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The prevalence and clinical significance of intestinal parasites in HIV-infected patients in Denmark

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Abstract

To investigate the prevalence and clinical significance of intestinal parasites in human immunodeficiency virus (HIV)-infected patients, faecal specimens from 96 HIV-infected patients were submitted to microbiological analyses, including microscopy and polymerase chain reaction for protozoa and enteropathogenic bacteria. Results of microbiological analyses were compared with self-reported gastrointestinal complaints collected using a validated questionnaire. Thirty-two (33%) patients were positive for parasites. However, opportunistic parasites (Isospora and Cryptosporidium) were detected in only 2 instances. Entamoeba dispar was detected in 10 cases, 9 of which represented men who have sex with men (MSM). Despite generally low HIV RNA loads and high CD4+ T-cell counts, 42% of the 76 patients reporting symptoms complained of diarrhoea, 31% of whom were parasite-positive. The presence of diarrhoea was not associated with the presence or absence of parasites; neither was it associated with receiving highly active anti-retroviral therapy (HAART) in general, or protease inhibitors (PI) in particular. A CD4+ T-cell count <200 cells/mm^3 was not associated with parasitic infection or with diarrhoea. The data show that diarrhoea is a common symptom among HIV-infected patients in Denmark, but do not indicate that the diarrhoea is due to intestinal parasites.

Introduction

Chronic or intermittent diarrhoea affects 50–80% of human immunodeficiency virus (HIV)-infected individuals in both developed and developing countries [1]. Several investigations and reviews regarding the possible causes and handling of chronic diarrhoea in this particular group of patients are available [1–5]. Intestinal parasites are more likely to be the cause of HIV-related diarrhoea in those with prolonged symptoms, weight loss and CD4+ T-cell counts <150/μl [6,7], and opportunistic, potentially diarrhoea-causing parasitic infections due to Cryptosporidium, Cyclospora, Isospora and microsporidia have been seen mainly in patients with CD4+ T-cell counts <200/μl [7–10]. However, the use of highly active anti-retroviral therapy (HAART) has markedly reduced the prevalence of opportunistic parasitic infections [1,2,7,11,12]. It is not exactly clear to what extent this is due to a stabilization of the CD4+ T-cell count, and to what extent HAART has a directly anti-parasitic effect [7,13]. Paradoxically, HAART in itself may be an aetiological factor in the development of diarrhoeal symptoms, and patients receiving protease inhibitors (PI) are at particularly increased risk of experiencing periods of diarrhoea [4,14].

Investigators have found intestinal parasites in 52–59% of men who have sex with men (MSM) in highly developed parts of the world such as Scotland, Australia and California [15–17]. The level of intestinal parasitism in Scandinavia is generally low compared to elsewhere [18], but little is known about the level of intestinal parasitism among HIV-infected individuals in this region.

The formol–ethyl acetate concentration technique (FECT) is probably the most applied method for

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detecting cysts and ova in faecal specimens. Recently it was reported that potentially symptomatic, curable, intestinal parasitosis apparently goes unnoticed by the FECT in approximately 80% of parasite-positive individuals in Denmark, despite the examination of multiple stool samples [18]. This is why molecular methods must be used, not only to increase diagnostic sensitivity, but also to be able to distinguish between pathogenic and non-pathogenic species of Entamoeba.

This study was a cross-sectional study to identify the prevalence and distribution of intestinal opportunistic and non-opportunistic parasites among HIV-infected patients in Denmark using the FECT, diagnostic polymerase chain reactions (PCRs) and stool culture. Goals also included comparing the distribution of parasites with self-reported complaints of diarrhoea, CD4 T-cell counts and HIV RNA viral load. Finally, we speculate on the potential role of parasites in the aetiology of diarrhoea in HIV-infected patients in Denmark.

Materials and methods

Study individuals

HIV-infected patients were recruited from the outpatient clinic at the Department of Infectious Diseases, Hvidovre Hospital, Denmark in the period of October 2007 to April 2009. Prior to participation, patients were informed orally and in writing. All patients were asked to complete a validated diarrhoea questionnaire [19]. Each patient received vials for the submission of faecal samples. Information on HAART, immune status (CD4+ T-cell counts – both nadir and status during the study period), HIV RNA, ethnic origin and HIV mode of transmission (homosexual, heterosexual, intravenous) were all obtained from the patient records (Table I). Information on gastrointestinal complaints such as diarrhoea (defined as 3 daily stool passages or more) was extracted from a validated questionnaire completed by participating patients.

Table I. Clinical characteristics of the 96 HIV patients in Demark analysed during the period October 2007 to April 2009.

Clinical data	No. of patients
CD4+ T-cell count, cells/μl	
<150	3
151–300	10
301–500	28
>500	47
Nadir count 1–100	13
Nadir count >100	73
Data not available[a]	8/10
HIV RNA load, copies/μl	
1–1000	10
>1000	74
Data not available	12
anti-HIV therapy	
HAART therapy	82
PI therapy	33
NNRTI	53
NRTI	81
HIV transmission	
Homosexual	58
Heterosexual	23
Intravenous	7
Bisexual	1
Data not available	7

HAART, highly active anti-retroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors.
[a]CD4+ T-cell count/CD4+ T-cell nadir count.

Laboratory analyses

At least 1 set of faecal specimens from each patient was tested, each set consisting of 3 samples. One sample was submitted to bacteriological examination, another sample was tested for parasites using standard parasitological examination (FECT), and the last sample was sent to the Statens Serum Institut (Copenhagen, Denmark) and submitted to Blastocystis-specific culture and to DNA extraction for subsequent PCR analysis of the following species: Entamoeba histolytica, Entamoeba dispar, Cryptosporidium sp., Giardia intestinalis, Dientamoeba fragilis, Blastocystis and microsporidia, using conventional and real-time PCR with species-specific probes [20–23]. Diagnosis of intestinal pathogenic bacteria was done by culture of faeces directly and from selenite broth on different selective media, agglutination with relevant specific antisera, toxin determination using ImmunoCard Toxins A&B (Meridian Bioscience Europe), and PCR for diarrhoea-provoking Escherichia coli.

Statistical analysis

Tests for various associations were performed using a 2-tailed Fisher's exact test with 2-tailed probabilities for small sample sizes. Due to the limited data in the present study, only pair-wise analyses were performed, and multivariate analyses were not possible.

Ethical considerations

Only patients who provided informed consent were included in the study and patients positive for parasites were offered treatment. Ethical permission to implement the study was granted by the 'Videnskabsetisk Komité, Region Hovedstaden' (Project No. H-D-2007–0021).

Results

Baseline characteristics of patients

In total, 96 HIV-infected patients (75 males, 21 females) agreed to participate in the study, none of whom had AIDS prior to inclusion in the study. Seventy-one patients (74%) were Danish by birth and 13 (14%) were of non-European origin. Fifty-nine (61%) were MSM, 23 (24%) were heterosexuals, 7 (7%) were intravenous drug abusers, and for the remaining 7 (7%), no data were available (Table I). Eighty-two (85%) of the patients received HAART treatment. Seventy-six patients submitted questionnaires with self-reported symptoms.

The median HIV RNA copy load based on 90 available observations was 40 copies/µl (IQR 40–40). Only 2 patients had CD4+ T-cell counts <100 cells/mm^3 and only 5 in total had CD4+ T-cell counts <200 cells/mm^3. The median CD4+ T-cell count was 515 (IQR 370–695) based on 88 available observations.

Prevalence of parasites

Thirty-two (33%) patients were colonized/infected with parasites (Table II). No helminth infections were detected. Opportunistic parasites were detected in only 2 instances: a female with a CD4+ T-cell count of only 79 cells/mm^3 and an HIV RNA viral load of >100,000 copies was positive for oocysts of Isospora belli, and a male with normal immune status was infected with Cryptosporidium sp.

E. dispar was detected in 10 cases, 9 of which were known to represent MSM; for 1 case of E. dispar no information on sexual orientation was available. Only 2/10 cases of E. dispar were detected by microscopy.

Blastocystis was found in 22 cases (23%), of which 11 cases represented MSM and 5 represented patients of heterosexual orientation. In the remainder of the cases no information on sexual orientation was available. Blastocystis was not more prevalent among MSM than among heterosexuals ($p = 0.76$).

The 7 patients known to have contracted HIV by intravenous drug abuse were all parasite-negative. In MSM, 20/59 (34%) were diagnosed with a parasitic infection, and 6/23 (26%) heterosexuals were parasite-positive ($p = 0.60$). However, when excluding patients positive for Blastocystis, only 1/18 (6%) heterosexuals was parasite-positive (I. belli), whereas 8/47 (17%) MSM were positive, and although not significant ($p = 0.42$), the trend of MSM having a higher prevalence of parasites than non-MSM patients became stronger.

Blastocystis

Of the 22 Blastocystis-positive patients, 11 were positive for Blastocystis sp. subtype 3 (ST3), 9 for

Table II. Prevalence and distribution of intestinal parasites and enteropathogenic bacteria in 96 HIV-positive individuals detected by FECT, Ziehl–Neelsen staining, PCR and culture in relation to CD4+ T-cell counts and HIV RNA viral load.

Parasite	No. of cases detected[a]	Mean CD4+ T-cell count, cells/µl (No. of observations)	Mean CD4+ T-cell nadir, cells/µl (No. of observations)	Median HIV RNA viral load, copies/µl (No. of observations)
Blastocystis sp.	22 (11, 5, 0, 6)	629 (17)	297 (16)	<40 (17)
ST1	9 (5, 2, 0, 2)	620 (7)	322 (7)	<40 (7)
ST3	11 (5, 3, 0, 3)	607 (9)	275 (8)	40 (9)
ST1+ST3	2 (1, 0, 0, 1)	893 (1)	300 (1)	<40 (1)
Cryptosporidium sp.	1 (1, 0, 0, 0)	400 (1)	250 (1)	<40 (1)
Dientamoeba fragilis	3 (1, 2, 0, 0)	770 (3)	313 (3)	<40 (3)
Entamoeba dispar	10 (9, 0, 0, 1)	626 (9)	267 (9)	<40 (2)
Giardia intestinalis	2 (2, 0, 0, 0)	259 (2)	187 (2)	<40 (2)
Isospora belli	1 (0, 1, 0, 0)	79 (1)	14 (1)	>100,000 (1)
Other[b]	8 (7, 0, 0, 1)	591 (7)	279 (7)	<40 (7)
Parasites detected	32 (20, 6, 0, 6)	575 (27)	268 (26)	<40 (27)
Parasites detected other than Blastocystis sp.	19 (14, 3, 0, 6)	566 (17)	247 (17)	<40 (17)
No parasites detected	64 (66)	535 (61)	235 (60)	<40 (62)
Intimin-producing Escherichia coli	5 (0, 3, 0, 2)	638 (3)	216 (3)	<40 (3)
Clostridium difficile	1 (1, 0, 0, 0)	407 (1)	50 (1)	200 (1)
Pseudomonas putida	1 (1, 0, 0, 0)	480 (1)	312 (1)	5910 (1)

FECT, formol–ethyl acetate concentration technique; PCR, polymerase chain reaction; ST1, subtype 1; ST3, subtype 3.

[a]Numbers in parentheses refer to the number of cases detected in patients who contracted HIV by homosexual, heterosexual, intravenous, and unknown transmission, respectively.

[b]Other parasites included Entamoeba coli, Entamoeba hartmanni, Endolimax nana and Iodamoeba bütschlii.

subtype 1 (ST1), and 2 for both ST1 and ST3 (Table I). Six complained of diarrhoea, 5 of whom were positive for ST3. Five of 7 patients with ST1 reporting recent travel had been travelling, whereas none of the 6 patients positive for ST3 reporting recent travel had travelled ($p = 0.02$).

Gastrointestinal complaints

In the 7-day period prior to completing the questionnaire, 29/76 (38%) patients reported having diarrhoea every day, 32/76 (42%) patients reported experiencing at least 1 episode of diarrhoea, 29/76 (38%) had experienced cramping or pain associated with bowel movements and pain or rectal spasms without passing stool, and 21/76 (28%) complained of faecal urgency.

Ten of 32 (31%) patients with diarrhoea were parasite-positive. Of all 32 parasite-positive individuals, 10 (31%) complained of diarrhoea, 19 did not; for 3/32 no information on diarrhoea was available (Table III). Parasite-positive patients were not more prone to reporting on diarrhoea than parasite-negative patients ($p = 0.47$). Reports on diarrhoea were not associated with HAART treatment ($p = 1.00$), neither was the absence of parasites ($p = 0.54$), and, specifically, patients receiving PI were not more prone to report on diarrhoea than those who did not receive PI ($p = 0.48$). A CD4+ T-cell count <200 cells/mm^3 was neither associated with diarrhoea ($p = 0.30$) nor with the presence of parasites ($p = 0.30$). Ethnic origin did not seem to be a risk factor for any particular parasitic infection, or parasitic infections in general. Leaving out the few patients with the lowest CD4+ T-cell counts, there was a tendency of reports of diarrhoea being associated with increasing CD4+ T-cell count, but this trend was not statistically significant (data not shown).

Prevalence of enteropathogenic bacteria

Potential enteropathogenic bacteria were detected in 7 cases, 5 of which were due to intimin-producing Escherichia coli (Table II). Four of these 5 cases reported on symptoms, and all 4 reported having diarrhoea ($p = 0.03$) (Table III).

Table III. Relationship between diarrhoea and data from clinical and microbiological examinations in HIV patients in Denmark.

Diagnostic parameter	Cases in which diarrhoea was reported (%)	Cases in which diarrhoea was not reported (%)	Total cases for which reports on diarrhoea were available (%)
Blastocystis sp.	6 (30)	14 (70)	20 (91)
ST1	1 (13)	7 (88)	8 (89)
ST3	5 (50)	5 (50)	10 (91)
ST1+ST3	0 (0)	2 (100)	2 (100)
Cryptosporidium sp.	0 (0)	1 (100)	1 (100)
Dientamoeba fragilis	0 (0)	3 (100)	3 (100)
Entamoeba dispar	3 (33)	6 (66)	9 (90)
Giardia intestinalis	1 (50)	1 (50)	2 (100)
Isospora belli	1 (100)	0 (0)	1 (100)
Other[a]	4 (50)	4 (50)	8 (100)
Parasites detected	10 (34)	19 (66)	29 (91)
Parasites detected other than Blastocystis sp.	7 (39)	11 (61)	18 (95)
No parasites detected	22 (47)	25 (53)	47 (73)
CD4+ T-cell count, cells/µl			
<150	3 (100)	0 (0)	3 (100)
151–300	2 (29)	5 (71)	7 (70)
301–500	8 (36)	14 (64)	22 (79)
>500	17 (44)	22 (56)	39 (83)
Nadir count 1–100	4 (44)	5 (56)	9 (69)
Nadir count >100	24 (40)	36 (60)	60 (82)
HIV RNA load, copies/µl			
1–1000	28 (44)	36 (56)	64 (77)
>1000	4 (40)	6 (60)	10 (91)
HAART therapy	27 (44)	35 (56)	62 (79)
PI therapy	11 (48)	12 (52)	23 (74)
Intimin-producing Escherichia coli	4 (100)	0 (0)	4 (80)
Clostridium difficile	1 (1)	0 (0)	1 (100)
Pseudomonas putida	0 (0)	0 (0)	0 (0)

ST1, subtype 1; ST3, subtype 3; HAART, highly active anti-retroviral therapy; PI, protease inhibitor.
[a]Other parasites detected included: Entamoeba coli, Entamoeba hartmanni, Endolimax nana and Iodamoeba bütschlii.

Discussion

This study shows that diarrhoea and other gastrointestinal symptoms affect a substantial proportion of HIV-infected patients who immunocompromised or only slightly immunocompromised. Where the prevalence of diarrhoea among HIV-infected outpatients has previously been reported to range from 0.9% to 14.0% [24], this study saw a much higher prevalence based on information collected via a validated questionnaire. However, no associations were found between reports on symptoms and the presence of parasites or HAART status, which indicates that such symptoms might be idiopathic or at least also attributable to other factors, such as viral (including HIV) or bacterial pathogens, or maybe non-infectious causes [1]. In this study, 4 cases of intimin-producing E. coli infection were detected, all of which were associated with diarrhoea. Kearney et al. [24] found that a 'new diagnosis', typically due to cytomegalovirus or microsporidia, could be established by adding endoscopy with mucosal biopsies to standard microbiological stool examination; however, the finding of previously unidentified pathogens by this procedure was almost exclusively limited to patients with CD4+ T-cell counts <100/µl [24]. In the present study, only 2% of the patients had CD4+ T-cell counts <100/µl. Moreover, all patients were stool-negative for Enterocytozoon bieneusi and Encephalitozoon spp.

The vast majority of patients included in the present study received HAART, which apart from indirectly restoring the CD4+ T-cell count may have a direct anti-parasitic effect [7]. Kurniawan et al. [9] found a CD4+ T-cell count <200 cells/mm^3 to be associated with a higher prevalence of intestinal protozoa; this finding could not be established in the present study. Only 5 patients had CD4+ T-cell counts <200 cells/µl, 1 of whom had an Isospora infection, and to this end, opportunistic enteroparasitic infections were observed in only 2/96 (2%) cases in total. Whether this low prevalence of opportunistic parasites among this HIV-infected population is directly due to extensive HAART administration or secondary to a stabilization of immune response cannot be established.

While opportunistic enteroparasites were rare, the overall prevalence of intestinal parasites among HIV-infected patients in Denmark was higher than in other patient segments, such as patients suffering from travel-associated or persistent diarrhoea [25]. The prevalence of Giardia in Denmark in patients with persistent or travel-associated diarrhoea is very low (1–2%) compared to other countries [25], and in the present study, only 2 persons (2%) had Giardia. However, in terms of E. dispar and Blastocystis, the situation was different. Ten cases (10%) of E. dispar were detected and presented almost exclusively in MSM. It appears that 15% of all 59 explicitly identified MSM had E. dispar. In a report by Stark et al. [15], E. dispar was detected in 26/1246 (2%) MSM (both HIV-positive and HIV-negative), but in none of 622 non-MSM males. Also E. histolytica and Entamoeba moshkovskii were detected in MSM only. Thus, the search for and speciation of cysts of Entamoeba in MSM is crucial. The fact that E. dispar cysts were only detected by microscopy in 2/10 cases highlights the relevance of supplementary PCR-based diagnostic analysis in routine parasitological examination.

In Denmark, since 2006, the incidence of HIV infection in MSM has again been increasing, and in 2008 the number of new cases that could be attributed to homosexual transmission was 2–3 times the number that could be attributed to heterosexual transmission [26]. The prevalence of enteric parasites in MSM in Denmark is somewhat lower than that reported in countries such as the UK, USA and Australia, where prevalence rates range from 49% to 81%. Still, the present data set may be limited to the extent that 8/10 E. dispar infections in the present study were missed by microscopy and only detected by real-time PCR. This led us to speculate on whether other amoebic cysts had been missed by microscopy. However, the prevalence of Entamoeba hartmanni, Entamoeba coli, and Endolimax nana in the present study were only 1%, 6%, and 1% respectively, and in accordance with previous reports from Denmark [25]. Since such parasites are cosmopolitan in distribution and have prevalence rates that roughly parallel those of pathogenic species [27], they are important surrogate markers and can be seen as indicators of faecal–oral transmission.

Whereas the proportion of Blastocystis-positive patients with travel-associated diarrhoea was recently reported to be 13% [28], it was 23% in the present study population, which means that the share of Blastocystis-positive individuals is higher among HIV-infected individuals than in any other patient segment investigated in Denmark. Although transmitted by faecal–oral route as, for example E. dispar, the presence of Blastocystis appeared to be independent of sexual orientation, thus contrasting with the data reported by Stark et al. (2007), who found a significantly higher proportion of Blastocystis-infected individuals among MSM compared to controls (non-MSM males) [15]. The background prevalence of Blastocystis in Denmark is high compared to other parasites [29], and it might therefore be that the prevalence of Blastocystis in the population is unaffected by sexual orientation and practices. However, this finding allows us to speculate on whether Blastocystis can be transmitted in ways other than faecal–oral. Whether Blastocystis causes diarrhoeal disease in either immunocompetent patients or in

HIV-infected patients is a matter of controversy [3]. The present data on Blastocystis do not support the hypothesis that a particular subtype is associated with symptoms, while others are not [30–32]. However, due to the remarkable extent of genetic diversity in Blastocystis, data on Blastocystis subtype distribution alone might not be enough to investigate whether certain strains are associated with the development of disease, while others are not. Therefore, more subtle tools for distinguishing between strains should be developed and much larger studies undertaken in order to clarify the potential role in disease of this extremely common parasite [31].

In conclusion, the prevalence of single-celled intestinal parasites in HIV patients in Denmark is high compared to other patient segments in the country. Despite generally low HIV RNA loads and high CD4+ T-cell counts, 42% of the patients reporting symptoms complained of diarrhoea. However, no single diagnostic or anamnestic feature could be associated with the presence of diarrhoea and thus, our data do not support the hypothesis that reports on diarrhoea are generally due to symptoms caused by intestinal parasites, or the hypothesis that HAART in itself induces diarrhoea.

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