Dermatophytosis in a Steller Sea Lion (*Eumetopias jubatus*)

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ABSTRACT. Serious dermatophytosis caused by *Trichophyton mentagrophytes* was found in a Steller sea lion (*Eumetopias jubatus*) at Yomiuri Land Marine Aquarium in Tokyo. The external clinical signs were extensive depilation and hyperkeratosis, as well as redness and depigmentation of the skin. Histopathological findings of the skin revealed PAS positive fungal hyphae with septa in the corneum layer of the epidermis. Further microscopic examination suggested that this lesion of the skin was typical chronic dermatophytosis. Based on morphological and growth characteristics, the isolate was identified as *Trichophyton mentagrophytes*. It was thought that the infection was due to some factors including species and individual specific and environmental factors and so on.—KEY WORDS: dermatophytosis, Steller sea lion, *Trichophyton mentagrophytes*.

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In common with terrestrial mammals, marine mammals sometimes suffer from dermatophytosis. *Microsporum canis* was isolated from a harbor seal, which showed depilation about the face and nose and subsequently developed pustular dermatitis [1]. As for *Trichophyton* isolated from marine mammals, two related reports have been published, one by Hoshina [3], which reported serious dermatitis in a bottlenosed dolphin (*Trusiops truncatus*) with multiple nodules on its posterior trunk. In the second report, several fungal species, including *Trichophyton*, were isolated from wild healthy northern fur seals (*Callorhinus ursinus*) [7].

The authors were able to isolate *Trichophyton mentagrophytes* from an adult male Steller sea lion (*Eumetopias jubatus*) suffering from severe dermatitis. This is the first report of a dermatophytosis in the Steller sea lion.

A male Steller sea lion was caught in Hokkaido, Japan in 1980 and subsequently kept for performances in Yomiuri Land Marine Aquarium in Tokyo. When the skin samples were taken it was an adult male approximately 13 years old, with an approximate body length of 2.5 m and weighing about 700 kg.

The animal was kept in a pool filled with freshwater from a well near the aquarium. An overflow system maintained water circulation. The pool was emptied and sterilized with sodium hypochlorite and refilled regularly.

The first skin lesion, noticed in April, 1988, consisted of a large number of round depilated areas, 2 to 3 cm in diameter, covering the head and back (Fig. 1). The depilated areas increased in number and size, spreading over the entire body, but when the molting period began, fur grew in the middle of the depilated areas, and by early September the areas were completely covered with fur. Over the next few years, the animal's skin condition seemed to improve during molting periods, but its condition subsequently deteriorated and in June, 1991 it lost almost all of its remaining fur. Applications of povidone-iodine and bathing in sea or salt water had been done as treatment but those were ineffective. In June, 1991, the external clinical signs of the animal's condition were depilation, hyperkeratosis, redness and skin depigmentation over the entire body. Though the clinical signs seemed very serious, the animal did not appear to feel much irritation and was generally healthy apart from the skin lesions.

Fig. 1. First skin lesions of the Steller sea lion, showing round depilation areas from the head to the back.

Skin samples were taken from the vertex area of the animal. The tissues were fixed in a 10% phosphate-buffered formalin solution and routinely processed to make paraffin sections stained with haematoxylin and eosin (H & E). Some sections also were stained with Giemsa and periodic-acid Schiff (PAS) reactions.

The epidermal Malpighian cells of the lesion showed apparent irregular hyperplasia protruding into the dermal layer (Fig. 2). Moreover, parakeratosis, microvesicules and severe necrosis were observed between the horny layer and the prickle layer of the epidermis.

PAS reaction revealed that numerous fungal hyphae had penetrated the parakeratotic and necrotic lesions (Fig. 3). The hyphae were branched, septate and approximately 1.2–3.7 μm in diameter. Although the hyphae were observed chiefly in the horny layer and the clear layer of the parakeratotic lesion, they occasionally penetrated the necrotized prickle layer. Many polymorphonuclear leucocytes (PMNs) infiltrated the lesions associated with the fungus. Multiple microabscesses also were

observed in the parakeratotic and necrotized lesions, some of which contained the fungus. Keratohyaline granules disappeared in the granular layer of the lesions associated with the fungus.

In hair follicles, numerous hyphae were observed destroying the hairs. Although the hyphae rarely appeared, keratohyaline granules were also observed in the granular layer of hair follicles. In contrast with the parakeratotic and necrotized lesions of the epidermis, no inflammatory responses were observed in the fungal lesions of the hair follicles.

No hyphae had penetrated into the dermal layer, in which there was infiltration by many lymphocytes and macrophages with a few PMNs that had mast cells.

The fungus was isolated from the lesional skin and placed on Sabouraud dextrose agar "Eiken" (SDA) supplemented with 500 μg/m*l* each of penicillin and streptomycin and Mycobiotic agar "Eiken" at 30°C. A typical isolated strain, NJA 9103, was used for all experiments.

When the fungus was incubated on Potato dextrose agar at 25°C, the colony initially appeared white buffered, powdery and downy, and later became pinkish or pale yellow in color. The colony reverse was yellow-brown. Hyphae ranged from 1.2 to 4.0 μm in diameter. The colonies rapidly developed a dense fluff containing many macroconidia. Abundant macroconidia were clavate and thin-walled and measured 35–61 × 7–10 μm, with five or six septa. Other microconidia, measuring 4–6 × 2–3 μm, were also abundant but were round and single-called (Fig. 4). There were many coiled spirals, "spiral bodies" among the hyphae (Fig. 5).

The characteristics mentioned above were similar to those of the species of *Trichophyton mentagrophytes, T. terrestre* and *T. rubrum. T. mentagrophytes* and *T. terrestre* are mainly distinguished by whether the fungi grow at 37°C. When the fungus was incubated on SDA at 30 and 37°C, the isolated strain developed well at both temperatures. Therefore, the fungus was separated from *T. terrestre*. On the other hand, the strain showed rapid growth, and did not produce a red pigment on corn meal glucose agar. The characteristics separated this fungus from *T. rubrum*. The fungus was identified as *Trichophyton mentagrophytes* (Robin) Blanchard 1896.

The sea lion was healthy with a good appetite and normal activity despite severe skin lesions. However, the fact that the hyphae had reached the granular layer indicates the severity of this dermatophytosis.

This aquarium has kept several other kinds of pinnipeds under the same conditions as the Steller sea lion. Similar skin lesions have been found among spotted seals (*Phoca largha*) and South American sea lions (*Otaria flavescens*). Dermatophytosis was only recognized in the latter species. After South American sea lions were introduced, dermatophytosis began to be found among these seals. It indicates the possibility that this species brought the fungus to the facility.

However, no skin lesions were observed in the California sea lions (*Zalophus californianus*). It is possible that some species have a specific agent which may affect the occurrence of the disease. Waldolf [8] mentions that the composition of fatty acids in the Northern fur seal's blubber has a high ratio of short-chained saturated fatty acids, some of which work as anti-fungal agents and the

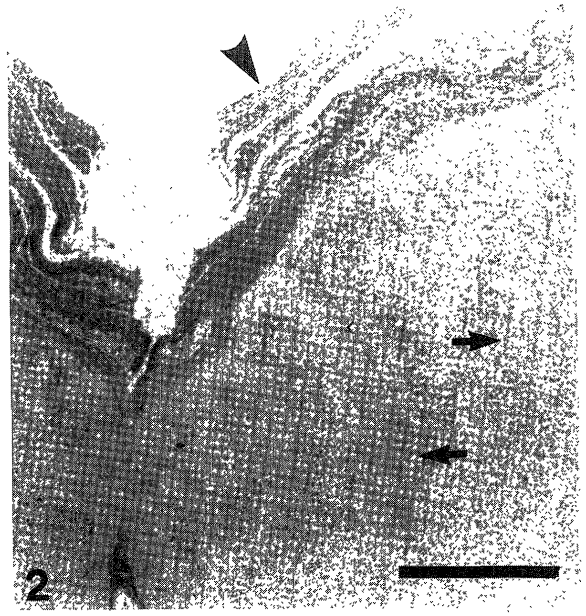

Fig. 2. Irregular hyperplasia of epidermal Malpighian cells (arrows) and parakeratosis (arrow head) in the skin lesion. H&E. Bar = 200 μm.

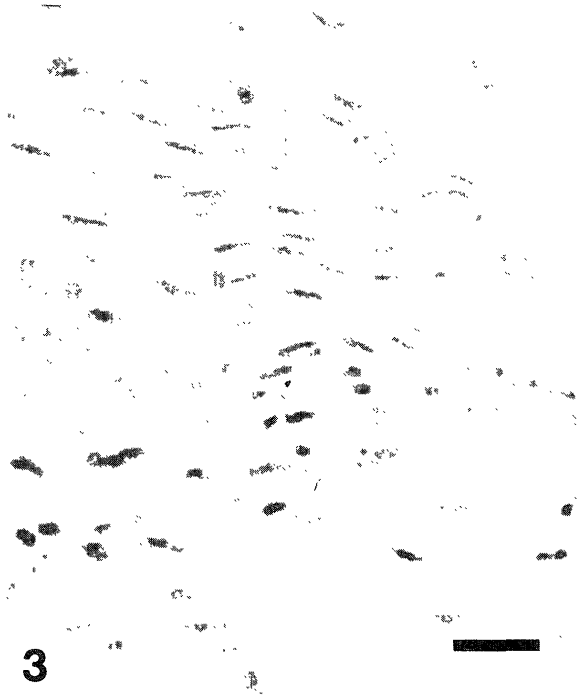

Fig. 3. Numerous fungal hyphae penetrating into the parakeratotic and necrotic skin lesion. PAS reaction. Bar = 10 μm.

Fig. 4. Abundant macroconidia (arrow) and microconidia (arrow head). Cotton blue. Bar = 20 μm.

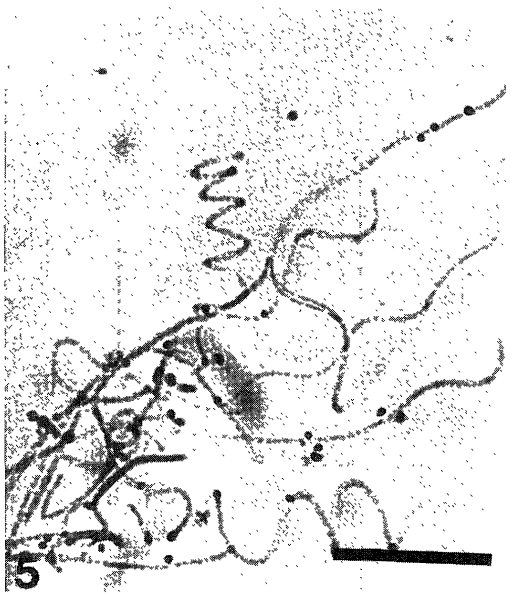

Fig. 5. Coiled spirals among the hyphae. Cotton blue. Bar = 20 μm.

differences between the species fatty acid compounds may affect the species resistance to fungal agents in the skin.

Superficial fungus infection due to *Trichophyton mentagrophytes* or *Candida albicans* is generally limited to the horny layer by a number of anti-fungal substances in serum protein in the deeper layer of the epidermis [4]. Takahashi *et al.* [6] also reported that keratohyaline in the granular layer has an anti-fungal effect. From these findings, it was suggested that the disappearance of keratohyaline and severe necrosis of the prickle layer evident in this case had induced the failure of the subject's self-defence mechanisms, with the result that the fungus isolated, *T. mentagrophytes*, had easily been able to invade a deeper layer.

On the other hand, histopathological findings indicate that anti-fungal substances were more easily maintained in the granular layer of the hair follicles than in the epidermal lesions. This indicates that the fungus could not invade beyond that layer, and thus could not induce the inflammatory cellular infiltration which was thought to be the normal response to foreign organisms invading through a non-specific self-defence barrier such as anti-fungal substances in the epidermis [5].

In addition, among the 55 Steller sea lions held in Japanese facilities, there have been no other occurrences of *T. mentagrophytes* infection. These findings also suggested a possible failure in the functional activity of the subject's self-defence mechanisms.

A further factor is that this sea lion was fed frozen fish which had been defrosted in fresh water, causing a great loss of nutrition than if they had been defrosted in sea water [2]. Food quality also affects the composition of fatty acids in blubber.

Pinniped's skin is kept soft and wet by constant soaking in water, thus increasing the potential for infection. This sea lion was kept in fresh water, where the fungal agents are more likely to survive in the keretin shed from the body surface of the sea lion than they are in sea water. Although many facilities keep pinnipeds in freshwater, their natural habitat is the ocean, and therefore fresh water may cause some stress on their skin. We believe that such environmental factors also might be the cause of diseases.

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