

Dual Infection of Respiratory Tract with an *Aspergillus* sp. and Bacilli in a Canary (*Serinus canarius*)

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Abstract: The present report documents a case of acute aspergillosis and bacillosis in a canary in which the bacillus and fungal colonization was confined to the trachea and lung. A male canary was presented to clinic with a history of the presence of watery diarrhea and swelling in the left intertarsal joint without weight bearing. Supportive care was attempted but the bird did not improve and 48 h after beginning of respiratory signs, the bird died. At necropsy, symmetric necrotic zone were seen in the junction of syrinx to lung. The trachea was edematous and congested. The intestine was edematous and dilated. Histopathologic examination of lungs and trachea revealed infiltrative reactions of aspergillosis. Fungal hyphae with dichotomous divisions characteristic of aspergilli were clearly demonstrated in the trachea and lung by PAS and H&E stains. The pinkish amorphous material in the spleen was demonstrated to be amyloid with Congo red stain. *Isospora* life stages in the enterocytes were detected.

Key words: Aspergillosis, amyloidosis, *Isospora* sp., histopathology, stage, spleen

INTRODUCTION

Aspergillosis is a most common mycotic infection in a wide variety of avian species worldwide and causing severe and life-threatening illness in birds (Greenacre *et al.*, 1992; Jones and Orosz, 2000; Salehi *et al.*, 2000). Aspergillosis was one of the first diseases described in wild birds when it was reported in a European Jay in 1815 and appears to be more common in parrots and mynahs than other pet birds (Redig, 1993; Bauck, 1994). Aspergillosis is frequently encountered in the lower respiratory tract of various birds (Keymer, 1982; Redig, 1993) and occasionally in other organs such as oral mucosa, brain, eye, intestine, liver, kidney, skin, nasal passages and bone (Keymer, 1982; Fitzgerald and Moisan, 1995; Richard, 1997; Akan *et al.*, 2002; Atasever and Gunussoy, 2004). Immunosuppression probably contributes to outbreaks of aspergillosis (Barton *et al.*, 1992; Jones and Orosz, 2000) because healthy birds can withstand inhalation of large numbers of spores (Whiteman and Bickford, 1989; Richard, 1997).

Coccidiosis is a disease of universal importance in poultry production with varying degrees of pathogenicity (Stadler and Carpenter, 1996; McDougald and Fitz-Coy, 2008) but *Isospora* sp. are common in Passerines and single cases are being reported (Clyde and Patton, 1996;

Greiner and Ritchie, 1994; Dorrestein, 2000). In cage birds, coccidiosis is rarely seen because current avicultural practices interrupt the infection cycle (Macwhirter, 1994; Clyde and Patton, 1996; Scott, 1996; Madill, 2000; Schmidt *et al.*, 2003) but although many species of *Isospora* have been listed, their clinical significance and life cycle has yet to be fully described (Todd, 1981).

Amyloidosis is a disorder that is characterized by extracellular deposition of proteinaceous material between cells in various tissues and organs of the body. Amyloidosis is generally divided into primary (AL) and secondary Amyloidosis (AA) (King and Alroy, 1996; Landman and Gruys, 1998; Schmidt *et al.*, 2003; Snyder, 2007). Only Amyloid A (AA) has been detected in birds (Landman and Gruys, 1998; Nakamura *et al.*, 1998) and commonly occurs in association with chronic infectious diseases (King and Alroy, 1996; Landman and Gruys, 1998; Kunkle, 2003; Schmidt *et al.*, 2003). The lesions appear to be irreversible and no successful treatment is known (Gelis, 2003). Amyloidosis is a rare disease, especially in birds except waterfowls (Zschiesche and Jakob, 1989; Hatai *et al.*, 2009). There are a few reports of natural amyloidosis in cage bird (Macwhirter, 1994; Schmidt *et al.*, 2003; Nouri *et al.*, 2011).

Although, aspergillosis has been reported in a variety of both captive and free living avian species (Bauck, 1994;

Redig, 1993) there is a report of amyloid accumulation related to mycotic infection in the liver and spleen of a female ostrich (*Struthio camelus*) (Akkoc *et al.*, 2009) and there is only a single report of combined acid-fast bacilli infection and aspergillosis (Kaliner and Cooper, 1973). The present report describes the clinical and pathological features of a canary with coccidiosis and dual infection of respiratory tract with an *Aspergillus* sp. and bacilli associated with amyloid accumulation.

CASE REPORT

Case history: A male canary was presented to clinic with a history of the presence of watery diarrhea and swelling in the left intertarsal joint without weight bearing. Supportive care was attempted but the bird did not improve and 48 h after beginning of respiratory signs such as open beak dyspnea, gasping and hyperpnea, the bird died and a complete necropsy was performed and was sent to Pathology Department, Faculty of Veterinary Medicine, The University of Tehran for more routine pathological studies.

Necropsy: On necropsy, gross lesions were observed in the lungs, trachea and intertarsal joint. Symmetric necrotic zone were seen in the junction of syrinx to lung (Fig. 1). The trachea was edematous and congested. The intestine was edematous and dilated. No significant lesions were noted in the liver, kidney or other internal organs.

Bacteriology: Because no fresh or frozen samples were kept, cultures to identify the causative species were not performed.

Histopathologic examination: Collected organs were preserved in 7.2% buffered formalin. Later, they were cut

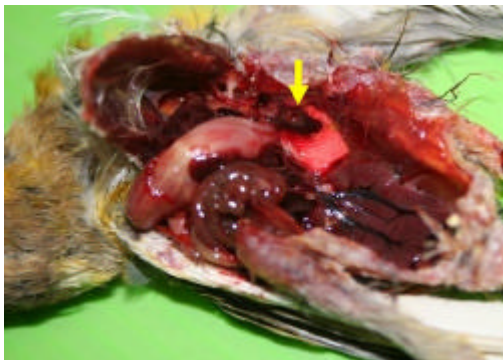


Fig. 1: Symmetric necrotic zone were seen in the junction of syrinx to lung (yellow arrow) in canary

5-6 μ m in thickness and stained with Hematoxylin and Eosin (HE), Congo red, Ziehl-Neelsen (ZN), modified silver and Periodic Acid-Schiff (PAS) techniques.

Histologically, the case showed a form of the infiltrative reactions on the same tissue section. Fungal hyphae with dichotomous divisions characteristic of aspergilli were clearly demonstrated in the trachea and lung by PAS and H&E stains. Dichotomously branching mycelia diffusely penetrated into the pulmonary parenchyma inducing an exudative cellular inflammation. The agent invaded into blood vessels and sometimes developed as large aggregates consisted of radiating hyphae toward the bronchial lumens (Fig. 2A). The blood vessels were congested and contained septic thrombosis and hyphae. A mass of fungal hyphae, bacilli and granulocytes partly occludes the tracheal lumen, too (Fig. 2B).

In spleen, marked deposits thickened the basement membranes of blood vessels and extended into the surrounding parenchyma and with Congo red staining amyloidosis were confirmed. Isospore life stages in the enterocytes were detected (Fig. 3). Mononuclear cells infiltration observed in lamina propria of the small

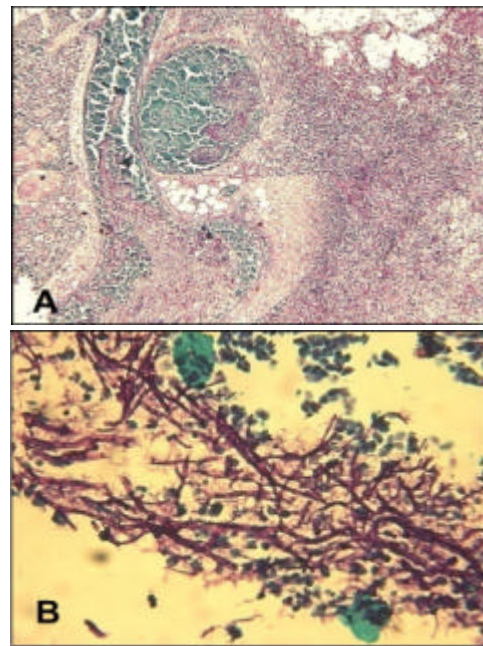


Fig. 2: A) *Aspergillus* sp. hyphae in the Trachea. PAS. 100x. B) *Aspergillus* sp. hyphae in the lung. The lesions were contained necrosis, numerous branched, septate hyphae radiating and clumps of bacilli in the center. Macrophage and small number of lymphocyte made up the intervening tissue H&E stain. 400x

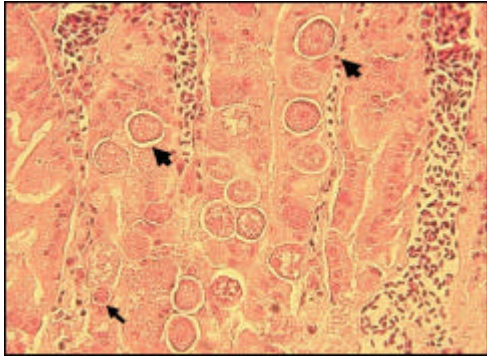


Fig. 3: Group of macrogamete (large black arrows) and microgametes (small black arrow) occupies the small intestinal epithelium. Minimal necrosis is seen. H&E stain. 50x

intestine. There was destruction of intestinal epithelium. Histology of the joints showed osteomyelitis with necrosis and inflammatory cells. In other examined organs, no significant lesions or fungal hyphae were observed.

DISCUSSION

Aspergillosis is a common cause of mortality in Passerines (Jones and Orosz, 2000) and cases of concurrent aspergillosis and bacterial infections have been recorded earlier by other researcher (Kaliner and Cooper, 1973; Dyar *et al.*, 1984). Two types of tissue reaction, infiltrative and granulomatous were seen in the lung associated with pulmonary aspergillosis (Tsai *et al.*, 1992). This finding was similar to that earlier described in other avian species (Tsai *et al.*, 1992). In view of these findings, an inhalation route of infection can be excluded in this bird. Richard (1997) speculated that because the spores of *A. flavus* larger than those of *A. fumigatus*, it is expected that the spores will settle out higher in the respiratory tract and be trapped within the mucociliary apparatus. This is a possible explanation for the location of the fungal colonization in the present case.

Canaries appear to be susceptible to the development of aspiration pneumonia and subsequent tracheitis which could be a predisposing factor to the development of mycotic disease. Both low humidity and excessive dust may interfere with the normal mucociliary activity of the respiratory ciliated epithelium and predispose the birds to respiratory aspergillosis (Zinkl *et al.*, 1977). Earlier research has demonstrated numerous conidia in macrophages recovered from the respiratory tract of turkeys within 15 min of aerosol exposure to *A. fumigatus* (Richard and Thurston, 1983). Subsequently, some of the intracellular conidia germinate and the infection

progresses with the development of tissue-invasive hyphae. In addition, the development of pneumonia was consistent with vascular spread of the infection (Kunkle and Rimler, 1996).

Most fungal infections are a result of poor husbandry including improper ventilation that permits accumulation of fungal spores (Jones and Orosz, 2000; Joseph, 2003). It is also known that trauma, toxicoses, antibiotics and corticosteroids, overcrowding, transport, mixing of groups, other illnesses, stress, malnutrition, unsanitary conditions and another injury to the respiratory system (e.g., smoke inhalation) are the contributing factors for outbreaks of aspergillosis in avian (Forbes *et al.*, 1992; Tsai *et al.*, 1992; Jones and Orosz, 2000; Akan *et al.*, 2002; Joseph, 2003). Researchers suggested that shipping stress and concurrent disease might be the most important predisposing factors for the establishment of mycoses in the present study.

Aspergillus sp. infection typically produces fungal nodules or plaques within the lungs and on the air sacs (Pal *et al.*, 1990; Whiteman and Bickford, 1989; Barton *et al.*, 1992; Richard, 1997; Akan *et al.*, 2002). In the present report, progressive severity of gross lesions in lungs and of microscopic lesions in both trachea and lung tissue were seen. Lesions were observed as bilateral sever congestion without any sign of fungal nodule or plaque. Histology of the lungs showed an acute pneumonia with many necrotic areas and large numbers of hyphae with dichotomous branching.

Coccidia sp. are not commonly seen in caged birds because transmission is via the ingestion of sporulated oocysts which require warm, moist environments in which to sporulate and become infective (Macwhirter, 1994; Clyde and Patton, 1996; Scott, 1996; Madill, 2000) and survival of oocyte in cage is limited to a few days because of the heat and the action of molds and bacteria and individual sanitary measures (Clyde and Patton, 1996; McDougald and Fitz-Coy, 2008; Nouri *et al.*, 2012).

This case was presented with a history of watery diarrhea. Enteric disease caused by species of *Isoospora* occurs when the epithelial cells are destroyed during asexual reproduction, causing mild to severe diarrhea (Patton, 1993; Greiner and Ritchie, 1994) such as this case. There was a variable nonsuppurative inflammatory response and necrosis indicating that the bird probably had a compromised immune system.

The coccidium of canaries is *Isoospora serini* and *Isoospora canaria* (Box, 1975; Schmidt *et al.*, 2003). Different species of coccidia have different trophisms for different portions of the intestinal tracts (Schmidt *et al.*, 2003; McDougald and Fitz-Coy, 2008). A disseminated infection may result from invasion of *I. serini* whereas

I. canaria usually results in the typical coccidian infection restricted to the intestinal epithelium (Joseph, 2003). Thus, on the basis of the location, size and invasive behavior, *I. canaria* were identified but further research is currently underway to characterize the parasite.

Amyloidosis is the general term applied to several diseases characterized by the deposition of amyloid AA in caged wild and domestic birds (Landman and Gruys, 1998; Nakamura *et al.*, 1998; Akkoc *et al.*, 2009). It is usually the result of chronic infections, especially viral and bacterial causes (King and Alroy, 1996; Landman and Gruys, 1998; Kunkle, 2003; Schmidt *et al.*, 2003). A few reports of amyloidosis in chickens have been made, although often in water birds such as Anatidae and swans, among others (Zschiesche and Jakob, 1989; Hatai *et al.*, 2009). However, researchers recently encountered incidental amyloidosis in a canary. In the present case, any lesions were not apparent grossly but amyloid deposits were confirmed histologically. The distribution of organ involvement varies from bird to bird (Landman and Gruys, 1998). In this case, the tissue distribution of amyloid was similar to that reported earlier in Passerines (Nouri *et al.*, 2011). Microscopically, the mild degree of amyloid deposition was most marked in the spleen. These hyaline substances stained positively with Congo red. Amyloidosis is less common in psittacine birds but when it does occur, generally involves both the spleen and the kidney (Schmidt *et al.*, 2003).

CONCLUSION

The present case has differences from the earlier published cases in that, first, it is about combined bacilli infection and aspergillosis and second, it was observed in a canary as an acute infection and eventually the lesions confined to the trachea and lung.

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