INTESTINAL PROTOZOAN PARASITES IN CHILDREN WITH DIARRHEA AND ASSOCIATED RISK FACTORS IN SELECTED HEALTH INSTITUTIONS IN IMO STATE

BY

JUDE CHIDIEBERE CHILEKE

(BSc. Microbiology, IMSU)

20134870678

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,

FEDERAL UNIVERSITY OF TECHNOLOGY OWERRI

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN ENVIRONMENTAL HEALTH BIOLOGY

DECEMBER, 2019

CERTIFICATION PAGE

This is to certify that this work, "Intestinal Protozoan Parasites in Children with Diarrhea and Associated Risk Factors in Selected Health Institutions in Imo State" was carried out by I, **Chileke Chidiebere Jude (Reg. no. – 20134870678)** in partial fulfilment for the award of the degree of Masters of Science in Environmental Health Biology in the Department of Biotechnology of the Federal University of Technology, Owerri.

Dr. Mgbemena, I. C. — Date: 17/12/2019

(Supervisor)

Prof. Ezejiofor, T. I. N. — Date: 17/12/2019

(Head of Department)

Prof. Ogbulie, J. N. — Date: 19/12/19

(Dean, School of Biological Science)

Prof. Mrs. Oti, N. N. — Date

(Dean, Post Graduate Studies)

Prof. Mrs. Comfort Etok — Date: 13-12-2019

(External Supervisor)

DEDICATION

I dedicate this research work to God, my family members, parents and to lovers of children. I also dedicate this piece of work to all friends of knowledge.

ACKNOWLEDGEMENT

I seize this opportunity to acknowledge God for His guidance throughout the time of this study. I say glory be to God.

I'm delighted to acknowledge Dr. Mgbemena, I. C., my project supervisor, for her super supervision. I also appreciate in a special way, my vibrant Head of Department, Prof. Ezejiofor, T. I. N., and my esteemed lecturers in whom I am well pleased: Prof. Iwuala, M. O. E., Prof. Nwaigwe, H. C., Prof. Okwujiakor I. A., Prof. Njoku, O. O., Prof. Opara, F. N., Prof. Ozoh, P. T. E., Dr. Onyeocha, I. O., Dr. Obasi, K. O., Dr. Udebuani, A. C., Dr. Ogbulie, T. E. My own Dr. Okereke, J. N., my PG coordinator and lecturer is not left out, thank you for your sacrifices and encouragement. These noble professors and doctors have shaped my thinking in the academic world.

I also acknowledge the unalloyed guidance and help of Mr. Nkem B. I. of Federal Medical Centre Owerri, for his unceasing sacrifice to make this research a success. He is a good resource person. I recommend him to other scholars. I specially thank Mr. Nnadozie A., for his super guidance.

Finally, thanks beyond the power of words to express go to my lovely parents and other family members for their effort to the success of this study.

To my school mates, Judith, Onyima, Mohammed and Joseph for being good friends, I thankfully recognize you all.

TABLE OF CONTENT

PRELIMINARIES

ABSTRACT

This study was carried out with a view of assessing intestinal protozoan parasites in children with diarrhea and the associated risk factors in selected health institutions in Imo State. Diarrheic stool samples were collected from consented subjects and analyzed in the laboratory applying standard parasitological techniques. Demographic and socio-economic data were collected using pre-tested researcher administered questionnaire. Of the 1,200 diarrheic stool samples examined, 404 (33.6%) yielded positive result. *C. parvum* (20.0%), *E. histolytica* (10.3%) and *G. lamblia* (3.3%) were isolated. Protozoan diarrhea occurred most in children of age group 5-8 years; age prevalence had no significant difference ($p>0.05$). Primary school subjects yielded highest prevalence (39.8%) with significant difference ($p<0.05$) among other school categories. Rural children had high prevalence (37.3%) than urban children (27.1%) with statistical significant difference ($p<0.05$). Significant difference was noted among the three zones of the study area, Orlu (41.8%), Owerri (30.6%) and Okigwe (28.9%) ($p<0.05$). The comparison of protozoan parasite prevalence among the selected hospitals was statistically non-significant ($p>0.05$). Social risk factors identified included poor hand hygiene, poor knowledge, attitude and practice on source of diarrheal transmission. Poor source of drinking water, dirty playing grounds and crowded areas especially in public schools were the other factors. This present study therefore calls for adequate campaign and public health enlightenment to be extended to children and their care-givers in this area. Environmental sanitation especially proper fecal disposal should be put in place and adequately monitored since the protozoan diarrhea is transmitted through fecal-oral routes.

Key Words: Prevalence, Protozoan Diarrhea, *C. parvum, E. histolytica, G. lamblia*, Risk factors

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND INFORMATION

Good health exalts a nation, and children are gifts to the nation. It is often said that, when you "keep a child healthy, you keep the nation healthy". Keeping children healthy is one of the important health duties of their parents, their care-givers, and the society at large, as some infantile diseases affect them, diarrhea inclusive. Measures of child health are useful indicators of the health of a nation, particularly for Nigeria where children constitute about 45% of the total population (Federal Official Statistics, 1997).

Be that as it may, diarrhea has been known as the most common disorder within the abdominal region and especially within the gastrointestinal tract (GIT). The gastrointestinal tract is a tube that transfers food to the organs of digestion (Ruppert *et al.,* 2004). GIT consists of the stomach, the small intestine and the large intestine. It is considered as a long muscular tube beginning from the mouth and ending at the anus.

Human protozoan parasites which are part of the human intestinal parasites live in the GIT and especially within the intestine, hence intestinal protozoan parasites. Over the years scientists have discovered that protozoan parasites are one of the groups of organisms causing gastrointestinal infections. Scientists have also mentioned some other protozoan species like *Dientamoeba fragilis, Balantidium coli* and *Sopora hominis*. Similar protozoan parasites have also been identified in different parts of the world including Nigeria.

The most important human parasitic infections worldwide are the intestinal parasitic infections (Adekunle, 2002). The intestinal parasites had been long recognized as an

important health problem (Oguoma, *et al.,* 2008). According to Akingbade, *et al* (2013), intestinal parasitic infection is important public health problem in the tropics, and developing countries. It has become a major health problem in many developing countries like Nigeria (Corry and Brett, 2004; Sayyan, *et al.,* 2005).

There are two main types of intestinal parasites and they are helminths and protozoa. In their adult form, helminths cannot multiply in the human body. Protozoa, however have only one cell, and can multiply inside the human body, which contributes to their survival and enables serious infections to develop (Sayyan, *et. al.,* 2005). Sometimes two or more protozoan can cause infection at the same time, a concept known as polyparasitism (CDC, 2009; Sayyan, *et. al.,* 2005). Several epidemiological studies indicated high prevalence rates of intestinal infections among Nigerian children (Oguoma, *et. al.,* 2008; Sayyan, *et. al.,* 2005).

However, from the review of literatures, majority of Nigerian children including Imo children, from low socioeconomic class have been found to be anemic, stunted with retarded growth and underweight due to malnutrition, and human intestinal parasites mainly are implicated. Our major concern in this work is not the helminthic intestinal parasitic infections, but assessing the intestinal parasitic protozoa that cause diarrhea in children.

1.2 PROBLEM STATEMENT

The World Health Organization (WHO, 2013) pegged diarrhea as the second most common cause of death and also reported that about 1.7 – 5 billion cases of diarrhea occur every year. Deaths resulting from diarrhea have become a global burden, and it has been known according to Kosek *et al* (2003) to cause high child mortality of about 21% in developing countries. Goodwill message by Dr. Robert Limlim (2008), the then acting UNICEF representative in Nigeria, during the national launch of hand washing campaign held at

Sheraton Hotel and Towers Abuja on May 22, 2008 stated that diarrhea prevalence rate in Nigeria is 18.8% and is one of the worst in Sub-Sahara Africa and above the average of 16%. Diarrhea accounts for over 16% of child deaths in Nigeria and an estimated 150,000 deaths mainly amongst children under five occur annually due to this disease mainly caused by poor sanitation and hygiene practices. Diarrhea was associated with up to 75% of all illnesses in young children as far back as 1987 when Huttly *et al* worked in 5 villages in Imo state.

Since then, several researchers continued the struggle to determine the cause of death in children in Imo State due to diarrhea. Researchers like Benjamin *et al* (2013) and Dozie *et al* (2011) who worked on *Cryptosporidium parvum* discovered that cryptosporidiosis pose a great public health challenge among children in Imo State.

A serious call has been made on prevalence of diarrhea and its negative effects by the Federal Government of Nigeria in her Family Support Programs called "Healthy Living". This campaign is aimed at educating mothers on how to help curb the spread of diarrheal infections in their children. This is in consonance with the fact that diarrheal infection has become a public health concern and challenge for all in our society, including Imo State.

Therefore, these present facts about diarrhea and its burdens in Imo State, parts of Nigeria and beyond are enough reasons to go into this research topic "intestinal protozoan parasites in children with diarrhea and associated risk factors in selected health institutions in Imo State".

1.3 OBJECTIVES OF THE STUDY

This research was aimed at assessing the intestinal protozoan parasitic agents of diarrhea and associated risk factors among children in Imo State, South Eastern Nigeria.

The specific objectives were;

- To determine the overall prevalence of diarrhea among the study population
- To isolate the intestinal protozoan parasites causing diarrhea among the study population
- To compare the prevalence of diarrhea among various age groups, school enrolment and sex
- To compare the prevalence of diarrhea according to occupation of mothers and areas of residence
- To determine the Knowledge, attitude and practice of the children and their care-givers

1.4 **RESEARCH HYPOTHESIS**

Both the Null (H□) and Alternative (H□) Hypothesis were tested. The following postulated Null Hypotheses were tested at 0.05% level of significance, either to accept the null hypothesis and reject the alternative or vice versa.

1. There is no statistical significant difference in the gender related prevalence of infection among the subjects.

2. There is no statistical significant difference in the age related prevalence of intestinal protozoan agents of diarrhea.

3. There is no statistical significant difference in the prevalence of intestinal protozoan agents of diarrhea according to school enrolment.

4. There is no statistical significant difference in the distribution of infection among children in relation to mothers' occupation.

5. There is no statistical significant difference in the prevalence of the parasites according to area of residence of the subjects.

6. There is no statistical significant difference in the prevalence of parasites according to the three senatorial zones in Imo State.

7. There is no statistical significant difference in the prevalence of parasites in the selected health institutions in Imo State.

1.5 JUSTIFICATION OF THE STUDY

Though previous researchers (Mordi and Ngwodo, 2007; Ajero *et al.,* 2008; Oguoma *et al.,* 2008; Chukwuma *et al*., 2009; Dozie *et al.,* 2011; Akingbade *et al.,* 2013; Benjamin *et al*., 2013*)* have noted the prevalence and public health significance of *Giardia lamblia, Entamoeba histolytica* and *Cryptosporidium parvum* in the area and other parts of the country, inadequate data still exist on intestinal protozoan causing diarrhea in children especially in Imo State, hence the present study which is designed to fill this gap. Information from this study will help the stake holders provide adequate control and preventive measures towards protozoan agents of diarrhea.

1.6 SCOPE OF THE STUDY

This study assessed protozoan agents of diarrhea including *C. parvum, E. histolytica,* and *G. lamblia* among Imo state children between 0-12 years suffering from diarrhea. The study determined the overall prevalence of protozoan associated diarrhea and the prevalence of other variables using standard parasitological methods.

1.7 RESEARCH QUESTIONS

To guide this public health research, the following questions were formulated:

1. Is there prevalence of protozoan associated diarrhea among children in Imo State?

2. If the answer to the first question above is in the affirmative, is the prevalence of protozoan associated diarrhea high or low?
3. Is *Cryptosporidium parvum, Entamoeba histolytica* and *Giardia lamblia* among the protozoan infective agents responsible for diarrhea among children in Imo State?
4. Is there significant difference of protozoan associated diarrhea among various sexes, age groups, school enrolments, occupations of mothers, residence and the selected health institutions attended by children in Imo state?
5. Are there risk factors, in Knowledge, Attitude and Practice (KAP) among children and their mothers/care-givers in Imo State predisposing chances of protozoan diarrheal infections?

CHAPTER TWO

LITERATURE REVIEW

Diarrhea is a disease that affects both the young and the old. It is characterized by loose, watery stools or a frequent bowel movement. It usually lasts a few days and often disappears without any treatment. It can be acute or chronic. But, for a better understanding of the research topic and why the need for its investigation, a quick review of diarrhea, its types, causes, effects, treatments, preventions, epidemiology, and the different protozoan parasites that are known to cause the leading diarrheal infections in Children would be paramount; thus, an assessment of such protozoan parasites in selected Imo health institutions.

2.1 DIARRHEA

Diarrhea is defined by World Health Organization as having three or more loose or liquid stool within 24 hours, or as having more stools than is normal for that person (Akingbade *et al.,* 2013). Acute diarrhea is defined as an abnormally frequent discharge of semisolid or fluid fecal matter from the bowel, lasting less than 14 days, according to World Gastroenterology Organization (WGO, 2011). Diarrhea is also the condition of having at least three loose or liquid bowel movements each day. It often lasts for a few days and can result in dehydration due to fluid loss. It is the consistency of the stools rather than the number that is most important (Sinclair *et. al.*, 2003). Signs of dehydration often begin with loss of the normal stretchiness of the skin and changes in personality. This can progress to decreased urination, loss of skin color, a fast heart rate, and a decrease in responsiveness as it becomes more severe. Loose but non watery stools in babies who are breastfed, however, may be normal (WHO, 2013).

Diarrhea infection is often acquired from food or water that has been contaminated by diarrheal stool, or directly from another person who is infected. It is frequent in poor populations (Akingbade *et al.*, 2013) and in immunocompromised individuals (Ferreira and Borges, 2002).

It may be divided into three types: short duration watery diarrhea, short duration bloody diarrhea, and if it lasts for more than two weeks, persistent diarrhea. The short duration watery diarrhea may be due to an infection by cholera. If blood is present it is also known as dysentery (WHO, 2013). A number of non-infectious causes may also result in diarrhea, including hyperthyroidism, lactose intolerance, inflammatory bowel disease, a number of medications, and irritable bowel syndrome (Doyle, 2013). In most cases stool cultures are not required to confirm the exact cause (Dupont, 2014).

According to research, about 1.7 to 5 billion cases of diarrhea occur per year (WHO, 2013; Doyle, 2013). It is most common in developing countries such as Nigeria, where young children get diarrhea on an average of three times a year (WHO, 2013). Diarrhea is a leading cause of morbidity and mortality globally; yet the overall burden of diarrhea in terms of duration and severity has not been quantified. As improvements in treatment lead to decreases in diarrheal mortality, it is important to understand the substantial impact of diarrhea morbidity on disability among children and adults worldwide (Laura *et al.*, 2012). Total deaths from diarrhea according to Global Burden of Disease, is estimated at 1.26 million in 2013 – down from 2.58 million in 1990 (GBD, 2013). In 2012, it is the second most common cause of deaths in children younger than five (0.76 million or 11%) (WHO, 2013; CDC, 2013). Frequent episodes of diarrhea are alsoa common cause of malnutrition and the most common cause in those younger than five years of age (WHO, 2013). Center for

Disease and Control in USA, also recorded that, other long term problems that can result include stunted growth and poor intellectual development (CDC, 2013).

2.1.1 TYPES OF DIARRHEA

There are different means through which diarrhea can occur and they include the following:

2.1.1.1 SECRETORY DIARRHEA: Secretory diarrhea means that there is an increase in the active secretion, or there is an inhibition of absorption. There is little to no structural damage. The most common cause of this type of diarrhea is cholera toxin that stimulates the secretion of anions, especially chloride ions. Therefore, to maintain a charge balance in the lumen, sodium is carried with it, along with water. In this type of diarrhea, intestinal fluid secretion is isotonic with plasma even during fasting (Webmd, 2011). It continues even when there is no oral food intake.

2.1.1.2 OSMOTIC DIARRHEA: Osmotic diarrhea occurs when too much water is drawn into the bowels. If a person drinks solutions with excessive sugar or excessive salt, these can draw water from the body into the bowel and cause osmotic diarrhea. Osmotic diarrhea can also be the result of mal-digestion (e.g., pancreatic disease or Coeliac disease), in which the nutrients are left in the lumen to pull in water. Or it can be caused by osmotic laxatives (which work to alleviate constipation by drawing water into the bowels). In healthy individuals, too much magnesium or vitamin C or undigested lactose can produce osmotic diarrhea and distention of the bowel. A person who has lactose intolerance can have difficulty absorbing lactose after an extraordinarily high intake of dairy products. In persons who have fructose mal-absorption, excess fructose intake can also cause diarrhea. High-fructose foods that also have high glucose content are more absorbable and less likely to cause diarrhea. Sugar alcohols such as sorbitol (often found in sugar-free foods) are difficult for the body to

absorb and, in large amounts, may lead to osmotic diarrhea (Webmd, 2011). In most of these cases, osmotic diarrhea stops when offending agent (e.g. milk, sorbitol) is stopped.

2.1.1.3 EXUDATIVE DIARRHEA: Exudative diarrhea occurs with the presence of blood and pus in the stool. This occurs with inflammatory bowel diseases, such as Cohn's disease or ulcerative colitis, and other severe infections such as *E. coli* or other forms of food poisoning (Webmd, 2011).

2.1.1.4 INFLAMMATORY DIARRHEA: Inflammatory diarrhea occurs when there is damage to the mucosal lining or brush border, which leads to a passive loss of protein-rich fluids and a decreased ability to absorb these lost fluids. Features of all three of the other types of diarrhea can be found in this type of diarrhea. It can be caused by bacterial infections, viral infections, parasitic infections, or autoimmune problems such as inflammatory bowel diseases.

2.1.1.5 DYSENTERY DIARRHEA: If there is blood visible in the stools, it is also known as dysentery. The blood is trace of an invasion of bowel tissue. Dysentery is a symptom of, among others, *Shigella*, *Entamoeba histolytica*, and *Salmonella* infections.

2.1.2 TRANSMISSION ROUTE: Infectious diarrhea is acquired by fecal-oral transmission that includes consumption of contaminated food or water, person-to-person contact, or direct contact with fecal matter. With regard to water-borne-diarrhea, transmission patterns occur when in-house water storage facilities or/and water sources are contaminated (corresponding to domestic domain and public contamination) (Jesen *et al.*, 2004, Jesen *et al.*, 2002). Most of transmission of diarrhea occurs in the domestic domain (Jesen *et al.*, 2004).

According to Curtis *et al.*, (2000), there are four transmission routes that the major infectious agents use to reach human host, namely human-to-human via the environment; human-to-

human multiplying in the environment; human-to-animal-to-human via the environment and animal-to-human via the environment. In situations where fecal contamination of the domestic environment is high, the majority of cases of endemic disease probably occur either by human-to-human transmission, or from the human-to-human transmission of pathogenic agents which have multiplied in the environment.

2.1.3 CAUSES OF DIARRHEA

Apart from parasites like the helminths and the protozoan, diarrhea could also be caused through the following:

2.1.3.1 POOR SANITATION: Open defecation is a leading cause of infectious diarrhea leading to death (WHO, 2014). Poverty is a good indicator and an associate of the rate of infectious diarrhea in a population. This association does not stem from poverty itself, but rather from the conditions under which impoverished people live. The absences of certain resources compromise the ability of the poor to defend themselves against infectious diarrhea. Poverty is associated with poor housing, crowding, dirty floors, lack of access to clean water or to sanitary disposal of fecal waste (sanitation), cohabitation with domestic animals that may carry human pathogens, and a lack of refrigerated storage for food, all of which increase the frequency of diarrhea. Poverty also restricts the ability to provide age-appropriate, nutritionally balanced diets or to modify diets when diarrhea develops so as to mitigate and repair nutrient losses. The impact is exacerbated by the lack of adequate, available, and affordable medical care (Jamison, 2006).

2.1.3.2 LACK OF CLEAN WATER: One of the most common causes of infectious diarrhea is lack of clean water. Often times, improper fecal disposal leads to contamination of groundwater. This can lead to widespread infection among a population, especially in the

absence of water filtration or purification. Human feces contain a variety of potentially harmful human pathogens (Brown, *et. al.,* 2013).

2.1.3.3 POOR NUTRITION: Proper nutrition is important for health and proper functioning of the body, including the prevention of infectious diarrhea. It is important especially to young children who do not have a fully developed immune system. Zinc deficiency, a condition often found in children in developing countries can, even in mild cases, have a significant impact on the development and proper functioning of the human immune system (Black and Sazawal, 2001). Indeed, this relationship between zinc deficiency reduced immune functioning corresponds with an increased severity of infectious diarrhea. Children who have lowered levels of zinc have a greater number of instances of diarrhea, severe diarrhea, and diarrhea associated with fever. Similarly, vitamin A deficiency can cause an increase in the severity of diarrheal episodes; however there is some discrepancy when it comes to the impact of vitamin A deficiency on the rate of disease. While some argue that a relationship does not exist between the rate of disease and vitamin A status, others suggest an increase in the rate associated with deficiency. Given that estimates suggest 127 million preschool children worldwide are vitamin A deficient, this population has the potential for increased risk of disease contraction (West, 2002).

But the major cause of diarrhea in the context of our research is the ones caused by intestinal protozoan, and we will look at them in brief soonest.

2.1.4 SYMPTOMS OF DIARRHEA

Though diarrhea is a symptom, not a disease, signs and symptoms associated with diarrhea may include:

- Loose, watery stools

- Abdominal cramps
- Abdominal pain
- Fever
- Blood in the stool
- Bloating
- Nausea
- Urgent need to have a bowel movement

If one suffers diarrhea for a few days, one may feel lightheaded or weak. This comes from rapidly losing the minerals, sugar, and water that the body needs. Normally, diarrhea won't cause one to lose control of the bowels – if this happens, one should consult a doctor.

Urinating less may also be observed. This is because the body is losing water through bowel movements instead of urine. One should see a doctor immediately if diarrhea lasts longer than 48 hours or there is a fever of 38.5ºC or higher or severe abdominal pain or vomiting that prevents fluid replacement by mouth. Acute diarrhea accompanied by fever and stools with blood can be signs of a potentially dangerous infection or parasite.

In children, particularly young children, diarrhea can quickly lead to dehydration. Call a doctor if a child's diarrhea doesn't improve within 24 hours or the baby becomes dehydrated, has a fever above 102 F (39 C) and has bloody or black stools. This information was culled from Medbroadcast statement, and Mayo Clinic Staff information about diarrhea in 2017.

2.1.5 TREATMENT OF DIARRHEA

These treatment methods were culled from Markus, (2017).

Correcting dehydration is the priority of diarrhea treatment.

Mild cases of acute diarrhea may resolve without treatment. Persistent or chronic diarrhea will be diagnosed and treated in addition to the symptoms of diarrhea.

For all cases of diarrhea, the first important step in treatment is to rehydrate, and this can be done in the following ways:

- Fluids can be replaced by simply drinking more fluids, or they can be received intravenously in severe cases. Children and older people are more vulnerable to dehydration.
- Oral rehydration solution/salts (ORS) - this is water that contains salt and glucose. It is absorbed by the small intestine to replace the water and electrolytes lost in the stool. In developing countries, ORS costs just a few cents; the World Health Organization (WHO) says ORS can safely and effectively treat over 90% of non-severe diarrhea cases.
- Oral rehydration products are available commercially - for example Oralyte and Rehydralyte. Zinc supplementation may reduce the severity and duration of diarrhea in children.
- Loperamide (Imodium, for example) is an antimotility drug that reduces stool passage.

- Bismuth subsalicylate (for example, Pepto-Bismol) reduces diarrheal stool output in adults and children and may be a safer alternative to loperamide. This drug can also be used to prevent traveler's diarrhea. There is concern that antidiarrheal medications could prolong bacterial infection by reducing the removal of pathogens via stools.

Antibiotics are only used to treat diarrhea caused by a bacterial infection. If the cause is a certain medication, switching to another drug might be possible.

Nutrition may be another way of treating diarrhea. Some nutritional tips by various nutritionists for treatment of diarrhea include:

- Sip on clear, still liquids such as fruit juice without added sugar, replacing lost water after each loose stool with at least one cup of liquid
- Do most of the drinking between, not during meals
- Consume high-potassium foods and liquids - examples include diluted fruit juices, potatoes (without the skin), bananas
- Use high-sodium foods and liquids - broths, soups, sports drinks, salted crackers

Other advice from the nutritionists is to:

- Eat foods high in soluble fiber to help thicken the stool - bananas, rice, oatmeal, for example
- Limit certain foods that may make diarrhea worse such as creamy, fried, and sugary foods

It is always advised that diarrheal patients stay away from certain food and drink that might make diarrhea worse. Such food and drink include:

- Sugar-free gum, mints, sweet cherries, prunes
- Caffeinated drinks and medication

- Fructose in high amounts, from fruit juices, grapes, honey, dates, nuts, figs, soft drinks, and prunes
- Lactose in dairy products
- Magnesium
- Olestra (Olean) - a fat substitute

2.1.6 PREVENTION OF DIARRHEA

As we have different means diarrhea could occur, so also we have different means of preventing it, as so many researches has shown. The following enumerates the different means.

2.1.6.1 SANITATION: Numerous studies have shown that improvements in drinking water and sanitation (WASH) lead to decreased risks of diarrhea (Wolf *et. al.,* 2014). Such improvements might include for example use of water filters, provision of high-quality piped water and sewer connections (Wolf *et. al.*, 2014).

In institutions, communities and households, interventions that promote hand washing with soap lead to significant reductions in the incidence of diarrhea (Ejemot *et. al.,* 2008). The same applies to stopping open defecation at community-wide levels and providing access to improved sanitation (United Nations, 2014; Plos one, 2014). This means use of toilets and implementation of the entire sanitation chain connected to the toilets (collection, transport, disposal or reuse of human excreta).

2.1.6.2 HAND WASHING: Basic sanitation techniques can have a profound effect on the transmission of diarrheal disease. The implementation of hand washing using soap and water, for example, has been experimentally shown to reduce the incidence of disease by

approximately 42–48% (Curtis and Cairncross, 2008; Cairncross *et. al.*, 2010). Hand washing in developing countries, however, is compromised by poverty as acknowledged by the CDC: Handwashing is integral to disease prevention in all parts of the world; however, access to soap and water is limited in a number of less developed countries (CDC, 2013). This lack of access is one of many challenges to proper hygiene in less developed countries. Solutions to this barrier require the implementation of educational programs that encourage sanitary behaviors.

2.1.6.3 WATER: Given that water contamination is a major means of transmitting diarrhea, efforts to provide clean water supply and improved sanitation have the potential to drastically cut down the rate of disease incidence. In fact, it has been proposed that we might expect an 88% reduction in child mortality resulting from diarrhea as a result of improved water sanitation and hygiene (Brown *et. al.*, 2013; Black *et. al.*, 2003). Similarly, a meta-analysis of numerous studies on improving water supply and sanitation shows a 22–27% reduction in disease incidence, and a 21–30% reduction in mortality rate associated with diarrheal disease.

Chlorine treatment of water, for example, has been shown to reduce both the risk of diarrheal disease, and of contamination of stored water with diarrheal pathogens (Arnold and Colford, 2007).

2.1.6.4 VACCINATION: Immunization against the pathogens that cause diarrheais a viable prevention strategy. However it does require targeting certain pathogens for vaccination. In the case of Rotavirus which was responsible for around 6% of diarrheal episodes and 20% of diarrheal disease deaths in the children of developing countries, use of a Rotavirus vaccine in trials in 1985 yielded a slight (2-3%) decrease in total diarrheal disease incidence, while reducing overall mortality by 6-10%. Similarly, a Cholera vaccine showed a strong reduction in morbidity and mortality, though the overall impact of vaccination was minimal as Cholera

is not one of the major causative pathogens of diarrheal disease. Since this time, more effective vaccines have been developed that have the potential to save many thousands of lives in developing nations, while reducing the overall cost of treatment, and the costs to society (Rheingans *et. al.*, 2009; WHO, 2010).

2.1.6.5 NUTRITION: Dietary deficiencies in developing countries can be combated by promoting better eating practices, and supplementation with vitamin A and/or zinc. Zinc supplementation proved successful showing a significant decrease in the incidence of diarrheal disease compared to a control group (Black, 2003). They majority of the literature suggests that vitamin A supplementation is advantageous in reducing disease incidence (Mayo-Wilson *et. al.*, 2011). Development of a supplementation strategy should take into consideration the fact that vitamin A supplementation was less effective in reducing diarrhea incidence when compared to vitamin A and zinc supplementation, and that the latter strategy was estimated to be significantly more cost effective (Chagan *et. al.*, 2013).

2.1.6.6 BREASTFEEDING: Breastfeeding practices have been shown to have a dramatic effect on the incidence of diarrheal disease in poor populations. Studies across a number of developing nations have shown that those who receive exclusive breastfeeding during their first 6 months of life are better protected against infection with diarrheal diseases (WHO, 2000). Exclusive breastfeeding is currently recommended during, at least, the first six months of an infant's life by the WHO (Sguassero, 2013). Others like Probiotics decrease the risk of diarrhea in those taking antibiotics (Hempel *et al.*, 2012).

2.1.6.7 MANAGEMENT: In many cases of diarrhea, replacing lost fluid and salts is the only treatment needed. This is usually by mouth – oral rehydration therapy – or, in severe cases, intravenously. Diet restrictions such as the BRAT diet are no longer recommended (King, *et. al.*, 2003). Research does not support the limiting of milk to children as doing so has no effect

on duration of diarrhea. To the contrary, WHO recommends that children with diarrhea who continue to eat as sufficient nutrients are usually still absorbed to support continued growth and weight gain, and that continuing to eat also speeds up recovery of normal intestinal functioning (WHO, 2005). CDC recommends that children and adults with cholera also continue to eat (CDC, 2010). Medications such as loperamide (Imodium) and bismuth subsalicylate may be beneficial; however they may be contraindicated in certain situations (Schiller, 2007).

2.1.6.8 FLUIDS: Oral rehydration solution (ORS) (slightly sweetened and salty water) can be used to prevent dehydration. Standard home solutions such as salted rice water, salted yogurt drinks, vegetable and chicken soups with salt can be given. Home solutions such as water in which cereal has been cooked, unsalted soup, green coconut water, weak tea (unsweetened), and unsweetened fresh fruit juices can have from half a teaspoon to full teaspoon of salt (from one-and-a-half to three grams) added per liter. Clean plain water can also be one of several fluids given. There are commercial solutions such as Pedialyte, and relief agencies such as UNICEF widely distribute packets of salts and sugar. A WHO publication for physicians recommends a homemade ORS consisting of one liter water with one teaspoon salt (3 grams) and two tablespoons sugar (18 grams) added (approximately the 'taste of tears'). Rehydration Project recommends adding the same amount of sugar but only one-half a teaspoon of salt, stating that this more dilutes approach is less risky with very little loss of effectiveness. Both agree that drinks with too much sugar or salt can make dehydration worse.

Appropriate amounts of supplemental zinc and potassium should be added if available. But the availability of these should not delay rehydration. As WHO points out, the most important thing is to begin preventing dehydration as early as possible. In another example of prompt

ORS hopefully preventing dehydration, CDC recommends for the treatment of cholera continuing to give Oral Rehydration Solution during travel to medical treatment (CDC, 2010).

Vomiting often occurs during the first hour or two of treatment with ORS, especially if a child drinks the solution too quickly, but this seldom prevents successful rehydration since most of the fluid is still absorbed. WHO recommends that if a child vomits, to wait five or ten minutes and then start to give the solution again more slowly.

Drinks especially high in simple sugars, such as soft drinks and fruit juices, are not recommended in children under 5 years of age as they may increase dehydration. A too rich solution in the gut draws water from the rest of the body, just as if the person were to drink sea water. Plain water may be used if more specific and effective ORT preparations are unavailable or are not palatable. Additionally, a mix of both plain water and drinks perhaps too rich in sugar and salt can alternatively be given to the same person, with the goal of providing a medium amount of sodium overall. A nasogastric tube can be used in young children to administer fluids if warranted (Webb and Starr, 2005).

2.1.6.9 EATING: WHO recommends a child with diarrhea should continue to be fed. Continued feeding speeds the recovery of normal intestinal function. In contrast, children whose food is restricted have diarrhea of longer duration and recover intestinal function more slowly. A child should also continue to be breastfed. The WHO states “Food should never be withheld and the child's usual foods should not be diluted. Breastfeeding should always be continued” and in the specific example of cholera, CDC also makes the same recommendation (CDC, 2010). In young children who are not breast-fed and live in the developed world, a lactose free diet may be useful to speed recovery (MacGillivray, *et. al.*, 2013).

2.1.6.10 MEDICATIONS: While antibiotics are beneficial in certain types of acute diarrhea, they are usually not used except in specific situations (De Bruyn, 2008). There are concerns that antibiotics may increase the risk of hemolytic uremic syndrome in people infected with Escherichia coli O157:H7 (Wong, *et. al.*, 2000). In resource poor countries, treatment with antibiotics may be beneficial (De Bruyn, 2008). However, some bacteria are developing antibiotic resistance, particularly *Shigella* (WHO, 2009). Antibiotics can also cause diarrhea, and antibiotic-associated diarrhea is the most common adverse effect of treatment with general antibiotics.

While bismuth compounds (Pepto-Bismol) decreased the number of bowel movements in those with travelers' diarrhea, they do not decrease the length of illness (Dupont, *et. al.*, 2009). Anti motility agents like loperamide are also effective at reducing the number of stools but not the duration of disease (Dupont, 2014). These agents should only be used if bloody diarrhea is not present (Pawlowski, *et. al.*, 2009).

Bile acid sequestrants such as cholestyramine can be effective in chronic diarrhea due to bile acid malabsorption. Therapeutic trials of these drugs are indicated in chronic diarrhea if bile acid malabsorption cannot be diagnosed with a specific test, such as SeHCAT retention (Wedlake *et al.*, 2009).

2.1.6.11 ALTERNATIVE THERAPIES: Zinc supplementation benefits children with diarrhea in developing countries, but only in infants over six months old. This supports the World Health Organization guidelines for zinc, but not in the very young (Lazzerini and Ronfani, 2013).

Probiotics reduce the duration of symptoms by one day and reduced the chances of symptoms lasting longer than four days by 60% (Allen, *et. al.*, 2010). The probioticslactobacillus can

help prevent antibiotic associated diarrhea in adults but possibly not children (Kale-pradhan, *et. al.,* 2010). For those who with lactose intolerance, taking digestive enzymes containing lactase when consuming dairy products often improves symptoms.

2.1.7 EPIDEMIOLOGY OF DIARRHEA: Worldwide in 2004 approximately 2.5 billion cases of diarrhea occurred, which resulted in 1.5 million deaths among children under the age of five. Greater than half of these were in Africa and South Asia. This is down from a death rate of 4.5 million in 1980 for gastroenteritis (Mandell, *et. al.,*2004). Diarrhea remains the second leading cause of infant mortality (16%) after pneumonia (17%) in this age group.The majority of such cases occur in the developing world, with over half of the recorded cases of childhood diarrhea occurring in Africa and Asia with 696 million and 1.2 billion cases respectively, compared to only 480 million in the rest of the world (WHO, 2013).

Infectious diarrhea resulted in about 0.7 million deaths in children under five years old in 2011 and 250 million lost school days (United Nations, 2015; Walker, *et. al.,* 2013). In the Americas, diarrheal disease accounts for a total of 10% of deaths among children aged 1–59 months while in South East Asia, it accounts for 31.3% of deaths (Walker, *et. al.*2012). It is estimated that around 21% of child mortalities in developing countries are due to diarrheal disease (Kosek, *et. al.,*2003).

Measures of child health are useful indicators of the health of a nation, particularly for Nigeria where children constitute about 45% of the total population (Fed. Off. Stat., 1997). The country's infant mortality rate of 114 per 1000 live births is among the highest in sub-Saharan Africa and mortality among children under five years of age is as high as 300 per 1000 live births in some parts of the country. Epidemiological evidence shows that diarrhea is a major problem, with estimate done-in-six children under the age of five years experiencing at least one episode every fortnight (Fed. Off. Stat., 1997). Children aged 4–24 months are at

the greatest risk of developing diarrhea from contaminated food and water. Normally, breast milk is the Main source of nourishment for children within their first months of life. The dependence on breast milk reduces their exposure to food-borne pathogens, and the anti-infective properties of breast milk also afford some protection. Between 4 and 6 months of age, however, complementary foods are given, and children are thus exposed to food-borne pathogens. Study of 454 children in eastern Nigeria showed that the incidence of diarrhea was highest among children aged between 6 and12 months, the age range which coincides with the usual weaning period in the region (Duche *et al.*, 2013).

Epidemiological study by Huttly *et al* (1987) recorded diarrhea in rural communities in Imo State, Nigeria to be high with acute diarrhea found to be associated with up to 75% of all illnesses in young children. The same study showed that the risk factors included lower socio-economic status, an unclean domestic environment, use of non-purified water, absence of soap, and feeding methods other than exclusive breast-feeding in the early months of infancy. From their research one could deduce poor domestic hygiene, and poor infant feeding as predisposing factors that have contributed to these findings.

Also, elsewhere in Imo State, a study carried out by Benjamin *et al* (2013) on cryptosporidiosis among children in some rural parts of Imo state Nigeria in September, 2013, showed that cryptosporidiosis poses a public health challenge in children found in rural parts of Imo State, though it has not attained epidemic proportion. In this study, an overall prevalence of 14.3% was recorded and the infection was more prevalent among children in primary schools with 16.4%, followed by those in daycare centers, (14.5%).

2.1.7.1 GLOBAL BURDEN OF DIARRHEAL DISEASE IN CHILDREN: Diarrhea as a global problem is more prevalent in developing countries like Nigeria. This burden is predominant in conditions of poor environmental sanitation, absence of good water

source/supply, poverty and limited education on the disease and associated risk factors (WHO, 2000). WHO says 99.2 million DALYs (disability adjusted life years) lost is caused as a burden as approximately 1 billion cases of diarrhea occur each year worldwide. It is well known that diarrheal disease is one of the leading causes of illness and death in young children in developing countries. Diarrhea accounts for 21% of all disease causing deaths at below five years of age and causes 2.5 million deaths per year, although diarrhea morbidity remains relatively unchanged, about 1 billion episodes or 3.2 episodes per child-year (Kosek *et al.,* 2003; WHO, 2000; Parashar *et al.,* 2003).

2.1.8 IMPACT OF DIARRHEAL DISEASE ON CHILDREN:

2.5 Million Deaths caused by diarrhea yearly, is a large burden. In addition, many time, this number have long-term lasting effects on nutritional status, growth, fitness, school performance and cognition (Kosek *et al.,* 2003; WHO, 2000). Some studies have revealed the impact of diarrhea on growth (Molbak, 2000; Checkley *et al.* 2003; Moffat, 2003). It is believed that diarrhea have a significant impact on growth due to reduction in appetite, altered feeding practices and decreased absorption of nutrients (WHO, 2000). Each day of illness due to diarrhea produces a weight deficit of 20 – 40 grams. Molbak, (2000) found that infants who spent more than 20% of their time with diarrhea had a weight deficit of approximately 370 grams at follow-up after 1 year of age.

Impacts on height has also been observed and the impact varied by age and sex. For instance, during infancy, boys who spent from 20% to less than 40% of their time with diarrhea were 5.1mm shorter than those that had no diarrhea, whereas the deficit in girls was negligible. At age of 1 – 4 years, with the same time spent with diarrhea, the deficit on height was 2.1mm and 3.0mm in boys and girls respectively (Molbak, 2000). As said earlier, diarrhea has impact on height by age. Diarrhea during the first 6 months of life resulted in long-term height

deficits that were likely to be permanent. In contrast, diarrhea after 6 months of age showed transient effects. Similarly, Molbak, (2000) indicated that after 6 months of age, the effect of diarrhea on growth was transient due to catch-up growth.The greatest impact of diarrhea on children's growth occurs in the first 3 years of life and particularly, during the second half of infancy (6 – 12 months) and in sound year of life.

2.2 PROTOZOA: In some biological taxonomy schemes, protozoa are a diverse group of mostly motile unicellular eukaryotic organisms (Edward and Jennifer, 2009). In classifications proposed by Thomas Cavalier-Smith and his collaborators, the group is ranked as a kingdom comprising seven phyla (Ruggiero *et. al.*, 2015). Historically, protozoa were defined as unicellular protists with animal-like behavior, such as movement. The group was regarded as the zoological counterpart to the Protophyta, which have plant-like behavior, e.g. photosynthesis.

Formal use of the term protozoan has been discouraged by some researchers, because modern ultra structural, biochemical, and genetic techniques have shown that the group does not form a clad, as required by phylogenetic systems of classification. In 2005, the members of the Society of Protozoologists voted to change the name of that organization to the International Society of Protistologists.

While use of the term protozoa has declined in the professional literature, the term is still used informally for single-celled and heterotrophic eukaryotes, such as ciliates and flagellate parasites (highly motile, non-photosynthetic organisms which, traditionally, have been regarded as "animal-like").

Most protozoa are restricted to moist environments, such as soils, mosses and aquatic habitats, although many form resting cysts which enable them to survive drying. Many

protozoan species are symbionts, some are parasites, and some are predators of faeces, bacteria and algae. There are an estimated 30,000 protozoan species (Nyle and Ray, 2009).

2.2.1 TYPES OF PROTOZOA

Different protozoan of special mention include the following:

2.2.1.1 ***Giardia lamblia:*** is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing Giardiasis. The parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission (Oxford, 2003). Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastrointestinal tract, but remains confined to the lumen of the small intestine. *Giardia* trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes. If the organism is split and stained, its characteristic pattern resembles the familiar "smiley face" symbol. Chief pathways of human infection include ingestion of untreated sewage, a phenomenon particularly common in many developing countries (Hogan, 2010); contamination of natural waters also occurs in watersheds where intensive animal grazing occurs.

Giardia infection can occur through ingestion of dormant microbial cysts in contaminated water, food, or by the fecal-oral route (through poor hygiene practices). The cyst can survive for weeks to months in cold water, (Huang and White, 2006) so can be present in contaminated wells and water systems, especially stagnant water sources, such as naturally occurring ponds, storm water storage systems, and even clean-looking mountain streams. They may also occur in city reservoirs and persist after water treatment, as the cysts are resistant to conventional water treatment methods, such as chlorination and ozonolysis (Huang and White, 2006). Zoonotic transmission is also possible, so *Giardia* infection is a concern for people camping in the wilderness or swimming in contaminated streams or lakes,

especially the artificial lakes formed by beaver dams (hence the popular name for Giardiasis, "beaver fever").

In addition to waterborne sources, fecal-oral transmission can also occur, for example in day-care centers, where children may have poor hygiene practices. Those who work with children are also at risk of being infected, as are family members of infected individuals. Not all *Giardia* infections are symptomatic, and many people can unknowingly serve as carriers of the parasite.

2.2.1.2 ***Entamoeba histolytica:*** is an anaerobic parasitic protozoan, part of the genus *Entamoeba* (Ryan and Ray, 2004). Predominantly infecting humans and other primates, *E. histolytica* is estimated to infect about 50 million people worldwide. Previously, it was thought that 10% of the world population was infected, but these figures predate the recognition that at least 90% of these infections were due to a second species, *E. dispar* (Ryan and Ray, 2004). Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission. The word *histolytic* literally means "Tissue destroyer".

The active (trophozoites) stage exists only in the host and in fresh loose feces; cysts survive outside the host in water, in soils, and on foods, especially under moist conditions on the latter. The cysts are readily killed by heat and by freezing temperatures, and survive for only a few months outside of the host (AWWA, 2006). When cysts are swallowed they cause infections by excysting (releasing the trophozoites) in the digestive tract. Infection can be asymptomatic or can lead to amoebic dysentery or amoebic liver abscess (Ryan and Ray, 2004). Symptoms can include fulminating dysentery, bloody diarrhea, weight loss, fatigue, abdominal pain, and ameboma. The amoeba can actually 'bore' into the intestinal wall, causing lesions and intestinal symptoms, and it may reach the blood stream. From there, it

can reach different vital organs of the human body, usually the liver, but sometimes the lungs, brain, spleen, etc. A common outcome of this invasion of tissues is a liver abscess, which can be fatal if untreated. Ingested red blood cells are sometimes seen in the amoeba cell cytoplasm.

2.2.1.3 *Cryptosporidium parvum:* *cryptosporidium parvum* is one of several protozoan species that cause cryptosporidiosis, a parasitic disease of the mammalian intestinal tract. Primary symptoms of *C. parvum* infection are acute, watery, and non-bloody diarrhea. *C. parvum* infection is of particular concern in immunocompromised patients, where diarrhea can reach 10–15L per day. Other symptoms may include anorexia, nausea/vomiting and abdominal pain. Extra-intestinal sites include the lung, liver and gall bladder where it causes respiratory cryptosporidiosis, hepatitis and cholecystitis (CDC, 2007).

Infection is caused by ingestion of sporulated oocysts transmitted by the fecal-oral route. In healthy human hosts, the median infective dose is 132 oocysts. The general *C. parvum* life cycle is shared by other members of the genus. Invasion of the apical tip of ilealenterocytes by sporozoites and merozoites causes pathology seen in the disease.

Infection is generally self-limiting in immunocompetent people. In immunocompromised patients, such as those with AIDS or those undergoing immunosuppressive therapy, infection may not be self-limiting, leading to dehydration and, in severe cases, death.

Cryptosporidium parvum oocysts are very difficult to detect and therefore diagnose. The oocysts in fecal samples would indicate if the parasite is present in the body but they have a very small size and so detection is made very difficult. Doing a fecal ELISA method to detect the presence of the parasite would eliminate the difficulty due to the small size and many

other methods are unable to distinguish between past and present infections; something serological ELISA would not allow to distinguish (Dupont, 2014).

C. parvum is considered to be the most important waterborne pathogen in developed countries. The protozoa also caused the largest waterborne-disease outbreak ever documented in the United States, making 403,000 people ill in Milwaukee, Wisconsin in 1993. It is resistant to all practical levels of chlorination, surviving for 24hrs at 1000 mg/L free chlorine. It is an obligate intracellular pathogen (Deng *et. al.,* 2004).

Transmission of agents that cause diarrhea, such as the ones aforementioned, are usually by fecal-oral route which include the ingestion of fecal contaminated water or food, person to person contact and direct contact with infected faeces (Andu *et. al*., 2002). Epidemiological studies of diarrhea have been reported from several African countries including Gabon (Presterl *et. al*., 2003) and Egypt (Rao *et. al*., 2003). It ranks second only to respiratory diseases and is a major cause of morbidity among notable diseases in some part of the world. The main cause of death from acute diarrhea is dehydration, which result from loss of fluid and electrolyte in stool. Another important cause of death is dysentery and under nutrition. Diarrhea is an important cause of under nutrition because patients eat less during diarrhea and their ability to absorb nutrients is reduced. Moreover, nutrient requirement is increased as a result of infection (Sinclair *et. al.,* 2003).

Risk factors that predispose children to diarrhea include poor sanitation, poor social and economic status and malnutrition (Andu *et. al*., 2002). Laboratory information is particularly useful to help distinguish invasive enteropathogens (which may require antimicrobial therapy) from non-invasive agents, such as viruses (rotavirus, adenovirus, calicivirus, and astrovirus) and parasites (*Giardia lamblia, Entamoeba histolytica* and *Cryptosporium parvum*).

CHAPTER THREE

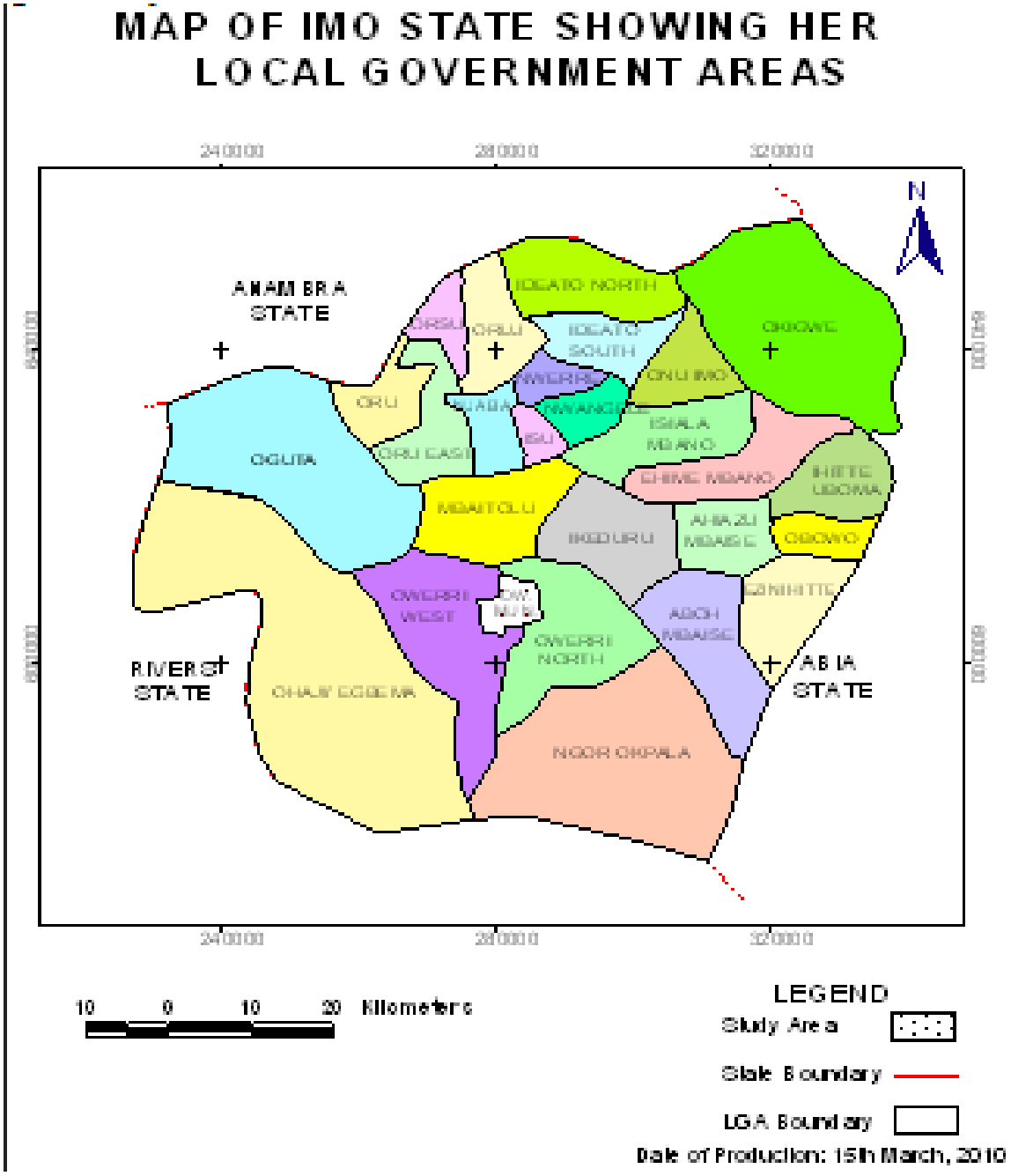
MATERIALS AND METHODS

3.1 MATERIALS: See appendix column at the back.

3.2 METHODS:

3.2.1 STUDY AREA: Imo State is one of the 36 states of Nigeria and lies in the South Eastern Nigeria with Owerri as its capital and largest city. Having been previously part of East-Central State, the state is named after the Imo River. The State is made up of three geopolitical areas namely Owerri, Orlu and Okigwe. The Orashi River has its source in this state. The local language is Igbo and Christianity is the predominant religion.

The state lies within latitudes 4°45'N and 7°15'N, and longitude 6°50'E and 7°25'E with an area of around 5,100 sq km. It shares boundary with Abia State on the East, River Niger and Delta State on the west, Anambra State on the north and Rivers State on the south. Besides Owerri, Imo State's major towns are Isu, Okigwe, Oguta, Orlu, Akokwa, Mbaise, Mbano, Mbaitoli, Mbieri, Orodo, Nkwerre, Ubulu, Ngor Okpala, Omuma, Mgbidi, Awo-Omamma and Orsu.(Imo State of Nigeria Statistical Year Book, 2000).



3.2.2 STUDY POPULATION: This scientific research covered a population of Imo State children of less than 12 years of age in selected health institutions in the state. The selected health institutions included General Hospitals in Aboh Mbaise, Arondizuogu, Awomamma, Ihitte Uboma, and Okigwe. Also, samples were drawn from Federal Medical Center Owerri

and Imo State University Teaching Hospital Orlu which are the major referral centers in the state. Approval from the hospital managements were obtained before sample collections.

3.3.3 SAMPLE COLLECTION: A total of 1,200 stool samples of the subjects were collected. Single faecal sample from each child was collected with sterile leak-proof sample containers, labeled and transported to the laboratory for analysis. Stool sample collection process was explained to the subjects, (study population), as in Ukaga and Nwoke (2007). About 10g of fluid/diarrheal stool was collected from each subject.

Serial numbers, date of collection, sex, and time stool was passed, were labeled against clean wide stool bottles. The samples were stored temporarily in 10% formal saline prior to laboratory investigation during transportation of specimen (Ochei and Kolhalkar, 2008).

3.3.4 STUDY VARIABLES: A detailed researcher's administered questionnaire was distributed during sample collection. This helped to ascertain their gender, age, socio-economic status, eating habit, source of drinking water, method of purifying drinking water, and clinical presentation.

3.3.5 LABORATORY ANALYSIS/EXPERIMENTATION: This examination was of two parts, the visual or macroscopic examination of faeces and the microscopic examination popularly called the wet mount preparation were employed.

3.3.5.1 MACROSCOPIC EXAMINATION OF FAECES: This involved a careful visual examination of the stool samples with the naked eyes. The color, constituency and presence of blood, pus, mucus, were checked and recorded as explained in Ochei and Kolhalkar, (2008). The aid of an applicator stick was employed in the check for consistency. Careful visual examination of stool was done thus:

- ✓ Color, (brown, reddish, black-tarry, clay, or green)

- ✓ Consistency, (formed, soft, loose or watery)
- ✓ Blood
- ✓ Pus
- ✓ Mucus, and labeled as explained in Ukaga and Nwoke, (2007).

*Applicator stick was used where necessary.

3.3.5.2 MICROSCOPIC EXAMINATION OF STOOL: Microscopic identification of parasites was based on diagnostic stages of the pathogens, i.e. morphology of trophozoites, cysts, oocysts, and ova. Each specimen was examined by direct saline wet mount. Formol–ether concentration and Modified Zielh-Neelsen staining technique were used to detect oocysts of *Cryptosporidium parvum* as explained in Ukaga, and Nwoke, (2007) and Henriksen and Polen, (1981) respectively.

The microscopic examinations were as follows:

- ➢ Saline Wet Mount was first prepared as follows:
    - ✓ A glass slide was cleaned thoroughly with dry cotton wool
    - ✓ It was labeled with wax pencil on the left with
        - Subject number
        - Date
    - ✓ A drop of saline was made on the center of the slide
    - ✓ With applicator stick a gram of the stool was collected
    - ✓ Mixed well with the drop of saline
    - ✓ Holding the cover slip at an angle
    - ✓ It was touched at the edge of the drop
    - ✓ And then gently lowered onto the slide
    - ✓ It was viewed under low power (X10) to detect agents and

- ✓ Then viewed with high power dry objectives to identify

For *Cryptosporidium parvum*, two techniques were used:

- Formol-ether concentration (Ukaga and Nwoke, 2007)
- Modified Zielh-Neelsen Staining (Henriksen and Polen, 1981).

FORMOL-ETHER CONCENTRATION IS THUS:

- With Pasteur's pipette, 10ml of 10% formalin was placed in a tube
- Then it was mixed well with 1g of stool using the applicator's stick
- It was stirred until slightly cloudy suspension was formed
- A gauze filter was fitted in a funnel and placed on a centrifuge tube
- Suspension was passed through the filter until 7ml mark was reached
- Filter was discarded and 3ml of ether was added to the suspension
- It was mixed well for 3mins
- Then transferred to the centrifuge and centrifuged for 1min
- The fatty plug (debris) formed was loosened with the applicator stick
- Then the supernatant was discarded by inverting the tube
- Tube was replaced in its rack
- Then the fluid on the side of tube was allowed to settle with the sediment
- It was mixed well and a drop was transferred onto a slide

MODIFIED ZIELH-NEELSEN STAINING IS THUS:

- A smear was made from slide of formol-ether conc.
- The smear was dried at 50°C in oven for 2mins
- Then fixed first with 96% methanol for 3mins
- And was passed briefly through a flame

- It was stained with Conc. Carbol Fuchsin (0.34% fuchsin & 4% w/v phenol)
- And stained for 20mins without heating
- The smear was later washed for 30sec. with 1% sulphuric acid sol.
- It was counterstained with 5% Malaquite green for 5min
- Rinsing was done with tap water
- It was then viewed under microscope at X800 magnification.

3.3.6 STATISTICAL ANALYSIS: Raw data were entered into Excel Table and analyzed with Statistical Package for Social Sciences (SPSS), version 20.0. Chi – Square Test (X^2) was used to determine statistical differences of the variables.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 RESULT

Out of the 1,200 subjects comprising of 516 (43.0%) male and 684 (57.0%) female examined for intestinal protozoan agents of diarrhea, 404 (33.6%) yielded positive result while 796 (66.3%) were negative. Table 1 shows the statistical significant difference of the gender related prevalence of the subjects.

Table 1: Gender Related Prevalence of Infection among the Subjects.

Gender	Number Examined	Number Uninfected	Number Infected	Prevalence (%)
Female	684	395	289	42.3
Male	516	401	115	22.3
Total	**1,200**	**796**	**404**	**33.6**

χ^2cal. = 26.85

χ^2tab. = 3.84, *df* =1

($p<0.05$); statistically significant.

Table 2: Age Descriptive Statistics of the Subjects.

Parameter	N	Minimum	Maximum	Mean	Standard Deviation
Age (Years)	1,200	1	11	4	3.051
Valid N (Listwise)	1,200				

S.D = 3.051

The mean age and standard deviation was noted as 4±3.05 as shown in table 2 below.

Table 3 shows the distribution and occurrence of the intestinal protozoan parasites isolated from the subjects. Of the three protozoan parasites isolated, *Cryptosporidium parvum* occurred most (20.0%) followed by *Entamoeba histolytica* (10.3%) while the least occurrence (3.3%) was noted for *Giardia lamblia*.

Table 3 Frequency Distribution of the Intestinal Protozoan Parasites Isolated

Parasite isolated	Frequency	Valid (%)
Cryptosporidium parvum	240	20.0
Entamoeba histolytica	124	10.3
Giardia lamblia	40	3.3
Negative subjects	796	66.3
Total	**1,200**	**100**

The age distribution of the subjects examined for intestinal protozoan agents of diarrhea is shown in Table 4. Age group of 5-8 years yielded the highest prevalence (37.5%) of infection while prevalence rates of 33.8% and 26.6% were noted for the age groups of 1- 4 years and 9-11 years respectively. No statistical significant difference was noted in the prevalence rates among the various age groups ($p>0.05$). Details are shown in Table 4 below.

Table 4: Age-related Prevalence of Intestinal Protozoan Agents of Diarrhea Isolated.

Age (Years)	Number examined	Number infected	Prevalence (%)
1 – 4	719	243	33.8
5 – 8	301	113	37.5
9 – 11	180	48	26.6
Total	**1,200**	**404**	**33.6**

χ^2cal. = 2.83

χ^2tab. = 5.99, *df* = 2

(p>0.05); statistically non-significant.

Table 5 shows the prevalence of parasitic agents of diarrhea according to school enrolment of the subjects. Subjects in primary school were infected most (39.8%) while the least infection (8.1%) was noted for subjects who were not enrolled in school. However, prevalence rates of 36.9%, 30.1% and 32.4% were recorded for subjects in Day-care, Pre-nursery and Nursery school respectively. Significant difference exist among the various prevalence rates according to school enrollment ($p<0.05$).

Table 5: Prevalence of Diarrhea According to School Enrolment

School enrolment	Number examined	Number infected	Prevalence (%)
Not enrolled	86	7	8.1
Day-care	237	87	36.9
Pre-nursery	186	56	30.1
Nursery	290	94	32.4
Primary	402	160	39.8
Total	**1,200**	**404**	**33.6**

χ^2cal. = 19.00

χ^2tab. = 9.49, *df* = 4

($p<0.05$); statistically significant.

Table 6 shows the distribution of the parasites according to occupation of the mothers. Children whose mothers are farmers were infected most (61%) followed by children of traders (55.6%) while those whose mothers are applicants yielded the least prevalence (17.5%). Prevalence rates of 8.0%, 20.2%, 32.6% and 19.7% were obtained for children whose mothers are artisans, civil servants, house wives and others respectively. Details are shown in Table 6 below. Statistical significant difference was noted for distribution of parasites according to occupation of the mothers.

Table 6: Distribution of Infection among Children in relation to Mothers' Occupation.

Occupation of Mothers	Frequency	(%)	N0. of Subjects Examined	Number Infected (%Prevalence)
Applicant	97	8.1	97	17(17.5)
Artisan	179	14.9	179	68(38.0)
Civil servant	262	21.8	262	53(20.2)
Farmer	115	9.6	115	71(61.7)
Housewives	181	15.1	181	59(32.6)
Trading	178	14.8	178	99(59.6)
Others	188	15.7	188	37(19.7)
Total	**1,200**	**100.0**	**1,200**	**404(33.6)**

χ^2cal. = 60.75

χ^2tab. = 12.59, *df* = 6

($p<0.05$); statistically significant

The prevalence of the protozoan parasites according to the area of residence of the subjects is shown in Table 7 below. Subjects resident in rural areas were infected most (37.3%) while those residing in urban areas yielded the least (27.1%) prevalence of infection. There was significant difference in the prevalence of parasites according to area of residence of the subjects by chi-square statistical analysis of data ($p<0.05$).

Table 7: Prevalence of the Parasites according to area of Residence of the Subjects.

Residence	Number examined	Number infected	Prevalence (%)
Urban	431	117	27.1
Rural	769	287	37.3
Total	**1,200**	**404**	**33.6**

χ^2cal. = 6.50

χ^2tab. = 3.84, *df* = 1

($p<0.05$); statistically significant.

Table 8 shows the prevalence rates of the parasites amongst the subjects according to the three Senatorial Zones. Prevalence rates of 30.6%, 41.8% and 28.9% were recorded for subjects from Owerri, Orlu and Okigwe respectively. Statistical significant difference was also noted in prevalence rates according to zones ($p<0.05$).

Table 8: Prevalence of Parasites according to the Three Senatorial Zones in Imo State.

Zone	Number examined	Number infected	Prevalence (%)
Owerri	422	129	30.6
Orlu	390	163	41.8
Okigwe	388	112	28.9
Total	**1,200**	**404**	**33.6**

χ^2cal. = 8.57

χ^2tab. = 5.99, *df* = 2

($p<0.05$); statistically significant.

Table 9 shows the prevalence of protozoan agents of diarrhea according to the selected health institutions in the area. Samples from Orlu Teaching Hospital yielded the highest (42.3%), followed by samples from General Hospital Awomamma (40.0%) while samples from Aboh General Hospital recorded the least prevalence (19.6%). Prevalence rates of 32.6%, 34.0%, 28.0% and 28.4% were also recorded for samples from General Hospital Arondizuogu, Federal Medical Center Owerri, General Hospital Ihitte Uboma and Okigwe General Hospital respectively. Chi-square analysis showed no significant difference in prevalence rates according to the health institutions sampled ($p>0.05$).

Table 9: Prevalence of Parasites according to the Selected Health Institutions Sample

Tertiary hospitals	Number examined	Number positive	Prevalence (%)
Aboh Mbaise	102	20	19.6
Arondizuogu	49	16	32.6
Awomamma	90	36	40.0
Federal medical Centre Ow.	320	109	34.0
IhitteUboma	75	21	28.0
Okigwe	264	75	28.4
Orlu	300	127	42.3
Total	**1,200**	**404**	**36.6**

χ^2cal. = 12.46

χ^2tab. = 12.59, *df* = 6

(p>0.05); statistically non-significant.

Table 10 shows the knowledge assessment of subjects and their care-givers on source of diarrhea infection. The result showed that out of the 1,200 subjects/care-givers, 19.58% responded yes to contaminated food as source of diarrhea infection. 14.17%, 2.83%, 4.42% and 27.33% of the subjects/care-givers responded to contaminated water, spiritual problem, witchcraft and mosquito bite respectively as sources of diarrhea infection. 31.16% had no idea to the source of diarrhea infection.

Table 10. Knowledge Assessment of Subjects/Care-givers on Source of Diarrhea Infection

Source of infection	Response (%)
Contaminated food	235(19.58)
Contaminated water	170(14.17)
Spiritual problem	34(2.83)
Witchcraft	53(4.42)
Mosquito bite	328(27.33)
No idea	380(31.67)
Total	**1,200(100)**

Table 11 shows the assessment of attitude/practice of the subjects and their care-givers to source of diarrhea infection. The result further showed that out of the 1,200 subjects/care-givers, 25.83% responded yes to hand washing after defecation while 74.17% responded no while 27.08% and 72.92% responded yes and no respectively to hand washing before each meal. Also 41.75% and 58.25% of the subjects responded yes and no respectively to defecation on/near play ground. The statistical difference in relation to the attitude and practice of the sources of diarrhea infection was tested ($p<0.05$).

Table 11. Attitude/Practice Assessment of Subjects and their Care-givers on Diarrhea Transmission

Attitude/practice on source of infection	Response (%)		Total
	Yes	**No**	
Hand washing after defecation	310(25.83)	890(74.17)	**1,200**
Hand washing before meal	325(27.08)	875(72.92)	**1,200**
Defecation on/near play ground	501(41.75)	699(58.25)	**1,200**

4.2 DISCUSSION

The result of this research confirmed that part of the causes of diarrheic infections among children in Imo State is due to protozoan parasites. Prevalence rate of 42.0% was recorded for female subjects while the male subjects yielded the least prevalence of 22.2% for intestinal protozoan agents of diarrhea. This may be attributed to difference in exposure to sources of infection. Previous researchers (Benjamin *et al*., 2013; Akingbade *et al*., 2013; Awolaju and Morenikeji, 2009) have reported similar results in Imo State, though Atu and Jimoh (2015) and Oguoma *et al* (2008) reported highest infection among male subjects in Benue state and Owerri respectively. Significant relationship exists between gender and prevalence of infection.

The intestinal protozoan parasites isolated from the subjects included *Cryptosporidium parvum, Entamoeba histolytica,* and *Giardia lamblia*. *Cryptosporidium parvum* known to cause cryptosporidiosis occurred most (20.0%), followed by *Entamoeba histolytica* (10.3%) and *Giardia lamblia* (3.3%).This means that *C. Parvum* still remains a major parasitic agent of diarrhea in Imo State as noted by Dozie *et al* (2011) and Benjamin *et al* (2013). Therefore continuous effort should be maintained by health care providers and stakeholders so as to eradicate the parasite from this part of the world. Conversely, the present result contrasts the report of Oguoma *et al* (2008) who recorded prevalence rates of 3.5%, 35.5% and 25% for *C. parvum, E. histolytica* and *G. lamblia* respectively.

Furthermore, the result of the present study also proved the work of Akingbade *et al* (2013) to be true that intestinal parasitic infections are important public health challenge in the tropics and developing countries especially in children. This evidence corroborates the report of Huttly *et al* (1987) that put diarrheal infection – which protozoan parasites are major contributors – at 75% cause of illness in young children in Imo State, especially in rural

communities. More so the present result further supports that of Kosek *et al* (2003) who noted that diarrhea was the cause of high child mortality of about 21% in developing countries, like Nigeria.

Furthermore, prevalence of infection varied according to the various age groups of the sampled population. The highest prevalence of 37.5% was recorded for age group of 5 – 8 years. While the age groups of 1 – 4 years and 9- 11 years yielded prevalence rates of 33.8% and 26.6% respectively. The highest prevalence of 37.5% noted among the age group of 5 – 8 years may be attributed to the fact that children of this age group indulge more in activities which favour hand-to-mouth transmission of these protozoan parasites, especially *C. parvum* and *G. lamblia.* It may also be linked to the fact that children of this age group are found mostly in primary schools with less care from their parents and care-givers at home and during school hours respectively. This may also be the reason for the high prevalence rate of 33.8% recorded for age group of 1 – 4 years. Conversely, the least prevalence of 26.6% recorded for the age of 9 – 11 years may be attributed to the fact that children of this age group are more mature than their counterparts in terms of hand hygiene. Similar reports have been noted by previous researchers (Simon-oke and Ogunleye 2015; Benjamin *et al.,* 2013; Oguoma *et al.,* 2008; Awolaju and Morenikeji, 2009)

Also, prevalence of infection among the subjects was noted according to school enrolment. Children of primary school age yielded the highest prevalence of 39.8% for intestinal protozoan agents of diarrhea, while the least prevalence (8.1%) was recorded for children not enrolled in school. Children in Day-care, Pre-nursery and Nursery yielded prevalence rates of 36.9%, 30.1% and 32.4% respectively. The least prevalence of 8.1% noted among children not enrolled in school may be attributed to more care on the children in terms of general hygiene by their care-givers. Secondly, this category of children has less contact with other children who may be carriers of the pathogens since they are not enrolled in school. High

prevalence rates noted among other categories may be attributed to the fact that they are more exposed to the pathogens via activities that predispose them to fecal-oral route transmission. More so, crowded environment like school pose a risk factor in disease transmission. This is evidenced by the fact that children in nursery and primary schools carelessly defecate around the school premises especially those in rural areas and some urban areas where toilet facilities and water supply are inadequate. By so doing, oocysts or infectious stage of these parasites easily disseminate within the school environment thereby making it easier for the pathogens to get the subjects via fecal-oral route since hand-to-mouth activities are predominant among these school categories. This result also supports that of previous researchers (Oguoma *et al.,* 2008). Significant difference was noted between the prevalence of infection among children not enrolled in school and their counterparts enrolled in school ($p<0.05$).

The present study further compared rate of infection according to occupation of the mothers. Children whose mothers are farmers yielded the highest prevalence (61.7%) followed by those whose mothers are traders (55.6%). Probably children of farmers do accompany their mothers to the farm for farming activities. In the process they may be forced by circumstance to indulge in hand-to-mouth activities or drink the nearby stream water which may serve as potential source of infectious stage of the protozoan parasites isolated in this study. The present result therefore shows that there is a link between prevalence of infection and the occupation of the mothers of the sampled population. Therefore adequate health education on the need for proper hand hygiene should be ensured among the mothers especially traders and farmers residing in Imo State. Government and stakeholders therefore, should map out program/enlightenment campaigns geared towards educating school children on the significance of fecal-oral route means of pathogen transmission, especially protozoan agents of diarrhea.

The result of this study also highlighted the prevalence rates among subjects resident in rural and urban areas. However, infection occurred most (37.3%) among subjects resident in rural areas while those resident in urban yielded the least (27.1%) prevalence of intestinal protozoan agents of diarrhea. This means that children in rural areas are more exposed to the infectious agents than their urban counterparts. Possibly, there are inadequate knowledge/measures on proper hand hygiene among the rural dwellers in the study area. However, government should ensure adequate measures geared towards closing this gap. This result further corroborates the reports of Dozie *et al* (2011) who reported highest prevalence of *C. parvum* among rural dwellers when compared with their urban counterpart in Imo State, South Eastern Nigeria. Also Odu *et al* (2010) gave a similar report in Rivers State, South-South Nigeria.

Zonal distribution of infection showed that subjects from Orlu senatorial zone were infected most (41.8%) followed by those in Owerri senatorial zone (30.6%), while Okigwe senatorial zone recorded the least (28.9%) prevalence. The high prevalence of 41.8% and 30.6% recorded for Orlu and Owerri zones respectively shows that human activities that favour the transmission of these protozoan pathogenic agents of diarrhea predominate in these areas. This may also be attributed to non-compliance and non-observance of the risk factors that predispose children to these pathogenic agents of diarrhea. However, this high prevalence of 41.8% recorded among subjects from Orlu zone is high when compared to the prevalence of 21.2% recorded by previous researchers (Dozie *et al.*, 2011) in the area, though their work centered on the prevalence of *Cryptosporidiosis* in Imo State. Conversely, 28.9% prevalence recorded for children resident in Okigwe zone depicts that infection is low in this area when compared to other zones like Owerri and Orlu zones. May be the protozoan pathogens studied in this research do not predominate in this zone. The reason for this low prevalence noted for children from Okigwe zone may also be that there is bettercompliance and

observance of the knowledge, attitude and practice on the transmission routes of these protozoan pathogenic agents. This result recorded for Okigwe zone contrasts that of previous researchers (Dozie *et al.,* 2011; Benjamin *et al.,* 2013) in this area.

The prevalence rates of protozoan parasites of diarrhea according to the samples drawn from the selected health institutions in the study areas were compared. The high prevalence rates of 42.3% and 40.0% from Orlu Teaching Hospital and General Hospital Awomamma respectively may be attributed to the fact that the children who attended these hospitals are more predisposed to the protozoan agents of diarrhea than their counterparts who attended other health institutions. Also, the reason may be that there is less hygienic care on these children by their parents at home and their care-givers in their individual schools against protozoan agents of diarrhea. More so, there may be poor knowledge on preventive measures among children and their care-givers, against contracting diarrhea, who attended these two health institutions than their counterparts that attended the other health institutions like Aboh General Hospital with least prevalence (19.6%).

However, the reason for low prevalence rates from children who attended General Hospital Arondizuogu, Federal Medical Center Owerri, General Hospital Ihitte Uboma and Okigwe General Hospital may be that, there is fair knowledge on predisposing factors among children and their care-givers on the cause of protozoan diarrheal infections.

Conversely, the least prevalence rate of protozoan diarrheal infection recorded for children who attended Aboh General Hospital may be that much hygienic care against the protozoan agents of diarrhea is given to these children than their counterparts who attended other hospitals. Also, knowledge, attitude and practice gap seen among the children and their care-givers who attended other health institutions may be less than the gap seen among children and their care-givers who attended Aboh General Hospital.

In the light of this discussion therefore, there is more work on the part of the government through her health workers to educate parents and care-givers from these risk areas that yielded high prevalence. Parents and care-givers on their own part should make sure they keep to the rules and practices against the factors that predispose children to these protozoan agents of diarrhea.

To ascertain the knowledge, attitude and practice of the sampled population,a pre-tested researcher administered questionnaire was employed. The result showed that larger percentage of the sampled population has poor knowledge on the source of diarrheal infection. This may be the reason why greater population of the sampled subjects yielded positive to protozoan diarrheal infection.

Furthermore, the assessment on the source of diarrheal infection (Table 11) showed that mosquito bite had the highest response (27.33%) as against contaminated food (19.58%), contaminated water (14.17%), spiritual problem (2.83%) and witchcraft (4.42%) of the categories viewed. 31.67% of the subjects/care-givers had no idea on the source of diarrheal infection. The result when critically viewed showed that poor knowledge exists among subjects on the source of protozoan diarrheal infection, because, the sum of the percentages of the subjects that responded negatively is greater than those that responded positively.

Also, the present study showed poor attitude to source of diarrheal infection on the part of the children and their care-givers. This further gave the reason for high prevalence of protozoan diarrhea among the sampled population. It is therefore deduced that poor personal hygiene exists among the subjects.These observations corroborate the work of previous researchers (Afolabi *et al.*,2016) that reported poor feeding habit as one of the major factor that predispose individual to diarrhea.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Summarily, the findings of this present research show that some intestinal protozoan parasites are the cause of diarrhea among children in Imo State. More so, prevalence of these parasites is very high in this area. This calls for urgent attention by the Government through Ministry of Health, so as to map out modalities aimed at proffering lasting solutions to the threat posed by these protozoan pathogens. Adequate campaign and public health enlightenment should also be extended to parents and care-givers in Primary schools and Nursery/Daycare centers in order to ensure adequate hygienic practices at homes and in schools.

Since the protozoan pathogens are transmitted through fecal-oral route, therefore proper environmental sanitation especially fecal disposal should be ensured in this area. Children should be discouraged from defecating indiscriminately around their play grounds, at homes and in school premises. When these measures are ensured, it will go a long way in reducing the prevalence of these protozoan pathogens in this part of the world.

5.2 RECOMMENDATION

Since prevalence is high, there is urgent call on Government to:

1. Map out modalities to alleviate the threat/burden
2. To organize adequate public health campaigns geared towards educating Imo State children and their care-givers on the menace of the protozoan associated diarrhea
3. To put up/enforce laws on adequate health hygienic practices

Since transmission is through fecal-oral route proper fecal disposal should be ensured by both the government at the federal, state and local levels and the Non-governmental organizations (NGOs) including both national and international NGOs.Children should be discouraged from defecating near play grounds at home and in school

REFERENCES

Adekunle, L. (2002). Intestinal Parasites and Nutritional Status of Nigerian Children. Ibadan medical communication Group. *African Journal of Biomedical Research.* **5***(3)*: 115-119.

Afolabi, O. J., Simon-oke, I. A. and Bakare, P. T. (2016). The prevalence of intestinal parasites among students in federal university of technology Akure, Nigeria. *International Journal of Tropical Disease and Health* **14***(2):* 1-9.

Ajero, C. M., Nwoko, B. E. B., Nwoke, E. A. and Ukaga, C. N. (2008). Human Amoebiasis: Distribution and Burden; and the Nigerian Environment. *International Science Research Journal.***1***(2):* 130 -134.

Akingbade, O. A., Akinjinmi, A. A., Ezechukwu, U. S., Okerentugba, P. O. and Okonko, I. O. (2013). Prevalence of intestinal parasites among children with diarrhea in Abeokuta, Ogun State, Nigeria. *Researcher,* **5** (9): 66 – 73.

Allen, S. J., Martinez, E. G., Gregorio, G. V. and Dans, L. F. (2010). Probiotics for treating acute infectious diarrhoea. Allen, S. J., ed. *Cochrane Database Syst. Rev.* 11: 30 - 48.

Andu, R., Omilabu, S.A., Peenze, I. and Steele, D. (2002). Viral diarrhea in young children in two districts of Africa. *Central African Journal for Medicine*, 48: 59- 63.

Arnold, B. F. and Colford, J. M. (2007). Treating water with chlorine at point-of-use to improve water quality and reduce child diarrhea in developing countries: a systematic review and meta-analysis. *The American Journal of Tropical Medicine and Hygiene* **76***(2):* 354–64.

Atu, B. O. and Jimoh, P. O. (2015). Determination of some Protozoan agents of Diarrhea and their relation with Socio-economic Factors and Hygienic Habits in Orphanages in Benue State. *Journal of Natural Sciences Research*. **5***(23):* 2224 – 3186.

Awolaju, B. A. and Morenikeji, O. A. (2009). Prevalence and intensity of intestinal parasites in five communities in south-west Nigeria. *African Journal of Biotechnology*. **8***(18):*4542-4546.

AWWA, (2006). Waterborne Pathogens. *American Water Works Association*. Retrieved, June-06-2015.

Benjamin, N., Chukwuocha, U., Dozie, I., Amajuoyi, O. and Nwoke, M. (2013). Cryptosporidiosis among children in some rural parts of Imo State, Nigeria. *Journal of Public Health and Epidemiology.* **5** *(11):* 440-444.

Black, R. E. (2003). Zinc deficiency, infectious disease and mortality in the developing world. *The Journal of nutrition* **133***(5):* 1485–9.

Black, R. E., Morris, S. S. and Bryce, J. (2003). Where and why are 10 million children dying every year? *Lancet* **361***(9376):* 2226–34.

Black, R. E. and Sazawal, S. (2001). Zinc and childhood infectious disease morbidity and mortality. *The British Journal of Nutrition*. **85** *(2):* 125–9.

Brown, J., Cairncross, S. and Ensink, J. H. (2013). Water, sanitation, hygiene and enteric infections in children. *Archives of Disease in Childhood* **98***(8):* 629–34.

Cairncross, S., Hunt, C., Boisson, S., Bostoen, K., Curtis, V. and Fung, I. C. (2010). Water, sanitation and hygiene for the prevention of diarrhoea. *International Journal of Epidemiology*. **39***(l 1):* 193–205.

Centre for Disease and Control. (2007). Cryptosporidiosis. Laboratory Identification of Parasites of Public Health Concern. Center for Disease Control. Retrieved, June- 06-2015.

Centre for Disease and Control. (2009). Intestinal Protozoan Parasites. Retrieved, June-07-2015.

Centre for Disease and Control. (2009). Intestinal Helminth Parasites. Retrieved, June-07-2015.

Centre for Disease and Control. (2013). Global Diarrhea Burden. Retrieved, June-09-2014.

Centre for Disease and Control. (2013). Diarrheal Diseases in Less Developed Countries. *CDC*. Retrieved, June-17-2015.

Checkley, W., Epstein, L. D., Gilman, R. H., Cabrera, L. and Black, R. E. (2003). Effects of acute diarrhea on linear growth in Peruvian children. *Am. J. Epidemiol.* **157** *(2)*: 166 – 75.

Chagan, M. K., Van den Broeck, J.,Luabeya, K. K., Mpontshane, N. and Bennish, M. L. (2013). Cost of childhood diarrhoea in rural South Africa: exploring cost-effectiveness of universal zinc supplementation. *Public Health Nutrition* **17***(9):* 1–8.

Chukwuma, M. C., Ekejindu, I. M., Agbakoba, N. R., Ezeagwuna, D. A., Anaghalu, I. C. and Nwosu, D. C. (2009). The Prevalence and Risk Factors of Geohelminth Infections among Primary School Children in Ebenebe Town, Anambra State, Nigeria, Middle-East. *J. Scientific Research*. **4***(3):* 211-215.

Corry, J. K. and Brett, V. S. (2004). Common Intestinal Parasites. *Am. Fam. Physician*. **69***(5):* 1111-1118.

Curtis, V. and Cairncross, S. (2008). Effect of washing hands with soap on diarrhoea risk in the community: a systematic review. *The Lancet Infectious Diseases* **3***(5):* 275–81.

Curtis, V., Cairncross, S. and Yonli, R. (2000). Review: Domestic hygiene and diarrhea - pinpointing the problem. *Trop. Med. Int. Health.* **5** *(11)*: 22 – 3.

De Bruyn, G. (2008). Diarrhoea in adults (acute). *ClinEvid.* Retrieved, June-08-2015.

Deng, M., Lancto, C. A. and Abrahamsen, M. S. (2004). *Cryptosporidium parvum* regulation of human epithelial cell gene expression. *International Journal for Parasitology* **34***(1):* 73–82.

Doyle, (2013). *Anesthesia for otolaryngologic surgery*. edited by Basem Abdelmalak, D. John Cambridge: Cambridge University Press. p. 282–287.

Dozie, I., Nkem, B. and Chukwuocha, U. (2011). Cryptosporidiosisin Imo State, Nigeria. *J. Rural Trop. Public Health* 10:106-110.

Duche, R. T., Omali, O. and Umeh, E. U. (2013). Bacterial contamination of children's weaning foods in Asase, North Bank, Makurdi. *International Journal of Scientific and Engineering Research.* **4** *(8)*: 2184 - 2225.

DuPont, H. L. (2014). Acute infectious diarrhea in immunocompetent adults. *The New England Journal of Medicine* **370***(16):* 1532–40.

DuPont, H. L., Ericsson, C. D., Farthing, M. J., Gorbach, S., Pickering, L. K. and Rombo, L. (2009). Expert review of the evidence base for self-therapy of travelers' diarrhea. *J Travel Med* **16***(3):* 161–71.

Edward A. I. and Jennifer, M. W. (2009). *Schaum's Outline of Microbiology*. McGraw Hill Professional. pp. 144.

Ejemot, R. I.,Ehiri, J. E., Meremikwu, M. M. and Critchley, J. A. (2008). Hand washing for preventing diarrhoea. Ejemot, R. I., ed. *Cochrane Database Syst. Rev.* (1): 42-65.

Federal Office of Statistics. (1997). Poverty and welfare in Nigeria. *Abuja, Federal Office of Statistics, National Planning Commission, World Bank Resident Mission.*p 12–13.

Ferreira, M. S. and Borges, A. S. (2002). Some aspect of Protozoan Infections in Immunocompromised Patients – A review. *Mem. Inst. Oswaldo Cruz.* **97** *(4)*: 443 – 457.

GBD, (2013). Mortality and Causes of Death, Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **385***(9963):* 117–71.

Henriksen, S. A. and Pohlens, J. (1981). Staining of C*ryptosporidium* by a modified Zielh-Neelsen Technique. *Acta Vet. Scand.* 22:594-596.

Hempel, S., Newberry, S. J., Maher, A. R., Wang, Z., Miles, J. N. and Shanman, R. (2012). Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA: The Journal of the American Medical Association* **307***(18):* 1959–69.

Hogan, C. M. (2010). Water pollution. In McGinley, Mark; Cleveland, C. Washington DC: National Council for Science and the Environment. *Encyclopedia of Earth.* Retrieved, June-07-2015.

Huang, D. B. and White, A. C. (2006). An updated review on Cryptosporidium and Giardia. *Gastroenterol. Clin. North Am.* **35***(2)*: 291–314

Huttly, S. R., Blum, D., Kirkwood, B. R., Emeh, R. N. and Feachem, R. G. (1987). The epidemiology of acute diarrhea in a rural community in Imo state, Nigeria. *Pubmed.* **81** (5): 865-70.

Imo State Of Nigeria Statistical Year Book, (2000). Land Areas and Population Density, 2nd Ed, Nigeria, Government Press. p 3.

Jamison, D. T. (2006). *Disease control priorities in developing countries* (2nd ed. ed.). New York: Oxford Univ. Press. p54.

Jesen, P. K., Jayasinghe, G., Van der Hoek, W., Cairncross, S. and Dalsgaard, A. (2004). Is there an association between bacteriological drinking water quality and childhood diarrhea in developing countries? *Trop. Med. Int. Health.* **9** *(11)*: 1210 – 15.

Jesen, P. K., Jayasinghe, G., Van der Hoek, W., Cairncross, S. and Dalsgaard, A. (2002). Domestic transmission routes of pathogens: the problem of in-house contamination of drinking water during storage in developing countries. *Trop. Med. Int. Health.* **7** *(7)*: 604 – 9.

Kale-Pradhan, P. B., Jassal, H. K. and Wilhelm, S. M. (2010). Role of Lactobacillus in the prevention of antibiotic-associated diarrhea: a meta-analysis. *Pharmacotherapy* **30***(2):* 119–26.

King, C. K., Glass, R., Bresee, J. S. and Duggan, C. (2003). Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *Centers for Disease Control Prevention. MMWR Recomm Rep* **52***(16):* 1–16.

Kosek, M., Bern, C. and Guerrant, R. L. (2003). The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the World Health Organization.* **81***(3)*: 197–204.

Laura, M. L., Christa L. F. W. and Robert, E. B. (2012) Systematic review of diarrhea duration and severity in children and adults in low- and middle –income countries. *BMC Public Health* 12:832

Lazzerini, M. and Ronfani, L. (2013). Oral zinc for treating diarrhoea in children". *The Cochrane Database of Systematic Reviews* 1:54

MacGillivray, S., Fahey, T. and McGuire, W. (2013). Lactose avoidance for young children with acute diarrhoea. *The Cochrane Database of Systematic Reviews* 10: 5433.

Mandell, G. L., Bennett, J. E. and Dolin, R. (2004). *Mandell's Principles and Practices of Infection Diseases*. 6th ed. Churchill Livingstone. In Diarrhoea: why children are still dying and what can be done.*WHO*. Retrieved, June-07-2015.

Markus MacGill (2017). What you should know about diarrhea: Reviewed by University of Illinois-Chicago, School of medicine, USA. *www.medicalnewstoday.com*. Retrieved, January-30-2017.

Mayo Clinic Staff (2017). Diarrhea symptoms and causes. *http://www.mayoclinic.org*

Mayo-Wilson, E., Imdad, A., Herzer, K., Yakoob, M. Y. and Bhutta, Z. A. (2011). Vitamin A supplements for preventing mortality, illness, and blindness in children aged under 5: systematic review and meta-analysis. *BMJ (Clinical Research Ed.)*343: 50-94.

Medbroadcast (2017). Diarrhea, The Facts. *http://www.medbroadcast.com*

Moffat, T. (2003). Diarrhea, respiratory infections, protozoan gastrointestinal parasites and child growth in Kathmandu, Nepal. *Am. J. Phys. Anthropol.* **122** *(1)*: 85 – 97.

Molbak, K. (2000). The epidemiology of diarrheal diseases in early childhood: a review of community studies in Guinea-Bissau. *Dan Med. Bull.* **47***(5)*:340-58

Mordi, R. M. and Ngwodo, P. O. A. (2007). A study of blood and gastro-intestinal parasites in Edo State. *African Journal of Biotechnology*. **6***(19):* 2201-2207.

Nyle, C. B. and Ray, R. W. (2009). *Elements of the Nature and Properties of Soils* (3rd Edition). Prentice Hall. p. 35.

Ochie, J. and Kolhatkar, A. (2008). *Medical Laboratory Science, Theory and Practice.* Tata McGraw-Hill Publishing Company Limited, New Delhi. p 919-945.

Odu, N. N., Maxwell, S. N., Nte, A. R. and Akujobi, C. O. (2010). Helminthiasis among school children in rural communities in Rivers State. Nigeria. *Nig. Journal Microbiology*. **24***(1):* 2219-223.

Oguoma, V. M., Anyasodor, A. E. and Mbata, T. I. (2008). Prevalence of Intestinal Protozoan parasites among children aged 5-12 years in Owerri Metropolis South Eastern Nigerian.*Trop. J. Health Sci*. **15***(2):* 43-48.

Oxford, (2003). Oxford textbook of Medicine**.** 4th ed. Oxford University Press. pp. 759–760.

Parashar, U. D., Bresee, J. S. and Glass, R. I. (2003). The global burden of diarrheal disease in children. *Bull. World Health Organ.* **81** *(4)*: 236.

Pawlowski, S. W., Warren, C. A. and Guerrant, R. (2009). Diagnosis and treatment of acute or persistent diarrhea". *Gastroenterology* **136***(6):* 1874–86.

Plos One, (2014). Open Defecation and Childhood Stunting in India: An Ecological Analysis of New Data from 112 Districts. *Plos One.* Retrieved, June-10-2015.

Presterl, E., Zwick, R. H., Reichmann, S., Aichelburg, A., Winkler, S., Kremsner, P. and Granigner, W. (2003). Frequency and virulence properties of diarrheagenic

Escherichia coli in children with diarrhea in Gabon. *American Journal of Tropical Medical Hygiene*. 69: 406-410.

Rheingans, R. D., Antil, L., Dreibelbis, R.,Podewils, L. J., Bresee, J. S. and Parashar, U. D. (2009). Economic costs of rotavirus gastroenteritis and cost-effectiveness of vaccination in developing countries. *The Journal of Infectious Diseases*. 200: 16–27.

Rao, M. R., Abu-Elyazeed, R., Salvarino, S. J., Naficy, A. B., Wierzba, T. F., Abdel-Messih, I., Shaheen, H., Frenck, R. W., Svennerholm, A. M and Clemens, J. D. (2003). High disease burden of diarrhea due to enterotoxigenic *Escherichia coli* among rural Egyptian infants and young children. *Clinical Microbiology Infectious* 41: 4862-4864.

Robert, L. (2008). Unicef Nigerian Representative Goodwill Message on the National launch of hand washing campaign in Abuja Nigeria. Retrieved March, 2017.

Ruggiero, M. A., Gordon, D. P., Orrell, T. M., Bailly, N., Bourgoin, T., Brusca, R. C., Cavalier-Smith, T., Guiry, M. D. and Kirk, P. M. (2015). A High Power (over 9000) Level Classification of All Living Organisms. *PLoS ONE* **10** (4): 48.

Ruppert, E. E., Fox, R. S. and Barnes, R, D. (2004). *Introduction to Eumetazoa. Invertebrate Zoology* 7th ed. Brooks / Cole. pp. 99–103.

Ryan, K. J. and Ray, C. G. (2004). *Sherris Medical Microbiology.* 4th ed. McGraw Hill. pp. 733–8.

Sayyan, A. A., Imanzadeh, F., Bagheri, Y., Karami, H. and Yaghoobi, M. (2005). Prevalence of Intestinal Parasitic Infections in the Islamic Republic of Iran. *Eastern Medi. Health J.* **11** *(3)*: 1-7.

Schiller, L. R. (2007). Management of diarrhea in clinical practice: strategies for primary care physicians. *Rev Gastroenterol Disord* **7** (l 3): 27–38.

Sguassero, Y. (2013). Optimal duration of exclusive breastfeeding: RHL commentary. *WHO*. Retrieved, June-20-2015.

Simon-Oke, I. A. and Ogunleye, E. (2015). Prevalence of *Entamoeba histolytica* among Primary School Children in Akure, Ondo State, Nigeria. *Journal of Public Health and Epidemiology.* **7** *(11)*: 346 – 351.

Sinclair, M. I., Harris, A. H., kirk, M. and Fairley, C. K. (2003). Cost of community gastroenteritis. *Journal of Gastroenterology Hepatology*. 18: 322 – 328.

Ukaga, C. N. and Nwoke, B. E. B. (2007). *Practical Medical Parasitology, for Biological Sciences and Medical Students*. Ed. 2nd. Megasoft Publishers, Ikenegbu L/out Owerri. p15-42.

United Nations, (2014). Call to action on sanitation. *United Nations*. Retrieved, June-14-2015.

Walker, C. L., Aryee, M. J., Boschi-Pinto, C. and Black, R. E. (2012). Estimating diarrhea mortality among young children in low and middle income countries. *Ed. Myer L*. 7 (1): 151.

Walker, C. L., Rudan, I., Liu, L., Nair, H., Theodoratou, E. and Bhutta, Z. A. (2013). Global burden of childhood pneumonia and diarrhoea. *Lancet* **381***(9875):* 1405–16.

Webb, A. and Starr, M. (2005). Acute gastroenteritis in children. *Australian Family Physician* **34***(4):* 227–31.

Webmd, (2011). The Basics of Diarrhea. *Webmd.com*. Retrieved, May-09-2011.

Wedlake, L., A'Hern, R., Russell, D., Thomas, K., Walters, J. R. and Andreyev, H. J. (2009). Systematic review: the prevalence of idiopathic bile acid malabsorption as diagnosed by SeHCAT scanning in patients with diarrhoea-predominant irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics* **30***(7)*: 707–17.

West, K. P. (2002). Extent of vitamin A deficiency among preschool children and women of reproductive age. *The Journal of Nutrition* **132***(9):* 2857–2866.

World Gastroenterology Organization. (2011). Practice Guideline - Acute diarrhea. *WGO.* Retrieved, May-20-2011.

World Health Organization. (2000). Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. WHO Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. *The Lancet* **355***(9202):* 451–5.

World Health Organization. (2000). Diarrheal disease Fact Sheet. *Institute*of one *World Health.* Retrieved January-30- 2017.

World Health Organization. (2004). Mortality and Burden of Disease Estimates for WHO Member States in 2004.*World Health Organization*. Retrieved, June-08-2015.

World Health Organization. (2009). Diarrhoeal Diseases. *World Health Organization*. Retrieved, June-07-2015.

World Health Organization. (2010). *Oral cholera vaccines in mass immunization campaigns* (PDF). *WHO*. pp. 6–8.

World Health Organization. (2013). Diarrhoeal disease Fact sheet N°330. *World Health Organization*. Retrieved, June-11-2015.

World Health Organization. (2014). WHO Diarrhoeal disease. *Who.int.* Retrieved May-27-2015.

West, K. P. (2002). Extent of vitamin A deficiency among preschool children and women of reproductive age. *The Journal of Nutrition* **132***(9):* 2857–2866.

Wolf, J., Prüss-Ustün, A., Cumming, O., Bartram, J., Bonjour, S., Cairncross, S., Clasen, T., Colford, J. M., Curtis, V., De France, J., Fewtrell, L., Freeman, M. C., Gordon, B., Hunter, P. R., Jeandron, A., Johnston, R. B., Mäusezahl, D., Mathers, C., Neira, M.

and Higgins, J. P. T. (2014). Systematic review: Assessing the impact of drinking water and sanitation on diarrhoeal disease in low- and middle-income settings: systematic review and meta-regression. *Tropical Medicine & International Health* **19***(8):* 928–942.

Wong, C. S., Jelacic, S., Habeeb, R. L., Watkins, S. L. and Tarr, P. I. (2000). The risk of the hemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections. *N. Engl. J. Med.* **342***(26):* 1930–6.

APPENDIX I

CHI-SQUARE TABLES FOR RESULT TABLES

TABLE 1

GENDER	NO EXPECTED		NO INFECTED		TOTAL	
	f_o	f_ε	f_o	f_ε	f_o	f_ε
MALE	684	728	289	245	973	973
FEMALE	516	472	115	159	631	631
TOTAL	**1,200**	**1,200**	**404**	**404**	**1,604**	**1,604**

χ^2cal. = 26.85

χ^2tab. = 3.84, *df* =1

($p<0.05$); statistically significant.

TABLE 4

AGE(YEARS)	NO EXPECTED		NO INFECTED		TOTAL	
	f_o	f_ε	f_o	f_ε	f_o	f_ε
1 - 4	719	717	234	236	953	953
5 - 8	301	311	113	103	414	414
9 – 11	180	172	48	56	228	228
TOTAL	**1,200**	**1,200**	**395**	**395**	**1,595**	**1,595**

χ^2cal. = 2.83

χ^2tab. = 5.99, *df* = 2

(p>0.05); statistically non-significant.

TABLE 5

SCHOOL ENROLMENT	NO EXPECTED		NO INFECTED		TOTAL	
	f_o	f_ε	f_o	f_ε	f_o	f_ε
NOT ENROLLED	86	70	7	23	93	93
DAY-CARE	237	242	87	82	324	324
PRE-NURSERY	186	181	56	61	242	242
NURSERY	290	287	94	97	384	384
PRIMARY	402	420	160	142	562	562
TOTAL	**1,200**	**1,200**	**404**	**404**	**1,605**	**1,605**

χ^2cal. = 19.00

χ^2tab. = 9.49, df = 4

($p<0.05$); statistically significant.

TABLE 6

OCCUPATION OF MOTHERS	NO EXPECTED		NO INFECTED		TOTAL	
	f_o	f_ε	f_o	f_ε	f_o	f_ε
APPLICANTS	97	85	17	29	114	114
ARTISANS	179	185	68	62	247	247
CIVIL SERVANTS	262	236	53	79	315	315
FARMER	115	139	71	47	186	186
HOUSEWIVES	181	180	59	60	240	240
TRADING	178	207	99	70	277	277
OTHERS	188	168	37	57	225	225
TOTAL	**1,200**	**1,200**	**404**	**404**	**1,604**	**1,604**

χ^2cal. = 60.75

χ^2tab. = 12.59, df = 6

($p<0.05$); statistically significant.

TABLE 7

RESIDENCE	NO EXPECTED		NO INFECTED		TOTAL	
	f_o	f_ε	f_o	f_ε	f_o	f_ε
URBAN	431	410	117	138	548	548
RURAL	769	790	287	266	1056	1056
TOTAL	**1,200**	**1,200**	**404**	**404**	**1,604**	**1,604**

χ^2cal. = 6.50

χ^2tab. = 3.84, df = 1

($p<0.05$); statistically significant.

TABLE 8

ZONE	NO EXPECTED		NO INFECTED		TOTAL	
	f_o	f_ε	f_o	f_ε	f_o	f_ε
OWERRI	422	412	129	139	551	551
ORLU	390	414	163	139	553	553
OKIGWE	388	374	112	126	500	500
TOTAL	**1,200**	**1,200**	**404**	**404**	**1,604**	**1,604**

χ^2cal. = 8.57

χ^2tab. = 5.99, df = 2

($p<0.05$); statistically significant.

TABLE 9

TERTIARY HOSPITALS	NO EXPECTED		NO INFECTED		TOTAL	
	f_o	f_ε	f_o	f_ε	f_o	f_ε
ABOH MBAISE	102	91	20	31	122	112
ARONDIZUOGU	49	49	16	16	65	65
AWOMAMMA	90	94	36	32	126	126
F. M. C. OWERRI	320	321	109	108	429	429
IHITTE UBOMA	75	72	21	24	96	96
OKIGWE	264	254	75	85	339	339
ORLU	300	319	127	108	427	427
TOTAL	**1,200**	**1,200**	**404**	**404**	**1,604**	**1,604**

χ^2cal. = 12.46

χ^2tab. = 12.59, df = 6

(p>0.05); statistically non-significant.

APPENDIX II

MATERIALS USED FOR THE RESEARCH:

The following materials were used through the course of this project. They included, applicator stick, cotton swab, hand gloves, glass slides, microscope, washing battle, wide mouth bottles with corks, cover slips, Pasteur's pipettes,

APPENDIX III

RESEARCHER ADMINISTERED QUESTIONNAIRE

INTRODUCTION:

1) This is a dissertation questionnaire on the topic: Intestinal Protozoan Parasites in Children with Diarrhea and Associated Risk Factors in Selected Health Institutions in Imo State.

   This dissertation is in fulfilment for the award of Master of Science in Environmental Health Biology, Federal University of Technology Owerri.
2) To achieve this aim, your cooperation is highly needed and your responses will be treated with all level of confidentiality.
3) Participants are required to supply stool samples and respond to the following questions on behalf of their wards.
4) Kindly tick only one response for each question except where otherwise instructed.

SECTION A: DEMOGRAPHIC DATA

1) Age (years) _____
2) Sex: A) Male ☐ B) Female ☐
3) Level of education: A) Not enrolled ☐ B) Daycare ☐ C) Pre-nursery ☐ D) Nursery ☐ E) Primary ☐
4) Occupation of parents/guardians: A) Applicant ☐ B) Artisan ☐ C) Civil servant ☐ D) Farmer ☐ E) Housewife ☐ F) Trading ☐ G) Others (specify) _____

SECTION B: HEALTH/CLINICAL STATUS

5) Stool consistency: A) Watery ☐ B) Semi-formed ☐ C) Formed ☐

6) Have your ward suffered diarrhea before? A) Yes ☐ B) No ☐

7) For how long? A) Days ☐ B) Weeks ☐

8) Was a clinic/hospital attended before treatment? A) Yes ☐ B) No ☐

9) State location of clinic/hospital: _____

10) Was any laboratory test done before treatment? A) Yes ☐ B) No ☐

11) Is he/she suffering diarrhea now? A) Yes ☐ B) No ☐

12) For how long now? A) Days ☐ B) Weeks ☐

SECTION C: SOCIO-ECONOMIC STATUS

13) Area of residence: A) Urban area ☐ B) Rural area ☐

14) Zone of Imo State stool was collected: A) Owerri ☐ B) Orlu ☐ C) Okigwe ☐

15) Selected/General Hospital stool was sampled: A) Aboh Mbaise ☐ B) Arondizuogu ☐

C) Awomamma ☐ D) FMC Owerri ☐ E) Ihitte Uboma ☐ F) Okigwe ☐

G) Orlu ☐

16) Type of residence: A) Bungalow ☐ B) Slum ☐ C) A room apartment ☐

D) Self contain room ☐ E) Flat apartment ☐ F) Crowded compound apartment ☐

G) Others (specify) _____

17) How many members in a home? A) 2-4 ☐ B) 5-7 ☐ C) 8-above ☐

18) Source of drinking/cooking water: A) Stream/River ☐ B) Pipe-borne ☐

C) Sumo/bore hole water ☐ D) Sachet water ☐ E) Water from tanker ☐

F) Rain water ☐ G) Others (specify) _____

19) Diarrhea is caused through: A) Contaminated food ☐ B) Contaminated water ☐

C) Spiritual problem ☐ D) Witchcraft ☐ E) Mosquito bite ☐ F) No idea ☐

20) How many square meals per day? A) 1 ☐ B) 2 ☐ C) 3 ☐ D) More than 3 ☐

21) Does your ward wash hand before and after meal? A) Yes ☐ B) No ☐

22) If yes, how often? A) Very often ☐ B) Frequently ☐ C) Always ☐ D) Not at all ☐

23) Does your child wash hand after defecation? A) Yes ☐ B) No ☐

24) What type of toilet is used at home? A) Pit toilet ☐ B) Water system ☐ C) Bucket toilet ☐ D) Open defecation ☐

25) Do you defecate on/near play ground in school and/or at home? A) Yes ☐ B) No ☐