

Granulomatous Pododermatitis in the Digits Caused by *Fusarium proliferatum* in a Cat

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ABSTRACT. To the best of our knowledge, we present here the first report of a case involving granulomatous pododermatitis caused by *Fusarium proliferatum* in a 10-year-old female cat. A cutaneous mass developed on the foot-pad of the right hind leg. Nodular granulomatous dermatitis with numerous macrophages and multinucleated giant cells containing cytoplasmic fungal structures were revealed on histological examination. Periodic acid-Schiff reaction and Fungi-Fluor staining clearly revealed irregular, septate fungal hyphae engulfed by macrophages and multinucleated giant cells. Polymerase chain reaction and sequence analysis targeting three domains of the extracted fungal DNA revealed 100% amplicon homology with *F. proliferatum*.

KEY WORDS: cutaneous mycosis, feline, fusariosis, *Fusarium proliferatum*.

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Fusarium species are common soil saprophytes and plant pathogens that produce toxins known to contaminate stored grain [14]. In humans, opportunistic infection by *Fusarium* species has reportedly led to disseminated infections [16, 17, 22] or superficial locally invasive infections, including keratitis, onychomycosis and subcutaneous mycoses [7, 13, 19, 23]. More than 50 *Fusarium* species have been identified, but only a few have been shown to infect humans [13].

Several cases of skin fusariosis have been reported in dogs and cats [2, 9–12, 20, 21]: however, *F. proliferatum* has not been identified as the causative agent. With a focus on the genetic identification of *F. proliferatum*, we describe here the histopathological features of feline granulomatous pododermatitis with *Fusarium* infection.

A 10-year-old, spayed female, mixed-breed cat living outdoors was brought to a local veterinary clinic due to debilitation. On physical examination, three masses were found as follows: one on the foot-pad of the right hind leg (2 cm in diameter); and one on each of the forelegs (both 1 cm in diameter). The cat had evident weight loss, chronic diarrhea and anemia. The crusted and ulcerated mass on the right hind leg was surgically removed, fixed with 10% neutral-buffered formalin and submitted to our laboratory for histopathologi-

cal examination (Fig. 1).

Macroscopically, the cut surface of the fixed mass was white, solid and lobulated (Fig. 2). It was embedded in paraffin wax, cut into 3- μ m-thick sections and stained with hematoxylin and eosin (H.E.). Intense and diffuse infiltration of macrophages and multinucleated giant cells in the dermis was revealed by light microscopy (Fig. 3). Macrophages and multinucleated giant cells frequently contained fungi seen within cytoplasmic vacuoles (Fig. 4). In addition, infiltration of some lymphocytes, plasma cells and neutrophils was observed in the lesion. Periodic acid-Schiff (PAS) reaction and Fungi-Fluor staining (Biomate Co., Ltd., Tokyo, Japan) clearly showed abundant, irregular, septate hyphae with dichotomous branching, budding and yeast-like organisms in the cytoplasm of numerous macrophages and multinucleated giant cells (Figs. 5 and 6).

For identification, DNA was extracted from paraffin-embedded tissue using a DEXPAT extraction kit (TaKaRa Bio Inc. Otsu, Japan). Polymerase chain reaction (PCR) and sequence analysis targeting the fungal D2 domain of large subunit rRNA of this extracted DNA was performed using the MicroSeq D2 rDNA fungal PCR/sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.). The DNA databank of Japan (DDBJ) was used to determine that the amplicon sequence had high homology with *Fusarium* (>99%) and *Gibberella* (data not shown) spp. The primer common to the ribosomal internal transcribed spacer region 2 (ITS2) of *F. oxysporum* and *G. fujikuroi* was constructed, and PCR was subsequently performed. The forward primer was 411F:5'-GCATATCAATAAGCGGAGGAAAAG-3', and the reverse primer was 415R:5'-GGTCCGTGTTTCAAGACG-3'. The amplicon was 100% homologous with *Fusarium* sp.

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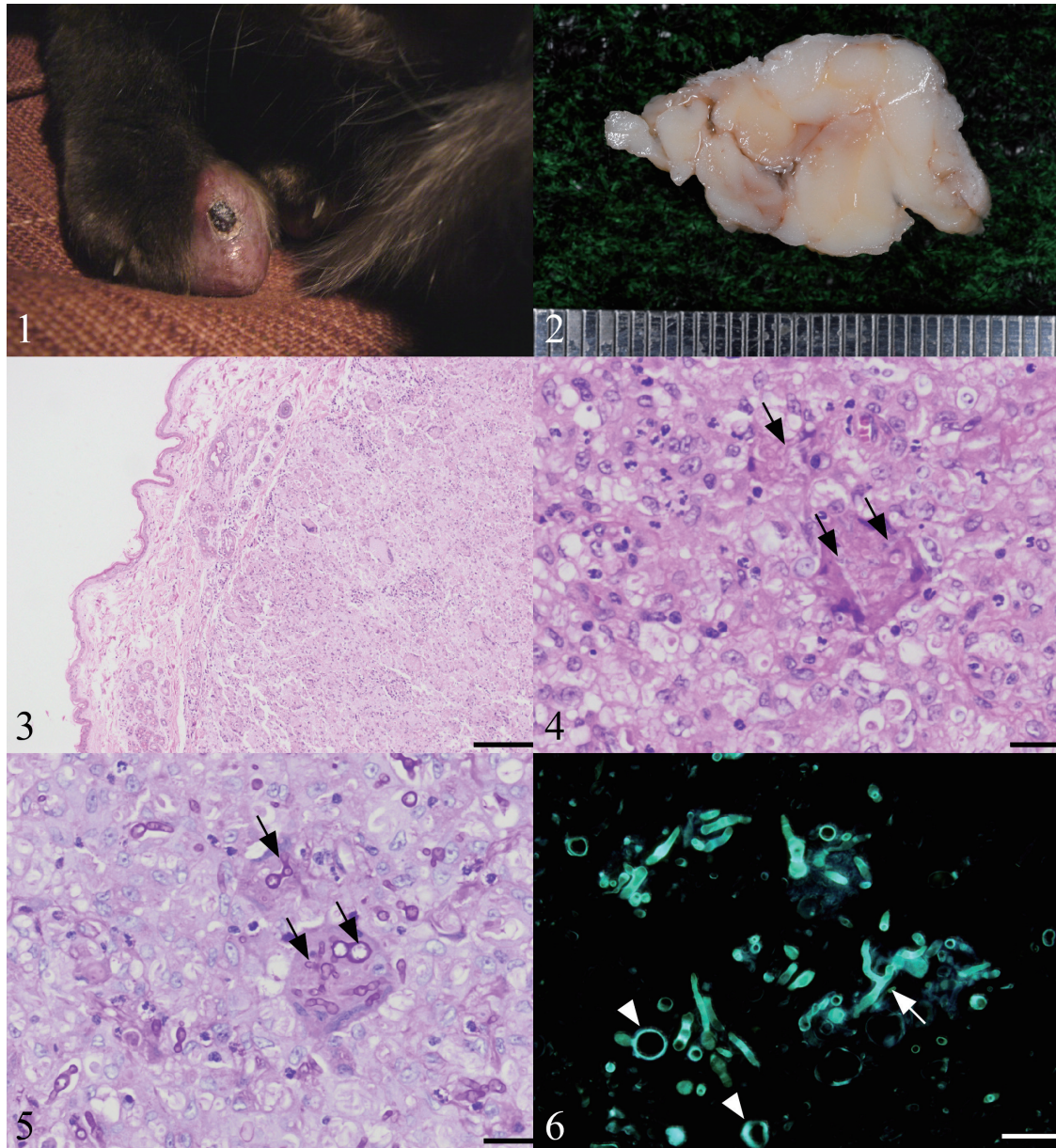


Fig. 1. Macroscopic findings of the mass in the right hind leg. The mass (approximately 2 cm in diameter) with the crusta forms on the foot-pads.

Fig. 2. The cut surface of formalin-fixed mass shown in Fig. 1. The cut surface of the fixed mass was white, solid and lobulated.

Fig. 3. Histological findings of the right hind leg mass. Intense infiltration of macrophages and multinucleated giant cells in the dermis. H.E. Bar: 200 μ m.

Fig. 4. Histological findings of the right hind leg mass. Macrophages and multinucleated giant cells include vacuoles with fungi in their cytoplasm (arrows). Lymphocytes, plasmacytes and neutrophils also scattered in the lesion. H.E. Bar: 20 μ m.

Fig. 5. Histological findings of the right hind leg mass. Numerous fungal hyphae were observed in cytoplasm of macrophages and giant cells (arrows). PAS reaction. Bar: 20 μ m.

Fig. 6. Histological findings of the right hind leg mass. Fungifluor stain clearly revealed the septate fungal hyphae with dichotomous branching (arrows), budding and yeast-like shape (arrowheads). Fungifluor stain. Bar: 10 μ m.

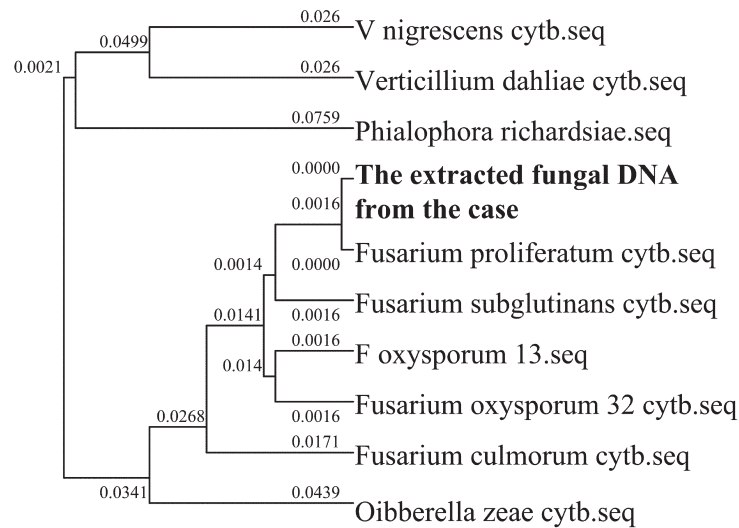


Fig. 7. Phylogenetic tree based on the partial DNA sequence of mitochondrial cytochrome b gene. The extracted fungal DNA was 100% homology with *F. proliferatum* (GenBank accession number, AB743574) and 99.7% with *F. oxysporum* and *F. subglutinans*.

QJC-1403 and 100% to 99.3% with different strains of *F. proliferatum* (data not shown). For further analysis, a primer was constructed to target a partial sequence of the *F. proliferatum* mitochondrial cytochrome b gene, and PCR was once again performed. The forward primer was 418F:5'-ATTTGAGGAGGTTTTAGCGT-3', and the reverse primer was 419R:5'-TACTATGGCAGGTGGTGT-3'. Results showed that the amplicon was 100% homologous with *F. proliferatum* (GenBank accession number, AB743574) and 99.7% with *F. oxysporum* and *F. subglutinans* (Fig. 7).

In the present case, we confirmed that the cutaneous mass was caused by *F. proliferatum* infection. Histologically, it is difficult to distinguish fusariosis from other hyalohyphomycosis, including aspergillosis. Fungal culture, *in situ* hybridization or immunostaining of paraffin-embedded tissue may lead to the correct diagnosis; however, precise species identification remains difficult and may therefore require DNA sequence analysis, which has recently been performed for both animals and humans [4, 5, 9, 10, 12]. In the present case, the fungal hyphae were histologically detected, and the final diagnosis and species identification were obtained by PCR and sequence analysis of the DNA extracted from paraffin blocks. This result supports the usefulness of a molecular biological approach to the diagnosis of fusariosis.

After the first operation, the cat in this case was treated with itraconazole (ITZ) (8 mg/kg/day). The dose was reduced after one week due to diarrhea. The masses on both forelegs were removed after treatment with ITZ and examined by light microscopy. Histologically, the two masses showed severe infiltration of lymphocytes and plasma cells and fewer numbers of macrophages in the dermis, but fungi were not observed (data not shown). In previous reports, the

treatment of ITZ to fusariosis was curative in cats [11, 20, 21], and it was suggested ITZ was also curative in this case. Their antifungal efficacy pattern is of interest to improve our knowledge of management of fusariosis in cats.

Immunodeficiency is an important predisposing factor to fusariosis in both animals and humans [3, 22]. In this case, neither prior use of corticosteroids nor history of either feline immunodeficiency virus (FIV) or feline leukemia virus (FeLV) infection was confirmed. However, the clinical symptoms observed in this case, including debilitation, weight loss, chronic diarrhea and anemia, suggested a decline in immune function as a predisposing factor for infection in this case. To the best of our knowledge, this is the first report of an *F. proliferatum* infection in an animal, although the infections have been reported in humans [1, 6, 8, 15, 18]. The histological findings in this case were consistent with fusariosis, such as *F. proliferatum* infection, in humans and animals [5, 9, 13, 18, 20, 21, 23]. *Fusarium* species are widely distributed in the environment, and a case of *F. proliferatum* infection in humans via a punctured finger from a plant has been previously reported [15]. In this case, *F. proliferatum* might infect from wounds on the feet. Therefore, *F. proliferatum* and other *Fusarium* species may not only be important pathogens in humans, but in animals as well.

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