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Review Articles

AVIAN POLYOMAVIRUS: A RECENT UPDATE

F. Padzil[1], A. R. Mariatulqabtiah[1,2*] and J. Abu[3]

[1]*Institute of Bioscience, Universiti Putra Malaysia, UPM Serdang, Malaysia*
[2]*Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM Serdang, Malaysia*
[3]*Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM Serdang, Malaysia*

SUMMARY

Avian polyomavirus disease is among the most common viral diseases of domesticated exotic birds as such in psittacine families. Caused by avian polyomavirus (APV) which possess a circular, double-stranded DNA which encodes for major structural virus protein 1 (VP1) and minor structural proteins VP2, VP3 and VP4, the disease is also known as Budgerigar fledgling disease polyomavirus (BFPyV), Papovavirus, and Psittacine polyomavirus. Infections from APV may lead to cutaneous haemorrhage, abdominal distension, feather abnormalities and even death. The APV virus has a broad avian host range and is known to cause acute chronic disease in several psittacine birds such as parrot, cockatoo, macaw, and budgerigar. The current status of APV epidemiology globally has not been fully recorded. Only the studies of the virus and disease caused within several countries are used as references, and few were done together with detection of beak and feather disease virus. Despite the common occurrence of APV among bird breeders in Malaysia, a very limited study has been done to evaluate the prevalence status of APV in Malaysia. In this review, we wish to disseminate knowledge, particularly to pet owners and bird breeders, on APV characterisations, its updated occurrence worldwide and prevention strategies. This information may be useful to trigger in depth study on the epidemiology of disease and better management practises among breeders.

Keywords: avian polyomavirus, psittacine birds, PCR

INTRODUCTION

Avian polyomavirus disease is among the most common viral diseases of domesticated exotic birds as such in psittacine families. Caused by avian polyomavirus (APV), the disease is also known as Budgerigar fledgling disease polyomavirus (BFPyV), Papovavirus, and Psittacine polyomavirus (Hsu *et al*., 2006). The infection of this virus is reported globally, and the first case was discovered in young budgerigars (*Melopsittacus undulatus*) in 1981 (Tomasek *et al*., 2007). The APV virus has a broad avian host range and is known to cause acute chronic disease in several psittacine birds such as parrot, cockatoo, macaw, and budgerigar. Many incidences of APV linked diseases have been reported in young captive birds with high mortality rates (Parrish, 2011). Even though the International Committee on Taxonomy of Viruses appointed the virus name as budgerigar fledgling polyomavirus, but due to its wide host range among avian species, it is now called APV (Katoh *et al*., 2009). The distinct biological and molecular traits of this virus making it being proposed to another different subgenus *Avipolyomavirus* within the genus *Polyomavirus* (Johne *et al*., 2000). The aim of this review is to disseminate knowledge on APV characterisations and its current worldwide occurrence.

Avian Polyomavirus Structure and Genome

APV is a virus under the family of *Polyomaviridae* and genus *Avipolyomavirus*. It is an unenveloped virus with icosahedral viral capsid containing circular double-stranded DNA, with a genome size of 4981 bp (Johne *et al*., 2000; Hsu *et al*., 2006). The DNA has covalently closed ends and complexed with cellular histones in supercoiled form (Johne and Muller, 2003; Parrish, 2011). The icosahedral viral capsid has T=7 symmetry, with diameter around 40-45 nm and comprised with 72 pentamers. The details on the structure of the polyomavirus particle have been well described using X-ray crystallography (Johne and Muller, 2003).

The APV genomes can be categorized into two regions; an early region which codes for tumour (T) antigen, and late region which encodes for structural proteins (Katoh *et al*., 2009). Tumour antigen encoded in the early genes of APV is responsible for viral genome replication as well as host cell transformation (Halami *et al*., 2010). The late region expression is complexed by two putative promoter regions, together with alternative and partial splicing activities (Johne *et al*., 2000). The genome of APV also contains the non-coding regions or regulatory controls which consist of origin of replication (ori), promoter-enhancer sequences and transcription start site (TSS). Starting from the short non-coding regulatory region, both of the early and late regions are being transcribed bi-directionally (Halami *et al*., 2010).

The structural proteins encode within the late region are recognised as major structural virus protein 1 (VP1) and minor structural proteins VP2, VP3 and VP4. VP1 is the major capsid protein of *Polyomaviridae*. It is expressed from VP1 gene of 1032-bp, identified from complete genome of APV at region 1899-2930 (NCBI Accession Number: NC_004764). A capsomer of polyomavirus particle is represented by five molecules of VP1 assembled into a pentametric complex which interacts with either, VP2 and VP3 (Johne and Muller,

**Corresponding author: Dr Abdul Razak Mariatulqabtiah (A. R. Mariatulqabtiah); Phone No : +603 89471938; Fax No :+603 89467590 Email: mariatulqabtiah@upm.edu.my*

2003). Virus-like particle (VLP) of APV has been successfully formed via chicken embryo (CE) cells permissive, where the replication of RNA polymerase 1-based recombinant influenza viruses induced the coexpression of VLP, which is correlated to the nuclear localization of VP1 (Johne and Muller, 2003).

The VP2 and VP3 are translated from the same open reading frame (ORF) but with a different start codon (Halami *et al.*, 2010). The structure of VP3 is similar to the C-terminal region of V2 (Johne and Muller, 2003). A distinct characteristic of avian polyomaviruses from other groups within the genus is the presence of VP4. In the upstream of the VP2 coding region in the avian polyomaviruses, an additional ORF is present which encodes VP4 (Halami *et al.*, 2010). The VP4 protein with molecular weight of 32 kDa is important for APV replication (Katoh *et al.*, 2009). It also interacts with VP1 and double stranded DNA (Johne and Muller, 2003). A leucine zipper motif has been recognised in VP4, which is known to regulate the DNA binding activity (Johne and Muller, 2003). In addition, the expression of V4 has been linked to the induction of apoptosis of the host cells (Johne *et al.*, 2000; Johne and Muller; 2003). An additional protein consisting of the 125 C-terminal amino acid residues of VP3 has been recognised in simian virus 40 (SV40) of mammalian polyomavirus and named VP4. However, the VP4 in SV40 has no similarities in terms of sequence, structure and biological properties compared to the VP4 of avian polyomaviruses (Daniels *et al.*, 2007).

Phylogenetic Relationships of Polyomavirus

The family *Polyomaviridae* are categorised as group 1 virus in the Baltimore classification system as they comprise double stranded DNA genomes (Neu *et al*., 2008). In 2015, taxonomy released by International Committee on Taxonomy of Virus documented 73 species within this family under four genera (*Alphapolyomavirus, Betapolyomavirus, Deltapolyomavirus* and *Gammapolyomavirus*). APV is assigned under the genus *Gammapolyomavirus*or *Avipolyomavirus* (Johne *et al*., 2011). *Polyomaviridae* shares the similar virion structure as *Papillomaviridae*, comparable genome organization, mechanisms of replication, cell cycle regulation as well as tumour induction (Parrish, 2011). Members of both families undergo replication in nucleus, where *Polyomaviridae* DNA is transcribed from both strands, while *Papillomaviridae* DNA is transcribed from only one stand (Johne *et al.*, 2000; Parrish, 2011). Several distinct features have been observed between mammalian polyomaviruses and APV as such in region encoding for large tumour (T) antigen, non-coding regulatory region as well as agnoproteins (Johne *et al*., 2000).

The family *Polyomaviridae* consists of different polyomaviruses that infect many species such as humans, monkeys, rats, hamsters, and many species of birds majorly in a host-specific interaction (Halami *et al.*, 2010; Johne *et al.*, 2006). The first member of *Polyomaviridae* described is the mouse polyomavirus (mPyV) in 1953 on the transmission of mouse leukaemia (Neu *et al*., 2008). Polyomaviruses are very host specific despite the flexible and wide range detection methods. There was no report of polyomavirus first discovered in an organism and later detected in another, except for APV and SV40. Human polyomaviruses are among the species that have been discovered in depth throughout these recent decades, which include human BK polyomavirus, JC polyomavirus, KI polyomavirus and WU polyomavirus (Johne *et al*., 2011). Several types of polyomaviruses have been identified to represent polyomaviruses of birds, which are APV, finch polyomavirus (FPyV), crow polyomavirus (CPyV), goose haemorrhagic polyomavirus (GHPyV) and canary polyomavirus (CaPyV) (Guerin *et al*., 2000; Halami *et al.*, 2010; Johne*et al.*, 2006; Stoll *et al.*, 1993). Based on nucleotide sequence identity of greater than 50%, it has been found out that CaPyV is most closely related to APV and FPyV (Halami *et al.*, 2010).

The phylogenetic, biological and structural data support the groupings of bird polyomaviruses in a separate group under the family *Polyomaviridae* (Halami *et al.*, 2010; John and Muller, 2003). Based on the sequence analysis of genome, it has been found out that avian polyomaviruses segregate independently from the mammalian ones (Parrish, 2011). This indicates a long-standing evolutionary divergence, where the viruses group might end up in different genera in the future.

Symptoms of Avian Polyomavirus Disease

APV is an etiologic agent of a lethal multisystemic disease in different bird species scrutinised around the world (Johne and Muller, 2003). Presumptive diagnosis of APV infection can be deduced according to the history, clinical findings, and histopathologic lesions and characteristic gross (Mamom *et al*., 2009). Records of natural and experimental infections of APV in budgerigars have shown major pathological symptoms such as hydropericardium, ascites and hepatitis (Halami *et al.*, 2010; Johne and Muller, 2007).APV also induces histologic lesions focal necrosis with enlarged clear basophilic intracellular inclusions in the spleen, liver and many other tissues (Bert *et al*., 2005; Parrish, 2011). Disease symptoms are associated with increased mortality of the young captive psittacines. APV infections may cause acute lethal death without premonitory signs, unlike mammalian polyomaviruses infections which are usually harmless yet persistent towards the non-immunocompromised hosts (Johne *et al.*, 2000). In most cases, the birds affected with APV were showing temporary clinical warnings such as paleness, lack of appetite, anorexia, subcutaneous haemorrhages and crop stasis before dying suddenly (Parrish, 2011). In critical form, APV disease results in full crops, reddened skin, feather dystrophy, scattered cutaneous haemorrhages, enlarged heart and liver, and eventually acute death Parrish, 2011).

The incidences of diseases associated with APV have been reported frequently from captive population. Statistical analysis showed that the APV infection in birds from shelters and rescue centres is much higher than in veterinary clinics or in their natural habitats (Dolz *et al.*, 2013). The birds living in captivity have closer proximity to each other, hence the virus could spread much easier

among the population. The fact that the virus could remain in the faecal for months increase the chance of infection to the new-born hatchlings or recently arrived captives. The number of affected nests raised during the breeding season, and the figures went higher as the nestling cycles continued (Tomasek *et al*., 2007). Nestlings in captivity between as early as 1 week to 5 months of age are most vulnerable to this virus, having a mortality rate up to 100% (Hsu *et al*., 2006; Tomasek *et* al., 2007). APV induced disease is usually characterised by deaths in hand-raised nestlings of non-budgerigar psittacine species (Tomasek *et* al., 2007). The infected birds can shed the virus in the faeces for up to 6 months (Parrish, 2011). A high prevalence of APV infection and virus shedding were recognised in asymptomatic cockatiels, which lasted for more than 18 months due to possible tolerance of the host to the APV (Tomasek *et al.*, 2007). The avian species that survived from the APV infection during young stage would develop feather ailment as a significant symptom (Johne and Muller, 2007).

The AVP disease may be linked with a milder disease of budgerigars called French moult, which results in chronic disorders of feather formation (Parrish, 2011). Subclinical infection of polyomavirus has been identified in zebra finches (*Poephila guttata*), kookaburra (*Dacelo novaeguineae*) and Ross's turaco (*Musophaga rossae*) (Parrish, 2011). While most of the birds found to be infected with APV were in grown in captivity, the only species shown to have APV infection in its own natural habitat is the sulphur-crested cockatoo (*Cacatua gallerita*) in Australia (Parrish, 2011).

Status of Avian Polyomavirus Worldwide

The current status of epidemiology of APV all over the world has not been fully recorded. Only the studies of the virus and disease caused within several countries are used as references, and few were done together with detection of beak and feather disease virus (BFDV) (Bert *et al*., 2004; Dolz *et al*., 2013; Hsu *et al*., 2006,). Clinical diagnosis and molecular analysis such as polymerase chain reaction (PCR) and rolling circle amplification (RPA) are among the methods used to verify and characterised the presence of this virus. The samples taken were from different sources, either veterinary clinics, captive breeders, rescue centres or shelters.

A total of 269 samples from 19 psittacine species were showing APV infection at the rate of 4.8 % (13/269) in Costa Rica between 2005 and 2009 (Dolz *et al.*, 2013). A prevalence of 19.7% of BFDV was detected in the same study, while 3.3% of the birds were found out to be infected with both APV and BFDV disease (Dolz *et al.*, 2013). Based on this study, birds infected with BFDV would have 6.24 times more chance to be infected with APV (Dolz *et al.*, 2013). Within the same duration of years in Poland, a total number of 751 symptom–free birds from 31 genera and 59 species were tested for BFDV and APV (Piasecki and Wieliczko, 2010). Results show that the occurrence of BFDV was 25.3% and APV was 22.2%, a percentage that is enough to spark a concern. However, in Italy, APV was detected in only 0.8% birds from a total of 877 samples (Bert *et al*., 2004). The samples were collected from 18 Italian psittacine breeding centres and four trade centres for over a 4-year period. High mortality of nestling cockatiels (*Nymphicus hollandicus*) was studied in a breeding flock of exotic birds in Central Slovakia, showing all nestlings in affected nests of 14 breeding adults died within 1 week of age (Tomasek *et al*., 2007). However, from a survey in Germany, there was no positive detection of APV infection in 85 symptom-free birds of 20 different psittaformers from 30 breeders all over the country (Rahaus and Wolff, 2004). This indicated that the occurrence of persistent APV infection among the captive psittacine populations in Germany is relatively low.

In South-East Asia region, several surveys have been conducted to screen the epidemiology of APV disease infection. In Taiwan, from a total of 165 psittacine birds belonging to 22 genera, 15.2% were found out to be positive of APV (Hsu *et al.*, 2006). A study done in Thailand showed that 8.1% or 10 over 124 samples were positive to APV via multiplex polymerase chain reaction on captive psittacine samples of 10 genera and 20 species (Fungwitaya *et al*., 2009). A recent local study in Klang Valley, Malaysia, has shown the presence of APV in six out of 30 pooled samples (20.0%) of psittacine birds from four different breeders (Zanon, 2016).

Vaccine against Avian Polyomavirus

After the discovery of APV, efforts on inventing an effective vaccine against the virus did not end up in success due to the presence of subclinical infections and acutely diseased carrier birds (Ritchie *et al*., 1998). The lack of knowledge in characterising the epidemiology and pathophysiology of the infection has affected the vaccine discovery too. Consequently, positive incidences APV in many studies have signified the spread of this virus worldwide. This resulted in substantial economic deficits and psychological distress for aviculturists and pet store owners every year. Hence the demand of preventing the virus infection and minimising its impact are increasing rapidly.

Virus neutralisation techniques as well as immunodiffusion have been utilised to establish anti-polyomavirus antibodies in psittacine birds since the 1980s. Several types of conventional adjuvants were evaluated to identify their effect on the immunogenicity of inactivated APV, which includes Equimune, E-3, Acemannan and mineral oil (Ritchie *et al*., 1994). The adjuvants were however found to cause muscular necrosis, and the oil-adjuvanted vaccines were correlated to death of the treated samples (Ritchie *et al*., 1998).

The introduction of inactivated APV vaccine by Biomune Company (USA) was proposed to be instrumental to cease the outbreaks of APV disease in 9 parrot nurseries (Phalen, 1998; Ritchie *et al*., 1998). The utilisation of the virus vaccine approved by USDA was reported to halt the mortality in each aviary within 4 weeks twice vaccinations. The inactivated avian polyomavirus vaccine safety was evaluated in 1823 psittacine birds from more than 80 species (Ritchie *et al.*, 1998). The study has also shown that the APV vaccine is

immunogenic and efficacious in mature and immature psittacines. The example of current vaccine available is Psittimune® APV by Creative Science (USA), which contains antigenic isolate of APV and adjuvant for enhanced response to vaccination. Primary immunization is given to young birds at 5 weeks of age, and the second dose is given after 2-3 weeks after the initial one.

In term of prevention, management practice itself is adequate to avert the introduction of APV into a nursery (Phalen *et al*., 2000). Up to now, there is no known treatment for APV disease. While vaccination is available, this option may cost as much as USD40-60 per bird and booster shots are essential each year. Certain psittacine species are still susceptible to the virus infection. Most of the adult psittacines are rarely to develop the disease, as the exposure towards the virus at heightened natural body immunity in correlation with age. Besides, the effectiveness of the vaccine in younger birds is in question, as the nestlings would still be having a risk of being exposed to the APV infection before they complete the full vaccination.

CONCLUSION

While typical infection of APV occurs in a well-fleshed juvenile and just before fledgling age, most of adult birds can act as a carrier of APV without showing any clinical warnings to other susceptible birds around them. Therefore, containment of the virus is relatively difficult. An advisable method of prevention would be to isolate the young birds until they complete their full vaccination. Clinical diagnosis and molecular analysis from swabs and faecal samples can also be done routinely to verify the circulation of this virus.

CONFLICT OF INTEREST

None of the authors of this paper has any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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