

Detection of *Cryptococcus neoformans* in faecal matter: a novel presentation of disseminated cryptococcosis

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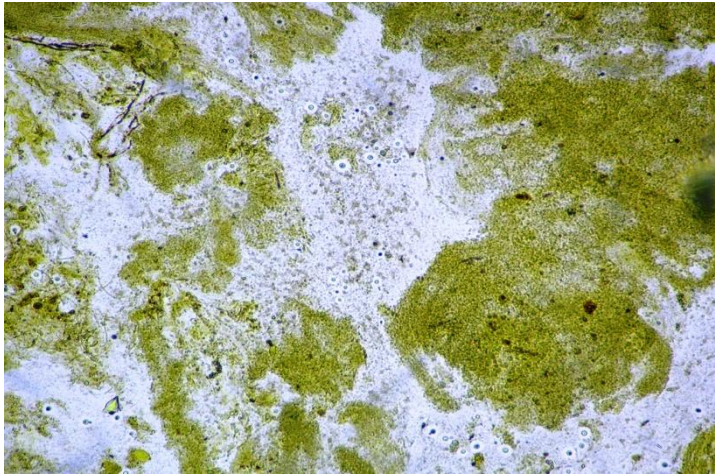
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Cryptococcosis is a fungal infection caused by *Cryptococcus neoformans* which primarily affects the central nervous system (CNS) and lungs. People with weakened immune systems, such as those suffering from AIDS, are generally more susceptible to this kind of infection [1]. Due to the predominant location of the disease in the CNS, an accurate diagnosis can be made by means of a microscopic examination using cerebrospinal fluid (CSF), allowing the round yeasts to be visualized more distinctly as well as permitting the isolation of the causative agent, *C. neoformans* [2]. Because of the wide spread of *C. neoformans*, the mycological study of other clinical samples, such as blood, bone marrow, scraping of skin lesions, respiratory secretions, or urine can also test positive for the infection [3]. The determination of the antigen polysaccharide capsular of *C. neoformans* can be performed in different biological fluids, increasing precision and effectiveness when diagnosing the disease [4]. Detailed in this case report is an unusual case of cryptococcosis found in a patient with AIDS, diagnosed from a stool sample sent to our laboratory for parasitological study. A 30-year-old man visited the Francisco J Muñiz Hospital for Infectious Diseases suffering from diarrhoea and a weight loss of 48 pounds over a period of two months. The patient was alert, oriented, and able to hold a conversation. He had been diagnosed with HIV-1 antibody nine years prior to his visit. The patient had a drug abuse history and was known to have shared needles with multiple partners.

Previous studies had excluded opportunist pathogens and other known causes of diarrhoea.

Nonspecific anti-diarrhoeal medications brought temporary relief of the symptoms. The patient went, on an outpatient basis, to the Parasitology Laboratory with a stool sample, collected over seven consecutive days in a solution of sodium acetate-acetic acid-formalin (SAF) [5]. Stool microscopy was requested for recovery of protozoal pathogens such as *Cryptosporidium* [6]. Although the patient's stool sample tested negative for parasites, faecal smears, microscopically examined, evidenced the presence of numerous yeasts. Many were of rounded form, with a thick wall and surrounded by a clear halo, while others were oval, possessed a thin wall, and lacked the aforementioned halo indicating the presence of *C. neoformans* (Figure 1). Furthermore, the addition of the India ink stain to the faecal concentrates revealed capsules in the thick-walled yeast (Figure 2), also compatible with *C. neoformans*. Additionally, as previously stated, samples of the blood, bronchoalveolar lavage (BAL), and CSF were also examined to test for the presence of *C. neoformans*. Immediately after the mycological diagnosis, the patient received intravenous amphotericin B, 1 mg/kg (IV) q24 h, which is our hospital's protocol for this mycosis [7]. However, due to the rapid deterioration of his general state, he was moved, the following day, to the Respiratory Intensive Care Unit, where he shortly died due to metabolic decompensation. The results obtained from cultures from the clinical samples taken from the patient tested positive for *C. neoformans* several days after he died. Polysaccharide antigen was not performed due to the positive results obtained with cultures. We were

Figure 1. Fresh microscopy of the fecal sample with 100x, revealing rounded yeasts with a clear halo (suspected as *Cryptococcus neoformans*).



unable to isolate the *C. neoformans* from the stool because the only available samples were those maintained in SAF solution.

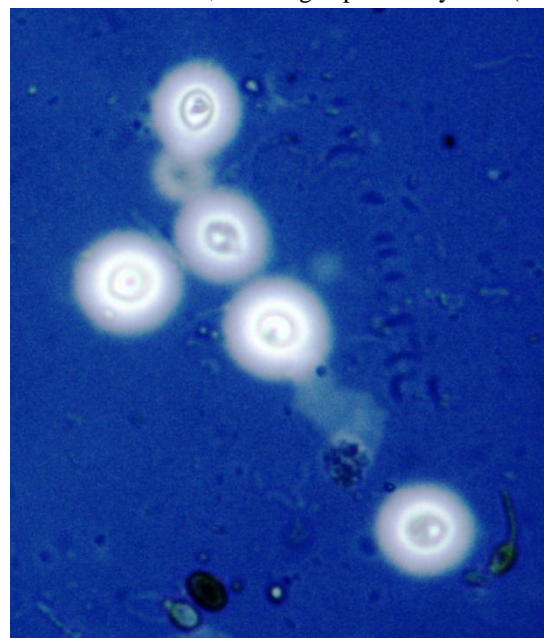
The presence of fungi in faecal samples is reserved almost exclusively to *Candida* yeasts, particularly *C. albicans*, which make up a small part of the microbiota of human and animal intestines [8]. However, there can be rare exceptions, including the presence of fungal pathogens such as *Cryptococcus neoformans* and *Paracoccidioides brasiliensis* as observed in the samples sent to us for parasitological study. The first was taken from a patient with cryptococcosis associated with AIDS [9] and the second from an individual with an acute disseminated form of Paracoccidioidomycosis [10]. Similarly, microscopic samples of concentrated faecal matter, stained using the Kinyoun technique, testing for *Cryptosporidium* sp., *Isospora belli* and *Cyclospora cayentaniensis*, in a patient with AIDS and disseminated nocardiosis, revealed the presence of unusual acid-fast filamentous bacteria, compatible with *Nocardia* [11]. Using this staining method on a number of other faecal samples of AIDS patients also revealed acid resistant bacilli connected with the morphology of *Mycobacterium* in disseminated tuberculosis [12]. In these cases, as in the former ones, the presence of these microorganisms in the stool samples has been linked to the swallowing of mucous secretions containing microbes ingested from air inhalation and/or ingestion of contaminated foods [13].

In some cases, during the process of microscopically examining the yeasts in the faecal samples, using techniques such as Kinyoun staining, we have occasionally observed the presence of a

clear halo around some of the cell walls, which were anomalies and were a result of an observational error and not because of the genuine existence of a capsule [personal observation, unpublished data]. The conservative solution, SAF, in which the samples were sent, did not alter the morphology of the microscopic yeasts. The presence of round capsulated yeasts in body fluids is normally considered to be *C. neoformans*, given that no other morphologically similar human pathogens are present.

In this reported case, the diagnosis of disseminated cryptococcosis was suggested from the detection of rounded yeasts in the stool, brought in,

Figure 2. Microscopic preparation of the fecal sample added with India ink, showing capsulated yeasts (400x).



initially, for parasitological study. Chronic diarrhoea caused by *C. neoformans* is rare and clinical suspicion is required. The observation that this disseminated infection can follow ingestion of *C. neoformans* in mice and monkeys suggests that the gastrointestinal tract could serve as a portal of entry for this pathogen. At present, the frequency and importance of the gastrointestinal route of infection in humans is unknown [14]. Taking into account the presence of *C. neoformans* in faecal samples has been, in our experience, always associated with the pulmonary localization of cryptococcosis and we believe that this phenomenon is produced by swallowing respiratory secretions. We have not observed the presence of *C. neoformans* in any other patients than those with pulmonary cryptococcosis. This unexpected find in the faecal matter was the first indication of the disseminated infection which was then later confirmed by the isolation of the fungi in the blood, CSF, and BAL.

To summarize, in HIV patients with chronic diarrhoea, fungal infections should be considered a differential diagnosis, including *C. neoformans*. The discovery of fungi in faecal samples, which is considered to be a rare exception, should not be underestimated by microbiologists. In many cases, as in the one which has been detailed, it can very well suggest and/or even diagnose a disease.

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