PREVALENCE OF GIARDIA LAMBLIA IN ASSOCIATION WITH BODY MASS INDICES OF CHILDREN 0-5 YEARS PRESENTING WITH GASTROENTERITIS IN KADUNA METROPOLIS, NIGERIA

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OCTOBER, 2016

DECLARATION

I declare that the work in this project dissertation entitled "Prevalence of *Giardia lamblia* In Association with Body Mass Indices of Children 0-5years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria" was carried out by me. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project dissertation was previously presented for another degree or diploma at this or any other Institution.

Sikiru, Monsurat Titilayo _____ _____

Signature Date

CERTIFICATION

This project dissertation entitled "Prevalence of *Giardia lamblia* In Association with Body Mass Indices of Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria" by SIKIRU, MONSURAT TITILAYO meets the regulations governing the award of the degree of Master of Science in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my beloved elder brother Dr.T.H.Sikiru and all my loved ones. Your love gave me the strength to carry on.

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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 0-5years presenting with gastroenteritis in association with body mass indices (BMI) in Kaduna Metropolis, Nigeria. Of 200 samples examined, 12 (6%) were positive for *Giardialamblia* 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 (χ^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 (χ^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 with p-value as follows [(p=0.005: p=0.012 and p=0.0007)].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%). There was a significant increase of infection as age increases (p=0.02797). 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.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Acute gastroenteritis presenting as infant diarrhoea remains a common illness among infants and children throughout the world. Among children in the United States, acute diarrhoea accounts for more than 1.5 million outpatients, 200,000 hospitalized inpatients and about 300 deaths per year (Zimmerman *et al*., 2001; CDC, 2014). 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, available report indicates that more than 315,000 deaths of preschool age children occur annually as a result of infantile diarrhoea disease with 80% of the population affected (Aminu *et al.*, 2008; Ayolabi *et al*., 2012).

Gastroenteritis or gastro 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 and parasitic pathogens. Intestinal parasites have a worldwide distribution and have been associated with gastroenteritis in children. One of the most common intestinal protozoa parasite is *Giardia lamblia* (Jelinek and Neifer, 2013).

Among the intestinal flagellates, *Giardia lamblia* and *Dientamoeba fragilis* are pathogenic to man (Monali *et al*., 2012). The highest rates of infection are therefore encountered 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).

About 3.5 billion people are infected worldwide and about 450 million people are ill due to these infections which are mainly in children of about 2-14 years. The infections cause iron deficiency anemia, growth retardation and other physical and mental problems in children (Nyamngee *et al*., 2006).

Giardia is an enteric protozoan that infects a wide range of vertebrate hosts, being the causative agent of gastrointestinal disease of humans in both developing and developed countries (Jombo *et al*., 2011). Giardial infection also has significant impact on livestock health, causing diarrhoea and loss of weight (Carmena *et al.*, 2012). Global *Giardia* infection rates have been reported by Carmena *et al*. (2012) in domestic animals in Spain in the range of 3%-58% for cattle, 25% for water buffaloes, 1%-56% for sheep, 12%-36% for goats, 17%-31% for pigs and 5% for alpacas. Similarly to human giardiasis, they reported that pathogenesis of *Giardia* in animals is also a multifactorial process involving both parasite and host factors. The combination of these factors results in malabsorptive diarrhoea and lower weight gain (Carmena *et al.*, 2012).

Evidence of zoonotic transmission among humans and cats living in the same community had been reported by Pereira *et al*. (2007). A prevalence as high as 67.5% of people under the age of 15 years infected by cross-species transmission including zoonotic transmission of *G. lamblia* near Kibale National Park in Western Uganda was reported by Jonhston *et al*. (2010). *G. lamblia* is not a life-threatening parasite, nevertheless, it is still considered as the most common water-borne diarrhoea-causing disease. It is important to understand the aetiology, frequency and consequences of acute diarrhoea in children (Dib *et al*., 2008).

Transmission is either direct, through the faecal-oral route or indirect through ingestion of contaminated water or food (Carmena *et al*., 2012). Giardiasis is associated with poor sanitary conditions, insufficient water treatment, daycare centers and with institutional facilities such as nursing homes (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 by the time of first presentation) and weight loss (Jason, 2003). Clinical manifestations are usually diarrhoea, abdominal cramps, nausea, bloating and loss of appetite. In chronic and complicated cases, cholecystitis and malabsorption may be observed (Naglaa *et al*., 2011). Age and body mass index were reported as risk factors associated to Giardial infection among street orphanage children 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 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 aetiology of growth failure is multifactorial, malnutrition and repeated infections in children have been documented as causative agents, among which *G. lamblia* is a hallmark cause (Amuta *et al*., 2009). Growth failure indicated by stunting, wasting and underweight conditions can be assessed by anthropometric indices of Height-for-Age (HAZ), Weight-for-Age (WAZ), and weight-for-height (WHZ) (Inabo *et al*., 2011).

1.2 Statement of Research Problem

Paediatric diarrhoea remains one of the major causes of death among infants. This is especially true in the third world countries such as Asia, Africa and Latin America, where it causes millions of death within the age group of 0 to 4 years. The main factors for high

occurrence of diarrhoea in children and mortality rate are unsafe water, inadequate sanitation and/or physiological conditions such as malnutrition (Giordano *et al.*, 2001).*G. lamblia* causes a worldwide health problem that requires better intervention; it was the causative agent of giardiasis, a disease which affected over 200 million people in 2002 (Lane and Lloyd, 2002). Giardiasis is the most frequently diagnosed water borne disease and the major public health concern of water utilities in the developed and developing nations (Al-Emarah and Al-Saad, 2014).

Several studies have revealed that a chronic infection of *Giardia* during childhood contributes to protein-energy malnutrition, vitamin A deficiency, iron deficiency anaemia, zinc deficiency and poor cognitive and educational performance in children (Seow *et al*., 2014). It has also been shown to have negative impact on growth and development in children and most often it occurs asymptomatic (Duran *et al*., 2010; Inabo *et al.*, 2011). Ample evidence suggests that *Giardia,* which has been found in all classes of vertebrates also, has great potential for zoonotic transmission (Caccio *et al*., 2005; Carmena *et al*., 2012).

1.3 Justification of the Study

The World Health Organization reported that 200 million people in Asia, Africa and Latin America have symptoms of giardiasis with some 500,000 new cases a year, especially among children. The infection may produce severe acute diarrhoea in children less than five years of age with chronic infections resulting in weight loss and growth retardation (WHO, 1996). Giardiasis has been recognized in 2004 as a neglected disease by the World Health Organization (Chakaroka, 2010).Giardiasis is rarely fatal; however, deaths can be caused by extreme dehydration, mainly in infants or malnourished children (Dib *et al.*, 2008).

Therefore, there is a need for more research on this protozoa parasite. Little is known on this parasite in children. More emphasizes are laid on bacterial and viral aetiology of diarrhoea than parasitic agents of disease in children under the age of five years old. Children under the age of five are given inadequate or no attention by most parents. Due to this reason most children tend to eat food/ drink water or have contact with companion animals like dogs, cats, pigs that are either infected or infested with parasites which has led to high prevalence of intestinal parasitosis of children in Northern Nigeria (Hamza and Biu, 2012; Muhammed *et al*., 2014). This research provides the current prevalence of *G. lamblia* and other intestinal parasites in children within the range of five years as well as the associated risk factors in Kaduna Metropolis.

1.4 Aim of the Study

The study aimed to determine the prevalence of *Giardia lamblia* in association with body mass indices in children under 0-5 years presenting with gastroenteritis in Kaduna Metropolis, Nigeria.

1.5 Objectives of the Study;

The objectives of the study were to:

1. Detect *Giardia lamblia* in stool specimen of the study population using microscopy and *Giardia* ELISA kit.
2. Determine the Body Mass Indices of children 0-5 years presenting with gastroenteritis in relation to giardiasis.
3. Determine the risk factors associated with the cause of giardiasis.

4. Identify other possible intestinal parasites in stool samples of studied population using microscopic examination.

CHAPTER TWO

2.0 LITRATURE REVIEW

2.1 Brief History of *Giardia Lamblia*

Giardia lamblia has been considered one of the most ancient and primitive eukaryotic organisms on the planet (Wensaas, 2011). This view has been challenged by resent research; still this parasite has been around for a long time unnoticed. Our knowledge about microorganisms was very limited up to the second half of the 19th century when a range of bacteria and other microbiological pathogens were described and linked to specific infections (Adam, 2001; Wensaas, 2011).

Giardia lamblia was first discovered by a Dutch microscopist Antonie Van Leeuwenhoek in 1681 (Jason, 2003; Wensaas, 2011). He observed the protozoan in one of his own diarrhoeic stools and in his own words, "wherein I have sometimes also seen animalcules a moving very pettily…….albeit they made a quick motion with their paws, yet for all that they made but slow progress". Van Leeuwenhoek's description is of the *Giardia* trophozoite (Jason, 2003). In 1883, the genus *Giardia* was named after Prof. A.M Giard of Paris (Monali *et al*., 2012).

In 1888, Blanchard suggested the name *Lamblia intestinalis*, which then changed to *G. duodenalis* in 1902 (Adam, 2001; Caccio *et al*., 2009). There continued to be controversy about the number of *Giardia* species for many years, with some investigators suggesting species names on the basis of host of origin and others focusing on morphology (Caccio *et al*., 2009). For example, over 40 species names had been proposed on the basis of host of origin. Filice (1952) published a detailed morphologic description of *Giardia* and proposed that three species names should be used on the basis of the morphology of the median body: *G. duodenalis*, *G. muris*, and *G. agilis* (Caccio *et al*., 2009). The species name *G. lamblia* became widely accepted through the 1970s. Since the 1980s, some have purposed the use of

the name *G. duodenalis*, and in the 1990s, the name *G. intestinalis* was purposed by other investigators. At this time there does not appear to be adequate reason to abandon the term *G. lamblia*, which has been widely accepted as still the official name according to the Integrated Taxonomic Information System (Wensaas, 2011).

G. lamblia is pear shaped and has one or two transverse, claw-shaped median bodies; *G. agilis* is long and slender and has a teardrop-shaped median body; and the *G. muris* trophozoite is shorter and rounder and has a small, rounded median body. *G. lamblia* is found in humans and a variety of other mammals, *G. muris* is found in rodents, and *G. agilis* is found in amphibians (Adam, 2001; Caccio *et al*., 2009).

2.2 *Giardia*

Giardia as a genus belongs to a member of the subphylum Sarcomastigophora, class Zoomastigophora. They are flagellated bianucleated protozoa that affect the intestinal tract of a wide range of vertebrate hosts, including mammals, birds, reptiles and amphibians (Carmena *et al*., 2012). It has a pear shaped which exists in two stages: an active trophozoite stage and the dormant cyst stage, which is the infective stage (Adam, 2001; Caccio *et al*., 2009). There are three identified species of *Giardia*; *Giardia lamblia, Giardia muris* and *Giardia agilis*. *Giardia lamblia* is the only species of them known to infect humans (Naglaa *et al.,* 2011).*Giardia* differs from other eukaryotes by the absence of peroxisomes and proper mitochondria, but contains mitochondria-like organelles called mitosomes (Cheesbrough, 2010; Wensaas, 2011).It is considered as the most common human intestinal protozoa, especially in the tropic (Jason, 2003). It ranges in clinical severity from asymptomatic to highly pathogenic. Both host factors (e.g. nutrition, immunity, co-infection with other agents) and pathogen factors (e.g. strain, infectious dose) are thought to contribute to the clinical

severity of giardiasis (Caccio *et al*., 2009). *G. lamblia* is also notable for cross-species transmission, including zoonotic transmission (Caccio *et al*., 2005).

It is also known that *G.lamblia (G. duodenalis, G. intestinalis)* consist of eight morphologically identical but genetically distinct genotypes or assemblages, designated A-H (Caccio *et al*., 2009; Wensaas, 2011). Assemblage A and B have been identified to infect humans and many other mammalian hosts, including domestic animals and wildlife (Espelage *et al.,* 2010). While assemblages C and D infect dogs, assemblage F infect cats, assemblage E infects hoofed livestock, assemblage G infects rats and assemblages H infects marine animals (Caccio *et al.,* 2005). With regards to clinical manifestation, studies on the correlation between the assemblages and clinical symptoms have reported controversial results (Seow *et al.,* 2014).

Sub-assemblages (also called subgroups) have been recognized within some assemblages (Adam, 2001). Three subassemblages - AI, AII and AIII – have been defined, to date, in assemblage A. Sub-assemblage AII is usually found in people, while sub-assemblage AI mainly occurs in livestock and pets (Caccio *et al*., 2009). However, this division is not absolute; sub-assemblage AI has been isolated occasionally from people and AII from animals (CDC, 2010; Carmena *et al.,* 2012). Sub-assemblage AIII has been detected in hoofed wild animals. As of 2012, it has not been found in humans (Carmena *et al.,* 2012). It is more difficult to define sub-assemblages in assemblage B, which is genetically diverse. Two subassemblages, BIII and BIV, were described by allozyme electrophoretic studies, but DNA sequence analyses do not support these 2 groups. Host-specific sub-assemblages have not yet been identified in assemblages C through G (Caccio *et al.,* 2005).

2.3 Morphology of *Giardia lamblia*

Giardia lamblia exist in two forms; the infective cyst and the *Giardia* trophozoite (CDC, 2014).

2.3.1 Morphology of *Giardia* cysts

The cysts of *G. lamblia* are 8-12µm in length and are ellipsoid in shape (Figure 2.1). They contain 4 nuclei which tend not to be obvious, longitudinal fibrils consisting of the remains of axonemes and parabasal bodies may also be seen (Cheesbrough, 2010). Cysts may appear to shrink from the cell wall. The cysts are infective as soon as they are passed. *Giardia* cysts can survive for long periods in the environment under cool moist conditions. They are susceptible to desiccation and direct sunlight and are destroyed more quickly under hot and dry conditions (Jason, 2003). Cysts remained viable in river water for nearly 3 months at 0-4°C and 1 month at 20-28°C (Adam, 2001). In soil, cysts are held at 4°C and almost 90% of cysts are still viable after 49 days. However, infectivity is lost within 7 days at 25°C. Cysts also survived for one week in solid cattle manure at 4ºC, but as long as 18 days in human faeces (Adam, 2001; CDC, 2010).

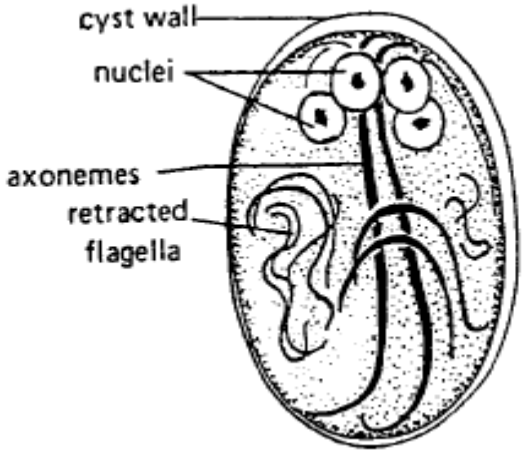

Figure 2.1: Cyst of *Giardia lamblia*

(Source: CDC, 2014).

2.3.2 Morphology of *Giardia* trophozoite;

The trophozoites of *G.lamblia* are flattened pear shape and are an average size of 15µm long, 9µm wide and 3µm thick (Figure 2.2). When stained, the trophozoite is seen to have 2-4 nuclei, 2 slender median rods (axostyles) and 8 flagella arising from the anterior end (Chiodini *et al.*, 2005). They have been described as looking like tennis rackets without the handle (they are often seen as having a comical face-like appearance when looking at the front view) (CDC, 2010).

The movements of *Giardia* trophozoites are described as tumbling leaf motility, using their 4 pairs of flagella for locomotion. They attach themselves to the surface of the jejuna or duodenal mucosa by their disc-like suckers which are found on their ventral surface, they multiply in the gut by binary fission (Cheesbrough, 2010).

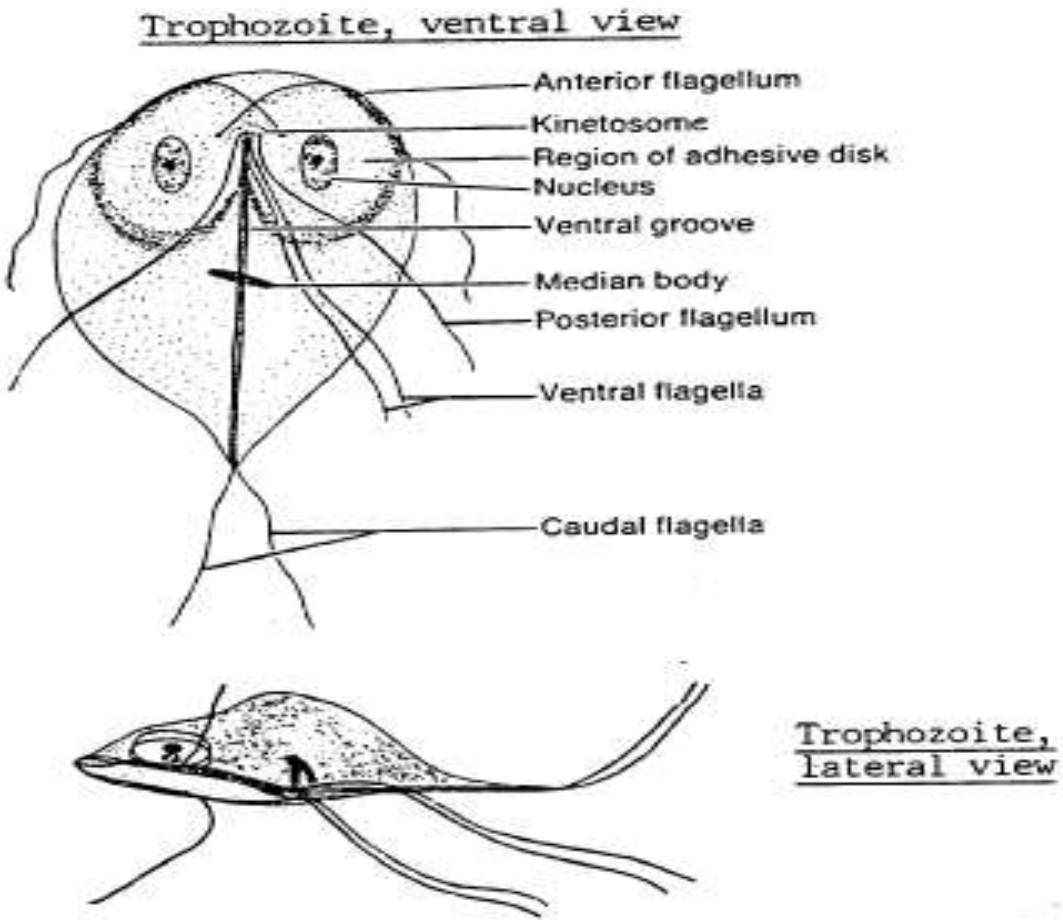

Figure 2.2: Diagram of *Giardia lamblia* trophozoite

(Source: CDC, 2014).

2.4 Life cycle of *Giardia*

The life cycle of *G. lamblia* comprises two main stages: a trophozoite stage which colonizes the intestinal epithelium of the host and causes disease and an infectious cyst stage which is resistant in the environment (Figure 2.3). *G. lamblia* trophozoites have been reported to rarely invade the mucosa of the duodenum and jejunum, but normally they are considered non-invasive and attach to the microvilli and baso-lateral membranes of the enterocyte (Adam, 2001). In addition, the normal villus structure is affected in some patients like in villus blunting (atrophy) and crypt cell hypertrophy and increases of crypt depth. The colonization of trophozoites in the small intestine results in a reduction in the height of the microvilli and therefore a loss of absorptive surface area (Jason, 2003). This loss of absorptive surface leads to mal-absorption of glucose, electrolytes and water, with reduced disaccharidase activity. This results in the small intestine filling with mucous and fluid, and ultimately mal-digestion and hypermotility, all responsible for the clinical manifestation of diarrhoea (Al-Emarah and Al-Saad, 2014).

For infection to take place, the host must ingest the cysts. Excystation is initiated when environmental stimuli like gastric acid and pancreatic enzymes are detected across the cyst wall. A single trophozoite containing four nuclei (4N each) is released; then divides into two without further DNA replication resulting in four daughter trophozoites under acidic condition in the jejunum. The trophozoites then encyst and are passed in the human stools (Miguel and Cabada, 2007).

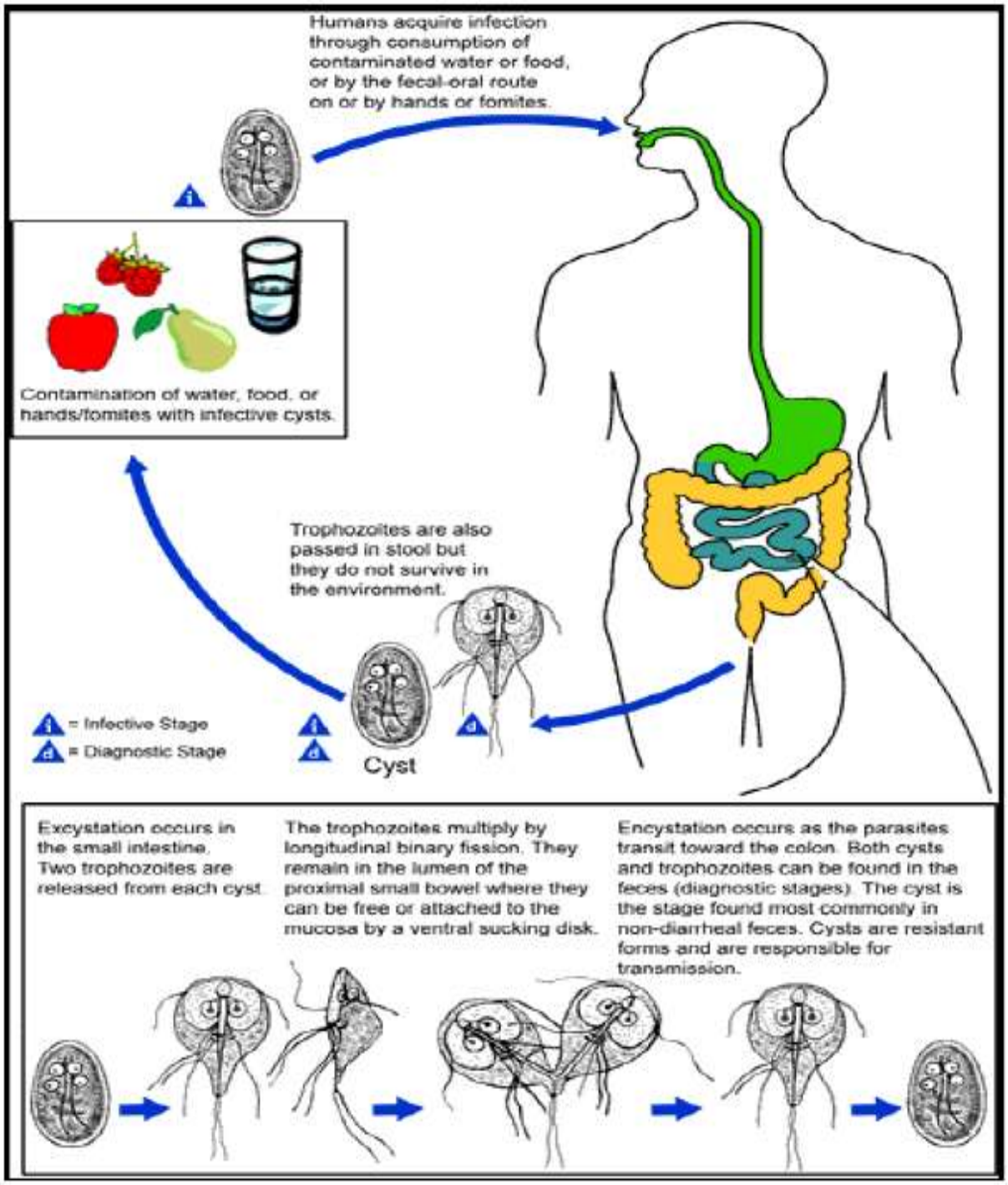

Figure 2.3: Life cycle of *Giardia*

(Source: CDC, 2014).

2.5 Pathogenesis of *Giardia lamblia*

As the acute stage of the disease lasts for only a few days, giardiasis is not always recognised. The differential diagnoses are acute viral enteritis, bacterial food poisoning, intestinal amoebiasis or an infection with toxigenic *Escherichia coli* (Monali *et al.,* 2012).

Once excystation occurs, *Giardia* trophozoite use their flagella to "swim" to the microvillus covered surface of the duodenum and jejunum, where they attach to enterocytes using a special disk located on their ventral surface (Jason, 2003). The attachment process damages microvilli, which interfers with nutrients absorption, rapid multiplication of trophozoite eventually create a physical barrier between the enterocytes and the intestinal lumen further interfering with nutrient absorption. This process leads to enterocyte damage, villus atrophy, crypt hyperplasia, intestinal hyperpermeability and brush border damage that causes a reduced disaccharidase enzyme secretion (Nain *et al.,* 1992). Research also demonstrates the process of cytopathic inducing substances such as glycoproteins, proteinases and lactins that may cause direct damage to the intestinal mucosa (Adam, 2001). *Giardia* trophozoites do not usually penetrate the epithelium, invade surrounding tissue nor enter the blood stream (Yakoob *et al.,* 2005). Thus, infection is generally contained within the intestinal lumen (Jason, 2003).

Studies in human patients with chronic giardiasis have now confirmed that infection with *Giardia lamblia* is indeed associated with increased rates of enterocyte apoptosis. *Giardia* can also prevent the formation of epithelial nitric oxide, a compound known to inhibit Giardial growth, by consuming local arginine, which effectively removes the substrate needed by enterocytes to produce nitric oxide (Jason, 2003). This mechanism may contribute to *Giardia*-induced enterocyte apoptosis, since arginine starvation in these cells is known to cause programmed cell death (Adam, 2001). A variety of enteropathogens are known to

cause epithelial apoptosis. Some reports have described a novel biological process in which sodium-coupled-glucosetransporter-1 (SGLT-1) activation may rescue enterocytes from lipopolysaccharide-induced epithelial cellapoptosis by enhancing glucose uptake (Buret, 2008).

2.6 Clinical Manifestation of *Giardia lamblia*

The role of *Giardia* in disease was unknown for centuries; well into the 20th century, but its ability to produce enteritis in man was suspected, yet not established (Wensaas, 2011). When the microbiologist Clifford Dobell in 1919 convincingly argued that Van Leeuwenhoek had been the first to identify the parasite, he made a point of congratulating the Dutch for not making the wrongful assumption that *Giardia* was the cause of his diarrhoea (Wensaas, 2011).

In the 1950's experimental studies showed that ingestion of *Giardia* cysts in capsules or drinking water led to infection in man, in the sense that cysts would later be found in stool samples (Adam, 2001). Still it could not be established that this caused clinical illness. Clinical accounts suggested that the infection may be followed by diarrhoea and other symptoms, but there was substantial controversy about the pathogenicity of *Giardia*. The main question was whether the parasite would cause disease, or merely be observed in greater numbers because the diarrhoea would constitute a more favourable environment in which *Giardia* would multiply (Espelage *et al.*, 2010; Wensaas, 2011).

During the last 40 years, *Giardia* has been isolated in several outbreaks of gastroenteritis in both Europe and North America providing arguments for its pathogenic potential. The experiment that formally established the pathogenicity of *Giardia* in humans by fulfilling the Koch postulates was published by Nash *et al*. (1987). Following thorough medical investigation 15 healthy volunteers, all men, were given sterile inocula with *Giardia*

trophozoites that had been grown from two different strains (GS/M and Isr) of cysts obtained from patients suffering from giardiasis. The trophozoites were administered by a tube into the small intestine. Later cysts would be detected in multiple stool samples from all ten men that received GS/M *Giardia*, as sign of infection. *Giardia* cysts were not detected in any of the five men that received the Isr strain. Of the ten men infected with *Giardia*,four developed typical diarrhoeal disease, which proved that *Giardia* can lead to both infection and clinical disease (Wensaas, 2011). Experimental and clinical studies have shown that people may be infected without developing symptomatic giardiasis (Inabo *et al.*, 2011).

The mechanisms by which *Giardia* causes disease are not fully understood. The diarrhoea frequently observed in symptomatic giardiasis is caused by combined malabsorption and hypersecretion that is the result of diffuse shortening of microvilli. These alterations are partly mediated by host T lymphocytes that are activated secondary to disruption of epithelial tight junctions and increased transepithelial permeability (Adam, 2001).

Children are more commonly affected than adults. The symptoms may be mild diarrhoea, flatulence, anorexia abdominal cramps, foul smelling diarrhoea, staetorrhoea and the malabsorption syndrome (Monali *et al*., 2012). No blood or mucus is normally seen. However, 50% *G. lamblia* infections are symptomless although several infections may develop in immunocompromised hosts (Miguel and Cabada, 2007). The factor that determines susceptibility of the host to the disease is poorly understood. After swallowing cyst for the first time symptoms commonly developed 2-6 weeks later (Thiongo *et al.*, 2012).

The incubation period can be shorter than the time that takes to start passing cyst in the stools. Diarrhoea lasts between 1 and 4 weeks. In majority of patients it is self limited and mild to moderate (Miguel and Cabada, 2007). However, a significant proportion of infected individuals are completely asymptomatic (Inabo *et al.*, 2011). In some individuals giardiasis

is short lasting and resolve spontaneously. In others, infection can be prolonged (Buret, 2008).

2.7 Host Defenses against *Giardia*

Host defenses against *Giardia* infection may be classified into two broad categories – non-immunological responses and immunological responses (Jason, 2003).

The body has a number of non-immunological mechanisms by which it responds to attempted infection by *Giardia* trophozoites. Nitric oxide (NO) can inhibit the growth of many pathogenic microorganisms, and enterocytes have been shown to produce and release nitric oxide into the intestinal lumen. NO has been demonstrated to inhibit trophozoite proliferation and differentiation *in vitro*. However, *Giardia* can prevent the formation of NO by actively taking up and metabolizing arginine from the intestinal lumen, which effectively removes the substrate enterocytes need to produce NO. Addition of extra arginine to the growth media has been shown to restore enterocyte nitric oxide production (Eckmann and Gilli, 2001; Jason, 2003).

Scavenging arginine may also affect mucosal integrity, as NO is involved in the regulation of mucosal barrier integrity (Mourad *et al.,* 1999; Jason, 2003). *G. lamblia* inhibits epithelial NO production by consuming arginine before epithelial cells can utilize it. This may partly explain the increase in intestinal permeability associated with Giardial infection. Supplement with arginine or the consumption of arginine-rich foods may be able to overcome this impediment and increased mucosal Nitric oxide production (Jason, 2003).

Another non-immunological response to *Giardia* is defensins small antimicrobial peptides released from intestinal epithelial cells. Paneth cells located within the crypts of the small intestine release α-defensins, while β −defensins are released by enterocytes. Both classes of defensins appear to insert themselves into cell membranes of pathogens, which creates pores

in the membrane and leakage of intracellular materials, ultimately resulting in cell lysis. In vitro research has demonstrated the ability of α-defensin to kill *Giardia* trophozoite (Adam, 2001).

Singer and Nash (2000) illustrated the importance of T-cells in the control of giardiasis. Neither Th1 nor Th2 cells were absolutely necessary for the clearance of *Giardia* infection (Jason, 2003). This suggests that in the absence of Th1 cells, Th2 cells are sufficient for clearance of the parasite, or that in the absence of Th2 cells, Th1 cells are sufficient. Alternatively, Th3 cells (mucosal T cells) may play the major role (Adam, 2001). However, in interferon-gamma deficient animals, parasite clearance was delayed when compared to controls. This suggests that Th1 response may be more substantial in controlling Giardial infections. T-cell cytokines may also induce the production and release of antigiardial defensins into the intestinal lumen (Jason, 2003).

2.8 Epidemiology of *Giardia lamblia*

Giardia is mainly spread through contaminated drinking water, but other pathways of transmission are also recognised (Wensaas, 2011). The first food-borne cases were detected in home-prepared salmon. Later other outbreaks have been linked to noodle salad, fruit salad and raw sliced vegetables (Caccio *et al*., 2009). A review of all known outbreaks associated with recreational waters in the United States between 1971 and 2000 concluded that in 97 of 259 registered outbreaks, protozoa 37.5% were the aetiologic agents (Wensaas, 2011).

Giardiasis has also been linked to interactive water fountains. The first report came from Florida in 2006 (Eisenstein *et al*., 2008). In 2003, 30 primary cases of giardiasis during a large outbreak in Boston, Massachusetts, were linked to exposure to a children's pool and as

many as 105 secondary cases probably resulted from person-to-person spread. Transmission from person to person is a well-known problem in child day care centers, and this was the site for the first outbreak of giardiasis in Norway, in Trondheim in 2006 (Wensaas, 2011). It was also reported that children are at risk a study that showed that nappy handling was associated with a four-fold increased risk of giardiasis (Fraser *et al.,* 2000).

The incidence was highest among children 1-4 years of age. A prevalence study in five Berlin kindergartens in 2006 identified *Giardia* in three of 202 children (Espelage *et al.,* 2010). A Meta analysis on 13 non-heterogeneous studies from the Nordic countries published before 2004 estimated a pooled prevalence of 5.8% among persons with and 3.0% among persons without gastrointestinal symptoms (Adeyemo *et al*., 2010). The high estimates for prevalence of *Giardia* infection in low-income countries are most uncertain. They are partly based on studies with few participants, restricted to patients with gastroenteritis or limited to children (Fraser *et al.,* 2000). In some instances there is no clear distinction between symptomatic or asymptomatic infection (Wensaas, 2011).

In developing countries, children have a high prevalence of giardiasis and frequent re-infections. People living with infected children will most likely get infected as well. Transmission of cyst and the disease occurs mainly by the feacal- oral route (Naglaa *et al*., 2011).

2.8.1 Epidemiology of *Giardia lamblia* in Nigeria

Asymptomatic excretion of *G. lamblia* is common in some populations, such as children attending day care centers have been reported by Inabo *et al.* (2011) with prevalence of 41.4% in asymptomatic children in two Local Government Areas of Zaria Kaduna State. While, in Guma Local Government Area in Benue State of Nigeria 40.4% prevalence of giardiasis was reported in 2006 where 18.7% of total infected children were, children

between the age group 0-4 years. Children of age group 5-9 years were reported to be most infected with giardiasis (Nyamngee *et al.*, 2006). Other researchers like Hamza and Biu (2012) reported 10.5% prevalence of giardiasis in children in Maiduguri, Borno State and Muhammed *et al.* (2014) reported a prevalence of 14.3% of giardiasis also in children in Borno State, Nigeria.

2.8.2 Epidemiology of *Giardia lamblia* in Africa

In Africa, a study from the rural Nile Delta of Egypt stools were analysed once a week as part of a six months investigation of 42 children and during this period *Giardia* was detected in 41 of the children in 42 of the specimens analysed (Mahmud *et al.*, 2001). In rural Ethiopia a prevalence of 25.8% among children was recorded in the dry season of 2005 and 39.8% in the wet season of 2006. A one year Zambian study on 100 pre-school children followed for one year with analyses of stool samples once a month showed that 75 of the children had been infected with *Giardia* during that year, but 21 of those had no episodes of diarrhoeal disease (Siwila *et al.*, 2011). A larger study from a district hospital in Mozambique found that only 2.5% of 529 children with diarrhoea were infected with *Giardia* and similarly Moyo *et al.* (2011) found *Giardia* in 1.9% of 280 children hospitalized with diarrhoea in Tanzania (Wensaas, 2011).

2.8.3 Epidemiology of *Giardia lamblia* in First World Countries

In the United State in 2012, a total of 15,223 cases were reported. In a study including 197 paediatric patients with acute non dysenteric diarrhoea in the United State, giardiasis was the cause of 15% of cases second only to rotavirus (Karin *et al.*, 2015). One report in Canada noted on adjusted incidence rate of 25.8 cases per 100,000 populations between 1990 and 1998. Almost 40% of cases occurred in travelers; other important sources of infection include unfiltered water and person to person transmission. A German study noted a prevalence of

giardiasis of 11.5 cases per 100,000 populations among twenty years old (Espelage *et al.*, 2010). The prevalence of human giardiasis in Spain has been mainly studied in paediatric population but also in hospital outpatients and inmate and immigrant subjects. Typical infection rate of giardiasis ranged from 3% - 7% and 13% - 25% for asymptomatic and symptomatic individuals respectively (Carmena *et al.,* 2012).

Early studies in Guatemala suggested that in the second year of life, *G. lamblia* infection affects growth (Sagi *et al.,* 1986). However, more recent studies showed that there is no effect of *G. lamblia* carriage on growth and disease rates in infants and children (Fraser *et al*., 2000). A report of weight and height percentile achievements of *G. lamblia*–positive children in 77 nursery children aged 3 months to 3 years was tended to be higher than those in the *G. lamblia*–negative group (Fraser *et al*., 2000). A questionnaire answered by the parents did not show increased prevalence of gastrointestinal symptoms in the *G. lamblia*–positive group. There were no significant differences in weight and height achievements between *G. lamblia*–positive and *G. lamblia* –negative children. *G. lamblia*–positive children tended to have fewer symptoms related to their respiratory tracts, as recorded in a weekly questionnaire. Thus, the effect of *G. lamblia* infection on growth is unclear (Fraser *et al.*, 2000).

2.8.4 Epidemiology of other intestinal parasites in Nigeria

Gastro-intestinal parasites are identified as a cause of morbidity and mortality throughout theworld particularly in the under developed countries. Wariso and Ibe (1994) reported 46.0% prevalence rate of intestinal parasite within some parts of Port Harcourt, Nigeria. Alison *et al*. (2004) reported 17.0% in Uganda. Mordi and Ngwodo (2007) reported 0.7% in all the eighteen Local Government Areas of Edo State,Nigeria. Okolie *et al.* (2008) reported a prevalence value of 75% among patients with appendicitis in Oguta, Imo State, Nigeria.

Chukwuma *et al.* (2009) in their study on the prevalence of parasitic geohelminth infection of primary school children in Ebenebe Town, Anambra State, reported a prevalence value of 53.6% in soil and 87.7% in stool. Awolaju and Morenikeji (2009) reported 48.4% among primary and post-primary school children in Ilesha West, Osun State and 50.80% among school children in Ilaje, Osun State, Nigeria. Chukwuma *et al*. (2009) also reported prevalence of geohelminth eggs/larvae in soil with respect to schools. They reported prevalence of geohelminth eggs/ larvae in Umuji primary school as 52.5%, Umuogbuefi primary school 83.3% and Obuno primary school 32.5% and overall prevalence in stool samples in the three schools to be 87.7% with distribution as follows; Umuji primary school, 87.5%, Umuogbuefi primary school, 97.5% and Obuno primary school, 75%. Alli *et al.* (2011) reported 49.4% in Ibadan, Oyo State, Nigeria. Odu *et al*. (2013) reported an overall prevalence of 15.7% among primary school children in Rivers State, Nigeria. Akingbade *et al*. (2013) reported prevalence of 25.8% in diarrhoeal diseases children in Abeokuta, Ogun State Nigeria.

2.9 Laboratory Diagnosis of *Giardia lamblia*

It is sometimes difficult to establish the diagnosis of giardiasis. Tests for parasitic antigen in stool are at least as sensitive and specific as good microscopic examination and are easier to perform (Eckmann and Gillin, 2001). All these methods occasionally yield false-negative results. The implications of the study are that no symptom complex is associated with the giardiasis. Giardiasis may present with abdominal pain alone and it should be considered even in the absence of diarrhoea (Yakoob *et al.,* 2005).

Diagnosis of *Giardia lamblia* infection by microscopic examination of stool for ova and parasites (O and P) is a laboratory process. Iodine- stained wet smears, trichrome – stained cyst concentration prepared by formalin-ethyl acetate centrifugation or by zinc sulphate flotation, and trichrome stained polyvinyl alcohol (PVA) preserved stools are standard

methods of stool preparation used to increase the sensitivity of *Giardia* detection (Chakaroka, 2010).

There are conflicting reports on findings by microscopy that direct smears without preservation are as low as 50% sensitive. While others suggest that there is minimal diagnostic gain from more invasive and expensive testing (El-Nahas *et al.,* 2013). Immunological testing of stool and serum has been reported as more sensitive means to diagnose giardiasis (copro-antigen diagnosis). The direct detection of antigens in stool was first demonstrated for *Giardia lamblia* by Craft and Nelson using counter immunelectrophoresis. Since then, the isolation of a *Giardia* specific antigen (GSA) 65 has facilitated the development of other antibody associated assay of antigen detection such as ELISA and immnofluorescence assay. The accuracy of this technique has been compared with that of microscopy in patients with gastrointestinal symptomatology with sensitivities and specificities of 95% to 100% and over 90% respectively. The technique's accuracy and simplicity have been cited as its major advantages while cost concerns appears to be the principal disadvantage (Behr *et al.,* 1996).

Giardiasis is diagnosed by signs and symptoms, as well as the presence of *Giardia* cysts and trophozoites in the stool (Al-Saeed and Issa, 2010). Sensitivity is poor when only a single sample is analysed, particularly if there is low quality, intermittent excretion of cysts or pigments (El-Nahas *et al.,* 2013). Stool examination can be unreliable, however, as organisms may be excreted at irregular intervals, which can produce a false negative test result (Ahmed *et al.,* 2014). Hence, definitive diagnosis may require repeated stool examination, feacal immunoassays, or even sampling of the upper intestinal contents. Two stool examinations will detect 80 – 90% of infections, while three samples detect 90% (Jason, 2003; El-Nahas *et al.,* 2013).

2.9.1 Stool Examination in the Diagnosis of *Giardia*

Stool examination in the most common means to diagnose *Giardia lamblia* cyst and trophozoite using various methods (El-Nahas *et al.,* 2013). Some of these methods are;

2.9.1.1 Wet mount

In bright-field microscopy, cysts appear ovoid to ellipsoid and usually measure 11 to 14µm (range 8 to 19µm). Immature and mature cysts have 2 and 4 nuclei respectively. Intracytoplasmic fibrils are visible in cysts (Cheesbrough, 2010).

2.9.1.2 Direct fluorescent antibody (DFA) assay:

This technique offers the highest combination of sensitivity and specificity and is considered the gold standard by many laboratories. For commercial DFA kits it is recommended that a concentrated stool specimen be used to increase the probability of detection of low numbers of cyst. However, special equipment (fluorescence microscope) and commercially available test kits are required and it does not provide a permanently stained slide that can be archived (CDC, 2014).

2.9.1.3 Enzyme immunoassay

The Enzyme immunoassay (EIA) does not rely on microscopy and is useful for screening large number of specimens. Borderline positives and questionable negatives obtained with this technique should be further confirmed by DFA. Antigens of *Giardia* are detected in the feaces using this method; therefore, specimens should not be concentrated prior to testing. However special equipment (microplate reader and commercially available test kits are required) (CDC, 2014).

2.9.1.4 Rapid immunochromatographic cartridge assays

The rapid cartridge assays may be used with preserved specimens and are quick and easy to perform. Antigens of *Giardia* are detected in the faeces using this method; therefore, specimens should not be concentrated prior to testing. Borderline positives and questionable negatives obtained with this technique should be further confirmed by DFA. No special equipment is required (CDC, 2014).

2.9.2 Other diagnosis

Duodenal biopsy is probably one of the most sensitive tests with values ranging between 82.5% and 100%. But its invasiveness limits the use of this test to clinical situations (Miguel and Cabada, 2007). Serology for *Giardia* IgG is useful for epidemiologic studies, since after infection, IgG antibodies level remain high for prolonged periods of time. It is not clear if testing for IgM antibodies is useful in the clinical management of giardiasis (Miguel and Cabada, 2007).

2.10 Treatment

Several drugs are effective against *Giardia*, but there is uncertainty about the optimal regimen. There is concern about the development of resistance to existing treatment, and research on new therapeutic drugs for giardiasis has been initiated (Wensaas, 2011).

i. The use of metronidazole has cure rate ranging from 80 to 95%. Cases of metronidazole resistant are uncommon.
ii. Albendazole is as effective as metronidazole when given for five days
iii. Furazolidon can cure 80 to 85% of infection.
iv. Nitazoxanide can cure 70 to 85% of infection.

v. Tinidazole is effective between 90 and 100% with single oral dose (Miguel and Cabada, 2007)

2.11 Prevention/Control

Proper food and water handling should be emphasized. Good hand hygiene should be encouraged and good sanitation should be encouraged. Chlorination, flocculation, sedimentation and filtration of public water supplies should be done properly. Treatment of asymptomatic carriers may be considered in special situations (Miguel and Cabada, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Kaduna State is found in the North-West geopolitical zone of Nigeria on a global location between latitude 90^{o}N and 140^{o}N of the equator and longitude 70^{o}E and 100^{o}E. It has 23 Local Government Areas and it is divided into three senatorial zones (Figure 3.1). It is bounded by other States like 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.

3.2 Study Design

This study was a descriptive cross sectional hospital based study and non probability sampling by snow- ball in the choice of hospitals 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. The name of hospitals, locations and number of samples collected from hospital is presented on Table 3.1

3.3 Collection of Data

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 (Appendix II). The information are child's source of drinking water, mode of breast feeding for children less than seven months, day care attendance, hands wash after defeacating and mothers/caregivers level of education.

Nutritional status was accessed by anthropometric indices of weight-for-age [Appendices VIII and IX (WHO, 2007)].

3.4 Study Population

The study population for this dissertation were paediatric in-patients and outpatients both males and females between 0-5 years that were presenting with gastroenteritis or diagnosed with the symptoms of gastroenteritis by the clinicians.

3.5 Inclusion Criteria

All paediatric in-patients and outpatients 0-5 years that were presented with diarrhoea, vomiting, and abdominal pain whose parents/caregivers gave their consent.

3.6 Exclusion Criteria

All paediatric in-patients and outpatients that were presented with diarrhoea, vomiting and abdominal pain above 5 years as well as those children who were less than 5 years whose parents/caregivers did not give their consent

3.7 Ethical Approval

Ethical approval was obtained from the Ministry of Health Kaduna State (Appendix I) 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.

Figure 3.1: Map of Kaduna State

(Source: NPC, 2006)

3.8 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.

$$S = \frac{t^2 P(1-P)}{m^2} \text{ (Kuta } et\ al., 2014)$$

Where;

S = sample size

t = confidence level at 95% = 1.96

P = National prevalence (14.3%)

M = Marginal error at 0.05

$$S = \frac{(1.96)^2 \times (0.143)(1-0.143)}{(0.05)^2}$$

$$S = 188.3$$

Sample size calculated was 188.3 and it was increased by 25% in order to minimize sampling error to make a total of 200. A total of 200 single stool samples were collected from both in-patients and outpatients children 0-5 years presenting with gastroenteritis in the four hospitals (Table 3.1).

Table 3.1 Hospitals Where 200 Samples Were Collected, Their Locations and Number of Samples Collected.

S/N	Hospitals	Location	No. of samples collected
1	Yusuf Danshoto memorial Hospital	Tudun Wada/Kaduna South	93
2	Barau Dikko teaching hospital	Kaduna central	35
3	St. Gerard Catholic hospital	Kachia road/ Kaduna South	20
4	Dr Gwamna Awan General hospital	Makera Kaduna South	52

3.9 Sample Collection and Processing

Two milliliter (2ml) of watery stool or semi formed stool were collected by the help of trained nurses from eligible children 0-5 years both in-patients and outpatients presenting with gastroenteritis during the course of the study into clean labeled screw capped tubes (universal bottles). Samples collected were transported on ice packs to the Parasitology Laboratory of the Department of Microbiology, Ahmadu Bello University Zaria. Stool samples were stored at 2°C – 8°C immediately and longer storage was done at -20°C under constant supply of power. Repeated freezing and thawing of samples were avoided (Muhammed *et al*., 2014). The two hundred stool samples were examined for *Giardia lamblia* using three methods of light microscopy technique and *Giardia* ELISA kit (Appendix III). The examination of *Giardia lamblia* from stool samples using microscopy was carried out in the Parasitology Laboratory of the Department of Microbiology, Ahmadu Bello University Zaria for five weeks. While the ELISA kit used to detect *Giardia* antigen in stool samples were done in the Molecular Laboratory of the same department for a day.

3.10 Light Microscopy Technique;

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

3.10.1 Unstained (normal saline faecal sample)

Faecal suspension of 0.1ml of each sample was placed on a glass slide and mixed with a drop of 0.9% solution of NaCl and the slide was covered with a glass cover slip and examined for the presence of parasites at 10x and 40x magnification using Olympus research microscope (optical co-ltd, Japan) (Cheesbrough, 2010).

3.10.2 Iodine stain faecal sample

Faecal suspension of 0.1ml of each sample was placed on a glass slide and mixed with a drop of Lugol's iodine and the slide was covered with a glass cover slip and examined for the

presence of parasites at 10x and 40x magnification using Olympus research microscope (optical co-ltd, Japan) (Cheesbrough, 2010).

3.10.3 Zinc sulphate (33% ZnSO4) Flotation Method

Faecal suspension of 0.1ml of each sample was added in a tube containing 9 ml of 33% $ZnSO_4$ 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. The slide was examined for the presence of parasite at 10x and 40x magnification using Olympus research microscope (optical co-ltd, Japan) (Chakaroka, 2010).

3.11 *Giardia* ELISA Principles and Procedures

Giardia ELISA is an in vitro immunoassay for the qualitative determination of *Giardia* antigen in feacal specimens (Diagnostic automation, 2013). The *Giardia* ELISA tests principle and procedures were as follows.

3.11.1 *Giardia* ELISA test principle

Giardia specific antigen present in the stool specimens are captured by monoclonal antibodies attached to the microwells. The wells are incubated and washed before anti-*Giardia* polyclonal antibodies conjugated to horseradish peroxidase are added. The enzyme conjugate will “sandwich” any antigen bound to the wells. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex. The stop solution ends the reaction and turns the blue color to yellow. If no antigen is captured, or if there is an insufficient level of antigen, no colored reaction takes place (Diagnostic automation, 2013).

3.11.2 *Giardia* ELISA test procedure

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 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 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 was 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 index finger for 15 seconds. The reaction was read within 5 minutes after stop solution was added in the Molecular Laboratory of the Department of Microbiology Ahmadu Bello University, Zaria. The results were read visually (Appendix VII) and also with ELISA plate reader (GF-M300 Microplate reader, B BRAN Scientific and Instrument Company England) at 450nm wave length (Diagnostic automation, 2013).

3.12 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).

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

3.12.1 Weight Measurement

For the measurement of weight and height with the help of trained anthropometrists, each child was asked to step up backward onto the scale and stand still over the center of the scale with body weight evenly distributed between both feet using the Heinz digital scale. The child's arms were hanging freely by the sides of the body, with palms facing the thighs. The child held his/her head up and face forward. Weight was recorded to the nearest 0.1 kilogram using the scale with a digital readout (CDC, 2014).

3.12.2 Height Measurement

For the measurement of height, each child was asked to stand with his/her back against the board. The weight of each child was evenly distributed on both feet. Each child was asked to place the legs together, bringing the ankles or knees together. They were instructed to stand erect (stand up straight and look straight ahead) and the position was verified from both the front and from the left side of the body. Next, each child's head was positioned in the Frankfort Horizontal Plane. 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 height measured using the recumbent length board by the trained anthropometrists. Children from 24 months of age and older who could stand unassisted had their standing height measured using stadiometer (CDC, 2014).

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. Those with BMI less than 14kg/m^2 were recorded as low BMI or underweight. While children with BMI of 14kg/m^2 and above were recorded as normal weight (Appendix IV and V).

3.13 Analyses of Data

The results obtained were analyzed using Open Epi info package version 6 developed by World Health Organisation for epidemiological studies. Pearson's chi square statistical test and odds ratio at 95% confidence interval (CI) was used to obtain p-values. Tables, charts and figures were formed where necessary using micro excel. Discussions and comments were drawn based on the results of the study.

CHAPTER FOUR

4.0 RESULTS

In this study, microscopic examination shows that 12 children were infected with *Giardia lamblia*. Only *Giardia* cysts were identified in all positive samples. While ELISA had 28 of children infected with giardiasis out of the total number of two hundred stool samples of children presenting with gastroenteritis in Kaduna Metropolis (Figure 4.1).

The comparism of sensitivity and specificity of the ELISA kit using microscopy as gold standard shows a percentage sensitivity of 100% and specificity of 91.5% respectively as presented on Table 4.1.

The prevalence of *Giardia lamblia* by gender among children 0-5 years presenting with gastroenteritis in Kaduna Metropolis shows the male children were at higher risk of infection with prevalence of 15.89% (17/107) children infected with giardiasis . While female counterparts had 11.83% (11/93) using the prevalence of giardiasis gotten from ELISA as presented on Table 4.2. The difference was statistically not significant [($p = 0.4092$, $\chi^2 = 0.6811$, df = 1)].

The prevalence of *Giardia lamblia* by age group (months) among children 0-5 years presenting with gastroenteritis in Kaduna Metropolis was shown on Table 4.3. The age group 49-60 months had the highest prevalence of 16.39% followed by the age group 37-48 months with 14.71%. The least was recorded among children in the age group 0-12 months with prevalence of 9.68%. The difference was statistically not significant [($p = 0.9319$, $\chi^2 = 0.848$, df = 4)].

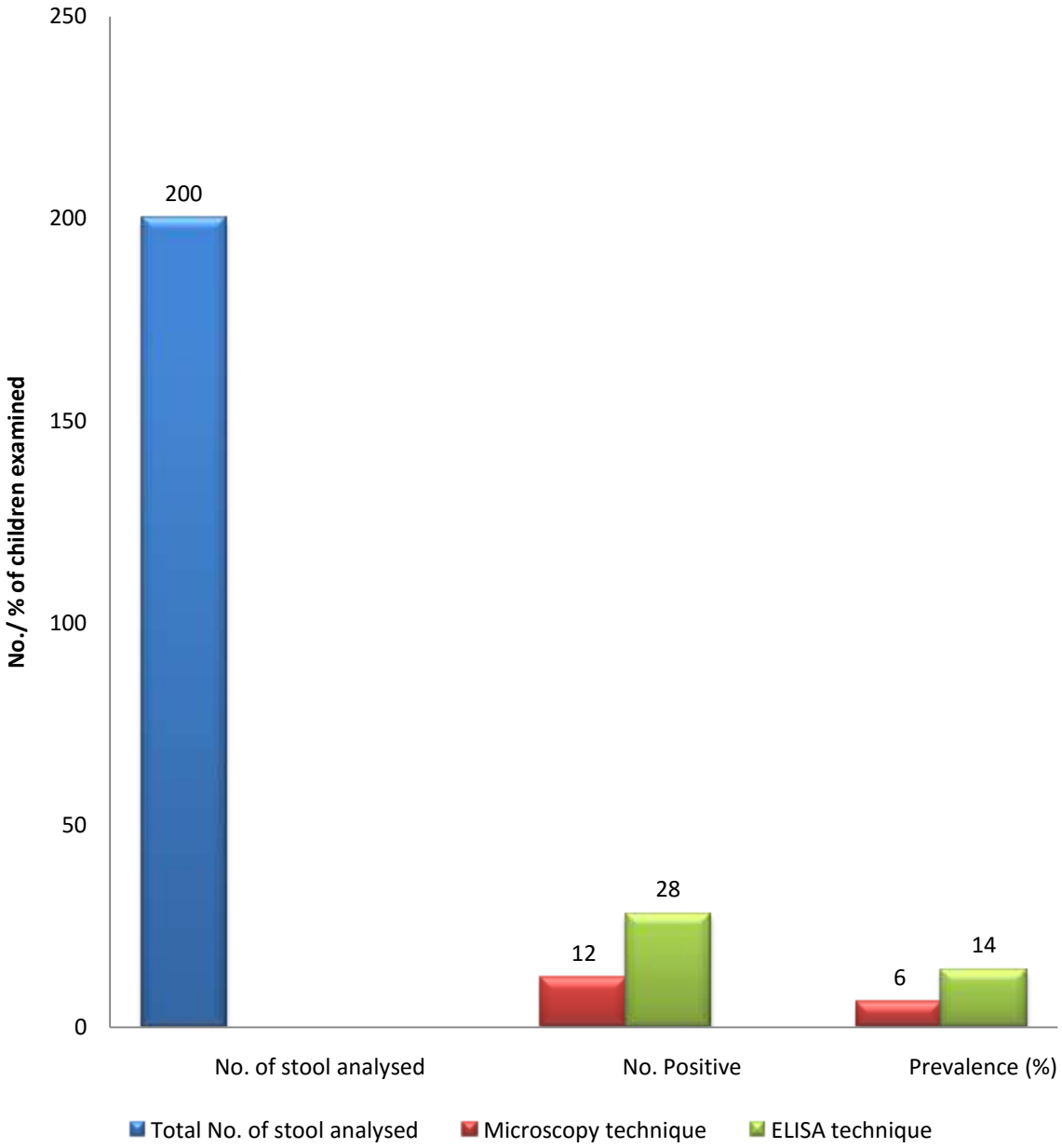

Figure 4.1: Prevalence of *Giardia lamblia* in by Microscopy and ELISA of Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria.

Table 4.1: Sensitivity and Specificity of ELISA versus Direct Microscopy for Diagnosis of *Giardia lamblia* in Stool Samples of Studied Area using Microscopy as Gold Standard

Microscopy	Total No.	ELISA positive	ELISA negative	Sensitivity %	Specificity %
Positive	12	12	0		
Negative	188	16	172	100	91.5
Total	200	28	172		

KEY

$$Sensitivity = \frac{No.ofpositivesamplesforbothMicroscopyandELISA}{TotalNo.ofpositivebyMicroscopy}$$
$$= \frac{12}{12} \times 100 = 100$$

$$Specificity = \frac{No.ofNegativesamplesforbothMicroscopyandELISA}{TotalNo.ofNegativebyMicroscopy}$$
$$= \frac{172}{188} \times 100 = 91.5$$

Table 4.2: 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
Total	200	28	14

$p = 0.4092, \quad \chi^2 = 0.6811, \quad df = 1$

Table 4.3: 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	14.71
25 – 36	32	4	12.50
37 – 48	42	6	14.29
49 – 60	61	10	16.39
Total	200	28	67.58

$p = 0.9319, \quad \chi^2 = 0.848, \ df = 4$

The body mass indices obtained from children 0-5 years presenting with gastroenteritis shows 33% children underweight, 66% normal weight and 1% overweight respectively in Figure 4.2 (Appendix VI).

The prevalence of underweight BMI in children according to age and sex is presented in Figure 4.3. Children between age group 49-60 months had the highest prevalence. The least prevalence was found in the age group 0-12 months.

BMI obtained in infected children with giardiasis presenting with gastroenteritis in Kaduna Metropolis according to sex is shown in Table 4.4. *Giardia* positive male and female children had 27.27% prevalence for underweight BMI. Children with normal weight for their ages and heights yet infected had 7.57% prevalence for BMI. Overweight children were found not infected with giardiasis in this study.

The mean weight of infected children that had normal or healthy BMI and those with low or underweight BMI is shown in Table 4.5. The p= 0.316172 shows no statistical difference between Giardial infected children and BMI in children 0-5 years presenting with gastroenteritis in Kaduna Metropolis, Nigeria. The percentage mean weight of both normal weight and underweight infected children with Giardiasis accounted for 58.08% and 41.92% respectively.

The risk factors associated with the cause of Giardiasis in children 0-5 years presenting with gastroenteritis Kaduna Metropolis, Nigeria is shown in Table 4.6. Those that drank well water had a high odd of acquiring the infection/disease and significantly associated with the cause of disease in these children [(OR= 4.222, 95% confidence interval CI= 1.712-10.41 and p= 0.0053)]. Those with severe malnutrition also had high odd of acquiring the infection and significantly associated with the cause of giardiasis in children 0-5 years presenting with gastroenteritis in Kaduna Metropolis [(OR= 4.187, 95% confidence interval CI= 1.577-11.11

and p= 0.012)]. Children whose mothers/caregiver that had quranic education alone and those that had mothers/caregivers with no formal education shows highest odd of acquiring the infection and statistically associated with [(OR= 7.261, 95% confidence interval CI= 1.951-27.02 and p= 0.0007)] respectively. Children that drank tap water, river water, not exclusively breast fed, none breast feeding, daycare and none school attendance, moderately malnourished and those children that did not have hands washed after toilet or defeacating all had the odd of acquiring the infection/disease but statistically shows no significant association in this study.

The prevalence of giardiasis with other intestinal parasites in children 0-5 years presenting with gastroenteritis in Kaduna Metropolis, Nigeria is shows in Table 4.7. The most common parasite was *Entamoeba coli* with the prevalence of 18% followed by *Giardia lamblia* with prevalence of 14%. The least found was *Strogyloides stercoralis* with prevalence of 0.5% in children 0-5 years presented with gastroenteritis Kaduna Metropolis. Protozoa and helminthes were identified in children 0-5 years presented with gastroenteritis in this study.

The prevalence of intestinal parasites in children 0-5 years presenting with gastroenteritis in Kaduna Metropolis in Nigeria according to sex is shown in Table 4.8. About half of the children had intestinal parasites with the prevalence of 44.5%. Male children were more infected than their female counterparts with a prevalence of 47.66% and 40.86% respectively.

Age group distribution of intestinal parasites among infected children 0-5 years presenting with gastroenteritis in Kaduna Metropolis, Nigeria is shows in Table 4.9. There is significant association between age group and infection R=0.869719 and p=0.020797. Children between the age group 49-60 months were more infected with intestinal parasites with a prevalence of 59.02% and the least infected age group were 0-12 months with a prevalence of 25.8%.

The prevalence of mixed infected children, infected children and uninfected children 0-5 years presenting with gastroenteritis in Kaduna Metropolis is shown on Figure 4.4 with the prevalence of 2.5%, 42% and 55.5% respectively.

Plate I to plate VIII shows the results of wet mount and iodine stained slides of all identified intestinal parasites in the study under 40x magnifications of microscope.

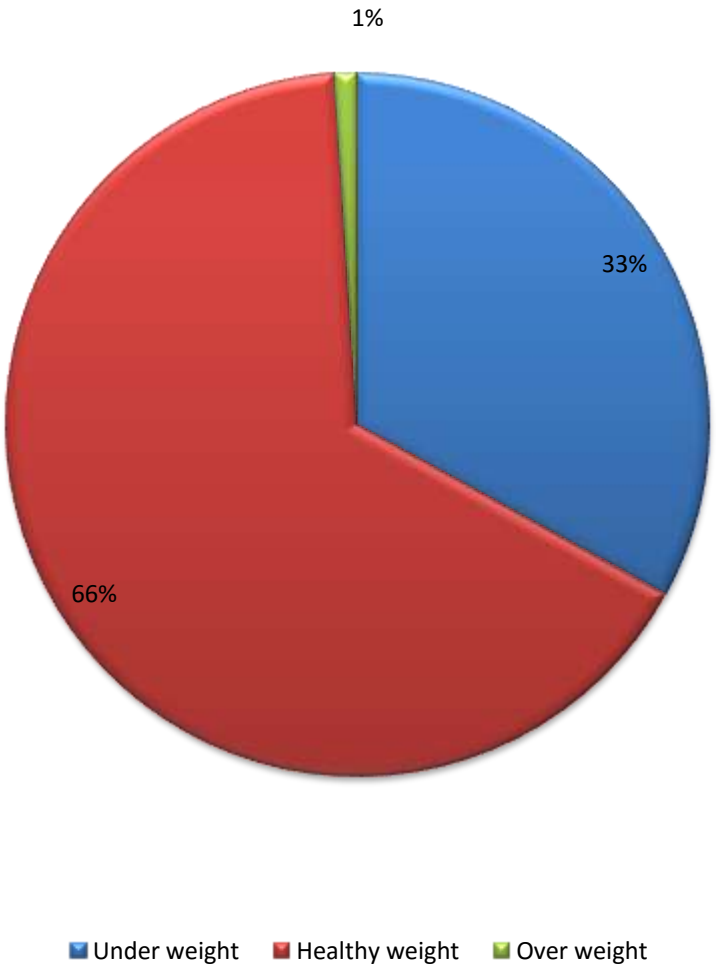

Figure 4.2: Body Mass Indices of Children 0 – 5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

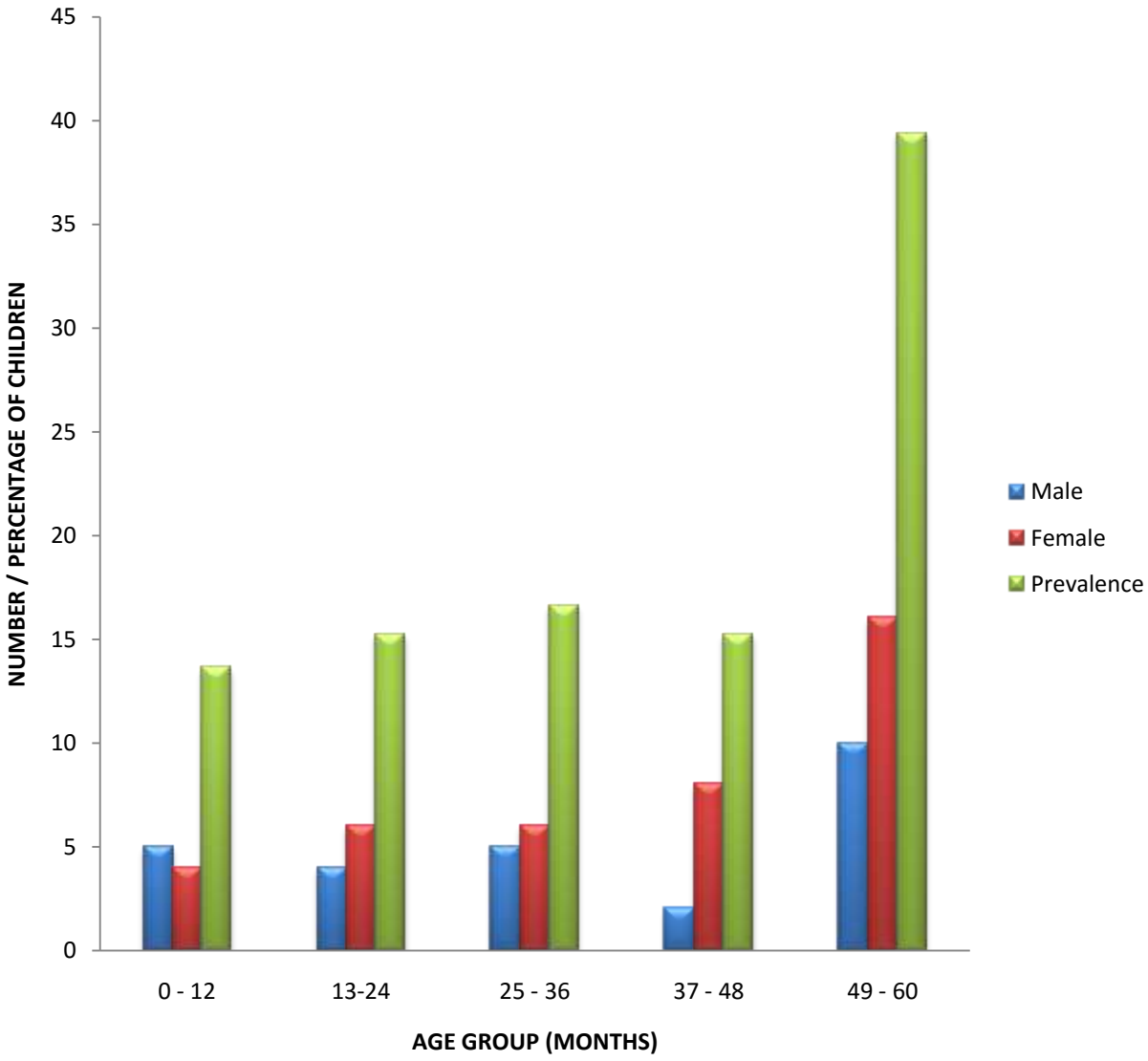

Figure 4.3: Body Mass Indices of Underweight Children According to Age and Sex in Children Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

Table 4.4: 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^2)	Total number for BMI	Positive male (%)	Positive female (%)	Total (%)
Underweight	66	10(15.15)	8(12.12)	18(27.27)
Healthy weight	132	7(5.3)	3(2.27)	10(7.57)
Overweight	2	0(0)	0(0)	0(0)
Total	200	17(8.5)	11(5.5)	28(14)

Table4.5: 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^2)	Percentage Mean Weight	Normal BMI Range (Kg/m^2)
Under weight	18	13.15	41.92	0-13.9 (<5th percentile)
Normal weight	10	18.22	58.08	14-18.5(5^{th}-85^{th} percentile)

p= 0.316172

Table 4.6: Risk Factors Associated with Giardiasis in Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

Risk factors	No infected	Total examined	Odds ratio(OR)	p. value	Lower-upper limit CI at 95%
Source of water					
Tap	9	46	1.728	0.3177*	0.7223, 4.136
Well	10	30	4.222	0.005389**	1.712, 10.41
Bore hole	5	45	0.7174	0.7182	0.2562, 2.009
Sachet water	4	70	0.2677	0.01794	0.08891, 0.8059
Bottle water	0	8	0.3905	0.99999	0.02169, 7.029
River	0	1	3.109	0.4057*	0.1019, 94.89
Feeding					
Exclusive breast feeding	0	11	0.2738	0.3435	0.0156, 4.806
Not exclusive breast	3	20	1.094	0.8919*	0.2988, 4.006
None breast feeding	25	169	1.62	0.4505*	0.4578, 5.736
School attendant					
Day care	2	10	1.577	0.8419*	0.3172, 7.839
Nursery	13	100	0.8467	0.8389	0.3803, 1.885
Primary	2	20	0.6581	0.8920	0.1441, 3.005
Non attendance	11	70	1.239	0.7546*	0.5452, 2.817
Nutrition (weight for age)					
Normal nutrition	13	139	0.3164	0.01050	0.1399 , 0.7154
Moderate malnutrition	7	38	1.516	0.5244*	0.5925, 3.88
Severe malnutrition	8	23	4.187	0.01243**	1.577, 11.11
Hand wash after Toilets					
NO	19	130	1.16	0.9105*	0.4947, 2.721
YES	9	70	0.8619	0.9105	0.3675, 2.021
Mother's highest education					
Quranic education only	8	20	5.333	0.0004120**	1.946, 14.62
Primary education	6	60	0.596	0.2872	0.2286, 1.554
Secondary education	4	55	0.3954	0.09131	0.1306, 1.197
Tertiary education	1	25	0.2284	0.1235	0.02964, 1.76
Quranic/ formal education	4	30	0.9359	0.9091	0.3, 2.92
None of the above	5	10	7.261	0.0007624**	1.951, 27.02

*OR $>$ 1 and **p$\leq$ 0.05

Table 4.7: Prevalence of Other Intestinal Parasites in Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

Intestinal parasites	No. Positive for parasite	Prevalence (%)
Protozoa		
Giardia	28	14
Entamoeba coli	36	18
Entamoeba histolytica	6	3
Total	70	
Helminthes		
Ancylostoma duodenale	17	8.5
Hymenolepis nana	7	3.5
Enterobius vermicularis	2	1
Ascaris lumbricoides	2	1
Strongyloides stercoralis	1	0.5
Total	29	

Table 4.8: Prevalence of Intestinal Parasites according to Sex in Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

Sex	No. Examined	No. Positive	Prevalence (%)
Male	107	51	47.66
Female	93	38	40.86
Total	200	89	44.5

Table 4.9: Prevalence of Intestinal Parasites According to Age Group in Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

Age group (months)	No. Examined	No. Infected	Prevalence (%)
0 – 12	31	8	25.81
13-24	34	10	29.41
25-36	32	15	46.88
37-48	42	20	47.62
49-60	61	36	59.02
Total	200	89	44.5

p= 0.020797

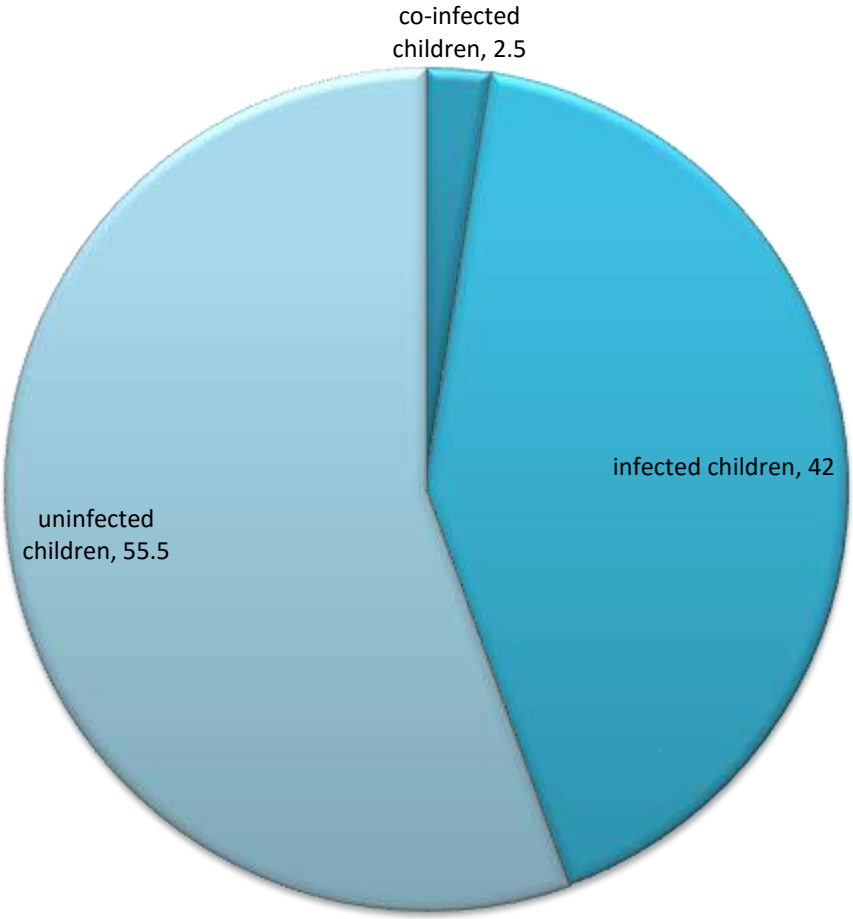

Figure 4.4: Prevalence of Mixed Infected, Infected and Uninfected Children 0-5 Years Presenting with Gastroenteritis in Kaduna Metropolis, Nigeria

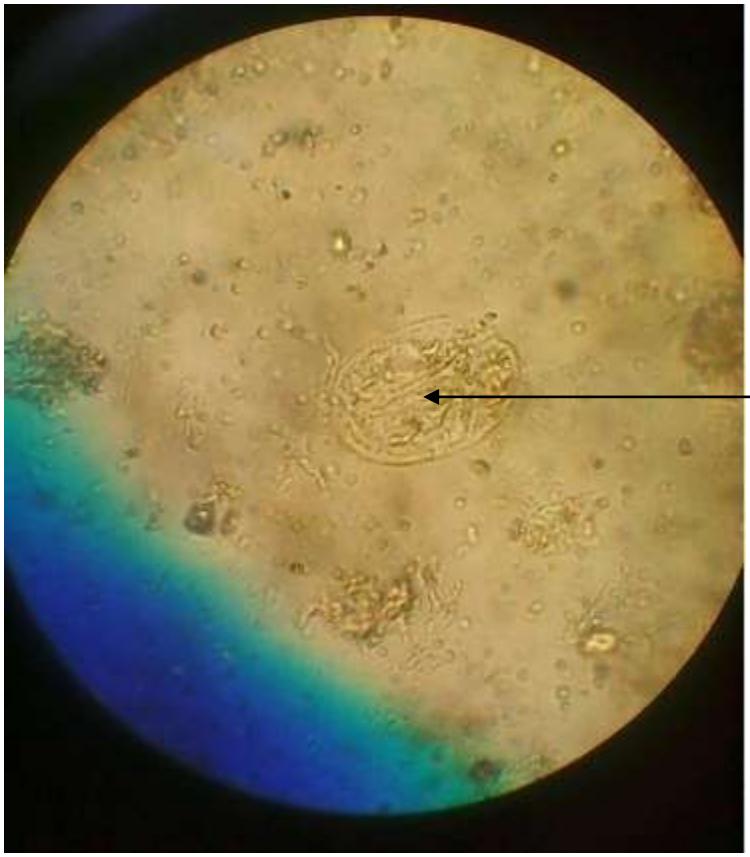

Plate I: Cyst of *Giardia lamblia* in Wet Mount under 40x Magnification

Plate II: Cyst of *Entamoeba coli* in Wet Mount under 40x Magnification

Plate III: Cyst of *Entamoeba histolytical* in Lugol's Iodine Stained under 40x Magnification

Plate IV: **Ovum (a) and Rhabdiform Larvae (b) of *Ancylostoma duodenale*in Wet Mount under 40x Magnification**

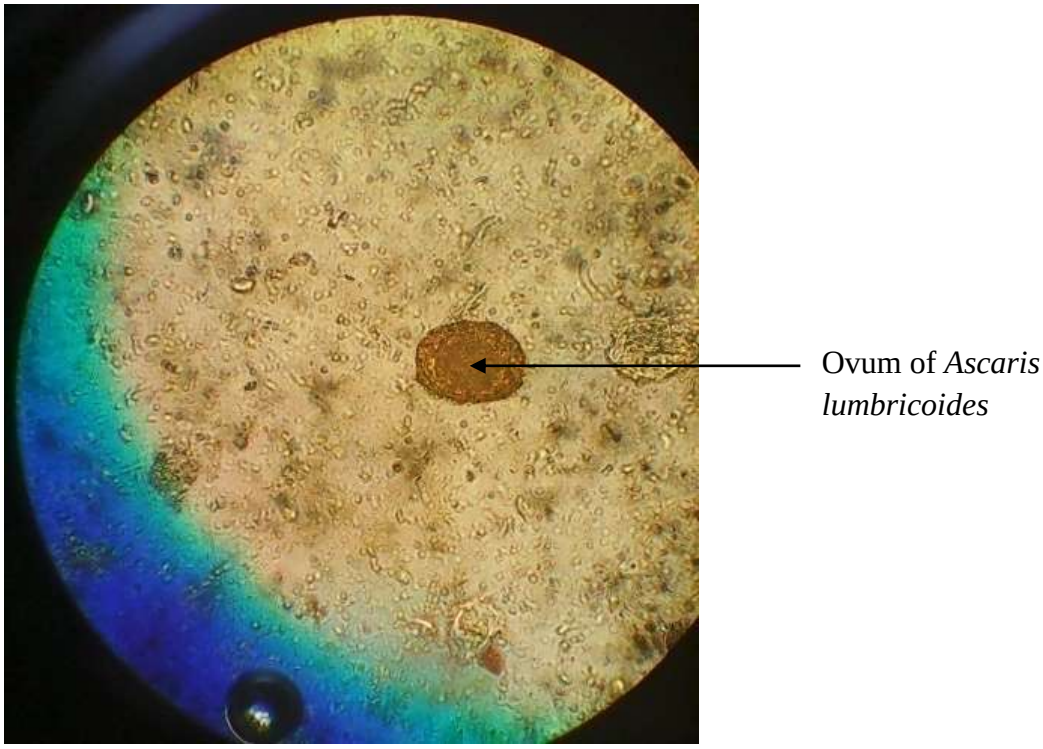

Plate V: Ovum of *Ascaris lumbricoides* in Wet Mount under 40x Magnification

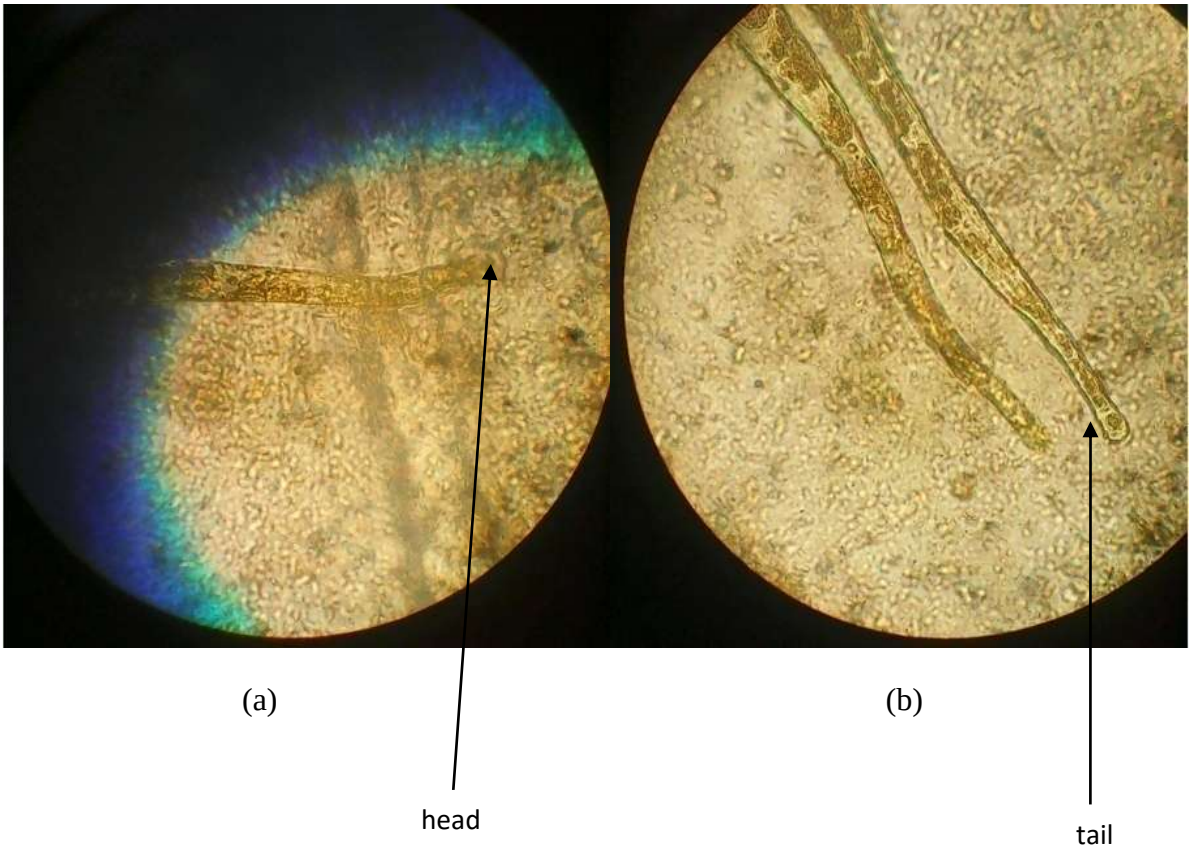

Plate VI: **Filariform of *Hymenolepis Nana* (a) Head (b) Tail in Wet Mount under 40xMagnification**

Plate VII: Ovum of *Enterobius vermicularis* in Wet Mount under 40x Magnification

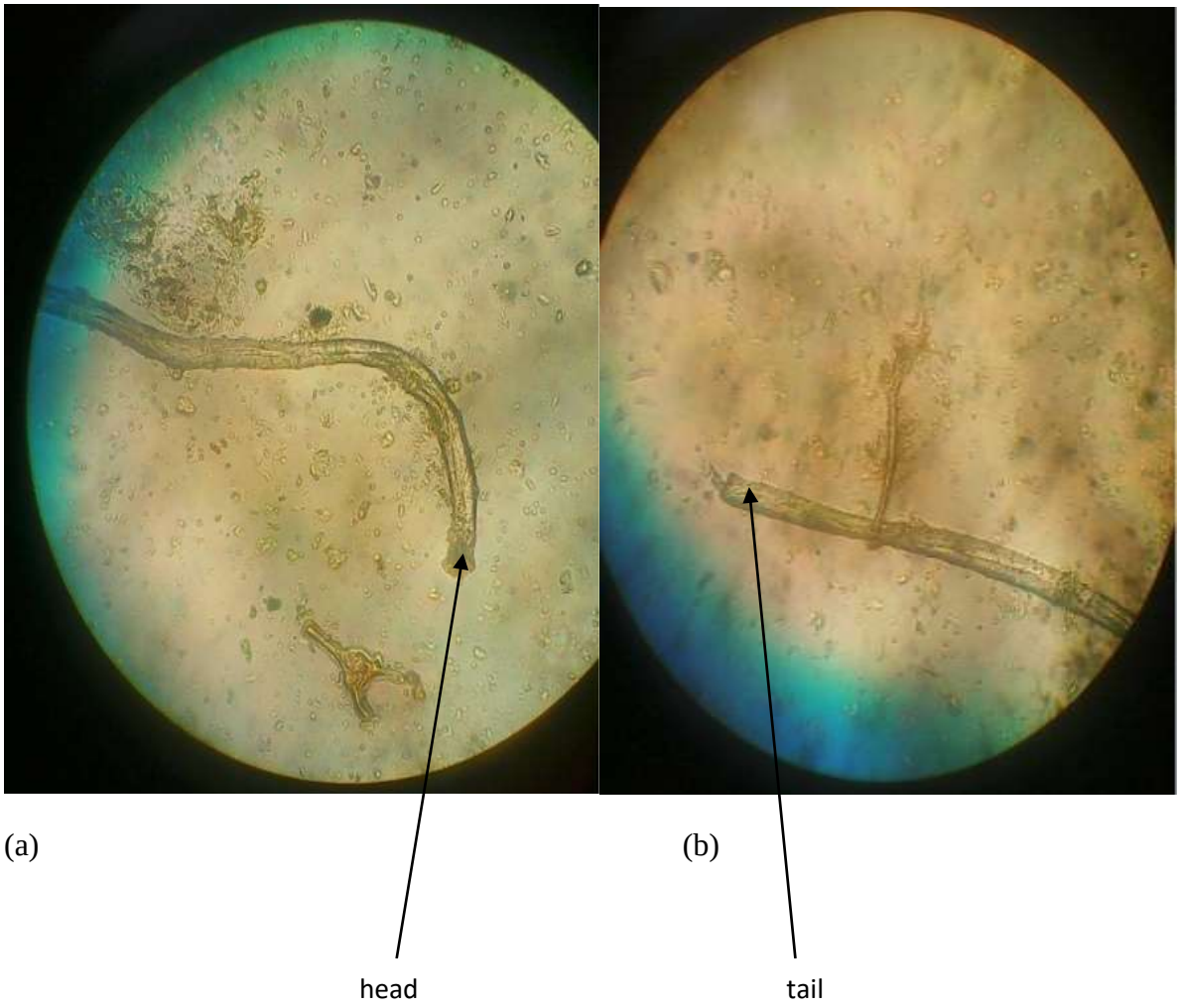

Plate VIII: Filariform of *Strogyloides stercoralis* (a) Head (b) Tail in Wet Mount under 40xMagnification

CHAPTER FIVE

5.0 DISCUSSIONS

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 a low sensitivity, therefore miss up to 50% of *Giardia* infections because of the intermittent shedding of the parasites which led to false negative result. 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).

The result of sensitivity and specificity of 100% and 91.5% respectively of the ELISA kit that was used taking microscopy as gold standard is in agreement with reports from other studies. Exactly the same result of 100% sensitivity and 91.5% specificity using RIDASCREEN ELISA kit a study carried out by Jahan *et al*. (2014) from children suffering from giardiasis was observed in India. Ahmed *etal*. (2014) in Bangladesh reported a sensitivity of 88.8% and 90.91% specificity on the detection of *Giardia lamblia* in ELISA assay. Al-Saeed and Issa (2010) had sensitivity and specificity of ELISA used as 76.4% and 100% respectively. A sensitivity and specificity of 100% and 94.8% respectively was also reported by Behr *et al*. (1996).

Direct microscopic examination needs experienced staff and is labour intensive. On the other hand, the benefit of direct examination is that it also detects other parasites. However, ELISA testing is easier, cheaper and faster compared with other serological tests and is useful for the rapid invesgation of a large number of stool specimens in laboratories (Al-Saeed and Issa, 2010).

The prevalence of giardiasis was higher in male children than in female for ELISA and microscopy respectively. This is in agreement with other researchers in Nigeria (Nyagnmee *et al*., 2006; Inabo *et al*., 2011). The higher prevalence in 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 outdoors activities like playing footballs and racing in a field infested with *Giardia* cysts. The prevalence of 14% in this study could be attributed to the study population used.

Giardiasis is said to be common in children than in adult but most literature revealed that is more in children between the ages of 5-10years. Due to constant exposure to infected surfaces as children advance in age, they have more contact with contaminants in the environment and lack personal hygiene. The result in this study is in accordance to the work of Thiongo *et al*. (2012) in Kenya, who reported 12.8% of Giardiasis in children below 5 years. In contrast, with Nyagnmee *et al*. (2006) in Nigeria reported 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.

These three studies have different settings, sample area and population study. One was hospital based study of children 0-5 years presenting with gastroenteritis in Kaduna Metropolis and the other was refugee camp dwellers of 0-19 years in Guma LGA in Benue State. Overcrowding, lack of personal hygiene, contaminated drinking water may be responsible for this higher prevalence of *Giardia lamblia*. The last studied on giardiasis in asymptomatic undernourished children from day care, nursery and primary school between the ages of 0-12 years in two Local Government Areas in Zaria Kaduna State. High risk of person to person transmission would have been the cause of the high prevalence. However,

similar result was reported by Hamza and Biu (2012) and Muhammed *et al*. (2014) with a prevalence of 10.5% and 14.3% respectively on the study of intestinal parasitic infections among patients attending a Tertiary Health Institution in Borno State, Northeastern part of Nigeria.

The prevalence of giardiasis among age group in this work shows no statistically significant. Although the age group 49-60 months were more infected with giardiasis and this was followed by the age group 37-48 months. This may be attributed to their increasing activities either at home or being in school at the playground more in contact with infected fomites and surfaces contaminated with *Giardia* cysts than the younger children. This finding is in agreement with Inabo *et al*. (2011) and previous reports or studies who reported higher prevalence of giardiasis in older children 5-10years. In contrast, the low prevalence of giardiasis observed in children of age group 0 -12 months may be explained by the reason of exclusive breast feeding of children less than 6 months old in this age group which reduces their chances of being infected by the disease. A hospital based surveillance study by Mahmud *et al.* (2001) in Egypt reported that breast feeding was protective against Giardial infection for infants up to 6 months of age.

The results of body mass indices in this study shows large number of children had normal weight for their height which is above the 5^{th} 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 as a result of differences in growth and development between a male child and a female child.

However, the mean weight of infected children in relative to their body mass indices statistically shows no significant differences. This finding is in agreement with Fraser *et*

al.(2000) who reported *Giardia lamblia* carriage in children not associated with higher rates of gastrointestinal or respiratory illness in Bedouin, Israel. 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 to *Giardialamblia* 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. 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 to the cause of infection of children to *Giardia lamblia* in this study were 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 to the cause of the infection/disease. Infected children that drank well water were found to have greater odd of acquiring giardiasis than others. Those that drank tap water and water from river also had an odd of acquiring the infection/disease but show no association. 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 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 but statistically not associated.

Children that had no exclusive breast feeding and none breast feeding also had the odd of being infected but shows no statistical association in this study. But children that were exclusively breasting fed show no odd of being infected by the disease. This result agreed

with the findings of Mahmud *et al*.(2001) in Egypt which said breast feeding can serves as a protection against Giardial infection. Children that attendance day care and those none attendance had the odd of being infected but statistically not associated in this study. It means day care and none school attendance are at risk of infection. Children that their mothers/caregivers did not wash their hands after defeacating or after the used of toilet showed odd of being infected but not statistically associated as well. This could be due to poor or lack of personal hygiene and can serve as a tool for source of infection in children.

Children whose mothers/caregiver had only quranic form of education alone and those with no any form of formal educational level had highest odd of acquiring the infection because they might lack good hygiene like washing of hands before eating and proper sanitations, changing of diapers and eating of contaminated fruit since their mothers/caregivers might lack the knowledge of hygiene and good environmental sanitation that could exposed them to the disease. Also, contamination from feeding utensils used by these mothers can also lead to the cause of infection in children.

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 *Entamoeba coli* with the highest prevalence followed by *Giardia lamblia* and the least was *Strongyloides stercoralis*. 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. 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

that had worked and recorded high prevalence of intestinal parasites in children. Similar result was also reported in Kenya with children above 3years found to be more infected with the above mentioned parasites (Thiongo *et al*., 2012). These parasites could cause iron deficiency anemia in children which could lead to loss of weight in children. Also, male were more infected than female children.

The result of regression indicates that there is a significant increase in infection prevalence with increase in age. This is probably because children are more at behavioural risks as they grow. Parental care becomes less in children above 2 years old compared to the infants. This is with respect to what they eat, school hygiene, water source and their increased in playing habit. Hence, the increase in intestinal parasitic infections in children is age dependant.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Giardia lamblia had the 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 sensitive than microscopy. Male children had higher prevalence than female. However, 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 age groups, 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 higher prevalence of underweight to healthy weight body mass indices.

The risk factors that were statistically associated to the cause of disease/infection of *Giardia lamblia* in this study were source of water, malnutrition and maternal/caregivers educational levels. Which had odd ratio greater than 1 and significantly associated at p-value ≤ 0.05

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

6.2 RECOMMENDATIONS

1. The use of ELISA for the diagnosis of *Giardia lamblia* in stool samples should be encouraged in the laboratories. It is cheaper and faster, does not need a long time experience or trained personnel.
2. Parents should be enlightened on the balance diet meal that should be given to children under five years in order to avoid malnutrition in children and reduce their susceptibility to infectious diseases.
3. Good hygiene and proper sanitation should be encouraged and emphasized in our localities through media and other communication devices.
4. Washing of children's hands at home and even in schools before and after children's break should be encouraged.
5. Further research should be carried out in this location on children above 5 years old and the possibilities of zoonotic transmission should be considered in order to confirm the level of cross species transmission of this infection in children.

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APPENDIX I: Ethical Approval Letter from Kaduna State Ministry of Health

MINISTRY OF HEALTH, KADUNA STATE

All Communication to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Telephone: 234-2480048
Website: ttp://www.kmoh.kd.gov.ng
Email: info@moh.kd.gov.ng

Independence Way,
P.M.B 2014
Kaduna
Kaduna State, Nigeria.

MOH/SOM/744/VOL.3/324

27th July, 2015

NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW

PREVALENCE OF [illegible] AND ITS ASSOCIATION WITH BODY MASS INDEX (BMI) IN PAEDIATRIC PATIENTS (0-5) YEARS SUFFERING FROM GASTROENTERITIS IN [illegible] METROPOLIS

Name of Principal Investigator: SHIRU, MONSURAT TITILAYO
Address of Principal Investigator: Dept. of Microbiology, Faculty of Science, Ahmadu Bello University Zaria, Kaduna State.
Date of receipt of Application: 12th June, 2015
Date of Ethical Approval: 15th July, 2015

This is to inform you that the Research described in the submitted Protocol, the Consent forms, advertisements and other participant information materials have been reviewed and given full approval by the Health Research Ethics Committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

DR. S. M. JATAU
Chairman
Health Research Ethics Committee

Appendix II: Sample of Questionnaire

DEPARTMENT OF MICROBIOLOGY. FACULTY OF LIFE SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA

PREVALENCE OF GIARDIA LAMBLIA IN ASSOCIATION WITH BODY MASS INDICES OF CHILDREN 0-5 YEARS PRESENTING WITH GASTROENTERITIS IN KADUNA METROPOLIS, NIGERIA

Name Parent/ Guardian: _____ Date: _____

Residential Address: _____

Name of Hospital: _____

PATIENT'S SERIAL NO.: _____ **CODE: _____**

1. INFORMATION ON THE CHILD

i. Sex (Please tick appropriately only once)

MALE ☐ FEMALE ☐

ii. Age of child (Please tick appropriately only once)

0-12months	13-24 months	25-36 months	37-48 months	49-60 months
☐	☐	☐	☐	☐

2. SOURCE OF DRINKING WATER

Please tick appropriately

Tap	Well	Bore hole	Sachet water	Table water	River water
☐	☐	☐	☐	☐	☐

3. FEEDING

Please tick appropriately only once

Exclusive breast feeding	No-exclusive breast fed	None breast feeding
☐	☐	☐

4. SCHOOL ATTENDANT

Please tick appropriately only once

Day care ☐ Nursery ☐ Primary ☐ Non attendant ☐

5. NUTRITION (WEIGHT FOR AGE)

Please tick appropriately only once

Normal nutrition ☐ Moderate malnutrition ☐ Severe malnutrition ☐

6. HAND WASH AFTER TOILETS

Please tick appropriately only once

No ☐ Yes ☐

7. MOTHERS/ CAREGIVERS LEVEL OF HIGHEST EDUCATION

Please tick appropriately only once

Quranic Education only ☐ Primary Education ☐

Secondary Education ☐ Tertiary Education ☐

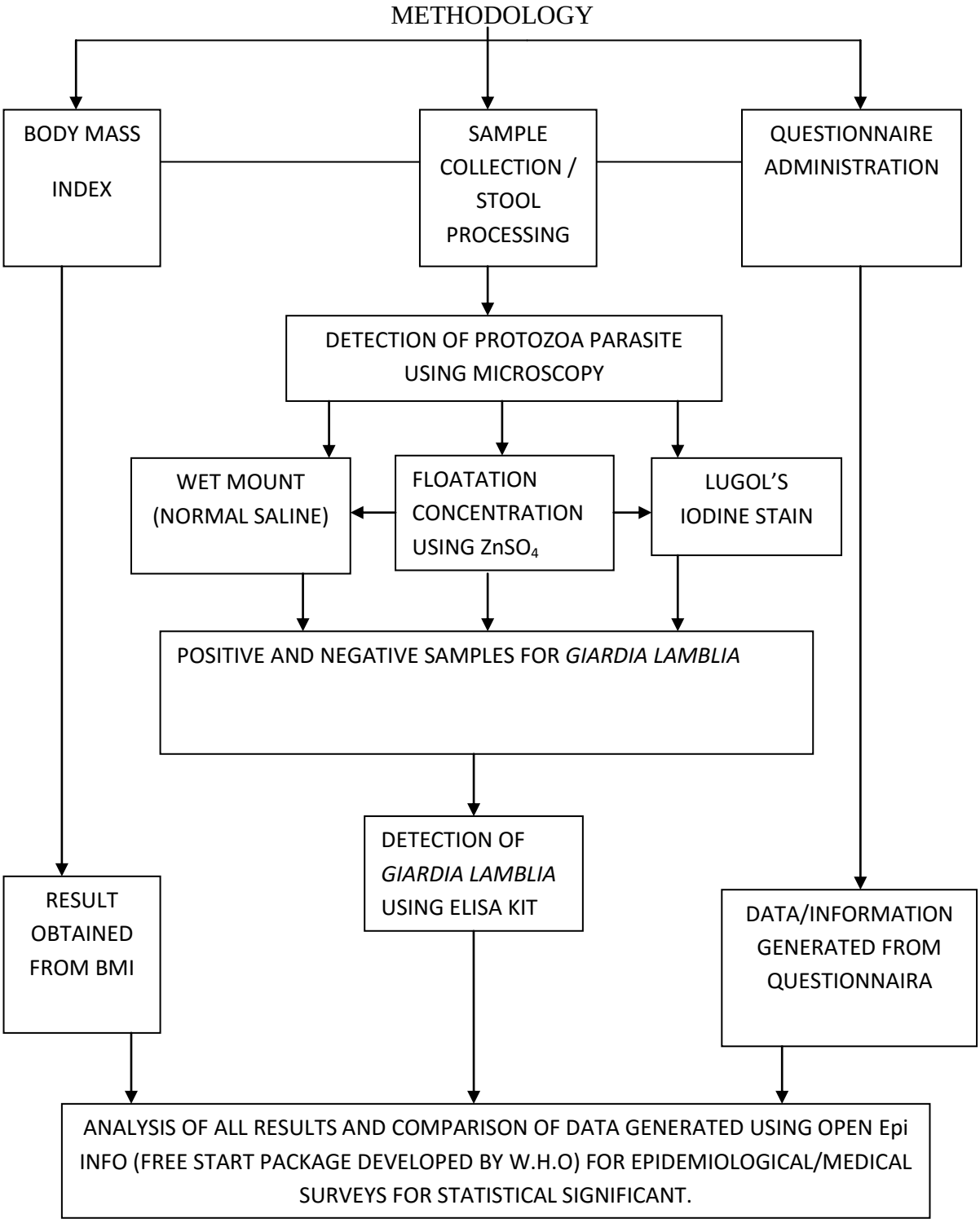
Quranic/ Formal Education ☐ None of the above ☐

8. ANTHROPOMETRIC MEASUREMENTS

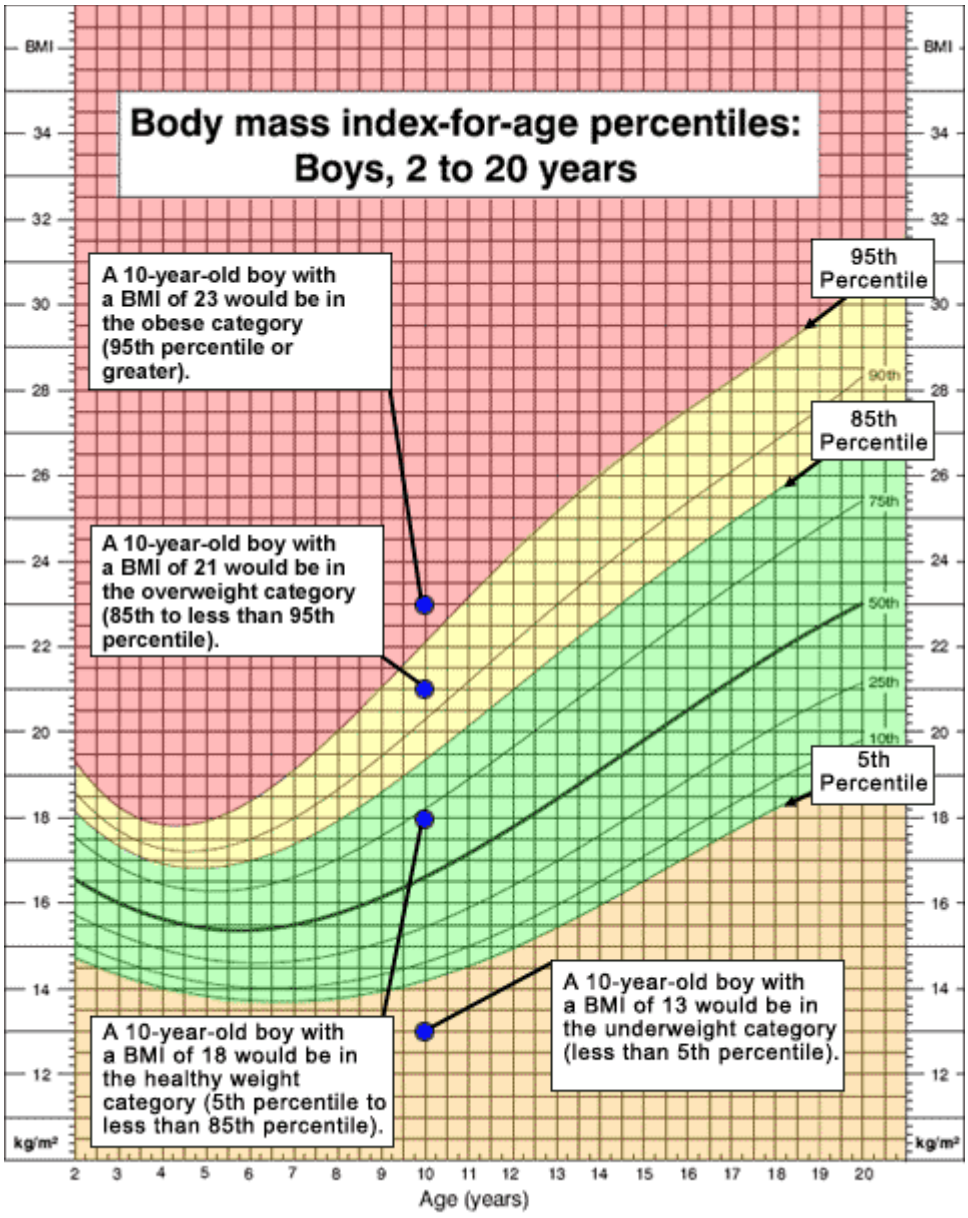
Weight of Child ☐ Height of Child ☐ BMI of Child ☐

Thank you for your help.

Appendix III: **Summary for Methods used.**

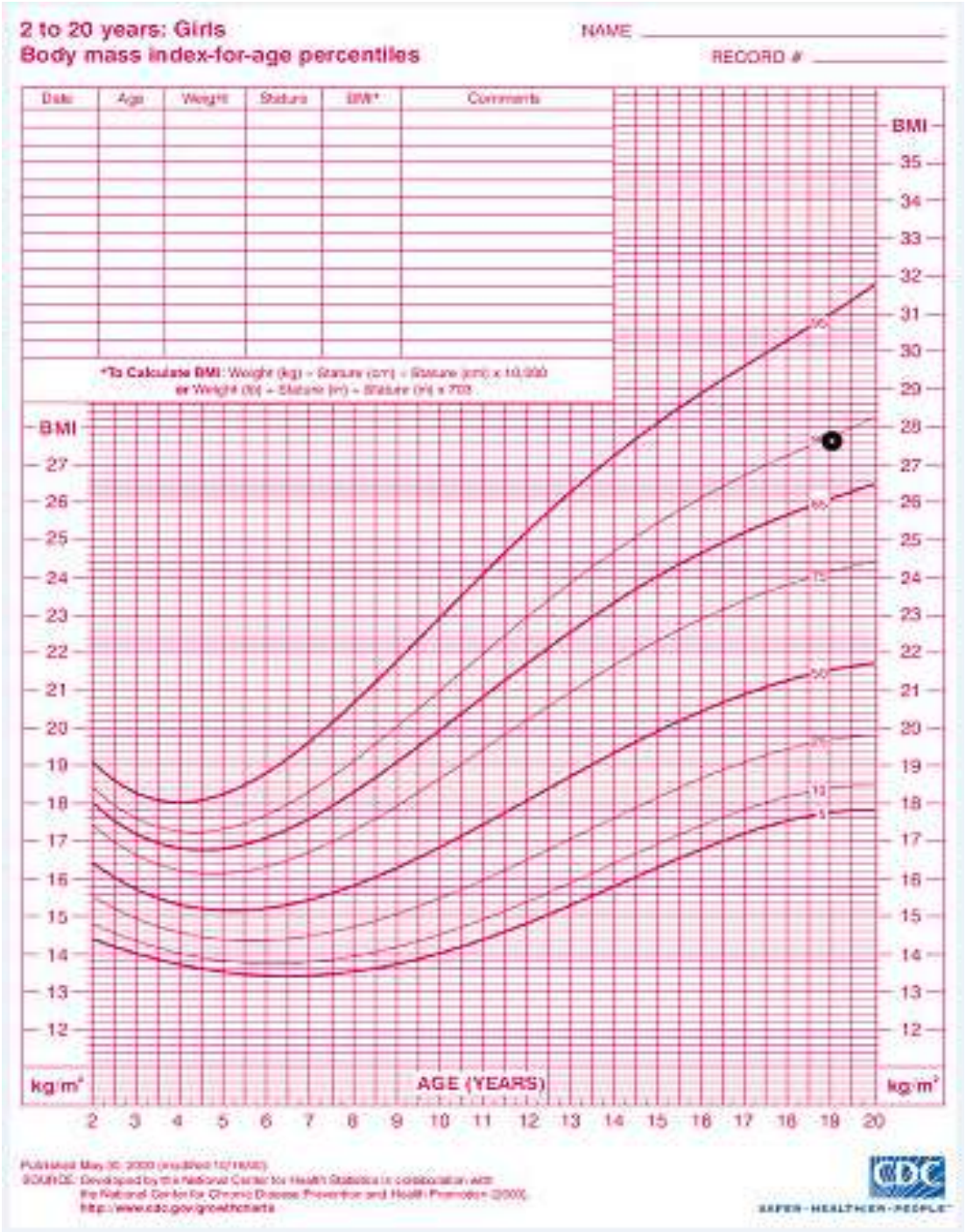


AppendixIV: **BMI Chart for Age Percentile for Boys**



(Source: CDC, 2014)

AppendixV: **BMI Chart for Age Percentile for Girls**



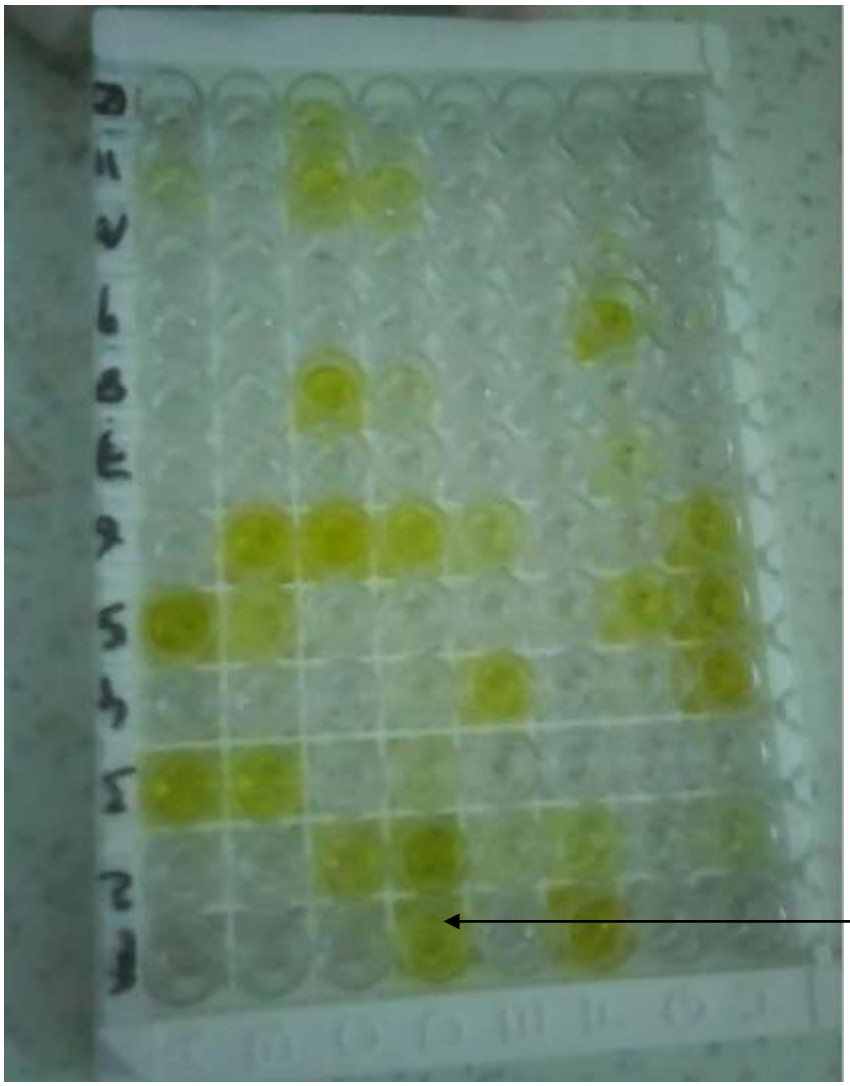
(Source: CDC, 2014)

AppendixVI: BMI-For-Age Weight Status Categories and Corresponding Percentiles

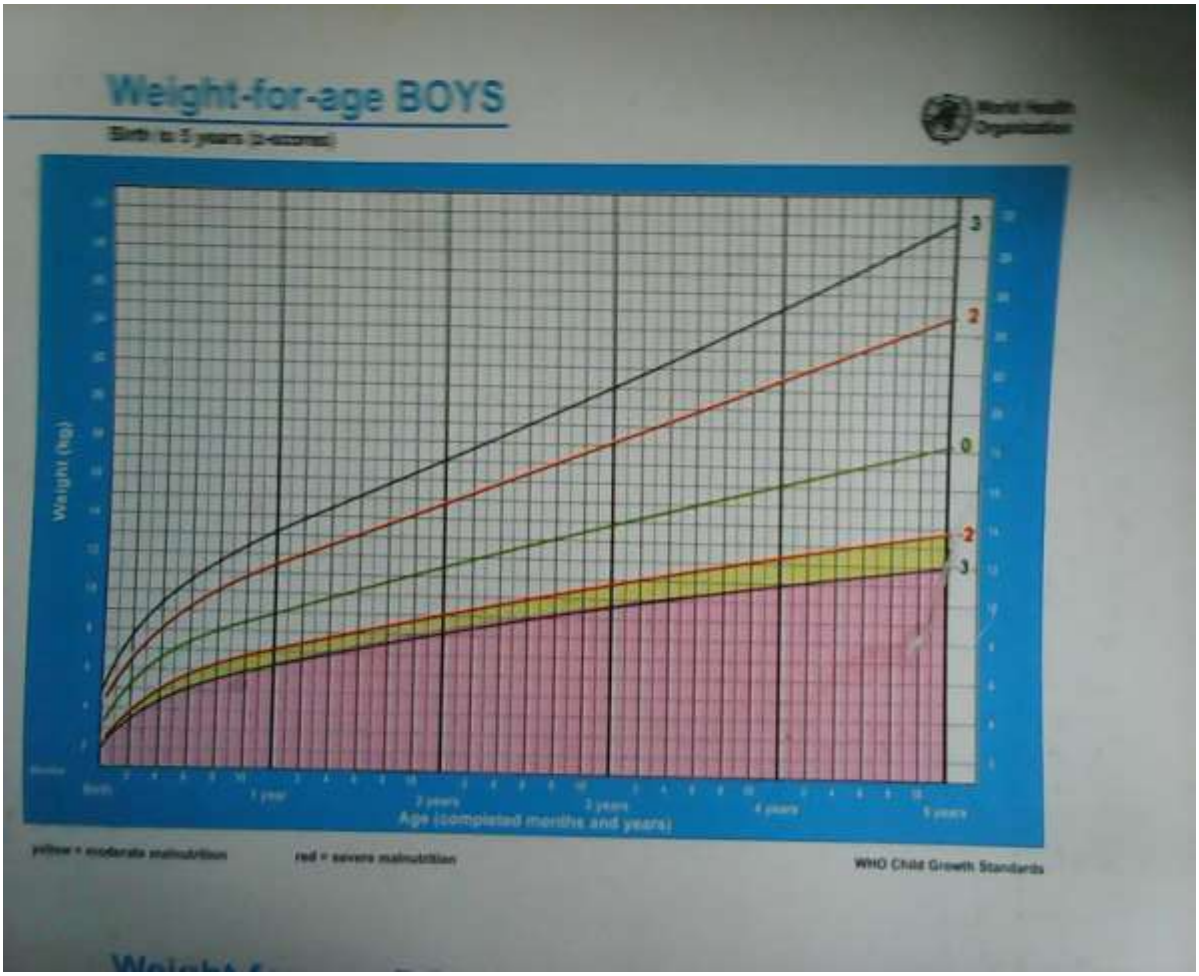
Weight Status Category	Percentile Range
Underweight	Less than the 5th percentile
Healthy weight	5th percentile to less than the 85th percentile
Overweight	85th to less than the 95th percentile
Obese	Equal to or greater than the 95th percentile

(Source: CDC, 2014)

AppendixVII: **ELISA Microplate Result of *Giardia* Positive Sample**



Appendix VIII: **Nutritional Status Using Weight for Age (Z Scores) for Boys**



(Source: WHO, 2007)

Appendix IX: Nutritional Status Using Weight for Age (Z Scores) for Girls.

Source: WHO, (2007)