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ORIGINAL ARTICLE

Prevalence, Risk Factors, and Molecular Analysis of *Giardia lamblia* Infection among Primary School Pupils in Kaduna Metropolis, Nigeria: A Cross-Sectional Study

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ABSTRACT

Giardia lamblia is an intestinal protozoan parasite causing giardiasis, a significant cause of diarrhoeal disease among children. Data on its occurrence and molecular distribution among Nigerian schoolchildren remain limited. This study assessed the prevalence, risk factors, and molecular profile of *G. lamblia* infection among primary school pupils in Kaduna Metropolis, Nigeria. A cross-sectional study was conducted among 183 pupils selected using stratified random sampling across four Local Government Areas. Stool samples were examined microscopically and analysed using enzyme-linked immunosorbent assay (ELISA) for *G. lamblia* antigen detection and polymerase chain reaction (PCR) for molecular analysis of genetic assemblages. A structured questionnaire assessed relevant risk factors. Data were analysed using descriptive statistics and chi-square tests at $p \leq 0.05$. Overall prevalence was 9.29% (17/183). Prevalence varied significantly across locations, with the highest at LGEA Primary School, Nasarawa, 17.02% (8/47), and lowest at LGEA Primary School, Kawo, 4.35% (2/46) ($p \leq 0.05$). Pupils aged 7–11 years had a prevalence of 11.84%, with lower prevalence among older groups. Significant associations were observed between infection and selected hygiene factors, including toilet type. PCR amplification produced a 272-bp product specific for *G. lamblia* Assemblage B, identified among the molecularly analysed samples. Assemblage B distribution was significantly associated with demographic and socioeconomic characteristics. *G. lamblia* infection occurs among primary school pupils in Kaduna Metropolis, with Assemblage B detected among molecularly analysed samples. The findings underscore the need for health education, improved sanitation, safe drinking water, and targeted school-based interventions to reduce giardiasis transmission.

Keywords: *Giardia lamblia*; Giardiasis; Prevalence; Molecular analysis; Assemblage B; Risk factors; Primary school pupils.

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1.0 INTRODUCTION

Giardia lamblia sometimes called *G. duodenalis or G. intestinalis* is one of the most widespread intestinal protozoa that infect humans all over the world causing giardiasis (Adam, 2021). It is one of the major causes of diarrhoea in humans worldwide, especially in children. According to the CDC (2024), giardiasis affects more than 280 million people worldwide and is transmitted by the faecal-oral route of transmission. The parasite inhabits the duodenum and ileum of humans where it causes significant pathologies primarily in children (Adam, 2021). According to Hooshyar et al (2019), the risk of giardiasis is probably higher in individuals with diarrhoea and it is a common problem in countries with poor sanitation, especially in poor and developing countries. The life cycle of *G. lamblia* is simple and it involves two stages. The infectious cyst form which is passed into the environment through defecation, and a trophozoite stage, which is found in the lumen of the infected individuals (Jex et al., 2020). Infection is contracted after ingestion of the cyst by a host, followed by the release of trophozoites into the intestine in a process known as excystation (Eric & Micheal, 2024). Prevalence of *Giardia* infection is reported to be higher in developing countries due to poor sanitary conditions which favours the contamination of water and food with parasite cysts (Belkessa et al., 2021). Giardiasis is currently included by the World Health Organization among the "neglected diseases initiative" due to its high prevalence among children in developing nations (Farzad et al., 2021). Infection is characterized by diarrhoea, flatulence, anorexia, abdominal pains, malabsorption and vomiting (Chelsea & William, 2024.). Other severe consequences of giardiasis observed in children may include retarded growth, poor cognitive function and detrimental effect on nutritional function (Prabakaran et al., 2023). According to Dunn & Juergens (2024), there is increased mortality in infants from giardiasis infection due to dehydration. Infection rate in asymptomatic children has been reported to be between 8% to 30% in developing countries and 1% to 8% in industrialized nations (Hooshyar et al., 2019). *G. lamblia* is considered a species complex of several assemblages, comprises eight distinct genetic groups known as assemblages (A-H) (Klotz et al., 2023). The majority of these assemblages are host specific, but only assemblages A and B are known to infect humans, with assemblage B being described as more common (Klotz et al., 2023; Dunn & Juergens, 2024). However, reports of studies on giardiasis in humans has not provided enough information on the geographic or socio-economic differences in the distribution of assemblages A and B in many parts of the world (Ryan et al., 2019).
Diagnosis of *G.lamblia* infection is carried out in a variety of ways depending on the availability of equipment. In most developing countries including Nigeria, the laboratory diagnosis of *G. lamblia* is mainly based on the finding and demonstration of microscopic cyst and trophozoites in stool samples. The present study aimed to investigate the prevalence,

identify the associated risk factors and distribution of *G. lamblia* infection among primary school pupils in Kaduna metropolis, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Study area

The study was conducted in Kaduna Metropolis, Kaduna State, Nigeria, covering Kaduna North, Kaduna South, Igabi, and Chikun Local Government Areas (LGAs). The study area lies approximately between 10°23′ and 10°43′ N latitude and 7°17′ and 7°37′ E longitude. The study was conducted from February 2023 to February 2024. These LGAs were selected because they encompass the major urban and peri-urban communities of Kaduna Metropolis, providing a representative setting for assessing *G. lamblia* infection among school-aged children.

2.2 Study population

The population for the study comprised primary school pupils who were selected by stratified random sampling technique. Stratified sampling entailed dividing the population into various strata of age and sex. This sampling technique therefore allowed selection to be conducted across the different classes in the schools.

2.3 Sample size determination

Sample size was calculated using the formula described by Naing et al. (2006), based on a previously reported *Giardia lamblia* prevalence of 41.4% among children in Zaria, Kaduna State, Nigeria (Helen et al., 2011), at a 5% precision level. The calculated minimum sample size was 180 pupils. A total of 183 pupils were recruited for the study.

$$N = \frac{Z^2 P(1 - P)}{d^2}$$

Where, N= Sample Size
Z= the normal standard deviation at 95% confidence interval.
P= the frequency of occurrence of G. *lamblia.*
d= degree of precision.

2.4 Inclusion and exclusion criteria

Primary school pupils aged 7–21 years who were enrolled in the selected schools, whose parents or guardians provided informed consent, and who assented to participate were eligible for inclusion in the study. Only pupils who provided stool samples suitable for laboratory analysis were included. The 7–21-year age range was adopted to include all pupils enrolled in the selected primary schools who met the study criteria, irrespective of age, since school enrolment status rather than chronological age defined the study population. Pupils outside the 7–21-year age range**,** those who did not adequately complete the questionnaire, those who declined to participate or whose parents/guardians did not provide consent, and those who were unable to provide and submit a stool sample were excluded from the study.

2.5 Ethical approval

Ethical approval for the study was obtained from the Health Research Ethics Committee (HREC) of the Kaduna State Ministry of Health (Approval No. MOH/ADM/744/VOL.1/1170; NHREC No. NHREC/17/03/2018)**.**

2.6 Administration of questionnaire

Questionnaires were administered to the pupils to obtain information on their socio-demographic data, health status (if they had diarrhoea and gastrointestinal symptoms), parents' educational status and occupation, sanitary facilities, and source of drinking water. Letters addressed to the parents and providing guide on how to collect early morning stool from their wards and questionnaire were sent to parents of pupils too young to complete the questionnaires on their own.

2.7 Sample collection and storage

One hundred and eighty-three (183) stool samples were collected from pupils from the selected primary schools in the study area. The primary schools were selected from each LGA to provide representation of the different administrative areas of Kaduna Metropolis and enable comparison of *G. lamblia* occurrence across the metropolis. Pupils were provided with sterile plastic sample bottles. The sample bottles have spatula and tight-fitting lid. The pupils were instructed on how to collect a spatula full of stool sample in the morning and transfer it into the labelled container. A single stool sample per child was collected. Samples collected from pupils were transported in sample trays immediately to the laboratory and were stored at −20°C until analysis.

2.8 Sample analysis

Samples were analysed at the Precision Biomedical Laboratory and National Eye Center, all located in Kaduna State. Samples brought to the laboratory were analysed immediately or stored at -20°C until analysis.

2.9 Microscopic examination

Fresh stool samples were examined using saline and iodine preparations. Normal saline (0.9%) was prepared by adding 9 g of pure sodium chloride (NaCl) to one litre of distilled water. Lugol's iodine was prepared by adding 5 g of iodine powder and 10 g of potassium iodide into 100 mL of distilled water following the standard procedure described by the World Health Organization (WHO, 2019). A working solution of Lugol's iodine was then made by diluting 1mL Lugol's iodine and 5mL of distilled water. About 2 mg of each stool sample was mixed with a drop of normal saline (0.9%) for saline preparation, on a clean glass slide (75×26 mm) and covered with a cover glass (22×22 mm). another 2mg of each stool sample was mixed with a drop of Lugol's iodine on the glass slide, covered with a cover glass and placed beside the saline preparation. This was placed carefully and then examined by light microscopy using (10×) and (40×) objective lens and then photographed (Appendix F).

2.10Detection of parasite antigen from stool sample using enzyme linked immunosorbent assay (ELISA)

The principle is based on the detection of *G. lamblia* antigen enzyme-antibody-antigen complexes that formed in the presence of *Giardia* antigen (Pouryousef et al., 2023). *Giardia lamblia* antigen was detected in stool samples using the AccuDiagnostic Giardia ELISA kit (Diagnostic Automation/Cortez Diagnostics, Inc., USA) according to the manufacturer's instructions. Briefly, stool samples were diluted with the provided dilution buffer, and aliquots of the diluted samples, together with positive and negative controls, were added to the ELISA wells. Following incubation and washing, conjugate and chromogenic substrate were added sequentially according to the manufacturer's protocol. The reaction was terminated with stop solution, and absorbance was measured using an ELISA reader. Samples were interpreted based on the manufacturer's specified criteria.

2.11 Extraction of parasite DNA from faecal stool sample.

DNA was extracted from *G. lamblia* positive samples using Phenol-chloroform faecal DNA extraction protocol (Faria et al., 2016). About 200 µL of sample was transferred to a 1.5 mL tube followed by the addition of 400 µL of lysis buffer and 10 µL of proteinase K to the tube, and incubated at 55°C for 10 to 60 min. After incubation, 400 µL of equilibrated phenol (pH 7.8) was added and vortexed for 1 min. The tube was then centrifuged at 12000 x g for 5 min and then decanted.

To the supernatant, 700 µL of Chloroform and Isoamyl alcohol (24:1) (672 µL Chloroform: 28 µL Isoamyl alcohol) was added, mixed well and centrifuged at 12000x g for 5 min. The upper aqueous layer was transferred to a fresh tube and 40 µL of 3M sodium acetate (pH 5.2) and 400 µL of 100% ethanol was added. This was then incubated at -20°C for 1 h. After incubation, it was centrifuged for 15 min at 4°C and at a speed of 14000 x g to pellet the DNA.

The supernatant was carefully removed without disturbing the DNA pellet and 150 µL of 70% cold ethanol was added and centrifuged at 4°C for 2 min at 14000 x g and the supernatant discarded. The DNA pellets were then dried at room temperature for 10 min and resuspended in 100 µL of Tris-EDTA buffer by pipetting up and down. It was centrifuged briefly to collect the sample in the tube which was then placed on ice.

2.11.1 DNA Amplification and Molecular Characterization

Molecular characterization of *G. lamblia* was carried out using assemblage-specific primer in a multiplex PCR protocol. PCR-relevant protocols, primers and probes are as shown in Table 1 were used (Faria et al., 2016). The genotyping analysis was performed using assemblage specific primers that amplify PCR-tpi gene. The procedure are as follows: To 10 µL of the master mix (Bioline UK), 3 µL of Molecular grade water

was added as well as 1ul each of the forward and reverse Primers. 5 µL of the template DNA was also added. The total solution for the PCR mix was 20 µL.The mixture was then transferred into Thermal cycler (Applied Biosystem USA). The condition used in the PCR machine is as follows; Initial Denaturation was carried out at 95°c for 1 min. Followed by a set of 40 cycles consisting of denaturation at 94°C for 30 seconds. Annealing at 50°C for 40 seconds, and Extension at 72°C for 1 min, followed by final extension at 72°C for 10min.

2.11.2 Gel Electrophoresis

Agarose (1%) was used for gel electrophoresis. This was prepared by transferring 1 g of agarose powder in 100 mL TBE Buffer. The suspension was stirred until it dissolved, then heated in microwave until it boils. It was then allowed to cool to 50°C and then 2 µL of Server DNA stain was added and then poured into a cast and allowed to solidify. It was then removed and put into the electrophoresis tank. The PCR products were loaded into the well. Electric current was applied at 100V for 40 seconds, the gel was then taken to Gel Documentation System and visualized under UV. DNA from axenic cultures of *G. lamblia* strains WBC6 (assemblage A) and GS (assemblage B) were used as positive controls, while ultra-pure water was included as negative control.

2.11.3 Sequence analysis

Deoxyribonucleic acid (DNA) sequences specific to the *tpi* gene was not done as there was no positive result on the *tpi* gene PCR analysis.

Table **1.** Primers for real-time PCR, assemblage-specific PCR, and *tpi* gene-based genotyping

Assay	Primer/Probe	Assemblage	Primer and Probe Sequences (5^0-3^0)	PCR Product Size (bp)	Reference
Assemblage specific PCR	4E1-HP	A B	Forward AAAGAGATAGTTCGCGATGTC Reverse ATTAACAAACAGGGAGACGTATG Forward GAAGTCATCTCTGGGGCAAG Reverse GAAGTCTAGATAAACGTGTCGG	165 272	Faria et al., 2012.
Tpi gene analysis	*Giardia* AL3543 *Giardia* AL3546 *Giardia* AL3544 *Giardia* AL3545	All assemblages	Forward AAATIATGCCTGCTCGTCG Reverse CAAACCTTITCCGCAAACC Forward CCCTTCATCGGIGGTAACTT Reverse GTGGCCACCACICCCGTGCC	605 530	Belkessa, 2021.

Sources: (Faria et al., 2012; Belkessa, 2021).

2.12 Data analysis

Data were analysed using IBM SPSS Statistics version 20. Descriptive statistics, including frequencies and percentages, were used to summarize the occurrence and distribution of *G. lamblia* infection across the study variables. Associations between *G. lamblia* infection and categorical demographic, socioeconomic, environmental, and clinical variables were assessed using the chi-square test of independence or Fisher's exact test where appropriate. Statistical significance was set at $p < 0.05$. Findings were presented in tables and bar charts.

3.0 RESULTS

3.1 Prevalence of *Giardia* through stool antigen detection

An overall prevalence of 9.29 % was obtained from 17 positive samples recorded. High prevalence, 4.37 (17.02 %) was recorded from 47 samples examined in LGEA Primary School, Nasarawa and lower prevalence, 1.09 (4.35) was recorded from 46 samples examined in LGEA Primary School, Kawo. 0.000 ($p < 0.05$) (Table 2)

3.2 Risk factors of Giardiasis among primary school children

Table 3 presents the distribution of *Giardia lamblia* antigen by gender. A prevalence of 6.01 (10.28 %) was recorded from 11 positive cases out of 107 females examined samples. Lower prevalence, 3.28 (7.89 %) was recorded in 76 males sample examined. The distribution of *Giardia lamblia* antigen by age group is presented in Table 4 Sampled population of age group, 7 – 11 recorded higher prevalence, 4.92 (11.84%) and lower prevalence, 0.55 (20.00%) from 1 positive sample out of 5 samples examined in age group 17 – 21.

3.3 Prevalence of *Giardia lamblia* by socioeconomic status

The distribution of *Giardia lamblia* antigen by socioeconomic, housing and educational status are presented in Table 5. Pupils whose parents were traders recorded higher prevalence, 3.28 (7.32 %) from 6 positive samples obtained out of 82 total population examined. Lower prevalence, 0.27 (15.15 %) was obtained from 5 positive samples out of 33 children of farmers examined. A significant chi-square value, 0.000 ($p \leq 0.05$) was obtained from different classes of socioeconomic backgrounds. Similar higher prevalence, 4.3 (19.51 % and 5.35 % respectively) were recorded from similar 8 positive samples each of those living in flat and rooming houses out of 41 and 126 respective populations examined. No prevalence, 0 (0.00 %) was recorded from 3 population of housing classified as other. Chi-square analysis for *Giardia lamblia* prevalence per housing was significantly different, 0.000 ($p \leq 0.05$). Based on educational status of parents, there was a very high prevalence, 4.92 (9.38 %) among those who had secondary education from which 9 positive samples were obtained out of 96 population examined. Also, lower prevalence, 1.09 (11.76 %) was recorded from 2 positive samples out of 17 population of pupils whose parents had non-formal education. There was a high significant difference, 0.000 ($p \leq 0.05$) chi-square value among education status examined.

Table **2.** Seroprevalence of Giardiasis among sampled people

Variable	Total No. Examined	No. of positive	% of positive	Prevalence	Statistics (χ^2)
Primary Schools					
LGEA Primary School Nasarawa (Chikun LGA)	47	8	17.02	4.37	0.000
LGEA Primary School K/Mashi (Kaduna South LGA)	48	3	6.25	1.64	
LGEA Primary SchoolKawo (Kaduna North LGA)	46	2	4.35	1.09	
LGEA Primary School Rigasa (Igabi LGA)	42	4	9.52	2. 19	
Total	183	17	9.29	9.29	

Table **3.** Distribution of *Giardia lamblia* antigen by gender

Variable	Total No. Examined	No. of positive	% of positive	Prevalence	Statistics (χ^2)
Sex					0.000
Male	76	6	7.89	3.28	
Female	107	11	10.28	6.01	
Total	183	17	18.17	9.29	

Table **4.** Distribution of *Giardia lamblia* antigen by age group

Variable	Total No. Examined	No. of positive	% of positive	Prevalence	Statistics (χ^2)
Age group					χ^2
7 – 11	76	9	11.84	4.92	0.000
12 – 16	102	7	6.86	3.83	
17 – 21	5	1	20.00	0.55	
Total	183	17	37.70	9.30	

Table **5.** Distribution of *Giardia lamblia* antigen by socioeconomic, housing and educational status

Variable	TotalNo.Examined	No. of+ve	% of+ve	Prevalence	Statistics(χ^2)
Socioeconomic background					
Civil servant	24	3	12.50	1.64	0.000
Farmer	33	5	15.15	0.27	
Trader	82	6	7.32	3.28	
Unemployed	19	2	10.53	1.09	
Others	25	1	4.00	0.55	
Housing					
Bungalow	13	1	7.69	0.55	0.000
Flat	41	8	19.51	4.37	
Rooming	126	8	6.35	4.37	
Other	3	0	0.00	0	
Educational status					
Non-formal	17	2	11.76	1.09	0.000
Primary	46	3	6.52	1.64	
Secondary	96	9	9.38	4.92	
Tertiary	24	3	12.50	1.64	

3.4 Distribution of *Giardia lamblia* by hygiene status, symptoms and local government area

Giardia lamblia distributed by hygiene status indicating toilet type, water source is presented in Table 6. A high prevalence of 6.01 (12.22 %) was recorded from 11 positive samples out of 90 population examined of those that use flush toilet and similar prevalence of 0.55 (11.11 % and 25.00 %) were recorded from 1 sample each for those who use bucket and others type of toilet. There was a high level of chi-square significance, 0.000 ($p \leq 0.05$) for all toilet types used and the distribution of *Giardia lamblia*. Higher prevalence of 6.01 (10.28 %) was recorded from 11 positive samples obtained out of 107 population that uses tap as their source of water. While those who use water from other sources was not prevalent, those whose water sources are borehole and stream were low in prevalence, 0.55 (3.45 % and 25.00 % respectively) of *Giardia lamblia* distribution. Chi-square analysis between *Giardia lamblia* and water sources was highly significant, 0.000 ($p \leq 0.05$). The distribution of *Giardia lamblia* by symptoms such as diarrhoea, abdominal pain and vomiting are presented in Table 7. High prevalence was recorded for those who had no symptoms of diarrhoea, 6.01 (7.24 %), abdominal pains, 6.56 (9.30 %) and vomiting, 7.10 (7.93 %) out of 152, 129 and 164 total population examined. The distribution of *Giardia lamblia* by local government area is presented in Table 8, Chikun Local Government Area recorded higher prevalence, 4.37 (17.02 %) from 8 positive samples out of 47 population examined. Lower prevalence, 1.09 (4.26 %) was recorded in Kaduna North Local Government Area from 2 positive samples obtained out of 46 total population examined. Chi-square value was significantly different, 0.000 ($p < 0.05$) between LGEAs and *Giardia lamblia* distribution.

Table **6.** Distribution of *Giardia lamblia* by hygiene status

Variable	Total No. Examined	No. of +ve	% of +ve	Prevalence	Statistics (χ^2)
Toilet type					
Bucket	9	1	11.11	0.55	0.000
Flush	90	11	12.22	6.01	
Pit	80	4	5.00	2. 19	
Others	4	1	25.00	0.55	
Water Source					
Borehole	29	1	3.45	0.55	0.000
Stream	4	1	25.00	0.55	
Tap	107	11	10.28	6.01	
Well	35	4	11.43	2.19	
Other	8	0	0.00	0	

Table **7.** Distribution of *Giardia lamblia* by symptoms

Variable	Total No. Examined	No. of +ve	% of +ve	Prevalence	Statistics (χ^2)
Diarrhoea					
Yes	31	6	19.35	3.28	0.000
No	152	11	7.24	6.01	
Abdominal pains					
Yes	54	5	9.26	2.73	0.000
No	129	12	9.30	6.56	
Vomiting					
Yes	19	4	21.05	2. 19	0.000
No	164	13	7.93	7. 10	

Table **8.** Distribution of *Giardia lamblia* by local government area

Variable	Total No. Examined	No. of +ve	% of +ve	Prevalence	Statistics (χ^2)
Chikun	47	8	17.02	4.37	0.000
Kaduna South	48	4	8.51	2.19	
Kaduna North	46	2	4.26	1.09	
Igabi	42	3	6.98	1.64	

3.5 Polymerase Chain Reaction

Plates 1 and 2 shows gel electrophoresis result indicating the presence of *Giardia* Assemblage B DNA in all tested samples (lanes 1–12) at 300 bp. Similarly, Gel electrophoresis carried out using TPI gene could not be amplified, hence no band was observed.

Distribution of *G. lamblia* assemblage B using Polymerase Chain Reaction (PCR) Table 9 shows the distribution of *Giardia lamblia* assemblage B by age and sex. High prevalence, 41.18 (77.78 %) from 7 positive samples obtained out of 9 total population, age range 7 - 11

examined. There was no positive sample, (0) recorded for those of age range ≥ 17. Chi-square value between distribution of *Giardia lamblia* assemblage B and age range was significantly different, 0.000 ($p \leq 0.05$). Males were highly prevalent, 35.29 (83.33 %) with 5 positive samples out of 6 total population examined and females' prevalence of 29.41 (45.45 %) which is lower. Chi-square value is significantly different, 0.000 (at $p \leq 0.05$). (Table 9). Distribution of *Giardia lamblia* assemblage B by socioeconomic background, housing and educational status are presented in Table 9. Result obtained from farmers was highly significant, 29.41 (40.00 %) from 2 positive samples out of 5 total population examined. Lower prevalence, 5.88 (100 %) was recorded from 1 positive sample out of 1 total population examined from socioeconomic background labelled as others. Chi-square analysis indicated high significance, 0.035 (at $p \leq 0.05$). Similar high prevalence of 47.06 (75 % and 50 %) each of *Giardia lamblia* assemblage B were recorded from population associated with flat and rooming housing respectively. Lower prevalence, 5.88 (0 %) was recorded from those in bungalow housing. Chi-square value between *Giardia lamblia* assemblage B and housing was significantly different, 0.033 (at $p \leq 0.05$) (Table 10). Pupils of parents with secondary education had higher prevalence with 47.06 (44.44 %) recorded from 4 positive samples out of 9 total population examined. All other classes of educational status, non-formal education, 11.77 (100 %), primary education, 11.77 (66.67 %) and tertiary education, 11.77 (66.67 %) had similar low prevalence out of the total population examined. Also, chi-square value was significantly different, 0.001 ($p \leq 0.05$).

Distribution of *Giardia lamblia* assemblage B by hygiene status (toilet type and water source) is presented in Table 11. Population who used flush toilet type were highly significant, 35.29 (54.55 %) from 6 positive samples out of 11 total population examined. Similar low prevalence, 5.88 (100 %) each were recorded for bucket and other toilet types. There is high significance, 0.005 ($p \leq 0.05$) chi-square value obtained between *Giardia lamblia* assemblage B and toilet type. High prevalence, 41.18 (63.64 %) was recorded from 7 positive samples out of 11 total population examined and borehole water source recorded lower prevalence, 5.88 (100 %) from 1 positive sample out of 1 total population examined. There is high significance, 0.013 (at $p \leq 0.05$) chi-square value obtained between *Giardia lamblia* assemblage B and water source. *Giardia lamblia* assemblage B distribution by symptoms (diarrhoea, abdominal pains and vomiting) are presented in Table 12. Population examined that are positive with no symptoms were highly prevalent (diarrhoea, 35.29 (54.55 %), abdominal pains, 41.18 (58.33 %) and vomiting, 47.06 (61.54 %)) from the total population examined. There is high significance, 0.002 (at $p \leq 0.05$) chi-square value obtained between *Giardia lamblia* assemblage B and symptoms

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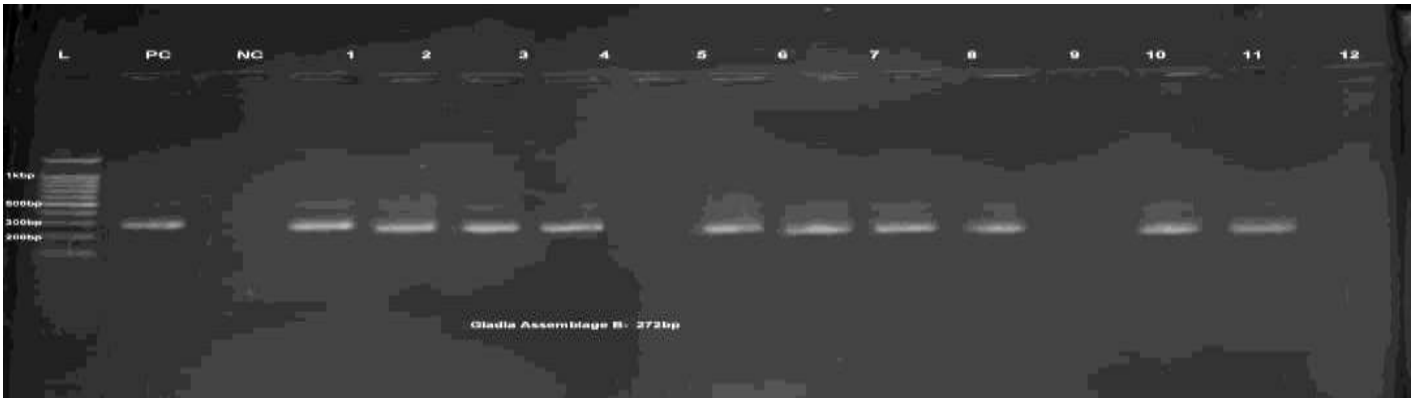


Plate **1.** Agarose gel electrophoresis of PCR amplicons showing the detection of *Giardia Assemblage B* DNA in all Tested Samples (lanes 1–12)
PC - positive control; NC – negative control; L – Ladder.

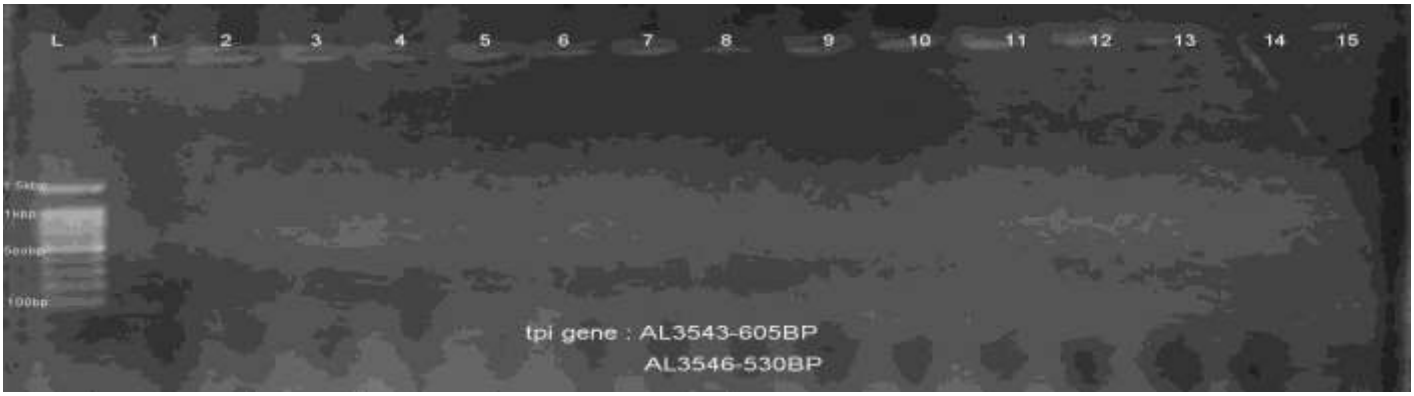


Plate **2.** Agarose gel electrophoresis of PCR products amplified using TPI gene-specific primers showing absence of detectable amplicons in all test samples.

Table **9.** Distribution of *Giardia lamblia* Assemblage B by age group and sex

Variable	**Total No. Examined**	**No. of positive**	**% of positive**	**Prevalence**	**Statistics (χ^2)**
Age range					
7 – 11	9	7	77.78	41. 18	0.002
12 – 16	7	3	42.86	17.65	
≥17	1	0	0.00	5.88	
Sex					
Male	6	5	83.33	35.29	0.002
Female	11	5	45.45	29.41	
Total	17	10	58.82		

Table **10.** Distribution of *Giardia lamblia* Assemblage B by socioeconomic background and educational status

Variable	Total No. Examined	No. of positive	% of positive	Prevalence	Statistics (χ^2)
Socioeconomic background					
Civil servant	3	2	66.67	11.77	0.035
Farmer	5	2	40.00	29.41	
Trader	6	3	50.00	17.65	
Unemployed	2	2	100.00	11.77	
Others	1	1	100.00	5.88	
Housing					
Bungalow	1	0	0.00	5.88	0.033
Flat	8	6	75.00	47.06	
Rooming	8	4	50.00	47.06	
Educational status					
Non-formal	2	2	100.00	11.77	0.001
Primary	3	2	66.67	11.77	
Secondary	9	4	44.44	47.06	
Tertiary	3	2	66.67	11.77	

Table **11.** Distribution of *Giardia lamblia* assemblage B by hygiene status

Variable	Total No. Examined	No. of +ve	% of +ve	Prevalence	Statistics (χ^2)
Toilet type					
Bucket	1	1	100.00	5.88	0.005
Flush	11	6	54.55	35.29	
Pit	4	2	50.00	11.77	
Others	1	1	100.00	5.88	
Water Source					
Borehole	1	1	100.00	5.88	0.013
Stream	1	0	0.00	0	
Tap	11	7	63.64	41. 18	
Well	4	2	50.00	11.77	

Table **12.** Distribution of *Giardia lamblia* assemblage B by symptoms

Variable	Total No. Examined	No. of +ve	% of +ve	Prevalence	Statistics (χ^2)
Diarrhoea					
Yes	6	4	66.67	23.53	0.001
No	11	6	54.55	35.29	
Abdominal pains					
Yes	5	3	60.00	17.65	0.001
No	12	7	58.33	41. 18	
Vomiting					
Yes	4	2	50.00	11.77	0.002
No	13	8	61.54	47.06	

4.0 DISCUSSION

The microscopic identification of *Giardia spp*. in faecal samples is considered the most common approach for the diagnosis of giardiasis. This method is performed to detect cysts and trophozoites. However, this study utilized molecular methods due to their sensitivity and specificity in differentiating multiple assemblages of *Giardia lamblia* (Nawaz et al., 2019). The decrease in the prevalence of giardiasis with increasing age could be based on a number of factors. One reason is increasing and developing immune system with age in humans. Younger children are known to have less developed immune responses, thus making them more vulnerable to infections like giardiasis. However, as they grow in age, their immune systems strengthen, thereby enhancing their ability to resist infections (Mohammed & Omudu, 2011) including giardiasis. Another reason is behavioural factors of younger children such as hand-to-mouth activities, inadequate hand washing, and close contact in group settings which increases their exposure to *G. lamblia* cysts. Older children typically adopt relatively better hygiene practices, and in turn reducing their risk of infection. Findings from this study agree with Ali et al. (2022) whose study in Duhok, Iraq, observed a peak infection rate among younger children, with prevalence significantly decreasing in children in higher ages, highlighting age as a critical determinant of infection risk. This study also agrees with Khanum et al. (2015) whose study reported that susceptibility to *G. lamblia* was higher among younger age groups, with a marked reduction in older children, likely due to immune adaptation and reduced exposure to high-risk environments. Furthermore, research by Abdulabbas et al. (2022) in Wasit Province and Mosul, Iraq, also found that the prevalence of *G. lamblia* was highest in children under ten years of age, with reduced infection rates in older age groups, underscoring the influence of age on susceptibility to the disease. These findings collectively suggest that as children grow and mature, the risk of *G. lamblia* infection diminishes due to factors including behavioural changes and physiological adaptations that bolster resistance to such infections. Higher prevalence of *Giardia lamblia* infection occurred in females than in males and almost double. This could be a result of multiple factors, such as environmental and hormonal differences. For instance, cultural or social practices that increase the female exposure to potentially contaminated water sources may be a contributory factor to females having higher prevalence than males. In regions where women and girls are more engaged in household activities such as water collection and food preparation, the likelihood of exposure to waterborne pathogens like *G. lamblia* may be higher (Dunn & Juergens, 2024). Biological factors, such as hormonal differences, may also affect susceptibility to infections, though further research is necessary to clarify this link. Moreover, disparities in health-seeking behaviour between genders, where females might be more likely to seek healthcare services including diagnosis, could lead to higher chances of discovery of the pathogen and consequently higher prevalence among females. These factors collectively suggest that the gender-related prevalence of *G. lamblia* may be multifaceted, rooted in both social exposure and biological predisposition. However, findings from this study disagree with Hamad (2024). The study which was carried out in Ramadi Teaching Hospital, Fallujah Teaching Hospital and Ramadi Teaching Hospital for Maternity and Children in Al-Anbar governorate of Iraq, found that males had a higher prevalence of *Giardia lamblia* infections compared to females. Specifically, the prevalence rates were 49.6% for males and 45.7% for females (Hamad, 2024).

Children of traders exhibited higher prevalence than those of farmers. This could be largely due to poor hygiene and exposure to contaminated water. Occupation therefore plays a role in the prevalence of *Giardia lamblia* infection. A study carried out by Hajare et al. (2022) conducted in Ethiopia at the Kochore Health Centre, found that occupations with frequent exposure to unsanitary environments, low income, and limited access to clean water were associated with higher *G. lamblia* prevalence. Additionally, research in Paris, France, revealed that sewage workers had a higher prevalence of intestinal parasites, including *G. lamblia*, compared to food handlers. The study showed that sewage-exposed workers had a significantly elevated risk of infection, likely due to the presence of protozoan cysts in raw sewage (Gautam et al., 2024). In Nigeria, a study examining occupational exposure to pathogens found that herdsmen, butchers, and farmers exhibited higher infection rates of *G. lamblia* and other enteric parasites compared to other groups. This prevalence was attributed to environmental exposure and inadequate hygiene practices associated with these occupations (Hajare et al., 2022). It can thus be argued that occupation and the associated socioeconomic background of parents contribute to the level of exposure to pathogens. Those that live in flats and houses demarcated in rooms have the same prevalence rate. There are no records of previous studies directly linking *Giardia lamblia* prevalence to particular housing type such as bungalows, flats or room arrangement. However, findings from this study on the spread of *G.*

lamblia suggest that living conditions associated with certain housing types could contribute to higher prevalence. For instance, flats and rooming arrangements often involve shared water facilities, bathrooms, and cooking areas, which can lead to increased contact with contaminated surfaces and water sources if sanitary conditions are not adequate. In densely populated living arrangements, the risk of waterborne or fomite-based transmission is heightened due to limited private access to sanitation, increased exposure to potential carriers, and possibly lower adherence to personal hygiene due to communal facilities. Research indicates that the lack of private sanitation facilities and higher levels of water and environmental contamination in crowded or shared housing increase the transmission risk of protozoan infections like *G. lamblia* (Ali et al., 2020). From the study, those that live in bungalows had the lowest prevalence. This lends credence to the role of increased hygiene associated with living in more private accommodation type with little or no sharing of facilities such as water, kitchens and toilets. The more educated a person is, the higher the chances of being economically empowered and also more enlightened about hygiene. Pupils whose parents had education up to tertiary level had a prevalence rate similar to those whose parents had primary education. On the other hand, those whose parents had secondary education had higher prevalence, while those with non-formal education had low prevalence. Though the results are not directly proportional, but there is a slight correlation between education and the prevalence of giardiasis. Thus, education remains a key in the eradication of not only giardiasis, but almost all other diseases known to man.

The type of toilet used at home plays a major role in the prevalence of *Giardia lamblia* infection. Findings from this study revealed that *Giardia lamblia* prevalence was highest among participants who used the flush toilet, followed by pit latrine, while those that used bucket toilets and other toilet type had lower prevalence each. These results can be traced to sanitation practices and cross-contamination risks, but they are not necessarily related to the type of toilet. Studies suggest that water-based sanitation systems, if not properly maintained, can lead to higher infection rates as they often involve shared plumbing networks that can become contaminated if there is inadequate infrastructure or poor maintenance system. In contrast, pit and bucket toilets, while often associated with unsanitary conditions, usually limit the spread of waterborne pathogens through direct water contamination pathways, though they present their own unique hygiene challenges (Saleh et al., 2018). A study conducted in Iraq found a correlation between the use of flush toilets and increased rates of *G. lamblia* infection, emphasizing that contamination from poorly maintained flush toilet systems was a significant factor (Saleh et al., 2018). A study conducted in Nigeria had highlighted that high infection rates among students were linked to shared toilets and poor sanitation in areas where people use flush toilets, where the risk of cross-contamination was increased by communal use and inadequate hygiene practices (Enitan et al., 2022). Conversely, research conducted in the Duhok region of Iraq suggested that while *G. lamblia* infection rates were higher in households with pit toilets, the infections were more likely due to overall low sanitation rather than the toilet type itself, indicating that hygiene practices and maintenance play more crucial roles than the specific type of sanitation facility (Ali et al., 2022).

Giardia lamblia is primarily transmitted through water, as its cysts are highly resistant to environmental conditions and can survive in various water sources, including lakes, rivers, and even inadequately treated tap water (Kumar et al., 2016; Dunn & Juergens, 2024). Of all the water sources analysed in this study, tap water was responsible for the highest prevalence. This high prevalence rate could be attributed to a number of factors, especially the inadequate treatment of water. Moreover, the infective cyst form of the parasite is known to be resistant to conventional water treatment methods such as chlorination. Consequently, it survives for longer periods in water sources including municipal water supply system. People become infected by ingesting contaminated water, often through drinking untreated or improperly treated tap water, or through participating in recreational water activities. The resilience of *G. lamblia* cysts makes them particularly problematic in areas lacking advanced water filtration systems, as they can survive typical disinfection processes. This is why filtration and additional treatment methods like ultraviolet (UV) radiation are often recommended in areas prone to contamination. Outbreaks of giardiasis are frequently linked to community water sources that become contaminated by human or animal faecal matter. This highlights the need for rigorous sanitation and water management practices to reduce the risk of widespread transmission. Chikun Local Government Area had the highest prevalence, followed by Igabi Local Government Area, Kaduna South and Kaduna North Local Government Area. *Giardia lamblia* is known to be mainly transmitted through water (Kumar et al., 2016). The transmission is also influenced by the hygienic condition of the environment. Out of the four local government areas in this study, Chikun and Igabi can be said to be the least in terms of the quality of hygiene and domestic water source (Sridhar et al, 2020). Thus, the findings tally with the standard of hygiene and water source. On the other hand, Kaduna North and Kaduna South Local Government Areas have relatively better hygienic conditions. PCR-based detection is widely regarded as a reliable method for identifying *Giardia* DNA due to its sensitivity and specificity. Gel electrophoresis results indicated the presence of *Giardia* Assemblage B DNA in all tested samples. The positive control (PC) confirms that the PCR amplification was successful, while the negative control (NC) suggests no contamination. The consistent band presence in all samples reflects successful amplification and potentially a widespread occurrence of *Giardia* Assemblage B in the samples that were tested. Studies have documented the high prevalence of *Giardia* across various environments, particularly in areas with significant human and animal interaction. *Giardia* is often found in environmental water sources, indicating contamination from human or animal waste. Ryan et al. (2019) reported that *Giardia* Assemblage B is frequently detected in water and soil samples, especially in urban areas where wastewater contamination is common. The consistency of positive results across all sample lanes in this study aligns with findings by Cacciò et al. (2018), who noted that *Giardia* Assemblage B tends to be more prevalent in densely populated or livestock-rich regions. This suggests that samples screened might have been collected from an environment with a significant presence of potential contamination sources, supporting the widespread detection of *Giardia* spp.

This study's use of PCR to amplify PCR fragment specific to *Giardia* Assemblage B mirrors methodologies used in studies by Feng & Xiao (2011) as well as Sulaiman & Xiao (2020). These studies confirmed the efficacy of PCR in identifying *Giardia* at low concentrations, making it a valuable tool in environmental and clinical samples. Lalle et al. (2016) found that targeting specific genes within *Giardia* Assemblage B often yields high specificity and can differentiate between assemblages. The successful detection in all sample lanes in this study suggests that the primer specificity in this PCR protocol was optimal for *Giardia* Assemblage B. The presence of *Giardia* Assemblage B in all samples has implications for public health, especially if these samples are from drinking water or areas frequented by humans and animals. Previous studies, like those by Einarsson et al. (2016), highlighted that *Giardia* is a common source of waterborne diseases. The widespread detection in analysed samples could point to a potential risk factor for human and animal health if contamination levels are high. Cotton et al. (2014) emphasized the zoonotic potential of *Giardia* Assemblage B, meaning it can infect both humans and animals. The presence of *Giardia* in the samples could indicate an environment where cross-species transmission is possible, especially if humans, livestock, or wildlife interact within the sampled area. As shown in Figure 4.2, there was no positive result in the PCR using tpi gene. This indicates that no other assemblage was detected in the positive samples aside assemblage B. Assemblage A which can be found in humans, was not detected as shown in Figure 4.1. Assemblages C-H are specific to human hosts (Adam, 2021). They were also not detected in this study.

Conclusion

This study highlights the prevalence, 17(9.29) of *Giardia lamblia* in Kaduna metropolis which was higher, 4.37 (17.02 %) in LGEA Primary School Nasarawa (Chikun LGA) and lower, 1.09 (4.35) in LGEA Primary School Kawo (Kaduna North LGA).

Higher infection rate was observed among younger age groups females, traders, farmers, and individuals using water-based sanitation systems and tap water as their source of water correlated significantly with their risk of infection ($p < 0.05$).

The distribution of G. lamblia assemblage B was observed to be highly prevalent among the following: age range 7 - 11, 41.18 (77.78 %); farmers, 29.41 (40.00 %); Population associated with flat and rooming housing respectively 47.06 (75 % and 50 %); Those with secondary education, 47.06 (44.44 %); Population who used flush toilet type, 35.29 (54.55 %); Population that are positive with no symptoms (diarrhoea, 35.29 (54.55 %), abdominal pains, 41.18 (58.33 %) and vomiting, 47.06 (61.54 %)).

Conflict of interests

The authors declare no conflicts of interest.

REFERENCES

Abdulabbas, A., Dhoha, R., & Hamza, A. (2022). Incidence of giardiasis among population at Wasit province. *Journal of Wasit for Science and Medicine*, 198, 1-7. https://doi.org/10.31185/jwsm.198.

Adam, R.D. (2021). *Giardia* duodenalis: Biology and Pathogenesis. *Clinical Microbiology* 15;34(4). doi:10.1128/CMR.00024-19.

Ali, A., Osman Ga'al, A., Osman, A., Said, M. A., & Elmi, I. Y. (2020). Prevalence of *Giardia lamblia* among children attended at SOS Hospital. *African Journal of Health and Medical Sciences*, 5(1), 47. https://doi.org/10.59067/afjhms.v5i1.47

Ali, I. Y., Mero, W. M. S., & Mohammed, A. B. (2022). Prevalence and risk factors of *Giardia lamblia* among infants and children in Duhok province/Kurdistan Region, Iraq. *Academic Journal of Nawroz University*, *11*(4), 1597. https://doi.org/10.25007/ajnu.v11n4a1597

Bature, A.S., Opotu, L.A. &Mounkaila, N. (2021). Evaluation of Water Supply and Sanitation Situation in Kaduna Metropolis in Northern Nigeria. *Public Health Research*, 11(1): 1-14

Belkessa, S., Ait-Salem, E., Laatamna, A., Houali, K., Sönksen, U.W., Hakem, A., Bouchene, Z., Ghalmi, F., and Stensvold, C.R., (2021). Prevalence and clinical manifestation of *Giardia* intestinalis and other intestinal parasites in children and adults in Algeria. *American Journal of Tropical Medical Hygiene*. 104(3): 910-916.

Brinkhoff, T. (2022). City population. Kaduna State in Nigeria. Population Statistics, Charts, Map and Location

Cacciò, S. M., La Rosa, G., Fratini, F., &Pozio, E. (2018). Genotyping of *Giardia duodenalis* from human and animal feces by PCR-RFLP analysis of the β-giardin gene. *Veterinary Parasitology, 167*(1), 32–40. https://doi.org/10.1016/j.vetpar.2009.08.004

Center for Disease Control and Prevention (CDC) (2024). Parasites Giardia. Available at: http://www.cdc.gov/parasites/girdia/pathogen.htmL

Chelsea, M. & William, A. P. Giardiasis. (2024). *MSD Manual professionalversion.*https://www.msdmanuals.com/professional/infectious-diseases/intestinal-protozoa-and-microsporidia/giardiasis

Cotton, J. A., Beatty, J. K., &Buret, A. G. (2014). Host parasite interactions and pathophysiology in *Giardia* infections. *International Journal for Parasitology, 44*(10), 925-933. https://doi.org/10.1016/j.ijpara.2014.07.004

Dunn, N., & Juergens, N.L. (2024). Giardiasis. Treasure Island (FL): StatPearls Publishing; https://www.ncbi.nlm.nih.gov/books/NBK513239/

Einarsson, E., Ma'ayeh, S., & Svärd, S. G. (2016). An up-to-date view on *Giardia* and giardiasis. *Parasitology, 143*(4), 439-455. https://doi.org/10.1017/S0031182015001654

Enitan, S. S., Oluchi, N. J., Digban, K. A., Akele, R. Y., & Olofinsae, S. (2022). Assessment of intestinal parasitic infections among school-aged children in relation to water, sanitation and hygiene (WASH) practices in [Region], Nigeria. *[Journal Name, e.g., International Journal of Health Sciences]*, *12*(4)

Eric, M.S. & Michael, H.P. (2024). Girdiasis. *Medscape.* https://emedicine.medscape.com/article/176718-overview?

Faria, C., P., Zanini, G., M., Dias, G., S., da Silva, S. & Sous- Mdo, C. (2016). Molecular Characterization of *Giardia lamblia*: First Report of Assemblage B in Human Isolates from Rio de Janeiro (Brazil). *PLoS One*. doi: 10.1371/journal.pone.0160762.

Farzad, M., Morteza S., Alireza, S., Laya, S., Mostafa, O., Ali, A., Soheil, H. & Amir, M.S. (2021). Global prevalence and associated risk factors of diarrhoeagenic *Giardia* duodenalis in HIV/AIDS patients: A systematic review and meta-analysis. *Microbial Pathogenesis*, 160(105202). https://doi.org/10.1016/j.micpath.2021.105202, https://www.sciencedirect.com/science/article/pii/S0882401021004769

Gautam, J., Parajuli, R.P. & Pandey, K. (2024). Prevalence and associated factors of intestinal parasitic infections in the Badi indigenous communities of Western Nepal. *Journal of Health Population and Nutrition* 43, 211 (2024). https://doi.org/10.1186/s41043-024-00694-1

Hajare, S. W., Betcha, A., Sharma, R., Bhosale, S. B., Upadhye, V., Kuddus, M., & Eriso, F. (2022). *Giardia lamblia* infection and associated risk factors among patients attending Kochore Health Center, Ethiopia. *Infectious Diseases Now*, 52(4), 321–328. https://doi.org/10.1016/j.idnow.2022.04.005

Hajare, S.W., Chekol, Y. & Chauhan, N.M. (2022). Assessment of prevalence of *Giardia lamblia* infection and its associated factors among government elementary school children from Sidama zone, SNNPR, Ethiopia. *PLoS One*. 15;17(3) doi: 10.1371/journal.pone.0264812.

Hamad, A. A. (2024). An Assessment of the Epidemiology and In vitro Susceptibility of *Giardia lamblia* Trophozoites and Cysts to a Variety of Plant Extracts. *Microbiology Research Journal International*, *34*(7), 113-123.

Helen, I. I., Binta, Y. & Sabo, Y. E. (2011). Asymptomatic Giardiasis and Nutritional Status of Children in Two Local Government Areas in Kaduna State, Nigeria. *Sierra Leone Journal of Biomedical Research*, 3 (3): 157-162.

Hooshyar, H., Rostamkhani, P., Arbabi, M., &Delavari, M. (2019). *Giardia lamblia* infection: review of current diagnostic strategies. *Gastroenterology and hepatology from bed to bench*, *12*(1), 3–12.

Jex, A. R., Svärd, S., Hagen, K. D., Starcevich, H., Emery-Corbin, S. J., Balan, B., Nosala, C., & Dawson, S. C. (2020). Recent advances in functional research in *Giardia* intestinalis. *Advances in parasitology*, 107, 97–137. https://doi.org/10.1016/bs.apar.2019.12.002

Khanum, H., Kadir, R., Arju, T., Ahmed, T., Karim, M. T., Sultana, A., & Huque, R. (2015). Detection of *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium* sp. infection among diarrhoeal patients. *Bangladesh Journal of Zoology*, *43*(1), 1–7. https://doi.org/10.3329/BJZ.V43I1.26132

Klotz, C., Schmid, M.W., Winter, K., Ignatius, R., Weisz, F., Saghaug, C.S., Langeland, N., Dawson. S., Lalle, M., Hanevik, K., Cacciò, S.M. & Aebischer, T. (2023). Highly contiguous genomes of human clinical isolates of *Giardia duodenalis* reveal assemblage- and sub-assemblage-specific presence-absence variation in protein-coding genes. *Microbial genomics*, 9(3), mgen000963, https://doi.org/10.1099/mgen.0.000963

Kumar, T., Majid, M.A.A., Onichandran, S. et al. (2016) Presence of *Cryptosporidium parvum* and *Giardia lamblia* in water samples from Southeast Asia: towards an integrated water detection system. *Infection Diseases of Poverty* 5, 3. https://doi.org/10.1186/s40249-016-0095-z

Lalle, M., Pozio, E., Capelli, G., Bruschi, F., Crotti, D., &Cacciò, S. M. (2016). Genetic heterogeneity at the β-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. *International Journal for Parasitology, 35*(2), 207–213. https://doi.org/10.1016/j.ijpara.2004.12.008

Mohammed, M., &Omudu, E. A. (2011). The prevalence of intestinal parasites among school children in rural and semi-urban areas of Makurdi, Benue State, Nigeria. *Sierra Leone Journal of Biomedical Research*, 3(3), 163–168.

Naing, L., Winn, T. & Rusli, B.N. (2006). Practical Issues in calculating sample size for prevalence studies. *Archives of feacal Sciences*, 1: 9-14

Nawaz, Z., Rasool, M. H., Siddique, A. B., Zahoor, M. A., Naz, A., Ali, S., & Mukhtar, M. U. (2019). Frequency and molecular detection of *Giardia* intestinalis in children attending pediatrics of Punjab, Pakistan. *Jundishapur Journal of Microbiology*, *13*(1).

Nuhu, H.T & Bartholomew, J. L. (2020). Impacts of Urban Sprawl on Livability in Kaduna Metropolis, Nigeria. *International Journal of Scientific Research in Science and Technology*, available at http://doi.org/10.32628/IJSRST207644.

Pouryousef, A., Fararouei, M. & Sarkari, B. (2023). Antigen-Based Diagnosis of Human Giardiasis: A Systematic Review and Meta-Analysis. *Iran Journal Parasitol*..doi: 10.18502/ijpa.v18i2.13180.

Prabakaran, M., Weible, L.J., Champlain, J.D., Jiang, R.Y., Biondi, K., Weil, A.A., Van-Voorhis, W.C. & Ojo, K.K. (2023). The Gut-

Wrenching Effects of Cryptosporidiosis and Giardiasis in Children. *Microorganisms.* 15;11(9):2323. doi:10.3390/microorganisms11092323.

Rigasa,Y.A., Badamasi, A.G. & Abdulkarim, B.I. (2015). The evolution of value chains and recycling opportunities in the informal management of municipal solid waste of Kaduna metropolis, Nigeria. *Biological and Environmental Sciences Journal for the Tropics*, 12(1): 794 – 905

Ryan, U., and Zahedi, A., (2019). A molecular epidemiology of giardiasis from a veterinary perspective. *Advanced Parasitology*, 106: 209-254.

Saleh, A. B., Ahmed, E. F., Mahmoud, M. S., & Abdel-Hafeez, E. H. (2018). Molecular and microscopic detection of *Giardia lamblia* in stool samples from symptomatic and asymptomatic children in Egypt. *Journal of the Egyptian Society of Parasitology*, *48*(1), 187–196.

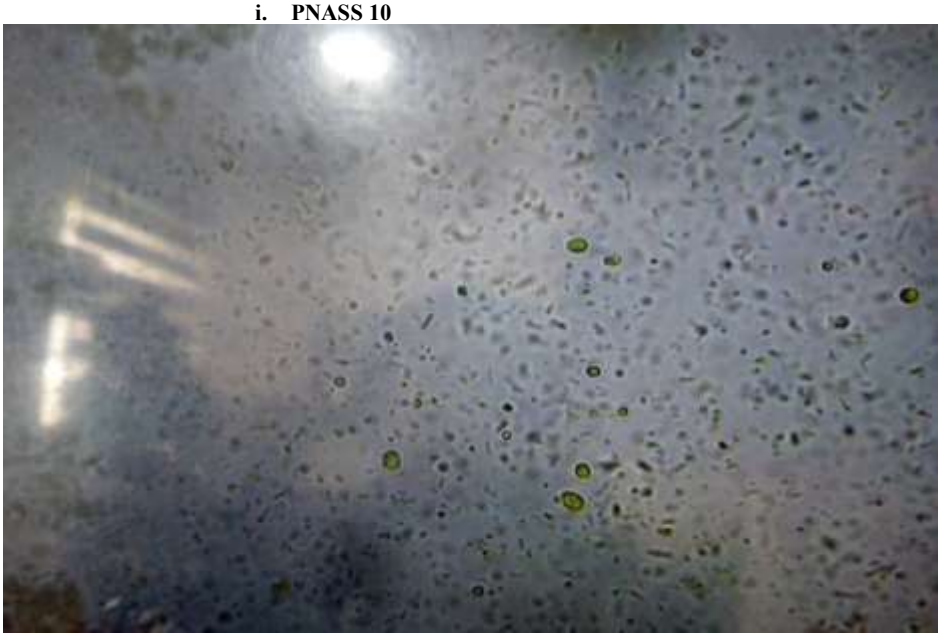
Sridhar, M., Okareh, O. T., and Mustapha, M. (2020). Assessment of Knowledge, Attitudes, and Practices on Water, Sanitation, and Hygiene in Some Selected LGAs in Kaduna State, Northwestern Nigeria. *Journal of Environmental and Public Health*, 1 (14), 10.1155/2020/6532512

Sulaiman, I. M., & Xiao, L. (2020). Molecular characterization of *Giardia* parasites in humans and animals in North America. *Journal of Parasitology Research, 104*(4), 387-392. https://doi.org/10.1155/2020/104389.

World Health Organization (WHO). (2019). *Bench aids for the diagnosis of intestinal parasites*. 2nd ed. Geneva: World Health Organization.

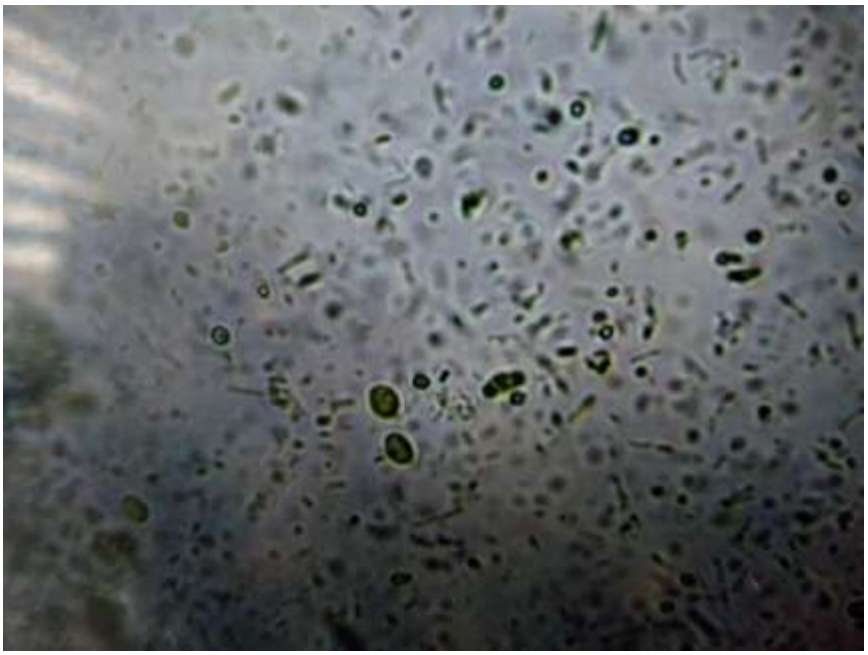
APPENDIX F
Microscopic pictures

i. **PNASS 10**



ii. **PKM15**

iii. **PK30**



iv. PR8.