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Original Research Article

Prevalence of *Giardia lamblia* and *Cryptosporidium parvum* co-infections among young adults in a private University in South-Western Nigeria

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Abstract: ***Background:*** *Giardia lamblia* and *Cryptosporidium parvum* are major causes of diarrhoeal diseases of humans worldwide and are included in the World Health Organization's 'Neglected Diseases Initiative'. ***Aim:*** This research was designed to assess the prevalence of *G. lamblia* and *C. parvum* co-infections among undergraduate Students of Babcock University, Ilishan-Remo, Ogun State, Nigeria. ***Methods:*** An aggregate of 120 faecal specimens were obtained from 120 partakers (60 females and 60 males) who met the inclusion criteria. The clinical and demographic data of the participants were compiled with the use of patterned survey. Diagnosis was carried out using standard laboratory methods for the detection of these parasites. ***Results:*** The outcome of this study shows that there was no record of *G. lamblia* and *C. parvum* co-infections among the study participants; however, *G. lamblia* and *C. parvum* mono-infection exist among the examined participants with a preponderance of 10% and 4.2%, respectively. There were no substantial differences (P>0.05) in the percentage occurrences of the two parasites established on the individual-level characteristics of the partakers. All participants who tested positive for mono-infection by each parasite had a history of diarrhoea (14.2%). Meanwhile, the 12(10%) participants who tested positive for *G. lamblia* mono-infection indicated abdominal pain (10), nausea and bloating (2.5%), presence of foul smelling watery stool (0.8%), presence of blood and mucus in stool (0.8%) and weight loss (0.8%). While all the 5 participants who tested positive for *C. parvum* indicated that they had only abdominal pain (4.2%). Significant risk factors associated with the occurrence of these parasitic infections include: lack of awareness, history of diarrhea, poor toilet hygiene, poor toilet-student ratio, as well as infrequent visits to the hospital for medical check-ups/laboratory tests. ***Conclusion:*** *Giardia lamblia* and *Cryptosporidium parvum* co-infection does not exist among undergraduate students of Babcock University, however, mono-infection does with a prevalence of 10% and 4.2%, respectively.

Keywords: Prevalence, Giardia lamblia, Cryptosporidium parvum, Young adults, South-western Nigeria.

INTRODUCTION

Diarrhoeal diseases, primarily caused by food borne or waterborne pathogens, are leading causes of illness and deaths, accounting for an estimated 1.9 million deaths annually at the global level (Bojuwoye et al., 2020). It is the eighth leading cause of mortality, responsible for more than 1·6 million deaths globally and about 90% (89·37%) mortality rate in South Asia and sub-Saharan Africa alone (Troeger *et al.,* 2017). The etiology of diarrhoea can be viral, bacterial or parasitic in nature. Two parasitic causative agents of diarrhoea of interest are *Giardia lamblia* and *Cryptosporidium parvum.*

Giardia lamblia (also known as *Giardia intestinalis* or *Giardia duodenalis*) is a bi-nucleate intestinal flagellate protozoan (Tzanidakis *et al.,* 2014). Morphologically, it exists in two forms; the pear-shaped trophozoite (vegetatitve and motile form) and the oval

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shaped cyst (resistant and infective form). *It causes* a major parasitic diarrhoeal disease known as Giardiasis, found throughout the world (Beer *et al.,* 2017). Giardiasis is associated with sub-acute or chronic diarrhoea and intestinal irritation. Giardiasis is typically characterized in humans by diarrhoea, steatorrhea, mal digestion, abdominal cramps, bloating and weight loss. In chronic disease there may be evidence for malabsorption of fat, vitamin A & B12, protein, D-xylose, iron and lactose (Ahmed *et al*., 2020). Infection is more common in children than in adults and infection can be asymptomatic, acute or chronic in nature (Huston *et al.,* 2006*).* The parasite has been found in as many as 80% of raw water supplies from lakes, streams, and ponds and in as many as 15% of filtered water samples *(Ryu et al., 2007). The parasite is naturally endowed with* various virulence factors including a large adhesive disc composed of microtubules to attach to the intestinal mucosa. During adhesion, the flagella of *G. lamblia* moves in a manner that draws fluid out from under the disc, resulting in an area of lower pressure, facilitating adhesion to epithelia (Buret *et al*., 2007).

Cryptosporidium parvum on the other hand, is a coccidian parasite. It is released in faeces as a colourless and spherical oocyst measuring about 4-5µm in diameter. It contains four crescent shaped non-encysted or naked sporozoites. The anterior end of the sporozoite is pointed and the posterior end is rounded and contains a prominent nucleus. The parasite causes a diarrhoeal disease known as cryptosporidiosis. Virulence factors include; circumsporozoite-like glycoprotein (CSL), with a molecular mass of 1,300 kDa, associated with the apical complex of sporozoites and merozoites (Langer *et al*., 2001), Glycoprotein 900 (gp900) located in micronemes and at the surface of invasive merozoites and sporozoites, as well as a sporozoite and merozoite cell surface protein gp15/40/60 complex (Cpgp40/15). These proteins present in their trails have been shown to play a role in parasite attachment, invasion, and motility (Wanyiri and Ward, 2006; (Arora and Arora, 2010b).

Susceptible host become primarily infected with these parasites on exposure to the cyst/oocysts, through the faecal-oral route including drinking of contaminated water, swallowing of water in pools, lakes and rivers during swimming activities, eating uncooked or under cooked contaminated food and through sexual practices such as the anal-oral sexual practice. The cyst/oocyst (the infective form to man as well as the diagnostic form excreted in the feaces) can survive for a prolonged period in the environment (lakes, ponds, streams and rivers). The oocyst of *C. parvum* in particular resists disinfection, including chlorination, and may still be present in post-treatment water supply. Several studies also suggest that flies may play an important role in the mechanical transmission of oocyst of *Cryptosporidium parvum* and *cyst of Giardia lamblia* (Painter *et al.,* 2015).

Relatively little is known about the epidemiology of cryptosporidiosis and Giardiasis in African countries, although a recent review of *Cryptosporidium* in Africa focussed on the epidemiology and transmission dynamics (Aldeyarbi, 2016). *Cryptosporidium* oocysts and *Giardia* cysts have been detected in a variety of African water sources including irrigation water in Burkina Faso (Kpoda *et al.,* 2015), stream, well, spring and lake in Cameroon, wastewater in Côte d'Ivoire, water from wells and the Kano river in Nigeria (Uneke and Uneke, 2007).

Giardia lamblia and *Cryptosporidium parvum* co-infections have been shown to be one of the major causes of diarrhoea, with varied morbidities across different geographical regions of the world. There are isolated studies on the epidemiology of cryptosporidiosis in Nigeria. In one of these studies, Useh and Jonah (1998) reported a prevalence of 4.4% for *C. parvum* amongst diarrhoeal subjects of all ages in Calabar, South Eastern Nigeria. In the same setting, Alaribe *et al*., (1998) documented a predominance level of 5.6% amidst patients suffering from diarrhoea. Infection was not reported among healthy control subjects with no history of diarrhoea. Elsewhere in North Central and South Western Nigeria, higher prevalence of cryptosporidiosis of 4.8% and 14% were reported among undernourished children and diarrhoeic patients by Ikeh *et al*., (2006) and Nwabuisi (1998), respectively. Primary symptoms of *C. parvum* infection are acute, watery, and non-bloody diarrhoea.

The prevalence of diarrhoeic infections among children below age 5 and above the age of 70 caused by *Giardia lamblia* and *Cryptosporidium parvum* has been analysed regionally in some areas and globally. But data on diarrhoea incidences of these parasites among young adult population is scarce, and studies that investigate the role of diarrhoea among young adults would be valuable. Literature search shows that no work has been done to assess the prevalence of mono- and co-infections of *Giardia lamblia* and *Cryptosporidium parvum* amidst undergraduate students of Babcock University, Ilishan-Remo, Ogun state, hence, the reason for this study.

METHODOLOGY

Study Design

This was a prospective institutional based-research to determine the prevalence of *Giardia lamblia* and *Cryptosporidium parvum* co-infections among undergraduate students of Babcock University, Ilishan-Remo, Ogun State.

Area of study

The study was done among undergraduate students of Babcock university, which is located in

Ilishan-Remo, Ogun State, South-Western Nigeria (coordinates; 6.8946^{o}N, 3.7174^{o}E), with about 10,000 student population.

Duration of study: The study proceeds for a session of two months (May-June, 2019).

Study Population

This cross-sectional institutional based study was carried out among undergraduate students of Babcock University, Ilishan-Remo, Ogun state, with history of diarrhoea.

Sample size Calculation

The proportional sampling used for the research was evaluated with the use of principle known as the single population proportion formula, which was formulated by Pourhoseingholi *et al.,* (2013).

Using the single population proportion formula;
$n=Z^2PQ/d^2$

Where**;**
n = Minimum proportional sampling required.
Z = Standard normal variant at 5% ($p<0.05$) error or 95% confidence interval is 1.96.
P = Percentage of diarrhoeic patients including *cryptosporidium parvum* and *Giardia lamblia* co-infections in distinction to preceding investigation.
Q = Proportion of diarrhoeic patients without *cryptosporidium parvum* and *Giardia lamblia* co-infections from previous study (1-P) and
d = Preferred degree of significance (0.05)

When calculating this, a 95% confidence interval was used, the error margin used was set at 0.05 and a P-value of 0.07 that is, a widespread presence of 7% for *Giardia lamblia* and *Cryptosporidium parvum* co-infections from previous study by Avik *et al.,* (2014). To make the study more robust and to minimise errors making an appearance from the probability of non-compliance, 20% of the sample size was included, giving a sample size of 120.

Sample size

The entire 120 faecal samples were assembled from 120 (60 males and 60 females) undergraduate students of Babcock university, Ilishan-Remo, Ogun state, Nigeria with record of diarrhoea.

Exclusion Criteria

Those without history of diarrhoea and those with history of antimicrobial therapy in the preceding two weeks, as well as the post-graduate students were exempted from the research.

Data Collection

Questionnaires were used to obtain clinical and demographic information from the test subjects, prior to specimen collection. Each opinion poll had a special participant identification number (PIDN). The foremost part of the questionnaire comprises of the biodata of the partakers, such as; name (optional), marital status, gender, age, level of study etc. The second part included clinical data of the subjects relating to brief history suggesting gastrointestinal infections e.g. symptoms such as fever, malaise, flatulence, passing of watery stool etc.

Specimen Collection, Transportation and Storage

Faecal specimens were requested from each participant in a clean, disposable, wide mouthed container with a tightly fitting lid and transported immediately to the laboratory for examination in a sealed plastic bag and processed within 15 minutes. Stool samples that were not processed immediately were preserved using formalin fixative (for wet preparation and Ziehl-Neelsen staining technique) and polyvinyl alcohol fixative (for Trichrome staining technique in a ratio of 3 parts of fixative to 1 part of faecal material). The rest of the sample were preserved in a tightly closed container, refrigerated at 2-8^{o}C to prevent desiccation.

Laboratory Diagnosis
Macroscopic Examination

The faecal specimens were examined macroscopically before being processed to check for colour, consistency and constituents (e.g., blood, mucus, pus and parasites).

Microscopic Examination
Saline and Iodine Wet Mount

A drop of normal saline was placed at the centre of the left and right half of a clean, grease-free glass slide, already marked with the respective PIDN. An applicator stick was used to pick up a small portion of the stool sample (about 2mg) and it was emulsified in the normal saline place on the slide. A drop of iodine was added to one of the suspensions and a coverslip was used to cover each suspension separately. Microscopic examination was done using a 10x objective lens to focus and 40x objective lens to magnify. Trophozoites of *Giardia lamblia* if present, showed a characteristic falling-leaf motility in wet mounts but they were non-motile in iodine wet preparation. Cysts of *Giardia lamblia:* If present, appeared ovoid to ellipsoid in shape and measured about11 to 14µm. Immature cysts have 2 to 4 nuclei. The median bodies of the cysts showed deep staining in iodine wet preparation. Oocysts of *Cryptosporidium parvum* are spherical or oval cysts, highly refractile and measuring about 4-5µm in diameter.

Concentration of Parasites

The formol-ether sedimentation technique as described by Ochei and Kolhatkar (2007) was used to concentrate the parasites in the faecal specimens.

Staining of Permanent Smears

Microscopic examination of permanent stained smears was used to confirm the identification of parasites seen in the wet preparation and concentration method described earlier.

Modified Ziehl-Neelsen Method Using 3% Acid Alcohol

A modified acid-acid fast staining was used for the detection of *Cryptosporidium parvum* oocysts in faecal specimens. A smear was made and allowed to air dry. It was fixed using ethanol for few minutes and flooded with basic carbol fuchsin, brought up to steam, but not boiled and left for 5 minutes. It was rinsed with distilled water and decolourised with 3% acid alcohol for 1 minute. It was further counterstained with 1% methylene blue and washed in distilled water. It was air dried and examined microscopically using immersion oil and 100x objective lens. Oocyst of *Cryptosporidium parvum* appeared pinkish red with blue background.

Trichome Staining Method

This is a rapid staining procedure used for the detection of the trophozoites and cysts of *Giardia lamblia* in stool specimens.

Procedure

Briefly, a thin smear was made at the centre of a clean-grease free, glass slide. It was placed in 70% ethanol-iodine for 10 to 20 minutes, when using PVA preserved samples, if not, it was fixed using Schaudinn's fixative for 1hr at room temperature or 5 minutes at 50^{o}C. It was placed in 2 changes of 70% ethanol for I minute each in fresh specimens and 4 minutes each in preserved specimens. It was flooded with trichome stain for 10 minutes. It was decolourised in 90% acid-ethanol for 10 seconds or until stain disappears from the smear. It was dehydrated in two changes of 100% ethanol for 1 minute each and it was cleared in xylene for 5 minutes. It was mounted using a cover slip and synthetic medium. Microscopic examination was done using the 100x objective lens and at least 200-300 oil immersion field were examined. Cysts: Stain slightly purple in colour and are ovoid to ellipsoid in shape. Trophozoites: Blue-green colouration, sometimes with a tinge of purple. Appear as pear-shaped organisms.

Statistical Analysis

Data gathered were compiled into Microsoft Excel. Analysis of data was carried out using SPSS Statistics Software Package (Version 18.0). Data gathered were statistically analysed using One-way Analysis of Variance and Turkey-Kramer Multiple Comparisons Test. The level of significance was determined at 95%. P values <0.05 were considered significant.

RESULTS

This present study investigated the prevalence of *Giardia lamblia* and *Cryptosporidium parvum* mono/co-infections among undergraduate students of Babcock University, Ilishan-Remo, Ogun state, Nigeria. The entire 120 students (60 males and 60 females), were recruited for the study.

The socio-demographic attributes of the research participants are presented in Table 1. Majority were within the age range of 16-21yrs (57.5%), followed by 22-25years (40.8%), 26-30years (0.8%), as well as 30-35years (0.8%). Most of them were Christians (89.2%), followed by Muslims (10%) and lastly traditional worshippers (0.8%). The Yorubas were in majority (70.8%), followed by the Hausas (16.7%) and then the Igbos (8.3%). On the basis of their study level, majority were 400 level students (33.3%), followed by the 500 level students (24.2%), 300 level students (21.7%), 200 level students (18.3%), 600 level students (1.7%) and lastly, 100 level students (0.8%). All participants were solitary on the footing of their marital status (100%).

Overall prevalence of *Giardia lamblia* and *Cryptosporium parvum* mono/co-infections amid the research participants is given using a pie chart (Figure 1). Out of the 120 participants examined, 10% of them were positive for *Giardia lamblia* mono-infection, while 4.2% were positive for *Cryptosporidium parvum* mono-infection. There was no record of *Giardia lamblia* and *Cryptosporidium parvum* co-infections among the study participants (0%).

Table 2 shows the prevalence of appearance of *G. lamblia* infection in connection to the socio-demographic attributes of the research partakers. On the basis of their gender, both the male (5.0%) and female (5.0%) participants were equally infected. Based on age range, the highest occurrence was recorded among age group 16-21years (6.7%), which was established to be considerably higher ($P<0.05$) than other age groups: 22-25years (3.3%), 26-30years (0%) and 30-35years (0%).

Based on their religion, the elevated appearance of *G. lamblia* infection was recorded among the Christian participants (9.2%), which was established to be considerably higher ($P<0.05$) when compared to that of traditional worshippers (0.8 %). *G. lamblia* infection was not recorded among the Muslim participants.

Furthermore; there were no considerably differences ($P>0.05$) in the appearance of *G. lamblia* infection among the research participants based on their study level. However, it should be noted that *G. lamblia* infection was not recorded among 100 and 600 level Students of the University examined (0%). Lastly, based on their marital status, all the 12 participants (10%) who tested positive for *G. lamblia* infection were singles, none was married.

The prevalence of appearance of *C. parvum* mono-infection in connection to the socio-demographic

attributes of the research partakers is given in Table 3. The female participants (3.3%) were established to be more infected with *C. parvum* than their male counterparts (0.8%). The difference in percentage positivity between the sexes was statistically significant (P<0.05). On the basis of their age range, all the participants who tested positive for *C. parvum*, were within the age range of 16-21yrs (4.2%). No infection was recorded among other age groups. Under religion consideration, the highest occurrence was found among Christians (3.3%), followed by Muslims (0.8%) and lastly Traditional worshippers (0%). The difference was statistically significant (P>0.0%). On the basis of their tribes, the Yorubas had the highest occurrence of *C. parvum* infection, while others showed 0% positivity. With regard to their class study level, the highest occurrence was found among 300 and 400 level students (1.7%, each) and followed by the 200 level students (0.8%). There was no record of *C. parvum* infection among the 100 level, 500 level and 600 level students (0%).

The risk factors associated with *G. lamblia* and *C. parvum* infections in connection to the socio-demographic attributes of the study partakers are given in Table 4. Lack of knowledge of parasites as diarrhoea causing agents, history of diarrhoea, toilet-student ratio, frequency of toilet washing and frequency of medical check-up and laboratory investigation when having diarrhoea are risk factors strongly (P<0.05) associated with the occurrence of *G. lamblia* and *C. parvum* amid the research partakers. 9.2% and 2.5% of the participants who tested positive for *G. lamblia* and *C. parvum*, respectively, had no knowledge of these parasites as diarrhoea causing agents. All of the participants who tested positive for both parasites had history of diarrhoea, 10% and 4.2%, respectively. Regarding toilet-student ratio, the highest occurrence of and *G. lamblia* and *C. parvum* was recorded among those who indicated a 1:8 toilet-student ratio, 6.7% and 3.3%, respectively, which was found to be statistically significant (P<0.05) when compared to others.

In addition to toilet-student ratio, another closely related risk factor was frequency of toilet washing. Those who indicated that they wash their toilet once weekly had the highest occurrence of *G. lamblia* and *C. parvum* infection, 5.8% and 4.2%, respectively with a p-value less than 0.05. Lastly, the frequency of medical check-up and laboratory investigation when having diarrhoea was also noted as another major risk factor. 5.0% and 2.5% of those who tested positive for *G. lamblia* and *C. parvum* infection, respectively, indicated that they only go for medical check-up and laboratory investigation sometimes when having diarrhoea.

The proportional appearance of symptomatic and asymptomatic infection among the study partakers is given with a bar chart in Figure 2. Ten of the participants (8.3%) had symptomatic *G. lamblia* infection, 2 (1.7%) had asymptomatic *G. lamblia* infection. On the other hand, 5 (4.2%) participants had symptomatic *C. parvum* infection, however, there was none with asymptomatic *C. parvum*.

Finally, the indications for parasitic gastroenteritis in relation to the occurrence of *G. lamblia* and *C. parvum* amid the research participants are given by a histogram (Figure 3). The 12 participants (10%) which were tested positive for *G. lamblia*, indicated abdominal pain, 3 (2.5%) indicated nausea and bloating, 2 (1.6%) had foul smelling watery stool, 1 (0.8%) person indicated presence of blood/mucus in stool and another person (0.8%) indicated weight loss. The only 5 (4.2%) participants who tested positive for *C. parvum* indicated that they had only abdominal pain.

Table 1: Socio-demographic attributes of the research participants

Characteristic	Class	Frequency (N)	Percentage (%)
Gender	Male	60	50.0
	Female	60	50.0
	Total	**120**	**100.0**
Age range	16-21yrs	69	57.5
	22-25yrs	49	40.8
	26-30yrs	1	0.8
	30-35yrs	1	0.8
	Total	**120**	**100.0**
Religion	Christianity	107	89.2
	Islam	12	10.0
	Traditional	1	0.8
	Total	**120**	**100.0**
Tribe	Yoruba	85	70.8
	Hausa	20	16.7
	Igbo	10	8.3
	Others	5	4.2
	Total	**120**	**100.0**

Characteristic	Class	Frequency (N)	Percentage (%)
Study level	100 Level	1	0.8
	200 Level	22	18.3
	300 Level	26	21.7
	400 Level	40	33.3
	500 Level	29	24.2
	600 Level	2	1.7
	Total	**120**	**100.0**
Marital status	Single	120	100.0
	Married	0	0
	Total	**120**	**100.0**

Table 2: The prevalence of appearance of *Giardia lamblia* mono-infection in connection to the socio-demographic attributes of the research participants

Characteristic	Category	No. of stool samples examined N (%)	No. Positive for *G. lamblia* N (%)	No. Negative for *G. lamblia* N (%)	P-Value	Pearson Chi-Square (χ^2)
Gender	Male	60 (50.0)	6 (5)	54 (35.0)	0.109	0.000
	Female	60 (50.0)	6 (5)	54 (45.0)	0.109	
	Total	**120 (100)**	**12 (10)**	**108 (90)**		
Age range	16-21yrs	69 (57.6)	8 (6.7)	61 (50.8)	0.042*	0.601
	22-25yrs	49 (40.8)	4 (3.3)	45 (37.6)	0.896	
	26-30yrs	1 (0.8)	0 (0.0)	1 (0.8)	1.000	
	30-35yrs	1 (0.8)	0 (0.0)	1 (0.8)	1.000	
	Total	**120 (100)**	**12 (10)**	**108 (90)**		
Religion	Christianity	107 (89.2)	11 (9.2)	96 (80)	0.046*	10.343
	Islam	12 (10)	0 (0)	12 (10)	0.999	
	Traditional	1 (0.8)	1 (0.8)	0 (0)	0.999	
	Total	**120 (100)**	**12 (10)**	**108 (90)**		
Tribe	Yoruba	85 (70.8)	8 (6.7)	77 (64.2)	0.133	5.588
	Hausa	20 (16.7)	1 (0.8)	19 (15.8)	0.997	
	Igbo	10 (8.3)	3 (2.5)	7 (5.8)	0.453	
	Others	5 (4.2)	0 (0)	5 (4.2)	0.999	
	Total	**120 (100)**	**12 (10)**	**108 (90)**		
Study level	100 Level	1 (0.8)	0 (0)	1 (0.8)	0.999	2.405
	200 Level	22 (18.3)	4 (3.3)	18 (15)	0.791	
	300 Level	26 (21.7)	2 (1.7)	24 (20)	0.805	
	400 Level	40 (33.3)	3 (2.5)	37 (30.8)	0.812	
	500 Level	29 (24.2)	3 (2.5)	26 (21.7)	0.851	
	600 Level	2 (1.7)	0 (0)	2 (1.7)	0.999	
	Total	**120 (100)**	**12 (10)**	**108 (90)**		
Marital status	Single	120 (100)	12 (10.0)	108 (90.0)	1.000	
	Married	0 (0)	0 (0)	0 (0)		
	Total	**120 (100)**	**12 (10)**	**108 (90)**		

Table 3: The prevalence of appearance of *Cryptosporidium parvum* mono-infection in connection to the socio-demographic characteristics of the study participants

Attribute	Class	No. of stool samples examined N (%)	No. Positive for *C. parvum* N (%)	No. Negative for *C. parvum* N (%)	P-Value	Pearson Chi-Square (χ^2)
Gender	Male	60 (50)	1 (0.8)	59 (49.2)	0.171	1.878
	Female	60 (50)	4 (3.4)	56 (46.6)	0.041*	
	Total	**120 (100)**	**5 (4.2)**	**115 (95.8)**		
Age range	16-21yrs	69 (57.5)	5 (4.2)	64 (53.3)	0.277	3.856
	22-25yrs	49 (40.8)	0 (0)	49 (40.8)	0.999	
	26-30yrs	1 (0.8)	0 (0)	1 (0.8)	0.999	
	30-35yrs	1 (0.8)	0 (0)	1 (0.8)	0.999	
	Total	**120 (100)**	**5 (4.2)**	**115 (95.8)**		
Religion	Christianity	107 (89.2)	4 (3.3)	103 (85.8)	0.736	0.614
	Islam	12 (10)	1 (0.8)	11 (9.2)	0.998	

Attribute	Class	No. of stool samples examined N (%)	No. Positive for *C. parvum* N (%)	No. Negative for *C. parvum* N (%)	P-Value	Pearson Chi-Square (χ^2)
	Traditional	1 (0.8)	0 (0)	1 (0.8)	0.999	
	Total	**120 (100)**	**5 (4.2)**	**115 (95.8)**		
Tribe	Yoruba	85 (70.8)	5 (4.2)	80 (66.7)	0.242	2.148
	Hausa	20 (16.7)	0 (0)	20 (16.7)	0.999	
	Igbo	10 (8.3)	0 (0)	10 (8.3)	0.999	
	Others	5 (4.2)	0 (0)	5 (4.2)	0.999	
	Total	**120 (100)**	**5 (4.2)**	**115 (95.8)**		
Study level	100 Level	1 (0.8)	0 (0)	1 (0.8)	0.999	2.278
	200 Level	22 (18.3)	1 (0.8)	21 (17.5)	0.998	
	300 Level	26 (21.7)	2 (1.7)	24 (20)	0.997	
	400 Level	40 (33.3)	2 (1.7)	38 (31.7)	0.997	
	500 Level	29 (24.2)	0 (0)	29 (24.2)	0.999	
	600 Level	2 (1.7)	0 (0)	2 (1.7)	0.999	
	Total	**120 (100)**	**5 (4.2)**	**115 (95.8)**		
Marital status	Single	120 (100)	5 (4.2)	115 (95.8)	1.000	
	Married	0 (0)	0 (0)	0 (0)		
	Total	**120 (100)**	**5 (4.2)**	**115 (95.8)**		

Table 4: Risk factors associated with *Giardia lamblia* and *Cryptosporidium parvum* infectionin relation to the socio-demographic attributes of the research participants

Attributes	Responses	No. of Participants examined N (%)	No. positive for *G. lamblia* N (%)	No. positive for *C. parvum* N (%)	No. negative for both *G. lamblia* & *C. parvum* N (%)	*P-Value*	Pearson Chi-Square (χ^2)
Knowledge of *G. lamblia* as a diarrhoea causing agent	Yes	37 (30.8)	1 (0.8)	4 (3.3)	32 (26.7)	0.062	8.519
	No	83 (69.2)	11 (9.2)	1 (0.8)	71 (59.2)	0.014*	
Knowledge of *C. parvum* as a diarrhoea causing agent	Yes	26 (21.7)	1 (0.8)	2 (1.7)	23 (19.2)	0.084	2.274
	No	94 (78.3)	11 (9.2)	3 (2.5)	80 (66.7)	0.032*	
History of diarrhea	Yes	97 (80.8)	12 (10)	5 (4.2)	80 (66.7)	0.046*	4.696
	No	23 (19.2)	0 (0)	0 (0)	23 (19.2)	0.999	
Consumption of food from the University Cafeteria	Often	45 (37.5)	2 (1.7)	0 (0)	43 (35.8)	0.969	8.980
	Sometimes	67 (55.8)	10 (8.3)	5 (4.2)	52 (43.3)	0.998	
	Never	8 (6.7)	0 (0)	0 (0)	8 (6.7)	0.998	
Consumption of food from the University community	Often	33 (27.5)	1 (0.8)	0 (0)	32 (26.7)	0.255	5.335
	Sometimes	85 (70.8)	11 (9.2)	5 (4.2)	69 (57.5)	0.181	
	Never	2 (1.7)	0 (0)	0 (0)	2 (1.7)	1.000	
Type of water consumed	Tap	8 (6.7)	0 (0)	0 (0)	8 (6.7)	0.998	3.961
	Portable	100 (83.3)	12 (10)	5(4.2)	83 (69.2)	0.411	
	Boiled & filtered	12 (10)	0 (0)	0 (0)	12 (10)	0.998	
Toilet-Student ratio	1 to 2	3 (2.5)	0 (0)	0 (0)	3 (2.5)	0.999	3.435
	1 to 4	16 (13.3)	0 (0)	0 (0)	16 (13.3)	0.999	
	1 to 6	50 (41.7)	4 (3.3)	1 (0.8)	45 (37.5)	0.073	
	1 to 8	51 (42.5)	8 (6.7)	4 (3.3)	39 (32.5)	0.028*	
Frequency of toilet washing	Daily	19 (15.8)	2 (1.7)	0 (0)	17 (14.2	0.382	2.556
	Once weekly	68 (56.7)	7 (5.8)	5 (4.2)	56 (46.7)	0.036*	
	Twice weekly	27 (22.5)	2 (1.7)	0 (0)	25 (20.8)	0.489	
	Thrice weekly	6 (5)	1 (0.8)	0 (0)	5 (4.2)	0.157	
Material used for anal cleaning	Tissue paper	14 (11.7)	0 (0)	0 (0)	14 (11.7)	0.999	5.110
	Water	34 (28.3)	6 (5)	2 (1.7)	26 (21.7)	0.276	
	Both	72 (60)	6 (5)	3 (2.5)	63 (52.5)	0.281	
Frequency of Hand washing	Always	90 (75)	6 (5)	4 (3.3)	80 (66.7)	0.258	6.798
	Often	25 (20.8)	4 (3.3)	0 (0)	21 (17.5)	0.119	
	Sometimes	5 (4.2)	2 (1.7)	1 (0.8)	2 (1.7)	0.999	
Frequency of medical check-ups & laboratory tests when having diarrhea	Always	5 (4.2)	1 (1.7)	0 (0)	4 (3.3)	0.081	9.837
	Often	14 (11.7)	5 (4.2)	2 (1.7)	7 (5.8)	0.076	
	Sometimes	88 (73.3)	6 (5.0)	3 (2.5)	79 (65.8)	0.004*	
	Never	13 (10.8)	0 (0)	0 (0)	13 (10.8)	0.998	

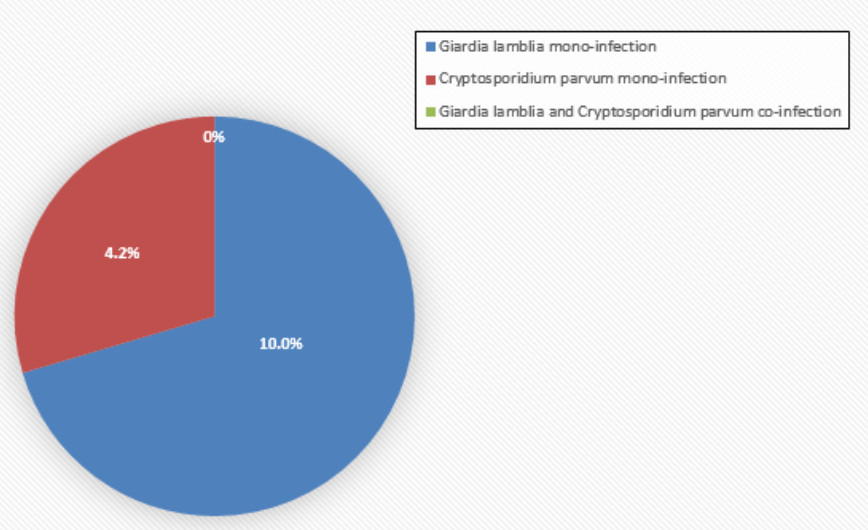

Figure 1: Pie chart showing overall appearance of *G. lamblia* and *C. parvum* mono/co-infection among the research participants

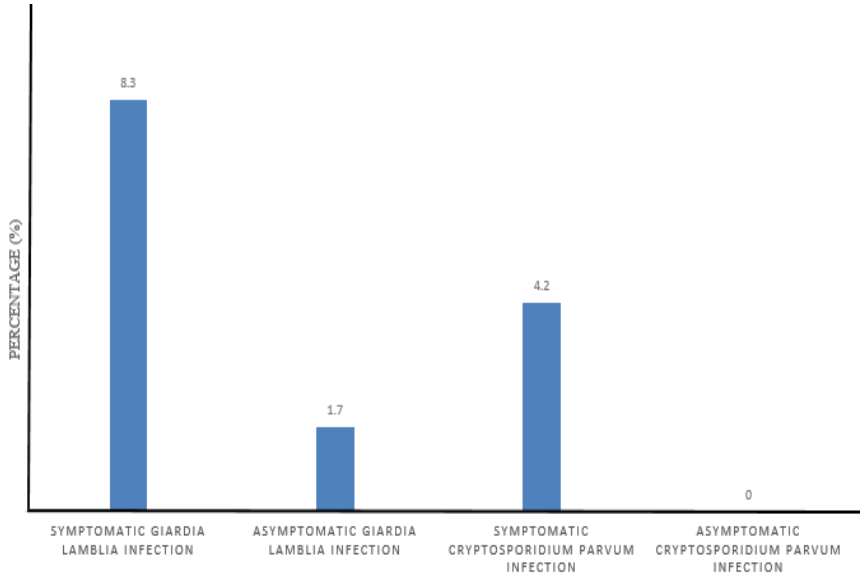

Figure 2: Bar chart showing the proportion of appearance of symptomatic and asymptomatic infection among the research participants

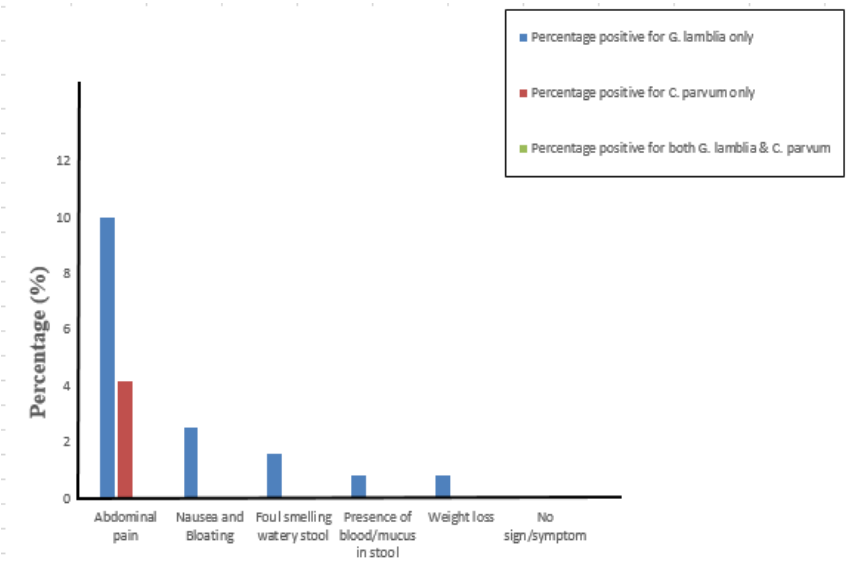

Figure 3: Bar chart expressing the manifestation for parasitic gastroenteritis in connection to the occurrence of *Giardia lamblia* and *Cryptosporidium parvum* among the study participants

DISCUSSION

Although there could be many other causes of diarrhoea, the enteric protozoa *Giardia lamblia* and *Cryptosporidium parvum* have been recognized as important causes of both out-break related and sporadic diarrhoea among the general populace in Africa, Nigeria in particular. Both immunocompetent and immunocompromised individuals could be victims of diarrhoeal diseases caused by these parasites (Adams, 1991).

Giardia lamblia and *Cryptosporidium parvum*, both of which are ubiquitous protozoa parasites, are medically important entero-parasites which have recently become very important particularly because of their increasing association with drinking water sources (Lim *et al*., 2009) especially in communities without proper sanitation and portable water (Ayalew *et al*., 2008; Helmi *et al*., 2011).

Principal methods of diagnosis include; saline and iodine wet preparation, concentration method, trichrome and iron-haematoxylin staining technique for the detection of *G. lamblia*, Ziehl-Neelsen and Safranin staining technique for the detection of *C. parvum.* Immunoassays, rapid diagnostic test kits, faecal leukocyte marker, flow cytometry, molecular techniques and duodenal aspirates are also used for the detection of Giardiasis and cryptosporidiosis.

To the best of our knowledge, no study has documented the prevalence of *G. lamblia* and *C. parvum* co-infections among undergraduate students of tertiary institution. This present study was therefore designed to investigate the presence of *G. lamblia* and *C. parvum* co-infections among undergraduate students of Babcock University, Ilishan-Remo, Ogun state, Nigeria using microscopic technique.

The result of this research shows that among the 120 participants surveyed, 10% of them were positive for *G. lamblia* mono-infection, while 4.2% were positive for *C. parvum* mono-infection. There was no record of *G. lamblia* and *C. parvum* co-infections among the study participants (0%). The 10% prevalence of *Giardia lamblia* mono-infection in this research was discovered to be less than the 14.3% reported by Atu *et al.,* (2014) in a research conducted in Benue State, Nigeria among people living in the senatorial districts of Benue State, using the quick immunochromatographic test strip method. This also agrees with similar studies from other parts of Africa. A higher prevalence of 15.4% and 36.9% was described by Tumwine *et al.,* (2003) in Uganda and Koffi *et al.*, (2014) in Cote d'Ivoire, respectively, in children using the polymerase chain reaction (PCR) method.

Furthermore, the 4.2% prevalence of *C. parvum* mono-infection discovered in this research was found to be three times less than the 13.2% described by Atu *et al.* (2014) in a study carried out in Benue State, Nigeria among people living in the Senatorial Districts of Benue State, using the quick immunochromatographic test strip method. In comparison with previous works done in other parts of Africa, on one hand, it was discovered to be greater than the 2.5% described by Tumwine *et al.,* (2003) in Uganda amid children using the PCR method; although, it was lower than the 20.9% described by Koffi *et al.,* (2014) in a study carried out in Cote d'Ivoire among children using PCR. Reasons for this disparity may be due to contrast in the geographical region, socio-economic status, level of awareness of the parasites, personal hygiene and environmental sanitation of the study participants, as well as the sensitivity and precision of the diagnostic methods used in detecting the parasites.

With regard to gender distribution, the prevalence of *G. lamblia* mono-infection was the same (5%) for both sexes, but the prevalence of C. *parvum* differs in males (0.8%) when compared to females (3.3%). This contradicts the job of Tashima *et al*., (2009) who described a higher frequency of *G. lamblia* mono-infection among male Brazilian children (25%) than in their female counterparts in their female counterparts (8%) using PCR. It also disagrees with the work of Aniesona and Bamaiyi (2014) children in the arid region of North-eastern, Nigeria, who reported a higher prevalence in males (34.8%) than in females (18.9%). Meanwhile, O'Donoghue (1995) and Bello *et al*., (2011) using the Ziehl-Neelsen technique, did not find any marked difference in infection between both sexes in separate studies carried out in Cuba among the general population. Tumwine *et al.,* (2003) also reported that both sexes were equally infected with the two parasites among children hospitalized for diarrhoea at the Mulago Hospital in Uganda using PCR.

Based on age range, the highest occurrence of *G. lamblia* mono-infection was recorded among age group 16-21yrs (6.7%), which was established to be considerably higher ($P<0.05$) than other age groups. Similarly, all the participants who tested positive for *C. parvum* mono-infection were within the same age range of 16-21yrs (4.2%). No infection was recorded among other age groups. This observation is not surprising as students within the younger age group are most vulnerable and tend to exhibit low hygienic practices, while the students within the higher age group might be more aware and enlightened on the need for good hygienic practice.

This study cannot tell whether infection is more common among young adults than in children or elderly, since undergraduate students of Babcock University were the only target population. Meanwhile, a previous study by Aniesona and Bamaiyi (2014), shows that the overall prevalence of cryptosporidiosis in children aged 0 to 15 years in the arid region of

Borno State, North-eastern Nigeria was higher (42.9%) than among older age groups. Similar observation was made by Wellington *et al.,* (2009), who reported that both *C. parvum* and *G.* lamblia were most common among children aged 0-10 years in a study carried out in Lagos, South-western Nigeria using the microscopic method and PCR. Higher prevalence rates particularly among age 16-21yrs could be due to their poor knowledge of parasites to cause diarrhoeic infections, low level of personal hygiene, as well as the type of water available in their local environment.

It is worth mentioning at this juncture that, although both parasites share the same habitats and modes of transmission, and therefore have equal chance of infecting their hosts, there was no record of *G. lamblia* and *C. parvum* co-infections among the participants (0%) in this current study. To the best of our ability, no earlier research have described co-occurrence of *Giardia lamblia* and *Cryptosporidium parvum* infection in human population, the sense for this conceivable and further study would be required.

Regarding the threats connected with frequency of *G. lamblia* and *C. parvum* infections amid the research participants; lack of knowledge of parasites as diarrhoea causing agents, history of diarrhoea, toilet-student ratio, frequency of toilet washing and frequency of medical check-up and laboratory investigation when having diarrhoea were found to be strongly ($P<0.05$) connected with the frequency of *G. lamblia* and *C. parvum* among the study participants. This observation is in accord with the findings of Ajjampur *et al.,* (2007), who described the same. Cognition and intelligence are essential epidemiologic tools in the prevention and control of communicable diseases from public health perspective. Majority of the study participants (69.2-78.3%) indicated that they have no knowledge of these parasites as causative agents of diarrhoea. Therefore, they couldn't have taken the necessary preventive measures in this regard.

Talking about history of diarrhoea, it is well documented that once an individual has been previously exposed to diarrhoea causing-agents like *G. lamblia* and *C. parvum*; the chances of re-occurrence exist especially in endemic regions, depending on the level of personal hygiene of the individual and degree of exposure to parasites as well as auto-infection in the case of cryptosporidiosis. Toilet-Student ratio and frequency of toilet washing are other important threats. Majority of the research participants who came back positive to whichever of these parasites stay in the oldest Halls of residence on the campus with high level of occupancy and a toilet-students ratio of 1:8, which we considered to be inadequate. This warranted environmental inspection of their Halls of residence. The physical conditions of some of the toilet facilities in the hostel as at the time of visit calls for serious health concerns as some students are in the habits of not flushing the toilets after use. It is well documented that Giardiasis and cryptosporidiosis are more prevalent especially in areas with poor environmental and personal hygiene (Painter *et al.,* 2015; Escobedo *et al.,* 2018).

With regards to the type of drinking water consumed, the 12 (10%) and 5 (4.2%) participants who tested positive for *G. lamblia* and *C. parvum* infection, respectively, indicated that they drink portable water. Although, portable water is considered safe for consumption by majority, the detection of these parasites among consumers of portable water suggest otherwise. This could be due to poor treatment of portable water as cysts and oocysts of these parasites naturally exist in water and have been reported to resist disinfection, including chlorination, and can survive for a prolonged period in the environment. Although some studies have shown that cysts of *G. lamblia* are easily inactivated by chlorination, the oocysts of *C. parvum* have been reported to be resistant to the usual concentrations of 0.2−1 mg/L chlorine used in communal drinking water (WHO, 2003; Allgood and Quick, 2008). And since these parasites are not eliminated by chlorination, they may still be present in post-treatment water supply as well as portable water (Semenza and Nichols, 2007; Chako *et al.,* 2010; Painter *et al.,* 2015).

To therefore ensure availability of safe water void of these parasites, other effective water treatment techniques in addition to chlorination, must be considered and employed. These include sterilization processes using steam, ethylene oxide, Sterrad 100 and other similar technologies which can inactivate up to 3 logs or more of *Cryptosporidium parvum* (Chako *et al*., 2010). Sedimentation and filtration has been documented to provide an effective barrier against *C. Parvum* (Semenza and Nicholas, 2007). Coagulators such as aluminium sulphate, iron (II) sulphate, or iron (III) chloride can also be used to neutralize the negatively charged oocysts, thus promoting their coagulation. Instead of using ineffective chemical means of water purification and very difficult filtration processes, there is a new trend towards reverse osmosis, membrane filtration, and electronic/radiation methods (King *et al.,* 2015).

Regarding the symptomatology of Giardiasis and cryptosporiodiosis among the study participants, 10 (8.3%) of the participants with *G. lamblia* infection were symptomatic, while 2 (1.7%) were asymptomatic. On the other hand, 5 (4.2%) participants had symptomatic *C. parvum* infection, while there was none with asymptomatic *C. parvum*. This is differ from the earlier work of Smith and Mank (2011), who reported 8-30% and 1-8% infection rate in asymptomatic children in developing and industrialized regions, respectively (Smith and Mank, 2011). Symptomatic infection has been reported in both

immunocompromised and immune-deficient patients of all ages, but however, once the primary infection has been established, the immune system of the host plays an important role in determining the duration and severity of the disease (White *et al.*, 2015).

In this research, we defined symptomatic infection as the discovery of parasite being of one or more clinical manifestations of gastroenteritis in the participants. Conversely, asymptomatic infection is the discovery of parasite in the absence of any signs and symptoms of gastroenteritis in the participants. The implication of being asymptomatic is that such individuals despite they display no outward clinical manifestations of the disease, they harbour and shed these parasites indiscriminately without knowing they constitute crucial reservoir of infection within the community. Convalescent and chronic carriers of these parasites must therefore be detected and properly handled in order to halt the pattern of contagion inside the university population. And following appropriate anti-parasitic therapy, clinical cure from *Giardia*sis and cryptosporidiosis is defined as absence of clinical manifestations persistent about these parasitic infections. On the other hand, immunologic cure is confirmed by non-detection of serum antibody and stool antigen, while microbiologic cure is confirmed by a negative stool microscopy.

Finally, regarding the indications for parasitic gastroenteritis among the study participants, most of them who tested positive for *G. lamblia*, indicated abdominal pain, nausea and bloating, foul smelling watery stool and presence of blood/mucus in stool. Whereas most of the participants who tested positive for *C. parvum* indicated that they had only abdominal pain. It therefore appears that *G. lamblia* is a more aggressive parasite than *C. parvum*, considering the pathologies reported among the study participants. This aggressiveness may be partly due to its virulent factors which include the sucking disc which acts as an organ of attachment, the numerous flagella for motility, lectins which interfere with nutrition absorption function of the host's microvilli and the long generation time of the parasite amongst others.

CONCLUSION

Co-infection of *Giardia lamblia* and *Cryptosporidium parvum* does not exist among undergraduate students of Babcock University, however, mono-infection does with a prevalence of 10% and 4.2%, respectively. Infected participants complained of abdominal pain, nausea and bloating, foul smelling watery stool, presence of blood/mucus in stool and weight loss amongst others. Identified threats connected with the incidence of *Giardia lamblia* and *Cryptosporidium parvum* among the research participants include: lack of awareness, history of diarrhoea, poor hand and toilet hygiene, high toilet-student ratio, and infrequent visits to the hospital for medical check-ups as well as laboratory tests amongst others. The detection of other intestinal parasites (such as eggs of *Fasciola hepatica*, larvae of *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Strongyloides stercoralis*, as well as microfilaria) among the study participants, apart from parasites of interest (*Giardia lamblia* and *Cryptosporidium parvum*), calls for serious concerns and therefore urgent public health intervention is needed to halt the cycle of transmission of these neglected parasitic diseases.

CONSENT

All authors state that 'written' informed approval was acquire from the participants with guarantee of anonymity and confidentiality before the beginning of the research.

ETHICAL APPROVAL

Ethical consent for the research was received from the Babcock University Health Research Ethics Committee (BUHREC) with ethical authorization registration number: BUHREC346/19.

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