Waterborne Outbreak of Intestinal Microsporidiosis in Persons with and without Human Immunodeficiency Virus Infection

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Among 1454 persons whose stool samples (n = 5692) were submitted to a reference laboratory for microsporidia assessment from 1993 to 1996, microsporidia were identified in 338 persons: 261 persons infected with human immunodeficiency virus (HIV), 16 transplant patients, and 61 others. Intestinal microsporidiosis appears to be an endemic disease in HIV-positive persons (prevalence, 0.1%) and a sporadic disease in HIV-negative persons (prevalence, <1/1 million). A waterborne outbreak in 200 persons (attack rate, 1% in HIV-positive patients/month) occurred in the 1995 summer, without evidence of fecal contamination of water. No explanation was found before the outbreak ended, several months before the antiretroviral era. Factors associated with microsporidiosis diagnosis were HIV infection, male homosexuality, low CD4 cell counts, and diarrhea. The major factor associated with a diagnosis of microsporidiosis during the outbreak was living in an area corresponding to one of the three water distribution subsystems of the town. Lake contamination was suspected.

Microsporidia are obligate intracellular protozoans of the phylum Microspora. Several hundred species cause diseases in a variety of invertebrates and vertebrates. Before the AIDS era, anecdotal human infections of keratoconjunctivitis, myositis, and peritonitis/hepatitis were attributed to microsporidia [1]. The first case of microsporidiosis in a person infected by the human immunodeficiency virus (HIV) was identified in 1985 and led to the description of a new species: Enterocytozoon bieneusi [2]. This species is associated with chronic diarrhea, unexplained weight loss, and cholangitis [3–16]. Another species, Encephalitozoon intestinalis (formerly Septata intestinalis), is associated with intestinal manifestations with or without disseminated symptoms [17].

The epidemiology of intestinal microsporidiosis remains largely unknown, and reported prevalence in AIDS patients with chronic diarrhea varies from 10% to 50%. In persons not infected by HIV, only anecdotal cases have been reported, mainly in immunosuppressed persons and travelers [16–25]. No known reservoir of the parasite has been identified, and the mode of transmission remains unclear. However, several factors, including the intestinal localization of parasites and limited data regarding a possible link with male homosexuality or bisexuality [26], argue for fecal-oral transmission. An association of infection with contact with water or fish has also been reported [27, 28], suggesting that microsporidiosis could be waterborne, although no seasonal trend in the prevalence of microsporidiosis was found in two studies in the United States and Brazil [29, 30]. The description and validation of a new assay for the identification of microsporidia in stool specimens [3] led us to implement this analysis in our parasitology laboratory in April 1993. Here we report the results of a retrospective review of stool analyses for microsporidia performed in our institution from May 1993 to December 1996.

Patients and Methods

Identification of patients and clinical data. Stool analyses for microsporidia have been performed in Lyon since May 1993 in a unique reference parasitology laboratory that receives samples from Lyon and surrounding areas (population >1.5 million). We retrospectively reviewed laboratory data from May 1993 to December 1996 and linked the findings with survey data for HIV infection in Lyon collected by the French Ministry of Health (DMI 2) [31]. Data collected for HIV-infected persons were age, gender, risk factors for HIV, weight, CD4 cell count, and the presence of diarrhea at the time of stool analysis. Data for other persons were obtained via direct contact with the clinical units that requested stool analysis; data for most of these patients was limited to age, gender, and confirmation of serologic status regarding HIV infection. No
data regarding the reasons for stool analysis or for specific analysis of microsporidia were available for these patients.

**Lyon’s water distribution system.** Drinking water treatment and distribution in Lyon are managed by a private company. The water is obtained from 300 hectares in the northeast of Lyon and its suburbs. The water comes from the Rhône River and is naturally filtered by alluvia where it is pumped through 114 wells. The water is treated by chlorination in 2 sites and then distributed in 3 independent subsystems corresponding to different elevations. The upper elevation system (north) is supplied by factory A and the 2 other systems (the medium elevation system in the east and south and the lower elevation system in the center and west) by factory B. At an additional site (factory C), surface water is pumped directly from a lake in a recreational area and then treated by flocculation, ozonation, and filtration. As needed, factory C can provide water independent of the 3 main distribution systems and is mainly active in summer. The location of patients’ residences in relation to the 3 water distribution subsystems was added to clinical data to test the hypothesis of a link between microsporidian infections and drinking water.

**Water safety data.** In accordance with French regulations, water samples are prospectively collected from different parts of the system and analyzed for water safety by an independent laboratory by use of standard parameters (turbidity, pH, conductivity, total coliform, fecal streptococci).

**Stool analysis for microsporidia.** Analysis for microsporidia was performed on fresh stools by a modified trichrome staining method [5] from May 1993 to October 1994. This technique was replaced by Uvitex 2B staining [32] from November 1994 to May 1995. The 2 techniques were then both done on all stool samples to increase both sensitivity for small numbers of spores and specificity [33].

**Definition of cases.** A diagnosis of intestinal microsporidiosis was suspected when structures compatible with microsporidia were identified on ≥1 stool sample. The diagnosis was confirmed when ≥2 stool samples were positive for microsporidia or when ≥1 stool sample was positive for microsporidia by the 2 identification techniques. Species identification by electron microscopy or polymerase chain reaction (PCR) [34–36] was performed in only few cases.

**Statistical analysis.** Descriptive statistics were based on percentages for categorical data and on mean ± SD for continuous variables. The statistical unit was the patient. Incidence was defined, as usual, as the number of new cases occurring in a given time. Incidence ratio was the number of cases divided by the total number of patients analyzed.

Univariate analysis was performed first on the entire population of patients, then only on the subset of HIV-infected patients. The purpose of univariate analysis was to select potentially explanatory variables. According to the types of data, comparisons were based on χ² tests with the appropriate number of degrees of freedom or, when appropriate, by the Mann-Whitney U test.

Multivariate models were built, using the logistic regression technique in 3 ways (i.e., forward, backward, and manually). The choice of the best explanatory model was made by assessment of the goodness of fit, with an entry level of 0.25 and a removal level of 0.15. All analyses were performed with SAS software (6.12; SAS Institute, Cary, NC) for the DEC digital vax machine (Digital Equipment Corporation, Maynard, MA, USA). Charts were generated by Excel software (Microsoft, Redmond, WA).

**Results**

**Demographics.** In total, 5692 stool samples from 1453 patients were assessed for microsporidia from May 1993 to December 1996. Mean age was 40.3 ± 13.5 years. There were 1102 men and 351 women. Serologic status for HIV infection was obtained for 1373 patients (HIV infected, 978 [71%]; not infected, 395 [29%]). No clinical information was available for non–HIV-infected persons. The main factor for HIV infection was male homo/bisexuality (52%), followed by heterosexual contact (23%) and intravenous drug abuse (12%). Diarrhea was reported at the time of stool examination by 37% of the HIV-infected patients. The mean CD4 cell count for HIV-infected patients was 149 ± 198/mm³. There was a trend for an increase in CD4 cells over time (mean CD4 cells/mm³, 71 ± 131 in the second quarter of 1993 vs. 215 ± 220 during the fourth quarter of 1996).

**Stool analysis for microsporidia.** In all, 6924 analyses for microsporidia were conducted on the 5692 stool samples: 2617 with modified trichrome staining, 1843 with Uvitex 2B staining, and 1232 with both staining methods. Structures compatible with microsporidia were visualized in 565 (15%) of 3849 samples stained with the modified trichrome and in 710 (23%) of 3075 samples stained with Uvitex 2B. Positive stools were obtained from 338 patients; of these, 200 (59%) were confirmed as defined. Because the distributions of confirmed and presumed cases over time were similar, both were considered for analysis. Species diagnosis was performed in only 24 patients and led to the identification of E. bieneusi in 14 patients and E. intestinalis in 10.

**Prevalence of microsporidia in stools and definition of outbreak.** The percentage of stools positive for microsporidia remained stable from May 1993 to May 1995 (median, 15% of tested stools; range, 5%–32%) with both of the 2 stains. There was a sudden increase in the percentage of positive stools in May 1995, >50% of all stools from June to September, and then the percentage decreased to levels similar to those in the first period (median, 9%; range, 1%–29%). This increase was related to an increase in new diagnoses of intestinal microsporidiosis during this period, leading us to define the period between 15 May and 30 November 1995 as an outbreak period. The period from 1 May 1993 to 14 May 1995 was defined as the preoutbreak period; the period from 1 December 1995 to 31 December 1996 was defined as the postoutbreak period. The preoutbreak and the postoutbreak periods were considered to be the endemic period.

**Prevalence of microsporidian infection and attack rate during the outbreak.** The median prevalence rate in HIV-infected patients was 0.1% during the preoutbreak period. During the outbreak, the attack rate peaked at 1% of HIV-infected patients.
in July and August 1995 and then decreased to 0.03% in the postoutbreak period. In patients not infected by HIV, the disease appeared sporadically (estimated prevalence, <1/1 million persons). In patients diagnosed during 1993, 1994, and 1996, 44% of the diagnoses were recorded in June or July, but there were too few patients to draw definite conclusions about a seasonal trend. The monthly incidence of intestinal microsporidiosis in HIV-infected persons followed up in Lyon’s hospitals (i.e., attack rate by month during the outbreak) and the percentage of stools positive for microsporidia by both techniques are shown in figure 1.

Infections in HIV-infected patients and in transplant patients. Microsporidia were identified in 261 (77%) HIV-infected patients, in 16 (5%) transplant patients (1 bone marrow, 2 heart-lung, 4 liver, and 9 kidney transplant recipients), in 52 patients (15%) without these types of immunodeficiency, and in 9 persons (3%) with unknown serologic status. Follow-up was limited for the majority of the transplant patients; stool samples were obtained for >6 days from only 6 patients. Of these 6 patients, 5 maintained microsporidia in stools throughout follow-up (6–857 days). Thirteen of the 16 transplant patients acquired microsporidia during the outbreak period.

Evolution of infection. Among positive patients, 85 (25%) had only 1 stool sample. Among patients with >1 stool sample, the mean duration of positive stools was 89 ± 167 days. Evolution of infection appeared to be shorter when acquired during the outbreak period (mean duration of positive stools, 32 ± 73 days) than during the endemic period (117 ± 208 days). Persistent microsporidiosis for >1 month occurred in 105 patients (41% of positive patients with >1 stool sample) both during the outbreak (47 patients) and during the endemic period (58 patients).

Factors associated with microsporidiosis diagnosis. Patients with microsporidiosis were more frequently infected by HIV than persons without microsporidiosis (80% vs. 68%; \( P = .0003 \), Fisher’s exact test), were significantly younger (38.5 ± 11.9 vs. 40.7 ± 13.9 years; \( P = .01 \), Mann-Whitney \( U \) test), and were more frequently male (84% vs. 79%; \( P = .002 \), Fisher’s exact test). No difference was observed between negative and positive patients by water distribution system.

In HIV-infected persons, factors associated with positivity were male sex, male homo/bisexuality (65% vs. 55%; \( P = .01 \), Fisher’s exact test), low CD4 cell count (120 ± 167 vs. 160 ± 207/mm\(^3\); \( P = .01 \), Mann-Whitney \( U \) test), and presence of diarrhea at the time of analysis (53% vs. 37%; \( P = .0004 \), Fisher’s exact test). Weight loss was not associated with positivity in this study.

In a logistic multivariate model, the only variable associated

![Figure 1](image-url)  
Figure 1. Monthly incidence of intestinal microsporidiosis in human immunodeficiency virus (HIV)-infected patients and % of stools positive for microsporidia by technique and by month from May 1993 to December 1996. Denominator for each month was derived from survey data for HIV infection in Lyon collected by the French Ministry of Health (DMI 2) [31]. Monthly incidence during June–November 1995 corresponds to monthly attack rate during same period.
with a diagnosis of microsporidiosis in HIV-infected and non-infected patients was HIV infection (odds ratio [OR], 2.04; 95% confidence interval [CI], 1.64–2.45; \( P = .004 \)). Other variables (age, sex, and water distribution subsystem) were not associated with microsporidiosis in these patients. In a second logistic multivariate model, the variables associated with a diagnosis of microsporidiosis in HIV-infected persons were younger age (OR, 0.98/1 year; 95% CI, 0.96–0.99; \( P = .04 \)), male homosexuality (OR, 1.59; 95% CI, 1.19–1.98; \( P = .02 \)), and fewer CD4 cells (OR, 0.99/1 cell/mm\(^3\); 95% CI, 0.99–0.99; \( P = .003 \)). Other variables (sex, weight loss, and water distribution subsystem) were not associated with microsporidiosis.

**Analysis of outbreak period.** By comparison with the endemic period, fewer persons diagnosed with intestinal microsporidiosis during the outbreak period were infected with HIV (73% vs. 88%; \( P = .002 \), Fisher’s exact test) or were male (78.5% vs. 88%; \( P = .04 \), Fisher’s exact test). Similarly, fewer HIV-infected persons diagnosed during the outbreak were male homosexual (57.5% vs. 74%; \( P = .01 \), Fisher’s exact test). For HIV-infected persons, other factors associated with a diagnosis of microsporidiosis during the outbreak were a higher CD4 cell count (140 ± 174 vs. 96 ± 155 cells/mm\(^3\); \( P = .006 \), Mann-Whitney U test), the absence of diarrhea (58% vs. 34%; \( P = .003 \), Fisher’s exact test), and lower weight loss (−6.3% ± 9.2% of initial weight vs. −10.1% ± 9.7% of initial weight; \( P = .004 \), Mann-Whitney U test). There was a strong association between a diagnosis of intestinal microsporidiosis during the outbreak period and living in the medium elevation water distribution system area (\( P = .01 \), \( \chi^2 \) test).

A logistic multivariate model was built to study the effect of these variables on the diagnosis of microsporidiosis during or outside the outbreak period in HIV-infected persons. The two variables associated with a diagnosis of microsporidiosis during the outbreak period were a lower weight loss (OR, 1.04/1% of loss of usual weight; 95% CI, 1.01–1.08; \( P = .01 \)) and living in the area corresponding to the medium elevation system (OR, 3.36; 95% CI, 2.58–4.14; \( P = .002 \)). Other variables (age, sex, HIV risk factor, absolute number of CD4 cells) were not associated with microsporidiosis.

**Water safety data.** During the whole study period, 4822 water samples from different parts of the water system were routinely analyzed for fecal contamination by an independent laboratory. No parameter was consistently associated with significant fecal contamination of water either during or outside the outbreak period. For example, only 1 sample (outside the outbreak) was positive for coliforms, and 6 samples (1 during the outbreak, 5 outside) were positive for fecal streptococci. No increase in cases of *Cryptosporidium parvum* or *Giardia intestinalis* infection diagnosed in our laboratory was observed before, during, or after the microsporidiosis outbreak.

**Discussion**

We believe that our study is the first large study of intestinal microsporidiosis in persons with and without HIV infection. This study has some intrinsic limitations, notably the retrospective collection of data and the lack of clinical data for persons not infected by HIV. However, since our laboratory is the reference laboratory for our town, we were able to obtain exhaustive data for the study period.

Patterns of infection outside the outbreak period were compatible with an endemic disease in HIV-infected persons. The severity of the disease appeared to be mainly related to immunologic status, since low CD4 cell count was the major independent variable, as described elsewhere [4, 6, 8, 13]. This could explain the latent infection [37] and paucisymptomatic disease [38] reported elsewhere in some HIV-infected patients. Evolution of microsporidiosis has also been correlated with excretion of parasites [39] and with highly active antiretroviral therapy [40–43]. However, there is no evidence that antiprotease drugs or specific treatment [44] could have influenced the evolution of the outbreak.

In persons not infected by HIV, the disease appears sporadic. Previous studies reported a high seroprevalence of *Encephalitozoon* species in immunocompetent subjects [45]. At least 5% of our study subjects were immunodepressed following organ transplantation, and one cannot exclude the possibility that some other persons not infected by HIV could have been immunodepressed for reasons such as corticosteroid treatment or chemotherapy. The real prevalence of intestinal microsporidiosis in persons not infected by HIV remains to be determined, but it could be a significant factor underlying morbidity in all immunodepressed patients.

Male homosexuality was independently associated with the diagnosis of intestinal microsporidiosis in HIV-infected subjects. This association has been reported in other studies [26, 27] and supports the hypothesis of a fecal-oral route of contamination for intestinal microsporidiosis.

The increase in new diagnoses of microsporidiosis observed at the end of the second quarter of 1995 is consistent with the occurrence of an outbreak. Factors associated with a diagnosis of microsporidiosis during the outbreak period were markedly different than during the endemic period, suggesting there was an extension of the infection to a larger-than-usual group of persons, possibly related to a common route of contamination. The clustering of cases in the medium elevation system suggests that the outbreak could be related to contamination of surface water treated in factory C. Factors that could potentially favor contamination of water include the small size of microsporidia, which could help them escape filtration, and the unknown potency of resistance of microsporidial spores to physical agents and disinfectants. Factors that may have contributed to contamination include pumping of surface water directly from a recreational area that is mainly frequented by swimmers in summer and treatment of water by flocculation, ozonation, and filtration instead of chlorination, as done in the 2 other treatment facilities. Similar reported links between contact with water and intestinal microsporidiosis include swimming in pools, rivers, ponds, and lakes [26, 27] and drinking unfiltered
tap [27] and well water [28]. Other reported factors include contact with sea-water and fresh-water fish [28] and the use of humidifiers [27]. The growing body of evidence suggests that intestinal microsporidiosis should be considered a potential waterborne disease.

Water safety data in our study were not suggestive of significant fecal contamination during the period of analysis. This indicates that markers that are validated for bacterial contamination are not appropriate for microsporidial contamination, as described elsewhere following a cryptosporidiosis outbreak [46]. Identification of water contamination by microsporidiosis is impossible to date, since no technique allows direct identification of microsporidia in water. A recent study described the detection of microsporidial DNA and the identification of *E. bieneusi* in surface water by PCR [47], but the usefulness of this assay in routine analysis requires additional study. Finally, the observed discordance between the 2 stains, the apparently less severe evolution of microsporidiosis when acquired during the outbreak, and the absence of species identification during or outside the outbreak cannot exclude the possibility that the outbreak could have been secondary to a microsporidial species other than *E. bieneusi* and *E. intestinalis*.

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References


