Isolation of *Rhodotorula mucilaginosa* from skin lesions in a Southern sea lion (*Otaria flavescens*): a case report

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ABSTRACT: This paper reports the isolation of *Rhodotorula mucilaginosa* from skin lesions in a Southern sea lion (*Otaria flavescens*). The microorganism was isolated from cutaneous lesions, identified by the commercial API 20 C AUX system, and confirmed by sequencing. Topical treatment with sertaconazol resulted in complete clinical recovery of the animal and repeat testing did not result in the recovery of the yeast from the healed lesion sites.

Keywords: dermatomycoses; rhodotorulosis; sertaconazol; pinnipeds; yeast

Skin disease is a common problem of captive marine mammals (Pollock et al., 2000), and can be recurrent in some animals. Although pinnipeds are relatively resistant to superficial mycoses (Montali et al., 1981; Frasca et al., 1996), there are several reports of superficial infections in seals, sea lions and elephant seals that are caused by yeasts (Dunn et al., 1984; Guillot et al., 1998; Nakagaki et al., 2000; Pollock et al., 2000) and moulds (Montali et al., 1981; Frasca et al., 1996; Pollock et al., 2000). Among the factors proposed to be associated with these kind of lesions are warm pool temperatures, excess pool chlorine levels that eliminate the normal bacterial flora of the skin, prior or concomitant bacterial or viral infections, prolonged antibiotic use, nutritional imbalances, the presence of skin abrasions, and various intrinsic and extrinsic stressors, including breeding season, moulting, transport or social isolation (Montali et al., 1981; Frasca et al., 1996; Guillot et al., 1998; Pollock et al., 2000).

This report describes the clinical and microbiological findings from a Southern sea lion with skin lesions, and the role of *Rhodotorula mucilaginosa* in the development of the clinical process. The identification of the yeast was achieved using standard techniques of mycological examination and confirmed by sequencing.

Case description

An 8½-year-old female Southern sea lion (*Otaria flavescens*) weighing approximately 115 kg presented with several skin lesions. The animal was housed in a non-chlorinated pool, with three other females of the same species, and a pair of gray seals (*Halichoerus grypus*). The water was recirculated through a series of sand filters and its temperature varied between 24 and 26°C. The salinity was maintained at 20 g/l and water pH was 8.2. Clinical antecedents of the animal were of no importance, apart from some mild episodes of keratitis and corneal ulcerations that occurred one and half years before the cutaneous problems described here. These episodes were treated with Chibroxin® coliria (norfloxacine, Laboratorios Thea, Barcelona, Spain). The only possible stress factor was that the animal could have been entering the breeding season, but this was not confirmed.

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The first skin lesions appeared in June 2008, as small (approximately 1 cm diameter), rounded, alopecic areas in the dorsum, face and flippers of the animal. Some of these alopecic areas presented ulcerations caused by the intense pruritus produced in the animal. The ulcers were solitary, well delineated, without formation of nodules. Their approximate number was around 100. Treatment was with povidone iodine gel (Betadine gel 10%; Meda Pharma, Madrid, Spain), three times a day, for seven days, but no remission of the skin lesions was detected. Instead, more ulcerated areas with intense pruritus appeared on the dorsum and the caudal flipper (Figure 1). Samples from different lesions were taken for microbiological examination. The surface of the skin was cleaned with water and decontaminated with a sterile gauze soaked in 10% povidone iodine solution (Betadine solucion dermica; Meda, Pharma, Madrid, Spain). Sterile swabs were rubbed over the lesions, placed in transport medium (Amies agar transport medium; Difco, Madrid, Spain) and maintained at 4°C until processing. While awaiting the results of the microbiological analysis, the animal was treated with an ointment containing triamcinolone acetonide, neomycin and nystatin (Positon ungüento; Faes Farma, Madrid, Spain), and two capsules a day of an essential fatty acid supplement (linoleic and linolenic acids; Glavaderm oral; Intervet, Salamanca, Spain).

The samples were cultured on Columbia agar with 5% sheep blood (TecLaim; Madrid, Spain), Sabouraud agar (Biomerieux; Marcy l'Etoile, France) and Sabouraud agar (Biomerieux) supplemented with 0.5 g/l chloramphenicol (Sigma; St. Louis, USA), and incubated at 30°C under aerobic conditions. At 48 h of incubation, a pure and profuse growth of small, salmon-pink, mucoid colonies was observed on Sabouraud agar with chloramphenicol (Figure 2). On microscopic examination, small rounded budding cells were seen, but no pseudohyphae formation was observed. On the two other media (Columbia blood agar and Sabouraud agar without antibiotics), high numbers of yeast colonies appeared in mixed culture with other microorganisms, specifically Gram-positive cocci and Gram-negative coccobacilli, identified as *Staphylococcus intermedius* and *Psychrobacter phenylpyruvicus*, respectively.

The identification of the fungal isolate was performed using the API 20 C AUX system (Biomerieux). According to its substrate assimilation profile, the yeast was identified as *Rhodotorula mucilaginosa* (formerly, *R. rubra*). To confirm this identification, the D1/D2 region of the large subunit (LSU) rRNA gene was amplified by a PCR using primers F63 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and LR3 (5'-GGTCCGTGT TTCAAGACGG-3') (Fell et al., 2000). The amplicon obtained was sequenced using an ABI Prism Big Dye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems; Foster City, USA) and analyzed on an ABI Prism 3730 sequencer (Applied Biosystems). The sequence obtained (615 bp) was compared with those in the Genbank databases using BLAST software (http://www.ncbi.nlm.nih.gov/blast). This search identified the yeast as *Rhodotorula mucilaginosa*, with 99% identity to the sequence of *R. mucilaginosa* TJY11b (Genbank accession number EU285542.1).

Based on the results of the microbiological analysis, this yeast was considered the etiologic agent responsible for the lesions, and a topical antimycotic treatment with Sertaconazole cream (Dermofix;

Figure 1. Skin lesions in the Southern sea lion at the moment of sampling for microbiological analysis

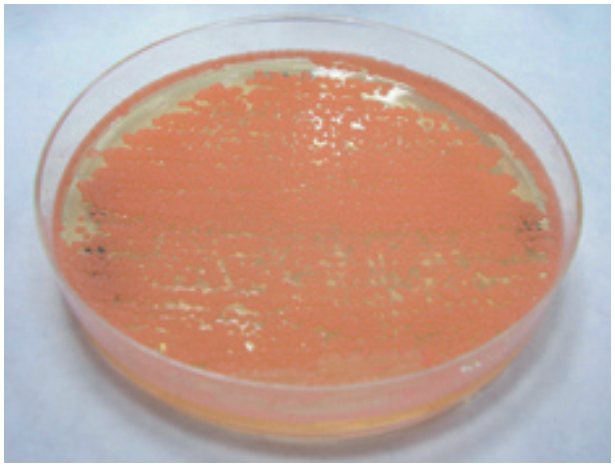

Figure 2. Growth in pure culture of *Rhodotorula mucilaginosa* on Sabouraud Agar with chloramphenicol

Ferrer Group, Barcelona, Spain) was initiated. Two months later, the animal showed evident recovery, with almost complete healing of the lesions (Figure 3). New samples were taken from the previously ulcerated areas and microbiological analysis was performed as previously described. Both *P. phenylpyruvicus* and *S. intermedius* appeared again on Columbia blood agar and Sabouraud agar without antibiotics; however, no growth of *R. mucilaginosa* or any other yeast was observed on any of the culture media used. Accordingly, treatment with sertaconazole was subsequently suspended. On the basis of the microbiological findings and the results of antifungal treatment, *R. mucilaginosa* was considered to be the etiological agent responsible for the lesions in the skin of the sea lion.

DISCUSSION AND CONCLUSIONS

Yeasts of the genus *Rhodotorula* are widely distributed in the environment (Galan-Sanchez et al., 1999; Preney et al., 2003; Gomez-Lopez et al., 2005), and can also be found in pools were marine mammals are kept in captivity (Buck, 1980). Moreover, different species of this genus are common commensals of terrestrial and aquatic animals (Ross and Morris, 1965; Bruce and Morris, 1973; Shotts et al., 1990; Cafarchia et al., 2006; Garcia et al., 2007). Although the pathogenicity of these basidiomycetous yeasts has been questioned, in recent years, an increase in the number of infections caused by *Rhodotorula* spp. in humans has been reported (Galan-Sanchez et al., 1999; Zaas et al., 2003; Cerikcioglu et al., 2005; Gomez-Lopez et al., 2005; Savini et al., 2008; Tuon and Costa, 2008), with *R. mucilaginosa* being the species most frequently isolated (Tuon and Costa, 2008).

Figure 3. Appearance of the previously ulcerated area in the Southern sea lion skin after a two-month topical treatment with sertaconazole

Among the few references to the pathogenicity of *Rhodotorula* spp. in animals, are several reports of outbreaks of cutaneous rhodotorulosis in chickens (Beemer et al., 1970; Page et al., 1976; Aruo, 1980). In one of these cases, the authors demonstrated that high doses of *R. mucilaginosa*, but not of other fungi isolated from the skin of healthy or diseased chickens, reproduced the dermatitis under experimental conditions (Beemer et al., 1970). More recently, *R. mucilaginosa* has also been isolated from skin lesions in cetaceans (Shotts et al., 1990), and reptiles (Kostka et al., 1997). However, no reports have been previously published on the isolation of *R. mucilaginosa* from this kind of lesion in pinnipeds. It should be borne in mind that different environmental factors could cause stress to the animal and contribute, directly or indirectly, to

the development of the lesions. In the present case, it was not possible to identify these factors.

In spite of the increasing importance of infections due to *Rhodotorula* spp., there are few publications reporting the *in vitro* susceptibility of *Rhodotorula* strains to antifungal agents with a standardized method (Gomez-Lopez et al., 2005; Tuon and Costa, 2008). These yeasts seem to be resistant to some therapeutic agents, especially to azoles and echinocandins (Galan-Sanchez et al., 1999; Preney et al., 2003; Zaas et al., 2003; Serena et al., 2004; Gomez-Lopez et al., 2005; Savini et al., 2008). Sertaconazole is a topical antifungal of the azole family (Carrillo-Munoz et al., 1999; Pfaller and Sutton, 2006). This agent is effective against a wide spectrum of fungi that cause superficial infections. Sertaconazole has two primary effects on cell function: (i) it inhibits ergosterol synthesis, which interferes with fungal cell growth; and (ii) it binds directly to nonsterol lipids in the membrane of fungal cells, altering the regulation of membrane permeability, and thereby contributing to immediate cell death (Carrillo-Munoz et al., 1999; Pfaller and Sutton, 2006). In the case reported here, topical treatment with sertaconazole resulted in complete clinical recovery of the animal, and prevented the progression of the superficial mycosis by removal of the yeast. In contrast, such treatment apparently did not have any effect on the growth of bacteria.

Finally, it is difficult to assess the possible participation of other microorganisms isolated from the skin lesions of the sea lion, as both *P. phenylpyruvicus* and *S. intermedius* are widely distributed in the environment. They are also common commensals of the animal skin and, under certain conditions, may act as opportunistic pathogens (Shotts et al., 1990; Bowman, 2006; Bowman et al., 2006; Gotz et al., 2006; Leung et al., 2006). Nevertheless, the microbiological findings and the outcome of the antifungal therapy indicate that *R. mucilaginosa* was the main agent responsible for the skin lesions in the sea lion.

To confirm this diagnosis, a skin biopsy should have been performed. However, the procedure for taking biopsy samples in sea lions usually requires advanced medical training (not available for this animal) or capture and sedation or anaesthesia of the animal. The resolution of the skin lesions made this awkward and risky procedure unnecessary.

In conclusion, *R. mucilaginosa* can be considered an opportunistic pathogen that may cause skin lesions in captive pinnipeds. In view of the literature reviewed, this is the first report on the identification of *R. mucilaginosa* from skin lesions in pinnipeds.

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