Trans R Soc Trop Med Hyg 2013; **107**: 631–638
doi:10.1093/trstmh/trt078

ORIGINAL ARTICLE

Infectious diarrhoea in antiretroviral therapy-naïve HIV/AIDS patients in Kenya

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Received 19 April 2013; revised 3 July 2013; accepted 17 July 2013

Background: Diarrhoea is a significant cause of morbidity and mortality in immunocompromised patients. The objectives of this study were to investigate the aetiological agents, risk factors and clinical features associated with diarrhoea in HIV/AIDS patients in Kenya.

Methods: Sociodemographic, epidemiological and clinical data were obtained for 164 HIV/AIDS patients (70 with and 94 without diarrhoea) recruited from Kenyatta National Hospital, Kenya. Stool samples were examined for enteric pathogens by microscopy and bacteriology.

Results: Intestinal protozoa and fungi were identified in 70% of patients, more frequently in those with diarrhoea ($p<0.001$). Helminths were detected in 25.6% of patients overall, and bacterial pathogens were identified in 51% of patients with diarrhoea. Polyparasitism was more common in patients with diarrhoea than those without ($p<0.0001$). Higher $CD4^+$ T-cell count (OR = 0.995, 95% CI 0.992–0.998) and water treatment (OR = 0.231, 95% CI 0.126–0.830) were associated with a lower risk of diarrhoea, while close contact with cows (OR = 3.200, 95% CI 1.26–8.13) or pigs (OR = 11.176, 95% CI 3.76–43.56) were associated with a higher risk of diarrhoea.

Conclusions: Multiple enteric pathogens that are causative agents of diarrhoea were isolated from stools of antiretroviral therapy-naïve HIV/AIDS patients, indicating a need for surveillance, treatment and promotion of hygienic practices.

Keywords: HIV/AIDS, Diarrhoea, Enteric pathogens, *Isospora*, *Cryptosporidium*, *Shigella*

Introduction

Diarrhoea is a significant cause of morbidity and mortality in people infected with HIV with or without AIDS.[1,2] Diarrhoea was reported to occur in up to 50% of patients with AIDS in developed countries prior to use of antiretroviral therapy (ART) and in up to 80% of those in resource-limited countries.[1,3,4] Whilst AIDS-associated diarrhoea can result from ART side effects and HIV/AIDS enteropathy, 30–60% of these patients[5] develop diarrhoea due to infection with enteric pathogens, including parasites, fungi, bacteria and other viruses.[1–3] The prevalence of these pathogens varies greatly depending on geographic region, season, age, socioeconomic status, sexual practices and immune status.[6] Concurrent infection with other pathogens, particularly helminths, can accelerate the rate of HIV progression by augmenting viral replication.[7]

Widespread use of ART has markedly reduced the prevalence of opportunistic infections in patients with HIV/AIDS in industrialised countries.[8,9] However, ART is not as widely available or affordable in resource-constrained countries and opportunistic pathogens remain a significant cause of diarrhoea in HIV/AIDS patients in these countries.[3,6,10] Protozoan parasites and fungi, including *Cryptosporidium* spp., *Isospora belli*, *Cyclospora cayetanensis*, *Giardia lamblia*, *Entamoeba histolytica* and microsporidia, are the most commonly identified intestinal pathogens in HIV-infected patients.[1,2,10–12] Bacterial infections are more frequent and severe in HIV/AIDS patients than in the general population and are responsible for >20% of diarrhoeal episodes in these patients.[1,3,13]

An estimated 6.2% (approximately 1.5 million) of the adult population in Kenya is infected with HIV.[14] Of these, only 400 000 (27%) are estimated to be on ART.[15] Diarrhoea is reported to occur frequently in this population,[16–18] but limited information is available regarding the aetiology, risk factors and clinical consequences of infectious diarrhoea in ART-naïve HIV-infected patients in Kenya.

The goal of this study was to document and describe rates of occurrence of intestinal pathogens in ART-naïve adults with HIV/AIDS and the associated sociodemographic, epidemiological and clinical features among those presenting with or without diarrhoea to the Comprehensive Care Clinic (CCC) of Kenyatta National Hospital (KNH) (Nairobi, Kenya) from June 2009 to July 2010.

Materials and methods

Study site and subject recruitment

This was a cross-sectional study conducted at KNH and the Kenya Medical Research Institute (KEMRI) in Nairobi, Kenya. The study was approved by the KEMRI Ethical Review Committee, the KNH Ethical Review Board and Tufts Medical Center Institutional Review Board. Subjects were enrolled through the CCC of KNH, a clinic that provides outpatient HIV/AIDS services. ART-naïve HIV-infected adults aged ≥18 years of age presenting to the CCC were eligible to be enrolled into the study. Consecutive patients presenting to the CCC were screened for study eligibility and informed consent was sought accordingly. Diarrhoea was defined as passage of three or more unformed stools per day for 72 h at the time of enrolment. 'No diarrhoea' at enrolment was defined as no diarrhoea for 6 months prior to enrolment. Cases were defined as patients with diarrhoea of any duration at the time of enrolment, whilst controls were defined as patients with no history of diarrhoea for 6 months prior to enrolment.

Acute diarrhoea was defined as a diarrhoeal episode lasting <14 days, persistent diarrhoea was defined as diarrhoeal episodes lasting 14–30 days and chronic diarrhoea was defined as diarrhoea lasting >30 days.

Data and sample collection

A standardised questionnaire was used to collect sociodemographic, epidemiological and clinical data including age, gender, marital status, site of residence, education, occupation, income, housing, past medical history, risk factors for diarrhoea, and presenting symptoms including self-reported weight loss, presence and duration of diarrhoea, vomiting, abdominal pain and fever. Physical examinations were performed by the attending physician at the CCC. Data were also obtained from patients' medical records at the CCC following enrolment in the study. At the time of enrolment, stool and blood were obtained from each patient.

Laboratory studies

$CD4^+$ counts

$CD4^+$ counts were determined using a CyFlow SL3 (Partec GmbH, Münster, Germany) at the CCC at KNH.

Detection of parasite ova and cysts

Diarrhoeal stools samples were processed within 1 h of collection or were stored in Cary–Blair transport medium at 4°C and were processed within 4 h of collection. For detection of ova and cysts, a wet mount was prepared in saline (0.85% NaCl) or Lugol's iodine and was examined by light microscopy. All stool samples were also concentrated with formalin–ether and were evaluated by Ziehl–Neelsen acid fast staining for detection of *Cryptosporidium* spp., *I. belli* and *C. cayetanensis*. To confirm infection with *C. cayetanensis*, modified safranin staining and UV fluorescence microscopy were performed. A modified trichrome stain with a high concentration of Chromotrope 2R was used to detect microsporidia.

Identification of bacterial pathogens in diarrhoeal stool samples

Stool samples were cultured following routine procedures and bacterial pathogens were identified using the API 20E system (bioMérieux, Inc., Marcy-l'Étoile, France) as per the manufacturer's protocol. Pathogenic *Escherichia coli* were identified using multiplex PCR.[19] Stool samples from patients without diarrhoea were not analysed for bacterial pathogens.

Statistical analyses

Statistical analyses were performed using Prism software v.5.0 (GraphPad Software Inc., San Diego, California, USA) and the Statistical Analysis Software package v.9.13 (SAS Institute Inc., Cary, North Carolina, USA). Student's t test was used to compare means between two groups for normally distributed continuous variables, while the Mann–Whitney test was used to compare medians of non-normally distributed continuous variables. Categorical variables were compared using the χ^2 test, with the exact method used when appropriate. Multivariate logistic regression analyses were performed using the stepwise method while considering potential confounders identified through clinical significance or statistical significance in bivariate analyses ($p<0.20$). Results of statistical analyses were considered significant when p-values were ≤0.05.

Results

Sociodemographic and epidemiological characteristics

Between June 2009 and July 2010, 167 study participants were enrolled into the study; 3 of the patients did not provide samples and were therefore not included in the analysis. The sociodemographic and epidemiological characteristics of the

Table 1. Sociodemographic and epidemiological characteristics of HIV/AIDS patients with and without diarrhoea

Characteristic	Diarrhoea (n = 70)	No diarrhoea (n = 94)	p-value
Age (years) (mean ± SD)	36 ± 10	39 ± 10	NS[a]
Female [n (%)]	46 (66)	45 (48)	0.03[c]
Married or remarried [n (%)]	28 (40)	40 (43)	NS[c]
Level of education completed [n (%)]			NS[c]
Primary education (standard 1–7)	17 (24)	31 (33)	NA
Secondary education (form 1–4) or vocational training	31 (44)	40 (43)	NA
High school (forms 5 and 6) or higher	22 (31)	23 (24)	NA
Household monthly income (Ksh × 1000) [median (IQR)]	28 (15–40)	25 (15–45)	NS[b]
No. of adults per household [median (IQR)]	2 (2–3)	2 (2–3)	NS[b]
No. of children per household [median (IQR)]	3 (2–4)	2 (1–3)	0.004[b]
No. of rooms in the house [median (IQR)]	3 (2–4)	4 (3–4)	NS[b]
Adults and children per room [median (IQR)]	1 (1–2)	2 (1–2)	NS[b]
Contact with dogs or cats [n (%)]	18 (26)	32 (34)	NS[c]
Contact with cows [n (%)]	23 (33)	9 (10)	0.0001[c]
Contact with pigs [n (%)]	12 (17)	2 (2)	0.0007[c]
Contacts with goats and sheep [n (%)]	10 (14)	17 (18)	NS[c]
Contact with other animals [n (%)]	3 (4)	5 (5)	NS[c]
Contact with poultry [n (%)]	20 (29)	32 (34)	NS[c]
Water supply			0.05[c]
Tap water supply, n (%)	53 (76)	54 (57)	NA
Bore well n (%)	8 (11)	19 (20)	NA
Open sources (rivers and dams) n (%)	9 (13)	21 (22)	NA
Water boiled/treated [n (%)]	40 (57)	70 (74)	0.02[c]
Obtained produce from:			
Own garden [n (%)]	18 (26)	40 (43)	0.026[c]
Open-air market [n (%)]	39 (56)	33 (35)	0.009[c]
Supermarket [n (%)]	13 (19)	21 (22)	NS[c]
Own garden and open-air market [n (%)]	15 (21)	12 (13)	NS[c]
Open-air market and supermarket [n (%)]	13 (19)	10 (11)	NS[c]
Less than 3 meals per day [n (%)]	15 (21)	25 (27)	NS[c]
Non-vegetarian diet [n (%)]	54 (77)	68 (72)	NS[c]
Dietary supplements [n (%)]	8 (11)	15 (16)	NS[c]

Ksh: Kenyan shillings; NA: not applicable; NS: not statistically significant.
[a]Student's *t*-test.
[b]Mann–Whitney test.
[c]χ^2 test.

study subjects are shown in Table 1. Study patients were from every province in Kenya but were predominantly from Nairobi province (56/164; 34.1%). Of the 164 participants, 91 (55.5%) were women and 73 (44.5%) were men. Moreover, 124/164 (75.6%) were Christian, 15/164 (9.1%) were Muslim and 25/164 (15.2%) were of other religions. Sexual orientation was heterosexual in 158/164 patients (96.3%) and homosexual in 6/164 patients (3.7%).

Overall, 70/164 patients (42.7%) presented with diarrhoea at the time of enrolment and 94/164 (57.3%) did not have diarrhoea within the past 6 months prior to enrolment. There were no significant differences between the two groups with regard to age, marital status, education, income, housing or number of adults per household. However, there were significantly more female patients with diarrhoea compared to those without ($p < 0.05$), and patients with diarrhoea had significantly more children per household ($p < 0.005$). Diarrhoea was significantly associated with close contact with cows ($p = 0.0001$) or pigs ($p < 0.001$).

Occurrence of diarrhoea was associated with well and open water sources ($p = 0.05$) and obtaining food produce from open-air markets ($p = 0.009$). Patients without diarrhoea were more likely to treat their water (by boiling, filtering, addition of chemicals or use of UV bottles) ($p = 0.02$) and to obtain food (farm produce and dairy) from their own gardens and dairy animals ($p = 0.026$). There were no significant differences between the two groups in dietary practices (vegetarian or non-vegetarian), use of dietary supplements (such as multivitamins or herbal supplements) or number of meals consumed per day.

Clinical and laboratory features

The clinical and laboratory characteristics of patients with and without diarrhoea are shown in Table 2. Of the patients with diarrhoea, 44/70 (63%) had acute diarrhoea (<14 days), 21/70 (30%) had persistent diarrhoea (14–30 days) and 5/70 (7%) had chronic diarrhoea (>30 days). Watery diarrhoea was reported in 28/70 patients (40%), mucoid diarrhoea in 18/70 patients (26%), frothy diarrhoea in 12/70 patients (17%) and bloody diarrhoea in 12/70 patients (17%). In addition, 26/70 (37%) of the patients with diarrhoea reported three or more episodes of diarrhoea in the previous 3 months, 56/70 (80%) reported three or more episodes in the previous 12 months, while only 3/70 (4%) reported that the current episode was the first in 12 months.

Patients with diarrhoea had significantly lower $CD4^+$ T-cell counts than patients without diarrhoea ($p<0.001$). This difference remained when considering only those with $CD4^+$ T-cells counts <200 cells/mm^3 ($p=0.003$). Patients with diarrhoea were more likely to report having fever ($p=0.004$), vomiting ($p<0.001$), abdominal pain ($p<0.001$) and weight loss ($p=0.018$).

Table 2. Clinical and laboratory characteristics of HIV/AIDS patients with and without diarrhoea

Characteristic	Diarrhoea (n = 70)	No diarrhoea (n = 94)	p-value
CD4 count (cells/mm^3) (mean ± SD)	193 ± 143	292 ± 194	<0.001[a]
CD4 cell count <200 cells/mm^3 [n (%)]	43 (61)	35 (37)	0.003[b]
Fever [n (%)]	32 (46)	23 (24)	0.004[b]
Vomiting [n (%)]	19 (27)	5 (5)	<0.001[b]
Abdominal pain [n (%)]	46 (66)	6 (6)	<0.001[b]
Self-reported weight loss [n (%)]	46 (66)	44 (47)	0.018[b]
Other opportunistic infection(s) [n (%)]	28 (40)	40 (43)	NS[b]
Pulmonary TB	13 (19)	7 (7)	0.031[c]
Oral candidiasis	3 (4)	10 (11)	NS[c]
Cryptococcal meningitis	1 (1)	1 (1)	NS[c]
Pneumonia (aetiology unknown)	10 (14)	5 (5)	0.049[c]
Kaposi's sarcoma-associated herpes virus	0 (0)	1 (1)	NS[c]
Herpes zoster	4 (6)	10 (11)	NS[c]
Herpes simplex	2 (3)	9 (10)	NS[c]

NS: not statistically significant.
[a]Student's *t*-test.
[b]χ^2 test.
[c]χ^2 test using Fisher's exact test.

A review of hospital records for opportunistic infections indicated that there was no difference between the two groups in the occurrence of non-gastrointestinal opportunistic infections including oral candidiasis, cryptococcal meningitis, Kaposi's sarcoma-associated herpes virus (KSHV), herpes zoster and herpes simplex infections. Diarrhoea was significantly associated with pulmonary TB ($p=0.031$) and pneumonia (aetiology not characterised) ($p=0.049$).

Factors associated with diarrhoea

The results of multivariate analyses are shown in Table 3. Factors associated with lower odds of having diarrhoea were higher $CD4^+$ T-cell count (OR = 0.995, 95% CI 0.992–0.998) and use of treated water (OR = 0.231, 95% CI 0.126–0.830). Factors associated with higher odds of having diarrhoea were history of close contact with cows (OR = 3.200, 95% CI 1.26–8.13) or pigs (OR = 11.176, 95% CI 3.76–43.56). Abdominal pain was significantly associated with diarrhoea (OR = 74.8, 95% CI 16.6–336.3).

Intestinal pathogens

Table 4 shows the occurrence of different intestinal pathogens identified in stools of patients with or without diarrhoea. Intestinal protozoa, predominantly *I. belli*, *Cryptosporidium* spp., *E. histolytica/dispar*, *G. lamblia* and *C. cayetanensis*, were detected in 115/164 patients (70.1%) overall and were more frequently identified in patients with diarrhoea compared to those without ($p<0.001$). Microsporidia were found in 7/70 patients (10%) with diarrhoea and 2/94 patients (2%) without diarrhoea. Patients with diarrhoea had significantly higher rates of infestation with *I. belli* ($p=0.04$), *E. histolytica/dispar* ($p=0.03$), *G. lamblia* ($p=0.01$) and microsporidia ($p=0.04$) and a trend towards statistical significance with *Cryptosporidium* spp. ($p=0.07$) and *C. cayetanensis* ($p=0.08$). *Isospora belli* infection was significantly associated with acute diarrhoea ($p<0.05$). *Cryptosporidium* spp. and *E. histolytica/dispar* were more commonly identified in patients with acute diarrhoea (18/44 [41%] and 9/44 [20%], respectively) and *G. lamblia* was more frequently identified in patients with persistent diarrhoea (6/21; 29%) but these differences were not statistically significant. Protozoa identified in patients with bloody diarrhoea included *I. belli* and *E. histolytica/dispar*. *Cryptosporidium* spp., *I. belli* and *C. cayetanensis* were identified in patients with watery diarrhoea. *Cryptosporidium* spp., *I. belli* and *E. histolytica/dispar* were identified in patients with mucoid diarrhoea and only *G. lamblia* in those with frothy diarrhoea.

Helminths were identified in 42/164 patients (25.6%) overall but there was no statistically significant difference between the two groups. The helminths identified were *Ascaris lumbricoides* (22/164; 13.4%), *Strongyloides stercoralis* (8/164; 4.9%), hookworm (6/164; 3.7%), *Schistosoma mansoni* (3/164; 1.8%), *Hymenolepis nana* (2/164; 1.2%) and *Enterobius vermicularis* (1/164; 0.6%). Hookworm (3/44; 7%), *H. nana* (1/44; 2%) and *S. mansoni* (1/44; 2%) were only isolated from patients with acute but not from those with persistent diarrhoea; however, the sample size was too small to show any statistically significant differences. *Strongyloides stercoralis* was significantly more prevalent ($p=0.024$) in patients with persistent diarrhoea. The only helminth isolated from patients with bloody diarrhoea was *E. vermicularis*. *Schistosoma mansoni*, *A. lumbricoides* and

Table 3. Multivariate logistic regression model showing factors associated with diarrhoea among patients with HIV/AIDS

Variable	Adjusted OR (95% CI)
$CD4^+$ T-cell count (cells/mm^3)	0.995 (0.992–0.998)
Water boiled/treated	0.231 (0.126–0.830)
Contact with cows	3.200 (1.26–8.13)
Contact with pigs	11.176 (3.76–43.56)
Abdominal pain	74.8 (16.6–336.3)

Table 4. Frequency [n (%)] of intestinal parasites isolated from stools of HIV/AIDS patients with and without diarrhoea

Parasite	Diarrhoea (n = 70)	No diarrhoea (n = 94)	p-value[a]
All intestinal parasites	59 (84)	43 (46)	<0.001
Protozoa			
Isospora belli	12 (17)	6 (6)	0.04
Cryptosporidium spp.	11 (16)	6 (6)	NS
Entamoeba histolytica/ dispar	11 (16)	4 (4)	0.03
Giardia lamblia	10 (14)	3 (3)	0.01
Cyclospora cayetanensis	4 (6)	1 (1)	NS
Fungi			
Microsporidia	7 (10)	2 (2)	0.04
Helminths			
Ascaris lumbricoides	10 (14)	12 (13)	NS
Strongyloides stercoralis	5 (7)	3 (3)	NS
Hookworm	4 (8)	2 (2)	NA
Schistosoma mansoni	1 (1)	2 (2)	NA
Hymenolepis nana	1 (1)	1 (1)	NA
Enterobius vermicularis	0 (0)	1 (1)	NA

NA: not applicable; NS: not statistically significant.
[a]Fisher's exact test was used to analyse all data.
The number of pathogens may exceed the number of patients as multiple pathogens were identified in some patients.

hookworms were isolated from those with watery and mucoid diarrhoea, while no helminth was isolated from those with frothy diarrhoea.

Polyparasitism (more than one parasite) was significantly more common ($p<0.0001$) in patients with diarrhoea (26/70; 37%) compared to those without (8/94; 9%).

$CD4^+$ T-cell counts and intestinal parasites

Comparison of $CD4^+$ T-cell counts among patients infected with different intestinal parasites is shown in Table 5. There was no significant difference in $CD4^+$ T-cell counts between patients with or without diarrhoea when individual parasites were considered. However, $CD4^+$ T-cell counts were significantly lower in patients with diarrhoea infested with any intestinal parasite ($p<0.005$), one or more protozoa ($p<0.05$), one or more helminth ($p<0.005$) and those with mixed pathogens ($p<0.005$) than in patients without diarrhoea.

Among the patients with diarrhoea, those with chronic diarrhoea had significantly lower ($p<0.05$) mean $CD4^+$ T-cell counts (103 ± 78 cells/mm^3) compared with those with acute diarrhoea (237 ± 144 cells/mm^3) or persistent diarrhoea (168 ± 155 cells/mm^3). Mean $CD4^+$ T-cell counts varied between those with watery (167 ± 152 cells/mm^3), mucoid (216 ± 153 cells/mm^3), frothy (180 ± 40 cells/mm^3) and bloody (166 ± 132 cells/mm^3) diarrhoea; however, there was no significant difference between them.

Bacterial pathogens in patients with diarrhoea

One or more bacterial pathogens were identified in the stools of 36/70 patients (51%) with diarrhoea. The most common were *Shigella* spp. (11/70; 16%) and *Salmonella* spp. (8/70; 11%), followed by enteroaggregative *E. coli* (7/70; 10%), *Klebsiella* spp. (5/70; 7%), *Campylobacter* spp. (5/70; 7%), enterotoxigenic *E. coli* (4/70; 6%), *Citrobacter freundii* (3/70; 4%), *Pseudomonas* spp. (2/70; 3%), enteropathogenic *E. coli* (EPEC) (1/70; 1%), *Morganella morganii* (1/70; 1%), *Aeromonas* spp. (1/70; 1%) and *Vibrio cholerae* (1/70; 1%). More than one bacterial species were found in 8/70 patients (11%) with diarrhoea. Among the patients with diarrhoea, *Salmonella* spp. (18/44; 41%), *Campylobacter* spp. (4/44; 9%), *V. cholerae* (1/44; 2%), *Aeromonas* spp. (1/44; 2%) and *M. morganii* (1/44; 2%) were all isolated in patients with acute diarrhoea but not in those with persistent diarrhoea. However, the differences were not statistically significant. Bacteria reported in patients with bloody diarrhoea included *Campylobacter* spp., *Salmonella* spp., *Shigella* spp. and EPEC. *Campylobacter* spp., *Shigella* spp., *C. freundii* and *M. morganii* were isolated from patients with watery diarrhoea. *Klebsiella* spp., EPEC, *C. freundii* and *V. cholerae* were isolated from those with mucoid diarrhoea, whilst only *Salmonella* spp. were isolated from patients with frothy diarrhoea.

Discussion

Although infectious diarrhoea is a major complication of HIV/AIDS that adversely impacts the quality of life, survival and associated healthcare costs, there are few studies that have evaluated diarrhoeal disease in patients with HIV/AIDS in Kenya. To the best of our knowledge, this is the first study in Kenya to characterise the risk factors, presenting symptoms and intestinal pathogens associated with diarrhoea in ART-naïve HIV-infected patients.

In this study most of the patients came from Nairobi province, most likely due to the proximity to KNH. Six patients reported that they were homosexual; however, it should be noted that homosexuality is illegal in Kenya and therefore there is likelihood of gross under-reporting. It has also previously been noted that the majority of those who report having had sex with men are in fact bisexual.[20] We found that overall, almost one-half of these patients presented with diarrhoea, most of them with recurrent acute watery diarrhoea. Patients with diarrhoea were significantly more immunocompromised (based on $CD4^+$ T-cell counts)

Table 5. Comparison of CD4+ T-cell counts among HIV/AIDS patients infected with different intestinal parasites

Parasite	Diarrhoea (n = 70)		No diarrhoea (n = 94)		p-value
	n (%)	CD4 count (cells/mm^3) [median (IQR) or mean ± SD]	n (%)	CD4 count (cells/mm^3) [median (IQR) or mean ± SD]	
Isospora belli	12 (17)	186 (65–229)	6 (6)	189 (178–352)	NS[a]
Cryptosporidium spp.	11 (16)	152 (53–398)	6 (6)	194 (83–273)	NS[a]
Entamoeba histolytica/dispar	11 (16)	201 (66–301)	4 (4)	269 (143–600)	NS[a]
Giardia lamblia	10 (14)	136 (53–211)	3 (3)	120 (77–155)	NS[a]
Ascaris lumbricoides	10 (14)	203 (165–260)	12 (13)	257 (227–304)	NS[a]
Microsporidia	7 (10)	189 (53–386)	2 (2)	450 (198–702)	NS[a]
Strongyloides stercoralis	5 (7)	156 (152–165)	3 (3)	430 (398–437)	NS[a]
Cyclospora cayetanensis	4 (6)	114 (76–212)	1 (1)	308 (308–308)	NS[a]
Hookworm	4 (6)	289 (216–358)	2 (2)	366 (212–520)	NS[a]
All protozoa	54 (77)	193 ± 20	33 (35)	270 ± 36	0.05[b]
All helminths	19 (27)	209 ± 25	27 (29)	359 ± 35	0.003[b]
Mixed infections	26 (37)	177 ± 22	8 (9)	378 ± 79	0.002[b]
Total parasites	59 (84)	201 ± 19	56 (60)	305 ± 26	0.002[b]

NS: not statistically significant.
[a]Mann-Whitney test.
[b]Student's *t*-test.

and were more likely to have associated symptoms such as abdominal pain.

The few studies on infectious diarrhoea in HIV/AIDS patients in Kenya focused mainly on the pathogens identified in patients with chronic diarrhoea. A study in Nairobi found that the most commonly identified pathogens in stool were *Shigella flexneri*, *Salmonella enterica* serovar Typhimurium and *Cryptosporidium parvum*, all of which were significantly more prevalent in HIV-positive compared with HIV-negative individuals.[21] A study by Mwachari et al. on 75 HIV-positive patients with chronic diarrhoea identified intestinal pathogens in 52% of patients, the most common being *Cryptosporidium* spp. (17%) and *S. typhimurium* (13%).[16] In that study, 41% of the patients died and detection of *Cryptosporidium* spp. was the single most significant predictor of death ($\chi^2 = 5.2$, $p<0.05$).[16] In a subsequent study of HIV-infected adults from the same group, intestinal pathogens were identified in stool samples of 35% of patients with chronic diarrhoea, with *Mycobacterium* spp. (13%), *Cryptosporidium* spp. (11%), *Salmonella* spp. (6%) and *Shigella* spp. (3%) being the most common.[17] However, none of these studies compared the occurrence, clinical features or risk factors for acquisition of intestinal pathogens in HIV/AIDS patients with or without diarrhoea.

In the present study, as expected, higher CD4$^+$ T-cell counts were significantly associated with lower rates of diarrhoea, confirming previous reports.[3] Treatment of drinking water was also associated with lower odds of developing diarrhoea. In a randomised control trial, water quality interventions were shown to result in a reduction in diarrhoeal disease in a population living with HIV/ AIDS.[22] Other factors associated with higher odds of having diarrhoea in this study included close contact with animals such as cows and pigs. Some of the parasites identified in this study, particularly *Cryptosporidium*, are zoonotic and animals may be the source of infection. Contact with animals has previously been reported to be a risk factor for acquisition of intestinal parasites such as *Cryptosporidium* in HIV/AIDS patients.[23] Abdominal pain was significantly associated with diarrhoea, as found in other studies.[6] Whilst fever, nausea and vomiting appeared significant in bivariate analyses, these were not independently associated with diarrhoea when adjusted for abdominal pain. Self-reported weight loss was not significantly associated with diarrhoea in the multivariate analysis in this study. This may be due to subjective reporting rather than actual measurements.

Among non-intestinal opportunistic infections, only TB and pneumonia of unknown aetiology were significantly more frequent in patients with diarrhoea compared to those without. TB is one of the leading causes of mortality among people with HIV in Kenya, with a prevalence of approximately 10%.[15] Pneumonia is also a significant cause of mortality in HIV-infected adults in Kenya.[15]

In the present study, intestinal protozoa were identified in 70% of all ART-naïve HIV-infected patients, mostly those with diarrhoea. Previous studies in HIV-infected patients with chronic diarrhoea from Kenya identified *Cryptosporidium* as the most common intestinal parasite.[16,17,21] In the current study, *Cryptosporidium* spp. were the second most common protozoan isolated. Surprisingly, *I. belli* was the most commonly identified intestinal protozoan in all patients in this study and was more common in patients with diarrhoea than in those without diarrhoea. This finding was unexpected as previously only one isolated

case of *I. belli* had been reported in an HIV-infected man with diarrhoea in Kenya.[24] The current study also documented a high incidence of microsporidia and *C. cayetanensis* in the patients. Microsporidia have not previously been reported in HIV-infected patients in Kenya, and only two cases of *C. cayetanensis* have been reported in humans in Kenya, one from an immunocompetent patient with abdominal pain and diarrhoea and the other from an HIV-positive patient with wasting and diarrhoea.[25] In this study, *G. lamblia* and *E. histolytica/dispar* infestations were significantly associated with diarrhoea. In Kenya these protozoa are common in patients with diarrhoea with or without HIV,[26] but it is not known whether these protozoans are more common in HIV-infected patients.

Helminths were isolated in almost one-third of the study population (both with and without diarrhoea). In a study by Walson et al. in HIV-infected ART-naïve adults in Kenya, hookworm species were the most prevalent (56.3%), followed by *A. lumbricoides* (17.1%), *Trichuris trichiura* (8.7%), *S. mansoni* (7.1%) and *S. stercoralis* (1.3%).[27] The lower prevalence of helminths in the present study could be due to the use of a single stool specimen for diagnosis, which is less sensitive than the use of three samples used in many surveys.[28]

The prevalence of opportunistic pathogens in HIV-infected patients without diarrhoea was remarkably high. Although there are no reports of these pathogens in HIV-infected patients without diarrhoea in Kenya, similar observations have been made in other studies in areas where these infections are endemic.[11,29] The reasons for this are not clear but may include protection from diarrhoea owing to a lower burden of infection or to protective memory immune responses acquired due to repeated exposure to these pathogens early in life before acquisition of HIV infection. Multiple intestinal pathogens occurred both in patients with and without diarrhoea although they were more prevalent in those with diarrhoea. This could be due to the fact that patients with diarrhoea were more immunocompromised than those without diarrhoea.

In more than one-half of the patients with diarrhoea, one or more bacterial pathogen was identified from their stool sample. This study is in agreement with other studies in Kenya where *Shigella*, *Salmonella*, *Campylobacter* and pathogenic *E. coli* species were the most frequently identified in HIV-positive patients with diarrhoea.[17,30] However, it is not known whether these bacteria are more commonly identified in patients with HIV/AIDS than those without HIV/AIDS.

Despite several limitations of this study, we have documented a diversity of pathogens that may be associated with diarrhoea in HIV/AIDS patients in Kenya, many of which were not previously recognised as important AIDS-associated pathogens.

Limitations

Owing to financial constrains, the presence of other organisms such as viruses and *Mycobacterium avium-intracellulare* that have the potential of causing diarrhoea[1,2] was not assessed in this study. In addition, we did not investigate bacterial pathogens in patients without diarrhoea. We were not able to measure viral loads or to perform PCR for detection of parasitic infections. Future longitudinal studies using sensitive and specific molecular and immunological assays are needed to document the presence and burden of intestinal pathogens, to understand the impact of these organisms on HIV progression and to design interventions that could reduce the burden of disease in populations that are most at risk of suffering adverse consequences caused by such organisms.

Conclusion

Multiple parasitic, bacterial and fungal pathogens that are causative agents for diarrhoea were isolated from stools of ART-naïve HIV-infected persons in Kenya. Infectious diarrhoea can lead to morbidity, affecting the quality of life, and may even lead to mortality. The fact that approximately 43% of the patients enrolled in this study presented with diarrhoea to the clinic indicates that diarrhoea is a major problem in this population. Results from this study indicate a need for opportunistic pathogen surveillance, treatment and promotion of hygienic practices such as treatment of drinking water and safe handling of animals.

Authors' contributions: JWW, HDW and RO conceived the study; JWW, HDW, CNW and GK designed the study protocol; JWW, HK and PN recruited the patients; JWW, HK, SM, DEW, TK and TW undertook the laboratory work; JWW and MK undertook analysis of the data. JWW and HW drafted the manuscript; all authors contributed to interpretation of the data, read the manuscript and approved the final manuscript. JWW and HDW are guarantors of the paper.

Acknowledgements: The authors wish to thank all of the study subjects who participated in this study. The authors also thank Margaret Okuku and Zipporah Mworia at Kenyatta National Hospital (Nairobi, Kenya) for help in recruiting the patients, and Joyce Nyambura of the Center of Microbiology Research (Kenya Medical Research Institute [KEMRI], Nairobi, Kenya) and David Warunge of the Center of Biotechnology Research and Development at KEMRI for help in the parasitological analysis and other aspects of the study. The authors also thank Dr Christine Wanke (Tufts University School of Medicine, Boston, Massachusetts, USA) for critical review of the manuscript. This work has been submitted with the permission of the Director, KEMRI.

Funding: This study was supported by a Fogarty International Clinical Research Fellowship [R24 TW007988] to JWW.

Competing interests: None declared.

Ethical approval: This study was approved by the Kenya Medical Research Institute Ethical Review Committee, the Kenyatta National Hospital Ethical Review Board and Tufts Medical Center Institutional Review Board.

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