A CASE OF LOBO'S DISEASE IN THE DOLPHIN *SOTALIA GUIANENSIS*

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Summary

Lobo's disease was diagnosed in a dolphin *(Sotalia guianensis* van Beneden) caught in the estuary of the Surinam river.
The lesions were located on the rostrum and underneath the right pectoral fin.
The clinical symptoms and the pathological reaction were essentially the same as those found in human patients and in the *Tursiops truncatus* discovered by Migaki & al. The morphology of the fungus was similar to that of *Loboa loboi* in man and in *Tursiops truncatus.*
Two fungi, *Glenospora graphii* Vuill. and *Torulopsis haemulonii* Van Uden & Kolipinski, were grown from the tissue, but were not regarded to be of etiological significance.
The *Glenospora graphii* isolate has been deposited in the collection of the Centraalbureau voor Schimmelcultures at Baarn as CBS 883.71, the *Torulopsis haemulonii* in the Yeastdivision at Delft as strain 6332.

Introduction

In 1931 Jorge Lobo described the first case of a chronic, cutaneous mycosis in man which he called Keloidal Blastomycosis. The patient came from the Amazon valley and showed characteristic skin lesions. Later clinically similar cases have been observed not only in Brazil, but also in Surinam, French Guiana, Costa Rica, Panama, Columbia and Venezuela (Emmons, Binford & Utz, 1963; da Silva Lacaz, 1967; Wiersema, 1971). Keloidal Blastomycosis, also known as Lobo's disease, Lobo's granuloma or Lobomycosis, is a rare disease 59 cases having been reported in the literature since 1931. The budding fungus is very abundant in the lesions. Its isolation seems to be impossible or at least extremely difficult. Reports on the successful isolation of the fungus are very scarce and doubtful. Hence its classification and morphology in vitro are still uncertain. Fonseca & Leao called the fungus *Glenosporella loboi* in 1940. Other names given to this fungus are *Paracoccidioides loboi* (Fonseca & Leao) Almeida & Lacaz, 1949, *Blastomyces loboi* (Fonseca & Leao) Langeron & Vanbreuseghem, 1952 and *Loboa loboi* (Fonseca & Leao) Ciferri & al. 1956, *Glenosporopsis amazonica* Fonseca Filho, 1943, is a doubtful synonym.

The inoculation into laboratory animals appears to be difficult. Only one author, Wiersema, reported in 1971 the successful inoculation into the foot pads of 2 golden hamsters.
Until 1971 Lobo's disease was only known as a mycosis of the human skin. In that same year, however, Migaki, Valerio, Irvine & Garner described a case in the skin of the tail stock and flukes of an Atlantic bottle-nosed dolphin *(Tursiops truncatus)*. The following report deals with a similar case in *Sotalia guianensis,* a dolphin caught in Surinam.

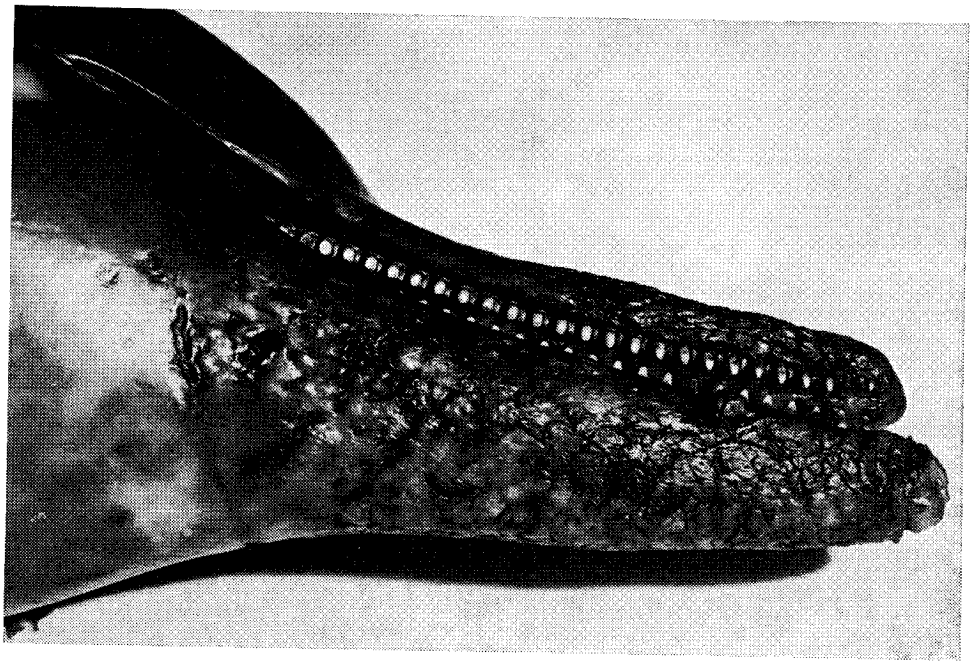

fig. 1.
View from below of the keloidal skin lesions on the beak of *Sotalia guianensis* (Courtesy of Rijksmuseum van Natuurlijke Historie, Leiden).

Case History

On May 19, 1971 the Rijksmuseum van Natuurlijke Historie at Leiden received a specimen of a pregnant, adult female of the dolphin species, *Sotalia guianensis* van Beneden. The animal measuring 182 cm in length had been caught in the estuary of the Surinam river at about 6° northern latitude and 55° western longitude between February 15, 1971 and April 13, 1971. Frozen immediately after having been unloaded in the harbour it was kept at -20°C

during the transport to Leyden. On receipt the upper and lower part of the rostrum and the area underneath the right pectoral fin appeared to be covered with keloidal skin lesions (fig. 1). These have later been examined in the Laboratory for Parasitology at Leyden. Because it was not possible to study the disease at once, a part of the affected skin was removed and put into a refrigerator at +2°C. Small subsamples were immediately fixed in Bouin-Hollande for histological processing.
When after about one month a study of tissue sections stained in haematoxyline-eosine showed that the subepidermal tissues were invaded by a budding yeastlike organism a mycosis was suspected. For this reason the skin specimen was sent to the Centraalbureau voor Schimmelcultures where the diagnosis 'Lobo's Disease' was established in June 1971.

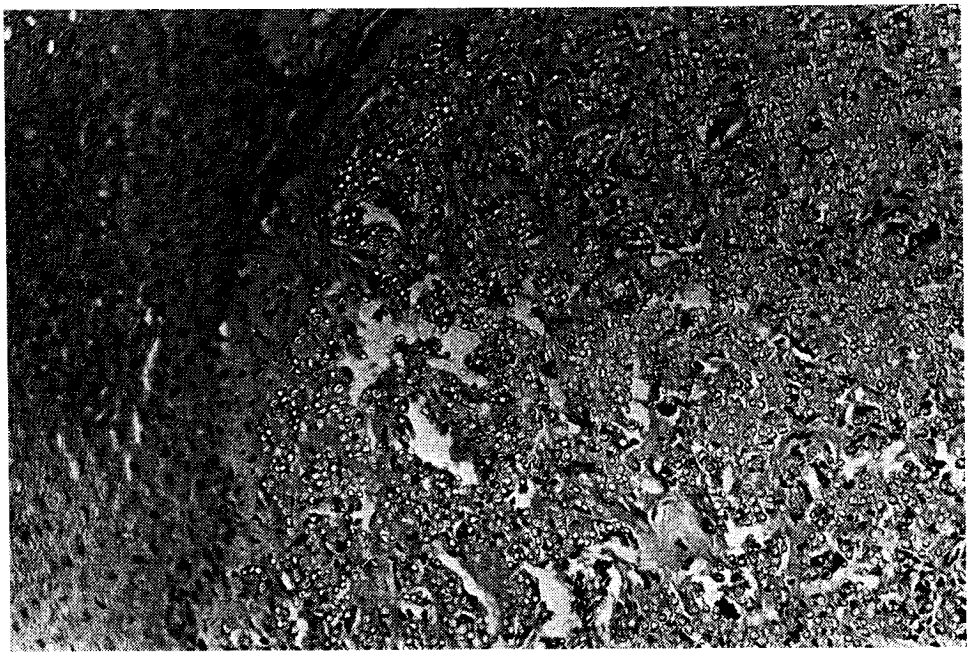

fig. 2.
Histological section of the skin showing the great abundance of the fungal cells in the dermis and their absence in the epidermis (x 100).

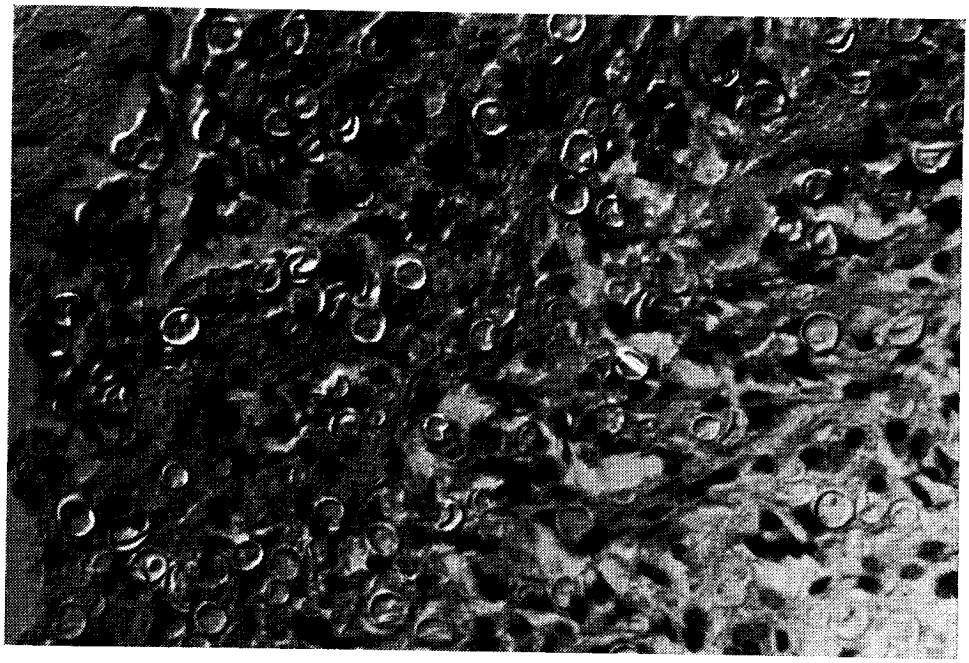

fig. 3.
Higher magnification of a part of the infected skin showing a fungal cell with a birefringent rod (x 540).

Pathology

In HE preparations of the skin lesions an extensive granulomatous reaction with giant cells and histiocytes was observed in the dermis which was the only tissue where the fungal cells were seen (fig. 2).
The epidermis was of unequal thickness, most likely due to a reactive proliferation of the epithelium. Granulocytes were rare. Some fibroblast proliferation may point to an advanced age of the lesions, which might be inferred from their large extension as well. Nuclear dust was observed in some areas. The giant cells were irregular in shape and size and contained 5 - 35 nuclei which were often densely packed together and usually located near the cell periphery. Fungal cells were present in varying numbers in almost all giant cells and histiocytes, but also in the intercellular spaces.

Mycology

The round to oval fungal cells were very abundant in the subepidermal tissues occurring either separate or connected with each other by short, narrow tubes in branched chains of 2-5 cells (fig. 3 and 4). Each cell of a chain had

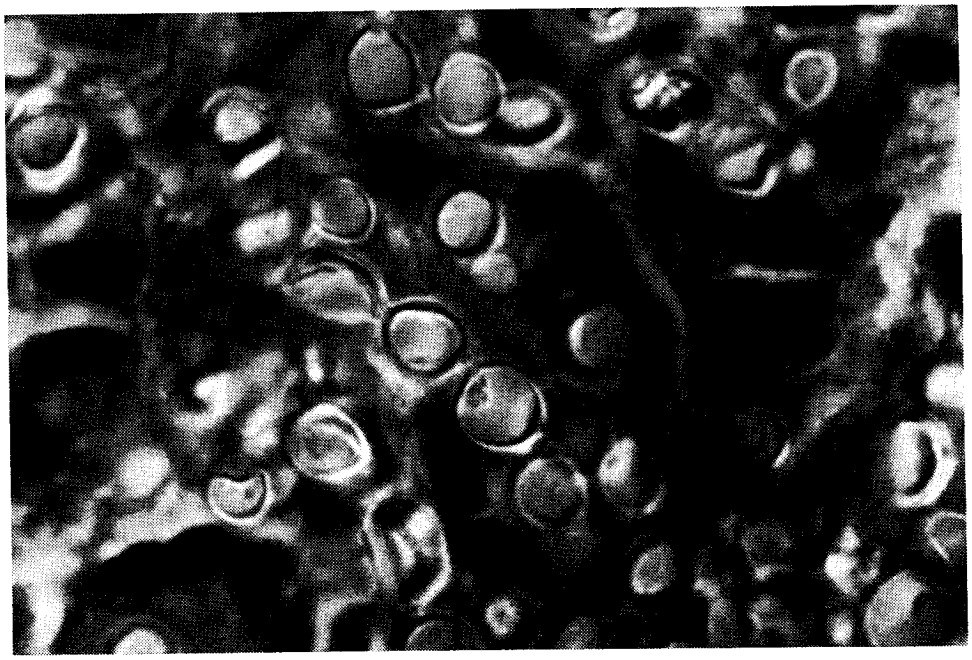

fig. 4.
High magnification of the fungus in the tissue showing a short chain in the middle and a giant cell containing one fungal cell at the bottom left (x 1350).

produced one, in case of branching 2 or even 3 buds. The cell diameter varied from 6 to 12μ with an average of 7,9μ. The PAS positive, highly refractile cell wall which did not stain with HE was usually 0,5μ and in some cases 1μ thick. Many fungal cells appeared to be empty, in others an eosinophilic cytoplasmic mass was seen. Collapsed cells were quite numerous. When HE stained tissue sections were observed between crossed nichols a conspicuous luminescent dotting of the dark microscopic field showed that many cells were birefringent. The luminescence was either distributed all over the cell surface or restricted to rod- or lense-shaped sections (fig. 3).

Although the very heavy bacterial contamination was not very promising for a successful isolation of the causative organism, small fragments of the skin specimen were inoculated on Sabouraud dextrose agar and malt agar (both media with 20 units penicilline and 40 microgram streptomycine per ml), Littman oxgall agar + 30 microgram streptomycine per ml and blood agar. The malt agar plates were incubated at 10, 15, 20, 25 and 28°C, the Sabouraud plates at 25 and 28°C, the Littman plates at 25°C and the blood agar at 28°C. No growth occurred at 10, 15 and 20°C; bacteria grew very

profusely at 28°C. After about 3 weeks of cultivation at 25°C one colony of a filamentous fungus which was later identified as *Glenospora graphii* Vuill. was observed on one of the Sabouraud agar plates. The colony which was moist and cream-coloured in the beginning developed aerial mycelium and a brown pigment upon aging. Microscopic examination showed hyphae, fertile coremia and branched chains of colourless budding cells the latter strikingly similar in size and shape to the fungus cells seen in the Sotalia skin. Because only one colony of *Glenospora graphii* was isolated, its etiological significance had to be regarded as doubtful.

After 5 weeks three colonies were observed of a yeast which was identified by D. Yarrow at Delft as *Torulopsis haemulonii* van Uden & Kolipinski a species with a marine distribution. The isolation medium in this case was malt agar with penicilline and streptomycine. As the cells of this yeast were much smaller than those of the Sotalia fungus, we did not regard it as the etiological agent either. Besides this yeast and *Glenospora graphii* no other fungi have been isolated.

Discussion

The very great resemblance of the cells of the fungus in the Sotalia skin to the cells of the young culture of *Glenospora graphii,* a fungus isolated from cases of mycosis of the cornea and external auditory canal in man, would suggest it to be the etiological agent. The fact, however, that only one colony was obtained from the clinical specimen which unfortunately was almost digested by bacteria would make this assumption speculative. The taxonomical status of *Glenospora graphii* is still a matter of discussion. Its relationship with *Scedosporium (Monosporium) apiospermum* (Sacc.) Sacc., the conidial state of *Petriellidium Allescheria) boydii* (Shear) Malloch, a fungus causing mycetoma in man, will be the subject of a future paper. The isolation of *Torulopsis haemulonii* is quite interesting because it is the third record of this apparently marine yeast. The type was isolated from the gut contents of a Blue-striped Grunt *Haemulon sciurus,* a fish caught at Biscayne Bay, Florida; a second strain originated from a sample of seawater collected near Lisbon, Portugal.

As said before this yeast was not regarded as the aetiological agent because its cells were much smaller than those of the fungus in the Sotalia skin. The possibility that it was a secondary invader could not be proved by demonstration of its cells in the tissue.

The size of the fungal cells in the Sotalia skin (6 - 12μ with an average of 7,9μ) agreed very well with the reports by Emmons & al. (about 8μ) and Wiersema (9-10μ) of human cases, by Migaki & al. (5-10μ) of the case in *Tursiops truncatus* and with our own observations (8-12μ) on cells in a HE-stained,

histological section of a human case of Lobomycosis sent as neotype to the Centraalbureau voor Schimmelcultures by da Silva Lacaz.

The frequent absence of cytoplasmic contents and collapse of the fungal cells is a character that is also observed in the *Tursiops truncatus* and in man. The thickness of the cell wall is the same as that of *Loboa loboi* in human skin. Another character common to both *Loboa loboi* in man and to the Sotalia fungus, but not seen in *Tursiops truncatus,* is the hyaline zone or capsule around the fungal cells. It has been regarded by the present authors as an artefact caused by shrinkage of either the fungus and/or the host tissue. The birefringence of several cells of the Sotalia fungus which has also been in human cases of Lobomycosis and in the *Tursiops* case is observed in other pathogenic fungi such as *Histoplasma duboisii, Histoplasma farciminosum* and *Paracoccidioides brasiliensis* as well. For the latter species this phenomenon has already been reported by Potenza, Lares Campos & Feo (1953).

The clinical picture and the pathology of the Sotalia disease and the microscopic morphology of the fungus agree all very well with Lobomycosis in man and in *Tursiops truncatus.* The PAS positive spines observed by Migaki & al. on the wall of older fungal cells in their *Tursiops* is regarded by the present authors to be of the same nature as the more evenly distributed PAS positive deposits on the cell wall of *Loboa loboi* in man and to be a reaction product between the antigens of the fungus and the antibodies of the host.

Considering the fact that in this report the diagnosis Lobomycosis has been based on the comparative study of the morphology of the fungus in the tissues and the clinical and pathological aspect of the disease, it has to be remarked that if no definite proof is given by cultivation of the etiological agent that one and the same fungus is involved in the three hosts, the possibility that Lobomycosis is caused by more than one species cannot be excluded.

Migaki & al. doubt that their infected *Tursiops* which had been caught in Florida had carried the disease all the way from Central or South America. According to these authors it could be possible that either the disease had spread slowly northward by contact between infected dolphins or was enzootic and yet undetected in these animals. The finding in the area where human Lobomycosis occurs of the same disease in *Sotalia guianensis,* a species restricted to the northern part of South America where it lives in fresh, brackish and salt waters in harbours and in rivers, can not corroborate one or the other hypothesis.

When we assume that the same fungus is involved in man as well as in the dolphins it would be conceivable that it grows either in- or outside these dolphins or other water-inhabiting hosts, being deposited during periods of flooding or high tides on the tropical vegetation thus forming a source of infection for the local population in the area where human cases of Lobomycosis occur.

However, the reverse may also be true, the fungus growing on the vegetation, being washed off by water and reaching the river or the ocean where it may infect the dolphins. The subsequent spreading of the infection amongst these animals may all least be partly explained when a better insight is gained in the migratory habits of the dolphin populations in the area of distribution.
That Lobomycosis has not been reported more often in dolphins might be explained by the observation that skin lesions in these animals are quite numerous. Mycotic keloidal skin lesions could very well have been mistaken for scarring tissue or some other type of skin alteration with a more or less similar aspect. It may therefore be usuful in this connection to make an appeal to all those who some way or other are dealing with Cetaceae to pay full attention to skin lesions that might be attributed to Lobomycosis. It is the opinion of the authors that only by a systematical study of more material and above all the isolation of the etiological agent in pure culture a deeper understanding of this interesting disease can be obtained.

Acknowledgement

The authors are grateful to Dr. A. M. Husson (Rijksmuseum van Natuurlijke Historie, Leiden) who first noticed the lesions on the dolphin's skin, and to Dr. E. Scheffer (Laboratory of Pathology, University Hospital, Leiden) for checking the description of the pathological anatomy.

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