

CASE REPORT

Zoonotic transmission of *Cryptococcus neoformans* from a magpie to an immunocompetent patient

K. LAGROU¹, J. VAN ELDERE¹, S. KEULEERS², F. HAGEN³, R. MERCKX¹, J. VERHAEGEN¹, W. E. PEETERMANS² & T. BOEKHOUT^{3,4}

From the Departments of ¹Immunology and Microbiology, and ²Internal Medicine, University Hospital Leuven, Herestraat, Leuven, Belgium, and ³Centraalbureau voor Schimmelcultures, CT Utrecht, the Netherlands and ⁴Department of Medicine, Division of Acute Medicine and Infectious Diseases, University Medical Centre, 3508 GA Utrecht, the Netherlands

Abstract. Lagrou K, Van Eldere J, Keuleers S, Hagen F, Merckx R, Verhaegen J, Peetermans WE, Boekhout T (University Hospital Leuven, Leuven, Belgium; and Centraalbureau voor Schimmelcultures, CT Utrecht, the Netherlands) Zoonotic transmission of *Cryptococcus neoformans* from a magpie to an immunocompetent patient (Case report). *J Intern Med* 2005; **257**: 385–388.

Abstract. We report a case of cryptococcal meningitis in an immunocompetent female patient with

exposure to a pet magpie (*Pica pica*). Genetically indistinguishable isolates were cultured from the cerebrospinal fluid of the patient and excreta of the bird. Our data strongly suggest zoonotic transmission of *Cryptococcus neoformans* var. *grubii* from a magpie to this patient.

Keywords: *Cryptococcus neoformans*, immunocompetence, magpie, transmission.

Introduction

Cryptococcus neoformans commonly infects immunocompromised patients, especially AIDS patients and organ transplant recipients and causes fungal meningoencephalitis. The yeast has been isolated from environmental sources, such as house dust and soil, and from the excrements of a variety of avian species (e.g. pigeons, chickens, parrots, sparrows, starlings, turtledoves, canaries and skylarks) [1]. Several reports have been published describing the development of cryptococcosis in patients after exposure to birds or avian excreta, suggesting that human infection can result from this source [2, 3]. With the use of molecular techniques, it has been possible

to demonstrate that isolates obtained from bird excreta on the one hand and isolates from patients on the other are closely related [4, 5]. This adds to the increasing evidence linking clinical isolates to those found in bird excreta. In 2000, Nosanchuk *et al.* [6] documented zoonotic transmission of *C. neoformans*, from a pet cockatoo to a renal transplant recipient patient, for the first time. We document a second case of probable zoonotic transmission of *C. neoformans*. Genetically indistinguishable isolates were cultured from the cerebrospinal fluid (CSF) of the patient and excreta of the magpie (*Pica pica*). Our data strongly suggest that the yeast was transmitted from a pet magpie to an immunocompetent patient causing meningitis.

Case report

A 44-year-old woman with epilepsy in the medical history was admitted to the hospital because of headache since 3 weeks. The patient suffered from anorexia, nausea and weight loss. The patient did not register her body temperature at home. Physical examination was normal. There was no nuchal rigidity or photophobia. Temperature was 37.5 °C. A nuclear magnetic resonance (NMR) scan showed a few small lesions in the white matter of the corona radiata, interpreted by the radiologist as old ischaemic lesions. There was no contrast enhancement. Three days after admission, fever went up to 39 °C and a lumbar puncture was made. The CSF showed 444 white blood cells per microlitre (4% granulocytes, 13% monocytes, 83% lymphocytes), a glucose content of 5 mg dL⁻¹ and a protein content of 2161 mg L⁻¹. CSF culture grew *C. neoformans*. The *C. neoformans* antigen titre was 1/512 in CSF and 1/256 in serum. Mycobacterial and viral CSF culture and PCR remained negative.

The patient was treated with amphotericin B plus flucytosine for 2 weeks followed by a treatment with fluconazole 400 mg day⁻¹ for 6 months. Initially, she developed intracranial hypertension, a transient paralysis of the abducens nerve and cerebellar ataxia. An NMR scan revealed a new cystic lesion with peripheral contrast enhancement in the cerebellum and bilateral choroiditis (Fig. 1). After discharge from the hospital, the patient did well under prolonged treatment with fluconazole.

The patient was HIV seronegative and had no history of an increased infection rate. White blood cell count, immunoglobulin A, G, M and E concentrations, complement factors C3, C4 and total complement, the CD4 cell count (504 cells mm⁻³, 46.9%) were all within normal limits. A lymphocyte stimulation test with *Candida*, tuberculin, tetanus toxoid antigen and pokeweed mitogen revealed high proliferation ratios. The hypothesis of a zoonotic transmission was suggested by one of us (SK) and an anamnesis for bird exposure was performed. The patient told a pet magpie bird was present at the veranda of her parents' home where she stayed the last 3 months before admission to the hospital. Excreta, collected from the bird's cage were brought to the microbiology lab for culture and also grew *C. neoformans*.

Both isolates utilized urea and were encapsulated. The isolates were further identified using the ID 32C system (bioMérieux, Hazelwood, MI, USA) and had identical biochemical profiles (code 5166764351). Both isolates were serotype A isolates (*C. neoformans* var. *grubii*), mating type α [7]. Pulsed field gel electrophoresis analysis performed following Perfect *et al.* [8] with some minor modifications, resulted in identical chromosomal DNA banding patterns (Fig. 2a). Analysis of genetic relatedness of the isolates as investigated by amplified fragment length polymorphism (AFLP) analysis [9], showed that the similarity between the isolate from the magpie MP and that from the patient PT was 99.2% (Fig. 2b), calculated by the unweighted pair-group method by

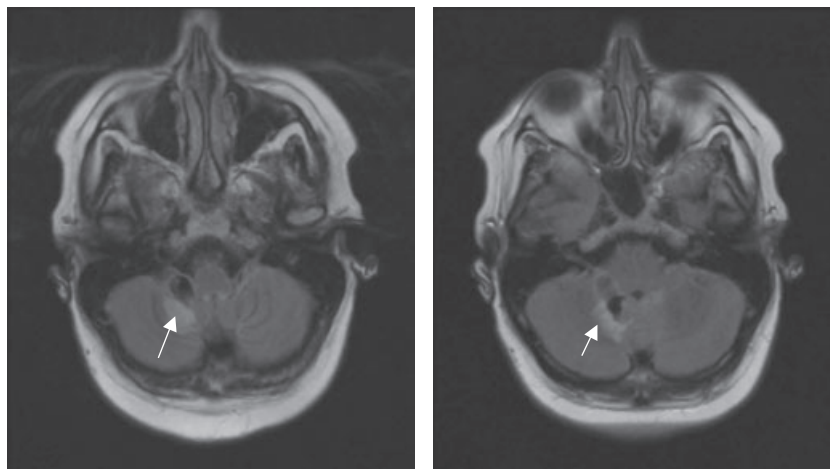


Fig. 1 Nuclear magnetic resonance scans of the brain showing a cystic lesion in the cerebellum (arrow).

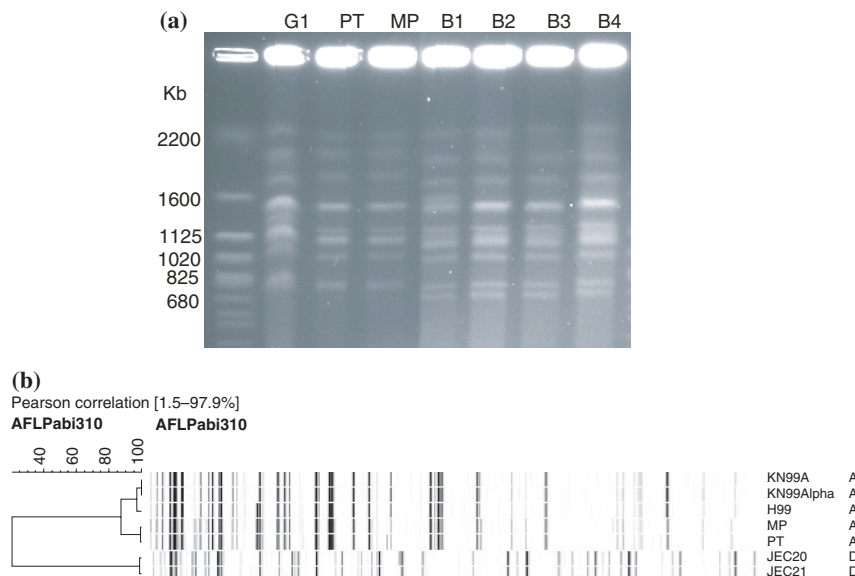


Fig. 2 Pulsed field gel electrophoresis and amplified fragment length polymorphism (AFLP) analysis of the clinical (PT) and bird (MP) isolate. (a) Electrophoretic karyotypes obtained by contour-clamped homogenous electric field (CHEF) analysis. DNA molecular size markers are indicated along the left margin. Other strains on the gel are: G1, serotype D strain of *Cryptococcus neoformans*; B1–B4, *C. neoformans* isolated from Belgian aviaries. (b) Dendrogram obtained by AFLP analysis showing high similarity between the clinical isolate PT and the isolate MP from the magpie droppings.

using average linkages (UPGMA, no optimization; 0.05% position tolerance). The clinical strain H99 (α A) and the congenic backcrosses KN99a (α A) and KN99 α (α A) were included, as well as the congenic serotype D strains JEC20 (α D) and JEC21 (α D) as control strains with high genetic similarity [10, 11]. Strain similarity by AFLP amongst the congenic control was 99.5% and 98.8% for the strains KN99a/KN99 α and JEC20/JEC21 respectively.

Discussion

We report the second case of human cryptococcosis in which a genetically indistinguishable *C. neoformans* isolate was recovered from excreta of a bird in the patient's environment. The isolate recovered from the excreta of the magpie and the isolate from the patient's CSF were shown to be identical by ID 32C identification and karyotyping. Similarity with AFLP analysis was 99.2%. Clinical isolates of *C. neoformans* exhibit great variation in chromosome number and size [12]. The high frequency of chromosome differences between strains makes electrophoretic karyotyping a highly discriminatory technique for distinguishing amongst isolates [8]. AFLP is a genotyping method with a high

discriminative power and reproducibility [9]. The available data strongly suggest that the magpie excreta were a reservoir for the strain of *C. neoformans* that infected the patient. However, we cannot exclude the possibility that both patient and the bird excreta were infected by an undetermined third source, although we consider this option less likely than transfer from the bird to the patient.

Except for some rare cases of primary cutaneous cryptococcosis, the infection is thought to result initially from inhalation of airborne fungi from an environmental source. Our case illustrates that prolonged contact with birds may not be required for exposure to *C. neoformans*. The magpie was not let out of its cage and the patient was not involved in cleaning the cage. Our patient's contact with the bird was limited to passing by the cage when entering home. Staib [13] showed that *C. neoformans* can be isolated from the ambient air near caged birds. This has to be kept in mind when asking patients for environmental exposure to birds. A thorough anamnesis aimed at the identification of bird contact (and not only handling of birds or cleaning bird cages) is indicated when a clinician is confronted with a patient with cryptococcosis.

To our knowledge, isolation of cryptococci from excreta of a magpie has not been reported yet. In contrast to the previous report of zoonotic transmission of *C. neoformans*, our patient is not immunocompromised. Whereas *C. neoformans* var. *gatii* infects immunocompetent individuals, *C. neoformans* var. *grubii* typically infects immunocompromised patients [14, 15]. Those patients at particularly high risk have defects in the cell-mediated immunity, such as patients with AIDS, lymphomas, and sarcoidosis and those receiving immunosuppressive therapy. Cryptococcosis also appears to occur with increased frequency in association with leucopenia, diabetes mellitus, rheumatoid arthritis, and hepatic cirrhosis [1]. Therapy with infliximab, a tumour necrosis factor alpha antagonist, may predispose patients to cryptococcal disease as suggested by Shrestha *et al.* [16]. This recent case also suggests the possibility of zoonotic transmission from a pet cockatiel, although they did not succeed in culturing *C. neoformans* from its faeces. Our patient did not have any of these risk factors mentioned. The basis of virulence in serotype A strains of *C. neoformans* strains infecting apparently immunocompetent individuals is not well understood yet, but may be attributable to mutations occurring in the yeast and/or underlying immune system impairment in patients [14].

Investigation of cryptococcosis cases using molecular tools may improve our understanding of the risk factors for this disease and identify particular disease causing exposures. This could lead to formulation of prevention strategies.

Conflict of interest statement

No conflict of interest was declared.

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Correspondence: Prof. Katrien Lagrou, University Hospital, Department of Microbiology, UZ Gasthuisberg, Dienst Laboratoriumgeneeskunde, Herestraat 49, Leuven 3000, Belgium.
(fax: 32 16 34 79 31; e-mail: Katrien.lagrou@uz.kuleuven.ac.be).