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CLINICAL AND PATHOLOGICAL FINDINGS OF ASPERGILLOSIS IN MAGELLANIC PENGUINS (*Spheniscus magellanicus)*

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ABSTRACT

We studied a series of fifteen fatal cases of aspergillosis in penguins (*Spheniscus magellanicus*), seen over a 4-year period at a rehabilitation center in Southern Brazil. The clinical and pathological findings based on the lesions found at necropsy are described herein. The majority of animals (11/15) had sudden death without clinical signs. In 33.3% (5/15) of the cases, aspergillosis was restricted to the respiratory system and 66.6% showed disseminated disease, with liver, kidney, adrenal gland and gastrointestinal tract involvement. Typical lesions were characterized as white-yellowish granulomatous nodules. To the best of our knowledge, this is the largest series of aspergillosis cases described in penguins in South America.

KEYWORDS: aspergillosis; avian pathology; mycosis; penguins; seabirds.

ALTERAÇÕES CLÍNICAS E PATOLÓGICAS DA ASPERGILOSE EM PINGUINS-DE-MAGALHÃES (*Spheniscus magellanicus)*

RESUMO

Uma série de 15 casos fatais de aspergilose em pinguins (*Spheniscus magellanicus*) foi observada durante um período de quatro anos em um centro de reabilitação no Sul do Brasil. Os achados clínicos e patológicos das lesões encontradas na necropsia são aqui descritos. A maioria dos animais (11/15) teve morte súbita sem evidenciar sinal clínico prévio. Em 33,3% (5/15) dos casos, aspergilose estava restrita ao trato respiratório e 66,6% demonstrou doença disseminada, com envolvimento hepático, renal, de glândulas adrenais e trato gastrointestinal. Lesões típicas se caracterizaram por nódulos granulomatosos branco-amarelados. De acordo com a literatura consultada, essa é a maior série de casos de aspergilose descrita em pinguins na América do Sul.

PALAVRAS-CHAVE: aspergilose; aves marinhas; micoses; patologia; pinguins.

INTRODUCTION

Aspergillosis is a common disease affecting captive penguins. Although the infection primarily involves the respiratory tract, a wide range of clinical presentations are seen, with high rates of associated mortality (ABUNDIS-SANTAMARIA, 2003; KEARNS & LOUDIS, 2003; TELL, 2005). Both acute

and chronic aspergillosis have been described in penguins. Acute cases are usually associated with a high inoculum and/or severe states of immunosuppression, which quickly progresses to death within 24-48 hours. Chronic aspergillosis has a prolonged clinical course, causing death in weeks or months (ABUNDIS-SANTAMARIA, 2003; KEARNS & LOUDIS, 2003).

Penguins undergoing rehabilitation are in a captive care situation until they can be released back to the wild. This condition is common in injured animals, as mainly oiled penguins (RUSSEL et al., 2003) are considered as predisposed to acquire the fungal infection. This study aimed to evaluate the clinical and anatomopathological findings of 15 Magellanic penguins (*Spheniscus magellanicus*) diagnosed with aspergillosis during the rehabilitation process.

MATERIAL AND METHODS

A series of cases of fatal aspergillosis in 15 Magellanic penguins was studied. The affected animals were penguins in a temporary captive care situation, while being rehabilitated for release back to the wild. During a 4-year period (January 2004 - December 2007) at a rehabilitation center in Southern Brazil (32ºS/52ºW), 52 penguins died and were submitted to post-mortem examination to determine the cause of death. Fifteen of them (29%) were diagnosed as aspergillosis cases and then selected to this study.

All these aspergillosis cases were confirmed through mycological and histopathological evaluation of the lung and other altered tissue collected during the necropsy. Cases were characterized as proved aspergillosis by the demonstration of hyaline and septate hyphae on direct microscopic exam using potassium hydroxide (KOH 20%) associated with the *Aspergillus* sp. isolation on culture (Sabouraud dextrose agar with chloranphenicol), and the fungal structures observed in histopathology (Hematoxylin-Eosin and Gomori-Grocott methods). Five of these cases were already cited by XAVIER et al. (2007).

A detailed necropsy was done in the 15 penguins and all macroscopic visual lesions from each animal were measured and technically described in separate records. Disease presentation was classified as localized or disseminated based on the lesions found at necropsy. Clinical data of these penguins that died due to *Aspergillus* infection, were collected evaluating the records of each animal provided by the rehabilitation center.

RESULTS AND DISCUSSION

Aspergillus fumigatus caused disease in 14/15 (93.3%) of the penguins included in this series. One case was associated with *A. flavus* infection. Direct microscopic exam and histopathology showed septate, hyaline and branching hyphae in tissues and complete asexual reproductive structure of *Aspergillus* in the air sacs involved. The vast majority of the animals suffered from sudden death, except four penguins that showed anorexia, voice change, and dyspnea right before death.

In 5/15 (33.3%) of the cases, aspergillosis was restricted to the respiratory system (Table 1). This was pathologically characterized by pulmonary congestion associated with multiple white-yellowish granulomatous nodules, ranging 0.1-1.0 cm in diameter. These nodules were diffusely distributed in the lungs. Thickening of air sacs was also observed, and a few fungal colonies were identified in these areas.

Table 1. Anatomical sites of aspergillosis in penguins

Magellanic Penguin	Lung / Air sacs	Syrinx	Disseminated			
			Liver	Adrenal	Kidneys	GI tract
1	GN	-	-	-	-	-
2	GN	-	-	-	-	-
3	GN / FC	-	-	-	-	-
4	GN / FC	-	-	-	-	-
5	GN / FC	GN	-	-	-	-
6	GN / FC	GN	GN / FC	-	GN	-
7	GN / FC	GN	GN	-	GN	GN
8	GN / FC	GN	-	-	GN	-
9	GN / FC	GN	-	-	GN	GN
10	GN	GN	-	-	GN	GN
11	GN	GN	-	-	-	GN
12	GN / FC	-	GN	GN	-	-
13	GN	-	-	GN	-	-
14	GN / FC	-	-	GN	-	-
15	GN	-	-	-	-	GN

GI: Gastrointestinal; GN: Granulomatous nodules; FC: Fungal colonies.

Disseminated aspergillosis was seen in 10/15 (66.6%) (Table 1). Severe involvement of the respiratory tract was observed in all of these cases. In some cases, adherence of the air sacs to the celomic wall was observed (Figure 1). There were abundant plaque-like caseous and necrotic debris covering the air sacs, with grayish-green fungal colonies on the surface of the air sacs. The pulmonary parenchyma showed hemorrhages with multiple granulomatous nodules and necrotic areas.

Adrenal involvement was observed in 3/15 (20%) of the cases, two occurring in the right adrenal gland and one affecting the left side gland. These occurred as large masses of 8-10 cm, formed by multiple granulomatous nodules (Fig. 1).

The kidneys were affected in 5/15 (33.3%) of the birds, in which a few up to multiple granulomatous nodules were seen. Cortical lesions were observed 3/5 (60%) of these animals. The other two penguins had friable necrotic lesions on the kidney, which distorted the anatomy of the organ.

Granulomatous nodules in the liver were observed in 2/15 (13.3%) of the cases and fungal colonies seen in one of them. The gastrointestinal tract showed granulomatous nodules ranging from 0.1-1.0 cm in 5/15 (33.3%) of the animals, involving the serosal layer of the esophagus, stomach, omentum and mesenterium.

Syrinx lesions occurred in 1/5 (20%) with pulmonary aspergillosis, in the penguin that showed voice change, and in 6/10 (60%) animals with disseminated disease. These lesions manifested by multiple granulomatous nodules, sometimes occurring as large masses of 5-10 cm, containing necrotic debris and caseous exudates. Partial or total obstruction of the air passage occurred in 4/7 (57.1%) cases (Figure 1).

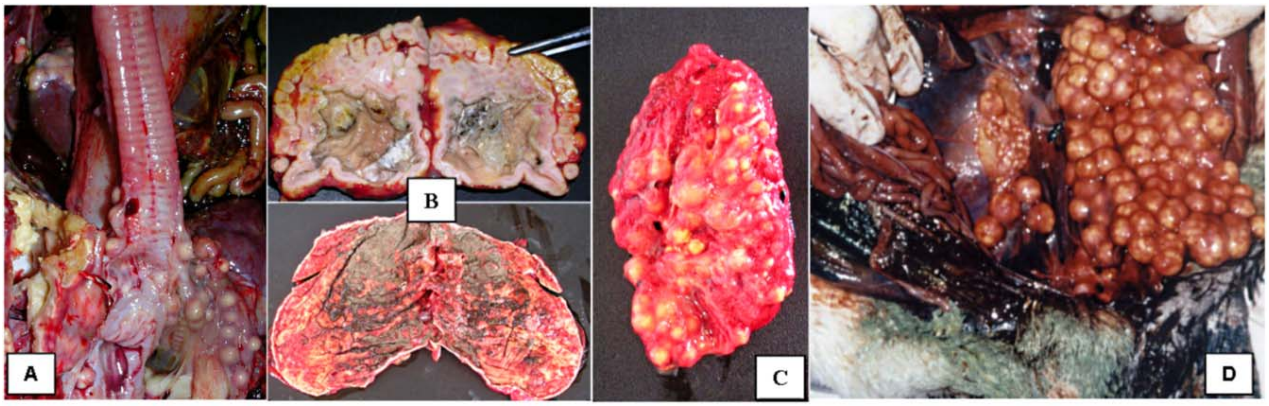

Figure 1. Disseminated aspergillosis in Magellanic penguins. A) Granulomatous lesions in the syrinx forming masses that partially obstructed the air passage; B) Air sacs thickening containing fungal colonies; C) Lung parenchyma with multiple nodules (arrows) and necrosis foci (large arrows); D) Multiple coalescent nodules resulting in masses in the adrenal gland.

Avian predisposition to aspergillosis is attributed to anatomical and physiological factors, related to the respiratory tract. These include absence of a diaphragm and epiglottis, limited distribution of pseudo-stratified ciliated columnar cells, lack of surface macrophages, and mainly the presence of air sacs. Air sacs are warm cavernous structures that contain high oxygen concentrations and few blood vessels to carry immune cells for host protection (BAUCK, 1994; TELL, 2005). Air sacs provide an optimal habitat for fungal growth, commonly showing the presence of reproductive phases of *Aspergillus* species, which is an inherent characteristic of avian aspergillosis (TELL, 2005). Accordingly, these tissues were affected in 100% of the penguins evaluated in this study.

In penguins the trachea bifurcates and, through lumen constriction, it promotes air turbulence. This allows fungal conidia to escape from the exhalation air-flow, to deposit on the epithelium and develop focal infection (BAUCK, 1994). During the interaction between conidia and host immune cells, necrotic debris and caseous exudates are deposited in these sites. This can obstruct the air passage, resulting in a range of clinical manifestations that include voice change and dyspnea. The syrinx, situated at the tracheal bifurcation, can be the only tissue infected in the host, characterizing the tracheal form of aspergillosis (ABUNDIS-SANTAMARIA, 2003; KEARNS & LOUDIS, 2003). However, in our study this presentation was not found, since 85.7% (6/7) penguins with syrinx lesions had disseminated aspergillosis, and 14.3% (1/7) had diffuse lower respiratory tract disease. It is possible that the infection was initiated on the syrinx in three severe cases, in which large masses were observed causing

air-flow obstruction. This speculation is based on the fact that larger lesions were observed in the syrinx in these cases, in relation to the other tissues affected.

Our study supports other descriptions in which the main sites involved in the disseminated disease were liver, other abdominal organs and the gastrointestinal serosae (REDIG, 1993; ABUNDIS-SANTAMARIA, 2003; KEARNS & LOUDIS, 2003). We also observed frequent involvement of adrenal glands in penguins with disseminated aspergillosis, which might be associated to the higher blood-flow to this organ. Although adrenal gland's involvement has been described in humans (MAATEN et al., 1995), this occurrence has not yet been described within the pertinent literature about aspergillosis in penguins.

The largest study on aspergillosis in captive wild birds showed 11 cases in three species of penguins (*Aptenodytes patogonica, Pygoscelis antartica,* and *S. magellanicus*) (AINSWORTH & REWELL, 1949). Air sacs and lungs were affected in all cases, but lesions were not found in other tissues. In our study, infection was restricted to the respiratory system in only 33.3% (5/15) of cases, with 66.6% (10/15) showing disseminated aspergillosis. Disseminated infections have also been reported in other wild bird species, some showing lesions on syrinx, liver, spleen and pericardium (AINSWORTH & REWELL, 1949). Studies in zoos documenting aspergillosis in penguins are usually associated with outbreaks, involving the respiratory tract (KHAN et al., 1977; FLACH et al., 1990) and the liver (KHAN et al., 1977).

In rehabilitation centers, aspergillosis does not occur as outbreaks. Instead, isolated cases are seen in immune-compromised animals (RUSSEL et al., 2003; XAVIER et al., 2007). Susceptibility to aspergillosis in wildlife hospitals is aggravated by stress due to habitat change, transport and handling, and also due to the underlying problem, which caused them to be in rehabilitation in the first place. In addition, animals are improperly treated with corticosteroids and antibiotics. Conditions like malnutrition, dehydration and oil fouling are not uncommon (RUSSEL et al., 2003; TELL, 2005; SILVA-FILHO & RUOPPOLO, 2006). On a publication from Southern Brazil, CARRASCO et al. (2001) reported the case of an oiled penguin diagnosed with aspergillosis. Disseminated granulomatous nodules were observed in the respiratory tract, liver, kidneys and stomach serosa. In another study with oiled seabirds in rehabilitation, aspergillosis lesions were also observed in the lungs, air sacs, liver and kidneys (BALSEIRO et al., 2005).

In contrast to the findings in captivity, aspergillosis is not common in free-ranging penguins. In a study investigating the causes of death in these animals, the 11 cases of aspergillosis were restricted to the respiratory tract and no dissemination was observed (HOCKEN, 2000). While captive penguins may present several factors favoring disease dissemination (HOCKEN, 2000; RUSSEL et al., 2003; TELL, 2005; SILVA-FILHO & RUOPPOLO, 2006), malnutrition is usually the only predisposing factor to opportunistic infections amongst wild seabirds.

This study shows the importance of the disease in penguin rehabilitation which has the tendency to cause disseminated lesions and do not be restricted to the respiratory system. To the best of our knowledge, this is the largest series of aspergillosis cases described in penguins in South America.

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