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Aspergillosis in free-ranging Magellanic penguins

Mariana Trápaga^{1,2}, Aryse Martins Melo³, Vanice Poester^{1,2}, Paula Lima Canabarro⁴, Andrine Silva⁵, Sérgio Estima⁶, Esteban Frere⁶, Melissa Orzechowski Xavier^{1,2}

¹Laboratory of Mycology, Federal University of Rio Grande, Rio Grande, Brazil
²Post-Graduate Program in Health Sciences, Federal University of Rio Grande, Rio Grande, Brazil
³National Institute of Health, Dr. Ricardo Jorge, Lisbon, Portugal
⁴Marine Animal Recovery Center (CRAM/FURG), Oceanographic Museum Prof. Eliézer de C. Rios, Federal University of Rio Grande, Rio Grande, Brazil
⁵Center for Environmental Education and Monitoring (NEMA), Rio Grande, Brazil
⁶Centro De Investigaciones Puerto Deseado -Unidad Academica Caleta Olivia – (UNPA) Universidad Nacional De La Patagonia Austral, Puerto Deseado, Argentina

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Aspergillosis is an opportunistic fungal disease caused by the *Aspergillus* genus, mostly by *Aspergillus* section Fumigati. Captive Magellanic penguins are vulnerable to *Aspergillus* infection, being this mycosis a limiting factor during the process of rehabilitation with a mortality rate of around 50%.

Objectives: Giving the scarce data regarding the occurrence of aspergillosis in non-captive penguins, we aimed to evaluate the proportional mortality by aspergillosis in free-ranging Magellanic penguins during their migration and reproductive season.

Methods: Carcasses of Magellanic penguins were collected from the Southern coast of Brazil between June 2017 and October 2019 between Barra do Chui beach (Southern RS, Brazil - 33°44'19.9"S 53°21'56.3"W) and the isthmus with the Lagoa do Peixe (Southern RS, Brazil - 31°26' S, 51°10' W 31°14'S, 50°54'W). In addition, in January 2019, penguins found dead in the reproducing colony in four islands localized in Puerto Deseado City (Santa Cruz, Patagonian, Argentina - 47°45'00"S 65°55'00"W) were collected. All animals were necropsied, and macroscopic alterations were observed. Samples of macroscopic lesions and/or respiratory systems were collected for histopathology and mycological culture. Only proven aspergillosis cases, defined by suggestive lesions at necropsy, associated with hyaline, septate, and 45° branched hyphae in histopathological slides and isolation of *Aspergillus* sp. in the culture were computed. Fungal isolates were identified by molecular techniques.

Results: A total of 98 Magellanic penguins were included in our study, being 80 recovered on the Southern RS beach, and 18 from the Patagonian colony. Two penguins collected in Southern Brazil were diagnosed with aspergillosis, both juveniles, one showing nodules in the lung parenchyma, and the other nodules and fungal colonies at the lung and air sac, resulting in a proportionate mortality rate of 2.5%. Both isolates were identified as *A. fumigatus sensu stricto*. Regarding the carcasses collected from the reproductive colony (Patagonian islands), no penguin had anatomopathological or mycological evidence of aspergillosis.

Conclusion: Given the already known importance of aspergillosis in seabirds undergoing rehabilitation, these data suggest that penguins may already arrive in these centers infected by *Aspergillus* spp. The absence of aspergillosis cases in the reproductive period could be attributed to the low number of carcasses included. Our study is an initial step to demonstrate aspergillosis as one of the causes of mortality also in free-living penguins, especially during the migration process, instigating more studies, in other routes of migration, as well as in reproductive colonies.

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Isolation of *Cryptococcus neoformans* and other yeast from pigeon droppings in Khartoum state, Sudan

Wisal Abdalla
 Central Veterinary Research Laboratory, Khartoum, Sudan

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Objectives: The aim of this study was to investigate the presence of *Cryptococcus neoformans* and other yeast species in pigeon droppings in Khartoum state, Sudan.

Methods: Sample collection A total of 120 samples were collected from pigeon droppings from Khartoum state. Pigeon droppings were collected using sterile wooden spatulas and placed directly in clean plastic bags.

Processing of sample
 Approximately 2 g of pigeon droppings were added to 10 ml of sterile saline. The samples were allowed to stand for 20 min with frequent vortex.

Isolation and Identification of yeast isolates
 A loopful of supernatant fluid from prepared samples was taken and streaked onto Sabouraud's dextrose agar media supplemented with 0.05 mg/ml chloramphenicol incubated at 37°C for 1-2 days. All yeast isolates were identified by direct microscopic examination using the lacto-phenol-cotton-blue stain India ink stain and Gram's stain. Further confirmatory tests were conducted using Corn Meal Agar (CMA) with tween 80, germ tube production test using horse serum, and urease test. Confirmation of identification was done using API 20C AUX and API ID 32C AUX (bioMérieux®, Madrid, Spain).

Results: *Cryptococcus neoformans* is the most common species isolated from pigeon droppings as shown in Table 1. Different *Candida* species have been isolated Figure 1.

Isolated yeast	Number
<i>Cryptococcus neoformans</i>	42
<i>Candida</i> spp	34
<i>Cryptococcus albidus</i>	5
<i>Stephanosacus cijerrii</i>	4
<i>Rhodotorula glutinis</i>	4
<i>Rhodotorula mucilaginosa</i>	3
<i>Geotrichum capitatum</i>	2
<i>Zygosaccharomyces</i> spp	1

Conclusion: The present study concluded that there is a potential role of pigeons as a reservoir for *C. neoformans* and other zoonotic yeasts in the environment that can affect humans and animals.