ASSESSMENT OF FACTORS ASSOCIATED WITH GASTROINTESTINAL PROTOZOAN INFECTIONS IN CHILDREN AGED BETWEEN 6 AND 59 MONTHS WITH DIARRHEA IN KISII TEACHING AND REFERRAL HOSPITAL, KENYA

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A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN PARASITOLOGY OF THE SCHOOL OF PURE AND APPLIED SCIENCES, DEPARTMENT OF BIOLOGICAL SCIENCES KISII UNIVERSITY

NOVEMBER 2020
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DEDICATION

I dedicate this work to my dear wife Olpha Kwamboka, Son Emmanuel, parents;
Zephaniah Ondara Ayiema and Jennipher Mbera, my sisters and brothers.
ACKNOWLEDGEMENT

Special gratitude to my supervisors, Dr. Nyanchong’i Benson Omweri and Dr Rael Jepkogi Masai whose tireless efforts and devotion has made me get to this far. Nonetheless, i wish to acknowledge Kisii University as an institution for giving me a chance to pursue my postgraduate studies not forgetting to as well recognize the management of Kisii Teaching and Referral Hospital for allowing me use their facility for the period of four month of research. I also wish to thank and acknowledge Mr. Alfred Mbaka and Mr. Anthony Maobe both working at Kisii Teaching and Referral Hospital as laboratory technicians MCH wing for their key contribution and assistance during stool processing and microscopic examination. Thanks to all my lecturers especially Dr Johnson Nyangeri and Dr Samuel Mong’are for their contribution, colleagues and friends for their moral support and encouragement shared. Lastly, I give gratitude to all who made the research successful, may God keep blessing you as you bless others.
ABSTRACT

Gastrointestinal protozoan infection is a global concern in health especially in most of the developing countries where fecal contamination of food and water cause high morbidity. High cases of gastrointestinal infections have been reported among adults in Kenya that includes the following studies; 19.0 % prevalence in Thika, 13.8% in Mukuru slums Nairobi and 13.6 % in Kisii but information on the prevalence among children under 5 years of age is scarce. This study was aimed at evaluating the status of gastrointestinal protozoan infections in children in the region by determining the general prevalence of the gastrointestinal protozoan infections, establishing the parasite densities for each identified gastrointestinal protozoa and evaluating the risk factors associated with gastrointestinal protozoan infections among the children aged between 6 months and 59 months presenting diarrhea symptoms seeking medication at the county referral hospital (K.T.R.H). A cross sectional study was carried out at Kisii Teaching and Referral Hospital (K.T.R.H) targeting children aged between 6 months and 59 months symptomatic of diarrhea for a period of four months. Microscopic examination of stool was carried out using direct wet mounts and formol-ether stool concentration techniques so as to diagnose the intestinal protozoans in stool. A simple designed questionnaire was issued to the caregivers of the children seeking medication at the facility to get more information on the hygienic practices, socio-economic status and the level of public awareness on gastrointestinal illnesses upon signing a consent form. For descriptive data, percentage was employed to determine the characteristics of the study population. Chi-square tests ($\chi^2$) were used to check on the associations between the variables by cross tabulations using the SPSS version 21. Thirty-four children (28.3%) were infected with either Entamoeba histolytica, Giardia lamblia or both parasites. There was a decreased infection index with advance in age though not significant ($p=0.337$). Female children were 1.5 times less likely to be infected with gastrointestinal protozoa than their male counterparts, ($p=0.392$). Notably, there was a high parasite density in co-infected individuals compared to single case infections. The drinking water source was identified as a major risk factor of the infection, ($P=0.030$). Hygienic practices like hand washing before meals highly reduced the risk of infection while unhygienic practices like finger sucking increased the risk of infection ($P<0.05$). However, economic status of caregivers and the practice of hand washing after toilet were not significant, ($P=0.312$). Rate of infection in children under 5 years was relatively higher in the region, thus the county government of Kisii and the relevant stakeholders tasked for water provision should increase the supply and access of clean treated tap water to inhabitants of Kisii County and formulate legislative policies on how to improve water quality at the source. Additionally, public health education and awareness should be intensified to encourage the hygienic practices such as hand washing before meals and the use of single human waste disposal unit per household and discourage unhygienic practices like finger sucking to significantly reduce the gastrointestinal protozoan infections.
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<table>
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<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>SDGs</td>
<td>Sustainable Development Goals</td>
</tr>
<tr>
<td>CBS</td>
<td>Central Bureau of Statistics</td>
</tr>
<tr>
<td>KDHS</td>
<td>Kenya Demographic and Housing Survey</td>
</tr>
<tr>
<td>FWBC</td>
<td>Fruit washing before consumption</td>
</tr>
<tr>
<td>GI</td>
<td>Gastro Intestinal</td>
</tr>
<tr>
<td>GWASCO</td>
<td>Gusii Water and Sanitation Company</td>
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<tr>
<td>HWAT</td>
<td>Hand washing after toilet</td>
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<tr>
<td>HWBM</td>
<td>Hand washing before meals</td>
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<tr>
<td>IFA</td>
<td>Immuno-Florescence Assay</td>
</tr>
<tr>
<td>KHPF</td>
<td>Kenya Health Policy Framework</td>
</tr>
<tr>
<td>KTRH</td>
<td>Kisii Teaching and Referral Hospital.</td>
</tr>
<tr>
<td>KNBS</td>
<td>Kenya National Bureau of Statistics</td>
</tr>
<tr>
<td>MDG</td>
<td>Millennium Development Goals</td>
</tr>
<tr>
<td>SID</td>
<td>Society International Development</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>SSA</td>
<td>Sub-Saharan Africa</td>
</tr>
<tr>
<td>UNICEF</td>
<td>United Nations International Children’s Emergency Fund</td>
</tr>
<tr>
<td>W.H.O</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WTBD</td>
<td>Water treatment and boiling before drinking</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>Chi square</td>
</tr>
<tr>
<td>Z-SCORE</td>
<td>Constant that corresponds to the confidence interval</td>
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<tr>
<td>WGO</td>
<td>World gastroenterology Organization</td>
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<td>IPI</td>
<td>Intestinal Parasitic Infections.</td>
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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Diarrhea remains a problem among the leading causes of death in children globally out of which, 27,400 mortality cases among the children with less than 5 years old associated to diarrhea and other diarrheal illnesses are recorded in Kenya (Bryce et al 2005). Globally, it is projected that approximately 2 million diarrhea cases and diarrheal related illnesses occur annually among children who have not reached their fifth birth anniversary out of these 50% prevalence is recorded in Africa, South Asia (38%), East Asia (9%) and Pacific (7%) respectively (Montresor et al., 2017).

The bulk of gastrointestinal related illnesses are self-limited and very specific, however, certain possible causal factors that includes nutritional deficiency, immune suppression, and early years of age prompts the development of tenacious diarrhea. Worldwide, 1.87 million deaths of under-fives occur from acute diarrheal illness. According to 2008-9 Kenya Demographic and Health Survey (Banke-Thomas, & Ameh, 2016) and the Kenya National Bureau of statistics, severe and acute type of diarrhea is the main cause of ill health and death among young children under five years old (Gruebner et al., 2015).

The outcome of the survey showed that 17 percent of the children experienced diarrhea within a range of a fortnight before surveillance and the occurrence of diarrhea peaks at ages 6-11 months. Furthermore, infant mortality was between 52 deaths to 74 deaths in every 1,000 live births describing mortality rate among children under five (Boschi-Pinto.,2005).
These statistics reveal substantial degeneration from the earlier Kenya Demographic Health Statistics. Of these high rates of under-five mortality, regional disparities exist. Infant death rate is 65 per 100 live births in the former Western province whereas the mortality index among children under the age five was 121 cases per 1000 live births and diarrhea emerges the second after clinical malaria that are accountable for the infections and deaths annually among the under 5’s in the western Kenya (KDHS 2003). Much of the efforts have been put on the better understanding of helminthic epidemiology and distribution (Ouattara et al., 2010), besides these much efforts it was found that there are relatively fewer studies compared to the other previous studies done on intestinal protozoan infections. The fewer studies in intestinal protozoa makes it of a huge concern in the world of parasitology since parasites like Entamoeba histolytica that causes intestinal amoebiasis is in third position of the protozoan illnesses that accounts for death globally that comes immediately next to clinical malaria then established schistosomiasis that is caused by Schistosoma spp that targets man as the definitive host. Intestinal amoebiasis accounts for an estimated number of 180 million people out of which the mortality ranges between an approximate of 40,000 to 110,000 every year.

Recent studies that have been done in the area for instance a study on the intestinal parasitic infections done in the former Kisii municipality in Kisii town, Kenya indicated that gastrointestinal protozoans were prevalent among the food handlers and are prevalent and were primarily transmitted via contaminated water (Nyarango et al., 2008), however, there is not much documentation on the diarrhea and other related diseases that accounts for the intestinal parasitic infections.
infections in the study area and its surrounding. Therefore, this study was aimed at assessing the contributing factors of intestinal protozoan infections in diarrheal children aged between 6 and 59 months old so that various preventive and control measures are put in place to reduce the infections and possibly prevent new case of infections.

1.2 Statement of the problem

Diarrhea is the principal cause of infections and deaths among children aged under 5 years old in most undeveloped nations. The epidemiology and distribution of diarrhea is global but intense in the African regions and Southern part of East Asia (Boschi et al., 2008). In the Republic of China, giardiasis affects 28.5 million people every year (Feng et al., 2011). In Nigeria, 13.8% of the school going children were confirmed positive of intestinal parasitism (Okpala et al., 2014). In Kenya, Kiambu County, protozoan infections were (14.6%) *Entamoeba histolytica*, (6.9%) *Giardia lamblia* and (5.8%) *Iodamoeba buschili*. The mortality among the children under age of 5 is recorded as 27,400 annually (Bryce et al 2005). However, the contributing factors to diarrhea and related illnesses are unclear in many parts inclusive of Kisii County, Kenya. The intention of this study was to determine contribution of various factors linked to gastrointestinal illnesses at KTRH a referral hospital in Kisii County, Kenya. Causative organisms associated with gastro intestinal protozoa are in many incidences identified in about 50% of all cases that are symptomatic to gastro intestinal illnesses. In settings where there are inadequate resources impartial reporting based on convenience is likely, and most of the information is always prejudiced (Tiwari et al., 2009). These Causative agents for Intestinal infections are usually transmitted through a range of routes whereby the epidemiology of gastro
intestinal illnesses are swayed by the virtue of their transmission modes that are varying between developed and developing countries (Klimo, & Schmidt, 2003). This study is essential as Kenya is struggling to achieve the vision 2030 global agenda contemporarily referred to as the Sustainable Developments Goals (SDGs) which include: MDG1; MDG 3 and MDG 7 (Watkins et al., 2017).

1.3 Purpose of the study
For many years, Kenya as a country has struggled to put a stable basis in an attempt to overcome crucial development barriers that affects her citizens, which include health. Various efforts have been made right from developing a health policy framework (KHPF 1994-2010), launch of the global commitment agenda commonly referred to Vision 2030, enactment of the new Constitution of 2010, to efforts of attaining the globally projected commitment agenda (SDGs) of the year 2030 which aims at driving Kenya towards ensuring a healthier population. Determining the factors associated with gastrointestinal protozoan infections among the children would aid in developing suitable prevention and control strategies of communicable diseases. This will in turn minimize the cost of treatment and hospitalization, ensure proper allocation of limited public health resources, enhance disaster management and administration all aimed at achieving national and global health goals.

1.4 Study objectives
1.4.1 Overall objective
To investigate the factors that are linked to gastrointestinal parasitic illnesses in diarrheal children of age 6-59 months who seek medication at Kisii Teaching and Referral Hospital (KTRH).
1.4.2 Specific Objectives

i. To determine the prevalence of the gastrointestinal protozoan infections among children aged 6-59 months with diarrhea.

ii. To evaluate the parasite densities of each identified gastrointestinal protozoa among children aged 6-59 months.

iii. To assess risk factors associated with gastrointestinal protozoan infections among children aged between 6 months and 59 months.

1.5 Null hypotheses

**H**ₐ. The infection prevalence of gastrointestinal protozoans among children of age between 6-59 months with diarrhea attending the Kisii county referral hospital is low.

**H**₂. The parasite densities of gastrointestinal protozoa among children aged between 6-59 months with diarrhea is considered to be “rare” (2-5 organisms in every five high power fields of view).

**H**₃. There is no association between the risk factors and the gastrointestinal protozoan illnesses in diarrheal children of age less than 5 years attending Kisii level six Hospital.

1.6 Scope of the study

The current study anticipated to assess how the contributing factors that include; hand washing before meals, hand washing after a visit to the toilet, fruit washing before eating, aspect of finger biting and sucking, the practice of sharing or not sharing a single disposal unit, the main source for water used in drinking and socio-economic conditions of homes from which the patients came affects the gastrointestinal protozoa infections among the diarrheal children of less than 59 months old in Kisii Teaching and Referral Hospital. The study was conducted
between the month of April to August 2017 at KTRH. The study targeted a population of 132, however, because of some logistics of the inclusion and exclusion criteria and non – compliance index, 120 children of the age between 6 months and 59 months old were sampled for this study.

1.7 Conceptual Framework
Operational definition of terms

**Enema**  Is the introduction of liquid or gas into the rectum so as to expel its contents.

**Jabia-**  Harvested rain

**Morbidity**  The number of infected cases of a specific disease in an area.

**Mortality**  The proportional number of deaths in a specific group of people over a given period of time.

**Nausea**  The feel to vomit

**Parasitosis**  Is a rare psychiatric condition where patients have a mistaken belief that they are infested or infected by parasites.

**Prevalence**  The number of disease cases in a particular population over a specified period of time.

**Sanitation**  Maintenance of high level of personal and environmental hygiene and how they affect people in a given area.

**Symptomatic**  Showing that a particular disease is present.

**Mixed infections**  This refers to a case where a single host is infected by a variety of parasite species simultaneously.
CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction
Intestinal protozoan infections are mostly classified into: Amoebae (rhizopoda), entero flagellates, ciliates and coccidian (de Silva, 2003); (Brooker et al., 2000) and are the ones for the substantial intestinal illness, malnutrition and death worldwide, mostly amid young children that lives in developing countries, (Feng et al., 2011); (Stanley, 2003).

There are three goals in the sustainable Development Goals previously referred to the Millennium Development Goals (MDG) that are linked with diarrheal diseases. They include, MDG1, MDG 3 and MDG 7 that are aimed at eradicating extreme poverty and hunger, provision of good health and well-being and increasing the number of people with access to sustainable and improved sanitation respectively (Watkins et al., 2017). It is only 31% of people who have access to improved hygiene with larger population at risk of contracting infectious diseases due to unimproved hygiene.

Causative agents for Intestinal infections are transmitted through a range of routes whereby the epidemiology of gastro intestinal illnesses are swayed by the virtue of their transmission modes that are varying between developed and developing countries (Klimo et al., 2003).
2.2 Diarrhea as a result of protozoan infections

Diarrhea is a common phenomenon in most populations globally and can be as a result of many causative agents among which are the viruses (rotavirus), helminths, bacteria, protozoa and many others. Two gastro intestinal protozoans have been linked to diarrhea and diarrhea related illnesses; *Entamoeba histolytica* causes amoebiasis and *Giardia lamblia* flagellate that causes giardiasis in humans while isosporans have been linked to individuals that are immunocompromised due to other co infections like HIV exposed patients. (Keiser *et al.*, 2002).

Ciliates like *Balantidium coli* has also been said to be predominant but less common cause of diarrhea. Other amoebae species including *Blastocystis horninis* and *Dientamoeba fragilis* previously have been identified in diarrheal stools obtained by patients seeking medication, however, it is not very clear on how they cause diarrhea and diarrhea related illnesses, interestingly their causative part is definitely clear. Additionally, microbes like *Entamoeba dispar* are nearly nonpathogenic hence most likely do not cause diarrhea (Ansari, 2012).

Diarrhea is characterized by the passage of watery or semi-formed stool for three or more times in a day due to use of contaminated food and drinks with various disease-causing microorganisms among which are the protozoa. Diarrhea can as well result due to certain food or drugs intolerance and occasionally stress (Hilbert *et al.*, 2003). Diarrhea is a common phenomenon that has been in existence since and has remained to be problematic in many unindustrialized nations (Reither *et al.*, 2007).

Additionally, some fundamentally basic conditions that are common and familiar in the societies we live includes malnutrition that in most cases may bring about
escalation in the likelihoods and risks of infections that results into diarrhea diseases in unindustrialized countries (Al-Agha & Teodorescu, 2000).

2.3 Distribution and spread of intestinal protozoa

Intestinal protozoa are globally spread with basic differences on the occurrence. Over an estimate of 3 billion individuals are affected by gastro intestinal parasites with people about 450 million ill and the infection proportion among individuals keeps on rising (WHO, 2004).

The parasitic infections invading the human intestinal tract can be classified into acute and chronic intestinal infections. Pathogenic microorganisms that includes; bacteria, viruses and parasites may cause chronic infections especially if the diagnosis is not done in time and then followed by appropriate treatment. Diarrhea can also be as a result of diseases that emerges due to internal dysfunctions of gastrointestinal tract for example Idiopathic bowel disease and Crohn’s disease among many others (Lamps, 2007).

A major concern for the intestinal protozoan infections is that they are self-limited, nonetheless, definite risk elements like malnourishment, compromised immunity, and tender age increase chances of developing persistent diarrhea (Heymann, 2008). The occurrence of the gastro intestinal infections in many of the developing countries is considerably high notwithstanding a noticeable decreasing trend in death rates.

Approximately 1.87 million children die of diarrhea or diarrhea related diseases before realization of their fifth birthday. Diarrheal illnesses averagely accounts for slightly less than a quarter of all child deaths out of which about 78% of the fatality cases are recorded especially in areas of the continents that are poverty stricken (Boschi-Pinto et al., 2008).
2.4 Epidemiology of gastrointestinal protozoa

As much as the gastro intestinal protozoa appear to have less impact on humans than the other diseases like AIDS and tuberculosis which has been put to priority while neglecting it among many other tropical diseases, they are an immense problem and set back in tropical regions and should as well be put to consideration in the aid of reducing the child death rate and generally improve child health in the struggle to meet the sustainable development goals (Utzinger et al., 1999). The effects of Intestinal parasites cause noteworthy ill health and death across the world which has made it a global problem, principally in unindustrialized countries where a larger population has other related ill health conditions. These effects results to a tremendous effect on socio-economic aspects in terms of high treatment and hospitalization costs.

It is evaluated that close to 1/8 of population is infected with Entamoeba histolytica (Farthing et al., 2003). Today, the increased infection cases of E. histolytica is accounted for essentially in nations of low financial class, while reduced E. histolytica infections is common in industrialized nations. More cases as a result of E. histolytica have been confirmed to be from the Sub-Saharan Africa (Li and Stanley, 1996). Cyst carriers are the reservoir hosts for E. histolytica disease. Disease materializes principally through the fecal-oral way by ingesting fecal material in water or anything edible by man that is contaminated by human feces.

Food borne illnesses are brought about by unsanitary treatment of rations of food, and during the cooking by infected people. Farming practices, for example, the utilization of crude residential sewage for vegetable development generally in urban areas can build the vulnerability of amoebiasis (Srikanth and Naik, 2004).
It has been disclosed that cultivating individuals are altogether progressively soiled with pathogenic intestinal protozoa because of the eating of crude vegetables developed on contaminated sewage with no post field treatment. Pandemics due to amoebiasis can likewise occur when crude sewer content infiltrates into water channels and subsequently soils water sources for drinking water (Farthing et al., 2003).

Significant transmitters for cyst forms of *G. lamblia* include person to person and untreated drinking water like the relinquished wells. *Giardia* cysts usually in normal occasions survive for long outside the host till they get into a feasible host that spreads its infective forms which are in most cases in surface water or water from abandoned wells and other sources. Research studies shows that it is not easy to control the survival of *Giardia lamblia* cysts in water treated by chemicals like chlorine since such cysts can't be killed by treatment (Abrar et al., 2015). Contaminated foods and water are other means of transmission, despite the fact that it is believed to be generally extraordinary. Equally, direct infections, individual to individual that occurs via fecal-oral transmission is shown in overcrowded set up that include, overpopulated schools, day care centers, ghettos and rehabilitation hubs (Minemo et al., 2003).

In the year 2013 -2014, WHO together with the World Gastroenterology Organization (WGO0 indicated that diarrheal disease is among the killer diseases in the tropics and it was ranked the second highest cause of illness and loss of life per 1000 lives among children in the developing world dissimilar to already developed nations of the world. Lack of public awareness among mothers or the guardians that takes care of the children when the parents are away concerning infant and childhood diarrhea play
a key part in the aid of controlling juvenile diarrhea, (Mwambete & Joseph, 2010). Diarrhea can be acute, chronic or persistent. Acute diarrhea is characterized by sudden inception of diarrhea in which watery loose stool is passed three or more times in a span of one day and may last for days to several hours, for instance diarrhea due to cholera infections or acute bloody diarrhea for the case of dysentery. Chronic or persistent diarrhea can last approximately two or more weeks (WHO, 2013).

Globally millions of infected individuals with gastro intestinal protozoans among which *Entamoeba histolytica* approximately affects over 50 million people all over the world and *Giardia lamblia* that is also estimated to have a general prevalence of about 2.8 million people. Additionally, more than one billion people do not have improved and quality water system and sources with more than three million people, majority children succumbing from water-related illnesses (Sobsey & Bartram, 2003).

### 2.5 Methods of transmission of intestinal protozoa

This is principally through ingestion of infective forms that can be obtained from polluted environment and poor personal hygiene for example, contamination with human excreta has demonstrated to be a potential transmission risk of gastro intestinal protozoa. The likelihood of pollution of edibles or water with protozoal infective forms by infected food and water vendors has been observed to be a noteworthy way of transmission of gastro intestinal protozoa to individuals (Idowu and Rowland, 2006). Gastro intestinal protozoa gotten through such a mode consist of *E. histolytica, G. lamblia* and *B. coli*
2.6 Predisposing factors gastro intestinal protozoa

2.6.1 Poor sanitation facilities

Poor water and sanitation in many areas, both in the countryside and urban set ups represent several intestinal diseases on a worldwide scale. For instance, *Entamoeba histolytica* is thought to infect 10% of the total general population globally and to yield a bigger number of those who die than some other parasites, notwithstanding from those brought about by intestinal ill health and schistosomiasis (Li and Stanley, 1996). Despite the fact that it has an overall dispersion, infection rates are most outrageous in warm environs and surpass half in regions where the aspect of hygiene is low, for instance, in city ghettos. For reasons apparently not associated to acquaintance, symptomatic amoebiasis (amoebic loose bowels) is less common in females and juveniles than men (Plorde, 1994). Poor sanitation additionally prompts the endemicity of most intestinal illnesses such as giardiasis, balantidiasis, and coccidiosis (Montresor *et al.*, 2015).

Water quality at the source refers to fundamental properties of water that defines its safety for use by humans; more than a half of Kenya’s population does not access clean treated water within their reach, probably because poor water quality and safety at the source. Decent level of hygiene includes public awareness on suitable hygiene and practices, endurable, inexpensive and affordable services of sanitation that ensures better health and wellbeing of people. Infections due the water contaminations and lack of access to improved water systems imposes much costs among which there is reduced income as a result of disease which subsequently affects the country’s GDP. Comparable to other developing
countries, intestinal protozoal infections are key health problems in Kenya and other countries in the tropics (Reither et al., 2007).

### 2.6.2 General environmental and personal level of hygiene

Poor collective and personalized hygienic behaviors responsible for the spread of quite a number of protozoan diseases. For instance, pathogenic amoebiasis is generally erratic, the consequence of direct transmissions from one individual to another mostly among the poor populations and the contamination of water and edible foods by asymptomatic individuals of infective parasitic forms are major determinants of the upsurge in the spread. Along these lines, man gets amoebiasis by ingesting contaminated food or water by human excreta, since there are no known animal harboring these cysts. Food borne illnesses are brought about by unsanitary treatment of rations of food, and during the cooking by diseased people. Through contaminated foods and water occurs sometimes in prevalent form. Such epidemics, though, less volatile compared to pathogenic intestinal bacteria (Cheesbrough, 1981; Chiodini, 2001); the issue of contact is linked to the how the host behave in its natural environment (Kightlinger et al., 1998).

### 2.6.3 Socio-economic factors

Many factors have been associated to high cases of parasite infections, some of which include, the interaction between biotic/abiotic factors and socioeconomic parameters (Magambo et al., 1998). The intestinal parasitic infections is most outrageous along the tropics relative to the regions where the aspect of hygiene is low, for instance, in city ghettos. In Sri Lanka, the occurrence of the intestinal protozoa and other intestinal parasites were on average of between seven to ten
times greater among the children from overcrowded environs than in children hailing from areas where there is not much overpopulation (Atukorala & Lanerolle, 1999). In situations where the toilets are not easy to clean, contamination level is high ) and when the toilet is provided and not used the transmission of intestinal parasitic infection is not effectively controlled through provision of toilets (Muchiri et al., 2001.

2.7 Life cycle and pathogenesis of gastro intestinal protozoan infections

Intestinal protozoa are many with diversities in terms of their life cycles and infective stages and pathogenicity, among the medically important gastro intestinal protozoa are *Giardia lamblia* that is globally distributed and is mostly prevalent in the tropical regions. Giardiasis, a parasitic disease that is caused by an intestinal flagellate *Giardia lamblia*, is a predominant cause of diarrhea that is fatal and affects growth and development of children (Simsek, Zeyrek, & Kurcer, 2004). *Giardia lamblia* effects on average 200 million people across the globe (Mineo et al., 2003) and it is spread via ingesting water and food that is contaminated with sewage matter and person to person contact (Bejon et al., 2008).

Ingestion of contaminated water and food serves as the main route of transmission for intestinal protozoa with a tendency of displaying similar life patterns comprising of two developmental stages that include cyst and trophozoite stages. The primary mode of transmission in man is through fecal-oral transmission route whereby the cysts form of these parasites are ingested via contaminated foods and water by an appropriate host, the cyst then change its form into a motile trophozoite in the host a form that displays an active metabolism (Haque et al.,
While the parasite is in a suitable host, it absorbs nutrients and then undertakes a sexual multiplication during its motile form; however, some of the trophozoites change into cysts that have got a resistant wall that remains dormant and finally gets excreted together with the feces (Fig 2.1).

The resistant wall formed by the cysts protect the parasite from dehydration in the external environment that is not favorable for the parasite form to replicate and continue with its lifecycle as it then undertakes a comparatively resting period in readiness for ingestion by the immediate host where it transforms into the infective forms. All the possible aspects that increase the chances of ingesting any contaminated substance with fecal debris that is key in the transmission of these intestinal parasites. In overall, circumstances encompassing direct transmissions in human, unhygienic environments and human hygienic practices encourages the parasite transmission to a suitable host (Haque et al., 2003).

Figure 2. 1: General lifecycle of gastrointestinal protozoa (Wiser & Linski, 2015)
2.7.1 Systematic sequence of infection by *Entamoeba histolytica* in man.

The infective cyst form is ingested by the host and once in the host it undergoes excystation in the small intestines. The mature cyst divides into 4 and then 8 trophozoites which move to colonize the colon. The formed cysts the forms another walls a process known as encystation before they are excreted off the host in feces that later gets ingested by the host.

Figure 2. 2:Lifecycle of *Entamoeba histolytica* (CDC,2010)
2.7.2 Pathophysiology of Entamoeba histolytica

Entamoeba species is dimorphic in nature with the trophozoite and cyst forms. The motile trophozoites feed on other microbes such as bacteria and host tissues, replicate, occupy and colonize the lumen and the mucosa of the large intestine, sometimes they may proceed to invade tissues and other organs (Stanley, 2003). Trophozoites are majorly found in the watery stools, however, they die as soon as they get out of the body of the host, in case they are ingested, they do not survive within the intestinal tract due to increased acidity that kills them. Some trophozoites in the colon along the lumen transforms to cysts that are later excreted in feces. E. histolytica trophozoites can get attached to the epithelial cells killing them and polymorphonuclear leukocytes that in turn results to dysentery. Trophozoites forms do secrete enzymes which eats into the extracellular organs and beyond. Trophozoites can be moved through the portal circulatory system and reaches the liver where they cause necrotic liver abscesses. From the liver they can proceed to the lung, skin, or sometimes can move to the brain and other organs via the blood stream.

In the formed stools, cysts predominate and after successful encystation they are able survive in the external environment. Contaminated food and water indirectly, play a great role in its transmission from one individual to another besides the direct transmission route. Interestingly Amoebiasis can be sexually transmitted via oral-anal contact (Chiodini et al., 2001).

2.7.3 Diagnosis and prevention of Entamoeba histolytica

Microscopy and identity of E. histolytica is based on the amebic trophozoites, cysts, or both in stool or tissues; however, there is a challenge in distinguishing pathogenic from non-pathogenic Entamoeba spp, due to their morphological similarity. Enzyme based Immunoassays such as polymerase chain reactions are usually carried out to confirm the diagnosis outcomes of direct wet mounts and other techniques because of its high sensitivity and specificity.
Enzyme immunoassays is the most widely used serologic test globally but sometimes it becomes impossible to distinguish between acute and persistent *E. histolytica* infections as it may proceed for months to years especially in endemic areas. Therefore, it is only advisable to use serological tests in situations where the previous infection is not common. For high sensitivity and specificity, more than two stool specimens from the same individual and concentration of the stool specimens may be required to enhance microscopic identification of intestinal amebas. Before stool examination, no probiotics, antacids, enemas and intestinal radiocontrast agents should be administered as they interfere with the intermittent excretion of the cysts. It is possible to differentiate *E. histolytica* from other amebas when using polymerase chain reactions-based assays and enzyme immunoassay for molecular analysis of antigens in stool.
2.7.4 Infection mechanism of *Giardia lamblia* in man.

Ingestion of viable cysts results to the removal of the cyst wall. The trophozoites enter the intestinal mucosa causing fatty diarrhea and while within the host after replication a cyst wall is reformed on the trophozoites resulting to the formation of the infective cyst forms in the colon and the area around the rectum. The cysts are then excreted with feces that may end up contaminating into water sources and food in case they come into contact.

Figure 2. 3:Lifecycle of *Giardia lamblia* (CDC,2010)
2.7.5 Pathophysiology of *Giardia lamblia*

Immediately after excystation, Giardia trophozoites use their locomotory apparatus to get into the microvillus of the small intestine, where they adhere themselves to the enterocytes using their ventral discs. The attachment damages the lining of the microvilli resulting into malabsorption of the nutrients. Giardia trophozoites undergoes through a rapid replication that ends up hindering the interaction between the enterocytes gastrointestinal lower part of the small intestines that affects nutrient uptake further. Once the gastrointestinal cells are damaged, it is followed by intestinal hyperpermeability and brush border that reduces disaccharidase enzyme secretion. Trophozoites do not infiltrate into the blood circulation through the epithelial cells (Katz *et al.*, 2001)

Osmotic diarrhea may result once the intestinal bush borders are damaged the respective enzyme activity drastically reduces affecting sugar absorption into the body from the lumen. Giardiasis in rodents accelerate intestinal movements that increases the smooth muscle contractility, both of which greatly contribute to giardial diarrhea

2.7.6 Laboratory diagnosis and treatment of *Giardia lamblia*

*Giardia intestinalis* is identified in slightly more than 50% of patients after only one fecal specimen is examined and sensitivity is high if three stool specimens of the same patient are obtained and examined in different days. Stool antigen enzyme linked immunosorbent assays are the best for screening in high incidences settings, however, they should not substitute stool microscopy. Stool antigen-based immunoassays could be vital if the stool tests for cysts are negative and giardiasis is still suspected. Microscopic examination has an added advantage since other parasites in stool can be identified besides *Giardia lamblia* in stool that can cause diarrheal related illness (Farthing *et al.*, 2004).

Giardiasis is potentially managed using a multiple nutritional measures and phototherapy. Orally administered probiotics affects the microflora of the proximal small intestines hence
directly inhibit the Giardia growth and also induce innate and immunological anti- giardial mechanism (Perez et al., 2001)

2.8 Multiple gastrointestinal protozoan infections

Mixed infections are common in developing nations since various contemporary study findings shows that multiple infection cases are common in areas that are categorized as poverty stricken zones including some parts in Africa, Asia and America, (Guignard et al., 2000); (Keiser et al., 2002); (Waikagul et al., 2002). Remarkably, amongst the few studies that have examined multiple infection cases, many focused on specific age groups. The limited number of studies undertaken in larger populations established that mixed infections rise with advance in age where the adolescents and young adults have the highest infection as the infection curve starts to decrease as one gets older (Keiser et al., 2002). The epidemiology of co-infected cases of intestinal parasites is intricate and many researchers have, customarily, focused on single case infections per host (Cox & Guthrie, 2001). There are quite a number of factors that contributes to co-infections, such include; lack of treated clean water safe for use by man, overcrowding and poor personal and environmental hygienic practices (Asaolu & Ofoezie, 2003)

2.9 Effects of intestinal protozoa

Gastro Intestinal protozoan infections in humans results to a variety of symptoms that include; acute or persistent diarrhea, dehydration, malnutrition, general malaise, abdominal cramps, passing of large amounts of fat in feaces and recurrent deaths. There are a variety of effects that the gastro intestinal protozoans impose on man as their definitive host. They are broadly classified into direct and indirect effects.
2.9.1 Human health

Ill health as a result of intestinal protozoa infections can be hazardous to human beings, they include; nutritional effects and effects on cognitive components. Nutritional effects that cause diseases accompanying intestinal parasitic infections are demonstrated mostly as dietary disruption, reduced food intake due to lack of appetite, impaired digestion and malabsorption. Tenacious infections can aggravate the lower bowel, results into passage of loose stools three or more time in a day and retarded growth and mental growth. Children are mostly affected when the infection becomes severe at a time when they need to grow and learn therefore imposing great risk to their entire development process. The most practical demo are due to the worm infections in children especially in cases where the worm burden is very high which may produce harmful effects on the intellectual functions hence affecting their achievement in education (Sternberg, 1997). Intestinal infections interfere with the child development for instance a child from a low socio-economic class underperform and never realize their full potential.(Roche & Layrisse, 1966). Iron-deficiency anaemia (IDA) in infants and children of a younger age has been found to significantly lower their performance on psychological tests (Walker, & Chang, 2000)

Studies in Tanzania showed that school children infected with intestinal parasites underperformed in some tests due to their harmful effects of intestinal parasites on their cognitive component, (Sternberg, 1997). Additionally, intestinal parasitic infections could have a devious impact upon the developmental process of children especially when it comes to making life decisions due to lack of interpersonal and emotional skills(Miguel & Kremer, 2002). Amoebiasis due Entamoeba histolytica may result to abdominal discomfort, gastrointestinal ulcerations and gastrointestinal obstruction (Kucik et al., 2004)
2.9.2 Social economic status

People of low economic status in many occasions live in areas of poor sanitation due to the inability to afford improved houses of residence with better hygiene conditions. This poses greater risks for infections, hence linked illness and death to the variety of factors, including contaminated water, lack of improved sanitation and foods contaminated with cysts of intestinal parasites (Al-Agha & Teodorescu, 2000). Contemporary studies have shown high frequency of pathogenic microbes, especially in children in the South Saharan Africa region when compared with the other parts of the world. This could be due to the fact that African as a continent is unduly affected by many intestinal illnesses and therefore understanding the epidemiology and endemicity of disease-causing microorganisms that affect the sub-Saharan African countries and understanding how this affects prevention and control measures is inevitable. The high profile and burden of HIV infection in countries in SSA populations have increased the risk of acute and persistent diarrhea.

There is much value in dealing with a variety of pathogens unlike single case infections since quite a number of them have similar ways of transmission and exposure. The exposure and preventive measures for each specific intestinal illness may influence a number of pathogens concurrently (Tiwari et al., 2009). Costs associated with secondary transmission and lost productivity show a positive correlation to parasitic intestinal infections. Additionally high costs are incurred due to outpatient treatment, hospitalization, medical claims and lost man hours and such infections can result into death (Muennig et al., 1999).

2.9.3 Clinical pathogenicity of gastro intestinal protozoa

The symptoms and signs produced by infections with pathogenic intestinal protozoa include: loss of appetite, diarrhea, malabsorption, vomiting, abdominal pains, blood and/or mucus in stool and diarrhea (Katz & Hotez, 2004). Intense bleeding diarrheic scenes are basic in intestinal diseases, for instance, Entamoeba histolytica. Greasy steatorrhoeic stools are likewise passed in intense diseases with Giardia lamblia (Heymann, 2008).
2.9.4 Electrolyte imbalance
Looseness of the bowels interrupts the normal food absorption resulting to upsets and corrosiveness in the bowels of the body causing hypokalaemia, hyponatraemia and hypochloraemia. These upsets result to seizures and intermittent loss of motion of the affected patients, particularly with extended proximity of worm contaminations which go untreated (Kucik et al., 2004); (Katz and Hotez, 2004)

2.9.5 General illness
General fatigue is characterized by cerebral pains, sickness and stomach irritations and is the condition related to specific timelines for few protozoan diseases, particularly for chronic and persistent situations. This is a common case that occurs in amoebiasis and giardiasis.

2.9.6 Malnourishment
Endless protozoan contaminations inhibit fat loss and besides causes defective ingestion of protein and fat-solvent nutrients and Folic Acid. This condition therefore leads to diarrhea and loss of nutrients to the environment in feces. Continued intestinal infections that results to nutrient loss could cause ill health in humans. For example, *Giardia lamblia* has been associated with malnutrition due to the debilitated fat absorption that prompts hindered development in children (Hilbert et al., 2003)

2.10 Laboratory diagnosis
The microscopic examination of gastrointestinal protozoan infections focuses on the identifying and distinguishing the trophozoites and/or cysts a in human fecal material by using direct wet mounts, Kato-Katz techniques or concentration/ sedimentation methods, and subsequent standard microscopic investigation (Marti & Escher, 1990). Patients ought to be sensitized to pee first before collecting the fecal sample so that no urine contaminates the stool; emphasis should be done to the patients to use gloves when handling stool and there after wash their hands to elude possible infections; patients should be given waste newspapers or plastic
basins to put under the toilet seat to defecate on after urination so that the stool sample to be collected does not come to direct contact with the toilet bowls which could contain germs or cleaning agents that may kill the somatic forms of parasitosis by altering the PH as well as ensuring that no tissue mixes with it.

If the patient has diarrhea, a plastic bag should be tapped to the toilet seat for ease collection of the stool samples. When a patient is constipated, should be given small enema; for young children, stool can be collected from diapers or from a small diameter glass tube inserted into the rectum held by the adult. This will improve medical service delivery, diagnostic capacity and minimize possible re infections of gastro intestinal protozoa.

2.11 Control of gastro intestinal protozoa

2.11.1 Personal and environmental hygiene

Suitable disposal of human wastes prevents contamination of foods and drinking water sources and therefore this mitigates human intestinal infections whose mode transmission is mainly fecal-oral pathway (Asaolu & Ofoezie, 2003). The removal of the possible contaminants from the areas around the water sources greatly safeguards the water at the source. Surface water run offs if not redirected away from the water sources, then it is possible for the human excreta and animal refuse gaining access into water bodies hence contaminating these water collection points. In cases where livestock rearing is done around the water sources, proper erection of barriers would be used to keep animals from contaminating the drinking water sources. After successful elimination of the possible contaminants from the water sources or around them then it becomes easier to safeguard these water sources by entirely treating them by carrying out regular laboratory analysis of water samples so as to ensure water quality at the source. Most springs used for drinking water needs continuous disinfection system to ensure the safety of drinking water by humans.
In addition, a structured policy on the standards of the type of affordable and descent human waste disposal unit that will minimize possible contaminations by human fecal material and any household waste that possibly can be a potential contaminant of water sources should be designed. Control of flies and other insects in food preparation and serving points prevents dispersal of infective stages of gastro intestinal protozoa to foods (Asaolu & Ofoezie, 2003). Studies indicate that washing of fruits; utensils and hands prevent cross-contamination of foods with cysts of gastro intestinal parasites (McPherson et al., 2000)

2.11.2 Public health education and awareness

People should be educated on proper sanitation, which involves promotion of the usage of properly constructed latrines via formulation and development of legislation public sensitization on health education. Gastro intestinal protozoan infections can be stopped or significantly reduced through many other interventions that are cheaper so as to avoid ingestion of infective forms of parasites and direct contact with contaminated foods. Food safety measures help prevent intestinal parasitic infections which are transmitted by fruits and vegetables. Health education involves the following: necessity for drug treatment for those already infected because they can act as reservoirs of gastro intestinal protozoan transmissions, sanitary and personal hygiene improvements to prevent reinfection and disrupts the life cycle in transmission of gastro intestinal protozoa from person to person through safe, proficient and hygienic food preparation methods, proper food storage and the practice of hand washing especially when one comes into contact with infected stool(Vardiman et al., 2009). Health educators should encourage individuals to adopt behaviors that will prevent auto-infections or reservoirs of other people’s infection and must be multidisciplinary where various stakeholders will be involved to educate the public on sanitation and personal hygiene in controlling gastro intestinal protozoan infections.
2.11.3 Food preparation and heat treatment of food stuffs

Adequate cooking of foods and boiling of water destroy all infective stages of intestinal parasites, however, microwave and some other cooking devices may not be relied upon since not all intestinal parasites in foodstuffs are destroyed for the reason that heating is uneven and cooler spots may allow survival of such parasites.

2.11.4 Filtration and disinfections

Filtration eradicates some intestinal protozoan cysts and Oocysts from water. Nevertheless, *Cryptosporidium* spp has been found in filtered water. Chlorination only eliminates some parasites from water leaving cysts because they are resistant treatment.(Heinrich *et al.*, 2003)
CHAPTER THREE
METHODOLOGY

3.1 Introduction
This chapter includes study site, study design, targeted population, determination of sample size, required equipment, materials and reagents, sample collection and processing and ethical consideration for this research.

3.2 Study site
Kisii Teaching and Referral Hospital in Kisii County, Kenya was used for this study. The hospital receives referrals from 10 sub counties and others from the surrounding counties whereby it treats mainly low-income patients. The hospital is located in southwestern Kenya on geographical coordinates of 0°41’S 34°46’E and an altitude of 1660m above sea level. Kisii County is one among many counties with resource-limited set ups with about half the population (49%) not accessing improved water sources (KNBS &SID 2013). Most of the households have pit latrines but maintenance of proper sanitation and hygiene is poor. The KTRH annual health records indicates that diarrhea and gastroenteritis presumed infections is second after clinical malaria among the top 10 diseases in children under five years. Diarrhea and gastroenteritis presumed infections is a major problem in Kisii County in children especially children less than 5 years old with feasible differences on the occurrence of certain intestinal protozoa in circulation in children. The Kisii county referral hospital has a catchment zone covering more than 1.3 million people as per the statistics of census 2009. Kisii level six hospital has a daily capacity of 200 patients admitted and 400 patients attending various outpatient clinics for health services. The study area has the second most populated town in Nyanza with a population density of 2862 per km² (CBS, 2009).
Figure 2.4: Map of Kisii town showing the location of Kisii Teaching and Referral Hospital (Google map)
3.3 Study design

Cross-sectional study covering different age cohorts, male and female diarrheal children in KTRH was conducted between 1st April and 30th August 2017.

3.4 Sample and sampling techniques

3.4.1 Target population

The research targeted children under five years symptomatic of diarrhea and diarrheal related illnesses attending Kisii Teaching and Referral Hospital (KTRH) for medication. This facility on average receives fifty cases daily from various hospitals within the County and other neighboring counties.

3.4.2 Sample size determination

The determination of the sample size was calculated using a formula developed by Cochran for representative proportions (Sapoka et al., 2006), in cases where the population is less than 10,000. Typically, the hospital approximately receives about 200 children with diarrhea symptoms monthly attending the facility for medication who hails from within the county and others from nearby counties.

Assumptions for Cochran’s formula

\[ n = \frac{Z^2 \times P \times (1-P)}{\bar{e}^2} \]

- \( n \) = the desired sample size for a population is less than 10 000
- \( Z \) = the standard deviation is 1.96, which corresponds to 95% confidence level.
- \( P \) = prevalence of the previous diarrhea cases among the children under age of 5 years in south Nyanza region is 8.5% (UNICEF/WHO, 2009).
- \( q = 1 - P \)

\( \bar{e} \) = is the level of precision taken as a percentage usually at 5%.

Using the Cochran developed formula, the sample size was derived as;

\[ n = Z^2 \times P \times (1-P) / \bar{e}^2 \]

\[ n = 1.96^2 \times 0.085 \times 0.915 / 0.052 = 120 \]
A total of 132 children were targeted for sampling to provide room for the 10% non-response rate due to the likely unwillingness to participate in research.

3.4.3 Inclusion criteria
This study only included the clinically symptomatic cases of diarrhea and diarrhea related illnesses, those who had resided within the locality of Kisii County for at least two weeks and the ones whose caregivers signed a written consent form.

3.4.4 Exclusion criteria
Asymptomatic or children who were on antimicrobial agent(s) such as metronidazole, tetracycline, erythromycin and antacids within a month prior to sample collection or had just completed investigation with barium meal/enema diarrhea were excluded from the study. 
Data was rejected where information was provided without sufficient details for identification and where the consent forms were not signed. Contaminated stool samples were disallowed.

3.4.5 Sampling technique
The children were randomly sampled out where all children aged between six months and nine months old with diarrhea attending Kisii Teaching and Referral Hospital had equal opportunity of being selected for study.

3.4.6 Questionnaire
A simple questionnaire was designed by the author in English and used for collecting information from the children under five years with diarrhea who attended the Kisii county referral hospital (KTRH) seeking medication within the period of study. The caregivers/guardians of the participants were on voluntary terms requested to confirm if their children had previously experienced diarrhea related symptoms such as passing loose watery stools, and bloody or mucoid, they were also asked if they have been on any antimicrobial drugs within the past one month. Recruited trained research assistants interrogated respondents on demographic data; socioeconomic background, behavioral risks, environmental sanitation
and living condition characteristics. The questionnaire was filled as the interviews were on by the trained research assistants upon consent by the caregivers.

**3.4.7 Validity**
A pilot study was conducted on 8% (10 children) of required sample size. Their stool samples were collected after the caregiver signed a written informed consent form at Iyabe Health Centre. Data on the age, sex and various possible risk factors were obtained through a questionnaire developed by the author that was pretested and later modified to make it an appropriate tool for quality data collection and validation of results. This pilot test was done for three days between 20th and 22nd of February 2017 in preparation for the actual study that commenced in April 2017.

**3.4.8 Reliability**
The instruments and tools selected for this study mainly included, a structured questionnaire for data collection, centrifuge machine and light microscope for sample examination which were pre tested and approved for use after they were found to be consistent in the results obtained from the 10 children recruited for pilot study at a nearby hospital (Iyabe level 4) Kisii County. A duo stool testing was also done for each of the 10 samples to ensure reliability, this was done by comparing the independent results of each sample, if the results of the tests had no significant discrepancies assuming that key factors remained constant then the tools were recommended for use since they could be reliable. This was to improve the quality on data collection and subsequent stool sample examination.

**3.4.9 Ethical considerations**
In the preparations for data collection, the study protocol (Reference Number: KSU/R&E/03/5/79,2015) was sort and approved by Eastern Africa, Baraton University Ethics Committee and the ethical clearance issued. Letter authorizing the research, Research permit, letter of acceptance to use the hospital for the research were all obtained in time from the relevant institutions and the caregivers were given an elaborate explanation for the objectives,
procedures, possible risks, and benefits associated with the study if any before the start of interviews and stool collection. Informed consent form was filled by the caregivers accompanying the children prior to data collection, appendix (vii). During the interviews, they were judiciously and procedurally assured of the confidentiality of any information that they may be required to give that will be crucial to the study, and they were free to pull out of the study without quoting reasons for quitting if they feel that the said agreement is breached.

3.4.10 Sample collection procedure
Clean dry fecal containers were issued to caregivers of each patient and instructed on how the samples were to be collected. Information on sex, age and personal and environmental aspects were recorded instantly upon submission of samples to the processing bench at the laboratory to avoid confusion.

3.4.11 General sample examination
All the fecal samples were observed macroscopically and microscopically. The color, odor, presence of mucus and/or blood stains were used for assessment of stools. The applicator sticks were used to determine the nature and consistency of stool. The stool specimens were then processed for direct wet mounts and concentration techniques before being observed under a microscope. Identity of the trophozoites/cysts was done based on morphological differences.

3.4.12 Microscopic examination procedure
Bold standard microscopic oval/cyst and adult parasite detection techniques were used in the examination and identity of the suspected organisms in stool was done within the first 30 minutes using the morphological diagnostic features for accurate and reliable results (appendix viii and ix) respectively.

The cysts and trophozoites of *Giardia lamblia* and *Entamoeba histolytica* were observed and distinguished. The parasite cyst /trophozoites in the stool specimen were counted and the parasite density of each species was expressed as ‘many’ (three cysts/trophozoites per high - power field; ‘moderate’ two cysts/trophozoites per high power field of view; ‘few’one
cyst/trophozoite per 2-3 high power fields of view and ‘rare’ (two to five cysts/trophozoites per mount)

3.4.12 Methods of data analysis

Raw data was keyed in Microsoft Excel, data cleaning done by an expert and only appropriate data was entered before being exported to the SPSS version 21 (SPSS, Chicago, IL, USA) tool. For all descriptive data, percentage was used to determine the characteristics of the population of study, including the occurrence of gastrointestinal protozoa identified.

Chi-squares tests ($\chi^2$) were used to check on the associations between the variables by cross tabulations. The parasite density was expressed as: rare, few moderate and many. For all the factors that were found to have significant statistical difference, a confidence interval was calculated at 95% (CI) and significant differences were defined $p \leq 0.05$ significance level.
CHAPTER FOUR

RESULTS

4.1 Introduction

One hundred and twenty children aged between 6 months and 59 months old were included in
the study and their stool specimen were diagnosed for gastro intestinal protozoa infections over
a period of four-months of which 34 (28%) children tested positive of either *Entamoeba histolytica*, *Giardia lamblia* or both parasites. The gastrointestinal protozoa were examined
microscopically under a light microscope and identification was done using the morphological
diagnostic features. The criteria for identifying the protozoa trophozoites was motility structures,
type of motility, and number of nuclei, karysome and chromatid bars (Heckendorn *et al.*, 2002).
Other structures such as cytoplasmic inclusions for example erythrocytes and yeast were used in
identifying amoebic trophozoites while structural details such as sucking disks and spiral groove
or filaments were used in identification of flagellate trophozoites. The number of nuclei in the
karysome and the thickness of the cyst wall were among diagnostic features used for distinguishing
cysts of *E. histolytica* from cysts of *G. lamblia* as mentioned in appendix viii. The following
shows plates obtained during microscopy (micrograph plates (1-4).

Plate1 (a): cyst for *Entamoeba histolytica*; Plate 2 (b): Trophozoite for *Giardia lamblia*;
Plate 3 (c): cyst for *Giardia lamblia*; Plate 4 (d): Trophozoite for *Entamoeba histolytica*
4.2.1 Gastro intestinal protozoan infections in relation to the age of children between the age of 6 months and 59 months with diarrhea

In this study children aged between 6-11 months had the highest infections (10.83 %) compared to other age groups of the children examined though not significant, (P=0.337). There was an interesting decreased infection trend with an advance in age (Table 4.1).

Table 4.1 Prevalence of gastro intestinal protozoan infections among children aged between 6-59 months in Kisii County Kenya

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Number of patients</th>
<th>Parasite species</th>
<th>Number infected (%) per cohort</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-11</td>
<td>50</td>
<td>E. histolytica</td>
<td>10(20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>2(4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>1(2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13(10.83) *</td>
<td></td>
</tr>
<tr>
<td>12-23</td>
<td>21</td>
<td>E. histolytica</td>
<td>6(28.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>2(9.5)</td>
<td>.337</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>2(9.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 (8.33)</td>
<td></td>
</tr>
<tr>
<td>24-35</td>
<td>24</td>
<td>E. histolytica</td>
<td>2(8.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>0(0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>2(8.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4(3.33)</td>
<td></td>
</tr>
<tr>
<td>36-47</td>
<td>12</td>
<td>E. histolytica</td>
<td>2(16.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>0(0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>1(8.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3(2.5)</td>
<td></td>
</tr>
<tr>
<td>48-59</td>
<td>13</td>
<td>E. histolytica</td>
<td>2(15.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>2(15.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>0(0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4(3.33)</td>
<td></td>
</tr>
<tr>
<td>Sub total</td>
<td>120</td>
<td>E. histolytica</td>
<td>22(18.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>6(5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>6(5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>34(28.3)</td>
<td></td>
</tr>
</tbody>
</table>

Additionally, E. histolytica and G. lamblia were the gastrointestinal protozoa identified in examined stool specimens with the percentage infections of 18.4 % and 6% respectively.
Remarkably, 5% of the total stool specimens examined were there were co-infected cases of both *E. histolytica* and *G. lamblia*.

### 4.2.2 Prevalence of gastro intestinal protozoan infections in relation to sex among children of age between 6 months and 59 months old with diarrhea

Fresh stool samples of 120 diarrheic children were examined for protozoal infections of which 71 children with a percentage of (59.1%) were males and 49 (40.1%) were females. Of the positive cases of either *Entamoeba histolytica*, *Giardia lamblia* or both parasites, males were more infected compared to females with 23 (67.6%) and 11 (32.4%) respectively. (Table 4.2)

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>6-11</th>
<th>12-23</th>
<th>24-35</th>
<th>36-47</th>
<th>48-59</th>
<th>Sub-total</th>
<th>% per sex</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (71)</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>23</td>
<td>32.39</td>
<td>0.392</td>
</tr>
<tr>
<td>Female (49)</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>11</td>
<td>22.44</td>
<td></td>
</tr>
<tr>
<td>Sub-total (120)</td>
<td>13</td>
<td>10</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>34</td>
<td>22.44</td>
<td></td>
</tr>
<tr>
<td>% per age group</td>
<td>10.83</td>
<td>8.33</td>
<td>3.33</td>
<td>2.5</td>
<td>3.33</td>
<td>28.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Evidently, males had higher proportion of infections than their female counterparts in each of the age group, though not statistically different (P=0.392).
4.3 Parasite densities among children attending Kisii Teaching and Referral Hospital

The densities are classified as: rare (2-5 organisms per 22 mm square cover slip), few (1 organism per 5-10 high power fields (40x)), moderate (1-2 organisms per high power field to as few as 1 organism per 2-3 high power fields.) and many (over 3 organisms in every high power field.) in that order (Table 4.3)

Table 4. 3: The parasite densities of gastro intestinal protozoa infections among children of age between 6-59 months old attending Kisii Teaching and Referral Hospital

<table>
<thead>
<tr>
<th>Type of parasite</th>
<th>Parasite densities</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rare</td>
<td>Few</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>2(5.9%)</td>
<td>3(8.8%)</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>5(14.8%)</td>
<td>13(38.2%)</td>
</tr>
<tr>
<td>Mixed infections</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>Subtotals</td>
<td>7(20.6%)</td>
<td>16(47.1%)</td>
</tr>
</tbody>
</table>

For the purpose of this study the parasite densities were further specified as follows;

**“Rare”** - three organisms per 22 mm square cover slip

**“Few”** - 1 organism per 5-10 high power fields (40x)

**“Moderate”** - 1 organism per high power field.

**“Many”** - Over 3 organisms in every high-power field.

The parasite density for *Entamoeba histolytica* was found high in the stool specimen with one organism per each field of view that was categorized as ‘few’ (38.2 %) followed by the specimen rated as (14.8 %), moderate (11.8 %) and in a category defined as many where there was no stool specimen with three or more organisms per field of view. The same occurrence was recorded for *Giardia lamblia*. Interestingly, for all co-infection cases, there were at least three organisms observed in every high-power field of view which was categorized as many when defining parasite density. In general, the densities of all the examined stool specimens was found to be significantly lower (P < 0.001) despite co-infected individuals having more than three organisms viewed under high power field.
4.4 Effect of hygienic practices on the gastro intestinal protozoan infection among children with diarrhea aged between 6-59 months old at KTRH

4.4.1 Gastro intestinal protozoan infections and the practice of finger sucking and biting
Eighty-six children were sucking fingers whereas 54 did not. Out of the ones who practiced finger sucking, 25 (74%) children had gastrointestinal protozoa whereas only 9 (10%) children of those children who did not practice finger sucking were infected \( P < 0.001 \), (Table 4.4.)

Table 4.4: Effect of hygienic practices on gastro intestinal protozoan infections among diarrheal children under age 5 years in Kisii County Kenya.

<table>
<thead>
<tr>
<th>Hygienic practice</th>
<th>Practice presence/absence</th>
<th>Parasite identified</th>
<th>Number infected (%)</th>
<th>( \chi^2 )</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand washing before eating meals</td>
<td>No 32</td>
<td>E. histolytica</td>
<td>14(44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>5(16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>3(9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes 88</td>
<td>E. histolytica</td>
<td>8(9)</td>
<td>34.789</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>1(1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>3(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit washing before eating</td>
<td>No 50</td>
<td>E. histolytica</td>
<td>11(22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>5(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>5(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes 70</td>
<td>E. histolytica</td>
<td>11(16)</td>
<td>11.758</td>
<td>0.508</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>1(1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>1(1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waste disposal</td>
<td>Single 66</td>
<td>E. histolytica</td>
<td>4(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>1(2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>1(2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multiple 54</td>
<td>E. histolytica</td>
<td>18(33)</td>
<td>28.072</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>5(9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>5(9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finger sucking</td>
<td>No 86</td>
<td>E. histolytica</td>
<td>7(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>1(1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>1(1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes 34</td>
<td>E. histolytica</td>
<td>15(44)</td>
<td>47.071</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. lamblia</td>
<td>5(15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>5(15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water source for drinking</td>
<td>Streams/rivers</td>
<td>E. histolytica</td>
<td>12(63)</td>
<td>18.479</td>
<td>0.030*</td>
</tr>
<tr>
<td></td>
<td>Unprotected springs</td>
<td>G. lamblia</td>
<td>6(32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>Both (E. histolytica and G. lamblia)</td>
<td>1(3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26</td>
<td></td>
<td>10(38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tap</td>
<td>E. histolytica</td>
<td>7(32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bore hole</td>
<td>G. lamblia</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>Both (E. histolytica and G. lamblia)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Economic status of caregiver(s.)</td>
<td>Low income 102</td>
<td>E. histolytica</td>
<td>27(23%)</td>
<td>3.395</td>
<td>0.758</td>
</tr>
<tr>
<td></td>
<td>High income 18</td>
<td>G. lamblia</td>
<td>7(39)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.4.2 Gastro intestinal protozoan infections and the practice of hand washing before meals

The information obtained from the caregivers concerning the practice of handwashing before any meal, it was found out that 32 children of the 120 did not directly or indirectly engage themselves in the practice of hand washing more often. Out of the total cases of infections, 35.3% of children who took meals often after their hands were washed were infected while 64.7% of the children did not regularly have their hands washed before any meal were infected. Notably, among those that were not regularly washing their hands or being assisted in hand washing before meals, 22 (67%) children were infected by gastrointestinal protozoans while 12 (14%) children were infected among the 88 children whose caregivers actively engaged them in hand washing before meals, only. Therefore, the practice of handwashing on regular basis greatly decreased the risk of infections in children who were involved in this study (P < 0.001).

4.4.3 Gastro intestinal protozoan infections and the mode of human waste disposal by households

The current study examined the effect of the mode of human waste disposal by households on gastro intestinal protozoan infection in children. It was found out that 66 (55%) children came from the households that did not share a common unit of human waste disposal while 54 (45%) children came from households where a single unit could be shared by multiple households. Of the total cases of infections, 82% of children infected came from those households that happened to have multiple households sharing a single unit of human waste disposal whereas 17.6% were infected among the ones who came from households that did not practice sharing of a single unit by more than one household. It was worth to note that out of those children that came from households that shared a single disposal unit, 28 (52%) of them were infected with either parasite compared to the 6 (9%) children that were infected among those who did not have the practice of sharing human waste disposal units, the aspect of not sharing a unit for
disposing human waste was found to have significantly reduced the infections $P<0.001$ (Table 4.4).

### 4.4.4 Gastro intestinal protozoan infections and the main water source for drinking

The major sources of water for drinking in households included, treated tap water, boreholes, streams/rivers, springs and others. 34 (28.3%) children were using treated tap water, 26 (22%) used water from boreholes, 19 (15.8%) were using water from the streams/ rivers, 19 (15.8%) children springs and 22 (18%) of children used water from other sources that included jabia, vendors and many others. It was noted that out that out of the 19 children that came from households that used water from rivers/streams, 12 (63%) were infected while out of the 26 children that came from households that used borehole water 7 (38%) children were infected. There were 22 children that used water from other sources including rain and water vendors out of which 7 (32%) children were infected whereas only 6 (32%) children out of the 19 from households that used water from the springs were infected. Moreover, 34 children used treated tap water and only 1 (0.8%) child was infected

Thirty four children of the 120 who were examined for intestinal protozoan had 28.3% positivity index out of which, 12 (35%) were from the households that used water from rivers/streams, 7 (21%) from those households that used water from the borehole, 7 (21%) who were infected came from the households that used water from other sources, 6 (18%) used water from the springs and lastly, only 1 (3%) child of the 34 infected came from the households that mainly used treated tap water for drinking. Notably, use of water drawn from the streams or rivers was highly related to the increased risk of infection by gastro intestinal protozoa comparable to other water sources in this study and the use of treated tap water was negatively associated to the risk of infections (Table 4.4) $P = 0.030$. 


4.4.5 Gastro intestinal protozoan infections and the practice of fruit washing

The study intended to establish whether the practice of washing the fruits and vegetables before eating had an impact on the risk of infection of gastro intestinal protozoa. Of the 120 children, 50 (42%) were not often washing the fruits all the time before eating while seventy children (58%) had the practice of regularly eating washed fruits and vegetables that are used as salads all the time. Of the infected children with gastro intestinal protozoa 21 (62%) who demonstrated that they ate unwashed fruits were infected while 12 (35%) children that often ate fruits that were washed tested positive. Further analysis on the proportion of infection revealed that 21 (42%) children were infected among the ones that ate fruits most of the times without being washed while only 12 (17%) were infected of those ones that frequently ate washed fruits and vegetable salads, therefore those who often directly or indirectly practiced fruit washing before eating were less vulnerable to gastrointestinal protozoan infections compared to those who did not (P=0.508).

4.4.6 Gastro intestinal protozoan infections and the economic status

The study anticipated to investigate the effect of economic status of the caregiver on the risk of gastro intestinal protozoa infection. Economic status was categorized into high/middle level income and low income. One hundred and two (85%) children came from the families that were classified as low-income earners and 18 (15%) were from the families that were categorized as high / middle income earners. Of the total positive cases of either intestinal protozoa identified in stool, 27 (79%) children were from low income earning families while 21% of the infected children came from families categorized as high /middle level income earners (P=0.758). Out of the 18 children who came from households that caregivers belonged to a high/middle income level, 7 (39%) were infected with gastro intestinal protozoa and remarkably there was relatively higher numbers of infection among those that came from the households in which caregivers were classified as low income class 27 (23%) though did not significantly reduce the risk of infections.
CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Prevalence of gastrointestinal protozoa in diarrheal children of age 6-59 months old

Out of one hundred and twenty examined stool specimens, 34 (28.3%) children were infected with *Entamoeba histolytica, Giardia lamblia* or both parasites with positivity index of 18.0 %, 5.0 % and 5.0 % respectively. The infections are higher than other sites in Kenya, a study in Kitui County, Kenya had intestinal protozoan infection at 12.6% (Nguhiu *et al.*, 2009). The infections in the current study were comparable to other study findings for instance in Mukuru an informal set up in Nairobi, where the prevalence was reported at 25.6% (Mbae *et al.*, 2013) and in Kitui County 38.6% (Kisavi, 2015). In contrast, the current study findings were higher than studies in other African countries that include Mozambique with a prevalence of 16% (Kneel *et al.*, 2018) and comparable to study findings in Tanzania 29.6% (Ngoso *et al.*, 2015) and in Nigeria with slightly high infections of 36.52% (Firdu *et al.*, 2014). These discrepancies could be explained by the differences in living conditions of the participants involved in this study, environmental sanitation, handwashing before meals an after toilet, the practice of finger sucking and biting and finally, usage latrines and mode of human waste disposal (Taye *et al.*, 2014). The high infections warrant attention and institution of measures to control and treat infected individuals.

This current study indicated that there was a decrease in the intestinal protozoa infections with the advance in age of children from 6-11 months (10.83 %) being the highest and the least among the children of age 48-59 months. Findings in Tanzania showed a different picture with children of age 6-12 months having the least and 12-24 months having the highest infections of intestinal protozoa (Ngoso *et al.* 2015). A The study in the southern part of Ethiopia on infectious protozoa diseases of poverty also agrees disagrees with the current
study findings as it reported a highest infections among children of age group between 24 - 36 months, while the age group ≤ 1 being least infected,(Mulatu et al., 2015). This study also differs with another study done by De Souza et al., (2007) who indicated that the intestinal infections was at the peak between 1-2 years. The vulnerability among the children of this age groups could be attributed to personal hygienic and behavioral factors that include unhygienic behavior during the introduction of solid foods and water due to the possible contamination and crawling on a contaminated surfaces which increases the chances of accessing filthy material into their mouths (Adnan et al, 2008). In addition, use of diapers may allow for the possible transmission of gastro intestinal protozoans because of auto-infection if not used appropriately.

In this study males 23 (67.6%) were more infected than females 11 (32.4%), which implies that a male child is 1.5 times more likely to be infected with gastro intestinal protozoa than their female counterparts. These findings were similar to other studies done in Nakuru Kenya (Chabalala and Mamo, 2001), Nigeria (Anosike et al., 2004; Adeyeba & Akinlabi, 2002) and South Korea (Nkengazong et al., 2009). Nevertheless, some studies have shown higher infections in females unlike to the case in males (Chukwuma et al., 2009). The male children being more infected than female children could be attributed to the differences in genetic and general behavioral aspects (Coutsoudis et al., 2001), generally males show a reduced immune responses compared to the females. This is attributed to the larger number of genes related to immune activities that are found on the X-chromosome. This phenomenon results to a high gene expression program that allows females to quickly respond to an extended immune system as compared to males hence females have an advantage of innate and adaptive immunity. These disparities usually attributed the differences in the ecological factors, differences in behavioral exposure or morphological patterns (Stanley, 2003 and Burgess, 2013).
5.1.2 Parasite densities of the gastrointestinal protozoans

This study findings concur with other studies in the study area where more than three parasites were seen under a high power field mount (Nyarang’o et al, 2008) and similar studies (Sohail and Fischer 2004; Stenzel and Boreham 1996). However, in Bali Island, parasite density was less than 3 parasites per field, (Luh et al, 2018). Interestingly, co-infected children presented a higher parasite density compared to single case where the parasite density was relatively lower. This concurred with a study in Uganda among malaria co-infected children where parasite densities of intestinal protozoa were lower and this was because intestinal parasitic infections especially helminths induce a Th2 host response that explains an immunomodulatory effect (Ayodele et al, 2015). A study in Mozambique among children of age 0-168 months also revealed co-infection cases of Entamoeba histolytica and Giardia lamblia where the parasite density was found highest among the immunocompromised children (lopez et al., 2015). In addition, co-infections were lower than other studies conducted in Ethiopia and Tanzania (Abate et al, 2012; Alemayehu et al., 2014). Socio-economic class has been positively correlated to polyparasitism. Low income earners and living in overcrowded areas like ghettos and slums where sanitation is poor and access to clean treated water predisposes individuals to health situations associated with polyparasitism (Saldiva et al., 1999). In the Northeastern part of Brazil, almost two-thirds of the 13% cases of infections were from families characterized by poor sanitation and environmental conditions. This scenario could be associated to variances in sanitation and environmental conditions in cases where contamination of water and food is common, different routes of infection and different exposures to the host (Harhay et al., 2010)
5.1.3 Effect of personal/ communal hygiene practices to the gastro intestinal protozoan infections among children aged between 6 months and 59 months old with diarrhea.

This study finding showed that the practice of regularly having their hands washed before any meal greatly minimized the risks of infections, it is in agreement with other studies. In Kilifi, Kenya, the practice of washing hands every time a visit to the toilet is made proved to significantly reduce the cases of acute bloody diarrhea. The aspect of not handwashing before preparing any meal was also attributed to the risk for dysentery in rural African communities with as high as 30 % (Njuguna et al., 2016). In addition, a study in Nepal was also similar to the current study where those who did not practice proper hand washing before eating was greatly associated to protozoan infection (Sah et al., 2016). The same case was witnessed in Benue, Nigeria (Ojiaku et al., 2014), Malawi (Morse et al., 2008) and in Atlanta, USA (Strunz et al., 2013), where in all these studies hand hygiene greatly reduced the protozoan infection by significantly minimizing the fecal contamination and improving health. Therefore, the practice of hand washing before eating meals elementally reduced the infection and this could be explained by the fact that improperly washed hands plays a major role in fecal – oral route transmissions in humans.

This study established that finger sucking and biting greatly heightened the risk of protozoan infections in children, P<0. 001. This study showed conformity with other studies including the habit of nail biting and finger sucking that was positively correlated with increased infection of intestinal protozoa in Sri Lanka. Similar results were witnessed in Nepal that both nail biting and sucking fingers were significantly associated factors in school children (Sah et al., 2014). In contrast, the practice of sucking fingers and nail biting was negatively associated with diarrhea and the intestinal infections in Benue, Nigeria, (Ojiaku et al., 2014). Cysts of gastro intestinal protozoa might bury under the surface of the nail and fingers after contacting contaminated food and water. This mainly contributes to transmission of the protozoa into human digestive system through oral route pathway (Lahiru 2016). Therefore, health education
to discourage the practice of finger sucking could be vital in minimizing the possible risks of intestinal protozoa infections in children.

This study findings indicated that, use of unprotected sources of water such as streams and rivers was found to be highly correlated to the increased risk of infection by gastro intestinal protozoa comparable to other water sources in this study and the use of treated tap water was negatively associated to the risk of infections. This findings agrees with a study conducted in Nepal, where gastrointestinal protozoan infection was higher in study population who used stream water for drinking compared to other water sources and the prevalence of protozoan infection was also seen to be low among the population using treated tap water before drinking in contrast to those did not treat though there was no significant difference (Sah et al 2016).

The present study findings were in agreement to a study in Northern Ethiopia and Cote d’Ivoire (Birhane et al., 2018; Koffi et al.,2014). Previously, use of water from wells and other unprotected water sources has been linked to amoebiasis and giardiasis in Saudi Arabia and for those who used clean treated water had low exposure to the risk of infection, (Omar et al.,1995).This phenomenon can be attributed to the possible contamination by protozoans due to surface run-offs that may result into the human excreta getting into the unprotected water sources hence contaminating them (Atu et al.,2014).

Proper disposal of human feces is said to possibly prevent the spread of intestinal protozoan parasites contained in feces in the home and the areas around the home environment (Curtis et al.,2000). Better conditions for the disposing human fecal matter could possibly reduce the risk contacting intestinal diseases by 32% (Fewtrell et al.,2005). The present study investigated the effect of human waste disposal by households on the risk of infection by gastro intestinal protozoa in children. It was established that households had practices of either not sharing toilets/latrines with more than one household 66 (55%) or using a single disposal unit by many households 54 (45%) and the infections were higher among the children who came from the
households that had the practice of sharing a unit of disposal by multiple households. This study is similar to the findings of (Adamu & Petros, 2006; Noor et al., 2007) in which intestinal parasitic infections were spread world widely and more adverse in areas characterized by poor living conditions that include; over-crowding, poor environmental sanitation and poor usage of pit/latrine. These factors are the causes of a major share of the burden of diseases and deaths in developing countries (Adamu et al., 2006). In Sri Lanka, the occurrence of the intestinal protozoa and other intestinal parasites were found more predominant among children living in overcrowded conditions characterized by poor improper disposal of human waste was found to be seven to ten times more than in children in areas that are not overcrowded (Atukorala & Lanerolle, 1999). In circumstances where the toilets are not easy to clean due to overcrowding, contamination level is high (Muchiri et al., 2001). Since giardiasis is common among children, the safe disposal of children’s excrements is vital in preventing the transmission of these protozoan diseases (Osman et al., 2016). Therefore, proper toileting/pit latrine usage may possibly increase the level of cleanliness and effectively reduce the risk of gastro intestinal protozoan infections.

Subsequently this study reveals that fruit washing greatly reduced the risk of gastrointestinal infections though not significant since those children whose caregivers ensured fruits are always washed before eating were found less vulnerable to gastrointestinal protozoan infections relative to those who didn’t. This study conquers with a study in Iran, among the aboriginal poor populations (Nader et al., 2008) where most of the children consumed raw foods especially fruits without washing them and interestingly didn’t show any significant association to intestinal infections but differs with a study in Rwanda where majority of the study population ate fruits and vegetables without washing them (Mushimiye et al., 2017). Transmission through contaminated food was primarily correlated to giardiasis. This can be attributed to the fact that fresh vegetables and fruits when eaten without being washed
as they serve as reservoirs for the intestinal protozoan infections (Guidetti et al. 2009). More infections are intestinal parasites are likely to occur in areas without improved water sources and with poor sanitation characterized by unhealthy food handling and lack of awareness on the risks of possible infections especially through fecal -oral route of infections (Rajeswari et al., 1994) (Al-Agha & Teodorescu, 2000). Consuming an improperly washed fruit poses one to great risk of infection by human pathogens and therefore poor hygienic practices related to planting, transportation and storage of fruits can become easily contaminated with parasites (Sia et al., 2012)

The economic status of caregiver did not show any significant difference with the risk of infections, however there was a decreased infection among the children who came from homes categorized as middle/high income. A similar study disclosed that intestinal parasites are usually associated with poverty though did not indicate any statistical difference between socioeconomic status and the intestinal parasitic infections (Osei et al., 2012). Remarkably, in Peru, Giardia spp was the predominant intestinal parasites among the population considered poor (Nundy et al. 2011). This could be because individuals categorized as middle/high income earners may have greater opportunity of access to health measures such as use handwash and sanitizers, treatment of water used at home, improved toilets and sanitation facilities while low income earners may not afford these measures (De silva et al., 2003) Therefore, though economic status of caregivers was not significantly associated to gastro intestinal protozoa, the study findings on the numbers of infections could be of importance in the aid of reducing poverty and improve the living standards of families that have extremely low socioeconomic status.
5.2 Conclusions
Gastrointestinal protozoa infections among children with diarrhea of age between 6 months and 59 months old in Kisii Teaching and Referral Hospital Kisii County, Kenya was 28.3% hence still prevalent in the region. Male children between the age of 6 months and 59 months old in Kisii Teaching and Referral Hospital, Kisii County are 1.5 times more vulnerable to gastrointestinal protozoan infections unlike to the case of their female counterparts. The parasite densities of each species in co-infected children were higher than singly infected children who participated in the study. The practice of children regularly taking meals once their hands are washed and the aspect of not sharing pit latrines/toilets for disposing human waste by more than one household was found to be negatively associated to intestinal protozoan infections while the practice of finger sucking heightened the risk of infection. Treated tap water greatly reduced the chances of children being infected but the use of water from unprotected water sources for drinking was positively correlated with infections.

5.3 Recommendations
Although these findings are hospital based, it may represent the population of the region in the study area since the hospital is a referral health facility that provides a wide range of health services, for this reason therefore, regular surveillance, prevention and treatment is recommended.

Health education and public health awareness programs on sanitation and proper personal/environmental hygiene should be regularly carried out by relevant authorities in Kisii County. The county government of Kisii and the stakeholders responsible for provision of water services should put mechanisms in place to increase accessibility of clean treated tap water to the inhabitants of Kisii County and formulate legislative policies on water quality at the source.
5.4 Suggestions for further studies

A study incorporating the diagnosis of other microbes causing diarrhea should be done to establish any possible link with intestinal protozoans.

Since mixed infections were strongly associated with high parasite densities, an in-depth study should be done to determine the cause of this phenomenon including immunological studies.
REFERENCES.


Feng, Y., Zhao, X., Chen, J., Jin, W., Zhou, X., Li, N.,... Xiao, L. (2011). Occurrence, source, and human infection potential of cryptosporidium and Giardia spp. in source and tap...


APPENDICES

APPENDIX (1) INFORMED CONSENT FORM

Kisii University, post graduate studies section, school of pure and applied sciences in the department of biological sciences.

Title: Prevalence and predisposing factors associated with gastrointestinal protozoa in children under age five with diarrhea, Kisii County.

Patient identity no._________________________

Consent to participate in this study

I greet you, I am ……………………….. Working on this research with an objective of determining the factors associated with gastrointestinal protozoa in diarrheal children under age five. The study intends to examine 120 diarrheal children under age five attending outpatient department of Kisii Teaching and Referral Hospital. I therefore request you to be part in this study since you are a patient having visit at this clinic. You have been randomly selected. I would like you to understand the intention of this study and your part so that you may take decision if you would like to join us in this study. If you accept to join, I will then ask you to sign this paper (or if you cannot read/ write, make your mark in front of a witness). Please ask for explanation of any information that you may have not understood.

Information about the research

If you accept to participate, I will interview you. I will ask you about your background and brief history of your illness. The interview will last at maximum 20 minutes. After the interview, I and my research investigators shall collect fresh stool sample from you for examination.

In case of the possible risks, I shall do my best to safeguard your privacy and study records. This interview shall be private. However, it is possible that others may learn that you have joined the research. Because of this, others may treat you dishonorably.
The interview questions may make you have some anxiety. You can reject to answer any question. You may also end the interview at any time without notice.

For the Possible benefits, this study has no one on one benefit but the findings of this study will help to improve interventions against diarrhea, gastro intestinal protozoa infections and other related illnesses. I may not provide any incentive for preventing or curing diarrhea and gastro intestinal protozoa if any but the interview may offer a good advice to you on how you can perhaps live diarrhea and gastro intestinal protozoa infections free life. If you decide not to be in the research you are free to decide if you want to take part in this research or not.

Confidentiality

I shall do all the best to protect information about you and your role in this research. I will also interview you in a private place. I will definitely not write your name on the interview form. I will use your form number to connect your interview response to our stool testing laboratory. You will not be named in any reports. Only the study staff and investigators will know your responses to the questions.

Compensation

You will not receive any cash by joining this study.

Leaving the research study

You may leave the research at any time. If you leave, it will not change the health attention you receive here. If you choose to take part, you can change your mind at any time and pull out. If so, please tell the research interviewer why you wish to leave.

Your rights as a participant: This research has been reviewed and approved by the Kisii University research and extension unit and NACOSTI.

If in case you have questions about this study, you should contact the Coordinator or the Principal Investigator ONDARA CALEB OKERI, Kisii University School of pure and applied sciences BOX, 408-40200.
Signature: …………………………..

Do you agree?

Participant Agrees

Participants disagree

I  --------------------- have read and understood the matters in this form. I agree to participate in this study.

Participant signature -------------------------------

Signature of witness (if can’t read) ---------------------- Signature of research assistant

---------------------- Date of signed consent -------
APPENDIX (II) DIRECT WET PREPARATION METHOD

Direct wet mounts were prepared to observe the presence or absence of protozoa cysts and/or trophozoites according to the conventional laboratory practice (Cheesebrough, 2001). The semi-formed and loose stools were then processed by putting a drop of normal saline on one end of a clean glass slide and a small quantity of the sample added using the applicator stick; both will be emulsified. Another emulsified mixture of normal saline and stool samples was then carried out at the opposite end of the same glass slide. Individual preparations were covered gently with glass cover slips and examined with X10 objective lens of the microscope. The X40 objective Len was used to identify any observed organism of interest under good contrast of the microscope. Dobell’s iodine was then added in drops to stain observed cysts for easy identification. The applicator stick was after used to carefully transfer watery stool samples onto the clean slides, covered with the cover slip and observed under the microscope, a drop of Eosine added with the Pasteur pipette, to aid the identification of motile. The criteria for identifying the protozoa trophozoites was motility structures, type of motility, and number of nuclei, karyosome and chromatid bars (Heckendorn et al., 2002). Other structures such as cytoplasmic inclusions for example erythrocytes and yeast were used in identifying amoebic trophozoites while structural details such as sucking disks and spiral groove or filaments were used in identification of flagellate trophozoites (Markell et al., 2008). Presence or absence of protozoan trophozoites or cysts and the respective parasite density was recorded for each specimen.
APPENDIX (III) FORMALIN-ETHER STOOL CONCENTRATION METHOD

The modified Ridley’s formol-ether stool concentration method was used to prepare concentrated stool specimen to determine the intensity of protozoan infection according to Garcia and Bruckener (2001). With an applicator stick, a pea sized faecal stool will be added into 10ml of formalin in a centrifuge tube (15 ml) stirred and brought into a suspension. The suspension was then strained through the 400µm sieve or two layers of surgical gauze pre wet with formalin into a new centrifuge tube and the gauze discarded. The volume was then adjusted to 10 ml by adding more 10% formalin. To this 3ml of Ethyl ether was then added to the suspension in the tube, capped and shaken vigorously for 10 seconds. The suspension was consequently centrifuged at 2000 rpm for 3 minutes, the contents of the tube from the centrifuge contained of 4 layers: Top layer of Ethyl ether, the second layer consisting of plug of fatty debris that adheres to the walls of the tube, then a layer of formalin, the bottom layer consists of the sediment. The plug of debris was gently loosened with an applicator stick by helical movement and the three top layers poured off in a single movement and allowed to drain, inverted for at least five seconds. The fluid left behind was mixed with the sediment. The sediments from each sample was then used to make smears for examination of *Entamoeba histolytica* and *Giardia lamblia* cysts/trophozoites. With a disposable glass pipette, a drop of suspension was subsequently transferred to a slide for examination under a cover slip; iodine stained suspension that was made especially for the cysts in case of any. The preparation was then examined with the X10 objective to bring the parasites to focus and for proper identification and detailed morphology of the material in question, higher power objectives of the microscope was there after used in a systematic manner for fine details of identification.
APPENDIX (IV): STRUCTURED QUESTIONNAIRE
N/B: (This part may be filled by the caregiver or the patient where applicable)

A. PATIENT’S BIO DATA

Patient no  ........................................................................................................................................

Age in months  .................................................................................................................................

Gender:  male   female

Relationship with caregiver (if any)  ..........................................................

County  ............................................................. sub county  .........................................................

B. SANITATION STATUS

1. What is the main source of water at home for the patients whose name is indicated above? (*Please tick where applicable*)

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>a</td>
<td>Streams/rivers</td>
</tr>
<tr>
<td>b</td>
<td>Springs</td>
</tr>
<tr>
<td>c</td>
<td>Tap</td>
</tr>
<tr>
<td>d</td>
<td>Bore hole</td>
</tr>
<tr>
<td>e</td>
<td>Others</td>
</tr>
</tbody>
</table>

2. How do you treat water at home for use (name of patient) (please tick appropriately)?

   i. Boiling  

   ii. Chlorination  

   iii. Filtration  

   iv. Not applicable  

3. Does the child always get the hands washed every time after visiting the toilet?

   Yes  

   No  

4. Does the child get his hands washed with soap? (Please tick what is applicable)
   Yes □
   No □

5. Does the child get his/her hands washed before meals?
   Yes □
   No □
   If yes, how often?
   (a) Always
   (b) Rarely
   (c) Never

6. Does the child always eat washed fruits? (Mark what is appropriate)
   Yes □
   No □

7. How many doors do the latrine(s) at the area of residence (child) have?
   i. Single door □
   ii. Double doors □
   iii. More than three □

8. Does the child come from homesteads where they share a single door latrine with other households? Yes □ No □ (Please tick what is applicable)

9. Does the patient suck or lick the fingers? Yes □ No □

10. Does the child have long finger nails? Yes □ No □

11. Does the patient attend school? Yes □ No □

12. Does the patient breastfeed? Yes □ No □

13. What is the highest level of education of the child’s mother? (mark where applicable (✓))
   □ None
   □ Primary
   □ Post primary
14. What is the occupation of the child’s parent(s)?
   □ Business
   □ Farmer
   □ Civil servant
   □ Peasant
   □ Other

15. What is the child’s (family) current area of residence?
   □ Urban
   □ Rural
APPENDIX (V) GASTRO INTESTINAL PROTOZOA AND THE POSSIBLE PRE-DISPOSING FACTORS

Likelihood Ratio Tests

<table>
<thead>
<tr>
<th>Effect</th>
<th>Model</th>
<th>Fitting Likelihood Ratio Tests</th>
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<tr>
<td></td>
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<tr>
<td></td>
<td>-2 Log</td>
<td>Likelihood Chi-Square df Sig.</td>
</tr>
<tr>
<td>Intercept</td>
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<td>.000 0 .</td>
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<td>MWS</td>
<td>96.457</td>
<td>7.978 9 .000</td>
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<tr>
<td>FWBC</td>
<td>93.142</td>
<td>4.663 3 .198</td>
</tr>
<tr>
<td>HWBM</td>
<td>98.330</td>
<td>9.851 3 .000</td>
</tr>
<tr>
<td>sucking</td>
<td>115.740</td>
<td>27.261 3 .000</td>
</tr>
<tr>
<td>HWD</td>
<td>99.972</td>
<td>11.493 3 .009</td>
</tr>
</tbody>
</table>

The chi-square statistic is the difference in -2 log-likelihoods between the final model and a reduced model.

a. This reduced model is equivalent to the final model because omitting the effect does not increase the degrees of freedom.
APPENDI (VI) RESEARCH PERMIT

THIS IS TO CERTIFY THAT:
MR. CALEB OGERI ONDARA
of KISII UNIVERSITY, 17-40206 NYAMORAMBE, has been permitted to
conduct research in KISII County
on the topic: GASTRO INTESTINAL
PROTOZOAN INFECTIONS AMONG
CHILDREN WITH DIARRHEA UNDER FIVE
YEARS IN KISII COUNTY: A COMPARATIVE
STUDY.

for the period ending:
30th September, 2015

Applicant’s Signature

Permit No: NACOSTI/P/15/0327/5057
Date Of Issue: 22nd May, 2015
Fee Received: Ksh 1,000

Director General
National Commission for Science,
Technology & Innovation
APPENDIX (VII) RESEARCH PERMIT

CONDITIONS

1. You must report to the County Commissioner and
   the County Education Officer of the area before
   embarking on your research. Failure to do that
   may lead to the cancellation of your permit.
2. Government Officers will not be interviewed
   without prior appointment.
3. No questionnaire will be used unless it has been
   approved.
4. Excavation, filming and collection of biological
   specimens are subject to further permission from
   the relevant Government Ministries.
5. You are required to submit at least two (2) hard
   copies and one (1) soft copy of your final report.
6. The Government of Kenya reserves the right to
   modify the conditions of this permit including
   its cancellation without notice.

RESEARCH CLEARANCE
PERMIT

Serial No. A 5156

CONDITIONS: see back page.
APPENDIX (VIII) RESEARCH AUTHORIZATION LETTER

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213775
2241549, 318249, 318245, 318249
Fax:+254-20-18245, 318249
Email: secretary@nacost.go.ke
Website: www.nacost.go.ke
When replying please quote:
Ref: No.

NACOSTI/P/15/8327/5057

22nd May, 2015

Caleb Okeri Ondara
Kisii University
P.O. Box 402-40800
KISIL

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on “Gastro intestinal protozoan infections among children with diarrhea under five years in Kisii County: A comparative study,” I am pleased to inform you that you have been authorized to undertake research in Kisii County for a period ending 30th September, 2015.

You are advised to report to the County Commissioner, the County Director of Education and the County Coordinator of Health, Kisii County before embarking on the research project.

On completion of the research, you are expected to submit two hard copies and one soft copy in PDF of the research report/thesis to our office:

DR. S.K. LANGAT, OGW
FOR: DIRECTOR GENERAL/CEO

Copy to:

The County Commissioner
Kisii County.

The County Director of Education
Kisii County.
APPENDIX (IX) KISII TEACHING AND REFERRAL HOSPITAL
AUTHORIZATION LETTER

MINISTRY OF HEALTH

DEPARTMENT OF RESEARCH
THE KISII TEACHING & REFERRAL HOSPITAL
P.O. BOX 92
KISII

REFERENCE NO.

DATE: 12th July, 2015

OINDARA CALEB

RE: RESEARCH AUTHORIZATION

This is to inform you that you have been authorized to extend your data collection on “Gastro-intestinal protozoan infection among children under 5 years with diarrhoea attending OPD at KISII Teaching and Referral Hospital” for 3 weeks with effect of 13th August 2015.

DR. E.B. MASANTA
- MBCHB (UON), MPH (KRU), FCOGH, FOCSTI
PGCFM (KISII) Assistant (Research & Bio (UON))

DEPARTMENT OF RESEARCH
APPENDIX (X) UNIVERSITY RESEARCH AUTHORIZATION LETTER

KISII UNIVERSITY
OFFICE OF THE REGISTRAR RESEARCH AND EXTENSION

REF. KSU/R&E/3/5/100 DATE: 16th April 2015

TO WHOM IT MAY CONCERN

RE: CALEB OKERI ONDARA, REG.NO.MPS11/70003/14

This is to confirm that the above mentioned, is a student of Kisii University currently pursuing a Degree of Masters of science in Medical Parasitology of Kisii University. He is embarking on a research topic, “Gastro Intestinal Protozoan Infections among Children with Diarrhea under five years in Kisii County, Kenya”

Kindly accord him any assistance in terms of a research permit.

Thank you.

[Signature]

Prof. Anakalo Shitandi, PhD
Registrar, Research and Extension

AS/gn
APPENDIX (XI) ETHICAL CLEARANCE CERTIFICATE.
APPENDIX (XII): MAP LOCATING KISII COUNTY, KENYA

Figure 3.1: map of Kisii County
APPENDIX (XIII): PLAGIARISM REPORT

ASSESSMENT OF FACTORS ASSOCIATED WITH GASTROINTESTINAL PROTOZOAN INFECTIONS IN CHILDREN AGED BETWEEN 6 AND 59 MONTHS WITH DIARRHEA IN KISII TEACHING AND REFERRAL HOSPITAL, KENYA

ORIGINALITY REPORT

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   1%