Dermatitis caused by Neosartorya hiratsukae in a hedgehog

Jae-Ik Han, Ki-Jeong Na

Department of Veterinary Medicine, Chungbuk National University, Cheongju 361-763, Republic of Korea

Abstract

This case report describes the fungal dermatitis caused by N. hiratsukae in a household hedgehog confirmed by microscopic examination of conidiophores and DNA analyses including ITS region, partial β-tubulin and calmodulin gene. It is the first report of a natural N. hiratsukae infection in animals.

A 1-year-old African pygmy hedgehog presented with progressive dermatosis on its back. The clinical signs started about 3 weeks previously. The animal had not been treated for the dermatosis prior to presentation. The hedgehog had been purchased from a local pet store about 10 months previously and was housed on commercial sawdust bedding in a plastic box. The hedgehog had never been in contact with other animals. It was fed with commercial dog food ad libitum.
At the first presentation, the hedgehog exhibited well-circumscribed and widely located alopecia on its back that was accompanied by severe scaling and pin-point hemorrhages (Fig. 1). However, the activity, appetite, defecation, and urination of the hedgehog were unaffected. Other abnormal signs were not present. The affected area was examined by scraping the squames and quills attached to the margins of the alopecic lesion and analyzing this sample by microscopy and culture. The stained debris contained many keratinized epithelial cells and septate hyphae. Consequently, on the day of presentation, daily topical application of econazole ointment was commenced as an empirical antifungal therapy. By the seventh day after presentation, while the scaling had not improved, the extension of the alopecic lesion had ceased. However, the treatment was discontinued on the basis of the owner’s decision.

In the meantime, the hair roots of the quills collected from the lesion were cultivated at 30°C for 7 days on Sabouraud dextrose agar. The cultured colonies were white with white to yellowish-white reverse and had an irregular, folded, velvety appearance that differed from the colonies produced by dermatophytes, which are a common causative agent of fungal dermatitis in hedgehogs (9). Upon microscopic analysis, conidial heads of the isolate that were similar to those of *Aspergillus* spp. were seen.
The isolate was identified by molecular typing. The genomic DNA encoding the ribosomal internal transcribed spacer (ITS) region consisting of ITS1, 5.8S and ITS2 were amplified by using primers ITS1 and ITS4 as described previously (11). Partial β-tubulin and calmodulin genes were also partially amplified by using primers Bt2a and Bt2b (4) and primers Cmd5 and Cmd6 (7) as described previously. All amplicons were sequenced by using a ABI prism™ Bigdye™ terminator cycle sequencing Ready reaction kit V.3.1 (PE Applied Biosystems, Foster City, Calif, USA). When compared to the sequences in GenBank, the ITS sequence of the isolate was found to be 97% similar to the *N. hiratsukae* sequence that had been deposited by the Bacteriology Division of Osaka prefectural Institute of Public Health in Japan (GenBank accession number AB185257). The β-tubulin sequences was found to be 100% similar to the *N. hiratsukae* sequences that had been deposited by the Korea Agricultural Culture Collection of the National Institute of Agricultural Biotechnology (GenBank accession number DQ534095.1) and the calmodulin sequence was found to be 98% similar to the *N. hirasukae* sequences that had been deposited by the Korea Agricultural Culture Collection of the National Institute of Agricultural Biotechnology (GenBank accession numbers AY870699.1 and DQ534169.1) (Fig. 2). Thus, we identified the species isolated from the hedgehog as *N. hiratsukae*. 

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Living cultures of the case strain have been deposited in the Korea Agricultural Culture Collection of the National Institute of Agricultural Biotechnology, Suwon (KACC 43533) and the Korean Collection for Type Culture of the Korea Research Institute of Bioscience & Biotechnology, Daejeon, Republic of Korea (KCTC 26423).

While species of the genus *Neosartorya* present ubiquitously, they have only rarely been reported to be pathogenic. To date, only eight cases of *Neosartorya* infections in humans have been reported in the medical literature (2, 5, 8, 10, 12, 14, 16). *N. hiratsukae* was first described in Japan, where it was isolated from air and pasteurized aloe juice (17). Only four case of *N. hiratsukae* infection in humans has been reported previously (5, 15). All case reports of *Neosartorya* infections suggest that these infections only occur in immunosuppressed or immunocompromised individuals. Thus, species of this fungus genus seem to infect humans in an opportunistic manner.

With regard to *Neosartorya* infections in animals, it has been reported that experimental administration of fischerin, a toxin produced by *N. fischeri var. fischeri*, induces lethal peritonitis in mice (3). However, natural infections of animals with this genus have not been reported previously. This report is thus the first to show that animals (in this case, a household hedgehog) can be naturally infected by *N. hiratsukae*. 


Fungal dermatitis is a common skin disease in animals (13) and almost all fungal dermatitis infections are caused by dermatophytes. In animals, its clinical signs include well-circumscribed alopecia, hyperkeratosis and scaling. There is usually no pruritus. Upon culture on Sabouraud dextrose agar, white to yellowish white colonies with variable appearance develop (21). The case described here evinced a similar clinical lesion and the fungal colonies generated early after culturing the quills extracted from the lesion were white. Consequently, we initially diagnosed the case as a dermatophytosis. However, we later observed under a microscope that the conidial heads of the cultured colonies differed from the characteristic features of dermatophytes and instead resembled *Aspergillus*. This led us to perform molecular typing to identify the isolate. These observations suggest that the real incidence of dermal aspergillosis in animals caused by species of the genus *Neosartorya* may be under-reported. This is because in the veterinary clinic, dermatological problems caused by pathogenic fungi are usually diagnosed and treated on the basis of the animal’s history and clinical signs alone. In addition, *Aspergillus* species collected from the skin surface in veterinary medicine are often discarded as contaminants. Guarro and coauthors have also suggested that *Neosartorya*-induced dermal aspergillosis in humans may occur more frequently than previously thought (5).
At present, *A. fumigatus* and the species of the genus *Neosartorya* has been conventionally identified on the basis of morphological and microscopic characteristics. However, such identification techniques are time-consuming and require a high level of expertise and thus are not practical for clinical laboratories (6, 8, 14, 19). Instead, multilocus gene typing for genes such as β-tubulin, calmodulin and actin have been used for species identification and phylogenetic analysis (1, 7, 18, 20). A recent taxonomic study of the species belonging to *Aspergillus* section *Fumigati* also suggests that sequencing the calmodulin and β-tubulin genes allows good species delimitation and recognition because all sequences of the ex type cultures of section *Fumigati* are available from specialized databases and GenBank (20). Here we show that maximum parsimony phylogenetic analyses using a set of β-tubulin and calmodulin sequences available in GenBank allowed us to identify the isolated strain as *N. hiratsukae* with a bootstrap of 100% and 99%, respectively (Fig. 3). Thus, this case was diagnosed on the basis of molecular typing as a *N. hiratsukae* infection. These observations confirm the potential of this fungus to cause animal disease.

The ITS region, calmodulin and β-tubulin nucleotide sequences of the isolate have been deposited in Genbank under accession numbers EU543210, EU543211, and EU543212, respectively.
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Fig. 1. On presentation, the hedgehog exhibited well-circumscribed complete alopecia, severe scaling and pin-point hemorrhages. Many quills were excluded from the hair follicles and were merely attached to the keratins in the lesion, thus permitting them to be easily removed from the skin.
Fig. 2. The homologies of the query sequences compared with β-tubulin (A) and calmodulin gene (B). The β-tubulin sequence of the isolate was found to be 100% similar to that of DQ534095 and calmodulin sequences of the isolate was found to be 98% similar to that of AY870699. Conservation bars mean the similarity of the sequence between query and type strains. Sequence alignment was performed using CLC Sequence Viewer v. 4.6.2.
Fig. 3. Phylogenetic tree based on the β-tubulin (A) and calmodulin (B) gene sequences of the type cultures in the GenBank databases. Indicates the fungus isolated from the hedgehog. The first series of letters/numbers mean GenBank database numbers and second series of letters/numbers mean the numbers of the type strains. Sequence
alignments were performed using CLUSTAL-X v. 1.8 and MEGA4 v. 4.0.2 was used for phylogenetic analyses.