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SJMLS-1-2016-18 Intestinal Parasites among HIV/AIDS Patients on ART Attending Specialist Hospital, Sokoto State, Nigeria

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Abstract

The increase Acquired Immunodeficiency in Syndrome (AIDS) patients in the world particularly in the tropics has also resulted in the increased prevalence of opportunistic infections including intestinal parasitic infections among the patients. This study was conducted to determine the prevalence of intestinal parasites among HIV/AIDS patients in Sokoto - Nigeria. Stool specimens from HIV/AIDS patients and control group were screened for intestinal parasites. Intestinal parasites were identified using direct microscopy, formalin-ether concentration and Ziehl Neelsen methods. A total of 398 participants (150 HIV/AIDS patients on ART, 148 HIV/AIDS patients not on ART and 100 HIVnegative individuals) were tested among patients consulted at the hospital. The prevalence of intestinal parasites was 37.6% (91/150) among HIV positive on ART, 37.1% (80/148) among HIV patients not on ART and 66% (66/100) among HIV negatives. The overall prevalence of intestinal parasites in respect to gender shows that, HIV infected male had 64.6% (104/161) and female had 48.9% (67/137) when compared to HIV seronegatives 59.3% (32/54) and 73.9% (34/46) respectively for males and females. It was observed that among protozoa, Entamoeba histolytica had the highest prevalence of 19.8% (59/298) in HIV patients with least prevalence of Balantidium coli of 3.4% (10/298), the most commonest helminthes was Hookworm with 29.9% (89/298) followed by Ascaris lumbricoides 11.1% (33/298). We recommend that provision of safe drinking water and health education on efficient environmental sanitation and improving personal hygiene could help prevent the contamination of water, food and help promote health and wellbeing among HIV/AIDS patients.

Keywords: Intestinal Parasites, HIV/AIDS, Patients, ART, Specialist Hospital, Sokoto State, Nigeria.

Introduction

Human immunodeficiency virus (HIV) infection still remains a global challenge, and Sub-Saharan African countries including Nigeria contribute a great proportion to this epidemic. Adults are among the most vulnerable group to HIV infection and acquire HIV infections mainly through unprotected sexual intercourse which constitutes 90% of the route of infection among other routes which include unscreened blood transfusion, sharing of sharp object like razor blade, syringes and needles. HIV complications are attributed to either opportunistic infections or direct effects of virus to tissues. Opportunistic infection include diarrhoea which result from the infestation of intestinal parasites and the activation of commensal organisms such E. coli thereby becoming parasitic in nature. In the era of Highly Active Anti-retroviral Therapy (HAART), there has been a reduction in the incidence of opportunistic infections with reduction of both morbidity and mortality, which has paved way for manifestation of non-infectious complications including renal complications (Flemming, 2000). Acute and chronic diarrhoea has been associated with different species of gastrointestinal parasites, which are responsible for considerable morbidity and mortality in HIV/AIDS patients (Oguntibeju, 2006).

Intestinal parasitic infections play an important role in the progression of HIV infection, by further disturbing the immune system while is already engaged in the fight against HIV (Framm, 2000). The gastrointestinal pathology associated with HIV infection comprises a significant enteropathy with increased levels of inflammation and decreased levels of mucosal repair and regeneration (Douek, 2008). HIV infection leads to loss of CD4⁺T cells, which leaves affected individuals susceptible to opportunistic infections. Many of the opportunistic infections that ultimately plague such individuals



involve infectious agents that are normally checked mucosal barriers which the include bv Cryptosporidium spp, Giardia lamblia, Entamoeba histolvtica. Ascaris lumbricoides. hookworm infection, Schistosoma spp and Strongyloides stercoralis are important cosmopolitan intestinal parasites that are common among children and immunocompromised individuals (Gbakima, 2007). The pathogenic intestinal parasites such as Cryptosporidium, Cyclospora, E. histolytica and Giardia, can last for months in patients with AIDS, malabsorption of nutrients, gradual causing debilitation through dehydration, and metabolic abnormalities and are responsible for severe diarrhoea episodes (Hunter, 2002).

The sources of parasitic infections in humans include contaminated soil, food and water sources with human faeces and poor sewage disposal such as the use of night soil as fertilizer (Mustafa, 2001). Faecal oral route is significant in the transmission of parasitic infections via poor personal hygiene and environmental contamination. Nosocomial outbreaks of cryptosporidiosis have also been described, where an individual gets infected in the hospital.

In developing countries, gastrointestinal parasite infections are mostly due to poverty characterized by poor hygienic habits, absence of potable and clean water, absence of good faecal disposal system and poor nutrition. Pathogens responsible for causing diarrhoea differ according to geographical location, therefore laboratory diagnostic evaluations are required to determine their prevalence in each population so as to provide guidelines for therapy and necessary data for planning and evaluation of HIV/AIDS patients care and management. Furthermore, areas with different agro-ecological zones could favor the development of different intestinal parasites. The aim of this study was to determine the intestinal parasites in both HIV/AIDS patients on ART and those not on ART attending specialist Hospital Sokoto State Nigeria.

Materials And Methods

Description of study Area

Sokoto State, Nigeria has land mass area of 563.861 hectares, located at latitude 13.05 degree North and longitude of 5.15 degree East, bordered with Niger Republic at the North, Chad Republic and Cameroun at North East, Benin Republic at the West, Atlantic ocean in the West, Cameroun in the South East. Being situated in the Savannah on the low land arid region in the Northern Western part of Nigeria, it has an average high temperature of 44 degree Celsius annually.

Questionnaire Administration

Questionnaire was used to collect sociodemographic data (age, gender, educational status, marital status and occupational group) and other information (when diagnosed, how long subjects have lived with HIV, life style and history of HAART use).

Study design and sample collection

Persons living with HIV/AIDS were randomly selected from among patients attending the Inpatient and Outpatient Departments of Specialist Hospital, Sokoto. Verbal informed consent was obtained from all subjects after counseling. The inclusion criteria included; confirmed HIV-positivity, and willingness to offer a verbal informed consent to participate in the study. The following individuals who did not meet the inclusion criteria were excluded; HIVnegative individuals and non-consenting HIV positive individuals. HIV-seropositive patients were defined as those who have tested and found positive for HIV-1 and 2 antibody using two consecutive rapid test (Abbott, USA) while AIDS patients in addition to being HIV-positive show clinical symptoms classified by WHO and CDC as symptoms for AIDS. Participants included 100 HIVnegative controls, 150 HIV patients on HAART and 148 HIV patients who were HAART naive.

Specimen Collection

Stool samples was collected into clean wide-mouth specimen containers from male and female patients attending Specialist Hospital Sokoto for clinical treatment. A total of 398 samples (150 HIV/AIDS patients on ART, 148 HIV/AIDS patients not on ART and 100 HIV-negative individuals). Subjects were aged 1–50 years.

Specimen Processing and Laboratory Analysis

Freshly voided stool specimens was processed and examined microscopically using low magnification (x10 and x40) objective lens for saline wet mount to detect motile trophozoites (Garcia, 2002). Formolether concentration was performed and the sediment examined as iodine wet mounts to detect ova, larva and cysts. Then x40 objective fields of the stool smears were examined before a slide was considered negative. Air-dried smears prepared from fresh stool samples were stained by a modified acid-fast stain (AFS) to detect *Cryptosporidium* and *Isospora* Occyst.



of intestinal parasite was found among those that have CD4 counts 201-500 cells/µl and 501-800 cells/ μ l 60.4% (61/101) and 64.4% (96/149)respectively. There was a statistically significant difference in the occurrence of intestinal parasites based on the CD4 count of the HIV infected patients (X^2 = 25.537, p < 0.05). The effect of ART treatment was investigated among the HIV-infected subjects. Table 3: shows the prevalence of intestinal parasite among HIV infected subjects on ART and not on ART as well as HIV negative participants. The prevalence of intestinal parasites were found to be highest among the HIV negative controls participants 66.0% (66/100) compared to HIVinfected subjects on ART 60.7% (91/150) and HIV positive subjects not on ART 54.1% (80/148). The difference however was not statistically significant $(X^2=3.66, p > 0.05)$. The distribution of the various intestinal parasites among the HIV Infected Subjects is shown in Table 4. Among the protozoan, Entamoeba Histolytica was found to be the most prevalent with 19.8% (59/298), while hookworm 29.9% (89/298) was found to be the most prevalent helminthes.

The prevalence of intestinal parasites among the HIV infected and HIV negative participants was compared based on marital status. Among the HIVinfected subjects, divorcees had the lowest prevalence 25% (3/12) while singles had the highest prevalence (X^2 =20.745, p <0.05%). Among the HIVnegative participants, the highest prevalence of infection was observed among the married 97.4% (38/39) while the singles had 45.9%(28/61) $(X^2=28.156, p < 0.05)$. Table 6 shows the prevalence of intestinal parasites among the HIV infected and HIV negative participants based on marital status. The prevalence of intestinal parasite was compared among the HIV infected and negative participants based on gender. Among the HIV infected subjects , males recorded the highest prevalence of infection 64.6% (104/161) compared to female, 48.9%(67/137).The difference was however not statistically significant ($X^2=2.377$, p >0.05). Among the HIV negative, females recorded higher prevalence of infection 73.9%(34/46) compared to male 59.3% (32/54). The difference was however not statistically significant ($X^2=7.455$, p >0.05). Table 5 shows the gender- related occurrence of intestinal parasites among the HIV infected and HIV negative participants. The prevalence of intestinal parasite was compared among the HIV infected and HIV negative participants based on their level of Educational attainment. Among the HIV-infected, the prevalence of intestinal parasites was highest

Preparation of Wet Mount

A drop of saline was placed in the centre of the left half of the slide, with an applicator stick 2mg of faeces was added to the saline (0.85%), mixed to obtain a uniform suspension, covered with a cover slip and examined.

Formalin-ether Technique

About 0.5g of stool was transferred to10% formalin (formaldehyde 10ml, distilled water 90mls), in a 15ml test tube. It was mixed and allowed to stand for 30 minutes for adequate fixation. the faecal suspension was Strained through two layers of gauze in a funnel into a centrifuge tube, 4ml of Diethyl ether was added into the tube, the tube was closed with a glass stopper and shaken for 30 seconds and the stopper was remove carefully. The tube was centrifuge for two minutes at 500g, the plug of debris was loosened from the side of the tube using an applicator stick, the tube was rapidly inverted to pour off the ether, the debris and the formalin. The sediment was transferred onto a slide covered with cover slip and examined for parasite, count the number of each type of parasites in the entire preparation and the report was based on the number of parasite per gram of feaces (Ochie et al., 2008).

Modified Acid Fast Stain

The strong Carbol Fuchsin, 3% Acid Alcohol and Methylene Blue was used. Fecal smear on the glass slide was allowed to dry and fixed with gentle heat, carbol fuchsin was applied and heated until steam was produced. It was washed with water, 3% acid alcohol was applied until colorless, wash with water and methylene blue was applied allowed for 30 seconds. The slide was washed with water and blotted and dried. Stained slides were examined at ×100 objective (Cheesbrough, 2007).

Results

Occurrence of intestinal parasites in HIV negative and HIV infected patients attending Specialist Hospital Sokoto were investigated for intestinal parasites. Table 1 shows the distribution of intestinal parasites based on age groups. Intestinal parasites have been found to be more prevalent in the HIV negative patients, 66% (66/100) compared to HIV infected, 57.4% (171/298). The difference however was not statistically significant (X²= 80.537, p > 0.05). The prevalence of intestinal parasites was compared among the HIV-infected subjects based on their CD4 lymphocyte count. Table 2: shows the occurrence of intestinal parasites in relation to CD4 counts of HIV infected patients attending Sokoto Specialist Hospital, Sokoto. The highest occurrence



among those educated up to the primary school level 79.7% (51/64) while the lowest prevalence was observed among those with no formal education 43.2% (51/118) (X^2 =14.47, p <0.05). Among the HIV-negative participants, the highest prevalence of infection was observed among those educated up to the primary educational level with 100% (13/13), while those with secondary education had the lowest prevalence 53.6% (15/28) (X^2 =29.754, p<0.05). The prevalence of intestinal parasites among the HIV infected and HIV negative participants was

compared based on their occupational groups. Among the HIV -infected, the highest prevalence was observed among students 27(69.2%) while the lowest prevalence was observed among teachers and commercial sex workers 0(0%) (X²=29.08, p< 0.05). In HIV-negative, the highest prevalence of infection was observed in the traders and civil with 100% (15/15) and 100% (15/15) servant respectively, while the lowest prevalence was observed among Teachers, farmers and among commercial sex workers 0% ($X^2=31.14$, p < 0.05).

	Table 1: Distribution of Intestinal Parasites Based on Age Groups.								
Age Group	Intestinal Parasites								
(Years)	HIV-Negative Number Tested	Number Positive	(%)	HIV-Positive Number Tested	Number (%) Positive				
1-5	0	0(0.0)		6	6(100.0)				
16-20	37	4(10.8)		41	23(56.1)				
21-25	47	47(100.0)		28	28(100.0)				
26-30	0	0(0.0)		68	31(45.6)				
31-35	0	0(0.0)		39	12(30.8)				
36-40	11	11(100.0)		52	28(53.8)				
41-45	5	4(80.0)		34	21(61.8)				
46-50	0	0(0.0)		30	22(73.3)				
Total	100	66(66.0)		298	171(57.4)				



CD4 counts/µl	Intestinal Parasites based on the CD4 Intest	tinal Parasites (%)
•	Number Examined	Number (%) Positive
< 200	9	0(0.0)
201-500	101	61(60.4)
501-800	149	96(64.4)
801-1100	39	14(35.9)
Total	298	171(57.4)

Table 2: Prevalence of Intestinal Parasites based on the CD4 count of HIV infected Subjects.

Table 3: Prevalence of Intestinal Parasite among HIV Positive Subjects on ART, not on ART and HIV negative participants.

HIV and ART Status	Intestinal Parasites				
	Number Tested	Number (%) Positive			
HIV Positive (On ART)	150	91(60.7)			
HIV Positive (Not on ART)	148	80(54.1)			
HIV Negative	100	66(66.0)			
Total	398	237(59.5)			

Table 4: Distribution of the various intestinal parasites among the HIV Infected Subjects

Intestinal Parasites	Intestinal Parasites				
	Number Examined	Number (%) Positive			
Protozoa					
Entamoeba histolytica	298	59(19.8)			
Giardia lamblia	298	17(5.7)			
Balantidium coli	298	10(3.4)			
Entamoeba coli	298	19(6.4)			
Cryptosporidium parvum	298	14(4.7)			
Isospora oocyst	298	15 (5.0)			
Helminthes					
Ascaris lumbricoides	298	33(11.1)			
Trichuris trichura	298	30(10.1)			
Enterobius vermicularis	298	4(1.3)			
Strongyloides stercorlaris	298	13(4.4)			
Teania solium	298	11 (3.7)			
Hook worm	298	89 (29.9)			



Table 5: Prevalence of Intestinal Parasite among the HIV infected and negative participants	based on
gender.	

Gender			Study	Groups		
	HIV-Infected Subjects		HIV-negative controls			
	Number	Number	(%)	Number	Number	(%)
	Examined	Positive		Examined	Positive	
Male	54	32(59.3)		161	104(64.6)	
Female	46	34(73.9)		137	67(48.9)	
Total	100	66(133.2)		298	171(113.5)	

Table 6: Prevalence of Intestinal Parasites among the HIV infected and HIV negative participants based on marital status.

Marital Status	Subject Groups				
	HIV-Infected Subjects		-	HIV-negative c	ontrols
	Number Examined	Number Positive	(%)	Number Examined	Number (%) Positive
Single	61	28(45.9)		53	43(81.1)
Married	39	38(97.4)		180	101(56.1 <u>)</u>
Divorced	0	0		12	3(25.0)
Separated	0	0		12	5(41.7)
Concubinage	0	0		41	19(46.3)
Total	100	66(143.3)		298	171(250.2)

Table 7: The prevalence of Intestinal Parasites among the HIV infected and HIV negative participants based on their level of Educational attainment.

Education		Subject Groups				
Status	HIV-Infected Subjects		HIV-negative controls			
	Number Examined	Number	(%)	Number	Number (%)
		Positive		Examined	Positive	
No formal	13	13(100)		118	51(43.2)	
Primary	13	13(100)		64	51(79.7)	
Secondary	28	15(53.6)		64	45(70.3)	
Post Secondary	46	26(56.5)		52	24(46.2)	
Total	100	67(302.4)		298	171(239.4)	

Occupational	Subject Groups						
groups	HIV-Infected S	-					
	Number Examined	Number Positive	(%)	Number Examined	Number Positive	(%)	
Unemployed	11	10(90.9)		43	29(67.4)		
Trading	15	15(100)		96	52(54.2)		
Students	59	26(44.1)		39	27(69.2)		
Civil servant	15	15(100)		40	16(40.0)		
Teaching	0	0		1	0(0)		
Farming	0	0		57	42(73.7)		
Commercial Sex Worker	0	0		5	0(0)		
Others	0	0		17	5(29.4)		
Total	100	66(250)		298	166(333.9)		

 Table 8: The prevalence of Intestinal Parasites among the HIV infected and HIV negative participants based on their occupational groups.

Discussion

HIV and AIDS patients have reduced immune response, thus are at high risk of acquiring gastrointestinal parasitic infections which are responsible for considerable morbidity and mortality in HIV and AIDS patients. The prevalence of intestinal parasites among HIV patients observed in this present study was 57.4%. Our observed prevalence is in consistent with similar studies carried out in Ethiopia 52.6% (Hailemarian, 2004), 59.8% (Assefa, 2009), 47% in Turkey (Buyukbaba, 2004), 50% in Thailand (Wiwanitkit, 2001) and 50 to 80% (Ramakrishna, 1999) in India. The difference in the intestinal parasitic infection could be related to individual personal hygiene, which could come from the water and food which is a major source of intestinal parasite, Among pediatrics, the most common source are the soil or floor during child play and through mechanical sources. The distribution of intestinal parasites varies according to the geographical location due to different climatic conditions. The reason for the high prevalence among HIV-infected in Sokoto, may be due to the fact that majority of the population are urban dwellers stricken with poverty characterized by unhygienic habits, absence of potable and clean water, absence of good faecal disposal system, low level of education and poor nutrition, hence increasing the rate of infection of intestinal parasites.

The gastrointestinal pathology associated with HIV infection comprises significant enteropathy with increased levels of inflammation and decreased levels of mucosal repair and regeneration (Douek, 2008). HIV infection leads to loss of CD4⁺T cells, individuals which leaves affected mortally susceptible to opportunistic infections. Many of the opportunistic infections that ultimately plague such individuals involve infectious agents that are normally checked by the mucosal barriers which include Cryptosporidium spp, Giardia lamblia, Entamoeba histolytica, Ascaris lumbricoides and hookworm infection. In this study, we observed that there is a significant difference in the occurrence of intestinal parasites and CD4 count of the HIV infected patients. There was significant different in the prevalence of intestinal parasites between individual with CD4 801-1100 and lower CD4 count. The difference could be as result of more better quality of life, and personal hygiene. A healthy immune system help resist the infestation of some of the intestinal parasites. One major effect of intestinal parasites is the atrophy of the villi and inflammatory reaction could also be a contributing factors to the mal-absorption process.

In this present study, we observed a high prevalence of 60.7% of intestinal parasites among HIV patients on ART when compared with those not on ART of 54.1% and when compared to HIV negative which had prevalence of 66%. The reason of a higher



prevalence among HIV-negative participants compared to HIV infected subjects may be due to poor personal hygiene of seronegative individual. Intestinal parasitic infections play an important role in the progression of HIV infection, by further disturbing the immune system which is already engaged in the fight against HIV (Framm, 2000). The gastrointestinal pathology associated with HIV infection comprises significant enteropathy with increased levels of inflammation and decreased levels of mucosal repair and regeneration (Douek, 2008). HIV infection leads to loss of CD4⁺T cells, which leaves the affected individuals mortally susceptible to opportunistic infections. Many of the opportunistic infections that ultimately plague such individuals involves infectious agents that are normally checked by the mucosal barriers which include Cryptosporidium spp, Giardia lamblia, Entamoeba histolytica, Ascaris lumbricoides. hookworm infection, Schistosoma spp and Strongyloides stercoralis are important cosmopolitan intestinal parasites that are common among children and immunocompromised individuals (Gbakima, 2007). The pathogenic intestinal parasites such as Cryptosporidium, Cyclospora, E. histolytica and Giardia, can last for months in patients with AIDS, malabsorption of nutrients, gradual causing debilitation through dehvdration, and metabolic abnormalities and are responsible for severe diarrhoea episodes (Hunter, 2002).

Majority of the intestinal parasites observed among HIV-infected subjects in this study were protozoan with few helminths. In the research it was noticed that the most commonest protozoa was Entamoeba histolytica (19%) while the least was Balantidium coli (3.4%). The most prevalent helminthes was hookworm (29.9%) while the least prevalent was E. vermiculeris (1.3%). We observed a prevalence of Isospora belli of 5% among HIV infected patients. Our observed prevalence of Isospora belli is consistent with work done in other parts of the world; 5% in Thailand by Wiwanikit and colleagues (2001) and 7% in Brazil (Silver et al., 2005). In two studies done in China, Isospora belli was not reported (Tian et al., 2012). In contrast, a low prevalence of 1.3% was observed in Yaounde (Safari et al., 2006). Similarly, 1.9% prevalence was reported in Congo (Wumba et al., 2010). Also Roka (2012). Our finding is also consistent with report among a cohort of HIV-infected subjects in Equatorial Guinea which reported intestinal parasitic infection rate of 85.5% with 83.8% being pathogenic helminthes and 55.4% being pathogenic protozoa. Different species of protozoa have been associated with acute and chronic diarrhea in HIV infection and AIDS, namely *cryptosporidium parvum*, *isospora belli*, *entamoeba hystoolitica and Giardia lamblia*. These pathogens account for significant number of cases of diarrhoea (Wiest et al., 2009). The environmental conditions which include temperature, wind and humidity in the arid region favor the viability and maturation eggs of helminths and also the cyst of the intestinal protozoan are relatively resistant against adverse environment which leads to high prevalence of infection.

It was discovered that male had high intestinal parasites in both sero-negative and HIV infected. The reason for this male gender predisposition is unknown. Poor personal hygiene and poor environmental sanitation leading to contamination of soil and water sources may be responsible for the high prevalence of intestinal parasites among the subjects in this study. The inhabitants of rural areas obtain untreated drinking water from the river which exposes them to high risk of contamination. Previous report indicates that poor personal hygiene and poor environmental sanitation leading to contamination of soil and water sources is responsible for high incidence of intestinal parasites (Rukmanee *et al.*, 2008).

The prevalence of intestinal parasites was higher among less educated subjects with no-formal education and primary education. The reason for this observation may be due to the fact that better educated individuals have access to more resources, have better access to safe drinking water and better quality of life compared to the less educated. Also better educated individual can make better informed decision about their health. Most less educated individuals are rural dwellers. They have problem of access to clean and portable drinking water. This predisposes them to infection with intestinal parasites. Less educated persons tend to live in congested houses which can compromise sanitation and nutrition. Low level of education especially in parents could lead to intestinal parasitic infection, because they lack information on the importance of practicing good hygiene and sanitation.

In this study, we observed a higher prevalence of intestinal parasites among singles HIV-infected and HIV-negative participants. The reason for this predisposition is unknown. However, the source where drinking water is taken has important value to health and to the quality of life. Sources like rivers, lakes, and even groundwater from which drinking water is taken may contain a wide variety of contaminants, both chemical and microbial, that can

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cause illness and disease. These water sources can be contaminated with parasitic elements that cause disease due to various unhygienic practices like defecating around water sources. These elements can then get their way back to water sources when washed down by rain water leading to water contamination. Also, basic hygiene is impossible to achieve if there is insufficient quantity of water.

Among the HIV-infected, intestinal parasites was more prevalent among traders and students. The reason for this occupational -related predisposition may be associated with their personal hygiene. Environmental sanitation and personal hygiene may probably play a role for the higher occupationalrelated prevalence rate. The source of drinking water can play a significant role in predisposition to intestinal parasites. Using water from a river and unprotected well/spring were risk factors for intestinal parasite infection. This may arise from the contamination of water with animals and human waste (Udonsi and Amabibi, 1992).

Conclusion

There was high prevalence of intestinal parasites among HIV positive patients. Provision of safe drinking water and health education on efficient environmental sanitation and improving personal hygiene can help curb water, food and individual contamination, thus promoting good health.

Recommendations

In view of the increase in intestinal parasite observed among HIV-infected subjects in this study, the following are recommended to reduce if not eliminate the infestation of intestinal parasites; increase in personal hygiene, food should be thoroughly washed with salt before consumption especially vegetables, care in handling young infants in their contact with the environment, regular stool analysis and creating of awareness and education.

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