



Disseminated fungal infection with *Aspergillus versicolor* and *Schizophyllum commune* in a dog

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ABSTRACT

A two-year-old neutered male Coton de Tulear presented with lethargy, anorexia, and tachypnea. Cystic masses noticed at the cranial mediastinal region were diagnosed as granuloma containing hyphae of *Aspergillus versicolor*. Despite antifungal treatment using itraconazole, fluconazole, and voriconazole, the lesions spread to the lung. After euthanasia, *Schizophyllum commune* was identified in the lung and splenic lymph node. This is the first case of fungal infection caused by *A. versicolor* and *S. commune* in a dog.

1. Introduction

Aspergillus spp. are not only ubiquitous filamentous fungi in the environment but also opportunistic pathogens in the humans and animals. While localized sinonasal aspergillosis commonly occurs, a disseminated form of aspergillosis was rarely observed in dogs involving various body organs such as intervertebral disks, bones, kidneys, spleen, lungs, lymph nodes, and central nervous system [1,2]. *A. terreus* and *A. deflexus* are the common causative agents, and *A. versicolor* was first reported in the USA in 2011 for systemic aspergillosis [2,3].

Schizophyllum commune is an environmental, wood-rotting basidiomycetous fungus, rarely involved in the human pathology and is usually associated with immunosuppressive conditions [4]. Three canine cases of *S. commune* have been reported in Japan; one had nodules in the neck, anterior mediastinum, and cranial lung lobes, and the remaining two suffered from osteomyelitis [5–7]. Localized fungal infection generally responds well to treatment with antifungal agents; whereas, systemic mycoses tend to become serious and have poor prognosis.

We report the first case of a disseminated fungal infection caused by *Aspergillus* and *Schizophyllum* in a young dog who was not immunosuppressed.

2. Case

A two-year-old neutered male Coton de Tulear dog weighing 6.3 kg was presented to the VIP Animal Medical Center with lethargy and

anorexia (day 0). On physical examination, the patient showed rapid and shallow breathing pattern with a respiratory rate of 70 breaths/min. Complete blood count revealed leukocytosis due to neutrophilia (16500/ μ L; reference range 2950–11640/ μ L) and monocytosis (1400/ μ L, reference range 150–1120/ μ L). Except an increase in the concentration of C-reactive protein (105.1 mg/L, reference range 0–10 mg/L), no other remarkable laboratory findings were observed. Thoracic radiography revealed a widening of the cranial mediastinum. Computed tomography (CT) images exhibited 4.37 \times 3.33 \times 5.69 cm sized cystic masses with a marginal enhancement in the cranial mediastinal region (Fig. 1A–C). Cranial vena cava was located between the masses and collapsed by the masses. A nodule of 2.5 mm in diameter with localized ground-glass opacity was visible in the cranial right lung lobe (Fig. 1D). Enlarged mediastinal lymph nodes (LN) and a right prescapular LN were also identified (Fig. 1E and F). There were no remarkable CT findings in the abdomen and skull.

Exploration and removal of the mass was planned via median sternotomy on day +11. Pre-oxygenation for 5 minutes and premedication with midazolam 0.1 mg/kg and fentanyl 0.003 mg/kg were intravenously (IV) administered. General anesthesia was induced with propofol 6 mg/kg IV and maintained with isoflurane in air oxygen mixture. Constant rate infusion of fentanyl 0.005 mg/kg/hr along with pre- and peri-operative cefazolin 30 mg/kg IV administration every 90 minutes was maintained. The patient was positioned in dorsal recumbency, and the entire ventral thorax area was aseptically prepared. Skin incision was made in the median thorax along the entire sternum length.

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Superficial and deep pectoral muscles were sharply incised at the median and separated from the sternum bilaterally. After sternotomy was performed with an oscillating saw and the sternum was retracted bilaterally with a finochietto retractor, multiple and adjoining mediastinal masses were identified (Fig. 2A). Each mass adhered to the right cranial lung lobe, cranial vena cava, and cranial part of pericardium, respectively. As complete mass removal could not be achieved due to strong adhesion between the masses and adjacent tissues and blood vessels, debulking surgery was alternatively performed. A cystic lesion with a purulent discharge was discovered during mass dissection (Fig. 2B), of which swab sample was taken for microorganism culture. The sternum incision was closed by a figure of 8 sutures with 2-0 prolene. A chest tube was placed to drain the accumulating fluid. Incised muscles, subcutaneous tissue, and skin were routinely closed. After 6 days of hospitalization, the drainage volume decreased to less than 10 ml per day, and the patient was discharged with normal surgical incision healing process. When the sutures were removed on postoperative day 14, the value of C-reactive protein was measured to be less than 10 mg/L (reference range 0–10 mg/L).

On histopathology, the mass was composed of abundant macrophages and necrotic cellular debris forming coalescing granulomas admixed with multinucleated cells and surrounded by numerous lymphocytes and plasma cells (Fig. 3A and B). Multifocally, the central area of the granulomas contain variable numbers of branching hyphae (Fig. 3C). Through the fungal culture on Sabouraud dextrose agar with chloramphenicol following a PCR amplification using universal internal transcribed spacer (ITS) primers: ITS1, 5'-TCCGTAGTGAACCTGCGG-3' and ITS4, 5'-TCCTCCGCTTATTGATATGC-3' and sequence analysis, *A. versicolor* was identified. The antifungal susceptibility testing was not performed. No bacteria were found. The patient revealed improvement in symptoms after taking itraconazole 5 mg/kg q24h for a month.

The patient was again presented with clinical signs including lethargy, tachypnea, and regurgitation 4 months later (day +150). On thoracic radiograph, the mass in the mediastinum was larger and caused severe tracheal elevation. The fungal hyphae were still identified in the

mass with the fine needle aspiration biopsy. Considering Aspergillosis based on the previous diagnosis, increased doses of itraconazole were administered, as for 5 mg/kg q12h for 2 weeks and 7 mg/kg q12h for 9 weeks, and eventually replaced with fluconazole 10 mg/kg q12h for 13 weeks; however, the mass had not reduced in size.

The patient was further referred to the Konkuk University Veterinary Medical Teaching Hospital to explore possible cures for the *Aspergillus*-related mediastinal mass on day +336 after first presentation. At re-examination CT, a mass sized $10.3 \times 4.6 \times 7.4$ cm included several cystic structures in the cranial left lung lobe, which had heterogeneous enhancement patterns and peripheral rim enhancement (Fig. 4A). An iso-attenuated soft tissue mass $4.9 \times 1.3 \times 4.4$ cm with homogenous contrast enhancement was observed in the cranial right lung lobe region (Fig. 4B). Besides, three heterogeneously enhanced masses including cystic structures in the pleural space were observed. Cranial vena cava and brachiocephalic artery passed between the masses and collapsed by them. Ground glass opacities were present in the cranial right lung parenchyma. Enlargement of the thoracic and abdominal LN including left prescapular, cranial mediastinal, splenic, pancreaticoduodenal, ileocolic, jejunal, and bilateral lumbar aortic LN along with honeycomb appearance of the spleen were identified on both CT and ultrasonography (Fig. 4C and D). Bronchoalveolar lavage fluid contained predominantly macrophages and lymphocytes, and sheets of epithelial cells were sparsely observed. No infectious agents were demonstrated for the bacterial and fungal culture. Fine needle aspiration was performed for the prescapular LN, spleen parenchyma, and splenic LN, in which numerous fungal hyphae were present. Therefore, disseminated fungal infection was demonstrated, and aspergillosis was suspected. No specific findings were observed for the microscopy of urine sediment and culture.

After the antifungal medication was changed to voriconazole at a dose of 4 mg/kg q12h for 3 weeks, the respiratory sign including tachypnea and dyspnea temporarily improved. However, the dog was eventually euthanized humanely due to the severity of the disseminated lesions and poor quality of life on day +363. Ultrasound-guided

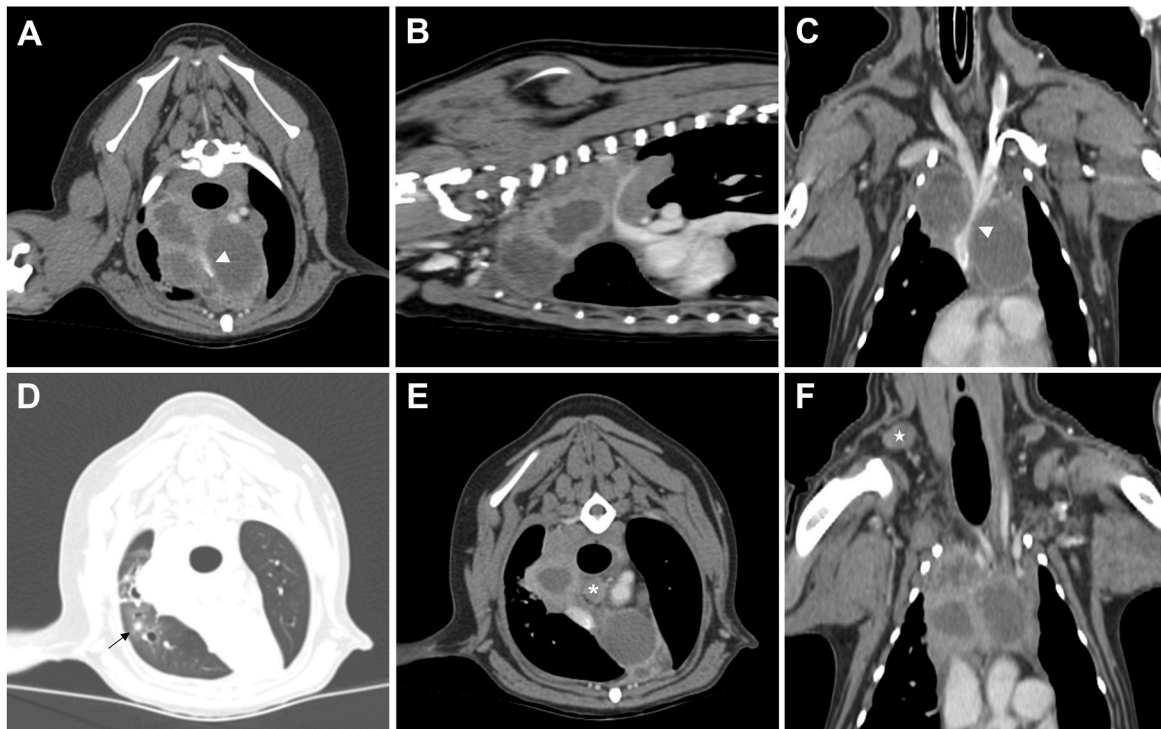


Fig. 1. Computed tomography images of thoracic cavity. Cystic masses sized with $4.37 \times 3.33 \times 5.69$ cm with marginal enhancement in cranial mediastinal region (A–C). Cranial vena cava located between the masses and collapsed by the masses (arrowhead). A nodule 2.5 mm in diameter (arrow) and localized ground glass opacity in the cranial right lung lobe (D). Enlarged mediastinal lymph node (asterisk) and right prescapular lymph node (star) (E,F).

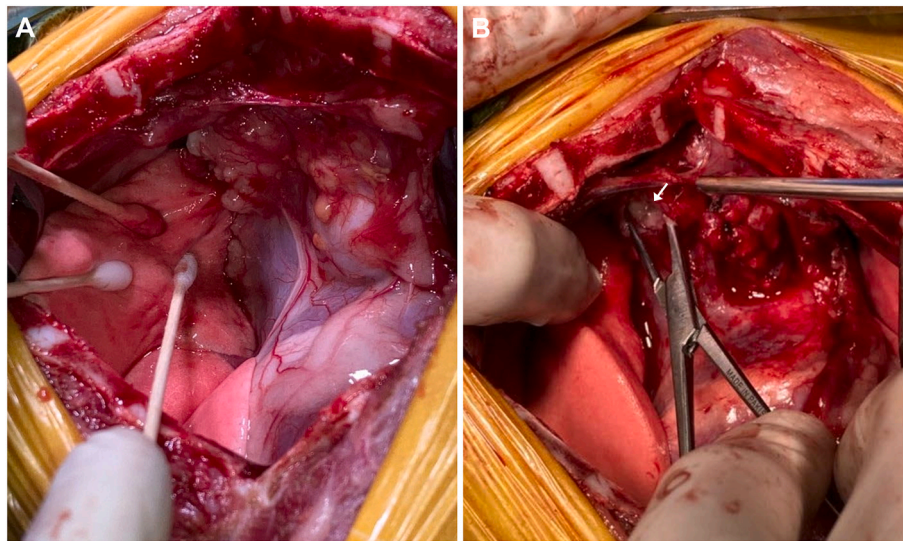


Fig. 2. Intraoperative macroscopic appearance of the lesion. Multiple and adjoining mediastinal masses adhered to right cranial lung lobe, cranial vena cava, and cranial part of pericardium (A). A cystic lesion including purulent discharge (arrow) (B).

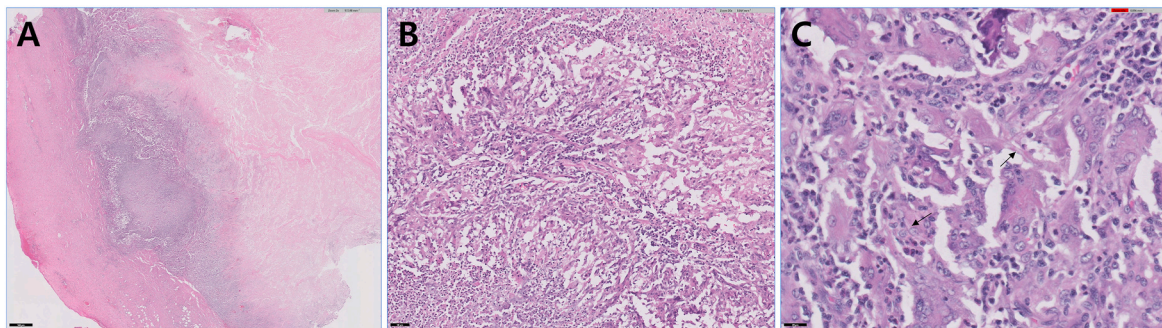


Fig. 3. Histopathology of mediastinal mass. Abundant macrophages and necrotic cellular debris forming coalescing granulomas admixed with multinucleated cells (A,B). Pyogranulomatous inflammation and intralesional fungal hyphae (black arrows) (C). Hematoxylin and eosin (H&E). A = 2 \times , B = 20 \times , C = 60 \times magnification.

percutaneous core needle biopsies of the mediastinal mass, lung parenchyma, and splenic LN were performed with the consent of the owners. Ribbon-like, septate fungal hyphae were found on the histopathological examination of all the three tissue specimens. The mediastinal mass was entirely composed of cellular debris and degenerated collagen admixed with abundant neutrophils and fewer macrophages, lymphocytes, and plasma cells; therefore, diagnosed as chronic, extensive, and severe pyogranuloma with necrosis and intralesional fungal hyphae (Fig. 5A). The lung sections consisted of variably dense collagen interspersed with small vessels, which surrounded low to moderate numbers of lymphocytes, plasma cells, and macrophages (Fig. 5B). The cortical and medullary architecture of splenic LN was largely effaced by sheets of macrophages, neutrophils, and multinucleated giant cells mixed with fewer lymphocytes, plasma cells, and histiocytes containing fungal hyphae (Fig. 5C and D). Both localized spread of the fungal infection and systemic spread were demonstrated. The biopsy samples were cultured on Sabouraud dextrose agar with chloramphenicol and a filamentous fungus grew after 7 days of incubation at 25 °C. Similar to the previous identification method of *A. versicolor*, the universal primers ITS1 and ITS4 were used to amplify the ITS region, and the sequence were analyzed through BLAST search in the NCBI database. Surprisingly, the fungal isolate from the lung and splenic lymph node was identified as *S. commune* and not *A. versicolor*.

3. Discussion

Disseminated aspergillosis has a poor prognosis despite aggressive antifungal agents and adjuvant treatment in dogs [1,2,8,9]. A case series study had reported that 17 of 30 dogs were euthanized within a week due to serious clinical symptoms including neurological signs, pain, and respiratory failure [2]. More than 3 years' survival have been documented in two German shepherd dogs after *Aspergillus*-affected lung lobectomy and removal of associated lymph node [10]. In the cases of extrapulmonary aspergillosis for the paranasal sinus, endocardium, myocardium, joint, esophagus, the American Society of Infectious Diseases recommends surgical resection of the affected lesions combined with antifungal therapy with voriconazole or a lipid formulation of amphotericin B [11]. Additional surgery was considered to reduce the mass effect and to facilitate drug treatment in our patient. However, it was not performed due to the enlarged multi-cystic masses that severely adhered other organs including esophagus. More than 50% of the lungs were affected on re-examination CT.

Antifungal resistance of aspergillosis is a newly emerging problem in human medicine, and itraconazole, voriconazole, posaconazole, and isavuconazole are being used in patients with systemic aspergillosis [11]. Of the 10 dogs with disseminated aspergillosis receiving posaconazole, 8 dogs relapsed after treatment was discontinued or during treatment [9]. In the present case, the size of granuloma in the thoracic cavity had not shrunk even with the antifungal treatments using itraconazole, fluconazole, and voriconazole; that was considered due to the

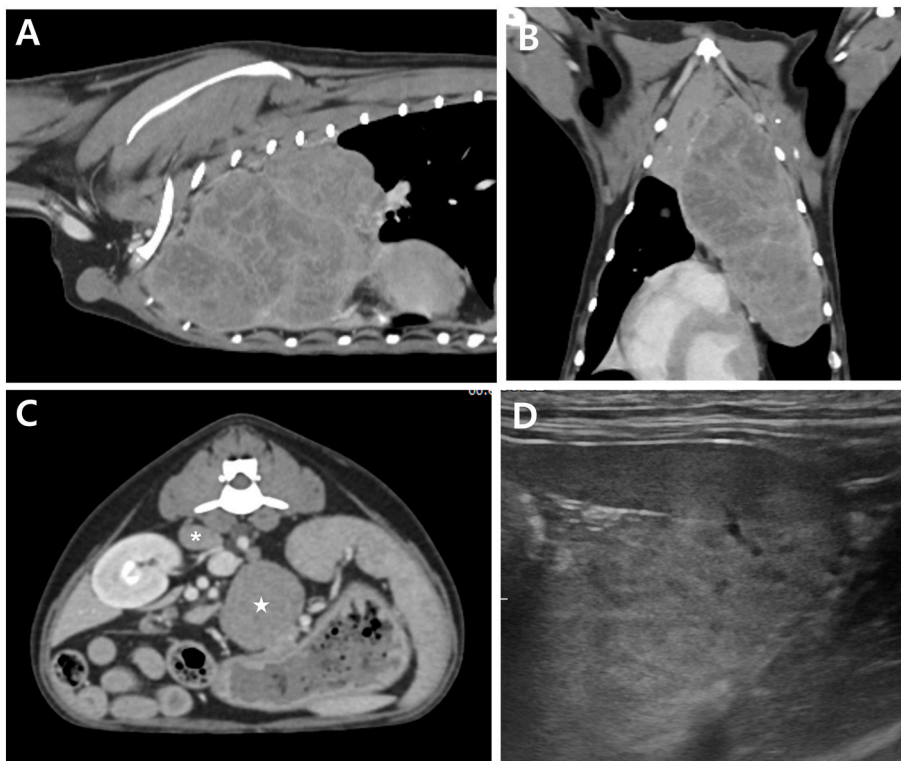


Fig. 4. Re-examination Computed tomography (CT) images and ultrasonography of spleen. Masses sized 10.3 × 4.6 × 7.4 cm including several cystic structures in the cranial left lung lobe, which had heterogeneous enhancement patterns and peripheral rim enhancement (A,B). An iso-attenuated soft tissue mass 4.9 × 1.3 × 4.4 cm with homogenous contrast enhancement in the cranial right lung lobe region (B). Enlargement of splenic lymph node (star) and right lumbar aortic lymph node (asterisk) (C). Honeycomb appearance of the spleen on both CT and ultrasonography (C,D).

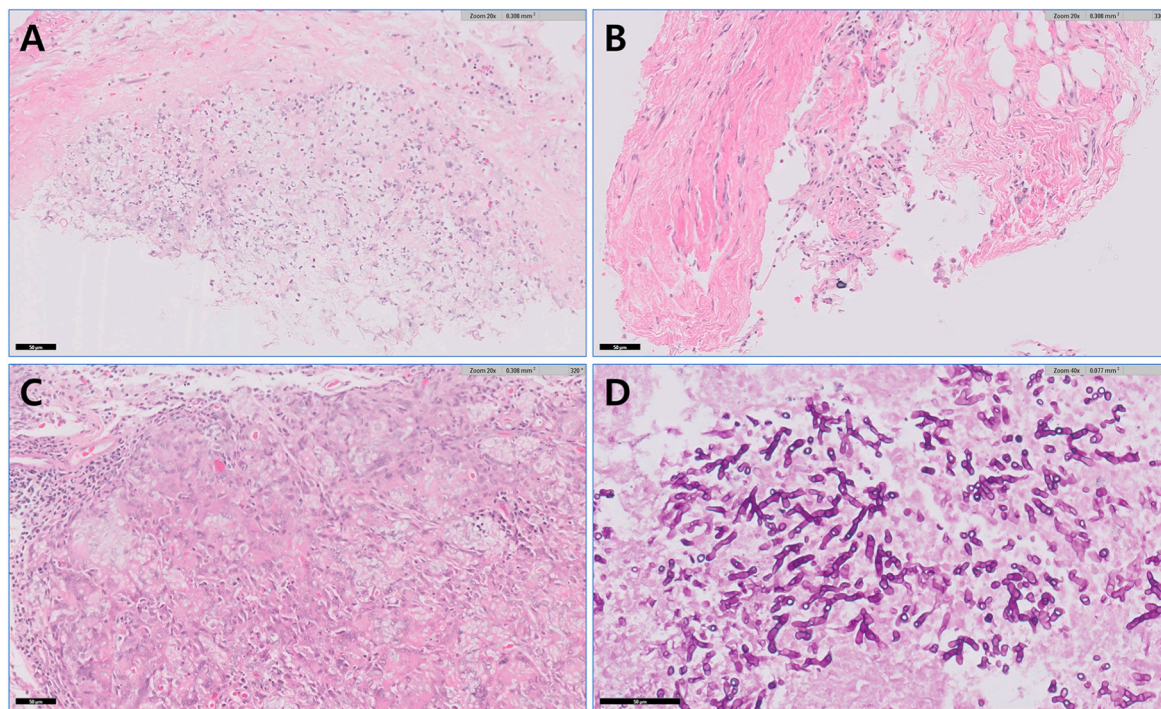


Fig. 5. Histopathology of mediastinal mass, lung, and splenic lymph node. Mediastinal mass composed of necrotic cellular debris and pyogranulomatous inflammation with nonstaining fungal hyphae (A). Lung comprised of fibrovascular stroma with lymphoplasmacytic and granulomatous inflammation, and hyphae (B). Pyogranulomatous lymphadenitis with fungal hyphae (C,D). A,B,C = Hematoxylin and Eosin (H&E), 20× magnification. D = Periodic acid-Schiff (PAS), 40× magnification.

antifungal resistance or massive lesion before postmortem. The species detected in the postmortem biopsies from the mediastinal mass, lung parenchyma, and splenic LN was not *A. versicolor* but *S. commune*. It could be caused by the limitation of the needle biopsies, which may not

be representative of the entire lesion. Final histopathological diagnoses from post-surgical or necropsy samples were different from the results from percutaneous needle aspiration biopsy [13]. It is unclear when the *S. commune* infected besides *A. versicolor*, whether at first presentation or

after the cure of initial aspergillosis; because antifungal susceptibility testing was not available and fungal isolation was not repeated while the mass size became larger. It is possible that the minor pathogen *S. commune* has been missed at the initial diagnosis owing to the fungal antagonism of *A. versicolor*, which refers to the action of fungi or bacteria that suppresses normal growth and activity of other species. In addition to *Trichoderma* spp., which has been widely used as antagonistic fungal agents, *Aspergillus* spp. are also identified as having potential action against plant fungal pathogen [14].

An in vitro study revealed that five strains of *S. commune* appeared susceptible to itraconazole [12]. Two canine patients with osteomyelitis and some human cases of *S. commune* infection have been completely treated with itraconazole [6,7]. The granulomatous lesion on the neck, where *S. commune* were isolated, and the nodule at the anterior mediastinum did not diminish in size with ketoconazole treatment in a dog [5]. Consequently, it could be inferred that disseminated and/or mixed mycosis could have caused the poor antifungal therapeutic response and exacerbation of illness in our patient.

Sites affected by disseminated aspergillosis included kidney, spleen, lymph nodes, bone, vertebra, liver, heart, pancreas, brain, lung, and eye [2]. The reported canine Basidiomycosis with *S. commune* involved subcutaneous tissue and bone marrow [5–7]. In humans, *Schizophyllum* took the form of many different diseases from the bronchopulmonary infection and sinusitis to extrapulmonary including onychomycosis, fatal brain abscess, meningitis, ulceration of the palate with perforation, and keratitis [15,16]. Severe necrotizing and granulomatous inflammation was seen in the eyes, lung, heart, and lymph nodes in a Harbor seal with *S. commune* [17]. The present case was the first canine *S. commune* isolated from the mediastinal mass, lung, and splenic lymph node. While bone and marrow are commonly involved in disseminated *Aspergillus* and *Schizophyllum* infection, the dog did not suffer from osteomyelitis.

Aspergillus and *Schizophyllum* are opportunistic pathogens, and cases are usually associated with immunocompromised states in humans [18, 19]. None of the previously reported cases with *S. commune* infections were related to underlying diseases [5,7]; except a Labrador retriever dog with pre-existing aortic stenosis and a history of impaling wound caused by a rotted bamboo stick [6]. In the present case, the initial chief complaint, including lethargy and anorexia disappeared as the patient's breathing had improved; therefore; it was suspected to be caused by respiratory distress. The dog had been abandoned and was adopted by the current owners. After that, there were no medical history of trauma, toxin, anesthesia, vaccination, and malnutrition, which have effects on immune system. Although specific testing evaluating the patient's immunologic function was not investigated, which is available in specialized research laboratories [20], no other suspected clinical symptoms and anything specific on laboratory work and diagnostic imaging of immunosuppression were observed, except for the findings stated in the case description. The dog lived indoors and did not take a walk in a grass. The environment was somewhat different from the common fungal infection seen in outdoor and farm dogs frequently exposed to the microorganism [21]. To the best of our knowledge, this is the first case report of a disseminated fungal infection caused by *Aspergillus* and *Schizophyllum*.

Declaration of competing interest

There are none.

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