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Case Report

Rhinitis due to *Aspergillus pseudoviridinutans* in an orange-winged Amazon parrot (Amazona amazonica)

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**ABSTRACT**

Species within the *Aspergillus viridinutans* complex are being increasingly recognized as pathogens of animals and humans. An orange-winged Amazon parrot (*Amazona amazonica*) was referred for a 6 month-history of a slowly developing swelling involving the right nostril. Abnormal physical exam findings included a mild firm swelling at the dorsolateral aspect of the right nostril with no nasal discharge. Computed tomographic examination showed mild deformation of the right nares and nasal conchae without distinct granuloma. A cryptic *Aspergillus* species in *Aspergillus* section *Fumigati* was cultivated and identified by PCR and comparative sequence analysis as *Aspergillus pseudoviridinutans*. Successful treatment was achieved using topical clotrimazole and systemic antifungals (itraconazole, terbinafine). This is the first report of *A. pseudoviridinutans* infection in a bird.

1. Introduction

Aspergillosis is an important infectious, non-contagous fungal disease affecting both free-living and captive birds [1,2]. Species in the ubiquitous opportunistic saprophytic genus *Aspergillus*, in particular *Aspergillus fumigatus sensu stricto*, are most commonly isolated [2–4]. Acute disease may develop following an overwhelming exposure to fungal spores. Chronic cases are typically caused by the fungal agent with concurrent predisposing factors such as host species predilection (*Amazona* sp., *Psittacus erithacus*, *Pionus* sp.), environmental conditions (poor ventilation, improper temperature and humidity), immunosuppression (disease, stress, hypovitaminosis A) or traumatic injury.

Avian aspergillosis most typically affects the lower respiratory system due to the unique anatomy of birds. The air sacs are usually the primary infection sites, since inhaled air reaches the caudal thoracic and abdominal air sacs prior to the epithelial surfaces of the lungs [1]. Nasal cavities are rarely affected [5], although affinity for the respiratory region of the nose is described [6].

Many *Aspergillus* species within section *Fumigati*, are known human and veterinary pathogens [7]. These strains are often misidentified in clinical samples, as they cannot be distinguished from *A. fumigatus sensu stricto* by conventional morphological analysis and sequencing methods [3]. Definitive species identification requires specific molecular techniques. This is crucial as these *A. fumigatus*–related species often display some level of intrinsic resistance to azoles and other antifungal drugs. We describe the first report of *A. pseudoviridinutans* in a bird.

2. Case

2.1. Presentation

A 15-year-old, female, orange-winged Amazon parrot was referred at day 0 for a 6 month-history of a slowly developing swelling involving the right nostril. The bird had recurrent right rhinitis treated with various topical and systemic antimicrobials by the referring veterinarian. The humidity level was generally low (<40%) in the room where the bird was housed with four healthy birds of various species (Monk parakeet (*Myiopsitta monachus*), blue-fronted Amazon parrot (*Amazona aestiva*), ringed-neck parakeet (*Psitacula krameri*), sun conure (*Aratinga solstitialis*)). Physical examination revealed a mild firm swelling of the cere immediately dorsolateral to the right nostril causing nasal asymmetry (Fig. 1). No nasal discharge was present but dust accumulation was visible against the operculum of the right nostril. Mild blunting of the left choanal papillae was noted.

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2.2. Differential diagnosis and initial diagnostic investigations

Differential diagnosis for the nasal lesion included chronic rhinitis secondary to resistant bacterial infection and/or concurrent fungal infection, neoplasia or less likely a rhinolith. Squamous metaplasia due to hypovitaminosis A and low environmental humidity were suspected as contributing factors to ease pathogen attachment and tissue invasion.

The initial diagnostic plan included complete blood count (CBC), plasma biochemical analysis and a nasal flush for cytological evaluation, aerobic bacterial culture and fungal culture. CBC was within normal limits. Biochemistry analysis showed triglycerides were mildly elevated (2.90 mmol/L). Cytology showed no evidence of inflammation with numerous basophilic ovoid structures compatible with proteinaceous debris or potentially yeasts. The bird was discharged pending culture results with instructions to the owner to flush the bird’s nostrils daily with 3ml of saline. Fluconazole (5mg/kg PO q24h for 3 weeks) was initiated to address a suspected yeast infection. On day 3, bacterial culture revealed *Enterobacteriaceae*. Tobraycin eye drop (1-drop q12h) was initiated in the right nostril along with a course of azithromycin (40 mg/kg q24h PO) for 10 days.

On day 21, the in-house microbiology laboratory identified an *Aspergillus*-like species on three inoculated spots with the nasal wash performed on day zero. The isolate had the phenotypic appearance of *A. fumigatus*, but did not grow at 50 °C. Therefore, it was sent to a provincial reference mycology laboratory for further identification. The sample was inoculated on 3 media (potato dextrose agar, czapek’s agar, malt agar) and incubated at 30 °C, 37 °C, 42 °C, 45 °C and 49 °C (Fig. 2) for 7 days. Fungal growth occurred on all three media with no growth above 45 °C. Definitive species identification was achieved by sequencing of ITS, partial β-tubulin (benA) and calmodulin (caM), as previously described [12]. Pending results, and given the finding of thermotolerance, the isolate was provisionally identified as a cryptic species in section *Fumigati*.

2.3. Therapeutic management and follow-up

To further investigate the nasal lesion [8], a computed tomographic (CT) examination of the head was recommended and performed in order to determine more precisely the extent of the lesions, to determine if a focal lesion could be debrided and to better monitor response to treatment. At day 55, CT scan revealed no discrete surgically correctible lesions. There was mild thickening of the soft tissues surrounding the right naris. The right nasal conchae were deformed, and the right nasal meatus was mostly occluded. The mucosa covering the right nasal conchae was mildly thickened as well, and there was medial displacement of the conchal margin. Soft tissue within the left infraorbital diverticulum, ventral to the left globe, was noted (Fig. 3). Itraconazole (10 mg/kg PO q24h) and terbinafine (30 mg/kg PO q12h) were implemented. Mechanical flushes of the right naris with saline twice daily were continued. Following nasal flushing with saline, 0.5 ml of clotrimazole 1% in polyethylene glycol was applied in the right naris.

At day 92, recheck examination revealed the right naris appeared more inflamed and narrowed. Biochemical analysis was performed to monitor for hepatic side effects and no significant findings were present.
Topical clotrimazole was discontinued and oral antifungals along with bi-weekly nasal flushes with saline were continued. At day 139, the right nostril was slightly more prominent dorsolaterally, but the naris was now depressible for the first time. The right nasal meatus was more similar in size compared to the contralateral one. No discharge was observed. Biochemical analysis was within normal limits. Nasal flush of the right naris was performed and submitted for fungal culture, which yielded no growth. It was decided to continue systemic antifungal therapy along with bi-weekly nasal flushes until two consecutive CT scans showed absence of progression or regression of nasal lesion and until two consecutive fungal cultures were negative.

At day 181, physical examination findings were unchanged compared to prior visit. Recheck CT scan showed reduced soft tissue within the right naris with unchanged deformation of the right naris and infraorbital diverticulum. The treatment regime was continued. At day 236, the owner reported that the bird had occasional nasal discharge. She had discontinued bi-weekly nasal flushes. On physical exam, dried discharge was present within the right naris. CBC and biochemical analysis showed no significant findings. Nasal flush submitted for culture yielded no fungal growth. Bacterial culture was positive for Enterobacter cloacae. The bird received a 10-day course of trimethoprim-sulfamethoxazole (30 mg/kg PO q12h). Systemic antifungals were continued and the owner was instructed to re-institute bi-weekly nasal flushes with saline.

At day 294, after 8 months of systemic antifungal therapy, the nostrils were almost symmetrical with no discharge present. Conclusions of the CT scan were unchanged with no signs of active infection identified (Fig. 4). Nasal flush for bacterial and fungal culture yielded no growth. Itraconazole and terbinafine treatments were discontinued and the owner was instructed to continue nasal flushes or showers at least once weekly for the remainder of the bird’s life.

At day 324, one month after discontinuing oral antifungals, the bird presented for swelling of the right nostril after boarding to a relative’s house. Nasal flushes ceased while boarding. No nasal discharge was present but the owner reported seeing white opaque material while flushing the nostril for the first time after returning home. A sample was collected for aerobic and fungal cultures. Fungal culture yielded no growth. Bacterial culture revealed Enterobacter cloacae. Trimethoprim-sulfamethoxazole (30 mg/kg PO q12h) was initiated along with ciprofloxacin 0.3% ophthalmic drop (1 drop in each nostril q12h). At day 345, bacterial culture confirmed resolution of the infection. The beneficial mechanical cleaning obtained through regular nasal flushes was re-emphasized. To this date, five years following discontinuation of all medication, the bird remained clinically healthy with no recurrence of upper respiratory infections.

2.4. Fungal identification and susceptibility tests

 Sequences were deposited in GenBank (ITS: MT102878, BenA: MT117003, CaM: MT117004). The isolate (LSPQ-01141) was identified as A. pseudoviridinutans based on comparative sequence analysis of ITS, betatubulin and calmodulin sequences with the NCBI nucleotide database. The isolate could not be speciated on the basis of ITS sequencing alone as it shared 100% nucleotide homology with isolates of A. fumigatus var sclerotiorum (Genbank accession no. MH860940), A. pseudoviridinutans (Genbank accession nos. KY08736 and KY08735) and A. fischeri (Genbank accession no. KF624799). The isolate was definitively identified based on 99.1% betatubulin homology (452/456 nt) with A. pseudoviridinutans (Genbank accession no. KJ914689) and 99.9% calmodulin homology (578/579 nt) with a human lung isolate of A. pseudoviridinutans (Genbank accession no. LT795933) and results of fungal susceptibility tests are presented (Table 1). Antifungal susceptibility testing was performed according to the CLSI M38-A3 standard for filamentous fungi.

3. Discussion

This case is the first report of A. pseudoviridinutans infection in a bird successfully managed medically.

A. pseudoviridinutans is part of the A. viridinutans species complex (AVSC), in section Fumigati, which includes nine other species (A. udagawae, A. acrenis, A. aureoles, A. wyomingensis, A. siamensis, A. felis, A. arcoverdensis, A. frankstonensis, A. viridinutans) [11].

<table>
<thead>
<tr>
<th>Drugs</th>
<th>MIC (ug/mL)</th>
<th>A. pseudoviridinutans</th>
<th>ECV (ug/mL)</th>
<th>A. fumigatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flucytosine</td>
<td>&gt;64</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>&gt;256</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Posaconazole</td>
<td>1</td>
<td>0.5</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*MIC: Minimum inhibitory concentrations, ECV: Epidemiological cutoff value
A. pseudoviridinutans has been isolated from clinical samples in humans (n = 5) and from the environment, specifically soil and cave sediment [11]. Cryptic Aspergillus species in section Fumigati have been recognized as occasional causes of human invasive aspergillosis in 3–6% of cases, but their actual prevalence may be underestimated because of their lack of recognition by conventional diagnostic approaches [3]. Until now, cryptic species of Aspergillus have not been identified to cause aspergillosis in birds – none were identified in molecular surveillance studies of avian aspergillosis in Australia or in California, although the number of birds sampled in these studies was relatively small [2, 12].

In birds, rhinitis caused by aspergillosis usually starts unilaterally, eventually invading the sinuses, blood vessels, turbinate cartilages and nasal bones [6]. In the case presented here, the infraorbital sinus and nasal conchae were involved. In mild non-invasive cases, the agent is eventually invading the sinuses, blood vessels, turbinate cartilages and usually confined to the superficial mucosal layer inducing focal and of birds sampled in these studies was relatively small [2, 12].

Application of intranasal clotrimazole in small animals is performed under general anesthesia. After sealing the rostral and caudal openings of the nasal cavity with Foley catheters, clotrimazole solution is instilled to fill the nasal cavity with an infusion time of one hour [20]. This technique was not elected in this patient due to the very rostral location of the lesion and the risks associated with prolonged anesthesia.

In conclusion, A. pseudoviridinutans should be included in the differential diagnosis of upper respiratory disease in psittacines. Accurate species identification of Aspergillus species is important to guide therapy. Antifungal susceptibility testing has not yet been performed considering differences in innate drug resistance might influence response to therapy, as seen for some cryptic Aspergillus species of the Fumigati section.

Conflict of interest

The authors declare no conflict of interests.

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References


