



## Case Report

Rhinitis due to *Aspergillus pseudoviridinutans* in an orange-winged Amazon parrot (*Amazona amazonica*)Langlois Isabelle<sup>a,\*</sup>, Barrs R. Vanessa<sup>b</sup>, Dufresne J. Philippe<sup>c,1</sup><sup>a</sup> Centre Hospitalier Universitaire Vétérinaire (Langlois), Université de Montréal, Faculty of Veterinary Medicine, 3200 Sicotte St, Saint-Hyacinthe, QC, J2S 2M2, Canada<sup>b</sup> Department of Veterinary Clinical Sciences, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong SAR, China<sup>c</sup> Laboratoire de santé publique du Québec (LSPQ), Institut national de santé publique du Québec, 20045 chemin Sainte-Marie, Sainte-Anne-de-Bellevue, QC, H9X 3R5, Canada

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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 naris 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-contagious 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

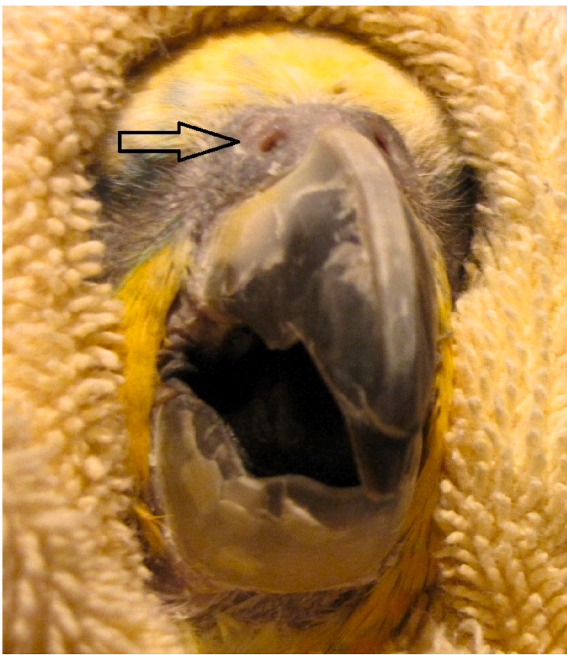
techniques. This is crucial as these *A. fumigatus*-related species often display some level of intrinsic resistance to azoles and other antifungal drugs. We describe the first report of *A. pseudoviridinutans* in a bird.

## 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 diet was adequate for the host species at the time of presentation. However, prior history revealed suboptimal nutrition. 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 (*Psittacula 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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**Fig. 1.** Photograph of an orange-winged Amazon parrot (*Amazona amazonica*). A mild firm swelling of the cere immediately dorsolateral to the right nostril causing nasal asymmetry (arrow) without nasal discharge is present. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

## 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

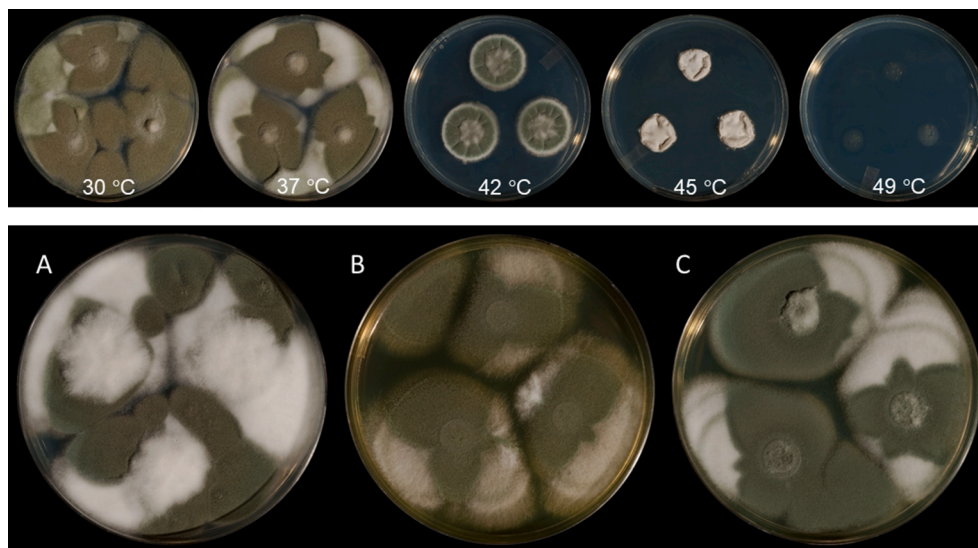
numerous basophilic ovoid structures compatible with proteinaceous debris or potentially yeasts. The bird was discharged pending culture results with instructions to the owner to flush the bird's nostrils daily with 3ml of saline. Fluconazole (5mg/kg PO q24h for 3 weeks) was initiated to address a suspected yeast infection. On day 3, bacterial culture revealed *Enterobacteriaceae*. Tobramycin 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  $\beta$ -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*.

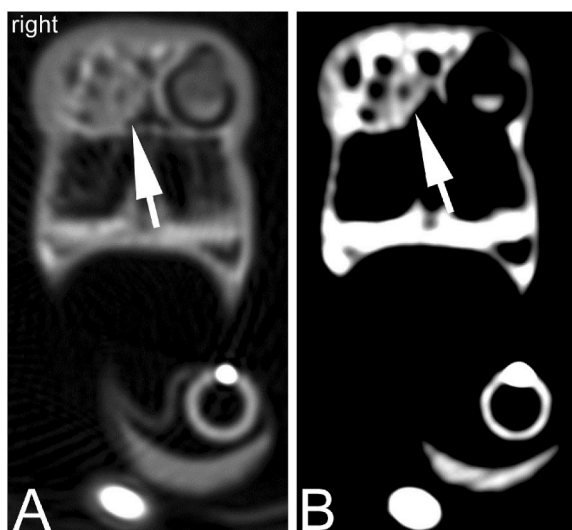
## 2.3. Therapeutic management and follow-up

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.



**Fig. 2.** Macroscopic morphology of *Aspergillus pseudoviridinutans* on Potato Dextrose Agar (PDA) at various temperatures (30 °C, 37 °C, 42 °C, 45 °C and 49 °C- top panel) and on Czapek's agar (A), Malt agar (B) and PDA (C) media at 30 °C (bottom panel).



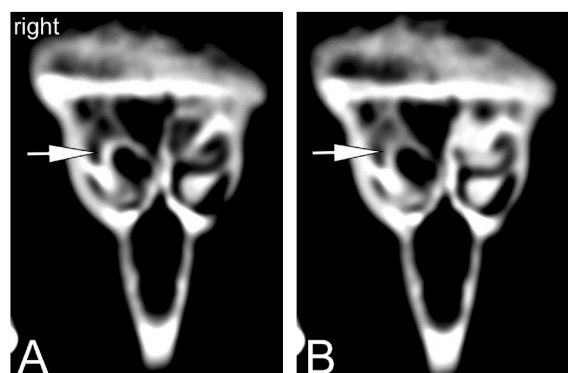
**Fig. 3.** Computed tomographic examination of an orange-winged Amazon parrot (*Amazona amazonica*) in a bone algorithm pre-contrast (A) and detail algorithm after contrast media administration (B). Mild thickening of soft tissue around the right naris (arrow) with the right nasal meatus mostly occluded is present (arrow). There is no discrete surgically correctible lesions.

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 yield no fungal growth. Bacterial culture was positive for Enterobacteriaceae. 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,



**Fig. 4.** Computed tomographic examination of an orange-winged Amazon parrot (*Amazona amazonica*) in a soft-tissue algorithm pre- (A) and post-contrast media administration (B). Persistent deformation of the right nasal conchae with no contrast enhancement (arrow) is present.

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. acensis*, *A. aureoles*, *A. wyomingensis*, *A. siamensis*, *A. felis*, *A. arcovendensis*, *A. frankstonensis*, *A. viridinutans*) [11].

**Table 1**

Antifungal susceptibility tests for *A. pseudoviridinutans* isolate from an orange-winged Amazon parrot (*A. amazonica*). The epidemiological cutoff value for *A. fumigatus* are presented for comparison.

Drugs	MIC (ug/mL) <i>A. pseudoviridinutans</i> Orange-winged Amazon	ECV (ug/mL) <i>A. fumigatus</i> [9,10]
Amphotericin B	4	2
Flucytosine	>64	-
Voriconazole	1	1
Itraconazole	2	1
Fluconazole	>256	-
Posaconazole	1	0.5

\*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 usually confined to the superficial mucosal layer inducing focal and multifocal lesions [6]. In cats and dogs, upper respiratory tract aspergillosis is subdivided into sinonasal aspergillosis (SNA) and sino-orbital aspergillosis (SOA). In dogs, SNA accounts for more than 99% of cases and is noninvasive, whereas in cats SOA is the most common form (65% of cases) and is invasive.

Definitive diagnosis of aspergillosis in birds is based on fungal identification with histopathological evidence of the causative agent in associated inflammatory lesions. However, nasal wash samples submitted for fungal culture and identification are usually diagnostic for aspergillosis if positive [13]. Interestingly, mild cellular reaction incited in situ is described with nasal aspergillosis [6], which correlate with the cytological assessment in this patient. It is also possible the dilution effect contributed to the initial cytological results. The morphological and physiological characteristics of the isolate were compatible with a cryptic species of *Aspergillus*, since *A. fumigatus* is thermotolerant, whereas cryptic species usually have a maximum growth temperature of between 42 and 45 °C.

Definitive species identification required comparative sequence analysis, utilizing not just the internal transcribed spacer (ITS) ribosomal DNA region, which is widely utilized as a universal “bar code” for identification of clinical filamentous fungi, but also the secondary markers betatubulin and calmodulin. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is promising to expedite species identification [14], although this method is currently available only for some *A. fumigatus*-related species.

Evidenced-based treatment protocols are not available in birds for upper respiratory aspergillosis. Currently recommended treatment regime to overcome the disease include topical therapy combined with systemic antifungal therapy [1]. The initial antifungal therapeutic plan in this bird was based on the tentative diagnosis of *A. felis*. Likewise, concurrent topical and systemic therapy is recommended when *A. felis*, a species highly correlated with invasive disease is identified in small animals [15]. At the time this case was managed, antifungal susceptibility results for 13 *A. felis* isolates from cat’s clinical specimens had been evaluated [8] and revealed a MIC of  $\leq 1$   $\mu\text{g}/\text{mL}$  for itraconazole, and a minimum effective concentration (MEC) of  $\leq 0.25$   $\mu\text{g}/\text{mL}$  for terbinafine. Therefore, a combination of systemic itraconazole and terbinafine was chosen. Antifungal susceptibilities to *A. pseudoviridinutans* have been assessed by determining the MIC values [16]. One isolate (*A. pseudoviridinutans* NIHAV1) showed high MICs to voriconazole and itraconazole while another (*A. pseudoviridinutans* NRRL 6106) showed MICs of  $\leq 2$   $\mu\text{g}/\text{mL}$ . Our isolate was susceptible to posaconazole and voriconazole. Although the minimum inhibitory concentration of itraconazole was higher than the epidemiological cutoff value for *A. fumigatus*, the value was not particularly elevated. This may have accounted for the positive response to treatment, since definitive breakpoints to antifungal agents are not yet fully established.

Terbinafine is useful in the treatment of refractory and systemic fungal infections, particularly aspergillosis [17]. In addition, synergistic activity is noted with other antifungals, notably triazoles.

Pharmacokinetics of terbinafine following administration of a single oral dose of 60 mg/kg have been reported in Hispaniolan Amazon parrots (*Amazona ventralis*) [18]. The peak concentrations achieved were within the range of the in vitro MICs for *Aspergillus fumigatus* (0.02–5.0  $\mu\text{g}/\text{mL}$ ). In the absence of pharmacokinetics and pharmacodynamics study regarding the safety and the effect of multiple doses, a dose of 30 mg/kg q12h was elected for long-term administration in this patient. Oral administration of doses of 15–30 mg/kg q12h have been used clinically for the treatment of upper and lower respiratory aspergillosis in psittacine birds with favorable results [19].

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 should always be 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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