EXAMINATION OF THE PREVALENCE AND SEASONAL VARIATION OF INTESTINAL MICROSPORIDIOSIS IN THE STOOLS OF PERSONS WITH CHRONIC DIARRHEA AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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Abstract. The epidemiology of human microsporidiosis is poorly understood and environmental factors affecting transmission of the organism have not been fully elucidated. Temporal variation in the prevalence of microsporidia in the stool of patients with human immunodeficiency virus (HIV) infection and diarrhea was studied to evaluate the role of water-borne transmission. From January 1993 to December 1996, 8,439 stools from HIV-infected individuals were examined for microsporidia spores in southern California. Yearly positivity rates were 8.8% in 1993, 9.7% in 1994, 6.6% in 1995, and 2.9% in 1996. An analysis for linear trend showed a statistically significant decrease in stool positivity rates over time ($\chi^2 = 81.9, P = 0.001$). No significant seasonal variation in the prevalence of microsporidiosis was seen over that time period. These results suggest the constant presence of microsporidia in the environment, rather than a seasonal association with recreational water use or seasonal contamination of the water supply, and a real decrease in yearly prevalence of microsporidia related diarrhea. Factors related to a progressive decrease in prevalence are subjects of future investigation.

Intestinal microsporidiosis is an important cause of diarrhea and wasting in persons with the acquired immune deficiency syndrome (AIDS). The epidemiology of human microsporidiosis is poorly understood and the environmental factors affecting transmission of the organism have yet to be fully elucidated. Water-borne transmission of a pathogen is suggested by temporal variation in the prevalence of the disease. In North America, seasonal variation in the prevalence of Cryptosporidium parvum has been linked to recreational water use, contamination of water supplies, and the rainy season. Microsporidiosis has not been studied as extensively. However, seasonal prevalence of both C. parvum and microsporidiosis was examined in Brazil, a developing country with a rainy season. In that study, an excess of C. parvum infection was noted during the rainy season, but no corresponding seasonal variation was noted in the prevalence of human microsporidiosis. These findings by Wuhib and others, who evaluated 295 stool specimens collected from 166 human immunodeficiency virus (HIV)-infected persons over an 18-month period, suggested that contaminated water was not likely to be a major source of microsporidial infection.

The goal of the present study was to examine the seasonal variation and prevalence of microsporidia in the stools of HIV-infected persons with chronic diarrhea in southern California over a four-year period.

MATERIALS AND METHODS

Stool specimens were collected as part of routine diagnostic care from HIV-infected persons and examined at the Microbiology Reference Laboratory in Cypress, California from January 1993 through December 1996. The samples came from more than 30 different clinical sites, including the Southern California Permanente Medical Group Central Laboratory, Smith-Kline Beecham Clinical Laboratories, and hospitals and medical groups throughout southern California from Bakersfield to San Diego. Approximately 90–95% of the stool specimens were submitted in 10% formalin, and the remainder were fresh specimens. Data were not available regarding prior antiparasitic therapy, T lymphocyte subset counts, or clinical stage of HIV disease for specific individuals.

This study was approved by the Kaiser Permanente Medical Care Program, Southern California Region, Institutional Review Board for the Protection of Human Subjects.

Diagnosis was performed by the use of a modified trichrome stain (MTS) and Fungi-fluor stain (FFS) (Polysciences, Inc., Warrington, PA). In year one, each specimen was evaluated by two experienced technicians for detection of microsporidia by both MTS and FFS. Agreement between the technicians and between MTS and FFS was 100%. Therefore, in years two through four, the technologists used FFS for rapid screening of specimens. Initially negative specimens were considered truly negative. However, initially positive specimens were confirmed by MTS and the second technician.

Seasonal variability was determined by evaluation of monthly prevalence, calendar seasons (winter, spring, summer, fall), and rainy versus dry seasons. The Wilcoxon test was used in all three cases to assess the statistical significance of differences in stool positivity rates. Yearly variation in prevalence was examined by a chi-square test for linear trend.

RESULTS

Eight thousand five-hundred fifty stool specimens from HIV-infected patients were examined for microsporidia between January 1993 and December 1996. One hundred eleven of the 8,550 specimens were repeated positive stools and therefore excluded from all analyses, leaving 8,439 specimens from 8,439 HIV-infected individuals. Overall, 558 (6.6%) of the 8,439 specimens were positive for microsporidia. Yearly positivity rates were as follows: 137 (8.8%) of 1,557 in 1993, 193 (9.7%) of 1,991 in 1994, 155 (6.6%) of 2,346 in 1995, and 73 (2.9%) of 2,545 in 1996. When as-
FIGURE 1. Prevalence (Y axis) of microsporidial spores in stool specimens of human immunodeficiency virus–positive patients, 1993–1996 by year (X axis). N = 8,439. Differences were analyzed using the chi-square test for linear trend ($\chi^2 = 81.9$, $P = 0.001$).

To assess potential seasonal variability in the proportion of stool specimens positive for microsporidia, three seasonal variables were constructed.

1) Month. Stool specimens were categorized according to the calendar month of their testing. Mean positivity rates for each of the 48 months comprising the study period were computed separately. To assess the statistical significance of the differences in positivity rates between the 12 calendar-year months, we combined the total number of specimens listed and the total number of positive specimens for each month across each of the four study years. Overall positivity rates for stool specimens ranged from a low of 5.0% (May) to a high of 9.6% (January). Nonparametric statistical tests indicated that although monthly positivity rates varied considerably over the short term, none of the differences was statistically significant (overall $\chi^2 = 2.16$, degrees of freedom [df] = 11, $P = 0.99$).

2) Calendar season. Differences in stool positivity rates were analyzed according to the season of the calendar year. Data for January, February, and March comprised the winter season; data for April, May, and June the spring season; July, August, and September the summer season; and October, November, and December the fall season. When assessed by season, overall positivity rates for specimens differed only slightly. The lowest positivity rate occurred in the spring months (6.0%), while the highest positivity rate occurred in the winter months (7.6%). The nonparametric analysis showed that positivity rates did not differ significantly between seasons in the study period ($\chi^2 = 0.59$, df = 3, $P = 0.90$).

3) Rainy versus dry season. Analysis of the data was performed after dividing each of the four years into rainy and dry seasons. In general, rainfall in southern California occurs between the months of December and February, with the other months falling into the dry season. In this four-year study period, the highest proportion of positive stools was detected during the rainy season, with 8.1% of specimens positive for microsporidia in the months of December, January, and February. In contrast, the mean positivity rate for the remaining dry months was 6.1%. The difference between these two proportions; however, was not statistically significant ($\chi^2 = 0.10$, df = 1, $P = 0.75$).

A chi-square test for linear trend was performed to assess whether yearly prevalence rates had significantly changed over the course of the study period. The result indicated a significant trend for less frequent microsporidia positive stool specimens over time ($\chi^2$ for linear trend = 81.9, $P = 0.001$) (Figure 1).

DISCUSSION

No significant temporal variation was observed in microsporidia-positive stool specimens from HIV-positive individuals over the four year period. Microsporidiosis in HIV-infected individuals does not appear to undergo either tem-
poral or seasonal variation. Monthly and seasonal variation in prevalence was random and no distinct patterns could be established. While there was a suggestion of increased prevalence during the rainy season, this trend was not statistically significant. The source of the increased prevalence in the rainy season was related to the unusually high number of microsporidial stools not only in November 1993 (dry season), but in January and February 1994 (rainy season). This pattern was not born out in the other years of the study, further demonstrating the random nature of the prevalence of microsporidia.

The results of our southern California study are similar to those reported in the Brazil study despite differences in climate, culture, and geography. No evidence for significant seasonal variation in stool positivity rates could be found in either the summer, when recreational water use could lead to excess infection or the rainy season, when heavy runoff could cause contamination of public water sources. This suggests microsporidial infection may be present at a constant level in the environment. Investigation of other determinants of clinically apparent microsporidial infection may be useful in the clarification of the epidemiology and environmental sources of microsporidiosis. These potential risk factors include but are not limited to oral-fecal, sexual, air-borne, zoonotic transmission, and recrudescence of previous quiescent disease in immunocompromised persons.

Our finding of a statistically significant decrease in yearly prevalence of microsporidia-related diarrhea in HIV-infected patients was an interesting, important, and unexpected outcome of this study. The decreasing prevalence of an important pathogen has far-reaching implications for both routine clinical care and the economics of health management of AIDS patients. Since clinical data other than HIV and diarrhea status of subjects was not known, the causes of the decrease in prevalence cannot be determined. The rapidity of the decrease from 1994 through 1995 to 1996 suggests (however speculative) one possibility. The use of multi-drug antiretroviral regimens and the use of protease inhibitors, a new class of antiretroviral agents, the first of which was licensed in 1995, became extensively used during the period of decreasing prevalence in microsporidia-positive specimens we observed. Whether the effect of these drugs and other factors are operative in this decreasing prevalence awaits further study.

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