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Aspergillosis in free-ranging Magellanic penguins

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

Objective: Given the scarce data regarding the occurrence of aspergillosis in non-captive penguins, we aimed to evaluate the proportion of mortality by aspergillosis in a free-ranging Magellanic penguin colony 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 Chuí beach (Southern RS, Brazil - 31°39'39.3"S 52°22'46.9"W) and the islands with the Lagoa do Peixe (Southern RS, Brazil - 31°24’S, 51°10’W 31°14’S, 50°34’W). In addition, in January 2019, penguins found dead in the reproducing colony on four islands located in Puerto Deseado City (Santa Cruz, Patagonia, Argentina: 47°4’0”S 65°51’56.3’W) were collected. All animals were necropsied, and macroscopic alterations were observed. Samples of microscopic lesions and/or respiratory systems were collected for histopathological and mycological analysis. Only proven aspergillosis cases, defined by negative culture at necropsy, associated with hyaline, septate, and 45° branched hyphae in histopathological slides and isolation of Aspergillus sp. in the culture were considered. Fungal isolates were identified by molecular techniques.

Results: A total of 98 Magellanic penguins were included in our study, being 89 necropsied 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 2.1%. Both isolates were identified as A. fumigatus sensu stricto. Regarding the carcasses collected from the reproductive colony (Patagonian islands), no penguin had anamorphological 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 colony could be attributed to the low number of carcasses included. Our study is an initial step to diagnose aspergillosis as one of the causes of mortality also in free-ranging penguins, especially during the migration process, instigating more studies, in other regions 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

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Objective: 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 samples
Approximately 2 g of pigeon droppings were added to 10 ml of sterile saline. The samples were allowed to stand for 20 mins with frequent remixing.

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/l of 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 and Gram’s stain. Further confirmatory tests were conducted using Crome Mural Agar (CMA) with vancomycin, gentamicin production test using horse serum, and assimilation test.

Confirmation of identification was done using API 20C AUX and API ID 32C AUX (BioMerieux, Madrid, Spain).

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

| Isolated yeast                     | Number |
|-----------------------------------|--------|
| Cryptococcus neoformans           | 42     |
| Candida spp                       | 34     |
| Cryptococcus albidus              | 5      |
| Aspergillus olivaceus             | 4      |
| Rhodotorula glutinis              | 3      |
| Rhodotorula mucilaginosa          | 3      |
| Geotrichum capitatum              | 2      |
| Zygosaccharomyces spp             | 1      |

Conclusion: The present study concluded that there is a potential role of pigeon as a reservoir for C. neoformans and other zoonotic yeasts in the environment that can affect humans and animals.
Objective: Aspergillosis causes a heavy burden on birds in captivity, such as Humboldt penguins. In recent years the colony of a Belgian zoo has experienced very high mortality rates and the zoo has already taken several measures to lower the burden. This study was set up to see if the penguins acquire A. fumigatus via the environment and if so, if additional measures can be taken to limit the incidence.

Methods: A total of 29 clinical strains collected from 2018 to 2022 were included in the study. From April 2021 until January 2022, four samplings have been performed, accounting for every season. A combination of sand, water, nest swabs, and air was analysed for the presence of azole-resistant A. fumigatus. In brief, air samples were collected at fixed locations in the penguin enclosures by impacting 1000 L on an agar plate [neat + chloramphenicol (MC) and MC + triazolamid (MC + T)]. A total of 100 mL water was collected and 10 mL water was filtered through a 0.22 μm filter and placed on an MC plate, and repeated with the other 50 mL on MC + T. A total of 9 mL 0.1% Tween 20 + 0.4% NaCl was added to 3 g of sand and vortexed for 1 min. Both MC and MC + T plates were incubated with 100 μL of this suspension. All plates were incubated at 40°C ± 1°C for 48 h. The phenotypical resistance pattern of all clinical isolates was determined using the EUCAST method.

Results: The phenotypical resistance pattern showed resistance in 7 isolates (24%) with 5 of them showing resistance to posaconazole, one was resistant against voriconazole, itraconazole, and posaconazole, and 1 showing pan-resistance. No A. fumigatus colonies could be detected from water samples, nor from the sand. One A. fumigatus isolate was retrieved from the nest swabs. In total 44 A. fumigatus colonies were isolated from air samples collected on MC + T medium. All have been subjected to EUCAST microbroth-dilution determination and 4 resistant isolates could be detected (all had a MIC value for posaconazole of 0.5 μg/mL and one strain showed additional resistance against itraconazole with a MIC of 4 μg/mL. Cyp51A sequencing of all resistant strains is ongoing and will give more insight in the molecular mechanisms involved to investigate the potential link with the environment.

Conclusion: This study showed high resistance rates in the clinical isolates. Four resistant isolates were found in environmental air samples. Sequencing of the cyp51A gene will give more information on a possible relation between the resistance mechanisms found in the clinical and the environmental isolates. More research should be done to investigate the origin of the resistant isolates in the environment.

Figure 1. Shows different Candida spp isolated from pigeon droppings

Figure 2. Capsule formation of Cryptococcus neoformans using India ink