Short Report

Two cases of HIV-associated cryptococcosis due to the variety *gattii* in Rwanda

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There are two varieties of *Cryptococcus neoformans*, the variety *neoformans* and the variety *gattii*. Both can be responsible of cryptococcosis. Cases due to the variety *neoformans* have been reported throughout the world, whereas infections due to the variety *gattii* remain restricted to delimited geographical areas mainly in tropical and subtropical countries. Variety *gattii* exists in southern California, South America, Africa, Asia and Australia. There are 4 different serotypes; two, serotypes A and D, belong to variety *neoformans*, and the other 2, serotypes B and C, belong to variety *gattii*. Serotypes B and C account for only 10·3% and 4% respectively of the clinical cases, the variety *neoformans* being by far the most common (BENNETT *et al.*, 1977).

It is well known that variety *neoformans* is almost always responsible for cryptococcosis associated with the acquired immune deficiency syndrome (AIDS) (BOTTONE *et al.*, 1987; RINALDI *et al.*, 1986; SHIMIZU *et al.*, 1986; SWINNE *et al.*, 1986). Nevertheless, since 1987, 4 cases caused by variety *gattii* have been reported (CLANCY *et al.*, 1990; KAPEND'A *et al.*, 1987; ROZENBAUM *et al.*, 1990; ST GERMAIN *et al.*, 1988).

We describe here 2 new cases of HIV-associated cryptococcosis due to *C. neoformans* var. *gattii* in Kigali, Rwanda. D-proline assimilation was used to determine the variety of the isolates (DUFAIT *et al.*, 1987).

Patient no.1, a 27 years old Rwandese woman without any remarkable medical history, was admitted to the Department of Internal Medicine of the Centre Hospitalier de Kigali with deterioration in general condition, intermittent fever, anorexia, weight loss and cough that had started developing progressively 3 weeks earlier. Physical examination disclosed pneumonia of the right lower lung field. A chest roentgenogram confirmed this finding and further revealed a right hilar adenopathy. HIV-1 serological tests (enzyme-linked immunosorbent assay [ELISA] and Western blot) were positive. Repeated examinations of sputum for acid-fast bacilli were negative. As the condition of the patient did not improve with penicillin or trimethroprim-sulphamethoxazole, fibrobronchoscopy combined with bronchoalveolar lavage (BAL) was carried out. *C. neoformans* was isolated from BAL culture and identified as variety *gattii*, serotype C. To our knowledge this is the first report of serotype C from Africa. Direct examination and culture of the cerebrospinal fluid (CSF) and examination of the CSF for soluble cryptococcal antigen were all negative. Blood culture and screening for cryptococcal antigen in the serum were also negative. The peripheral CD_4 lymphocyte count and percentage were 448/mm^3 and 20% respectively and the CD_4/CD_8 ratio was 0·62. The patient was initially treated with oral ketoconazole 400 mg/d for 35 d. As the patients's condition deteriorated and cavitation appeared in the right lung field, ketoconazole was replaced by oral itraconazole 400 mg/d and antituberculosis drugs were added to the antifungal treatment. Unfortunately the patient left the hospital against the physician's advice 5 d after starting the last treatment and was lost for follow-up.

Patient no.2, a 27 years old Rwandese man without known medical history, was admitted because of headache, lumbar pain, fever and cough of 2 weeks duration. He had been treated with antimalarial drugs without success. Physical examination revealed neck stiffness. A spinal tap was performed which removed clear CSF with 4 cells/mm^3 and protein and glucose levels of respectively 51 and 24 mg/dl. A wet mount and an India ink preparation revealed encapsulated yeasts confirmed as *C. neoformans* by culture on Sabouraud medium. The same pathogen was isolated from blood. Both isolates belonged to the variety *gattii*, serotype B. Intravenous amphotericin B, 0·7 mg/kg/d, was started but the patient died 4 h after the onset of treatment. HIV-1 serological tests (ELISA and Western blot) were positive. CD_4 lymphocyte counts could not be done.

The description of these 2 new cases of HIV-associated cryptococcosis due to variety *gattii* in Central Africa are all the more interesting as variety *gattii* has practically disappeared from Central Africa, where it was common before 1980. We studied 12 clinical isolates in Zaire between 1951 and 1979, 6 of which belonged to the variety *gattii* (SWINNE *et al.*, 1986). In contrast, in the same country, only one of 204 isolates from AIDS-associated cryptococcosis cases collected after 1980 was variety *gattii* (KAPEND'A *et al.*, 1987). In Rwanda, only 2 of 482 strains isolated from CSF and/or blood since 1983, corresponding to the cases described here, have been identified as variety *gattii*.

We could not find saprophytic isolates of *C. neoformans* var. *gattii* in the environment of the patients, even if they were living in country where *Eucalyptus camaldulensis*, the natural habitat of this variety, is common (ELLIS & PFEIFFER, 1990).

It is noteworthy that 2 of the 6 *gattii* isolates, one of ours and that of CLANCY *et al.* (1990), were recovered from the lungs without any evidence of further involvement. However, despite moderate immunodeficiency the clinical course of the meningoencephalitis in our second patient was as fulminant as in some cases of *C. neoformans* involvement of the central nervous system.

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Short Report

Detection of clinically and epidemiologically significant strains of *Escherichia coli* in faeces from young children in the tropics

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We recently concluded a prospective 3 years study of *Escherichia coli* isolated from a cohort of Aboriginal children less than 5 years old in remote communities in the tropical north-west of Australia (GUNZBURG *et al.*, 1992a). Five isolates of *E. coli* from each faecal sample were either tested for hybridization with gene probes for heat-labile (LT) and heat-stable (ST) enterotoxins, Verotoxins 1 (VT1) and 2 (VT2), enteroadherence factor (EAF) and O157:H7 pilus or were examined biochemically for resemblance to enteroinvasive *E. coli* (EIEC). Enterotoxigenic *E. coli* (ETEC), Verotoxigenic *E. coli* (VTEC) or enteroadherent *E. coli* (EAF^+) were isolated from almost 40% of diarrhoeal stool samples, compared with 12·7% for *Salmonella* spp., the next commonest pathogen; no invasive *E. coli* was detected (GUNZBURG *et al.*, 1992b).

Detection of *E. coli* virulence factors is specialized and expensive, so it would be helpful to have guidelines about which faecal specimens should be examined for diarrhoeagenic strains. Patterns of isolation of *E. coli* strains with virulence factors in our study differed with season and children's ages, and this information could help to develop rational guidelines for investigation of *E. coli* strains from children in similar tropical environments.

In our prospective study (GUNZBURG *et al.*, 1992b) we found that ST^+ strains were isolated in both wet and dry seasons but were significantly associated with diarrhoea only in children aged 6–18 months. LT^+ strains were associated with diarrhoea only in the wet season in children aged 18–24 months. Acute diarrhoea was associated with VT1-producing VTEC but not VT2-producing strains, and only in children younger than 18 months; there was no seasonality of VT1-associated diarrhoea. VTEC strains capable of actin accumulation in HEp-2 cells, a screening test to identify strains belonging to enteropathogenic serogroups (KNUTTON *et al.*, 1989), were significantly associated with diarrhoea ($P<0·001$). EAF^+ strains were related with diarrhoea in children under 18 months old only, and tended to be more frequent in the late dry–early wet season. No faecal sample contained visible blood or mucus, and all *E. coli* strains biochemically similar to EIEC were negative in the Séreny test.

Our experience suggests the following guidelines for examination of faecal samples from young children in tropical environments, similar to north-west Australia, where infectious diarrhoea is endemic. LT^+ strains should be sought in specimens from children 18–24 months old and confined to the wet season. Throughout the year ST^+ strains should be sought in children 6–18 months old, and EAF^+ strains in children below 18 months. VT-producing strains should be tested for in children aged below 18 months. Enteroinvasive strains need not be considered unless there is evidence of blood or mucus in children's stools or unless the area is known to have sporadic or epidemic incidents of dysentery. Pooled EPEC sera may be useful in identifying Verotoxigenic and enteroadherent strains. Adoption of such an approach should help in identification of *E. coli* strains of clinical and epidemiological importance, as well as reducing the effort and cost involved in screening all faecal samples.

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