

## FUNGI ISOLATED FROM THE EXCRETA OF WILD BIRDS IN SCREENING CENTERS IN PELOTAS, RS, BRAZIL

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### SUMMARY

The identification of the fungal species belonging to the healthy microflora in animals is a precondition for the recognition of pathological processes causing them. The aim of this study was to investigate the presence of potentially pathogenic fungi in the feces of wild birds collected in Screening Centers. Samples were collected from the feces of 50 cages with different species of birds. The samples were processed according to the modified method STAIB and the plates incubated at 32 °C for up to ten days with daily observation for detection of fungal growth. The isolation of the following species was observed: *Malassezia pachydermatis*, *Candida albicans*, *C. famata*, *C. guilliermondii*, *C. sphaerica*, *C. globosa*, *C. catenulata*, *C. ciferri*, *C. intermedia*, *Cryptococcus laurentii*, *Trichosporon asahii*, *Geotrichum klebahnii*, *Aspergillus* spp., *A. niger* and *Penicillium* spp. Knowing the character of some opportunistic fungi is important in identifying them, facilitating the adoption of preventive measures, such as proper cleaning of cages, since the accumulation of excreta may indicate a risk for both health professionals and centers for screening public health.

**KEYWORDS:** Birds; Fungi; Excretions.

### INTRODUCTION

Zoonoses transmitted by wild animals kept as pets have been seen as a public health problem. Opportunistic mycoses such as cryptococcosis, aspergillosis, and candidiasis are increasing in their frequency due to immunosuppressive diseases. Moreover, the easy dispersion of spores by wind renders the fungi airborne, causing diseases in humans. Among the opportunistic mycoses that can occur in humans are onychomycosis, otitis, ceratomycoses, and fungemias<sup>8</sup>.

However, the increasing number of conservation programs involving wildlife translocation, release, and reintroduction into their natural environments involves the risk of contagion by the possible transmission of infectious agents to humans<sup>5</sup>. And in this sense, it is estimated that close to fifty million live animals are confined in cages in Brazil, many of them from illegal catches<sup>13</sup>, and they require constant handling.

The opportunistic fungi are generally present in normal microbiota of humans and animals without causing disease processes in healthy individuals, but when a disruption occurs, usually resulting from predisposing factors (pathological, physiological, immunological and mechanical) there is an increase in the invasion and multiplication of these organisms in the tissues, causing infectious conditions<sup>11,21</sup>.

The keeping of wild or exotic animals is a common activity in Brazil;

passerines and Psittaciformes are very popular as pets<sup>14</sup>. However, the excreta of these birds represent a source of contamination of domestic and public environments by the species *Cryptococcus neoformans*, making it a risk factor for the occurrence of cryptococcosis. Aspergillosis is an opportunistic mycosis par excellence, caused by different species of fungi of the genus *Aspergillus*, especially *A. fumigatus*<sup>3,16</sup>.

And although the fungal diseases are primarily associated with immunosuppression and inter-current illnesses, understanding the factors involved in the epidemiology of the disease is crucial<sup>12</sup>.

The Centers for Wildlife Screening (CETAS) are places which welcome, identify, triage, treat, and allocate the wild animals rescued or seized by the inspectors, as well as wild animals received from individuals that were holding animals captive in the domicile illegally<sup>2</sup>. Given this context, the aim of this study was to investigate the presence of potentially pathogenic fungi in the feces of wild birds collected at the Center for Rehabilitation of Wild Animals of the Institute of Biology (IB) of the Universidade Federal de Pelotas (UFPEL).

### MATERIAL AND METHODS

Over six months, samples of excreta of birds in 50 cages housed at the Núcleo de Reabilitação da Fauna Silvestre (IB/UFPEL) were collected. Five surveys (five cages) were carried out per week from June 2010 to

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July 2011, totaling 50 samples. The bird species that were observed in the NURFS: *Saltator aurantiirostris* (beak-hard), *Pipraeidea bonariensis* (Tanager-papa-orange), *Stephanophorus diadematus* (Basking Tanager), *Paroaria coronata* (Cardinal Red), *Saltator similis* (straight rail), *Ramphocelus bresilius* (tie-blood), *Lanio cuculattus* (Fret-king), *Turdus rufiventris* (Rufous-bellied Thrush), *Turdus flavipes* (thrush-una), *Mimus saturninus* (Chalk-field), *Gubernatrix cristata* (Cardinal-yellow) *Sicalis flaveola* (canary-the-earth), *Amblyramphus holosericeus* (cardinal-the-plated), *Cyanoloxia brissonii* (Bluebird), *Pitangus sulphuratus* (bem-te-vi), *Myiopsitta monachus* (Monk Parakeet), *Ramphastos toco* (toucan's black-billed), *Guira guira* (anu-white) and *Columba livia* (pigeon-domestic).

The birds are housed in individual cages, and these are organized according to species and families. Usually the animals are housed in the NURFS for a period necessary for recovery. The cages are cleaned daily using the disinfectant chlorhexidine gluconate.

Samples were collected before the daily cleaning of the site, in individual sterile jars, around five grams of feces per cage. The material was immediately sent to the Laboratório de Micologia, Departamento de Microbiologia e Parasitologia, Instituto de Biologia, UFPel for processing.

The excreta samples were processed according to the modified method STAIB<sup>10,15</sup>. In laminar flow, a volume of 1 g of sample was homogenized with the aid of a mortar and pestle, and was then transferred to a sterile falcon tube with 10 mL of sterile saline. The tubes were homogenized by vortexing for three min and kept at rest for 30 min until decantation of the supernatant was obtained. Then a dilution was made of each sample by transferring 1 mL of the supernatant into another sterile falcon tube containing 9 mL of sterile saline and 5 mg of chloramphenicol (10-1 dilution). The tubes were again homogenized in a vortex and then 100 µL aliquots of this solution were seeded in duplicate by scattering technique with the aid of sterile Drigalski loop in Petri dishes containing Sabouraud agar with chloramphenicol and also agar niger (AN). The plates were incubated at 30 °C for up to seven days with daily observation for detection of fungal growth.

The identification of filamentous fungi was performed from the macro- and micromorphology through visualization of the characteristics of the colonies and the production of blades with visualization Lactophenol blue cotton and carrying microculture between blades when needed. Yeasts were identified by microscopy with conducting Gram stained smears, technical germ tube, microculture, and biochemical tests using a Vitek 2 system (Table 1).

## RESULTS

Of the 50 samples collected from 50 cages, 45 (90%) showed some fungal species. In 13 (26%) samples there was growth of filamentous fungi, 41 (90%) were isolated from yeast samples, and in ten (20%) of the samples growth of both filamentous fungi and yeasts occurred.

The following yeast species were observed: *Candida albicans* (31%), *C. famata* (11%), *C. guilliermondii* (4%), *C. catenulata* (4%), *C. intermedia* (2%), *C. sphaerica* (4%), *C. cifferri* (6%), *Trichosporon asahii* (2%), *Rhodotorula* sp. (2%), *Geotrichum klebahnii* (4%), *Cryptococcus laurentii* (4%), *Candida globosa* (2%) and *Malassezia pachydermatis*

**Table 1**  
Biochemical tests performed by Vitek 2 System for identification of yeasts

Test	Quantity/ well
L-Lysine ARYLAMIDASE (LysA)	0.0228 mg
L-MALATE assimilation (IMLTa)	0.15 mg
Leucine -ARILAMIDASE (LeuA)	0.0234 mg
GP Arginine (ARG)	0.15 mg
ERYTHRITOL assimilation (ERYa)	0.3 mg
GLYCEROL assimilation (GLYL a)	0.16 µL
Tyrosine ARYLAMIDASE (TyrA)	0.0276 mg
BETA-N-ACETYL-GLUCOSAMINIDASE (BNAG)	0.0408 mg
ARBUTIN assimilation (ARBa)	0.3 mg
AMYGDALIN assimilation (AMYa)	0.3 mg
D-GALACTOSE assimilation (dGALa)	0.3 mg
GENTIOBIOSE assimilation (GENa)	0.3 mg
D-GLUCOSE assimilation (dGLUa)	0.3 mg
LACTOSE assimilation (LACa)	0.96 mg
METHYL-D-GLUCOPYRANOSIDE assimilation (MAdGa)	0.3 mg
D-CELLOBIOSE assimilation (dCELa)	0.3 mg
GAMMA-GLUTAMYL TRANSFERASE (GGT)	0.0228 mg
D-MALTOSE assimilation (dMALa)	0.3 mg
D-RAFFINOSE assimilation dRAFa	0,3 mg
PNP-N-acetyl-BD-galactosaminidase (1 NAGA1)	0.0306 mg
D-MANNOSE assimilation (dMNEa)	0.3 mg
D-MELIBIOSE assimilation (dMELa)	0.3 mg
D-MELEZITOSE assimilation (dMLZa)	0.3 mg
L-SORBOSE assimilation (ISBEa)	0.3 mg
L-RHAMNOSE assimilation (IRHAa)	0.3 mg
XYLITOL assimilation (XLTa)	0.3 mg
D-SORBITOL assimilation (dSORa)	0.1875 mg
SUCROSE / SUCROSE assimilation (SACa)	0.3 mg
UREASE (URE)	0.15 mg
ALPHA-GLUCOSIDASE (AGLU)	0.036 mg
D-TURANOSE assimilation (dTURa)	0.3 mg
D-TREHALOSE assimilation (dTREa)	0.3 mg
NITRATE assimilation (NO3a)	0.03 mg
L-ARABINOSE assimilation (IARAa)	0.3 mg
D-GALACTURONATE assimilation (dGATa)	0.15 mg
ESCULIN hydrolysis (ESC)	0.225 mg
L-GLUTAMATE assimilation (IGLTa)	0.15 mg
D-XYLOSE assimilation (dXYLa)	0.3 mg
DL-LACTATE assimilation (LATA)	0.15 mg
ACETATE assimilation (ACEa)	0.15 mg
CITRATE assimilation (SODA) (CITa)	0.15 mg
GLUCURONATE assimilation (GRTas)	0.15 mg
L-PROLINE assimilation (IPROa)	0.15 mg
2-KETO-D-GLUCONATE assimilation (2KGa)	0.15 mg
ACETYL GLUCOSAMINE assimilation (NAGA)	0.15 mg
D-GLUCONATE assimilation (dGNTa)	0.15 mg

(2%), and also the following filamentous genera: *Aspergillus* spp. (8%), *A. niger* (4%), *Penicillium* spp. (6%) and *Mucor* sp. (4%).

Among the birds of the family Thraupidae (*Saltator aurantirostris*, *Pipraeidea bonariensis*, *Stephanophorus diadematus*, *Paroaria coronate*, *Saltator similis*, *Ramphocelus bresilius*, *Lanio cucullatus*) the following growth of yeast was observed (Chart 1): *M. pachydermatis* *Rhodotorula* sp., *C. laurentii*, *G. klebahnii*, *C. globosa*, *C. famata*, *C. albicans*, *C. guilliermondii*, *C. sphaerica*, and *C. catenulata*, and the following intermediate filamentous fungus: *Aspergillus* sp., *Penicillium* sp. and *Mucor* sp.

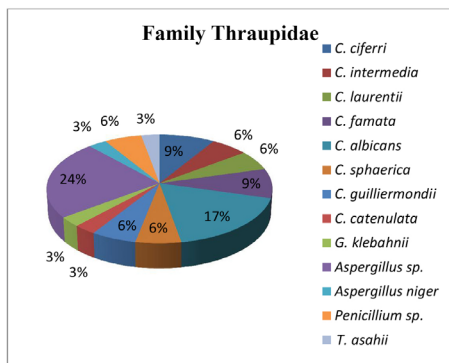


Chart 1 - Fungi isolated in Family Thraupidae.

As for the family Turdidae (Chart 2): *Turdus rufiventris* and *Turdus flavipes* the following was found: the filamentous fungus *A. niger* and the yeasts *G. klebahnii*, *C. albicans* *C. cifferri*.

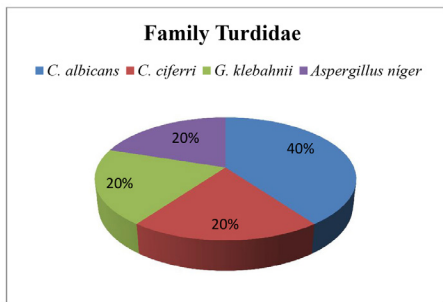


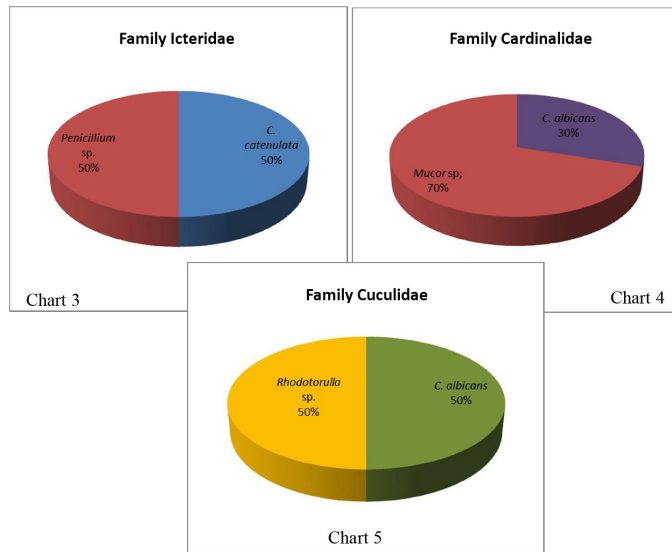
Chart 2 - Fungi isolated in Family Turdidae.

In the *Amblyramphus holosericeus*, family Icteridae *Penicillium* sp. and the yeast *C. catenulata* were isolated. In the *Cyanoloxia brissonii* family Cardinals found *Mucor* sp. and *C. albicans*. In Cuculidae (*Guira guira*): *C. albicans* and *Rhodotorula* sp. (Charts 3, 4 and 5).

In the family Tyrannidae (*Pitangus sulphuratus*), in Psittacidae (*Myiopsitta monachus*), Emberizidae (*Gubernatrix cristata* and *Sicalis flaveola*) and Ramphastidae (*Ramphastos vitellinus*) there were only isolated strains of *C. albicans*, as in Mimidae (*Mimus saturninus*). Finally, in Columbidae (*Columba livia*), there was no fungal growth.

## DISCUSSION

The fragmentation and degradation of habitats, the isolation of



Charts 3, 4 and 5 - Fungi isolated in Families Icteridae, Cardinals and Cuculidae.

populations, and greater proximity to humans and their pets has hampered the health of wild animals<sup>6</sup>. Furthermore, animals held in captivity or transported even for a short period can be exposed to a variety of pathogens, and become potential carriers of infectious diseases<sup>1</sup>.

From an epidemiological standpoint, poultry birds have an especially important role in contamination of the environment and the spread of pathogens by depositing their excreta. The behavior of birds frequently approaching the population as they are searching for food and shelter, contributes to the transmission of human pathogens, such as fungi and parasites<sup>19</sup>. *C. neoformans* is common in pigeon excreta, as can be seen in FARIA *et al.* (2010), but were negative in this experiment, justified by the low number of specimens of the species. *Cryptococcus* sp. passerines in the study<sup>12</sup> have also been found. SANTOS *et al.* (2009), isolated *C. neoformans* and *Candida* spp. in the feces of captive parrots and passerines.

Yeasts and moulds now rank among the ten most frequently isolated pathogens in febrile patients with an impaired immune system. Fungi are mainly opportunistic pathogens that only invade the body if a severely weakened natural defense permits them to do so. Most factors facilitating an invasive fungal infection are unavoidable because they are directly connected to the underlying diseases as well as to their treatment<sup>11,21</sup>.

The relevance of avian zoonoses is that, being asymptomatic infections in birds that are wrongly viewed as healthy, they hinder a possible diagnosis and treatment, thus increasing the chances of transmission to owners<sup>12</sup>. Maintenance of hygiene on the premises, wearing rubber gloves so that there is no direct contact with excreta, washing and sanitizing hands thoroughly after contact and handling of the birds are key practices to avoid possible transmission of zoonoses<sup>17</sup>.

The fungi, such as *Aspergillus* spp., are ubiquitous and airborne in the environment; they are microorganisms classified among the most abundant, and globally distributed, and can be isolated from soil, air, water, foods, plants, and the surfaces of decomposing material<sup>20,23</sup>. The main clinical form of aspergillosis in birds is the respiratory one, and the

infectious source, *Aspergillus* conidia from the environment, penetrates into the host organism mainly by air, thus affecting the respiratory system of these animals<sup>10</sup>.

The fungi found in this study are known to be potentially infectious pathogens by characteristics such as their ability to produce enzymes, adhesion capacity to the host cell, resistance to antifungal agents, and are associated with the production of hyphae that may contribute to the infectious process<sup>9,22</sup>.

With regard to hygiene and health, it is essential to remove the feces and urine of animals in captivity daily, preventing the proliferation of bacteria and fungus in the environment. Perches and nests should be free of droppings; drinking and feeding troughs should not be located beneath the perches so that the birds do not defecate in them. Cages must be washed and brushed every day and disinfected frequently. In this sense, it can be seen that cleaning, sanitizing, and disinfection are critical in maintaining any captivity. In the study area, the cages, drinkers, feeders, bowls, perches, nests, and even the utensils and equipment were cleaned daily; however a high percentage of organisms that are potentially pathogenic to humans and animals<sup>4</sup> were still observed.

Potentially pathogenic fungi are present in the feces of wild birds housed in rehabilitation centers and could pose risks to human health and animals. Thus, care with cleaning, sanitizing, and disinfecting are critical in maintaining any captivity.

## RESUMO

### Fungos isolados de excretas de aves silvestres em centro de reabilitação em Pelotas, RS, Brasil

A identificação das espécies de fungos pertencentes à microbiota saudável em animais é pré-condição para o reconhecimento dos processos patológicos causados por esses. O objetivo deste estudo foi investigar a presença de fungos potencialmente patogênicos nas fezes de aves selvagens coletadas em Centros de Triagem. Foram coletadas amostras de fezes de 50 gaiolas com diferentes espécies de aves. As amostras foram processadas de acordo com o método modificado STAIB e as placas foram incubadas a 32 °C por até dez dias, com observações diárias para detecção do crescimento dos fungos. Observamos o isolamento de espécies: *Malassezia pachydermatis*, *Candida albicans*, *C. famata*, *C. guilliermondii*, *C. sphaerica*, *C. globosa*, *C. catenulata*, *C. ciferri*, *C. intermedia*, *Cryptococcus laurentii*, *Trichosporon asahii*, *Geotrichum klebahnii*, *Aspergillus* spp., *A. niger* e *Penicillium* spp. Conhecendo o caráter de alguns fungos oportunistas resalta-se a importância para identificá-los, facilitando a adoção de medidas preventivas, como a limpeza adequada das gaiolas, uma vez que a acumulação de excrementos pode indicar um risco tanto para os profissionais de saúde e centros de triagem de saúde pública.

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