

ISOLATION OF SAPROPHYTIC *CRYPTOCOCCUS NEOFORMANS* VAR. *NEOFORMANS* IN KINSHASA, ZAÏRE

by

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Summary — *Cryptococcus neoformans* var. *neoformans*, which is responsible for cryptococcosis associated with AIDS (CN + AIDS) in Kinshasa, was isolated outdoors from pigeon and chicken excreta and indoors from air and dust collected in houses of «CN + AIDS» patients and also in houses of apparently healthy persons. It is shown for the first time that cockroaches may harbour the yeast in their digestive tract. Our results indicate that contact with saprophytic *C. neoformans* var. *neoformans*, which constitutes the source of infection, is probably more common than generally accepted.

KEYWORDS: Cryptococcosis; *Cryptococcus neoformans*; AIDS; Zaïre.

Introduction

Cryptococcosis is a non-contagious, air-borne yeast infection, usually acquired by inhalation (19). There are two varieties of *C. neoformans*, the variety *neoformans* and the variety *gattii* and both are incriminated in the etiology of cryptococcosis. Both varieties can be differentiated on the basis of their serotype (5, 21), physiology (1, 4, 8) and morphology (20).

They also differ from an epidemiological point of view:

- so far only the variety *neoformans* has been isolated from saprophytic sources, mainly from bird droppings, pigeon excreta in particular (14,15);
- cases of cryptococcosis due to the variety *neoformans* have been reported throughout the world, whereas infection due to the variety *gattii* has been described only in some well delimited geographical areas (9), for instance Zaïre (6). Surprisingly, this variety could not be isolated again from Zaïre after 1969. Forty isolates obtained since then from patients originating from this country (18) and 18 additional isolates which we recently acquired, belong exclusively to the variety *neoformans*. As all the cases seen after 1980 (i.e. 35) were suspected or proven for AIDS, we decided to look for this variety in the environment in order to determine the possible sources of infection of AIDS patients in Kinshasa.

Material

All the samples were taken in the city of Kinshasa.

We collected 35 soil samples in the streets, 83 samples of bat (*Tadarida (Mops) condylura*) droppings under the corrugated iron roofs of houses taken at random, 144 chicken, 62 pigeon, and 71 sparrow excreta.

Indoors dust and air samples were also collected in 79 houses, of which 74 were occupied by apparently healthy persons and 5 had been occupied by cryptococcosis associated with AIDS (CN + AIDS) patients. The 74 houses were chosen at random but not in the neighbourhood of the 5 others.

We examined the digestive contents of 113 cockroaches (*Periplaneta americana*), a common species living in the domestic environment in tropical countries. They were trapped at random from 16 other houses of persons without any history of «CN + AIDS».

Methods

Soil and droppings

A suspension of about 1 cm³ in 9 ml sterile water was made and inoculated directly with a swab on a selective medium.

Dust

In one case, two suspensions of 0.5 cm³ dust in 4.5 ml sterile water were shaken and inoculated directly with a swab on the selective medium. In 78 other houses RODAC (Replicating Organisms Direct Agar Contact) plates filled with a selective medium were applied directly on the floor or on various furnitures.

Five RODAC/house were taken in 4 houses of «CN + AIDS» patients but, due to financial limitations, only 2 RODAC/house were taken in the 74 other houses.

Air

We had no opportunity to take air samples in one of the five houses of «CN + AIDS» patients. So, samples were taken only in 78 houses with a «Biotest R.C.S. Centrifugal Air Sampler». Known volumes were sucked into a drum and set in rotation by means of impeller blades. The particles in suspension were impacted onto a plastic strip containing a selective medium. One hundred l/house were taken in the 4 houses of «CN + AIDS» patients i.e. 5 plastic strips or 5 × 20 l air, but due to financial limitations, only 40 l/house were taken in the 74 other houses i.e. 2 plastic strips or 2 × 20 l air.

Cockroaches

The digestive contents removed aseptically from the autopsied insects were directly inoculated onto a selective medium.

Media

- selective medium for isolation: we used a modified *Guizotia abyssinica* medium on which *C. neoformans* colonies turn brown (13). Results were read after 8 days at 25°C;
- variety of the isolates: the canavanine-glycine-bromothymol blue medium (8) was used to determine the variety of the isolates. Results were recorded after 2, 4 and 6 days at 25°C.

Results

Soils and droppings

The results are summarized in table 1. *C. neoformans* var. *neoformans* was only isolated from chicken (1.4%) and pigeon droppings (11.3%).

TABLE 1
Isolation of *C. neoformans* var. *neoformans* from soil and droppings

	n° positive/ n° specimens	% positive
Soil	0/35	—
Droppings:		
bats	0/83	—
chickens	2/144	1.4
pigeons	7/62	11.3
sparrows	0/71	—

Dust and air

C. neoformans var. *neoformans* was isolated in 5 houses: 2 out of 5 houses of «CN + AIDS» patients and 3 out of 74 other houses.

The positive results are summarized in table 2.

TABLE 2
Isolation of *C. neoformans* var. *neoformans* from air and dust of five houses

Site	Sample	Method	n° positive/ n° specimens	C.F.U.*
1. CN + AIDS house	dust	susp.	2/2	2 & 4
2. CN + AIDS house	dust	RODAC	1/5	3
3. Other house	dust	RODAC	1/2	3
4. Other house	air	R.C.S.	1/2	2
5. Other house	air	R.C.S.	1/2	1

* Colony forming units.

Cockroaches

Two isolates of *C. neoformans* var. *neoformans* were recovered for the first time from the intestinal content of 113 cockroaches trapped from two different houses.

Discussion

Before the existence of AIDS in Central Africa was established, an unusually high number of cases of cryptococcosis were recorded in the Mama Yemo Hospital in Kinshasa (10). It has since been noticed that the prevalence of disseminated cryptococcosis in AIDS patients living in or recently emigrated from Central Africa, can be as high as 13 to 35% (2, 7, 12). These figures are much higher than those reported from the USA where apparently only 6% of AIDS patients acquire this opportunistic infection (3, 11). The high prevalence of *C. neoformans* var. *neoformans* infections in this group of AIDS patients is still unexplained. It is apparently not related to differences in virulence of the organism since our experimental data indicate that isolates of *C. neoformans* var. *neoformans* from Central Africa are not more pathogenic than isolates from elsewhere (17). More frequent exposure to saprophytic sources of *C. neoformans* var. *neoformans* might be another possibility.

Our results indicate that *C. neoformans* var. *neoformans* can be isolated outdoors from pigeon and chicken droppings and indoors in the domestic environment from dust and air. Cockroaches can harbour the yeast in their digestive tract and consequently we suspect that they may perhaps play a role in the dissemination of the yeast in nature.

Even if *C. neoformans* var. *neoformans* is highly prevalent in pigeon droppings (11.3%), it is unlikely that these play a real part in its dissemination in Kinshasa since contacts with pigeon habitats are not that frequent.

Contacts with chicken habitats are probably more frequent, but only 1.4% of chicken excreta harboured this yeast.

Therefore, it seemed logical to look for the presence of *C. neoformans* var. *neoformans* in the immediate daily, domestic environment.

Since, due to cost limitations, different numbers of dust and air samples were collected from the houses of patients with and without «CN + AIDS», a direct comparison between the rates of isolation cannot be made, but our results indicate that contacts with this organism are probably more common than generally accepted. However, more extensive studies are needed to determine the prevalence and natural sources of *C. neoformans* var. *neoformans*.

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Isolément de *Cryptococcus neoformans* var. *neoformans* d'origine saprophytique à Kinshasa, Zaïre.

Résumé — *C. neoformans* var. *neoformans*, agent de la cryptococcose associée au SIDA (CN + SIDA) à Kinshasa a été isolé dans l'environnement extérieur à partir de déjections de pigeons et de poules. Il a également été isolé à partir de prélèvements de poussière et d'air récoltés dans des maisons dont certaines avaient été occupées par des patients «CN + SIDA». Nous avons observé, pour la première fois, la positivité du contenu digestif de blattes.

Nos résultats montrent que les contacts avec *C. neoformans* var. *neoformans* d'origine saprophytique, qui sont à l'origine de la contamination des individus, sont probablement plus faciles qu'on ne le suppose.

Afzondering van saprofytaire *Cryptococcus neoformans* var. *neoformans* in Kinshasa, Zaïre.

Samenvatting — *C. neoformans* var. *neoformans*, verwekker van met AIDS geassocieerde cryptococcose (CN + AIDS) werd afgezonderd in de omgeving uit duiven en kippenmest. Hij werd ook in huisstof en lucht aangetoond in woningen van onder andere «CN + AIDS» patiënten. Deze gist werd ook voor het eerst gekweekt uit de darminhoud van kakkerlakken. Derhaive is het contact met mogelijke saprofytaire infectiebronnen veel groter dan tot nog toe vermoed werd.

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