Isolation of *Fusarium* sp. from a Claw of a Dog with Onychomycosis

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**ABSTRACT.** An 8-year-old male Golden Retriever had lameness and claw abnormality in the second digit of the left forelimb. Radiography revealed osteomyelitis in the distal phalanx bone of the affected limb. Microscopic examination of the claw revealed numerous hyphae in the claw matrix. Fungal DNA fragments coding the ribosomal internal transcribed spacer region (ITS) were detected from the claw matrix as well as fungal colonies of the clinical isolates by PCR. Nucleotide sequencing revealed that the amplicons shared > 99% homology with *Fusarium* sp. Therapy including oral itraconazole resulted in regrowth of a new claw, in which no hyphae were detected. To the authors’ knowledge, this is the first case report of canine onychomycosis in which *Fusarium* sp. was isolated from the affected claw.

**KEY WORDS:** canine, claw, *Fusarium*, infection, onychomycosis.

Onychomycosis is a term for claw infection caused by dermatophytes, nondermatophyte molds or yeasts. The incidence of onychomycosis is very rare in dogs. Scott *et al.* reported that onychomycosis accounted for 7 out of 196 (3.6%) dogs affected with claw diseases [24]. Dermatophytes such as *Trichophyton mentagrophytes* and *Microsporum gypseum*, nondermatophyte molds such as *Geotrichum candidum* and *Blastomyces dermatitidis* and yeasts such as *Candida* sp. and *Malassezia* sp. have been isolated from claws affected with onychomycosis in dogs [1, 24, 25]. Conversely, to date, there have been no reports describing that *Fusarium* sp. were isolated from onychomycotic claws of dogs. This report describes a dog with onychomycosis, in which genetic evidence of *Fusarium* sp. was demonstrated in the affected claw as well as in fungal colonies of the clinical isolates.

The case was an 8-year-old, intact male Golden Retriever, presented with a 3-month history of lameness and claw abnormality in the second digit of the left forelimb. According to the owner’s declaration, the dog has no apparent history of trauma on the affected claw prior to development of the claw abnormality. Simultaneous with the development of claw changes, a solitary tumor developed on the lumbar area of the skin. The general condition of the dog seemed to be good except for the claw abnormality and the cutaneous tumor.

On physical examination, the affected claw exhibited macronychia and onychorrhexis, and the claw matrix seemed to be dull, brownish and partly brittle (Fig. 1A). Paronychia and hyperkeratosis of the footpad were also recognized in the affected digit. Besides the affected area, other claws and digits showed no abnormalities. The cutaneous tumor on the lumbar area consisted of a solitary, well circumscribed and firm tumor that was approximately 5 cm in diameter (data not shown).

Radiographic analysis of the affected digit revealed bone destruction with cortical disruption on the distal phalanx bone (Fig. 1B). Complete blood counts, serum chemistry and thoracic as well as abdominal radiographs yielded unremarkable results. Microscopic examination of the claw samples revealed numerous hyphae in the claw matrix (Fig. 2), suggesting fungal infection of the claw. For fungal culture, the affected claw was initially disinfected with alcohol to avoid contamination of microorganisms present on the claw surfaces, and a sample was then cultured on a Sabouraud’s dextrose agar and potato dextrose agar at 27°C for 2 weeks. Fungal colonies with a flat, cinnamon color and a cottony texture developed within 1 week. Microscopic examinations of the isolates revealed branching hyphae, but no microconidia or macroconidia were recognized. Therefore, the fungal species of the clinical isolates was not identified by the morphological characteristics of the cultured isolates.

To confirm the fungal species of the isolates by molecular analysis, PCR to amplify the ribosomal internal transcribed spacer (ITS) region (5.8 rRNA gene, ITS1 and ITS2) was performed. Extraction of genomic DNA samples and PCR analysis were preformed as previously reported [16]. The sequences of the primers for the ribosomal ITS region were constructed based on sequences reported previously [14]: the forward primer was ITS5 (5’-GGA AGT AAA AGT CGT AAC AAG G-3’), and the reverse primer was ITS4 (5’-TCC GCC GCT TAT TGA TAT GC-3’). The ampli-
cons were directly sequenced by the dideoxy chain termination method using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.). Comparative sequence analyses with the ITS region in GenBank showed that the queried sequence of the clinical isolate was >99% homologous with *Fusarium sp.* (GenBank accession number, EF611096), with only a single nucleotide being replaced (351C to T; Fig. 3), probably due to a single nucleotide polymorphism in the cultured isolate. PCR amplification was also performed using DNA extracted directly from the affected claw, and nucleotide sequencing revealed that the amplicon had 100% homology with *Fusarium sp.* (GenBank accession number, EF611096) and *Gibberella avenacea,* a teleomorph of *Fusarium sp.* (GenBank accession number, EF610300; data not shown). Thus, these findings provided genetic evidence of *Fusarium sp.* in the affected claw. Genetic evidence of dermatophytes (*e.g.*, *Microsporum sp.* and *Trichophyton sp.*) was not identified in the affected claw or cultured isolates. Bacterial culture isolated *Escherichia coli* from the affected claw.

Under general anesthesia, the cutaneous tumor on lumbar area was removed surgically. As osteomyelitis caused by fungal and/or bacterial infection was considered as a differential diagnosis, amputation of the affected digit was recommended. However, as the owner did not agree with the amputation, the affected claw was removed at the proximal end at the time of surgical removal of the lumbar tumor. To determine whether or not neoplasia or bullous diseases were recognized in the distal phalanx of the affected digit, onychobiopsy of the affected digit was also performed according the method reported previously [19]. Histopathologically, the tumor consisted of a subcutaneous and dermal mass comprising spindle and polymorphic cells arranged in a cartwheel pattern with abundant collagen in a part of the mass (data not shown). The histopathological findings were consistent with those in hemangiopericytoma. Histopathology of an onychobiopsy sample revealed marked epidermal acanthosis with hyperkeratosis, dilatation of blood vessels with perivascular-to-diffuse infiltration of mononuclear cells and extravasation of red blood cells in the superficial dermis of the claw fold (Fig. 4). These findings may support the clinical finding of paronychia. No neoplasia or bullous diseases were recognized in the claw fold sample. The claw matrix was not recognized in multiple histological sections, probably due to complete removal of the claw matrix by...
Based on these findings, the claw lesion of the present dog was diagnosed as onychomycosis caused by *Fusarium* infection. The dog was treated with oral itraconazole (6.6 mg kg⁻¹, q24h) and marbofloxacin (5 mg kg⁻¹, q24h); the latter was selected according to antibiotic sensitivity tests of the isolated *E. coli*. At 40 days after initiation of therapy, regrowth of a new claw was recognized, although the regenerated claw did not have a normal shape, probably due to resorption of bone marrow in the distal phalanx bone (Fig. 5). Claw scraping at 40 days after initiation of therapy yielded no fungal organisms in the claw matrix (data not shown). The present dog did not exhibit lameness at 40 days after initiation of therapy.

*Fusarium* sp., which are ubiquitous saprophytic fungi and important plant pathogens, can also be opportunistic pathogens causing onychomycosis in mammals [2–7, 9, 10, 12, 13, 17, 18, 20–23, 26–28]. In dogs, *Fusarium* sp. have been isolated in cases with fungal meningoencephalitis [11], pyelonephritis [8], dermatomycosis [15] and disseminated infection including kidney and subcutis infection [16], but no reports have described canine onychomycosis caused by *Fusarium* sp. to date. In the present case, genetic evidence of *Fusarium* sp. was demonstrated in the affected claw. The present dog was strongly suspected as having onychomycosis caused by *Fusarium* sp., as numerous hyphae were present in the claw matrix of the affected claw, while no genetic evidence of dermatophytes was found.

As the claw is the body site that contacts directly with soil, it might have greater opportunity to be contaminated with saprophytes. In addition, as the tip of the claw is completely isolated from immune systems, it might be easily infected by saprophytes, even through trivial flaws.

In conclusion, to the authors’ knowledge, this is the first case report of canine onychomycosis in which *Fusarium* sp. was isolated from an affected claw. Our findings also indicate...
cated that the combination of microscopic analysis, fungal culture and PCR would help to identify causative fungi of infections in dogs with onychomycosis. Identification of underlying diseases affecting immune status is also important to understand a patient’s condition and manage the infection.

REFERENCES


