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Intestinal protozoa in HIV-infected patients in Apulia, South Italy

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SUMMARY

Protozoa are important enteric pathogens in patients with human immunodeficiency virus (HIV) infection. In this study the prevalence of intestinal protozoa in 154 HIV-infected patients, with or without diarrhoea, in our region (Apulia, South Italy) was evaluated between December 1993 and February 1998. In the majority of patients CD4+ T cell count was below 200/μl. The overall prevalence of intestinal protozoa was 43/154 (27·92%). Twenty-eight (43·08%) out of 65 patients with diarrhoea and 15 (16·85%) out of 89 non-diarrhoeic patients were parasitized. In particular, in the group of 65 patients with diarrhoea the following protozoa were identified: *Cryptosporidium parvum* in 14 (21·54%), *Blastocystis hominis* in 7 (10·77%), microsporidia in 6 (9·23%), *Giardia lamblia* in 4 (6·15%) and *Isospora belli* in 1 (1·54%). Three patients were *Cryptosporidium parvum*-microsporidia co-infected. In patients without intestinal symptoms, prevalence was 3/89 (3·37%) for *Cryptosporidium parvum*, 9/89 (10·11%) for *Blastocystis hominis*, 1/89 (1·12%) for microsporidia and 2/89 (2·25%) for *Giardia lamblia*. A significant ($P < 0·001$) correlation was observed between protozoan infection and the presence of diarrhoea. In particular, *Cryptosporidium parvum* and microsporidia infections were significantly ($P < 0·001$) and $P = 0·046$, respectively) associated with diarrhoeal illness. Moreover, the majority of cases of cryptosporidiosis were first diagnosed in the periods of heaviest rainfall. Therefore, drinking water contamination may be a possible source of human infection in our area.

INTRODUCTION

Diarrhoea is a relatively common complication in symptomatic HIV-infected subjects and may have multiple aetiology [1], but opportunistic protozoa infections usually account for most of the disease and have a significant impact on survival in these patients [2, 3].

A previous study carried out by our group during the period June 1986–July 1991 in 51 HIV-infected patients with diarrhoea showed a high prevalence (33·3%) of cryptosporidiosis in our region (Apulia, Southern Italy) [4]. As regards the prevalence of cryptosporidiosis in immunocompetent population, in a sample restricted to hospitalized subjects only, we found *Cryptosporidium parvum* in 6/359 (1·67%) children under 14 years of age with enteritis in the

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period June 1987–July 1994 [5]. The purpose of the present investigation was to evaluate the overall prevalence of intestinal protozoa in HIV-infected patients with and without diarrhoeal illness in our region.

MATERIALS AND METHODS

Patients

In the period between December 1993 and February 1998, 154 HIV+ adult patients (109 males and 45 females), referred to the Infectious Diseases Clinic of the University of Bari (Apulia, Southern Italy), were examined for the presence of intestinal protozoa. They included a group of 65 patients with diarrhoea (at least 4 liquid or semiformed bowel movements per day for more than 3 days) and 89 non-diarrhoeic patients. They were not treated with combination antiretroviral therapy at the time of stool examination. CD4+ T lymphocyte count was available at the time of stool submission from all but two patients. The mean CD4+ cell count was $101{\cdot}05 \pm 163{\cdot}49$ S.D./μl in all examined subjects, $115{\cdot}58 \pm 166{\cdot}65$ S.D./μl in diarrhoeic patients and $90{\cdot}49 \pm 161{\cdot}28$ S.D./μl in patients without diarrhoea. The examined subjects included 95 intravenous drug users, 23 HIV+ partners, 11 homosexuals, 10 blood transfused, 6 promiscuous heterosexuals, 6 subjects with multiple risk factors and 3 without any identified risk factor for HIV infection. Patients in whom stool examination was positive for bacterial or viral enteropathogens were excluded from this study.

Stool specimens

From each patient three faecal samples were collected without fixatives every other day and transported to the laboratory, where they arrived and were processed within 2 h.

Parasitological examination

Specimens were fixed with 10% formalin for 30 min and then concentrated by a formalin-ether sedimentation technique, according to Ritchie [6], with a modification in the centrifugation step (500 ***g*** for 10 min), to avoid loss of *Cryptosporidium* oocysts. Obtained sediments were then examined as wet mounts in saline and iodine. Microsporidial spores were recovered from filtered fresh faeces by a water ether sedimentation method [7], which included a centrifugation step at 700 ***g*** for 2 min. Permanent stained smears were performed only for intestinal coccidia and microsporidia, by a modified Ziehl–Neelsen technique [8] and a modified trichrome stain, according to Ryan [9], respectively. Microsporidial spores were identified by the ovoid shape, by the refractile, bright pinkish red stain and by the characteristic belt-like stripes.

Statistical analysis

Statistical evaluation was performed by the χ^2 test.

RESULTS

Parasitological stool examination revealed the presence of potentially pathogenic protozoa in 43 (27·92%) out of 154 examined subjects. In particular, the following protozoa were identified: *Cryptosporidium parvum* in 17 patients (11·04%), *Blastocystis hominis* in 16 (10·39%), microsporidia in 7 (4·55%), *Giardia lamblia* in 6 (3·90%) and *Isospora belli* in 1 patient (0·65%). Three patients were co-infected with *Cryptosporidium parvum* and microsporidia and one with *Blastocystis hominis* and microsporidia.

In 37/43 parasitized patients, diagnosis was performed by the examination of the first stool sample. In six cases a second sample was necessary, for the identification of *Cryptosporidium* (2 cases), microsporidia (2 cases), *Isospora belli* (1 case) and *Giardia* (1 case). Results from the third sample were all in agreement with those from the first two.

Twenty-seven out of 109 males and 16 out of 45 females were parasitized, without significant differences between sexes ($P = 0{\cdot}246$)). A significant ($P < 0{\cdot}001$) correlation was observed between protozoan infection and the presence of diarrhoea at the time of stool submission. In fact, 28 (43·08%) out of 65 patients with diarrhoea and 15 (16·85%) out of 89 patients without diarrhoea were parasitized. In particular, *Cryptosporidium parvum* and microsporidia infections were significantly ($P < 0{\cdot}001$ and $P = 0{\cdot}046$, respectively) associated with diarrhoeal illness. No correlation was found between the other isolated protozoa and intestinal symptoms, even if the limited number of these cases does not allow statistically valid

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Table 1. *Intestinal protozoa identified in 43/154 HIV-infected patients* (*65 diarrhoeic patients and 89 without diarrhoea*)

Protozoa	Total infected patients		Diarrhoeic patients		Asymptomatic patients		Correlation with diarrhoea
	n	%	*n*	%	*n*	%	*P*
Cryptosporidium parvum	17	11·04	14	21·54	3	3·37	< 0·001
Blastocystis hominis	16	10·39	7	10·77	9	10·11	0·890
Microsporidia	7	4·55	6	9·23	1	1·12	0·046
Giardia lamblia	6	3·90	4	6·15	2	2·25	0·415
Isospora belli	1	0·65	1	1·54			
Total*	43	27·92	28	43·08	15	16·85	< 0·001

* Three patients with diarrhoea were *Cryptosporidium parvum*-microsporidia co-infected and one was *Blastocystis hominis*-microsporidia co-infected.

conclusions. Protozoa identified and '*P*' derived from the χ^2 test indicating correlation with diarrhoea are listed in the Table 1.

The unique case of isosporiasis and 7 out of the 17 cases of cryptosporidiosis were the first markers of AIDS.

The mean CD4 cell count was $27{\cdot}47 \pm 29{\cdot}55$ S.D./μl in *Cryptosporidium parvum*-infected subjects, $107{\cdot}5 \pm 177{\cdot}76$ S.D./μl in *Blastocystis hominis*-infected patients and $15{\cdot}67 \pm 18{\cdot}23$ S.D./μl in microsporidia-infected subjects, except for 1 patient with microsporidiosis, but without diarrhoea, in whom CD4 cell count was 387/μl.

DISCUSSION

In this study we evaluated the prevalence of intestinal protozoa in 154 HIV infected subjects in the Apulia region of Southern Italy, where such data are lacking. Moreover, the association between the identification of protozoa and the presence of diarrhoea was investigated. Overall, 43 (27·92%) patients were parasitized; intestinal protozoa were detected in 28 (43·08%) patients with diarrhoea and in 15 (16·85%) without intestinal symptoms.

In 86% of case parasites were detected by the first stool specimen and in 100% by the second specimen; this suggests that two samples may be sufficient for the parasitological diagnosis in HIV patients, as indicated by other authors for the diagnosis of cryptosporidiosis by a modified acid-fast stain in patients with AIDS and diarrhoea [10].

Among the protozoa identified, *Cryptosporidium parvum* was found most frequently, followed by *Blastocystis hominis*, microsporidia, *Giardia lamblia* and *Isospora belli*. No cases of amoebic infection were found, but it should be mentioned that our method did not include permanent stained smears or culture for amoebae.

Interestingly, the majority of cases of cryptosporidiosis (11/17) were first diagnosed in the period December–March, which are the months of heaviest rainfall in our area. In particular, 7 out of 17 new cases of cryptosporidiosis were recorded in the period January–March 1996. In the light of our results, possible sources of human infection, even including drinking water contamination, must be investigated in our region.

In other Italian regions, studies from Northern Italy indicated infection rates for *Cryptosporidium parvum* of 7·8% in 408 HIV-infected patients [11] and 9·7% in 144 HIV-infected patients with diarrhoea [12]. In Central Italy, 6·6% of 376 patients with AIDS [13] and 9·8% of 457 HIV patients with diarrhoea were infected [14]. Indirect evidence of higher rates of infection in Central Italy comes from a serological survey by means of an oocyst soluble antigen in an ELISA, which demonstrated the presence of specific IgG in 15·8% of 82 HIV-positive patients [15] and from a study on cell-mediated immune response to an oocyst antigen in healthy volunteers [16]. Moreover, a large outbreak of cryptosporidiosis was reported in Italy between January and February 1995, which included 294 out of 1731 members of a community for the rehabilitation of drug users. The attack rate of clinical cryptosporidiosis was 13·6% among HIV-negative individuals and 30·7% among HIV-positive subjects. *Cryptosporidium* oocysts were found in the sandy sediment collected from the bottom of two water storage tanks serving the community [17].

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In other countries, many investigations have been carried out on the prevalence of cryptosporidiosis in HIV-infected patients [18–32]. A review of 100 investigations involving 133175 patients (see [33]), reported rates of *Cryptosporidium parvum* infection among HIV-infected patients with diarrhoea as 14% in developed areas and 24% in developing areas.

In our study *Blastocystis hominis*, which has recently been placed within the stramenopiles by phylogenetic analyses of 16S-like rRNA gene sequences [34], was the most frequent intestinal protozoa, apart from *Cryptosporidium parvum*, without any significant association with diarrhoeal illness. In fact, it was present in 7 (10·77%) out of 65 diarrhoeic patients and in 9 (10·11%) out of 89 patients without diarrhoea. Similar prevalence of this organism has been observed in the USA in a large survey performed in immunocompetent symptomatic or asymptomatic subjects [35]. Reconciling even potential pathogenicity with the essentially equal prevalence of this organism in symptomatic and asymptomatic subjects seems difficult, even if the existence of strains of *Blastocystis hominis* with different virulence deserves further investigation.

As regards the prevalence of microsporidia infection, previous studies of HIV-associated chronic diarrhoea by coprodiagnostic techniques indicate a prevalence of intestinal microsporidiosis of 9–16% [36], even if a higher prevalence has been reported in Germany [37] and in developing countries [32, 38]. It is unclear whether this range in prevalence represents true geographic variation due to different risk factors for microsporidial infection or differences in ability of various laboratories to identify spores. We have demonstrated a rather low prevalence of microsporidia in our region, which were present in 7/154 (4·55%) HIV patients and 6/65 (9·23%) diarrhoeic patients, three of whom were coinfected with *Cryptosporidium parvum* and one with *Blastocystis hominis*. This may be due in part to different risk factors for HIV infection in the Italian population, which included more drug users than homosexuals, even if in the limited number of our microsporidia-infected patients, no correlation with any particular risk factor for HIV infection was found. Our patients with microsporidiosis exhibited low CD4 cell counts, with the exception of one subject who had asymptomatic infection and CD4 cell counts of 387/μl. In this respect, microsporidiosis in patients with relatively high CD4 counts has been described [39]; the frequency of asymptomatic enteric carriage by microsporidia, which has been demonstrated mainly by examining intestinal biopsies [40] and stools [37, 41] from HIV-infected patients, deserves further investigation with coprodiagnostic techniques sensitive enough to detect low-level infection.

Moreover, the low prevalence (0·8%) of isosporiasis in our patients is in accord with the results of a recent large survey in other Italian regions, which demonstrated a prevalence of 0·5% of *Isospora belli* infection in HIV-infected subjects [42].

Finally, it should be stressed that our examined patients exhibited low CD4 cell counts and were not treated with combination antiretroviral therapy including a protease inhibitor, at the time of stool examination. In fact, this therapy could restore the immunity to enteric protozoa [43–47], thus decreasing the prevalence of protozoan infection.

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