



**Prevalence of *Giardia lamblia* in Association with Body Mass Index in Children (0-5) Years with Symptoms of Gastroenteritis Attending Selected Hospitals in Kaduna Metropolis, Nigeria**

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**ABSTRACT**

*Giardia* is a major causative agent of gastrointestinal disease in humans and causes giardiasis. The present study utilised microscopy and ELISA copro-antigen to determine the prevalence of *Giardia lamblia* in stool samples of children 0-5 years presenting with gastroenteritis in association with body mass indices (BMI) in Kaduna Metropolis, Nigeria. Of 200 samples examined, 12 (6%) were positive for *Giardia lamblia* using microscopy and 28 (14%) using ELISA kit. The prevalence of giardiasis was higher in males (15.89%) than females (11.83%). There was no significant difference in the level of infection in both sexes ( $\chi^2=0.6811$ ,  $df= 1$ ,  $p= 0.4092$ ). The highest prevalence of giardiasis was observed in 4-5 years (16.39%) while the lowest was in the 0-1 year (9.68%). There was no significant difference in the prevalence of giardiasis among the age groups ( $\chi^2= 0.848$ ,  $df= 4$ ,  $p = 0.9319$ ). There was no association between giardiasis and BMI ( $p = 0.3161$ ). The study showed that 33% of the children were underweight, while 66% and 1% had normal weight and overweight respectively. There was a strong association between giardiasis and source of drinking water (well water), nutritional status, and mother's level of education. The most common parasite identified in this study was *Entamoeba coli* (18%) followed by *Giardia lamblia* (14%) and the least was *Strongyloides stercoralis* (0.5%). Based on the above results, it can be concluded that the prevalence of *Giardia lamblia* in children 0-5 years presenting with gastroenteritis has no association with their BMI. Therefore it is important that children 0-5 years should be fed with a balanced meal. Improved personal hygiene should be encouraged and treated water should be protected from re-contamination.

**INTRODUCTION**

Acute gastroenteritis presenting as infant diarrhoea remains a common illness among infants and children throughout the world. It has been established that in the very poor countries of Africa, Asia, and South America a child suffers up to 15 to 19 episodes of diarrhoea with 4.6 million to 6 million deaths annually (Grote *et al.*, 2011).

In Nigeria, the available report indicates that more than 315,000 deaths occur annually as a result of infantile diarrhoea disease with 80% of preschool-age children affected (Aminu *et al.*, 2008; Ayolabi *et al.*, 2012).

Gastroenteritis is an illness caused by infection and/or inflammation of the digestive tract. It is characterized by nausea, vomiting, diarrhoea and/or stomach cramps. Other symptoms may include fever, headache, blood, or pus in the faeces, loss of appetite, bloating, lethargy, and body aches (CDC, 2014). Infectious gastroenteritis is caused by a variety of viral, bacterial, parasitic pathogens. Intestinal parasites have a worldwide distribution and have been associated with gastroenteritis in children.

Among the intestinal flagellates, *Giardia lamblia* and *Dientamoeba fragilis* are pathogenic to man (Monali *et al.*, 2012). The burden of Giardial disease is highest in developing countries (10-30% in young children), while in developed countries, infections occur mostly in persons living in closed communities, homosexual men, immigrants and of increasing importance, travelers returning from highly endemic countries (Carmena *et al.*, 2012; Jelinek and Neifer, 2013). The infections often result in iron deficiency, anemia, growth retardation, and other physical and mental problems in children (Nyamngee *et al.*, 2006). The combination of these factors results in malabsorptive diarrhoea and lower weight gain. Transmission is either direct, through the faecal-oral route, or indirect, through the ingestion of contaminated water or food (Carmena *et al.*, 2012). Giardiasis is associated with poor sanitary conditions, insufficient water treatment, daycare centers, and institutional facilities such as nursing homes (Pereira *et al.*, 2007). Evidence of zoonotic transmission to humans from cats living in the same community had been reported by Pereira *et al.* (2007).

Giardiasis infections can often be distinguished from viral or bacterial gastrointestinal (GI) infections by the longer

duration of illness (often 7-10 days) and weight loss (Jason, 2003). Age and body mass index were reported as risk factors associated with Giardial infection among street children orphanage in Peru by Bailey *et al.* (2013). Body mass index (BMI) is a factor calculated from a child's weight and height. It is a reliable indicator of body fitness for most children and teens and is also used as a screening method for weight categories that may lead to health problems (CDC, 2014). Growth failure indicated by stunting, wasting, and underweight conditions is associated with increased morbidity and mortality in children and it is estimated that as many as 182 million children in developing countries are affected. Although the etiology of growth failure is multifactorial, malnutrition and repeated infections in children have been documented as indices as well as *G. lamblia* infection. Growth failure can be assessed by anthropometric indices of Height-for-Age (HAZ), Weight-for-Age (WAZ), and weight-for-height (WHZ) (Inabo *et al.*, 2011). Thus the work is aimed at determining the prevalence of *Giardia lamblia* in association with body mass index in children within the age of 0 to five years suffering from gastroenteritis in Kaduna metropolis, Nigeria.

## MATERIALS AND METHODS

### Study Area/Location:

The study was conducted in Kaduna Metropolis location between latitude 90°N and 140°N of the equator and longitude 70°E and 100°E (NPC, 2006). The State has 23 local government areas and it is divided into three senatorial zones; Kaduna North, Kaduna Central, and Kaduna South local government area. It is bounded by Katstina state to the North, Kano state to the Northeast, Bauchi state to the East, Plateau state to the Southeast, FCT to the South, Niger state to the Southwest and West, and Sokoto state to the Northwest.

### Study Design:

This study was a descriptive cross-sectional hospital-based study and non-

probability sampling by conveniences in the choice of hospital selection. Samples were collected from fifty paediatric in-patients and one hundred and fifty out-patients presenting with gastroenteritis in each hospital within a period of seven weeks (September- October 2015) in Kaduna Metropolis, Nigeria. Questionnaires were administered to the mothers/caregivers of children who are literate and there were interactive sections for the mothers/caregivers of children who are illiterate to generate information on risk factors. The information is child's source of drinking water, mode of breastfeeding for children less than seven months, daycare attendance, hands wash after defecating and mothers/caregivers level of education. Nutritional status was accessed by anthropometric indices of weight-for-age (WHO, 2007). Ethical approval was obtained from the Ministry of Health Kaduna State and was taken to the various hospitals where samples were collected. Informed consent forms were given to mothers/caregivers of each child before samples were collected.

#### **Study Population:**

The study populations were paediatric in-patients and outpatients both male and female between 0-5 years that were presented with gastroenteritis or diagnosed with the symptoms of gastroenteritis by the clinicians. Symptoms include diarrhoea, vomiting, and abdominal pain whose parents/caregivers gave their consent.

#### **Sample Size Determination:**

The sample size for this study was determined using the formula by Kuta *et al.* (2014) at 95% confidence level and a reported 14.3% prevalence obtained for *Giardia lamblia* in children hospitalized in Maiduguri Teaching Hospital by Muhammed *et al.* (2014) in Borno State.

$$S = t^2 P(1-P) / m^2$$

$$S = 1.96^2 * 0.143 * 0.857 / 0.05^2 =$$

$$0.47059584 / 0.0025 = 188.31677 \sim 188 \text{ samples}$$

The sample size calculated was 188.3 and it was increased by 25% in order to minimize sampling error to make a total of 200.

#### **Sample Collection and Processing:**

Stool samples were collected from

eligible 0-5 years old children hospitalized for gastroenteritis during the course of the study research into clean labeled screw-capped tubes (universal bottles). Samples were collected and transported immediately on ice packs to the Parasitology Laboratory of Microbiology Department, Ahmadu Bello University Zaria. Stool samples were stored at 2°C – 8°C immediately after collection and processed within 72 hours. Longer storage was done at -20°C.

#### **Detection of *Giardia lamblia* ova in Stool**

##### **Samples of The Study Population:**

The three-light microscopy techniques used to examine *Giardia* and other intestinal parasites were:

##### **Unstained Smear:**

For the unstained smear, 0.1g or 0.1ml of the faeces was emulsified in one drop of normal saline on a grease-free slide. The slide was covered with a glass coverslip and examined for the presence of parasite or cysts.

##### **Iodine Stained Faecal Smear:**

The smear was prepared by emulsifying 0.1g or 0.1ml of the faecal material in a drop of Lugol's iodine on a microscope slide.

##### **Zinc Sulphate (33% ZnSO<sub>4</sub>) Flotation Method:**

Faecal suspension of 0.1ml of each sample was added in a tube containing 9 ml of 33% ZnSO<sub>4</sub> and centrifuged at 2000 rpm for 3 minutes using MSE centrifuge (made in England). A loopful of faecal suspension was removed from the surface of the liquid using a wire loop and placed on a glass slide.

All slides were examined for the presence of parasite at 10x and 40x magnification using Olympus research microscope (optical co-ltd, Japan) (Cheesbrough, 2010).

#### **Detection of *Giardia lamblia* ova in Stool**

##### **Samples of The Study Population:**

*Giardia* ELISA, an in vitro immunoassay for the qualitative determination of *Giardia* antigen in faecal specimens (Diagnostic automation) was used in accordance with the manufacturers. The stool samples were diluted to the ratio 1:7 in the dilution buffer provided in the kit. All

other reagents were allowed to come to a room temperature 25°C. A blank with the dilution buffer was placed in well 1, 100 µl of negative control was placed in well 2, 100 µl of positive control was placed in well 3 and 100 µl of diluted samples were added to each of the remaining wells. The plates were incubated for 60 minutes at room temperature 25°C and then vigorously washed five (5) separate times without the formation of bubbles using the washing buffer provided by the manufacturer. After the last wash, the well was slapped out on a clean absorbent towel to remove excess wash buffer. Two drops (100µl) of enzyme conjugate was added to each well and incubated for 30 minutes at room temperature 25°C. It was then vigorously washed again five (5) separate times without the formation of bubbles with the washing buffer. After the last wash, the well was slapped out on a clean absorbent towel to remove excess wash buffer. Two drops 100µl of Chromogen were added to each well and incubated for 10 minutes at room temperature 25°C. Two drops 100µl of stop solution was added to each well and the wells mixed gently by tapping the side of the strip holder with the index finger for 15 seconds. The reaction was read within 5 minutes after the stop solution was added in the Molecular Laboratory of the Department of Microbiology Ahmadu Bello University, Zaria. The results were read visually and also with ELISA plate reader (GF-M300 Microplate reader, B BRAN Scientific, and Instrument Company England) at 450nm wavelength.

#### **Standardized Measurement Procedure for Body Mass Index:**

The standard measurement procedure for body mass index (BMI) can be calculated using the formula below (CDC, 2014).

$$BMI = \frac{\text{(Weight in kilograms)}}{\text{(Height in meters} \times \text{Height in meters)}}$$

For the measurement of weight and height with the help of trained anthropometrists. Weight was recorded to the nearest 0.1 kilogram using the scale with a digital readout (CDC, 2014). The measurement of height was performed by simply

asking each child to stand with his/her back against the board. The measurement was recorded to the nearest 0.1cm and converted to meters using the Holtan stadiometer. Infant and children younger than 24 months of age have the height measured using the recumbent length board while those aged 24 months of age and older who could stand unassisted had their standing height measured using a stadiometer (CDC, 2014).

#### **Data Analysis:**

The results obtained were analyzed using the Open Epi info package developed by WHO for epidemiological studies. Pearson's chi-square statistical distribution test and odds ratio were calculated to obtain p-values. The body mass indices of all the children were calculated using the formula above as recommended by CDC and interpreted using the BMI chart CDC standard 2014.

#### **RESULTS**

The result showed that 12 (6%) and 28 (14%) of the children were positive for *Giardia lamblia* by Microscopy and ELISA respectively (Fig. 1). The prevalence of *Giardia lamblia* was higher in males (15.89%) as compared with the females (11.83%) by the ELISA result (Table 1). The difference was however not statistically significant ( $p, \chi^2 = 0.6811$ ). The children aged 49-60 months had the highest prevalence of 16.39% while those 0-12 months had the least prevalence of 9.68%. The difference was statistically not significant [ $(p = 0.9319)$ ] (Table 2).

The general body mass indices (BMI) of the children showed that 66 (33%) children underweight out of which 18 (27.27%) harbor the parasite with a mean weight of 13.15kg/m<sup>2</sup> (Table 3). On the other hand, 132 (66%) had normal weight with 10 (7.57%) infected with *Giardia* and a mean weight of 18.22Kg/m<sup>2</sup>. None of the overweighted children harbored the parasite (Table 4). There was no statistically significant difference between *Giardia* infected children and BMI of the children ( $p= 0.316172$ ). The high-risk factors identified as associated with the acquisition of *Giardia* in the children include use of well

water (OR= 4.222), use of open running / river water (OR = 3.109), severe malnutrition (OR= 4.187) and those whose mothers/caregivers had no formal education (OR= 7.261) (Table 5). Other factors with a relative risk of acquiring the parasite include the use of tap water, failure to exclusively breastfed, none breastfeeding, use of daycare, none school attendance, moderately malnourished, and those children that did not

have hands washed after the toilet or defecating. The study also discovered other parasites in varying prevalence such as *Entamoeba* (18%), *Entamoeba histolytica* (3%), *Ancylostoma duodenale* (8.5%), *Hymenolepis nana* (3.5%), *Enterobius vermicularis* (1%), *Ascaris lumbricoides* (1%) and *Strongyloides stercoralis* (0.5%) as presented in Table 6.

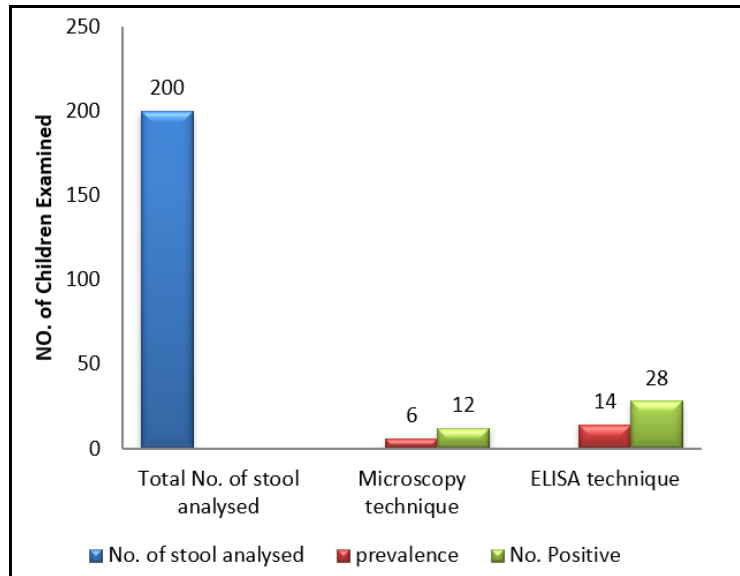


Fig. 1: Prevalence of *Giardia lamblia* by Microscopy and Elisa techniques

Table 1: Prevalence of *Giardia lamblia* by Gender among Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria using ELISA

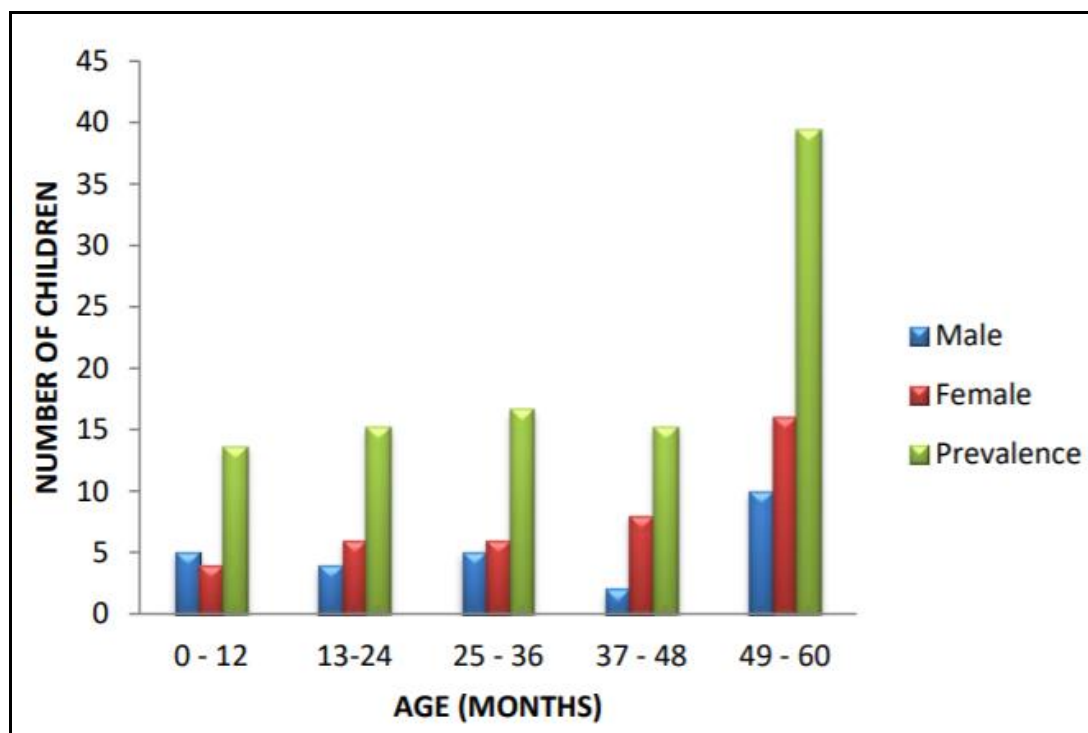
Gender	No. Examined	No. Positive	Prevalence (%)
Male	107	17	15.89
Female	93	11	11.83
<b>Total</b>	<b>200</b>	<b>28</b>	<b>14</b>

Significant at  $p \leq 0.05$  ( $\chi^2 = 0.6811$ ,  $p = 0.4092$ ;  $df = 1$ )

Table 2: Prevalence of *Giardia lamblia* by Age Group in Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

Age group (Months)	No. Examined	No. Positive	Prevalence (%)
0 – 12	31	3	9.68
13 – 24	34	5	14.7
25 – 36	32	4	12.5
37 – 48	42	6	14.29
49 – 60	61	10	16.39
<b>Total</b>	<b>200</b>	<b>28</b>	<b>67.58</b>

Significant at  $p \leq 0.05$  ( $\chi^2 = 0.848$ ;  $p = 0.9319$ ;  $df = 4$ )



**Fig. 2:** Body Mass Indices of Underweight Children According to Age and Sex in Children Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

**Table 3:** Body Mass Indices Obtained in Relation to Infected Children with Giardiasis in Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

BMI (Kg/m <sup>2</sup> )	Total number for BMI	Positive male (%)	Positive female (%)	Total (%)
Underweight	66	10(15.15)	8(5.3)	18(27.27)
Healthy weight	132	7(5.3)	3(2.27)	10(7.57)
Overweight	2	0(0)	0(0)	0(0)
<b>Total</b>	200	17(8.5)	11(5.5)	28(14)

**Table 4:** Mean Weight of Underweight and Normal Weight of Infected Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria.

BMI Classification	No Infected	Mean Weight (Kg/m <sup>2</sup> )	Percentage Mean Weight	Normal BMI Range (Kg/m <sup>2</sup> )
Under weight	18	13.15	41.92	0-13.9 (<5 <sup>th</sup> percentile)
Normal weight	10	18.22	58.08	14-18.5(5 <sup>th</sup> -85 <sup>th</sup> percentile)

Significant at p.value  $\leq 0.05$

(p.value =0.316172)



**Table 5:** Risk Factors Associated with Giardiasis in Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

Risk factors	No uninfected	No infected	Total examined	Odds ratio	Confidence interval at 95%	
					Lower Limit	Upper limit
<b>Source of water</b>						
Tap	37	9	46	1.728	0.7223	4.136
Well	20	10	30	4.222	1.712	10.41
Bore hole	40	5	45	0.7174	0.2562	2.009
Package water	66	4	70	0.2677	0.08891	0.8059
Table water	4	0	8	0.3905	0.02169	7.029
River	1	0	1	3.109	0.1019	94.89
<b>Feeding</b>						
Exclusive breast feeding	11	0	11	0.2738	0.0156	4.806
Non exclusive breast	17	3	20	1.094	0.2988	4.006
None breast feeding	144	25	169	1.62	0.4578	5.736
<b>School attendance</b>						
Day care	8	2	10	1.577	0.3172	7.839
Nursery	87	13	100	0.8467	0.3803	1.885
Primary	18	2	20	0.6581	0.1441	3.005
Non attendance	59	11	70	1.239	0.5452	2.817
<b>Nutrition (weight for age)</b>						
Normal nutrition	126	13	139	0.3164	0.1399	0.7154
Moderate malnutrition	31	7	38	1.516	0.5925	3.88
Severe malnutrition	15	8	23	4.187	1.577	11.11
<b>Use of Toilets</b>						
NO	111	19	130	1.16	0.4947	2.721
YES	61	9	70	0.8619	0.3675	2.021
<b>Mother's highest education</b>						
Quranic education only	12	8	20	5.333	1.946	14.62
Primary education	54	6	60	0.596	0.2286	1.554
Secondary education	51	4	55	0.3954	0.1306	1.197
Tertiary education	24	1	25	0.2284	0.02964	1.76
Quranic/ formal education	26	4	30	0.9359	0.3	2.92
None of the above	5	5	10	7.261	1.951	27.02

\*Significant at OR > 1 and \*\* p value ≤ 0.05

**Table 6:** Prevalence of Other Intestinal Parasites in Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

Intestinal parasites	No. Positive	Prevalence (%)
<b>Protozoa</b>		
<i>Giardia</i>	28	14
<i>Entamoeba coli</i>	36	18
<i>Entamoeba histolytica</i>	6	3
<b>Helminthes</b>		
<i>Ancylostoma duodenale</i>	17	8.5
<i>Hymenolepis nana</i>	7	3.5
<i>Enterobius vermicularis</i>	2	1
<i>Ascaris lumbricoides</i>	2	1
<i>Strongyloides stercoralis</i>	1	0.5
<b>Total</b>	<b>99</b>	

## DISCUSSION

In this current study, the prevalence of giardiasis was found to be 6% by microscopy and 14% by ELISA kit. The low prevalence in microscopy could probably be due to the single stool sample that was used in this study. And it has been established that diagnosis via microscopic examination of a single stool specimen has low sensitivity, therefore miss up to 50% of *Giardia* infections because of the intermittent shedding of the parasites which led to a false-negative result. The microscopic examination of three consecutive stool specimens is required to reach a sensitivity of over 90%. Enzyme immune-assay (EIA) for detection of the specific antigens in stools has developed into an efficient diagnostic technique in the detection of *Giardia lamblia* (Thiongo *et al.*, 2012; Jelinek and Neifer, 2013). Thus the ELISA result was used for determining the infection with the sociodemography of the patients.

The prevalence of giardiasis was higher in male children than in the female. This is in agreement with other researchers in Nigeria (Nyagnmee *et al.*, 2006; Inabo *et al.*, 2011). The higher prevalence in a male could be probably due to the higher activities in male children. Male children can easily come in contact with contaminants in the environment during outdoor activities like playing football and racing in a field infested with *Giardia* cysts. The result in this study is in accordance with the work of Thiongo *et al.* (2012) in Kenya, who reported 12.8% of Giardiasis in children below 5 years. A similar result was reported by Hamza and Biu (2012) and Muhammed *et al.* (2014) with a prevalence of 10.5% and 14.3% respectively. In contrast, with Nyagnmee *et al.* (2006) in Nigeria reported a higher prevalence of giardiasis as 40.4% in children living in the refugee camp of Guma, Guma LGA, Benue State and Inabo *et al.* (2011) reported 41.4% in asymptomatic children in two Local Government Areas of Zaria, Kaduna State respectively.

The results of body mass indices in this study show a large number of children had normal weight for their height which is above the 5th percentile. Infection/malnutrition could have attributed to underweight or low body mass indices recorded in other children in this study. The number of female children with low body mass indices tends to be higher than that of the male children in this study. This could be a result of differences in growth and development between a male child and a female child.

However, the mean weight of infected children relative to their body mass indices statistically shows no significant differences. This finding is in agreement with Fraser *et al.* (2000). In contrast to the report of Bailey *et al.* (2013) in Peru and Duran *et al.* (2010) in Venezuela who reported low body mass index is associated with *Giardia lamblia* infection. The low body mass indices in those infected with giardiasis could be attributed to malabsorption syndrome associated to chronic *Giardia* infected patients which reduces the digestion of food. This could lead to weight loss in children. A similar report was recorded by Inabo *et al.* (2011) in Zaria, Nigeria. However, other intestinal parasites could have accounted for the low body mass index in children as well in this study.

The risk factors associated with the cause of infection of children to *Giardia lamblia* in this study were the source of drinking water, nutritional status, and educational status of mothers which was also reported by Fraser *et al.* (2000). These were all statistically associated with the cause of the infection/disease. Infected children that drank well water were found to have greater odd of acquiring giardiasis than others. This could be explained because most well waters are easily contaminated and not treated before drinking by many people in this part of the country. And it has been established that contaminated water can be served as a vehicle for the transmission of giardiasis. Also, children that are not properly fed can



easily be susceptible to all sort of infection as they lack the ability to resist infection. This explained why those that had severe malnutrition had greater odd of acquiring the infection/disease than those that had moderate malnutrition.

Children that had no exclusive breastfeeding and none breastfeeding also had the odd of being infected but shows no statistical association in this study. But children that were exclusively breastfed show no odd of being infected by the disease. This result agreed with the findings of Mahmud *et al.* (2001) in Egypt which said breastfeeding can serve as a protection against Giardial infection.

Morbidity due to intestinal parasites has always been an important public health problem in the Tropics, but the incidence and severity may vary depending on the location and period of time (Akingbade *et al.*, 2013).

The prevalence of intestinal parasites in children in this study revealed other parasites such as *Entamoeba coli* with the highest prevalence followed by *Giardia lamblia* and the least was *Strongyloides stercoralis*. This finding is in agreement with reports from previous researchers, Awolaju and Morenikeji (2009) in Osun State, Chukwuma *et al.* (2009) in Rivers State, Alli *et al.* (2011) in Oyo State and Hamza and Biu (2012) in Borno State of Nigeria. This could be due to contaminants from eating food/drinking water, malnutrition, lack of personal hygiene, and lack of maternal education which predisposed most children to infection/disease in this area of study.

## CONCLUSIONS

*Giardia lamblia* had a prevalence of 6% and 14% by microscopy and ELISA respectively. The use of ELISA as a means for diagnosis seems to have more advantages and sensitivity than microscopy. Male children had a higher prevalence than females. However, the difference was statistically not significant. Age-wise distribution of Giardial infection showed the highest and lowest prevalence to be 16.39% and 9.68% among the 49 – 60 months and 0-12 months respectively.

The prevalence of *Giardia lamblia* was found not associated with low body mass indices in children 0-5 years presenting with gastroenteritis in Kaduna metropolis, Kaduna State, Nigeria. The Body Mass Indices obtained with relative to infection shows no significant difference  $p= 0.316172$ . However, infected children with giardiasis had a higher prevalence of underweight to healthy weight body mass indices.

The risk factors that were statistically associated with the cause of disease/infection of *Giardia lamblia* in this study were the source of water, malnutrition, and maternal/caregivers educational levels. Which had an odd ratio greater than 1.

Other intestinal parasites found in children in this study were *Entamoeba coli*, *Ancylostomaduodenale*, *Entamoeba histolytica*, *Hymenolepis nana*, *Enterobius vermicularis*, *Ascaris lumbricoides*, and *Strongyloides stercoralis*. *Entamoebacoli* were found to be the most common infection among the children followed by *Giardia lamblia*. The prevalence of infected children with intestinal parasites was 44.5% and infection progresses as age increases due to increased behavioural risks as children grow.

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