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Epidemiology of infections with intestinal parasites and human immunodeficiency virus (HIV) among sugar-estate residents in Ethiopia

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Intestinal parasitic infections could play an important role in the progression of infection with human immunodeficiency virus (HIV), by further disturbing the immune system whilst it is already engaged in the fight against HIV. HIV and intestinal parasitic infections were investigated in 1239, randomly selected individuals, aged 15–54 years, living on a sugar estate in central Ethiopia. Intestinal parasites were identified in faecal samples (one/subject) using direct, concentration, and (for *Strongyloides stercoralis* larvae) Baermann methods. HIV serological status was determined using ELISA, with ELISA-positive samples confirmed as positive by western blotting. Most (70.1%) of the subjects were infected with at least one intestinal parasite and 3.1% were seropositive (but asymptomatic) for HIV. The intestinal parasites identified in the study population were amoebic parasites (*Entamoeba histolytica/Enta. dispar*) (24.6%), hookworms (23.8%), *Ascaris lumbricoides* (22.2%), *Trichuris trichiura* (19.5%), *S. stercoralis* (13.0%), *Taenia saginata* (4.5%), *Giardia lamblia* (3.0%), and *Enterobius vermicularis* (1.3%).

Overall, the HIV-positives were no more or less likely to carry intestinal parasites than the HIV-negatives (76.2% *v.* 69.9%; $P > 0.05$). However, when each parasite was considered separately, amoebic parasites were found to be more common in the HIV-positives than the HIV-negatives (43.7% *v.* 24.0%; $P < 0.05$). This difference remained significant in a multivariate analysis, after controlling for the socio–demographic characteristics of the study participants. In conclusion, there was moderate interaction between intestinal parasites and HIV at the asymptomatic stage of HIV infection. The observed association between amoebic and HIV infections requires confirmation in a prospective study, allowing for the analysis of biological mechanisms involved in the association.

Intestinal parasites have been identified as possible aetiological agents of the diarrhoea seen in patients, from the industrialized world (Laughon *et al.*, 1988; Smith *et al.*, 1988;

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Eeftinck Schattenterk *et al.*, 1991) and developing countries (Malebranche *et al.*, 1983; Sewankambo *et al.*, 1987; Colebunders *et al.*, 1988; Conlon *et al.*, 1990), who have the acquired immunodeficiency syndrome (AIDS). The most frequent opportunistic parasites are intracellular protozon, such as *Cryptosporidium parvum*, *Isospora belli*, members of the order Microsporidia, and *Cyclospora* (DuPont and Marshall, 1995). Other protozoans, such as *Entamoeba histolytica* and *Giardia lamblia*, and helminths, including *Strongyloides stercoralis*, hookworm, *Ascaris lumbricoides*, *Taenia saginata* and *Trichuris trichiura*, are considered non-opportunistic, except on rare occasions (Gompels *et al.*, 1991; Hung *et al.*, 1999). However, in co-infected individuals, they could play an important role in the progression of infection with human immunodeficiency virus (HIV), even at the asymptomatic stage of HIV infection, by further disturbing the immune system which is already engaged in the fight against HIV, or by stimulating HIV replication (Croxson *et al.*, 1988; Actor *et al.*, 1993; Bentwich *et al.*, 1995; Rizzardini *et al.*, 1996). In addition, the already high morbidity and mortality associated with intestinal parasites in the developing world (Walsh and Warren, 1979) could increase were intestinal parasites more common and/or more symptomatic in HIV-positives than in HIV-negatives.

Very few studies have examined the presence of intestinal parasites in asymptomatic, HIV-infected subjects (Esfandiari *et al.*, 1995; Lindo *et al.*, 1998). The present study was therefore undertaken, to examine the prevalence of infection with these parasites in HIV-positive and HIV-negative individuals living on a sugar estate in central Ethiopia. Ethiopia has been experiencing a severe HIV/AIDS epidemic for the past 15 years (Fontanet *et al.*, 1998) and is an endemic area for many species of intestinal parasite (Tesfa-Yohannes and Kloos, 1988).

SUBJECTS AND METHODS

The Wonji–Shoa sugar estate, established in 1953 by a Dutch company, is 107 km south–east of Addis Ababa, the capital city of Ethiopia. The total resident population of the estate during the June 1995 census was 24 262, including 12 620 adults aged 15–54 years. Residents were defined as all individuals who had been living for more than 6 months on the estate at the time of the census. They consisted mainly of two groups: the residents of the two villages, Wonji and Shoa, who worked mainly in the sugar factories; and the residents of the camps, some of whom were migrant workers from southern Ethiopia who come every year to cut the sugar cane. Wonji village, in the north of the estate, has several daily connections by bus to the nearby city of Nazareth, which is on the main trading road between Addis Ababa and commercial ports on the Red Sea.

Infection with intestinal parasites is among the principal causes of morbidity at the local health facilities, and increasing annual numbers of patients seen at Wonji hospital since 1992 have been diagnosed as HIV-positives (W. Masho, unpubl. obs.).

In preparation for a cohort study on the natural history of HIV infection, two surveys were conducted on the estate, to assess HIV prevalence, the stability of the population, and the acceptability of a long-term research project on HIV/AIDS (Sahlu *et al.*, 1998). A first survey was conducted from November 1995 to April 1996. During this initial survey, 8% of the 10 989 non-migrant workers aged 15–54 years were randomly selected from the census list, so that the precision around the estimated HIV prevalence would be $\pm 2\%$. To get a precise estimate of HIV prevalence among the 1347 migrant workers, the sampling fraction was increased to 25% in their two camps. The second survey, carried out between June and September 1996, included all the 544 factory workers aged 18–45 years who lived in Wonji village.

Following pre-test counselling and informed consent, each study participant provided a blood sample for serological analysis of HIV-1, and a stool sample for diagnosing intestinal parasitic infections. Serum samples were tested for HIV-1 antibodies using a Vironistika ELISA (Organon, Boxtel, The Netherlands). Sera reacting positive in this ELISA were confirmed as positive using another commercial test, based on western blotting (HIV Blot 2.2; Genelabs, Redwood City, CA). Intestinal parasites were identified by the following methods: direct examination in saline and iodine; concentration in formalin–ethyl-acetate; Baermann for *St. stercoralis* larvae (based on warming up of stools in water at 37°C, so that any larvae emerge and sediment, followed by centrifugation and microscopic examination; Watson and Al-Hafidh, 1957); and Kato thick smear for schistosome eggs. Because they cannot be distinguished microscopically, no differentiation was made between *Enta. histolytica* and *Enta. dispar*. Quality control was organized through visits to the Wonji Hospital laboratory by an experienced parasitologist (T.W.) from the Department of Parasitology of the Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa, and by re-analysing 10% of the Kato thick smears at EHNRI. Patients found to be infected with intestinal parasites were treated according to Ethiopia's national treatment guidelines. The study protocol was approved by institutional and national ethical-clearance committees.

Statistical Analysis

The proportions and means reported for the total study population were adjusted for the stratified sampling design and study compliance. This means that, for the prevalences of intestinal parasitic and HIV infections, the actual fractions and the reported percentages were different. To simplify the presentation, only the percentage values for these adjusted prevalences are reported in the Results section. Univariate and multivariate analysis of risk factors for each intestinal parasitic infection and for HIV infection were performed using logistic regression models, again adjusted for the stratified sampling design and study compliance.

RESULTS

During the two surveys, 1750 individuals were randomly selected using the census list. Of these, five had died since the completion of the census, 20 were sick and thus not able to come to the study clinic, 60 had changed address (including those who had left the estate), 97 were non-eligible because they fell outside of the age limits, and 329 refused to participate in the study. Thus, 1239 subjects were finally enrolled. They consisted of 890 males and 349 females, with a mean age of 30 years, and ranging in age from 15–60 years (two individuals above the cut-off of 54 years were inadvertently enrolled in the study). Most of the study participants were asymptomatic (since those too sick to go to the study clinic refused to participate).

The prevalence of (detected) infection with any intestinal parasite in the study population was 70.1%. Amoebic (*Enta. histolytica/Enta. dispar*) infection was the most common (24.6%), followed by hookworm (23.8%), *A. lumbricoides* (22.2%), *Tr. trichiura* (19.5%), *St. stercoralis* (13.0%), *Ta. saginata* (4.5%), *G. lamblia* (3.0%), and *Enterobius vermicularis* (1.3%). The prevalence of schistosome infection, based on the presence of *Schistosoma mansoni* eggs in stools, was 31.4%; data on this infection have already been presented in detail (Fontanet *et al.*, 2000). Socio–demographic factors associated with the presence of the five most prevalent intestinal parasites are shown in Table 1. As expected, the prevalence of many of the parasites decreased with age (*A. lumbricoides*, *Tr. trichiura)*, and educational level (*St. stercoralis*, hookworm, *A. lumbricoides*), and was relatively high among camp residents (*St. stercoralis*, hookworm, *A. lumbricoides*, *Tr. trichiura*) and/or field workers (*St. stercoralis*, hookworm). Exceptions to these patterns included the increasing prevalence of *St. stercoralis* infection with age, and the relatively high prevalence of amoebic parasites in the subjects who lived in the villages. Most of these associations remained after controlling for other socio–demographic characteristics, as shown in Table 2.

HIV seroprevalence was 3.1% overall. It was higher (see Table 1): in the older age-groups than the younger (5.8% in those aged ≥ 40 years *v.* 2.2% in those aged < 40 years;

TABLE 1

Prevalence of infection with intestinal parasites and HIV prevalence, by socio–demographic characteristics and, for the intestinal parasites, by HIV serological status, on the Wonji–Shoa sugar estate in 1996

	No. and (%) of subjects	*Prevalence (%)*					
		Strongyloides stercoralis	*Hookworm*	Ascaris lumbricoides	Trichuris trichiura	*Amoebic parasites*	*HIV*
All	1239 (100)	13.0	23.8	22.2	19.5	24.6	3.1
GENDER							
Male	890 (61)	15.5	29.4	19.6	16.7	26.2	3.4
Female	349 (39)	8.9*	15.1*	26.2*	24.0*	22.0	2.5
AGE (years)							
15–19	199 (24)	7.0	26.8	27.1	25.1	23.0	0.6
20–29	324 (28)	9.4	22.0	21.3	18.5	30.0	2.9
30–39	384 (24)	16.9	21.2	22.2	19.2	20.3	2.9
> 39	332 (25)	19.2†	25.6	18.2†	15.5†	24.0	5.8†
EDUCATIONAL GRADE‡							
< 1	295 (25)	16.8	25.0	25.6	19.2	21.2	5.5§
1–6	389 (30)	18.4	27.2	23.3	22.0	21.8	2.2
7–11	391 (33)	7.3	23.1	22.5	19.5	29.3	2.3
> 12	164 (12)	6.6†	14.7†	11.4†	14.1	25.7	2.1
RESIDENCE							
Wonji village	390 (21)	5.9	1.5	12.4	10.2	32.8	5.8
Shoa village	116 (14)	9.4	4.8	23.9¶	36.8¶	30.6	1.1
Camps	733 (65)	16.0¶	35.0¶	24.9¶	18.7¶	20.7¶	2.6¶
DURATION OF RESIDENCE (years)**							
0–4	37 (3)	1.4	24.8	19.0	24.8	37.6	9.9
5–9	105 (7)	10.5	21.9	22.9	14.6	26.2	3.9
10–14	112 (7)	12.4	24.5	21.3	20.8	31.4	1.3
15–19	981 (83)	13.7†	23.9	22.0	19.6	23.4	2.9
FIELD WORKER?							
Yes	425 (67)	20.7	44.0	24.0	17.7	19.1	3.0
No	814 (33)	9.2*	14.0*	21.3	20.4	27.2*	3.1

TABLE 1 *(continued)*

		Prevalence (%)					
	No. and (%) of subjects	Strongyloides stercoralis	*Hookworm*	Ascaris lumbricoides	Trichuris trichiura	*Amoebic parasites*	*HIV*
HOUSEHOLD SIZE (no. of members)							
1	77 (5)	9.5	28.7	22.6	11.3	26.1	3.6
2–4	442 (29)	15.0	27.5	22.4	17.0	23.4	4.0
5–8	513 (43)	12.3	18.1	19.1	20.3	25.1	2.6
> 8	207 (22)	12.0	29.1	27.7	23.2†	24.7	2.6
MIGRANT?							
Yes	298 (76)	19.8	34.9	30.5	11.4	17.8	3.0
No	941 (24)	12.0*	22.2*	21.0*	20.7*	25.6*	3.1
HIV-POSITIVE?							
Yes	52 (3)	12.5	16.5	11.1	10.7	43.7	
No	1187 (97)	13.0	24.1	22.5	19.8	24.0*	

*,†,§,¶, Significantly different ($P < 0.05$), in a logistic model, adjusted for the stratified sampling design and study compliance, using the intestinal parasite infection (or HIV infection) as the dependent variable (*), for an increase of one category of the independent variable (†), comparing no education to all other categories of education combined (§), or using Wonji village as the reference (¶).

‡ Grades 1–6, 6–12 and > 12 correspond to primary, secondary and tertiary education, respectively

** Data only available for 1235 of the 1239 subjects.

TABLE 2

Odds ratios and confidence intervals for factors independently associated with infection with intestinal parasites among 1239 residents of the Wonji–Shoa sugar estate, during 1996

	*Odds ratio and (95% confidence interval) for:**				
	Strongyloides stercoralis	*Hookworm*	Ascaris lumbricoides	Trichuris trichiura	*Amoebic parasites*
Male gender	1.38 (0.75–2.54)	1.70 (0.98–2.95)	0.64 (0.41–1.00)	0.70 (0.43–1.15)	1.76 (1.14–2.70)
Age (per 10-year increase)	1.11 (0.83–1.48)	0.67 (0.51–0.87)†	0.70 (0.53–0.91)†	0.78 (0.59–1.04)	1.02 (0.82–1.27)
Educational level (per increase of one category)	0.73 (0.54–0.98)†	0.81 (0.63–1.04)	0.74 (0.58–0.95)†	0.79 (0.61–1.02)	1.03 (0.82–1.28)
RESIDENCE					
Wonji village	1	1	1	1	1
Shoa village	1.57 (0.66–3.71)	2.55 (0.53–12.2)	1.97 (1.02–3.79)†	4.80 (2.55–9.01)†	0.97 (0.58–1.65)
Camps	1.96 (0.98–3.94)	21.7 (6.06–77.5)†	1.64 (0.94–2.87)	1.75 (0.95–3.19)	0.69 (0.44–1.08)
Duration of residence (per 5-year increase)	1.06 (0.90–1.25)	1.08 (0.93–1.24)	1.06 (0.93–1.22)	1.04 (0.74–1.43)	0.92 (0.81–1.05)
Field worker	1.64 (0.91–2.97)	2.91 (1.75–4.86)†	1.39 (0.83–2.34)	1.47 (0.85–2.55)	0.60 (0.37–0.98)†
Household size (per additional member)	0.97 (0.90–1.04)	1.06 (0.99–1.14)	1.03 (0.97–1.10)	1.01 (0.94–1.07)	0.99 (0.92–1.05)
Migrant	0.81 (0.48–1.36)	0.68 (0.45–1.04)	1.95 (1.24–3.06)†	0.55 (0.33–0.94)†	0.76 (0.48–1.21)
HIV-positive	0.79 (0.27–2.30)	0.74 (0.27–2.03)	0.53 (0.18–1.57)	0.62 (0.15–2.52)	2.34 (1.07–5.13)†

* From a multivariate logistic model, using the intestinal parasite infection as the dependent variable, and all other socio–demographic characteristics plus HIV infection as the independent variables, adjusted for the stratified sampling design and study compliance.

† $P < 0.05$.

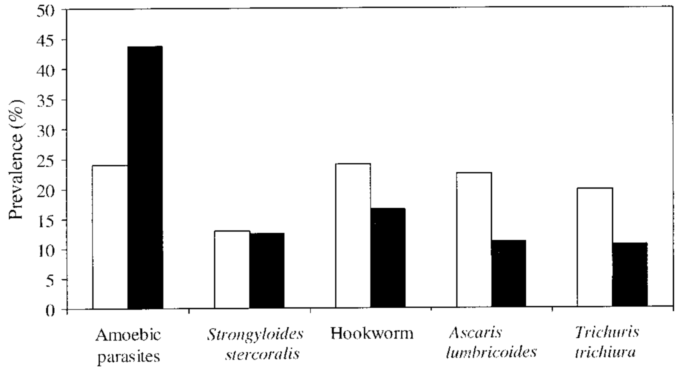

Fig. The prevalences of infection with various intestinal parasites, among the HIV-negative (□) and HIV-positive (■) subjects from the Wonji–Shoa sugar estate.

$P<0.05$); in the subjects with no education than in those with any (5.5% *v.* 2.2%; $P<0.05$); and in those living in Wonji village than in all other subjects (5.8% *v.* 2.4%; $P<0.05$). The prevalence of any intestinal parasitic infection was no different between the HIV-positive and HIV-negative subjects (76.2% *v.* 69.9%; $P>0.05$). However, when each parasite was considered separately, amoebic parasites were found to be significantly more common among the HIV-positives than among the HIV-negatives (43.7% *v.* 24.0%; $P<0.05$; see Fig.). This difference remained significant after controlling for socio–demographic characteristics (Table 2): the adjusted odds ratio (95% confidence interval) for the association between amoebic and HIV infections was 2.34 (1.07–5.13).

DISCUSSION

The prevalence of intestinal parasitic infections was high in this study population, with 70.1% of all study participants having at least one such infection. Such a high prevalence is not unusual for developing countries, however, and similar values have been documented before in Ethiopia (Tesfa-Yohannes and Kloos, 1988). Parasites acquired by walking barefoot in stool-contaminated soil, such as *St. stercoralis* and hookworm, were more commonly found in low-educated people, in people living in the camps (where hygienic standards are lower than in the villages), and in field workers (who are most likely to walk barefoot). Amoebic parasites had a different epidemiological distribution, being more common in the villagers than in the camp residents. It is unclear whether food or water was the main source of human infection with the amoebic parasites. That the usually recognised risk factors for intestinal parasitic infections were also found in the present study is reassuring in terms of the validity of the diagnostic methods used, even if only one stool examination was performed for each participant.

HIV was apparently introduced into Ethiopia in the mid-1980s (Tsega *et al.*, 1988). It is currently more common in the urban areas of the country, including the capital city, where a prevalence rate of 17% among antenatal care attenders has been documented (Fontanet *et al.*, 1998). Few relevant data are available for rural areas, although prevalences ranging from 0%–6% were documented in six surveys carried out in 1993 (Anon., 1998). The HIV prevalence in the Wonji–Shoa sugar estate was 3.1% at the time of the present survey. It was relatively high in Wonji village (5.8%), probably because of this village's close connections with the nearby city of Nazareth, on the trading road between the capital city and the commercial ports of the Red Sea.

There was little interaction apparent between intestinal parasitic and HIV infections in the present survey. Of all the parasites described, only amoebic parasites were significantly more common among the HIV-positive individuals than among the HIV-negatives. A study done in the same population (Wonji sugar estate), using stool culture and zymodeme analysis, found that the vast majority (27/29) of the amoebic parasites recovered were the non-pathogenic *Enta. dispar* (Gatti *et al.*, 1998). It is likely, therefore, that most of the amoebic parasites observed in the present study were also *Enta. dispar*, particularly since most participants were asymptomatic.

The absence of any other association between intestinal parasites and HIV infection argues against strong interactions, such as, for instance, a several-fold increase in the susceptibility of HIV-infected individuals to intestinal parasites infection, during the asymptomatic stage of HIV infection. Indeed, the present study had $>80\%$ power to detect a statistically significant 2-fold increase/decrease in the prevalence of intestinal parasites in HIV-positives compared with that in HIV-negative individuals (at least, for any parasite with a prevalence $>15\%$ in the HIV-negatives).

The apparent association between HIV infection and amoebic parasites is unlikely to be the result of confounding by socio–demographic characteristics, since it remained in the multivariate analysis controlling for age, gender, residence, site of work, and other characteristics. Confounding by sexual behaviours might have occurred if, as observed in the homosexual communities of industrialized countries (Phillips *et al.*, 1981), individuals who have multiple sexual partners, and who are therefore at higher risk of HIV infection, also have sexual practices that increase faeco–oral contact and therefore put them at higher risk of amoebic infection. However, such mechanisms would be more likely to result in substantial confounding in a community with low background (i.e. water- or food-borne) transmission of amoebic parasites, such as that of homosexuals in industrialized countries, than in a community with high background transmission of amoebic parasites, such as the Wonji community. The observed association may, instead, be related to an increased susceptibility of HIV-positive individuals to amoebic infection, as a consequence of their immunosuppression. In a prospective study among factory workers in Tanzania, HIV-positive participants were found to have higher incidences of amoebic infection than their HIV-negative counterparts (21.4 *v.* 13.4 infections/100 person-years; $P<0.05$; Borgdorff, 1994). A prospective study is currently on-going at the site of the present investigation, and may eventually confirm this association between amoebic and HIV infections, as well as the role of incident intestinal parasitic infections on the progression of HIV infection and its markers.

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