

Pulmonary Aspergillosis Secondary to Open Fracture of Pneumatic Bone in a Brown Hawk Owl (*Ninox scutulata*)

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(Accepted: June 10, 2013)

Abstract : A flightless brown hawk owl (*Ninox scutulata*) weighing 180 g was rescued and referred to the teaching veterinary hospital with humerus open fracture. On physical examination and radiography, open fracture of the left humerus was found. After 3 days, orthopedic surgery was operated with intramedullary pins and wires. The bird died 2 days after surgery with anorexia. On necropsy, multiple green nodules with 2-3 mm in diameter were observed at the surface and inside of the left lung. Numerous conidial heads and spores were seen in the center of foci in the histopathological examination. The mycelia penetrated the surrounding pulmonary parenchyma, showing inflammation and necrosis. The fungus was isolated from the lung and cultured on Sabouraud Dextrose Agar at 30°C for 7 days. The colony was blue-green color with a powdery surface. The fungus was identified as *Aspergillus fumigatus* by DNA analysis, including the internal transcribed spacer region, partial β -tubulin, and the calmodulin gene. This case was diagnosed as pulmonary aspergillosis secondary to open fracture of pneumatic bone in a brown hawk owl.

Key words : *Aspergillus fumigatus*, pulmonary aspergillosis, humeral fracture, Brown hawk owl (*Ninox scutulata*).

Introduction

Aspergillosis caused by Genus *Aspergillus* (*A.*) has been reported in mammals and birds (3). In humans, it is second common fungal disease after candidiasis (5). *A. fumigatus* is the most common causative agent, but *A. flavus*, *A. niger*, *A. glaucus* and *A. nidulans* have also been isolated. *A. fumigatus* is world widely distributed saprophyte involving in the recycling of carbon and nitrogen. Because of high sporulation capacity, the fungus has high density in the air ranging 1 to 100 conidia/m³ and makes a polypeptide allergic antigen, fungal toxin or β 1 & 3-glucan which has been known as immunomodulator. The spore of the fungus usually reaches to the alveoli, thus it causes the infection in the pulmonary epithelium. In healthy state, the alveolar macrophage or type 2 pneumocyte phagocytes the fungal conidia whereas the fungus makes germinated hypha invading into the pulmonary epithelium in the immunosuppressive state (13). As wet and warm environment such as feedstuff or sawdust bed stored in wet storage box facilitates the growth of *Aspergillus* species, domestic birds are always exposed to the danger of the infection. The stress, poor nutrition, predisposing disease or chronic glucocorticoid administration reducing immune response can also cause aspergillosis (1,8). Aspergillosis is classified into

two types; chronic and acute infection. Long-term aspiration of low concentration of conidia in immunosuppressive state provokes chronic infection with fungal granuloma in the entire respiratory tract. Acute infection is caused by aspiration of massive amount of conidia. This type of infection can be occurred even in healthy condition and causes severe respiratory distress and sudden death after widespread lung invasion (7). Some birds of prey such as goshawks, rough-legged hawks, red-tailed hawks, golden eagles or snow eagles have been reported to have a predisposition to the infection (4). In South Korea, aspergillosis has been reported in several mam-

Table 1. Complete blood count of the brown hawk owl

Parameter (units)	Values	Reference interval ^a
WBC ($\times 10^3/\mu\text{l}$)	1.1	6.1-19.8
RBC ($\times 10^6/\mu\text{l}$)	1.18	1.64-2.64
PCV (%)	28	34-43
Hb (g/dl)	5.6	9.6-13.8
MCV (fL)	237	178
MCHC (g/dl)	20	29

WBC, white blood cells; RBC, red blood cells; PCV, packed cell volume; Hb, hemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration

^aIt is based on Eurasian eagle owl (*Bubo bubo*) which comprises of the same family Strigidae, as brown hawk owl from International Species Information System (<http://www2.isis.org>).

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mals and birds such as goats, dogs, ostriches, golden eagles, sea gulls, penguins, whooper swans, barnacle geese and snow geese (2, 9-12,14,16-18,20)

This report describes a case of fatal aspergillosis after open fracture surgery of humerus in a brown hawk owl.

Case

History and physical examination

A young brown hawk owl (Korean natural monument No. 324-3) weighing 180 g was referred to Chungbuk National University Veterinary Medical Center for unable to fly. Physical and radiographic examination revealed open fracture of left humerus (Fig 1). Peripheral blood was collected from wing vein and stored into a heparinized tube. Manual complete blood count was performed and the result was interpreted with reference interval of eagle owls because of no data for brown hawk owls. The CBC revealed decrease of leukocyte count, RBC concentration, PCV, hemoglobin and MCHC, and increase of MCV suggesting iron-deficiency and regenerative anemia due to severe hemorrhage probably caused by humerus open fracture.

Treatment

For the first 3 days, the patient was stabilized with feeding half of chick in a dark and calm cage. As an empirical antibiotic therapy, cefazolin 100 mg/kg (Cefazoline, Chong Kun Dang Pharmaceutical, Seoul, Korea) was administrated intramuscularly twice a day. After stabilization, the fracture was fixed with intramedullary pin and wire under general anesthesia with isoflurane (Forane, JW Pharmaceutical, Seoul, Korea). However the patient was unexpectedly dead in 2 days after the surgery.

Necropsy and histopathologic examination

On necropsy, 2-3 mm green patches with ground glass appearance were widespread on the surface and inside of left lung (Fig 2). After dissection of the left lung, the cutting surface was imprinted on the glass slide and stained with Diff-Quik. The stained smear revealed several fungal hyphae and

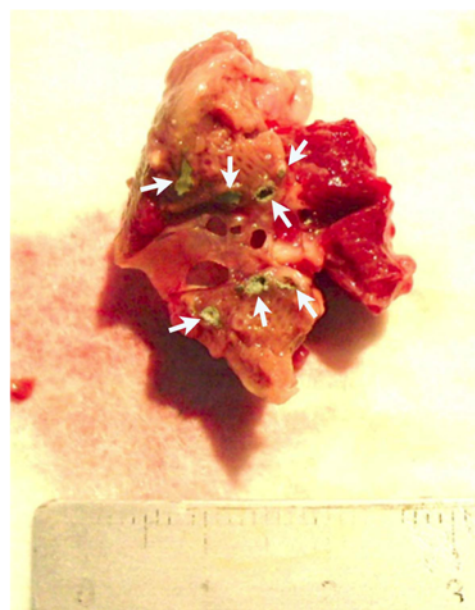


Fig 2. Left lung on necropsy revealing blue-green colonies with velvet appearance into the bronchi (arrows).

conidia. The lung tissue was fixed with 10% neutered formalin and processed for histopathologic examination. The tissue section revealed that several conidial heads and hyphae were grown into the loci of alveoli and penetrated into the bronchioles and lung parenchyma. The areas invaded by the fungal materials were filled with numerous inflammatory cells (Fig 3).

Fungal identification

The green patch obtained from the surface of lung was cultivated on Sabouraud dextrose agar (Difco, NJ, USA) at 30°C for 7 days and blue-green colonies with velvet-like appearance were identified suggesting Genus *Aspergillus*. For identification, molecular analyses of ITS region, β -tubulin and calmodulin genes were performed as described previously (6). After the amplification of the genes, the amplicons were sequenced by ABI Big Dye Terminator Cycle Sequencing

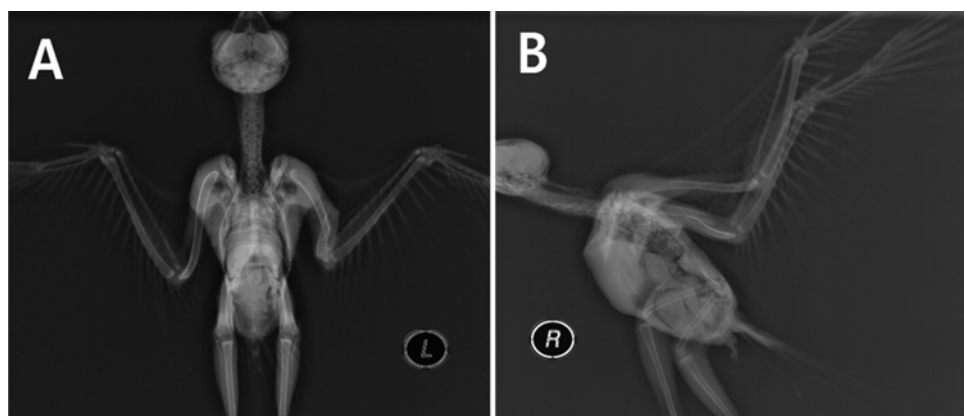


Fig 1. Ventrodorsal (A) and right lateral (B) radiographs revealing left humerus fracture.

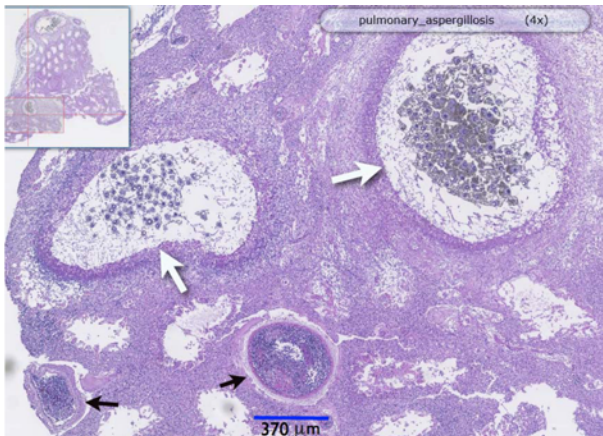


Fig 3. Histopathologic examination revealing conidial heads and spores in the parabronchial lumen (white arrows) and mycelia invaded into the lung parenchyma. The blood vessels were filled with inflammatory cells (black arrows). H&E stain.

Ready Reaction Kit v3.1 (PE Applied Biosystems, Foster City, CA, USA). In the comparison of the obtained sequences, the ITS region was 100% similar to the ITS region of *A. fumigatus* deposited by Faculte de Medecine et de Pharmacie in France (GenBank accession number GU594751). The sequence of β -tubulin or calmodulin was 99% or 100% similar to that of *A. fumigatus* deposited by Korea University in South Korea (GenBank accession number FJ436789) or National Center for Agricultural Utilization Research in USA (GenBank accession number EF669915), respectively. Based on the results, the isolated fungus was identified as *A. fumigatus*.

Discussion

This report describes a case of sudden death after open fracture surgery without any specific signs in a wild brown hawk owl. By necropsy and histopathologic examination, left lung aspergillosis was confirmed. Considering that bird humerus is a pneumatic bone which has a connection to clavicle air-sac, it is presumed that the pulmonary aspergillosis is caused by the open fracture of the humerus.

Diagnosis of aspergillosis is difficult because of non-specific and a variety of clinical signs. In the laboratory examination, typical changes of aspergillosis are monocytic leukocytosis, lymphopenia, hyperproteinemia with hyperglobulinemia and non-regenerative anemia (7). The present case showed decreased number of leukocyte and macrocytic hypochromic anemia, suggesting acute inflammation and hemorrhage. Vitamin B₁₂ or folic acid deficiency and bone marrow activation after hemolysis were ruled out because of no specific change of RBC on blood film reading. Typical radiographic findings of aspergillosis are bronchial pattern, asymmetric air-sac, increased soft tissue density of oropharynx, supraorbital space, trachea, syrinx, lung and air-sac, sharpened outline of air-sac by ascites fluid, hepatomegaly or renomegaly. Those findings however are not easy to find in early stage of

disease. At the first radiographic examination for the present case, we also failed to find any abnormal finding, suggesting that the infection was not severe or not present before the fracture.

As the aspergillosis is not easy to diagnose in early stage, it is important to prevent the infection. To reduce the possibility of the infection, feedstuff or sawdust bed should be stored in clean and dried place (15). When finding open fracture of pneumatic bone, empirical antifungal therapy with oral itraconazole (10-20 mg/kg twice a day) or sprayed clotrimazole (20 mg/kg for 2 weeks) can be considered (19).

Acknowledgements

This report was supported by 2011 Chungbuk National University Research fund.

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솔부엉이의 함기골 개방골절에 의한 속발성 폐 아스퍼질러스 감염 증례

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요 약 : 비행곤란을 나타내는 솔부엉이가 구조되어 충북대학교 동물의료센터에 의뢰되었다. 신체검사와 방사선 검사에서 좌측 상완골의 개방골절을 확인하였다. 3일 후 수내정과 와이어를 이용해 골절부 정복을 실시하였으나, 수술 후 2일 만에 급사하였다. 부검시 2~3 mm 크기의 녹색 결절이 왼쪽 폐의 표면과 내부에 다발성으로 발생하였다. 조직검사 결과 포자와 균사가 폐포에 다량 증식되고, 일부가 폐 실질에 침입하여 염증과 괴사를 유발하였다. 진균을 분리하여 Sabouraud dextrose 배지에 30°C에서 7일간 배양한 결과, 청녹색의 뿔뿔 양상을 나타내는 곰팡이를 관찰하였다. ITS, β -tubulin 및 calmodulin 유전자를 증폭하여 동정한 결과 *A. fumigatus*로 확인되었다. 최종적으로 함기골 개방골절에 따른 이차적인 폐 아스퍼질러스증으로 사망한 것으로 진단하였다.

주요어 : *Aspergillus fumigatus*, 폐 아스퍼질러스증, 함기골 개방골절, 솔부엉이