



Lymphadenopathy Associated with Disseminated Aspergillosis in a Jindo Dog in Korea

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Abstract A 2-year-old outdoor, spayed, female Korean Jindo dog was referred for thoracic and abdominal masses. Diagnostic imaging revealed generalized lymphadenopathy and a significant amount of pleural effusion. The cytological evaluation of the pleural effusion and fine-needle aspiration of the mediastinal lymph nodes confirmed the presence of fungal hyphae. The histopathological examination of the mediastinal lymph nodes stained with Grocott's methenamine silver revealed fungal mycelia, and *Aspergillus terreus* was identified based on fungal DNA sequencing. Thus, the dog was diagnosed with disseminated canine aspergillosis caused by *A. terreus*. This case report describes a rare case of disseminated canine aspergillosis caused by *A. terreus* in a Korean Jindo dog.

Key words *Aspergillus terreus*, disseminated aspergillosis, dog, lymphadenopathy, systemic fungal infection.

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Introduction

Aspergillosis in humans and animals is caused by saprophytic filamentous fungi of the genus *Aspergillus*, usually found in natural habitats (1,14). The genus *Aspergillus* was recently classified into eight distinct subgenera, including *Aspergillus*, *Fumigati*, *Circumdati*, *Terrei*, *Nidulantes*, *Ornati*, *Warcupi*, and *Candidi*. These subgenera were further divided into 22 sections, each encompassing several related species (8). Opportunistic infections are usually caused by the inhalation of airborne spores and can be divided into two types: nasal aspergillosis, which affects only the nasal and frontal sinuses, and disseminated aspergillosis, in which an invasive infection occurs due to hematogenous spread (1,13).

In canine disseminated aspergillosis, *Aspergillus terreus* and *A. deflexus* are the most common species, which spread hematogenously to many organs, including the kidney, liver, spleen, lymph nodes, muscles, pericardium, bone, brain/central nervous system, intervertebral discs, and joints (13,14). Clinical signs of disseminated aspergillosis vary depending on the organ system. Disseminated aspergillosis occurs most commonly in German Shepherd breeds, suggesting that genetic factors play a significant role in their vulnerability and pathogenesis (2,6,14). Infections often occur in immunocompromised individuals, especially those recently prescribed immunosuppressive drugs, because the host defense system plays a critical role (2). The prognosis of disseminated aspergillosis is poor in animals, even with proper antifungal therapy and supportive treatment (1,3). Herein, we report a rare case of disseminated canine aspergillosis caused by *A. terreus* in a Korean Jindo dog.

Case Report

A 2-year-old, outdoor, spayed female Korean Jindo dog was referred to the Veterinary Medical Teaching Hospital at Gyeongsang National University, presenting with dyspnea, vomiting for 2 weeks. The primary veterinary hospital confirmed thoracic and abdominal masses, and prednisolone (1 mg/kg, PO, SID) and antibiotics were prescribed because of the high likelihood of a tumor. However, they were unresponsive, and the symptoms of dyspnea, vomiting, and pain worsened.

The physical examination revealed dyspnea with expiratory distress, fever (40.1°C), cold extremities, unmeasurable blood pressure, weak femoral pulsation, delayed capillary refill time, and pale and dry mucus membrane. The dog's level of consciousness was obtunded, and nervous behavior, such as vocalization, was observed. On physical examination, abdominal pain was confirmed. Initial laboratory analysis showed severe neutropenia ($0.16 \times 10^9/L$; reference interval, $2.95\text{--}11.64 \times 10^9/L$) with degenerative left shift (3,979 of band neutrophils/ μL), indicating immature neutrophils outnumbered mature neutrophils, and accompanied by many neutrophil toxic changes; hyperlactatemia (9 mmol/L; reference interval, 0–2.5 mmol/L) was also observed. Based on

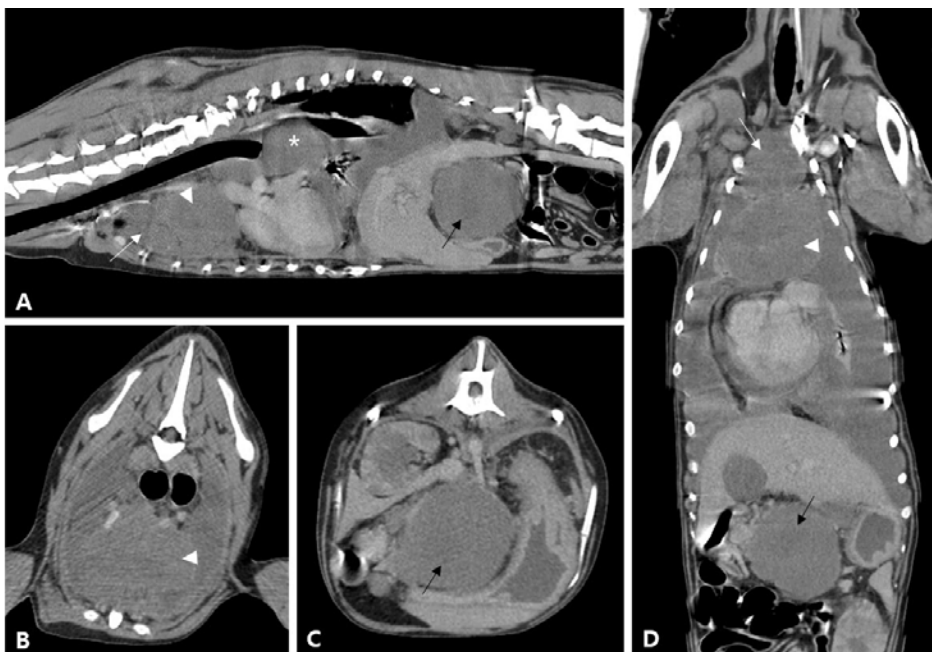


Fig. 1. Computed tomography (CT) images: sagittal (A), axial (B, C), and coronal (D) post-contrast CT images. CT revealed generalized lymphadenopathy involving the sternal (white arrow), cranial mediastinal (arrowhead), tracheobronchial (asterisk), and hepatic (black arrow) lymph nodes in the thoracic and cranial abdomen. A significant amount of pleural effusion is observed.

these findings, the dog was resuscitated for decompensatory shock due to systemic inflammation or sepsis.

A computed tomography (CT) scan (Aquilion lightning 160, Canon Medical Systems, Ōtawara, Japan) was performed without anesthesia, and contrast medium (Iohexol, Omnipaque 300[®], GE Healthcare, Annsgrrove Cork, Ireland) was manually administered intravenously at a dose of 1 mL/kg. CT revealed generalized lymphadenopathy with mild contrast enhancement in the thorax, including the sternal and tracheobronchial lymph nodes. A severely enlarged cranial mediastinal lymph node was identified, resulting in compression of the cranial vena cava (Fig. 1). Significant pleural effusion was observed. Multifocal atelectasis was observed in the lung parenchyma. Abdominal CT imaging also revealed generalized lymphadenopathy, particularly marked enlargement of the hepatic lymph nodes, resulting in compression of the portal vein and caudal displacement of the small intestine. In addition, multiple infarctions were found in the bilateral renal cortex, and multiple small hypoattenuating nodules were found in the splenic parenchyma.

When the pleural effusion examination was performed via

thoracentesis, the pleural effusion was bloody and turbid and was classified as an exudate (total protein, 5.5 g/dL; total nucleated cells, 21,000/ μ L). The cytological analysis of the pleural effusions revealed suppurative inflammation accompanied by linear substances that appeared to be fungal hyphae (Fig. 2). Fungal hyphae were also observed on fine-needle aspiration cytology of mediastinal lymph nodes. Histopathological examination by tru-cut biopsy of the mediastinal lymph node stained with Grocott's methenamine silver revealed dense mat/plaques of fungal hyphae are non-pigmented, 5-7 μ m diameter with parallel sides, frequent septa, and branch dichotomously at 45° angles, most suggestive of *Aspergillus* spp. (Fig. 3). For a definitive diagnosis, fungal culture was performed using Sabouraud dextrose agar, and the result was *Aspergillus* spp. based on the microscopic morphological features of the hyphae and conidial heads. For DNA-based identification of the fungal isolate, polymerase chain reaction amplification and DNA sequencing of the internal transcribed spacer (ITS) of the fungal ribosomal DNA and translational elongation factor (TEF) 1-alpha genes were performed (7), which resulted in *A. terreus* as the best-match species. The

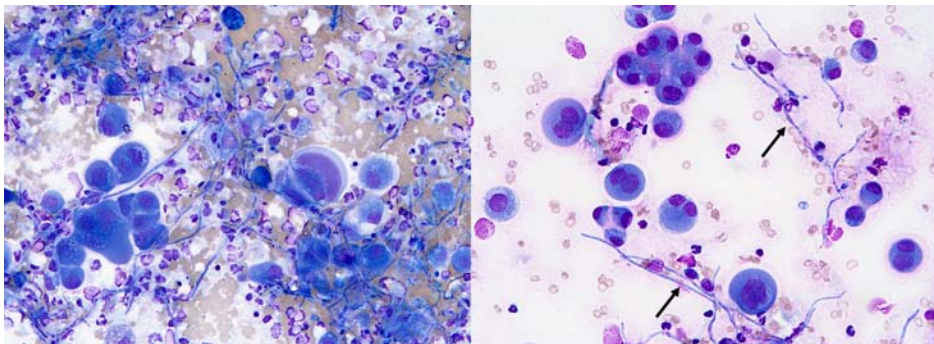


Fig. 2. Pleural effusion cytology. Mesothelial cells, numerous neutrophils, and inflammatory cells are observed, accompanied by linear substances that appeared to be fungal hyphae (black arrows), suggesting suppurative inflammation related to fungal infection; $\times 400$, Diff-Quik stain.

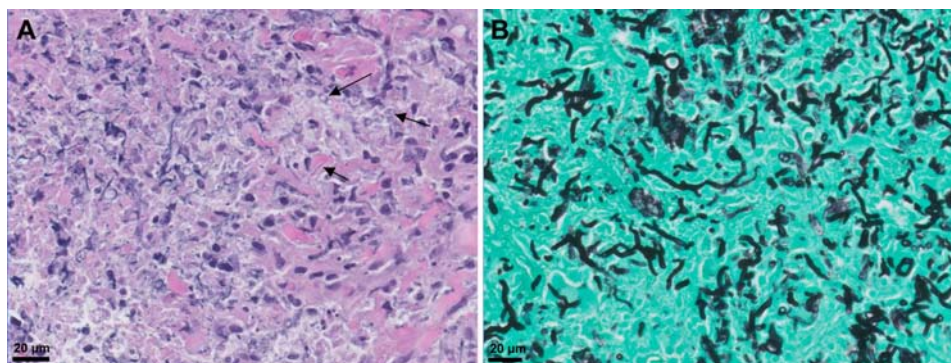


Fig. 3. Histopathological examination by tru-cut biopsy of the mediastinal lymph node. (A) Locally extensive necrosis with mixed inflammation and intralesional fungal organisms (arrows, $\times 60$, hematoxylin and eosin stain); (B) dense mat/plaques of fungal hyphae are non-pigmented, 5-7 μ m diameter with parallel sides, frequent septa, and branch dichotomously at 45° angles, with features most suggestive of *Aspergillus* spp.; $\times 60$, Grocott's methenamine silver stain. Bar size is 20 μ m.

fungal DNA sequences of *ITS* and *TEF1*-alpha were 99-100% matched to *A. terreus* DNA compared with the GenBank database (GenBank accession no. MT316343 and KM921970, respectively). Accordingly, the case was confirmed to be disseminated canine aspergillosis caused by *A. terreus*.

Despite shock resuscitation with intravenous fluids, vasopressors, oxygen supplementation, and thoracocentesis, there was no improvement in vital signs and symptoms, and cardiopulmonary arrest eventually occurred. Necropsy was not performed per the owner's discretion.

Discussion

Disseminated aspergillosis is a fatal disease in dogs. Since the first report of disseminated aspergillosis in dogs by *A. terreus* in 1978 (15), additional findings have been reported regarding the etiology, pathology, clinical course, and prognosis associated with *A. terreus* infection (1,8,14). *A. terreus* is distinguished morphologically from other *Aspergillus* species, such as *A. fumigatus*, *A. flavus*, and *A. niger*, by the presence of accessory conidia or aleuriospores produced directly from the hyphae, in addition to the common phialoconidia that arise from conidiophores (9). Aleuriospores that are globose-shaped can be utilized in fungal dissemination from the initially infected nasal/respiratory tissues into the internal organs via hematogenous spread, as demonstrated in experimental mice with *A. terreus* infection (10); thus, invasive disseminated *Aspergillus* infection occurs more frequently associated with *A. terreus* in dogs (11). It has been shown in many studies that young, female, German Shepherd dogs are predisposed to disseminated aspergillosis (1,3,14).

In a study of 30 dogs with disseminated aspergillosis, 20 were German Shepherds, and 77% (23 of 30) were females. In addition, dogs infected with *A. deflexus* or *A. terreus* were more likely to be female than those infected with other *Aspergillus* species (14). Comparative studies of serum immunoglobulin concentrations in healthy dogs indicate that the IgA level in the German Shepherd dog is significantly lower than that in other breeds (4,5). Although the dog in this report was not a German Shepherd, it exhibited these common characteristics because the patient was a female dog and infection occurred at a young age. Since the dog developed a systemic infection, it was assumed that there was a deficiency in the innate immune system. Although *A. terreus* is a common saprophyte, isolates from disseminated infections in humans have higher growth rates and virulence in mice than the saprophytic forms (12). Immunocompromised patients are at risk of pulmonary colonization, leading to tissue damage, uncontrolled fungal growth, and potential

dissemination via vascular invasion (1). Several case reports have described the recent use of immunosuppressive drugs in dogs with disseminated aspergillosis (2). For the dog in this report, clinical symptoms began to deteriorate rapidly after taking an increased dose of steroids for 10 days before visiting the hospital. Pleural and pericardial effusions, which had not been confirmed in previous local hospital examinations, were also confirmed. A local veterinarian prescribed an immunosuppressive dose of steroids because of lymphadenopathy, which may have played a significant role in disease progression.

Affected lymph nodes can enlarge two to three times their normal size, and even up to five times their normal size in some cases (6). This finding is consistent with the prominent lymph node enlargement observed in this case. Similarly, other cases of disseminated aspergillosis with severe lymph node enlargement have been reported (13-15). However, clinicians should be aware that lymphadenopathy can be found in dogs not only with primary lymphoid or metastatic neoplasia but also with infection. Fungal infections may not respond to antibiotics; therefore, the identification of the primary cause of lymphadenopathy must be evaluated.

In addition, concerning the kidney and spleen lesions found on CT imaging, it was strongly suspected that they were involved, as these organs are commonly affected (1,6,14). There is a possibility that the persistent nervous behavior in the dog, including vocalizations, may be related to the musculoskeletal and nervous systems. Unfortunately, in this case neurological and/or magnetic resonance imaging examinations could not be performed because of severe dyspnea upon admission and expiration during the full examination.

Conclusions

This report describes a rare case of disseminated canine aspergillosis caused by *A. terreus* in a Korean Jindo dog. This report demonstrates that infectious diseases such as disseminated aspergillosis must be included in the differential diagnosis of patients with severe lymphadenopathy, and caution is needed for dogs when infectious diseases are not excluded because steroid prescriptions can be fatal to dogs with lymphadenopathy.

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Conflicts of Interest

The authors have no conflicting interests.

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