A case of tuberculosis and aspergillosis in a Long-Legged Buzzard (Buteo Rufinus)

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Introduction

Aspergillus is a cause of fungal disease usually in the respiratory system of wild poultry but also located in the whole body, resulting in general infections [2, 14, 16, 18]. Diseases caused by Aspergillus fumigatus can be both in acute and chronic forms [3, 7, 16, 18]. In birds in wildlife can also have the two forms [2, 7, 9, 14, 18]. Acute form of the disease is responsible for usually mortality in mature birds and brooder pneumonia in newly hatched chickens [16, 18]. Aspergillus was first seen and reported in a crow (European Jay) in 1815 [14, 20]. Aspergillus is also common in birds reared in cages [3, 7, 10, 16, 20]. Not only birds reared in cages but also birds living on water and birds fed on carrion are sensitive to the disease [7, 10, 14, 16, 18]. Although several paths have been known in literature, spread of the infection through respiratory path is more prominent [14, 16, 18].

Tuberculosis is a chronic disease and manifests itself with characteristic caseous necrotic lesions in liver, spleen, and gastrointestinal tract of poultry [17, 20, 21]. Waste water containing tuberculosis bacillus, contaminated rubbish, and manure comprise the infection sources for tuberculosis in birds fed on carrion and other wild birds [17, 20, 22]. Sensitivity to the disease increases in case of poor environmental conditions including malnutrition [20, 21, 22]. Poultry tuberculosis is observed only in mature birds due to the subclinical and chronic development of the disease. Mortality rate of tuberculosis among wild birds varies [4, 9, 15, 19]. It has been known that wild birds are more sensitive to M. avium due to their more primitive immune system compared with other endothermic birds [5, 10, 20, 21, 22].

There is no work in the literature reporting both tuberculosis and aspergillosis together in a Long-legged Buzzard (Buteo rufinus). Therefore, the main objective of this work was to report a case of both tuberculosis and aspergillosis together seen in a Long-legged Buzzard (Buteo rufinus) with clinical, pathological, and microbiological findings.

Materials and Methods

A four-year old female Long-legged Buzzard (Buteo rufinus) was brought to Erciyes University School of Veterinary Medicine to receive treatment for removal of a mass under...
the right wing joint. The bird was down on its sternum with no movement at all. The bird was also emaciated i.e. very weak and thin pectoral muscles. Although the mass was successfully removed, the bird did not recover from the anesthesia and died.

**Macroscopic examination:** The removed mass was fluctuant, 4x5.5x3 cm in size, and weighted 95 g with skin included (Figure 1A). After the palpation of left and right sides and outsides of the mass, a fluctuant, serous, colourless, and odourless liquid came out. Cystic and lobular formations as well as 0.2-0.5 mm necrotic centers in diameter with yellowish orange color were observed in cross sections (Figure 1B).

**Necropsy:** Spleen was enlarged and included grey-white necrotic lesions in serosa of cross sections. The appearance of ileal serosa was similar to that of the spleen. Intestinal lumen was full of parasites. There were many lesions in the lungs with a high degree of density and yellow-brown in color and 1-2 mm in diameter. The air sacks of the lungs were thickened and dim. Systemic organ samples were fixed in 10% buffered formalin. Following trimming and blocking in paraffin, 5-6 thick cross sections were prepared and stained with haematoxylin-eosin. Part of the cross sections were prepared at 37°C for 3-4 weeks of incubation [6].

For fungal isolation, 20 IU/ml penicillin G potassium (1,000,000 IU, I.E. Ulagay, Turkey) and 40 µg/ml streptomycine sulphate (1 g, I.E. Ulagay, Turkey) were added to a medium containing Sabouraud Dextrose Agar (SDA) (Oxoid, Hampshire, England). Czapex-Dox Agar (Oxoid, Hampshire, England) was used for identification [11, 12]. Incubation conditions were as follow: 25°C with aerobic media for 3-4 weeks. 10% KOH (Merck, Darmstadt, Germany) was used for direct microscopic examination of organ samples, whereas lactophenol cotton blue (GBL, Dudullu, Istanbul) was used to stain isolated colonies. Macroscopic and microscopic examinations of colonies were carried out using the method described by Arda [1]. In addition, Blood Agar Base (Difco, Detroit, USA) containing 5% sheep blood was used in order to detect also, if any, infection factor under the incubation conditions at 37°C with both aerobic and anaerobic media.

**Results**

**MICROSCOPICAL EXAMINATION**

Wide regions of necrotic granulomatous inflammations were observed in the cross sections of the mass from the wing as well as in spleen, lungs, and intestines (Figure 2A, 2B). Granulomas comprised giant cells around necrotic regions, heterophil leucocytes, cell infiltrations with macrophages, and fibrous tissue from the outside. Also observed were hyphae with septum and conidiophore structures characteristics for fungi in bronchial lumina and surrounding mesobronchus in some necrotic regions of the lung. For the differential diagnosis, Ziehl-Nielsen and Gridley’s staining were performed. Gram (+) and acid-fast bacteria using Ziehl-Nielsen staining in the mass from the wing, lungs, intestines, and spleen (Figure 3A), and also hyphae and spores of fungi using Gridley’s staining in the lung were identified (Figure 3B). Although both diseases were not observed in the liver and heart, granulomatous-type inflammation was observed in the intestine (ileum).

**BACTERIOLOGICAL AND MYCOLOGICAL EXAMINATION**

Acid-fast bacteria (dark red, rod) using Ziehl-Nielsen staining were identified. Colonies prepared in Löwenstein-Jensen media with glycerin (Merck, Darmstadt, Germany) were detected on day 12 with being white colored, with moisture, adhesive, and able to suspend in serum physiology. The mentioned colonies were determined to be *Mycobacterium* sp. based upon microscopic and macroscopic examinations.

Fungal elements were found after direct examination of the lung. Incubation with Sabouraud Dextrose Agar on the lung resulted with growth on the 10th day, whereas there was no growth on any other organ incubations. With microscopic examination, also observed were hyphae with septum and short and strait conidiophore structures, vesicles, followed by one-rowed sterigmat, and conidia. In the solid media, colonies first white followed by green and thin granules were detected. The findings indicated that the microorganism was A. *fumigatus*. No other microorganism was identified after incubation on Blood Agar.

**Discussion**

Aspergillosis is a disease usually which locates in the respiratory systems of such wild poultry as bald eagle, African fish eagle, Abyssinian tawny eagle, and wild duck causing acute or chronic form or the infection [2, 3, 7, 14, 16, 18]. Lung aspergillosis is known to expose itself simultaneously as a complication of other resistant-breaking diseases [16, 18]. Clinical signs of aspergillosis include anorexia, stagnation, drowsiness, dyspnea, increased breathing, diarrhea, sometimes convulsions, torticollis, and paralysis [14, 16, 17, 20]. In our case, the general signs of aspergillosis were similar in the Long-legged Buzzard, showing chronic symptoms in accordance with the literature. It was difficult to tell that the first disease was a complication of the other although it might have been seen that pulmonary aspergillosis was a complication of tuberculosis. In necropsy, it is worth to note that in the lungs there are common milier yellow and hard granulomas, the air sacs of the lungs are thickened and dark, and there is also vivid greenish-yellow exudate oozing out of the cross section of the lungs [2, 3, 14, 16, 18].

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Microscopically, necrotic granulomatous inflammations exist in the air sacs and the lungs with assorted sizes and shape of straight circles [16, 18]. At the centre of big granulomata, there are wide eosinophilic necrotic regions, giant cells toward outside, macrophages, heterophil leucocytes, and scattered fungal hyphae [2, 3, 7, 14, 16, 18]. Bronchus and mesobronchus lumina are full of mucus, fibrin, nucleus fragments, cell crumblings, leucocytic cell inflammations as well as hyphae and conidiophores [2, 7, 14, 16, 18]. Hyphae through penetration to parenchyma of the lung result in an exudative cellular inflammation and formation of necrosis [2, 16, 18]. In our case, necropsy and microscopic examinations revealed findings parallel with the literature.

Poultry tuberculosis is a chronic disease characterized by caseous necrotic tubercles in the liver, spleen, and gastrointestinal track of old poultry [17, 20, 21]. The disease is seen very often in wild poultry of North America and also rarely seen in birds reared in cages as well as in gulls and crows [8, 9, 10, 15, 19, 22]. Turkeys, pheasants, quails, geese, cranes and certain birds of prey are susceptible to the disease more than that of water birds. Tuberculosis is deadlier for captive waterfowl compared with other poultry [8, 9, 15, 17, 22]. It was hard to interpret our results since there was no incidence of tuberculosis reported on Long-legged Buzzard (Buteo rufinus) but in African fish eagle in the literature, although it has been know that tuberculosis is a chronic bacterial disease with a high incidence causing death of domestic and wild poultry [4, 8, 9, 15, 19, 20, 21, 22]. In terms of clinical findings, besides diarrhea and lameness, clinical symptoms of our case were similar to that of the literature including emaciation, stagnation, drowsiness, muscle atrophy, diarrhea, lameness, and unthrifty appearance [9, 10, 19, 20, 21, 22].

Tuberculosis is typically manifesting itself with lesions of nodular structures which are solid, yellowish-white or grey in color, and 0.5 mm - 1 cm in size [4, 17, 19, 20, 21, 22]. Lesions are located usually in the liver but also in the lungs, spleen, and intestines [4, 10, 15, 17, 19, 20]. Abscess and nodular proliferations located around the eyes, on the wings and leg joints, the face, and the base of the beak in poultry [8, 10, 15, 20]. Typical lesions are enlarged liver and spleen which are easy to crumble [9, 15, 19, 20]. The location of these lesions indicates the path of spreading bacillus [20, 21, 22]. It is thought that intestinal contamination of M. avium occurs through water and feedstuffs, and respiratory contamination through inhalation [15, 17, 19, 20]. Necropsy findings of our case were all similar to the literature reported for domestic and wild poultry [4, 9, 10, 17, 19, 20, 21, 22], except that we did not observe any liver lesions.

Poultry tuberculosis has been reported to be common in wild birds such as sparrows and starlings, and other birds such as crows and gulls that are in a close contact with wild birds feeding on carrion [10, 15, 19]. In our case, the Long-legged Buzzard living in a park under a protection program is likely to be in contact with wild birds including sparrows, starlings, and crows but not gulls as other investigators reported [9, 10, 15, 19, 20, 21]. Wild birds and other birds
fed on carrion become contaminated with tuberculosis through being exposed to \textit{M. avium} containing contaminants such as manure, contaminated water, and sewage \cite{17, 20, 22}. Therefore, the Long-legged Buzzard of our case might have been contaminated with tuberculosis through one of the contamination sources above. However, this conclusion cannot be drawn unless more cases to be found.

References