Acta Scientific Gastrointestinal Disorders (ISSN: 2582-1091)

Volume 6 Issue 5 May 2023

Research Article

Genetic Assemblages of *Giardia lamblia* Infection among School Children in Ibadan Municipality

Imo S George*

Cell Biology and Genetics Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria

***Corresponding Author:** Imo S George, Cell Biology and Genetics Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria.

Received: March 20, 2023

Published: April 10, 2023

Abstract

Giardia lamblia is the etiologic agent of giardiasis in humans and other mammals worldwide. The burdenof disease is high among children in developing countries where there is poor or inadequate sanitation. *Giardia* is a neglected tropical parasite and so the epidemiology and genetic diversity of this parasite is poorly understood. The aim of this study was to determine the genetic diversity of *Giardia* assemblages inasymptomatic children in public primary schools in Ibadan and to determine novel polymorphisms in arginine deiminase of *Giardia lamblia.* A total of 322 fresh faecal samples were collected from school children between August and December 2017, May and July, 2018. *Giardia* parasite was detected by microscopy and multiplex PCR was used to determine genetic assemblage of *Giardia*. ARGDE (arginine deiminase) gene was amplified by PCR and amplicons sequenced using Sanger sequencing. Statistical analysis was done using Fisher's exact test, Pearson's chi squared test, T test and stepwise regression at significant level of $p < 0.05$. Sequences were analyze using BLAST tool on NCBI, Bioedit and mega 7 software. The overall prevalence of *Giardia lamblia* was 18.9% (61 out of 322) as detected by microscopy.Multiplex PCR detected only 18.0% (58 out of 322). Assemblage A was the most prevalent (83.6%) compared to assemblage B (1.6%). 9.8% (6 out of 61) had mixed infection of assemblages A and B. Exposed drinking water and untreated drinking water were significant risk factors for *Giardia* infection. All sequenced samples (7 out of the 61 amplicons) showed no significant similarity with reference sequenceof arginine deiminase of *Giardia lamblia* on the NCBI database. These findings indicate that there is a needto carry out national screening programs aiming to detect asymptomatic *Giardia* infection to minimize the spread of giardiasis and further studies using accurate molecular typing tools are imperative for unravelling the intricate molecular epidemiology of giardiasis.

Keywords: Giardiasis; Molecular Epidemiology; Intestinal Parasites; *Giardia lamblia*

Introduction

Giardiasis is one of the most common parasitic gastrointestinal diseases worldwide and it has been included as one of the neglected tropical disease being a significant cause of diarrhoea in community settings [9]. *Giardia* infection is caused by the parasitic protozoa *Giardia lamblia* (synonymous with *G. duodenalis* and *G. intestinalis*) that infects human and other animals worldwide [2,21]. *Giardia lamblia* isa cosmopolitan parasite with the highest prevalence rate occurring in the tropics and subtropics. Travellers to tropical Africa, Mexico, South-east Asia and Western South America are at risk of acquiring giardiasis. *Giardia lamblia* infects 200 million people worldwide and may produce symptoms in 500,000 individuals worldwide [38]. In developing countries like Nigeria, *Giardia lamblia* is one of the first pathogen to infect infants and peak prevalence rate of 15-20% occur in children under 10

years [38]. The prevalence rate of *Giardia* infection is higher in the developing countries (20-30%) compared to 2-5% in industrialized countries as a result of poor sanitary conditions which favours the contamination of food and water with thecyst [38]. Giardiasis has been included in the neglected disease initiative by World Health Organization (WHO) since 2004 due to its impacts on human health [40].

Giardia lamblia has a direct life cycle in which the parasite alternates between the cyst and the trophozoite stages that are responsible for the occurrence and transmission of the disease, respectively. The cyst stage is stable, resistant to environmental conditions, chlorination, freezing and ultraviolet exposure, and remainsviable for months preferably in cold and wet environments as water temperature favours *Giardia* survival. The presence of moisture has protective effect on *Giardia* survival. Contamination of potable water with the stool of infected individuals or domestic animals and consumption of raw vegetables are known to easethe transmission of *G. lamblia* [5].

Ingestion of cysts paves the way for trophozoites to emerge from the cysts and attach to the small intestinalmucosa of the host, resulting in asymptomatic to symptomatic infections. Although 200 million individualsacquire symptomatic giardiasis every year, the majority of human giardiasis is asymptomatic [50].

Giardia infection is significant with public health impacts because of its potency to cause major emergencyresponses, outbreaks and effect on growth in children. Clinical symptoms of this disease in human are diverse, ranging from asymptomatic to acute/chronic diarrhoea, dehydration, abdominal pain, nausea, vomiting and weight loss [16]. The severity of giardiasis is determined by the interaction between the host factors such as developmental, nutritional and immunological status and virulence factor of the parasite [37]. Different factors are thought to contribute to the wide variation in clinical symptoms, including the virulence of the *Giardia* strain, the number of cysts ingested, the age of the host, and the state of the host'simmune system at the time of infection [2]. It is documented that *G. lamblia* is considered multispecies witha broad host range [21,40] with members showing little or no variation in their morphology but present an unusual genetic variability. The evolutionary genetic recombination among isolates may affect the variability of the disease, as it reinforces the parasitic resistance to host immunity and anti-protozoan treatments [28].

Molecular characterization and phylogenetic analysis has shown at least eight major assemblages (A-H) of*Giardia lamblia* with different host ranges and specificities [40]. Assemblage A and B are known to infectshumans and also animals in rare cases; Assemblages C and D occur predominantly in Dogs and other canids; Assemblage E occur in hoofed livestock; Assemblage F occur in Cats; Assemblage G in rats; Assemblage H in marine mammals. Assemblage A is subdivided into three assemblages AI-AIII: AI is found in animals,AII is commonly found in humans although it was reported in a few studies in animals and AIII is exclusively found in animals. The host distribution of assemblage B is subdivided into two sub assemblagesBIII and BIV and are most prevalent in human population and less common in animals [40,43].

Regarding giardiasis, it has been shown that symptoms are more associated with assemblage A as reportedin a study carried out by [24] in Bangladesh while a study by [22] in Ethiopia found out that assemblage Binfections are more likely to be symptomatic. Studies by [35,36] in a group of children from La Habana, Cuba found out that children harbouring assemblage B of *Giardia lamblia* were more likely to have symptomatic infections than children with isolates from assemblage A. *Giardia lamblia* is able to degrade the amino acid arginine as source of energy through arginine dihydrolasepathway [44] and two of the enzymes arginine deiminase (ADI) and ornithine carbamoyltransferase (OCT) are released upon *Giardia*-intestinal epithelial cell (IEC) interaction [44]. *Giardia* rapidly reduce the amountof arginine in the growth medium during *in vitro* growth [45] resulting in reduced proliferation of IECs withan assemblage A isolate leading to a reduction of nitric oxide (NO) production by these cells [15] since arginine is also a substrate for NO synthases. *Giardia* arginine deiminase has been identified as a protein responsible for a reduced NO response in *in-vitro* setups [45]. In vitro, NO acts as cytostatic against *G. lamblia* trophozoites and inhibits encystation and excystation [15], the two processes essential for infection.It plays an important role in muscle relaxation and thus in mechanical parasite elimination by peristalsis [28,29]. Therefore, the reduction of the NO response of the host is in favour of *Giardia* growth.

The application of assemblage specific primers coupled with the use of more than one molecular marker has been employed to accurately assess the occurrence of mixed infections in clinical samples and to improve the detection of the assemblages

[4,21,23]. Although a number of studies has been carried out on the distribution and prevalence of *Giardia* species in different part of the country, little or no research has been carried out on molecular epidemiology of giardiasis in Ibadan to determined genetic assemblages and sub assemblages of *Giardia lamblia* in Nigeria; hence there are no epidemiological data. The high burden of giardiasis among children in developing countries especially where sanitation is inadequate is of public health importance [48]; Sanitation provision in this area is grossly deficient; most children do not have access to safe or treated drinking water, hygienic toilet and large amount of untreated faecal waste is discharged into the environment, this is likely to have a significant impact on infectious disease burden and quality of life. However, environmental risk arises from a wider array of sources that include air pollution from vehicles, land and water pollution from soils and untreated sewage disposal which have caused directand immediate negative impact on human health and safety especially for the poor in this area [31]. The warm humid climatic conditions in Ibadan are conducive for the parasite survival [48].

There is a need to carry out a study to identify the assemblages of *Giardia lamblia* infection and the associated transmission factors in the high-risk group being the children in Ibadan municipality in order to control and eradicate this disease. The increasing population that is a factor associated with the disease prevalence [14], poor sanitation and personal hygiene [17] makes it necessary for this study 'genetic assemblages of *Giardia lamblia* infection among school children in Ibadan, Nigeria.

Methodology

Study area

Samples were collected from four areas across tree local government of Oyo State; Orogun Akinyele local government (latitude 7°27′3.9528″N longitude latitude 3°55′6.0528″N), Moniya, Akinyele local government area (latitude 7°31′42″N longitude 3°54′41″E); Sango area Boluwaji Ibadan South East Local government area (latitude 7°22′33″N longitude 3°55′51″N) and Yemetu Ibadan North Local Government (latitude 7°22′33″N longitude 3°55′51″N), Oyo state, Nigeria.

Study design

This was a cross sectional study and 322 asymptomatic school children in the urban settings of Ibadan municipality, Oyo state participated in the study. This study was carried out between August 2017 to December 2017 and between May 2018 to July 2018.

Ethical approval

Ethical approval for this study was obtained from Oyo State ministry of Health ethical approval committee (AD/13/479/537). Permission was obtained from the Headmaster and Headmistress of each school to carry out the research. The objectives of the study were explained to parents or guardians of the children before collection of the samples; written and/or oral consent from a parent or guardian of each participant was obtained before samples were collected from the children. Participation in the study was voluntary and refusal to participate did not attract any penalty. The participants were given opportunity to ask questions before the commencement of the study.

Collection of stool sample

The school pupils were given an explanation on giardiasis, causes, symptoms and mode of infectionand on the process of stool sample collection. After the explanation, the pupils were provided with a transparent sterile stool bottle without preservative into which they placed their samples. The stool bottles were label appropriately with codes corresponding to the individuals for easy identification of the samples. Fresh stool samples collected were placed on ice and taken within a period of one hour to the laboratory inthe Department of Zoology, University of Ibadan for laboratory analysis.

Questionnaire administration

Semi-structured questionnaire either Yoruba or English language was administered to 322 respondents to elicit information on each child based on their demographics (e.g., age, sex, height, weight and number of household members), environmental (e.g., availability and type of toilets in the household, type of water supply, garbage disposal methods, and presence of domestic animals), WASH (Water, Sanitation and hygiene) e.g., washing hands before eating, after defecation, and after playing with animals, washing vegetables and fruits before consumption, boiling and filtering of water before drinking, and bathing place, and general health status of the respondent (i.e., symptoms related to intestinal parasitic infections such as diarrhoea,, nausea, vomiting, and abdominal pain

and a history of receiving anthelmintictreatment), WHO [50]. The questionnaire were pretested and simplified for easy interpretation of the information. Symptomatic children are those children who have at least one of the symptoms mentioned above and included in the questionnaire, while the asymptomatic children are those with complete absenceof symptoms but are at risk of infection.

Detection of *Giardia Lamblia* in stool samples

Formol-ether concentration technique was used to concentrate and recover *Giardia lamblia* cysts. As described by [11], 1-5g of the faecal sample was homogenised in 7 mL of 10% formalin in a beaker and sieved with surgical gauze. The sieved suspension was transferred to a 15 mL centrifuge tube and 3 mL of ethyl acetate was added. After vigorously shaking, the centrifuge tubes containing the mixture were centrifuged for 3 min at 3000 rpm. A pipette was then used to loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ethyl acetate, faecal debris, and formol water. A pipette was used to transfer the sediment to make two drops on a plain microscope slide. Lugol's iodine solution and normal saline was added to both sides of the drops respectively and covered with a cover slip. The slide was then examined microscopically using the 10x and 40x objective (Olympus GX21) to view cysts of *Giardialamblia* and other parasites.

DNA extraction

DNA was extracted directly from *Giardia* positive samples using a commercial DNA extraction kit (DNA QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's instructions) with slight modifications.

Briefly, 220-250mg of stool was place into a 2 mL microcentrifuge tube. 1 mL InhibitEX buffer was then added to each sample and vortexed thoroughly until the samples were well homogenised. The samples were then incubated at 95°c for 5 minutes to break the cysts, followed by centrifugation at 13,000 rpm for 1 minute. 20µL of proteinase K was put into a new 2mL centrifuge tubes, 500 µL of the supernatant were transferred to a new microcentrifuge tube containing proteinase K. 500 µL of buffer AL was added to the mixture and vortexed for 1 minute for proper mixing and then incubated again at 70°C for 10 minutes. 500µL of ethanol was added to the lysate and vortexed again. 600 µL of the resultant lysate were transferred tothe QIAmp spin column and centrifuge at 13,000 rpm for 1-minute into 2 mL collection tubes. The collection tubes were discarded. The spin column enables the DNA to bind to the column membrane whilethe other particle moves into the collection tube. This step was repeated until all the resultant lysate was centrifuged through the spin column. The DNA in the spin column was first washed with buffer AW1 (500 µL), centrifuge at 13,000 rpm for 3 minutes and the collection tube containing the filtrate was discarded. Buffer AW2 (500 µL) was added to the column to wash the DNA again, centrifuged at 13,000 rpm for 3 minutes, discard the collection tubes, replaced new collection tubes and then centrifuge for 3 minutes. 200µL of buffer ATE (elution buffer) was added directly to the QIAmp membrane and left to incubate at room temperature for 5 minutes. Lastly, the QIAmp spin columns were centrifuged at 13,000 rpm for 3 minutesinto a new microcentrifuge tube to elute DNA. The extracted DNA were then stored at -20°C until PCR analysis.

PCR reactions

A Multiplex PCR-relevant protocol was used to determine the assemblages of *Giardia* samples [47]. The genotyping analysis was performed using an assemblage specific primer that amplifies 165-bp fragment for assemblage A and 272-bp fragment for assemblage B at 4E1-HP loci [47]. The PCR reactionswere conducted in a 50µL reaction mixture for 4E1-HP loci containing 25 µL of Master Mix (containing; PCR buffer, dNTPs, $MgCl_2$, Taq polymerase), 0.1 µL of each primer, 5 µL of DNA template (DNA extracted from *Giardia lamblia* positive samples), and 19.6 µL of nuclease free water to make up 50 µL volume. PCR was performed on Eppendorf 96 well Master-cycler (Inqaba biotech) using One Taq (R)2x Green Master mix (New England Biolabs). Concerntration of primers in PCR reactions was 10pM each and sequences of primer pairs are reported in table 1.

All reactions began with an activation-denaturation step at 94°C for 5minutes and were carried out for 40 cycles each consisting of 94°C for 30 seconds, an annealing temperature of 56°C for 30 seconds and 72°Cfor 30 seconds. A final extension step was carried out at 72°C for 7 minutes. The amplified DNA (PCR products generated) in each sample were confirmed by gel electrophoresis using 2% agarose in 1xTAE buffer and run at 60 Volt for 90 minutes (Vanni., *et al*. 2012). The gel was stained with ethidium bromide (0.5 µg/mL) and viewed using both a tabletop gel viewer and GelMax Imager.

PCR amplification of arginine deiminase (ARGDE)

Arginine deiminase gene was amplified using primers that is specific for both assemblages A and B Amplification of arginine deiminase gene was performed with primers ARGDEF (GAGAAGCTTGCACAGGCAAC) for forward primers and ARGDER (TCAACTGGCTCGCCATTCTT) for reverse primer. The PCR reactions were conducted in a 50µL reaction mixture containing 25 µL of Master Mix (containing; PCR buffer, dNTPs, $MgCl_2$, Taq polymerase), 0.1 µL of each primer, 5 µL of DNA template (DNA extracted from *Giardia lamblia* positive samples), and 19.8 µL of nuclease free water to make up 50 µL volume. PCR was performed on Eppendorf96 well Master-cycler (Inqaba biotech) using One Taq (R)2x Green Master mix (New England Biolabs).

All reactions began with an activation-denaturation step at 95°C for 5minutes and were carried out for 35 cycles each consisting of 95°C for 30 seconds, an annealing temperature of 48°C for 30 seconds and 72°Cfor 30 seconds. A final extension step was carried out at 72°C for 5 minutes. The amplified DNA (PCR products generated) in each sample were confirmed by gel electrophoresis using 2% agarose in 1xTAE buffer and run at 60 Volt for 90 minutes. The gel was stained with ethidium bromide (0.5 µg/mL) and viewed using both a tabletop gel viewer and GelMax Imager. The amplicons generated was further sequenced by Inqabal using Sanger sequencing.

Sequencing protocol (Sanger Sequencing)

PCR products was cleaned with ExoSAP; the Exo/SAP master mix was prepared by adding 50.0 µL of Exonuclease I (NEB M0293) 20U/ul and 200.0 µL of Shrimp Alkaline Phosphatase (NEB M0371) 1U/uL to a 0.6mL micro-centrifuge tube. The following reaction mixture was prepared by adding 10.0µL of PCR Mixture and 2.5µL of Exo/SAP Mix. The two mixture was mixed well and incubated at 37°C for 30 minutes. The reaction was stop by heating the mixture at 95°C for 5 minutes.

The generated sequences were blasted using the BLAST tool on NCBI, BIOEDIT software was used to editeach of the generated sequences and the Mega 7 software was used to align the sequences generated with that of the database to check for polymorphisms in arginine deiminase of *Giardia lamblia*.

Statistical analysis

Data collected was entered into the computer using MS excel 2010 and analysed using SPSS (Chicago, IL, USA). Fisher's exact test was used to test the degree of association that exist between the distributions of *Giardia assemblages*. Pearson's chi squared test was used to test the association between the *Giardia* prevalence and different risk factors investigated using questionnaire (independent variables), T test was used to compare the difference between two variables, stepwise regression analysis was used toascertain independent variables with highest significance associated with *Giardia* infection. The level of statistical significance was set as $p < 0.05$ (95% confidence interval). Bioedit, BLAST tool on NCBI, mega 7 and CLUSTAL W were used for sequence comparison, alignment and detection of novel polymorphism.

Assay	Assemblage	Primer	PCR product (bp)
4E1-HP	A	For AAAGAGATAGTTCGCGATGTC Rev ATTAACAAACAGGGAGACGTATG	165
	B	For GAAGTCATCTCTGGGGCAAG Rev GAAGTCTAGATAAACGTGTCGG	272

Table 1: Primers for assemblage specific PCR and sizes of the amplicon.

Results

Demographics of the study population

The study included 153 males and 169 females. 185 (57.4%) of the students fall within 10-13 years of age. Table 2 shows the demographic distribution of the students.

Prevalence of *Giardia lamblia* infection by microscopy

Out of the 322 students sampled, 61 (18.9%) of them were positive for *Giardia intestinalis*. Other parasites like *Ascaris lumbricoides, Entamoeba species* and *Trichiuris trichiuria* were detected in some samples.

Prevalence of *Giardia lamblia* assemblages

Of the 61 positive samples, only 58 (95.1%) of the samples were successfully amplified at 4E1-HP loci. The *Giardia lamblia* assemblages were successfully determined (figure 1). Assemblage

A was found in 51 samples (83.6%), assemblage B in 1 (1.6%) sample and mixed assemblages A and B in 6 samples (9.8%). Table 3 shows the summary of assemblage specific PCR results and distribution of the assemblagesin the study population according to age, sex and municipality.

PCR amplification of arginine deiminase (ARGDE)

Thirty three (33) DNA samples amplified showed positive bands for ARGDE amplification (Figure 2).

Sequencing result

Ten (10) positive PCR samples from ARGDE amplification were sequenced, this include 1 assemblage B, 5 assemblages A and 4 mixed assemblages A and B. Only positive PCR samples were successfully sequenced while three failed quality control test. The sequences returned included 1 assemblages B, 5 assemblages A and 1 mixed assemblage A and B. Of the seven samples successfully sequenced, none of the sequences showed any similarity with reference sequence of *Giardia* arginine deiminase on the database; instead, some of the generated sequences showed significant alignment/similarity with *Bacteroides dorei* isolates, *Bacteroides ovatus* strain. There was no alignment atall with arginine deiminase on the database; hence, there was no record of polymorphisms in arginine deiminase of *Giardia lamblia* in this study in Ibadan.

Prevalence of *Giardia lamblia* infection with sex

Table 4 shows the prevalence of *Giardia lamblia* infection in children in relation to sex. The resultshows that of the 322 samples collected, 61 were found to be infected giving an overall prevalence of 18.9%. Out of eighty-seven (154) samples collected from males, 26 (16.9%) were positive for *Giardia lamblia* and out of 168 samples collected from females, 35 (20. 8%). There was a negative significant association ($p < 0.05$) between prevalence and sex.

Prevalence of Giardia lamblia infection according to age

The result shows that there was a significant association ($p = 0.03$) between age and infection rate (table 5) and was statistically significant at Yemetu ($p < 0.05$). 4 out of 24 children above the age of 13 wereinfected compared to just 5 out of the 19 children under the age of 5. Most of the children infected in this study fall within the age of 10 – 13. The result was statistically significant ($p = 0.03$).

Prevalence of giardia lamblia infection according to BMI

Students T- test showed no significant difference in the BMI between infected and non-infected children. The relation between infection rate and BMI showed a significant association ($p < 0.05$).

Association between some risk factors and prevalence

Figure 3 shows that, there was an association between contact with domestic animals and infection($p < 0.05$). 56.1% (101) of the study participants use tap as their source of water, 71% agreed to not treatingtheir water at home, 89.4% of the students wash their hands after defecating, and this had a negative correlation with prevalence of *Giardia lamblia* infection ($p < 0.05$). 71% of the study population use drumsfollowed by clay pots (10%) for water storage.

Generally, drinking water source, current use of flagyl, consistency of flagyl use, use of dustbin and water storage do not have statistical significant difference in this study ($p > 0.05$) but there was a correlation between drinking water source and infection in Orogun ($p = 0.03$); there was a correlation between water treatment and infection in Yemetu ($p < 0.05$). Washing of hand after toilet use, washing fruits before consumption, Presence and type of toilet facility was statistically significant ($p < 0.05$). However, Pearson'schi- square test showed a negative correlation between use of dustbin and infection in Yemetu ($p < 0.05$) andthere was a negative correlation between consistency in flagyl use and infection rate in Orogun.

Figure 1: PCR amplification of the 4E1-HP marker from 61 human fecal samples positive for *Gardia lamblia*.

Figure 2: PCR amplification of arginine deiminase from 33 human fecal samples positive for *Giardia lamblia*.

Figure 3: Prevalence of *Giardia lamblia* infection and contact with animals $p < 0.05$.

Characteristics	Yemetu N = 113 (100%)	Orogun N = 67 (100%)	Moniya N = 109 (100%)	Sango N = 33 (100%)	Total 322
Age Group 0-2	0(0%)	0(0%)	0(0%)	1(3.0%)	1
3-5	7(6.2%)	1(1.5%)	3(2.7%)	8(24.2%)	19
6-9	39(34.5%)	22(32.8%)	23(21.1%)	9(27.3%)	93
10-13	62(54.9%)	41(61.2%)	74(67.9%)	8(24.2%)	185
14-16	5(4.4%)	3(4.5%)	8(7.3%)	5(15.1%)	21
17-20	0(0%)	0(0%)	1(0.9%)	2(6.1%)	3
Sex Male	63(55.8%)	24(35.8%)	49(44.9%)	17(51.5%)	153
Female	50(44.2%)	43(64.2%)	60(55.0%)	16(48.5%)	169
Total	113	67	109	33	322

Table 2: Demographics of the study population.

Characteristics	Assemblage A N = 51	Assemblage B N = 1	Assemblage A+B N = 6	Total
Age groups 0-2	1	Nil	Nil	1
3-5	4	Nil	1	5
6-9	17	Nil	4	21
10-13	26	1	1	28
14-16	3	Nil	Nil	3

17-20	0	Nil	Nil	0
Sex Male	22	1	2	25
Female	29	Nil	4	33
Municipality Yemetu	18	1	1	20
Orogun	7	Nil	5	12
Moniya	20	Nil	Nil	20
Sango	6	Nil	Nil	6
Total	51	1	6	58

Table 3: Summary of assemblage specific PCR results and distribution of *G. lamblia* assemblagesaccording to age groups, sex and municipality.

	Infection		Total
	Positive	Negative	
Sex Male	26	128	154
Female	35	133	168
Total	61	261	322

Table 4: Prevalence of *Giardia lamblia infection* in children in relations to sex ($p < 0.05$).

Infection	Age						Total
	0-2	3-5	6-9	10-13	14-16	17-20	
Positive	1	4	19	33	4	0	61
Negative	0	15	74	152	17	3	260
Total	1	19	93	185	21	3	322

Table 5: Prevalence of *Giardia lamblia* infection with age ($p = 0.03$).

Discussion

Giardiasis causes a major public and veterinary health concerns worldwide. Transmission is either direct, through the faecal oral route or indirect through ingestion of contaminated water or food. In developing country like Nigeria, *Giardia*sis is acquired during early infancy and its prevalence peaks up to30% in children under 10 years of age. Apart from diarrhoea, giardiasis in children in Nigeria can result infaltering of long-term growth and impairment of cognitive function [21].

Recently, different molecular methods are available to distinguish the different *Giardia* assemblages and this is done mainly by nested PCR followed by RFLP or DNA sequencing or by real-time PCR [21]. These protocols are based on amplifying a gene segment with a primer that binds specifically to the DNA sequences that are conserved in the two assemblages of *Giardia* species. Molecular genotyping techniqueshave been used to study the epidemiology of giardiasis, including the aspect of zoonotic transmission, the occurrence of mixed infection in humans, the potential for genetic exchanges between parasite isolates and the correlation between clinical symptoms and type of assemblage.

This study was carried out among public primary school children who have been shown to be at risk for giardiasis and other intestinal parasites [34]. A total of 342 children were selected but only 322 participated in the study. This study determines the prevalence and genetic diversity of *G. lamblia* among school children using multiplex assemblage specific PCR assay in four areas within three local governments within Ibadan municipality and to detect novel polymorphism in arginine deiminase of *Giardia lamblia*. Faecal samples collected from children were screened by both microscopy and Multiplex PCR. Although microscopy and PCR had similar sensitivities in a Dutch study [42] as well as in this present study. [48] reported higher infection rate by PCR. Superior sensitivity of PCR in detecting *G. lamblia* has been shown in Danish patients [46] and Rwanda children [26]. Hence, repeated microscopic examination or the use of other diagnostic tools such as immunoassays could have reduced the discrepancy between PCR and microscopy in clinical setup.

Previous studies demonstrated that *Giardia* is the principal intestinal protozoan that infects children around the country with prevalence rates reported from 10 to 55% [10,19,32]. This is higher when compared to a recent study conducted by [48]. In this study, the microscopy based prevalence of *G. lamblia* infection in school children was 18.9% (61 out of 322) and this is in line with peak prevalence rate of 15-20% in children under 10 years and 18-30% in developing country [7,40]. This is also in agreement with a study conducted by [36] in asymptomatic and symptomatic children from La Habana with an overall prevalence of giardiasis at 22%. On the other hand, this present finding is slightly lower than the prevalence of 17.6% reported by [8] in Albania, 15% reported by [38], 10.8% reported by [48] in children in Central Ethiopia, 11.7% reported by [1] among primary school children in Chenchen town, Southern Ethiopia and 11.9% reported by [37] among children from La Habana, Cuba. These variations could be due to differences in hygiene practices, quality of drinking water source, differences in environmental condition of the study localities and parental socioeconomic status of the study participants.

Figure 3 show that, there was an association between contact with domestic animals and infection explaining the reasons why frequent contact with domestic animal leads to increase in infection. 35.7% (115) of the study participants use tap as their source of water and there was no association between drinking water source and prevalence ($p > 0.05$) but there was a correlation in Orogun ($p = 0.03$). Lack of water treatment in Yemetu lead to an increased rate of infection as 75% of all the participants agreed to not treating their water at home and this is in agreement with a study carried out by [3], where they reported large inhabitants in Ibadan relying on well water as their source of drinking water, making them predisposed to water-borne diseases. Current use of flagyl and its consistency reduces the rate of infection as seen among participants in Orogun. The high prevalence of *Giardia* infection (18.9%) in Ibadan municipality may be due to risk factors like lack of current use of flagyl, lack of consistency of flagyl use, uncovered drinking water storage, untreated drinking water.

So far, little or no information is available on genetic assemblages of *Giardia* infection in Ibadan and Nigeria in general. This study showed a PCR based prevalence of 18.0%. There was no significant difference among the study areas. *G. lamblia* infection in children was higher in Moniya (60.6%) than Orogun (17.9%). This could be attributed to similarity of quality of drinking water source, personal hygiene and environmental sanitary conditions observed in the study areas. The difference in prevalence of infection by sex and age was significant in Yemetu. The study participants were asymptomatic carriers and thus, the prevalence of pathogens like *Giardia lamblia* might be found at equal proportions in healthy individuals [49]. This may explain the reason why there was no significant difference in the BMI of infected and non- infected children.

Not washing of hands after defecation, not washing fruits before consumption and lack of toilet facility (open defecation) favours the persistence and transmission of this infection. Although this study could not show strong correlation between some risk factors like drinking water storage and infection. Majority of the participants' parents are traders and this had no correlation with *Giardia lamblia* infection in this study. The prevalence of *Giardia* is strongly associated with risk factors related to host such as socio-demographic, environmental, and zoonotic conditions [41]. Since water plays a major role in *Giardia* transmission, the quality of drinking water is an essential parameter for risk factor assessment in giardiasis. Apart from this, hygienic behaviour of children must be considered as a main risk factor of this disease.

Multiplex PCR method adopted in this study ensures a more specific form of detection of the different *Giardia* assemblages. The 61 samples that were found to be positive for *Giardia lamblia* were subjected to PCR detection. 58 samples were amplified at 4E1-HP loci (figure 2). The overall prevalence of *Giardia lamblia* in this study by PCR detection was 18.0% compared to 18.9% prevalence by microscopy.

This study detected the presence of Assemblage A as the most prevalent assemblage in asymptomatic children (table 3). As to global distribution of assemblage A and B among humans, assemblage A is more prevalent than assemblage B as reviewed by [22] in Ethiopia, [25] in Egypt and [20] in Brazil. Studies shows that majority of *G. lamblia* infections identified in Parana by [12], Sao Paulo by [33] were assemblage B. Recently, [13] showed no significant difference between assemblage distribution while in Cuba, [36] found out that the two assemblages were detected at equal frequencies. The prevalence of each assemblage varies greatly according to region and within the same country but

assemblage A seems to be more common in Ibadan and other countries than B [20]. Assemblage B is associated with more severe illness, in children from Cuba, assemblage B was associated with higher frequency of diarrhoea, flatulence,and abdominal pains [36] and association between assemblage B and flatulence was also observed in children from Sweden [30]. This study was conducted on asymptomatic children and Assemblage A commonly occurs in asymptomatic people and/or shows a relatively mild symptom as seen in this study and this is in agreement with reports from other places like Saudi Arabia [6], Cuba [36].

The seven successfully sequenced samples showed no significant similarity with reference sequence of arginine deiminase of *Giardia lamblia* on the database but one out of the seven generated sequence showed significant alignment with *Bacteroides dorei isolate*, *Bacteroides ovatus* V975 and *Bacteroides ovatus* strain. There was no alignment at all with arginine deiminase on the database; hence, there was no record of polymorphisms in arginine deiminase of *Giardia lamblia* in this study. Based on the observation of thisstudy, the sequences generated range from 148 base pair to 407 base pairs indicating that the sequences were not rich enough compared to reference sequence of about 1743 base pair on the database and this maylikely be the reason for lack of alignment. It is recommended that the annealing temperature time should be increased from 30 seconds to 5 or 7 minutes to enable proper and specific annealing.

Conclusion

Determination of *Giardia* assemblages is a valuable approach to understanding the complex dynamics underlying transmission of *Giardia* infections. System of portable water is of great concern for transmission of *Giardia* infection and other protozoan infections. In this study, assemblage A was the mostpredominant genetic group among Ibadan schoolchildren than assemblage B. Logistic stepwise regressionanalysis indicated that untreated drinking water source and uncovered water storage were significant risk factors of acquiring *G. lamblia* infection. Monitoring of parasites in drinking water should be accompanied by analysis of *Giardia* to access the quality standard of drinking water. The findings of present study indicate that there is a need to carry out national screening programs aiming to detect asymptomatic *Giardia* infection to minimize the spread of giardiasis and further studies using accurate molecular typing tools are imperative for unravelling the intricate genetic assemblages of *Giardia* infection in children.

Bibliography

1. Abossie, A., Seid, M. 2014. Assessment of the prevalence of parasitosis and associated risk factorsamong primary school children in Chencha town, Southern Ethiopia. *BMC Public Health*.14:166.

2. Adam, R.D. 2001. Biology of *Giardia lamblia*. *Clinical Microbiology Review*, 14: 447–75.

3. Adetunji, V. O. and Odetokun, I. A. 2011. Groundwater contamination in Agbowo community, Ibadan Nigeria: Impact of septic tanks distances to wells. *Malaysian Journal of Microbiology*, *7*.3:159-166.

4. Almeida, A., Pozio, E., Cacciò, S. M. 2010. Genotyping of *Giardia duodenalis* cysts by new real-time PCR assays for detection of mixed infections in human samples. *Applied and Environmental Microbiology*.76.6:1895–901.

5. Anuar, T. S., Azreen, S. N., Salleh, F.M., Moktar, N. 2014. Molecular epidemiology ofgiardiasis among orang Asli in Malaysia: application of the triosephosphate isomerase gene. *BMC InfectiouseDisease*, 14:78.

6. Al-Mohammed, H. I. 2011. Genotypes of *Giardia intestinalis* clinical isolates of gastrointestinal symptomatic and asymptomatic Saudi children. *Parasitology Research*, 108:1375–81.

7. Arora, D. R., and Arora B. B.2004. Medical Parasitology. Third edition. SDR. Printers (Pvt Ltd) Delhi-110094.

8. Berrili, F., Di Cave, D., D'Orazi, C., Orecchia, P., Xhelilaj, L., Bejko, D., Caca, P., Bebeci, D., Cenko, F., Donia, D., Divizia, M. 2006. Prevalence and genotypying of human isolates of *Giardia duodenalis* from Albania. *Parasitology International,* 55:295-297.

9. Caccio, S. M., Thompson, R, C., Mchlauchlin, J., Smith, H. V. 2005. "Unravelling Cryptosporidium and Giardia epidemiology". *Trends in Parasitology*, 21.9:430- 437.

10. Canete, R., Diaz, M. M., Garcia, R. A., Martinez, P. M., and Ponce, F. M. 2012. "Intestinal parasitics in children from a day care centre in Matanzas City, Cuba," *PLOS ONE*, 7.12: e51394.

11. Cheesbrough, M. 2009. District Laboratory Practice in Tropical Countries Part 1.2nd edition, Cambridge University press, Cambridge, 195-216.

12. Colli, C. M., Bezagio, R. C., Nishi, L., Bignotto, T. S., Ferreira, E. C., Falavigna-Guilherme, A. L., 2015. Identical Assemblage of *Giardia duodenalis* in humans, animals and vegetables in an urbanarea in Southern Brazil indicates a relationship among them. *PLOS ONE*, 10.30: e0118065

13. David, B.E., Guimaraes, S., de Oliveira, P. A., de Oliveira-Sequeira, G. C., Bitterncourt, N.G., Nardi, M. R., Eduordo, P., Ribolla, M., Franco, B. M., Branco, N., Tosini, F., Bella, A., Pozio, D.,Cassio, M.,S. 2015. Molecular Characterization of intestinal protozoa in two poor communities inthe state of Sao Paulo, Brazil. *Parasites and Vectors*, 8.1:1-12.

14. Davis, A., Pawlowski, Z. S. 1985. Amoebiasis/Giardiasis and its control. Bullentin of the world health organization, 63.3:417-26.

15. Eckmann, L., Laurent, F., Langford, T., Hetsko, M., Smith, J., Kagnoff, M., Gillin, F. 2000. Nitricoxide production by human intestinal epithelial cells and competition for arginine as potential determinants of host defense against the lumen-dwelling pathogen Giardia lamblia. *Journal of Immunology, 164: 1478-1487.*

16. Eckman, L. 2003. Mucosal defences against *Giardia*. *Parasite Immuunol*ogy, 25:259-70.

17. Egbinola, C. N., Amanambu, A. C. 2015. Water supply, sanitation and hygiene education in secondary schools in Ibadan, Nigeria. *Bulletin of Geography, Socio-economic series*, 29.29:31-46

18. Egbinola, C. N., Amanambu, A. C. 2015. "Geogenic contamination of groundwater in shallow aquifers in Ibadan, south-west Nigeria", Management of Environmental Quality: *An InternationalJournal*, 26. 3:327-341.

19. Escobedo, A. A., Canete, R., and Nunez, F. A. 2007. "Intestinal protozoan and helminth infections in the Municipality Suan Juany Martinez, Pinar del Rio, Cuban," *Tropical Doctor*, 37. 4:236-238.

20. Faria, C. P., Zanini, G. M., Dias, G, S., da Silva, S., Sousa, M. 2016. Molecular characterization of*Giardia lamblia*: First report of Assemblage B in human isolates from Rio de Janeiro (Brazil). PLOS ONE, 11.8: e0160762.

21. Feng, Y, and Xiao, L. 2011. "Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis," *Clinical Microbiology Reviews*, 24. 1: 110–140.

22. Gelanew, T., Lalle, M., Hailu, A., Pozio, E., Caccio, S. 2007. "Molecular characterization of human isolates of *Giardia duodenalis* from Ethiopia, *Acta Tropica*, 102.2:92-99.

23. Geurden, T., Casaert, S., Vercruysse, J., Claerebout, E., Berkvens, D., Geldolf, P. 2008. Mixed *Giardia duodenalis* assemblage A and E infections in Calves. *International Journal of Parasitology*, 35:307-13.

24. Haque, R., Roy, S., Kabir, M., Stroup, S., Mondal, D., Houpt, E. 2005. *Giardia* assemblage A infection and diarrhoea in Bangladesh, *Journal of Infectious Diseases*, 192.12:2171-2173.

25. Helmy, M. F., Abel-Fattai, H. S., Rasheed, L. 2009. Real-time PCR/RFLP assay to detect *Giardia intestinalis* genotypes n human isolates with diarrhoea in Egypt. *Journal of Parasitology*, 95:1000-4.

26. Ignatius R, Gahutu JB, Klotz C, Steininger C, Shyirambere C, Lyng M. 2012. High prevalence of *Giardia duodenalis* assemblage B infection and association with underweight in children, Rwandan. *PLOS Neglected Tropical Disease*, 6: e1677.

27. Kosuwin, R., Putaporntip, C., Pattanawong, U., Jongwutiwes, S. 2010. Clonal diversity in *Giardia duodenalis* isolates from Thailand: evidences for intragenic recombination and purifying selectionat the beta giardin locus. *Gene,* 449:1–8.

28. Li, E., Zhou, P., Singer, S. M. 2006. Neuronal nitric oxide synthase is necessary for elimination of Giardia lamblia infections in mice. *Journal of Immunology, 176: 516-521.*

29. Li, E., Zhao, A., Shea-Donohue, T., Singer, S.M. 2007. Mast cell-mediated changes in smooth muscle contractility during mouse giardiasis*. Infection and Immunity, 75: 4514-4518.*

30. Lebbad, M., Petersson, I., Karlsson, L., Botero-Kleiven, S., Andersson, J. O., Svenugsson, B. 2011.Multilocus genotyping of human *Giardia* isolates suggests limited zoonotic transmission and association between assemblage B and flatulence in children. *PLOS Neglected Tropical Disease,* 5.8: e1262.

31. Makinde, O. O. 2012. Housing: Central City Slums, a case study of Ibadan*. Journal of Environmentand Earth Science.*2: 9.

32. Núñez, F. Á., Lopez, J. L., De la Cruz, A. M., Finlay, C. M. 2003 "Factores de riesgo de la infección por *Giardia lamblia* en niños de guarderías infantiles de Ciudad de La Habana, Cuba," Cadernos de Saúde Pública, 19. 2:677–682.

33. Oliveira-Arbex, A. P., David, E. B., Oliveira-Sequeira, T. C., Bittencourt, G. N., Guimaris, S. 2014. Genotyping of *Giardia duodenalis* isolates in asymptomatic children attending day care centre: evidence of high risk for anthroponotic transmission. *Epidemiology and Infection*, 1-11.

34. Ouattara, M., N'Guéssan, N. A., Yapi, A. and N'Goran, E. K. 2010. Prevalence and spatial distribution of *Entamoeba histolytica/ dispar* and *Giardia lamblia* among schoolchildren in Agboville area (Côte d'Ivoire). *PLOS neglected tropical diseases* 41: e574.

35. Pelayo, L., Nundez, F., Rojas, P. 2008. *Giardia* infections in Cuban children: the genotypes circulating in a rural population, *Annals of Tropical Medicine and parasitology*, 102.7:585-595.

36. Puebla, L. J., Nunez, F.A., Nunez, Y. A. Fernandez. 2014. "Correlation of *Giardia duodenalis* assemblages with clinical and epidemiological in Cuban children," Infection, *Genetics and Evolution,* 23:7-12.

37. Puebla, L. J., Nunez, F.A., Silva, I. M., Rivero, L. R., Gonzalez, M. M., Sutil, Y. M., Valdes, L. A.,Millan, I. A.,Muller N. 2015.Molecular Characterization and risk factors of Giardia duodenalisamong school children from La Habana, Cuba. *Journal of parasitology research.*2015.378643:8

38. Ramos, J. M., RodriguezValero, N., Tisiano, G., Fano, H., Yohannes, T., Gosa, A., Fruttero, E., Reyes, F., Gorgolas, M. 2014. Different profile of intestinal protozoa and helminthic infection among patients with diarrhoea according to age attending a rural hospital in Southern Ethiopia. *Tropical Biomed*icine, 31.2:392-7.

39. Rayani, M., Unyah, N. Z., Hatam, G. 2014. Molecular Identification of *Giardia duodenalis* isolatesfrom Fars Province,Iran. *Iranian Journal of Parasitology*, 9.1:70-8.

40. Ryan, U., and Caccio, S. 2013. "Zoonotic potential of Giardia", *International Journal for Parasitology*, 43.12-13:943-956.

41. Savioli, L., Smith, H., Thompson, R. 2006. "*Giardia*, and *Cryptosporidium* join the 'Neglected Diseases Initiative", *Trends in parasitology*, 22.5:203-208.

42. Schuurman, T., Lankam, P., van-Belkum A., Kooistra, Smid M., van-Zwet, A. 2007. Comparisn of microscopy, real-time PCR and a rapid immunoassay for the detection of *Giardia lamblia* in human stool specimens. *Clinical Microbiology Infection*, 13:1186-91.

43. Sprong, H., Cacciò, S. M., Van Der Giessen, J. W. 2009. Identification of zoonotic genotypes of

Giardia duodenalis. *PLOS Neglected Tropical Disease*, 3.12:1–12.

44. Stadelmann, B., Hanevik, K., Andersson, M.K., Bruserud, O., SvaÈrd, S. G. 2013. The role of arginine and argininemetabolizing enzymes during *Giardia* host cell interactions in vitro. *BMC Microbiology,* 13: 256.

45. Stadelmann, B., Merino, M. C., Persson, L., Svard, S. G. 2012. *Arginine consumption by the intestinal parasite Giardia intestinalis reduces proliferation of intestinal epithelial cells. PLOS One, 7: e45325-10.1371.*

46. Stensvold, C., Nielson, H. 2012. Comparison of microscopy and PCR for detection of intestinal parasites in Danish patients supports an incentive for molecular screening platforms. *Journal of Clinical Microbiology,* 50:540-1.

47. Vanni, I., Cacciò, S. M., van Lith, L., Lebbad, M., Svard, S. G., Pozio, E., tosini, F. 2012 "Detectionof *Giardia duodenalis* assemblages A and B in human faeces by simple, assemblage-specific PCR assays," *PLOS Neglected Tropical Diseases,* 6.8, e1776.

48. Wagayehu, T., Karim, M R., Li, J., Adamu, H., Erko, B., Zhang, L., Tilahun, G. 2016. Prevalence and Genetic Characterization *Cryptosporidium* species and *Giardia duodenalis* in lambs in Oromiaspecial zone, central Ethiopia*. BMC Veterinary Research Disease,* 13.1:1-7.

49. Walker, C. L., Sack, D., Black, E. R. 2010. Etiology of diarrhea in older children, adolescent and adults: a systemic review. *PLOS Neglected Tropical Disease,* 4.8:e768.

50. WHO, 1998. Basic Laboratory manuals in medical parasitology. In: Library W, editor: *NLM Classification.* Geneva. World Health Organization.