We report what is, to our knowledge, the first study in which microsporidial infection was detected in elderly human immunodeficiency virus (HIV)–negative patients. Of the 60 elderly patients studied, 47 had diarrhea. Intestinal microsporidiosis due to Enterocytozoon bieneusi was diagnosed in 8 patients (17.02%) by use of Weber’s chromotrope-based stain and polymerase chain reaction with species-specific primers. The mean age of these 8 patients was 75 years; 7 had chronic diarrhea and 1 had nonchronic diarrhea. Six of the patients with chronic diarrhea had no other pathogens isolated. In our opinion, elderly patients, because of their special immunological characteristics, should be considered a group at risk for the acquisition of intestinal microsporidiosis.

Microsporidia are ubiquitous parasites that are capable of causing infection in almost all groups of animals. Since the first well-documented case of microsporidial infection in humans was reported in 1959, this parasitosis mainly has been associated with patients with AIDS. There have been few reports of microsporidial infection in HIV-negative persons [1, 2]. However, travelers have recently emerged as a group considered to be at risk for such infection [3].

The number of genera implicated in microsporidiosis in humans has increased at the same rate that improvements in diagnostic techniques and interest in this group of parasites have increased. Enterocytozoon bieneusi, which is the microsporidium that most frequently causes infection in humans, is principally associated with chronic diarrhea, unexplained weight loss, and cholangitis [4]. The epidemiology of intestinal microsporidiosis is not yet well known, and most studies have focused on chronic diarrhea occurring among patients with AIDS (incidence, 7%–50%) [4].

Although many questions about microsporidiosis remain unanswered, in recent years, new data have accumulated that have begun to change general conceptions about this parasitosis. For example, E. bieneusi was considered to be a parasite found only in humans, but, at present, its zoonotic potential is a focus of discussion [5, 6]. E. bieneusi also has been detected in surface water and has been associated with a waterborne outbreak of infection [7]. Furthermore, the species has been isolated in 10% of respiratory samples obtained from 46 asymptomatic HIV-positive patients and in
33% of such samples obtained from 15 symptomatic HIV-positive patients, findings that reinforce the possibility that respiratory spread is a mode of transmission [8].

Aging involves a diminishment of immunological capacity, leading to what is called “immunosenescence.” This process is mainly associated with a dysfunction in cell-mediated immunity [9], correlating with the great importance that cell immunity has in the control of microsporidial infection. This is seen not only in animal models [10] but also, in patients with AIDS [11]. In the present study, we report the detection of microsporidial infection in HIV-negative elderly patients who, because of their special immune status, could be considered a group at risk for microsporidiosis.

MATERIALS AND METHODS

A total of 193 samples (including 134 stool and 13 urine samples, 1 bile fluid sample, 1 pleural and 2 colon biopsy specimens, 29 sputum specimens, 8 bronchoalveolar lavage samples, and 5 bronchoalveolar aspirates) were obtained from 60 elderly patients (35 men and 25 women) who presented at the outpatient geriatric clinic at the Hospital do Meixoeiro (Vigo, Spain). The mean patient age was 73.5 years (range, 65–78 years). Of the patients (35 men and 25 women) who presented at the outpatient 5 bronchoalveolar aspirates) were obtained from 60 elderly pa-

RESULTS AND DISCUSSION

Stool samples and colon biopsy specimens obtained from 8 elderly patients contained a low number of spores (size, 0.9–1.2 μm), which appeared to be pinkish red with use of Weber’s chromotrope-based stain. These spores had the ovoid shape and the clear vacuole-like polar zone that are characteristic of microsporidia; the typical beltlike stripe of these organisms was also displayed by some of the spores. The respiratory samples obtained from the 15 elderly patients who had symptoms of respiratory disease tested negative for parasites, including microsporidia. Twenty (33.3%) of the 60 elderly patients had other pathogens identified, as shown in table 1.

Amplification of the DNAs isolated from stool and biopsy specimens, by use of PCR with E. bieneusi–specific primers, revealed a diagnostic band of 607 bp in the agarose gels. When the templates obtained were assayed with ECLD–, E. hellem–, or E. intestinalis–specific primers, no amplification was obtained for any of the samples studied. No PCR inhibitors were detected in templates obtained by DNA purification of any of the patient samples studied.

Of the group of 47 geriatric patients who had diarrhea, 8 patients (17.02%) were found to be positive for microsporidia; 7 patients had microsporidia spores identified in fecal samples, and 1 patient had such spores identified in a colon biopsy specimen but not in a fecal sample. E. bieneusi was the microsporidia implicated as the cause of all cases of diarrhea, as confirmed by PCR. No microsporidia were observed in respiratory samples obtained from patients with respiratory symptoms, although 2 of the patients had diarrhea with seeding of microsporidia spores at the time the study was done. The clin-

Table 1. Pathogens other than microsporidia isolated in 20 elderly HIV-negative patients.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Specimen</th>
<th>No. (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas hydrophila</td>
<td>Feces</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td>Bacteroides fragilis group</td>
<td>BAL</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Urine</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td>Clostridium difficile toxin</td>
<td>Feces</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Feces/sputa</td>
<td>6 (10)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Blood*</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>BAL/sputa</td>
<td>3 (5)</td>
</tr>
</tbody>
</table>

NOTE. BAL, bronchoalveolar lavage.

* For hemoculture.
clinical features of the 8 patients for whom test results revealed the presence of microsporidia are shown in Table 2. These patients were 4 men and 4 women (mean patient age, 75 years), 6 of whom lived in a rural area. Seven of the patients had chronic diarrhea and 1 had nonchronic diarrhea. Although no other pathogens were isolated from 6 of the patients with chronic diarrhea, Pseudomonas aeruginosa was detected in 1 of the remaining patients and Aeromonas hydrophila, Streptococcus pneumoniae, and Bacteroides of the fragilis group were isolated in the other.

Other studies of immunocompetent individuals basically have referred to isolated cases or cases among groups of young individuals who live in tropical countries [19]. In most of these studies, E. bieneusi was the microsporidia identified. Several studies have also shown that microsporidia may be implicated as a cause of traveler’s diarrhea [3]. However, to our knowledge, the present study is the first to investigate microsporidiosis in HIV-negative elderly patients.

The diminishment in immune response that occurs as a result of aging is reflected in an increase in the frequency of infectious diseases, a higher pathogenicity of disease and a higher mortality rate, a decrease in resistance to such chronic infections as tuberculosis, a loss of cutaneous reactivity to common antigens, and an alteration of the recognition and regulation system in association with loss of the capacity to respond to external agents. Finally, aging is related to an increase in the rate of autoimmune processes [20]. The origin of these alterations, which lead to what is called immunosenescence, is not well known and is, in most cases, only partially understood.

On the other hand, the importance of a competent immunosystem for protection against microsporidiosis is well documented. Cell immunity plays a prominent role in the control of microsporidial infection, as has been shown in animal models [10]. The age-related involution of the thymus, which is also a well-known process related to T lymphocyte differentiation and maturation, results in an altered T lymphocyte response. Therefore, it may be reasonable to suspect that elderly patients have a higher risk of developing microsporidiosis than do healthy immunocompetent HIV-negative individuals.

The results of the present study show that 8 (17.02%) of 47 elderly patients with diarrhea were found to have E. bieneusi infection. Exposure to human microsporidia may be a common phenomenon, as new data have suggested [6, 7]. For this reason, our findings could be explained by a relatively common latent carriage of microsporidia within human hosts, because microsporidial infections have been recognized in asymptomatic hosts [21], and because symptomatic microsporidial disease might, in many cases, represent increasing parasitic burden due to alterations in the immune system. We do not know whether the patients who we studied acquired infection through person-to-person transmission or from another source (e.g., water, food, or contact with animals), but it is of interest to note that 6 patients (75%) lived in a rural area. Further studies involving larger groups of elderly patients should be done to obtain conclusive results regarding this matter.

The data obtained in the present study suggest that chronically ill, elderly individuals may be an additional group at risk for acquisition of microsporidial infection. Whether infection with E. bieneusi is associated with symptomatic disease in elderly patients needs to be defined in further studies; however, it is noteworthy that E. bieneusi was the only pathogen isolated from 6 patients with chronic diarrhea. In our opinion, a search for microsporidia should be included in routine stool examinations performed for chronically ill, elderly individuals, at least when diarrhea is present and no other pathogens have been isolated.

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