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Spontaneous *Mycobacterium avium* serovar 2 infection in a Muscovy duck (*Cairina moschata*) - a case report

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ABSTRACT

Avian tuberculosis is an avian disease of worldwide importance. It is typically caused by serovars 1, 2 and 3 of *Mycobacterium avium* subsp. *avium*. This paper describes spontaneous, subclinical tuberculosis in a 5-year-old male Muscovy duck. Necropsy revealed a characteristic distribution pattern of granulomatous lesions involving the liver and spleen. Histopathological analysis confirmed the formation of tubercles containing a large number of giant and epitheloid cells, while PCR analysis (according to insertion sequence IS901) and an agglutination test detected *M. avium* serovar 2 as the causative agent of this condition.

Key words: avian tuberculosis, *Mycobacterium avium* serovar 2, Muscovy duck

Introduction

Avian tuberculosis is an important disease in captive, exotic and free-ranging wild birds all over the world. Of the mycobacterium genera, the *Mycobacterium avium* subspecies avium is most widely distributed (THOEN and BARLETTA, 2004). A variety of different factors can influence the pathogenesis of this disease, i. e. age, stress or immune status. The synergy of these factors and different *M. avium* serovars can result in a variety of

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forms of disease expression. Muscovy ducks (*Cairina moschata*) are the only domestic ducks that are not derived from mallard stock. These large ducks originate from Brazil and can vary from black and white in colour in the wild, to different colours produced during domestication (DONKIN, 1989). There are only a few reports in the scientific literature on diseases in Muscovy ducks (DAVISON et al., 1993; TAKAHASHI et al., 1996; WOOLCOCK et al., 2000; CAMPAGNOLO et al., 2001; PALYA et al., 2003). In this paper, pathological, PCR and serological evidence of spontaneous, sub-clinical tuberculosis in a male Muscovy duck is presented.

Material and methods

During September 2004, ten Muscovy ducks were killed as part of a selection process in extensive hobby rearing. At post-slaughter examination several granulomatous lesions were observed in one male. This 5-year-old male had been imported into the breeding stock two years earlier. Samples of liver, spleen and testes were taken for histopathological analysis and stained routinely by haematoxylin-eosin and additionally by Ziehl-Nielsen staining for detection of acid-fast bacilli (ŠVOB, 1974). At the same time, samples of liver, spleen and testes were cultured and recovered mycobacteria were identified by the standard procedure (KENT and KUBICA, 1983).

In the process of *Mycobacterium* identification by means of the polymerase chain reaction (PCR), two primers were used: TB1 (5'-GAG-ATC-GAG-CTG-GAG-GAT-CC-3') and TB2 (5'-AGC-TGC-AGC-CCA-AAG-GTG-TT-3') (HANCE et al., 1989). Five µL of extracted DNA were amplified in a 50 µL reaction mixture containing 1 unit of enzyme Amplitaq Gold DNA polymerase (Applied BioSystems, USA), 4 µL of nucleotide mixture (dATP, dCTP, dGTP, TTP; each in a concentration of 200 mM), buffer solution to 5 µL, $MgCl_2$ to 4 µL (Applied BioSystems, USA) and distilled water. The reactions were amplified with 33 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds and extension at 72 °C for 60 seconds, with a final extension at 72 °C for 5 minutes (GeneAmp PCR System 2700, Applied BioSystems, USA).

Identification of pathogenic *M. avium* serovars (1, 2 and 3) of the mycobacteria in question was performed according to the possession of the insertion sequence IS901 (KUNZE et al., 1992). For that purpose, a pair of primers, P1 FR300 (5'- CAG-CCA-GCC-GAA-TGT-CAT-CC-3') and P2 FR300 (5'- CAA-CTC-GCG-ACA-CGT-TCA-CC-3') was used. Two µL of extracted DNA were amplified in 20 µL of reaction mixture containing 10 µL of HotStarTaq Master Mix (Qiagen GmbH, Germany), distilled water to 7.9 µL and 0.1 µL of primers (each in a concentration of 10 mM). The reactions were amplified with 33 cycles of denaturation at 94 °C for 60 seconds, annealing at 63 °C for 45 seconds and extension at 72 °C for 90 seconds, with a final extension at 72 °C for 3 minutes.

The amplified products were detected by gel electrophoresis using 2% - agarosa gel, using UV transluminator and camera (Bio-Capt, Vilbert Lourmat, France).

Finally, the agglutination test was applied to identify the respective serovar of *M. avium* (SCHAEFER, 1965; SCHAEFER, 1979).

Results and discussion

Post-mortem examination revealed an enlarged right lobe of the liver with two focally distributed granulomatous lesions (Fig. 1).

Fig. 1. Focally distributed granulomatous lesions on the liver (marked with A) and spleen (marked with B) of a presented Muscovy duck

A similar lesion was observed on the spleen. The lesions were firm, greyish-white in colour and rose above the surrounding parenchyma. The clinical history provided was that the animal showed no signs of disease prior to slaughter. The first presumptions were directed to avian tuberculosis or neoplasia. However, the characteristic distribution of the lesions in this case (liver and spleen) pointed directly to tuberculosis as the cause of this condition (FRANCIS, 1958). Furthermore, additional histopathological analysis revealed typical granulomatous lesions in the liver (Fig. 2) and spleen, while the testes showed no signs of lesion development. Considering the fact that Mycobacteria are intracellular bacteria, granuloma formation is an attempt by the host to localize the disease process and to allow the immune mechanisms to destroy the bacilli (THOEN and BARLETTA, 2004). This is the crucial moment for the shift of the disease into the more chronic, or in other cases, the acute, generalized type. Development of the first microscopical lesions in the liver and spleen occurs in ducks 12 days after infection, while they become visible on the liver 133

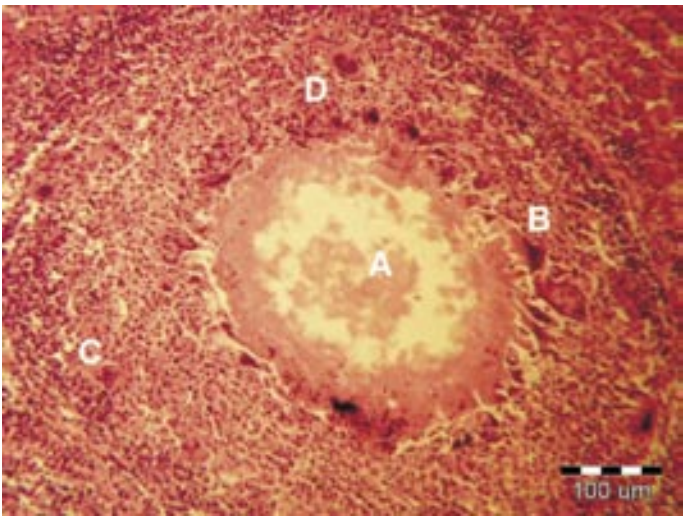

Fig. 2. A section of a tubercle. Necrotic centre (A) was surrounded with different types of inflammatory cells. Note the presence of both, Langhans (B) and the foreign body (C) type of giant cells. Among them, epitheloid cells were present (D). H&E; × 16, scale bar = 100 µm.

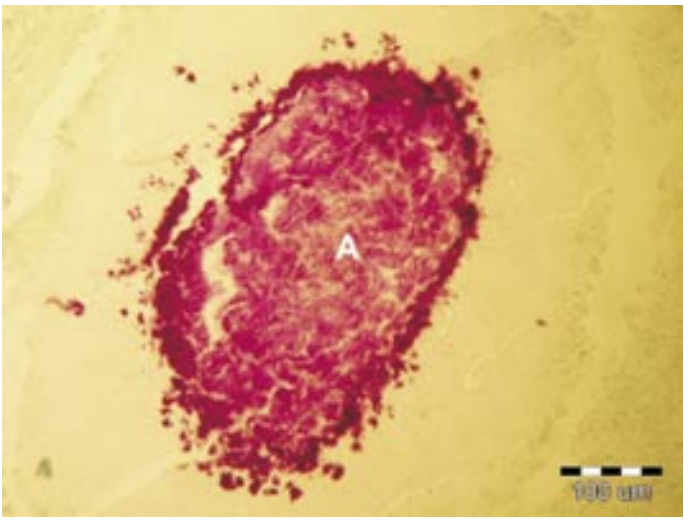

Fig. 3. Additional staining revealed numerous acid-fast bacilli located mainly in the centre of a tubercle. Ziehl-Nielsen; × 16, scale bar = 100 µm.

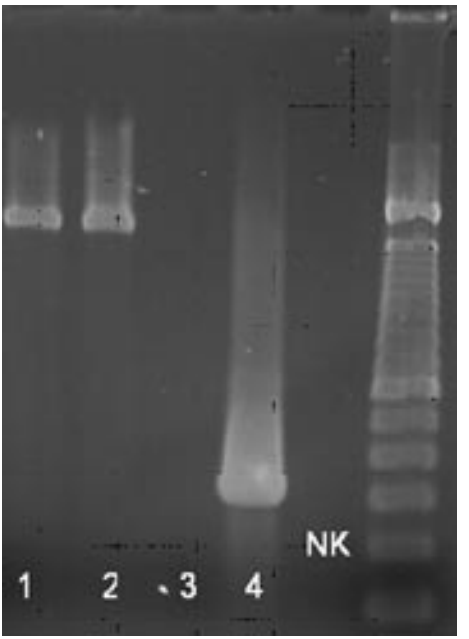

Fig. 4. The second PCR reaction has confirmed that tuberculous lesions were caused by bird-pathogenic *Mycobacterium avium*. Legend: 1) material from examined duck (1700 bp); 2) *Mycobacterium avium* serovar 2 (1700 bp); 3) Mycobacterium bovis; 4) *Mycobacterium avium* serovar 4 (300 bp); NK) negative control.

days after per-oral infection (CERNY, 1982; HEJLICEK and TREML, 1995), allowing us to assume that the course of the disease was longer. As seen in Fig. 2, the necrotic centre of the lesion was surrounded by a large number of inflammatory cells, i.e. lymphocytes, macrophages, epitheloid and giant cells. Among the giant cells, both Langhans and foreign-body types were present, indicating the presence of delayed-type hypersensitivity (DTH). Special, Ziehl-Nielsen staining revealed a large number of acid-fast bacilli, localized in the centre as well as on the periphery of the lesions (Fig. 3).

M. avium subsp. *avium* and the taxonomically closely related *M. intracellulare*, both referred to as *M. avium* complex, can be divided into a large number of different serovars (FULTON and THOEN, 1997; CVETNIĆ et al., 2001; THOEN and BARLETTA, 2004). Among them, birds are susceptible to *M. avium* serovars 1, 2 and 3, whereas other serovars produce only a minimal level of disease following intravenous or intraperitoneal inoculation (THOEN and BARLETTA, 2004). The first applied PCR reaction, in which we used primers TB1 and TB2 (HANCE et al., 1989), confirmed on the molecular level that our isolates belong to the Mycobacterium genera. The second PCR reaction revealed that the lesions in the liver

and spleen of the Muscovy duck in question were caused by the *M. avium* (KUNZE et al., 1992) (Fig. 4). Finally, by means of the agglutination test we confirmed that the lesions on the liver and spleen of this Muscovy duck were caused by *Mycobacterium avium* serovar 2. CVETNIĆ et al. (2001) have reported that the most frequent cause of avian tuberculosis in Croatia is *M. avium* serovar 3 (52.63%), that serovar 2 is involved in 23.68% of cases, while serovar 1 is mainly found in wild birds. Even though serovars 2 and 3 are the most frequent, extremely resistant to environmental conditions and highly transmissible among birds, they do not pose a great risk for human health. According to FALKINGHAM III (1994), serovars 2 and 3 were not recovered from human patients in the United States. FULTON and THOEN (1997) have also stated that *M. avium* serovar 2 is rarely isolated from humans. Furthermore, they observe that the majority of cases of avian tuberculosis in humans are acquired through human-to-human contact.

Therefore, we can conclude that even though the necropsy procedure is usually sufficient to diagnose tuberculosis, it cannot provide us with a final diagnosis of the serovars of the causative mycobacteria. In order to understand the epidemiological meaning of avian tuberculosis it is necessary to apply other more sophisticated methods, i. e. an agglutination test or PCR.

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SAŽETAK

Avijarna tuberkuloza je značajna bolest ptica diljem svijeta, najčešće uzrokovana serovarom 1, 2 ili 3 bakterije *Mycobacterium avium* subsp. *avium*. Ovaj rad opisuje spontanu, supkliničku tuberkulozu u 5 godina

starog mužjaka mošusne patke. Pri razudbi je uočen karakterističan raspored granulomatoznih tvorbi po jetri i slezeni. Patohistološkom pretragom potvrđeno je formiranje tuberkula s velikim brojem divovskih i epitelnih stanica, a PCR-om (sekvenca IS901) i aglutinacijskim testom potvrđeno je da je bakterija *M. avium* serovar 2 uzročnik opisane bolesti.

Ključne riječi: avijarna tuberkuloza, *Mycobacterium avium* serovar 2, mošusna patka