Artykuł przeglądowy *Review*

Avian polyomavirus infections in Amazon parrots

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Summary

According to the available literature, budgerigars are the most susceptible to being infected with avian polyomavirus (APV), whereas this infection is very rare in Amazon parrots. Although the same virus is responsible for the disease, clinical symptoms in the Amazon parrot are considerably different than those observed in budgerigars. APV is transmitted primarily bird-to-bird but it is also thought to be transmitted via the egg. Many affected young amazon die, while most infected adult birds develop lethargy, poor appetite and diarrhoea, with the surviving birds developing antibodies to the virus. However, despite the common misconception, that adult birds are more resistant, the adult amazon are readily susceptible to infection, can become ill and some may die. The main clinical symptoms of APV infection in the amazon parrot include hepatomegaly, ascites and hydropericardium. Necropsy often show spleno- and hepatomegaly with irregular red and yellow mottling of the liver, while histopathological examination present pathognomomic lesions as multifocal necrosis in the liver and kidney, enlarged nuclei and enlarged amphophilic intranuclear inclusions in the liver, kidney and spleen. Procedure against APV infection in an outbreak requires vaccinating the adults and neonates to stimulate flock immunity, as well as cleaning and disinfecting the contaminated facility.

Keywords: amazon parrots, avian polyomavirus, amphophilic intranuclear bodies, PCR (Polymerase Chain Reaction)

The avian polyomavirus (APV) is one of the most significant viral pathogens of cage birds which leads to substantial economic losses for aviculturalists and pet store owner each year. Since the APV was identified as an etiologic agent of disease in psittacine birds, the virus has spread throughout aviaries and the pet trade to a point where it is a leading cause of death in psittacine birds (2, 9). It should be noted that while the polyomaviruses that infect budgerigars, finches and larger psittacine birds like the amazon exhibit similar clinical presentation, the distribution of lesions and problems that the viruses causes within a flock are dramatically different among susceptible species (14).

Amazon parrot is the common name for parrots of the genus *Amazona*. These are medium-size parrots native to the New World ranging from South America to Mexico and the Caribbean. Most Amazon parrots are predominantly green, with accenting colours that depend on the species and can be quite vivid. Many amazon parrots have a remarkable ability to mimic human speech and other sounds. Partly because of this they are popular as pets or companion parrots and a small industry has developed in breeding parrots in captivity for this market (7, 28).

Properties of APV and infections aetiology

APV belong to the group of DNA viruses and has been classed as a papovavirus. The family *Papovaviridae* no longer exists, it has been divided into two families: papillomaviruses and polyomaviruses, with APV being one of the latter (18). The infection was called first budgerigar fledgling disease (BFD) but is now called APV infection because of its broad host range (20). APV is a 40- to 50-nm diameter icosahedral non-enveloped virion with a 4.8- to 5.5-kb circular double stranded DNA genome (15, 18). APVs are thermostable, can withstand freeze-thawing and heating at 56°C for 2 hours. APV is also resistant to organic solvents. The environmental stability of the virus causes a considerable problem in birds because persistently infected birds can shed the virus in their excrement or feather dust. Manual removal of any contaminated organic material followed by the application of a disinfectant is required to prevent or interrupt a disease outbreak (29).

BFD was first noted as a clinical syndrome in 1976 and was first reported as a disease affecting budgerigars (*Melopsittacus undulatus*) in the USA and Canada

in 1981. APV infections have been described throughout the world (4, 8, 10, 11, 14, 15, 17, 20-23, 27), (Phalen D. N.: Avian polyomavirus: my thoughts. www. blackstone – aviaries. com/polyom. 2007). Characteristic morphological lesions associated with the virus have been demonstrated in companion birds *inter alia* from Canada, USA, Italy, Japan, Hungary, Australia, Germany and Poland (5, 8, 17, 18, 21-23, 27). Based on the literature, the described virus is relatively frequent and serious cause of morbidity and death in birds cages and zoos (1, 7, 9). This virus is spread among budgerigars and causes BFD (14, 16, 21). However, APV appear to infect a wide variety of Psittaciformes (parrots), Passeriformes (weaver finches, canaries) and also gallinaceous birds, including chickens and turkeys (22, 27, 29).

Pathogenesis and the course of infection

The clinical pattern is referred to as „French moult" and parrots with such symptoms as „race parrots". Such names arise from feather abnormalities, frequently caused not only by polyomaviruses, but also by circoviruses (7, 11). According to literature data, the feather abnormalities that are common with polyomavirus infections in budgerigars are described less frequently in amazon parrot (2, 14, 21, 23). It seems that APV are probably capable of causing disease in all psittacine species. However, nestling and juvenile birds are most susceptible (21, 14, 17, 22). As the authors report, most adult amazons infections are asymptomatic and go unrecognized (3, 23). The majority of birds that die of APV infection are hand-raised nestlings (regardless of whether those are budgerigars, more susceptible to infection, or much more resistant Amazon parrots) (23). Interestingly, parent-raised amazon (not budgerigars or lovebirds) do not seem to become diseased, but excrete the virus for up to 12 weeks (22). The course of the disease depends on the species, age and the condition of birds' immune system. Young parrots are more susceptible to infection due to their poorly-developed immune system. The exact incubation time is unknown, but its estimated duration ranges from 1 to 2 weeks. A high mortality rate is observed in budgerigars (20% to 100% if the virus appears in the flock for the first time) between the 15th and 19th day of life, whereas in amazons, the disease symptoms can be observed between day 20 and day 56 of their lives, with a lower mortality rate (8, 21).

Some parrots are more susceptible to APV infections, while in others the disease never occurs (14, 23, 27). The most susceptible to infection include: budgerigars, aras, conures, lovebirds and rose-ringed parakeets (7, 11, 14). The disease is observed much less frequently in cockatiels and amazons (27, 28). The occurrence of symptoms, their intensity and character, depend on the parrot's species and age at which it was infected. It has been shown that APV infection does not always manifest itself with clinical symptoms. Young birds are more frequently infected than adult ones; the most susceptible amazons are those before 14th week of life, and a bird dies within 48 hours of the symptoms' appearance (1, 4). According to literature reports, 99.9% of infections in adult birds are asymptomatic. Such birds may be a source of infection, but they themselves never show any symptoms (3).

Cases of acute APV in adult amazons have been described. Such course of the disease is frequently caused by immunosuppression, this in turn being caused by infection with PBFDV (Psittacine Beak and Feather Disease Virus); the following are usually mentioned as inducing the appearance of clinical symptoms: stress associated with changes in weather, diet, breeding or concomitant disease – tab. 1 (2, 21).

Horizontal transmission is the major method of infection. Many birds are infected subclinically and they spread the virus through feces, urine and respiratory secretions. Infection persists in the kidneys of carrier birds and the virus can be excreted intermittently in the droppings, probably during times of stress (19). A carrier can shed the virus while showing no signs of disease, infecting any susceptible birds that it encounters. Interestingly, it is rarely the amazons that die from APV that are the source of the virus, but rather it is the birds that remain normal that are the likely carriers of the virus and are responsible for introducing it into a nursery and pet shop. APV may multiply in feather follicle and is thus disseminated with feather powder, especially during a period of increased susceptibility to stress, e.g. in the breeding season.

Tab. 1. Incidence of Avian polyomavirus (APV) infection and co-existence of APV and Psittacine Beak and Feather Disease Virus (PBFD) in amazona from Poland (samples were collected between 2006-2009 from symptom-free birds, by means of a PCR assay) (21)

Genus	Species	Number of birds tested	Number of APV positive birds	Number of APV and PBFD positive birds
Amazona	Amazona aestiva	36	9	0
	Amazona amazonica	25	4	0
	Amazona autumnalis	5	0	0
	Amazona barbadensis	8	6	2
	Amazona brasiliensis	1	0	0
	Amazona farinosa	5	1	0
	Amazona festiva	7	0	0
	Amazona finschi	4	0	0
	Amazona leucocephala	4	1	0
	Amazona ochrocephala	14	2	0
	Amazona oratrix	4	0	0
	Amazona sp.	23	2	0

Tab. 2. Avian polyomavirus infection of amazon diagnosed by routine histopatology, DNA in situ hybridization, and DNA amplification with Southern or dot blot analysis (8)

Species	Age	Nuclear inclusions	Karyomegaly	Hybridization	Amplification/ blotting	Causes of hepatic necrosis
Blue-fronted Amazon parrot	3,5 years	–	–	–	–	Gram-negative rods
Double yellow-headed Amazon parrot	2 months	+	+	+	+	APV
Red-lored Amazon parrot	Not determined	+	+	+	+	APV

Infection may spread both along the horizontal and vertical route – asymptomatically infected birds hatch infected chicks, thereby disseminating the virus (1, 8, 22). The parents may transmit the virus to their offspring while feeding them with crop secretions, containing exfoliated epithelial cells. It has been observed that the older the infected birds are, the shorter the period of virus spreading is; for example, adult hyacinth macaw disseminate the APV virus for six weeks or less (7, 29).

The clinical pattern of the disease and anatomopathological lesions

According to literature data, amazon parrots may die suddenly without signs of illness or die after showing depression, anorexia, weight loss, delayed crop empting, regurgitation, diarrhoea, dehydration, subcutaneous haemorrhages, ataxia and paralysis. Clinical signs are common at weaning and infected fledglings often die 12-48 hours after the development of clinical signs (22, 23). A chronic form of APV is also thought to exist which causes body mass loss, intermittent anorexia, polyuria, recurrent bacterial and fungal infections (8). There may be a lack of down feathers on the back and abdomen or symmetrical feather abnormalities characterized by abnormally formed primary and tail feathers (2, 9).

Anatomopathological lesions which occur in amazon parrots in the course of APV infection include the crop filled with food, hydroperitoneum, enlarged liver with light focal lesions, splenomegaly, enlarged pale kidneys, extravasations in the central nervous system, fluid in the pericardial sac, extravasations under the epicardium, pulmonary oedema, numerous extravasations in skeletal muscles and skin and generalized pallor (4, 5, 9).

Microscopic pattern of internal organs

The most typical changes for the APV infections affect the liver (3). They include extravasations, karyomegaly and enlarged amphophilic intranuclear inclusions, multifocal necrosis with cellular infiltrations consisting of heterophiles and plasmatic cells. In the amazon parrot, inclusion bodies are found only in the spleen, kidney and liver. In cells with karyomegaly, the nucleus is enlarged, has marginated chromatin and often contains a large slightly amphophilic inclusion (5, 8).

Polyomavirus inclusion bodies can also frequently be detected in tissues from persistently infected, clinically normal adult amazon. According to literature reports, histopathological analyses of kidney sections in some cases show different degrees of renal tubules epithelium necrosis as well as interstitial infiltrations of inflammatory cells and extravasations with hyperaemia (12, 19). Decreasing number of lymphocytes has been observed in the spleen and in the bursa of Fabricius (3).

Diagnosis

A presumptive diagnosis of APV infection can be made from the history, clinical and pathological features. However, histopathological, bacteriological and serological investigations should be used to rule out differential diagnoses.

There are a number of methods which make it possible to detect APV infection. These include: antibody assay, virus isolation, the presence of intranuclear amphophilic incrusions, observation of virions under an electron microscope as well as polymerase chain reaction (PCR) (tab. 2). Their utility varies. Virus isolation is expensive and time-consuming, similar to immunohistochemical examinations (10, 13). Serological examination is based on the assay of plasma or serum antibody level, which grows rapidly, reaching the maximum concentration within 4 to 6 weeks of infection. Antibodies persist in the blood for months or even years, depending on the bird species. In practical terms, such analyses are being given up as they only inform about the presence of infection itself and not about the virus dissemination. Moreover, a number of researchers maintain that it is T lymphocytes rather than antibodies that are necessary to eradicate the virus from the bird's body (10, 22).

The test of choice is PCR, whose popularity is growing. It makes it possible to detect a small amount of the virus' DNA. Its sensitivity is also both the method's greatest advantage and disadvantage, because even the smallest contamination of a sample in a laboratory may cause falsely positive results (12, 16).

Unfortunately, the presence of amphophilic intrusions in hepatocytes' nuclei does not make BFD diagnosis certain, because other viruses such as adenovirus, herpesvirus and psittacine beak and feather disease virus can produce amphophilic to basophilic intranuclear inclusions in psittacine tissues.

Disease prevention

In aviaries where the amazon parrot are being raised, the aviculturalist should be encouraged not to keep and breed budgerigars, lovebirds or cockatiels. All new birds entering the aviary must be quarantined and tested for APV by PCR before they are put in with the breeding birds (12). Preventing the polyomavirus infections within a flock requires the vaccination of two groups of birds: the breeding flock and the young birds. Adults are vaccinated to reduce the spread of the virus among the resident population in aviary. Neonates are vaccinated to protect them before they leave the aviary and are exposed to other birds which may be shedding the virus (23, 24). Amazon parrots in which a polyomavirus vaccine has been evaluated for safety are: blue-fronted Amazon parrots, green--cheeked Amazon parrots, Hispaniolan Amazon parrots, lilac-crowed Amazon parrots, mealy Amazon parrots, orange-winged Amazon parrots, red-lored Amazon parrots, spectacled Amazon parrots, tucuman Amazon parrots, yellow-crowned Amazon parrots, yeallow-headed Amazon parrots and yellow-naped Amazon parrots (24-26). Vaccination play a pivotal role in reducing the incidence of APV infection. However vaccination shouldn't be expected to completely combat the deleterious effects of poor management or hygiene. Aviculturists, with aviaries where the disease does not occur should be encouraged to maintain a closed flock with strict quarantine and hygiene procedures. This includes eliminating exposure to free-flying wild birds and regulating food, utensils and humans with access to the birds.

APV most probably remain infectious under the fingernails for long periods. Incubators and brooders must be capable of being thoroughly disinfected and cleaned between clutches (using 2% Virkon S). New stock should be obtained from seronegative and APV--free aviary flocks. Birds must be held in quarantine and confirmed as APV-free preferably both by serology and DNA-probe before being incorporated into the breeding flock (26).

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