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## Prevalence of Intestinal and Urinary Helminth Parasite Infections among Residents of Communities around Lake Alau, Maiduguri

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Intestinal and urinary helminth infections affect up to a quarter of the world's population, with the potential of causing significant public health and economic burden. The occurrence of helminth parasites in stool and urine of residents, and water and soil samples of some communities around Lake Alau, Maiduguri, Nigeria was determined using direct microscopy, floatation and sedimentation techniques. A total of 349 (34.7%) residents were infected out of the 1,005 examined, consisting of 220 (21.9%) males and 129 (12.8%) females, with significant ( $p < 0.05$ ) variation based on gender. Similarly, the infection was significantly ( $p < 0.05$ ) higher among young individuals 197 (19.6%) than the adults 152 (15.1%). Among the ten communities (Logajiri, Melari, Dumbari, Alau Limanti, Awa Isari, Limanti, Gaskeri, Attakeri, Chellomi and Gomari Karekəri) examined, the infection was significantly ( $p < 0.05$ ) highest at Gomari Karekəri 57 (5.7%) and Alau Limanti 55 (5.5%) and least at Gaskeri 19 (1.9%) and Chellomari 20 (2.0%). Intestinal parasites encountered consist of *Ascaris lumbricoides* 234 (23.3%), *Trichuris trichiura* 24 (2.4%), *Strongyloides stercoralis* 10 (1.0%), *Schistosoma mansoni* 8 (0.8%), and *Taenia* species 1 (0.1%) ( $p < 0.05$ ). *Schistosoma haematobium* 72 (7.2%) was the only helminth parasite recovered from urine samples examined. Environmental (water and soil) contamination with parasite stages was predominantly with pre-parasitic nematode larvae 50 (25%), eggs of *Strongyloides stercoralis* 50 (25%), *Ascaris lumbricoides* 30 (15%), *Strongyle* spp. 30 (15%) and cysts of protozoans; *Entamoeba histolytica* 30 (15%) and *Giardia intestinalis* 10 (5%). The results suggest that infection with intestinal and urinary parasites is common among the residents of the study area and that socio-cultural habits may favour the survival and transmission of these parasites in the area.

**Keywords:** Communities; Intestinal parasites; Lake Alau; Maiduguri; Residents; Urinary parasites**INTRODUCTION**

Infections due to pathogenic organisms like viruses, bacteria, protozoa and helminths are the most common health problems usually associated with drinking water (Nwosu and Hamza, 2005). An estimated over 2 billion people worldwide are infected with soil-transmitted helminths, which are responsible for Disability Adjusted Life Years (DALYs) of approximately 39 million (Mardu *et al.*, 2019). This indicates a huge economic burden on the world's population, especially in the regions of Asia, Africa and South America. Helminthic infections are generally production limiting and insidious in nature, and may be neglected especially on mild presentation. They often thrive well in settings where there is absence or low level of hygiene, poverty and defective socio-economic standards as obtain in rural communities.

In Nigeria, about 64% of the population live in rural communities (Reed and Mberu, 2014), while over 70% of households in rural communities do not have access to improved water supply as they rely solely on self-water supply such as rivers, perennial streams, water ponds and unprotected means that may predispose to water-borne diseases (Ishaku *et al.*, 2011). The usage of unsafe water has been a major factor responsible for the spread of water-borne and related diseases in rural communities (Udoh, 1982; Odikamnor and Ozowara, 2014).

Despite Government's effort in providing basic social/health care facilities, many communities in Borno State are left to source water for drinking and other domestic purposes from streams, wells, ponds, and water vendors from unknown sources. This results in frequent outbreaks of diseases with associated high morbidity and sometimes mortality due to a combination of inadequate health care, lack of potable water

supply, poor sanitation services and unhygienic practices in these rural communities.

In the communities around Lake Alau, potable safe drinking water source is completely absent, as such this study was designed to determine the variety and prevalence of intestinal and urinary parasitic infections among the communities around the lake, and to assess the socio-cultural habits of the residents that may contribute to the contamination of the environment with parasitic stages. The study equally evaluated the level of contamination of the environment (land and water) with parasitic organisms infective to man and domestic animals. Furthermore, the absence of previous data on urinary and intestinal helminthes from these communities partly informed the need for the conduct of the study.

## MATERIALS AND METHODS

### Study Population and Area

This study was carried out in the communities (Logajiri, Melari, Dumbari, Alau Limanti, Awa Isari, Limanti, Gaskeri, Attakeri, Chellomi and Kerakiri) on the fringes of Lake Alau, near Maiduguri. Maiduguri is the capital and largest urban centre of Borno State located in the North-eastern corner of Nigeria with an area of 69,436 square Kilometres and a population of about 4,151,193 million people (NPC, 2006). The city gets its major water supply from the lake water. The vegetation of the area is semi-arid, characterized by a short rainy season of four to five months (June to September or October), followed by a prolonged dry period for the rest of the year (Hess *et al.*, 1995).

### Ethical Consideration

Ethical clearance for the study was sought and obtained from the Ethics Committee of the Ministry of Health Borno State, Nigeria.

### Sample Collection

Verbal consent of the residents that participated in the study was sought and obtained before the commencement of the study. Consent for participation was the only inclusion criterion for all the participating individuals. A total of 10 communities were selected based on convenience for the study. Fresh faecal (5g) and urine (5ml) samples were collected from the respondents directly into appropriately labelled containers and transported to the laboratory for immediate examination, while soil samples (20g) each were collected directly into appropriately labelled containers from 10 different sites, 200 meters apart, within each study community according to standard criteria (Soulsby, 1982). Water samples (500 ml) were collected into a beaker for parasitological examination from different randomly selected points 200 meters apart in the lake from which residents of the area either wash up, bathe, draw water for drinking or other domestic purposes. A total of 1,005 each of urine and stool were collected, while one hundred (100) samples each of soil and lake water were similarly collected.

### Stool and Soil Samples Examination

The stool and soil samples collected were examined using both the direct microscopic examination and the floatation methods (Fleck & Moody, 1988; MAFF, 1997).

### Examination of Urine Samples

Collected urine samples per individual were placed in a test tube and centrifuged at 1500 g/sec for 2 minutes. The supernatant was carefully decanted and the sediment reconstituted with a drop of the remaining urine sample. A drop of the reconstituted urine sample was prepared and examined microscopically as described earlier (Fleck and Moody, 1988; MAFF, 1997).

### Lake Water Examination

Water samples were left to stand on the laboratory bench for about two hours to allow all particulate matter and parasite stages to sediment to the bottom of the container. The volume of the water was then reduced to about 20 ml without disturbing the sediments. The remaining quantity was then dispensed into centrifuge tubes and centrifuged at 500 rpm for 5 minutes (MAFF, 1997). The supernatant solution was carefully decanted and the sediments reconstituted with a few drops of the remaining water. These were then transferred onto a Petri dish or clean microscope slide. A drop of Lugol's iodine was added to each sample to enable visualization of the parasite stages present. In each case, the samples were carefully examined microscopically using the x10 and x40 objective lenses of a stereo or light microscope. Some of the slides were also air-dried and the preparation stained with Giemsa stain to further examine and identify protozoan stages present. In this case, the smears were fixed with absolute methanol before placement in staining containers containing Giemsa stain, and allowed to stain for 30 minutes. Thereafter, they were washed with buffered saline solution and allowed to air-dry. The slides were then examined for parasite stages using x100 (oil immersion) objective lens of the microscope (MAFF, 1997).

### Data Analysis

Data generated from the study were analysed using SPSS version 17 (SPSS, 2011). Significant variation among and between variables was tested using chi square, while  $p < 0.05$  was considered significant throughout the study.

## RESULTS

The results showed that out of the 1,005 individuals examined in the ten communities around the Alau lake area, 349 (34.7%) were infected with at least one urinary or intestinal parasite (Table 1). Between the sexes, 220 (31.2%) males were infected out of the 705 examined, and this was significantly ( $p < 0.05$ ) higher than the infection in females, where 129 (43.0%) individuals were infected among the 300 examined. Similarly, young persons were more infected 197 (37.8%) than the adults 152 (39.5%). Among the ten communities involved in the study, the highest frequency of infected individuals were from Gomari Karekari 57 (50.9%) and Alau Limanti 55 (29.1%), while the least infections were recorded from Gaskeri 19 (26.8%), Chellomari 20 (24.7%) and Altakeri 21 (33.9%).

Table 2 shows the distribution of recovered parasite species based on the sexes and ages of infected residents. The intestinal parasites recovered consisted of *Ascaris lumbricoides* found among 234 (23.3%) residents, *Trichuris trichiura* in 24 (2.4%), *Strongyloides stercoralis* in 10 (1.0%), *Schistosoma mansoni* in 8 (0.8%) and *Taenia* species

1 (0.1%) infected residents ( $p < 0.05$ ). In general, individual intestinal parasites were relatively more common in male than female individuals ( $p < 0.05$ ). Also, young residents were more commonly infected with individual intestinal parasites than adults ( $p < 0.05$ ) except for *Schistosoma mansoni* that was more prevalent in adults than young persons. The only parasite recovered from urine samples was *Schistosoma haematobium* which occurred in 72 (7.2%) of the individuals examined during the study with similar prevalence between the sexes and age groups.

The prevalence of the recovered parasite species among the study communities is presented in Table 3. *Ascaris lumbricoides* occurred in a relatively high ( $p < 0.05$ ) prevalence in all the ten communities involved in the study, ranging from 39.7% in Dumbari to 12.6% in Awa Isari. The other parasites that were also recovered in relatively high prevalence in some of the communities included *Schistosoma haematobium* and *Trichuris trichiura*. On the other hand, *Schistosoma mansoni*, *Strongyloides stercoralis* and *Taenia* species were recorded in very low prevalence and only in a few of the communities.

**Table 1:** Prevalence of Intestinal helminth parasites among residents of communities around Lake Alau, Maiduguri, Northeastern Nigeria

Parameters	No. examined	No. infected (%)	P-value
<b>Sex</b>			
Male	705	220 (31.2) <sup>a</sup>	0.000
Female	300	129 (43.0) <sup>b</sup>	0.000
<b>Age</b>			
Adult	385	152 (39.5) <sup>a</sup>	0.015
Young	620	197 (37.8) <sup>b</sup>	0.015
<b>Community</b>			
Logojiri	71	36 (50.7) <sup>a</sup>	0.005
Meleri	126	37 (29.4)	0.211
Dumbari	78	35 (44.9) <sup>b</sup>	0.050
Alau Limanti	189	55 (29.1)	0.086
Awa Isari	95	23 (24.2) <sup>f</sup>	0.032
Limanti	120	46 (38.3)	0.434
Gaskeri	71	19 (26.8)	0.182
Attakeri	62	21 (33.9)	0.884
Chellomari	81	20 (24.7) <sup>f</sup>	0.047
Gomari Karekari	112	57 (50.9) <sup>d</sup>	0.000
<b>Total</b>	<b>1,005</b>	<b>349 (34.7)</b>	

Values with different superscripts in 3<sup>rd</sup> column differed significantly ( $p < 0.05$ )

## DISCUSSION

The result of this study indicated an overall prevalence (pooled) of 34.7% for intestinal and urinary parasitism, similar to the 34.64% reported by Oluwole *et al.* (2018) among school pupils in Ogun State, South-western, Nigeria. Also, the species (*Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Schistosoma mansoni*, *Taenia* spp, *Schistosoma haematobium* *Strongyle* spp, *Entamoeba histolytica* and *Giardia intestinalis*) of parasites identified are similar to those reported by Muhammad *et al.* (2014) from Maiduguri. These types of parasites are the most commonly encountered in rural communities where prevalence is often associated with poverty, ignorance, poor environmental hygiene, and impoverished health services; and where infection poses serious threat to healthy living.

The findings in this study of a significantly higher prevalence amongst males than females agree with Muhammad *et al.* (2014) who sampled patients at a tertiary health facility in Maiduguri, and this may be attributed to variations in

Table 4 shows the frequency of recovery of parasite stages from the lake water and soil samples examined in the communities around the Alau lake area during the study. A total of five (*Ascaris*, *Strongyloides*, *Strongyle* type, *Giardia intestinalis* and *Schistosoma mansoni*) different parasites ova were recovered from soil and water samples examined during the study. This is addition to *Entamoeba histolytica* cyst and pre-parasitic nematode larvae. The eggs of *Strongyloides stercoralis* were the most prevalent and were present in 50 (50%) of the soil samples, while *Strongyle* type eggs were present in 30 (30%) of the samples. *Ascaris lumbricoides* eggs were present in 20 (20%) of the soil samples, followed by pre-parasitic nematode larvae and arthropod larvae found in 10 (10%) of the soil samples in each case. Furthermore, 40 (40%) of the examined water samples harboured pre-parasitic nematode larvae, while *Entamoeba histolytica* cysts were found in 30 (30%) of the water samples. *Giardia intestinalis* cysts, *Ascaris lumbricoides* eggs and *Schistosoma mansoni* eggs were each found in 10 (10%) samples.

frequency and intensity of occupational exposures. It however, disagreed with Biu *et al.* (2009) who observed a higher prevalence in females than males, which may be related to the socio-economic disposition of the studied population. Most of the parasite's ova were recovered from soil (50.0%) compared to water (23.1%) samples ( $p > 0.05$ ) in this study, which agrees with Anosike *et al.* (2006) who observed that geo-helminths are very common in tropical areas. This may relate to the fact that parasites ova in soil have reduced chances of translocation, compared to those found in water which are constantly taken from one place to another due to moving water bodies.

Also, in this study, young residents were more infected (19.6% ( $p < 0.05$ )) than the adults (15.1%), giving credence to the earlier finding of Mardu *et al.* (2019) which showed an increase in infection rate from childhood to young adulthood and a decline in adulthood.

**Table 2:** Species distribution of helminth parasites among infected residents of communities around Lake Alau, Maiduguri, Borno State based on sex and age

Parasites	Variable	No. examined	No. (%) infected	P-Value
<i>Ascaris lumbricoides</i>	Male	705	141 (20.0) <sup>a</sup>	0.000
	Female	300	93 (31.0) <sup>b</sup>	0.000
	<b>Age</b>			
	Young	385	139 (22.4) <sup>a</sup>	0.000
	Adult	620	95 (24.7) <sup>b</sup>	0.000
<i>Trichiuris trichiura</i>	<b>Sex</b>			
	Male	705	13 (1.8) <sup>a</sup>	0.132
	Female	300	11 (3.7) <sup>a</sup>	0.132
	<b>Age</b>			
	Young	385	18 (2.9) <sup>a</sup>	0.000
	Adult	620	6 (1.6) <sup>b</sup>	0.000
<i>Schistosoma mansoni</i>	<b>Sex</b>			
	Male	705	8 (1.1)	0.064
	Female	300	0(0.0)	
	<b>Age</b>			
	Young	385	2 (0.3) <sup>a</sup>	0.680
	Adult	620	6 (1.6) <sup>a</sup>	0.680
<i>Schistosoma haematobium</i>	<b>Sex</b>			
	Male	705	50 (7.1) <sup>a</sup>	0.892
	Female	300	22 (7.3) <sup>a</sup>	0.892
	<b>Age</b>			
	Young	385	31 (5.0) <sup>a</sup>	0.463
	Adult	620	41 (10.6) <sup>a</sup>	0.463
<i>Strongyloides stercoralis</i>	<b>Sex</b>			
	Male	705	8 (1.1) <sup>a</sup>	0.736
	Female	300	2 (0.7) <sup>a</sup>	0.736
	<b>Age</b>			
	Young	385	2 (0.7) <sup>a</sup>	0.802
	Adult	620	4 (1.0) <sup>a</sup>	0.802
<i>Taenia</i> spp.	<b>Sex</b>			
	Male	705	0(0.0)	0.125
	Female	300	1 (0.1)	
	<b>Age</b>			
	Young	385	1 (0.2)	0.204
	Adult	620	0(0.0)	

Values with different superscripts in columns differed significantly ( $p < 0.05$ )

**Table 3:** Distribution of parasite species among residents of various communities around Lake Alau, Maiduguri

Community	<i>Ascaris lumbricoides</i>	<i>Trichuris trichiura</i>	<i>Schistosoma mansoni</i>	<i>Schistosoma haematobium</i>	<i>Strongyloides stercoralis</i>	<i>Taenia</i> species	Total
Logojiri	10 (14.1) <sup>a</sup>	0(0.0) <sup>a</sup>	3 (4.2) <sup>a</sup>	5 (7.0) <sup>a</sup>	0(0.0) <sup>a</sup>	0 (0.0) <sup>a</sup>	18 <sup>a</sup>
Meleri	28 (22.2) <sup>b</sup>	3 (2.4) <sup>b</sup>	2 (1.6) <sup>b</sup>	4 (3.2) <sup>b</sup>	2 (1.6) <sup>b</sup>	0 (0.0) <sup>a</sup>	39 <sup>b</sup>
Dumbari	31 (39.7) <sup>c</sup>	1 (1.3) <sup>b</sup>	0(0.0) <sup>b</sup>	4 (5.1) <sup>b</sup>	0 (0.0) <sup>a</sup>	1 (1.3) <sup>b</sup>	37 <sup>b</sup>
Awa Isari	12 (12.6) <sup>a</sup>	8 (8.4) <sup>c</sup>	1 (1.0) <sup>a</sup>	4 (4.2) <sup>b</sup>	0 (0.0) <sup>a</sup>	0 (0.0) <sup>a</sup>	25 <sup>c</sup>
Limanti	28 (23.3) <sup>b</sup>	3 (2.5) <sup>b</sup>	0 (0.0) <sup>b</sup>	16 (13.3) <sup>c</sup>	1 (0.8) <sup>b</sup>	0 (0.0) <sup>a</sup>	48 <sup>d</sup>
Gaskeri	18 (25.4) <sup>d</sup>	1 (1.4) <sup>b</sup>	0 (0.0) <sup>b</sup>	2 (2.8) <sup>d</sup>	0 (0.0) <sup>a</sup>	0 (0.0) <sup>a</sup>	21 <sup>c</sup>
Attakeri	17 (27.4) <sup>d</sup>	1 (1.6) <sup>b</sup>	0 (0.0) <sup>b</sup>	5 (8.1) <sup>a</sup>	0 (0.0) <sup>a</sup>	0 (0.0) <sup>a</sup>	23 <sup>c</sup>
Chellomari	17 (21.0) <sup>d</sup>	0 (0.0) <sup>a</sup>	1 (1.2) <sup>a</sup>	4 (4.9) <sup>b</sup>	0 (0.0) <sup>a</sup>	0 (0.0) <sup>a</sup>	22 <sup>c</sup>
Alau Limanti	33 (17.5) <sup>c</sup>	7 (3.7) <sup>c</sup>	1 (0.5) <sup>a</sup>	11 (5.8) <sup>c</sup>	5 (2.6) <sup>c</sup>	0 (0.0) <sup>a</sup>	57 <sup>f</sup>
Gomari Karekari	40 (35.7) <sup>c</sup>	0 (0.0) <sup>a</sup>	0 (0.0) <sup>b</sup>	17 (15.2) <sup>c</sup>	2 (1.8) <sup>b</sup>	0 (0.0) <sup>a</sup>	59 <sup>f</sup>
<b>Total</b>	234	24	8	72	10	1	349

Values with different superscripts in columns differed significantly ( $p < 0.05$ )

**Table 4:** Prevalence of parasite stages in lake water and soil samples from communities around Lake Alau, Maiduguri

Parasite Stage	Prevalence No. (%)		
	Soil n=100	Water n=100	Total n=200
<i>Ascaris ova</i>	20 (20.0) <sup>a</sup>	10 (10.0) <sup>a</sup>	30 (15.0) <sup>a</sup>
<i>Strongyloides stercoralis</i> ova	50 (50.0) <sup>b</sup>	0 (0.0) <sup>b</sup>	50 (25.0) <sup>b</sup>
<i>Strongyle type</i> ova	30 (30.0) <sup>c</sup>	0 (0.0) <sup>b</sup>	30 (15.0) <sup>a</sup>
<i>Entamoeba histolytica</i> cyst	0 (0.0) <sup>d</sup>	30 (30.0) <sup>c</sup>	30 (15.0) <sup>a</sup>
<i>Giardia intestinalis</i> ova	0 (0.0) <sup>d</sup>	10 (10.0) <sup>d</sup>	10 (5.0) <sup>c</sup>
Preparasitic nematode larvae	10 (10.0) <sup>c</sup>	40 (40.0) <sup>c</sup>	50 (25.0) <sup>b</sup>
<i>Schistosoma mansoni</i> ova	0 (0.0) <sup>d</sup>	10 (10.0) <sup>a</sup>	10 (5.0) <sup>c</sup>

Values with different superscripts in columns differed significantly ( $p < 0.05$ )

This may often be attributed to involvement in socio-economic activities by this age group which in turn leads to higher exposure to sources of infection. Also, high level of

hygienic practices commonly found among adults is also associated with decreased prevalence of infectious agents, contrary to what is obtained in children.

Furthermore, the higher prevalence rates of infection observed in Gomari Karekari, Alau Limanti and Limanti communities compared to others agree with Mbanugo and Onyebuchi (2002) who reported that different prevalence levels among villages could be attributed to poor sanitary conditions of these areas, socio-economic status of the inhabitants, and their relative population size. The presence of *S. stercoralis* infections among the residents may be responsible, in part, for the finding of pre-parasitic larvae in both water and soil samples examined during the study. Some of the larvae recovered may also be of other parasitic nematode species like the hookworms or contributed to by free-living species. *Strongyloides stercoralis* eggs were generally common in both water and soil samples examined in the study area (Adamu *et al.*, 2012). This indicates the fact that this parasite easily thrives well as free-living nematode in the soil or water (Bhatia *et al.*, 2006). Additionally, they are usually common in dogs and pigs (Bhatia *et al.*, 2006) such that, contamination of the water or soil could have been through human and/or animal sources (Nwosu *et al.*, 1999). However, the absence of pigs in the study area limits the animal source to be likely dogs which were prevalent in the area during the study. Similarly, the *strongyle* type eggs found in soil samples could have originated from faecal contamination with human and animal (dog) faeces freely deposited in the open environment (Ahmed *et al.*, 1994; Adamu *et al.*, 2012). Several studies in this and other geographical areas have shown the contamination of public places with human and canine nematode stages through indiscriminate defecation in such places (Nwosu *et al.*, 1999; Nwosu and Damagum, 2003). It is therefore possible, that, this may be the case in the present study as stray dogs were very common and seen to scavenge around for food in the study area during the period. In addition, human and animal faeces were noted in several open spaces in the study area and some of them very close to the water bodies from which the people usually draw their drinking water.

The common habit of the inhabitants of the examined communities of generally eating well cooked food may be responsible for the very low prevalence of *Taenia* species in the various communities. *Taenia* species are known to be very prevalent among people who eat meat either raw or in the undercooked state (Nwosu *et al.*, 1999). The few inhabitants who had the infection with *Taenia* species may have acquired them through eating beef (suya) since this may not be roasted well enough to kill the metacestodes (*Cysticercus bovis*) present in the meat (Bhatia *et al.*, 2006). Although no attempt was made to determine the exact species of the *Taenia* eggs recovered during the study, the absence of the pig intermediate host of *Taenia solium* in the study, suggests that the *Taenia* eggs recovered during the study may not have been those of *Taenia solium*. Consequently, it is concluded that the *Taenia* eggs seen during the study were exclusively those of *Taenia saginata* since most of the roasted meat '(suya)' eaten in the area were predominantly prepared with beef.

The recovery of cysts of protozoan parasites consisting of *Entamoeba histolytica* and *Giardia intestinalis* freely from the water samples examined in the study, suggests that parasite stages might have reached the water sources through faecal contamination. These parasites have also been

previously recovered from people and pet animals in this geographical area of the country (Nwosu *et al.*, 1990; Nwosu and Hamza, 2005; Muhammad *et al.*, 2014). Since most of the residents defecated freely in open places, runoff water during the rains carries the deposited parasite stages into the various ponds and lake water that constitute the major sources of drinking water for the residents. When such contaminated water is consumed by the residents, especially the young ones, the infections frequently manifest, in part, as the diarrhoea commonly noted by the residents as a major sign of intestinal parasite infection in the area. This transmission cycle may be a means of maintaining the parasite in the communities. The cycle may also be contributed to, in the case of *Strongyloides stercoralis*, by the cycle in the stray dogs (Soulsby, 1982).

In conclusion, the study showed that urinary and intestinal helminth parasites, which are pathogenic were highly prevalent among residents of the study communities and that their socio-cultural behaviours such as eating habit and indiscriminate defecation and urination in the environment, non-availability of tap water favour the development, survival and transmission of these parasites in the area. There is the need for improved personal and environmental hygiene and provision of basic health and social amenities if prevention and control of these parasites would be achieved.

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#### Conflicts of Interest

The authors declare that they have no conflict of interest

#### Authors' Contributions

CON, EN and AWM conceived and designed the study. AH collected samples and CON analysed the generated data. CON and AWM supervised the process of sample analysis. JL and AH drafted the manuscript. All authors read, corrected and approved the final version of the manuscript.

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