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Aspergillosis in a colony of Humboldt penguins (*Spheniscus humboldti*) under managed care: a clinical and environmental investigation in a French zoological park

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objectives: Aspergillosis is an opportunistic fungal infection due to *Aspergillus* spp., and primarily *A. fumigatus*. The disease is pervasive in avian populations, especially those under managed care. The incidence and the management of avian aspergillosis can be critically impacted by exposure to high levels of spores via environmental contamination and by strains

resistant to azole drugs. The present study aimed at assessing the impact of environmental contamination on the clinical incidence of aspergillosis in penguins managed under human care. ZooParc de Beauval was chosen for the investigations, because this zoological facility hosts a large colony of Humboldt penguin (*Spheniscus humboldti*) and it is geographically located in the rural countryside of the Loir-et-Cher district, France, surrounded by numerous large crop fields

Material and methods: The fungal contamination in the environment was assessed through three serial sessions of surface sampling in bird nests: all isolates were counted, identified by DNA sequencing, and then systematically screened for resistance mutations and MICs (minimal inhibitory concentrations) elevation for the *A. fumigatus* strains. In addition, the clinical incidence of aspergillosis was evaluated in the penguin population over a 3-year period. A microsatellite-based analysis tracked *A. fumigatus* isolates circulation.

Results: Environmental investigations highlighted a substantial increase in the fungal load during the Autumn season (>12-fold vs. the other timepoints) and a large overrepresentation of species belonging to the *Aspergillus* section *Fumigati* (ranging from 22.7 to 94.6% (Fig. 1)). Only one cryptic species (*A. nishimurae*) and one isolate exhibiting resistance mutation (G138S in the *cyp51A* gene; MIC itraconazole >4 µg/ml) were detected. The overall incidence of aspergillosis was measured at ~3.4% case-years and was observed mostly in juvenile penguins (Fig. 1). The analysis of microsatellite polymorphism revealed a high level of genetic diversity among *A. fumigatus* isolates, except for one strain that was largely over-represented during the Autumn sampling session. A limited number of isolate genotypes was collected from clinical cases and from the environment.

Conclusions: Fungal environmental contamination and incidence of aspergillosis in penguins appeared variable depending on seasons, but the rural location of the penguin habitat did not seem to influence the emergence of resistant strains.

