Infection with the mushroom *Schizophyllum commune* (MykoWeb; http://www.mykoweb.com/) causes an emerging mycosis in humans [1–3, 5, 8–10]. Thus far, more than thirty human cases have been reported worldwide, including Japan. Although mushroom hyphae have two different morphologies, primary mycelium and dicaryotic mycelium, the reported cases in Japan were mostly caused by primary mycelia. Present mycological techniques fail to identify specific characteristics of the primary mycelia, making diagnosis difficult. It is likely that the burden of disease caused by *S. commune* is much greater than currently reported.

In humans, mainly respiratory organs are affected, indicating respiratory tropism of this organism. Even the one exceptional case of mycelium brain abscess was likely the result of dissemination from a granulomatous lesion in the lungs. Among canines, thus far only one report has been published, a cervical granulomatous lesion due to *S. commune* infection [6]. As is the case with humans, identification of the pathogen is difficult. Appropriate treatment, however, demands a definitive diagnosis. Here, we describe a rare case of a Labrador retriever that suffered from *S. commune* osteomyelitis and was successfully treated.

A six-year-old female Labrador retriever was admitted to Tanaka Animal Hospital in Tottori prefecture in Japan with presenting with claudication of the left hindlimb. The animal suffered from pre-existing aortic stenosis, and previously had been impaled by a rotted bamboo stick to her inguinal area.

In addition to general physical tests, the dog received X-ray examination. The X-ray examination revealed atypical osteogenesis with marked bone resorption (Fig. 1). The biopsy samples were collected under the presumptive diagnosis of neoplasia, and processed for smear, culture, and pathological examination. The Giemsa stain (Diff-Quik stain, Dade Behring, U.S.A.) for the smear samples revealed unstained numerous hyphae under light microscope. Additionally, samples for the pathologic analysis were preserved in the 10% neutral buffered formalin, processed for paraffin embedding and stained with routine hematoxylin & eosin and Periodic Acid Schiff (PAS) method. Microscopically, numerous hyphae within necrotic and granulomatous osteomyelitis were seen using hematoxylin-and-eosin staining (Fig. 2a). Furthermore, the PAS stain clearly visualized the morphology of the hyphae (Fig. 2b); that is, a thin cell wall 1.5 µm-3 µm wide without other specific features. These findings confirmed the diagnosis of a mycotic disease.

By fungal culture test, no growth was seen on Sabouraud Agar media incubated at 37°C during the first month, but white, flocculating fungi appeared thereafter. Two months later, the culture strategy was changed. Following two weeks incubation at 25°C, the fungal colony on potato dextrose agar was moderately fast-growing, flocculating and white with a maximum diameter of 73 mm (Fig. 3). These colonies, however, could not grow at 42°C. They released a methane-like smell, and hyphae were septate, branched and hyaline. Conidia were not observed. These findings classified the organism as a basidiomycete.

Under the suspicion of *S. commune* infection, we performed mating tests using several tester *S. commune* strains. The isolated organism formed clamp connection with some of tester strains, IFM45818, IFM46099, IFM46102, IFM46103, and IFM46101 (Fig. 4a) [1]. And this organism made fruiting bodies with IFM46100 (Fig. 4b). Moreover, to verify this organism as *S. commune*, we utilized set primers for the region of internal transcribed spacer (ITS) and D1/D2 domain, and performed PCR and sequences on the extracted fungal DNA (InstaGene Matrix; BioRad, CA, and DNA tests showed the pathogen as the mushroom *Schizophyllum commune*. Antibiotic sensitivity testing also revealed susceptibility to itraconazole, which was used to successfully treat the dog. This is a rare case of canine basidiomycosis with *S. commune* as the etiologic agent.
The PCR primers were ITS4: TCCTCCGCTTAT-GATATGC, ITS5: GGAAGTAAAGTCGTAA-CAAGG, and NL1: GCATATCAATAAGCGGAAGA, NL4m: GTCCGTGGTTTCAAGACG. Sequence reactions and analysis were performed by BigDye Terminator v3.1 Cycle Sequence kit (Applied Biosystems, CA, U.S.A.) and 3130x Genetic Analyzer (Applied Biosystems). The PCR-amplified DNA was specific for *S. commune* with 99% homology. These findings made confirmation of this organism as *S. commune*.

We medicated the dog with itraconazole (orally, 150 mg/day), which led to resolution of symptoms within one month. Assessment of the antibiotic sensitivity of this *S. commune* against amphotericin B, flucytosine, fluconazole, itraconazole, miconazole, and micafungin revealed its susceptibility to amphotericin B, itraconazole, and miconazole (data not shown). These findings supported the effectiveness of our treatment with itraconazole.

*S. commune* is a mushroom that colonizes rotting wood and has a wide global distribution. It maintains itself via a complex life cycle that includes fruiting bodies (mushrooms), basidia, basidiospores, homokayons, dikaryons [7]. This case demonstrates extra-respiratory infection of *S. commune* in a dog is possible, confirming a previous report. We hypothesize that the mycelia invaded through the puncture wound with the rotted bamboo and subsequently reached the bone marrow via infiltration into the circulation system. This case has important implications for the tropism of the organism and the etiology and pathogenesis of this disease, and suggests greater possibilities for exposure and infection than previously realized.

Deep mycoses are typically associated with a poor prog-

Fig. 1. X-ray examination. Atypical osteogenesis with marked bone resorption on her left hindlimb.

Fig. 2. Pathological examination. (a) Severe necrotic osteomyelitis in the dog. Bone marrow is occupied by necrotic foci with massive granulomatous reaction. H&E stain. × 100. (b) Numerous PAS-positive hyphae in the bone marrow. PAS stain. × 400.
nosis. The present case, however, shows that timely treatment with itraconazole can be successful. The S. commune was rarely detected than other pathogenic fungus, but general anti-mycotic medicine such as amphotericin B, itraconazole, and miconazole were effective. As shown in our study, routine mycological testing cannot identify this organism. It will be necessary to develop more clinically applicable tools to veterinary medicine and one tool showing great promise may be the polymerase chain reaction to make rapid and definitive diagnoses [4].

REFERENCES


