GIARDIASIS AMONG PRIMARY SCHOOL CHILDREN IN RURAL AND URBAN AREAS OF BENIN CITY, EDO STATE

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THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES,UNIVERSITY OF BENIN,BENIN CITY, EDO STATE IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE IN MEDICAL LABOURATORY SCIENCE

APRIL, 2021

DECLARATION

I declare that this thesis titled "***Giardiasis* among primary School children in rural and urban areas of Benin City, Edo State"** was written by me and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where states otherwise by reference or acknowledgement the work presented is entirely my own.

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Ijanmi .O. Morine **Date**
Student

CERTIFICATION

This is to certify that this thesis titled "*Giardiasis* among primary School children in rural and urban areas of Benin City, Edo State'' was carried out by Ijanmi Omoikhose Morine with matriculation number PG/BMS171499 under my supervision: DR. F.O. AKINBO of the department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, Edo State; Nigeria.

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DEDICATION

This work is dedicated to the Almighty God, the author of knowledge and wisdom, for his countless love.

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My utmost thanks go to God Almighty for the life He has given unto me and the grace to overcome throughout the period of my research work.

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May almighty God bless you all.

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ABSTRACT

Giardiasis is a disease of public health importance, a common cause of acute and chronic human diarrheal. In some developing countries, infection and mortality rates among young children can be quite high. This study was carried out to determine *Giardia lamblia* infection among children in rural and urban areas in Benin City, Edo State, A comparative study was done, using the microscopy method and ELISA technique, 288 primary school children consisted of (194) males and (94) females were enrolled in this study, Stool specimen was collected from each participant into a sterile universal bottle and sent for analysis to the laboratory department of City of Hope Medical Centre, Benin city. The age of participants ranged from 6-14years. A well-structured questionnaire bothering on the bio-data and socio-demographic characteristics was administered on each participant prior to the collection of Specimen. Informed consent was obtained from the parent or guardian of each participant. The stool specimens were analyzed using microscopy and ELISA technique. A prevalence of 5.6% of *Giardia lamblia* infection was observed among children in the Study Areas. Comparative studies of the microscopy method and ELISA techniques showed a p-value of < 0.05 (0.001) which was significant. There was a significant difference in the age group 10-11 years old presenting with the highest prevalence of 13.7% in comparison to other age groups with p-value < 0.05 (0.014). Hand hygiene was highly prevalent (12.5%) with p-value <0.05(0.000) which was significant, which showed that those who observed poor hand hygiene are with the high odds of being infected than those who practice good hand hygiene. Epidemiological factors such as Residential locations, settlement type, type of toilet, proximity of kitchen to toilet facility, diarrhoea, and Food source significantly affected the prevalence of *G. lamblia* infection. Increased Awareness, personal hygiene and good sanitary practices, particularly among mothers and children, should be encouraged as this would curb the spread of giardiasis. ELISA with sensitivity of 95% to 100% and specificity over 90% when compared with direct microscopy provides a relevant alternative method to the routine ova and parasite examination in diagnosing giardiasis. Based on this, detection of giardiasis should not solely be based on microscopy; more effective methods such as ELISA could be employed.

CHAPTER ONE
1.0 INTRODUCTION

1.1 Background of study

Giardia Intestinalis is synonymous to *Giardia lamblia* and *Giardia duodenalis* a cosmopolitan flagellated parasite, is of utmost importance based on the fact that it's the common cause of acute and chronic human diarrheal illness worldwide, the causative agents for giardiasis which colonizes and reproduce in the small intestines resulting to significant morbidity and mortality in the world, particularly in developing ones ,occurring more in children than in adults has become a major concern in public health.(Huston *et al*., 2006; Simner and Kraft 2017; Runsey and Waseem, 2019). Some of the recommended ways to reduce diarrhoeal disease are providing safe drinking water, safe waste removal especially the use of facilities to dispose faeces in a sanitary way and improved hygienic standard (WHO, 1992).

History of the *Giardia lamblia*

In 1681, the first description of *Giardia* was by Antonie van Leeuwenhoek who in a letter to Robert Hooke referred to "animalcules" resembling Giardia trophozoites in his stool. In 1859 Vilém Lambl a Czech physician discovered something similar, he observed and saw *Gardia* trophozoites in the stool of his pediatric patient, Lambl termed the organism *Cercomonas intestinalis* which was later published. Furthermore, in 1888 Raphaël Blanchard renamed the parasite *Lamblia intestinalis* in Lambl's honor. In 1915, the organism was later renamed

by Charles Stiles, *Giardia lamblia* in honor of both Lambl and Professor Alfred Mathieu Giard of Paris. In 1921, A detailed description of the parasite morphology was published by Charles E. Simon. (Feely *et al* ., 2013; Maria 2014; Ford 2015 ; Despommier *et al*.,2019)

Epidemiology

Giardiasis is the most common cause of parasitic gastro-intestinal disease and it is estimated that up to two hundred million people are chronically infected with *Giardia lamblia* globally in the world (Feng and Xiao, 2011). Giardiasis prevalence rates have been reported to vary from (2% - 5%) in developed countries to (20%- 30%) in developing countries, Factors such as geographic area, urban or rural setting of the society are at risk where this living areas have an history of drinking tap water

(Filtered or unfiltered surface water), age group composition such as ages 0–9 years and 45–49 years. surveillance data have shown that this bimodal age distribution are more susceptible to giardiasis without gender preferences and socio-economic conditions (Kotloff *et al*., 2013, Painter *et al*., 2015; Pijnacker *et al*., 2016).

This variable makes the illness consistently high among young children from day-cares and nurseries, infecting children under 5 years old and their caregivers (Gagnon *et al*., 2006; Psaki *et al,* 2014).

Transmission

The reproductive cycle of Giardia includes non-motile cysts and motile trophozoites, both the trophozoites and cyst may be excreted in stool, these stages are responsible for clinical illness and transmission of the disease. The cysts can survive long-term and become immediately infectious when released into the environment via faeces and can remain infectious for up to almost 3 months, thriving and reproducing in cool, damp areas, especially in river or any water source (Ordóñez-Mena *et al*., 2018). Just like other parasites; the cysts are responsible for transmission of Giardia enteritis infection.

A Survey showing, an investigation of 242 outbreaks, affecting 41,000 persons, reported that most outbreaks resulted from waterborne (74.8%), foodborne (15.7%), person-to-person (2.5%), and animal contact (1.2%) transmission, with waterborne outbreaks having the highest number of incidence rates, in terms of number of cases per outbreak (Adam *et al*., 2016). This depicts that waterborne transmission is the most common cause of *Giardia lamblia* infections that can easily be transmitted, the standard industrialized world model for delivery of safe drinking water and sanitation technology, is not available in most of the developing world (Yusuf *et al*., 2007). it can also be transmitted by fecal-oral transmission with contaminated food or direct fecal-oral contact among family members, person-to-person contact, and sexual transmission (oral-anal contact or animal-to-person transmission) which may be presented either as an acute

condition, which may later advance to chronic illness. Those that can easily be vulnerable or be at high risk of having giardiasis, include

- Diaper-age children who attend day-care centers ;
- adults that work in child-care organizations or day-care centers ;
- institutionalized individuals such as nursing homes ;
- Homosexual individuals (i.e. men who have sex with men) without using condoms
- Immunocompromised individuals such as chronic variable immunodeficiency, hypogammaglobulinemia, HIV, immunosuppressed individuals, cystic fibrosis (Beltrami *et al*., 2005; Mascarini and Donaliso, 2006 ; Duffy *et al*., 2013)

Fecal-oral transmission

This is also a significant mechanism of transmission; it is the majorly responsible for the outbreaks in day-cares and nurseries with poor sanitary conditions. This outbreak is significantly high between young children, who have close contact or are more likely to pass the parasite fecal-orally at day-cares than at home. For example, in the Netherlands, where around half of preschool children are cared for in day-care centers, a mean of 2.5 days a week, children at day-care centers are twice as likely to test positive to *Giardia lamblia* as their home-care counterparts, infecting around 4.2% of children (Enserink *et al*., 2014; Pijnacker *et al*., 2016).

Sexual transmission

Giardia lamblia infection in the form of oral-anal transmission and fecal-oral transmission predominantly occurs among men who have sex with men. Currently, a large body of publications exists that have led to a deeper insight or understanding of how giardiasis can easily be a sexually transmitted infection. These studies have proved that the prevalence rates of giardiasis among men who have sex with men ranges from 2 - 30% (Escobedo *et al*., 2014). Although giardiasis is not a major cause of AIDS-associated diarrhea, however the prevalence of giardiasis, and the chronicity of symptoms, is susceptible more in patients whose immunity has been immunocompromised like AIDS patients, especially in developing countries (Espelage *et al*., 2010).

Some infected individuals may remain asymptomatic, the most common symptoms vary from foul-smelling diarrhea, greasy stools, nausea and vomiting, to flatulence, bloating and abdominal cramps; Laboratory diagnosis is confirmed through stool sampling (Shane *et al.,* 2017).

Impacts of giardiasis on children.

Globally in the world, *Giardia lamblia* is one of the most common parasitic human diseases affecting about 280 million people worldwide with symptomatic giardiasis, with rates as high as 7% in developed world and 30% in the developing world (Esch and Peterson, 2013). However, giardiasis is endemic and commonly reported in children aged 1-10 years old, infecting more children than adults, particularly those that are malnourished, where sanitation and health care services are relatively poor (kotloff *et al*., 2013; Minetti *et al.*, 2016) Children are more

likely to come in contact with feces, especially those that wear diapers, those that are in toilet training or those that spend time in a child care center.

Giardiasis, is a parasitic infection caused by *Giardia lamblia* which uses enzymes that break down proteins to attack the villi causing enterocytes damage and loss of the epithelial cells, this alters the epithelial barrier thereby affects the digestive tract (stomach and intestines), The microscopic parasite attaches itself to the lining of the small intestines in humans, where it interferes with the body's absorption of fats and carbohydrates from digested foods, the pathology results in nutrients malabsorption, diarrhea, steatorrhea, vomiting and abdominal pain. This intestinal parasite can be spread to children on dirty hands, on the soiled surfaces of toys and bathroom fixtures, and in contaminated water or food (Pires *et al*., 2015). However, giardiasis can occasionally lead to severe dehydration and other dangerous complications. The illness usually begins with severe watery diarrhea, without blood or mucus. Infants, children with chronic illnesses and children taking immune-suppressing medications are more susceptible and vulnerable to the infection (Donowitz *et al*., 2016).

1.2 Statement of problem

Giardia lamblia is the major cause of acute diarrhea resulting in acute to chronic symptoms leading to about 5% mortality rate in children and this disease is recorded worldwide. However, a high number of infected persons are recorded in overcrowded developing countries where there is poor sanitary condition and quality water are not put in place. Majority of the residents of Benin metropolis,

Edo State, do not have access to portable water provided by the government. Hence they depend on their personal provision for drinking water, There is need for this study to be extensively highlighted and research done as there is little known about the occurrence and prevalence in Benin city; Edo state. This study provides broad insight on the history, transmission, impact, prevalence, prevention and associated risk factor of *Giardia lamblia* amongst primary school children in rural and urban areas of Benin City, Edo State.

1.3 Justification of the study

They are few reported data on Gardiasis in Nigeria (Chijioke *et al.,* 2018). Therefore, this research is carried out to fill the existing knowledge gap by determining Gardiasis among Primary school children in Rural and Urban areas of Benin City, Edo State.

1.4 Research hypothesis

i. NULL HYPOTHESIS: There are no significant differences between the children of primary school age with a higher prevalence of *Giardia lamblia*.

ii. ALTERNATE HYPOTHESIS: There are significant differences between the children of primary school age with a higher prevalence of *Giardia lamblia*.

i. NULL HYPOTHESIS: There are no significant differences in the risk factors associated with *Giardia lamblia* infections in Benin metropolis.

ii. ALTERNATE HYPOTHESIS: There are significant differences in the risk factors associated with *Giardia lamblia* infections in Benin metropolis.

i. NULL HYPOTHESIS: There are no significant differences in the microscopy method in comparison to the ELISA technique for *Giardia lamblia* infection.

ii. ALTERNATE HYPOTHESIS: There are significant differences in the microscopy method in comparison to the ELISA technique for *Giardia lamblia* infection.

1.5 Aim of study

The aim of this study was to determine the prevalence of *Giardia lamblia* infection among children in rural and urban areas in Benin City, Edo State.

1.6 Specific objectives

The specific objectives of this study were to:

1. determine the prevalence of *Giardia lamblia* among children in rural and urban areas of Benin City, Edo State.
2. determine the risk factors associated with *Giardia lamblia* infections in Benin metropolis.
3. Compare the microscopy and ELISA techniques for the laboratory diagnosis of *Giardia lamblia* Infection

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of *Giardia*

Giardia first description was attributed to the microscopist Antonie van Leeuwenhoek (1632 to 1723), Vilem Dusan Lambl (1824 to 1895), a Czech physician, was credited with the discovery in 1859 of the flagellate *Giardia*. The name *lamblia* was given to the species by Blanchard in 1888 (Kotloff *et a*l., 2013). Protozoan parasites within the genus *Giardia* have a long history within Veterinary Medicine. Most species that infect domestic animals were initially described as separate species in the1920s: *Giardia caprae* (Tzanidakis *et al*., 2014) from sheep and goats; *Giardia bovis* from cattle; *Giardia equi* from horses; *Giardia canis* (Bouzid *et al*., 2015) from dogs; and *Giardia felis* from cats synonym, *Giardia cati* (Sprong *et al*., 2009; Bouzid *et al*., 2015).

During the first 50 years that these parasites were known to infect animals, it was difficult to assess their effects because of the numerous gastrointestinal parasites co-inhabiting these hosts. As the prevalence of other enteric parasites ‘declines, the effects of *Giardia* infection alone are becoming better understood. Interest in this group of protozoa began only, when *Giardia lamblia* was isolated from mammal, bird and amphibian hosts (Kulda and Nohynkova, 1978). Initially, assignment of a species name to *Giardia* was based on the animal host species from which the organism was isolated. Filice (1952) rejected this concept of host specificity and proposed to use the morphology of the

trophozoite microtubular organelles known as the median body to classify species into three groups: (i) the amphibian group (*G. agilis*), which has a long teardrop-shaped median body; (ii) the rodent and bird group (*G. muris*), which has two small, rounded median bodies; and (iii) the human group Gardia duodenalis = lamblia= intestinalis), in which the single or double median bodies resembles the claw of a claw hammer.

2.2 Morphology of Giardia

Giardia lamblia has two morphological stages: the trophozoite and the cyst. The trophozoite is pear shaped, with a broad anterior and much attenuated posterior. It is 10-12µm long and 5-7µm wide, bilaterally symmetrical, and has two nuclei. It is also relatively flattened, with a large sucking disk on the anterior ventral side, which serves as the parasite‘s method of attachment to the mucosa of the host (Ryan, 2018).

The trophozoite also has two median bodies and four pairs of flagella (anterior, caudal, posterior and ventral). The *G. lamblia* cyst is egg-shaped, and measures 8-14µm by 7-10µm. After encystation, each organelle duplicates, so each cyst contains four nuclei, four median bodies, eight pairs of flagella--although these organelles are not arraigned in any clear pattern. Upon excystation, each cyst produces two trophozoites (Cernikova *et al*., 2018), the cysts secreted in fecal material are effectively protected from environmental stress like UV by the cyst wall and can survive several weeks in fresh water (Einarsson *et al*., 2015).

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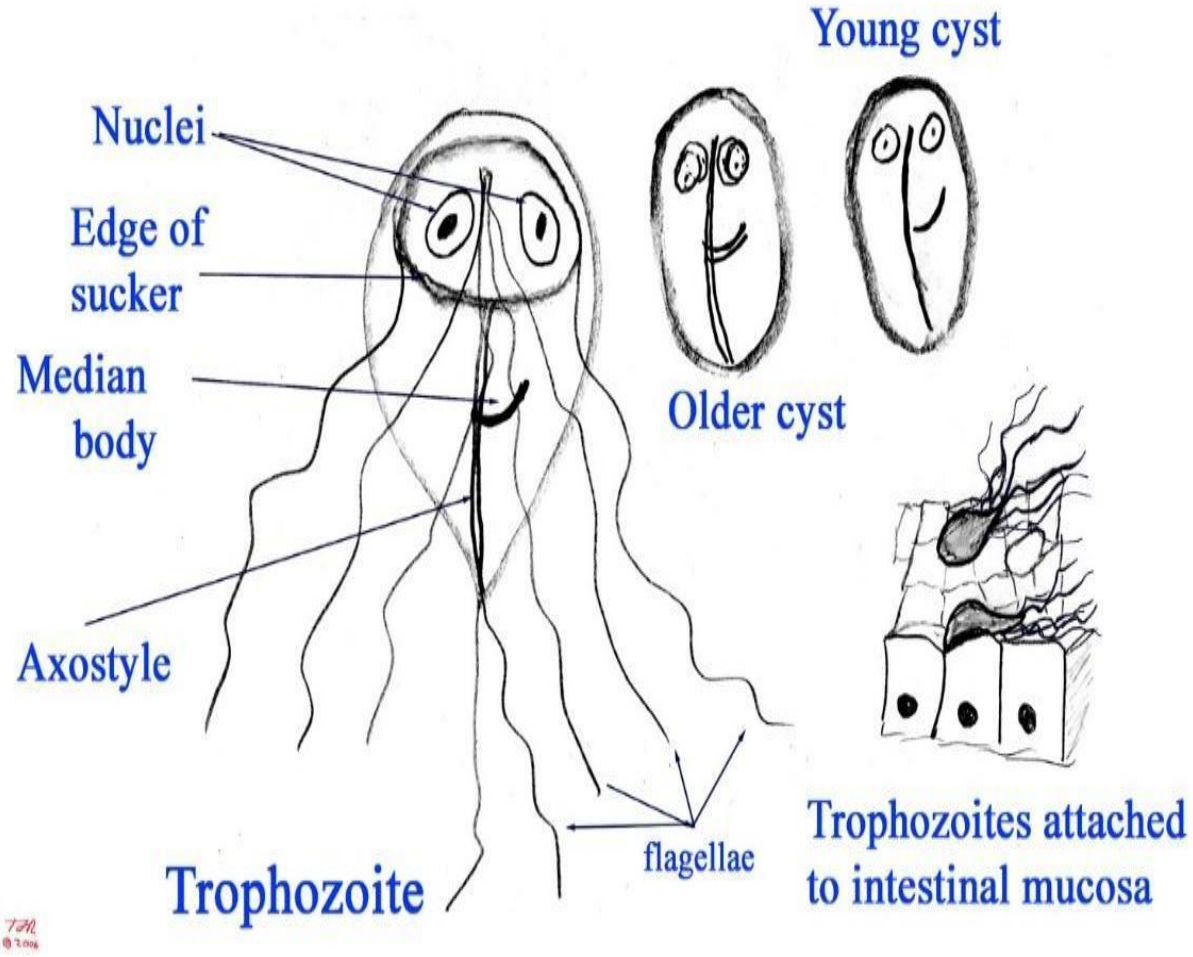

Plate 2.1: *Giardia* trophozoite and cysts (CDC, 2010).

2.3 Taxonomical Classification of *Giardia*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Subphylum	Mastigophora
Class	Zoomastigophora
Order	Diplomonadida
Family	Hexamitidae
Genus	*Giardia*
Species	*lamblia*

(Monis *et al*., 2009)

2.4 Pathology of *Giardia*

Pathology and clinical signs result from both the direct action of the parasite and the body‘s response to it (Kirkpatrick, 1987). When signs occur, they are related to mal-digestion and malabsorption. Studies of pathogenesis in animals are limited, most of our assumptions are deduced from the knowledge of human infections (Barr, 2006). Proposed mechanisms include epithelial cell apoptosis, barrier dysfunction. The histopathological changes occurring at the mucosal sites range from minimal to severe enough to cause enteropathy with enterocyte damage, villus atrophy, and crypt hyperplasia (Ferguson *et al.,* 1990.). The reasons for these variations are similar to those mentioned above as possible

factors contributing to the variation of clinical manifestation. Shortly after the trophozoites leave the stomach of their new host in response to low pH, excystation will take place (Faso and Hehl, 2011). Using their flagella and ventral disc, trophozoites released in the upper part of the small intestine move to the microvillus-covered surface of the duodenum and jejunum, where they attach themselves (Einarrson *et al.,* 2016), and play a role in the onset of the pathology (Inge et al.,1986; Arora and Arora, 2010).The suction force created by this mode of attachment may damage the microvilli and interfere with the process of food absorption. Eventually, the rapid multiplication of the trophozoites by binary fission creates a physical barrier between the intestinal epithelial cells and the lumen of the intestine, interfering with the process of absorption of nutrients (Hill and Nash, 2011).

2.4.1 Host Immunity against *Giardia*

Accumulating experimental evidence suggests that Giardia infections are also capable of modulating pro-inflammatory responses to other stimuli via several mechanisms. Observations that *Giardia* infections can protect against the development of diarrheal disease are consistent with the immunomodulatory capabilities of the parasite. Indeed, acute gastrointestinal inflammatory responses represent a collection of cellular and humoral effector responses and involve a variety of different cell types and mediators; several of these have been shown to contribute to the development of diarrheal disease. For instance, infection with entero-haemorrhagic *Escherichia.coli* causes chloride hyper-secretion, a major

driving force for diarrheal disease, via mechanisms that require Polymorphonuclear infiltration (Elliot *et al*., 1994; Scott *et al*., 2004).

Research has demonstrated that certain *Giardia* infections are capable of attenuating recruitment of pro-inflammatory leukocytes and decreasing nitric oxide production (Lee *et al*., 2012). In addition, evidence is accumulating that Giardia infections may modulate other pro-inflammatory events. However, these mechanisms have not been fully characterized. The following sections will describe the immunomodulatory mechanisms of Giardia and describe how this may result in the attenuation of diarrheal disease during *Giardia lamblia* co-infection (Wampfler *et al*., 2014).

2.4.2 *Giardia* and the Intestinal Mucus Layer

The entire gastrointestinal tract is lined with a layer of mucus of varying thickness with a structural backbone comprised of mucin glycoproteins dissolved in luminal water. In the colon, this layer can be further subdivided into two separate layers: a dense, inner mucus layer largely devoid of bacterial populations and an outer, loosely packed outer layer containing various bacterial populations (Johannson *et al*., 2008). In the intestinal tract, the primary mucus constituent is the mucin-2 (MUC2) protein (Johannson *et al*., 2011; McGuckin *et al*., 2011). Preliminary research has demonstrated in vivo Giardia assemblage B GS/M isolate infections in mice damages the small intestinal mucus layer by degrading the MUC2 protein and inducing the hyper secretion of mucus in the small intestine and colon, resultantly leading to mucin depletion from goblet cells; this culminated in a

weakened mucus layer and facilitated disease (Pestechian *et al*., 2014). Furthermore, studies monitoring mucus disruption during in vivo Giardia GS/M infections have observed an increase in bacterial translocation across the epithelial barrier, but this was not associated with an increase in pro-inflammatory markers at the point of acute infection (Hasnian *et al*., 2010; Chen *et al*., 2013). Separate in vivo studies have demonstrated those pro-inflammatory enteropathogens, such as *H. pylori, Entamoeba histolytica*, and *Trichurismuris*, alter the mucus layer and this contributes to the initiation or exacerbation of gastrointestinal disease (Tanaka *et al*., 2003; Kissoon *et al*., 2013). Similarly, modulation or aberrant assembly of the mucus layer is often associated with intestinal inflammation and increased expression of pro-inflammatory cytokines including interleukin (IL)-1β, IL-4, IL-6, CXCL8 IL-13, and TNF-α (Song *et al*., 2003).

Finally, in vivo studies using mice devoid of Muc2 revealed that the mucus layer plays an important role in protection against *Gardia lamblia* infection from pro-inflammatory enteropathogens, such as *Entamoeba histolytica* and *Trichuris trichuria.* Deletion of this gene results in exacerbated intestinal inflammatory responses (Song *et al*., 2003; Kisson *et al*., 2013). Similarly, disruption or aberrant expression of MUC2 has been observed in patients with chronic intestinal inflammatory disorders, such as ulcerative colitis (Heazlewood *et al*., 2008; Larsson *et al*., 2011). Collectively, these results demonstrate that the disruption of the intestinal mucus layer is majorly associated with gastrointestinal inflammation. It remains to be determined why disruption of the mucus layer during *Giardia*

infections fails to elicit pro-inflammatory intestinal responses. Moreover, it remains to be seen how *Giardia* co-infections may alter host pro-inflammatory responses or alter susceptibility to co-infecting gastrointestinal pathogens (Murphy *et al.,* 2016).

2.4.3 *Giardia* and Neutrophil Recruitment

The Accumulation of tissue polymorphonuclear leukocytes or neutrophils (PMNs) is a hallmark of numerous bacterial, viral, and parasitic *Giardia lamblia* infections. Polymorphonuclear are myeloid-derived innate immune cells that are essential to the host defense system against a varieties of bacterial and fungal pathogen, they possess various anti-microbial mechanisms, including the ability to phagocytose infectious agents, secrete anti-microbial proteases, and release neutrophil extracellular traps (Amulic *et al*., 2012).

In the absence of pro-inflammatory stimuli, Polymorphonuclear leukocytes or neutrophils are kept in a non-activated state within the bone marrow; they also remain inactivated in circulation. During an acute inflammatory response, there's an increased expression and production of Polymorphonuclear chemo-attractants promotes Polymorphonuclear neutrophils activation and recruitment into tissues, including the gastrointestinal tract (Van Haastert and Devreotes, 2004). Certain polymorphonuclear chemoattractants are capable of inducing the transepithelial migration of Polymorphonuclear neutrophils; this process occurs following Polymorphonuclear neutrophils have contact with the basolateral surface of the intestinal epithelium which results in the functional changes to both

Polymorphonuclear neutrophils or leucocytes and the intestinal epithelial cells (Chin and Parkos, 2007).

However, it is important to note that Polymorphonuclear neutrophils or leucocytes infiltration can induce pathophysiological responses that result in water and solute loss, hence it causes diarrheal disease. In vivo and in vitro experiments have suggested that this may involve Polymorphonuclear neutrophils or leucocytes-mediated intestinal barrier dysfunction or anion secretion (Weissmuller *et al*., 2008; Cotton *et al*., 2014). These collective results have demonstrated the importance of Polymorphonuclear neutrophils or leucocytes contributing to diarrheal disease.

Recent studies have also shown that *Giardia* infections may attenuate intestinal Polymorphonuclear neutrophils or leucocytes recruitment. Notably, these observations have been recorded with assemblage A, the genotype that has been postulated not to induce overt intestinal pro-inflammatory responses (Cotton *et al*., 2014). For example, *Giardia* assemblage A decreased granulocyte infiltration and cytokines and chemokines involved in Polymorphonuclear neutrophil recruitment after intra-rectal instillation of pro-inflammatory *Clostridium difficile* toxin A/B; These effects were not observed with in vivo Giardia assemblage B GS/M infections (Cotton *et al*., 2014). This study was also the first to demonstrate that co-incubation of *Giardia* trophozoites with inflamed colonic mucosal biopsy tissues from patients with active Crohn's disease decreased supernatant levels of numerous pro-inflammatory mediators, including those involved in PMN

recruitment (Cotton *et al*., 2014). Further studies went on to identify potential immunomodulatory molecules involved in this process. The findings demonstrated that assemblage A *Giardia* cathepsin B (catB) cysteine proteases degraded CXCL8 induced by pro-inflammatory interleukin-1β, or by *Salmonella enterica* serovar Typhimurium, and attenuated CXCL8-induced PMN chemotaxis; these effects were not observed with assemblage B GS/M trophozoites at early time points and, potentially, occur via different mechanisms (Cotton *et al*., 2014). These studies highlight a hitherto unidentified anti-inflammatory capability for *Giardia* infections and, more specifically, *Giardia* cat B proteases. Another recent study shows that these catB cysteine proteases may also be implicated in the degradation of epithelial villin (Bhargava *et al*., 2015).

2.5 Lifecycle of *Giardia*

The lifecycle of *Giardia lamblia* completes in a single host (Man), no intermediate host is required. The lifecycle consists of two stages: the cyst and trophozoites. Infection is acquired orally by ingestion of cysts from contaminated water, food or surfaces.

Excystation occurs in the stomach and duodenum in the presence of gastric acid, pancreatic enzymes (chymotrypsin and trypsin).An acidic environment with the pH 1.3-2.7 is required for excystation. Each cyst excysts to produce two trophozoites in the duodenum within 30 minutes of ingestion. The trophozoites which is the vegetative form of *Giardia lamblia* multiplies in the intestine by binary fission, adhere to enterocytes by means of their ventral

sucker mediated possibly through surface mannose binding lectin present on the surface of the trophozoites, some of the trophozoites are passed down to the large intestine where they again encyst in the presence of neutral pH and bile salts. The trophozoite is almost exclusively found in the human intestine, its primary niche (Despommier *et al.,* 2019).

The process of encystation begins with the appearance of Encystation specific secretory vesicles (ESVs), in the cytoplasm of the trophozoites, within 24hrs after appearance the trophozoites is covered with the cyst wall proteins, resulting in the formation of the cyst. The cysts are the infective form of parasite, Formation of cyst begins by shortening of flagella followed by condensation of cytoplasm and secretion of thick hyaline cyst wall. The encysted trophozoites then undergo another phase of nuclear division and produces quadrinucleated mature cyst, and the lifecycle is repeated. The cyst is highly resistance against chlorination, ozonolysis, and can survive in cold water between 4-8^{0}C (Huang and White, 2006).

2.5.1 Acquisition and excystation of *Giardia*

Acquisition of *Giardia* is primarily through the cyst form, in the fecal-oral route or, in unindustrialized regions, through contaminated water. The cyst is protected against the host‘s gastric acid with its cyst wall. Studies have suggested that its passage through the stomach of the host provided an acidic environment that

contributes to initiation of excystation, transformation from the cyst form to trophozoite. (Adam, 2001; Elmendorf *et al*., 2003) Excystation is also facilitated by pancreatic protease, and cysteine protease CP2 and encystation process is essential for transmission and survival of the parasite *Giardia intestinalis*.

Encystation starts when the parasite is swept away further down in the small intestine, and the process can be divided into an early and late phase.

In addition, evidence has been gathered to show that excystation may be inhibited by antibody to the cyst wall, calmodulin antagonists TFP and W7 and by wheat germ agglutinin (Meng *et al*., 1996; Bernal *et al*., 1998; Cotton *et al*., 2015).

2.5.2 Attachment and reproduction of *Giardia*

The trophozoite adheres to the wall of the small intestine using its ventral disc, and replicates within the lumen. Attachment was thought be accomplished by the hydrodynamic force generated under the disc using the ventral flagella (Holberton and Marshall, 1995; Hagen *et al*., 2011). Another proposed mechanism for attachment involves a surface lectin, found on the ventral disc of the trophozoites,that binds to mannose (Elmendorf *et al*., 2003).

Reproduction and encystations (formation of cyst) are carried out in the host mid- jejunum (small intestine). The metabolic activity of the trophozoite may cause diarrhoea, malabsorption and weight loss- a condition known as Giardiasis. In some patients, no symptom may develop. The reason of this is unknown,

but it is believed that the parasite strain and host factors may play a role (Thompson, 2001; Veenemans *et al*., 2011).

2.5.3 Encystation of *Giardia*

Encystation is observable under the microscope, and detailed mechanisms have been characterized by biochemical methods. Encystation occurs at mild alkalotic pH of 7.8 with the presence of fatty acid conjugated with bile salts (Elmendorf *et al*., 2003). In vitro studies have shown that encystation is induced by cholesterol and exposure of bile salts and fatty acids (Allian *et al*., 2017). Encystation starts when the parasite is swept away further down in the small intestine, and the process can be divided into an early and late phase. There are two phases of encystation.

In the early phase, Cell wall proteins are synthesized and transported to the periphery. Increased cell wall protein synthesis renders a Golgi-like structure readily visible with electron microscopy (Allian *et al.*, 2017). The proteins, containing a leucine-rich signature, are redirected to Encystation-specific transport vesicles (ESV), visible under light microscopy (Morf *et al*., 2010).

The late phase involves the assembly of the cyst wall and morphological change. The assembly begins with cyst wall filaments, then the filamentous layer of the cell wall (Adam, 2010). During the course of late-phase encystations, the trophozoite loses motility. Eventually a non-adhering cyst enclosing two physically joined trophozoites with four nuclei results (Allian and Buret, 2020)

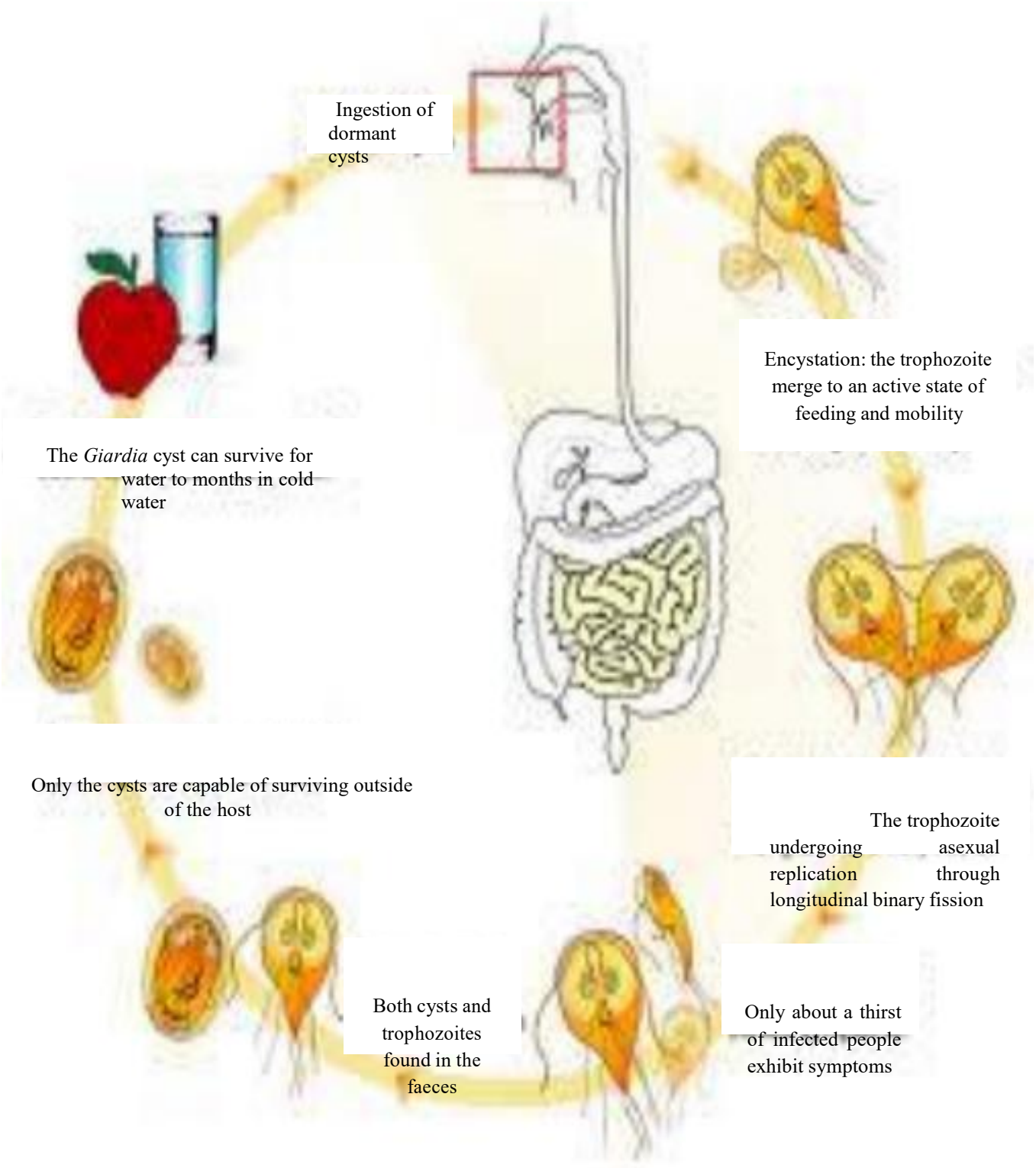

Plate 2.2: Lifecycle of *Giardia* (CDC, 2010)

2.6 Risk Assessment for Giardiasis in Water Sources.

Rose *et al*. (1991) used an exponential model to evaluate risks of *Giardia* infection from estimated exposures to *Giardia* in drinking water in the United States. Drinking water exposures were obtained from survey data describing the occurrence of *Giardia* in polluted and pristine water sources and considering average removals and inactivation of cysts with various types of water treatment (Moreira and Bondelind, 2017). The same approach was used in the development of the SWTR where performance-based standards for the control of *Giardia* were used to meet the EPA‘s recommended public health goal of no more than one *Giardia* infection per 10,000 persons from drinking water exposures (U.S. EPA, 1989; Karanis *et al*., 2011). The EPA felt that this goal could be maintained by achieving 99.9% reductions of *Giardia* cysts through filtration and disinfection in all water systems. Several small foodborne outbreaks of giardiasis have been associated with the contamination of ice and foods by infected food service workers, restaurant-associated transmission of *Giardia* appear to be a significant public health problem for children (Quick *et al.,* 1992; Kosek *et al*., 2017).

2.7 Global giardiasis and geographical distribution

Giardia lamblia is one of the major causes of waterborne diseases worldwide (CDC, 2017). Giardiasis is a disease of public health importance. In some developing countries, infection among young children can be quite high.

2.7.1 Distribution of giardiasis in Africa and the Middle East.

Mortality rate is greater in African countries where death risk is 4.3×10^{-1}, these data justify the main risk factors where unsafe water and integrity of hygienic environmental conditions are been compromised, which has resulted to malnutrition and immunosuppression, although some communities are been marginalized by some invariable characteristics (Heredia *et al*., 2015).

Giardia prevalence for countries in Africa and the Middle East conducted a survey of the prevalence of intestinal parasites in primary school children in Nimo, Nigeria. Of the 1,536 stool samples collected from eight primary schools, only 0.1% was positive for *Giardia lamblia* examined by light microscopy after formalin ether centrifugation. Ejiofor *et al.* (2011) reported a prevalence rate of 10.1% in Children Presenting with Diarrhea at Secondary Health Facility in Awka, South-East Nigeria. A survey in Pikine (Senegal) of five groups of children from ecologically representative sections of the town confirmed the high prevalence of *Giardia* (43.7%) in urban areas of Africa (Uneke and Uneke, 2007).

Okojukwo and Inabo (2012) reported a prevalence of *Giardia lamblia* in stool samples of children in day-care centres, nursery and primary schools in two Local Government Areas, Sabon-Gari and Zaria, in Kaduna State, Nigeria. Of 374 samples examined, 150 (41.45%) were positive for *Giardia lamblia*. The prevalence of giardiasis was higher in males (50.3%) than females (49.7%). The highest

prevalence for *Giardia* (56.8%) was found in children living on the outskirts of the town. Giardia cysts were identified in over 50% of the studies (73.7%; 14/19). Prevalence rates ranged from 2% (3/150) (Amaechi *et al*., 2016) to over 99% (159/160) (Chijioke *et al*., 2018). The regional prevalence in North, West and East Africa ranged from 3 to 84%, 2 to 99% and 8 to 28% respectively waterborne transmission is a major mode of transmission for *Giardia*. Climate change represents a major threat for access to safe drinking water in Africa which has more climate sensitive economies than any other continent (Bain *et al*., 2013).

A study by done by Abougrain *et al.* (2010) in libya showed that Intestinal parasites such as *Giardia lamblia* was been detected in food such as fresh fruits and vegetables, Similar studies was also carried in Ethiopia, Egypt, Ghana, Sudan (El said, 2012; Duedu *et al*., 2014; Tefera *et al.*, 2014; Mohamed *et al*., 2016). Giardia cysts have been detected in a variety of African water sources including irrigation water in Burkina Faso (Kpoda *et al*., 2015) a stream, well, spring and lake in Cameroon (Ajeaga, 2013), wastewater in Côte d'Ivoire, packaged drinking water in Ghana, tap water, drinking water treatment plants, canals, tanks and swimming pools in Egypt (Osei *et al*., 2013; Yapo *et al*., 2014; El-kowrany *et al*., 2016). Water from wells and the Kano River in Nigeria (Uneke and Uneke, 2007).

The overall prevalence of *Giardia* infection was 19.4% with more urban children (21.1%) passing cysts than rural children (16.7%). A survey of 770 households in

Behera, Egypt, selected by a random cluster sampling technique (Mohamed *et al*., 2016), found *Giardia lamblia* in 24.7% of children, aged 6 months to 12 years; faecal analyses from direct smear and the Kato-Katz examination techniques were available from 1844 and 1783 children respectively. A two-year study of the etiologic agents associated with episodes of diarrhoea in a family cohort population was conducted in eight villages of rural north-eastern Egypt; 3,243 stool specimens from 3,513 episodes of diarrhoea were analyzed for enteropathogens (Ali *et al.,* 2004). The most commonly identified agent in persons with diarrhoea was *Giardia lamblia* (44%); 60% of *Giardia*-positive stool specimens occurred in children 6 to 24 months of age. The isolation of *Giardia lamblia* from stool specimens was similar for persons with diarrhoea and those without, and no discernible seasonal pattern was found for infection found *Giardia* in 10.7% and 19.7% of 271 children who attended a clinic in Alexandria, Egypt, between December 1991 and July 1992 using conventional trichrome staining and immunofluorescent methods, respectively (Stazzone *et al*., 1996). Ahmed, (2010) surveyed 1426 apparently healthy Saudi children, finding *Giardia lamblia* to be the most common pathogenic parasite (3.6%).

2.7.2. Distribution of giardiasis in Asia.

Giardia prevalence for countries in Asia.

Xiao *et al*. (2013) found stool specimens positive for *Giardia lamblia* in 4% of 83 children under three years of age who were treated for persistent diarrhoea in the gastroenterology unit of the Institute for the Protection of Children's Health in Hanoi. Kang *et al.* (1998) determined the prevalence of intestinal protozoal infection in 78 members of 15 families from a rural village in India. Stool specimens from all subjects were examined on alternate days for one month; the overall prevalence of parasitic infections was 97.4%, with only 2 of 78 subjects not excreting parasites in any of fifteen specimens. Eighteen (23.1%) persons had only one type of parasite, while 58 (74.3%) excreted multiple parasites.

2.7.3 Distribution of giardiasis in Europe.

Gray and Rouse (1992) found that 23.7% of index cases of giardiasis in Bristol, England, were in preschool children; the remainder in travellers abroad and persons aged 10 years or more engaged in water recreation. Olszok and Kucharz (1996) found about 10% of adults and 20% of children in Poland infected with *Giardia lamblia* only a small percentage of infected persons were symptomatic. Skorochodzki *et al.* (1998) studied a sample of 112 children hospitalized with chronic abdominal pain from 1992 to 1993 in north-eastern Poland; based on the results of the duodenal fluid examination, *Giardia lamblia* infection was diagnosed in 77 (68%) children. Nikolic *et al*. (1998) surveyed intestinal parasitism among 5981 school children in central Serbia during the period 1984-1993. The

study included 2887 females and 3094 males, 7-11 years old representing 10% of the total age-matched population in the region. *G. lamblia* was detected in 6.8% of the children.

Carmena *et al*. (2006) conducted faecal analysis in school-age children aged 5-14 years olds in Centro de Salud de Motadel Cuervo, Cuenca, Spain, The study included 297 children of whom 133 (44.8%) were found infected with parasites. *G. lamblia* was the parasite most frequently detected intestinal parasite (36.4%). There was no difference in prevalence between boys and girls. During 1994 to 1996, Perez *et al.* (1997) found the overall prevalence of intestinal parasites (27.1%) in 1,917 children without symptoms, 6 to 10 years of age, living in 20 villages in the Guadalquivir valley of Spain to be similar to that found in other regions of Spain; *G. lamblia* (5.1%) was the second most commonly identified parasite in these children. Garcia *et al*. (1989) studied diarrhoea and the prevalence of enteropathogens for a one-year period in a group of 144 children in Spain, the prevalence rate of *Gardia lamblia* infection was 4%. Buchrieser *et al*. (1988) identified *Gardia lamblia* in 28.7% of stool specimens collected on the Cape Verde Islands; 90% of the samples came from children aged between 6 and 14 years.

2.7.4 Distribution of giardiasis in North America.

In the United States, *Giardia* is the most frequently identified parasite in stool specimens submitted for ova and parasites, and the overall prevalence for all age groups ranges from 4.0% to 12% depending on the year and state (Kappus *et al.,* 1994). In 39 states, *Giardia* was found to be the most frequently identified parasite every month of the survey periods; no information was reported specifically for prevalence in children. Harter *et al*. (1982) reviewed the results of randomly collected stool specimens in two counties of Washington State and found that 37 (7.1%) of 518 healthy 1- to 3-year-old children were positive for *Giardia* cysts. Caeiro *et al*. (1999) determined the etiology of acute, non-dysenteric diarrhea among 147 children between 2 and 11 years of age from nine out-patient clinics in Texas, New York, Michigan, Florida, New Jersey, Utah, and Pennsylvania. A recognized etiologic agent was detected in the stools of 89(61%) of the children. *Giardia lamblia* was detected in 22 (15%) children with a spring peak; most of the cases of Giardiasis were identified in Houston, Texas, and Levittown, New York (Bartelt and Platts-Mills, 2016).

In an epidemiological study of endemic cases of giardiasis reported from 1983 to 1986 in Vermont, children aged 1-4 years had the highest incidence rate for symptomatic *Giardia* infection more than any age group including adults (Birkhead and Vogt, 1989). The incidence of symptomatic giardiasis was almost four-fold higher than for infants less than 1year of age and children 5-9 years

of age. Among children aged 1-4years, the incidence was 50% greater for boys (approximately 1 200 cases/100,000/year) than girls (135/100,000/year). Among infants less than 1 year of age, symptomatic giardiasis rates were much lower, but the incidence was 40% greater for boys (55 cases/100,000/year) than girls (40/100,000/year). Incidence rates among girls and boys were similar after the age of 4 years: 40 cases/100,000/year for ages 5 to 9 years and 18 cases/100,000/year for ages 10-19. Schuster *et al.* (2005) reviewed records from 2,186 *Giardia*-positive patients in British Columbia, Canada, and also found that the majority of *Giardia* infections were in the 1 to 5 year age group. Schuster *et al.* (2005) reported the incidence of *G. lamblia* infection in Colorado among children age 0 to 15 years to be 3.05 per 100,000 per year.

Giardiasis is often considered to be a disease of developing countries due to poor sanitation and lack of portable water supply (Minetti *et al*., 2011). The disease is worldwide in distribution, to the extent that even in developed nations where portable water could be contaminated with small amounts of sewage particularly if septic systems are built too close to water supply (Torgerson *et al*., 2015). The transmission of the cyst of *Giardia lamblia* could occur from person to person or by fecal-oral route, but commonly it is by contaminated drinking water and food. Infrequently transmission can occur by recreational activities involving contaminated water like during swimming (Pires *et al*., 2015). Apart from that,

children tend to commonly put their fingers or objects in their mouth and may even swallow pool water and mostly may not wash their hands well thereby increasing the chances of acquiring the infection (Stuart *et al.,* 2003).

2.8 Risk Factors of Giardiasis

Giardia lamblia has been recognized as a common causative parasite of diarrhoea and nutritional disorders (Al-Mekhlafi *et al.,* 2010). In developed countries, giardiasis is considered a travel related disease. However, routine surveillance data from Germany indicate that >50% of infections were acquired indigenously (Werner *et al.,* 2010). Studies on risk factors for sporadic giardiasis have reported that in developed nations, giardiasis is significantly associated with drinking contaminated tap water, recreational exposure to fresh water and swimming pools, and travelling to developing countries (Hoque *et al.,* 2001; Donowitz, 2016).

In New Zealand, nappy changing was associated with a high risk of *Giardia* infection (Hoque *et al.,* 2003). However, in developing countries, socio-demographic factors play an important role in *Giardia* infections (Cifuentes *et al.,* 2004). In Malaysia, giardiasis is an endemic disease predominantly among children with prevalence ranging from 2.6-25% (Al- Mekhlafi *et al.,* 2010). Epidemiological studies have emphasized poverty, poor personal hygiene and lack of proper sanitation as factors playing major roles in the high prevalence of

giardiasis. Although the Malaysian government has been very proactive in dealing with poverty, providing basic amenities and proper sanitation, infection rates have not reduced much since the 1970s (Sahmini *et al*., 2018). *Giardia* is frequently spread directly from person to person, especially among young children attending day-care centers, nurseries, institutions, children living in areas with poor sanitation and hygiene, and children with siblings (Choi *et al*., 2014) In institutions, crowding, faecal incontinence and poor personal hygiene may promote the transmission of infections among children (Lal *et al*., 2013). Children can also become infected from ingestion of contaminated drinking water and the accidental ingestion of water while swimming or other water recreation (Fink and singer, 2017).

Infected infants and children, either symptomatic or asymptomatic, may transmit infection to other children or adults, especially family members or other care-givers. Higher incidence rates of symptomatic giardiasis and hospitalized cases of giardiasis in women of child bearing age may be related to increased exposure to infected children (Oliveira-Arbex *et al*., 2016). There is no evidence that *Giardia* is transmitted from mother to fetus (Hall, 1994), although infants can acquire infections at an early age suggesting that mothers can infect their children very soon after childbirth (Salit *et al*., 2009; Carrerro *et al*., 2020).

2.8.1 *Giardia* in water.

Giardia lamblia cysts are distributed worldwide in surface waters, even those of excellent quality and have been found in surface waters from the Arctic to the tropics (Amoros *et al.*, 2010). Cysts occur in surface waters throughout all months of the year. Waterborne outbreaks have been reported, and some have resulted in a large number of cases of illness (U.S. EPA, 2016). In 26 waterborne outbreaks associated with drinking water in the United States, levels of *Giardia lamblia* cysts ranging from <1/100L to 580,000/100L were detected from either treated or source water (U.S. EPA, 2016). Children, as well as adults, have been affected by outbreaks associated with drinking water systems and recreational waters (Borchardt *et al*., 2003). Endemic waterborne giardiasis in adults and children has also been associated with drinking unfiltered surface water or shallow wells and swimming (U.S. EPA, 2016).

Giardia lamblia has been the most commonly identified pathogen in waterborne outbreaks reported in the United States since 1971. During 1965 to 1996, 133 waterborne outbreaks and almost 28,000 cases of giardiasis were reported in the United States, primarily in unfiltered surface water systems (U.S. EPA, 2016). Ten (8%) of these outbreaks were associated with the use of individual drinking water systems or non-potable water sources, and 108 (81%) outbreaks were associated with public water systems; 14 (11%) outbreaks were associated with accidental ingestion of water during recreation. Unfiltered surface water systems were

responsible for 56% of the reported waterborne giardiasis outbreaks in the United States (Soller *et al*., 2010). Communities with unfiltered surface water systems experienced a waterborne outbreak rate that was eight times greater than communities where surface water is both filtered and disinfected (Ryu and Abazadegan, 2008). Children were included among the reported cases in these outbreaks, but limited information is available on the number of cases or attack rates for children. In a waterborne outbreak in Berlin, New Hampshire, 38% of children under 10 years of age were infected; an infection rate of 60% was found in children 10-19 years (Lopez *et al.,* 2003). The infection rate among adults also ranged from 38 to 62%.

2.8.2 *Giardia* in pets.

Pets such as dogs, cats, snakes and other animals have been thought to present a risk for children, but there has been little epidemiological evidence that they pose a significant risk even though dogs and cats are often found infected. *Giardia* infection was found in 153 (77%) of 200 dogs and 9 (3%) of 300 cats tested in Minnesota (Bemrick, 1961). Similar prevalence was reported in Spain (Lopez-Brea, 1982) and Japan (Asano *et al.,* 1991). Kirkpatrick and Green (1985) reported the prevalence of *Giardia* infection in cats to range from 1 to 11% in the United States. Franco and Cordeiro (1996) studied possible transmission of *Giardia* from pets in a study in Campinas, Brazil, but faecal examinations of the domestic animals were

negative for *Giardia*. Chute *et al*. (1987) found no elevated risk of giardiasis associated with household cats or dogs in a study conducted in New Hampshire. DeSa Cardoso *et al*. (1995) found no association between *Giardia* infection in children under 5 years of age and domestic animals in a study in Aracaju, Brazil.

Giardia lamblia is the most frequently identified etiologic agent causing waterborne outbreaks in public water systems in the United States. Epidemiological studies of endemic giardiasis and reported waterborne outbreaks have identified higher risks among persons using unfiltered surface water. An estimated 155 million people in the United States continue to use unfiltered surface water from municipal water systems (U.S. EPA, 2016).

Public water systems that use surface water sources without filtration and that do not meet provisions of the Sewage Water Treatment Regulations (U.S. EPA, 2016) are considered at a very high risk for waterborne transmission of giardiasis. Persons using shallow surface water are also at a higher risk of giardiasis (Efstratiou *et al*., 2017). It is not known how many persons in the United States use shallow well water, and an estimate of children exposed to shallow surface water is not available. Outbreak investigations and epidemiological studies show that *Giardia lamblia* is also transmitted during swimming and other water recreational activities and the ingestion of contaminated water while attending picnics, camping, and hiking (Traub *et al.*, 2004). Accidental ingestion of contaminated water while swimming

and water play is also an important waterborne risk for young children. It is not known how many children may be exposed to potentially contaminated swimming pools, wading pool, lakes, and streams (*Geurden et al*., 2010). Dog might get infected by contact with infected faeces from another dog, Rolling and playing in contaminated soil, licking its body after contact with a contaminated surface (for instance, a dirty litter box, dog cage or crate) and drinking water from a contaminated creek, pond, or other body of water (Traub *et al.*, 2004; Efstratiou *et al*., 2017).

2.8.3 Clinical Signs of Giardiasis

The clinical diagnosis of giardiasis is difficult since symptoms are nonspecific and resemble those of a number of other gastrointestinal ailments (John *et al*., 2007). Common signs and symptoms of *Giardia* infection (in both humans and pets) are diarrhoea, gas, abdominal discomfort, nausea, and vomiting (Buret and Cotton, 2011; Donowitz *et al*., 2011). However, it is possible to be infected and have no signs or symptoms of illness. Moreover, the symptoms observed vary with the life cycle stage of the parasite. The incubation period may last 12 to 19 days and is marked by the first detection of cysts in the faeces (Traub *et al.*, 2004).The clinical signs of parasitic infections in dogs are varied and occasionally some infected animals may present no symptoms (Muhsen and levine, 2012). These factors, coupled with inadequate information by dog keepers on the risks of disease

transmission, control of zoonosis transmitted by domestic animals, control of stray dogs and poor of hygiene has resulted in an increased risk of exposure to zoonosis transmitted by these animals (Sowemimo, 2008). Dog are infected from swallowing the *Giardia* cysts, usually in contaminated water. Water can be contaminated from faeces from infected dogs or other wild animals. *Giardia* is not transmitted from casual contact *Giardia* is more easily spread when the environment remains wet as dry environments do not allow the cysts to persist (Sowemimo, 2008; Hill *et al*., 2011).

2.9 Transmission of giardiasis between animals and humans.

There have been cases under certain circumstances in which the human assemblage has been found in dogs (Tangtrongsup and Scourza, 2010). *Giardia lamblia* assemblage A has been recovered from humans and dogs living within the same locality (Robertson, 2007). In an urban setting in Japan, a mix of human and dog-specific assemblages were recovered from dogs in breeding kennels and households (Ballweber *et al.*, 2010; Balderrama-Carmona *et al*., 2015). In contrast, a study of an Australian aboriginal community found dogs to harbor purely dog-specific assemblages (Chase *et al.*, 2012). Questions clearly linger regarding the amount of crossover that actually occurs between these different assemblages and their hosts under conditions that allow transmission (Fletcher *et al.,* 2011).

In addition, the general public is aware of human giardiasis as a disease entity, and clients may refuse to accept an explanation of it being non-zoonotic. Thus, the simple response to the question of treatment is to treat the infected animals and thereby remove any potential risk for both humans and other animals (Robertson, 2007; Ahmed *et al*., 2013).

Hill *et al*. (2011) showed that viable cysts, from symptomatic human donors can cause infection in the beaver and muskrat and concluded that the beaver and muskrat were possible intermediate reservoirs for *Giardia* that infects humans. Cyst-bearing faeces of rats, mice, dogs, cats and deer may occasionally reach drinking water, these animals do not, as beavers and muskrats do, by nature defecate in water. The beaver has also been implicated as a source of contamination in waterborne outbreaks (Feng and Xiao, 2011).

2.10 Environmental occurrence of *Giardia*.

2.10.1 *Giardia* in surface water.

Cysts have been found all months of the year in surface waters from the Arctic to the tropics in even the most pristine of surface waters. Occasionally, seasonal variations are reported. Cyst levels are generally higher in rivers or streams influenced by agricultural (e.g., cattle or dairy farming) or residential such as Sewage discharges activities (Singh, 2007). Cysts occur in surface waters

throughout the year; In North America, levels are generally higher in the late summer to early winter (Mons *et al*., 2009). Generally, no relationship is seen between cyst levels and bacterial indicators of water contamination. In the United States, levels of *Giardia* in water are somewhat lower than *Cryptosporidium*; in Canada, surveys have found higher levels of *Giardia* than *Cryptosporidium* (Sowemimo, 2008).

2.10.2 *Giardia* in waste water.

The concentrations of *Giardia* in human wastewater vary seasonally and daily, depending on the water use practices and health status of the community; the levels of endemic disease in the locality affect the rate at which the pathogens are shed in feces, therefore, their concentrations in wastewater (Atherholt *et al*., 1998; Eisenberg *et al*., 2002).

Based on this, examination of *Giardia* in raw sewage has been suggested as a way to assess the prevalence of *Giardia* infection and detecting possible outbreaks in communities. Only one study has examined the relationship between cysts in sewage and illness in the community; a correlation was found between raw sewage cyst levels and reported cases of giardiasis in 11 cities in the United States (Jakubowski *et al.,* 1999; Eisenberg *et al*., 2002). All of the raw sewage samples were positive for *Giardia*, 48% of secondary effluent samples contained

from 1- 44 cysts/L, 80% of the sludge samples contained from 70-30,000 cysts/L (Santos *et al.,* 2004; Carmena, 2010).

2.10.3 *Giardia* in ground waters.

Itagaki *et al.* (2005) did not detect *Giardia* in any of 17 samples from six wells in Wisconsin. Hibler (1988) found *Giardia* cysts in 19% of springs and 3% of wells sampled. Lee (1993) reported the contamination of two wells in Pennsylvania by surface streams less than 100 feet from the wells; *Giardia* was recovered from all samples collected from the wells. Hancock *et al.* (1997) collected 463 groundwater samples from 199 sites in 23 states in the United States; *Giardia* cysts were found in 14% of the springs, 1% of the vertical wells, 36% of the horizontal wells, and 25% of the infiltration galleries. The mean levels in positive water samples was 8 cysts/100 L (range = 0.1 to 120/100 L) (Itagaki *et al.*, 2005).

2.10.4 *Giardia* in contact surfaces.

Hermann (2015) evaluated a method for detecting *Giardia* cysts from environmental surfaces, and field tested the method in six commercial child day-care centers. The method was capable of recovering spiked cysts from Formica® surfaces when they were inoculated with 10 to 190 cysts on a surface area of 50 cm^2 or with 10 to 20 cysts/400 cm^2. Recoveries from stainless steel surfaces were lower and false negatives were higher. Cysts were not recovered from wood and

fiber glass surfaces spiked with 190 cysts/400 cm^2. In two day-care centers; two fiber-glass chairs (6%) and one Formica® table (2%) surface were found to be positive for *Giardia* cysts (Hermann, 2015).

2.10.5 *Giardia* in soil.

The wide distribution of cysts in human and animal populations suggests that soil may be contaminated with *Giardia* through faecal deposition, irrigation and sewage treatment practices (Noor *et al*., 2007). In soil held at 4°C, almost 90% of cysts were still viable after 49 days; however, infectivity was lost within 7 days. At 25°C, cysts survived for one week in solid cattle manure; At 4ºC, cysts survived for as long as 18 days in human feces.

A progress report from the Cornell University Whole Farm Planning Scientific Support Group (1993) discussed development and evaluation of a soil sampling protocol and detection method to evaluate the prevalence and transport of *Giardia* cysts on dairy farms within the New York City watershed (Escobedo *et al.*, 2016).

2.10.6 *Giardia* in food.

There are lack of quantitative data on the occurrence of *Giardia* cysts in foods, and improvements are needed in both sampling and analysis. Although foodborne giardiasis outbreaks in the United States have involved fish, sandwiches, vegetables, fruit and noodle salad, no information was available for *Giardia* levels

in these foods, four techniques that were originally developed for clinical specimens and had been adapted to foods (Itagaki *et al.*, 2005; Farzan *et al*., 2011).

2.11 Prevention of *Giardia lamblia*

Good hygiene, such as hand washing, reduces the risk of acquiring *Gardia lamblia*, or transmitting it to others; Efforts should especially be aimed at minimizing contact with feces (human or animal) , contaminated food ,untreated water that may be ingested orally, contaminated surfaces or items (such as soil, and fomites); should be avoided (Traversa *et al*., 2012).

Fecal contact with skin or mucous membranes should be avoided during sexual activity, both soil and irrigation water can contain Giardia, and cysts have been detected on crops. Vegetables and fruits should be washed before eating them. In higher risk situations, they should also be peeled if they will be eaten raw, as washing may not remove all organisms (Farzan *et al*., 2011).

Environmental modifications, by providing alternative sources of drinking water and restricting access to streams and other surface waters, can decrease faecal contamination of watersheds by livestock; People with giardiasis should not swim in recreational water for at least two weeks after the symptoms end (Santin *et al*., 2012).

On contact surfaces

Disinfection can be used to minimize the spread of infection which includes

- *Giardia lamblia* cysts on surfaces are susceptible to 5% sodium hypochlorite at a 1:30 dilution, as well as to some disinfectants including most quaternary ammonium solutions.
- In laboratories, 6% H2O2 can also be used to disinfect surfaces or decontaminate spills. Leaving disinfectants on contaminated surfaces for 5-20 minutes before rinsing helps ensure inactivation. Giardia cysts are susceptible to ultraviolet (UV) light, as well as to heat (e.g., steam) and desiccation.

In water

Gardia lamblia cysts can be killed by filtration through an absolute pore size of at least one micron (e.g., a filter that has been National Safety Foundation rated for cyst removal). Giardia cysts are relatively resistant to chlorination (Ngj *et al*., 2011). Treatment conditions reported to inactivate cysts in 5°C water include 4 mg/L chlorine for 60 minutes, at pH 6-8; 8 mg/L chlorine for 10minutes, at pH 6 or 7; and 8 mg/L chlorine for 30 minutes. Likewise, the effectiveness of iodination depends on the temperature, pH and turbidity of the water, as well as contact time with the chemical; Ozone and UV light can also inactivate cysts in water (Ngj *et al*., 2011).

2.12 Treatment of *Giardia*.

Fluid replacement is an important aspect of treatment associated with illness that comes with diarhhoea; Anti-giardial drugs are also important in the management of individual cases of giardiasis, but they may not prevent frequent reinfection of children who attend day-care centers or live in communities where *Giardia lamblia* exposures are routine. Chemotherapeutic agents used for treatment of giardiasis include metronidazole, tinidazole, quinacrine, furazolidone, albendazole, and ornidazole (Chandy and Mccarthy, 2009; Shane *et al*, 2017; Ordonez-Mena *et al* 2018). Various doses and treatment periods are recommended for each drug; these agents have different effectiveness in their ability to clear *Giardia*, and side-effects should be considered. Drug resistance or re- infection may occur. Paromomycin is recommended for pregnant women, but the cure rate may be low (Itagaki *et al.*, 2005). Supportive care, such as fluid and electrolyte management, may also be necessary; Symptoms can recur for a variety of reasons, such as drug resistant organisms, reinfection or post-*Giardia* lactose intolerance (Mejia *et al*., 2013). In some cases, a lactose-free diet may be needed for several months.

Asymptomatic carriers do not usually need treatment, but they may be treated to reduce transmission of the organism. Whether or not treatment is recommended can vary with the situation and risk of reinfection (Chandy and Mccarthy, 2009).

2.13 Public Health Action of *Giardia*

This study provides data to educate public health practitioners and health-care providers about the scope and magnitude of giardiasis in the Benin City, Edo State. These data can be used to establish research priorities and to plan future prevention efforts (Maikai *et al*., 2013). Estimates have been extrapolated from published data collected by United States with active giardiasis surveillance, laboratory surveys, and waterborne-disease outbreak reports. Giardiasis is the most frequently reported diarrheal disease in Africa (Chijioke *et al.,* 2018), and from 1983 through 1986, it was the most common reportable disease in Vermont (Bouzid *et al*., 2008). *Giardia* also was the most prevalent protozoan parasite in Arkansas during 1997 (Daly *et al.,* 2011) as well as the most commonly reported enteric pathogen in Wisconsin during the years 1983-1986 (Minnetti *et al.*, 2016)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was conducted between two Local Government Areas- Ikpoba Okha and Oredo, Benin City, Edo State. Benin City is the capital of Edo State having the largest urban Center, consists of 18 Local Government Areas namely, Akoko Edo, Egor, Esan Central, Esan North East, Esan South, East, Esan West, Etsako central, Etsakor East, Etsakor West, Igueben, Oredo, Orhionwon, Ovia North East, Ovia South West, Owan East, Owan West and Uhunmwode. Ikpoba okha has an area of 862km with a population of 487,400 whereas the Oredo has an area of 249km with a population of 490,600. Edo State has a population of 4,235,600 which is mainly a civil service state, their main means of ecking a living is through trade, transportation and commerce with a few artisans.

3.2 Study Population

The Study was conducted among primary school children in (Idogbo primary school) rural and urban (Ivbiotor primary school) schools of Ikpoba Okha and (Uwa primary school) rural and urban (Asoro primary school) schools of Oredo Local Government Areas. A total of 288 primary school children consisting of (194) males and (94) females were enrolled in this study. The age of participants ranged from the ages of 6-14years. A well-structured questionnaire bothering on the bio-data and socio-

demographic characteristics was administered on each participant prior to the collection of specimens. Informed consent was obtained from the parent or guardian of each participant.

3.3 Sample Size

Sample size determination was calculated using a previously described formular of Daniel (1999)

At 95% confidence level -5, 0, z=1.96

Sample size $n = Z^2pq/d^2$

Where:

n= minimum sample size

d=is the desired level of precision (i.e margin of error)

p=is prevalence

q= 1-p

Z= (1.96) Standard deviation at 95% confidence interval

Then,

Assuming a previous prevalence of *Giardia lambia* infection is 10%

P=5%; q=1-p

=1-5

=4

$n = 1.\ 96 \times 0.4 \times 0.5 / 0.\ 5^2$

n = 288

A minimum of 288 sample size will be used for this study

3.4 Inclusion Criteria.

Pupils that are attending primary school and those within the age range 6-14years whose parents or guardian consented to their participation were included in this study.

3.5 Exclusion Criteria

Children not in primary schools and those above 6-14years whose parents or guardian refused consent were excluded from this study. Primary children that are on anti-parasite agents were equally excluded.

3.6 Ethical approval

The protocol for this study was approved by the Ethics and Research Committee of the Ministry of Health, Benin City, Edo State (REF. FMOH/HREC/32/1164)

3.7 Specimen collection

A total of 288 stool samples were collected from primary school children with a number of 159 in rural area and 129 samples from primary children in urban settings using random sampling. Freshly voided stool was collected in a sterile universal bottle and sent to the Laboratory Department of City of Hope Medical Centre, off km 7 Sapele road, Benin City for analysis.

3.8 Specimen Processing

Samples collected were analyzed for parasite presence using microscopy and ELISA techniques.

Freshly voided faecal sample was collected from each pupil, into a clean universal screw capped container properly labeled. The parent (s) or guardians were properly educated on how to collect the specimen. (Lindo *et al*., 1998).

3.9 Stool microscopy

The consistency of the stool was observed, classified and recorded as loose, semi-formed, formed, mucoid, slimy, or watery. Each sample was divided into:

1. A portion of the feacal specimen was preserved in 10% formal saline for microscopy and
2. the other stored at – 20°C and used for ELISA technique

3.10 Macroscopic examination

Samples were examined macroscopically for color, consistency, mucus, blood and parasite.

3.11 Microscopic examination

Wet preparation for microscopy

A drop of fresh physiological saline was placed on one end of a clean grease-free slide and a drop of iodine on the other end. Using an applicator stick, a small amount of the faecal sample, about 2mg (matchstick head) was emulsified in Saline. Similar

step was taken and emulsified in iodine solution. Each of the preparation was covered with a clean cover slip and examined using x10 and x40 objective lenses. The presence of cyst, ova and parasite was taken as positive while the absence was taken as negative (Lindo *et al*.,, 1998).

Concentration technique

About 1g of faeces was emulsified in 4 ml of 10% formol saline in a screw-cap bottle. To this; 4ml of 10% formol saline was added, shaken and sieved. The filtrate was transferred into a tube and 4ml of diethylether was added and mixed well for 1minute. This mixture was spun at 3000rpm for 1min and the pipette was used to loosen the layer of faecal debris on the side of the tube and the suspension discarded leaving the sediment. The sediment is resuspended and a drop each was added to saline and iodine separately on a clean grease free slide and examined under microscope using x10 and x40 objective lenses.

3.12 Enzyme immunoassay method

Faecal samples stored at -20°C was analysed using the Wampole TM Giardia/Cryptosporidium ELISA kit (Tech Lab Inc.) following the manufacturer's instructions. This immunoassay is based on the detection of Giardia-specific antigen 65 (GSA 65) in aqueous extracts of stool specimens with monospecific polyclonal sera (Savioli *et al*., 2016).

Briefly, faecal specimens were diluted (1:20 [vol/vol]) in Trizma-based specimen dilution buffer to create a stool eluate. Specimen eluates (200 uI) were added to antibody-coated assay tubes in duplicate and incubated for 1 hr at room temperature. The tubes were washed thrice with phosphate-based wash buffer using 2ml per wash. Enzyme conjugate (200 uI) was added to each immunoassay tube and incubated for 1 h at room temperature. The tubes were washed four times using 3 ml of wash buffer per wash and immediately added 200ul of o-phenylenediamine-peroxide buffer substrate solution. After 10min, reactions were stopped with 50ul of 2 N sulfuric acid solutions, and the reaction was read visually.

Any detectable color generation was regarded as a positive assay outcome. Following visual assessment of positivity or negativity, the contents of the tubes were transferred to 96-well polystyrene plates and spectrophotometrically read at A490 on an MR 580 MicroElisa plate reader. Spectrophotometric data was presented as a means to minimize subjectivity of interpretation during this study as well as to provide quantitative measurement of exactly how much color development there was for each reaction. Controls was included each time, each test was run (Carrero *et al*., 2020).

Results was read both visually by assessing the color formed in each well of a microtiter plate and quantified by measuring the absorbance at 450nm on a microplate ELISA reader (Labsystems Multiskan MS). The recommended values

(cut-off points) are< 0.150 OD 450, for the negative control, and ≥ 0.500 for the positive control. In the test wells, any value ≥ 0.150 was considered positive, and recorded.

3.13 Statistical Analysis

All data was entered into a statistical package (Graphpad prism). Descriptive analysis including computation of chi-square, odd ratio and percentages were done on the study variables. The descriptive statistical analysis was used to determine significant difference as appropriate. A P-value of less than 0.05 was considered statistically significant at confidence limit was set at 95%.

CHAPTER FOUR

4.0 RESULTS

A total of 288 children from the studied areas participated in the study. One hundred and forty-eight (148) children were examined in Oredo and One hundred and forty (140) children in Ikpoba-Okha. Out of the 288 children examined, 18(6.3%) of them were infected with *G. lamblia.*

Table 4.1: Showed the comparison of microscopy and Elisa methods in detecting *Giardia lambia* in the stool samples. *Giardia lamblia* was more diagnosed in the Elisa method (5.6%) when compared with the microscopy method (0.7%). The Elisa method significantly diagnosed *G. lamblia* than the microscopy method (OR= 8.412; 95% CI= 1.916, 36.928; P<0.0001).

The study sites significantly affected the distribution of *G. lamblia* infection between Ikpoba-Okha and Oredo LGAs with children resident in Oredo LGA having the highest prevalence (8.6%) of *G. lamblia* when compared with the Ikpoba-Okha LGA (2.7%) (OR= 3.375; 95% CI = 1.062, 10.727; P=0.030) (Table 4.2).

More males were infected (6.7%) than their female counterparts (3.2%) with *G. lamblia* infection in this study. However, gender did not significantly affect the prevalence of *G. lamblia* infection (OR= 2.18; 95% CI= 0.605, 7.839; P=0.223) (Table 4.3).

Children in the age group of 10-11 years old had the highest prevalence of *G. lamblia* infection (13.7%), followed by 6-7 years age group (6.2%), 8-9 years age group (2.2%) whereas the those that 12 years and above did not report any incidence of the parasite. In addition, age of children significantly influenced the prevalence of *G. lamblia* infection in this study (P=0.014) (Table 4.4).

Children that reside in the rural settings had more *G. lamblia* infection (11.3%) than those in the urban (4.7%) and semi urban (3.1%). The type of settlement strongly impacted on the *G. lamblia* infection in this study (P=0.074) (Table 4.5).

Table 4.1: Comparison of microscopy and Elisa method in detecting *Giardia lamblia* among children in Ikpoba-Okha and Oredo LGAs

Method	No. Examined	No. Positive	% Positive	OR	95%CI	PValue
Microscopy	288	2	0.7	8.412	1.916-36.928	0.001
ELISA	288	16	5.6			

Table 4.2: Comparative distribution of giardiasis in Ikpoba-Okha and Oredo Local Government Areas

LGA	No. Examined	No. Positive	% Positive	OR	95%CI	P Value
Oredo	140	12	8.6	3.375	1.062-10.727	0.030
Ikpoba Okha	148	4	2.7			
Total	**288**	**16**	**6.3**			

Table 4.3: Comparative distribution of giardiasis in relation to gender among children in the study area

Gender	No. Examined	No. Positive	% Positive	OR	95%CI	P Value
Male	194	13	6.7	2.18	0.605-7.839	0.223
Female	94	3	4.1			
Total	288	16	6.3			

Table 4.4: Comparative distribution of giardiasis in relation to age among children in the study area

Age Group (Years)	No. Examined	No. Positive	% Positive	X^2	P Value
6-7	112	7	6.2	10.546	0.014
8-9	91	2	2.2		
10-11	51	7	13.7		
>12	34	0	0.0		
Total	288	16	6.3		

Table 4.5: Prevalence of Giardiasis in Ikpoba Okha and Oredo Local Government Areas in Relation to some Epidemiological Factors.

Parameters	No. Examined	No. Infected (%)	OR	95% CI	P value
Settlement type					
Urban	129	6 (4.7)			0.074
Semi urban	97	3 (3.1)			
Rural	62	7 (11.3)			
Drinking water					
Tap water	80	8 (10.0)			0.189
Borehole	19	0 (0.0)			
Satchet	100	4 (4.0)			
Boiled	89	4 (4.5)			
Toilet type					
Water closet	184	6 (3.3)			0.018
Open field	75	9 (12.0)			
Pit	29	1 (3.4)			
Contact with animals					
Yes	147	10 (6.8)	1.642	0.581 - 4.645	0.345
No	141	6 (4.3)			
Proximity of kitchen and toilet					
Yes	118	13 (11.0)	5.571	1.547 - 20.059	0.001
No	170	3 (1.8)			
Diarrhoea					
Yes	115	11 (9.6)	3.554	1.201 – 10.518	0.015
No	173	5 (2.9)			
Food vendor					
Yes	156	13 (8.3)	3.909	1.089 – 14.028	0.025
No	132	3 (2.3)			

Continuation of table 4.5

Hand hygiene					
Yes	168	1 (0.6)			0.000
No	120	15 (12.5)	23.857	3.106 – 183.276	

Giardia lamblia infection was not prevalent among those who used boiled water (4.5%) as compared to those that used tap water as their source of drinking water (10.0%), however the difference was not statistically significant in both local government area (P=0.189) (Table 4.5).

Individual that use the open field defecation presented with the highest prevalence of *G. lamblia* infection (12.0%), followed by the pit latrine (3.4%) and water cistern (3.3%). Open field defecation significantly influenced the transmission of *G. lamblia* infection in this study (P=0.018) (Table 4.5).

Association of children with domestic animals did not strongly affect prevalence of *G. lamblia* infection in this study (P=0.345) with those that have contact with animals having the highest prevalence (6.8%) of giardiasis when compared with children that did not have association with animals (4.3%) (Table 4.8).

Children whose have a close proximity of kitchen and toilet facility presented with the highest prevalence of 11.0% of *G. lamblia* infection when compared with those that have their kitchen situated away from the toilet (1.8%). In addition, close proximity of toilet facility to kitchen in homes of participants significantly impacted on the prevalence of *G. lamblia* infection (OR=5.571; 95% CI= 1.547, 20.059; P<0.001) (Table 4.5).

In this study, diarrhoea among children was a risk factor for acquiring *G. lamblia* infection as it strongly associated with prevalence of *G. lamblia* infection (OR=3.554; 95% CI= 1.201, 10.518; P=0.015). Children with diarrhoea had the highest prevalence of giardiasis (9.6%) when compared with those without diarrhoea (2.9%) (Table 4.5).

Children that eat from food vendors have 1 to 8 fold risk of acquiring giardiasis in this study. Eating from food vendors strongly affected the infection of *G. lamblia* among children (OR=3.909; 95% CI= 1.089, 14.028; P=0.025) with those that eat from food vendors presenting with a prevalence of 8.3% of *G. lamblia* when compared with those that do not eat from food vendors (2.3%) (Table 4.5).

Non washing of hands after using the toilet strongly associated with giardiasis among children in Ikpoba-Okha and Oredo LGAs with those that do not practice safe hand hygiene presenting with the highest prevalence of giardiasis (12.5%) compared with those practiced safe hand hygiene (OR= 0.0059; 95% CI= 3.106, 0.277; P=0.000) (Table 4.5)

CHAPTER FIVE

5.0 DISCUSSION

Enteric parasitic disease lies among the many health problems observed in economically disadvantaged populations of the poorest countries. Intestinal parasitism has clear social and environmental determinants, with high prevalence in regions with a deficiency in sanitation, portable water supplies, education, and adequate dwelling conditions (Anibal *et al*., 2007). *Giardia lamblia* is one of the most common agents of travelers' diarrhea infections, Giardia enteritis is seen throughout the world, and one of the most common protozoan infections (Haliez and buret, 2013).

In this study, an overall prevalence of 5.6% of *G. lamblia* infection was observed among children. This prevalence was lower than the 19.3% reported by Waldram *et al*. (2017) among households in Northwest England in the United Kingdom and 18.5% observed by Obiukwu *et al*. (2008) among children in Abagana, Anambra State. The reason for the difference in the prevalence of giardiasis in our work and that of other previous workers may be due to the observation and practice of good personal hygiene which includes frequent hand washing by the study population as taught and enforced by the parents of these children.

Two local government areas were studied with 288 stool samples obtained and tested for the presence of *Giardia lamblia* stool samples obtained were microscopically studied for the detection of *Giardia lamblia* using the concentration method and

ELISA technique respectively. The total percentage prevalence of giardiasis was 5.6% with the ELISA upon comparison with microscopy. This observation is in tandem with previous studies of Chan *et al.* (2000), Ozekinci *et al.* (2005) and Jahan *et al.* (2014) where ELISA technique was reported to have been more sensitive in the detection of *G. lamblia*. The diagnosis of giardiasis is majorly based on detection of trophozoites or cysts microscopically in stool specimens. Microscopic examination of a single stool specimen has been reported to present a low sensitivity (46%) as a result of intermittent shedding of the cysts. Hence, at least three faecal samples have to be taken and examined over a period of 3-5 days to achieve 94% accuracy in diagnosing positive giardia cases (Hanson and Cartwright, 2001; Weitzel *et al.,* 2006). The serious drawback of microscopic detection of this parasite is that it is time and labour intensive and depends on the skill of an experienced microscopist. In addition, the parasite may be hidden by bile pigments and not visualized by wet mount examination (Koneman *et al.,* 1992; Gharayi *et al.*, 2005). Although conventional microscopy of three stool samples (with or without concentration techniques) is still being recommended as "gold standard" to diagnose infections caused by *G lamblia*, but its sensitivity is found to be low (50-70%) even after multiple examinations (Barazesh *et al.,* 2010). ELISA with sensitivity of 95% to 100% and specificity over 90% when compared with direct microscopy provides a relevant alternative method to the routine ova and parasite (O & P) examination

method in diagnosing giardiasis with the added advantage of increased sensitivity required to confirm infections in patients with low parasite numbers and diagnose the disease even if the live parasite is absent in the faecal samples (Addiss *et al*., 1991; Chappell and Matson, 1992; Jelinek and Neifer, 2013).

Children from Oredo LGA had the highest prevalence of giardiasis (8.6%) when compared with those that reside in Ikpoba-Okha LGA (2.7%). Children that reside in Oredo LGA have a 1-8 fold risk for acquiring giardiasis than those from Ikpoba-Okha LGA OR= 2.928; 95% CI = 1.015, 8.439; P=0.038). The reason for this observation might be socio-economic status, improved standard of living and increased awareness on safe hygiene practice.

Gender did not associate strongly with the prevalence of giardiasis although, more ales were infected (6.7%) than their female counterparts (3.2%) with *G. lamblia* infection in this study (OR= 2.179; 95% CI= 0.605, 7.839; P=0.223). Similar finding was observed by Claudia *et al*. (2012) among children in Portugal where males were more infected (52.8%) than their female counterparts (48.2%). The reason for this finding might be due to the outdoor activities of the male children where they might be involved in playing football and some other events that expose them to the infectious cysts of this parasite.

Age of children significantly affected *G. lamblia* infection among children in this

study with the age group of 10-11 years old presenting with the highest prevalence of 13.7%, followed by 6-7 years age group (6.2%), 8-9 years age group (2.2%) whereas the those that are 12 years and above did not report any incidence of the parasite (P=0.014). This observation is consistent with the report of Choy *et al.* (2014) in Malaysia that observed increased prevalence of giardiasis among children that are less than 12 years of age. This might be so because children less than 12 years of age are more likely to come in contact with soil and surfaces and objects that have been contaminated with the viable cysts of *G. lamblia* in the course of their recreational activities. This might be partly responsible for our finding in this study.

Children that reside in the rural settings had more *G. lamblia* infection (11.3%) those in the urban (4.7%) and semi urban (3.1%). In addition, settlement type significantly impacted on the *G. lamblia* infection in this study (P=0.074). The pattern of distribution depends mainly on the availability of certain conditions required by parasites, such conditions may include suitable climate, human activity, unavailability of portable water, population movement and poor sanitation (Azazy and Raja'a, 2003). It is recommended that personal hygiene be encouraged and taught to these children more by their parents in order to reduce the effect of parasitic infections among children.

The increasing environmental contamination has been reported to affect the quality of water and may raise the risk of waterborne intestinal infections particularly

giardiasis (Taus *et al.,* 1998). Drinking piped water has been identified as a significant risk factor among the aboriginal population in Pahang, Malaysia (Choy *et al.,* 2014). Giardiasis infections have also been associated with drinking of contaminated tap water, fresh water and the movement of individuals from a nonendemic region to an endemic region (Isaac-Renton *et al.,* 1994; Hoque *et al.,* 2002). Children that use tap water as source of water had the highest prevalence of *Gardia lamblia* infection (10.0%) however, source of water did not strongly influence *G. lamblia* infection in our study (P=0.189). Similar finding was observed by Choy *et al*. (2014) in among residents of rural communities in Malaysia where drinking of non-boiled water was a predictor in acquiring giardiasis.

Individuals that use the open field defecation presented with the highest prevalence of *G. lamblia* infection (12.0%). In addition, open field defecation significantly influenced the transmission of *G. lamblia* infection in this study (P=0.018). It has been observed that living in homes where there is no functional toilets increase risk of acquiring giardiasis (Ngui *et al*., 2011; Choy *et al*., 2014). The defecation in indiscriminate places particularly rivers and bushes are common occurrence with places where adequate toilet facilities are lacking. This might be the reason for our finding.

In this study, children that are associated with domestic animals did not significantly affect the prevalence of *G. lamblia* infection (P=0.345). Those that have contact with

animals had the highest prevalence (6.8%) of giardiasis when compared with children that did not have association with animals (4.3%).

Children residing in homes where the kitchen is at close proximity to toilet facility were observed to have been more infected (11.0%) with *G. lamblia* infection when compared with those that have their kitchen situated away from the toilet (1.8%)this might be due to contaminated hands or contact surfaces. In addition, close proximity of toilet facility to kitchen in homes of participants significantly impacted on the prevalence of *G. lamblia* infection (P<0.0001). This might be due to personal hygiene, good hygiene measures should therefore be taken, monitored, taught and properly observed in homes.

In this study, diarrhoea among children was a risk factor for acquiring *G. lamblia* infection as it strongly associated with prevalence of *G. lamblia* infection (OR=3.554; 95% CI= 1.201, 10.518; P=0.015). Children with diarrhoea had the highest prevalence of giardiasis (9.6%) when compared with those without diarrhoea (2.9%). The reason for this finding may be due to the fact that individuals that just finished using the toilet may walk into the kitchen for food or water during which the door handle or other objects may be contaminated.

Children that eat from food vendors have 1 to 9 fold risk of acquiring giardiasis in this study. Eating from food vendors strongly affected the infection of *G. lamblia* among children (P=0.025) with those that eat from food vendors presenting with the

highest prevalence of 8.3% of *G. lamblia* infection. This finding is similar to previous study of Akinbo *et al.* (2013) that observed no significant association between source of food and *G. lamblia* infection. Food vendors are more likely not to keep high personal hygiene as they move their food from street to street exposed to contamination. The reason for our finding might be due to the ingestion of the cyst from contaminated objects before ingestion of the meal.

Children that do not practice washing of hands after using the toilet reported higher prevalence of giardiasis (12.5%) in Ikpoba-Okha and Oredo LGAs (P=0.000). Personal hygiene is recommended to be taught both at home and outside and it is a key in controlling giardiasis. Hand hygiene remains an important factor to consider in the control of most parasitic infections, especially infections of fecal-oral routes because *Giardia lamblia* is usually contracted through contact with contaminated water, food, soil and transmitted fecal-orally, also by direct person-to-person or animal-to person transmission presenting classically as an acute condition, which may also become chronic. Intestinal parasitic infections have a worldwide distribution with high prevalence found in people with low socio-economic status and poor living conditions as well as people in over-crowded areas with poor environmental sanitation, improper garbage disposal, unsafe water supply and unhygienic personal habits, these factors are the causes of a major proportion of the

burden of disease and death in developing countries (Adamu *et al*., 2006; Noor *et al*., 2007).

CONCLUSION

A prevalence of 5.6% of *Giardia lamblia* infection was observed among children in Ikpoba-Okha and Oredo LGAs of Edo State. Residential locations, age, settlement type, type of toilet, proximity of kitchen to toilet facility, diarrhoea, food source and hand hygiene significantly affected the prevalence of *G. lamblia* infection.

RECOMMENDATIONS

Based on this study indicated that detection of Giardiasis should not solely be based on microscopy as more sensitive detection such as ELISA could be employed. Furthermore, increased awareness on good sanitary practices should be taught, properly observed and monitored particularly among mothers and children as this would decrease the risk of *Giardiasis*. Lastly in order to avert potential health implications associated with the outbreak of this infection, authourities concerned should ensure that good environmental and sanitary conditions are strictly adhered to. The present study, therefore, would be baseline for giardiasis among primary school children in Ikpoba Okha and Oredo local government, Benin city and other researchers that might be interested, as it was observed that there was no research done on giardiasis in this communities, the need for further research to be done on the study area should also be encouraged.

CONTRIBUTION TO KNOWLEDGE

This study has contributed to knowledge in the following ways:

1. The high prevalence of 5.6% of *G. lamblia* infection observed among the children in Ikpoba-Okha and Oredo LGAs of Edo State will draw the attention of the authority concerned to curb the spread of this infection.
2. The effectiveness of ELISA method shows that it should be included in the analysis of *G.lamblia* infection
3. Outdoor activities, mostly by the male children should be properly monitored and strict hand hygiene practices should be enforced and adhered to.

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PLATE 3: 96 well polystyrene Elisa plates showing positivity (Deep yellow) and negativity (no intensity) of the aqueous extract of the stool specimen.

PLATE 3b: 96 well polystyrene Elisa plates showing positivity (Deep yellow) and negativity (no intensity) of the aqueous extract of the stool specimen.

PLATE 3c: 96 well polystyrene Elisa plates showing positivity (Deep yellow) and negativity (no intensity) of the aqueous extract of the stool specimen.