The present study describes the isolation of *Fusarium sporotrichioides* from a canine cutaneous ulceration. A 2-year-old male Beagle dog weighing 8.6 kg, with a history of immune-mediated hemolytic anemia (IMHA), had been treated with prednisone for 9 months. Physical examination revealed cutaneous ulceration on the left foreleg. Histopathological examination of skin samples from the ulcerative area revealed many branching hyphae surrounding neutrophils. Since itraconazole (ITZ) is recommended for miscellaneous fungal infections, the dog was treated with ITZ. However, the ulcerative lesions did not improve and after 3 weeks of treatment the dog died due to renal failure. No autopsy was performed. Since the isolate recovered from the biopsy specimen was identified as *Fusarium* species by morphological characteristics, the animal was diagnosed as having an infection caused by this mould. The dog’s prior prednisone treatment may have played a role in establishing the fungal infection. Comparative sequence analyses of the ITS regions of the clinical isolate with those in GenBank showed that it was 100% identical to *F. sporotrichioides* and less than 96% similar to ITS of other *Fusarium* species. Based on these findings, *F. sporotrichioides* was established as the etiologic agent of the canine infection, a situation that has not been previously reported in dogs, as well as humans.

**Keywords** cutaneous ulceration, dog, *Fusarium sporotrichioides*, internal transcribed spacer (ITS) region

Canine cases of *Fusarium* infection have rarely been reported [5], but there is a description of a *Fusarium solani* systemic infection [6].

The present study describes the isolation of *Fusarium sporotrichioides* from a canine cutaneous ulceration. The fungus was identified by molecular techniques using the internal transcribed spacer (ITS) region of the ribosomal DNA [2].

**Case history**

A 2-year-old male Beagle dog weighing 8.6 kg was referred to the Nihon University Animal Medical Center due to a cutaneous ulceration on the left foreleg. It had a history of immune-mediated hemolytic anemia (IMHA) that had been treated with prednisone (1–2 mg/kg PO twice a day) for 9 months. The cutaneous ulceration did not respond to antibacterial chemotherapy and had increased in size over the last 5 months. Physical examination revealed cutaneous...
ulceration (elbow to pad) on the left foreleg (Fig. 1a) but there was no fever. Hematology studies indicated slight anemia and serum biochemical analysis showed no abnormal findings except for high levels of alkaline phosphatase (ALP; 261 U/L). Urinalysis showed bilirubinuria, proteinuria (100 mg/dl) and polyuria (SG: 1.008). Hepatomegaly and splenomegaly were detected on the lateral view of a radiograph. High densities of the liver and spleen were observed at ultrasound examination. Histopathological examination of skin samples from the ulcerative lesion revealed numerous branching hyphae around neutrophils in the dermis (Fig. 1b).

Since itraconazole (ITZ) is recommended for miscellaneous fungal infections [1], the dog was treated with ITZ at 20 mg/kg orally daily. However, the owner was not cooperative in monitoring the case and the ulcerative lesions did not improve. The dog died due to renal failure 3 weeks after treatment. No autopsy was performed at the owner’s request.

Mycological examination

Two biopsy specimens from deep tissues of the cutaneous lesion were cultured on Sabouraud’s dextrose agar at 27°C for 2 weeks with fungal colonies developing within 1 week of inoculation.

Several hundred cells of the isolate recovered from the samples were lysed with 1 mg of zymolyase-100T (Seikagaku Corporation, Tokyo, Japan) in lysis buffer containing 0.1 mM EDTA, 1% sodium dodecyl sulfate (SDS), 10 mM Tris hydrochloride (pH 8.0) and 0.3% 2-mercaptoethanol, at 37°C for 16 h. High molecular weight DNA obtained from these samples by phenol and chloroform extraction were dissolved in TE buffer (10 mM Tris-hydrochloride, pH 8.0 and 1 mM EDTA) for polymerase chain reaction (PCR) amplification.

The sequences of the primers for the ITS region were constructed based on previous reports [7], i.e., forward primer ITS5 (5’-GGA AGT AAA AGT CGT AAC AAG G) and reverse primer ITS4 (5’-TCC TCC GCT TAT TGA TAT GC). The PCR products were sequenced by the dideoxy chain termination method using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Results and discussion

The colony of the sub-cultured clinical isolate was flat and white in color and had a cottony texture after 2-weeks incubation on Sabouraud’s dextrose agar at 27°C (Fig. 2a). There were numerous microconidia and macroconidia (Fig. 2b). Microconidia were hyaline, oval, and non-septated, measuring 10–15 by 2–5 μm, while macroconidia were 40–50 μm long, hyaline, moderately curved, septated with generally three or more cells. Based on these findings, the isolate was identified as a *Fusarium* species [3] and the dog was diagnosed as having a *Fusarium* infection, possibly predisposed by the previous prednisone treatment.

Comparative sequence analyses with the ITS region in GenBank showed that the queried sequence of the clinical isolate was 100% identical to *F. sporotrichioides* (GenBank accession nos. FJ426386 and AF414972) and less than 96% similar to other *Fusarium* species. Therefore, the isolate was identified as *F. sporotrichioides* by molecular analysis. The sequences reported in this paper have been deposited in the GenBank database [accession no. *Fusarium sporotrichioides* genes for 18S rRNA,
ITS1, 5.8S rRNA, ITS2, partial and complete sequence, AB558531].

Based on these findings, the diagnosis of this canine case was refined to be *F. sporotrichioides* infection, which had not been previously reported in dogs and humans.

We treated an earlier canine case of systemic *F. solani* infection with ITZ at 5 mg/kg orally once a day combined with amphotericin B (AMB) at 0.15 mg/kg IV without success [6]. The prognosis of *Fusarium* infection in dogs might be regarded as poor.

Though the *in vitro* MIC of *F. solani* and *Fusarium oxysporum* against ITZ were 0.5–>16 μg/ml [8], the MIC values of *F. sporotrichioides* against this antifungal remains unknown. The combination therapy with AMB and ITZ was recommended to the dog’s owner who refused the treatment in hospitalization. Therefore, ITZ at the high dosage (20 mg/kg orally daily) was administered to the dog.

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**References**