Identification of fungi microflora in the ear conducts of rhesus macaques (*Macaca mulatta*) kept in captivity

Thais Lins BROTTO[1]
Márcia Cristina Ribeiro ANDRADE[2]
Miguel Ângelo Brück GONÇALVES[2]
Flávio GIMENIS[1]
Alexandre PINA[1]

1- Faculdade de Medicina Veterinária da Universidade do Grande Rio, Duque de Caxias - RJ
2- Centro de Criação de Animais de Laboratório do Departamento de Primatologia da Fundação Oswaldo Cruz, Rio de Janeiro - RJ

Correspondência para:
MÁRCIA CRISTINA RIBEIRO ANDRADE
Centro de Criação de Animais de Laboratório
Departamento de Primatologia
Fundação Oswaldo Cruz
Avenida Brasil, 4365 – Manguinhos
21045-900 - Rio de Janeiro - RJ
tlbrotto@hotmail.com

Received: 16/12/2003
Accepted: 15/03/2005

Abstract

Several predisposing factors could lead to auricular diseases caused by a saprophytic microflora. Identification of the microflora of fungi could help in the diagnosis and treatment of mycoses that can become pathogenic in case of homeostasic unbalance. This report aimed to identify the saprophytic fungi microflora in the middle ear conduct of clinically healthy rhesus monkeys used for biomedical research. Forty rhesus macaques were divided into two groups. Group I was formed by adult animals, housed in individual cages inside special experimentation containers with controlled temperature and humidity. Group II, originated from the colony, was formed by young animals, which were maintained in the natural environment, without temperature and humidity control. Cerumen of the middle ear conduct of the animals was collected through swabs. Cultivation of the samples was performed in Petri plates with Sabouraud agar with cloramphenicol 1%, sealed with adhesive tape and incubated at room temperature. In the 20 animals from group I, we found the following: *Aspergillus* (80%), *Candida* (60%), *Cladosporium* (5%) and *Rhodotorula* (5%). Group II presented a major diversity of fungi: *Candida* sp. (95%), *Aspergillus* (20%), *Cladosporium* sp. (60%), *Penicillium* sp. (30%), *Rodotorulla* sp. (15%), *Trychophytum verrucosum* (5%), *Epidermophyton flocosum* (5%), and *Scopulariopsis* sp. (5%). These data will be useful for diagnoses and treatments of otites and suggest that climatic factors could be responsibles for the great number of fungi present in the animals from group II, which were exposed to natural climatic conditions.

Key-words
Fungi microflora.
Non-human primates.
Rhesus monkey.
Ear conduct.

Introduction

Primates are potential transmitters of different diseases for hosting a number of different microorganisms and being highly susceptible to infections common in humans[1]. In primate colonies, dermatophytoses are generally not reported. However, due to their great infectivity, some agents can involve humans and animals. There are reports on saprophytic fungi that can become pathogenic and spread endemically from animal to animal or even to humans[2].

Fungi are part of the normal microflora of the external ear canal of humans and animals. Sometimes these, fungi are associated with external otitis[3]. The normal microflora could benefit the host by avoiding excessive growth of noxious microorganisms through a competitive process. Opportunistic pathogens are organisms that normally do not cause disease in their natural habitat in a healthy animal. Frequently, due to immunodeficiency of the host or even transfer of the microorganism from one to another symbiosis partner, this

competitive process could get out of balance[4].

The most common causes of hearing impairment in humans, laboratory animals and other species are otitis media, inflammatory processes or infections resulting in accumulation of fluid in the middle ear, which interferes with the tympanic vibrations. If the tympanic membrane gets immobilized due to fluid accumulation in the middle ear, the sound resonates backwards instead of penetrating the middle ear[5].

In the present paper, we identified the microflora of saprophytic fungi in the ear conduct of rhesus monkeys (*Macaca mulatta*) in order to find possible predispositions for ear diseases in these animals and to aid the diagnosis and combat of fungi, which can become pathogenic due to a homeostasic disorder.

Materials and Methods

Animals

Forty clinically healthy rhesus monkeys destined to biomedical research were used from the Laboratory Animal Breeding Center, Department of Primatology, Oswaldo Cruz Foundation (FIOCRUZ). These animals were divided into two groups with 20 (twenty) animals each.

Group I was composed of adult animals, one male and 19 (nineteen) females, housed in individual cages in special containers. Temperature and humidity in these containers were controlled according to the Guide for the Care and Use of Laboratory Animals[6]. The equipment used provided action-specific control of internal pressure, internal and external temperature, air input and output and humidity.

Group II was composed by 19 young males and one female, aged between 12 and 17 moths, from the breeding stock of the Department and destined to future research activities. Although shelter was available, these animals were kept in environments with natural climatic conditions, e.g. temperature and humidity were not controlled.

Results obtained from the two selected groups living in different environments were compared and possible organic dysfunctions caused by stress due to confinement in the container and the experiment itself taken into consideration. Analysis of results also considered the differences in temperature, humidity, sex, and age.

Collection of material from the middle ear conduct

Earwax was collected from the auditory canal of the animals using 40 sterile swabs. During sample collection from group I, the temperature registered in the container was 22,3° C and humidity was 73%. The collection of material from group II took place on a hot day with a temperature of approximately 30° C and high humidity. Seen that no thermostat or other specific equipment was used for registering temperature or humidity of the environment of group II, no exact data regarding these variables were available.

Sample processing

Swabs were kept at natural temperature and immediately sent to the Laboratory of Preventive Medicine, Department of Infecto-contagious Diseases of Domestic Animals of the University of Grande Rio (UNIGRANRIO), where were cultured. Each sample was cultured in Petri dishes containing Agar Sabouraud with 1% chloramphenicol, sealed with adhesive tape and incubated at room temperature (30ºC) for 7-14 days. After this incubation period, the agents were identified. Filamentous fungi were identified by their morpho-colonial characteristics using the adhesive tape technique[7], where the gummed side of a 13 cm long transparent adhesive tape is brought into contact with the fungi colony. The adhesive tape is then put on microscopy slides containing a drop of phenol cotton blue for examination under optic microscopy at 40x magnification.

Yeasts were identified according to their morpho-colonial characteristics. Yeasts of the genus *Candida* were isolated using the chromogenic medium CHROMagar Candidaâ[8]. A sample from the colonies was collected, cultured in the above-mentioned medium and incubated for 24 hr at 37º C in a microbiological incubator. Fungi were identified according to previous descriptions [9,10].

Discussion and Conclusions

In this report *Candida* sp. was found in 35% of rhesus monkeys from group I and in 45% from group II. This agent integrates the saprophytic microflora of humans[11] and it does not constitute a disease-producing factor in dogs[12]. In fact, the animals under study attacked by *Candida* sp. did not present any clinical alteration, showing that it is not pathogenic for the primate species under study.

In the 40 analyzed rhesus monkeys, 25% presented *Aspergillus flavus*, 32,5% *Aspergillus fumigatus* and 40% *Candida* sp. but no external otitis was found in the animals. Urrutia[13] detected 45,8% *Aspergillus flavus*, 0,8% *Aspergillus* fumigatus and 24,6% *Candida* sp. in 118 human patients with external otitis. The fact that saprophytic fungi can become pathogenic in immunodeficient hosts[1,14] suggests that none of the studied animals had any immunological problem at the time the otological samples were taken.

A great number of mycotic infections in primates kept in confinement, when straw or hey was used as bedding material. Besides, mycoses are quite frequents in animals submitted to prolonged treatment with antibiotics, which favors Candidiases as secondary infections. In simians kept next to avicultures, there is high incidence of Aspergilosis[15]. The primates studied in this paper were not kept under such a condition and were not treated with antibiotics; nevertheless, high incidence of different fungi in the auditory canal was detected. Although not having been kept next to an aviculture, in 50% of the animals different *Aspergillus* were found in the ear conduct.

The yeast *Malassezia* integrates the cutaneous microflora of humans and animals[16]. Nobre et al.[17] showed an incidence of *Malassezia pachidermatis* in healthy dogs and dogs with otitis of 25% and 80.7% respectively. Also, *Malassezia* sp. in the auditory canal of clinically healthy marmosets (*Callithrix jacchus* e *C. penicillata*) was verified[18]. Although the auditory canal is constituted by cutaneous tissue, no type of *Malassezia* was found in the analyzed animals suggesting that this yeast does not make part of the cutaneous fungi microflora of rhesus monkeys.

Grono and Frost[19] reported the presence of yeasts and fungi in both the ear canal of healthy dogs and dogs with external otitis, including presence of *Aspergillus* sp. in the affected canals. In this report we found an incidence of 97,5% of yeasts and fungi in the auditory canal of the 40 healthy analyzed rhesus monkeys, including 25% *Aspergillus flavus* and 32.5% *Aspergillus fumigatus*.

Some species of saprophytic fungi typically colonize warm and humid places with poor hygienic conditions, which would occur colonization and posterior infection of an animal kept in such an environment [20].

Presence of fungi is associated with climatic factors. High humidity and temperature favor the growth of fungi, so the external auditory canal can become an ideal environment for their proliferation[13]. Humidity results in maceration of the epithelium, increasing the probability of infection especially by fungi[21]. Humidity is an ideal condition for the proliferation of *Aspergillus*[14]. In the observations made in the primates at FIOCRUZ, the animals of group I were housed in a climatically controlled environment with a temperature of 22º C and 73% of humidity. The animals of group II were kept in a natural environment and collection of earwax was performed on a hot and quite humid day. The different climatic conditions in which the two groups were kept could explain the higher incidence of fungi found in group II.

Fungi from the ear conducts of rhesus monkeys (*Macaca mulatta*) of the group I. Rio de Janeiro, 2003

Animal	Ear conduct	
(Identification)	Left	Right
S32	Candida tropicalis	Aspergilus flavus
P15	Candida albicans	Candida albicans
O20	Aspergillus fumigatus	non-growth[a]
N32	Aspergillus flavus	Aspergillus fumigatus
N38	Candida albicans	Candida sp.
		Aspergillus flavus
N18	non-growth[a]	Candida tropicalis
M38	Candida sp.	Candida albicans
		Aspergillus flavus
L34	Aspergillus flavus	Candida albicans
	Aspergillus fumigatus	Aspergillus flavus
L46	Aspergillus fumigatus	Aspergillus fumigatus
I20	Candida sp.	Aspergillus fumigatus
	Rhodotorula sp.	
	Cladosporium sp.	
I4	non-growth[a]	Aspergillus flavus
H8	Candida albicans	Aspergillus flavus
	Aspergillus fumigatus	
F22	Candida sp.	Aspergillus fumigatus
F10	Candida tropicalis	Candida albicans
E14	Aspergillus fumigatus	non-growth[a]
C32	Aspergillus fumigatus	Aspergillus flavus
D10	Aspergillus fumigatus	non-growth[a]
D18	Candida sp.	Candida albicans
	Aspergillus flavus	Aspergillus fumigatus
		Aspergillus flavus
A4	Candida sp.	Aspergillus fumigatus
N8	Candida tropicalis	Candida sp.

a Mycotic growth did not occur

The lipid exudates of the auditory canal can increase the possibility of infection, seen that the growth of lipophilic yeasts can be enhanced by a lipid environment in the auditory canal, creating an outstanding environment for microbial proliferation[21]. During earwax collection, we observed a greater quantity of otological secretion in the adult animals of group I and very little secretion in the animals of group II. However, the greater quantity of secretion found in the animals of group I was not indicative of increased proliferation of fungi in relation to group II, suggesting natural alterations of the epidermis to be the result of the aging process.

There are no indications in literature for differences in the incidence of fungi between sexes. In the animals of FIOCRUZ, no significant differences

between sexes were observed. However, it was observed that the younger animals showed higher incidence of *Candida* sp., while the adult animals showed higher incidence of *Aspergillus* sp. These data are similar to those found in humans, indicating that this species of primates could be a suitable animal model for studying human otites. Mucocutaneous candidiasis, for example, is frequent in immunodefficient children, however in most cases the infection is harmless and solves with age, without needing treatment[22]. On the other hand, aged AIDS patients are more susceptible to *Aspergillus* infection[23].

Otitis is a very frequent pathological process with high incidence in children, causing relevant clinical-therapeutic problems[24]. Otitis is also an important disease in cats and dogs. Some of the factors, which predispose an animal to otological infections, include presence of crust and polyps in the canal, different allergies, seborrhea, neoplasias and nutritional and hormonal conditions. The infections can be primary or secondary to these predisposing factors, which individually or collectively, can lead to a favorable environment for infections[21]. In the rhesus monkey breeding unit of FIOCRUZ no cases of otites were registered, showing that, on the contrary to what is observed in cats, dogs and even in humans, otites have no clinical significance in the species *Macaca mulatta*.

Acknowledgments

We would like to thank Dr. Antônio da Mota Marinho for his incentive towards studies in the field of Primatology.

Identificação da microbiota fúngica em condutos auditivos de macacos rhesus (*Macaca mulatta*) mantidos em cativeiro

Resumo

Inúmeros fatores predisponentes podem acarretar doenças auriculares a partir de uma microbiota saprófita. A identificação da microbiota fúngica poderia auxiliar no diagnóstico e tratamento de micoses que possam se tornar patogênicas mediante um desequilíbrio homeostásico. Este trabalho objetivou identificar a microbiota fúngica saprófita no conduto auditivo médio de macacos rhesus (*Macaca mulatta*) clinicamente saudáveis, destinados à pesquisa biomédica. Quarenta macacos rhesus foram divididos em dois grupos. O grupo I foi formado por animais adultos, alojados em gaiolas individuais localizadas em containeres especiais de experimentação com temperatura e umidade controladas. O grupo II, originado da colônia de criação, foi formado por animais jovens, mantidos em ambientes livres, sem controle de temperatura e umidade. O cerúmen do conduto auditivo médio dos animais foi coletado através de swabs. A semeadura das amostras foi feita em placas de Petri contendo Ágar Sabouraud com cloranfenicol 1%, lacradas com fita adesiva e incubadas à temperatura ambiente. Nos 20 animais do grupo I, foi encontrado o seguinte: *Aspergillus* (80%), *Candida* (60%), *Cladosporium* (5%) e *Rhodotorula* (5%). O grupo II apresentou uma diversidade maior de fungos: Can*dida* sp. (95%), *Aspergillus* (20%), *Cladosporium* sp. (60%), *Penicillium* sp. (30%), R*odotorulla* sp., (15%), *Trychophytum verrucosum* (5%), *Epidermophyton flocosum* (5%) e *Scopulariopsis* sp. (5%). Estes dados serão úteis nos diagnósticos e tratamentos de otites e sugerem que os fatores climáticos podem ser responsáveis pelo grande número de fungos presentes nos animais do grupo II, que se encontram expostos às condições climáticas naturais.

Palavras-chave
Microbiota fúngica.
Primatas não-humanos.
Macaco rhesus.
Conduto auditivo.

References

1 ANDRADE, M. R. et al. Prevalence of antibodies to selected viruses in a long-term closed breeding colony of rhesus macaques (*Macaca mulatta*) in Brazil. **American Journal of Primatology**, v. 59, n. 3, p. 123-128, 2003.

2 DINIZ, L. S. M. **Primatas em cativeiro, manejo e problemas veterinários:** enfoque para espécies neotropicais. São Paulo: Ícone, 1997. p. 111-117.

3 KUTTIN, E. S.; MÜLLER, J. The fungal flora of zoo animals' ears. **Mycoses,** v. 37, n. 1, p. 59-60, 1994.

4 TORTORA, G. J.; FUNKE, B. R.; CASE, C. L. **Microbiologia**. Porto Alegre: Artmed, 2000. 827 p.

5 DUKES, F. A. D.; SWENSON, M. J.; REECE, W. O. **Fisiologia dos animais domésticos.** Rio de Janeiro: Guanabara Koogan, 1993. 856 p.

6 WOLFLE, T. L.; ROZMIAREK, C. M.; GROSSBLATT, N. G. **Guide for the care and use of laboratory animals.** Washington – D.C.: National Academy Press, 1996. 125 p.

7 CRUZ, H. L. **Micologia veterinária.** Itaguaí: Imprensa Universitária, 1998. 202 p.

8 SIDRIM, J. J. C.; MOREIRA, J. L. B. **Fundamentos clínicos e laboratoriais da micologia médica**. Rio de Janeiro: Guanabara Koogan, 1999. 287 p.

9 LA MAZA, L. M.; PEZZLO, M. T.; JO BARON, E. **Atlas de diagnóstico em microbiologia.** Porto Alegre: ARTMED, 1999. 216 p.

10 KONEMAN, E. W. et al. **Diagnóstico microbiológico**. Rio de Janeiro: MEDSI, 2001. 1465 p.

11 BARRINUEVO, C. E. et al. Contribuição ao estudo bacteriológico da flora nas otites crônicas e suas complicações. **Folha Médica**, v. 97, n. 1, p. 41-46, 1988.

12 MAGALHÃES, M. J.; SILVA, N.; MARQUES JÚNIOR, A. P. Otite externa em cães atendidos no Hospital Veterinário da UFMG: etiologia, freqüência e sensibilidade antibiótica. **Arq. Bras. Méd. Vet. Zootec.**, v. 37**,** n. 4, p. 333-341, 1985.

13 URRUTIA, R. G. et al. Estúdio microbiológico y epidemiológico de otomicosis. **Ver Na ORL Max,** v. 45, n. 3, p. 114-117, 2000.

14 LUCENTE, F. E. Fungal Infections of the external ear. **Otolaryngologic Clinics of North America,** v. 26, n. 6, p. 995-1006, 1993.

15 KINDLOVITS, A. **Clínica e terapêutica em primatas neotropicais**. Minas Gerais: Ufjf, 1999. p. 75-77.

16 CRESPO, M. J.; ABARCA, M. L.; CABAÑES, F. J. Otitis Externa Associated with *Malassezia sympodialis* in two cats. **J of Clinical Microbiology,** v. 38, n. 3, p. 1263-1266, 2000.

17 NOBRE, M. et al. *Malassezia pachydermatis* e outros agentes infecciosos nas otites externas e dermatites em cães. **Ciência Rural,** v. 28, n. 3, p. 447-452, 1998.

18 BRASIL-VIANNA, C. A. et al. Prevalência de fungos isolados do conduto auditivo de sagüis (*Callithrix jacchus*) e (*Callitrhix penicilata*) em cativeiro. In: CONGRESSO BRASILEIRO DE ZOOLOGIA, 24. 2002, Rio de Janeiro. **Anais...**

19 GRONO, L. R.; FROST, A. J. Otitis externa in the dog: the microbiology of normal and affected external ear canal. **Australian Veterinary Journal**, v. 45, n. 9, p. 420-422, 1969.

20 PEREZ, M. R. et al. **Levantamento de fungos superficiais e cutâneos de animais selvagens mantidos em cativeiro no Zoológico Quinzinho de Barros.** In: ENCONTRO DA ASSOCIAÇÃO BRASILEIRA DE VETERINÁRIOS DE ANIMAIS SELVAGENS, 10., 2001, São Paulo. **Anais...** p. 84.

21 KOWALSKI, J. J. The microbial environment of the ear canal in health and disease. **Veterinary Clinical of North American Small Animal Practice,** v. 18, n. 4, p. 743-754, 1988.

22 COHEN, S. R.; THOMPSON, J. W. Otitic candidiasis in children: an evaluation of the problem and effectiveness of ketoconazole en 10 patients. **An Otol Rhinol Laryngol**, v. 99, n. 6, p. 427-431, 1990.

23 GONZALÉS, J. J. et al. Aspergilosis invasiva en pacientes infectados por el virus de la inmunodeficiencia humana diagnosticados por necropsia: aportación de 4 casos y revisión de la bibliografía. **Enferm Infecc Microbiol Clin.,** v. 17, n. 2, p. 69-73, 1999.

24 LACOSTA, J. L. et al. Microbiologia de la otitis seromucosa. **Anales Otorrinolaringológicos Ibero - Americano**, v. 22, n. 5, p. 439-448, 1995.