

Original Research Article

Laboratory and Sonographic assessment of Intestinal parasitic load of peasant farmer in Emekuku Village in Nigeria

1. INTRODUCTION

Intestinal parasites (IP) are organisms which live and strife within the host for harm or mutual benefit¹. 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, 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 males⁶. 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, 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 hygiene⁹. They are very vulnerable in 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 samples¹². There are two main classes of parasites that infect the human intestine: protozoa and helminthes. Entamoeba. histolytica, G. lamblia, N. americanus, A. doudenale, hookworm, ascariasis and trichuriasis¹³. 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 faeces owing to very poor hygiene¹⁴, 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 stool¹⁵.

Depending on the parasitic species in question, various methods of quantification allow scientists to measure the numbers 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 development¹⁶.

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, determined and comparison made based on the biographic data of peasant farmers. Do the peasant farmers in Emekuku have high or low PL level 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, IP species and compare the PL distribution based on 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. 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 faeces 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 Concentration method using formal ether sedimentation technique for the concentration of stool parasites were used for comparison. Subjects who tested positive to IP were further examined using 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



Fig 1: Picture of materials used

2.2.1 Wet preparation method: 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 Dobbel's iodine was placed at the other end of the slide.
- An applicator stick was used to take 1g of the sample (faeces)
- The samples were emulsified in the drop of the saline and Dobbel's iodine.
- Smears of the saline and the iodine were covered with cover slip gently avoiding air bubbles and over floating.

- The smears were examined microscopically with X10 and X40 objective lens.

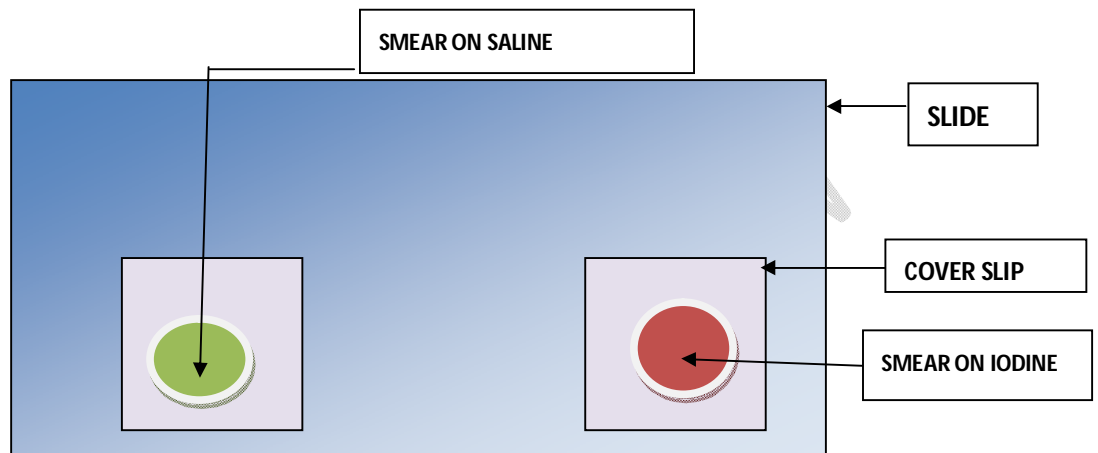


Fig 2: Illustration of Slide with the Saline and Iodine Smears

2.2.2. 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 faeces and placed it into a centrifuge tube containing 7ml of 10% formalin.
- The faeces 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.

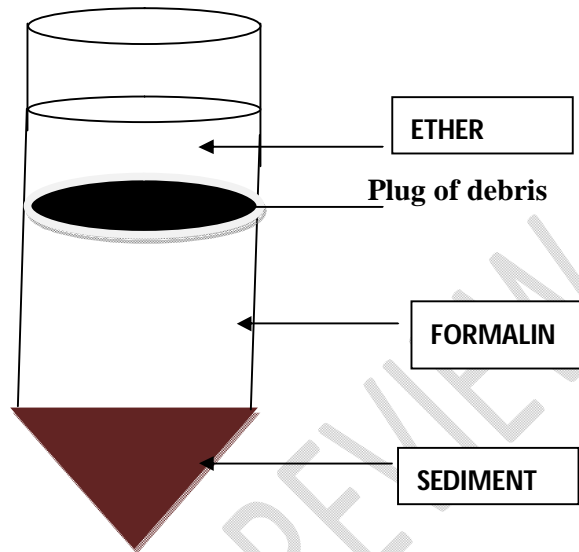


Fig 3: test tube using the concentration technique

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, Hyperechogenicity/hypoechoogenicity, Motility of IPs and morphologic changes in intestinal wall thickness¹⁹.

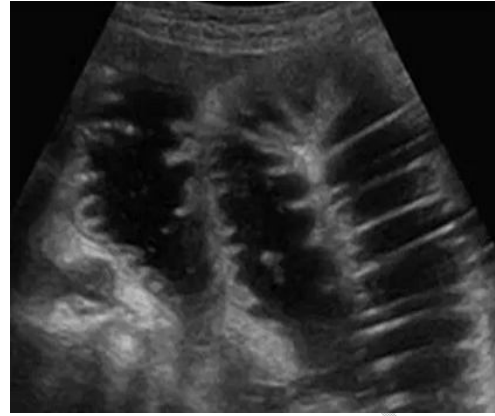
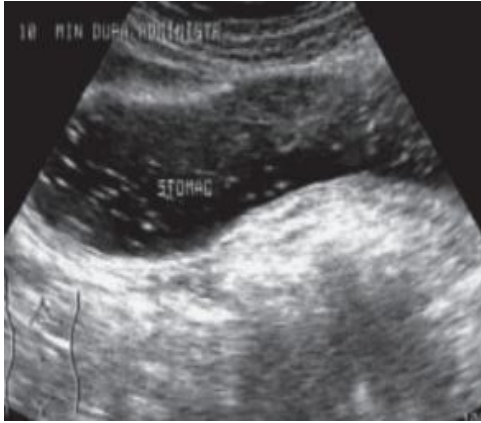


Fig4: Sonogram of the stomach filled with water Fig 5: Sonogram of the small intestine filled with water

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

3.0 RESULTS

3.1 Demography of subjects

Age and sex frequency distribution table is shown below. It shows the age range of subjects whose stools were studied and percentage. The age group 20 to 29 years subjects did not return the specimen bottles with samples, they were 5. 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 50% (63).

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

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. P value is < 0.05. Significant at P < 0.05

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
Hook worm	-	-	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

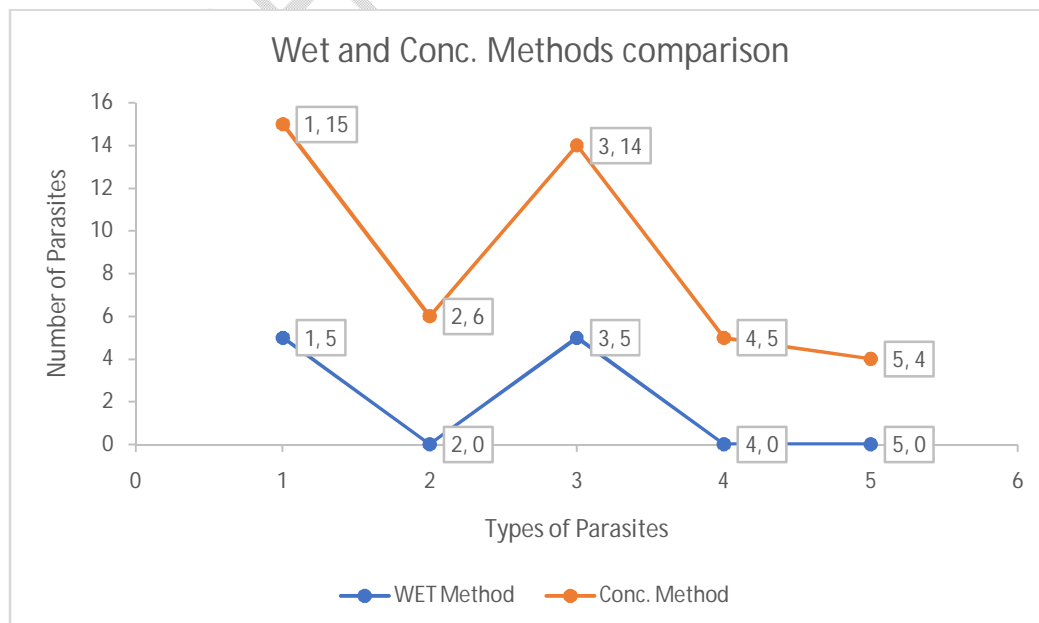


Fig 6: Scattered plot of the two methods

Table 3: Method comparison

Methods	No of Parasites seen	No 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. P value is < 0.05. Significant at P < 0.05

3.3 Parasites and age range distribution with Concentration method

Table 4 shows the parasites distribution 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 age group 30-39 years had 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.

Table 4: Parasite and age range distribution

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

3.4 Gender and parasite distribution

Table 5 shows the parasite and sex distribution. Positive test result indicates the presence of parasites in the stool specimen while negative test indicates absence of parasites. Female subjects had higher positive test for IP of 26 (41.27%) while the

male subjects with positive test result 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 $P < 0.05$.

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

Type of Parasites	Male (63)	%	Female (63)	%	Total	% n=44
<i>Ascaris Lumbricoides</i>	5	7.94	10	15.87	15	34.09
Hook worm	-	-	6	9.52	6	13.64
<i>E. Coli</i>	9	14.3	5	7.94	14	31.82
<i>Trachonionas Hominis</i>	-	-	5	7.94	5	11.36
<i>Entamoeba Histolytica</i>	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	
<i>Ascaris L.</i>	5	(6.38)	{0.30}	10	(8.62)	{0.22}	15
Hookworm	-	-	-	6	(4.02)	{0.97}	6
<i>E. Coli</i>	9	(5.96)	{1.55}	5	(8.04)	{1.15}	14
<i>T. Hominis</i>	-	-	-	5	(3.45)	{0.70}	5
<i>E. Histolytica</i>	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 $P < 0.05$.

3.5 Transabdominal sonography of the GIT to identify, characterize and confirm the presence of IP.



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

The most prevalent IP is the *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 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 absence

4.1 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 result 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 higher positive result of 26 (41.27%), the male had 18 (28.57%), $P = 0.039967$, at the level of $P < 0.05$ significance. This finding corroborates the findings of Sunil et al.,⁵; Hillegass et al.,⁶; Hotez et al.,⁹; Ragunathan et al.,²⁰ and Pullan et al.,²⁰. This implies that women are more at risk of intestinal parasitic infection than men and this could be as 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}

Table 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 level. 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 helminthes STHs globally²³.

Ascaris lumbricoides infected female peasant farmers more than the male in the present study. In table 5, 10 (15.87%) of female subjected 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 most in the age group 80 to 89 years, 10 (7.94%), 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 Julia²⁵; Silva et al.,²⁶. This implies that the geriatrics in the locality are more prone to IP. This could be as a result of the aging process with decreased immunity, hormones, farm stress, poor sanitation and nutrition and ineffective and inadequate anthelmintic 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 in the use of high frequency ultrasound techniques to complement the laboratory findings Njemenze et al.,^{19, 27}.

4.2 CONCLUSION

The geriatrics, especially the Female peasant farmers in Emekuku are more prone to IP, with *Ascaris lumbricoides* being most prevalent amongst other IP such as hookworm, *trachonionas hominis* and *entamoeba histolytica*. Sonography complement 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*.

4.3 LIMITATION OF STUDY

1. Low sample size was used because the majority of the peasant farmers were skeptical about participation in the study based of their belief and bias.
2. The age group 20 to 29 years subjects did not return the specimen bottles with samples, they were 5.

4.4 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 Concentration method.
4. Sonography is suggested to give more detailed findings and complications of IP

REFERENCE

1. WHO, Human Intestinal Parasites. Geneva, Switzerland: WHO Report; 2016 (Google Scholar).
2. Brooker, A. C. A. Clements, and D. A. P. Bundy (2006), "Global epidemiology, ecology and control of soil-transmitted helminth infections," *Advances in Parasitology*, vol. 62, pp. 221–261.
3. World Health Organization. Informal consultation on intestinal helminthes infection. Geneva, Switzerland: WHO; 2000. pp. 16–18. [Google Scholar].

4. Opara K, Udoidung Okon D, Edosomwan E, Udoh A. The Impact of Intestinal Parasitic Infections on the Nutritional Status of Rural and Urban School-Aged Children in Nigeria. *International Journal of MCH and AIDS*. 2012;1(1):73–82. [PMC free article] [PubMed] [Google Scholar].
5. Sunil T., Robe K, Nitin M and Feleke E. Prevalence of Intestinal Parasite Infections and their Associated Factors among Food Handlers Working in Selected Catering Establishments from Bule Hora, Ethiopia. *Biomed Res Int*. 2021; 2021: 6669742
6. Hillegass, M.A., Waterman, J.M., Roth, J.D. (2008). The influence of sex and sociality on parasite loads in Africans. *Behavioral Ecology*. Vol 19 (5) pp. 1006-1011.
7. Tessema A., Gelaye K., Chercos D. Factors affecting food handling practices among food handlers of Dangila Town food and drink establishments, north-west Ethiopia. *BMC Publish Health*. 2014;14(1):571-575.
8. NIAID. World NTD Day- Ending the Neglect of Neglected Tropical Diseases January 29, 2021.
9. Hotez, D. H. Molyneux, A. Fenwick (2007), “Control of neglected tropical diseases,” *The New England Journal of Medicine*, vol. 357, no. 10, pp. 1018–1027.
10. Edelman, Marc (2013) What is a peasant? What are peasantries? A briefing paper on issues of definition. United Nations Human Rights. Retrieved 11 September, 2019
11. Amuta E, Olusi T, Houmsou R. Relationship of intestinal parasitic infections and malnutrition among school children in Makurdi, Benue State, Nigeria. *The Internet Journal of Epidemiology*. 2013;7(1):20–24.
12. Prudhomme O'Meara W, Remich S, Ogutu B, et al. Systematic comparison of two methods to measure parasite density from malaria blood smears. *Parasitology research*. 2006; 99(4):500-504.

13. Zhang N, Harolyn W (2013). Classification of intestinal parasites. *Clinical infectious diseases*, 2:289-290.
14. WHO. Soil-Transmitted Helminth Infections. Geneva, Switzerland: WHO Fact Sheet; 2020. (Google Scholar).
15. Gabriella Cancrini. General Aspects of Helminths. *Encyclopedia of Infection and Immunity*. 2022 (2): 544-559
16. Garcia L.S., Arrowood M, Kokoskin E et al., 2018. Practical Guidance for Clinical Microbiology Laboratories: Laboratory Diagnosis of Parasites from the Gastrointestinal tract. *Clinical Microbiology Rev*. 2018 Jan; 31(1): e00025-17.
17. Njemanze PC, Anozie J, Chukwu C, Skelton A, Ogaraku AN. Ultrasound imaging characteristics of common protozoal and helminthic abdominal lesions. *The American Journal of Tropical Medicine and Hygiene*. 2001; 65:205-206
18. Njemanze PC, Njemanze J, Skelton A, Anoka A, Akagha O, Chukwu AA, et al. High-frequency ultrasound imaging of the duodenum and colon in patients with symptomatic giardiasis in comparison to amebiasis and healthy subjects. *Journal of Gastroenterology and Hepatology*. 2008;23:e37-e42. DOI: 1111/j.1440-1746.200705056.
19. Njemanze Phillip, Josephine T. Njemanze, Clara C. Ofoegbu, Chinwendu C. Darlington, Esther Nneke, Ijeoma A. Onweni, Uchechi V. Ejiogu, Chinenye U. Mgbenu, Nneoma E. Ukeje, Anthonia C. Amadi and Doris C. Amaefule. High-Frequency Ultrasound Imaging of the Intestine in Normal Subjects and Patients with Intestinal Parasites. 2019, DOI: 10.5772/intechopen.8465.
20. Ragunathan L, Kalivaradhan SK, Ramadass S, Nagaraj M, Ramesh K.(2010). Helminthic infections in school children in Puducherry, South India. *J Microbiol Immunol Infect*; 43:228-232.
21. Pullan RL, Brooker SJ. (2019). The global limits and population at risk of soil-transmitted helminth infections in 2010. *Parasit Vectors*; 5: 81-87.
22. Pullan, J. L. Smith, R. Jasrasaria, and S. J. Brooker, (2010) "Global numbers of infection and disease burden of soil transmitted helminth infections in 2010," *Parasites and Vectors*, vol. 7, no. 1, article 37.

23. Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, Xiao S. (2004). Hookworm infection. *N Engl J Med*; 351:799-807.
24. Forman E., Esy M. What makes the Elderly Prone to Parasitic Infection? *Asian Journal of Research in Infectious Diseases* 2021, 6(1):24-31.
25. Maria M and Julia M. High Prevalence of *Strongyloides stercoralis* Infection among the Elderly in Brazil. *Rev Inst Ttop Sao Paulo*. 2013,55(5):309-313.
26. Silva NR, Guyatt HL, Bundy DA. (1997). Worm burden in intestinal obstruction caused by *Ascaris lumbricoides*. *Trop Med Int Health*; 2:189-190.
27. Suthar P, Doshi R.P, Mehta C. and Khyati P. V. Incidental detection of ascariasis worms on USG in a protein energy malnourished (PEM) child with abdominal pain. *BMJ Case Rep*. 2015; 2015: bcr2014206668.

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