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Psittacine Beak and Feather Disease, Budgerigar Fledgling Disease and Aspergillosis in an African Grey Parrot (*Psittacus erithacus*)

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Abstract : A five-month-old African grey parrot was presented with alopecia, yellowish diarrhea, depression, and paralysis in the veterinary medical center, Chungbuk National University. The patient died 3 h later after hospitalization. For the accurate diagnosis, necropsy was performed and fungi were detected in the air sac. PCR was done for the viral detection which caused the alopecia, and for the species identification of fungi. Final diagnosis was a multi infection with avian circoviruses that caused psittacine beak and feather disease (PBFD), avian polyomavirus cause budgerigar fledgling disease (BFD), and *Aspergillus fumigatus*. This is the first report of a multi infection in South Korea.

Key words **:** avian circovirus, avian polyomavirus, aspergillus, psittacine beak feather disease.

Introduction

PBFD in over 35 species of Old World and New World *Psittaciforms* and these viruses belong to the family Circoviridae. It has small, icosahedral shape, and is non-enveloped. They contain single-stranded circular DNA, approximately 1.7-2.0 kb in length (14,18).

PBFD is a chronic, progressive and irreversible viral disease. However, it can cause severe, acute, disease syndrome in nestlings and African grey parrots (*Psittacus erithacus*) (8). Its clinical signs include symmetrical feather loss and dystrophy, and occasionally, beak deformities (14).

Avian polyomavirus (APV) causes BFD in psittacine birds (7). APV has icosahedral shape and has no envelope. It contains circular double-stranded DNA of 4981 bp. DNA can divide into two parts: early region and late region, by its function. The early region encodes large T and small t antigen. The late region encodes structural proteins, such as VP1, VP2 and VP3, and protein with unknown function (12). The clinical signs of BFD are acute death, abdominal distention, and a feather abnormality known as French molt. It was detected by the lack of a down feather of back and abdomen, piloplumes of head and neck and subcutaneous hemorrhage of nestling budgerigars. In older birds, it is a chronic disease with feather abnormality (8).

Aspergillosis is a fungal disease caused by genus Aspergillus (*A.*), in particular, *A. fumigatus* and *A. flavus*: it has been described in many wild bird species, both in the natural and captive states (3). These fungi are ubiquitous, so they can be detected in soil, feeds as grains and decomposing vegetables (3). They make the spores, which are scattered in the environment and these spores penetrate aerial apparatus, which offers the ideal conditions to germinate. Then these fungi produce the vegetative forms and invade the tissue. The target organs are the lungs and air sacs. However, these fungi can also affect the liver, the kidneys, the bones, the skins and the eyes (1).

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Case

A five-month-old African grey parrot was presented with alopecia (Fig 1), yellowish diarrhea, depression, and paralysis in the veterinary medical center. The patient started eating grain instead of weaning food. It lived in the house with a Bengalese finch in each cage. Its appetite and vitality were in the normal condition three days before presentation in the veterinary medical center. The owner observed depression and yellowish diarrhea two days before that. An alopecia of dorsal and tail feathers and paralysis of right leg was noticed during the physical examination. The initial supportive care was a fluid therapy (Hartman with 5% dextrose 6 ml) and antibiotic administration (cefotaxime natrium, 100 mg/kg IV bid). Despite these supportive care, the patient died a few hours afterwards. To define the cause of death, necropsy and molecular diagnosis were performed.

At necropsy, gross lesions were detected in the air sacs. The white to yellow fungal plaques germinated in air sac were found to be located in the right thoracic area. Fungal hyphae were observed on the imprint of the lung dissection (Fig 2). A part of fungal organism was taken from the colony in air sac for a polymerase chain reaction (PCR) to accurately diagnose the species of fungi. According to the clinical signs and regions growing feathers were taken from the alopecia area

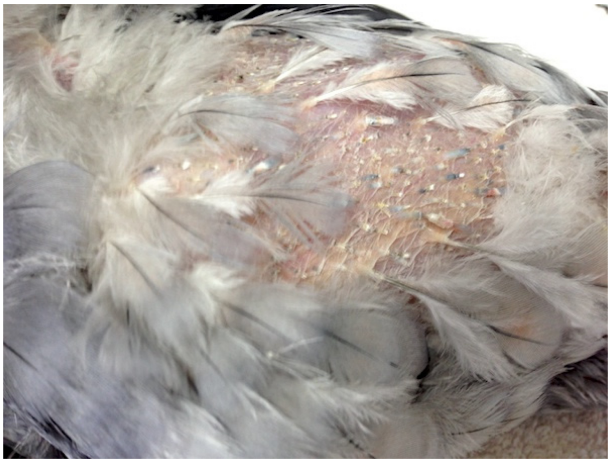

Fig 1. The African grey parrot patient with alopecia.

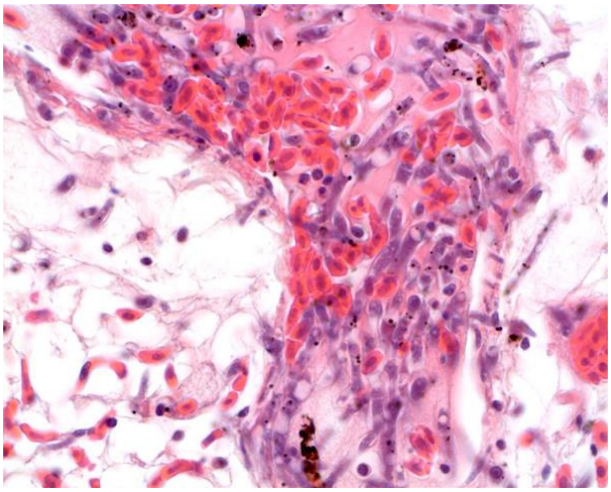

Fig 2. Fungus hyphae on slide imprint of the lung dissection. Many hyphae were detected around alveolar with red blood cells. H&E stain, × 1000.

for PCR for the accurate diagnosis of the viral disease (PBFD and BFD).

To identify the fungi, Ambion MagMAX™ (Life technologies, USA) total nucleic acid isolation kit was used for DNA extraction following the manufacturer's protocol. The i-Star-Taq™ DNA polymerase (iNtRON Biotechnoogy, South Korea) and Takara PCR Thermal Cycler Dice (Takara Bio Inc., Japan) were used for PCR. The primer sequence (ITS 1: 5'-TCCGTAGGTGAACCTGCGG-3', ITS 4: 5'-TCCTCCGC-TTATTGATATGC-3') was associated with fungal ITS region (10). Reactions were composed of initialization (94°C, 30 s), denaturation (94°C, 30 s), annealing (56°C, 30 s), extension step (72°C, 30 s) and final elongation (72°C, 3 m). Reactions were done for 40 cycles. BLAST search indicated that the sequence of the product matched up 100% with *A. fumigatus* gene for 18S ribosomal RNA, partial sequence, isolated from *A. fumigatus* Za79 (Accession No. AB976024.1)

Viral DNA was extracted from the feathers, which were collected from the patient. Ambion MagMAX™ total nucleic acid isolation kit (Ambion®, USA) was used for DNA extraction following the manufacturer's protocol.

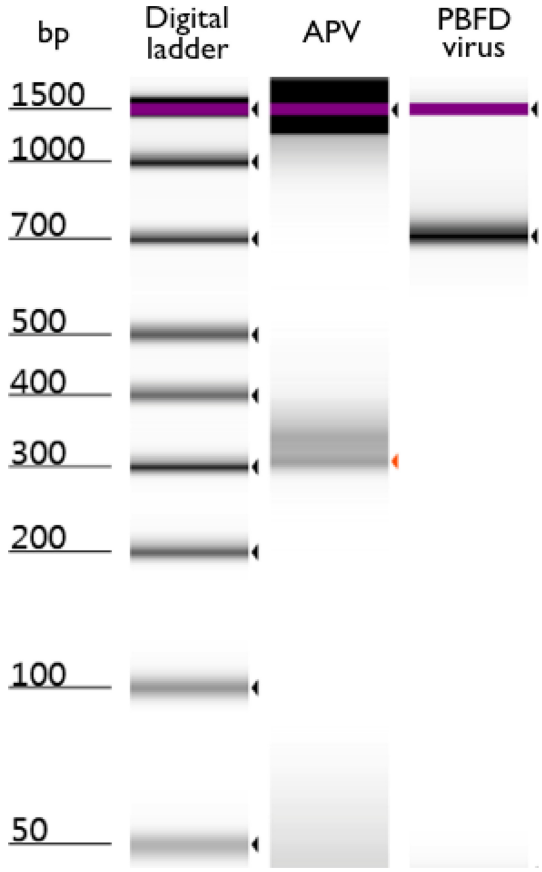

Fig 3. Electrophoresis of PCR products with 2200 TapeStation. PBFD (psittacine beak and feather disease) virus band was strong and appeared around 710 bp and APV (avian polyomavirus) band was weak and appeared around 310 bp.

To identify PBFD virus, primers were PBFDV2-f (5'-AAC CCT ACA GAC GGC GAG-3') and PBFDV4-r (5'-GTC ACA GTC CTC CTT GTA CC-3') that were open reading frame 1 (ORF1) within the genome of PBFD virus. The primer was already reported and PCR amplicon was 710 bp (16,17). Reactions were composed of initialization (94°C, 2 m), denaturation (94°C, 30 s), annealing (58°C, 30 s), extension (72°C, 1 m) and final elongation (72°C, 5 m). Reactions were done for 40 cycles.

To identify APV, primers were APV A-f (5'-CAA GCA TAT GTC CCT TTA TCC C-3') and APV B-r (5'-CTG TTT AAG GCC TTC CAA GAT G-3') that were small t antigen encoding and large T antigen gene intron region of the APV (12,13). Reactions were composed of initialization (94°C, 2 m), denaturation (94°C, 30 s), annealing (58°C, 30 s), extension (72°C, 30 s) and final elongation (72°C, 3 m). Reactions were done for 40 cycles. PCR amplicon size was 310 bp.

Electrophoresis about the PCR product of PBFDV and APV was performed with 2200 TapeStation (Agilent Technologies, Inc., USA). PBFD virus (710 bp) and APV (310 bp) PCR amplicons were identified (Fig 3).

The sequenced PBFD virus and APV amplicon matched up 100% budgerigar fledgling disease polyomavirus (Accession No. KT203769.1, KT203764.1, AB453164.1, FJ385773.1) and 98% beak and feather disease virus (Accession No. KM-

409545.1), respectively.

Discussion

Avian patient with alopecia is a very common case in animal hospital and there are many reasons for this condition, including viral diseases, bacterial diseases, fungal diseases and psychological problems (15). Bacterial and fungal diseases can be ruled out by the microscopic examination about alopecia area. Samples are easily taken by a cotton swab or the taping method. Viral diseases are also easily detected by PCR. Growing feathers in the alopecia area are very good samples for PCR to detect a viral disease. PBFD and BFD are considered to be common viral diseases that can cause alopecia in avian species (6).

PBFD virus and APV were detected in the feather samples from the patient by PCR. Co-infection rates with the PBFD virus and APV are vary across countries (2,4,6,11,14). Although relevant data were not published, we detected the PBFD virus in the five ill psittacine patents. Co-infection with APV was only the case presented in this study. PBFD and BFD are chronic diseases, but can cause sudden death after the onset of clinical signs onset. We supposed that acute progress was due to Aspergillus infection. In necropsy, aspergillosis was found in air sac of the right thoracic area. Although lung looked like a clean in necropsy, the spores and hyphae were observed on the imprint slide-glass of the lung dissection. A germinated fungus hyphae can invade capillary beds. It makes a critically worse status of the patient when co-infected with other diseases (1,9).

Recently, PBFD was detected in all over the country with a great frequency because of the international trade of the birds (14). Its clinical signs, especially alopecia, are very similar to those of BFD. It is necessary to diagnose these two viral diseases (5,18). In this situation, PCR is a very useful diagnostic method. It is definite to compare with a certain positive sample of each disease, making it possible to give a quick report for prompt clinical treatments.

Conclusion

The African gray parrot was diagnosed with a multi infection with PBFD virus, APV, and *A. fumigatus*. This case is the first report of such infection in South Korea.

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