Reprinted from the JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION, Vol. 179, No. 11, Pages 1198-1202.

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Cyclic Dermatitis Associated with *Fusarium* sp Infection in Pinnipeds

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SUMMARY

Dermatitis associated with *Fusarium* sp infection developed in 3 California sea lions *(Zalophus californianus)* and 3 gray seals *(Halichoerus grypus)* at the National Zoological Park in Washington, DC. The lesions were papular or nodular and were distributed mainly on the face, trunk, and flippers. One sea lion died 6 weeks after extensive cutaneous involvement. The lesions regressed after 1 mild exacerbation in the other 2 sea lions. In the gray seals, the skin condition appeared to worsen during the summer and to regress during the winter, despite oral and topical treatment with miconazole and thiabendazole.

Fusarium sp was repeatedly isolated from biopsy specimens of lesions. Hyperplasia of epidermal and follicular epithelium was associated with acute and chronic inflammation and fungal hyphae. The species of the fungus in 1 of the gray seals was determined to be *F solani,* a type occasionally associated with keratitis and opportunistic infections in human beings.

Initial excessive chlorination and high fluctuating pool temperatures attributed to a faulty water treatment system were considered as factors in promoting fungal growth.

CUTANEOUS DISEASES apparently are common in pinnipeds, but their causes are not always known. Some specific conditions include sealpox, affecting California sea lions *(Zalophus californianus),* South American sea lions *(Otaria byronia),* and harbor seals *(Phoca vitulina),*[1] and dermatophilosis in South American sea lions.[2] Both of these diseases produce nodular lesions. Other agents incriminated include *Corynebacterium phocae,* reported in gray seals in Britain,[3] and demodicosis[4] and calici virus infection in California sea lions.[5] To our knowledge, specific cutaneous diseases caused by fungi have not been reported.

The purpose of this report is to describe the clinical and pathologic features of dermatitis in California sea lions and gray seals associated with *Fusarium* sp infection.

History

The skin disease affected 2 different species of pinnipeds that were exhibited at the National Zoological Park at 2 different times. First involved were 3 of 4 adult California sea lions, including a 5-year-old male (No. 1), a 5-year old female (No. 2), and a 14-year-old female (No. 3). The animals were acquired from a sea lion exchange in 1969 and were exhibited in a pool containing chlorinated fresh city water that was changed weekly. They were fed a variety of whole frozen fish, including butterfish, trout, and herring, and the diet was supplemented with a salt and vitamin preparation.[a] They remained healthy until late summer of 1974, when circular eruptions were noticed over the trunk of sea lion 3. The lesions were firm papules that eventually became alopecic. Keratotic plugs could be expressed from what appeared to be cystic hair follicles. The lesions spread over the entire body of sea lion 3. There was less involvement of sea lion 1, with lesions affecting only the neck and flipper regions. Sea lion 2 had only a few scattered lesions on the trunk. The 4th sea lion had died 1 year earlier of trauma; skin lesions in this animal were never recorded.

Lesions from 2 of the affected animals (sea lion 1 and sea lion 3) were cultured and biopsied as follows: The sea lions were placed in a restraining squeeze cage. A wedge-shaped biopsy specimen was taken after local anesthesia with 2% lidocaine. The biopsied tissue was subdivided and portions were placed in brain-heart infusion medium[b] and on media for fungal isolation.[c,d] Fresh smears of some of the portions of the tissue were prepared with

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The authors thank Ms. M. Serre, Bethesda Naval Hospital, and Dr. Kwon-Chung, National Institute of Allergy and Infectious Diseases, for assistance with fungal identification, and Dr. C. Booth, Commonwealth Mycological Institute, Surrey, England, for speciation. We also thank Dr. W. Kaplan, Center for Disease Control, Atlanta, Ga, for performing fluorescent antibody evaluations, and aquatic vertebrate keepers, National Zoological Park, for data on temperature and chlorine concentrations in the pools.

[a]Sea-tabs, Pacific Research, El Cajon, Calif.
[b]Bacto-brain heart infusion, Difco Laboratories, Detroit, Mich.
[c]Sabouraud dextrose agar, Difco Laboratories, Detroit, Mich.
[d]Mycosel agar, Bioquest, Cockeysville, Md.

Fig 1—Lesions on the face of gray seal 1 are confluent and depigmented.

Fig 2—Lesions on the flipper also involve nail beds (gray seal 2).

Giemsa and acid-fast stains, and the remaining portions were placed in buffered 10% formalin for histologic evaluation. Scrapings of normal skin were made from sea lion 2 and were stained with Giemsa. Two of these sea lions were treated with griseofulvin at the rate of 1,500 mg daily for 6 weeks, and tetracycline at the rate of 3 g daily for 2 weeks. Results of this treatment were equivocal. Sea lion 3 died 6 weeks after the onset of the skin condition and was necropsied. The skin lesions eventually regressed in sea lion 1 and sea lion 2 and were healed approximately 3 months after the onset. A few lesions were reported in sea lion 1 during the spring of 1975. After that, both animals were sent to another zoo in the Midwest, where sea lion 2 died of "pneumonia" in March of 1977, and sea lion 1 died of "unknown causes" in April of 1979. There was no report of any skin disorders at the time of death.

In 1979, the aquatic exhibit was replaced by 2 large pools with a water treatment system designed to chlorinate, filter, and cool recirculated fresh water. Five California sea lions acquired from a marineland in California have been kept in 1 pool since January 1979 and they have remained healthy. Three gray seals, including a 6-year-old male (No. 1), a 5-year-old male (No. 2), and a 6-year-old female (No. 3) that eventually were affected with skin lesions, were put in another pool at the same time. The chlorine content for the 1st 1.5 years ranged between 1 and 2 ppm and thereafter was kept between 0.4-0.5 ppm. Water temperature was allowed to fluctuate with the ambient temperature throughout the winter, but during the summer, attempts were made to maintain it at 70 F (21.1 C). Because of a faulty chiller, however, in 1979 and 1980 the pool temperature frequently reached 80 F (26.6 C) or more. The gray seals were acquired from an aquatic mammal center in California, and upon their arrival, they were closely monitored for parasites by repeated fecal examination, the results of which were always negative. Hemograms were considered to be within normal limits, and although there was never any evidence of dirofilariasis, all seals were given diethylcarbamazine[e] prophylactically (5 mg/kg, daily) during the mosquito season. They were fed a variety of frozen fish, including herring, mackerel, butterfish, and smelt at the rate of from 6% to 10% of their body weight daily. The diet was supplemented with thiamin, vitamin E, and sodium chloride.

In June 1979, gray seal 1 was noticed to have raised circular lesions on the head, face, and flippers. One month later, the lesions had become confluent on the face (Fig 1) and some were spreading over the back and trunk. Similar lesions

[e]Nemacide, Med-Tech Inc, Elwood, Kan.

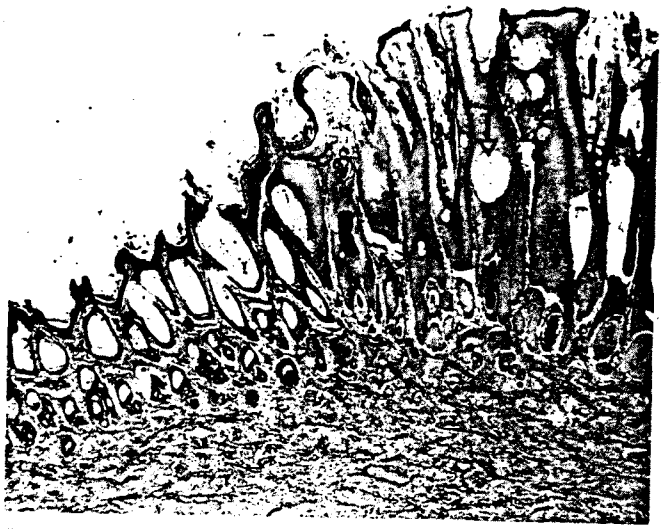

Fig 3—Photomicrograph of skin lesion from sea lion 3, with epidermal and follicular hyperplasia and intraepithelial abscesses (arrow). H&E stain; ×12.

developed in gray seal 2, but these were predominantly over the trunk and flippers (Fig 2). Gray seal 3 had only a few lesions on the body, and they never became progressive.

Between 1979 and 1981, the lesions appeared to regress during the winter and to recur during the summer. During that time, biopsies and cultures were performed 5 times from gray seal 1 and 3 times from gray seal 2. Several regimens of treatment were instituted (to be discussed subsequently). The methods for biopsy and culture were similar to those used for the California sea lions, with 1 exception: During the last biopsy procedure for sea lion 1, two skin segments were taken, 1 from a site that was cleansed with a surgical scrub and 70% ethyl alcohol; the other site, as in previous biopsies, was not surgically prepared. Fragments of tissues from both surgically prepared and unprepared sites were aseptically placed on fungal media[c,d] and sent to the Bethesda Naval Hospital, the Laboratory of Clinical Investigation at the National Institute of Allergy and Infectious Diseases, the National Institute of Health, as well as to our own Clinical Pathology Laboratory at the National Zoo. Tissue was also fixed in buffered 10% formalin for routine histologic preparation, as well as to provide paraffin sections that were sent to the Mycology Unit at the Center for Disease Control, Atlanta, Ga, for fluorescent antibody screening for various fungi. Biopsy tissue was also fixed in a glutaraldehyde-formalin fixative for processing for electron microscopy to screen for poxvirus and other pathogens.

Results

Cultures—Skin lesions from sea lion 1 and sea lion 3 contained *Fusarium* sp, as well as *Proteus* sp and several other types of enteric organisms that were not considered pathogens. *Fusarium* sp was also isolated repeatedly from the biopsied lesions from gray seal 1 and gray seal 2. The organism was

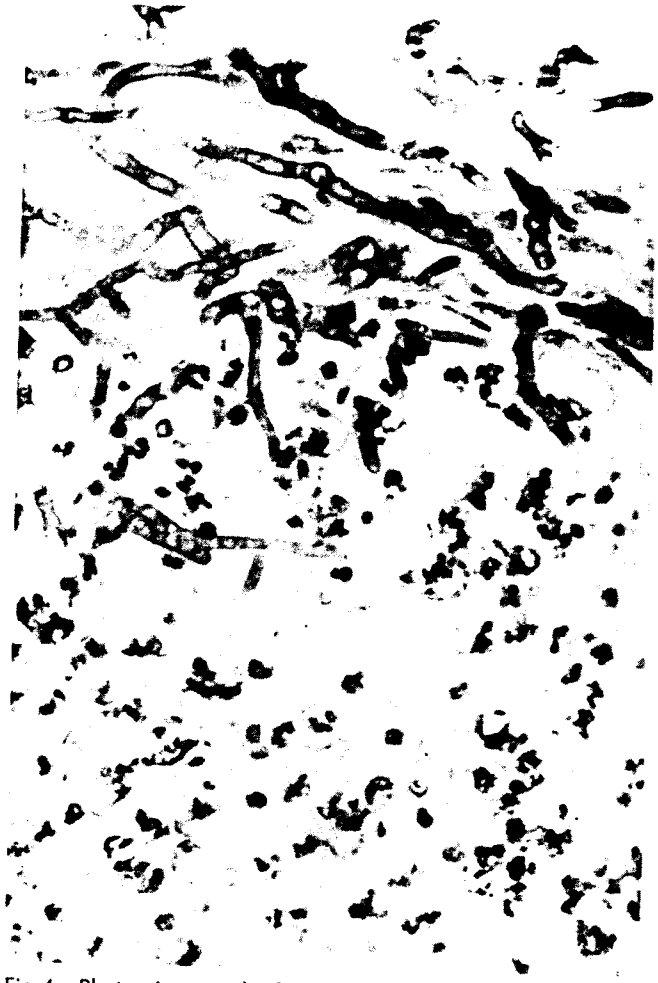

Fig 4—Photomicrograph of specimen from near intraepithelial abscess from gray seal 1 shows *Fusarium* hyphae surrounded by neutrophils. Periodic acid-Schiff stain; ×150.

isolated in pure culture from the biopsy site that was surgically prepared, whereas the sites that were not prepared also contained a variety of bacteria, including a coagulase-positive *Staphylococcus* sp (once), noncoagulase-positive staphylococci, *Serratia marcescens,* and *Proteus vulgaris*. Saprophytic fungi, including *Penicillium* sp, *Cladosporium* sp, and *Candida lipolytica,* were inconsistently recovered on several occasions from the unprepared sites. The *Fusarium* was determined to be *F solani.*

Pathology—Histologic examination of the skin lesions from both the sea lions and gray seals revealed acanthosis, with marked hyperkeratosis of both surface and follicular epithelium and distention of hair follicles with degenerate keratotic material (Fig 3). In many areas there were intraepithelial abscesses and a moderate acute-to-subacute inflammatory reaction in the dermis (Fig 3). There were septate branching hyaline fungal hyphae within the outer layers of the hyperplastic epithelium of both the epidermis and distended hair follicles. The hyphae varied from 2μm to 5μm in width and generally had parallel sides. Numerous hyphal elements were also free within the keratotic material in hair follicles. Occasionally, hyphae were associated with the intraepithelial abscesses (Fig 4),

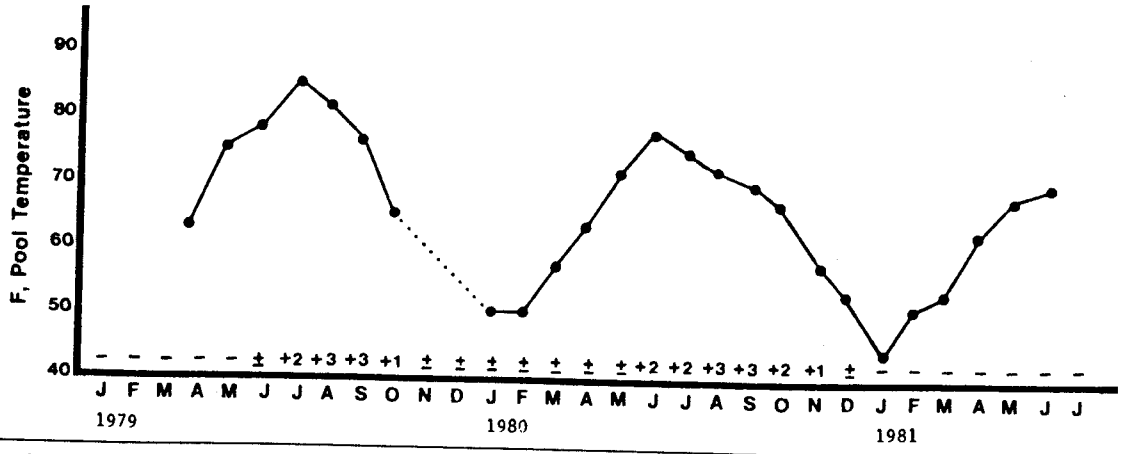

Fig 5—Graph correlating the severity of cutaneous *Fusarium* infection in gray seal 1 with pool temperature recorded over 3 summer seasons. . . . = temperature not recorded; +3 = skin lesions, marked; +2 = skin lesions, moderate; +1 = skin lesions, mild; ± = few scattered lesions; − = normal.

and rarely a hyphal fragment could be seen in a dermal peg adjacent to an inflamed follicle. The fungi were not always plentiful, but they could be readily identified with periodic acid-Schiff or Gomori's methenamine silver stains. Examination of gram-stained biopsied sections revealed a few gram-negative and gram-positive organisms on the surface of the lesions, but bacteria were not evident within the lesions or associated with the inflammatory reaction. Stains for acid-fast bacteria were also negative. Scrapings of normal regions on the skin of sea lion 2 were negative for fungi, in contrast to the numerous hyphae that were evident in the scrapings from the lesions in sea lion 3.

Candida and *Aspergillus* spp, and phycomycetes were eliminated as possible etiologic agents by examination of paraffin sections of the biopsied sites, using fluorescent antibody techniques.

Ultrastructural studies were negative for pox or other viruses.

Although the cause of death could not be determined for sea lion 3, the necropsy showed no evidence of any systemic involvement with any fungal species.

Treatment—Gray Seals—On the basis of the fungal nature of the lesions, several regimens of antifungal treatment were instituted in the gray seals. Right after the 1st onset of the disease (June 1979), gray seal 1 and gray seal 2 were treated with 1.5 g of miconazole given in fish 3 times a day for several 3-week periods, but with little or no improvement. During the next cycle the following summer, a solution made up of 500 mg of miconazole for IV use plus 100 ml of dimethyl sulfoxide plus water QS 480 ml was applied topically to the seals that were denied immediate access to the pool. There was little or no improvement with two 2-week periods of this treatment. Finally, thiabendazole (40 mg/kg, OD) was given for several 10-day periods, along with daily topical applications of a mixture of 1 part suspension of 108.6 g of thiabendazole powder suspended in 1 L of water to 2 parts of propylene glycol.

Discussion

The repeatedly positive cultures of the lesions for *Fusarium* sp, along with evidence of tissue invasion with mycelial elements having the configuration of *Fusarium* sp, indicate that this fungus was acting as a pathogen in the California sea lions and gray seals. Although the lesions grossly resembled those reported for sealpox and dermatophilosis, none of the histologic hallmarks of either of these conditions was seen in any of our cases. Fluorescent antibody studies tended to rule out *Aspergillus* and *Candida* spp, which are more commonly encountered as opportunistic pathogens in animals.

Fusarium spp are usually considered to be saprophytes, but some species are reported to be pathogenic in man and other animals. *Fusarium solani,* for example, occasionally causes mycotic keratitis in human beings,[6] and it and other species have been associated with infections in diabetics, cancer patients taking cytotoxic drugs, and burn patients.[7] In animals, *Fusarium* spp have been reported mostly as causes of infections of the eyes and integument of reptiles.[8] Inasmuch as cutaneous infections with a *Fusarium* sp had not been previously reported as a specific entity in pinnipeds, the disease in our pinnipeds may have been associated with some factor related to captivity. There was no morphologic evidence of immunodeficiency, at least not in the California sea lion that died with severe cutaneous involvement. The lesions appeared to be refractory to topical as well as systemic antifungal treatment, and the regression of the lesions that did take place seemed to be seasonal and probably was not related to the therapy. The lesions seemed to be at their worst during the hot summer months and resolved mostly by late winter. These impressions are based on observations of the gray seals over a 2.5-year period. Prior to their arrival, the gray seals had access to pools supplied with fresh ocean water. Transition of pinnipeds from salt water to chlorinated fresh water is commonly done, and usually the animals adapt well without serious problems.[9] Ideal water conditions for pinnipeds are 68–70 F

(20–21.1 C) at approximately 0.5 ppm of chlorine. The coliform count for marine mammals should be less than 1,000 colonies/100 ml of water.[10] Due to some difficulty in regulating the new water treatment plant for our facility, chlorination in our gray seal pool for the first 1.5 years ranged between 1 and 2 ppm and was adjusted to between 0.4 and 0.5 ppm thereafter. During these higher levels of chlorination, coliform counts were zero, whereas at the lower values, they were usually less than 100 colonies/100 ml of water. There was also difficulty with the water chiller, and it is quite possible that the fluctuation (Fig 5) in the water temperature may have promoted fungal growth. Generally, pinnipeds are relatively resistant to superficial mycoses, particularly the dermatophytes. Some of this resistance in the northern fur seal *(Callorhinus ursinus)* has been attributed to fatty acid secretions, which apparently act as natural barriers to fungal penetration.[11] Focal dermatitis with a seasonal prevalence has been reported in California sea lions, with histologic features similar to ours, but the cause was unknown. It also appears to be a self-remitting process.[4]

It is possible, then, that the initial high chlorine content may have altered sufficiently the bacterial flora and that, coupled with the increased pool temperatures, may have provided the proper setting for fungal invasion, probably through small skin abrasions that commonly occur in captive pinnipeds.

Although subjected to similar conditions in another pool, the new group of California sea lions never developed any serious skin problems, suggesting that the gray seals may have been more sensitive to these environmental alterations.

As of this writing (early August 1981), pool temperatures have stayed closer to the recommended ranges and only minor exacerbations of the skin lesions have developed.

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