LETHAL CONCURRENT AVIAN MALARIA AND ASPERGILLOSIS IN MAGELLANIC PENGUIN (*Spheniscus magellanicus*)

Concurrencia letal de malária aviar y aspergilosis en pingüino de Magallanes (*Spheniscus magellanicus*)

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RESÚMEN. - Malaria aviar (*Plasmodium* spp.) y aspergilosis (*Aspergillus* spp.) se consideran las dos enfermedades infecciosas más relevantes para los pingüinos en cautiverio. Aquí presentamos el caso de un pingüino de Magallanes adulto (*Spheniscus magellanicus*), que murió mientras se somete a rehabilitación. Resultados de la necropsia fueron característicos de la aspergilosis y sugerente de la malaria aviar, ambos fueron confirmados más tarde por medio de histopatología, histoquímica, cultivo micológico y microscopía electrónica.

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Magellanic penguins (*Spheniscus magellanicus*) are native to Argentina and Chile, and during winter they may swim northwards towards the Uruguayan and Brazilian coast. Every year, a fraction of these penguins becomes beach-cast in Argentina, Uruguay and Brazil, particularly if oiled or injured, being then sent to rehabilitation centers (Garcia Borboroglu *et al.* 2008). During rehabilitation as well as when maintained in permanent captivity, these penguins are susceptible to a number of infectious diseases, among which avian malaria and aspergillosis are considered the most relevant (Fowler & Fowler 2001).

Avian malaria results from the infection by *Plasmodium* sp., protozoans that are transmitted to birds by mosquitoes (*Culicidae*). Although Plasmodium infections tend to be relatively harmless for most birds, some taxa such as penguins are highly susceptible (Jones & Shellam 1999, Lapointe *et al.* 2012). The two most commonly reported species infecting penguins are *P. relictum* and *P. elongatum* (Jones & Shellam 1999), however there also reports involving *P. juxtanucleare* (Grim *et al.* 2003) and *P. cathemerium* (Luera- Carbo 1965). Pathogenesis of avian malaria is complex, and develops from the infection of circulating cells (erythrocytes) and tissues (endothelial cells, hemopoietic system, lymphoid-macrophage system), and the secondary effects associated to the hemolysis, vascular rupture and thromboembolism (Soni & Cox 1975, Atkinson 2008).

Aspergillosis is a fungal infection acquired through the inhalation of *Aspergillus* spp. conidia; this fungal genus is a ubiquitous, saprophytic and opportunist mold (Ainsworth & Rewell 1949). Infection is generally thought to be opportunistic, being mostly limited to indivi-

duals with impaired immunity (Verstappen & Dorrestein 2005). *Aspergilus fumigatus* is the species most frequently reported infecting penguins, but A. flavus also occurs occasionally (Carrasco *et al.* 2001, Xavier *et al.* 2007, 2011). Pathogenesis of aspergillosis is associated to the fungal colonization of the air sacs, lungs, trachea, bronchi and/or nasal cavity, with associated heterophilic and granulomatous responses (Femenia *et al.* 2007, Xavier *et al.* 2011). In some cases, hematogenous dissemination to non-respiratory tissues may also occur (Xavier *et al.* 2011).

An adult female Magellanic penguin (*Spheniscus magellanicus*) was found beach-cast in the coast of Rio de Janeiro, Brazil, and was transferred for rehabilitation at the Centro de Recuperação de Animais Marinhos (CRAM-FURG – 32°02'S 52°05'W), Museu Oceanográfico Prof. Eliézer de Carvalho Rios, Universidade Federal do Rio Grande (Rio Grande - RS, Brazil). The penguin had not been oiled and did not present external lesions. Upon admission to CRAM-FURG on 13/11/2005, the bird was molting and had a body mass of 2.9 kg, whereas healthy free-ranging adult Magellanic penguins molt in February-April and weight approximately 4 kg (Cranfield 2003). On 30/01/2006, with a body mass of 2.6 kg, the penguin presented apathy, pale mucosa, anorexia and dyspnea, dying within 24 hours.

After death of the animal, the necropsy findings included: a large blood clot was contained within the omentum and serosa of spleen and syntopic organs (about 15 x 6 x 3 cm.) (Figure 1a and 1b.); marked increase in the spleen (approximately 7 x 4 cm.) and rupture cracks; severe hepatomegaly; marked pulmonary congestion and edema; slightly thickened (≤ 1 mm), cranial thoracic air sacs with small multifocal white firm nodules (≤ 5 mm) (Figure 1c).; a white solid nodule (approx. 25mm) is also connected to the airbag adjacent to a blood clot spleen (Fig. 1a).

Histological examination revealed (Fig. 2): severe granulocytic diffuse fibrino-hemorrhagic splenitis, with large areas of hemorrhage; moderate to severe granulocytic interstitial pneumonia, with moderate to severe lung congestion and edema; presence of occasional tissue meronts compatible with Plasmodium in the lung; moderate mixed multifocal to coalescent periportal hepatitis, with multifocal foci of extra-medullar hematopoiesis; mild granulocytic myocarditis; mild multifocal tubular renal necrosis. Furthermore, air sacs were thickened by large layers of necrotic-granulocytic material, surrounded by giant cell formation and heterophilic infiltrate (piogranuloma); silver staining (Gomori-Grocott) revealed the presence of abundant fungal hyphae branching at 45° angles within the necrotic material.

Ultrastructural examination of the blood clot revealed the presence of intracytoplasmatic inclusions in-

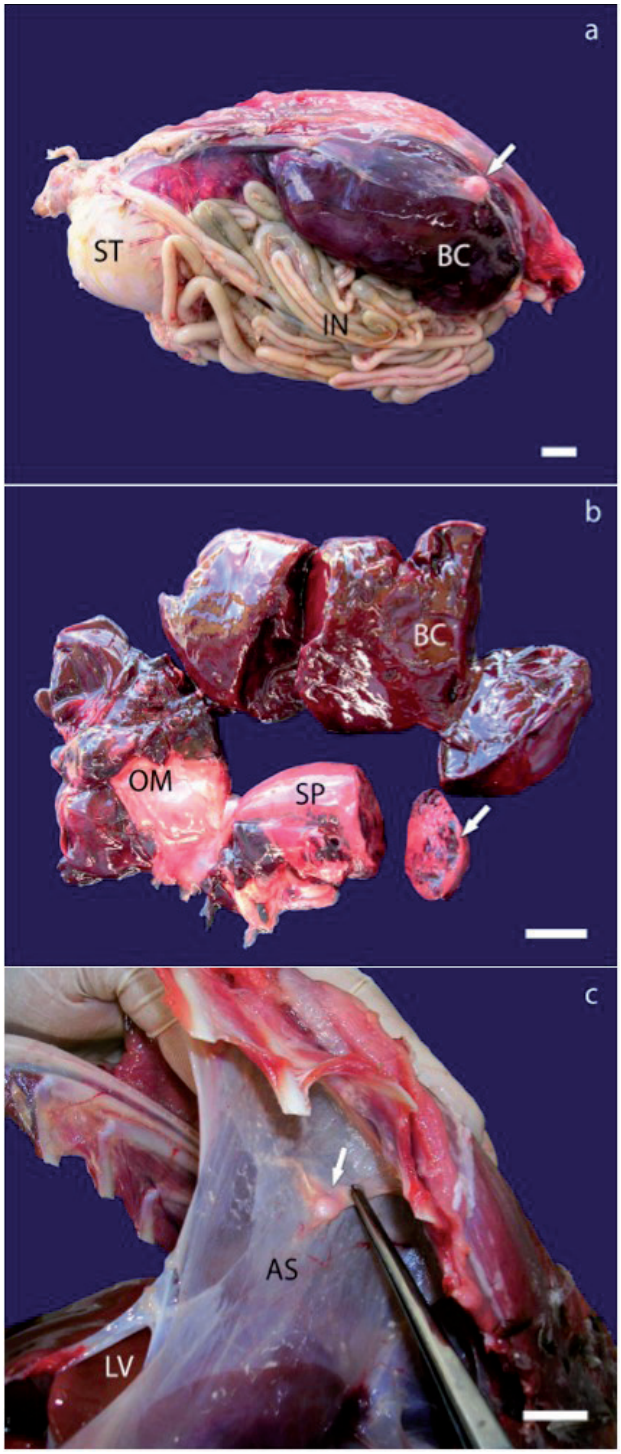

Figure 1. Gross necropsy findings. (a) A large blood clot (BC) surrounds the spleen, with a white nodule is attached to its surface (arrow); the intestines (IN) and caudal stomach (ST) are also visible. (b) Dissection of the large blood clot (BC) reveals it surrounds the spleen (SP), which has numerous areas of hemorrhage are visible on the cut surface of the spleen (arrow); parts of the omentum (OM) may also be seen. (c) Mildly thickened right cranial thoracic air sac (AS), with a white nodule compatible with fungal colonization (arrow); an area of the liver (LV) may also be seen. Scale bars represent 2 cm.

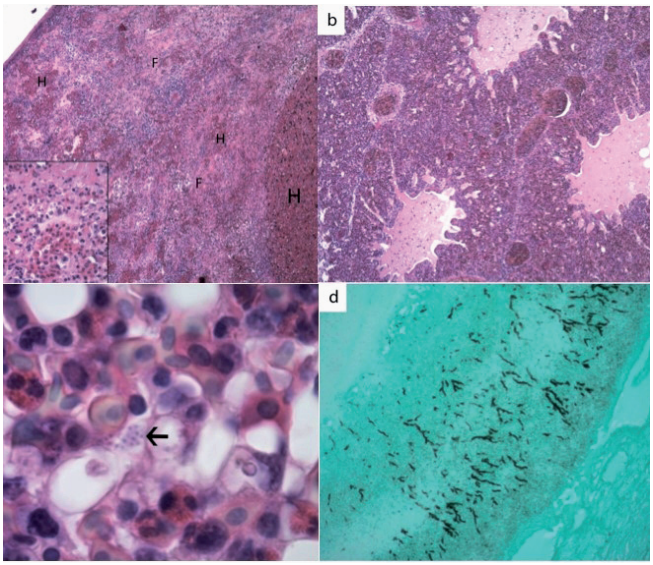

Figure 2. Select histopathological findings. (a) Spleen (H.E., 40x) with numerous areas of hemorrhage (H) and fibrin deposits (F); fibrin deposit are shown on the detail (H.E., 400x). (b) Lung (H.E., 40x) with marked diffuse congestion and edema. (c) Lung (H.E., 1000x) with a tissue meront (arrow). (d) Air sac (Gomori-Grocott, 100x) with numerous fungal hyphae (stained in black) within the layers of necrotic tissue.

side the erythrocytes, which were compatible with early parasitic stages of *Plasmodium* spp. (Fig. 3). Swabs were retrieved from the nodular air sac lesions and were cultured at 37 °C in Sabouraud dextrose; after 48 hours, fungal colonies consistent with *A. fumigatus* were observed (macroscopy: filamentous green-bluish surface; microscopy: pyriform vesicle and uniseriate phialides).

Their epidemiology, however, is profoundly distinct: avian malaria is a vector-borne protozoan infection that produces sporadic outbreaks with high mortality (Stoskopf & Beier 1979, Bueno *et al.* 2010), whereas aspergillosis is an opportunistic fungal respiratory infection that produces chronic and progressive clinical disease and high mortality (Xavier *et al.* 2007, 2011).

In the studied case, blood volume lost associated to hemorrhage from the spleen rupture was considerable and in association to the pulmonary congestion and splenic parenchymal hemorrhage, rendered hypovolemic/ distributive circulatory shock syndrome the most likely cause of death, probably due to avian malaria. Moreover, oxygenation was likely severely compromised by the extensive interstitial pneumonia and also, to a lesser extent, due to the thickening and fungal colonization of the air sacs. Red blood cell counts, hemoglobin concentration or hematocrit could not determined, but anemia is also likely to have occurred due to parasite-induced hemolysis (Soni & Cox 1975). Considering the apparent relative importance of these factors, it seems likely that avian malaria played a more decisive role in leading to death than did aspergillosis.

Concurrent aspergillosis in penguins deceased due to *Plasmodium* infection has been described occasionally. Both Scott (1927) and Rewell (1948) reported cases of avian malaria in king penguins (Aptenodytes patagonicus) at the London Zoological Gardens which had concurrent mycosis; although the etiological agent was not specified, it is fair to presume the authors referred to aspergillosis. Grünberg & Kutzer (1963) found that two of the eighteen penguins (*Spheniscus humboldti* and *S.*

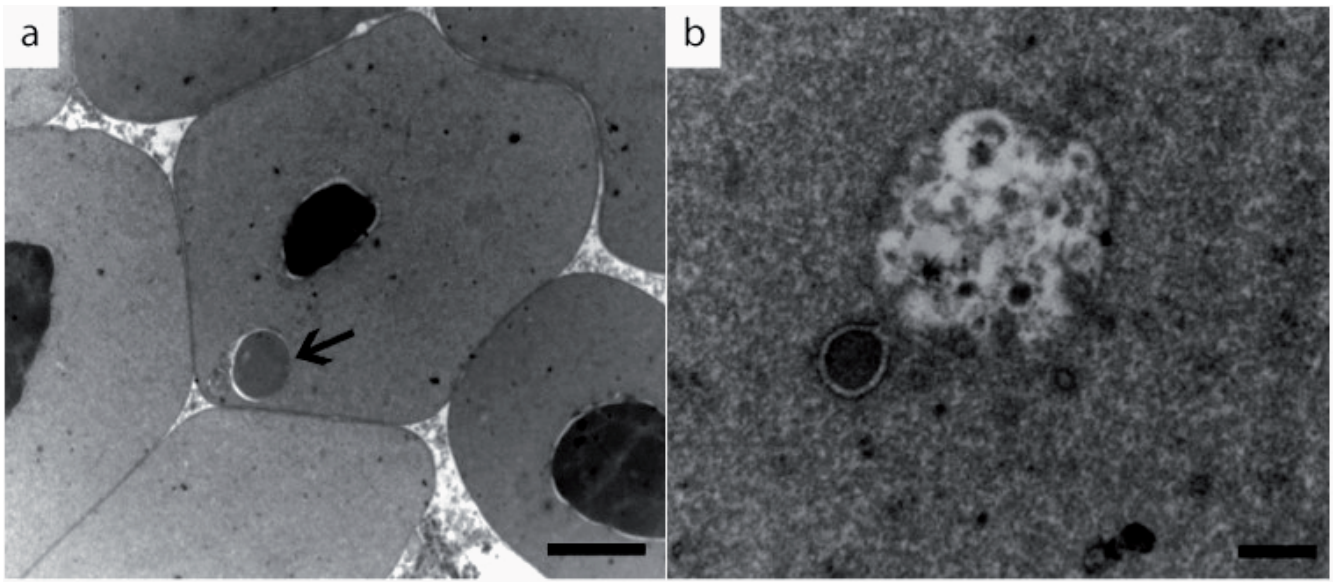

Figure. 3. Ultrastructural examination. (a) Parasitized red blood cell with an intracitoplasmatic merozoite (arrow) (scale bar = 2 µm). (b) Internalized merozoite and in the early stages of parasitophorous vacuole formation (scale bar = 200 nm).

demersus) from the Schönbrunn Zoo that died during an outbreak of avian malaria also had lesions associated to aspergillosis. Because of the gravity of the lesions associated with avian malaria and the celerity with which this disease can produce mortality in penguins, the concurring *Aspergillus* infections in these cases were generally interpreted as secondary necropsy findings. Our necropsy and histopathological findings are coherent with the interpretation that aspergillosis had a longer term and more chronic development than avian malaria. As such, it may be speculated that the animal would have had a slow and progressive *A. fumigatus* infection until the much more abrupt *Plasmodium* sp. infection developed and produced death. The potential role of Aspergillus as a predisposing condition, however, should not be disregarded. Even though *Plasmodium* is highly pathogenic to penguins and may cause the death of healthy individuals, subclinical infections are known to occur, particularly in individuals that were previously exposed to the pathogen (Cranfield *et al.* 1994). There are also a number of cases of non-vaccinated juvenile Magellanic penguins at CRAM-FURG in which malarial infection was clinically asymptomatic and failed to produce mortality. Furthermore, considering that experimental vaccines may reduce the severity of avian malarial infections in penguins (Grim *et al.* 2004) and that corticoid treatment may induce parasite recrudescence (Cranfield *et al.* 1994), it is clear that immune status plays a role in determining the susceptibility of penguins to avian malaria. Because *Aspergillus* elicits a strong immune response, with a marked involvement of heterophils and macrophages (Femenia *et al.* 2007), it is likely that a corresponding shift in cytokine secretion and immune cell recruitment/proliferation occurs in the infected penguins, as is known to occur in human and murine aspergillosis (Hebart *et al.* 2002). In this sense, the immune modulation associated to the chronic aspergillosis could result in increased susceptibility to malarial parasites, in an interaction analogous to that demonstrated between nematode infection and malarial parasites in mice (Su *et al*. 2005).

The studied case presented a splenic rupture, a finding that is atypical in aspergillosis and that called attention on the possibility of a concurrent pathological process. In these cases, because the gross findings of aspergillosis are exuberant and promptly detected, institutions sometimes bypass histopathology or complementary diagnostic tests and conclude aspergillosis was the cause of death, thus potentially underestimating the prevalence of avian malaria and other pathological processes. This case therefore illustrates and emphasizes the importance of conducting histopathology and complementary diagnostic tests regardless how exuberant the aspergillosis necropsy findings.

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