Feline Digital Phaeohyphomycosis due to *Exophiala jeanselmei*

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(Received 23 May 2008/Accepted 12 August 2008)

**KEY WORDS**: feline, granulomatous dermatitis, phaeohyphomycosis.

**ABSTRACT.** The black nodule measuring 1 cm in diameter developed in the base of nail of an 8-year-old Japanese domestic male cat. Histological examination of the excised nodule revealed a granulomatous lesion extending from the epidermis to adjacent bone. The lesion was consisted of diffuse infiltration of macrophages with epithelioid cells and multinucleated giant cells. These macrophages contained a few to numerous yeast-like brown pigmented fungus cells with a spherical shape and dark thick wall. The PCR amplification with universal primers of the 28S ribosomal RNA gene yielded a 628-bp fragment and the direct sequence confirmed that the diagnosis of the lesion was phaeohyphomycosis caused by the pathogenic dematiaceous fungus, *Exophiala jeanselmei*.

Phaeohyphomycosis is used to describe mycotic infections caused by various phaeoid fungi that form brown-pigmented yeast-like cells and hyphae [2]. Phaeoid fungi infection has been frequently described in human skin and soft tissues in the worldwide including Japan [12]. Fungi inhabiting soil and decomposed plants enter tissues through skin wound. Lesions are usually localized in subcutaneous tissues, but the fungus may enter into circulation, especially of immunosuppressed patients and result in systemic dissemination [1, 15]. There have been several reports of feline cutaneous phaeohyphomycosis, and fungal species were successfully determined by culture from a cutaneous granuloma or abscess [1, 4]. However, culture preparation requires a fresh sample and takes weeks to obtain the isolates. This short communication describes histopathologic features of the feline case of *E. jeanselmei* infection in Japan as well as molecular identification of fungal species by PCR and sequence technique.

An 8-year-old Japanese domestic male cat was presented to the private animal hospital because of staggering gait and nasal discharge. The symptoms were slightly improved by antibiotics and forced feeding for a short period. However the cat developed clinical signs of fever, anorexia, nasal discharge, vomiting and depression afterward. A blood examination revealed aplastic anemia and hyperglobulinemia. The cat was negative for FIV and FeLV by SNAP FIV/FeLV test (IDEXX Laboratories Inc, Maine, U.S.A.). The cat was suspected the FIP infection although it was not confirmed as a definite diagnosis. The cat was treated with peroral medication of antibiotic and subcutaneous infusion with corticosteroids. As the cat’s condition improved, the dose of steroid medication was reduced gradually. At that time, a veterinarian found a black nodule, the size of 1 cm in diameter, at the base of nail in the left 2nd digit. The mass was easily bleeding when the cat was clawing. The mass was surgically excised and subjected to histological examination. No recurrence was observed after complete excision.

The mass was fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin (HE) stain, periodic acid-Schiff (PAS) stain, the potassium permanganate-oxalate (bleaching) method, and Fontana Masson stain. The genomic DNA was extracted from a paraffin-embedded tissue and used for the PCR amplification. Three to 5 slices of the thin section were deparaffinized with xylene and digested in lysis buffer (1 M Tris-HCl, 0.5 M EDTA, 5 M NaCl, 10% SDS) with proteinase K (20 μg/ml). After phenol/chloroform extraction, the DNA was resuspended with Tris-EDTA buffer and applied to PCR. The universal primers for fungal species were constructed based on sequences of the internal transcribed spacer and 28S ribosomal RNA gene [5, 10]. The following was the forward and reverse primer sequences respectively; 5’-CGATATCAATAAGCGGAGGAAAAG-3’ and 5’-GGTCCGTTTTCAAGACG-3’. The PCR amplification was carried out for 35 cycles of the following conditions; 94°C for 30 sec, 53°C for 30 sec, and 72°C for 1 min. The PCR product was electrophoresed on 1.5% agarose gel and then single gel band sized 630 bp approximately was excised from the gel. The DNA of gel slice was recovered with the filter cartridge (SUPRECTM-01 TaKaRa, Tokyo, Japan) and used for sequence analysis with BigDye® Terminator Cycle Sequencing Kit and ABI 3100 DNA sequencer (Applied Biosystems, CA, U.S.A.). The sequence data was subjected to BLAST (The Basic Local Alignment Search Tool) search in the NCBI genebank.

Histological examination of the excised nodule revealed a granulomatous lesion extending from the epidermis to adjacent bone and roughly demarcated from normal tissue. The lesion was consisted of diffuse infiltration of macrophages.
with epithelioid cells, multinucleated giant cells, polymorphonuclear neutrophils, fewer lymphocytes and plasma cells accompanied with hemorrhage. These macrophages contained a few to numerous yeast-like brown pigmented fungus cells. The fungus cells had a spherical shape and dark thick wall ranged from 3 to 15 \( \mu m \) in width (Fig. 1). There was a few budding bodies but were no hyphal elements in the lesion (Fig. 2). The most of fungus cells were intracellularly localized. The margin of the fungal cells was positive with PAS reaction and Fontana-Masson stain. The brown pigments were eliminated by bleaching method. This result indicated the brown pigment was composed by melanin.

The amplified product of 628-bp fragment was excised from the biopsy sample and a reference strain of \textit{E. jeanselmei} (Accession number AF050271) except a transversion of G for T at the nucleotide 975 [16]. These results confirmed that the diagnosis was phaeohyphomycosis caused by the pathogenic dematiaceous fungus, \textit{E. jeanselmei}.

The morphological features of causative agents distinguish phaeohyphomycoses from other brown pigmented fungi: chromoblastomycosis, characterized by spherical fungus cells in tissues (sclerotic cells) and mycetoma, with tissue formation of black grains [1]. The present case showed the fungal cells with a budding body uncharacteristic of the sclerotic cells. The observation of distinctive dematiaceous hyphae is important to confirm phaeohyphomycosis. However, the fungi in our case were mostly present as a yeast-like form and no hyphal formation was noted in the lesion. In addition to the morphological confirmation, fungal DNA was successfully extracted from the formalin-fixed granulomatous lesion in the feline digit and the nucleotide sequence revealed more than 99% homology with \textit{E. jeanselmei}. This is the first feline case of phaeohyphomycosis diagnosed by molecular analysis.

The diagnosis of phaeohyphomycosis has been done obtained by direct examination of an infected tissue with KOH and histological specimens. For identification of the fungal specie, classical culture isolation has been long considered as the only reliable technique. In our case whole excised tissue was fixed in formalin for histological examination and unfortunately it was impossible to perform a classical mycological examination. Like our sample, the several cases in literature were not supported by culture isolation [1]. With the advance of molecular technique, the extraction of genomic DNA recovered from the formalin-fixed samples and the PCR including DNA sequencing may contribute to identify the pathogen, even without a fresh sample.

The listing in the text book cites 60 genera and 109 species as aetiological agents of phaeohyphomycosis [13]. Phaeohyphomycosis shows a broad spectrum of infections ranging from superficial, cutaneous or subcutaneous, to systemic or disseminated. Most feline cases were reported of cutaneous phaeohyphomycosis, but there was systemic involvement caused by \textit{Ochroconis gallopava}, \textit{Cladophialophora bantiana} and \textit{E. jeanselmei} [4, 9]. Although we suspected the cat under immunosuppressive condition by a use of corticosteroids, phaeohyphomycosis has been known to occur in immunocompetent hosts [4, 6]. In the most feline cases, diagnostic tests failed to reveal an underlying immunosuppression including a viral infection [3, 7, 8, 11, 14].

Little information has been available about the treatment of phaeohyphomycosis. The management of cutaneous and subcutaneous infection usually involves surgical treatment with or without an antifungal agent although antifungal therapy may result in variable prognosis even within strains of a single species [13]. Some phaeoid fungi resist antifungal drugs and develop a recrudescent clinical course or an aggressive course as systemic infection [1, 4, 9]. The
present case had a single lesion and no recurrence after a surgical resection, and recovered completely.

REFERENCES


