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Clinical Application of PCR-RFLP for the Differentiation of *Trichophyton mentagrophytes* var. *erinacei* in the Facial Dermatitis of Household African Pygmy Hedgehog (*Erinaceus albiventris*)

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Abstract : This report describes a case of severe and prolonged dermatophytosis in a hedgehog that was diagnosed by PCR-RFLP, a rapid and usefulness technique for identification of many causative agents and hereditary characters. A 5-month-old female hedgehog was presented with grade 2 facial pruritus, scaling, encrustation and hemorrhage. Cytology of exudates on the face showed a suspected fungal infection. A culture and tape imprint test of the cultured colony showed many hyphae and microcornidia, suspected to belong to the *Trichophyton* species. In the PCR-RFLP with *Mva*I and *Hinf* I, *Trichophyton mentagrophytes* var. *erinacei* was finally identified as a causative agent. The patient completely recovered after application of nystatin cream for 17 days.

Key words **:** African pygmy hedgehog, *Trichophyton mentagrophtes*, PCR-RFLP

Introduction

The hedgehog has been reported as a mediator spreading dermatophytes, mainly *Trichophyton*, which is normal flora on the quills of the hedgehog. Therefore, this species has been considered important in terms of public health. However, dermatophytosis of the hedgehog has rarely been reported. Previous reports indicate that the hedgehog with dermatophytosis showed mild to moderate scaling and recovered spontaneously. The most frequent agents of infection in the hedgehog are the members of the *Trichophyton mentagrophytes* complex, followed by *Arthroderma benhamiae* (3-5,9-12).

The PCR-RFLP (Restriction fragment length polymorphism) technique is a more accurate, rapid, and efficient tool than the other diagnostic tools, such as morphologic classification with microscopic examination, serologic tests, and PCR against the pathogenic organism itself. In the previous study using standard strains, RFLP analysis with enzymes *Mva*I and *Hinf* I of the amplified ITS1 and ITS2 has shown differentiation among the several dermatophytes, including *Arthroderma* species, *Trichophyton* species and *Epidermophyton floccosum* (8).

This report describes a clinical case of severe and prolonged dermatophytosis in a hedgehog due to *Trichophyton mentagrophytes* var. *erinacei*, which was identified by PCR-RFLP using *Mva* I and *Hinf* I.

Case report

A 5-month-old female hedgehog was presented with facial dermatitis. The patient had been purchased from a local pet store about 4 months previously, and was housed on sawdust bedding in a plastic box. It was fed with commercial dog food *ad libitum* and lived with another hedgehog that had no dermatologic signs.

On physical examination, severe scaling, encrustation and hemorrhage were found on the entire field of the face, and exposed skin on the face was lichenified (Fig 1). About 1

Fig 1. Photograph of the anesthetized patient at first presentation. Severe scaling, encrustation and hemorrhage were apparent on the entire field of the face. Hypotrichosis was also identified on the lesions.

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Fig 2. Cytology using an exudate from the lesions. Many round shaped pathogens, suspected arthroconidia, were identified in squames. Wright-Giemsa stain. × 1000.

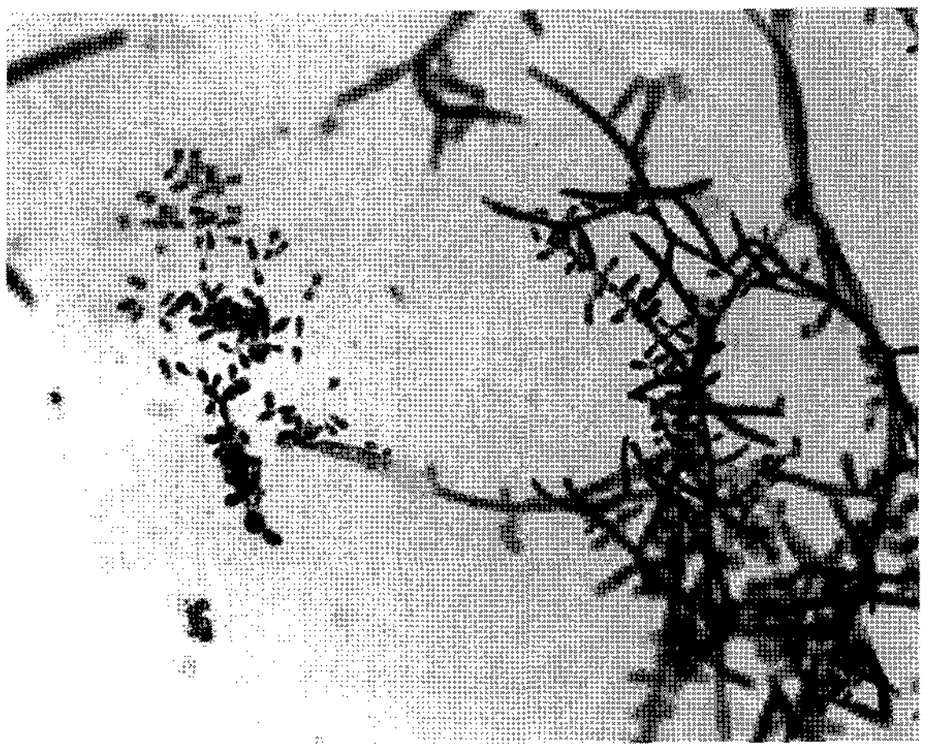

Fig 4. The imprint cytology of the incubated colony. Growing fungal hyphae and microcornidia like seeds are visible. Diff-Quik stain. × 1000.

month prior, some serous exudates were firstly detected on the face of the patient and encrustation appeared suddenly at 1 day before presentation. According to the itching scale developed by Colombo et al., the grade 2 pruritus on the face was identified (1).

The cytologic examination using exudates from the lesions showed neutrophilic inflammation with many round shaped pathogens in squames (Fig 2). Laboratory findings included mild lymphopenia (1,500 /μL; reference range: 2,400~7,500 /μL) and hyperglobulinemia (0.62 g/dL; reference range: 2.0~3.6 g/dL). In a fungal culture using crusts, a white color fungal colony was grown on the Sabraoud dextrose agar (Fig 3). A tape imprint test of this colony showed that hyphae were growing as well as many microcornidia suspected to be *Trichophyton* spp (Fig 4).

For identification of the fungal species, PCR-RFLP was performed. The universal fungal primer pair ITS1 (5'-TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC) were used. Restriction enzymes *Mva*I and *Hinf* I were selected according to previous research performed by Mochizuki et al. that proved the efficacy of these enzymes in the standard strains (*Arthroderma* species, *Trichophyton* species and *Epidermophyton floccosum*) and some human cases that had proved as *Trichophyton tonsurans* infections. Each restriction enzyme digestion was performed at 37°C for 1hr. Finally, the incubated pathogenic colony was identified as *Trichophyton mentagrophyte* var. *erinacei* according to the restriction patterns in the study performed by Mochizuki et al. (8, Fig 5).

The treatment prescribed included topical nystatin cream (Oridermyl®, Vetoquinol, Lure Cedex, France) once a day.

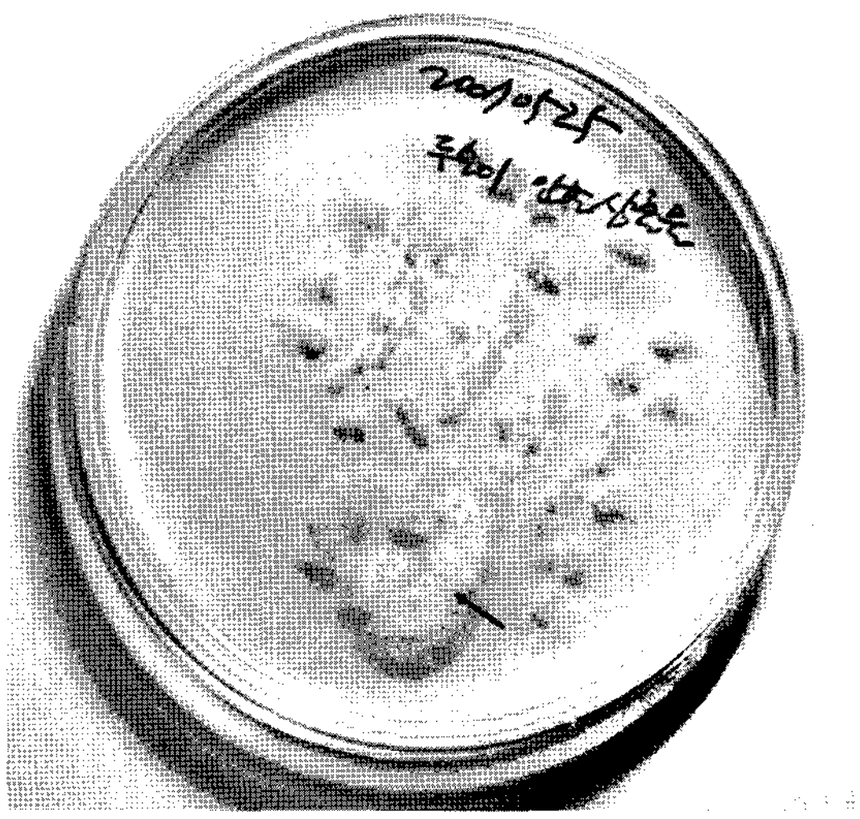

Fig 3. The result of incubation using crusts on Sabraoud dextrose agar. A white color fungal colonies are seen (arrow).

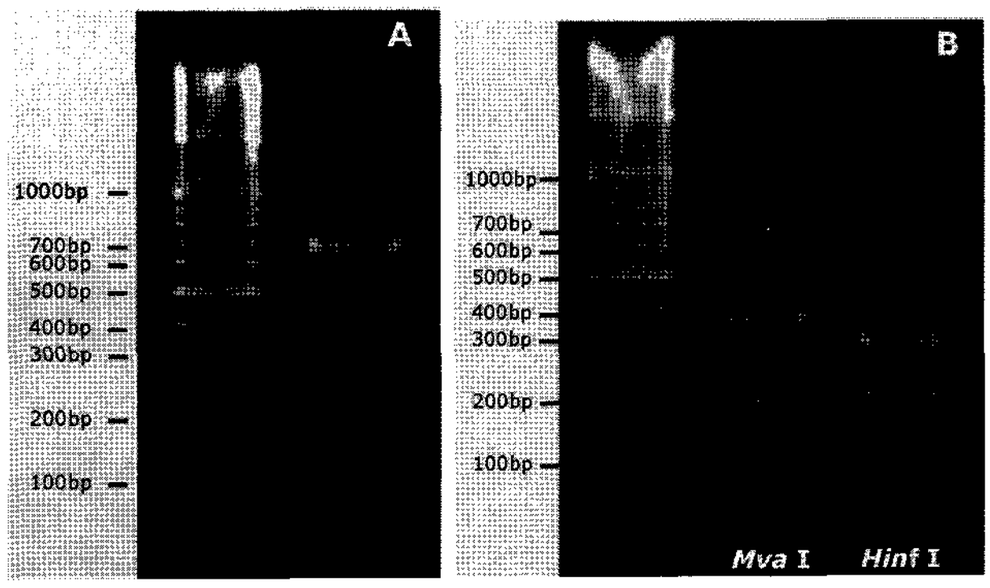

Fig 5. The result of PCR-RFLP of the incubated fungal colony. (A) The electrophoretic patterns of the PCR product by the universal fungal primer and 100bp ladder. Amplicon size is approximately 690bp. (B) The electrophoretic patterns of the restriction enzyme after digestion with *Mva*I and *Hinf* I and 100bp ladder. In *Mva*I, amplicon sizes are 360bp and 157bp. In *Hinf* I, amplicon sizes are 307bp, 200bp and 163bp. Each restriction fragment indicates that the fungal colony is *Trichophyton mentagrophyte* var. *erinacei*.

Fig 6. Photograph of the patient after 17 days of therapy. All pathologic features on the lesion are completely disappeared except for alopecia.

After 5 days of therapy, the lesions had gradually improved. After 17 days of therapy, the patient's lesions had completely resolved and the pruritus had disappeared (Fig 6). Treatment was discontinued at that point and the patient has no dermatologic signs.

Discussion

In general, young hedgehogs under 1 year old rarely get dermatophytes (although other young animals are sensitive to *Microsporum canis*), and persistent and periodic exposure to dermatophytes is needed for developing dermatophytosis.[2] In the present case, the patient is young and there were no clinical signs when she was purchased; this means that the disease was likely transmitted to the patient from the external environment. The owner had several sporadic papulations with mild pruritus on the medial forearm at the time the infection began in the hedgehog, but this clinical sign disappeared spontaneously after 1 week.

A study on the transmission of dermatophytes performed in New Zealand reported that *Caparinia erinacei* (a common mite found on hedgehogs in Kenya) mediates the transmission of dermatophytosis. In the study performed by Gregory et al. (4) in England, however, there was no evidence that *Caparinia erinacei* is involved in the transmission of dermatophytes (9). In the present study, the patient also showed no evidence of mite existence or mediation.

Hedgehogs have dermatophytes on the quills as normal microbial flora. A habit of sleeping in communal nests, as well as direct contact in the wild, results in the transmission of dermatophytes. However, dermatophytosis in the hedgehog has rarely been reported and almost all reports indicate that dermatophytes in the hedgehog are transmitted to other species, especially humans. Therefore, in terms of public health, the hedgehog has been considered as an important mediator for transmitting dermatophytosis (5,10-12). Therefore, rapid monitoring techniques are needed. However, at present, the definitive diagnostic techniques of fungal infections, such as incubation of clinical samples, serologic tests and PCR against the pathogenic organism itself, are time-consuming, complicated and inefficient. Because of its simplicity and rapidity, PCR-RFLP is widely used to differentiate genetic characters, as a substitute for sequencing. In the diagnosis of fungal infections, different restriction patterns created by restriction enzyme with PCR amplification may be used and several researches have been progressed in the human medicine (2,6,7). In the present case, *Mva*I and *Hinf* I were used to create the restriction fragment of the causative fungus and, as a result, *Trichophyton mentagrophytes* var. *erinacei* infection was identified. Also the result accord with microscofic findings.

Nystatin is a polyene antifungal agent. This agent binds to ergosterol, a major component of the fungal cell membrane, and forms pores in the membrane that lead to K^+ leakage and the death of the fungus. Because it is not absorbed across intact skin, this agent is considered a relatively safe drug for treating fungal infection (13). In the present case, nystatin was applied once a day; after 17 days of therapy, complete efficacy was proved without adverse effect. This result of therapy suggests that nystatin may be a safe drug for fungal infections in small exotic animals.

Acknowledgement

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