<u>Original Research Article</u>

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 Saharan Uganda is poorly understood. The aim of the study was to determine 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 and stool samples were obtained using sterile stool containers and laboratory analysis was carried out using modified Ziehl-Neelsen technique (ZN). Ethical clearance was acquired and the consent of the patients was sought.

Results and discussion: Coccidian prevalence of 5.4% in HIV patients was shown and the most prevalent coccidian species that were identified included *Cryptosporidium parvum* (3.6%) and *Isospora belli* (1.8%) and these were most prevalent in females 2.7% and 0.9% respectively. The major risk factors associated were shown to be mainly consumption of tap and bore hole water. Community lifestyle patterns are major contributing factors to the epidemiology of the condition. HIV patients on co-trimoxazole and drinking boiled water were shown to have a low prevalence of coccidian parasite diarrhea i.e. 1.9% and 2.6% respectively due to posive effects of co-trimocazole on coccidia as compared to HIV patients not on therapy. Patients taking co-trimoxazole and boiling water were shown to be associated ($P < 0.05$) with low infections.

Conclusion and recommendations: The study further highlighted the importance to control secondary infections in HIV patients regardless of age, gender and social status especially in HIV patients living in rural communities.

Keywords: “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-Saharan Africa [1]. Diarrhea has been identified as a major presenting complaints in HIV-infected patients. Because of the delayed diagnosis of these pathogens in HIV infected patients, the patients usually take medication without prescription from clinicians as well as local medications for treatment of signs and symptoms, therefore the disease is not treated especially in sub Saharan Uganda due to the liberalization of the medical drug industry and poorly regulated herbal therapies [1] [2] [3]. The epidemiology of coccidian parasites in HIV patients of sub Saharan origin is poorly understood. In a recent study in Ethiopia [4], prevalence of gastro intestinal parasites was shown to range between 18% - 40%. 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 [5].

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 parvum* (*C. parvum*) and *Isospora belli* (*I. belli*) were shown to be 20.8% and 7.9% respectively in HIV patients [6]. In a recent study in Kenya [7], 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, 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 [7]. Contamination of water with coccidian spp has been reported national water storage facilities [8]. Infection rates are highest in children living in sub-Saharan Africa and clinical cases are expected to be higher than reported due to limited infrastructure and research in the region [9] [10]. The current control strategies are towards community drug delivery of anti-helminthic drugs against intestinal parasites but there is none against coccidian parasites [3]. Stimulating research and development in rural communities through support of clinical trials to improve treatment, in addition to securing and increasing drug availability, needs governmental funding and resources that do not presently exist in most sub Saharan health care facilities [10]. 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 [2]. 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 boarder 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 and urban attending ARRH and presenting with diarrhea. The exclution 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 was 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 was used and classified into children (10-19 years), middle age (20-39 years) and adults (40-69 years). A control group of 31 participants (11 children, 10 adults and 10 elderly) who were HIV positive but had no diarrhea were randomly chosen for the study. Structured questionnaires was administered and stool samples were obtained using sterile stool containers and 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 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. The slides were viewed under x100 objective and recorded. Data obtained was recorded as frequency and expressed as percentages. Descriptive analysis using 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 a Coccidian prevalence of 6 (5.4%) in HIV patients, no coccidian parasites were identified in the test group as shown in **Table 1.** The most prevalent coccidian species that were identified included *Cryptosporidium parvum* and *Isospora belli* i.e. (3.6%) and (1.8%) respectively. There was no *cyclospora cayetensis* identified as shown in **Table 2**. From **Table 3**, out of the study group it was shown that the distribution of coccidian parasites was greater in females with an occurrence of 4.5% as compared to 0.9% among the male population. Statistical analysis showed there existed no significant relationship between coccidian parasite and gender (p= 0.19). It was further shown that coccidian parasites were more common in children and less in middle age and the adults i.e. 2.7%, 1.8% and 0.9% respectively as shown in **Table 3.** Further analysis showed that there no significant relationship (p = 0.15). From those that had coccidian parasites, further demographic data analysis showed that c. parvum was more prevalent among females with 50% and less in males with 16.7% as compared to *I. belli* which occurred 33.3% of the female and none in males as shown in **Table 4**. Statistical analysis showed there existed no significant relationship between gender and the coccidian species identified i.e. (p = 0.19). **Table 4** further showed that among the population with coccidian parasites, *c. parvum* was 50% more common in middle aged than in children (16.7%). As regards to *I. belli,* it was the least common species with 16.7% in both adults and the elderly none in children. From **Table 5,** 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.3.6% and 1.8% respectively and further statistical analysis showed there existed no significant relationship (p = 0.19). Most people collected their water from taps (27.9%) and bore hole (66.7%) compared to river (1.8%) and community wells (3.6%) as shown in **Table 5**. From the study group, HIV patients who drunk raw water and were not taking co-trimoxazole had a higher prevalence of coccidian parasites of 4.5% and 4.5% respectively as compared to a low prevalence in those that took boiled water (0.9%) and were on co-trimoxazole (0.9%) as shown in **Table 5.** Further analysis showed there exists a strong relationships ($P < 0.05$) in HIV patients taking boiled water and co-trimoxazole. There were different causes of diarrhea in the study however, the researchers focused on coccidian parasites as the major element of study because of its ability to cause chronic diarrhea in immune suppressed persons.

Table 1 Showing prevalence of coccidian parasites

	Frequency (%)		
	Coccidian	No coccidian	Total
Study group	6 (5.4)	105 (94.6)	111 (100)
Control group	0 (0)	31 (100)	31 (100)
Total	6 (4.2)	136 (95.8)	142 (100)

Table 2 Showing prevalence of coccidian parasites in the study population

Species	Frequency (%)
C. parvum	4 (3.6)
I. belli	2 (1.8)
C. cayatenensis	0 (0)
Total	6 (5.4)

Table 3 Showing demographic distribution of coccidian parasites in the study group

	Frequency (%)		
	Coccidia	No Coccidia	Total
Female	5 (4.5)	65 (58.6)	70 (63.1)
Male	1 (0.9)	40 (36)	41 (36.9)
Total	6 (5.4)	105 (94.6)	111 (100)
Children	3 (2.7)	19 (17.1)	22 (19.8)
Middle aged	2 (1.8)	57 (51.4)	59 (53.2)
Adults	1 (0.9)	29 (26.1)	30 (27)
Total	6 (5.4)	105 (94.6)	111 (100)

Table 4 Showing demographic distribution of coccidian parasite species in the study group

	Frequency (%)			
	C. parvum	*I. Belli*	*C. cayatensis*	Total
Female	3 (50)	2 (33.3)	0 (0)	5 (83.3)
Male	1 (16.7)	0 (0)	0 (0)	1 (16.7)
Total	4 (66.7)	2 (33.3)	0 (0)	6 (100)
Children	1 (16.7)	0 (0)	0 (0)	1 (16.7)
Middle aged	3 (50)	1 (16.7)	0 (0)	4 (66.7)
Adults	0 (0)	1 (16.7)	0 (0)	1 (16.7)
Total	4 (66.7)	2 (33.3)	0 (0)	6 (100)

Table 5 Showing risk factors associated with diarrhea in coccidian parasitic infection in the study group.

		Frequency (%)		
		Coccidia	No Coccidia	Total
Water source	Bore hole	2 (1.8)	72 (64.9)	74 (66.7)
	Tap	4 (3.6)	27 (24.3)	31 (27.9)
	River	0 (0)	2 (1.8)	2 (1.8)
	Community well	0 (0)	4 (3.6)	4 (3.6)
	Total	6 (5.4)	105 (94.6)	111 (100)
Water quality	Drink boiled water	1 (0.9)	38 (97.4)34.2	39 (35.1)
	Drink raw water	5 (4.5)	67 (93.1)60.4	72 (64.9)
	Total	6 (5.4)	106 (94.6)	111 (100)
Prophylaxis treatment	Taking co-trimoxazole	1 (0.9)	51 (98.1)45.9	52 (46.8)
	Not taking co-trimoxazole	5 (4.5)	54 (91.5)48.6	59 (53.2)
	Total	6 (5.4)	106 (94.6)	111 (100)

4. DISCUSSION

The study showed a prevalence of 5.4% which was common in middle aged and children in females as compared to males and the most prevalent coccidian species identified was *C. parvum* as shown in **Table 1** and **Table 2**. A study in central Uganda revealed a prevalence of 25% in a population of over 1000 children suffering from diarrhea due to *C. Parvum* [11]. The major risk factor associated with coccidian parasites in the study was consumption of raw drinking water from the bore hole and the taps **(Table 3).** Community lifestyle patterns such as poor hygiene, poor nutrition standards and challenges associated with preparation of safe drinking water such as scarcity of fuel (fire wood) and transport to collect fire wood from distant woods are responsible for the laziness amongst community members to prepare safe drinking water which is in agreement with a recent study [3]. Poor sanitation habits such as failure to clean water collecting jerricans and water collection areas over long periods of time is a major factor observed leading to contamination of water collected from bore holes in the communities [4]. Due to challenges of financing which is characteristic of sub-Saharan African local government, servicing of water pipes is hardily carried out thus leading to sporadic leakages and contamination of the water [10]. 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 due to positive therapeutic effects of co-trimoxazole on coccidian intestinal parasites [3]. However one of the patients who was taking co-trimoxazole and had coccidian parasite infection could probably have stated taking medication shortly before the study and not all they parasites had completely been eliminated in the body. Drug resistance could also have been the cause of identified coccidian parasites in that patient on diarrhea treatment with co-trimoxazole. Co-trimoxazole drug resistance can occur as a result of irregularities in taking of the drug leading to genetic mutation in the parasite. It is there fore recommended to take co-trimoxazole as a prophylactic treatment in management of diarrhea in immune suppressed patients [12]. 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 [7]. 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 [13]. 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 [14] [15] [16]. Children are associated with a weak immunity and coupled with poor nutritional habits. Inferential analysis showed there existed 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 [17]. Therefore, this research might be help a community with these kind infections to know the risk factors associated with the disease spread, the major risk group, importance of taking prophylactic treatment and importance of early diagnosis before the disease can become chronic and cause body or muscle wasting, dehydration and death.

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 those that had diarrhea but did not have coccidian parasites, it is possible that some of these cases could have been infected with bacterial pathogens, enteric viruses or other protozoan parasites The prevalence of coccidian parasites in HIV patients was shown to be 5.4% and the major risk factors identified were consumption of un-boiled water from the taps and bore holes. HIV patients on prophylactic treatment were shown to have an added advantage than those who were not. The study further highlighted the importance to control secondary infection in HIV patients regardless of age and social status. Proper 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 gastro intestinal tract thereby rendering it pathogenic.

The major causes of coccidian infection is consumption of un-hygienically dirty or contaminate water with coccidian parasites, not taking prophylactic treatment in HIV disease and the risk group include majorly immune suppressed persons.

Therefore, 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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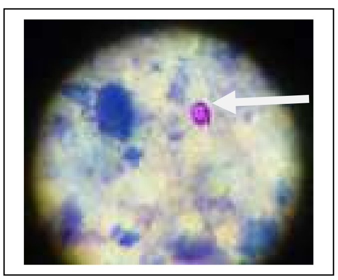
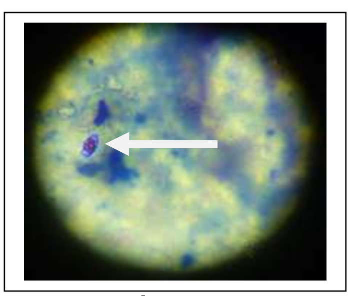
APPENDIX

Microscopic identification of the parasites



a



b

Fig. 1 Oocysts of *Cryptosporidium parvum* (a) x40 objective lens and *Isospora belli* (b) x40 objective lens in modified ZN staining