Original article

Isolation of *Cryptococcus neoformans* in Antwerp Zoo's nocturnal house

Cryptococcus neoformans im Nachttierhaus des Antwerpener Zoos

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Summary

Cryptococcosis was diagnosed postmortem in a striped grass mouse (*Lemniscomys barbarus*) housed in the nocturnal department of Antwerp Zoo. Eight of the remaining mice in the cage were captured. *Cryptococcus neoformans* was isolated from the lung of one animal. Two mice had an elevated serum cryptococcal antigen titre. On examination of the pooled faecal samples collected from 17 animal species housed in 23 cages of the nocturnal department, the pathogenic yeast was isolated from the faeces of the striped grass mice and a degu (*Octodon degus*). Numerous *Cr. neoformans* colonies were isolated from a tree-trunk, tree-stumps, and decaying wood collected from a hollow tree used to decorate the animals cage. Subsequent examination in four other cages of the nocturnal department revealed that all the sampled tree-trunks were colonized by *Cr. neoformans*. The fungus was isolated from the air sampled in the cage of the degu. Air samples collected in the public and service corridors remained negative. All the isolated strains were identified as *Cr. neoformans* var. *neoformans* serotype A.

Zusammenfassung

Bei einer afrikanischen Streifengrasmaus (*Lemniscomys barbarus*. untergebracht in einem Nachttierhaus des Antwerpener Zoos, wurde post morten eine *Cryptococcus neoformans*-Infektion festgestellt. Im gleichen Käfig wurden 25 weitere Tiere gehalten. Bei einer Grasmaus von 8 getöteten wurde *Cr. neoformans* aus der Lunge isoliert. Bei zwei weiteren Mäusen war *Cryptococcus*-Antigen im Bluserum nachweisbar. *Cr. neoformans* wurde auch aus den gesammelten Exkrementen der Grasmäuse sowie eines Degu (*Octodon degus*) isoliert. Aus den gesammelten Exkrementen aus 23 käfigen des Nachttierhaus die mit 17 verschiedener Tiergattungen belegt waren, wurde *Cryptococcus* zweimal isoliert; aus die Exkrementen der Grasmäuse sowie eines Degu (*Octodon degus*). Hingegen wurde *Cr. neoformans* wiederholt aus einem Baumstumpf, aus Baumresten und vermodertem Holz angezüchtet, das zur Dekoration des Tierkäfigs diente, ebenso auch aus Baumresten von vier weiteren Käfigen. Auch war der Pilz in der Luft des Degu-Käfigs nachweisbar, dagegen nicht in den Luftsammelproben aus den Laufgängen für das Publikum und für das Personal. Alle Isolate wurden als *Cr. neoformans* var. *neoformans* Serotyp A identifiziert.

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Introduction

Cryptococcosis caused by the basidiomycetous yeast *Cryptococcus neoformans*, has been reported sporadically in animals, including several exotic species.[1–4] The infection has a predilection for the central nervous system and is generally believed to be acquired through inhalation of airborne yeast cells.[5] The fungus consists of two varieties: *Cr. neoformans* var. *neoformans* with serotypes A, D, and AD and *Cr. neoformans* var. *gattii* with serotypes B and C. The two varieties differ in geographic distribution: *Cr. neoformans* var. *neoformans* has a worldwide distribution, whereas *Cr. neoformans* var. *gattii* occurs predominantly in the tropics and the southern hemisphere.[6,7] *Cryptococcus neoformans* var. *neoformans* utilizes the creatinin present in avian droppings as a nitrogen source.[8] Therefore, old pigeon excreta and soil contaminated with avian faecal material are the most commonly reported natural substrates for this fungus.[9,10] The natural occurrence of *Cr. neoformans* var. *gattii* is associated with *Eucalyptus* spp. and other tropical trees.[11]

This paper reports on cryptococcosis in striped grass mice housed in Antwerp Zoo. The subsequent investigations for the infectious source by examining the decorative elements in the cages and air sampling are presented.

Materials and methods

History

In October 2001, the corpse of a striped grass mouse (*Lemniscomys barbarus* L.) housed in a nocturnal cage at Antwerp Zoo, was presented for necropsy. The body, in early state of decomposition, was found on the soil, covered with withered leaves and bark mulch. Samples of the lung, liver, brain and intestine collected during necropsy were submitted for aerobic bacteriology and mycology. Subsequently, eight of 25 mice housed in the same cage were captured and killed. Blood, tissue samples and intestinal content were collected for laboratory investigation.

Examination of the cages

For enrichment purposes, the nocturnal cages were decorated with a mixture of plant materials. Withered leaves and pine bark mulch were used as substrate. Dried sods, tree-stumps, tree-trunks and tree-hollows, completed the cage furnishing. Only materials of native origin, collected in the woods (e.g. oak, birch and beech), were used. Four samples of soil, dried leaves and grass, were collected in the cage of the mice. Samples of wood collected by scraping decomposing parts, or sand-papering the bark of stumps and trunks, were gathered on three different places in the cage of the striped grass mice and from four other cages in the nocturnal department (Table 1). In addition, faecal material was collected from 16 other animal species housed in 22 cages of the nocturnal department.

Laboratory investigations

Tissue samples from the corpses of the striped grass mouse and the killed mice were examined microscopically and inoculated onto blood agar, McConkey agar and Sabouraud glucose agar. Faecal material and environmental samples collected in the cages were processed by moistening and homogenizing (10% w/v) with sterile physiological water (SPW). Sterile swabs were used to inoculate modified Guizotia abyssinica (MGA) medium.[12] Brown colonies on MGA medium, or yeast colonies isolated on Sabouraud glucose agar (SDA) turning brown on MGA medium, were identified positively as *Cr. neoformans* according to the classical methods of Barnett *et al.*[13] The D-proline assimilation test was used to determine the variety of the isolates.[14] Serotyping was carried out with the cryptocheck test (Iatron Laboratories, Inc., Tokyo, Japan). Serum cryptococcal antigen titres were determined with the latex crypto antigen detection system (IMMY; Immuno-Mycologics, Inc., Norman, OK, USA).

Air sampling

The sampler used was an aerobiocollector (air IDEAL™, bioMérieux, Marcy l'Etoile, France), operating according to the impaction principle, with sampling rate of 100 l air min^{-1}. The agar plates with MGA medium were used. A total of 600 l air was sampled on four different spots in the service-passage behind the cages and in the public corridor. Inside the cages mentioned in Table 1, 150 l air was sampled.

Cage/animal species	Sample examined	Isolation rate
Striped grass mice (*Lemniscomys barbarus*)	Sod	–
	Heather	–
	Withered oak leaves	+
	Pine bark mulch	–
	Decaying hollow tree	++++
	Tree-stump	+++
	Tree-trunk	++
Ringtail (*Bassariscus astutus*)	Tree-trunk	++++
Brush-tailed phalanger (*Trichosurus vulpecula*)	Tree-trunk	+++
Fat-tailed dwarf lemur (*Cheirogaleus medius*)	Tree-trunk	+++
Degu (*Octodon degus*)	Tree-trunk	++++

Isolation rate: number of *Cr. neoformans* colonies isolated on *Guizotia abyssinica* agar.
+, 1–10 cfu plate^{-1}; ++, 10–50 cfu plate^{-1}; +++, 50–100 cfu plate^{-1}; ++++, more than 100 cfu plate^{-1}.

Table 1 Isolation of *Cryptococcus neoformans* var. *neoformans* from the cage decoration in the nocturnal department of Antwerp Zoo.

Results

The stomach and intestine of the striped grass mouse corpse contained haemorrhagic fluid. The lungs were congested and the liver and kidneys showed postmortal degeneration. Microscopic examination of the brain, lung, liver and intestinal content, revealed numerous encapsulated yeast-like organisms. Cultures on SDA yielded yeast colonies that were identified as *Cr. neoformans*. Pathogenic bacteria were not isolated. Microscopic examination of the lungs and brains of the killed mice revealed no yeast-like cells. After inoculation on SDA, *Cr. neoformans* could be isolated from the lung of one animal. Serum cryptococcal antigen titres at 1 : 2 and 1 : 2000 were detected in two of the collected blood samples.

Numerous *Cr. neoformans* colonies were isolated from a tree-trunk, tree-stumps and decaying wood sampled in a hollow tree in the cage of the striped grass mice. Comparative examination in other cages of the nocturnal department, revealed that all the sampled tree-trunks were colonized (Table 1). *Cryptococcus neoformans* colonies were isolated from pooled faecal samples collected in the cages of the striped grass mice and the degu (*Octodon degus*). No yeast colonies were isolated in the air samples collected in the public corridor or the service section. Two *Cr. neoformans* colonies were isolated from the air sampled in the cage of the degu. All the isolated strains were identified as *Cr. neoformans* var. *neoformans* serotype A.

Discussion

There are few records of cryptococcosis in zoo animals. In San Diego Zoo, lesions of cryptococcosis were observed in eight primates, three artiodactyles, two carnivores, one pinniped and three birds, necropsied between 1964 and 1977.[15,16] Parsons *et al.*[17] reported on six cases in small animals at the Zoological Society of London during 1984–1986. At the National Zoological Park in Washington, the disease was diagnosed in nine mammals between 1975 and 1990. In the same zoo, 20 animals died during an outbreak of cryptococcosis in tree shrews (*Tupaia tana, T. minor*) and elephant shrews (*Macroscelides proboscides*) in 1991–1993.[18] In the stated cases the variety of the fungus was not determined. However, an environmental source for the fungus was not apparent, in contrast to cyptococcosis in koala's (*Phascolarctos cinereus*), which is related to *Eucalyptus* trees, one of the ecological niches for *Cr. neoformans* var. *gattii*.[19]

Cryptococcosis has never been diagnosed in Antwerp Zoo before. Probably because no selective culture media for the recovery of *Cr. neoformans* have been used in the past. For more than 10 years, several generations of striped grass mice, lived in the enclosure. Their exact total number and state of health of individuals was not known because of the dense decoration of the cage. After the isolation of *Cr. neoformans* from the corpse of a striped grass mouse, the decision was made to kill and examine a part of the mice in the enclosure. The yeast was also isolated from faecal samples collected in the cages of the mice and the degu. This faecal material may have been contaminated by the heavily colonized tree-stumps and -trunks in both cages. Otherwise, it is known that wild mice inoculated *per os* with *Cr. neoformans* shed viable yeast cells in their faeces for up to a year whilst showing no clinical symptoms.[20] In 1985, the occurrence of *Cr. neoformans* was

studied in the aviaries of Antwerp Zoo. On this occasion, *Cr. neoformans* var. *neoformans* was isolated from the nesting boxes and the role of wood as a habitat for this pathogenic yeast was suggested.[21] Staib reported that a wide variety of plant substrates supported *in vitro* colonization by *Cr. neoformans*. Dried samples of leaves and stems were suitable for growth of the fungus under laboratory conditions.[22] The isolation of both mating types of *Cr. neoformans* var. *neoformans* from wooden boards in a house at Bujumbura was considered as an argument in favour of the possibility that wood could play a real role in the sexual cycle of this heterothallic species.[23] Subsequently, *Filobasidiella neoformans*, the teleomorphic phase of the yeast, has been obtained under laboratory conditions by streaking sexually compatible isolates on the inner surface of autoclaved pieces of *E. leucoxylon* bark.[24] Decaying wood in tree-trunk hollows was found to be a natural substrate for *Cr. neoformans* var. *neoformans* and other yeast-like fungi.[25] In Brazil, Lazera *et al.* isolated *Cr. neoformans* var. *neoformans* from decaying wood inside the trunk hollows of several different living trees over a long period suggestive for colonization.[26] In contrast with *Cr. neoformans* var. *gattii*, the environmental habitat of *Cr. neoformans* var. *neoformans*, is not a particular tree. Similar to creatinin in avian excreta, it is believed that the by-products of wood decomposition may support saprophytic growth of *Cr. neoformans*. The yeast produces laccase, a phenoloxidase enzyme forming a melanin-like pigment when growing on a substrate containing polyphenolic compounds such as wood pulp lignin.[27,28] High number of *Cr. neoformans* colonies were isolated from tree-trunks and -stumps in the enclosures of the nocturnal animals in Antwerp Zoo. These materials were collected in native woods. Although only clean materials were used to decorate the cages, a possible contamination with bird droppings containing the pathogenic yeast could have occurred. The stable climatic conditions in the cages, protected from direct sunlight and an environment rich in organic matter, is suitable for survival and colonization. Only two *Cr. neoformans* colonies were isolated from the air sampled in one of five cages examined with positive wood samples. Low results of air sampling around positive trees were also reported by Lazera.[26] These findings suggest that the fungus is substrate-bound and airborne infection poses no potential threat to zoo visitors and personnel. The infected cages were cleaned and redecorated. As a precautionary measure, the zoo personnel have been instructed to wear masks and gloves when cleaning the cages.

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