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Case Report

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First Report of Oral Candidiasis by *Candida lusitaniae* in a Red Kite (*Milvus milvus*, Lineu 1758)

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Abstract

A free-living red kite (*Milvus milvus*, Lineu 1758) adult male was admitted to the CERAS - Centro de Estudos e Recuperação de Animais Selvagens (Castelo Branco, Portugal) debilitated and with injuries caused by lead projectiles. During the physical exam small lesions, not larger than 7 mm, were observed in the distal portion of the soft palate, laterally to the choanae, compatible with oral candidiasis. *Candida lusitaniae* was the agent isolated from the exudate that was removed from the lesions. This is the first reported case of *C. lusitaniae* associated to oral candidiasis and the first time this bird species was associated with this agent.

KEYWORDS
Candida lusitaniae, Milvus milvus, Yeast, Candidiasis

CLINICAL CASE

The red kite (*Milvus milvus*, Lineu 1758) is a migratory medium-large diurnal bird of prey from the family Accipitridae, who resides in the Western Palearctic region of Europe and northwest Africa (Almeida *et al*., 2005;). This species is classified as Least Concerned (LC) according to the IUCN Red List of Threatened Animals (The IUCN Red List of Threatened Species 2020, 2020). In Portugal the resident population is classified as Critically Endangered (CR) according to the Livro Vermelho de Vertebrados de Portugal (Almeida *et al*., 2005). The species has suffered a decrease in its population and distribution area, due to habitat destruction, direct persecution (shotgun) and poisoning (Almeida *et al*., 2005).

Candidiasis is placed among one of the most common infectious diseases in birds of prey (Deem, 2003). The most common species responsible by candidiasis in rapine birds are *Candida albicans, C. parapsilosis, C. rugosa, C. krusei, and C. tropicalis* (Deem, 2003). It is an opportunistic agent that is present in the gastrointestinal tract of these animals (Deem, 2003; Ford, 2010). Disease manifestation usually occurs in animals that are held in captivity associated to stress, immunocompromised animals, nutritional disorders, prolonged antibiotic therapy, or linked to other primary disease (Deem, 2003; Ford, 2010). It is one of the biggest impediments to successful recovery of birds of prey in Wildlife Rehabilitation Centres (Deem *et al*., 1998; Deem, 2003).

Candida lusitaniae is an extremely rare yeast (Hawkins and Baddour, 2003). It has been recently associated to some nosocomial infections in human hospitals, particularly in patients with cancer (Guinet *et al*., 1983; Minari *et al*., 2001; Tikotekar *et al*., 2008; Wawrysiuk *et al*., 2018; Apsemidou *et al*., 2020). This agent is known for its resistance to amphotericin B (Guinet *et al*., 1983; Asner *et al*., 2015).

An adult red kite (*Milvus milvus*) was admitted to CERAS - Centro de Estudos e Recuperação de Animais Selvagens (Castelo Branco, Portugal) with a normal body condition (2.5 on a scale of 0 to 5), weighing 772 g (within the expected range) and a mild degree of dehydration (6%). It presented a closed and comminuted fracture in the diaphysis of the right ulna. It had perforating wounds, with entrance to the muscle in the right knee, tibiotarsus area, left femur, at the level of the 3rd phalanx of the 3rd digit of the left hind limb. Feathers in the anatomical region of

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both femurs were damaged at the level of the beards and with a burnt appearance and thickened skin tissue in that region (groin). The animal was submitted to a radiographic examination where it was possible to observe numerous six lead projectiles, dispersed in various locations of the animal's body.

In the oral cavity two lesions were observed (not larger than 7 mm), in the distal portion of the soft palate, laterally to the choanae. One of them perforated the mucosa, with little depth and with greyish pasty content inside, and the other with a nodular shape (cauliflower appearance), grey in colour and haemorrhagic to the touch.

Swabs of the lesions in the oral cavity were collected and sent for fungal culture at INNO – Veterinary Laboratory (Braga, Portugal). The sample was plated onto solid culture media CHROMID® Candida/Sabouraud Gentamicin Chloramphenicol 2 Agar (REF 43464, BióMerieux, France) and incubated at 30-35°C during 48 h. On CHROMID® *Candida* Agar blue, dome-shaped and glistening colonies with 2-3 mm of diameter were observed. Sabouraud Gentamicin Chloramphenicol 2 Agar white, dome-shaped and glistening colonies with 2-3 mm of diameter were observed (Fig. 1). Species identification was made using the isolated colonies by VITEK® 2 Compact (YST ID Car REF 21343, BioMérieux, France). The identified agent was *Candida lusitaniae*. Susceptibility of the yeasts to antifungal agents was performed using FUNGITEST Kit (REF 60780, BioRad) by broth dilution method. The isolated agent was susceptive to all six antifungal agents: amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, ketoconazole and miconazole.

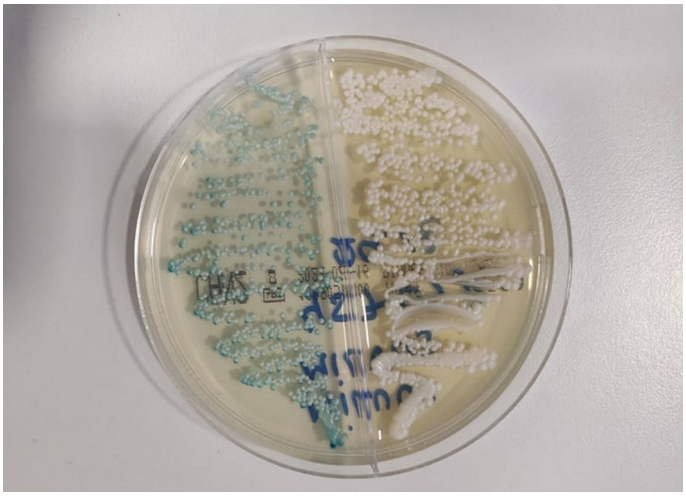

Fig. 1. *Candida lusitaniae* grown onto in solid culture media CHROMID® *Candida*/Sabouraud Gentamicin Chloramphenicol 2 Agar (REF 43464, BióMerieux, France) incubated at 30-35° C during 48h. On CHROMID® *Candida* Agar were observed blue, dome-shaped, and glistening colonies with 2-3 mm of diameter; and on the Sabouraud Gentamicin Chloramphenicol 2 Agar were observed white, dome-shaped, and glistening colonies with 2-3 mm of diameter.

Treatment of gunshot injuries included supportive therapy (fluid therapy with 0.9% Sodium Chloride and vitamin supplementation with Duphalyte®), anti-inflammatory (meloxicam 1 mg/kg, IM, SID), analgesic for pain management (buprenorphine 0.25 mg/kg, IM, SID and tramadol 10 mg/kg, PO, BID), antibiotic (amoxicillin and clavulanic acid 125 mg/kg, PO, BID), fracture stabilization (figure of 8 bandage), soft tissue wound care and excision of the projectiles. *Candida*-associated lesions in the oral mucosa were scrubbed with chlorhexidine 0.2% and pieces of growth were removed with tweezers and swabs, during the recovery period of the animal (3 months). When the animal was released back to its habitat, it only showed mucosal erosion at the lesion site (Fig. 2).

Fig. 2. Mucosal erosion in the oral cavity of red kite (*Milvus milvus*) after recovery from candidiase by *Candida lusitaniae*.

C. lusitaniae, the agent identified in this case, is an opportunistic agent, especially when the immune system of the host is compromised or previous primary infection occurs (Apsemidou *et al*., 2020; Minari *et al*., 2001). Although rare, it has been isolated in the oral and digestive flora of birds, such as pigeons (Pakshir *et al*., 2019). Candidiasis has been described in several species of animals (domestic and non-domestic) (Seyedmousavi *et al*., 2018), but in birds the oral and gastrointestinal forms are the most common (Deem, 2003; Seyedmousavi *et al*., 2018), as observed in this case.

Infections by *Candida* species in animals are usually caused by strains that commensally exist in the microbiota flora of the host rather than by vertical or longitudinal transfer (Seyedmousavi *et al*., 2018). In the case presented, the most likely scenario is that this yeast already existed in the oral cavity as commensal and the manifestation of the disease was due to immunosuppression caused by its wounds. Nevertheless, we cannot eliminate the possibility that this pathogen has been acquired from other animals (by contact with other individuals of the same specie or its preys).

Although *C. lusitaniae* is known for its resistance to amphotericin B, with some reviews reporting a prevalence of resistance as high as 60% (Apsemidou *et al*., 2020; Hawkins and Baddour, 2003; Tikotekar *et al*., 2008) that was not observed in the present case. In this case no treatment with antifungal agents was necessary, only the cleaning of the lesion and recovery of the animal health. The main cause of admission of the animal was not the candidiasis, but the injuries (wing fracture) inflicted by the shotgun. The lesion in the oral cavity was not fatal, and probably would not develop if the animals was not under stress and immunosuppressed. The observation of these lesions in this animal was a causality, that if not treated, considering the condition of the animal, could probably have disseminated to the internal organs.

Treatment of this type of lesion caused by *Candida* species before release of the animals into the wild is essential, thus avoiding contamination of the environment and other animals. The zoonotic potential of these yeasts is low, but it should be always considered (Deem, 2003; Seyedmousavi *et al*., 2018).

To the author´s knowledge, this is the first reported case of candidiasis by *C. lusitaniae* in a red kite (*M. milvus*). This report is vital to improve the understanding of pathogenic yeast in rapine birds and reinforces the importance of including infections by *Candida* species as a differential diagnosis of bacterial infections,

parasitosis and hypovitaminosis A, that should be considered when birds do not respond to antibiotic treatment.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to declare.

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