AVIAN BORNAVIRUS - AN UPDATE AND IMPLICATIONS FOR AUSTRALIA

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This manuscript is a modification of the fact sheet: Avian Bornavirus and Proventricular Dilation Disease which can be found here. It has been reprinted with the permission of the Australian Wildlife Health Network.

Introduction

Proventricular dilatation disease (PDD) has been the scourge of avian veterinarians and bird owners in many parts of the world since its recognition in the 1980's. It has been such a devastating disease because it can occur at any time in a birds life, it can occur in aviary birds and in pets, it can occur in individual birds or as outbreaks, is difficult to diagnose, challenging and at times impossible to treat, and until the discovery of its aetiologic agent the Avian Bornaviruses (ABVs), there were no tools available to manage it out of a collection. ABVs have been introduced into Australia and PDD has been diagnosed in pet and aviary birds in Eastern Australia since 1997. Yet it is the author's impression, that it is not making the same impact on pet and aviary birds as it has made in other countries. Our knowledge of ABVs is increasing at an exponential rate and it clear that novel ABVs exist in many species of bird, including ducks, geese, swans gulls, and passerines and these ABVs have the potential to have a wide geographic distribution in wild birds and in captive birds. These findings have significant implications for Australian Avian Veterinarians and wildlife biologists.

The purposes of this paper are to review the current understanding of Avian Bornaviruses, to look at the strengths and weakness of testing for detecting ABV infection in diseased and subclinically infected birds, and to encourage practitioners to look for evidence of ABV infection in species other than parrots.

Aetiology

The aetiologic agents of proventricular dilatation disease in parrots and other related diseases in parrots and other species of birds are Avian Bornaviruses. Avian Bornaviruses are RNA enveloped viruses that replicate in the nucleus of infected cells. There are 7 genotypes that have been detected in psittacine birds with genotype 2 and 4 being the most common (Gancz et al., 2008; Honkavuori et al., 2008). Another genotype (Avian Bornavirus- Canada Goose [ABV-CG] has been isolated from Canada Geese (*Branta canadensis*) (Delnatte et al., 2011) and it appears that other ABVs are also present in waterfowl (I. Tizzard pers. com 2014). Recent studies have found 3 new genotypes of ABV in canaries (Serinus canaria) from Germany that genetically map into a separate cluster these have been proposed to be called ABV-C1, ABV-C2, and ABV –C3 (Weissenbock et al., 2009b; Rinder et al., 2012). A similar genotype has also been identified in a Bengalese finch (*Lonchura striata*) and may represent a 4th genotype within the canary subgroup (Rubbenstroth et al., 2013).

Natural Hosts

Parrots (Psittaciformes): Avian Bornaviruses have either been detected in or suspected to occur, based on histological lesions, in over 80 species of parrot. Avian Bornavirus has never been detected in wild parrots, but have been detected in species whose original range would have included the Indopacific, Asia, Africa and Central and South America (Gancz et al., 2013, Heffels-Redmann et al., 2011). It is very likely that the number of species susceptible to infection is much larger than this and will be documented as more extensive testing occurs. Australia species known to develop PDD or have been detected with ABV infection include the sulphur-crested cockatoo (Cacatua galareta), palm cockatoo (*Probosciger atterimus*), red-tailed black cockatoo (*Calyptorhynchus magnificus*), galah (*Eolophus roseicapillus*), gang gang (*Callocephalon fimbriatum*), cockatiel (*Nymphicus hollandicus*), red-capped Parrot *(Purpureicephalus spurius*), and eclectus parrot (*Eclectus roratus*) (Gancz et al., 2013, Heffels-Redmann et al., 2011). It is highly likely that many native Australian species of parrots are susceptible to infection.

Passerine birds

Three gentotypes of ABV (C1, C2, and C3) have been detected in canaries and clinical disease and microscopic lesions resembling PDD have also been described in canaries (Rubbenstroth et al., 2013). A forth similar genotype has been found in a Bengalese finch (Rubbenstroth et al., 2013). Proventricular Dilation Disease has described in a greenfinch (*Carduelis chloris*), a long-wattled umbrellabird (*Cephalopterus penduliger*), a bearded barbet (*Lybius dubius*), honey creeper, and weaver finch (Perpinan et al., 2007).

Swans, Geese and Ducks

Proventricular Dilatation Disease-like lesions were first reported in Canada geese in 1991 (Dauost et al., 1991). Avian Bornavirus CG has been detected in wild Canada geese and trumpeter swans (*Cygnus buccinator*) and feral mute swans (*Cygnus olor*) in North America (Payne et al., 2011b; Delnatte et al., 2011, Guo et al., 2012; Delnatte et al., 2013). Some of these birds were healthy and others were ill and had PDD-like lesions. Additional studies have shown that ABV-CG can also be detected in snow geese (*Chen caerulescens*) and Ross's geese (*Chen rossii*) (Payne et al., 2012a, Payne et al., 2012b). ABV-CG has also been identified in mallards both domestic and wild (I. Tizard pers. com. 2013). Avian Bornavirus-CG has been isolated from fibroblasts derived from Pekin duck eggs and viral RNA has been detected in Pekin duck eggs from commercial sources, suggesting that ABV-CG is present in these commercial flocks (Payne et al., 2012b). Pekin ducks were inadvertently infected with ABV genotype 4 when exposed to infected cockatiels (Gray et al., 2009). Pekin ducks have been imported into Australia and could potentially be infected with an ABV genotype.

Other species

Avian Bornavirus RNA has been detected in three species of North American gull (herring gull [*Larus argentatus*], ring-billed gull [*L. delawarensis*] and laughing gull [*L. atricilla*]) (Payne et al., 2012b). The virus from the herring gull has been sequenced and is ABV-CG. Avian Bornavirus-CG has also been detected in the brain of a bald eagle (*Haliaeetus leucocephalus*) that died with encephalitis and from a Mississippi kite *(Ictinia mississippiensis*) (Payne et al., 2012b). Lesions resembling PDD have also been described in two other raptors, a red-tailed hawk (*Buteo jamancensis*) and peregrine falcon (*Falco peregrinus*) (Shivaprasad 2005). The host range of ABV-CG and Bornaviruses in general may be fairly extensive as it ABV-CG has recently been detected in a razorbill (*Alca torda*), and a yellow-crowned night heron (*Nyctanassa violacea*) (I. Tizard pers. com. 2013) and PDD like lesions have been described in a toucan (*Rhamphastos* sp.), and roseate spoonbill (*Platalea ajaja*) (Gregory et al., 1994). Screening of wildlife suggests that prevalences of infection will vary from year to year, and, even in the same species, infection may be common in some locations, but completely absent in others.

World distribution

Parrot genotypes of ABV have been documented in North America, South America, Europe, Africa, the Middle East, Japan and Australia and it is likely that these genotypes have disseminated globally as the result of the international trade in wild caught and domestically raised parrots (Kistler et al., 2008; Honkavuori et al., 2008; Marietto-Goncalves et al., 2009; Weissenbock et al., 2009a; Heffels-Redmann et al., 2011; Ogawa et al., 2011). ABV is a principal threat to the recovery of last significant population of Spix macaws currently housed in the Middle East (Wyss et al., 2009)

The ABV CG genotype has only been identified in waterfowl and other species in North America (Delanatte et al., 2011; Guo et al., 2012).

The canary genotype has only been described in canaries from Germany, but it is also likely to have a global distribution. The finch genotype was identified in a common avicultural species of finch in Japan (Rubbenstroth et al., 2013).

Occurrences in Australia

There have been no reports of ABV in Australian wild birds. PDD was first reported in Australia in 1997 in a captive green-winged macaw (*Ara chloroptera*) imported into Australia in 1993 (Sullivan et al., 1997). Proventricular Dilatation Disease has subsequently described in four other imported species including a red-sided eclectus parrot (*Eclectus roratus polychloros*), Moluccan cockatoo (*Cacatua moluccenensis*), sun conure (*Aratinga solstitialis*) and African grey parrot (*Psittacus erithacus erithacus*). An informal survey of a group of Australian avian practitioners in late 2008 indicated that 7 of 35 had cases of PDD that were documented by histopathology (Phalen unpublished information 2008).

Avian Bornavirus-2 was sequenced from tissues from a Moluccan cockatoo from a Queensland aviary (Weissenbock et al., 2009a). Avian Bornavirus - 4 has been detected in the tissues of a psittacine bird with PDD. Uncharacterized genotypes of ABV have also been detected in droppings from two other subclinically infected psittacine birds in aviaries on the East Coast of Australia (Phalen unpublished data 2013).

Epidemiology

There is increasing proof that ABVs are the cause of PDD. In a small study of experimentally infected parrots, Koch's postulates where fulfilled (Gray et al., 2010). Disease has also been produced in other less stringently controlled infection studies (Gancz et al., 2009; Lierz et al., 2012; Piepenbring et al., 2012).

The epidemiology of ABV in parrots is complex. Infected birds may develop disease or may not (Payne et al., 2011a). Incubation periods between infection and the development of disease may range from a few weeks to many years. Subclinically infected birds may shed virus for years, possibly their entire life (Payne et al., 2011). Infected birds may shed virus continuously, intermittently, rarely, and it is possible that some may never shed virus. Virus shedding has been detected in oral secretions and in droppings and virus has been shown to be shed in urine. Viral RNA has also been detected in feathers (Hoppes et al., 2010; Raghav et al., 2010). Given that there are so many genotypes of ABV and infection can occur in so many species, it is likely that both host and virus factors will ultimately be found to determine the frequency of virus shedding and the percentage of birds that will ultimately develop disease. It is also possible that infection with a second genotype may precipitate the onset of disease in subclinically infected birds (Mirhosseini et al., 2011; Payne et al., 2011a).

In a very limited study, ABV infection was induced with oral inoculation suggesting that virus ingestion is one route of infection. Inhalation of aerasolized particles contaminated with virus is another possible but unconfirmed route of infection.

Viral RNA has been detected in the eggs of infected parrots but not in others (Monaco et al., 2012 and Kerski et al., 2012), so it is possible that some but not all ABV infected parrots can vertically infect their offspring. Horizontal transmission after hatch is also likely (Kerski et al., 2012). Avian Bornavirus was found growing in fibroblasts derived from commercial duck eggs also suggesting that vertical transmission may be possible (Payne et al., 2012b).

Current studies suggest that infection prevalence's within flocks of avicultural species can vary substantially form no birds infected up to 28% birds infected (Heffels-Redmann et al., 2011). Disease rates can also vary substantially with no know history of disease in some infected collections to the loss of the majority of a collection over a period of a few years in others. Preliminary data in canary flocks suggests that infection prevalences vary from flock to flock and as do rates of morbidity and mortality (Rubbenstroth et al., 2013).

Very little is known about ABV epidemiology in wild birds, other than the increasing evidence that infection in swans, geese and ducks in North America is relatively common, but again prevalences will vary between populations.

Clinical signs

Many, perhaps most, ABV infections do not result in disease. When disease occurs, it is the result of damage to the nervous system that may be viral induced or the result of the host response to infection. Signs are typically divided into those caused by damage to the central nervous system and those associated with damage to the nerves controlling the motility of the digestive tract. Signs involving one or both systems may be present in diseased birds.

Signs caused by disease of the central nervous system are typically slow to develop and are progressive. They include, mentation changes, ataxia, a progressive weakness developing into paralysis, and rarely seizures (reviewed in Hoppes et al., 2010; Gancz et al., 2013). Blindness, although rare, can occur as the result of ocular or neurologic disease (Steinmetz et al., 2008).

Damage to the nerves of the digestive system results in alterations in gut motility and even paralysis of the gut, this intern impacts the bird's ability to digest food. Signs of alteration of digestive function include passage of whole seeds in the droppings, diarrhoea, regurgitation, delayed crop emptying and the resultant weight loss. Many birds are emaciated on presentation.

Diagnosis and laboratory procedures

Diagnosis of ABV infection in the live bird by molecular techniques.

Multiple polymerase chain reaction (PCR)-based assays have been described that have been used to detect ABVs in samples collected from live birds. The primers used in these assays need to be designed to detect the specific virus that is expected to be found in the species of bird that is being tested. In some cases, testing with multiple primers may be necessary if all possible ABV genotypes are to be detected (Kerski et al., 2012; Hoppes et al., 2013).

Avian Bornaviruses have been detected in oral and tracheal swabs, cloacal swabs, crop biopsies, skin, blood and feathers (Raghav et al., 2010, Kerski et al., 2012). A full comparison of the sensitivity and specificity of PCR examination of these different tissue samples from the same bird has not been done. Testing is also complicated by the fact that virus shedding can be extremely variable in frequency and amount, and it is likely that many birds shed virus intermittently, rarely, or not at all. In a study by Raghav et al., (2010) of five birds with confirmed ABV infection, over a 5 day period of sampling droppings, one never shed virus, one shed virus only on 1 day, one shed virus 3 of 5 days and two shed virus all 5 days. Current recommendations for testing include repeated testing of cloacal swabs or droppings (a minimum of 3 samples), possibly in conjunction with PCR analysis of feathers (reviewed in Gancz et al., 2010; Hoppes et al., 2013).

Diagnosis of ABV infection in the live bird by detecting antibodies.

Some, but not all birds infected with ABV will develop antibodies. Others will go long periods without developing circulating antibodies and then will suddenly develop them (Hoppes et al., 2010). It has been postulated that the sudden onset of antibody production may indicate the onset of clinical signs. In contrast many seropositive birds do not show signs of PDD (de Kloet and Dorrestein 2009). Several diagnostic tests have been developed to detect antibodies in the blood of infected birds (de Kloet and Dorrestein 2009, Villanueva et al., 2010). ABV produces two proteins (the N and P protein) to which their host may produce antibodies. Studies have shown that antibodies are most likely to be produced against the N protein (Villanueva et al., 2010). These antibodies have been detected using Western blot assays and enzyme-linked immunoassays. Immunofluorescent assays using cells infected with ABVs have also been used to detect antibodies (Villanueva et al., 2010). If the ultimate goal is to identify infected individual birds, testing sensitivity is improved if both PCR-based testing and antibody testing is done (de Kloet et al 2007; Hoppes et al., 2013).

Diagnosis of PDD

Neither the digestive nor neurological signs exhibited by birds with PDD are sufficiently specific to make a diagnosis; therefore other diagnostic testing is required. In advanced cases, plain radiographs demonstrate distention of the proventriculus and ventriculus. These organs may be massively distended. Dilation of the intestines may also occur, although less frequently. The presence of gas in any part of the digestive tract is abnormal and an indication of altered gastrointestinal (GI) motility. Contrast studies using repeated radiographs or fluoroscopy has proved a very effective way of detecting both subtle and severe changes in GI motility (reviewed in Hoppes et al., 2010; Gancz et al., 2012).

While signs and imaging findings may be highly suggestive of PDD, currently a diagnosis of PDD can only be made by demonstrating specific inflammatory lesions in affected nerves. Crop biopsies are the easiest and safest means of obtaining tissue that may contain diagnostic lesions, unfortunately only approximately 50% of birds with PDD will have lesions (Graham 1984; Doolan 1994; Gregory et al., 1996). Biopsy of a nerve on the serosal surface of the proventriculus or ventriculus is a much more sensitive, but also a much more risky procedure (Graham 1984).

Isolation of Avian Bornaviruses

Avian Bornaviruses are readily grown in cell culture. Cells of quail, chicken, and duck origin have been used to grow Bornaviruses (Hoppes et al 2010). Recently, the quail fibroblast cell line CEC-32 has been shown to be an efficient system for isolation of ABVs from parrots (Rubbensroth et al., 2012). Avian Bornavirues do not cause cytopathic effects in cells, therefore their presence must be detected PCR assays or immunohistochemistry.

Clinical pathology

Specific haematologic and blood chemistry changes are not associated with ABV infection or the development of PDD. Birds with advanced PDD are typically anaemic and hypoproteinemic. White blood cells counts can either be normal or elevated, depending on whether there is an overgrowth of bacteria and yeasts associated with the stasis of the digestive tract or there may be a secondary infection present in other body systems. Uric acid levels may be elevated if the bird is unable to get to its water source and becomes dehydrated.

Pathology

Typical necropsy findings include an emaciated bird with a massively distended proventriculus and ventriculus containing ingesta. Myenteric nerves to the proventriculus and ventriculus may be grossly enlarged. Dilation of some or all of the intestines may also occur. Dilatation of the gastrointestinal tract, however, is not always present. Microscopic lesions include a nonsuppurative encephalomyelitis, enlargement and lymphoplasmacytic infiltration of the myenteric nerves of ventriculus, proventriculus, crop and intestines in decreasing order of frequency with an associated lymphoplasmacytic infiltration. Similar lesions are found in peripheral nerves (Berhane et al., 2012). Inflammation of the nerves of the heart and lymphoplasmacytic infiltration of the adrenals occurs infrequently. Bornavirus can be identified in brain sections and many other tissues, using immune histochemical testing, if the necessary primary antibody is available (Lierz et al., 2009; Ouyang et al 2009; Rinder et al., 2009; Raghav et al., 2010). Currently this antibody is not available in Australia.

Differential diagnoses

Many chronic diseases can resemble PDD. Bacterial and fungal infections of the upper gastrointestinal tract can result in a failure of crop emptying and decreased gastrointestinal motility. Diseases of the ventriculus including fungal and bacterial infections, and cancer can also result in whole seeds being passed in the faeces. Intestinal obstructions can also result in dilatation of the ventriculus and proventriculus (reviewed in Gancz et al., 2010; Hoppes et al., 2013).

Zinc and lead intoxication cause stasis of the digestive tract in all species and dilatation of the proventriculus and ventriculus in some species of waterfowl. Zinc and lead intoxication will also cause central nervous system signs that resemble those seen in birds with PDD. Vitamin E, thiamine, and vitamin A deficiencies can also cause central nervous system signs resembling that seen in birds with PDD. Microscopically, paramyxovirus 1, 2 or 3, and arboviruses, including West Nile virus can produce brain lesions similar to those caused by ABV (reviewed in Gancz et al., 2010, Hoppes et al., 2013).

Laboratory diagnostic specimens

In the live bird, blood, droppings, oral swabs and feathers can all be submitted for PCR testing for ABV RNA. All samples should be kept chilled from the time they are collected until the time they are tested. A crop biopsy containing a section with a prominent blood vessel can be used to detect PDD lesions in nerves (Gregory et al., 1996; Gancz et al., 2010). A second smaller section can be submitted for PCR testing. This section should be frozen until it is tested.

In post-mortem specimens, a complete set of tissues should formalin-fixed and submitted for histopathology. Transverse sections of all levels of the digestive tract should be made. Half the brain can be formalin-fixed and the remainder frozen for PCR testing. Given that not all birds with PDD have ABV present in the brain, a section of ventriculus and proventriculus should also be saved frozen for PCR testing.

Cloacal and phyarngeal swabs, blood, and tissues (brain, crop, proventriculus, and ventriculus) have all been used to isolate ABVs. Samples should be tested immediately or frozen (-80 0C) until testing.

Treatment

Currently there are no treatments available that will cure a bird of infection. Treatments, however, have been developed that reversed the signs of PDD. These treatments are most effective in the early stages of the disease, and are unlikely to work in birds with advanced disease (Gancz et al., 2010).

Both Celecoxid (Dahlhausen et al., 2002) and Tepoxalin (Zubrin; Schering Plough, Union, NJ, USA) (Gancz et al., 2010) which are nonsteroidal anti-inflammatory drugs have been shown to reverse the clinical signs and microscopic lesions in birds with PDD. Dosage rates of 20 mg/kg Celcoxid once a day and 40/mg/kg Tepozalin once a day have been recommended.Treatment durations of up to many months may be necessary for resolution of signs. There is anecdotal evidence that the antiviral drug amantadine hydrochloride (20/mg/kg placed in food once a day) may also contribute to the recovery of birds with PDD when combined with Celecoxid (Gancz et al., 2010).

Prevention and control

Preventing the dissemination of ABV infection in captive collections of birds is challenging and may be impossible with current diagnostic techniques. It would require that all birds entering a collection be repeatedly tested by PCR assays and also tested for the presence of circulating antibodies. The later test is not available in Australia at this time. Even with this intensive level of screening, infected birds have been shown to be negative on both assays so introduction of positive birds into a collection may still occur.

ABV isolates are thought to be stable at neutral pH and can withstand alkaline and acid solutions, inactivated by heat (560C) for 3 hours, but are stable at 50C for 3 months. Their ability to survive in the environment is not known. The virus is enveloped and is assumed to be susceptible to commonly used disinfectants including chlorhexidine, phenolics, quaternary ammonium products and bleach (reviewed in Hoppes et al., 2013).

Implications for Australian aviculture and wildlife

At least two genotypes of ABV infecting parrots are present in Australia and were likely introduced during the period of time that legal importation of parrots into Australia occurred in the 1990's. Based on the author's diagnostic histopathology case load and experience, ABV infections resulting in PDD have not become as common as would be expected based on experiences in other countries. The reasons for this are uncertain, but may reflect different practices of aviculture in Australia. Outdoor aviaries are far more common in Australia than they are in cooler climates. Given that ABV is likely sensitive to sun light and other environmental conditions, this may impact its route of spread. Also, breeding of the species that are the most likely to disseminate it is fairly restricted because of their cost and this may also reduce its dissemination rate. The fact that infected birds are still around over 17 years after its initial recognition, implies that it is enzootic in at least some aviaries and that potential for much wider spread exists.

As mallard ducks and Pekin ducks have been both found to be infected with ABV, but to experience little disease, it is very likely that ABV could have enter Australia in these species in the past. It is also likely that imported passerine species, such as the canary, may have introduced passerine genotypes into Australia. Recent findings of ABV in gulls and other waterfowl suggest that ABV genetic strains might also be found in native gulls and waterfowl in Australia.

It would seem prudent that ABV be considered as an important differential diagnosis in any wild or captive bird exhibiting neurological signs, including stasis of the digestive tract. Increased screening and reporting of these cases, will be the only way that the full impact of the ABVs on Australian aviculture and wildlife will ever be determined.

Conclusions

Avian Bornaviruses are composed of a genetically heterogeneous population of viruses that infect a wide range of avian species. Their impact on captive-raised parrots has been, at times, catastrophic and they threaten captive breeding and reintroduction programs for endangered species of parrots. Avian Bornavirus 2 and 4 have been introduced into Australia as the result of the parrot trade and it is likely that other ABVs have also been introduced by importation of passerine species including the canary and ducks such as the Pekin duck and the mallard. Release of these viruses into wild populations of native Australian species or transfer to captive breeding populations of endangered species could have significant negative consequences.

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