NOTE *Clinical Pathology*

Systemic infection of *Mycobacterium avium* subspecies hominissuis and fungus in a pet dog

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ABSTRACT. A 3-year-old neutered female poodle with a long history of dermatophytic skin disease was presented with lethargy, anorexia and progressive weight loss. Abdominal ultrasonography revealed markedly enlarged mesenteric lymph nodes and multiple hypoechoic foci in the spleen. Cytology of the mesenteric lymph nodes and spleen showed granulomatous inflammation with fungal organisms and negatively stained intracytoplasmic bacterial rods consistent with *Mycobacteria* spp. Based on culture, multiplex polymerase chain reaction and sequence analysis, the bacterium was identified as *Mycobacterium avium* subspecies hominissuis. Despite treatment with antibiotics, the dog's condition deteriorated, and it died approximately 3 weeks after first presentation.

KEY WORDS: canine, dermatophyte, fine needle aspiration, granuloma, *Mycobacterium avium* subspecies hominissuis.

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Tuberculosis infection caused by nontuberculous mycobacteria (NTM) species, referred to as atypical mycobacteria, has been regularly detected worldwide [11]. In past decades, NTM has attracted the interest of human and veterinary medicine because of the public health threat it presents for the potential transmission of tuberculosis [1]. Of the NTM family members, *Mycobacterium avium* complex (MAC) is an environmental mycobacteria ubiquitously present in water, soil, food, plants and animals [9].

The present report describes a pet dog with an *M. avium* subspecies hominissuis infection diagnosed by clinical examination, cytology, culture, multiplex PCR assay and direct sequencing of the PCR product.

A 3-year-old neutered female poodle was presented to a local veterinary hospital with the clinical signs of lethargy, anorexia, mild fever (39.2°C) and progressive weight loss. The dog had undergone ovariohysterectomy, and approximately 5 weeks after the surgery, it began to undergo episodes of dermatophytosis. The skin disease was responsive to antifungal therapy with ketoconazole (10 mg/kg, IV, q12hr for 5 weeks), itraconazole (5 mg/kg, IV, q12hr for 10 weeks) and terbinafine hydrochloride (40 mg/kg, IV, q24hr for 6 weeks). A physical examination revealed sporadic alopecia affecting the trunk, neck and extremities and a palpable mass in the abdominal cavity. Complete blood counts and serum biochemical tests were within normal limits, except for mild hyperglycemia. Thoracic radiographs were not significant. Abdominal ultrasonography revealed splenomegaly with global distribution of multiple hypoechoic areas (Fig. 1A) and marked enlargement of the mesenteric lymph nodes (Fig. 1B).

Exploratory laparotomy was undertaken in an attempt to remove the affected spleen and lymph nodes. During the surgery, multiple white nodules measuring approximately 0.5 to 1 cm in diameter were noted in the spleen (Fig. 2A). The presence of enlarged mesenteric lymph nodes was detected (Fig. 2B). Fine needle aspirates were taken from both tissues and stained with Diff-Quik (Merck, Darmstadt, Germany). Cytologic examinations revealed a moderate number of individualized macrophages and epithelioid cells with occasional multinucleated cells. Within the cytoplasm of the macrophages and free in the background, there were numerous negatively stained bacterial rods (Fig. 3A). In the background, small lymphocytes and plasma cells were occasionally present along with many erythrocytes. Splenic and mesenteric lymph nodal aspirates were subjected to acid-fast (Ziehl-Neelsen) and periodic acid-Schiff (PAS) staining (Sigma-Aldrich, St. Louis, MO, U.S.A.) as described previously [4]. Direct Ziehl-Neelsen staining revealed a high number of strongly acid-fast positive rod-shaped bacteria within macrophages (Fig. 3B). PAS staining showed moderately pink-colored linear bacteria within the macrophages and linear structures with oval to elongate yeast-like swellings, measuring 1 to 2 μm in width and 2 to 6 μm in

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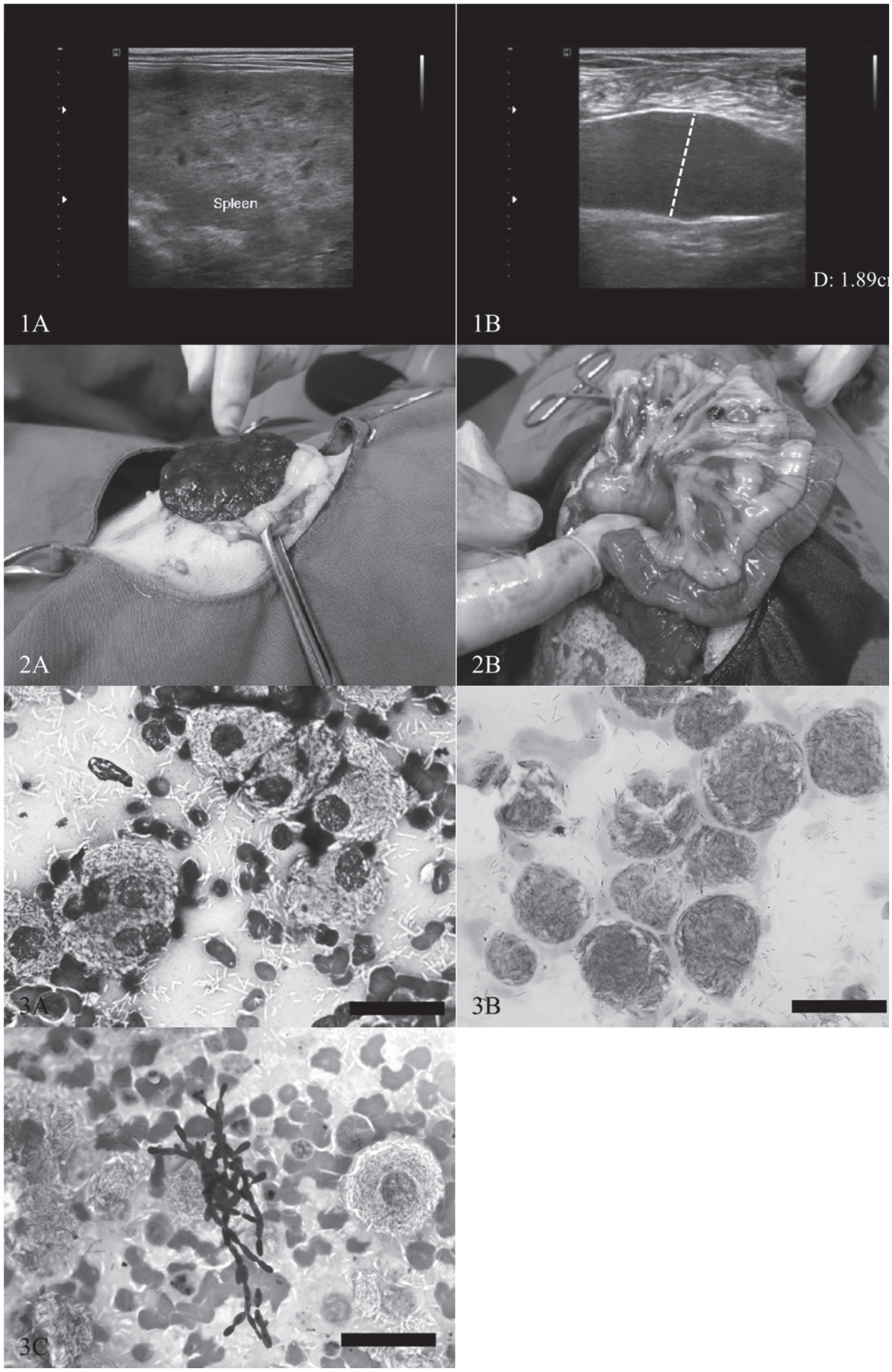

Fig. 1. Abdominal ultrasonography of the spleen and mesenteric lymph node of the dog. (A) Multiple hypoechoic areas were identified in the spleen and (B) enlarged mesenteric lymph node.

Fig. 2. Gross morphology of the spleen and mesenteric lymph node of the dog. (A) Multiple white nodules were noted in the spleen. (B) The mesenteric lymph node was grossly enlarged.

Fig. 3. Fine needle aspirates of the spleen and enlarged mesenteric lymph node of the dog. (A) Macrophages contain unstained rod-shaped mycobacteria in the spleen. Aqueous-based Wright, HP oil, × 1,000. Scale bar: 20 μm, enlarged. (B) Large numbers of acid-fast-positive intracytoplasmic bacilli were identified in the mesenteric lymph node. Ziehl-Neelsen stain, HP oil, × 1,000. Scale bar: 20 μm, enlarged. (C) PAS-positive oval to elongated hyphal structures were identified in the spleen. Periodic acid-Schiff stain, HP oil, × 1,000. Scale bar: 20 μm, enlarged.

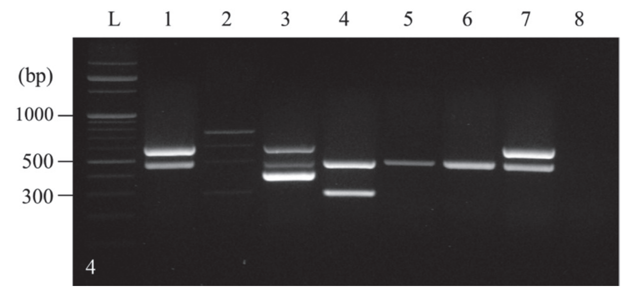

Fig. 4. Identification of *mycobacterium avium* subspecies hominissuis using gel electrophoresis separation of multiplex PCR products. Lane M, molecular ladder; lane 1, spleen from the patient; lanes 2–7, *M. avium* subspecies avium ATCC 35716, *M. avium* subspecies paratuberculosis ATCC 19698, *M. intracellulare* ATCC 13950, *M. bovis* AN5, *M. tuberculosis* H37Rv and *M. avium* subspecies hominissuis 104, respectively; and lane 8, distilled water (negative control).

length, that were similar in morphologic appearance to the dermatophytes (Fig. 3C). The cytological diagnosis was granulomatous splenitis and lymphadenitis with intracytoplasmic bacteria, which was consistent with *Mycobacterium* spp. Although fungal culture and identification were not performed, based on the history of persistent skin disease and the presence of fungal hyphae in the internal organs, systemic dermatophyte infection was considered highly likely.

The aliquots of aspirates from the spleen and mesenteric lymph nodes were subjected to bacterial culture and identification. The tissue samples were homogenized and decontaminated by a 10% oxalic acid solution for 10 min or 1.5% 1-hexadecylpyridinium chloride for 30 min at room temperature. After centrifugation at 1,000 × *g* for 10 min, the supernatant was inoculated on a growth medium containing Lowenstein-Jensen slants with and without glycerol. The inoculated media were incubated for 8 weeks at 37°C. Genomic DNA was extracted from visible colonies using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, U.S.A.) according to the manufacturer's instructions. Multiplex PCR assays were carried out as described previously [16]. The primer sets were generated based on 16S rRNA and IS1311 sequences obtained from GenBank that were designed to discriminate the *Mycobacterium avium* subspecies hominissuis from MAC organisms [15]. The reactions generated a 484 bp product of 16S rRNA and a 608 bp product of IS1311, and subsequent direct sequencing of these products identified the bacteria as *Mycobacterium avium* subspecies hominissuis (Fig. 4).

The patient was discharged and prescribed an antibiotic regimen composed of enrofloxacin (10 mg/kg, IV, q24hr), clavulanate/amoxicillin (12.5 mg/kg, IV, q12hr) and doxycycline (5 mg/kg, IV, q12hr) for 3 weeks. The antibiotic treatment appeared to be effective, resulting in improved appetite, clinical signs and remission of the enlarged mesenteric lymph nodes and splenic lesions detected on abdominal ultrasonography. Approximately 3 weeks later, however, the dog was brought to the hospital for evaluation of recurrent lethargy, anorexia and notable weight reduction. The patient had pyrexia (40.1°C). Hematologic analysis revealed anemia, severe thrombocytopenia and inflammatory leukocytosis (45,600 cells/μl) with left shift. The patient was administered a combination of rifampicin (10 mg/kg, IV, q12hr) and enrofloxacin (Baytril, 5 mg/kg, IV, q12hr). Euthanasia was recommended due to the poor prognosis and concern for zoonotic risk, but the dog died due to the deterioration of its condition.

The global incidence of NTM infection is increasing, and various MAC organisms have been identified using the advanced multiplex PCR assay [11, 14, 16]. *M. avium* subspecies hominissuis has only recently been identified, and it has mainly been reported in pigs and humans, with occasional cases in horses, a pet parrot and dogs [2, 7, 10, 12, 17]. The virulence of *M. avium* subspecies hominissuis in birds is low, but it causes severe disease in humans and veterinary species [12]. In humans, MAC, including *M. avium* subspecies hominissuis, causes respiratory diseases in adults, lymphadenopathy in children and disseminated infections in immunocompromised patients [3, 9, 14].

Dogs are known to be potential sources for the dissemination of atypical tuberculosis to humans and other animals [1, 18]. Although dogs are less susceptible to the disease due to their inherent resistance [6], sporadic MAC-associated diseases have been reported [4, 5, 7, 8]. In contrast to infection by direct contact between typical mycobacteria and a host, MAC infection predominantly occurs indirectly by exposure to environmental contaminants, such as water and soil [9]. Following infection through the oral or respiratory tract, a large proportion of canine cases have generalized granulomatous inflammation involving multiple organs, such as the mesenteric lymph nodes, liver and spleen [8, 9]. One of the important underlying factors predisposing an animal to MAC diseases is immune status, and immunosuppression in young dogs may play an important role in the clinical development of a MAC infection [1, 2, 4, 7, 8]. Although the immune status of the present dog was not critically evaluated, the dog was most likely immunocompromised. Chronic and recurrent skin disease and long-term treatment with antifungal agents potentially caused immunosuppression. The presence of dermatophytic fungal hyphae in the spleen and mesenteric lymph node following long-term treatment is very rare; however, it may have been associated with the suppressed immune status of the patient.

M. avium subspecies hominissuis infection is rare in dogs and has been reported in only 3 canine cases [2, 7]. This is the first case of *M. avium* subspecies hominissuis infection in a pet dog from South Korea, a country with an intermediate tuberculosis burden that is exhibiting a rapid upward trend in NTM disease incidence [13]. Furthermore, to the best of our knowledge, the present case report describes systemic coinfection with mycobacterium and fungus in a dog for the first time. The present case is valuable as an example of the potential public health risk of *M. avium* subspecies hominissuis infection in dogs, particularly for children and persons with immunosuppressed conditions. Caution should be exercised regarding interspecies transfer of the causative agent from an infected pet dog. Given the light of increasing interaction between human and companion animals, the present case report encourages veterinary practitioners to increase the awareness of potential mycobacterial infection and emphasizes the need for rapid identification and administration of an appropriate drug regimen for mycobacteria.

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