NOTE *Pathology*

Deep Granulomatous Dermatitis of the Fin Caused by *Fusarium solani* in a False Killer Whale (*Pseudorca crassidens*)

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ABSTRACT. A 10-year-old female false killer whale (*Pseudorca crassidens*) developed skin lesions in the left breast fin. Histopathologically, the lesions consisted of multiple granulomas spread diffusely into the deep dermis and bone; characteristically, each granuloma had septate, branching fungal hyphae and chlamydospores surrounded by eosinophilic Splendore-Hoeppli materials. Macrophages, epithelioid cells and multinucleated giant cells in the granulomas reacted mainly to anti-SRA-E5 antibody against human macrophage scavenger receptor type I. *Fusarium solani* was isolated and its gene was detected from the skin samples. Mycotic skin lesions by *Fusarium* spp. reported so far in marine mammals were regarded as superficial dermatitis; therefore, the present case is very uncommon in that the lesions spread deeper into the skin.

KEY WORDS: deep granulomatous dermatitis, false killer whale, fungus, *Fusarium solani*, Splendore-Hoeppli.

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Fusarium spp. including *F. solani* and *F. oxysporum* are common soil saprophytes classified under the order of ascomycota hypocreales; they are important plant pathogens causing diseases such as crown rot, head blight, and scab in cereal grains [12–14]. *Fusarium* spp. possess virulent mycotoxins including trichothecene. The mycotoxins suppress both humoral and cellular immunity and may cause tissue injury [13]. Fusariosis has been reported with an increasing frequency as an opportunistic infection in humans and animals such as turtles, sharks, dolphins and pinnipeds [2, 5, 7, 11].

In humans, fusariosis tends to develop in patients who are immunocompromised due to HIV infection, leukemia or organ transplantation; the disseminated infection shows refractory fever, skin lesions at infected sites and sinopulmonary lesions. Fusarial skin lesions occur at any site, particularly with predominance in the extremities. The skin gross lesions are recognized as nodule, ulcer, mycetoma, and intertrigo; histologically, the lesions consist of necrosis, panniculitis or granuloma [6, 13]. In the lesions, hyaline acute-branching septate hyphae are seen, invading the dermis and occasionally extending into the blood vessels with thrombosis [4]. Recently, *Fusarium* keratitis associated with contamination of contact lens solution has been reported [3].

In marine mammals, *Fusarium* spp. are most likely opportunistic pathogens of the skin. Animals with compromised immune systems presumably due to stress or illness are most susceptible [7, 8]. Damage to the integument due to excessive chlorination of the water and high fluctuating pool temperatures may play important roles in skin mycosis of marine mammals [11]. *Fusarium*-induced dermatitis has been reported in a group of captive California sea lions, Atlantic white sided dolphins, harbor seals and a pygmy sperm whale [7, 11]; all cases were diagnosed as superficial dermatitis and the lesions developed as raised, firm, erythematous plaques or nodules in the face, trunk, and flippers. Hence, we describe pathological characteristics of deep granulomatous dermatitis with unique Splendore-Hoeppli phenomenon caused by *F. solani* in a false killer whale.

A 10-year-old female false killer whale (*Pseudorca crassidens*), that was captured and kept in an aquarium, developed dermatitis in the left breast fin and the skin lesions have gradually spread. Biopsies of skin lesions were performed by scrapings, and histopathologically, the lesion was diagnosed as granulomatous dermatitis caused by fungal infection. Two months later, the dolphin died suddenly. At necropsy, the skin lesions were grayish-red, raised nodules with occasional ulcers; on the cut surface, the lesions spread diffusely into the deep dermis (Fig. 1). Besides the skin lesions, severe accumulation of foamy fluid in the trachea and lung, ulcers in the esophagus and rumen, injured head skin and cerebral subdural hemor-

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rhage were observed. The tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections of 4 μm thick were cut and stained with hematoxylin and eosin (HE). Selected sections were also stained with periodic acid-Schiff (PAS) reaction and Grocott's methenamine silver nitrate stain (GMS). For immunohistochemistry, sections from skin lesions and ulcerated rumen lesions were incubated with a rabbit polyclonal antibody against *Candida* spp. or *Aspergillus* spp. (provided by Dr. M. Kubo, NIAH, Ibaraki, Japan, 1:100), mouse SRA-E5 (Trans Genic Inc., Kumamoto, Japan, 1:200) against human macrophage scavenger receptor type I protein, mouse AM-3K (Trans Genic Inc., 1:100) against human macrophage hemoglobin/haptoglobin complex receptor, rabbit anti-CD3 (Dako Corp., Glostrup, Denmark, 1:200), rabbit anti-CD20 (Thermo Fisher Scientific Inc., Fremont, CA, U.S.A., ready-to-use) and mouse anti-α-smooth muscle actin (α-SMA; Dako, 1:1,000) for 16 hr at 4°C. Bound antibodies were detected with horse radish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibody (Histofine Simplestain MAX-PO; Nichirei, Tokyo, Japan) and 3,3'-diaminobenzidine tetrahydrochroride (DAB) (Vector Laboratories Inc., Burlingame, CA, U.S.A.) as chromogen.

Histopathologically, there were multiple granulomas in the fin lesions, and the granulomatous lesions expanded diffusely into the deep dermis (Fig. 2), partly bone tissues (Fig. 2; insert). Each granuloma consisted of fungal hyphae at the center surrounded by neutrophils, macrophages, epithelioid cells, multinucleated giant cells, lymphocytes and plasma cells; the fibrous connective tissues consisting of α-SMA-positive myofibroblasts enclosed and separated each of granuloma. Fragmented fungal hyphae were also observed within the cytoplasm of macrophages and multinucleated giant cells. Neither invasion of fungal hyphae into the blood vessels nor thrombus was found.

All fungal hyphae were septate, branching fungal hyphae irregular in diameter and thick-walled large spherical spores (chlamydospores) were also observed; the organisms were surrounded by intensely eosinophilic amorphous materials that often showed a clubbed or radiating appearance known as Splendore-Hoeppli phenomenon (Fig. 3). The hyphae were basophilic or amphophilic in HE-stained sections (Fig. 3), and stained red with PAS reaction (Fig. 4a) and brown-black with GMS stain (Fig. 4b). The Splendore-Hoeppli materials were intensely stained red with PAS reaction, but not by GMS stain. The hyphae were negative for antibodies against *Aspergillus* spp. and *Candida* spp. Immunohistochemically, main components of granulomas were SRA-E5 positive macrophages, epitheioid cells, multinucleated giant cells (Fig. 5a) and CD20 positive B lymphocytes (Fig. 6a). Fewer AM-3K positive macrophages (Fig. 5b) and CD3 positive T lymphocytes (Fig. 6b) were also observed. The multinucleated giant cells were negative for AM-3K (Fig. 5b). Esophagus and rumen developed erosion or mild ulcerative lesions, and fungal hyphae with slight infiltration of neutrophils were observed confined to the squamous epithelium. The hyphae were recognized by GMS stain and PAS reaction, and were immunopositive for *Candida* spp. and negative for *Aspergillus* spp. In the cerebrum, subdural and subpial hemorrhage corresponding to the gross finding and glial reactions in surrounding tissues were observed. Histological findings seen in other organs included severe pulmonary congestive edema and lymphopenia in the lymph nodes and spleen. *F. solani* were not detected in any visceral organs.

Samples of the kidney, spleen, heart, pancreas, lymph node, cerebrum, cerebellum and skin lesions were collected for fungal culture. These samples were inoculated onto potato dextrose agar (PDA, Difco, Detroit, MO, U.S.A.) plates at 35°C for 14 days. White floccose colonies were sprouted from the fin samples. Colonies on Sabouraud dextrose agar (SDA) containing 2% dextrose (Wako Chemical Co., Ltd., Osaka, Japan), 1% neopeptone (Difco) and 1.5% Bacto agar (Difco) and PDA showed white floccose colonies with pale red pigmentation into the media. Curved macroconidia consisted of 4 to 6 cells and chlamydospores were detected in the micro culture on PDA. DNA was extracted from the isolates and tissue samples. The internal transcribed spacer (ITS) 1-5.8S-ITS2 regions of ribosomal RNA gene sequences (ITS gene) consisted of 549 base pairs from both isolates obtained from the fin samples were 100% identical to the GenBank accession No. FN598930 derived from *F. solani* by BLAST (Basic Local Alignment Search Tool) searches. As a result, *F. solani* was identified only from the cultures of skin lesions. In addition, a partial sequence of ITS gene consisted of 226 base pairs obtained from a tissue sample by a nested PCR also showed 100% identity to No. FN598930.

Based on the pathological findings (characterized by multiple granulomas spread diffusely into the deep dermis and bones), as well as mycological studies and molecular analyses, the present skin lesion was diagnosed as deep granulomatous dermatitis caused by *F. solani*. *Fusarium*-induced dermatitis in marine mammals has been reported to be confined to the epidermis and sub-epidermal area (diagnosed as superficial dermatitis) [7, 11]. Interestingly, the present granulomas often involved Splendore-Hoeppli phenomenon around the fungal hyphae. The phenomenon is characterized by the presence of radiate, star-like, asteroid or club-shaped intensely eosinophilic materials around infectious or non-infectious agents [9]. Although the exact pathogenesis of the Splendore-Hoeppli phenomenon is not well understood, it has been thought to represent deposition of antigen-antibody complexes and cellular debris from inflammatory cells such as epithelioid cells and multinucleated giant cells. Development of Splendore-Hoeppli phenomenon has been reported in the occasional cases of fungal infections such as sporotrichosis, blastomycosis, zygomycosis, candidiasis and aspergillosis in humans and animals [9]. In a human fusariosis patient, the phenomenon was reported in association with mycetoma due to *F. moniliforme* [1]. To the best of our knowledge, the present case is the first report of *Fusarium* spp. infection in marine mammals accompanied with the Splendore-Hoeppli phenomenon.

The present study revealed that granulomas due to *F. solani* infection were composed mainly of SRA-E5-positive

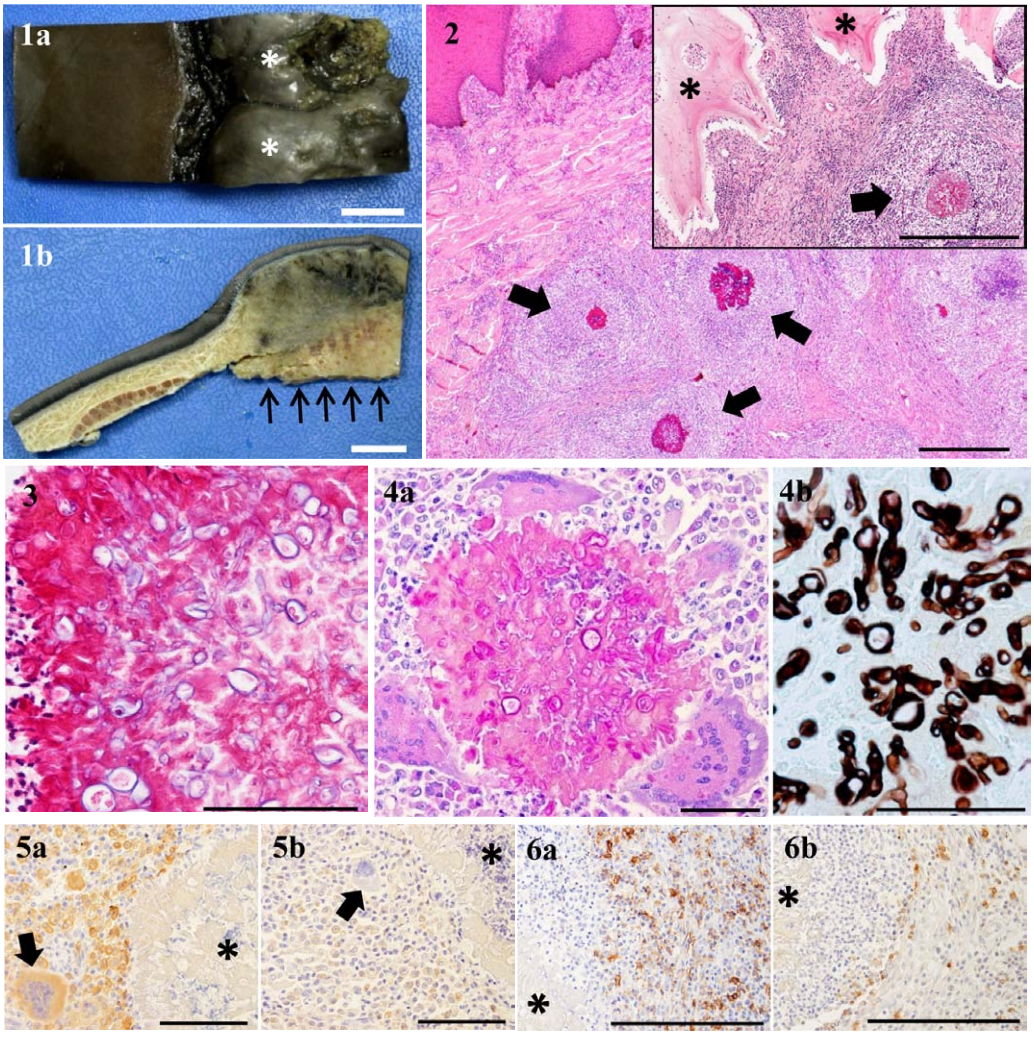

Fig. 1. Raised, nodular lesions (asterisks) with ulcer in the skin of the fin (1a). On the cut surface (1b), the lesions spread diffusely into the deep dermis (arrows). Bar=1 cm.

Fig. 2. Multiple granulomas (arrows) around clumps of fungal hyphae in the fin. Insert: the lesions spread diffusely into the deep skin, partly bone tissue (asterisks). HE. Bar=500 μm.

Fig. 3. The fungal hyphae are surrounded by intensely eosinophilic amorphous materials that sometimes show a clubbed or radiating appearance known as Splendore-Hoeppli phenomenon. HE. Bar=100 μm.

Fig. 4. The fungal hyphae are stained red with PAS reaction (4a) and brown-black with GMS (4b). Septate, branching fungal hyphae irregular in diameter and thick-walled large spherical spores (chlamydospores) are observed (4a and 4b). Bar=50 μm.

Fig. 5. Numerous SRA-E5-immunopositive macrophages, epitheliod cells and multinucleated giant cells (arrow) are seen in the granuloma (5a). Fewer AM-3K positive macrophages (5b) are also observed. Asterisks: the clumps of fungal hyphae. Bar=100 μm.

Fig. 6. In the granulomatous lesions, numerous CD20 positive lymphocytes (6a) and fewer CD3 positive lymphocytes (6b) are observed. Bar=200 μm.

macrophages, epithelioid cells and multinucleated giant cells; in contrast, AM-3K positive macrophages were very small in number. Similar immunohistochemical reactivities have been reported in human granulomatous diseases such as tuberculosis, sarcoidosis and foreign body reactions [10]. It is interesting to investigate different functions of macrophage populations participating in the granuloma formation, because macrophages are responsible for induction of T cells or B cells through antigen presentation in both cellular and humoral immunities.

The cerebral hemorrhage might have been due to accidental bruise on the head; judging from severe pulmonary congestive edema, under this condition the dolphin might have aspirated sea water and presumably died in a drown-

ing accident. Lymphopenia in the lymph nodes and spleen suggested decreased immunocompetence of the dolphin. Although the cause of immunosuppression of the present case was not determined, deeply spread mycotic granulomatous dermatitis caused by *F. solani* and candidiasis in the esophagus and rumen might have been related to decreased immunocompetence.

In conclusion, this is the first report of deep granulomatous dermatitis caused by *F. solani* in a False Killer Whale with a characteristic finding of Splendore-Hoeppli phenomenon.

REFERENCES

1. Ajello, L., Padhye, A. A., Chandler, F. W., McGinnis, M. R., Morganti, L. and Alberici, F. 1985. *Fusarium moniliforme*, a new mycetoma agent. Restudy of a European case. *Eur. J. Epidemiol.* **1**: 5–10. [Medline] [CrossRef]
2. Cabañes, F. J., Alonso, J. M., Castellá, G., Alegre, F., Domingo, M. and Pont, S. 1997. Cutaneous hyalohyphomycosis caused by *Fusarium solani* in a loggerhead sea turtle (*Caretta carreta* L.). *J. Clin. Microbiol.* **35**: 3343–3345. [Medline]
3. Chang, D. C., Grant, G. B., O'Donnell, K., Wannemuehler, K. A., Noble-Wang, J., Rao, C. Y., Jacobson, L. M., Crowell, C. S., Sneed, R. S., Lewis, F. M., Schaffzin, J. K., Kainer, M. A., Genese, C. A., Alfonso, E. C., Jones, D. B., Srinivasan, A., Fridkin, S. K. and Park, B. J. 2006. Multistate outbreak of *Fusarium* keratitis associated with use of a contact lens solution. *JAMA* **296**: 953–963. [Medline] [CrossRef]
4. Cocuroccia, B., Gaido, J., Gubinelli, E., Annessi, G. and Girolomoni, G. 2003. Localized cutaneous hyalohyphomycosis caused by a *Fusarium* species infection in a renal transplant patient. *J. Clin. Microbiol.* **41**: 905–907. [Medline] [CrossRef]
5. Crow, G. L., Brock, J. A. and Kaiser, S. 1995. *Fusarium solani* fungal infection of the lateral line canal system in captive scalloped hammerhead sharks (*Sphyrna lewini*) in Hawaii. *J. Wildl. Dis.* **31**: 562–565. [Medline]
6. Dignani, M. C. and Anaissie, E. 2004. Human fusariosis. *Clin. Microbiol. Infect.* **10**: 67–75. [Medline] [CrossRef]
7. Frasca, S. Jr., Dunn, J. L., Cooke, J. C. and Buck, J. D. 1996. Mycotic dermatitis in an Atlantic white-sided dolphin, a pygmy sperm whale, and two harbor seals. *J. Am. Vet. Med. Assoc.* **208**: 727–729. [Medline]
8. Higgins, R. 2000. Bacteria and fungi of marine mammals: a review. *Can. Vet. J.* **41**: 105–116. [Medline]
9. Hussein, M. R. 2008. Mucocutaneous Splendore-Hoeppli phenomenon. *J. Cutan. Pathol.* **35**: 979–988. [Medline] [CrossRef]
10. Komohara, Y., Hirahara, J., Horikawa, T., Kawamura, K., Kiyota, E., Sakashita, N., Araki, N. and Takeya, M. 2006. AM-3K, an anti-macrophage antibody, recognizes CD163, a molecule associated with an anti-inflammatory macrophage phenotype. *J. Histochem. Cytochem.* **54**: 763–771. [Medline] [CrossRef]
11. Montali, R. J., Bush, M., Strandberg, J. D., Janssen, D. L., Boness, D. J. and Whitla, J. C. 1981. Cyclic dermatitis associated with *Fusarium* sp infection in pinnipeds. *J. Am. Vet. Med. Assoc.* **179**: 1198–1202. [Medline]
12. Nelson, P. E., Dignani, M. C. and Anaissie, E. J. 1994. Taxonomy, biology, and clinical aspects of *Fusarium* species. *Clin. Microbiol. Rev.* **7**: 479–504. [Medline]
13. Nucci, M. and Anaissie, E. 2007. *Fusarium* infections in immunocompromised patients. *Clin. Microbiol. Rev.* **20**: 695–704. [Medline] [CrossRef]
14. Zhang, N., O'Donnell, K., Sutton, D. A., Nalim, F. A., Summerbell, R. C., Padhye, A. A. and Geiser, D. M. 2006. Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. *J. Clin. Microbiol.* **44**: 2186–2190. [Medline] [CrossRef]