Original Research Article

Epidemiology of Coccidian Parasites in HIV Patients of Northern Uganda

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ABSTRACT

Aim: The epidemiology of coccidian parasites in HIV patients of sub-sahara Africa is poorly understood. This study aimed at determining the epidemiology of coccidian parasites and their associated risk factors. This was a cross sectional study carried out in Arua district in West Nile region of Northern Uganda for a period of five months.

Materials and methods: Participants in the study included HIV positive patients presenting with diarrhea. A total of 111 patients were included and classified into children, middle aged and adults. A structured questionnaire was administered, stool samples were obtained using sterile stool containers and laboratory analysis carried out using modified Ziehl-Neelsen technique (ZN). Ethical clearance was acquired and the consent of the patients was sought.

Results and discussion: Prevalence of Coccidian parasites among HIV patients was found to be 5.4% and *Cryptosporidium parvum* showed more prevalence than *Isospora belli and cyclospora cayatenensis* i.e. (3.6%), (1.8%) and (0.0%) respectively. Most C*ryptosporidium parvum* infections occurred in children (13.6%) compared to adults (3.3%); with a significant relationship of (p = 0.02). The infection was higher in females (7.1%) than males (2.4%) (p = 0.19). The major risk factors associated with the disease were mainly consumption of contaminated and un-boiled water from taps and boreholes.

HIV patients who took co-trimoxazole and drunk boiled water were shown to have a low prevalence of coccidian parasites of 1.9% and 2.6% respectively (*$p<0.05$)*. This is because co-trimoxazole is a prophylactic drug for opportunistic infections and proper boiling of drinking water kills coccidian parasites.

Conclusion and recommendations: The study highlighted the importance and need to screen for coccidian parasites and emphasis on regular taking of prophylactic treatment as a way of controlling opportunistic infections in HIV patients. Future prevalence studies of Coccidia amongst healthy, HIV sero-negative children and adults of similar age groups in similar settings are recommended to ratify the relationship.

Keywords: "Epidemiology of coccidia in Humans," "Coccidia in HIV patients," "Coccidia risk factors," "Cryptosporidium in Uganda," "Diarrhea in HIV patients."

1. INTRODUCTION

Coccidian parasitic infections have altered the epidemiology and outcome of human immunodeficiency virus (HIV) patients in sub-sahara Africa [1]. Diarrhea has been identified as a major presenting complaint in HIV-infected patients. It is estimated worldwide that about 3.5 billion people are infected, and that 450 million are ill as a result of intestinal parasites (coccidian parasites) and protozoan infections and that the majority are pre-school and school going children [2]. It is basically children from poor countries that are often more prone to these intestinal parasitic infections due to high poverty levels, poor sanitation and low literacy levels in the region [3].

A recent study in Kenya has shown that *Entamoeba histolytica /Entamoeba dispar (E. histolytica/E. dispar)*, 225 (36.7%), *Cryptosporidium* spp. 187, (30.5%), *Giardia lamblia*, 98 (16%) were higher in children (<5 years) and that *E. histolytica/E. dispar,* and *Giardia. lamblia (G. lamblia)* were higher among outpatients than inpatients (13.8% vs 1.3% $p < 0.001$ and 5.8% vs 1.3% $p < 0.049$) respectively [4]. Intestinal parasitic infections have enormous effects on the general health of an HIV infected person [5] and Uganda being part of sub-sahara Africa is already overburdened by HIV infection. These patients often suffer from frequent episodes of diarrhea that is accompanied with severe dehydration, loss of weight and muscle wasting which can be fatal [6]. Because of delayed diagnosis of these pathogens, patients usually take self-medication or local herbs without prescription from a qualified health worker which has resulted into improper management of the disease. Liberalization of the medical drug industry and poorly regulated herbal therapies by governments with in sub-sahara Africa is probably linked to UN ending self-medication in sub-sahara Africa [1, 7, and 8].

Generally, the epidemiology of coccidian parasites in HIV patients of sub-sahara origin is still poorly understood. In a recent study in Ethiopia [9], prevalence of gastrointestinal coccidian parasites was shown to be 18% - 40% among patients that presented with diarrhea. A similar study in Uganda in HIV sero negative children 9-36 months revealed that out about 930 fecal samples that were examined, 116(12.5%) were *Cryptosporidium* positive [10]. Infection with *Cryptosporidium* spp*.* was found to be associated with lowered immunity and the major risk factors were absence of toilets, water source and poor standards of living [4]. In a previous study in Ethiopia also, the prevalence of *Cryptosporidium* spp*.* and *Isospora belli* (*I. belli*) were shown to be 20.8% and 7.9% respectively in HIV patients [11]. In a recent study in Kenya [12], it was shown that there was a prevalence of 50.9% of enteric parasites which were waterborne. The major risk factors identified in the study were; place of residence, agro-ecological, water source, family size, location, reliability to treatment and diarrheal status probably due to poor environmental sanitation and personal hygiene, food and individual contamination probably due to poor management and care of HIV patients [12]. Contamination of water with coccidian species has been reported at national water storage facilities [13]. Infection rates are highest in children living in sub-sahara Africa and clinical cases are expected to be higher than reported due to limited infrastructure and research in the region [14, 15]. The current control strategies are towards community drug delivery of anti-helminthic drugs against intestinal parasites but there is none against coccidian parasites [8]. Stimulating research and development in rural communities through support of clinical trials to improve treatment and increasing drug availability, needs governmental funding and resources that do not presently exist in most sub-sahara health care facilities [15]. Coccidian parasites are well recognized and account for about 20% of diarrheal episodes in children in developing countries and up to 9% of episodes in developed settings and causes a considerable amount of diarrheal illness in young farm animals worldwide [7]. Sporadic outbreaks among children in developed countries have been reported due to fecal-oral transmission [1]. Epidemiological variations have been observed in the socioeconomic and geographical effects of the distribution of coccidian parasites in humans that may influence the sources and routes of transmission. The study was carried out to highlight the importance of screening for intestinal coccidian parasites among HIV patients and also to emphasize the necessity of increasing awareness among clinicians regarding the occurrence and management of these parasites in the region.

2. MATERIAL AND METHODS

This was a cross sectional study carried out in Arua regional referral hospital (ARRH) in Arua district of Northern Uganda for a period of five months (January to May 2013). Arua district is located in a corner of West Nile region of Uganda and it borders both South Sudan and the Democratic Republic of the Congo. Arua District has five counties which are all served by Arua regional referral hospital majorly. Being at the border of two countries, the major economic activity in the region is cross-border trade. There is also a high influx of refugees from South Sudan and now the natural environment in the district has been severely stressed in some areas and levels of hygiene have declined due to increase in population. Participants in the study included HIV positive patients both rural, urban or refugees who attended ARRH and presented with diarrhea. The exclusion criteria included participants who did not present with diarrhea and were HIV sero negative. The entry point to the study was Arua hospital HIV clinic with in ARRH. The criteria for choosing participants in the study were based on hospital records for their HIV status and only those presenting with diarrhea were selected into the study after their consent. For children, consent was sought from their guardians/parents after explaining to them the aim of the study and stool collection procedure was explained to them. A total of 111 participants were included and classified as children (10-19 years), middle age (20-39 years) and adults (40-69 years). A control group of 31 participants (11 children, 10 middle aged and 10 adults) who were HIV positive but had no diarrhea were randomly chosen for the study. Structured questions like "Where do you collect water for domestic use?" "Do you boil drinking water?" were administered to individual participants in a language best understood to them. Procedures for stool collection were well explained and stool samples were collected in sterile stool containers. Laboratory analysis was carried on fresh stool samples 2 hours after stool collection using formol ether concentration technique and modified Ziehl-Neelsen.
Briefly; 10 ml of 10% formol-saline was added to approximately 2mg (matchstick head size) of semi formed/diarrheic faeces in a centrifuge tube, stirred using an applicator stick and filtered into another centrifuge tube. 3 ml of ether was added, mixed well and centrifuged at 3,000 rpm for 5 minutes. The sediment was then re-suspended after removing supernatant by tapping the bottom of the tube, mixed well and transferred to a slide for microscopic examination under a cover slip and viewed under microscope x10 objective and the findings were recorded. A small portion of the stool sediment that was concentrated was taken and a smear made on a clean slide. The smear was allowed to air dry then fixed with absolute methanol. The smear was stained with strong carbol fuchsin for 30 minutes, decolorised with 1% acid alcohol and rinsed with water and counter stained with 0.1% methylene blue (alkaline). The slides were viewed under x100 objective and recorded. Data obtained was recorded as frequency and expressed as percentages. Descriptive analysis using statistical Package for Social Scientists (SPSS) version 20 was carried out to determine associations and a p-value < 0.05 was considered statistically significant. All participants' results and details were confidentially kept by the researchers. A copy of this research report was submitted to the Department of Medical Laboratory Sciences and Mbarara University of science and technology research Ethical Committee for approval. Permission was sought from the hospital director, laboratory in-charge and head of HIV clinic Arua regional referral hospital. The purpose of this study including the procedure of specimen collection was explained to the participants. Consent of the patients was sought prior to recruitment for the study and the consent form was filled and signed by the patients. The consent form was translated into the local language and all participants/patients understood all the details of the study. Laboratory results of the patients were given to the clinicians concerned and all patients/participants were guided on how to get their results or any help from the hospital.

3. RESULTS

The study showed Coccidian prevalence of 6 (5.4%) in HIV patients with diarrhea. No coccidian parasites were identified in the control group of HIV positive participants without diarrhea as shown in **Table 1.** The most prevalent coccidian species that were identified included *Cryptosporidium* spp. and *Isospora belli* i.e. (3.6%) and (1.8%) respectively. There was no *Cyclospora cayetensis* identified as shown in **Table 1**. From **Table 2**, out of the study group it was shown that the distribution of coccidian parasites was greater in females with an occurrence of 7.1% as compared to 2.4% among the male population. For both *Cryptosporidiosis* and *I. belli*, univariate analysis did not show any significant differences in infection between males and females, (P = 0.19), however, HIV seropositive children with diarrhea (10-19) were three times more likely to be infected with coccidian parasites than the HIV seropositive age groups 20-39 and 40-69 years i.e. 13.6%, 3.4% and 3.3% respectively. Further analysis showed that age was significantly associated with *Cryptosporidium* spp*.* infection, whereby the prevalence was tending to be highest in children aged 10-19 years being twice more than occurrence in participants of age group 20-39 years (at 95% CI, P = 0.021) as shown in **Table 3** that *Cryptosporidium* spp*.* was 3/3 (100%) in 10-19 age group and 1/2 (50%) among ages 20-39 years. As regards to *I. belli,* it was the least common species with 1/1 (100%) occurrence in age group 40-69 years, ½ (25%) in ages 20-39 and none in children 0/3 (10-19). There was no significant relationship between *I. belli* and age (P = 0.15). From **Table 4,** the risk factors associated with diarrhea among those with coccidia were shown to be majorly in those who consumed raw drinking water from taps and bore holes i.e.12.9% and 2.7% respectively and further statistical analysis showed there existed no significant relationship (P = 0.19). Most people collected their water from bore holes (74/111) and taps (31/111) compared to river (2/111) and community wells (4/111) as shown in **Table 4**. From the study group, HIV patients who drunk raw water and were not taking co-trimoxazole had a tendency towards higher prevalence of coccidian parasites of 6.9% and 8.5% respectively as compared to tendency towards a lower prevalence of those who took boiled water (2.6%) and were on co-trimoxazole (1.9%) as shown in **Table 4.** Further analysis showed there is a strong relationship (P < 0.05) in HIV patients who take boiled water as well as prophylactic treatment. There were different causes of diarrhea that were identified during the study however emphasis of the researchers was maily put on coccidian parasites. Some of the incidental findings included; *E. histolytica/E. dispar, G.lamblia, Ascaris lumbricoides (A.lumbricoides), Escherichia coli (E. coli), Trichuris trichiura (T. trichiura)* and *Hook* worms. Some of the patients presented with mixed infections and commonest parasite found was *G. lamblia* and *E. histolytica/E. dispar,*. Tendency to show higher prevalence was seen to be among children (10-19) as shown in **Table 5**.

Table 1. Prevalence of ccoccidian parasites among HIV positive patients with diarrhoea compared to HIV positive controls without diarrhoea

Species	Coccidian	No coccidian	Total
	Frequency (%)		
Study group	6 (5.4)	105 (94.6)	111 (100)
Control group	0 (0)	32 (100)	32 (100)
Cryptosporidium spp.	4 (3.6)	107 (96.4)	111 (100)
I. belli	2 (1.8)	109 (98.2)	111 (100)
C. cayatenensis	0 (0)	111 (100)	111 (100)
Total	6 (5.4)	105 (94.6)	111 (100)

Table 2. Distribution of coccidian parasites by sex and age

		Frequency (%)		
		Coccidia	No coccidia	Total
Sex	Female	5 (7.1)	65 (92.9)	70 (100)
	Male	1 (2.4)	40 (95.6)	41 (100)
	Total	6 (5.4)	105 (94.6)	111 (100)
Age	10-19	3 (13.6)	19 (86.4)	22 (100)
	20-39	2 (3.4)	57 (96.6)	59 (100)
	40-69	1 (3.3)	29 (96.7)	30 (100)
	Total	6 (5.4)	105 (94.6)	111 (100)

Table 3. Distribution of coccidian species by sex and age

		Cryptosporidium spp.	*I. belli*	Total
Sex	Female	3 (60)	2 (40)	5 (100)
	Male	1 (100)	0 (0)	1 (100)
	Total	4 (66.7)	2 (33.3)	6 (100)
Age	10-19	3 (100)	0 (0)	3 (100)
	20-39	1 (50)	1 (50)	2 (100)
	40-69	0 (0)	1 (100)	1 (100)
	Total	4 (66.7)	2 (33.3)	6 (100)

Table 4. Risk factors associated with coccidian parasite infection

		Frequency (%)		
		Coccidia	No coccidia	Total
Water source	Bore hole	2 (2.7)	72 (97.3)	74 (100)
	Tap	4 (12.9)	27 (87.1)	31 (100)
	River	0 (0)	2 (100)	2 (100)
	Community well	0 (0)	4 (100)	4 (100)
	Total	6 (5.4)	105 (94.6)	111 (100)
Water quality	Drink boiled water	1 (2.6)	38 (97.4)	39 (100)
	Drink raw water	5 (6.9)	67 (93.1)	72 (100)
	Total	6 (5.4)	106 (94.6)	111 (100)
Prophylaxis treatment	Taking co-trimoxazole	1 (1.9)	51 (98.1)	52 (100)
	Not taking co-trimoxazole	5 (8.5)	54 (91.5)	59 (100)
	Total	6 (5.4)	106 (94.6)	111 (100)

Table 5. Incidental findings in the study

		Parasite						
		E. histolytica /E. dispar	*G. lamblia*	*A. lumbricoides*	*E. coli*	*T.trichiura*	*Hook* worms	**TOTAL**
		Frequency (%)						
Sex	Male	2 (25)	3 (37.5)	0 (0)	1 (12.5)	0 (0)	2 (25)	8 (100)
	Female	3 (30)	4 (40)	1 (10)	0 (0)	1 (10)	1 (10)	10 (100)
	Total	5 (27.7)	7 (38.9)	1 (5.6)	1 (5.6)	1 (5.6)	3 (16.7)	18 (100)
Age	10-19	4 (36.4)	3 (27.3)	1 (9.1)	0 (0)	1 (9.1)	2 (18.2)	11 (100)
	20-39	1 (25)	2 (50)	0 (0)	1 (25)	0 (0)	0 (0)	4 (100)
	40-69	0 (0)	2 (66.7)	0 (0)	0 (0)	0 (0)	1 (33.3)	3 (100)
	Total	5 (27.7)	7 38.9)	1 (5.6)	1 (5.6)	1 (5.6)	3 (16.7)	18 (100)
Water quality	Boil water	2 (33.3)	2 (33.3)	0 (0)	1 (16.7)	1 (16.7)	0 (0)	6 (100)
	Do not boil water	3 (25)	5 (41.7)	1 (8.3)	0 (0)	0 (0)	3 (25)	12 (100)
	Total	5 27.7)	7 (38.9)	1 (5.6)	1 (5.6)	1 (5.6)	3 (16.7)	18 (100)

4. DISCUSSION

Intestinal parasitic infections are classified today as the leading causes of mortality and morbidity among patients infected with HIV [6]. Studies from Iran, Ethiopia and Australia have showed that *cryptosporidiosis* is a common opportunistic infection in HIV disease [16, 4, 17]. In a recent research, a comparative study was done comparing prevalence of gastrointestinal protozoa among HIV positive and HIV-negative men in Australia. A total of about 1,868 inpatients was recruited for study; stool specimens collected from them were analysed for presence of gastrointestinal parasites. From the results of the study, it was observed that *C. Parvum* cases occurred more frequent in HIV-positive patients [17].

In most developing countries, co-trimoxazole is used to prevent opportunistic infections; therefore there has been a general decrease in the occurrence of gastrointestinal parasites since *Cryptosporidium* spp., *I. belli* and *C. cayetanensis* are sensitive to this treatment (16).

This study showed prevalence of coccidian parasites of 5.4%; *Cryptosporidium* spp. was twice more prevalent (66.7%) than *I. belli* (33.3%). *Cryptosporidium* spp. were more in age group 10-19 years i.e. 3/3 (100%) of those positive. A study in central Uganda revealed a prevalence of 25% in a population of over 1000 children suffering from diarrhea due to *Cryptosporidium spp.* [18]. Children being major suffers is linked to the mode of transmission of these intestinal parasites (feaco-oral) in relation to personal and community hygiene, because of inadequate knowledge in this group, they tend to suffer the consequences of intestinal parasites.

The major risk factor associated with coccidian parasites in the study was consumption of raw drinking water from the bore hole and the taps**.** Community lifestyle patterns such as poor health hygiene and poor nutritional standards and low education levels have contributed to increased disease burden in rural communities and also the fact that there was scarcity of fire wood, firewood collection sites were far have also promoted challenges in preparation of safe drinking water which is in agreement with a recent study [8]. Poor sanitation habits such as failure to clean water collecting jerricans and water collection areas over long periods of time were some of the factors observed that lead to contamination of water collected from taps and bore holes in the communities [9]. Due to challenges of financing, which is characteristic of sub-sahara African local government, servicing of water pipes is hardily carried out thus leading to sporadic leakages and contamination of the water [15]. This has subsequently led to increased episodes of infections in rural communities that are often forced to share the limited water sources especially in the dry seasons. Patients actively on co-trimoxazole treatment were found to have a low prevalence of coccidian parasites because it is a prophylactic treatment in HIV infection [8]. Due to reduced immunity in acquired immune deficiency syndrome (AIDS) disease, prophylactic treatment was given to HIV clients to prevent opportunistic infections such as chronic diarrhea due to gastrointestinal parasites however, abuse of this drug causes resistance to bacteria and coccidia parasites which could have been the reason as to why there were some coccidian parasites detected in a patient on prophylactic treatment. It is therefore recommended to take co-trimoxazole in its full dosage as a prophylactic treatment in management of diarrhea in immune suppressed patients [19].

The major risk factors attributed to diarrheal diseases are place of residence, agro-ecological, water source, family size, location, reliability, treatment and diarrheal status probably due to poor environmental sanitation and personal hygiene [12]. Arua being at the boarder of Uganda with Sudan and Congo has contributed to likelihood of poor sanitation due to an increasing population and the area also having few hospitals which cannot handle these growing numbers of population effectively. A recent census in Uganda has shown than the population of Arua district alone has increased from 559,075 persons in 2002 to 785,189 persons in 2014 census [20]. Adult females of reproductive age and children in developing countries are more likely to suffer from poor nutrition habits due to shortage of enough food as a result of population raise hence leading to low immunity and being susceptible to secondary infections [21,22,and 23]. Children are associated with a weak immunity and coupled with poor nutritional habits. Inferential analysis showed there is a stronger relationship in drinking boiled water and co-trimoxazole. This would be due to the added advantage of boiled water where by the eggs and parasites are killed thus breaking the lifecycle. Research has shown that consumption of unboiled water is a likely risk factor to water borne diseases [24]. The consequences associated with coccidian parasite infection are the ability to cause chronic diarrhea which leads to severe muscle wasting, dehydration and even death. There is need for the government to strengthen the health system in management and creation of awareness of this disease to all clinicians and immune suppressed persons.

Major constraints to the study included; small sample size and limited number of diagnostic tools used due to severe financial constraints.

5. CONCLUSION AND RECOMMENDATION

From the study, it was shown that there was generally a reduced prevalence of coccidian parasites as compared to previous studies in Uganda, Kenya and Ethiopia which can be assumed that perhaps most HIV patients in West Nile region of Uganda do take prophylactic treatment for gastrointestinal diseases.

Routine diagnosis of intestinal coccidia will obviate unnecessary treatment especially in the children who are more likely to suffer from side-effects of anti-parasitic drug therapy well as erroneous treatment with the antibiotics might augment antibiotic resistance amongst the bacterial population as well as altering the normal flora that is usually present in the human gastrointestinal tract thereby rendering it pathogenic.
Further studies to look at prevalence of coccidia among healthy children and adults without HIV in corresponding ages should be considered.
Patients with HIV living in rural communities where it is difficult to access safe drinking water should be encouraged to take prophylactic treatments seriously. A further study should be conducted in the region using a wider array of laboratory diagnostic tools like polymerase chain reaction (PCR) and larger sample size in order to determine the scale of diarrheal diseases in HIV patients in the region.

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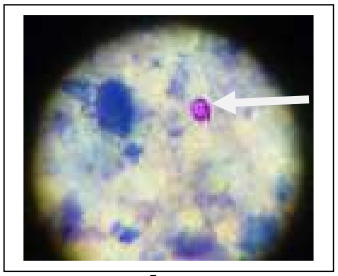
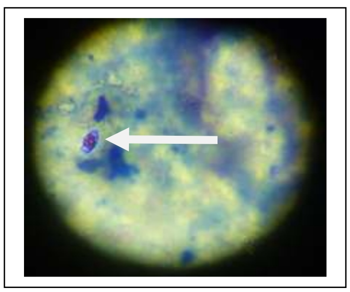
APPENDIX

Fig. 1. Coccidian oocysts in fecal smears from patients with diarrhea in Uganda, stained with modified Ziehl-Neelsen. A. *Cryptosporidium* spp. B. *Isospora belli*. X100.



A



B