

AIDS-associated cryptococcosis in Bujumbura, Burundi: an epidemiological study

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Cryptococcus neoformans var. *neoformans* was isolated from domestic dust in 54% of houses occupied or recently occupied by AIDS+ cryptococcosis patients and in 20% of control houses randomly chosen in Bujumbura, Burundi. It was not isolated from wards of similar patients in the University Hospital. This seems to prove that patients could not contaminate their own environment and that the presence of *C. neoformans* var. *neoformans* had another origin.

In 40% of the cases, patients admitted contact with pigeons in the city and pigeon coops are frequently positive.

We are convinced that the pigeon coops of the city play a part in the contamination of HIV+/AIDS patients; however, some patients denied any contact with pigeons and in these cases, the only possible source of contamination identified thus far is domestic dust, although the origin of the yeast remains unknown.

Previous studies have shown that HIV+/AIDS patients in Central Africa are more exposed to *Cryptococcus neoformans* var. *neoformans*, the variety responsible for cryptococcosis in AIDS, than they are in Europe. The incidence of cryptococcosis in association with AIDS in different central African countries is so high that this mycosis is considered by many authors to be an AIDS-indicator in Central Africa. A survey performed in Kinshasa, 3 years ago, showed that the variety could not only be isolated outdoors from pigeon and chicken droppings but also indoors in the domestic environment from dust and air [12].

A preliminary study in Bujumbura (1988) permitted the isolation of the yeast from the domestic environment of seven out of 20 patients with AIDS-associated cryptococcosis [11]. These observations prompted us to undertake a follow-up study.

METHODS

Samples

Attempts were made to isolate *C. neoformans* var. *neoformans* from a total of 800 samples taken from the environment.

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Three hundred and seventy-five dust samples (250 contact plates and 125 loose dust samples) were taken from 25 houses occupied or having recently been occupied by 24 AIDS+ cryptococcosis patients (one patient had two houses).

Highly positive samples were obtained from one of these 25 houses, where we performed a supplementary study. Another 20 contact plates and 20 samples of diverse materials (dust from walls, floor, furniture and beams, spider webs, . . .) were taken. We tried then to link cryptococcosis in AIDS patients to contact with pigeons. Each patient was asked if he had had any contact with pigeons. Whenever it was possible, five samples of pigeon droppings per coop were taken, giving a total of 30 samples in six coops.

Fifty contact plate samples were taken from 10 Internal Medicine wards in Kamenge University Hospital (five samples per ward). Four were occupied by AIDS+ cryptococcosis patients; two were occupied by AIDS patients without cryptococcosis and four were temporarily vacant.

In order to evaluate the probability of *C. neoformans* var. *neoformans* propagating from a focal point, given the favourable climatic conditions of the dry season, nine randomly selected houses in the vicinity of a pigeon coop from which the yeast had been isolated in high concentrations were examined (100 contact plates and 50 loose dust samples).

Given the high number of patients living in one particular area (five out of the 24 AIDS+ cryptococcosis patients), a control series of 10 houses from the same neighbourhood was randomly picked and examined using the same procedure: 10 contact plates and five loose dust samples per house.

Medium

Staib's medium on which *C. neoformans* colonies turn brown [9], slightly modified [6], was used as a selective medium. Its composition is as follows.

Guizotia abyssinica aqueous extract: 50 g ground 500 ml⁻¹ distilled water; boil 20 min.; filter.

Agar: 1.5% (for contact plates: 2%); glucose: 0.5%; creatinine: 0.1%; KH₂PO₄: 0.1%; MgSO₄: 0.05%; chloramphenicol: 0.1%; biphenyl: 0.1% (1 g 20 ml⁻¹ absolute ethanol).

Methyl violet: 0.2 ml from stock sol. 1%.

Distilled water q.s. 1000 ml.

Sampling

Contact plates (RODAC type*) filled with the selective medium were applied directly to the floor or against various pieces of furniture. For loose dust samples, as well as for pigeon droppings, 1 ml of material was suspended in 9 ml sterile distilled water and inoculated directly with a swab onto the selective medium. Results were read after 6 days of incubation at 25°C.

*Replicating Organisms Direct Agar Culture.

Variety of the *C. neoformans* isolates

The assimilation of D-proline was used to determine the variety of isolates (3). Results were read after 4 days of incubation at 25°C.

RESULTS

C. neoformans var. *neoformans* was isolated from the environment of 13 out of 24 patients. Eighteen contact plates out of 250 collected in 12 different houses were positive (max. 3 pos./10 plates/house). In all the cases, no more than 1 or 2 c.f.u. were found per 250 cm² furniture. Ninety-six contact plates were overgrown by moulds and were unreadable. Two loose dust samples out of 125, collected in two houses, were positive. The first sample contained 50×10^3 CFU g⁻¹ and the second one, 10^6 c.f.u. g⁻¹. In neither case could the precise nature and source of the dust material be identified. In the first house, 2/8 contact plates were also positive (two contact plates were unreadable). The high positivity of this house prompted us to take 40 more samples: six contact plates out of 20 and one sample of diverse material out of 20 were positive. This last sample of unknown material was taken at the same place as the positive one, in the first inquiry. The results are summarized in Table 1.

TABLE 1. Positive areas in the house of an AIDS+ cryptococcosis patient

A—Patient's bedroom (contact plates)	
Floor:	300 c.f.u. 25 cm ⁻²
Chair:	1 c.f.u. 25 cm ⁻²
Table:	15 c.f.u. 25 cm ⁻²
Suit-case:	3 c.f.u. 25 cm ⁻²
B—Floor of the living room (contact plates)	
	10 c.f.u. 25 cm ⁻²
	1 c.f.u. 25 cm ⁻²
C—Kitchen (loose dust sample)	
	10^6 c.f.u. g ⁻¹

In 10 out of the 24 cases, the patients had contact with pigeons. In six cases, pigeon dropping sampling was possible: *C. neoformans* var. *neoformans* was found in five pigeon coops out of six and in all samples (5/5) showed confluent growth.

Attempts were then made to isolate *C. neoformans* var. *neoformans* from wards of hospitalized AIDS patients. No *C. neoformans* var. *neoformans* was isolated from any of the 10 wards that were occupied either by AIDS patients with (4), or without (2) cryptococcosis or from the empty control wards (4).

Nevertheless, for three out of the four hospitalized AIDS + cryptococcosis patients, the yeast had been isolated at home. The dissemination of *C. neoformans* var. *neoformans* from a positive pigeon house to the immediate surroundings was evaluated and Fig. 1 gives us the location of the houses where sampling was performed as well as the degree of positivity.

C. neoformans var. *neoformans* was isolated from house H1 on the roof of which was situated the positive pigeon-coop P. The yeast was also isolated from old droppings D2 sampled in the pigeon-coop of the next-door neighbour, approximately 20 m away and unoccupied for 3 years. It was finally found in house H8, 100 m away from the positive pigeon-coop and from soil D5 collected at about 70 m distance. Finally, attempts were made to isolate *C. neoformans* var. *neoformans* from dust sampled in 10 control houses.

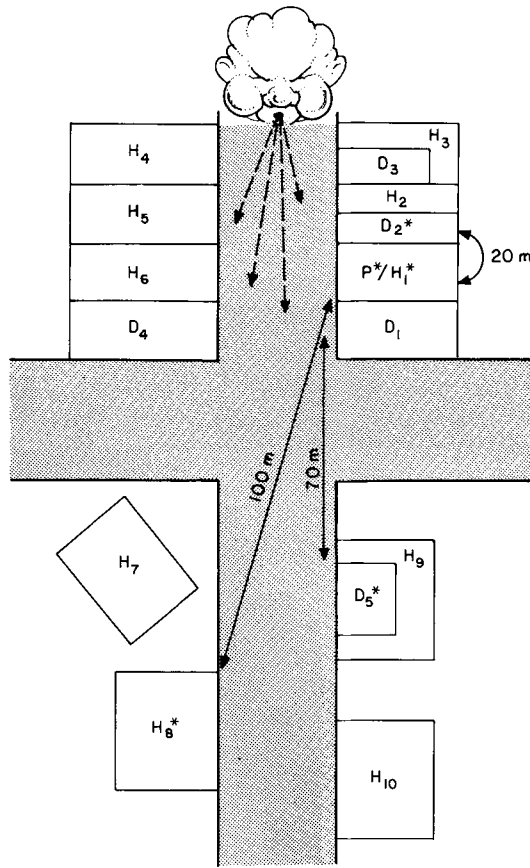


FIG. 1. Dissemination of *C. neoformans* var. *neoformans* from a positive pigeon-house. 'P': pigeon-house; 'H': houses (H1: 36 c.f.u. 250 cm⁻²/furniture and H8: 3 c.f.u. 250 cm⁻² furniture); 'D': dust (D2 2 × 10⁵ c.f.u. g⁻¹ pigeon droppings and D5: 150 c.f.u. g⁻¹ soil). (* = positive.)

Figure 2 summarizes the results. *C. neoformans* var. *neoformans* was found in two out of 10 control houses, C4 and C8, situated not far from those of three AIDS+ cryptococcosis patients, P2, P3 and P4. These three houses, P2, P3 and P4 are three out of the 25 houses occupied or having recently been occupied by the 24 AIDS+ cryptococcosis patients initially sampled. House P2 had been found to be positive earlier, whereas, houses P3 and P4 were negative.

DISCUSSION

The results obtained in this study confirm those of 1988, namely that *C. neoformans* var. *neoformans* can be isolated from domestic dust in Bujumbura. Not only was it isolated from 54% of houses occupied or recently occupied by patients with AIDS-associated cryptococcosis but also from 20% of randomly chosen control houses.

All the isolates from the environment were of the variety *neoformans* which is responsible for AIDS-associated cryptococcosis not only in Central Africa but also the World [5, 8, 13]. Indeed, for reasons still unknown, the variety *gattii* is only exception-

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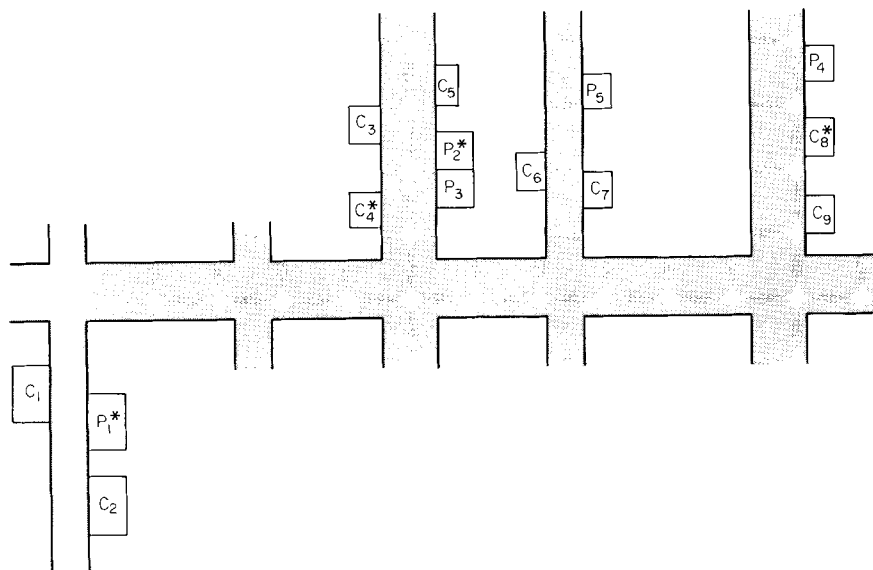


FIG. 2. Attempts to isolate *C. neoformans* var. *neoformans* from dust sampled in 10 control houses. 'P': AIDS+ cryptococcosis patient's house. 'C': control houses. C4, living-room positive: 306 c.f.u. 125 cm⁻² of wooden boards and 5 × 10⁴ c.f.u. g⁻¹ loose dust. C8, kitchen positive: 10⁴ c.f.u. g⁻¹ loose dust. (* = positive.)

ally isolated from AIDS+ cryptococcosis cases [4, 7]. It is well known that the variety *neoformans* in Central Africa, as well as elsewhere in the world, is commonly present in bird droppings, especially those of pigeons, where it multiplies [10].

Ten of our patients (40%) admitted contact with pigeons and in six cases (25%) sampling of the avian source could be performed. Strongly positive results were obtained in five cases implying that those five patients had been exposed to high doses of *C. neoformans*. We are convinced that the pigeon coops of the city play a part in the contamination of HIV+ or AIDS patients although the dissemination of spores from positive environments is more restricted than initially believed.

In 14 of the cases, the patients denied any contact with pigeons and the only possible source of contamination identified so far is domestic dust.

It appears that AIDS+ cryptococcosis patients cannot contaminate their own environment as negative results were obtained in their wards in the University Hospital of Kamenge, raising the question; what is the source of *C. neoformans* in domestic dust? We do not know but the isolation of the yeast from wood in a control house once again raises the problem of the possible existence in the environment of the teleomorph with a natural biotope of wood.

We conclude that HIV+/AIDS patients in Bujumbura are, as elsewhere in Central Africa, more frequently in contact with *C. neoformans* var. *neoformans* than generally accepted.

On the one hand, they are contaminated from contacts with pigeon droppings, which are frequently positive, but in quite a considerable number of cases they can inhale the yeast at home from domestic dust.

This highlights the problem of either HIV+ patients living in a domestic environment containing *C. neoformans* or AIDS+ cryptococcosis patients who after successful

treatment return home, where both can be (re-) contaminated. Therefore, like other workers [1, 2], we would like to stress the importance of systematically detecting the cryptococcal antigen levels in each HIV+/AIDS patient, first in the serum and later, if necessary, in the cerebrospinal fluid to detect new cases or reinfection as early as possible.

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