Lobomycosis in Atlantic bottlenose dolphins from the Indian River Lagoon, Florida

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The natural habitat of the organism is believed to be aquatic or associated with soil and vegetation, and recent characterization of ribosomal DNA from a dolphin lesion supports the hypothesis that *L. loboi* is a fungus. However, *L. loboi* cells infecting dolphin tissues are smaller than those infecting human tissues, suggesting that strains of the organism may differ.

Although *L. loboi* has been experimentally transmitted to armadillos, tortoises, mice, and hamsters, dolphins and humans are the only species known to be naturally susceptible to infection. Lobomycosis has been identified in bottlenose dolphins (*Tursiops truncatus*) and a marine-riverine species of dolphin (*Sotalia fluviatilis*). In humans, lobomycosis, sometimes referred to as keloidal blastomycosis, is characterized by the appearance of cutaneous nodules that grow slowly over many years. These cutaneous nodules may be smooth, ulcerated, verrucous, or plaquelike and develop on exposed areas of the body, particularly the extremities, ears, face, and buttocks. Lesions increase in size and distribution over time and may spread locally and by autoinoculation.

Human lobomycosis is endemic in tropical portions of Central and South America, especially in remote areas and in communities along rivers in the Amazon and Orinoco basins. The organism appears to be introduced at the site of local trauma from insect bites, animal bites, cuts, or abrasions. Occupational exposure to water, soil, and vegetation is thought to be responsible for cases involving rubber workers, miners, farmers, and fishermen. In industrialized countries, human lobomycosis has been documented only 5 times and has been identified only in persons who have had a history of travel to areas in which the organism is endemic.

Lobomycosis was first identified in bottlenose dolphins from the Gulf and Atlantic coasts of Florida in the early 1970s and was subsequently identified in dolphins from the Suriname River estuary, Spanish-French coast, South Brazilian coast, and Texas coast of the Gulf of Mexico. Dermal lesions in dolphins are

**Objective**—To determine the prevalence of lobomycosis, a mycotic infection of dolphins and humans caused by a yeastlike organism (*Lacazia loboi*), among dolphins in the Indian River Lagoon in Florida.

**Design**—Cross-sectional study.

**Animals**—146 Atlantic bottlenose dolphins.

**Procedure**—Comprehensive health assessments of bottlenose dolphins in the Indian River Lagoon of Florida (*n = 78*) and in estuarine waters near Charleston, SC (*n = 71*), were conducted during 2003 and 2004. Bottlenose dolphins were captured, examined, and released. Skin lesions were photographed and then biopsied. Tissue sections were stained with H&E and Gomori methenamine silver stains for identification of *L. loboi*.

**Results**—9 of 30 (30%) dolphins captured in the southern portion of the Indian River Lagoon had lobomycosis, whereas none of the 45 dolphins captured in the northern portion of the lagoon or of the 71 dolphins captured near Charleston, SC, did. Affected dolphins had low serum alkaline phosphatase activities and high acute-phase protein concentrations.

**Conclusions and Clinical Relevance**—Results suggest that lobomycosis may be occurring in epidemic proportions among dolphins in the Indian River Lagoon. Localization of the disease to the southern portion of the lagoon, an area characterized by freshwater intrusion and lower salinity, suggests that exposure to environmental stressors may be contributing to the high prevalence of the disease, but specific factors are unknown. Because only dolphins and humans are naturally susceptible to infection, dolphins may represent a sentinel species for an emerging infectious disease. (J Am Vet Med Assoc 2006;228:104–108)
similar to those described in humans but may be more extensive, covering large areas of the body. Sites of predilection include the dorsal fin, flukes, head, and peduncle. Lesions may be associated with sites of apparent previous trauma, such as shark bite scars.

Most reports of lobomycosis in dolphins describe single cases. However, a recent review of pathologic findings in 17 dolphins that had stranded and died in the Indian River Lagoon of Florida between 2001 and 2002 reported that 3 had cutaneous lobomycosis. In addition, examination of photographs taken of dolphins in the Indian River Lagoon between 1996 and 2003 suggests that there may be a high prevalence of dermal lesions consistent with lobomycosis in this population.

These observations suggest that lobomycosis may be more common among dolphins in the Indian River Lagoon ecosystem than previously thought. However, previous studies involved only low numbers of dolphins or identified lesions visually or from photographs, rather than on the basis of histologic examination of biopsies specimens. The purpose of the study reported here, therefore, was to determine the prevalence of lobomycosis among bottlenose dolphins in the Indian River Lagoon ecosystem.

Materials and Methods

Study site—The Indian River Lagoon is a unique shallow-water ecosystem that comprises 40% of Florida's central east coast. The lagoon is an aggregate of 3 estuarine water bodies—the Indian River, Banana River, and Mosquito Lagoon—and extends 250 km from Ponce De Leon Inlet in the north to Jupiter Inlet in the south (Figure 1). The Indian River Lagoon is connected to the Atlantic Ocean by 5 small, widely spaced inlets and a single lock. The exchange of water between the lagoon and the ocean is limited by the size of these inlets and the shallow nature of the lagoon. As a consequence of this limited tidal exchange, the lagoon is particularly vulnerable to the influx of pollutants. Water quality in the Indian River Lagoon has changed substantially during the past 5 decades because of watershed alterations and changes in land drainage patterns. The major cause of the decrease in water quality is fresh- and storm-water discharges that alter salinity and water clarity and introduce nutrients and pollutants into the system. All of the sugarcane grown in Florida and approximately 38% of the citrus and 42% of the vegetable crops grown in the state are grown in areas that drain into the Indian River Lagoon.

Bottlenose dolphins represent a key component of the Indian River Lagoon ecosystem and, as apex predators, serve as sentinels for the health of the environment. Dolphins in this ecosystem have had high seasonal stranding rates since 1996, but in 2001, at least 30 dolphins in the northern portion of the Indian River Lagoon died of unknown causes during a 2-month period. None of the animals that died had evidence of lobomycosis, although most carcasses were badly deteriorated.

Dolphin health assessment procedures—Comprehensive health assessments of bottlenose dolphin populations in the Indian River Lagoon and in estuarine waters near Charleston, SC, were conducted during 2003 and 2004. All procedures involving dolphins were approved by the National Marine Fisheries Service as part of the permit process. Bottlenose dolphins in the Indian River Lagoon were captured and examined during June 2003 and 2004; dolphins in estuarine waters near Charleston, SC, were captured and examined during August 2003 and 2004.

Dolphins were captured in 2 separate areas of the Indian River Lagoon. The northern capture area included the Mosquito Lagoon and portions of the Indian river and Banana river north of latitude 28°15'0"N. The southern capture area included the St Lucie inlet, the north and south forks of the St Lucie river, and portions of the Indian River south of latitude 27°25'0"N.

Dolphins were captured by encircling them with a net in < 2 m of water to facilitate safe animal handling. Once a dolphin was encircled, experienced animal handlers were used to manually restrain the animal. Each dolphin was held in the water by 3 to 6 handlers to facilitate collection of blood samples. The dolphin was then placed on a stretcher and hoisted onto a processing boat for the remainder of the sample collection process. Heart rate and respiratory rate were monitored at 5-minute intervals, and animals were bathed continuously with seawater and kept under a shaded canopy until released.

A comprehensive, standardized health assessment that included a complete physical examination; ultrasonography of the thorax and abdomen; measurement of length, girth, and weight; and collection of blood, urine, and various tissues for hematologic, serum biochemical, microbiologic, and
immunologic testing and for evaluation of an extensive suite of biomarkers was performed. Age was estimated by examination of postnatal dentine layers in an extracted tooth. Skin lesions were photographed, and representative areas were biopsied after being disinfected with chlorhexidine and anesthetized with 3% meperidine. Biopsy specimens were removed with a sterile scalpel and forceps and preserved in neutral-buffered 10% formalin. Following completion of the health examination, dolphins were released in the same area where they had been captured.

Biopsy specimens were routinely processed and sectioned at a thickness of 5 µm. Sections were stained with H&E or Gomori methenamine silver and examined for *L.loboi*.

Statistical analysis—Student *t* tests were used to determine whether differences in age existed between affected and unaffected dolphins and between dolphins captured in the northern and southern portions of the Indian River Lagoon. A *χ²* test was used to determine whether the prevalence of lobomycosis among male and female dolphins differed. Statistical analyses were performed with standard software. Values of *P* < 0.05 were considered significant.

**Results**

During June 2003, 42 bottlenose dolphins in the Indian River Lagoon were captured and examined. During June 2004, an additional 33 dolphins were captured and examined, along with 6 dolphins that had been captured during 2003. Nine of the 75 (12%) dolphins captured in the Indian River Lagoon had histologically confirmed lobomycosis, including 6 of the 42 (14%) captured during 2003 and 3 of the 33 (9%) captured during 2004. None of the 71 dolphins captured in estuarine waters near Charleston, SC, had histologic evidence of lobomycosis.

During 2003, 23 dolphins were captured in the northern portion of the Indian River Lagoon, none of which had lobomycosis, and 19 dolphins were captured in the southern portion, of which 6 (32%) had lobomycosis. Similarly, none of the 22 dolphins captured in the northern portion of the lagoon during 2004 had lobomycosis, whereas 3 of the 11 (27%) dolphins captured in the southern portion did. Overall, 9 of 30 (30%) dolphins captured in the southern portion of the lagoon had lobomycosis, but none of the 45 dolphins captured in the northern portion did.

One dolphin with lobomycosis that was captured in 2003 was recaptured in 2004; lesions had persisted and become more extensive. None of the 5 dolphins without lobomycosis captured during 2003 that were recaptured in 2004 had evidence of the disease when recaptured.

Of the 30 dolphins captured in the southern portion of the Indian River Lagoon, 14 were male and 16 were female. The proportion of males with lobomycosis (4/14 [29%]) was not significantly different from the proportion of affected females (5/16 [31%]).

Ages of 60 dolphins captured in the Indian River Lagoon and 64 dolphins captured in estuarine waters off Charleston were determined by means of histologic examination of postnatal dentine layers in an extracted tooth. Mean ± SD ages of dolphins captured in the northern and southern portions of the Indian River Lagoon and near Charleston were 11.0 ± 5.0 years, 13.9 ± 6.3 years, and 14.7 ± 8.1 years, respectively. Mean age of dolphins with lobomycosis (20.6 ± 5.5 years; *n* = 5) was significantly higher than mean age for dolphins without lobomycosis (13.0 ± 7.0 years; 119).

For dolphins captured in the southern portion of the Indian River Lagoon, mean age of dolphins with lobomycosis (20.6 ± 5.5 years; *n* = 5) was significantly higher than mean age for dolphins without lobomycosis (11.5 ± 4.8 years; 14).

Dermal lesions in dolphins with lobomycosis consisted of multiple, firm, raised white nodules and plaques on the leading edges of the dorsal and pectoral fins, the head, the fluke, and the caudal peduncle (Figure 2). In severely affected animals, there were multiple aggregations of confluent lesions measuring > 30 cm in the broadest dimension. The lesions had a typical verrucous appearance with occasional ulcerations and crusts (Figure 3). Satellite lesions were observed extending from central foci.

Histologically, the lesions consisted of multifocal, subepidermal histiocytic granulomas between the skin and subcutaneous tissue. Granulomatous inflammatory changes extended deep into the dermis and subcutaneous layers of the blubber. The lesions were characterized by acanthosis with associated foci of chronic granulomatous inflammation. Inflammatory infiltrates contained numerous histiocytes and multinucleated giant cells, along with round to ovoid yeastlike bodies (7 to 12 µm in diameter) with thick refractile walls and small, central basophilic bodies. Numerous yeastlike cells were arranged in single chains connected by
sensitive to environmental perturbations, whether of mental changes. Marine mammals are particularly sensitive; dolphins in this region may be responding to environmental changes occurring in epidemic proportions among dolphins in the Indian River Lagoon. Reasons for the emergence of lobomycosis in the dolphin population of the Indian River Lagoon may reflect variations in water temperature, salinity, or vegetation and other agricultural or industrial contaminants introduced through runoff or point sources of pollution. Alternatively, the marked gradient in the distribution of lobomycosis from the southern to the northern portion of the Indian River Lagoon may reflect variations in water temperature, salinity, or vegetation and enhanced survival of the organism in the marine environment. Because the organism has not been cultured in the laboratory, its growth requirements are not known. Our data suggest that advanced age may be a risk factor for lobomycosis. Several dolphins with lobomycosis had high concentrations of α1-globulin, α2-globulin, or both, compared with values reported for healthy dolphins. No consistent hemato-logic abnormalities were observed in dolphins with lobomycosis.

Discussion

Results of the present study suggest that even though L loboi has been endemic in Florida waters since at least the early 1970s, lobomycosis may now be occurring in epidemic proportions among dolphins in the Indian River Lagoon. Reasons for the emergence of this rare disorder are unclear, but an implication is that dolphins in this region may be responding to environmental changes. Marine mammals are particularly sensitive to environmental perturbations, whether of anthropogenic or natural origins.

Cases of lobomycosis in the present study were clustered in the southern portion of the Indian River Lagoon, an area characterized by freshwater intrusion through the St Lucie River. Although dolphins are able to move long distances in a short time, Indian River Lagoon dolphins exhibit a degree of site fidelity in their home ranges. Photo-identification survey methods have been used to identify > 500 individual dolphins in the Indian River Lagoon, and a site-fidelity ratio (ie, the number of survey years during which a dolphin was sighted divided by the total number of years it could have been sighted) of 91% was calculated for 67 dolphins sighted ≥ 8 times between 1996 and 2001. Evidence for residency of ≥ 20 years comes from continued sightings between 1996 and 2004 of 20 dolphins that were freeze-branded between 1979 and 1981. Additionally, preliminary analyses have indicated that dolphin ranging patterns exhibit geographic partitioning within the major regions of the Indian River Lagoon.

Progressive infections with mycotic and fungal organisms are often associated with depressed cell-mediated immunity. Immunodeficiency appears to be a component of lobomycosis in humans, as manifested by delayed skin allograft rejection, failure to sensitize to dinitrochlorobenzene, and energy to Candida albicans and other common antigens. A case of lobomycosis in a Florida dolphin described in 1984 was accompanied by marked hypogammaglobulinemia and suspected acquired immunodeficiency. In the present study, affected dolphins had low serum activities of alkaline phosphatase, a marker associated with a poor prognosis in critically ill adult bottlenose dolphins and Pacific white-sided dolphins. Several dolphins with lobomycosis also had high concentrations of α1-globulin and α2-globulin, and increases in serum concentrations of these acute phase proteins are consistent with the presence of an acute inflammatory process. Preliminary results also suggest that some dolphins captured in the Indian River Lagoon in 2003 and 2004 had depressed lymphocyte proliferation responses to mitogens and impairments of humoral and cell-mediated immunity.

The evidence of impaired immune function in affected dolphins suggests that lobomycosis may be associated with an immunosuppressive cofactor of environmental origin, such as exposure to pesticides or other agricultural or industrial contaminants introduced through runoff or point sources of pollution. Alternatively, the marked gradient in the distribution of lobomycosis from the southern to the northern portion of the Indian River Lagoon may reflect variations in water temperature, salinity, or vegetation and enhanced survival of the organism in the marine environment. Because the organism has not been cultured in the laboratory, its growth requirements are not known. Our data suggest that advanced age may increase the risk of lobomycosis in dolphins, but differences in population age distribution did not appear to account for the localization of lobomycosis to the southern portion of the Indian River Lagoon.

Bottlenose dolphins in the Indian River Lagoon may serve as a sentinel for a potential public health hazard. Limited evidence exists to suggest that lobomycosis may be transferred from infected animals to people. In the 1970s, for instance, a dolphin with lobomycosis caught accidentally in the Bay of Biscay off the French-Spanish coast was transferred to an aquarium in Holland and a male aquarium attendant subsequently developed the disease several months after regular contact with the animal and the pool in which it was housed. Lobomycosis has not been reported in humans living in Florida counties that adjoin the Indian River Lagoon. However, the high prevalence of lobomycosis in the dolphin population of this coastal waterway, which is used extensively for recreational purposes, raises concerns for zoonotic or common-source transmission. Veterinary personnel...
and animal handlers who had contact with infected dolphins during capture and release applied 3% chlorhexidine scrub and 62% ethyl alcohol foam to exposed areas of the skin after handling infected animals. No dermal lesions developed in these personnel.

In summary, we report the emergence of a rare fungal disease in Florida bottlenose dolphins in epidemiologic proportions. Dolphins and humans are the only species known to be naturally susceptible to infection with *L. loboi*. These findings extend our earlier observations from photo-identification records and pathologic examinations of stranded dolphins and confirm that the prevalence of this rare disease is unusually high among dolphins in the Indian River Lagoon. The basis for the apparent emergence of lobomycosis in dolphins of the Indian River Lagoon remains unknown.


b. SAS system for Windows, version 9.1, SAS Institute, Cary, NC.

c. Calgon-Vestal Alcare alcohol foam, Steris Corp, Mentor, Ohio.

References


