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Original Article

Yeasts and filamentous fungi in psittacidae and birds of prey droppings in midwest region of Brazil: a potential hazard to human health

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Abstract

Birds of prey and from Psittacidae family are host to fungal microbiota and play an important role in the epidemiology of zoonoses. Few studies in the literature have characterized mycelial and yeast fungi in the droppings of these birds and correlated the isolates with the zoonotic potential of the microorganisms. Droppings from 149 birds were evaluated and divided into two groups: captive: *Rhea americana araneipes, Primolius maracana, Ara ararauna, Ara chloropterus, Anodorhynchus hyacinthinus*, *Amazona aestiva, Ara macao macao*, *Ramphastos toco, Sarcoramphus papa*, *Busarellus igricollis*, *Bubo virginianus nacurutu, Buteogallus coronatus, Buteogallus urubitinga urubitinga, Spizaetus melanoleucus, Spizaetus ornatus ornatus, Buteo albonotatus, Geranoaetus albicaudatus albicaudatus, Rupornis magnirostris magnirostris* and *Harpia harpyja*, and quarantined birds: *Amazona aestiva* and *Eupsitulla aurea*. The fungal isolates were identified according to macroscopic (gross colony appearance), micromorphological and biochemical characteristics. Among birds displayed in enclosures, *Aspergillus niger* (41.1%) and *Candida kefyr* (63.8%) were the fungi most frequently isolated in *Harpia harpyja* and *Ramphastos toco*, respectively. For quarantined birds, the following percentages were observed in *Eupsittula aurea*, (76.6%) *C. krusei*, (84.4%) *C. kefyr* and (15.2%) *C. famata,* while in *Amazona aestiva*, (76.2%) *C. krusei* was observed. These findings indicate potentially pathogenic species in the bird droppings assessed, which constitute a risk of exposure for keepers and individuals who visit the zoo. Birds of the Cerrado and Pantanal of Mato Grosso (Central Western region of Brazil) could act in the epidemiological chain of important zoonoses.

Keywords: fungal microbiota, central western region, Brazil, birds, zoological gardens.

Leveduras e fungos filamentosos em excretas de psittacideos e aves de rapina em cativeiro na região centro-oeste do Brasil: um risco potencial para a saúde humana

Resumo

Aves de rapina e psitacídeos são hospedeiras de uma rica microbiota fúngica e desempenham um papel importante na epidemiologia de zoonoses. Poucos estudos na literatura têm caracterizado fungos micelianos e leveduras nos excrementos de pássaros e correlacionados estes isolados com o potencial zoonótico dos microrganismos isolados. Excrementos de 149 aves foram divididas e avaliados em dois grupos: Em cativeiro: *Rhea americana araneipes, Primolius maracana, Ara ararauna, Ara chloropterus, Anodorhynchus hyacinthinus*, *Amazona aestiva, Ara macao macao*, *Ramphastos toco, Sarcoramphus papa*, *Busarellus nigricollis*, *Bubo virginianus nacurutu, Buteogallus coronatus, Buteogallus urubutinga urubitinga, Spizaetus melanoleucus, Spizaetus ornatus ornatus, Buteo albonotatus, Geranoaetus albicaudatus albicaudatus, Rupornis magnirostris magnirostris e Harpia harpyja* e aves em quarentena: *Amazona aestiva* e *Eupsittula aurea*). Os isolados de leveduras e de fungos micelianos foram identificados em observações

macroscópicas (aspectos das colônias), características micromorfológicas e bioquímicas. Entre as aves indicadas em compartimentos, *Aspergillus niger* (41,1%) e *Candida kefyr* (63,8%) foram os fungos mais isolada em *Harpia harpyja* e *Ramphastos toco*, respectivamente. Para as aves em quarentena, os seguintes percentuais foram observados em *Eupsittula aurea* (76,6%) *C. krusei,* (84,4%) *C. kefyr* e (15,2%) *C. famata,* enquanto em *Amazona aestiva* (76,2%) de *C. krusei* foi observada. Estes resultados indicam a presença de espécies potencialmente patogênicos nas excretas das aves avaliadas, constituem um risco a exposição para os criadores e pessoas que visitam o zoológico. Aves do Pantanal e do Cerrado de Mato Grosso (região Centro-Oeste do Brasil) poderia atuar na cadeia epidemiológica das zoonoses importantes.

Palavras-chave: microbiota fúngicas, região centro-oeste, Brasil, aves, jardim zoológico.

1. Introduction

Several studies have highlighted the importance of bird dropping as a suitable substrate for the growth of yeasts and filamentous fungi (Elhariri et al., 2015; Mendes et al., 2014; Chryssanthou et al., 2011; Brilhante et al., 2010; Marinho et al., 2010; Rosário et al., 2010; Lugarini et al., 2008; Baroni et al., 2006; Pereira, 2006; Mancianti et al., 2002; López-Martínez and Castañón-Olivares, 1995; Caicedo et al., 1999). This is because dried bird droppings are generally very fertile ground for fungal species growth, due to high concentrations of nitrogenous bases, and because as the droppings age, they contain higher concentrations of fungi than when recently eliminated (Elhariri et al., 2015; Mendes et al., 2014; Silva and Paula, 1963).

Several fungal species are commonly detected in bird faeces, especially yeasts of the genera *Cryptococcus* Vuill, *Candida* Berkh, *Trichosporon* Behrend and *Rhodotorula* Harrison (1927), in addition to filamentous fungi belonging to the genera *Aspergillus* Michelli, and *Penicillium* spp (Elhariri et al., 2015; Mendes et al., 2014; Santos et al., 2009; Fraga et al., 2011).

Studies concerning microbiota found in the gut, cloaca and excreta of birds have contributed new knowledge about the ways by which pathogenic fungi are dispersed (Fraga et al., 2011; Brilhante et al., 2010; Marinho et al., 2010; Rosário et al., 2010; Hein-Gonzáles et al., 2010; Baroni et al., 2006; Cafarchia et al., 2006; Mancianti et al., 2002; Caicedo et al., 1999; López-Martínez and Castañón-Olivares, 1995).

Many species of birds are kept as household pets and a wide variety of wild birds are housed in rehabilitation centers, zoos and at breeding sites (Brilhante et al., 2010; Cafarchia et al., 2006). The richness and composition of the birds provide valuable data to understand the sensitivity and resistance of species, nesting and migratory sites (Manica et al., 2010) that may be adversely affected by opportunistic infections. These infections can be developed in the birds themselves through environmental contamination of their habitats (Fraga et al., 2011; Lugarini et al., 2008; Cafarchia et al., 2006; Mancianti et al., 2002).

This study aimed to characterize the fungal microbiota (yeast and mycelial forms) of birds of prey and from Psittacidae in the central west region of Brazil that were maintained in small enclosures and to assess their importance in the epidemiological chain of zoonotic fungal other animals and the birds themselves.

2. Material and Methods

2.1. Animals

The study involved birds of prey and from Psittacidae family maintained in indoor public enclosures and under quarantine. Samples were collected from 109 birds (total of 18 species) on display for visitors, which were housed in 16 enclosures located at the regional animal zoological garden of the Cuiabá city - Federal University of Mato Grosso, central west region of Brazil, and 40 birds (2 species) maintained in 3 quarantine pens that were closed to visitors. These birds had serious health problems, had been ill-treated, or presented immunological compromise and required observation and medical veterinary care. There was no manipulation of animals only of stool samples so that the approval of the animal ethics committee was not necessary.

2.2. Sample collection

From November 2012 to February 2013, excreta were collected in triplicate from the public enclosures (n = 16) in which the birds (n = 109) were displayed for visitors; and from pens (n = 3) where birds (n = 40) were maintained in quarantine. In these enclosures and quarantine pens, kraft paper (1.80 × 1.80 cm) with autoclaved aluminum foil was extended underneath the perches where the birds slept and feed. After preparing the surface for excreta deposition, they were collected 8 hours later using sterile spatulas and placed in sterile containers. Samples that appeared to be humid were subjected to drying in an oven at 28 °C and collected after 24 hours.

2.3. Mycological procedures

2.3.1. Isolation

All the samples were carefully transported to the Medical Mycology Laboratory of the Federal University of Mato Grosso, for processing and identification of the microorganisms isolated.

One gram of each fecal samples was ground in a sterile petri dish to obtain a fine powder. The resulting powder was transferred to labeled Erlenmeyer flasks containing 30 mL of saline solution (0.9% NaCl) and 0.012g of chloramphenicol. The suspension was then vigorously agitated for 5 min to achieve homogenization. The resulting separated mixture was decanted after 30 min.

Following decanting, 1:30 dilution (1 mL of the supernatant + 29 mL of sterile saline + 0.012 g of chloramphenicol) was

performed. Next, the resulting solution was agitated for a further 5 min, followed by seeding of 100 µL of the supernatant onto 150 × 90 Petri plates containing Sabouraud dextrose agar (DIFCO) supplemented with chloramphenicol (100 mg/L) (Marinho et al., 2010).

The plated Petri dishes were maintained for 5 to 7 days in an environmental chamber at 25-27 °C. Following fungal growth, the colonies were counted and selected for seeding in tubes containing Sabouraud dextrose agar (DIFCO) supplemented with chloramphenicol, for isolation and subsequent identification.

2.3.2. Identification

Filamentous fungi: ***Aspergillus* and *Penicillium* genera**

Specific media were used to observe and identify the macroscopic characteristics of the colonies: CYA 25 (Czapeck Agar Yeast Extract 25), CYA 37 (Czapeck Yeast Extract Agar at 37), CY 20S (Czapeck Yeast Extract Agar with 20% sucrose), and MEA (malt extract agar), as previously established (Tell, 2005; Klich, 2002; Samson and Pitt, 2000; Pitt, 2000; Pitt and Hocking, 1997).

Agar culture medium was inoculated with spore suspensions on solid agar (Klich, 2002; Samson and Pitt, 2000; Pitt, 2000) at three equidistant points in the Petri dish. The cultures were incubated at 27 °C and observed after 7 days. Species identifications were made according to distinguishing features (Samson and Pitt, 2000).

The culture media used to facilitate the isolation, growth, and sporulation of *Fusarium* species were carnation leaf agar (CLA), banana leaf agar (BLA) and potato dextrose agar (PDA). The macroscopic structures of the colonies were identified by mycology standards atlas (Nelson et al., 1994; 1983).

In general, the identification of filamentous fungi was based on classic taxonomy (macro and microscopic characteristics). The surface and the reverse of the colonies were observed, as well as diameter, conidial color, texture and presence of soluble pigments (Tell, 2005; Klich, 2002; Samson and Pitt, 2000; Pitt, 2000; Pitt and Hocking, 1997).

2.3.3. Yeasts species

Cryptococcus spp: isolates were initially grown on niger agar and sequentially subjected to Christensen medium. The phenoloxidase production test was conducted in dopamine and CGB media (canavanine glycine bromothymol blue) (Hoog et al., 2000). Biochemical characteristics (fermentation and carbohydrate assimilation) were evaluated (Kurtzman et al., 2011).

Candida spp: Purification of isolated colonies was performed in chromogenic medium, Chromagar (DIFCO). Thereafter, pure colonies were tested based on classic biochemical and microscopic features.

Rhodotorula spp: macroscopic analysis was performed using colony staining. The urea test was performed in Christensen medium, together with a biochemical (sugar assimilation and fermentation tests) profile (Kurtzman et al., 2011).

Trichosporon spp: Colony macroscopic characteristics were observed, followed by observation of yeast morphology using a microscope with glass slides and coverslips (40X magnification). Assimilation tests were conducted on the isolates in addition to the Christensen test (Kurtzman et al., 2011).

For all the yeasts, analysis was performed in the Vitek 2 Compact System (bioMerieux) to confirm species identification using the classic method.

2.4. Statistical analysis

To determine associations between the risk exposures of categorical variables, the Pearson Chi square test was used. Differences were considered statistically significant when the value of p was ≤ 0.05.

3. Results

Droppings from 149 birds were evaluated and divided into two groups: captive (n = 109): *Rhea americana araneipes* (15), *Primolius maracana* (10*), Ara ararauna* (11), *Ara chloropterus* (10*), Anodorhynchus hyacinthinus* (8*), Amazona aestiva* (18), *Ara macao macao* (7), *Ramphastos toco* (4), *Sarcoramphus papa* (4), *Busarellus nigricollis* (2), *Bubo virginianus nacurutu* (3), *Buteogallus coronatus* (1), *Buteogallus urubitinga urubitinga* (3), *Spizaetus melanoleucus* (2), *Spizaetus ornatus ornatus* (3), *Buteo albonotatus* (4), *Geranoaetus albicaudatus albicaudatus* (1), *Rupornis magnirostris magnirostris* (2) and *Harpia harpyja* (1), and quarantined birds (n = 40): *Amazona aestiva* (32) and *Eupsitulla aurea* (8) according to Clements et al. (2017).

In birds fecal samples contained in public enclosures (n = 66), a total of 1,280 filamentous colonies were isolated from different species (Table 1). *A. niger* Van Tieghen was the most prevalent (40.1%) from order Acciptriformes; *Harpia harpyja* (Linnaeus, 1758). Regarding yeasts (n = 393), *Candida kefyr* Beij was the most frequent species (53.8%) isolated from the family Ramphastidae; *Ramphastos toco* (Statius Muller, 1776).

From a total of six samples (three samples in each enclosure), a total of 1,337 CFU/mL has been detected from the birds kept in quarantine environments, 1,080 corresponding to filamentous fungi and 257 to yeasts. Regarding birds maintained in quarantine, the predominance of the yeast species *Candida krusei* (Castellani) Berkhout (76.6%), *Candida kefyr* Beij (84.4%) and *Candida famata* (Zopf) Lodder & Kreger-van Rij (15.2%) was observed in specimens of Peach-fronted parakeet; *Eupsittula aurea* (Gmelin, 1788). For the species of Turquoise-fronted parrot, *Amazona aestiva* (Linnaeus, 1758), the most frequently detected was *C. krusei* (76.2%). In summary, for the two species of birds maintained in quarantine (n = 40), a total 1,080 CFU/mL were isolated, corresponding to four genera and three species. *C. krusei* was the most frequently isolated (480 CFU/mL) followed by *C. kefyr* (380 CFU/mL) (Table 2).

Of total colonies isolated in two environments evaluated, filamentous fungi amounted to 76.5% in those exposed to public viewing and 19.2% in environments quarantined

Table 1. Frequency distribution of the absolute values of CFU/mL of fungi isolated faeces of the bird species maintained in public enclosures.

BIRDS	Family	Filamentous fungi (n=1,280)				Yeasts (n=393)								Total
		Aspergillus niger		*Aspergillus flavus*		*Cryptococcus albidus*		*Rhodotorula mucilaginosa*		*Candida kefyr*		*Trichosporon inkin*		
		N	%	N	%	N	%	N	%	N	%	N	%	
Amazona aestiva (Linnaeus, 1758)	Psittacidae	160	13.7	-	-	-	-	-	-	30	23.1	-	-	**190**
Busarellus nigricollis (Latham, 1790)	Accipitridae	-	-	-	-	-	-	48	61.5	-	-	60	40	**108**
Buteo albonatus Kaup, 1847	Accipitridae	90	7.7	15	13.6	-	-	-	-	-	-	-	-	**105**
Geranoaetus albicaudatus (Vieillot, 1816)	Accipitridae	-	-	21	19.1	-	-	-	-	-	-	-	-	**21**
Harpia harpyja (Linnaeus, 1758)	Accipitridae	470	40.1	31	28.2	-	-	-	-	-	-	-	-	**501**
Buteogallus coronatus Vieillot, 1817	Accipitridae	290	24.8	-	-	-	-	-	-	-	-	-	-	**290**
Primolius maracana (Vieillot, 1816)	Psittacidae	90	7.7	-	-	-	-	-	-	-	-	-	-	**90**
Ramphastos toco Statius Muller, 1776	Ramphastidae	-	-	-	-	-	-	-	-	70	53.8	60	40	**130**
Rhea americana (Linnaeus, 1758)	Rheidae	-	-	6	5.4	-	-	-	-	-	-	-	-	**6**
Rupornis magnirostris (Gmelin, 1788)	Accipitridae	70	6.0	10	9.1	-	-	30	38.5	30	23.1	-	-	**140**
Sarcoramphus papa (Linnaeus, 1758)	Cathartidae	-	-	18	16.4	-	-	-	-	-	-	30	20	**48**
Spizaetus melanoleucus (Vieillot, 1816)	Accipitridae	-	-	-	-	23	65.7	-	-	-	-	-	-	**23**
Buteogallus urubitinga (Gmelin, 1788)	Accipitridae	-	-	9	8.2	12	34.3	-	-	-	-	-	-	**21**
Total		**1,170**	**100**	**110**	**100**	**35**	**100**	**78**	**100**	**130**	**100**	**150**	**100**	**1,673**

environments. However, isolated from samples collected from the group of animals kept in quarantine yeasts constituted 80.8% in contrast to 23.5% of the detected open house environment (Table 3).

The birds that remained exposed to the public showed 13 times more chance of isolation of filamentous fungi when compared to the birds that remained in quarantine (OR = 6.13; CI95% = 11.4-16.3; p <0.0001); however, the first environment behaved as a protective factor for the isolation of yeasts in the same birds (OR = 0.07; CI95% = 0.06 to 0.08; p <0.0001).

4. Discussion

The current literature contains few studies that have evaluated fungal microbiota isolated from bird droppings, the majority focus on surveying yeasts, particularly those belonging to the *Cryptococcus* complex, is a life-threatening systemic mycosis affecting a wide range of animals and humans (Danesi et al., 2014; Sykes and Malik, 2012). Concerning filamentous fungi, species belonging to the genus *Aspergillus* were most commonly reported regarding isolates from psittacine droppings (Fraga et al., 2011).

Knowledge concerning the microbiota present in a population of birds is essential for identifying microorganisms that act as reservoirs for the transmission of likely zoonoses. The zoological gardens usually attract large-scale movement of visitors and the keepers are continuously exposed to bird droppings.

In the Midwest region of Brazil, the States of Mato Grosso and Mato Grosso do Sul comprise the territorial extension of the Pantanal. This biome has specific and

Table 2. Frequency distribution of the absolute values of CFU/mL of fungi isolated from faeces of the bird species maintained in quarantine.

	Eupsittula aurea		*Amazona aestiva*		Total
	N	%	N	%	CFU/mL
Yeast					
Candida krusei (Castellani) Berkhout	-	-	480	76.2	**480**
Candida kefyr Beij.	380	84.4	-	-	**380**
Candida famata (Zopf) Lodder & Kreger-van Rij	70	15.6	-	-	**70**
Geotrichum candidum Link	-	-	30	4.8	**30**
Pichia anomala Redaelli	-	-	30	4.8	**30**
Trichosporon inkin (Küchenm. & Rabenh.) Vuill.	-	-	90	14.2	**90**
Total	**450**	**100**	**630**	**100**	**1,080**
Filamentous fungi					
Absidia cylindrospora Hagen	-	-	9	10.9	**9**
Acremonium kiliense Grütz	45	25.9	50	60.2	**95**
Aspergillus fumigatus Fresenius	-	-	12	14.4	**12**
Fusarium culmorum (Wm.G.Sm.) Sacc.	3	1.8	-	-	**3**
Fusarium dimerum Penz.	30	17.2	-	-	**30**
Fusarium solani (Mart.) Sacc.	60	34.5	-	-	**60**
Fusarium tabacinum (J.F.H. Beyma) W. Gams,	-	-	3	3.6	**3**
Penicillium citrinum Thom.	6	3.4	9	10.9	**15**
Penicillium glabrum (Wehmer) Westling	30	17.2	-	-	**30**
Total	**174**	**100**	**83**	**100**	**257**

CFU: Colony Forming Units.

Table 3. Distribution of yeasts present in CFU/ml from the faeces of birds in maintained quarantine and in public enclosures.

Yeast (n=1,506)	CFU/mL ≤ 90 [n (%)]	CFU/mL > 90 [n (%)]
Candida kefyr Beij.	--	510 (33.9)
Candida krusei (Castellani) Berkhout	--	480 (31.9)
Candida famata (Zopf) Lodder & Kreger-van Rij	70 (4.7)	--
Saccharomyces ellipsoideus Reess	30 (2.0)	--
Geotrichum candidum Link	30 (2.0)	--
Pichia anomala Redaelli	30 (2.0)	--
Trichosporon inkin (Küchenm. & Rabenh.) Vuill.	--	240 (15.9)
Sporobolomyces salmonicolor Fell & Tallman	3 (0.2)	--
Rhodotorula mucilaginosa (A. Jörg.) F.C. Harrison	78 (5.2)	--
Cryptococcus albidus (Saito) C.E. Skinner	35 (2.3)	--
Total	**276**	**1,230**

CFU: Colony Forming Units.

diverse wildlife, with many species on display for visitors to the zoo of the Federal University of Mato Grosso.

Considering the variety of birds found in the Brazilian fauna in the Cerrado (Manica et al., 2010; Telles and Dias, 2010) and Pantanal (Figueira et al., 2006), we were surprised that our review of the literature uncovered no reports of studies involving the fungal microbiota from droppings of the species assessed in this work. This fact hinders detailed comparison of the results obtained, since to our knowledge, this is the first work specifically assessing birds of the biome Cerrado (Savannah) and Pantanal in Mato Grosso.

These findings clearly show that the yeast microbiota detected among quarantined birds was more expressive. Although these microorganisms act as commensals, the number of immunocompromised patients in society has substantially increased and *Candida* species have been identified in various infections, particularly in fungemia (Kurtzman et al., 2011). Some authors have also reported systemic infections by less frequent agents, such as *Trichosporon* spp (Wille et al., 2013) and *Rhodotorula* spp (Yamamoto et al., 2013), affecting neutropenic patients, including neonates, patients requiring central venous catheters and those presenting haematological malignancies (Chitasombat et al., 2012).

The isolation of *Cryptococcus* spp. in excreta of psittacine birds (Filiú et al., 2002), members of the orders Passeriformes (Marinho et al., 2010; Lugarini et al., 2008),

Columbiformes (Cichon et al., 2011), and Accipitriformes (Cafarchia et al., 2006) has been consistently reported in the literature and often associated with the bird's habit of scraping and fragmenting pieces of wood and branches (Filiú et al., 2002).

Nascimento et al. (2017) recorded the occurrence of yeasts belonging to the *Cryptococcus* complex (*C. albidus* var. *albidus* = *C. albidus*) in the cloacae of 40 parrots (*Amazona aestiva)* maintained in a private farm located in the City of Jundiai, São Paulo, Brazil. Ninety percent of the isolates corresponded to the species *C. albidus*; and 10% to the species *C. laurentii*. The results obtained in this research confirm the role and the relevance of the parrots from the genus *A. aestiva* as a source of dissemination of yeasts in the environment.

In contrast to the findings of Nascimento et al. (2017), considering the droppings of the same species (*Amazona aestiva*) housed at the UFMT zoological garden, *C. albidus* was not isolated, but the presence of yeasts (*Pichia anomala*, *Candida krusei* and *Trichosporon* spp) was observed, together with other genera of filamentous fungi.

Pereira (2006) reported the isolation of *Cryptococcus neoformans* in *Aratinga mitrata* residing at a zoo. This finding raises awareness concerning the isolation of this species in exotic birds.

Reports from the literature indicate the occurrence of *Cryptoccoccus* yeasts in a number of distinct materials. Cryptococcus species are widely distributed in nature and can be isolated from various environmental sources such as air, soil, bird excreta, water, animals and decomposing wood (Villar et al., 2012; Leite-Junior et al., 2012; Pedroso et al., 2009; Wuczkowski et al., 2005). In birds, such reports occur: swallowtail feces (*C. neoformans*) (Hedayati et al., 2011), passerine and psittacine droppings (*C. neoformans*), droppings of birds in captivity (*C. neoformans and C. gatii*) (Hein-Gonzáles et al., 2010; Lugarini et al., 2008; Pereira, 2006; Mancianti et al., 2002), pigeon droppings and fragments of *Eucalyptus* spp. trees (Montenegro and Paula, 2000). Chryssanthou et al. (2011) reported isolating yeasts of the genus Cryptococcus (*C. neoformans, C. gattii and C. albidus*) in seabirds and highlighted the role of birds in the maintenance of yeasts in the environment.

In environmental samples, numerous records indicate the presence of yeasts, though not necessarily related to the presence of birds or bird droppings. Among these studies, the work by Baroni and colleagues (2006) is worth mentioning, since they demonstrated the presence of yeast (*C. neoformans* and *C. albidus*) in pigeon excreta, air inside church towers, and surrounding areas.

In Southern Brazil, Santos and colleagues (2009) reported the presence of *Cryptococcus neoformans* and *Candida* spp in the droppings of captive psittacine and passerines. They collected 29 samples, 15 from the oral cavity and 14 from the cloacae of these birds. Nine samples were obtained from *Amazona aestiva* which *Candida famata* was isolated. In this work, *Candida famata* and *Candida krusei* were also isolated.

Candida famata is no more considered as a pathogen for humans and *C. krusei*, although rare, is important because it is intrinsically resistant to azoles, the most widely used antifungals.

Brilhante and colleagues (2010), reported the characterization of the gastrointestinal microbiota of cockatiel (*Nymphicus hollandicus,* Kerr, 1792, Psittacidae family), emphasizing its potential danger to human health. Among the isolated yeasts, the genera *Candida* spp., *Cryptococcus* spp., *Rhodotorula* spp. and *Trichosporon* spp. The species *C. famata* and *C. albidus* were isolated, in agreement with the results obtained for *Amazona aestiva* (Santos et al., 2009).

Considering research conducted outside Brazil, the following studies should be mentioned: López-Martínez and Castañón-Olivares (López-Martínez and Castañón-Olivares, 1995), Mexico, who reported the isolation of *C. neoformans* (9.45%) in the bird droppings at San Juan Aragon zoological garden; and Caicedo et al. (1999), Colombia, who reported the isolation of *C. neoformans* (0.9%) from the droppings of 259 birds, subdivided into 12 orders, housed at the zoological garden of the city of Cali. More recently, Elhariri and colleagues (2015) evaluated excreta species lovebirds and cockatiels concluded that the excreta of these birds can play a role as a risk reservoir of *C. neoformans* in domestic and public environments and enhance their zoonotic importance to humans.

In Italy, was described the occurrence of yeasts in parrots located in family residences. Among the birds surveyed, *Amazona aestiva* presented isolates of the species *Candida krusei*, *Candida famata* and *Geotrichum* spp (Mancianti et al., 2002).

In Spain, were isolated *C. neoformans*, *C. uniguttulatus*, *C. laurentii* and *C. albidus*, obtained from pigeon cloacae (Rosário et al., 2010). Another group of researchers in Italy described the occurrence of yeasts in material obtained from the cloacae of migratory birds (Cafarchia et al., 2006). Their results show the same species (*C. albidus*, *C. famata* and *Trichosporon* spp) isolated from the droppings of birds from the Cerrado and Pantanal in Mato Grosso.

In Chile, also were isolated yeasts (*Candida* spp, *Cryptococcus* spp and *Rhodotorula* spp) in the cloacae of parrots in a rehabilitation centers (Hein-Gonzáles et al., 2010). Both *C. famata* and *C. albidus* were isolated, in agreement with data obtained in this work. Due to the different number of samples used in each study, the percentages detected are not comparable. The Chileans authors suggested that it would be interesting to conduct studies to verify whether there are significant differences in the yeast microbiota of birds maintained in zoological gardens and in wild birds, as well as the virulence factors associated with these yeasts.

The relevance of avian zoonosis is worth mentioning because these are infections that frequently remain asymptomatic in birds, mistakenly viewed as healthy, making it difficult to determine the correct diagnosis and subsequent treatment, thus increasing the chances of transmission to bird keepers, zoo visitors, and pet

owners. Thus, apparently healthy looking birds can act as hosts for pathogenic microorganisms by defecating and contaminating the environment with various yeast genera that show zoonotic potential (Nascimento et al., 2017).

Sedgwick et al. (1975) and Fowler (1993) stated that with respect to animals in captivity, despite efforts by professionals in maintaining strict sanitary management, the zoological environment remains conducive to the spread of a range of diseases, many of them zoonotic.

More currently, in 2014, Mendes and researchers reported the presence of fungi isolated from the excreta of wild birds in screening centers in Pelotas, South region of Brazil. The isolation of the following species was observed: Candida spp., Cryptococcus spp., *Aspergillus* spp. *and Penicillium* spp.

Regarding the isolation of filamentous fungi, a variety of species of the genus *Aspergillus* were isolated in this study; the genus *Aspergillus* predominated, followed by *Penicillium* spp. and *Fusarium* spp.

Conceição et al. (2010) indicated that species of the genus *Aspergillus* (*A. flavus* and *A. fumigatus*) were isolated in sunflower seed marketed in Aracaju state, Brazil. This fact deserves mention because sunflower seeds are used as a power source for parrots. Thus, there may be possible that parrots acquire aspergillosis by eating seeds contaminated with Aspergillus fungi. Acute fungal aspergillosis is characterized by respiratory disorders and the formation of caseous plaques in the lung and air sacs (Rousseaux and Dalziel, 1981). This type of aspergillosis occurs most frequently in young birds and causes high mortality. In contrast, chronic aspergillosis is more common in adult birds. This condition is mainly caused by *A. fumigatus* and *A. flavus* (Dagenis and Keller, 2009).

According to Tell (2005), the susceptibility of birds to aspergillosis is mainly because their respiratory tract has no diaphragm. However, they possess air sacs, which are great locations for fungal colonization, with optimal conditions of temperature and oxygen, as well as poor vascularization. The clinical signs are usually nonspecific and related to anorexia, weight loss, lethargy, or respiratory system impairment (rhinitis, dyspnea and changes in vocalization). Initially, colonization occurs and, depending on other related factors, the birds progress to infection and disease.

Other researchers, reported the occurrence of one or two fungal infections described in psittacine birds: cockatiel/*Nimphicus hollandicus* (Vasconcelos et al., 2011; Brilhante et al., 2010), lovebird/*Agapornis fischeri* (Nouri and Kamyabi, 2010), Australian parakeet/*Melopsittacus undulatus* (Silva et al., 2014), Turquoise-fronted parrot/*Amazona aestiva* (Nascimento et al., 2017; Yamamoto et al., 2013; Carrasco et al., 1998), sulphur-crested cockatoo/*Cacatua sulphurea sulphurea* (Anderson, 1993).

Maintaining hygiene in the public enclosures that house the birds at the UFMT zoological garden is critical, together with the use of personal protective equipment (PPE) by the keepers. These actions are important in order to prevent the exposure of the birds themselves and individuals who are engaged in professional activities and/or visiting the zoo from the infectious propagules contained in the environment.

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