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<증례보고>

Mycobacterium avium subsp. *avium* infection in a lineolated parakeet (*Bolborhynchus lineola*)

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Abstract : A 2-year-old lineolated parakeet (*Bolborhynchus lineola*) was presented with abdominal distention and respiratory distress for two months. The bird was poorly fleshed and the liver was enlarged on coelomic palpation. Plain and contrast radiographic examinations exhibited hepatomegaly and distended intestinal loop, which compromised the air sacs. Multifocal hyperechogenecity was observed in the liver on ultrasonography. Postmortem gross examination revealed hepatomegaly with numerous pinpoint tan foci in the hepatic parenchyma and distended small intestine filled with adult ascarids. Microscopically, granulomatous hepatitis and enteritis infected by intrahistiocytic acid-fast bacilli were evident. Polymerase chain reaction indicated that the acid-fast bacilli were *Mycobacterium avium* subsp. *avium*.

Keywords : avian mycobacteriosis, *Mycobacterium avium* subsp. *avium*, parakeet

Mycobacterial species are opportunistic environmental saprophytic microorganisms with wide vertebrate host range, which are commonly found in soil with fecal contamination or other organic debris [2,10]. Avian infection with some mycobacterial species causes avian mycobacteriosis, a chronic disease that affects all species of birds [12]. For example, *Mycobacterium* (*M.*) *avium* complex (MAC) and *M. genavenes* are most commonly associated with avian mycobacteriosis [5, 12] and *M. tuberculosis* and *M. bovis* have been also reported as causative microorganisms in some psittacine cases [1, 12]. Although no direct transmission of those microorganisms from birds to humans have been reported, indirect transmission is possible to cause human diseases such as tuberculosis or hypersensitivity pneumonitis [6]. Therefore, avian mycobacteriosis is a potentially important zoonotic disease.

A 2-year-old female lineolated parakeet (*Bolborhynchus lineola*) weighing 50 g was presented for progressive episodes of abdominal distention and respiratory distress dating back 2 months. The bird had been housed with a male parakeet in a stainless-steel cage. Physical examination revealed emaciation, poor feather condition, wide stance, and open-mouth breathing. The bird passed normal forms of urine, but the feces were watery. The caudal edge of the liver was palpable in the cranial coelom. Large numbers of ascarid eggs were detected on fecal floatation. Whole body radiographs revealed an extended coracoid-acetabulum line and compromised caudal thoracic and abdominal air sacs (Fig. 1A). In addition, poor serosal detail of the caudal

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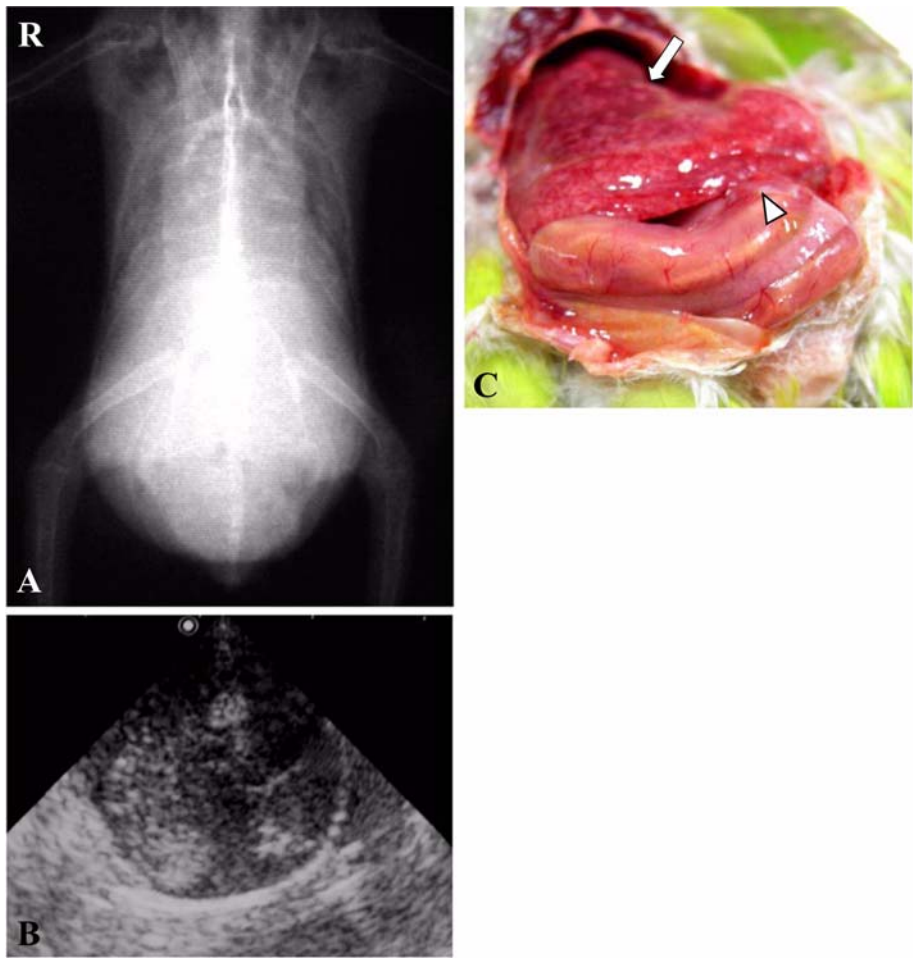

Fig 1. Widening of the cardiohepatic angle and compromised air sacs are indicated on ventrodorsal radiographic finding (A). These results imply organomegaly. And multifocal hyperechoic lesions of the hepatic parehchyma are identified on ultrasonographic examination (B). Hepatomegaly with generalized tan 1-2 mm foci on the surface of the liver (arrow) are observed on necropsy of a parakeet. Also, distended small intestine due to ascarial impaction (arrow head) is identified (C).

coelom was observed. Hepatic ultrasound examination identified hepatomegaly with multifocal hyperechoic lesions (Fig. 1B), which raised suspicion of hepatic granuloma or neoplasia.

The bird expired 2 days after presentation and a postmortem examination was performed. At necropsy, the liver was enlarged with generalized 1-2 mm diameter tan foci throughout the lobes. The small intestine was distended and filled by large numbers of adult nematodes that were consistent with ascarids (Fig. 1C). For microscopic examination, tissue samples were collected, fixed in 10% neutral buffered formalin, processed by routine procedures, and sectioned. The slides were stained with hematoxylin and eosin and Ziel-Nielsen (ZN) staining solutions. Microscopically, the liver had multifocal necrosis that was often accompanied by infiltration of a few macrophages and surrounding intense heterophils and occasional lymphocytes. ZN stain revealed moderate to large numbers of acid-fast bacteria (Fig. 2). Small intestinal villi, often at the tip, were infiltrated by moderate numbers of macrophages and multinucleated giant cells. Similar acid-fast bacteria were observed by ZN stain. Based on the gross and histopathologic findings, mycobacteriosis and ascarial impaction were diagnosed.

For molecular diagnostics, DNA was extracted from the paraffin-embedded tissue using a NucleaoSpin Kit (BD Biosciences Clontech, USA) according to the

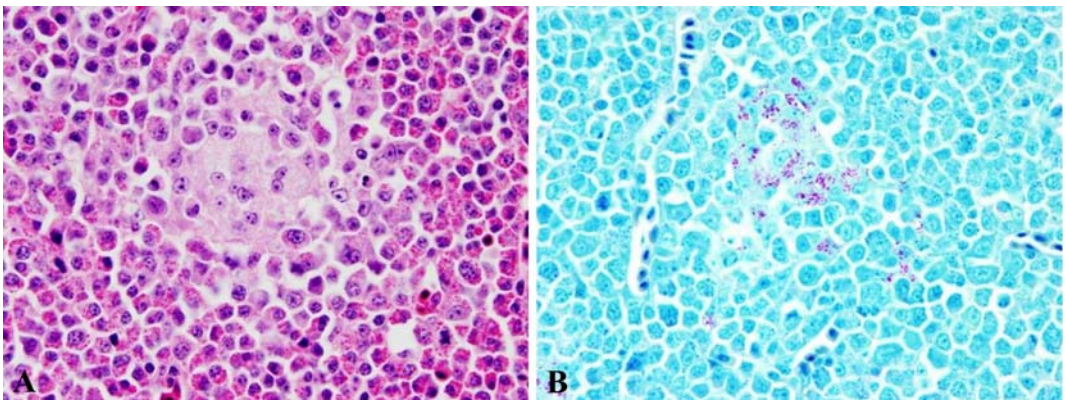

Fig. 2. Histopathologic findings of the liver. (A) Discrete granulomatous hepatitis with centrally located macrophages and surrounding heterophils are noted. H&E stain, ×400. (B) Intrahistiocytic acid-fast bacilli are evident on specific stain. Ziel-Nielsen, ×400.

manufacturer's recommendations. Polymerase chain reaction (PCR) was performed with a PCR Master Mix Kit (Qiagen, USA) and two primer sets previously described for differentiation of MAC and *M. avium* subsp. *avium* [3, 8]. PCR conditions were 94°C for 1 min, 30 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min, and 72°C for 10 min. DNA of *M. avium* subsp. *paratuberculosis* was used as the positive or negative control depending on the targets. The amplified products were resolved by 1.5% agarose gel electrophoresis and visualized by ethidium-bromide staining. As shown in Fig. 3, our PCR results revealed that the DNA sample was positive for *M. avium* subsp. *avium* with two strongly positive bands at 427 bp (IS*1245*, representing for MAC) and 577 bp (IS*901*, representing for *M. avium* subsp. *avium*).

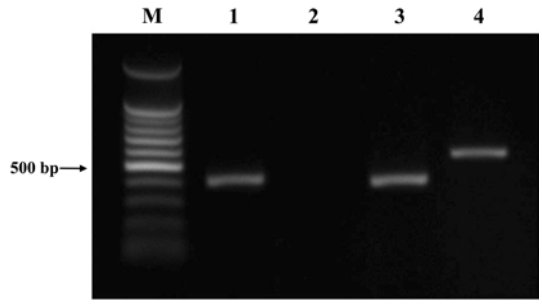

Fig. 3. Detection of mycobacterial species in a bird by polymerase chain reaction. M = 100 bp ladder; Lane 1 = *Mycobacterium* (*M.*) *avium* subsp. *paratuberculosis* (IS*1245*, positive control to the MAC); Lane 2 = *M. avium* subsp. *paratuberculosis* (IS*901*, negative control to the MAA); Lane 3 = liver sample (IS*1245*); Lane 4 = liver sample (IS*901*).

Previously, only a few cases of avian mycobacteriosis have been reported in Asia [9, 10, 13]. Here we reported that avian mycobacteriosis was diagnosed from a captive bird in Korea. This bird was bred in captivity and lived with a mate in a single cage kept indoors. The mate was free from mycobacteriosis based on histopathologic examination. Although the source of infection in this case remains unknown, the pathogen might have been disseminated to and so posed a threat to humans during the infectious period of mycobacteriosis in a bird.

MAC is a nontuberculosis mycobacterial species and *M. avium* and *M. intracellularis* are included. *M. avium* consists of four subspecies; *M. avium* subsp. *avium, M. avium* subsp. *hominissuis, M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *silvaticum* [7]. MAC have also been detected in a wide range of hosts, including birds (mainly *M. avium* subsp. *avium*), pigs (more frequently *M. avium* subsp. *hominissuis*), and small vertebrates [7]. Among the MAC subspecies, *M. avium* subsp. *avium* (MAA) is the most commonly infected pathogen in acquired immunodeficiency syndrome (AIDS) patients. MAA isolated from AIDS is known to have specific genetic determinants that allow bacterial proliferation in the host and contribute to the immunodeficiency [4]. The primary source of MAA infection in AIDS patients is most likely domestic and environmental water [11]. The current case implies that additional source of MAA infection may exist closely, namely pet birds. Moreover in a recent study [7], *M. avium* subsp. *hominissuis* has been isolated from patients diagnosed as MAC infection. This result

suggested that *M. avium* subsp. *hominissuis* could be directly transmitted to humans. However, research about the MAA infection between humans and birds has not been sufficient. Therefore, epidemiological studies are needed to evaluate MAA infection in birds as a source of human infection.

Antemortem diagnosis of avian mycobacteriosis is difficult and blood tests are not specific for differentiation [1, 6, 10]. Thus, direct ZN stain and culture are useful for diagnostic tools. Furthermore, PCR and specific serologic tests have been commonly employed for definite diagnosis and subspecies identification [6, 12]. Culture-based methods are limited by the fact that mycobacterial growth does not frequently occur or requires an extended incubation period ranging from several weeks to six months depending on mycobacterial species [2, 10]. Therefore, definitive diagnosis depends on necropsy and histopathological examinations. Radiographic and ultrasonographic examinations can be useful for diagnosis in some cases like ours [2, 5].

A avian mycobacteriosis associated with fatal lesions and ascarid infestation was diagnosed in a lineolated parakeet based on radiographic, ultrasonographic, and histopathologic findings. Avian mycobacteriosis is potentially zoonotic and, therefore, further research is necessary to examine the origin and distribution of avian mycobacteriosis in Korea.

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