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Prevalence and Risk Factor of Intestinal Helminthiasis and Lymphatic Filariasis (Elephantiasis) Co-Infections in Idumuje-Unor Community, Delta State, Nigeria

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Abstract

This study was undertaken to ascertain the prevalence and risk factors for intestinal helminthiasis and lymphatic filariasis co-infection in Idumuje-Unor, a rural community in Delta State. Sedimentation and formalin ethyl acetate concentration was carried out for the determination of helminth eggs in the stool samples. The Filarial Test Strip (Alere™) and rapid diagnosis test were used to confirm positivity for lymphatic filariasis. DNA extraction and PCR amplification of *Wuchereria bancrofti* () genes was carried out. The study showed that out of the 231 samples examined, 149(64.5%) were positive for at least one parasitic infection. From the 149 positive cases, 5(3.4%) co-infection of intestinal helminthes and lymphatic filariasis was observed. *Ascaris lumbricoides* was the most prevalent (60.4%). Sex based prevalence recorded 23(15.4%) cases in male and 67(45.0%) cases in female for *A. lumbricoides* ($P > 0.05$) in the study area. *T. trichuira* was also significantly higher ($P > 0.05$) in female compared to male. Multiple infections were recorded for both male and females for *A. lumbricoides* and *T. trichuira* respectively. Female recorded higher prevalence of 3(60%) followed by male with 2(40%) for lymphatic filariasis based on sex. Prevalence was higher in age group 16 – 25 (60%). Risk Factors related to these parasitic infections in the study area showed that individuals whose water source is the hand dug well have a higher prevalence 95(63.8%) than those who use water from borehole 54 (36.2%). Risk factors for lymphatic filariasis showed that participants living around stagnant water had the highest prevalence of 3(60.00%). These data documented the baseline information on prevalence of intestinal helminthes and lymphatic filariasis co-infection in the study area.

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Introduction

Intestinal parasites are a medical and public health issues with growing significance in rural communities of tropical regions and developing country like Nigeria (Akpan and Abraham, 2011). Infections caused by intestinal parasites are a public health problem while poor socio-economic environment is a major factor facilitating the prevalence of the disease (WHO, 2016). Four notable species of intestinal helminth parasites viz; *Ascaris* sp. (roundworm), *Trichuris* sp (whipworm), *Ancylostoma* sp. and *Necator* sp (hookworms) termed geohelminths and soil transmitted helminths (STHs) are major causes of helminth infections. These infections are most prevalent in tropical and subtropical regions of developing countries with poor water and sanitation facilities (Tefera *et al.,* 2015). Helminth infections are major health problem in developing countries infecting about one-sixth of the global population (WHO, 2016). The prevalence rate of intestinal parasites varies considerably in different parts of Nigeria (Lemy and Egwunyenga, 2017). Studies had shown that *A. lumbricoides* is the most prevalent, followed by hookworms, *T. trichiura* and *Strongloides stercoralis* (Samaila *et al.,* 2016). However, in some parts of Nigeria, hookworm has been reported as the most prevalent (Nmorsi *et al.,* 2009).

Lymphatic filariasis infection (commonly known as elephantiasis), is a neglected tropical disease which occurs with the transmission of filarial parasites to humans through mosquitoes. This infection is usually caused by the filarial nematode such as *Wuchereria bancrofti, Brugia malayi* and *Brugia timori* usually vectored by mosquitoes of the genera *Anopheles, Aedes, Culex* and *Mansonia* with the genera *Anopheles* and *Culex* reported as the main vector of *W. bancrofti* in Africa (Okonofua *et al.,* 2021). The infection is usually acquired at childhood stage, causing hidden damage to the lymphatic system. This is later manifested visibly as lymphoedema, elephantiasis and scrotal swelling with painful disfiguring leading to permanent disability (WHO, 2021).

Globally, approximately 1.3 billion people across countries have been reported recently to be at risk of *W. bancrofti* causing lymphatic filariasis. The World Health Organization (WHO) report of 2018 affirmed that this disease affects more than 120 million people across 81 countries with over one third of the global burden of lymphatic filariasis recorded across 39 African countries. Nigeria was rated the second most endemic country worldwide and the country with the largest population at risk of infection with lymphatic filariasis caused by *W. bancrofti* in Africa (Oluwabiyi *et al.,* 2016). Filarial infections have been reported in the coastal and rainforest zones of Nigeria (Okonofua *et al.,* 2014). The disease has been reported across the six geo-political zones in Nigeria with positive cases recorded across five hundred and seventy four Local Government Areas. Also, approximately one hundred and fourteen million Nigerians are at risk of this disease (Federal Ministry of Health, 2015).

Factors enhancing exposure to parasitic helminths most especially *A. lumbricoides* eggs identified by previous studies include the lack of adequate toilet facility, poor defecation practices, poor sanitation practices in households and poor socioeconomic status of community dwellers (Al-Delaimy *et al.,* 2014). According to the World Health Organization (WHO, 2016), walking barefooted in hookworm endemic areas causes exposure to helminth parasites. The recent trend in urbanization, rural-urban migration, poor environmental hygiene in forms of improper/inadequate waste disposal facilities coupled with the derailing and deteriorating sanitary conditions in our local communities and some urban areas has constantly created enabling environment and breeding sites for mosquito vectors (Hussaini *et al.,* 2020). There is paucity of information on the prevalence of helminth parasites and associated risk factors in some rural communities. Also, accurate data on the burden of hydrocele due to lymphatic filariasis is unknown in most rural settings. Research conducted in the past has mainly dependent on conventional methods, ignoring the efficacy of molecular analysis using polymerase chain reaction (PCR) methods. Based on the aforementioned, there

is need for more preliminary assessment of the burden of lymphatic filariasis in rural communities. Hence, this study is therefore undertaken to ascertain the prevalence and possible risk factors for intestinal helminthiasis and lymphatic filariasis co-infections in Idumuje-Unor, a rural community in Delta State.

Materials and methods

Study area

The study was carried out at Idumuje Unor, in Aniocha North Local Government Area, Delta State. The community lies geographically between 6º20' 01.4" N and 6º 22' 06.5" N and between 6º 25' 01.4" E and 6º 27' 06.6" E. The area falls within the rainforest vegetation with a mean annual rainfall of 1500mm. The occupation of the people is mainly farming. Several unhygienic practice such as open defecation, poorly managed waste dumpsites and bushy vegetation capable of supporting mosquito breeding dominated the study area. The community also has poor drainage systems.

Ethical permission

Before the commencement of the research, ethical permission was obtained from Delta State Ministry of Health Ethical Committee and Faculty of Science, DELSU Ethical Committee. Also, prior notification and consent was obtained from Idumuje-Unor Primary Health Center which served as clinic for sampling. Informed consent was obtained from participants, parents/guardian.

Community mobilization

Prior to commencement of the study, meeting was held with the community head to explain the purpose and procedures of the study. With the consent of the community head, town crier was mandated to request interested participants to assemble at the community primary health center (PHC).

Sampling and demographic data collection

The stratified random sampling technique was used with random selection of participants across different streets in the local government area (LGA), and then enrolment of all dwellers in each of the houses. Questionnaire was administered to obtain demographic information (age, sex, education, occupation) from participants. Each enrolled participant was given a sterile specimen bottle for stool sample collection, which after collection was transported immediately to the Department of Animal and Environmental Biology Laboratory of the Delta State University, Abraka, Nigeria, for examination for the presence of helminth parasites.

Parasitological

Qualitative analyses, namely sedimentation technique and formalin Ethyl Acetate Concentration was carried out for the determination of helminth eggs in the stool samples using standard laboratory methods as described by Cheesbrough (2005).

Clinical examination and rapid diagnosis test for lymphatic elephantiasis

The eligible individuals enrolled for the study presented themselves for physical examination during the day to ascertain symptoms and signs of lymphatic filariasis. Chronic involvement of the genitalia in males and of the limbs in both sexes was examined. Using the Filarial Test Strip (Alere™), rapid diagnosis test was carried out in the field. The participant positive to lymphatic filariasis later presented themselves at night between 23:00 and 02:00 for collection of blood samples.

DNA Extraction

DNA extraction was done at Inqaba Biotec west Africa limited Ibadan, Oyo State, Nigeria. DNA extraction was done on the five (5) Lymphatic filariasis positive blood sample only. Genomic DNA was extracted from the blood samples using the Quick-DNA Miniprep Plus Kit.

PCR Amplification of genes and Gel Electrophoresis

The five (5) eluted DNA extracts were amplified for WBAN genes by PCR using the following primers sequence F – CGTGATGGCATCAAAG and R - AAATAAGGTTATACCAAGCA. Amplifications were performed using Eppendorf Master cycler nexus gradient 230 (Germany). After PCR amplification, 2ul

of each PCR product was run on 1% agarose gel, stained with SafeView Red (5ul) and photographed using a gel documentation system (E-BOX, Vilber Lourmat, Italy).

Data analysis

Statistical analysis was carried out using PAST (Paleontological Statistics) software package (version 4.10) for the analysis of data.

Results

Helminths prevalence by sex of respondents

The study showed that out of the two hundred and thirty one (231) individual samples collected and examined, 149(64.5%) were positive for at least one parasitic infection. From the 149 positive cases, 5(3.4%) were positive for both intestinal helminthes and lymphatic filariasis co-infection.

Table 1 shows the percentage prevalence of helminthes infection among males and females enrolled for the study. From the one hundred and forty nine (149) positive cases recorded, the results showed that higher number of females were positive with total number of one hundred and seventeen (117) with prevalence of 78.5% while male recorded positive cases of thirty two (32) with prevalence of 21.5% (Table 1).

Table 1. Percentage prevalence of helminthes infection among males and females.

Age Group	Total Number Examined			Number Infected		Total Number Infected
	Male	Female	Total	Male	Female	Total
1 – 15	10	23	33	9	23	32
16 – 25	13	28	41	6	20	26
26 – 35	11	28	39	8	21	29
36 – 45	6	21	27	3	18	21
46 – 55	11	31	42	2	16	18
≥56	13	36	49	4	19	23
Total	64(27.7%)	167(72.3%)	231	32(21.5%)	117(78.5%)	149
	231			149		149(64.5%)

The prevalence of intestinal helminthes in Idumuje Unor community Aniocha North Local Government Area of Delta State is presented in Table 2.

The result showed that *Ascaris lumbricoides* was the most prevalent accounting for 60.4% while *Trichuris trichuira* accounted for 39.6%. Based on sex, *Ascaris lumbricoides* was the most prevalent with 23(15.4%) cases in male and 67(45.0%) cases in female. This was followed by *Trichuris trichuira* which recorded prevalence of 18(12.1%) in male and 41(27.5%) in female. *A. lumbricoides* was significantly higher ($P > 0.05$) in the study area as and mostly in female compared to male. *T. trichuira* was also significantly higher ($P > 0.05$) in the study area as and mostly in female compared to male. Multiple infections were recorded for both male and females for *A. lumbricoides* and *T. trichuira* respectively.

Table 2. Prevalence of intestinal helminthes in Idumuje Unor community Aniocha North Local Government Area of Delta State.

Helminth Parasites	Total Number Infected	Male		Female		*p*-value
		No. of Positive Cases	% Prevalence	No. of Positive Cases	% Prevalence	
Ascaris lumbricoides	149	23	15.4	67	45.0	0.001
Trichuris trichuira	149	18	12.1	41	27.5	0.003
Multiple infections	149	11	7.4%	27	18.1	0.111
Overall prevalence	149	52	34.9	137	90.6	0.000

The percentage prevalence of lymphatic filariasis among rural dwellers in Idumuje-Unor community, Aniocha North Local Government Area of Delta State based on sex showed that female recorded higher prevalence of 3(60%) followed by male with 2(40%) for LF based on sex.

Age-range prevalence of lymphatic filariasis among rural dwellers in Idumuje-Unor community, Aniocha North Local Government Area of Delta State showed that prevalence was higher in age group 16 – 25 accounting for 3(60%) of the positive cases while 36 – 45 and ≥56 accounted for 1(20%) each prevalence of the total positive cases (Table 3).

Helminths prevalence by age and sex of respondents

The prevalence of helminths infection with age relationship for male and female in Idumuje Unor community Aniocha North Local Government Area of Delta State are presented in Table 4.

Table 3. Prevalence of lymphatic filariasis by age group among rural dwellers in Idumuje-Unor community, Aniocha North Local Government Area of Delta State.

Age Group	Total Number Infected		Number Infected		Total Number Infected
	Male	Female	Male	Female	Total
1 – 15	9	23	-	-	-
16 – 25	6	20	-	3	3
26 – 35	8	21	-	-	-
36 – 45	3	18	1	-	1
46 – 55	2	16	-	-	-
≥56	4	19	1	-	1
Total	32(21.5%)	117(78.5%)	2(40%)	3(60%)	5
	149		5		5(3.4%)

The study showed that children within age 1 – 15 were more infected followed by age 26 – 35 and 16 – 25 respectively. This was followed by age group >56, 36 - 46 while those within age group 46 -55 recorded the least in terms of helminth infections. Females accounted for higher prevalence across the different age groups.

Helminths Prevalence by occupation of respondents

The prevalence of helminth infections among the respondents based on occupation in Idumuje Unor community Aniocha North Local Government Area of Delta State is presented in Table 5. The result showed that students had higher burden of intestinal helminthes compared to other groups of occupation.

Table 4. Age and sex prevalence of intestinal helminthes in Idumuje Unor community Aniocha North Local Government Area of Delta State.

Helminth Parasites	Total Number Examined	Male		Female	
		No. of Positive Cases	% Prevalence	No. of Positive Cases	% Prevalence
1 – 15	149	9	6.0	23	15.4
16 – 25	149	6	4.0	20	13.4
26 – 35	149	8	5.5	21	14.1
36 – 45	149	3	2.0	18	12.1
46 – 55	149	2	1.3	16	10.7
≥56	149	4	2.7	19	12.8
Total	149	32	21.5	117	78.5

Helminths Prevalence by toilet facilities of respondents

The prevalence of helminth infections by toilet facilities of respondents in Idumuje Unor community Aniocha North Local Government Area of Delta State are presented in Table 6. The result showed that people using pit latrine had higher parasitic burden for both *A. lumbricoides* and *T. trichuiris* for both male and female. This was followed by water closet users while those using bush as their means of

defecation had less burden of helminth parasite infestation. *A. lumbricoides* recorded 14(9.4%) for male and 34(22.8%) for female; *T. trichuris* recorded 9(6.0%) for male and 24(16.1%) for female among pit latrine users. *A. lumbricoides* recorded 6(4.0%) for male and 21(14.1%) for female; *T. trichuris* recorded 5(3.4%) for male and 11(7.4%) for female among water closet users. The least was recorded among those using bush for defecation with *A. lumbricoides* 3(2.0%) for male and 12(8.1%) for female; *T. trichuris* recorded 4(2.7%) for male and 6(4.0%) for female respectively (Table 6).

Table 5. Prevalence of intestinal helminthes based on occupation in Idumuje Unor community Aniocha North Local Government Area of Delta State.

Helminth Parasites	Total Number Examined	Male		Female	
		No. of Positive Cases	% Prevalence	No. of Positive Cases	% Prevalence
Student	149	8	5.4	24	16.1
Farmer	149	7	4.7	21	14.1
Trader	149	6	4.0	20	13.4
Self Employed	149	3	2.0	16	10.7
Civil Servant	149	4	2.7	14	9.4
Paid Worker	149	2	1.3	13	8.7
Retired	149	2	1.3	9	6.0
Total	149	32	21.4	117	78.6

Risk factor for lymphatic filariasis

Risk factor related data of lymphatic filariasis in Idumuje Unor community Aniocha North Local Government Area of Delta State shows that individuals who live around stagnant water had the highest prevalence of 3(60.00%) followed by bushy environment and close to refuse dump with prevalence of 1(20.0%) respectively. Similarly, individuals who use dug pit as their refuse dumping site had a higher prevalence 4(80.0%) than those who dump their refuse openly 1(20.0%) (Table 7).

Polymerase chain reaction (pcr) detection

. Showed Polymerase chain reaction result to identify *Wuchereria bancrofti* on 1.0% agarose gel electrophoresis stained with SafeView Red (5ul). The gene was detected in all the five (5) samples with bands at 192bp.

Table 6. Prevalence of intestinal helminths by toilet facilities in Idumuje Unor community Aniocha North Local Government Area of Delta State.

	Male			Female		
	Ascaris	*Trichuris*	Multiple Infections	*Ascaris*	*Trichuris*	Multiple Infections
Water closet	6(4.0)	5(3.4)	4(2.7)	21(14.1)	11(7.4)	8(5.4)
Pit Latrine	14(9.4)	9(6.0)	5(3.4)	34(22.8)	24(16.1)	14(9.4)
Bush	3(2.0)	4(2.7)	2(1.3)	12(8.1)	6(4.0)	5(3.4)
p – value	0.000	0.000	0.001	0.000	0.000	0.000

Discussion

The study recorded high prevalence of intestinal helminthes of 64.5% in the study area. This high prevalence rate calls for public health concern taking into consideration the possible ill-health effects caused by helminth parasites infections with special reference to children (Taiwo *et al.,* 2017).

The prevalence of helminthiasis recorded in this study was higher compared to 6.6– 25.8% reported in Abeokuta, Ogun State by Sam-Wobo *et al.* (2012). Similar prevalence has previously been reported by Egwunyenga and Ataikiru (2005), Nmorsi *et al.* (2009) and Ito and Egwunyenga (2017). Also, higher prevalence of intestinal helminthiasis infection has also been reported by Ugbomoiko *et al.* (2006) who conducted a study in Oba Ile, Osun State and reported helminth prevalence of 95.7%. Other studies across Nigeria had reported prevalent values of helminths between 75% and 90% (Ejima and Ajogun, 2011).

Table 7. Risk factor related data of Lymphatic Filariasis in Idumuje Unor community Aniocha North Local Government Area of Delta State.

	No Examined	Positive	% Prevalence
Environment:			
Bushy	22	1	20.00
Clean	69	-	
Close to refuse	33	1	20.00
Stagnant water	25	3	60.00
Waste Dump:			
Dug pit	55	4	80.00
Open space	94	1	20.00

The percentage prevalence of lymphatic filariasis among rural dwellers in Idumuje-Unor community, Aniocha North Local Government Area of Delta State based on sex showed that male recorded higher prevalence of 3(60%) followed by female with 2(40%) for LF based on sex. The overall prevalence 3.4% reported in this study is lower than other related studies conducted in other parts of Nigeria with prevalence ranging between 15 and 19% as reported by Mbah and Njoku (2000) in Anambra, State, Anosike *et al.* (2005) in Ebonyi State and Ojunrogbe *et al.* (2010). The low prevalence of infection could be attributed to living conditions of the people. However, the prevalence of high occurrence of lymphatic filariasis could be attributed to housing conditions such as mud walls, thatched roofs and are without ceilings. Proximity to various breeding sites of the parasite's vectors seen in their poor environmental and unhygienic conditions is also implicated (Okonofua *et al.,* 2014).

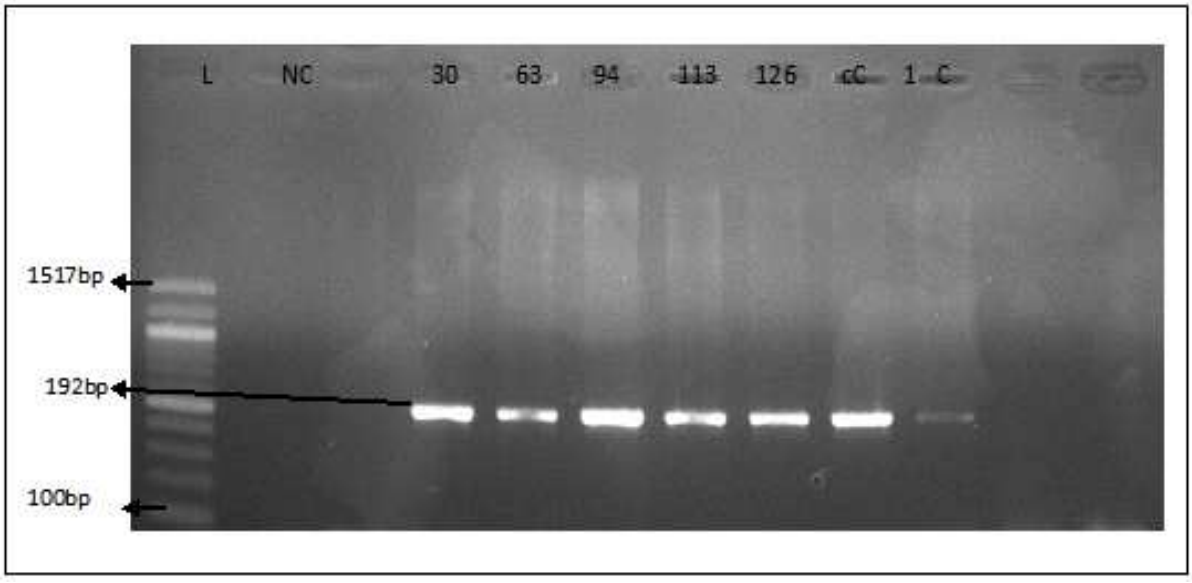

Fig. 1. Polymerase chain reaction results for detection of gene from five (5) *Wuchereria bancrofti* positive samples on 1.0% agarose gel electrophoresis stained with SafeView Red (5ul). L is 100bp -1517bp DNA ladder (molecular marker) while NC is a no DNA template control. Gel documentation system: E-BOX, Vilber Lourmat, Italy.

The study reported that *Ascaris lumbricoides* was the most prevalent accounting for 60.4% while *Trichuris trichuira* accounted for 39.6%. Based on sex, *Ascaris lumbricoides* was the most prevalent with 23(15.4%) cases in male and 67(38.3%) cases in female. This was followed by *Trichuris trichuira* which recorded prevalence of 18(12.1%) in male and 43(28.9%) in female. The results of this study is higher compared to

previous studies of Ogbe and Odudu (1990) who recorded prevalences of 71.1% for Ascaris, 59.2% for Trichuris, and 14.7% for hookworm among children attending primary schools, with the highest prevalences of Trichuris and hookworm infection in children aged 8–10 years. Ijabone and Olagunju (2006) reported a 72.0% prevalence of intestinal helminth infection in 533 pupils, aged 6–17 years, from Osun state. *Ascaris* (47%) were relatively common, and *Trichuris* (0.18%) was present but rare. The occurrence of multiple infection of *Ascaris lumbricoides* and *Trichuris trichuira* in the present study was recorded.

Lymphatic filariasis prevalence was higher in age group 16 – 25 accounting for 3(60%) of the positive cases while age group ≥56 accounted for 2(40%) prevalence of the total positive cases. The prevalence of age-related infection rates recorded in this study corroborate with previous studies which showed that prevalence rises with age (Okonofua *et al.,* 2014). The increase in infection spanned to the very older group 60 years corroborates to some reports that observed lower prevalence in the group (Anosikw *et al.,* 2005; Okon *et al.,* 2010). The occurrence of infection among adults within age group ≥56 years in our study indicates higher proximity of older age groups to the mosquito vectors of infection. Moreover, PCR amplification of gene to identify *Wuchereria bancrofti* among the five serological tested blood samples was also positive (100%) at the band 192 base pair as illustrated in the plate 1.0. This in line with work of Zhong *et al.* (2016) where all some serological tested blood sample were positive to gene at band 188bp.

Conclusion

The study reported a high prevalence of intestinal helminth and lymphatic filariasis co-infection in the study area. Demographic parameters showed that helminth infections prevalence was higher in female compared to males for intestinal helminth infections with *A. lumbricoides* as most prevalent compared to *T. trichuira*. The study also showed that lymphatic filariasis was more prevalence in male compared to female. The study also showed that PCR analysis identified the species *Wuchereria bancrofti* as the causal agent of lymphatic filariasis (elephantiasis) among the positive cases in the study area.

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