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Μια περίπτωση πνευμονικής ασπεργίλλωσης σε λευκούς πελαργούς

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■ A case of pulmonary aspergillosis in white storks

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ABSTRACT. Aspergillosis is a fungal infection affecting respiratory system both in mammals and avian species. It is more commonly encountered in birds, in comparison with its mammalian counterpart. Mostly isolated strains are *Aspergillus fumigatus* (95%) and *Aspergillus flavus* (5%). Affected lungs and air sacs reveal miliary to gross lesions like gray-yellowish or white-grayish granulomatous foci surrounded by white halos indicative of inflammatory infiltration. Five storks found dead in the rural areas near Istanbul were submitted to our faculty between years 2008 and 2014. Two of them were thought to be younger than 1-year-old and the other three were older than one year of age. Necropsies were performed right after their submissions. Aspergillosis lesions were observed in the lungs and thoracic air sacs of the first four storks. In addition to these changes the lesions were detected at the aortic bifurcation and on the testicular and renal capsule of the fifth stork. Histopathology revealed encapsulated granulomas with foci of caseous necrosis at the center surrounded by numerous macrophages, heterophil leukocytes, lymphocytes and foreign body giant cells in all the storks. Following the gross, histopathological and mycological examinations the agents were detected as *Aspergillus fumigatus*.

Although, the number of reported deaths due to Aspergillosis is not high in storks, we believe that these birds are quite susceptible to the disease and stress factors such as migration increases the risk of pathogenicity. This report was designed as a contribution to literature since there is only one reported case available with respect to aspergillosis associated death in storks and stress factors such as migration may also predispose storks to the disease.

Keywords: *Aspergillus fumigatus*, avian aspergillosis, stork, histopathology, necropsy

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INTRODUCTION

Aspergillosis is a fungal infection affecting respiratory system both in mammals and avian species. Most common clinical manifestations occur in trachea, bronchioles, lungs and air sacs. Furthermore, eye, brain, skin, joints and visceral organs are involved, as well (Atasever and Gumussoy, 2004, Beyaz et al., 2008, Cacciuttolo et al., 2009). Aspergillosis is more commonly encountered in birds, in comparison with its mammalian counterparts. Avian aspergillosis is frequently seen in turkeys and chickens followed by ducks, geese, quails, ostriches, parrots, canaries, pigeons, flamingos and penguins, respectively (Atasever and Gumussoy, 2004, Tell, 2005). Acute and chronic diseases develop in birds. Acute form of the infection affects mostly young animals with high morbidity and mortality rates. Chronic disease develops in adults and in turkey chicks (Cacciuttolo et al., 2009, Tell, 2005).

Aspergillus spores are found in large numbers within the soil, decomposing meat, forage, hay and any kind of food (Arda, 1980). Mostly isolated strains are *Aspergillus fumigatus* (95%) and *Aspergillus flavus* (5%) (Tell, 2005). Affected lungs and air sacs reveal miliary to gross lesions like gray-yellowish or white-grayish granulomatous foci surrounded by a layer of darker zone, which is an evidence of inflammatory infiltrations. Histopathology reveals encapsulated granulomas with foci of caseous necrosis at the center, surrounded by numerous macrophages, heterophil leukocytes, lymphocytes and foreign body giant cells. Fungal elements like septate or aseptate hyphae and spores are visualized by special stains scattered around within these lesions (Cacciuttolo et al., 2009, Tell, 2005).

Despite the frequency of the cases of aspergillosis in poultry and waterfowl (Atasever and Gumussoy, 2004, Beernaert et al., 2008, Beyaz et al., 2008, Cacciuttolo et al., 2009, Carrasco et al., 2009, Tell, 2005, Yokota et al., 2001), a very small number of cases were reported in white storks (Garcia et al., 2007, Olias et al., 2010). There are only two articles available with the former pointing out aspergillosis associated death in white storks (Olias et al., 2010), and the latter describing the occurrence of aspergillus strains obtained by tracheal swabs in live animals (Garcia et al., 2007). In our country, although aspergillosis has

been reported in poultry and waterfowl (Akan et al., 2002, Akkoc et al., 2009, Atasever and Gumussoy, 2004, Beyaz et al., 2008), it has not yet been reported in storks. Turkey is located both on migration routes of the storks through from North Europe to North Africa and it is one of their breeding grounds. Istanbul Bosphorus is also seated upon this important migration route. Thus, we aimed to report the detection of aspergillosis in five storks.

MATERIALS AND METHODS

Five storks were found dead in the rural areas near Istanbul and were submitted to our faculty between the years 2008-2014. The common submission period for all the birds is during the end of October and the beginning of November. Two of them were thought to be younger than 1-year-old and the other three were older than one year of age. Necropsies were performed just after submissions of the birds. Different portions of lungs, air sacs, spleen, kidneys, heart and liver were collected from all of the birds and all the samples were fixed in 10% buffered formalin and then submitted for histopathology. For this purpose, the samples were embedded in paraffin and cut at 3-5 μm thickness and then the sections were placed onto slides and stained with Hematoxylin-Eosin (H&E), periodic acid Schiff (PAS), Grocott and Ziehl-Neelsen. Spleen samples were stained also with Kongo-Red. Portions of lesioned lungs were stained with Gram stain and lactophenol in addition to the other stains. Fresh specimens of the mentioned organs were submitted for mycological culture examination, as well. Lung samples were cultured on a 7% defibrinated sheep blood agar plate (Oxoid), on a MacConkey agar plate and on two Sabouraud Dextrose Agar plates (SDA). Blood agar plate, MacConkey Agar plate and one of the SDA plates were incubated at 37 °C and the other SDA plate was incubated at 25°C. The blood agar plate and MacConkey agar incubated for 5 days while the two SDA plates incubated for 7 days (De Hoog et al. 2000).

RESULTS

Postmortem findings were similar in the first four birds examined: Numerous white nodules measuring from 0.5 cm to 5 cm in diameter were observed

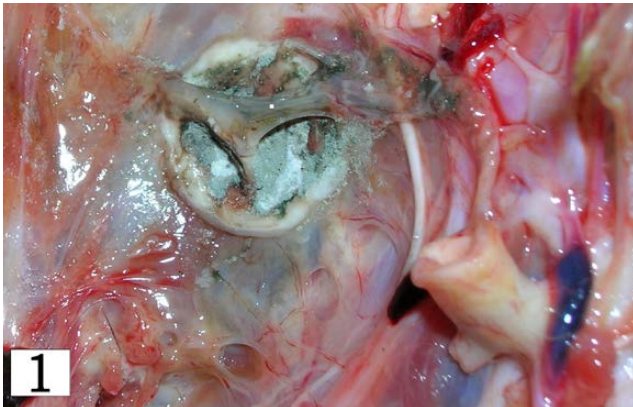


Figure 1. Moldy, greyish white depositions that resemble craters showing typical appearance of Aspergillosis in the lungs.



Figure 2. White round pyogranulomatous nodules filled with pus.

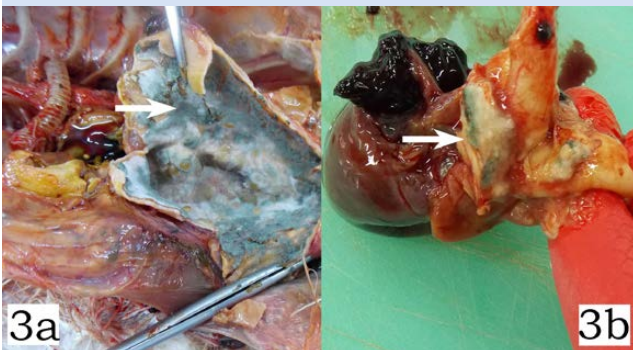


Figure 3
a. Aspergillosis. Greenish- gray moldy depositions in the abdominal air sac in the fifth stork.
b. Aspergillosis. Greyish dust like material at the aortic bifurcation in the fifth stork.

both in the lungs and thoracic air sacs. At the center of some of the nodules, there were craters covered with grayish dust like material that were surrounded

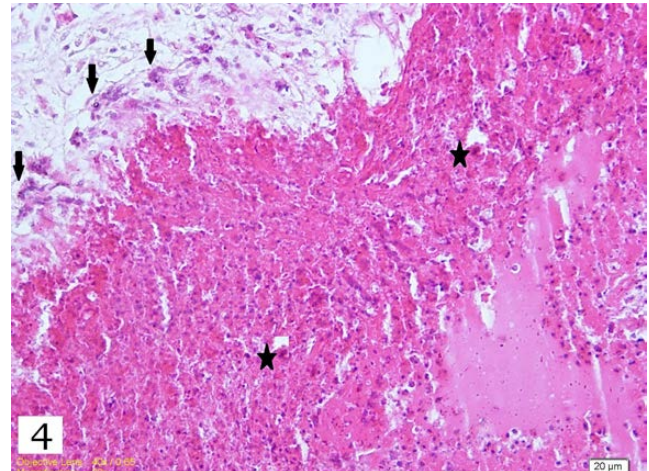


Figure 4. Focus of caseification necrosis (star) surrounded by a demarcation zone that consists of numerous macrophages, heterophil leukocytes, lymphocytes, foreign body giant cells (arrows) and fibrocytes and fibroblasts in the lung (H&E).

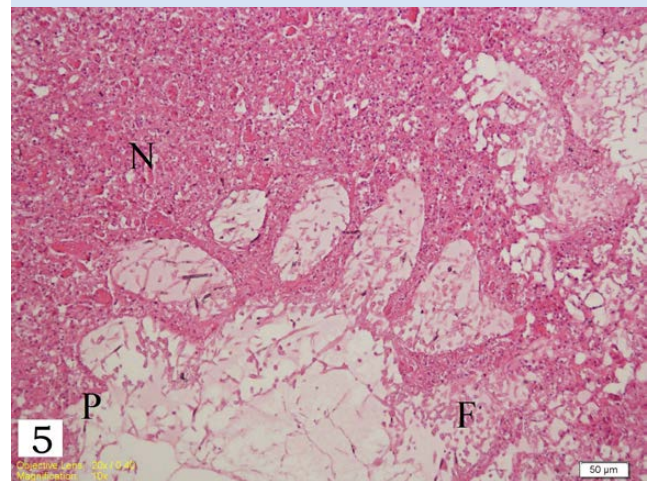
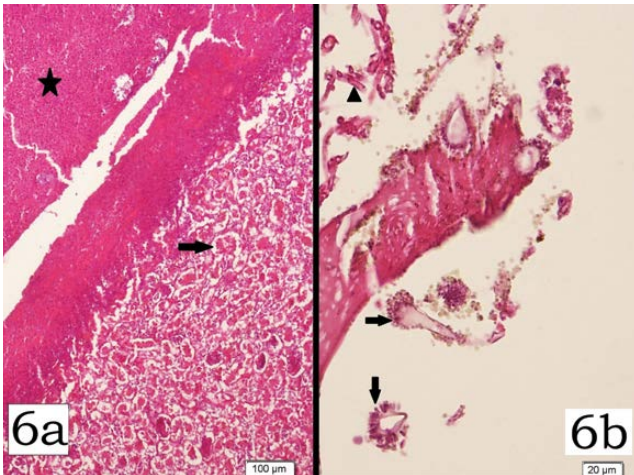


Figure 5. Necrotic pyogranulomatous areas (N) and numerous septate and non-septate hyphae (F) were present in the lumens of parabronchioles (P) (H&E).

by white halos (Fig.1). There were many nodules also on the cut surfaces of the lungs (Fig.2). The kidneys were congested. In the fifth stork, grayish dust like material identical to that seen in the lungs were observed also in the abdominal air sacs (Fig. 3a) and at the aortic bifurcation (Fig.3b).

Gram staining of the lungs revealed no evidence of acid resistant bacilli. However, lactophenol staining demonstrated numerous fungal hyphae.

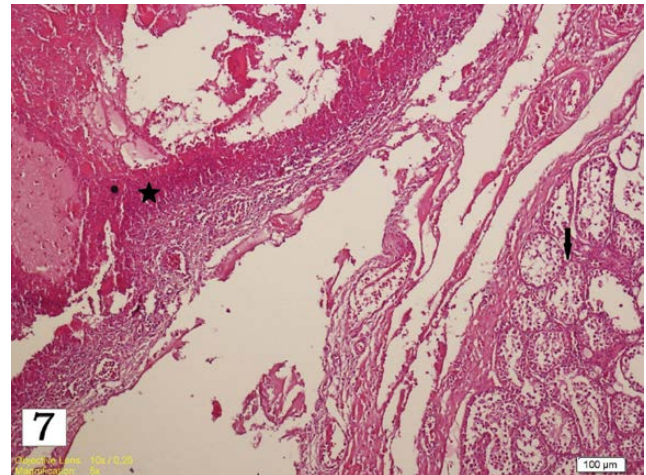
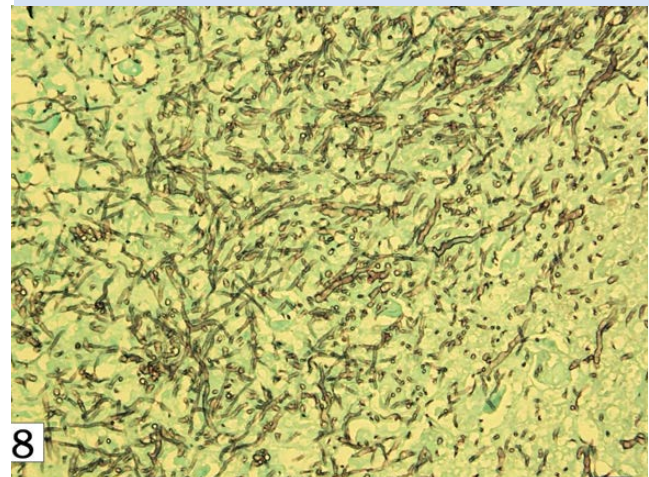
Histopathological examination of the organs revealed an exudative cellular inflammation composed of heterophil leukocytes, macrophages and foreign body giant cells in the lungs (Fig. 4). There

**Figure 6.**

- a.** Caseification necrosis (star) in air sacs adjacent to the kidney (arrow),
b. Conidiophores of *Aspergillus fumigatus* (arrows) and hyphae (arrowhead) (H&E).

were necrotic pyogranulomatous areas containing several septated and non-septated hyphae and conidia. Numerous septated and non-septated hyphae were also present in the lumina of parabronchioles (Fig 5). Furthermore, necrotic areas with numerous septated and non-septated hyphae and conidia and foreign body giant cells were observed in the abdominal air sacs, kidneys (Fig. 6a, 6b) and testis (Fig.7) of the fifth stork. In the slides that were stained with Grocott stain; hyphae and conidia of the aspergillosis were detected clearly (Fig. 8). Slides that were stained with Ziehl-Neelsen revealed no acid-fast bacilli. There were granulomatous lesions and foreign body cells in the air sacs, as well. In two young birds, there was urate crystal deposition in the kidneys. Hyalinization of trabeculae and amyloid like material deposition in the follicular areas were observed in the spleens. However, the Congo red stain was negative. Mononuclear inflammatory cells were seen around the portal veins and in the mid-zonal areas in the liver in both young birds and one of the adults.

There was no bacterial growth after a 7-day incubation period. Growth of fungal colonies was observed in the SDA plates that were incubated at 37 °C and 25 °C for 7 days. The microscopic examination revealed fungal colonies that were green at the center and white at the periphery. Lactophenol cotton blue staining revealed typical septated hyphae of *aspergillus*. Round vesicles, sterigmata and spores

**Figure 7.** Caseification necrosis (star) in air sacs adjacent to the seminiferous tubules (arrow) (H&E).**Figure 8.** Several fungal hyphae in the parabronchi (Grocot Stain-GMS)

were also seen. Single lined sterigma and conidia that were seated upon them were clearly observed. According to the gross and microscopic findings the agent was detected as *Aspergillus fumigatus*.

DISCUSSION

Pulmonary aspergillosis is among the most frequently seen mycotic disease in captive and free-ranging birds all around the world (Cacciuttolo et al., 2001, Charlto et al., 2008, Garcia et al., 2007). It is more common in birds than mammals due to the differences in their anatomic and immunocellular mechanisms (Garcia et al., 2007, Olias et al., 2010). Anatomic characteristics that might predispose birds to this disease include the lack of an epiglottis

that prevents particulate matter from entering the lower respiratory tract, the lack of a diaphragm which results in inability to produce a strong cough reflex and a limited distribution of pseudostratified ciliated columnar cells throughout the respiratory tract (Tell, 2005). Cellular characteristics that might predispose birds to respiratory aspergillosis include the lack of surface macrophages for phagocytizing *Aspergillus* spp. conidia and dependence on heterophils that use cationic proteins, hydrolases, and lysozymes rather than myeloperoxidase and oxidative mechanisms for killing fungal hyphae (Harmon, 1998). The most characteristic lesions in *Aspergillus* infections occur mainly in lungs and in air sacs. However, they can also be seen in the liver, spleen, myocardium, bones, brain, glandular stomach, bursa fabricius and eyes (Atasever and Gumussoy, 2004, Caciuttolo et al., 2009, Carrasco et al., 2009). In the present report, we detected numerous conidia and hyphae in both lungs and air sacs of the five white storks. And the mycological examination revealed *A. fumigatus* only in those organs. In two of the young birds, mononuclear cells were present in the liver and uric acid crystals were present in the kidneys and there was hyalinization in the white pulp of the spleen but there was no indication of Aspergillosis. Olias et al. (2010) reported mycotic pneumonia due to *A. fumigatus* in the necropsy of 22 storks and they also detected fungi only in the lungs and air sacs. They indicated two different types of pneumonia in the lungs: pneumonia of grade I was characterized by multifocal poorly circumscribed aggregates of epitheloid macrophages and multinucleated giant cells surrounding filamentous fungal structures while pneumonia of grade II was characterized by heterophilic granulomas with central necrosis and

degenerate heterophils surrounded by intact heterophils and a layer of epitheloid macrophages and multinucleated giant cells. In our cases, we detected the histopathological lesions of both grade I and grade II.

Garcia et al. (2007) reported that they took tracheal swab samples from 10 storks in a wildlife rehabilitation center and they detected *Aspergillus* spp. in one stork and *Candida* spp. in another one. Both of the storks survived despite the fungal infection. Nordani et al. (2006) reported that many birds are able to host the spores of the *Aspergillus* spp in the lungs and air sacs, leading to a dormant or chronic infection, with no clinical symptoms or apparent anatomopathological lesions. All these reports prove that *Aspergillus* spp. are facultative pathogens. They become pathogenic with the existence of predisposing factors such as stress, migration conditions and captivity of the wild birds, toxic substances and immunodeficiency. Two of the storks in our case were younger than 1 year-old and the other three were older than one year of age. They all died at the end of October and in November, which is the migration season, and thus we believe that Aspergillosis infection in our cases was related with stress, poor weather and environmental conditions during migration as it was reported in the literature (Caciuttolo et al., 2009, Tell, 2005).

In conclusion, although the number of reported deaths due to Aspergillosis is not high in storks, we believe that these birds are quite susceptible to the disease and stress factors such as migration, increases the risk of pathogenicity.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. ■

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