The birds were given amantadine PO q24h for eight weeks. Throughout the duration of the study, fecal samples were PCR-tested weekly for the presence of ABV; all the tests came back positive. It was evident that the birds had been shedding the ABV in their feces for several months. Seven months after the amantadine trial, they begin a new trial, this time increasing the doses of ribavirin over an 8-week period. The feces were tested bi-weekly and all results were positive throughout the trial period.[9]

In a 2012 controlled study by Sharman Hoppes, meloxicam and cyclosporine (an immunosuppressant) were tested on twelve cockatiels. They were infected with the" known pathogenic isolate, genotype ABV-4."[10] Four birds received meloxicam, four others were given cyclosporine, and the last four were the control group. Both treatment groups were given the medications PO q12h beginning on the 20$^{th}$ day post infection.[10]

The four birds treated with meloxicam displayed clinical signs of ABG/PDD. One died on day 61, and the other three were euthanized on days 78, 95, and 117. Histopathological results yielded evidence of ABG/PDD lesions. The four treated with cyclosporine revealed "subtle and unusual lesions" of ABG/PDD." [10] These were less severe than the ones found in the meloxicam-treated birds. The control birds and the cyclosporine birds "maintained their weight and appeared clinically normal throughout the study. In post-mortem findings, two control birds showed no histopathological ABV lesions, and two had subtle lesions." [10] All twelve birds tested PCR-positive for ABV-4 in several organs. **The researchers determined that neither of the two drug treatments prevented infection with the Avian Bornavirus. In addition, the birds treated with meloxicam actually displayed more intense symptoms and had "more severe lesions" than the control birds.** [10]

***It is important to note that, while some drugs, including meloxicam, continue to be dispensed to PDD-affected birds, only one, Celebrex, has shown continual, long-term relief from both gastrointestinal and Central Nervous System signs.***

## CURRENT TREATMENT PROTOCOLS

Once the cytokine storm begins, the infection cannot be stopped, and clinicians are unable to treat it, so they attempt to halt the action of the gangliosides in order to calm the inflammation. Cox-2 inhibitors (*celecoxib*) interrupt the cytokine production and reduce the ganglioside sensitivity. These drugs reduce inflammation and thus diminish the symptoms of ABG/PDD; however, they proved to be "less effective in maintaining this state of remission of the disease" over long periods of time. [21] Some birds respond to these medications and some do not, depending on the individual's immune system and its auto-immune reaction.[15]

## RECOMMENDED FORMULARY

- Celebrex: Cox-2 inhibitor for GI tract signs, pain, inflammation, and peripheral neuritis
- Robenacoxib: Cox-2 inhibitor, injectable form of celecoxib, for pain and inflammation
- Metoclopramide: GI *prokinetic* agent, for nausea and vomiting, easing digestive discomfort
- Gabapentin: for self-mutilation, seizures, neurological/neurogenic pain
- Cisipride: GI prokinetic agent for improving transit in birds with GI tract involvement, particularly early in the course of therapy.
- Leuprolide acetate and Deslorelin implants: for managing hormonal increases which occur with the onset of breeding activity.

***(Specific dosages may be obtained by contacting either R. Dahlhausen or S. Orosz.)***

## SUPPLEMENTS

Many supplements have been shown to aid in the improvement of the quality of life of affected birds:

- ✓ Antibacterial and antifungal therapy is advised to "control overgrowth of intestinal anerobes, yeasts, and *Macrorhabdus*.[4] GI tract disease "alters the intestinal microbiome," so probiotics and prebiotics may aid in restoring normal the intestinal environment.[4]
- ✓ Prokinetics are more effective early in the course of therapy.
- ✓ Omega fatty acids are helpful in reducing inflammation
- ✓ Semi-elemental diets, such as ***Lafeber's*** Emeraid Omnivore and Carnivore Critical Care Nutrition, "require minimal digestion and provide a readily absorbable source of essential nutrients and Omega fatty acids."[4]
- ✓ Herbal supplements like Silymarin (Milk Thistle), Gaia herbs, and ginger are "helpful in reducing inflammation, preserving hepatic function, and improving GI tract transit."[4] The liver is often affected by the bacteria coming from the abnormal intestinal environment."

Although some dietary supplements have been used to treat Avian Bornaviral Ganglioneuritis (PDD), none has shown consistent, positive results. (Dahlhausen, personal communication) The addition of the supplements has proven useful in alleviating some of the symptoms, but their benefits decrease over time.

## GUILDELINES FOR CURRENT DIAGNOSIS OF AVIAN BORNAVIRAL DISEASE

Recently, G. Dorrenstein has advanced the following recommendations. The avian practitioner is encouraged to consult these guidelines when diagnosing ABV/ABG/PDD:

- A bird is considered negative for ABV if he is healthy, has had repeated negative blood tests, and has had no contact with any other ABV-positive birds.
- A bird is considered positive for ABV if he is ill with clinical symptoms of Avian Bornaviral Ganglioneuritis (PDD), has had blood tests which are positive for antibodies against ABV, and the results can be confirmed by PCR.
- A bird that is ill, and is suspected of having ABV, is considered free of ABV if the serology is negative. He has probably not contracted the disease, and the PCR should validate that.
- A bird that is clinically healthy, but serologically positive, is considered a carrier of ABV. If repeated PCR testing results are negative, he may be considered "clean." [6]

"AVIAN BORNAVIRAL DISEASE SHOULD BE CONSIDERED AS ONE OF THE DIFFERENTIAL DIAGNOSES IN ANY BIRD DISPLAYING GASTROINTESTINAL OR CENTRAL NERVOUS SYSTEM SIGNS."

R. Dahlhausen

## CONTINUING RESEARCH

**For Reference Concerning Continuing Research, See Appendix E, p. 31**

## RECOMMENDATIONS TO CLINICIANS

- Prolonged therapy is advised to prevent future attacks.[4]
- Wait before euthanizing a bird in order to give treatment protocols a chance to work.
- Be careful when choosing the lab for ABV PCR testing and test interpretation due to the inability of some tests to detect all genotypes.[2] Also, some labs test at temperatures for dogs and cats and results may be incorrect because of that since birds' temperatures are higher. (Dahlhausen, personal communication)
- Screen collections to aid in preventing the spread of ABV in existing flocks and averting future outbreaks of the disease. [6]
- Repeat testing in affected flocks. Testing of apparently negative birds is strongly suggested for the highest reliability in diagnosis of ABV infection.[22]

## PREVENTION

Long-term survival does not mean that the bird has been cured. It still carries the virus and is a potential source of transmission to other birds. [2] Although the virus only lives eight hours outside the host, one cannot be too careful. (Dahlhausen, personal communication) To minimize the chances of ABV-ABG/PDD spreading within the home or aviary, clinicians should advice their clients to observe the following recommendations:

- Avoid overcrowding.
- Pay attention to hygiene. This is particularly important for owners who keep multiple birds. Scrupulous, constant attention must be paid to cleanliness.
- Immediately remove feces and dust and disinfect all inanimate objects with which the bird has come in contact. This is vital in the prevention of the spread of the disease. [22]

- Clean inanimate surfaces, such as toys and food/water dishes, frequently, to avert transmission through fomites.
- Take proper precautions to clean any exposed skin (particularly the hands and arms) and change clothing after handling the affected bird.
- Keep the aviary or home adequately ventilated.
- Provide superior nutrition and clean water.
- Avoid stressful conditions, particularly in aviary collections and in shelters.
- Do not smoke around the bird. Second-hand smoke and the use of other tobacco products inhibit the immune system. Smokers should not handle the bird unless they have cleansed their arms and hands thoroughly and changed clothing; the toxins on the skin can cause pododermatitis and lesions on the legs and skin.
- Isolate or remove any ABG/PDD-exposed, ABV-positive birds from the rest of the population in high-density situations, and separate them in home environments.[2]
- Do not wait to euthanize until the bird is nearly dead. The proventriculus can rupture, causing a quick but extremely painful death.

## CONCLUSION

Although clinicians have been aware of Avian Bornavirus for decades, and ABV has been proven to be one of the viral pathogens responsible for Avian Ganglioneuritis (PDD), many people, including some practitioners, have very little knowledge about the deadly consequences of ABV infection. This is a multi-faceted and devastating viral disease. Informed breeders and bird owners are capable of curbing the spread of this disease by closely monitoring their flocks and preventing or discontinuing the reproduction of ABV-positive birds. Hopefully, bird-keepers have gained sufficient awareness about the dangers of ABV and ABG/PDD to be vigilant regarding the signs and to have their birds tested before the disease progresses to a critical stage. And by offering their birds a quality diet, providing correct medications, and decreasing stress levels, they will afford the ABV-positive bird extended longevity and considerably enhanced quality of life.

## GLOSSARY

**Allodynia:** pain felt from sensations that are not ordinarily painful
**Antibody:** a protein that fights infection; this protein is produced by B-cells in the body in response to the presence of an antigen, e.g. a bacterium or virus. Antibodies are a primary form of immune response in resistance to disease and act by attaching themselves to a foreign antigen and weakening or destroying it.
**Antigenemia:** the presence of an antigen in the circulating blood.
**Antigen:** a substance which enters or is on the surface of the body and evokes the production of one or more antibodies. The body recognizes it as foreign and forms antibodies in the blood; this leads to an immune response.
**Apoptosis**: programmed, natural cell death
**Ataxia:** lack of muscle coordination, abnormal gait, and proprioceptive defects.
**Auto-immune reaction:** An autoimmune disorder is a condition that occurs when the immune system mistakenly attacks and destroys healthy body tissue. The result is an immune response that destroys normal body tissues.
**Brachial vein:** This is the large ulnar vein in the wing.
**Celiac ganglia:** nerve tissue in the upper abdomen

**Choana*:*** the area at the of the bird's mouth which has a slit or opening leading to the nasopharynx and sinuses
**Chorioretinitis**: the inflammation of the retina and choroid, which is the thin pigmented vascular coat of the eye.
**Clinical**: showing signs of the disease
**Cloaca:** the common cavity above the vent into which the intestinal, urinary, and reproductive canals open in birds
**Cytokines**: key communicators for immune cells in many chronic diseases. They are any of a group of small, short-lived proteins that are released by one cell to regulate the function of another cell, thereby serving as intercellular chemical messengers. They play a role in the immune system's defense against disease-causing organisms. Cytokines effect changes in cellular behavior which are important in injury repair.
**Cytolysis:** cell death resulting from a rupture in the cell's membrane.
**Effector:** a bodily organ, muscle or gland, which becomes active or that contracts or secretes in direct response to nerve impulses. It can also be a cell in the immune system that *mediates* an immune function.
**Encephalitis:** Encephalitis is irritation and swelling (inflammation) of the brain, most often due to infections.
**Encephalomyelitis:** inflammation of the brain and spinal cord
**Enveloped virus**: A virus that has an outer wrapping or envelope. This envelope comes from the infected cell, or host, in a process called "budding off." During the budding process, newly formed virus particles become "enveloped" or wrapped in an outer coat that is made from a small piece of the cell's plasma membrane. The envelope may play a role in helping a virus survive and infect other cells. Enveloped viruses can cause persistent infections.
**Epithelial:** Epithelial tissues line the cavities and surfaces of structures throughout the body and also form many glands.
**Etiology**: the study of causation or origination
**Extraneural tissues:** nerve tissues and the tissues around them
**Fundus:** the bottom or base of an organ, or the part of a hollow organ farthest from its mouth
**Ganglion:** a cluster or group of nerve cell bodies located outside the central nervous system
**Ganglioneuritis:** infection or inflammation of a nerve or nerve ganglion.
**Gangliosides**: Gangliosides are fatty lipid substances found within the brain and nerve cells and red blood cells and are abundant in nerve tissue. Gangliosides are involved in a variety of functions, including serving as antigens, receptors for bacterial toxins, mediators of cell adhesion, and mediators and modulators of signal transduction. Gangliosides mediate communications between cells and are sensitive to hormones which control cell behavior.
**Genome:** the entirety of an organism's hereditary information.
**Genotype, or genogroup*,*** refers to the genetic constitution of an individual. It can refer to a group or class of organisms having the same genetic constitution. It also describes a sub-group of a virus.
**Histopathology:** The study of the microscopic, anatomical changes in diseased tissue.
**Homogenate: a** substance that has been made uniform in composition
**Homology:** a structural similarity based on a common origin.
**Hyperalgesia:** abnormal heightened sensitivity to pain
**Hyperesthesia:** abnormal sensitivity to sensory stimuli.
**Immune-mediated disease**: Immune-mediated diseases are conditions which result from abnormal activity of the body's immune system; the immune system attacks the body.
**Immunohistochemistry:** IHC is the process of detecting antigens (proteins) in tissue cells.
**Inappetence:** lack of appetite
**Innervation**: the distribution or supply of nerves to a body part

**Lymphocytic ganglioneuritis**: inflammation of nerves caused by invasion of ganglions by infected lymphocytes.
**Lymphocytic, plasmacytic ganglioneuritis or lymphoplasmacytic ganglioneuritis:** Lymphocytes and plasmacytes are types of white blood cells that secrete antibodies and are involved with the immune system. Ganglioneuritis refers to an inflammation of a cluster (ganglion) of nerves.
**Lymphoid or lymphocytic infiltrations or lymphoplasmacytic infiltrations**: Any foreign body entering the lymph system.
**Macrophage:** a type of white blood cell that ingests/destroys foreign material.
**Macrorhabdus**: Originally thought to be a bacterium, the organism has recently been identified as a comycetous yeast and has recently been renamed *Macrorhabdus ornithogaster*. Although the host range and incidence of megabacteriosis are increasing, knowledge of the disease has been inhibited by the difficulty in growing the organism for subsequent study. Megabacteriosis is an important fungal disease of certain avian species, especially budgerigars, cockatiels, and finches.
**Mediate**: to mediate is to indirectly cause and action or change, as in stimulation by a hormone, to serve as a medium for causing a result; to effect or convey as an intermediate agent or mechanism.
**Myenteric plexus:** a group of nerve fibers inside the muscle tissue of the esophagus, stomach and intestines.
**Myocytes**: the cells responsible for generating the electrical impulses that control the heart rate.
**Natural reservoir host:** a species which harbors the pathogen but shows no ill effects and serves as a source of infection.
**Neurotropic**: having and affinity for nerve cells or tissues; capable of infecting nerve cells selectively, thus evading the immune system
**Non-cytopathogenicity:** the virus does not kill or take over the cell
**Non-suppurative:** non-pus-forming
**Pathogenesis**: production and development of a disease
**Parenteral:** administration of a medication in a way other than by mouth
**Paresis:** weakness and partial loss of voluntary movement
**Perivascular cuffing**: lymphocytes or plasma cells accumulate in a dense mass around the brain; this is an indication of inflammation or of an immune reaction.
**Phospholipid molecules**: also called Phosphatide; any member of a large class of fat-like, phosphorus-containing substances that play important structural and metabolic roles in living cells.
**Phosphoprotein:** Any of a group of proteins, such as casein, containing chemically bound phosphoric acid.
**Plexus (plural: plexi):** A plexus is a branching network of axons outside of the central nervous system.
**Pneumogastric nerve:** Cranial Nerve X, controlling the heart, lungs, digestive tract and other organs
**Polyphagia:** excessive eating or hunger
**Polyuria:** excessive urination
**Prokinetic:** a drug which increases motility by causing contractions of the GI muscles; it also aids in preventing regurgitation
**Proprioception**: awareness of the body's position and movement
**Proprioceptive defects:** difficulty of the bird in knowing where he is in space around him or knowing where his body parts are.
**Proprioceptor**: a sensory receptor, found chiefly in muscles, tendons, joints, and the inner ear, that detects the motion or position of the body by responding to stimuli arising within the organism.
**Proventriculus**: The first part of the avian stomach, the gastric stomach
**Ribonucleoproteins (RNP):** substances composed of both protein and ribonucleic acid, or RNA.20; RNA is in a family of large biological molecules that perform multiple vital roles in the coding, decoding, regulation, and expression of genes

**Seroconversion:** the development of detectable antibodies in the blood that are directed against an infectious agent or antigen, such as a virus or vaccine; a change in serologic test results from negative to positive as antibodies develop in reaction to an infection or vaccine. This gives the individual protection against the disease in the future.
**Serology**: the scientific study of blood serum and other bodily fluids. In practice, the term usually refers to the diagnostic identification of antibodies in the serum
**Sinoatrial node**: The sinoatrial node is the normal natural pacemaker of the heart and is responsible for the initiation of the heartbeat. It spontaneously generates an electrical impulse, which after conducting throughout the heart, causes the heart to contract.
**Transduction:** the transfer of genetic material from one microorganism to another by a viral agent (as a bacteriophage)
**Ventriculus**: second part of the avian stomach, the grinding stomach.
**Viremia:** The presence of viruses in the blood
**Viral matrix proteins:** structural proteins linking the viral envelope with the virus core.

## APPENDIX A: STUDIES PERTAINING TO THE ETIOLOGY AND PATHOGENESIS OF ABV/PDD

With the use of qPCR and randomly amplified RNA extracted from the brains of seven birds (three of which were confirmed with BDV, Honkavuori's group verified the presence of BDV in tissues of the brain, proventriculus, plasma, crop, ventriculus, duodenum, liver, lung, kidney, spleen, retina, cerebrum, cerebellum and adrenal glands of the three birds with ABG/PDD but not in the tissues of the four not suffering from ABG**/**PDD. [2] He found the highest concentrations of the virus in the eye, brain, spinal cord, and crop. Lower levels were present in the proventriculus and intestine, and the lowest viral concentrations were discovered in the pancreas and adrenal gland. [2] Using ABV-specific PCR analysis, Kistler et al. found that ABV detection rates among ABG/PDD cases were as much as 71% compared to the control group, which had 0%. [2] This is a high statistic; most population studies reveal a much lower rate of infectivity (usually around 40%) in birds which appeared healthy and normal. [2]

In 2009, Drs. Sharman Hoppes and Patricia Gray from Texas A&M were able to successfully culture ABV from the brain tissue of seven birds with confirmed ABG/PDD, but when culturing the brain tissues of non-infected birds, they found no virus. [6] This, too, substantiated the ABV- ABG/PDD link.

Another researcher, Dr. Ady Gancz et al., used tissue from ABV-positive birds to inoculate three healthy cockatiels *(Nymphicus hollandicus)*. "He achieved experimental transmission of ABV by using simultaneous inoculation of brain *homogenate* from a confirmed ABV *genotype*, ABV-4, through the *parenteral* and mucosal routes."[20, 6] All three developed "gross and microscopic ABGPDD lesions, and two exhibited overt clinical signs." [6] The third showed no symptoms; however, at necropsy, all three birds "showed the characteristic lymphoplasmacytic infiltrates in the myenteric ganglia and variable degrees of lesions in the brain and spinal cord."[20] In addition, ABV-4 RNA was found in many tissues. *Immunohistochemistry* (IHC) revealed Central Nervous System (CNS) effects in all three inoculees. [6]

More proof of the ABV-ABG**/**PDD connection was provided by Herbert Weissenbock et al. in an eleven-year study using RT-PCR and IHC. He examined tissue samples from 31 psittacine birds from Eastern Europe. These samples, collected from 1999 to 2008, tested positive for Avian Bornavirus, having viral antigens in their neural and extraneural tissues.[23] Analysis resulted in the following data:

- ✓ The presence of viral antigens within the brain and vegetative nerve *plexus* of the GI tract and "tissue lesions consistent with ABG/PDD (found with the IHC test) provided strong evidence for a causative role of the Avian Bornavirus in the development of (ABG/PDD)."[23]
- ✓ "Partial sequences of nucleoprotein (N-protein) and *viral matrix* protein genes in brain tissues of ABV cases…showed that the virus in most of the cases belonged to the ABV-2 and ABV-4 groups among the 5 genogroups" described up to that time. [23]

## APPENDIX B: GANGLIOSIDES AND ANTI-GANGLIOSIDE ANTIBODIES

Gangliosides are complex compounds naturally produced in the body, present on cell surfaces, and found mainly in the nervous system." [21] They are the mediators between the antibodies and *cytokines*; they "mediate nerve fiber injury and induce the lymphoid infiltration." [6] These *phospholipid molecules* are found in the brain and red blood cells and are abundant in nerve tissue. They serve as antigens, receptors for bacterial toxins, mediators of cell adhesion, communicators between cells, and modulators of signal *transduction*. These molecules "are the most commonly recognized autoimmune markers." [6]

### Studies by Rossi and Pesaro

In 2008, in an effort to confirm their hypothesis of a connection between ABG/PDD and the auto-immune system, researcher Dr. Giacomo Rossi et al., at the University of Camerino, Italy, "proposed a possible auto-immune mechanism for the pathogenesis of ABG/PDD." [2] Using purified gangliosides from the peripheral nervous systems of parrots, he inoculated six healthy cockatiels. After six weeks, 100% of the inoculated cockatiels and 33% of those which had received oral doses exhibited the classic clinical signs most often seen in cases of ABG/PDD. [21] Four of the symptomatic, inoculated birds also exhibited lesions most often seen in crop biopsies, and their tissues tested positive for anti-ganglioside antibodies. [2] Crop biopsies showed the that the purified gangliosides brought about the same distinguishing ganglioneuritis as was present in samples from naturally infected ABG/PDD subjects.[2]

This suggested that even naïve birds had developed ganglioneuritis, even though there was no virus involved; their immune systems attacked the newly introduced gangliosides despite lack of ABV infection. Rossi and Pesaro determined that the cockatiels could show the same symptoms of ABG/PDD if injected only with proteins from the birds' own nerves, not the bornavirus.[15] They confirmed that this syndrome is an auto-immune response to the gangliosides, thereby discovering the mechanism by which ABV infiltrates the body.[2,15]

ABG/PDD is triggered by an infection which leads to the production of specific antibodies. These antibodies bind to gangliosides, and this leads to immune-mediated damage to peripheral nerves; this damage results in clinical symptoms. [21]

The pathological name for ABG/PDD is "psittacine *encephalomyelitis* with infiltrative splanchnic neuropathy."[6] This refers to the *lymphocytic infiltrations* that have been detected in the "peripheral nerves and *perivascular cuffing* in the brain."[6] Lymphocytes and/or plasma cells accumulate in a dense mass around the brain, indicating inflammation and immune reaction. Rossi et al. found antibodies to gangliosides present in the sera of psittacine patients which were clinically ill with ABG/PDD. If only antibodies are found, this might mean that the disease is in a dormant state or that the bird has had a previous infection. [2]

## APPENDIX C: CYTOKINES AND CYTOKINE STORMS

*Cytokines* are small, short-lived proteins that are released by one cell to regulate the function of another cell; thus, they serve as intercellular chemical messengers for immune cells in many chronic diseases. As part of the immune system's defense against disease-causing organisms, cytokines effect the changes in cellular behavior required for injury repair. When the immune system encounters an immune stimulus, it produces cytokines. Once induced, they determine how the immune system should respond and to what degree. They may be pro-inflammatory compounds, anti-inflammatory compounds, or growth factors. Capable of inducing the production of *effector* molecules for various cell receptors, they sometimes cause the proliferation of other types of immune-system cells that are needed to prolong the immune response.[4]

Cytokines contribute to the symptoms of autoimmune disorders by releasing toxic chemicals which damage cells. Depending on their type and function, they can also trigger and perpetuate inflammation. On the other hand, they can lessen the immune system's response, thus reducing the duration and intensity of flare-ups of a disease. As part of the immune response, cytokines influence leukocytes and are synthesized in response to another cytokine. Once secreted, the cytokine binds to a specific protein molecule (known as a receptor) on the surface of the target cell—an event that triggers a "signaling cascade" inside that cell. The signal reaches the nucleus; there, protein production may be stimulated or inhibited. [4]

ABV causes ABG/PDD by creating a "cytokine storm", resulting in an infiltration of infected lymphocytes into the proventriculus, ventriculus, and parts of the small intestine. The resulting damage leads to maldigestion. It may even cause the severely dilated, thin wall of the proventriculus to rupture; this results in undigested food spilling into the abdominal cavity, causing severe infection and immediate death.[4]

The cytokine proteins are intended to heal the damage; instead, they bring about leakage of the ganglioside proteins into the nerves that have been damaged by the virus, causing nerve-fiber injury. This leads to "lymphoid infiltration into the brain or proventriculus and other intestinal organs, thus preventing proper digestion to occur and causing thinning of the wall of the GI tract."[15]

Cytokines can act on selected cells, or they can to react with various cell types. They can function together or synergistically, or they can operate antagonistically, working to reduce the activity of other cytokines. A cytokine storm, or hypercytokinemia, is a potentially fatal immune reaction consisting of a positive feedback loop between cytokines and immune cells. In this loop, the cytokine levels are highly elevated. [15]

The purpose of the lymphocytes is to destroy the invading virus, but because they are infected, they are instead producing the inflammation-causing antibodies. The neighboring cells are now becoming inflamed. This then becomes the cytokine storm: a massive inflammation of the nerve cells (T.J. Miesle, personal communication).

### VIRAL INVASION

- Ingestion: The body ingests the Avian Bornavirus, and the virus enters via the nerve cell without destroying it; therefore, the immune system doesn't recognize it immediately. ABV then infects more nerve cells.
- Infection: After the virus infects the lymphocytes and plasma cells, it takes over the immune system, leading to an auto-immune disorder.

- The ABV-infected lymphocytes and leukocytes produce antibody secretions which attack the infection.
- The antibodies bind to the gangliocytes; this triggers ganglioside activity in the nerve cell membrane, irritating the nerves and causing nerve damage.
- Gangliocytes trigger a cytokine release. The cytokines sense these antibodies and tell the cell to produce more cytokines to fight the inflammation.
- The cytokine release starts the inflammation by drawing a response from the lymphocytes. The defenders, the lymphocytes, come as they are called, but they are already infected and bring more infected lymphocytes to the cell.
- These produce more inflammation, which creates more antibodies, and so the cycle repeats and the inflammation spreads.

*The body is now engaged in discrete series of events from which it cannot escape. This continuous loop repeats itself until the bird succumbs to the disease.* (T.J. Miesle)

## APPENDIX D: VERTICAL TRANSMISSION

Michael Lierz et al., in a 2009 study, investigated the distribution of ABV in tissues of ABV-positive birds; the study "demonstrated ABV antigen in follicular cells, which may point to vertical transmission." [13] He examined thirty, dead-in-shell embryos which came from a "variety of psittacine species brought in from ABV-infected flocks with a history of Avian Bornaviral Ganglioneuritis (PDD)."[13] He analyzed the brain and proventriculus of each embryo for the presence of ABV RNA by using two different real-time, reverse transcription PCR's.[13] "Histopathological examinations and *immunohistochemistry* (IHC) testing of the complete embryo were carried out with antibodies directed against the viral *phosphoprotein* and X-proteins." [13]

In two of the thirty embryos examined, "ABV RNA was identified with 1034 PCR testing. None of the embryos exhibited histopathological lesions most often seen with an ABV infection. IHC did not reveal any positive results, except for in an ABV-positive Amazon embryo, and that result was ambiguous. [13] Three sets of parents of these embryos were also tested using swab specimens, but the results were inconsistent. When only two of the embryos tested positive, and the parents' results showed positive for some tests and negative for others, Lierz attributed the questionable results to the following possibilities:

- False-negative results might have been caused by increased incubation time by the breeder to ensure embryonic death; thus, the ABV RNA might have been degraded in some cases.
- The eggs may have originated from ABV-negative parents.
- The poor quality of the samples might have hidden the lesions.
- More than 30% of the birds could be infected and the virus shed only intermittently in ABG/PDD-affected, ABV-positive flocks." [13]

As a result of this experiment, Lierz came to the following conclusions:

- ABV-infected parents can most likely produce infected offspring.
- Embryonic infection that does not result in embryonic death is a basic requirement for successful vertical transmission. [13]

Therefore, at that time, he concluded that vertical transmission was not proven.[13]

Drs. Erin Monaco and Ian Tizard tested “66 eggs from various aviaries in which ABG/PDD was established and the presence of ABV had been verified by fecal PCR.” [14] Researchers tested egg contents for “the presence of ABV by real-time PCR assay, employing primer sets designed to detect the ABV-M protein.”[14]

Egg status ranged from eggs that were seemingly infertile to those in which there was some embryonic development. [14] Of the eggs tested, thirteen contained evidence of ABV. The virus was also found in the brain tissue of three embryos. At that time, he concluded that these tests did not provide absolute proof of vertical transmission, but they did suggest a strong possibility. [14]

A similar study conducted by J. Smith involved an outbreak of ABV-ABG/PDD in a nursery in which neonate cockatoos, African greys, and macaws were being hand-fed. Although yeast and gram-negative bacterial GI infections may be mistaken for ABV infections since they display the same clinical signs, they usually clear up within ten days with the proper medications. When Smith observed no improvement in the neonates, she tested them for ABV, suspecting immunosuppression or another underlying primary disease. Some died naturally during this time, and others were euthanized and sampled for ABV using PCR testing. The first four birds that were tested came from the same clutch or brooder, again suggesting vertical transmission. [19]

In later tests found eggs from infected hens to test ABV-positive by PCR assays. Researchers have confirmed that vertical transmission occurs; however, exactly when transmission occurs during egg development is unknown. [4]

## APPENDIX E: CONTINUING RESEARCH

- The identification of *natural reservoir hosts* of these viruses is crucial. Host species harbor the pathogen and serve as a source of infection while remaining asymptomatic. Some species other than psittacines have been identified as natural hosts, but others may exist. Infected, yet asymptomatic, birds are serving as virus reservoirs. [23]

- Researchers use RT-PCR in their search for viral nucleic acids, but these primer sets most probably cannot “detect all circulating ABV strains, since unknown ABV strains with divergent genomes may exist.” [20] Whether the PCR assay is sensitive enough to detect ABV infections prior to the onset of clinical signs is not known. Use of serological assays may provide better results. Therefore, more sensitive and specific assays are needed. [20]

- Epidemiological data shows that researchers in other countries are finding ABV in their psittacine population. However, most are unaware of the “true extent of the virus distribution” and the medical issues associated with it.[20] Since non-psittacine species are becoming infected, it is possible that ABV, not BDV, is responsible for the infections in these other birds and animals. PCR primers are needed that are able to “clearly distinguish between genetic material from ABV and that of BDV.” [20]

- It has not been proven that Avian Bornaviral Ganglioneuritis (PDD) can be induced by simply using the pure virus, not just the brain tissue or cell culture containing the virus. Other viral agents may be present in the biopsied tissues. [6]

- Researchers have attempted to develop a protective vaccine against ABV. However, because it "reproduces in a non-cytopathic manner in the host nucleus and persists due to mechanisms that evade the host immune system, ABV infections are considered chronic and life-long. Seroconversion is not possible with this virus. It is therefore unlikely that anti-viral or vaccine therapy will effectively eliminate the viral-infected state." [23]

- Research is needed into the development of a "method of accurately measuring systemic inflammatory factors," including cytokine involvement. "These serological assays could aid in better understanding and identifying inflammatory factors that stimulate an acute phase reaction." [1]

***The author is indebted to Robert Dahlhausen D.V.M., M.S. for his assistance and advice and for his review and approval of this document, and to Terrence J. Miesle M.S. for his advice, clarifications, and contributions to this document.***

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