

Avian Tuberculosis in Kayseri Zoo

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Summary : In this study, wild birds died at Kayseri Be tepeler Zoo then presented for necropsy to Faculty of Veterinary Medicine at Erciyes University were investigated for pathological, bacteriological and mycological aspects from October 2004 to March 2005. Of 12 wild birds four were pigeons (*Columba domestica*), three were long-legged buzzards (*Buteo rufinus*), two were mandarin ducks (*Aix galericulato*), two were turkeys (*Meleagris gallopavo*), and one was pheasant (*Phasianus colchicus*). On postmortem examinations, tuberculosis lesions on the liver, lungs, spleen, and small intestine were observed. Histopathological examinations of lesions revealed granulomas with acid-fast bacilli within typical multinucleated giant cells of the granulomas. *Mycobacterium avium* subsp. *avium* was isolated and identified by bacteriological culture and BACTEC rapid radiometric technique, respectively. In conclusion, the incidence of avian tuberculosis is high among wild birds in Kayseri zoo.

Key Words: Isolation, *Mycobacterium avium*, tuberculosis, wild bird

Kayseri Hayvanat Bahçesinde Kanatlı Tüberkülozisi

Özet : Bu çalı mada, Kayseri Be tepeler hayvanat bahçesinde Ekim 2004-Mart 2005 tarihleri arasında ölen yabancı kanatlılar patolojik, bakteriyolojik ve mikolojik yönden incelendi. Bu süre içinde 4 güvercin (*Columba domestica*), 3 kızıl ahin (*Buteo rufinus*), 2 mandarin örde i (*Aix galericulato*), 2 hindi (*Meleagris gallopavo*) ve 1 sülün (*Phasianus colchicus*) ölü olarak Erciyes Üniversitesi Veteriner Fakültesine getirildi. Nekropside karaci er, akci er, dalak ve ince ba ırsaklarda tüberküloz lezyonları gözlemlendi. Lezyonların histopatolojik incelemesinde çok çekirdekli dev hücrelerinde asido-rezistans basillerin bulundu u granulomlara rastlandı. Kültürel yoklama ve BACTEC hızlı radiometrik teknikle ölen tüm hayvanlardan *Mycobacterium avium* subsp. *avium* izole ve tanıya edildi. Sonuç olarak, Kayseri hayvanat bahçesinde yabancı kanatlılar arasında tüberkülozun insidensinin yüksek oldu unu saptandı.

Anahtar Kelimeler: zolasyon, *Mycobacterium avium*, tüberkülozis, yabancı kanatlı

Introduction

Avian tuberculosis (ATB) most commonly caused by *Mycobacterium avium* is a chronic wasting disease characterized by progressive development of tubercles in any tissue or organ of the body in birds (13). Natural infection of many domestic and wild avian species was reported in several countries (1,3,5,6). A few ATB cases were also reported in Turkey (7,10,11,12). Infection is transmitted by ingestion and inhalation of aerosolized infectious organisms from feces (4). The gross lesions are suggestive of tuberculosis, but a definitive diagnosis is based on bacteriological isolation and identification of the organism (9). In this study, the pathological and bacteriological features of avian tuberculosis were described.

Material and Methods

Four pigeons (*Columba domestica*), two mandarin ducks (*Aix galericulato*), three long-legged

buzzards (*Buteo rufinus*), one pheasant (*Phasianus colchicus*) and two turkeys (*Meleagris gallopavo*) died unexpectedly without any clinical signs of the disease at Kayseri Be tepeler Zoo between October 2004 and March 2005. Then they were presented to Faculty of Veterinary Medicine, University of Erciyes. Ducks, pheasant and turkeys at the same aviary while pigeons and buzzards were housed in separate aviaries.

In this study, five different species of total 12 wildlife birds were examined. Complete postmortem examination was performed. According to gross visual lesions, tuberculosis was suspected. Lungs, liver, spleen and cloacal swap samples were collected for subsequent bacteriological and histopathological examinations. Sixteen feces from litter, 5 feed and 3 water samples were also collected from the aviaries to determine the source and the transmission route of the infection.

Histopathological examinations

Tissue samples were fixed in 10% neutral-buffered formalin. The pieces of preserved organs were

embedded in paraffin, sectioned (5-6 μm), and mounted on glass slides, stained with haematoxylin and eosin (H&E) and Ziehl-Neelsen (ZN) "acid-fast stain" (8).

Bacteriological examinations

The smears prepared from the tissues, cloacal swaps and aviary samples were stained by ZN technique. The samples were decontaminated by a commercial kit (Or-Set 03, Or-Bak, Ankara, Turkey), inoculated on Lowenstein-Jensen (LJ) medium (Merck, Darmstadt, Germany) supplemented with glycerine, and in Middlebrook 7H12 (BACTEC 12B) medium (Becton Dickinson, Maryland, USA). Identification of isolates of mycobacteria was based on growth on LJ medium, colony morphology and following biochemical tests such as catalase, nitrate reduction, urease, pyrazinamidase and niacin accumulation (9). Furthermore, tissue samples were inoculated on Sabouraud Dextrose Agar (SDA) (Merck, Darmstadt, Germany) for differentiation of mycotic agents.

Results

Necropsy revealed disseminated white-yellowish, firm, nodular granulomas ranged from 1 mm to 4 cm in diameter throughout the spleen, liver, intestinal serosa and lungs (Figs 1a, 1b and 1c)



Figure 1. Varied sized white yellowish caseous foci in avian tuberculosis **1a)** Almost all the spleen parenchyma become caseous granuloma. Caseous foci in abdominal wall (white arrows), intestine (black arrows) **1b)** Caseous foci in liver, intestine (black arrows) **1c)** Cut surface of spleen

Almost the entire splenic parenchyma was replaced by caseogranulomas which were yellowish in colour and 4 x 3 x 2 cm in diameter in mandarin ducks. In addition, miliary foci on kidneys and proventriculus, and nodular foci on abdominal wall were observed in a pigeon. In histopathological examinations, characteristic

granulomas were seen in the sections of liver, spleen, lungs, and serosa of small intestines of all animals. Non-mineralized caseous necrosis at the centres of the granulomas, and epithelioid cells, multinucleated giant cells and macrophages at the periphery of the granulomas, were observed (Figs 2a, 2b and 2c). In the tissue sections stained by ZN, acid-fast bacilli were detected within multinucleated giant cells as well as in necrotic areas (Fig. 2d).

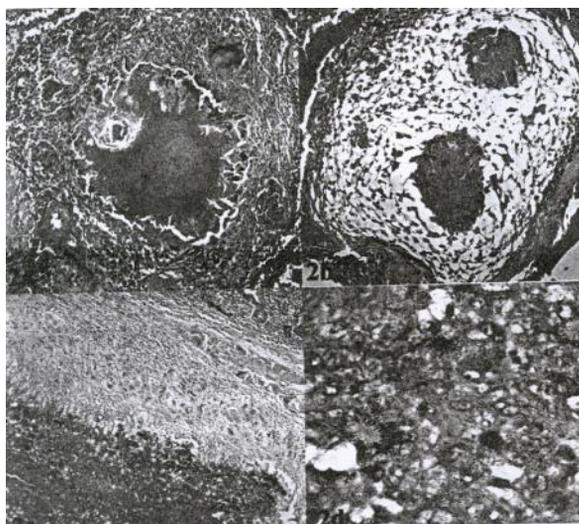


Figure 2. Typical caseous granulomas **2a)** Spleen, HE stain, x 200 **2b)** Lung, HE stain, x 200 **2c)** Intestine, HE stain, x 200 **2d)** Acid-fast bacilli within multinucleated giant cells at periphery of granuloma and necrotic areas, ZN stain, x1000.

Acid-fast bacilli (AFB) were seen in the smear prepared from the necropsy samples and cloacal swaps while the smears from aviary samples were negative for AFB. *Mycobacterium avium* subsp. *avium* was isolated and identified from the necropsy samples and cloacal swaps by the conventional procedure and by the BACTEC rapid radiometric technique. However, *M. avium* was not isolated from aviary samples. No growth on the SDA was occurred.

Discussion

Mycobacterium avium has a wide spectrum of hosts including poultry, pigeons, raptors, ratites, wild birds, psittacines, and passerines (3,5,6,7). Disease associated with *M. avium* has occurred mostly within captive settings and does not appear to occur commonly in free-living birds. The difficulties for the elimination of the disease in zoological gardens arise from the captivity of the

animals, improper cleaning and disinfection of the contaminated aviaries, survival of the tuberculosis' agents in the soil for a long time (13). In the present study, the factors that increase the susceptibility to tuberculosis were present, and the wildlife bird species in which the disease seen were similar to the species indicated elsewhere (3,7).

Ingestion of the bacterium in contaminated feed and water is the most common means of disease transmission (4,6). Furthermore aerosol route may also play an important role in the transmission of the disease (2). In the present study, the etiologic agent and lesions present in the liver, spleen, small intestines, lungs and cloacal swaps. Although agent and lesions were detected in the liver, spleen and lungs, isolation of the agent from small intestine and cloacal swaps supported the initial occurrence of the infection in the intestinal tract (13). In contrast to the findings of Özcan et al. (10) who found granulomas rarely in the lungs, in this report lesions were common in the lungs and showed that aerosol route may be effective in the transmission of the infection from bird to bird. Inability to isolate the causative agent from feces, feed and water samples collected from aviaries supported the suggestion of the aerosol transmission.

Various methods are used for the isolation and identification of the agent (9). In this study, both conventional method and rapid colorimetric technique were used. The findings of this study showed that BACTEC rapid radiometric technique can be a rapid indicator of the presence of tuberculosis agents in tissues. BACTEC system is a sensitive test for *M. avium* detection and it reduced detection time in tissue samples. In conclusion, to the author's knowledge, this is the first reported outbreak of tuberculosis in wild birds in Kayseri. These results demonstrated that the incidence of avian tuberculosis is high at zoo aviary in Kayseri, Turkey.

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