A Review of Circovirus, Polyomavirus and Adenovirus in Psittacine Birds and a Future Direction for Australian Research

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INTRODUCTION

Psittacine circovirus (Beak and feather disease virus) and polyomavirus are able to cause illness and death in captive and wild psittacine birds (Katoh et al., 2010). Relevant literature recognises that these viruses have a worldwide distribution (Shearer et al., 2008; Katoh et al., 2010). Comparatively, the distribution and effects of psittacine adenovirus, particularly on wild populations, but also captive psittacine birds, is still largely unknown, despite some case study literature (Wellehan et al., 2005). This paper reviews these three diseases and highlights the need to readily identify birds ailed by these viruses both clinically and subclinically. It presents a novel direction of study being conducted by a final year Veterinary Science student at the University of Melbourne and the issues associated with conducting such an investigation.

PSITTACINE CIRCOVIRUS

Psittacine beak and feather disease is caused by psittacine circovirus. This virus was first recorded in 1903 in the sulphur-crested cockatoo (*Cacatua galerita*) population in Australia (Raidal, 2012). It has since been documented to occur on a worldwide basis and is now recognised as the most common disease of captive and wild psittacine birds (Raidal, 2012). Over 60 species of captive and free-ranging parrot species have been found to be susceptible (Ha et al., 2007).

Aetiology and epidemiology

Psittacine circovirus is a member of the *Circoviridae* family (Ypelaar et al., 1999; Ritchie et al., 2003). The smallest of viruses yet described, belong to this family of viruses (Greenacre, 2005). The virus particles are housed in a non-enveloped, icosahedral capsid measuring between 5 and 26nm in diameter (Ritchie et al., 2003). The capsid contains a single strand of circular DNA, approximately 2000 nucleotides in size (Ypelaar et al., 1999; Ritchie et al., 2003).

This virus is exceptionally resistant to the elements, being likely to remain infective for months to years (Greenacre, 2005). Transmission occurs in a horizontal fashion; via inhalation, ingestion or even movement across the bursal epithelium (Phalen, 2006). Diseased and carrier birds shed the virus in their faeces, crop secretions and feather dander (Ritchie et al., 2003; Phalen, 2006). Contaminated fomites can be a mechanism of indirect horizontal transmission. In the nesting situation, feather dander and crop secretions have a high concentration of virus, and given the immature immune system in a nestling (Ritchie et al., 2003), recently hatched and juvenile birds are the most at risk age group (Phalen, 2006). In some instances, viral DNA has been detected in embryonated eggs,

suggesting the possibility of vertical transmission (Greenacre, 2005).

Pathogenesis and clinical signs

Psittacine circovirus targets the host's cell mediated immune system (Ritchie et al., 2003). Replication occurs in the bursa of Fabricus, thymus, gastro-intestinal associated lymphoid tissue, and circulating lymphocytes (Ritchie et al., 1989; Phalen, 2006). The ultimate site this virus targets is the epidermis and feather follicles (Raidal, 2012).

There is an acute and a chronic clinical presentation associated with psittacine beak and feather disease. Signs present within 21-28 days of infection and often the only sign is sudden death (Raidal, 2012). Alternately, nestlings may be depressed, regurgitate, develop a green diarrhoea, be systemically ill, and if any haematology is conducted, a leucopenia may be identified (Greenacre, 2005, Raidal, 2012). Newly developing feathers will have annular constricting bands and they can easily break, be pulled out or bleed (Phalen, 2006).

The chronic form is the one which is most familiar to clinicians. Chronic infections in the cockatoo (*Cacatua*) family present in parrots under three years old (Phalen, 2006). It is more insidious, progressing only with each subsequent molt (Ritchie et al., 1989; Greenacre, 2005). Incubation time is highly variable depending on which stage of the molt the parrot is in at the time of infection. Eventually, dystrophic feathers will replace normal ones. The down and contour feathers are often the first affected, with the primary feathers succumbing to changes later (Greenacre, 2005). Growing feathers are short, having a clubbed appearance, with evidence of past or recent haemorrhage in the shaft (Phalen, 2006). Clinically, this manifests as a symmetrical feather dystrophy and loss (Katoh et al., 2008, Ritchie et al., 1989). Beak lesions are common in cockatoos and their relatives. A lack of powder down dust on the beak is one of the first signs of beak and feather disease (Phalen, 2006). Lesions progress with beak elongation, fractures and eventual necrosis (Ritchie et al., 1989).

Members of the cockatoo (*Cacatua* spp.) Family and the galah (*Eolophus roseicapilla*) are the most diverse in their clinical presentations. African grey parrots (*Psittacus erithacus*) show signs similar to the cockatoo species (Phalen, 2006). Comparatively, lovebirds (*Agapornis* spp.) have no clinical disease and can eliminate the virus. Fledgling budgerigars (*Melopsittacus undulatus*) tend to lose primary and secondary wing feathers, making its distinction from polyomavirus difficult (Phalen, 2006). In Australian rainbow lorikeets (*Trichoglossus haematodus*), approximately one third will show persistent feather abnormatilites, one third die before their first molt, and the remaining develop normal feathers after their molt (Phalen, 2006). Other species may only have very easily pluckable feathers or yellow feathers replacing normally green feathers (Raidal, 2012). Affected parrots often succumb to secondary infections because of the immunosuppressive effects of the virus (Ritchie et al., 2003).

Diagnosis

Circoviral infection in psittacine birds is diagnosed by a combination of methods. Clinicians often reach a presumptive diagnosis based on clinical signs (Phalen, 2006). Should the affected bird be fatally affected, or if a clinically affected bird is submitted for post mortem examination, histopathological examination should reveal large basophilic intranuclear and intracytoplasmic inclusion bodies within macrophages, keratinocytes, hepatocytes and other tissues (Ritchie et al., 1989; Raidal, 2012). Alternatively, serological methods (haemagglutination assay, haemagglutination

inhibition assay and virus neutralisation) can be employed on feathers, blood and faecal matter. Enzyme-linked immunosorbent assay (ELISA), DNA *in situ* hybridisation and PCR have also been utilised (Kennedy, 2005; Katoh et al., 2010). A PCR assay on heparinised blood will often enable confirmation of viral infection (Phalen, 2006).

Prevention and control

Psittacine circovirus is highly resistant to desiccation in the environment and to common disinfectants. Products that are often suitable for inactivating environmentally resistant viruses, such as glutaraldehyde (Raidal, 2012) and Virkon-S are recommended when attempting to disinfect contaminated surfaces (Dahlhausen, 2010). Where disinfection procedures cannot safely or readily be employed, control is most often limited to preventing introduction of a shedding bird into a naive environment. Appropriate quarantine, biosecurity and routine screening of new birds should enable identification of infected birds (Raidal, 2012). Such birds should be immediately removed as they shed massive amounts of virus (Phalen, 2006). Any birds exposed to an infected bird should also be quarantined. In-contact birds can be tested initially, and after one month in quarantine, which allows sufficient time for viraemia to develop and be detected (Phalen, 2006). An inactivated vaccine has been produced in Australia, although it is not commercially available (Raidal, 2012).

PSITTACINE POLYOMAVIRUS

Avian polyomavirus (APV) is known as budgerigar fledgling disease and French molt (Davies, 2000). It has significant implications for breeders, as it can cause up to 100% mortality in affected nestling budgerigar and non-budgerigar psittacine birds (Rott et al., 1988; Phalen, 2006). It can simultaneously occur with circovirus, and potentiate the effects of this virus (Phalen, 2006, Raidal, 2012).

Aetiology and epidemiology

APV has a double stranded circular DNA that is 4981bp in length (Rahaus and Wolff, 2005; Katoh et al., 2010). The DNA is housed in a non-enveloped, icosahedral capsid of 45-50um in diameter (Rott et al., 1988; Rahaus and Wolff, 2005). It belongs to the *Polyomaviridae* family (Katoh et al., 2010), however, in recent years, there has been some controversy over its taxonomy.

The mode of transmission of APV is likely to be via a respiratory route, although vertical transmission has been speculated in budgerigars (*Melopsittacus undulatus*) (Phalen et al., 1991; Greenacre, 2005). It is suspected that captive and wild birds with subclinical infection act as a reservoir (Greenacre, 2005). The presence of infected nestlings and juvenile parrots perpetuates the infection cycle, as they shed virus for up to 6 months post infection (Phalen, 2006). Under times of stress, a recrudescence of viral shedding is likely to occur (Rahaus and Wolff, 2005; Phalen, 2006). Virus particles are shed via faeces, in feather dander and in exfoliated skin (Phalen, 2006, Raidal, 2012). APV is known to be thermostable, suggesting particles remain infective in the environment for a significant amount of time (Greenacre, 2005).

Pathogenesis and clinical signs

Nestling and juvenile parrots are the most at risk age group for becoming infected (Raidal, 2012), mainly due to their immature immune system (Gerlach, 1994). However, this does not limit infections to naive young birds, and all age groups should be considered susceptible (Raidal, 2012). Once

infected, the virus appears to have at least a 2-14 day incubation period (Greenacre, 2005). Infected birds are viraemic for 7-10 days with replication occurring in the liver, spleen, renal tubular epithelium, cerebellum, heart, skin and feather follicles (Phalen, 2006).

The outcome of infection with APV is dependent on the species, age and immune status of the host. Infected budgerigar (*Melopsittacus undulatus*) and *Neophema* nestlings will usually develop abdominal distension, pallor, distended full crops, subcutaneous haemorrhages, ataxia and head tremors (Katoh et al., 2010, Rahaus and Wolff, 2005). Death usually occurs between 10 and 25 days old (Davies, 2000). Severe feather abnormalities do not have time to develop prior to death (Phalen, 2006). At necropsy, lesions include hepatosplenomegaly, hydropericardium, ascites and haemorrhages in body cavities (Davies, 2000). In non-budgerigar psittacine birds, especially conures, macaws and eclectus parrots, dead nestlings may be the only clinical finding (Davies, 2000, Phalen, 2006). Other non-specific signs such as depression, anorexia, and weakness have been noted (Davies, 2000). Some young parrots have been observed to make a full recovery from infection (Davies, 2000).

If a budgerigar or neophema parrot is an older nestling or a juvenile at the time of infection with APV, feather abnormalities are the main clinical sign (Davies, 2000). Most notably, these birds fail to develop primary and secondary wing feathers. Contour feathers may lack barbs, be absent on the head and neck and down feathers might be sparse on the back and abdomen (Raidal, 2012). This presentation is considered to be the chronic form of the disease and results in a state of persistent infection (Rahaus and Wolff, 2005). Sub-clinical carrier birds exist and shed polyomavirus intermittently for life (Greenacre, 2005).

Juvenile, non-budgerigar psittacine birds which survive the initial polyomavirus infection, may develop chronic ascites and generalised oedema (Phalen, 2006). Affected birds fail to thrive and eventually die or are euthanased (Phalen, 2006).

The majority of adult parrots infected with polyomavirus are usually completely asymptomatic, but shed the virus for a few weeks (Phalen, 2006). If ill-effects are observed, it is likely that they have a concurrent infection. Interestingly, cockatoo (*Cacatua*) species appear resistant to the disease, showing little, if any, clinical signs, despite being able to contract an infection (Raidal, 2012).

Diagnosis

Suspicion of polyomavirus infection can be based on the clinical presentation in young birds, however, definitive diagnosis of infection is by pathological investigation or laboratory testing of clinical samples (Bert et al., 2005). In cases where birds have died from APV infection, a necropsy may show gross lesions such as haemorrhages, ascites, hepatomegaly and splenomegaly. Histopathologically there are enlarged nuclei and nuclear inclusions in cells in the spleen, liver, kidney and feather follicles (Greenacre, 2005, Katoh et al., 2010). Various PCR assays exist to test both tissue samples from dead and alive birds. Other techniques such as serology, electron microscopy, virus isolation and fluorescent antibody staining have also been employed to arrive at a diagnosis (Phalen et al., 1991, Raidal, 2012).

Prevention and control

A vaccine is available in North America (Greenacre, 2005), however, in Australia, prevention of APV outbreaks in aviaries requires repetitive testing, strict quarantine and biosecurity. The first step

towards preventing an APV outbreak in a psittacine flock is to ensure the virus does not exist enzootically within the aviary (Phalen, 2006). This is especially important for aviaries breeding budgerigars (*Melopsittacus undulatus*). Testing individual young birds or the aviary environment is vital (Raidal, 2012). If the aviary is free of APV, then maintaining a strictly closed flock or only allowing seronegative new birds into an open flock will prevent introduction of this virus (Phalen, 2006). Should elimination be required, all breeding should cease for a minimum of 6 months. Adult birds need to be relocated and the breeding aviary totally disinfected. Ideally, nesting boxes and all organic materials should be destroyed (Phalen, 2006). Following a six month reprieve from breeding, birds can be returned and breeding recommenced.

For parrot hand rearing nurseries, only the chicks from APV negative flocks should be raised in the nursery. Should polyomavirus be introduced by an apparently non-infected nestling or on contaminated equipment or clothing, further nestling introductions should stop. In this situation, allowing the parent birds to raise all future chicks for the season will prevent disease spread and development (Phalen, 2006). Following the outbreak, all surviving chicks need to test negative or be removed from the facility. Thorough disinfection of the nursery is required prior to the subsequent breeding season (Raidal, 2012). Given the stability of polyomaviruses in the environment, destruction of infective particles is only achieved using synthetic phenol, sodium hypochlorite, chlorine dioxide or 70% ethanol solutions (Ritchie et al., 1993).

PSITTACINE ADENOVIRUS

Adenoviruses are a diverse group of viruses present in a vast array of animal hosts. They have been identified in mammals (including humans), reptiles, fish, amphibians and avian species (Wellehan et al., 2005; Katoh et al., 2009). Information pertaining specifically to psittacine adenovirus (PsAdV) is largely limited to case study based literature. Comparatively, a significant amount of study has been conducted on Fowl Adenovirus (FAV) strains which have pathogenic effects and are of concern to the poultry industry (Hess, 2000). More investigation into PsAdV is needed to further the understanding of this virus.

Aetiology and epidemiology

There are three genera within the *Adenoviridae* family which have been identified in avian species; aviadenovirus, atadenovirus and siadenovirus (Wellehan et al., 2005). Phylogenetic analysis of adenoviruses and their hosts have suggested co-evolution, however, switching hosts has been documented (Wellehan et al., 2005, Wellehan et al., 2009). These viruses have a non-enveloped icosahedral capsid with a diameter of approximately 70um (Katoh et al., 2010). They have medium-sized, linear, double stranded DNA genome of 26-45,000 base pairskbp (Raue et al., 2005, Wellehan et al., 2009). As a family, adenoviruses are generally transmitted via the feco-oral route (Greenacre, 2005). In chickens, vertical transmission has been demonstrated (Greenacre, 2005) and this may also be the case for PsAdV. Shedding can be intermittent in latently infected birds (Ritchie, 1995).

Pathogenesis and clinical signs

The manner in which PsAdV disseminates through an avian host is not fully understood. Early work investigating the pathogenesis of fowl adenoviruses suggests that following ingestion of the virus, replication occurs in the intestinal tract (Ritchie, 1995). The host becomes viraemic and replication commences in a range of tissues including the liver, spleen, lung, kidney, central nervous system and

lymphoid tissues (Ritchie, 1995). In the case of PsAdV, replication has been confirmed to occur, in hepatocytes, enterocytes (Greenacre, 2005) and splenic lymphoid cells (Katoh et al., 2009). The virus is known to interact with the hosts immune system via a surface hexon protein (Katoh et al., 2009).

Clinically, many different signs have been identified in parrots later diagnosed with adenoviral infections. Adenovirus infection has been diagnosed in clinically normal birds (Wellehan et al., 2005; Luschow et al., 2007). More often, the presenting signs are vague, such as weight loss and diarrhoea, which is subsequently followed by acute death (Gomez-Villamandos et al., 1992, Ramis et al., 1992, Wellehan et al., 2009). Some other clinical signs described include depression, anorexia, conjunctivitis and cloacal hemorrhage (Greenacre, 2005, Wellehan et al., 2009). Feather changes do not appear to be a common presentation with clinical adenovirus disease but have be noted (Katoh et al., 2009). Necropsy is reported to revealhepatitis, enteritis, splenitis, interstitial pneumonia and other organ changes (Ramis et al., 1992, Wellehan et al., 2005, Wellehan et al., 2009).

Diagnosis

Unlike the other two viruses discussed, the clinical signs associated with adenoviral disease in psittacine birds are much more variable. This makes presumptive diagnoses based on clinical signs problematic. The most common mechanism for establishing a diagnosis appears to be via necropsy and being able to demonstrate adenovirus-like inclusion bodies on histopathology (Ramis et al., 1994; Droual et al., 1995, Katoh et al., 2009). In North America, DNA *in situ* hybridisation is the method of choice for diagnosing PsAdV (Greenacre, 2005). Virus isolation, electron microscopy, immunohistochemistry, serological and PCR assays have also been utilised (Wellehan et al., 2005; Luschow et al., 2007). Literature searches suggest that in Australia, identification is restricted to histopathological findings.

Prevention and control

Currently, there are no vaccines available for PsAdV. Consequently, control of infection is limited to prevention of spread of virus particles and appropriate disinfection of fomites. PsAdV has the ability to withstand environmental conditions for a prolonged period (Greenacre, 2005, Ritchie, 1995). Infectious adenovirus particles are possibly able to be neutralised with an hour's exposure to formalin, aldehydes or iodophores (Greenacre, 2005). To limit the introduction of adenovirus into an aviary, all access of wild birds should be prevented and strict quarantine and biosecurity employed by personnel entering the premises (Greenacre, 2005, Ritchie, 1995).

RATIONALE FOR INVESTIGATING VIRUS PREVALENCE IN VICTORIAN CAPTIVE PSITTACINE BIRDS

Although knowledge on the role of these viruses in disease in psittacine birds is constantly improving, there are gaps in the prevalence information of these diseases in Australia in captive populations and how frequently these viruses co-infect a host. At present, the literature reports psittacine circovirus DNA positive rates of 23% in Australia (Khalesi et al., 2005), 14.8-28% in New Zealand (Ha et al., 2007), 40.4% in Germany (Rahaus and Wolff, 2005), 8% in Italy (Bert et al., 2005), 41.2% for Taiwan (Hsu et al., 2006), and 18.5% in Japan (Katoh et al., 2010). For Psittacine polyomavirus, DNA positive rates are documented as being 0.8% for Italy (Bert et al., 2005), 2.7% for Japan (Ogawa et al., 2006) and 15.2% in Taiwan (Hsu et al., 2006). One serological survey for APV in sulphur-crested cockatoos in Australia identified 64.4% of this wild population was positive for neutralising antibody (Raidal et al., 1998). No such data appears to exist for PsAdV.

The Australian and New Zealand datasets focus on several species in wild populations. The largest dataset collected in Australia included a range of psittacine species, however, it focused on psittacine circovirus and the samples were submitted for testing by referring veterinarians based on a suspicion of disease (Khalesi et al., 2005). Comparatively, the overseas data (except New Zealand) have been surveys conducted on captive populations, with the majority of birds not necessarily showing any clinical signs. The limited information available on the prevalence of these viruses in Australian aviaries and the recent diagnosis of adenovirus infection in a case of sudden death in a captive parrot, has lead to this investigation, which is being conducted as a final year veterinary science student project. This investigation aims to provide an indication of the infection rates of psittacine circovirus, polyomavirus and adenovirus in captive parrot populations in Victoria and the frequency of co-infection with two or more of these viruses.

CONSIDERATIONS FOR SURVEYING CAPTIVE FLOCKS FOR THESE VIRUSES

Attempting to gain DNA positive rates for psittacine circovirus, polyomavirus and adenovirus in Australian aviaries has a number of obstacles. One issue was how to perform sampling in a manner that did not compromise animal ethics legalities. Qualified veterinary clinicians can readily perform such sampling if the tests are for a 'diagnostic purpose'. However, subjecting animals to handling and sample collection for research purposes requires animal ethics approval, which can take many months to obtain. Given that this is a final year student project, with a limited time frame to be conducted in, it was not practical to go through the animal ethics approval process. Therefore, sampling from aviaries was completed in a way where the birds did not need to be directly handled.

As all three viruses are known to be shed in faeces and/or feather dander, attaining feather and faecal samples from test subjects was thought to be appropriate under the circumstances. However, obtaining these from specific test subjects would require some form of handling to pluck a feather and collect individual faecal samples. This would be unacceptable to many aviculturists and violate the ethical considerations. It was, therefore decided, that recently molted feathers and fresh faecal samples from aviary floors would have to be sufficient. Where possible, faecal samples were collected by waiting for the bird of interest to defecate in their usual environment and this was then collected with a sterile swab.

Sample collection in such a manner is not ideal as rarely is there a single inhabitant in an aviary. This creates some ambiguity about the original owner of samples, as contamination of the aviary floor could not be excluded. As samples could not be directly attributed to an individual bird, it has been accepted that a positive result must be attributed to an aviary, not an individual.

How to readily and reliably detect these viruses was the next consideration. Given that the literature describes several polymerase chain reaction (PCR) protocols for identifying these viruses (Katoh et al., 2008), that PCR is often used in the clinical diagnosis of circovirus (Ypelaar et al., 1999) and polyomavirus (Phalen et al., 1991), and at the University there is ready access to a PCR macine and reagents, it was the preferred option. This technique is rapid, and simultaneously offers a high level of sensitivity and specificity, providing the primer choice is appropriate (Luschow et al., 2007). Primer sequences were selected based on published literature and preliminary setup of the PCR protocols were performed by post-graduate students at the University of Melbourne.

The availability of clinically normal birds for routine sampling was another consideration. Contact with an avian veterinarian and local aviculture society provided an avenue for involving aviculturists,

who were happy to volunteer their aviaries for sampling. Ultimately, aviaries sampled spanned along the eastern Victorian coastline from Mornington to Torquay. Aviculturists did stipulate the conditions of anonymity and an assurance that no harm would come to their birds during the sampling process. This reinforced the need for sampling to be conducted in a non-invasive, yet still informative manner.

The risk of sample collectors transporting viruses between aviaries was minimized by employing strict hygiene and disinfection methods before and at the completion of each aviary visit. This included the use of a clean dry pair of coveralls for each aviary, wearing disposable gloves, disinfection of footwear with either F10 or a 1% Virkon solution and application of hand sanitiser at the completion of the visit.

CONCLUSION

Investigating psittacine circovirus, APV and PsAdV in Australian captive flocks will enable further understanding of their epidemiology, prevalence and co-infection rates in aviaries. Published prevalence rates for each of these viruses could be of benefit to Australian clinicians, as it may assist presumptive diagnoses of these conditions. This investigation may also assist in understanding the possible interactions between these pathogens. Ideally, this data will also assist aviculturists by supplying information about how frequently these diseases occur in Australian collections, and the importance of good aviary biosecurity. It is hoped that data from the Victorian survey will be published later this year.

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