An outbreak of avian tuberculosis in peafowl (*Pavo cristatus*) and pheasants (*Phasianus colchicus*) in a zoological aviary in Turkey

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ABSTRACT: Avian tuberculosis was diagnosed histopathologically and microbiologically in two pheasants (*Phasianus colchicus*) and two peafowl (*Pavo cristatus*) kept in the same aviary. The incidence of avian tuberculosis in the aviary was 6%. Non-mineralized caseogranulomas were present in the liver (3 cases), spleen (3 cases), intestine (2 cases), lung (2 cases), and cloaca (1 case). Granulomas in the lung were present only in peafowl. The presence of granulomas in the lung of both infected peafowl suggests that peafowl were exposed to the agent via the respiratory route rather than the alimentary route. Histopathologic findings were typical of avian tuberculosis, including acid fast bacilli and centrally located caseo-necrosis surrounded by epitheloid macrophages, lymphocytes, and multinucleated giant cells. *Mycobacterium avium* subsp. *avium* was isolated from tissue samples of all infected birds.

Keywords: avian tuberculosis; pathology; peafowl; pheasant; zoonosis

Mycobacterium avium subsp. *avium* causes a chronic infection in several species of birds that is characterized by granuloma formation in various organs (Kutsal and Saglam, 1988; Butcher et al., 1990; Shane et al., 1993; Singbeil et al., 1993; Sanford et al., 1994; Sato et al., 1996; Thoen, 1997; Doneley et al., 1999; Marco et al., 2000; Gonzalez et al., 2002). The frequency of the disease in pheasants (*Phasianus colchicus*) is unclear due partially to the limited number of reports (Hejlicek and Treml, 1993; Singbeil et al., 1993; Hejlicek and Treml, 1995b). Similarly, information on avian tuberculosis (ATB) in peafowl (*Pavo cristatus*) is limited to the reports of few experimental and natural infections (Hejlicek and Treml, 1995a,b; Thoen, 1997). Because of insufficient data, the pathological characteristics of natural avian mycobacteriosis in both species as well as their susceptibility are yet to be outlined in detail.

Avian tuberculosis in birds living in zoological aviaries can cause serious economical losses including the death of rare and unusual species of birds (Singbeil et al., 1993; Sato et al., 1996; Marco et al., 2000). In addition, because of the zoonotic potential of the microorganism, exposure to infected birds may cause infection in humans especially those with immunocompromised diseases such as HIV/AIDS (Butcher et al., 1990; Thoen, 1997; Doneley et al., 1999; Martin and Schimmel, 2000). In 50–60% of HIV/AIDS patients, *M. a. avium* infection is encountered (Birkness et al., 1999; Martin and Schimmel, 2000).

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The present report describes the pathologic and bacteriologic findings of natural ATB in two pheasants and two peafowl collected from a zoological aviary in Turkey.

MATERIAL AND METHODS

Animals

Avian tuberculosis as a flock problem was encountered in a zoological aviary in Turkey during a period of three months (from 08/1998 to 10/1998). The aviary housed the 66 birds of six different species; duck (*Anas platyrhynchos*) (n = 12), goose (*Anser anser*) (n = 14), red-legged partridge (*Alectoris rufa*) (n = 5), pigeon (*Columbia livia*) (n = 28), pheasant (*Phasianus colchicus*) (n = 4) and peafowl (*Pavo cristatus*) (n = 3). The incidence of avian tuberculosis in the aviary was 6%. A ten-month-old ringneck pheasant, raised in this zoological aviary, died following clinical signs of emaciation and listlessness for three weeks. Following an immediate necropsy procedure, the suggestive diagnosis was made of mycotic infection, avian tuberculosis or neoplasia. Clinical signs of anorexia, progressive emaciation and decrease in egg production prior to death had also been recorded in another pheasant and two peafowl over several months. These emaciated birds were euthanasied and necropsied systematically. The remaining two pheasants and a peafowl in the same shelter along with the other species of bird exhibited no clinical sign.

Laboratory examinations

For histopathological examination, visceral organs were collected systematically and fixed in 10% neutral formalin. Tissues were processed routinely, embedded in paraffin and sectioned at a thickness of 5 μm. Paraffin sections were stained with hematoxylin and eosin and Ziehl-Neelsen's stain. For microbiological examination, samples of liver and lung lesions were collected, treated with 4% NaOH and ground in a sterile mortar and pH was set at 7.4. After centrifuging at 5 000 rpm, the precipitates were cultured in tubes with Lowenstein-Jensen Medium (BBL, Kansas City, Missouri, USA) for 8 weeks. Samples not treated with 4% NaOH were cultured on Sabouraud dextrose agar (Oxoid, Hampshire, UK) for mycologic examination.

RESULTS

Necropsy findings

The organ distribution of the granulomas is indicated in Table 1. The first pheasant died had emaciated chest musculature. Extensive miliary and nodular granulomas were present in the liver, spleen and intestinal serosa. The liver was markedly enlarged and its parenchyma was replaced by well demarcated caseogranulomas, which were tan to yellow in colour and 0.5 cm to 1.5 cm in diameter (Figure 1). The second pheasant (male, age unidentified) had also miliary granulomas in the liver and spleen.

Cachexia with severe atrophy of the breast muscles was observed at necropsy of the first peahen whose age was undefined. A granuloma, 6 × 4 × 3 cm in size, was present in the right lung (Figure 2). Several smaller granulomas of various sizes (up to 0.5 cm in diameter) were also present in the spleen parenchyma.

The second peahen with an undefined age had caseogranulomas in the serosa of the right cecum (4.5 × 4 × 2.5 cm) and colon (4 × 3 × 2 cm). Another granuloma was present in the cloaca that nearly

Table 1. The organ distribution and severity of macroscopic and histopathologic findings

Case No.	Species	Macroscopic/histopathologic findings				
		liver	spleen	intestines	lung	cloaca
1	pheasant	+++/+++	+++/+++	++/+++	–/–	–/–
2	pheasant	+/++	+/++	–/–	–/–	–/–
3	peahen	–/–	++/++	–/–	+++/+++	–/–
4	peahen	–/–	–/–	–/++	+++/+++	+++/+++

– none, + mild, ++ moderate, +++ severe

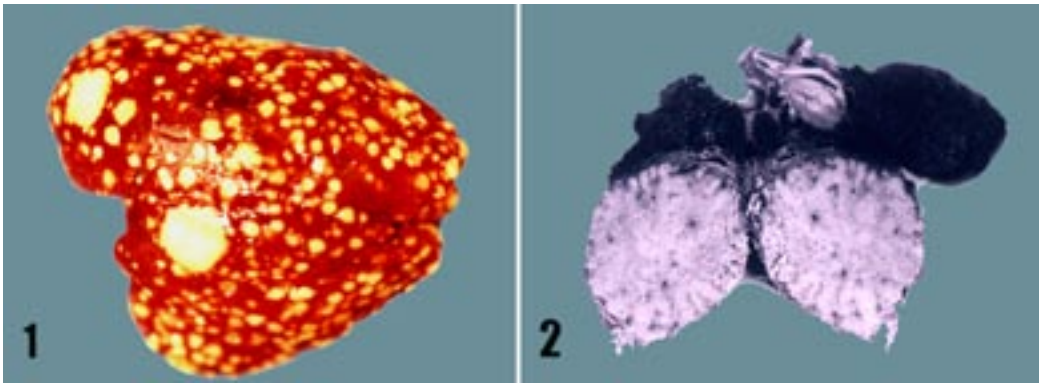

Figure 1. Gross view of multinodular granulomas in the liver in a pheasant

Figure 2. A large granuloma in the right lung of a peahen

occupied the entire pelvic cavity (4 × 2 × 1.5 cm). Several miliary nodules in the liver surface and a nodule (1 × 1.5 × 0.5 cm) in the right lung were observed. The serous membranes of the air sacs had a cloudy appearance and contained smaller diffuse nodules with a size of 0.1 cm to 0.5 cm.

Histopathological findings

Histopathological findings in all four cases were typical of avian tuberculous granulomas. Multiple granulomas observed in the liver, spleen, lung, cloaca and intestinal serosa (Table 1) were characterized by non-mineralized caseous necrosis at the centre, which was surrounded by a layer of epithelioid cells, multinucleated giant cells, and macrophages (Figures 3 and 4). Lymphocytes and macrophages were located at the peripheral margins of the granulomas. In tissue sections stained with Ziehl-Neelsen's stain, a large number of acid-fast bacilli were present within multinucleated giant cells as well as free in the necrotic areas.

Microbiological findings

M. a. avium was isolated from samples of all four cases and identified according to its phenotypic characteristics. Mycological cultures were all negative.

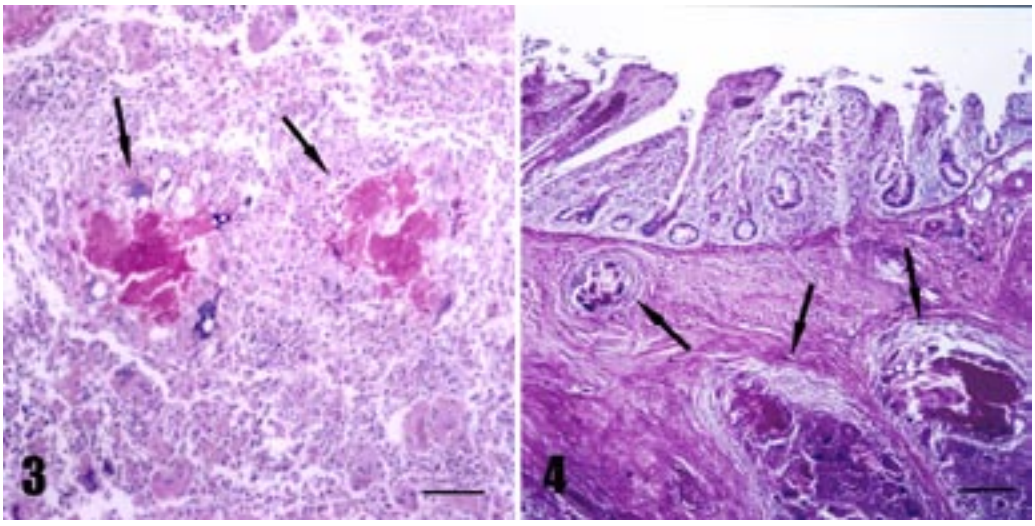

Figure 3. Typical caseogranulomas (arrows) in the lung of a peahen. Bar = 100 μm, hematoxylin and eosin

Figure 4. Typical caseogranulomas (arrows) in the serosa, extending to the muscular layer and submucosa of the intestine. Bar = 240 μm, hematoxylin and eosin

DISCUSSION

Avian tuberculosis is considered as a sporadic disease of birds in the wild (Kutsal and Saglam, 1988; Sato et al., 1996; Thoen, 1997). However, its frequency may increase if birds are held together in captivity (Singbeil et al., 1993; Sanford et al., 1994; Marco et al., 2000). In our study, ATB was a flock problem. Two peafowl and two pheasants kept in the same aviary exhibited severe clinical symptoms for a period of up to two months. The remaining two pheasants and one peafowl were not clinically influenced by the disease which suggests that individual response to the disease varies among the birds of the same species.

Pathological changes in avian tuberculosis are characterized by non-calcified granulomas, which are usually localized in the spleen, liver and intestinal serosa (Butcher et al., 1990; Hejlicek and Treml, 1993; Shane et al., 1993; Singbeil et al., 1993; Thoen, 1997; Doneley et al., 1999; Marco et al., 2000; Gonzalez et al., 2002). Likewise, granulomas in the liver (in 3 cases), spleen (in 3 cases), and intestine (in 2 cases) were also typical findings in the present study (Table 1). This type of an organ distribution of lesions is explained by an initial localization of the bacteria to the intestinal mucosa and its subsequent ingestion through the intestinal system. Eventually, bacteria are carried to the liver and spleen via blood drainage and lymphatics (Sanford et al., 1994; Thoen, 1997; Doneley et al., 1999; Marco et al., 2000). In the present study, we also observed a large granuloma in the cloaca of a peafowl and in the lung of both peafowl. Interestingly, in the peafowl with a large granuloma in the cloaca, the other intestinal granulomas were localized only in the lower gastro-intestinal tract, the cecum and colon. It is possible that initial contamination might have occurred in the cloaca. Infection even through the skin has been reported (Sanford et al., 1994). Exposure of the cloacal mucosa to contaminated soil may have induced formation of a local granuloma. *M. a. avium* can survive in the soil for years and contaminated soils can be a source of infection (Doneley et al., 1999).

Granulomas in the lung are rare in ATB (Sanford et al., 1994; Sato et al., 1996; Marco et al., 2000; Gonzalez et al., 2002). This is partially attributed to the inconsistent binding ability of *M. avium* to different types of epithelia. It binds to intestinal epithelia with much higher affinity compared to respiratory epithelia. Thus, the gastrointestinal tract is considered as the usual route of ATB, and the respiratory pathway is less frequent (Birkness et al., 1999). However, in the present study, granulomas in the lung were present in both peafowl, and one peafowl had no lesions in the liver and intestines at all. Such a distribution of lesions may be indicative of exposure through the respiratory system. Despite the fact that the number of peafowl in the present study is not sufficient to make a strong conclusion, we think that peafowl may be more sensitive to the respiratory system contamination than to the alimentary system.

Variations in sensitivity among different species of birds in response to *M. a. avium* infections have been reported (Hejlicek and Treml, 1993; Singbeil et al., 1993; Hejlicek and Treml, 1995a,b; Thoen, 1997). Experimental studies concluded that the pheasant is sensitive, guinea fowl is less sensitive, goose and duck are resistant and pigeon is very resistant to *M. a. avium* (Hejlicek and Treml, 1993, 1995b). Our study also indicated that there is variation in response to *M. a. avium* among different species of birds. Ducks, geese, partridges and pigeons did not exhibit any clinical sings of the disease although they had been sheltered in the same aviary. Importantly, the present study indicated that peafowl and pheasant are infected naturally with *M. a. avium* on which the number reported in the literature was so scarce.

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