Asian Journal of Research in Infectious Diseases

Volume 13, Issue 2, Page 15-25, 2023; Article no.AJRID.98727
ISSN: 2582-3221

Laboratory and Sonographic Assessment of Intestinal Parasitic Load of Peasant Farmers in Emekuku Village in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRID/2023/v13i2260

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/98727

Original Research Article

Received: 22/02/2023
Accepted: 24/04/2023
Published: 05/05/2023

ABSTRACT

Background: Intestinal parasites (IP) are organisms that live and strife within the host for harm or mutual benefit, is a global concern infecting over 3 billion people and causing morbidity in about 450 million people worldwide of which the developing countries are most affected, the majority being school children, rural dwellers, and peasant farmers. Parasitic load (PL) in our locality has not been assessed, or determined, and comparisons made based on biographic data of the peasant farmers.
Objective: The study was aimed at determining the PL of peasant farmers in Emekuku by Laboratory and Sonographic examinations.

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Methods: A cross-sectional study design with a purposive non-probability sampling technique was used to select 126 subjects within the age group of 20 years to 90 years of both sexes. Stool specimens were collected, Wet preparation and Concentration Methods were used for laboratory investigation and comparisons were made. Sonography was used for the confirmation and characterization of IP. Data were analyzed with simple descriptive statistics and chi-square.
Results: Wet prep technique recorded 10 (7.94%) positive cases while the Concentration method recorded 44 (34.92%) positive cases (P < 0.05). *Ascaris lumbricoides* amongst other IPs had the highest manifestation in the study, most prevalent in the age group 80 to 89 years,10 (62.5%) and more occurring in the female 10 (15.87%) and significant (P =0.039967). Sonographic indices for confirmation of IP were 4 (80.0%) out of 5.
Conclusion: Sonography complements the role of Laboratory stool analysis for the presence, characterization, location, and complications of IP such as *Ascaris lumbricoides.*

Keywords: Laboratory; sonography; intestinal parasites; peasant farmers.

1. INTRODUCTION

Intestinal parasites (IP) are organisms that live and strife within the host for harm or mutual benefit [1]. Intestinal Parasitic infection is a global concern infecting over 3 billion people and causing morbidity in about 450 million people worldwide [2,3] Developing countries are the most affected, the majority being school children, rural dwellers and food handlers, and peasant farmers [3,4,5]. In the adult population, females frequently carry higher parasite loads than males [6]. This sex bias has been attributed to inadequate health/hygiene enlightenment, typical hand-mouth activity, uncontrolled fecal activity, differences in movement patterns, habitat choice, diet, body size, and poverty [5,7]. The National Institute of Allergy and Infectious Diseases (NIAID) listed many IP as Neglected Tropical Diseases (NTDs) because they generally afflict the world's poor and historically have not received as much attention as other diseases [8,9].

Peasant farmers are rural poor, rural residents, serfs, and agricultural laborers, and are the common or simple people living in a community such as Emekuku in the southeastern area of Nigeria, where there is deprivation of urban activities such as proper hygiene[9]. They are very vulnerable to having different species of IP [10,11].

Parasite load (PL) is a measure of the number and virulence of the parasites that a host organism harbors, while quantitative parasitology deals with measures to quantify PL in samples of hosts and to make statistical comparisons of parasitism across host samples [12]. There are two main classes of parasites that infect the human intestine: protozoa and helminths. Entamoeba. histolytica, G. lamblia, N. americanus, A. doudenale, hookworm, ascariasis, and trichuriasis [13]. For example, Ascaris eggs live in soil that is contaminated by feces, the egg gets into the body through the mouth, and it can spread from person to person via the infected feces owing to very poor hygiene [14], which can be seen in the peasant farmers with the following signs and symptoms of nausea, vomiting, diarrhea, weight loss and visible worms in the stool [15]. Depending on the parasitic species in question, various methods of quantification allow scientists to measure the number of parasites present and determine the PL of an organism. Quantifying IP often requires dissection, extraction, and counting of the parasites. While other laboratory techniques do not require dissection; such as fecal examination which includes fecal smears and flotation methods. Fecal floats can detect reproductive forms of endoparasites organisms (eggs, larvae, oocysts, and cysts) that are passed through the digestive system in feces. Microscopic examination of these parasites using a simple light microscope enables one to see their staging development [16].

Complementing laboratory investigations for IP, sonography of IPs has been improved upon using high-frequency, high-resolution ultrasound duodenography and colonography with and without water contrast to detect the presence of IP within the GIT by using the classification features of IP such as Spatial reflector, Hyper-echogenicity/hypo-echogenicity, Motility of IPs and morphologic changes in intestinal wall thickness where the IP are found, also detected in the biliary system [17,18,19].

The PL in this locality has not been adequately assessed, and determined and comparisons

made based on the biographic data of peasant farmers. Do the peasant farmers in Emekuku have high or low levels in their GIT? What is the prevalence and different species of IPs afflicting the peasant farmers in this community? The aim of the study is to determine the prevalence, PL, and IP species and compare the PL distribution based on the age and sex of the peasant farmers, and the types of investigative methods.

2. MATERIALS AND METHODS

2.1 Study Design and Materials

A cross-sectional study design was adopted. A purposive non-probability sampling technique was used to select a sample size of 126 subjects (peasant farmers) at Ubowalla, Emekuku in Owerri North Local Government of Imo State, Nigeria. Consent and confidentiality of subjects were obtained. Symptomatic and asymptomatic adult subjects, 63 males and 63 females from the age of 20 to 90 years were studied.

Sterile stool containers were given to the subjects and were instructed to defecate early in the morning without eating and put about 5 grams of feces into the sterile container and cover it immediately. The specimens were collected and labeled with their names, sex, and age. Wet preparation using physiological saline and Dobbel's iodine method and the Concentration method using formal ether sedimentation technique for the concentration of stool parasites were used for comparison. Subjects who tested positive for IP were further examined using a high-frequency transducer (7-10MHz), high-resolution ultrasound imaging modality to perform a Transabdominal Sonography of the GIT to identify, characterize and confirm the presence and morphology of IP.

2.2 Procedures for Laboratory Investigation

2.2.1 Wet preparation method

The procedure was carried out as follows:

- A drop of physiological saline was placed at one end of a clean grease-free slide and one drop of Dobbl's iodine was placed at the other end of the slide.
- An applicator stick was used to take 1g of the sample (feces)
- The samples were emulsified in the drop of the saline and Dobbl's iodine.
- Smears of the saline and the iodine were covered with a cover slip gently avoiding air bubbles and over floating.
- The smears were examined microscopically with X10 and X40 objective lens.

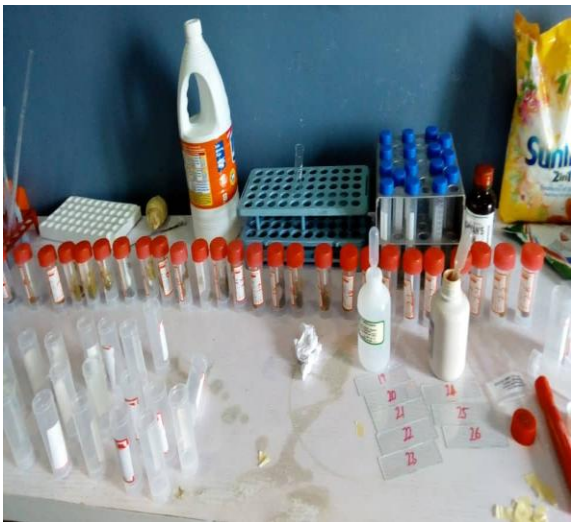

Fig. 1. Picture of materials used

2.2.2 The concentration method

The concentration method procedure required the use of ether (ethyl acetate) which was used as a lipid-removing agent and formalin as a fixative as follows:

- An orange stick was used to pick 1 gram of feces and placed it into a centrifuge tube containing 7ml of 10% formalin.
- The feces were emulsified in the formalin and filtered through gauze into the dish.
- The filtrate was then transferred into a boiling tube and 3ml of ether was added and mixed well by hand for 1 minute.
- The filtrate was then transferred back to the centrifuge tube and centrifuge at 3,000 rpm for 1 minute.
- The fatty plug was loosened with an applicator stick and the supernatant is poured away quickly by inverting the tube.
- The fluid on the side of the tube was allowed to drain onto the deposit and mixed well.
- Then a drop was transferred to a slide for examination under a cover slip.

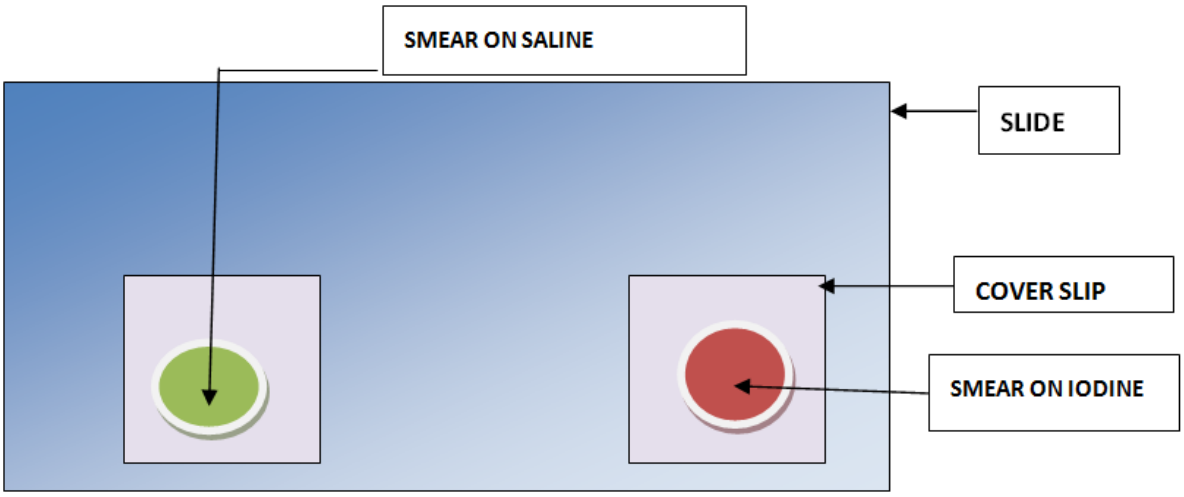

Fig. 2. Illustration of Slide with the saline and iodine Smears

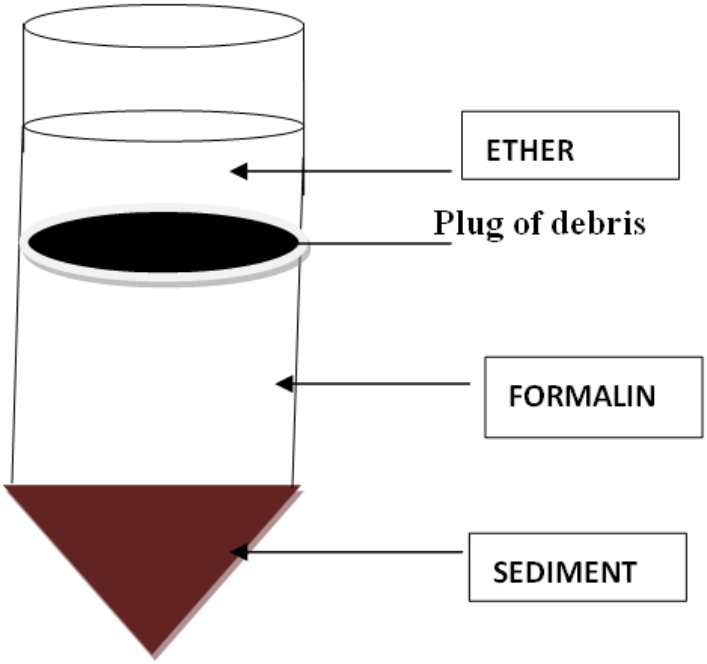

Fig. 3. Test tube using the concentration technique

2.3 Procedures for Sonographic Investigation

Subjects are urged to drink about 1.5 liters of water in order to fill the stomach, duodenum, and the other distal parts of the GIT (hydrographic technique), this is to create a better acoustic window to enable better visualization of the mucosa lining and intramural contents of the GIT.

A systematic approach of scanning the whole intestine is adopted which begins in a relaxed ventral position; beginning from the medial to the right and left anterior superior iliac spine and the pelvis. Elevation of the arms is done to spread the rib spaces to improve visualization of the hepatic and splenic flexures. These sonographic techniques are used to detect the presence of IP within the GIT by using the classification features of IP such as Spatial reflector, Hyperechogenity/hypoechogenicity, Motility of IPs, and morphologic changes in intestinal wall thickness [19].

Data were analyzed using simple descriptive statistics such as mean and percentage.

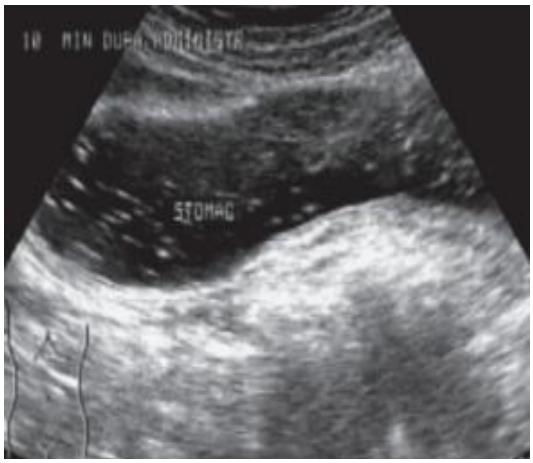

Fig. 4. Sonogram of the stomach filled with water

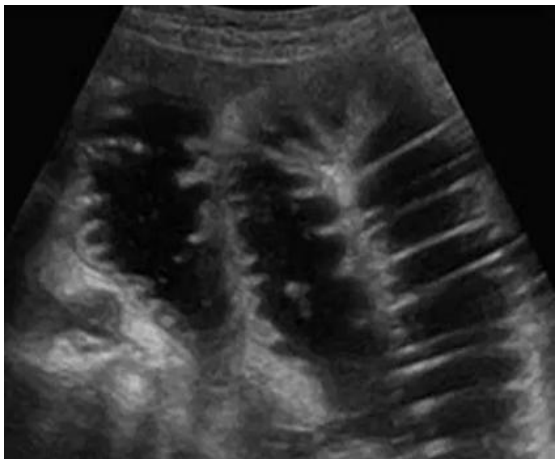

Fig. 5. Sonogram of the small intestine filled with water

3. RESULTS

3.1 Demography of Subjects

All the peasant farmers studied had the highest educational background in Primary school. No holders of the higher education record. The age and sex frequency distribution table is shown below. It shows the age range of subjects whose stools were studied and their percentage. The age group of 20 to 29 years of subjects did not return the specimen bottles with samples, they were 5. The result showed that the subject age range of 30 to 39 years was the highest in the study with 19.8% (25) while the least age range was 80 to 89 years which recorded 12.6 % (16).

The mean age range was 50 to 59 years. Male and female subjects were equal to 50% (63).

3.2 Parasites Frequency Distribution in both Methods Used

Table 2 shows the types of parasites seen in the study and their distribution in the different techniques used. The wet prep technique revealed 5(3.97%) *Ascaris lumbricoides* and 5(3.97%) *Entamoeba.coli.* The Concentration method revealed more parasites with 34.92% being positive and 65.08% negative. Table 3 shows Chi-square statistics was 27.246 with 1 degree of freedom. The p-value is < 0.05. Significant at $P < 0.05$.

3.3 Parasites and Age Range Distribution with Concentration Method

Table 4 shows the distribution of the parasite with age range. This shows the prevalence of a particular parasite within the age group studied. Subjects within the age range of 80-89 years had the highest prevalence of *Ascaris* 10 (62.5%). While the age group 30-39 years had the highest prevalence of *E. coli* 9 (36.0%). The parasite *Entamoeba H.* which occurred least was found in the age group 60-69 years (4 (18.2%)) and this group had two types of parasites.

3.4 Gender and Parasite Distribution

Table 5 shows the parasite and sex distribution. The positive test result indicates the presence of parasites in the stool specimen while the negative test indicates the absence of parasites. Female subjects had a higher positive test for IP of 26 (41.27%) while the male subjects with positive test results recorded 18 (28.57%). The total positive and negative tests result was 44 (34.92%) and 82(65.08%) respectively. *Ascaris lumbricoides* had the highest manifestation in the female subjects 10 (15.87%) while *E. coli* manifested more in male subjects 9 (14.3%). Table 5 shows that P-value is 0.039967. The result is significant at $P < 0.05$.

3.5 Transabdominal Sonography of the GIT to Identify, Characterize and Confirm the Presence of IP

The most prevalent IP is *Ascaris Lumbricoides* and which is the largest worm identified, characterized, and located by transabdominal sonography (TAS). Table 7 shows the sonographic indices for confirming the presence of AL. Four indices out of 5 meet the criteria for the confirmation and characterization of AL in the GIT.

Table 1. Age and sex distribution of subjects who were sampled

Age (Years)	Frequency	%	Sex	
			Male	Female
20 – 29	0	0	0	0
30 – 39	25	19.8	12	13
40 – 49	21	16.7	14	7
50 – 59	23	18.3	11	12
60 – 69	22	17.5	10	12
70 – 79	19	15.1	9	10
80 – 89	16	12.6	7	9
Total	126	100	63	63

Table 2. Parasites frequency distribution in wet prep and concentration methods

Types of parasites seen	Wet prep method (n = 126)	%	Concentration method (n = 126)	%
Ascaris lumbricoides	5	3.97	15	11.90
Hookworm	-	-	6	4.76
E. coli	5	3.97	14	11.11
Trichomonas hominis	-	-	5	3.97
Entamoeba histolytica	-	-	4	3.17
Number of Parasites seen (Positive +ve)	10	7.94	44	34.93
Number of Parasites not seen (Negative -ve)	116	92.1	82	65.08

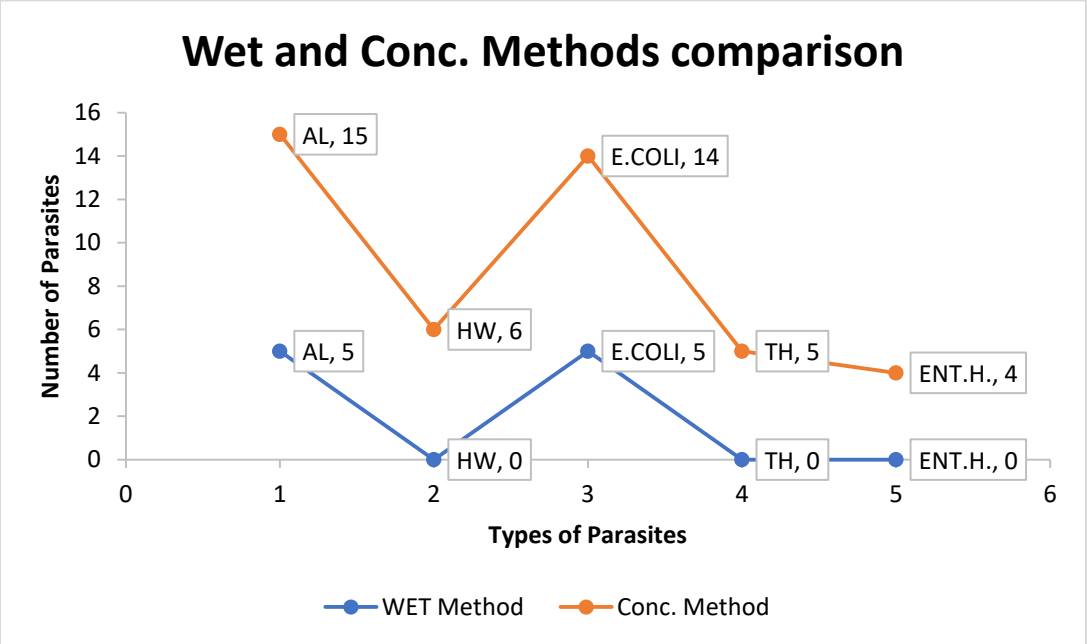

Fig. 6. Scattered plot of the two methods

Table 3. Method comparison

Methods	Number of Parasites seen	Number of Parasites not seen	Total
Wet method	10	116	126
Conc. method	44	82	126

Chi-square statistics is 27.246 with 1 degree of freedom. The p-value is < 0.05. Significant at P < 0.05

Table 4. Parasite and age range distribution

Age range (years)	Type of parasites	Frequency	% n = number of subjects in the age range	% n = 126
20 – 29	-	-	-	-
30 – 39	*E. coli*	9	36.0 (25)	7.14
40 – 49	Ascaris	5	23.8 (21)	3.97
50 – 59	Hookworm	6	26.1 (23)	4.76
60 – 69	*E. coli*	5	22.7 (22)	3.97
	Entamoeba H	4	18.2 (22)	3.17
70 – 79	*Trachonionas hominis*	5	26.3 (19)	3.97
80 – 89	Ascaris	10	62.5 (16)	7.94

Table 5. Gender and parasite distribution (Positive and Negative results)

Type of Parasites	Male (63)	%	Female (63)	%	Total	% n=44
Ascaris lumbricoides	5	7.94	10	15.87	15	34.09
Hookworm	-	-	6	9.52	6	13.64
E. coli	9	14.3	5	7.94	14	31.82
Trachonionas hominis	-	-	5	7.94	5	11.36
Entamoeba histolytica	4	6.35	-	-	4	9.09
					n = 126	
Positive result	18	28.57	26	41.27	44	34.92
Negative result	45	71.43	37	58.73	82	65.08

Table 6. Comparison of sex with types of IP

Types of Parasites	SEX						Row total
	Male			Female			
	No	X^2	P-value	No	X^2	P-value	
Ascaris L.	5	(6.38)	{0.30}	10	(8.62)	{0.22}	15
Hookworm	-	-	-	6	(4.02)	{0.97}	6
E. Coli	9	(5.96)	{1.55}	5	(8.04)	{1.15}	14
T. Hominis	-	-	-	5	(3.45)	{0.70}	5
E. histolytica	4	(2.13)	{1.65}	-	-	-	4
Column total	18			26			44

The Chi-square statistic is 10.0275. The p-value is 0.039967. The result is significant at P < 0.05

Table 7. Sonographic characterization and confirmation of the presence of IP indices

Sonographic features (Indices) of IP	Present	Absent
Spatial reflector	+	-
Hyperechogenity	+	-
Hypoechogenicity	-	+
Motility of IP	+	-
morphologic changes in intestinal wall thickness	+	-
Number of + and - indices	4 (80%)	1 (20%)

+ means presence while – means the absence

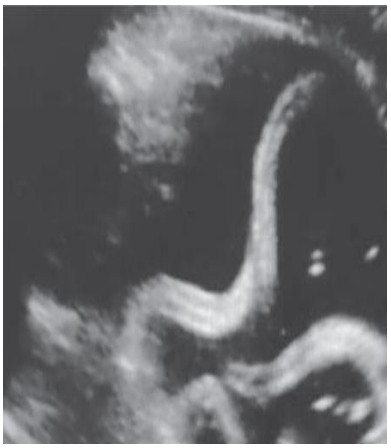

Fig. 7. Ascaris moving within the GIT

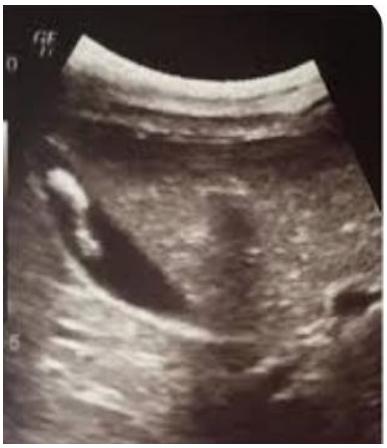

Fig. 8. Ascaris in the gall bladder

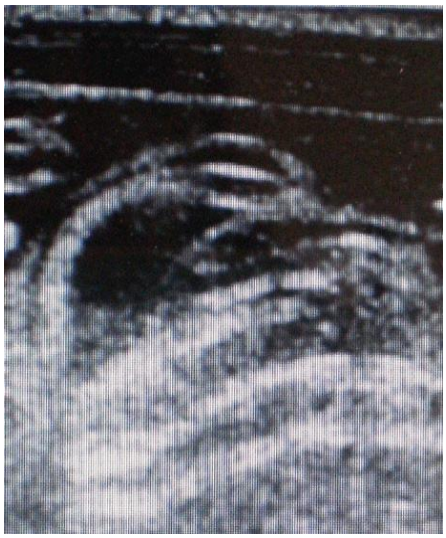

Fig. 9. Sonogram of IP with Linear transducer

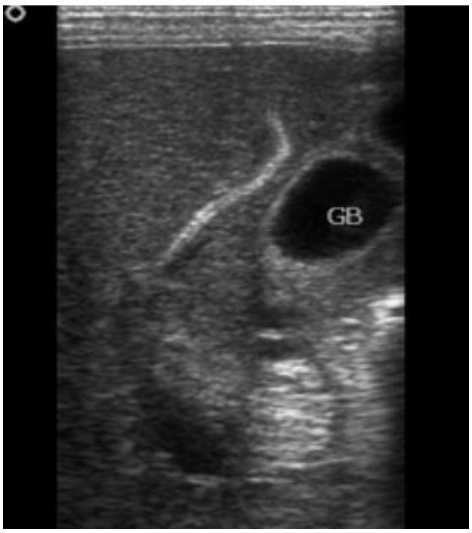

Fig. 10. Sonogram of IP in the biliary tree

4. DISCUSSION

In the present study, female peasant farmers in Emekuku are more vulnerable to intestinal parasites than male peasant farmers. Table 2 shows that the laboratory concentration method test yielded more positive results than the wet preparation method, 44 (34.93%) as against 10 (7.94%) respectively and significant at $P < 0.05$. The concentration test method which has a better yield result was therefore used for the study analysis. Female subjects had a higher positive result of 26 (41.27%), and males had 18 (28.57%), $P = 0.039967$, at the level of $P < 0.05$ significance. This finding corroborates the findings of Sunil et at. [5]; Hillegass et al. [6]; Hotez et al. [9]; Ragunathan et al. [20] and Pullan et al. [21]. This implies that women are more at risk of intestinal parasitic infection than men and this could be a result of poor sanitary and personal hygiene, lack of education, excessive farming work seen in the locality, and lack of proper regular treatment of intestinal parasites with the recommended therapy [1,7,14]. In the present study, all the peasant farmers studied had a highest educational background of Primary school. No holders of the higher education record. This may also imply that they are peasant farmers because of a lack of higher education.

Tables 4 and 6 in the present study showed the parasite frequency distribution which indicated that *Ascaris lumbricoides* 15 (34.09%) and *Entamoeba.coli* 14 (31.18%) are the commonest parasites in the locality. Other parasites such as hookworm, *Trichomonas hominis,* and *Entamoeba histolytica* were also discovered in the study but with low prevalence levels. This

finding agrees with the findings of Pullan et al. [22] who stated that an estimated 439 million people were infected with hookworm, 819 million with *Ascaris lumbricoides*, and 465 million with *Trichuris trichiura*, *Ascaris* being the leading soil-transmitted helminths STHs globally [23].

Ascaris lumbricoides infected female peasant farmers more than the male in the present study. In Table 5, 10 (15.87%) of female subjects were infected with *Ascaris lumbricoides*, this figure is twice the figure recorded in the male farmers 5 (7.94%). On the other hand, infected male subjects mostly had *Entamoeba. coli* 9 (14.3%). In Table 4, *Ascaris lumbricoides* were recorded mostly in the age group 80 to 89 years, 10 (7.94%), and 10 subjects out of 16 within this age group were infected (62.5%). This result agrees with the results of Forman and Esy [24]; Maria and Julia [25]; Silva et al. [26]. This implies that the geriatrics in the locality are more prone to IP. This could be a result of the aging process with decreased immunity, hormones, farm stress, poor sanitation and nutrition, and ineffective and inadequate anthelminthic therapy [14,24].

Sonography plays a complementary role to Laboratory investigation to confirm the presence of IP, location, and complications of the parasitic load. Table 7 shows the Sonographic characterization and confirmation of the presence of IP indices. *Ascaris lumbricoides* meet 4 (80%) of the criteria for confirmation. This corroborates the reports of various researchers on the use of high-frequency ultrasound techniques to complement the laboratory findings of Njemenze et al. [19,27].

5. CONCLUSION

The geriatrics, especially the Female peasant farmers in Emekuku are more prone to IP, with *Ascaris lumbricoides* being most prevalent amongst other IPs such as hookworm, *trichomonas hominins, and entamoeba histolytica*. Sonography complements the role of the Concentration method of Laboratory stool analysis better than the Wet Preparation method for the presence, characterization, location, and complications of IP such as *Ascaris lumbricoides.*

6. LIMITATION OF THE STUDY

1. Low sample size was used because the majority of the peasant farmers were skeptical about participation in the study based on their beliefs and bias.
2. The age group of 20 to 29 years subjects did not return the specimen bottles with samples, they were 5.
3. All the peasant farmers studied had the highest educational background of Primary school. No holders of higher education recorded.

7. RECOMMENDATION

The following are recommended:

1. Good general sanitary and hygiene, washing of hands before and after eating. And proper washing of food items such as vegetables, fruits, etc. with warm-salt water before eating.
2. Use of appropriate antihelminth regularly at least every 4 to 6 months.
3. Use of appropriate laboratory stool test techniques such as the Concentration method.
4. Sonography is suggested to give more detailed findings and complications of IP

CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Consent forms and explanations were given to the participants to solicit their willingness to participate in the study. Confidentiality of the results of the participant was also ensured. The laboratory and sonographic reports were given to the participants free of charge on an individual based in order to seek medical attention especially when the results are positive.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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