SHORT REPORT: PREVALENCE OF FECAL ENCEPHALITOZOOZ SP. SPORES AMONG HOSPITALIZED PATIENTS IN NEPAL

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Abstract. During the months of June and July 1998, stool samples from 341 hospitalized patients (mean age, 32.7 ± 16.3 years; range, 1–86 years) from Kathmandu, Nepal, were screened for the prevalence of Encephalitozoon sp. by use of anti-Encephalitozoon sp. monoclonal antibody 3B6-based immunofluorescence assay. The cross-sectional study revealed the presence of Encephalitozoon spores in 0.6% (2 of 341) patients. By use of direct microscopic examination, 27% (93 of 341) of patients were diagnosed with various gastrointestinal pathogens, among which Ancylostoma lumbricoides and Ancylostoma duodenale were the most commonly found, with prevalence rates of 8.8% (30 of 341) and 7.6% (26 of 341), respectively. To our knowledge, this is the first study to report the presence of Encephalitozoon sp. among humans in Nepal.

Encephalitozoon sp. (phylum Microsporidia) are rapidly gaining recognition as important opportunistic human pathogens. Three species of microsporidial parasites within the genus Encephalitozoon are known to infect humans: E. intestinalis, E. cuniculi, and E. hellem.1–4 Intestinal microsporidiosis due to Encephalitozoon (Septata) intestinalis and Enteroctytotozoon bieneusi are most frequently reported among immunocompromised people, such as patients with acquired immune deficiency syndrome (AIDS).1,2 Encephalitozoon cuniculi and Encephalitozoon hellem are less prevalent among immunodeficient patients.3–5 Although the most common clinical symptoms related to encephalitozoonosis among immunodeficient patients are chronic diarrhea and malabsorption, they can also cause disseminated diseases. Encephalitozoon hellem is primarily associated with keratoconjunctivitis, but it can also cause sinusitis, tracheobronchitis, intestitial nephritis, and hepatitis.1,6

The studies examining the prevalence of encephalitozoonosis have been limited to patients in Europe and the Americas who are positive for human immunodeficiency virus (HIV) or who have AIDS. Thus, the global epidemiology of human encephalitozoonosis is poorly understood. However, the significance of encephalitozoon as an important cause of morbidity among immunocompromised patients is clear. In Europe and the Americas, the prevalence of microsporidial infections among HIV-infected patients or patients with AIDS with chronic diarrhea has been reported to range 5–60%.2,7–10 Recently, a few studies have examined the prevalence of encephalitozoonosis among immunocompetent, non-AIDS populations. For example, a serological study found high antibody titers for E. intestinalis in 8% (24 of 300) of Dutch blood donors and in 5% (13 of 276) of pregnant French women.11 A similar study conducted in Slovakia showed that sera of 5.1% (5 of 98) of slaughterhouse employees were positive for Encephalitozoon cuniculi.12 Another study reported microsporidial spores in 4 immunocompetent, HIV-negative travelers, 2 of which were identified as E. intestinalis by polymerase chain reaction.13 A study conducted among 2 Mexican village populations identifying fecal spores by immunofluorescence assay revealed an 8% (20 of 255) prevalence of Encephalitozoon sp.14

Epidemiological data on Encephalitozoon infections among the Nepalese population are unknown. To assess the presence of Encephalitozoon sp. in Nepal, we examined 341 stool samples from hospitalized children and adults from Bir Hospital, Kathmandu, Nepal. Fecal spores were identified by use of monoclonal antibody 3B6-based immunofluorescence assay.

Situated 1,230 km (4,100 feet) above sea level, Kathmandu has a temperate climate with rainy summers and dry winters. This cross-sectional study was conducted during the months of June and July 1998. Bir Hospital is the largest, centrally located public hospital in Kathmandu and provides service to ~1 million residents in Kathmandu valley. All 341 patients were informed of the study, and consent was obtained before enrollment in this study. Guidelines for human experimentation of the U.S. Department of Health and Human Services and the University of Arizona Institutional Review Board (Human Subjects Committee) were approved and followed in the conduct of the present study. Five milliliters of fecal material was obtained from inpatients who submitted such samples at Bir Hospital. A 500-μL aliquot of each fecal sample was collected in sterile containers and screened for protozoan and helminth parasites by direct light microscopy, including Ancylostoma lumbricoides, Trichuris trichiura, Giardia lamblia, Entamoeba histolytica, and Ancylostoma duodenale. The remaining 4.5 mL of each fecal sample was preserved in 10% formalin and later concentrated by ethyl acetate sedimentation for Encephalitozoon sp. screening. Concentrates were fixed onto poly-L-lysine–coated 10-well diagnostic slides (0.01% vol/vol; Sigma Chemical, St. Louis, MO) and blocked with bovine serum albumin (2%, Sigma). Slides were incubated sequentially with anti-Encephalitozoon–specific mouse monoclonal antibody 3B6,15 followed by fluorescein isothiocyanate–labeled goat anti-mouse immunoglobulin G (Kirkegaard and Perry, Gaithersburg, MD).

Demographic data such as age and sex of patients were also obtained from clinical charts. Results were analyzed by the software Epi Info version 6.0 (CDC, Atlanta, GA). Correlation analysis was performed between age and sex variables, and presence of fecal Encephalitozoon spores.
TABLE 1
Prevalence of gastrointestinal pathogens identified by immunofluorescence assay and direct microscopy among 341 inpatients at Bir Hospital, Kathmandu, Nepal

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Infected patients, n (%)</th>
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<tbody>
<tr>
<td>Ascariasis lumbricoides</td>
<td>30 (8.8)</td>
</tr>
<tr>
<td>Ancylostoma duodenale</td>
<td>26 (7.6)</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>17 (5)</td>
</tr>
<tr>
<td>Giardia lambia</td>
<td>12 (3.5)</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>9 (2.6)</td>
</tr>
<tr>
<td>Hymenolepis nana</td>
<td>4 (1.2)</td>
</tr>
<tr>
<td>Encephalitozoon sp.</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Strongyloides stercoralis</td>
<td>1 (0.3)</td>
</tr>
</tbody>
</table>

Among the 341 inpatients, 54% (n = 178) were male subjects and 46% (n = 153) were female subjects. Their average age was 32.7 ± 16.34 years (range, 1–86 years). Various gastrointestinal parasites were identified in feces of 27% (n = 93) of all 341 patients (Table 1). Ascariasis lumbricoides and Ancylostoma duodenale eggs had the highest prevalence rates of 8.8% (n = 30) and 7.6% (n = 26), respectively. The prevalence of Giardia lambia cysts, Entamoeba histolytica cysts, Trichuris trichiura eggs, and Hymenolepis nana eggs was 5% (n = 17), 3.5% (n = 12), 2.6% (n = 9), and 1.2% (n = 4), respectively. Strongyloides stercoralis larvae, yeast, and spores of Encephalitozoon sp. were found in <1% of the samples. There were no correlations between prevalence of the parasites and either sex or age of the patients.

Several Encephalitozoon seroprevalence studies have contributed to the epidemiological evaluation of Encephalitozoonosis among non-AIDS populations.

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Several Encephalitozoon seroprevalence studies have contributed to the epidemiological evaluation of Encephalitozoonosis among non-AIDS populations. However, serological methods have inherent limitations because they measure exposure to the parasite, but not the actual presence of spores in feces. Also, it is not known for how long after exposure anti-Encephalitozoon sp. serum antibodies remain detectable. Encephalitozoon sp.—specific immunofluorescence assay, on the other hand, provides a direct identification of Encephalitozoon sp. spores in concentrated samples. Although the monoclonal antibody 3B6 strongly recognizes Encephalitozoon sp., it does not allow us to microscopically differentiate between E. cuniculi, E. hellem, and E. intestinalis. A better understanding of the global prevalence of encephalitozoonosis is needed for establishing prevention and control guidelines to minimize risk of infection among both immunocompetent and immunodeficient human populations. In addition, this knowledge may help doctors and health care workers to become aware of microsporidiosis because several chemotherapeutic agents, such as albendazole, have been successful in the treatment of E. hellem and E. intestinalis.

Further epidemiological studies designed to investigate the prevalence of Encephalitozoon sp. among general populations are needed to understand and control the infections these protozoans cause.

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REFERENCES