Molecular diagnosis of lobomycosis-like disease in a bottlenose dolphin in captivity

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We report the diagnosis and molecular characterization of lobomycosis-like lesions in a captive bottlenose dolphin. The clinical picture and the absence of growth in conventional media resembled the features associated with Lacazia loboi. However sequencing of ribosomal DNA and further phylogenetic analyses showed a novel sequence more related to Paracoccidioides brasiliensis than to L. loboi. Moreover, the morphology of the yeast cells differed from those L. loboi causing infections humans. These facts suggest that the dolphin lobomycosis-like lesions might have been be caused by different a different fungus clustered inside the order Onygenales. A successful treatment protocol based on topic and systemic terbinafine is also detailed.

Keywords lobomycosis, Onygenales, bottlenose dolphin, molecular diagnosis

Introduction

Lobomycosis (Jorge Lobo’s disease) is a cutaneous and subcutaneous disease described in humans and dolphins and caused by the yeast Lacazia loboi, of the order Onygenales. First discovered in a human patient from Brazil in 1930, it is considered endemic in some tropical and subtropical regions of South America, especially the Amazon area [1]. In addition, isolated cases have been reported in the USA [2], Canada [3], The Netherlands [4], Spain [5] and South Africa [6].

Lobomycosis-like disease in cetaceans was first identified in a bottlenose dolphin (Tursiops truncatus) stranded on the coast of Florida [7]. Since then, the river dolphin (Sotalia fluviatilis) is the only other cetacean species in which lobomycosis-like lesions have been reported [8]. A dolphin handler developed a clinical picture of lobomycosis several months after working with a dolphin with lobomycosis-like lesions, suggesting that transmission of the disease from dolphins to humans is possible [5]. At the same time, the probability of transmission appears to be low, since a physician who cut himself with the same scalpel that he had used to cut a lobomycosis-like lesion in a bottlenose dolphin did not contract the disease [9].

The clinical picture of Jorge Lobo’s disease is characterized by chronic and localized lesions in the skin that are nodular, solid, with a smooth, shiny surface with a keloidal appearance. The lesions may also be infiltrative, gummatous, ulcerative, verrucous, tumoral, sclerodermiform, macular, or in plaques [1]. Although no systemic involvement has been reported and the patients’ overall health is good, clinical consequences may include restricted movement, significant esthetic damage, secondary infections and, in sporadic cases, cancer [1].

The features of the lesions provide presumptive diagnosis of lobomycosis, with definitive conclusions to be made by direct visualization of the yeast structures [10] or by histopathology, including yeast staining procedures. This organism cannot be grown in artificial media, based on several unsuccessful attempts using different approaches [1]. The few molecular studies published were done with infected humans and have identified the organism as a member of the order Onygenales [11]. Unfortunately, there are no published reports comparing the sequences of the etiologic agent found in cases of involving lobomycosis-like lesions of humans and
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dolphins, so it is unclear whether the same yeast is involved.

Case report

A bottlenose dolphin born in the wild was brought from Cuba to an aquarium in Spain in 2002. This animal arrived with two localized, slightly prominent whitish dermal lesions on the ventral side of the tip of the left pectoral fin. During the winter of 2009, these lesions became active and ulcerated, taking on a nodular and hyperplastic appearance (Fig. 1, panel A). Cytology and histopathology analyses of two biopsies of the skin lesions showed the presence of yeast-like structures joined in chains, resembling those described for lobomycosis (Fig. 1, panel B). The yeast cells had a mucilaginous envelope that disappeared after tissue homogenizing. The cellular wall measured approximately 1 μm. The chains were formed by a yeast cell that was 9×6 μm, followed by daughter cells that measured 6×6 μm, and lower numbers that were 5×5 or 3×3 μm.

In order to rule out the presence of a fungus that could be grown in artificial media, portions of the biopsies were inoculated onto Sabouraud dextrose agar but after four weeks of incubation, no fungi were recovered.

Molecular identification of the yeast was performed by a PAN-fungal PCR covering a variable length from the 18S ribosomal RNA gene, through the internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, and into the 28S ribosomal RNA gene [12]. Briefly, a 0.5 g sample was macerated in 5 ml of PBS with tissue lysis buffer in an automatic homogenizer (Bertin technologies). The homogenate was incubated overnight with proteinase K at 72°C. DNA extraction was performed with the High Pure PCR Template Preparation Kit (Roche Diagnostics), following manufacturer’s instructions, with an initial digestion with lysozyme. PCR was performed as described previously [12].

Products of PCR were electrophoresed on 2% agarose gels, stained with Sybr Green. The specific bands were excised and sequenced. Purification of DNA products in positive samples was performed using GFX PCR DNA and gel band purification kit (Amersham Biosciences), following manufacturer’s instructions. Sequencing was performed in triplicate using an ABI Prism 3100 sequencer (Applied Biosystems). To confirm the results sequenced products were compared with sequences available in GenBank using the Blast search.

Sequencing revealed that the organism belonged to the order Onygenales (Fig. 2) but the sequence obtained showed high homology to that of Paracoccidioides brasiliensis (p-distance 0.011) and much lower homology to L. loboi sequences obtained from human patients (p-distance: 0.180). Unfortunately no sequences from this genomic region have been reported for L. loboi in cetaceans. A neighbor-joining phylogenetic tree based on p-distances was performed with seven different sequences of P. brasiliensis, one from L. loboi (human isolate), one from Emmonsia crescens and a sequence of Aspergillus flavus as outgroup member (Fig. 2). The phylogenetic tree classified the sequence obtained within the P. brasiliensis group.

The treatment of the lesions involved different phases. Initially it was based on the use of itraconazole (2.5 mg/kg PO BID; Itraconazole Merck, caps 100 mg, Lab. Merck Generic), based on previous experiences (James McBain, personal communication.), and topical treatment with ketoconazole (Panfungol, Lab. Esteve). Since there was no reduction in the size of the lesions on this treatment protocols, it was changed to PO itraconazole (2.5 mg/kg PO BID) and terbinafine PO (2 mg/kg SID, Lamisil® compr 250 mg, 107

![Fig. 1](image_url) (A) Lobomycosis-like disease on the dorsal fin of a bottlenose dolphin (Tursiops truncatus). (B) Presence of yeast-like organisms forming chains (magnification ×400). Scale bar: 30 μm.

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different strains may be involved in the disease in these two hosts [14]. The morphology of the yeast cells found in dolphin samples is similar to those observed in the present case. The authors of the previous study recommend phylogenetic investigations to differentiate, if possible, the *L. loboi* involved in humans and dolphins.

Results of sequencing the rDNA region in the present case revealed that it was more similar to *P. brasilensis* than *L. loboi* observed in human patients. Ribosomal DNA sequences, including ITS regions may be considered the ‘gold standard’ for molecular identification of yeasts and molds [12].

One previous molecular study of yeast-like structures from a bottlenose dolphin with lobomycosis-like lesions, reported that a partial sequence of the 28S rRNA gene showed high homology to sequences of *P. brasiliensis* (p-distance 0.03) [15]. This assumes that the sequence from the dolphin in the study was from *L. loboi* involved in humans and dolphins.

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Molecular approaches are essential in order to compare the yeasts found in infected dolphins and humans, and to establish their relationship to other members of the order *Onygenales*. Although dolphin lobomycosis-like lesion may be caused by a different yeast-like fungus than that previously described for human lobomycosis, the zoonotic potential of this yeast should be considered, especially related to captive animals. Additionally, the fact that animal movement can introduce disease into new areas highlights the importance of developing diagnostic tools for the early and specific diagnosis of lobomycosis-like lesions to prevent the spread of the disease.

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