Pesq. Vet. Bras. 28(7):341-344, julho 2008

Screening of antigenemia and isolation of *Cryptococcus neoformans* and *C. gattii* from cloaca and crop of birds in the state of Paraná, Brazil[1]

Camile Lugarini[2]*, Larissa A.Z. Condas[3], Grazielle C.G. Soresini[4], Renata C.F. Santos[3], Marisol D. Muro[5], Margaret Ono[5], Marconi R. Farias[4] and Fabiano Montiani-Ferreira[3]

ABSTRACT.- Lugarini C., Condas L.A.Z., Soresini G.C., Santos R.C.F., Muro M.D., Ono M., Farias M.R. & Montiani-Ferreira F. 2008. **Screening of antigenemia and isolation of *Cryptococcus neoformans* and *C. gattii* from cloaca and crop of birds in the state of Paraná, Brazil.** *Pesquisa Veterinária Brasileira 28(7):341-344*. Departamento de Medicina Veterinária, Rua dos Funcionários 1540, Juvevê, Curitiba PR 80035-050, Brazil. E-mail: camilelug@gmail.com

Cryptococcus neoformans and *C. gattii* are associated with dry bird excreta but rarely recovered from birds' digestive tract. The objective of the present study was (1) to verify the existence of *C. neoformans* and *C. gattii* in crop and cloaca of wildlife and captivity birds hypothesizing about a possible primary source of this yeast in the excreta, and (2) to determine the fungi's invasive capability in avian species through latex agglutination. For that purpose, 172 cloacal and 77 crop samples of domestic pigeon, Passerine, and Psittacine birds were collected. None of these samples was positive, suggesting that the yeast is not saprobiotic in the digestive tract of these birds. Only one out of 82 serum samples collected from pigeons and Psittacine birds was positive (title 1:2) showing that *Cryptococcus* sp. probably has a low invasive capability in birds, and is thus considered only a dry excreta colonizer.

INDEX TERMS: Ecology, birds, yeast, *Cryptococcus neoformans, Cryptococcus gattii.*

RESUMO.- [**Antigenemia e tentativa de isolamento de *Cryptococcus neoformans* e *C. gattii* a partir da cloaca e inglúvio de aves no Estado do Paraná.**] *Cryptococcus neoformans* e *C. gattii* são frequentemente isolados de excretas de aves, entretanto ocorre pouca recuperação desse fungo a partir do trato gastrintestinal. Os objetivos desse estudo foram verificar a existência de *C. neoformans* e *C. gattii* no inglúvio e na cloaca de aves de vida livre e cativeiro, avaliando uma possível fonte primária desta levedura nas excretas e determinar a capacidade invasiva do fungo em aves por meio da aglutinação em látex. Para tanto, foram coletadas 172 amostras de cloaca e 77 de inglúvio de pombos-domésticos, Passeriformes e Psittaciformes. Nenhuma amostra se mostrou positiva, sugerindo-se que o fungo não é saprobiótico do aparelho digestório destas aves. Das 82 amostras de soro colhidas a partir de pombos-domésticos e Psittaciformes, somente uma obtida a partir de pombo-doméstico se mostrou positiva (titulação 1:2), demonstrando que *Cryptococcus* sp. apresenta baixa capacidade invasiva em aves, sendo, portanto, considerado somente um colonizador de excretas ressequidas.

TERMOS DE INDEXAÇÃO: Ecologia, aves, fungo, *Cryptococcus neoformans, Cryptococcus gattii.*

INTRODUCTION

Cryptococcosis is an opportunistic fungal disease mainly caused by *Cryptococcus neoformans*. Immunocompro-

[1] Received on April 15, 2008.
Accepted for publication on July 18, 2008.

[2] Instituto Chico Mendes de Conservação da Biodiversidade, Parque do Bom Menino, anexo ao Núcleo de Educação Ambiental, Av. Alexandre de Moura s/n, Centro, São Luis, MA 65045-470, Brazil. *Corresponding author: camilelug@gmail.com

[3] Departamento de Medicina Veterinária, Universidade Federal do Paraná, Rua dos Funcionários 1540, Juvevê, Curitiba PR 80035-050, Brazil.

[4] Departamento de Medicina Veterinária, Pontifícia Universidade Católica do Paraná, Rodovia BR376 Km14, Costeira, São José dos Pinhais, PR 83010-500, Brazil.

[5] Serviço de Análises Clínicas, Hospital de Clínicas, Universidade Federal do Paraná, Av. General Carneiro 181, Curitiba, PR 80060-900.

mised conditions such as those found in AIDS patients, post-organ transplant surgery, or hematological malignancies constitute the main factors in the development of this disease (Mitchell & Perfect 1995, Casadevall & Perfect 1998).

C. neoformans was traditionally subdivided into three varieties and five serotypes: *C. n.* var. *grubii* (serotype A) (Franzot et al. 1999), *C. n.* var. *neoformans* (serotype D), serotype AD - considered a hybrid (Boekhout et al. 2001) - and *C. n.* var. *gattii* (serotypes B and C) (Franzot et al. 1999). Recently, based on its morphological, biochemical, and molecular differences it has been suggested that the *C.n.* var. *gattii* be reclassified as a new species: *C. gattii* (Know-Chung et al. 2002).

C. neoformans is found in a variety of environmental sources, although it is mainly associated with avian droppings, especially those of domestic pigeons (Kobayashi et al. 2005, Granados & Castañeda 2005), Passerine, and Psittacine birds (Filiú et al. 2002, Abegg et al. 2006). *C. gattii*, on the other hand, is usually isolated from decaying wood (Lazéra et al. 1998, Lazéra et al. 2000, Granados & Castañeda, 2005), although it has recently been isolated from avian droppings (Abegg et al. 2006).

Although appearing in large numbers in avian environments, *Cryptococcus* spp. rarely cause clinical diseases in birds, because yeast cannot either grow at the bird's temperature or survive its passage through the intestinal tract, beyond its low invasive capability in birds (Mitchell & Perfect 1995, Casadevall & Perfect 1998, Filiú et al. 2002). Clinical infections with a subsequent elimination of the yeast from the body by intact cell immunity can also occur (Connolly et al. 1999) and, in this case, birds can be transitory carriers of the disease (Bauwens et al. 1986, Mitchell & Perfect 1995). In humans, clinical diseases are uncommon, but contact and sensitivization to antigens are more prevalent with antibodies detected in 20% of the adult population (Mitchell & Perfect 1995, Malik 2003). We assumed that the same applies to pigeons and Psittacine birds. They can be in contact with the yeast and eliminate the agent spontaneously. The carrier state might precede the establishment of infection, and the positive antigenemia might confirm the contact with *Cryptococcus* spp.

The objective of the present study was to verify the existence of *C. neoformans* and *C. gattii* in crop and cloaca of wildlife and captivity birds hypothesizing about a possible primary source of this yeast in the excreta, and to determine the fungi's invasive capability in avian species through latex agglutination.

MATERIALS AND METHODS

A total of 77 crop and 172 cloaca samples were obtained with uretral swabs (Bionete, Biolog, São Paulo, Brazil) from Passerine, Psittacine birds, and pigeons (Table 1). Passerine, Psittacine samples were obtained from birds received in

Table 1. Cloaca, crop and serum samples obtained from domestic pigeons, Passerine, and Psittacine showing the number of samples by species

Commun name	Scientific name	Order	Number of cloaca samples	Number of crop samples	Number of serum samples
Pigeon	*Columba livia*	Columbiforme	53	53	53
Black-throated Grosbeak	*Saltator fuliginosus*	Passerine	0	1	0
Ultramarine Grosbeak	*Cyanocompsa brissonii*	Passerine	4	1	0
Buffy-fronted Seedeater	*Sporophila frontalis*	Passerine	10	0	0
Chopi Blackbird	*Gnorimopsar chopi*	Passerine	7	0	0
Double-collared Seedeater	*Sporophila caerulescens*	Passerine	7	0	0
Epaulet Oriole	*Icterus cayanensis*	Passerine	1	0	0
Green-winged Saltator	*Saltator similis*	Passerine	4	3	0
Hooded Siskin	*Carduelis magellanica*	Passerine	2	1	0
Lined Seedeater	*Sporophila lineola*	Passerine	2	0	0
Red-crested Finch	*Coryphospingus cucullatus*	Passerine	3	0	0
Rufous-bellied Trush	*Turdus rufiventris*	Passerine	2	1	0
Rufous-collared Sparrow	*Zonotrichia capensis*	Passerine	2	0	0
Saffron Finch	*Sicalis flaveola*	Passerine	11	1	0
Trush [a]	*Turdus* sp.	Passerine	1	0	0
Uniform Finch	*Haplospiza unicolor*	Passerine	1	0	0
Violaceous Euphonia	*Euphonia violacea*	Passerine	1	0	0
Yellow-legged Trush	*Turdus flavipes*	Passerine	2	0	0
Blue and yellow Macaw	*Ara ararauna*	Psittacine	4	0	2
Orange-winged Parrot	*Amazona amazonica*	Psittacine	4	0	0
Plain Parakeet	*Brotogeris tirica*	Psittacine	4	4	0
Red-cappped Parrot	*Pionopsitta pileata*	Psittacine	2	2	0
Red-tailed Amazon	*Amazona brasiliensis*	Psittacine	1	0	0
Scaly-headed Parrot	*Pionus maximiliani*	Psittacine	5	2	3
Turquoise-fronted Parrot	*Amazona aestiva*	Psittacine	28	8	24
Vinaceous-breasted Parrot	*Amazona vinacea*	Psittacine	11	0	0
Total			172	77	82

[a] Species identified at genus level.

Centro de Triagem de Animais Silvestres (CETAS) PUCPR/IBAMA, located in Parana state, Brazil. The pigeons were divided into two groups: 20 samples from captivity and 33 from wildlife pigeons. Psittacine birds were tranquilized with midazolam (*União Química Farmacêutica Nacional*, Minas Gerais, Brazil) before starting the procedure for collecting crop samples. Other samples were obtained with manual restraint only.

The samples were plated onto birdseed (*Guizotia abyssinica*) agar plates or tubes and incubated at 30ºC for up to 7 days. Dark brown colonies suggestive of *C. neoformans* were then subcultivated on Sabouraud´s peptone dextrose agar plates and identified as *C. neoformans* or *C. gattii* by standard morphological and biochemical methods: the ability to produce melanin in birdseed agar, cycloheximide sensitivity, thermotolerance at 37ºC, urease production, and carbon and nitrogen assimilation profiles.

Blood was collected from 29 Psittaciformes, 20 captivity pigeons, and 33 wildlife pigeons - weighing more than 300g (Table 1). For this purpose, the animals were captured and manually restrained. Blood was obtained from the radial or ulnar vein, located in the medial face of their wing, centrifuged at 2000 rpm for 15min, and the serum was separated and stored in glass tubes at -20°C till serology was done. Blood samples of Passeriformes were not collected because their size makes it impossible to obtain the serum volume necessary for carrying out the test.

Antigenemia was carried out with the *Latex-Cryptococcus Antigen Detection System* kit (Immuno-Mycologics, Inc., Norman, OK, USA) following the manufacturer's standards. This test aims at the qualitative and semi-quantitative detection of capsular antigens *C. neoformans* and *C. gattii* in liquor and serum and detects a minimum of about 3.2ng/ml of capsular antigens.

RESULTS

All 249 cloaca and crop samples were considered negative for *Cryptococcus neoformans* and *C. gattii* isolation. A variety of other fungi grew on the bird seed agar, but those were not further classified. In what concerns the antigenemia, most serum samples presented a homogeneous suspension of particles without visualization of lump formation after mixing with latex, and were thus considered negative. The only positive sample came from a wildlife pigeon and the title was low (1:2).

DISCUSSION

This is the first study performed in Brazil with the aim to isolate *Cryptococcus neoformans* and *C. gattii* from bird's digestive tract. Despite *C. neoformans* isolation being frequent in excreta from avian species (Mitchell & Perfect 1995, Filiú et al. 2002, Kobayashi et al. 2005, Granados & Castañeda 2005, Abegg et al. 2006), the fungus is hardly isolated from the digestive tract of those birds (Rosario et al. 2005, Cafarchia et al. 2006a,b), as seen in the present study. Rosario et al. (2005) isolated only 1.81% of *C. neoformans* from cloacal samples of pigeons, and Cafarchia et al. (2006a) did not obtain any positive results for *C. neoformans* or *C. gattii* in cloacal samples of 421 migratory birds. Cafarchia et al. (2006b) did not manage to recover either *C. neoformans* or *C. gattii* from any sample of digestive system tract segments of any dead bird of prey and obtained 2.2% of *C. neoformans* var. *grubii* from cloacal swabs.

It is possible that such low or nule isolation rate is due to bad growth conditions for the fungus, because of the birds' high temperature and the high concentration of ammonia in fresh excreta, alkalinizing the medium (Sorrel & Ellis 1997). Mancianti et al. (2001) could not find *C. neoformans* or *C. gattii* in isolates of yeasts of 325 samples of fresh excreta of Psittaciformes.

The fast growth of contaminant fungi, especially Zygomycetes, makes it possible to obtain false-negative results (Swinne-Desgain 1975, Kobayashi et al. 2005). It is known that the number of colonies of *C. neoformans* and/or *C. gattii* is inversally proportional to the number of other yeast colonies contained in Petri plates (Swinne-Desgain 1975).

The mechanism through which the excreta become infected is still uncertain (Sorrel & Ellis 1997, Filiú et al. 2002). However, the development of *C. neoformans* in birds' excreta can be attributed to the large quantity of fungic cells in soil or air, dispersed by wind, which found a rich environment in excreta for their proliferation. It is widely known that non-infected pigeon excreta become infected when exposed to air containing aerosolubilized cells of *C. neoformans* (Casadevall & Perfect 1998). Besides, when transferred to places with high environmental prevalence of *C. gattii*, koalas (*Phascolarctos cinereus*) without nasal colonization by *Cryptococcus* spp. become persistently colonized (Krockenberger et al. 2002). The results obtained in the present study, together with the claims made by the cited authors, confirm the assumption of environmental contamination of the excreta. Nevertheless, for a more precise analysis of this hypothesis it would also be necessary to collect samples from the environment, air, and food in order to identify the main environmental source of *Cryptococcus* spp. Furthermore, additional studies are required to evaluate relation of cloacal and crop colonization with host, virulence and pathogenicity of the agent (Krockenberger et al. 2002), thus demonstrating the real importance of these factors in the understanding of the relationship between *Cryptococcus* spp. and various avian species.

The latex agglutination test is usually used for diagnosing cryptococosis in humans, pets and birds (Medleau et al. 1990, Mitchell & Perfect 1995, Raso et al. 2004). It demonstrates high sensitivity and specificity, and presents quick results (Medleau et al. 1990, Mitchell & Perfect 1995, Casadevall & Perfect 1998); however, rare negative results may be obtained when the disease is localized (Medleau et al. 1990).

No antigenemia was observed in most investigated birds, suggesting that *Cryptococcus* spp. do not invade the birds'organism, consequently discarding possible subclinical infections and the status of carrier. Negative results were also obtained for the antigenemia of dogs, cats, and

koalas with nasal cavity colonization by *C. neoformans* demonstrating a low invasive capability of the fungus in immunocompetent animals (Malik et al. 1997, Connolly et al. 1999). The only animal with positive antigenemia had a low title. In this case, it is suggested that there was a systemic invasion or a localized infection by active penetration, which can occur in birds (Malik et al. 2003). As no detailed clinical exam and no necropsy were carried out in the animals in order to confirm or discard the presence of induced lesions by *Cryptococcus* sp., crypto-coccosis cannot be discarded in that specific animal. It is worth pointing out that this individual was a wildlife bird and that in such environment birds are exposed to a higher quantity of inoculum, because of the contamination of urban agglomeration with pigeon excreta. Birds kept in captivity were given a daily hygienic treatment and had a lower susceptibility to develop cryptococcosis or to contact the yeast.

CONCLUSIONS

The results obtained in the present investigation led us to suggest that *Cryptococcus* spp. are not endosaprobiotic in the avian digestive tract, and that their primary source might be the environment, and also that they find a favorable environment for their development in birds' excreta. Furthermore, the yeast has a low invasive capability in the species included in this study.

Acknowledgments.- We thank CAPES and Fundação Araucária for their financial support; IBAMA for having licensed the project; the trainees, technicians and employees of CETAS PUCPR/IBAMA; the Curitiba Zoo and the UFPR Veterinarian Hospital, especially Lucyene Giselle Popp Brasil Queiroz, for their invaluable help in carrying out the project.

REFERENCES

Abegg M.A., Cella F.L., Faganello J., Valente P., Schrank A. & Vainstein M.H. 2006. *Cryptococcus neoformans* e *Cryptococcus gattii* isolated from the excreta of psittaciformes in a southern Brazilian zoological garden. Mycopathologia 161(2):83-91.

Bauwens L., Swinne D., Vroey C. & Meurichy W. 1986. Isolation of *Cryptococcus neoformans* var. *neoformans* in the aviaries of the Antwerp Zoological Gardens. Mykosen 29(7):291-294.

Boekhout T., Theelan B., Dias M., Fell J.W., Hop W.C.J., Abeln E.C.A., Drome F. & Meyer W. 2001. Hybrid genotypes in the pathogenic yeast *Cryptococcus neoformans*. Microbiol. 147:891-907.

Cafarchia C., Camarda A., Romito D., Campolo M., Quaglia N.C., Tullio D. & Otranto D. 2006a. Occurrence of yeast in cloacae of migratory birds. Mycopathologia 161(4):229-234.

Cafarchia C., Romito D., Iatta R., Camarda A., Montagna M.T. & Otranto D. 2006b. Role of birds of prey as carriers and spreaders of *Cryptococcus neoformans* and other zoonotic yeasts. Med. Mycol. 44(6):485-92.

Casadevall A. & Perfect J.R. 1998. *Cryptococcus neoformans*. American Society for Microbiology Press, Washington, DC. 541p.

Connolly J.H., Krockenberger M., Malik R., Canfield P.J., Wigney D.I. & Muir D.B. 1999. Asymptomatic carriage of *Cryptococcus neoformans* in the nasal cavity of the koala (*Phascolarctos cinereus*). Med. Mycol. 37:331-338.

Filiú W.F.O.F., Wanke B., Agüena S.M., Vilela V.O., Macedo R.C.L. & Lazera M. 2002. Cativeiro de aves como fonte de *Crytococcus neoformans* na cidade de Campo Grande, Mato Grosso do Sul, Brasil. Revta Soc. Bras. Med. Trop. 35(6):591-595.

Franzot S.P., Salkin I.F. & Casadevall A. 1999. *Cryptococcus neoformans* var. *grubii*: separate varietal status for *Cryptococcus neoformans* serotype A isolates. J. Clin. Microbiol. 37:838-840.

Granados D.P. & Castañeda E. 2005. Isolation and characterization of *Cryptococcus neoformans* varieties recovered from natural sources in Bogotá, Colombia, and study of ecological conditions in the area. Microb. Ecol. 49:282-290.

Know-Chung K.J., Boekhout T., Fell J.W. & Diaz M. 2002. Proposal to conserve the name *Cryptococcus gattii* against *C. hondurianus* and *C. bacillisporus* (Basidiomycota, Hymenomycetes, Tremellomycetidae). Taxon 51:804-806.

Kobayashi C.C.B.A., Hasimoto e Souza L.K., Fernandes O.F.L., Brito S.C.A., Silva A.C., Sousa E.D. & Silva M.R.R. 2005. Characterization of *Cryptococcus neoformans* isolated from urban environmental sources in Goiânia, Goiás, Brazil. Revta Inst. Med. Trop. 47(4):203-207.

Krockenberger M.B., Canfield P.J. & Malik R. 2002. *Cryptococcus neoformans* in the koala (*Phascolarctos cinereus*): colonization by *C. n.* var. *gattii* and investigation of environmental sources. Med. Mycol. 40:263-272.

Lazéra M.S., Cavalcanti M.A.S., Trilles L., Nishikawa M.M. & Wanke B. 1998. *Cryptococcus neoformans* var. *gattii*: Evidence for a natural habitat related to decaying wood in a pottery tree hollow. Med. Mycol. 36:119-122.

Lazéra M.S., Salmito Cavalcanti M.A., Londero A.T., Trilles L., Nishikawa M.M. & Wanke B. 2000. Possible primary ecological niche of *Cryptococcus neoformans*. Med. Mycol. 38:379-383.

Malik R., Wigney D.I., Muir D.B. & Love D.N. 1997. Asymptomatic carriage of *Cryptococcus neoformans* in the nasal cavity of dogs and cats. J. Med. Vet. Mycol. 35:27-31.

Malik R., Krockenberger M.B., Cross G., Doneley R., Madill D.N., Black D., Mcwhirter P, Rozenwax A, Rose K, Alley M, Forshaw D, Russel-Brown I, Johnstone A.C., Martin P., O'Brien C.R. & Love D.N. 2003. Avian cryptococcosis. Med. Mycol. 41:115-24.

Mancianti F., Nardoni S. & Ceccherelli R. 2001. Occurrence of yeasts in psittacines droppings from captive birds in Italy. Mycopathologia 153:121-124.

Medleau L., Marks M.A., Brown J. & Borges W.L. 1990. Clinical evaluation of a cryptococcal antigen latex agglutination test for diagnosis of cryptococcosis in cats. J. Am. Vet. Med. Assoc. 196(9):1470-1473.

Mitchell T.G. & Perfect J.R. 1995. Cryptococcosis in the era of AIDS – 100 years after the discovery of *Cryptococcus neoformans*. Clin. Microbiol. Rev. 8(4):515-548.

Raso T.F., Werther K., Miranda E.T. & Mendes-Giannini M.J.S. 2004. Cryptococcosis outbreak in psittacine birds in Brazil. Med.Mycol. 42:355-362.

Rosario I., Hermoso de Mendonza M., Déniz S., Soro G., Álamo I. & Acosta B. 2005. Isolation of *Cryptococcus* species including *C. neoformans* from cloaca of pigeons. Mycoses 48:421-424.

Sorrell T.C. & Ellis D.H. 1997. Ecology of *Cryptococcus neoformans*. Revta Iberoam. Micol. 14:42-43.

Swinne-Desgain D. 1995. *Cryptococcus neoformans* of saprophytic origin. Sabourandia 13:303-308.