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# Identification and genotyping of *Enterocytozoon bieneusi* among human immunodeficiency virus infected patients

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**KEYWORDS**
Microsporidia;
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**Summary** Microsporidia cause diarrhea among human immunodeficiency virus (HIV) infected patients worldwide. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the most common species infecting HIV patients. Various genotypes of *E. bieneusi* are transmitted from human to human (anthroponotic route) or from animal to human (zoonotic route). However, there is no study from India on genotypes of *E. bieneusi* among infected hosts. Therefore, we aimed to (a) study the prevalence, clinical symptoms, and species identification of microsporidia among HIV infected patients and (b) perform a genotypic analysis of *E. bieneusi* and a phylogenetic interpretation of the transmission of different genotypes among infected hosts. Two hundred and twenty-two HIV-infected patients and 220 healthy controls (HC) were tested for the presence of microsporidia using modified trichrome (MT) staining and PCR. Demographic, clinical and laboratory parameters were studied. Species identification was performed using PCR-RFLP. All *E. bieneusi* isolates were subjected to genotypic and phylogenetic analysis. Patients with HIV [$n = 222$, age 37.4 ± 10.4 y, 169 (76%) male] were more commonly infected with microsporidia than the HC [$n = 220$, age 34.5 ± 6.5 y, 156 (71%) male], using MT stain and PCR [4/222, 1.8% vs. 0/220, $p = 0.04$]. Patients infected with microsporidia more commonly presented with diarrhea than those not infected with microsporidia [4, 100% vs. 98/218, 45%; $p = 0.04$]. *E. bieneusi* was detected in all patients with microsporidia. Four novel genotypes (Ind1 to Ind4) were identified. Ind1 showed 95%

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similarity with genotype L (AF267142.1) reported in cats (Germany). Genotypes Ind2 to Ind4 showed 94—96% similarity to host-specific genotype A (AF101197.1) reported in humans. Phylogenetic analysis mainly showed an anthroponotic route of transmission (3/4), while the zoonotic route (1/4) was also observed. The prevalence of microsporidia among HIV-infected patients was 1.8%. Patients with microsporidia commonly present with diarrhea. *E. bieneusi* is the most common species infecting the study population. Four novel genotypes of *E. bieneusi* were identified, suggesting presumptive transmission mainly through the anthropological route.

## Introduction

Microsporidia commonly cause intestinal microsporidiosis among HIV-infected patients worldwide, with a prevalence ranging from 0.1% to 50% [1]. It is still under-reported in HIV patients from a developing country such as India, with a prevalence ranging from 1% to 15.9% [2—5]. Diarrhea is the most common clinical manifestation of intestinal microsporidiosis among HIV patients with CD4 cell counts $\leq$100 cells/$\mu$l [6,7].

*Enterocytozoon bieneusi* (E. bieneusi) and *Encephalitozoon intestinalis* are the most common species of microsporidia causing intestinal microsporidiosis in humans [5]. Intestinal microsporidiosis caused by *Encephalitozoon hellem* has been diagnosed in very few cancer patients [8]. *E. bieneusi*, the most common species, is often associated with chronic diarrhea in patients infected with HIV [6]. The drug of choice, albendazole, is not very effective against *E. bieneusi*. Therefore, emphasizing strategies to prevent infection in humans would be useful. The establishment of preventive measures depends upon tracing the route of transmission. Spores of *E. bieneusi* infecting humans, animals and birds are shed in the host's feces. Therefore, the transmission route may be person to person (anthroponotic) or animal to human (zoonotic). Genotyping of *E. bieneusi* isolates is a valuable tool for epidemiological investigations [9]. The genotyping is widely based on analysis of an internal transcribed spacer (ITS) of rRNA genes of microsporidia. To date, approximately 90 genotypes of *E. bieneusi* infecting humans and animals have been identified [10]. Some of the genotypes are host specific, infecting only humans, while others can infect humans as well as animals and thereby have zoonotic potential [11]. *E. bieneusi* is the most common species infecting animals and humans. Its transmission route among humans is still unclear in a developing nation such as India. To date, no study has been reported on genotypes of *E. bieneusi* among HIV-infected patients in India. Understanding the molecular epidemiology of *E. bieneusi*, including the route of transmission, would help to establish prevention strategies in India.

Accordingly, we aimed to (a) study the prevalence, clinical symptoms and species identification of microsporidia among HIV-infected patients and (b) perform genotypic analysis of *E. bieneusi* and phylogenetic interpretation of the transmission of different genotypes among infected hosts.

## Methods

### Study population and protocol

Two hundred and twenty-two consecutive HIV-infected patients (with and without diarrhea) attending the Immunology unit of a tertiary care institution were tested for the presence of microsporidia during March 2010 to November 2014. Patients infected with HIV were included in the study as per National AIDS Control Organization (NACO) guidelines [12]. Patients without HIV infection were excluded. Two hundred and twenty age- and sex-matched healthy controls without any history of apparent illness within the last 4 weeks were included in the study. Data on demographic, clinical and laboratory parameters were recorded in a standard questionnaire for each patient. Patients were asked if they purify potable water (filtered water) or consume the potable water without any purification (unfiltered water). The study protocol was approved by the Institutional Ethics Committee (PGI/DIR/RC/1085/2007).

### Sample collection

Three consecutive fresh stool samples were collected from each patient and healthy control. The samples were subjected to routine stool microscopy

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