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Clinicopathological Findings on Naturally Occurring Aspergillosis in Pigeons

Güvercinlerde Doğal Olarak Şekillenmiş Aspergilloziste Klinikopatolojik Bulgular

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Abstract: Aspergillosis is a serious fungal diseases and it affected all of the animals including birds. The aim of this study is to present clinicopathological findings of severe aspergillosis and treatment of the diseases in a pigeon flock. In this study, 1-1.5 month's old 17 pigeons from a flock of 85 presented with symptoms of respiratory distress, inappetence, torticollis, incoordination and 20% mortality were examined macroscopically and microscopically. At necropsy granulomatous lesions were observed mainly in air sacs and lungs. Microscopic examination of the squeeze preparation of the air sacs and lungs revealed the presence of typical conidiophores of an *Aspergillus sp.* Histopathologically, necrosis with heterophil leukocyte, lymphocyte, macrophage and giant cells were observed in the granulomatous lesions. Although severe nervous symptoms were observed there were no macroscopical lesions seen in brains and cerebellums, slight gliosis was diagnosed at histopathological examinations. Immunohistochemical examination of central nervous system for Newcastle Disease was negative. Fungal agents were demonstrated in lesions in lungs and air sacs with periodic acid Schiff (PAS) methods. After diagnosis the tiny house were ventilated remained pigeons without neurological but with general symptoms were treated with 10.3 mg/kg itraconazole and multivitamin complexes.

Key words*:* Aspergillosis, airsacculitis, nervous symptoms, pigeon, treatment.

Öz: Aspergillosis kuşlar dâhil bütün hayvanları etkileyen bir mantar hastalığıdır. Bu çalışmanın amacı bir güvercin sürüsünde karşılaşılan şiddetli bir aspergillozis hastalığında klinik ve patolojik bulguların incelenmesidir. Bu çalışmada, solunum yolu problemleri, iştahsızlık, tortikollis, inkoordinasyon ve %20 mortalitenin olduğu, 85 hayvanlık bir sürüden 1-1.5aylık, 17 güvercin makroskobik ve mikroskobik olarak incelendi. Nekropside, hava keseleri ve akciğerlerde granülomatöz lezyonlar gözlendi. Akciğer ve hava keselerinden yapılan sürme preparatlarda tipik *Aspergillus sp.* konidioforları saptandı. Histopatolojik olarak granülomatöz lezyonlarda heterofil lökositler, lenfositler, makrofajlar ve dev hücreler gözlendi. Şiddetli sinirsel semptomlara rağmen beyin ve beyincikte makroskobik lezyon gözlenmedi, histopatolojik yoklamada hafif gliozis dikkati çekti. Merkezi sinir sisteminin Newcatle hastalığı yönünden yapılan immunhistokimyasal incelemesi negatifti. Fungal etken akciğer ve hava keselerinde periodic acid Schiff (PAS) method ile gösterildi. Teşhisten sonra kümes havalandırıldı ve sinirsel semptomu olmayan ancak genel semptomu bulunan güvercinler 10.3 mg/kg itraconazole ve multivitamin kompleksleriyle tedavi edildi.

Anahtar sözcükler*:* Aspergillosis, airsakkülitis, sinirsel semptomlar, güvercin, tedavi.

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Introduction

Aspergillosis is a mycotic disease that affects many bird species. Although the term "aspergillosis" usually refers to pulmonary form, the disease may also manifest as systemic, ophthalmic or encephalitic syndromes (Chute and Richard, 1997). The most common cause of aspergillosis is *Aspergillus fumigatus* and *A. flavus*, the other causative agents are *A. niger, A. terresus, A. glavucus* (Clark et al., 1954; Beckman et al., 1994; Chute and Richard, 1997; Shivaprasad, 2002). Aspergillosis is a disease of economic importance in the poultry industry (brooder pneumonia), and is a frequent cause of respiratory disease in companion, aviary and free-ranging birds (Bauck, 1994; Shivaprasad, 2002). The disease may be chronic and insidious, or it may cause peracute death. Established aspergillosis infections are clinically challenging to resolve. Aspergillosis is occasionally described in pigeon flocks with one report listing a prevalence of 2.4%. Mouldy straw is a particularly common source of numerous fungal pathogens including *Aspergillus sp*. (Bauck, 1994).

In systemic cases, lesions are typically found in lungs, air sacs, heart muscle, liver and abdominal viscera. Grossly, lesions in all areas are similar. A cream colored granuloma or plaque is present with or without grey or white "cotton- wool" mycelia masses (Bauck, 1994; Chute and Richard, 1997). Histopathological examination of granulomas generally shows a necrotic foci surrounded by macrophages, heterophils and giant cells, sometimes within a connective tissue capsule. *Aspergillus sp.* are among the three most common mycotoxigenic genera (Chute and Richard, 1997). The aim of this study was to describe pathological findings in naturally infected pigeons with pulmonary aspergillosis and neurological symptoms and treatment of this severe mycotic infection with itraconazole, improved environmental condition and multivitamin complexes.

Material and Methods

Seventeen 1-1.5 months' old pigeons from a flock of 85 were presented with symptoms of respiratory distress, opistotonus, torticollis, incoordination, and approximately 20% mortality. The birds were housed in a small tiny house as a very crowded flock. The environment was humid, airless, dusty and sunless. In addition to this bad condition, feed for cattle and sheep that contaminated with mould was also stored in this part of the tiny house together with the pigeons. Birds that different age and species including poultry were kept in same flock. Owner stated that he put the pigeons this place two weeks ago for to prevent winter cold.

Necropsy was performed and samples were taken from all organs. Tissues were fixed in 10% buffered formalin and processed routinely for light microscopy. Five micron thick sections were taken from paraffin embedded tissues and stained with Hematoxylin-Eosin (HE) and periodic acid Schiff (PAS). At the same time squeeze preparations of air sacs were also made. Brain samples were sent to Istanbul University, Veterinary Faculty, and Department of Pathology for to immunohistochemical examination for Newcastle Disease.

After aspergillus diagnosis, remained birds without neurological symptoms in the flock were treated with 10.3 mg/kg itraconazole and multivitamin complexes for to modulate immune system. Itraconozole was given via the drinking water, every 24 hours during 14 days. At the same time the tiny house was aired, cleaned and enlarged.

Results

All of the 17 death pigeons were young and showed mild to severe neurological symptoms together with respiratory symptoms (Fig.1). Owner stated there were slight general symptoms like as depression at the remained birds. At necropsy, gross lesions were observed in air sacs and lungs. Thick, yellow cheese-like deposits and plaque formations were seen on the air sacs and lungs of all of the 17 pigeons (Fig.2). In four pigeons, yellowish- white foci were seen on peritoneum and mesenterium.

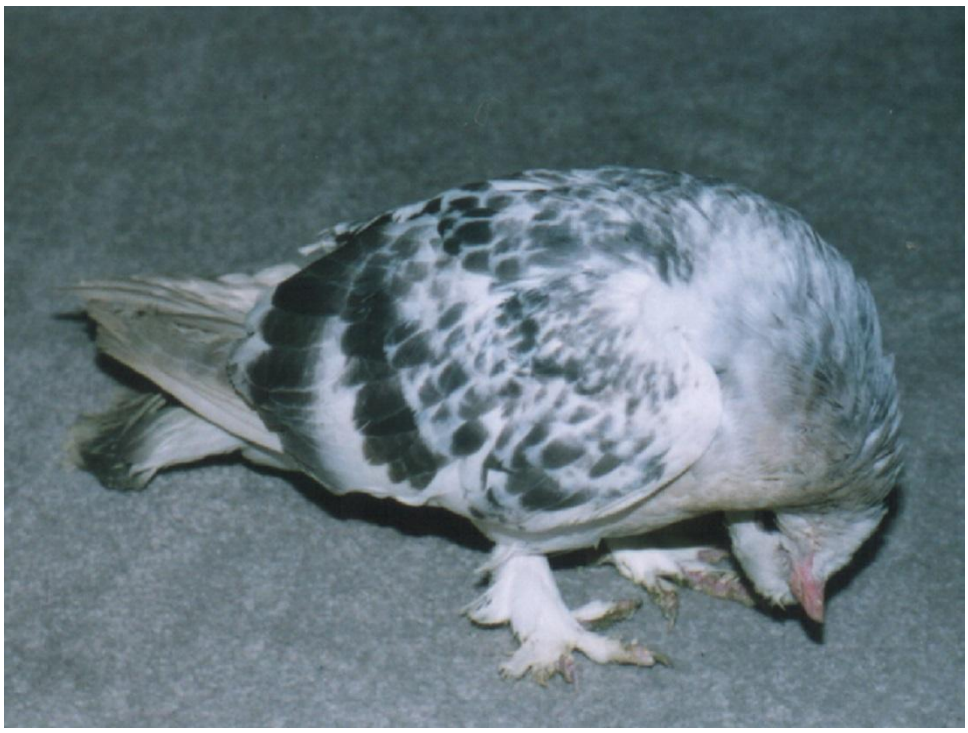

Fig.1. A pigeon with neurological symptoms suffer from Aspergillosis.

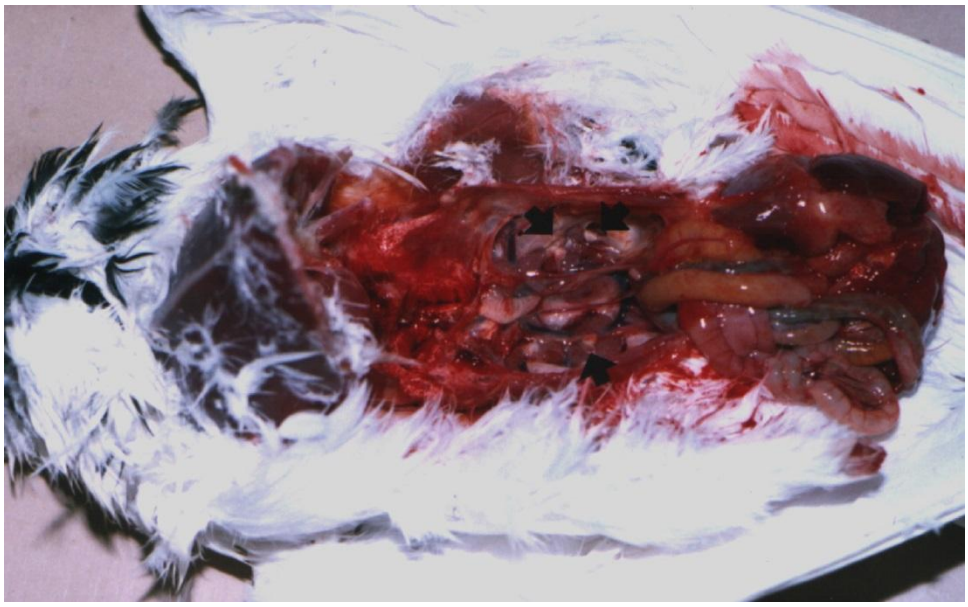

Fig.2. Numerous granulomatous lesions on air sacks (arrows).

Microscopic examination of the squeeze preparations of air sacs revealed the presence of typical conidiophores of an *Aspergillus sp*. Histopathological lesions were located mainly in air sacs and lungs. In air sacs membranes severe hyperaemia, oedema, heterophil leukocyte, lymphocyte, plasmacyte and epitheloid macrophage infiltration were observed in some pigeons. The pleural and subpleural spaces were markedly thickened with infiltrates of large numbers of heterophils and moderate numbers of lymphocytes, macrophages, and plasmacytes admixed with fibrin and homogenous proteinic material. Typical lesions in lungs were characterized by granulomatous inflammation with necrosis, hemorrhage and fungal elements in the centre of granulomas that were locally invasive in PAS stained sections. They were scattered throughout the pleura and subjacent lung tissue. Many of the granulomas had either heterophilic centers or central necrosis. Multinucleate giant cells were readily seen in granulomas (Fig.3). Fungal elements were demonstrated with PAS methods in granulomas as red filamentous structures (Fig. 4). Moderate numbers of fungal elements were present in granulomas. At the histological examination, branched hyphae morphologically similar to *Aspergillus sp.* were detected in the air sacs and lungs in PAS stained sections. Hyphae aligned themselves in a radial pattern around the beads fixed in alveoli. The involved alveoli and bronchiolar luminae were filled with heterophil leukocytes and necrotic including nuclear debris. There were no prominent reaction at the brain and cerebellum except slight gliosis at the microscopical examination. Immunohistochemical examination of brains for Newcastle Disease was negative.

After improvement of the environment and 2 weeks itraconazole - multivitamin treatment, all of the general symptoms were recovered and no new neurological symptoms were observed.

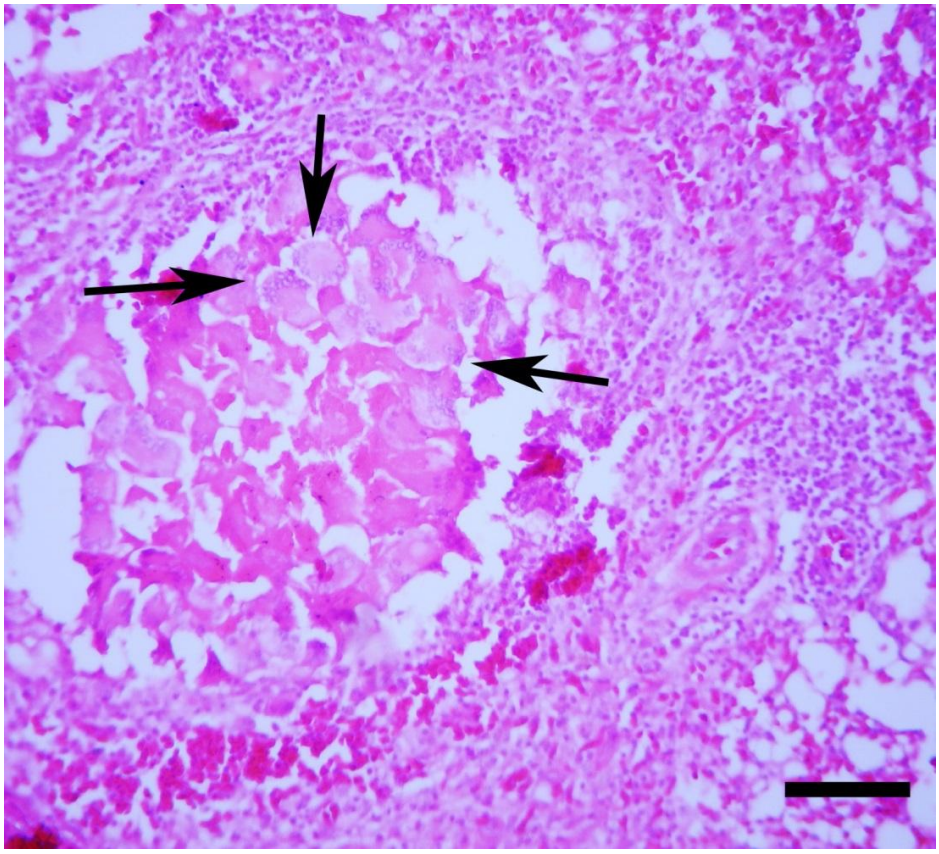

Fig.3. Multinucleate giant cells (arrows) in the granulomatous lesion in lungs, H.E. Bar= 100 μm.

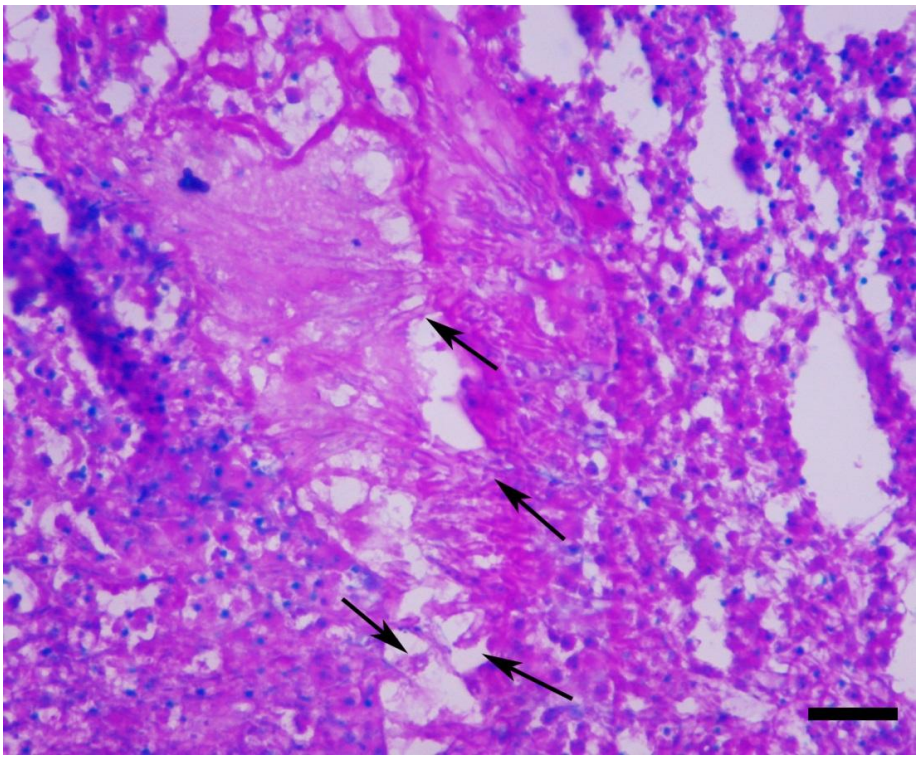

Fig.4. Demonstration of fungal elements in granulomas in lungs, PAS. Bar= 100 μm.

Discussion

Aspergillosis is a spectrum of disease of humans and animals caused by members of the genus *Aspergillus*. These include mycotoxicosis due to ingestion of contaminated foods, allergy and sequel to the presence of conidia, colonization without extension in performed cavities and debilitated tissues, invasive, inflammatory, granulomatous, necrotizing disease of lungs and other organs and rarely systemic fatal disseminated disease. The type of disease and severity depend upon the physiologic state of the host and the species of *Aspergillus* involved (Chute and Richard, 1997; Jones et al., 1997). Aspergillosis occurs as an acute disease with high morbidity and mortality rates in young birds (Chute and Richard, 1997; Clark et al., 1954). In this study, especially young pigeons were affected from the disease and possible cause of the disease was overcrowded flock, contaminated food and humid environment. While young birds died from the illness, the old ones recovered after improving the environment and treatment.

Healthy birds can generally withstand exposure to a high concentration of spores. Aspergillosis infections are generally divided into local and systemic diseases. Lesions frequently originate in one system or area and later advance into adjacent organs and systems

as the disease progresses. Acute cases occur when spores germinate in a particularly vital area or when multiple lesions germinate at once. Air sac infections in mature birds may progress for weeks, or can induce granulomas that are present for months (Bauck, 1994). Although the lesions were mainly localized in air sacs the disease was acute and combined neurological symptoms, the mortality rate was high in this case. Severity of the disease in this study attributed the rabid environmental change and bad management.

Avian aspergillosis usually affects pulmonary system. For that reason, the disease is also known as mycotic pneumonia and brooder pneumonia (Chute and Richard, 1997). However, reports about the encephalitic or meningoencephalitic aspergillosis in poultry are also present (Alexandrov et al., 2001; Raines et al., 1956). Raines et al. (1956) were firstly described encephalitic aspergillosis in turkeys. They observed necrotic foci in the cerebrum and cerebellum in naturally infected 18-days-old poults. The same authors also described that the typical lesions on the air sacs, lungs, livers, kidneys and serous surface of the small intestines. *Aspergillus sp.* has common mycotoxins and toxins can cause necrosis in brain. Some toxins of *Aspergillus sp*. are immunosuppressive (Chute and Richard, 1997). Although the nervous symptoms were present in this study there were no prominent lesions in brain or cerebellum. The possible cause of these nervous symptoms was attributed to toxins of the agent. High mortality was also attributed to immunosupperative effect of the toxins.

Aspergillus commonly grows in damp soils, decaying vegetation, organic debris and free grains. High numbers of spores (called conidia) are released in to the atmosphere and are inhaled by humans, birds and other animals. These spores travel through the upper respiratory tract to the lungs. If the spores colonized the lungs, the fungi may be disseminated to the other parts of the body and disease often leading to death occurs (Powel et al., 1994; Wobeser, 1997). The diagnosis can be confirmed by demonstration of the characteristic organisms with their slender dichotomously branching septate hyphae, in tissue section (Chute and Richard, 1997; Jones et al., 1997; Marks et al., 1994). In this study, similar findings were observed and they were suggested that transmission of infection by inhalation. The diagnosis was made on microscopic examination of the squeeze preparations of air sacs and characteristic histopathological findings. Morphology of the agents was similar to those in previous studies (Chute and Richard, 1997; Pal, 1992).

Aspergillus is ubiquitous, and the infections should always be considered to occur secondarily to an immunosuppressive event. It has been suggested that healthy birds exposed

to high concentrations of spores are generally resistant to infections, while immunocompromised hosts exposed to small concentrations of spores are frequently infected. Factors that influence the susceptibility of a bird to aspergillosis include shipping, overcrowding, malnutrition, poor ventilation, very young or old age, antibiotic therapy, corticosteroid administration, respiratory irritants or concomitant disease. The type of disease induced by aspergillosis is thought to be dependent on the source and number of spores contacted and the general condition of the bird (Chute and Richard, 1997). This study also showed that the environment is very important for mycotic infections and the disease is usually affected young ones and together with antifungal treatment, immunostimulation and improving the environment can be effective for treatment of aspergillosis.

Vitamins are organic trace nutrients that have either cofactor or hormone functions in the body (Roudybush, 1996). Studies done for several years have confirmed that vitamins are necessary for the proper functioning of the immune system. The action of immune system has been shown to be undetermined by a variety of nutrient deficiencies. Supplementation with higher than normal levels of certain vitamins (e.g. vitamin A ad vitamin E) has been shown to enhance the immune competence of poultry. Stress has been shown to increase the need for a number of vitamins, especially vitamin C (Jordan and Pattison, 1999). Dietary manipulations of some nutrients result in immunoregulatory consequences due to the participation of the nutrient or its products in communication within and between leukocytes (Klasing, 1997). For the treatment general supportive measures are indicated in very sick patients which must be kept in a quite, warm, clean and dry environment. Immuno-stimulants have been used for respiratory system infections (Wobeser, 1997). This study also showed that vitamins are effective to immununodulation and they can be used for preventive aim and supportive treatment of fungal infections together with antifungal drugs and improved environment.

The diversity of immune failure suggests that many lines of immunity are implicated in the *Aspergillus* elimination process. Non specific immunity plays a major role in the defense against *Aspergillus fumigatus,* including three major lines; anatomical barriers, immune system and phagocytic cells (Aldebert et al., 2001). The evidence of rodlet layer in conidia of aspergillosis may play an important role in the physiopathology of the disease in eliminating the neutrophils and macrophages of hosts on the early stage of the infection in mammalians (Shibuya et al., 1999). The respiratory tract plays a significant role; toxins and immunity are also important factors (Chute and Richard, 1997). The pathogenesis of

aspergillosis in birds is poorly understood but possible physiopathology may be similar. In this study, while severely affected bird died, but the birds on early and general symptom stage were recovered by antifungal drug, supportive immunomodulatory treatment together with environmental improvement.

Aspergillosis is not contagious (it will not spread from bird to bird) agent commonly grows in damp soils decaying vegetation, organic debris, and feed grains. High numbers of conidias are released in to the atmosphere and inhaled by humans, birds, and other animals. Failure to maintain clean environment often leads to severe outbreaks (Friend and Franson, 1999). Numerous antifungal drugs were reported for treatment of the disease but generally an effective therapy for avian aspergillosis in severely affected bird is not available (Chute and Richard, 1997). Changing the environment and administering supportive treatment with multivitamin complexes together with antifungal treatment were found effective in aspergilllosis in pigeon in this study.

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