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Case Report

Pulmonary Aspergillis in the dolphin (*Pontoporia blainvillei*)

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Abstract

Fungal infections that affect marine mammals, like most fungal infections, can be secondary to environmental stress or other infectious diseases. This article describes a case of pulmonary infection by *Aspergillus* spp. in a specimen of *Pontoporia blainvillei* found dead near the town of San Clemente de Tuyu and near the bay of Samborombón, Buenos Aires, Argentina. The lung was collapsed with necrotic foci, fragments were collected, fixed in 10% formalin and submitted to histopathological examination. Microscopically, a marked change of the pulmonary architecture was observed, with total alveolar collapse, extensive necrotic areas, where septal hyphae were observed weakly, when stained with H-E and clearly evidenced with Grocott staining. Since the potential causes of immunosuppression were not evident, the authors consider it probable that infection by *Aspergillus* spp. was established in the lungs that presented a primary, possibly bacterial pneumonia, as a result of some degree of immunosuppression.

Key words: fungal, marine mammals, histopathology.

Introduction

Marine mammals suffer fungi infections, most mycotic infections can be secondary to environmental stress or other infectious diseases. The diagnosis is based on clinical signs and is confirmed by the identification of the organism in biopsy or culture (10, 13).

Fatal pulmonary aspergillosis has been diagnosed in various species of cetaceans, including nose dolphins and orcas, and in various pinnipeds, such as Antarctic marine lobes (*Arctocephalus gazella*), seals and sea lions (4).

This case report describes a case of pulmonary infection by *Aspergillus* spp. in a specimen of *Pontoporia blainvillei* found dead near the town of San Clemente de Tuyu and near the bay of Samborombón, Buenos Aires, Argentina. At Samborombón Bay Rio de La Plata flows with a high load of contaminants.

Case Report

A dead specimen of "Franciscana" *Pontoporia blainvillei* was found close to the village of San Clemente del Tuyu, Buenos Aires, Argentina (36°22'S, 56°27'W), where an "in situ" necropsy was conducted. Grossly, a pleural bilateral stroke was observed, and lungs were condensed with some soft areas of necrotic appearance (Fig. 1). Cerebral edema and a slight hepatomegaly were also identified, while the remaining of the organs did not show macroscopic changes. A 10 x 15 cm fragment of the lungs was extracted and samples from the spleen, heart, skeletal muscle, and brain were also collected, measuring approximately 2.5 x 3 cm and 1 cm thick. All samples were fixed with 10 % buffered formaldehyde solution and sent to the Laboratory of Immunology and Pathology of Aquatic Organisms (LIPOA). In the laboratory, smaller samples of approximately 2 x 2.5 cm and 0.8 cm thick were taken from the lungs' fragment and these were post-fixed in 10% buffered formaldehyde. All tissues were then included in Paraplast. The histological sections were stained with Hematoxylin and Eosin, PAS, while for lung we also stained with Grocott.



Figure 1. Collapsed pulmonary parenchyma with extensive areas of necrosis (arrows). Bar: 1 cm.

Microscopically, a marked alteration of the pulmonary architecture was observed, with total alveolar collapse, extensive necrotic areas, these lesions were observed in the entire lung fragment studied. Granulomas were not observed. Septal hyphae were observed weakly when stained with H-E and clearly evidenced with Grocott staining. Hyphae were divided by dichotomous branching at an acute angle and occasionally presented localized dilations (Figs. 2, 3 and 4). Perineuronal edema and glial proliferation was observed in the brain and isolated hemorrhage foci were found in the liver. No significant alterations were found in other organs.

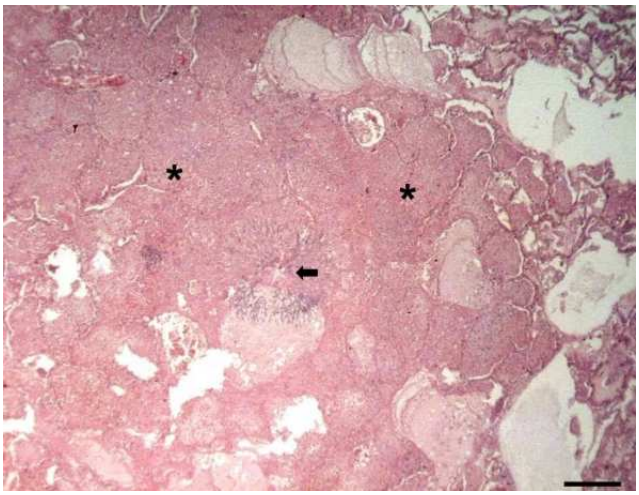


Figure 2. Pulmonary parenchyma with its modified architecture, where extensive areas of necrosis occupying the alveolar light (*) are observed in the center, structures that appear to be mycotic hyphae (arrow). H-E, Bar: 200 µm.

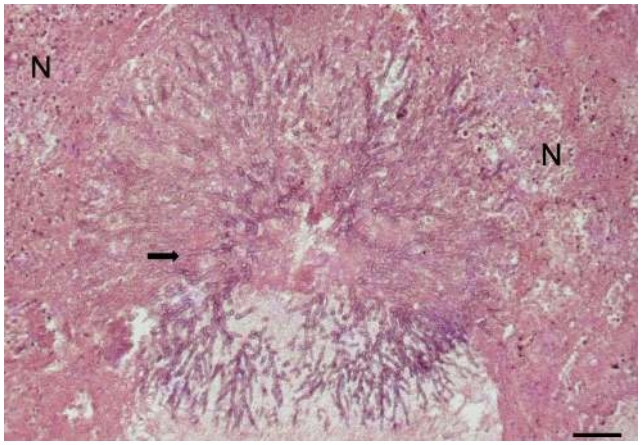


Figure 3. A greater increase in the anterior image, where pulmonary necrosis (N) and mycotic hyphae of septal aspect (arrow) are observed. H-E, Bar: 100 µm.

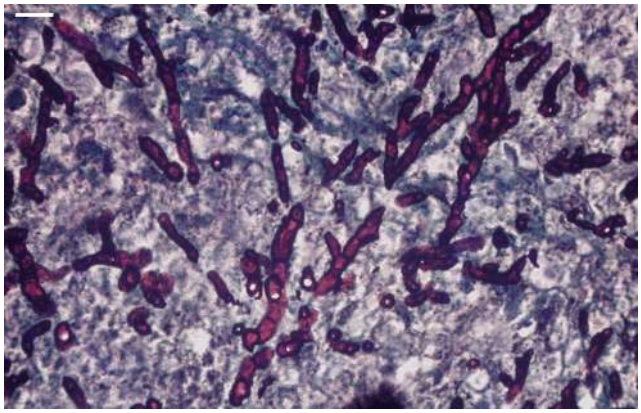


Figure 4. Pulmonary parenchyma with Grocott positive hyphae, septate, compatible with *Aspergilius* spp. Grocott Bar: 20 µm.

Discussion

Mycoses in marine mammals already diagnosed and reported are usually associated with a secondary chronic infectious process in cetaceans. In a review of 143 cultivated and wild marine mammals, 24 specimens of animals with mycotic infections, affected by 19 different fungal species, were found. Sixty-nine (48%) of these animals were stranded and 24 of them had a mycotic disease (2, 11, 14). Water contamination can generate immunosuppression in these animals, which can contribute to the occurrence of massive infections, both parasitic and bacterial, and mycotic (3).

Aspergillosis is one of the best-known mycotic pathogens that produce pneumonia in marine mammals (12). In this case report, the dolphin was infected with *Aspergillus* spp. In a contaminated aquatic environment, the fungus enters the body through the respiratory tract and can soon spread to distant sites. Systemic aspergillosis may originate at any of the primary sites of infection, especially the skin, lung, nasal sinus, and digestive system (9).

It is possible that the fungus would initially infect through the skin, causing multiple fungal pyogranulomas, which may be the primary site of the infection, then spread and end up circulating through the blood (12).

Mycotic infections are observed especially in immunodepressed animals, immunodepression may be caused by a contaminated environment.

It has already been demonstrated that the mortality of common tonines (*Phocoena sp*.) due to mycotic diseases in the waters around England and Wales, in addition to fungal pathogens, also presented higher concentrations of mercury in the liver (1), compared to those affected by non-infectious diseases, which suggests that they may have been immunocompromised by this contamination. Some authors suggest that polychlorinated biphenyls may also predispose aquatic mammals to diseases (7). The present case is a dolphin found near the bay of Samborombón, where La Plata River flows, which drags contaminants transported through the rivers Paraná and Uruguay, from the city of Buenos Aires, greater Buenos Aires and the Salado River Basin, one of the main basins with agricultural activity in Argentina, where pesticides are used abundantly. It has been demonstrated that the effects of contaminants in the waters of the Rio de La Plata in the Atlantic Sea generate immunodepression in animals living in these waters (5, 6, 8). Since the potential causes of immunosuppression were not evident, the authors consider it probable that infection by *Aspergillus* spp. was established in the lungs that presented a primary, possibly bacterial pneumonia, as a result of some degree of immunosuppression.

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