PREVALENCE OF *Cryptosporidium Species* AND *Giardia lamblia* INFECTION IN PATIENTS ATTENDING SIAYA COUNTY REFERRAL HOSPITAL, KENYA

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MAY, 2016

DECLARATION AND RECOMMENDATION

DECLARATION

I declare that this research thesis is my original work and has not been presented for the award of degree in any other University.

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Reg. No. SM17/2433/09

Signature _____ Date _____

RECOMMENDATION

We confirm that this research thesis was prepared under our supervision and has our approval to be presented for examination as per the Egerton University regulations.

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DEDICATION

This work is dedicated to my wife Esther Koskei and my sons Ivan, Nathan and Reuben for their unconditional support and motivation in all aspects throughout the study period.

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I wish to thank God for enabling and renewing my strength throughout the entire process of my studies at Egerton University. I thank Egerton University, especially Department of Biological Sciences for moulding me to be the person I am today. I would also like to thank the management of Kenya Medical Research Institute (KEMRI) Kisumu for allowing me to use their facilities and Dr. Peter Odada Sumba for his invaluable advice during the entire period of my research study. Thanks to my supervisors Dr. Jane Mburu and Prof. Rose Odhiambo for their guidance throughout the proposal development till completion of the thesis. My sincere thanks go also to KEMRI employees in microbiology department in particular David Omar Chemjor for their help with laboratory reagents.

ABSTRACT

Cryptosporidiosis and giardiasis are common infections in humans in Kenya and Cryptosporidiosis in particular is an opportunistic infection in HIV-infected individuals. Diarrhoeal disease caused by these parasites is a major public health problem particularly in countries with poor socioeconomic status. The study on *Cryptosporidium species* and *Giardia lamblia* was carried out in Siaya county referral hospital and was conducted to assess the prevalence and risk factors associated with these two parasitic infections. Estimation of $CD4^+$ T-lymphocyte count was also performed to determine immune status of the patients. A single stool and blood specimen was collected from each of the 384 patients attending hospital laboratory with diarrhoea. The stool specimens were processed for *Cryptosporidium species* and *G. lamblia* using ELISA (Giardia II/Cryptosporidium II) test and microscopy while the $CD4^+$ T-lymphocyte count was estimated using flow cytometry. Structured questionnaire was used to collect data on demographic information and risk factors associated with these infections. Overall prevalence of *G. lamblia* was 20.3% while *Cryptosporidium species* was 7.6%. There were no significant difference observed in Cryptosporidium species and *G. lamblia* infection in relation to sex and age of the patient ($p>0.05$). Risk factors such as immune status, level of education, source of water and waste disposal were not significantly associated with *Cryptosporidium species* and *G. lamblia* infection ($p>0.05$). However, hand washing practice using soap was significant ($p<0.05$). It was concluded that *Cryptosporidium* and *G. lamblia* are prevalent in patients attending Siaya county referral hospital with diarrhoea. In relation to immune status of the patients it was concluded that patients were not at risk of developing chronic diarrhoea. There is need for public health education to be enhanced on personal hygiene especially washing hands with soap which greatly reduces giardiasis and cryptosporidiosis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

THE LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
CD4^{+}	Cluster of differentiation 4 T-lympocytes
ELISA	Enzyme Linked Immunosorbent Assay
EPA	Environmental Protection Agency
HAART	Highly Active Anti-Retroviral Therapy
HIV	Human immunodeficiency virus
MOFP	Ministry of Finance and Planning
MOPHS	Ministry of Public Health Services
NCAPD	National co-ordinating Agency for Population Development
PCR	Polymerase Chain Reaction
WHO	World Health Organization
ZN	Ziehl Neelsen
Phi	Is a chi-square based measure of association

CHAPTER ONE

INTRODUCTION

1.1. Background Information

Diarrhoea is a significant health problem worldwide, especially in the developing world where adequate water and sanitation are lacking. This condition, which can lead to life threatening dehydration, is a common symptom of gastrointestinal infection. The pathogens (viruses, bacteria and parasites) that cause diarrhoea are spread through contaminated food or drinking water, and from person to person through poor hygiene and inadequate sanitation (*Lim et al.,* 2008, O'Reilly *et al*, 2012;).

Globally diarrhoea disease account almost 20% of all deaths in children below five years of age, with an estimated 2.2 million deaths annually (Gascon, 2000). Epidemiological studies of diarrhoea have been reported from several African countries including Nigeria, Ethiopia, Uganda, Mali and Democratic Republic of Congo (WHO, 2008). *Shigella*, *Salmonella*, *Campylobacter jejuni, Clostridium difficile, Cryptosporidium, G. lamblia* and some helminths are known to cause diarrhoea and are found throughout the world (Sang *et al.,* 2012). *Cryptosporidium* and *G. lamblia* are protozoa parasites that cause human diarrhoea in both children and adults. They are among the most common causes of gastroenteritis worldwide and lead to significant morbidity and mortality (WHO, 2005; Espelage, 2010).

Of all the reported gastrointestinal protozoa parasites, *Cryptosporidium* is the most morbid due to its ability to cause severe diarrhoea in immunocompromised and even in immunocompetent individuals (Adamu *et al.*, 2006). *Cryptosporidium* is a zoonotic protozoan parasite that is responsible for cryptosporidiosis (a gastro-intestinal disease) in human and animals. Generally cryptosporidiosis affects patients with several chronic health conditions that may have depressed their immunity. These include acquired immune deficiencies such as HIV/AIDS, diabetes and malnourished children (Awole *et al.*, 2003, Adamu *et al.*, 2006). The disease is typically self-limiting in immunocompetent individuals but may be life-threatening in immunocompromised individuals or malnourished children. In resource-poor countries, *Cryptosporidium* is the second leading cause of moderate-to-severe diarrhoea in children < 5 years and is associated with an increased risk of death in children < 2 years (Gatei *et al*, 2006).

Humans are thought to acquire the parasites by ingestion of cysts or oocysts, which are shed in the stool of infected humans or animals. Some of the recommended ways to reduce diarrhoeal diseases include providing safe drinking water, safe waste removal, especially the use of facilities to dispose faeces in sanitary way and improved hygienic standards.

The impact of diarrhoea illness caused by the two parasites is more severely felt in sub-sahara Africa (Molina *et al*, 2011). The prevalence of these parasitic etiologic agents is due to poor environmental and personal hygiene, largely caused by poor sewage disposal and inadequate treatment of water supply. In sub-Saharan Africa the prevalence of diarrhoea caused by *G. lamblia* is between 2.6-4% while 5.7-8.4% is caused by *Cryptosporidium* (Hamer *et al.,* 1998). *Cryptosporidium* is the leading cause of enteric diseases especially in children in Kenya, accounting for 4% (Gatei *et al.,* 2006) and 4.2% (Nyamwange *et al*., 2012), while *Giardia* accounts for 2% (Estambale *et al*., 1989).

Previous studies in Siaya have documented the prevalence of intestinal helminths (Thiong'o *et al.,* 2001) and risk factors associated with infection of some traditionally recognized agent of diarrhoea including bacteria and viruses (Brooks *et al*, 2003). However, epidemiological information on *Cryptosporidium* and *G. lamblia* infection is not available.

This study was meant to highlight current gastro-intestinal infections involving *Cryptosporidium* and *G. lamblia* in patient's visiting Siaya county referral hospital so that they can be given the correct medication. It was also carried out to determine risk factors associated with infections with the two parasites among patients presenting with diarrhoea in this hospital.

1.2. Statement of the problem

Diarrhoeal disease caused by *Cryptosporidium species* and *G. lamblia* is a major public health problem in several tropical and sub-tropical countries like Kenya with poor socio-economic status and therefore inadequate clean water and sanitation. Records from clinics show that episodes of diarrhoeal diseases in Siaya County have posed serious health problems especially, among young children and immunocompromised patients leading to dehydration and sometimes death. There exist little information on the prevalence of *Cryptosporidium* and *Giardia lamblia* based on few studies on cryptosporidiosis and giardiasis in Kenya. In addition there is no data on risk factors and immune responses against *Cryptosporidium* and *Giardia* infection in Kenya.

The present study was undertaken to establish prevalence and associated risk factors of *Cryptosporidium* and *Giardia* infection in patients presenting with diarrhoea in Siaya county referral hospital.

1.3. Objectives

1.3.1. General Objective

To determine $CD4^+$ T-lymphocyte count and prevalence of *Cryptosporidium* species and *G. lamblia* infection and their associated risk factors in patients attending Siaya county referral hospital.

1.3.2. Specific objectives

1. To assess the prevalence of *Cryptosporidium* species and *G. lamblia* in patients attending Siaya county referral hospital segregated by age and gender.
2. To determine the $CD4^+$ count of the patients presenting with diarrhoea in Siaya county referral hospital.
3. To assess the risk factors associated with *Cryptosporidium* species and *G. lamblia* infection in patients presenting with diarrhoea in Siaya county referral hospital.

1.4. Hypotheses

1. There is no association between *Cryptosporidium* species and *G. lamblia* infection and age and gender in patients attending Siaya Referral Hospital
2. There is no association between immune status and *Cryptosporidium* and *G. lamblia* infection
3. There is no association between *Cryptosporidium* species and *G. lamblia* infection and the risk factors that include source of water, level of education, washing hand without using soap and mode of faecal disposal.

1.5. Justification

In Kenya, the fast rate of expansion of new settlement which lack supply of piped water or properly managed wells or boreholes, especially in urban communities, leave large communities to consume untreated water. This has consequently increased the risk of water-borne infection, including giardiasis and cryptosporidiosis in some parts of country. Although no major outbreak of these diseases has yet to be reported in Kenya, increasing high incidences of the diseases

reported in our hospitals suggest that many people are consuming contaminated water. In Siaya information on the prevalence of *Giardia* and *Cryptosporidium* and their impact on public health are not available. This study therefore provided a platform to study prevalence of *Cryptosporidium* and *Giardia* and enumerate $CD4^+$ T- lymphocyte in patients presenting with diarrhoea. The finding of the study will provide data on prevalence and extend of immune response in the study population. The study will also provide useful insight information on risk factors associated with cryptosporidiosis and giardiasis in Kenya and thus will enable formulation of disease management and control strategies especially in immunocompromised patients.

Diagnosis in most hospitals in Kenya emphasize on demonstration of the presence of *G. lamblia* and other intestinal parasites in stool but not on *Cryptosporidium* species. Therefore there is need to include diagnosis of this parasite in routine laboratory stool examination and also include immunoassay (ELISA) techniques for the diagnosis of the two parasites.

CHAPTER TWO

LITERATURE REVIEW

2.1. *Cryptosporidium* Species

Cryptosporidium is an obligate enteric parasite of the phylum Apicomplexa and an important cause of diarrhoea worldwide. The first case of human cryptosporidiosis was reported in 1976 with more awareness of the organism increasing by the 1980s due to its association with HIV infection (Kosek *et al*, 2001). The parasite is found in soil, food, water or surfaces that have been contaminated with faeces from infected humans or animals. Currently there are sixteen species of *cryptosporidium* species (Table 1).

Table 1: *Cryptosporidium* species that infect mammals and other animals.

Species	**Host Animal**
C. hominis	Humans
C. parvum	Cattle, humans
C. felis	Cats
C. baileyi	Poultry
C. andersoni	Cattle
C. meleagridis	Turkey, humans
C. muris	Rodents
C. nostrum	Fish
C. serpentis	Snakes
C. wrairi	Guinea pigs
C. canis	Dogs
C. galli	Birds
C. suis	Pigs
C. bovis	Cattle
C. molnari	Fish
C. scopthalmi	Fish

Source: Xiao *et al.,* 2004

2.1.1. Taxonomy of *Cryptosporidium*

The taxonomy of *Cryptosporidium,* like the taxonomy of most organisms, remains controversial (Xiao *et al.*, 2004). The current taxonomic position of the genus *Cryptosporidium* is as shown in Table 2.

Table 2: Taxonomic classification of *Cryptosporidium*.

Phylum	Apicomplexa
Class	Conoidasida
Subclass	Coccidiasida
Order	Eucoccidiorida
Suborder	Eimeriorina
Family	Cryptosporidiidae
Genus	*Cryptosporidium*

Source: Tigabu *et al*. (2010)

Site of infection is commonly the small intestine although other areas of digestive tract or respiratory tract can be affected (El-Helaly *et al*, 2012). The infection is transmitted by faecal-oral route through contaminated food, water, touching mouth with contaminated hands and exposure to human faeces through sexual contact. The infection is initiated when sporozoites released from oocysts in the intestinal tract attach to and invade mucosal epithelial cells (Jex *et al*., 2008). In susceptible immuno-competent humans, mainly children, infection leads to a self-limiting diarrhoea. However, in immuno-compromised individuals, for example acquired immune deficiency syndrome, cancer patients, organ transplant patients who are taking certain immunosuppressive drugs and those with congenital immune deficiencies, chronic diarrhoea frequently develops. This may lead to considerable loss of fluids and also weight and even sometimes death (Hashim *et al*, 2006).

2.1.2. Biology and Life Cycle of *Cryptosporidium* species

The life cycle of *Cryptosporidium* species (Figure 1) begins following ingestion of the oocyst by a susceptible host. The oocysts are spherical in shape measuring 3-6 mm in diameter and it may be either thick-or thin walled. Thin walled oocysts may exist within the same host and start a new life cycle (auto-infection). This can lead to heavily infected intestinal epithelia and result in malabsorptive or secretory diarrhoea. Thick walled oocysts contain 4 infective sporozoites which penetrate individual epithelial cells. The excystation of these parasites to release infective sporozoites is triggered by stomach acid and bile salts (Stevens and Adam, 2004).

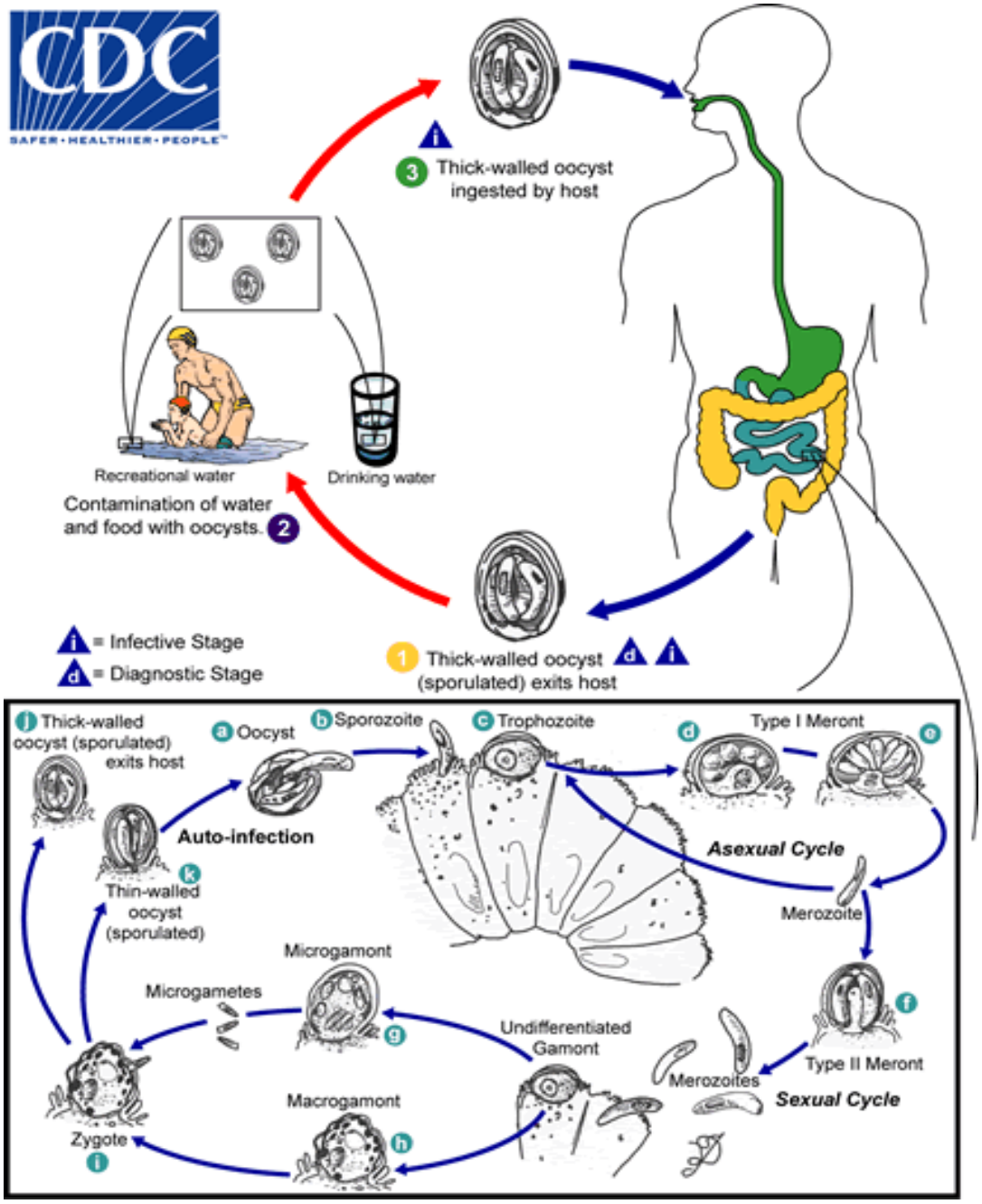

Figure 1: Life Cycle of *Cryptosporidium* species.

Source: CDC (2005) http://www.dpd.cdc.gov/dpdx

The freed sporozoites attach to epithelial cells and become enclosed within parasitophorous vacuoles. The trophozoites then undergo asexual proliferation by merogony and form two types of meronts; type I meronts and type II meronts. Type I meronts then invade

nearby cells and develop into Schizonts or trophozoites to complete the reproductive cycle (Nyamwange *et al.,* 2012). Type I meronts are capable of recycling indefinitely and, thus, the potential exists for new Type I meronts to arise continuously within the host. However, Type I meronts can sometimes be triggered into forming a second type of meront (Type II meront), which contains only four merozoites. Once liberated, the type II meronts constitute the initial stages of the sexual reproductive cycle. The Type II merozoites enter cells, where some enlarge and differentiate into macrogametes (macrogametocytes), while others undergo multiple fission inside the cells to form microgametocytes that contain 16 microgametes. The microgametocytes rupture to release microgametes, which then penetrate macrogametes, thus forming a zygote on which a resistant oocyt wall is formed. The resultant zygotes undergo further asexual development (sporogony) to form sporulated oocysts containing 4 sporozoites. Most oocyts are thick-walled and are excreted from the host in faecal material or perhaps via respiratory secretions (Siwila, 2010).

Development of *Cryptosporidium* occurs more rapidly, and each generation can develop and mature in as little as 2 days. Due to the fastness of the life cycle, and the auto-infective cycles, enormous numbers of organisms can colonize the intestinal tract in several days. As a result, the ileum soon becomes crowded and secondary sites such as the duodenum and large intestine are often infected (Al-Shamiri, 2010). *Cryptosporidium* lacks tissue specificity and has been found to be infecting the biliary tract, the respiratory system, middle ear, pancreas and the stomach particularly in immuno-suppressed individuals (Basher, 2006).

Patent period, which is the length of time Oocysts are shed in the faeces, generally lasts 6-18 days (4-10 days of diarrhoea) in immune-competent individuals but may be prolonged in immune-suppressed patients. However, some individuals shed Oocysts but appear asymptomatic (Putignani and Menichella, 2010). In a study by Certard (2007), the report indicate that in experimentally infected animals the prepatent period is generally 4 days but sometimes 3 days in heavy infections. However, in human outbreaks where lower numbers of Oocysts are probably ingested, 4-6 days is the patent period (Tarazona *et al*, 1998)

2.1.3. Epidemiology of *Cryptosporidium* species

Cryptosporidium infection is the most common intestinal protozoan parasite isolated worldwide in both immunocompetent and immunocompromised humans and has been reported from 3 days to 95 year old. It is responsible for both epidemic as well as endemic levels of

intestinal disease (Hunter, 2007). Infection is most frequently spread by direct person to person through faecal oral route or by zoonotic from cattle and sheep. Indirectly it is spread through the environment through water (Ribes *et al*, 2004).

Cryptosporidium species occur in both human and animals, and therefore the zoonotic nature of cryptosporidiosis is of great significance from the public health point of view. At least 16 *Cryptosporidium* species are considered valid species (Fayer, 2007), and of these, 6 are known to infect both humans and animals (Xiao *et al*, 2004). All these species have the potential to infect humans through contaminated food, recreational water, drinking water or direct ingestion of infective oocysts (Xiao *et al*, 2004).

In human, clinical disease appears to occur in all ages, but is most common among children and immunocompromised individual. Infection have been reported among children, parents of infected children and people who drink from shallow, unprotected wells and swimmers who swallow water while swimming in pools, lakes, rivers, ponds and streams (Adesiji *et al*, 2007). It can also be considered as an occupational and environmental hazard, among individuals who are involved in farming practices such as lambing and calving, veterinarians who come in contact with farm animals. People living in densely populated urban areas are most likely to be infected by *Cryptosporidium* whose oocysts may remain viable for several months, especially under moist conditions (Xiao *et al.,* 2004).

From prevalence studies, excretion rates vary between 1% - 3% in industrialized countries and 10% in less industrialized countries. Worldwide, it is estimated that 0.6 – 4.3% of humans are infected with *Cryptosporidium* (Jafari, *et al*, 2014). *Cryptosporidium* infection was reported in 10% - 15% of children with diarrhoea and 30% - 50% of AIDS patients with chronic diarrhoea in the developing world. In contrast, when samples from asymptomatic individuals were examined, prevalence ranged from 0 – 2% in developed countries compared to 0 – 9.8% in developing countries (Al- Shamiri, 2010).

Cryptosporidiosis prevalence varies considerably across sub-saharan Africa within certain subsets of the population. Prevalence of cryptosporidiosis in children with diarrhoea in West Africa ranges between 7.5% in Liberia and 12.5% in Guinea- Bissau (Musa *et al*, 2014). In one study done in the Democratic Republic of Congo, prevalence of cryptosporidiosis among children with diarrhoea recruited from hospitals was 22.2% (Wumba *et al*, 2014).

A study conducted in some East African countries found that among HIV-positive children with diarrhoea, prevalence varied between 13% in Tanzania and 73.6% in Uganda (Mor and Tzipori, 2008). The astonishingly high prevalence in Uganda is due to the sensitive PCR-based assay used.

There are limited studies on the epidemiology of cryptosporidiosis in Kenya and virtually nothing is known about the occurrence of the disease in Siaya district hospital. Simwa *et al*. (1989) observed that 3.8% of the 1420 faecal samples examined from children of five years and below presenting with diarrhoea in Kiambu district in central Kenya were positive for *Cryptosporidium* oocysts. In a separate study, Estambale *et al*. (1989) observed that 3.8% of the 133 stool samples sent from out-patient department and also from wards for routine microscopic examination at the Kenyatta National Hospital (KNH) had *Cryptosporidium* oocysts, and that both immunocompromised and immunocompetent individuals were infected. Mirza *et al*. (1994) observed that 42% (23/45) of the HIV/AIDS patients seen at KNH had *Cryptosporidium* infections whereas, only 8.6% (2/23) of the HIV negative individuals examined during the same period were positive for the parasite. It appears *Cryptosporidium* is a common infection in humans in Kenya, and may be an important opportunistic infection in HIV-infected individuals.

In a study in which stool samples were sent for routine laboratory microscopic examination from Narok District Hospital, Thika District Hospital, Machakos Provincial General Hospital, Gertrude's Gerdens children Hospital, Aga Khan Hospital and Kenyatta National Hospital, the overall prevalence of *Cryptosporidium* infection was established to be 4% (183/4899) (Gatei *et al*., 2006). The prevalence for individual laboratories studied was 7% (7/99) in Narok District Hospital, 4% (45/1,118) in Thika District Hospital, 4% (69/648) in Machakos Provincial General Hospital, 4% (30/690) in Kenyatta National Hospital, 3.5% (17/480) in Agan Khan Hospital and 2% (15/863) in Gerdrud's Gardens children Hospital.

2.1.4. Pathology and clinical features

The pathogenic mechanisms by which *Cryptosporidium* causes diarrhoea, malabsorption and wasting are poorly understood. Whatever these mechanisms may be, the initial host parasite interactive attachment and invasion are the critical primary events in pathogenesis. In general, it indicates that, epithelial cells are damaged as a result of *Cryptosporidium* species infections in two ways. This involves (a) cell death as a direct result of parasite invasion, multiplication, and

extrusion and (b) cell damage that could occur through T cell- mediated inflammation, producing villus atrophy and hyperplasia of the crypt (Chunge, *et al*, 1991). Although symptoms of cryptosporidiosis differ greatly between immuno-competent and immuno-compromised individuals, the most common clinical manifestation is profuse and watery diarrhoea, often containing mucus but rarely blood or leucocytes and symptoms includes abdominal cramps, low grade fever, nausea and vomiting (Xiao *et al.*, 2004).

In immune-competent patients, the disease is an acute self-limiting diarrhoea lasting approximately 1-2 weeks. In immunocompromised individuals, the disease is much more severe and symptoms include watery diarrhoea with stool frequency of up to 10 times a day with a mean volume of one litre (Mor and Tzipori., 2008).

2.1.5. Prevention and Control

In the absence of effective and specific therapy against infection with *Cryptosporidium* species, preventive measures are of great importance. Identifications of the most common routes of transmission and a better understanding of the species risk factors for exposure that lead to infection of *Cryptosporidium* would greatly facilitate development of more targeted prevention strategy (Huruy *et al*, 2011). Since most infections of *cryptosporidium* are initiated through ingestion of oocysts, control of this stage limits the spread of the disease. Strategies for prevention of *Cryptosporidium* infections are those usually recommended for avoiding any pathogen transmitted by the faecal oral route (Adamu *et al*, 2006).

2.1.6. Laboratory diagnosis

There are several methods used in the diagnosis of cryptosporidiosis (Mark-Carew *et al*., 2010), and these are characterized broadly into microscopy, immunological and molecular methods. Microscopy is considered to be the 'gold standard' for laboratory diagnosis of *Cryptosporidium* against which all other methods are evaluated because of its high specificity and sensitivity (Den Hartog *et al*., 2013).

Apart from using microscopy for screening, permanently stained prepared slides can be used for demonstration and teaching purpose (Mehraj *et al*., 2008). Microscopy is used widely at many hospitals in developing countries (Adjei *et al*., 2004; Wellington *et al*., 2009) because its application is comparatively affordable. The other methods have rather been used in most cases

for epidemiological studies or research purpose than routine diagnosis at the hospitals and clinics.

The standard immunological methods used to increase the sensitivity of *Cryptosporidium* detection include Immunofluorescence (IF) and enzyme linked immunosorbent assay (ELISA. These methods, which depend on antigens of the parasites present in stool samples for detection of infection are faster and more sensitive (Alsaeed and Issa, 2010), especially in cases where infection is very low. A number of test kits whose principle of diagnosis is based on immunological principles have been produced commercially for use (Weitzel *et al*., 2006). In recent years, rapid diagnostic tests that use antigen detection methods have been widely employed. These tests are both highly specific and sensitive when compared with microscopy method (Wegayehu *et al*., 2013). Some of these commercial kits upon evaluation however, have shown to be less sensitive than the conventional microscopy method (Weitzel *et al*., 2006).

The recent application of molecular tools in the detection of *Cryptosporidium* in stool sample (Asher *et al*., 2011) has not only improved upon diagnosis of these parasites but also helped to determine sources of infection through genotyping procedure (Insulander *et al*., 2013). The polymerase chain reaction (PCR) has been used extensively in the diagnosis of enteropathogens. The method since its introduction into medical research, keeps improving as researchers continue to make modifications in the original protocol to achieve better results. In spite of the advantages of PCR, many laboratories in developing countries find it difficult to use it because of cost of installing molecular laboratory facility and reagents for running tests (Essid *et al*., 2008).

There is evidence that, based on the type of diagnostic tool used, the results obtained from screening stool sample for *Cryptosporidium* species may differ significantly from one another (Roberts *et al*., 2011) or may agree without significant difference (Den Hartog *et al*., 2013, Tumwine *et al*., 2003). To determine prevalence accurately therefore, it will be very useful if each sample is tested by more than one diagnostic method. The challenge of using more than one diagnostic method however, for screening samples in developing countries will be the cost of running some of the tests which may not be easily affordable. For research purposes, many studies conducted in the fields of molecular epidemiology of cryptosporidiosis in developing countries have combined microscopy, which is cheaper with PCR (Gatei *et al*., 2006, Ajjampur

et al., 2009). In all of these studies, initial screening of all stool samples were done using microscopy, and only the positive samples used PCR.

2.1.7. Treatment

Cryptosporidiosis is known to be resistant to antimicrobial drugs unlike diseases such as *Toxoplasma gondii*, *Eimeria* and *Plasmodium* (Rossignol, 2010). There is no effective anti-microbial treatment available for cryptosporidiosis in man and mammals currently, reason being the unique compartment (parasitophorous vacuole) it establishes within the host cells, which is morphologically different from other related parasites. This vacuole may shelter the parasite from anti-microbial drugs (Hunter and Nicholas, 2002).

Treatment options of cryptosporidiosis depend largely on the immune status of the host. Since the disease is self- limiting, in immunocompetent individuals there is no need of specific therapy; however, supportive care with oral fluids and electrolyte replacement due to diarrhoea is beneficial in alleviating dehydration. In immunocompromised hosts, particularly AIDS patients with CD4 cells counts below 200 cells/µl, cryptosporidiosis can be threatening and must be treated (Rossignol, 2010).

In patients with AIDS, the ideal treatment involves partial restoration of immune functions with HAART (Highly Active Anti-Retroviral Therapy). In addition to HAART therapy, a number of antibiotics such as paromomycin, nitazonxanide, azithromycin that have partial efficacy against cryptosporidiosis are available on trial, of these paromomycin is the only agent so far that has been found to have efficacy in animals and humans in the treatment of intestinal cryptosporidiosis (Gargala, 2008).

2.2. *Giardia lamblia*

G. lamblia (also known as *G.lamblia* or *G. intestinalis*) is a unicellular flagellated intestinal protozoan parasite of humans isolated worldwide and is ranked among the top ten parasites of man (Nkrumah, 2011). The organism has been found in more than 40 animal species. There are five species of *Giardia* known to infect different animal species; *G. lamblia* infect mammals including man, rodents, reptiles; *G. muris* in rodents; *G. agilis* in amphibians; *G. ardae* in the great blue heron; *G. psittaci* in budgerigar (Adamu, 2006).

Giardia was originally identified by Van Leeuwenhoek in the 1681. Although it was the first protozoan parasite described, its role as pathogenic organisms was not recognized until

1970's. Prior to that time, the organism was thought to be harmless commensal of the intestine (Adam, 2001). *G. lamblia* has been identified as one of the most common causative agent of epidemic and diarrhoeal illness throughout the world (Huh, 2009).

2.2.1. Taxonomy of *G. lamblia*

The current taxonomic position of the genus *Giardia* is as shown in Table 3.

Table 3: Classification of *G. lamblia*

Kingdom	Animalia
Sub-kingdom	Protozoa
Phylum	Sarcomastigophora
Class	Zoomastigophora
Order	Diplomonadida
Family	Hexamitidae
Genus	*Giardia*
Species	*lamblia*

Source: Adam (2001)

2.2.2. Biology and Life cycle of *G. lamblia*

G. lamblia is a flagellated binucleated microaerophilic protozoa that inhabit upper part of the small intestine of its host and reproduces by binary fission. This is a type of reproduction in which one cell divides into two new cells by mitosis. During a growth cycle, the components of the cell multiply so that each daughter cell is complete copy of the parent cell. The cell then pinch off from each other and a complete reproduction cycle occurs. This parasite has a simple direct life cycle (Figure 2) consisting of an infective cyst and a vegetative trophozoite (Adimasu *et al*., 2014).

The cyst of *G. lamblia* is elliptically shaped, range in sizes from 6 – 10 microns and contains two to four nuclei. The structure of the cyst makes the organisms very resistant to environmental factors and disinfections and it is the transmittable form that causes the infection.

The cysts possess a thin protective wall that allows them to survive in faeces for weeks or in cold water for months (Xiao *et al*, 2004). Giardiasis is acquired by ingestion of contaminated water or food. The cysts pass through the stomach and enter the small intestine. The protective wall allows the cysts to survive the acidic conditions of the stomach until the cyst reaches the small intestine, where the conditions are alkaline.

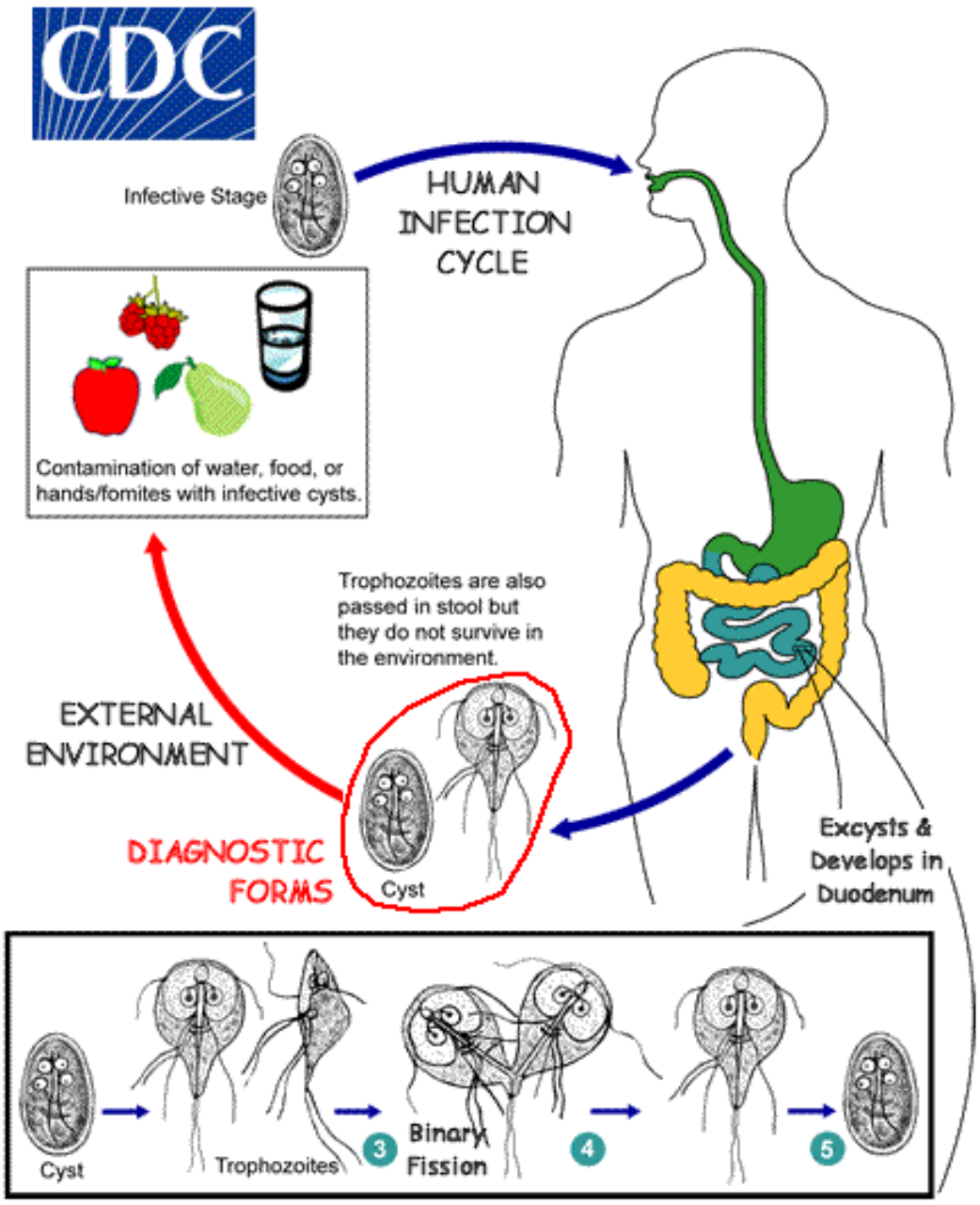

Figure 2: Life cycle of *G. lamblia*

Source: CDC (2005). http://www.dpd.cdc.gov/dpdx

The alkaline environment then triggers excystation process. During excystation, the cysts wall erupts at the pole opposite to the nuclei, so that flagella and other projections emerge from the rupture point. The cyst wall is then completely shed and the microbes enter into the trophozoite stage (Molina *et al*, 2011).

The trophozoite stage is approximately 12 -15 micrometers by 6 – 8 micrometers. The organism has a pointed elongated median body with two symmetric nuclei and four pairs of flagella. It resembles a "human face" on stained preparation (Ford, 2005). The trophozoite is the reproducing and motile stage of *Giardia* that attaches to the intestinal wall via its ventral disc and causes the symptoms of giardiasis. In severe cases, the trophozoites can become so numerous along the intestine that they cover it as "carpet" (Johnston, *et al*, 2010).

While the trophozoite is attached to intestinal mucosa, it not only absorbs but blocks nutrients from being transported across the epithelial lining of the intestine. It inhibits the absorption of fats, carbohydrates, vitamin and folic acid. Trophozoites are rarely infective because they are not resistant to gastric acid and die rapidly outside the body. The trophozoite then undergoes encystations (Adam, 2001).

Encystations take place as trophozoites pass to the posterior regions of the small intestine. Cyst wall formation is completed within approximately 44 – 70 hour and appears to be initiated by the presence of bile salts in the lower small intestine (Butt *et al*, 2004).. The most visible overall change during encystations is that trophozoites gradually round up, detach and lose mobility. Cyst formation is essential for the survival of *Giardia* outside the host intestines and for the transmission of the parasite among susceptible hosts (Wensaas *et al*, 2009).

The cysts then leave the body and are transmitted directly from person to person by contact with infected faeces or picked up by another host via contaminated water or food (Mor and Tzipori, 2008). Although infection after the ingestion of only one cyst is theoretically possible, the minimum number of cysts shown to infect a human under experimental conditions is ten (Adamu *et al*, 2006). Generally the cyst stage of *G. lamblia* causes the infection while the trophozoite causes the symptoms of giardiasis.

2.2.3. Epidemiology of *Giardia lamblia*

G. lamblia is the most common protozoan intestinal parasite isolated worldwide as one of the causative agents of diarrhoea. Epidemiological studies suggest that the parasite is responsible for about 5% of acute diarrhoea and 20% of chronic diarrhoea illness in the world. The incidence of

diarrhoea associated with *Giardia* is generally higher in developing countries as compared with developed nations where access to clean water and basic sanitation is lacking (Pinheiro *et al*, 2011). The prevalence of *G. lamblia* in developed countries is around 2 – 5% but in developing countries may be up to 20 – 30%. Nearly all children in developing countries will acquire *Giardia* at some point in their childhood (Adam, 2001)

Based on stool positivity, the prevalence of *G .lamblia* infection in children has been found to range from 1% to 72%, depending on the age group. Prevalence varies from country to country and among populations within countries. In some developing countries, infection among young children can be quite high. In the United States, United Kingdom, and Mexico, endemic *Giardia* infection most commonly occurs among children under five years of age and adults aged 25-39 years (Butt *et al*., 2004, Ignatius *et al*., 2012).

Giardiasis is common among children in day care centres, nursery and primary schools, orphanage, and other social institutions, as well as slums (Mehraj *et al*, 2008). In each of all 10 schools selected randomly for a study at western Tajikistan, in Asia, children were noted to be infected with *G, lamblia*, with a prevalence of 26.4% (Matthys *et al*, 2011). Similarly, among children of some three marginal urban districts of Trujillo in Peru, *G. lamblia* was identified as the most frequent parasite with a prevalence of 23.8% (Hollm-Delgado *et al*, 2008).

In a retrospective study conducted in 2007 in Havana, Cuba, medical records revealed that a total of 185 children were hospitalized for giardiasis at the Academic paediatric hospital (Escobedo *et al.*, 2011). Clinical information which accompanied the data also indicated that the mean length of hospital stay was 4.9 days (Mor and Tzipori, 2008). The study provided an opportunity for the clinician to observe the clinical significance of symptomatic giardiasis, and also obtained information on prevalence of hospitalized cases, which according to the authors was limited to Cuba.

A hospital based surveillance of enteric parasites at Kolkata, India revealed that *G. lamblia* was associated with diarrhoea in the area (Mukherjee *et al*., 2009). The study was conducted among children admitted to the Infectious Disease hospital at Kolkata between the period of November, 2007 and October, 2008 for complaints of diarrhoea. Until the study was carried out, the burden of enteric parasitic infection in and around Kolkata was not reported. The prevalence of *G. lamblia* infection in this study was 13.3%. At the gastro-enterology outpatient clinic of Aga Khan University hospital in Karachi, Pakistan, Patients screened for *G. lamblia*

infection by microscopy and polymerase chain reaction (PCR) showed that the infections were common (Yakoob *et al.*, 2010). Prevalence was 6.3% and 8.7% by microscopy and PCR respectively for this parasitic infection (Taylor and Hong, 2000).

In several studies conducted in African countries varying rates of prevalence for *G. lamblia* have been reported. In most of these studies however, the prevalence could have been underestimated as microscopy which has a lower detection rate have been used (Tesfalem *et al.*, 2012). In Lagos, Nigeria, prevalence of *G. lamblia* was 4.8% (Wellington *et al*., 2009, Uneke and Uneke, 2008). Mandomando *et al* (2007) reported prevalence of *G. lamblia* of 2.5% in Mozambique children hospitalized for diarrhoea. Also, in Chobe district of Botswana, *G. lamblia* was identified to be contributing significantly to recurrent diarrhoeal cases (Alexander *et al*., 2012).

In East Africa, a study conducted in the North western Ethiopian town of Pawi revealed a prevalence rate of 26.6% which was quite high and was attributed predominantly to the lack of potable water (Eyasu *et al*., 2010). A study conducted in rural communities in southern Rwanda reported a prevalence of 60% which exceeded figures reported from other parts of the world (Ignatius *et al.* (2012).

In a study conducted in Kiambu districts hospital, *G. lamblia* prevalence reported was 26.1% (Chunge *et al.*, 1992). In another study in a rural community of Nderu, Kiambu district, a prevalence of 44.7% was reported (Chunge *et al*. (1991).

From the foregoing discussion it is evident that most of the information available on cryptosporidiosis and giardiasis in Kenya is based on a few field-based or hospital based studies undertaken in or around Nairobi and Eldoret in the former Rift valley province. There is no data on the occurrence of *G. lamblia* infection in Siaya County and in particular Siaya county referral hospital.

2.2.4. Pathology and Clinical Features

As in any parasitic infections, host parasite interaction is the initial steps in the pathogenesis of giardiasis. In this interaction, first the *Giardia* trophozoites attach to the cell surface of villi by means of a disk on their posterior or ventral surface. Lectin, a protein on the trophozoite lining, recognizes specific receptors on the intestinal cell and may be partly responsible for the tight attachment between the parasite and the villi (Buret, 2007). Following attachment of trophozoites, there will be major structural and functional abnormalities in the

small intestines. Some of these abnormalities include mucosal damage as a result of mechanical obstruction or blockage of the intestine by a large number of parasites, the release of cytopathic substances such as thiol proteinase and lectins from *Giardia* trophozoites, the stimulation of a host immune response with release of cytokines and mucosal inflammation and deconjugation of bile salts (Troeger, 2006).

Although symptomatic infection causes a broad spectrum of clinical manifestations, *Giardia* infection results in a symptomatic carrier state in a majority of cases. The asymptomatic infections are most common in children and people with prior exposure to a source of infection (Adam, 2001). When this disease occurs, it can result in occasional days of acute watery diarrhoea with abdominal pain, or patients may experience a protracted, intermittent, often debilitating disease, which is characterized by passage of foul-smelling stools associated with flatulence, abdominal distension, and anorexia (Kosek *et al*., 2001).

2.2.5. Treatment

There are effective treatments against *Giardia* species but still preventive measures are of great importance. As in most diarrhoea causing agents, disease out-breaks can also be prevented by testing of purified and unpurified water to check for the presence of cysts of the parasites. Other preventive measures include boiling water intended for consumption, thoroughly washing hands before handling food, maintaining good personal cleanliness and proper disposal of faecal materials and information dissemination through print media to educate the public regarding the dangers of giardiasis (Eshete, 2008).

Currently there are different groups of drugs available to treat giardiasis. Based on different age group, endemicity of the parasite and pregnancy among other things, the use of anti-*Giardia* therapy varies (Gardner, 2001). In developed countries, unlike the developing countries, all patients who have *G. lamblia* in stool should be treated (Karabay *et al*, 2003). The most commonly used anti-*Giardia* drugs include metronidazole, Furazolidone and paromomycin. Metronidazole is the most common drug used for the treatment of giardiasis worldwide. Unlike other drugs, it is quickly and completely absorbed and penetrates body tissues and secretions such as saliva, breast milk, semen, and vaginal secretions. Of the common anti *Giardia* therapeutics, Furazolidone is the only one available in a liquid suspension and is an important therapeutic agent worldwide and it has been widely used in paediatric populations. Paromomycin

has been proposed as a treatment for *G. lamblia* in resistant infections and during pregnancy (Rossignol, 2010).

2.3. Immunity to *Cryptosporidium* and *G. lamblia* infection

Presently $CD4^+$ T-lymphocyte count has been shown to be an excellent indicator which defines the degree of immune suppression (Raytekar *et al*., 2012).The immune system is divided into three components, namely, nonspecific immunity (for example skin and other mucosal barriers), the innate immune system (soluble factors and cells), and the adaptive immune system. The adaptive immune response induced by specific antigens recognized by T- and B-cells is generally required to eliminate rapidly proliferating or virulent microbial pathogens. T-cells activate B-cells to proliferate and differentiate into plasma cells that produce antibodies (Kalia *et al*., 2006). Both $CD4^+$ and $CD8^+$ cells contribute to resistance to and clearance of acute cryptosporidiosis and giardiasis (Panterburg *et al*., 2008). The innate system is limited to pattern recognition immune responses based on a diverse array of sensors which detect invading pathogens. Thus, infection and /or persistent infection/carriage is established by the interplay between the host (immune system) and the pathogen (Dwivedi, *et al.*, 2007). Any component of immune system can be functionally or genetically abnormal as a result of acquired (e.g., through HIV infection, lymphomas, or high-dose steroids or other immune-suppressive medication) or congenital illness that either affect humoral immunity or compromise T- cell function (Borad and Ward, 2010).

Immunosuppression may also occur in malnourished persons, patients undergoing chemotherapy for malignancy, and those receiving immunosuppressive therapy. However, for parasitic infections, cell-mediated (T-cell) abnormalities predominate. These patients tend to have an increased risk of acquiring common pathogens (e.g., *Cryptosporidium*). In addition, these patients are at risk of infection by non-pathogenic parasites (those that do not cause disease in normal hosts) (Tuli *et al*., 2008).

Developing an infection with enteric protozoan parasites is dependent on absolute $CD4^+$ cell count, with lower counts associated with more severe disease, more atypical disease, and a greater risk of disseminated disease. In patients with relatively high $CD4^+$ count (>200 cells/µl) the infection may be asymptomatic or result in mild diarrhoea. However, patients with $CD4^+$ count less than 200 cells/µl can develop persistent or intractable diarrhoea, emphasizing importance of the host immune response in controlling the disease (Pattanapanyasat, 2012).

Previous studies have revealed that $CD4^+$ T-lymphocyte cells of < 200 cells/µl was associated with increased risk of parasitic infection (Akinbo *et al*., 2011). Although the outcome and severity of infection is critically dependent on the immune status of the host, the nature of the immune response in cryptosporidiosis, particularly in humans, is poorly understood.

2.4 Risk factors associated with *Cryptosporidium* and *G. lamblia* infections

A number of factors have been found to be associated with the incidence of giardiasis and cryptosporidiosis. The identification of these factors enables health authorities to initiate preventive and control measures, in order to reduce incidence within communities in which the diseases are endemic. Examples of such risk factors which generally vary from one population to the other include age, gender, geographic location, season, family history, breastfeeding habits, domestic and social settings, as well as environment and zoonotic factors (Pereira *et al.,* 2007).

There are seemingly conflicting reports on the nature of association that exists between gender, age, geographical location of people, or seasonal variations and the incidence of giardiasis and cryptosporidiosis among people and in particular children worldwide. Among Brazilian children hospitalized for diarrhoea, Pereira *et al* (2007) observed that age of child was positively associated with the odds of *G. lamblia* infection; and that the odds of giardiasis increased about 1.18 for each additional year of age. The majority of infected children were between 24-48 months of age (19.7%). Sex and weight however were not found to be associated with giardiasis in their population of study. Season was also not associated with G. lamblia infection. Among Brazilian children from a day care centre in Sao Paulo, however, there was higher prevalence of giardiasis in boys than girls (Tashima *et al*., 2009).

In contrast to observation made by Pereira *et al*., (2007), significantly more giardiasis cases identified in Cuban children were boys than girls (Escobedo *et al*., 2011). This difference was attributed to gender-associated difference in exposure to *Giardia.* In other words, the behaviour and recreational exposure of boys differ from those of girls in some settings. Infection occurred most among children of age 1-4 years. Additionally, rate of infection during hot season was higher than the cold season. This could be due to the fact that cold weather kills the infective cysts (Oda *et al*, 2005).

Other behavioural factors that could be involved include greater consumption of water and drinks in hot weather which may be sources of infection. Bello *et al.* (2011) reported that Cuban children aged 5 years and over, appeared to be at greater risk of *G. lamblia* infection than

younger ones, but gender had no marked effect on risks. Children who lived in rural areas appeared to be at a 3.1 fold greater risk of *Giardia* infection. In India, the age group 5-10 years was predominantly infected with G. lamblia (Mukherjee *et al*., 2009). Regarding seasonality, Murkherjee *et al*. (2009) observed that occurrence of *G. lamblia* remained almost unchanged throughout the year

Cryptosporidium infections have been reported to be most common among children of age 2 years and below (Lindo *et al.,* 1998). However, an epidemiological investigation of cryptosporidiosis in Cuban children, it was observed that there was no gender or age difference (Pelayo *et al*., 2008). The inclusion of children more than 5 years in the study (5-8 years) indicated that, in Cuba, cryptosporidiosis is a disease not only of infants but also of children of school age.

Generally, information obtained on risk factors for giardiasis and cryptosporidiosis in Africa show similar trends with those reported from other developing countries worldwide. In a study done in Guinea-Bissau, West Africa, among children of age 5 years and below with diarrhoea, children aged 6-11 months had the highest risk of infection with *Cryptosporidium* (Perch *et al*., 2001). In Kwara state, Nigeria, cryptosporidiosis was common among children of age less than 2 years in a study in which children of age ≤14 years with diarrhoea were screened for intestinal parasites (Gambo *et al.* 2014). In Lagos, Nigeria, the age with highest infection was 4-5 years old for both giardiasis and cryptosporidiosis (Wellington *et al*., 2009).

In East Africa, a study conducted in Ethiopia showed significantly high prevalence of giardiasis occurred among the females than males (Eyasu *et al*., 2010). Additionally, prevalence of *G. lamblia* and *Cryptosporidium* infections were not significantly different among the age groups. Similarly, *G. lamblia* infections was reported among all age groups of children studied in Botswana (Alexander *et al*., 2012), but the study also indicates that *cryptosporidium* infection was predominantly higher in children less than 2 years. In Kenya, cryptosporidiosis was reported to be most common among children of age13-24 months and least among 48-60 months of age (Gatei *et al*., 2006).

Homes overcrowded with people are common phenomenon in most developing countries for socioeconomic and cultural reason. Unfortunately, the situation could promote high level of inter personal transmission of infectious diseases. Sanitation level could worsen in overcrowded homes that lack modern or adequate toilet facilities, as occurs in many rural communities where

defaecation occur in open area leading to high levels of contamination of the environment. Giardiasis and cryptosporidiosis have a common source of infection, which is through the consumption of water contaminated by cysts and Oocysts of parasites (Eyasu *et al*., 2010; Ayalew *et al*., 2008).

The supply of water to community is an important risk factor for giardiasis and cryptosporidiosis infection (Isaac-Renton *et al*., 1999). Several outbreaks have resulted from the contamination of municipal water supplies with *Giardia* cyst and *Cryptosporidium* Oocysts (Robertson *et al*., 2007). This is partly because the normal chlorine level used to kill bacteria in municipal water supplies will not inactivate *Giardia* cysts or *Cryptosporidium* oocysts. The source of water to people in communities vary from one community to the other, and examples include piped water, wells, rivers, boreholes and bottled mineral water. Studies conducted on surface have revealed contamination with *Cryptosporidium* and *G. lamblia* (Helmi *et al*., 2011). In a study carried out in selected villages of Pawi special district, Northwestern Ethiopia, Eyasu *et al*. (2010) screened children who were drinking water from different sources for the oocysts and cysts of *Cryptosporidium* and *Giardia* respectively.

To ensure that children are adequately cared for at home, personal hygiene and heath education are critical. Unfortunately, it appears that parents who are not well educated either do not pay much attention to them or are completely ignorant of basic hygiene practices that could prevent infection of *Cryptosporidium* and *Giardia* infections. A number of studies have shown a close association of infections of both *G. lamblia* and *Cryptosporidium* with the level of education of parents (Adamu, 2006). Among Iraqi children suffering from acute diarrhoea, it was observed that the rate of *G. lamblia* was significantly associated with level of education of mothers as follows: illiterate or incomplete primary education (26.9%), complete primary or secondary education (22.3%), and university education (9.7%). As indicated, infection was higher in children whose mothers had low level of education.

CHAPTER THREE

MATERIALS AND METHODS

3.1. The Study Area and population

3.1.1. Siaya County referral hospital

The study was conducted at Siaya county referral hospital in Siaya County. The hospital is located within the town and is accessed by people from all parts of the county. Siaya County is one of the 12 counties that comprised of former Nyanza Province. It is bordered by Busia county to the north, Vihiga and Butere-Mumias county to the north-east, and kisumu County to the south east (Figure 3). The Total area of the county is approximately 1,520 km^2, the district lies between latitude 0^0 26' and 0^0 18' north and longitude 33^0 58' and 34^0 33' west.

Siaya county has a population of 520,000 and is divided into seven administrative sub-counties namely; Yala, Wagai, Karemo, Ugunja, Boro, Uranga and Ukwala. Ukwala is the largest covering an area of 319.5km^2 while Boro sub-county is the smallest covering an area of 180.1km^2. The county has three major geomorphologic areas namely; Uplands, Moderate lowlands and Yala swamp. The altitude of the county rises from 1,140m in the Eastern parts to 1,400m above the sea level in the West. There are few hills found in the county namely; Mbaga, Odiato, Akala, Rega and Nyambare. River Nzoia and Yala traverse the district and enter Lake Victoria through Yala Swamp (MOFP, 2002).

Poverty levels have generally been increasing over the years, from 41% in 1994 to 58.02% in 2002. The main pockets of poverty can be found in the lower parts of Boro, lower Ukwala, Uranga and karemo sub-counties which are characterized by low rainfall and poor soil. High altitude areas forming Yala, Ukwala and Ugunja sub-counties have higher rainfall and hence suitable for agriculture and livestock keeping. The main food crops grown are maize, sorghum, beans, cassava, sweet potatoes and vegetables, while the main cash crop grown are sugar cane, cotton, Robusta and Arabica coffee (MOFP, 2002).

3.1.2. Climate

The county experiences bimodal type of rainfall. The district is drier in the western part towards Bondo District and is wetter towards the higher altitude in the eastern part. On the highlands the rainfall ranges between 800 – 2000 mm. The lower areas receive rainfall ranging from 800 –1600 mm. The long rains fall between March and June while the peak is realised in

April and May. The short rains occur between August and November. The temperature also vary with altitude. The mean minimum temperature is 15^{o}C while the mean maximum temperature is 30^{o}C (NCAPD, 2005).

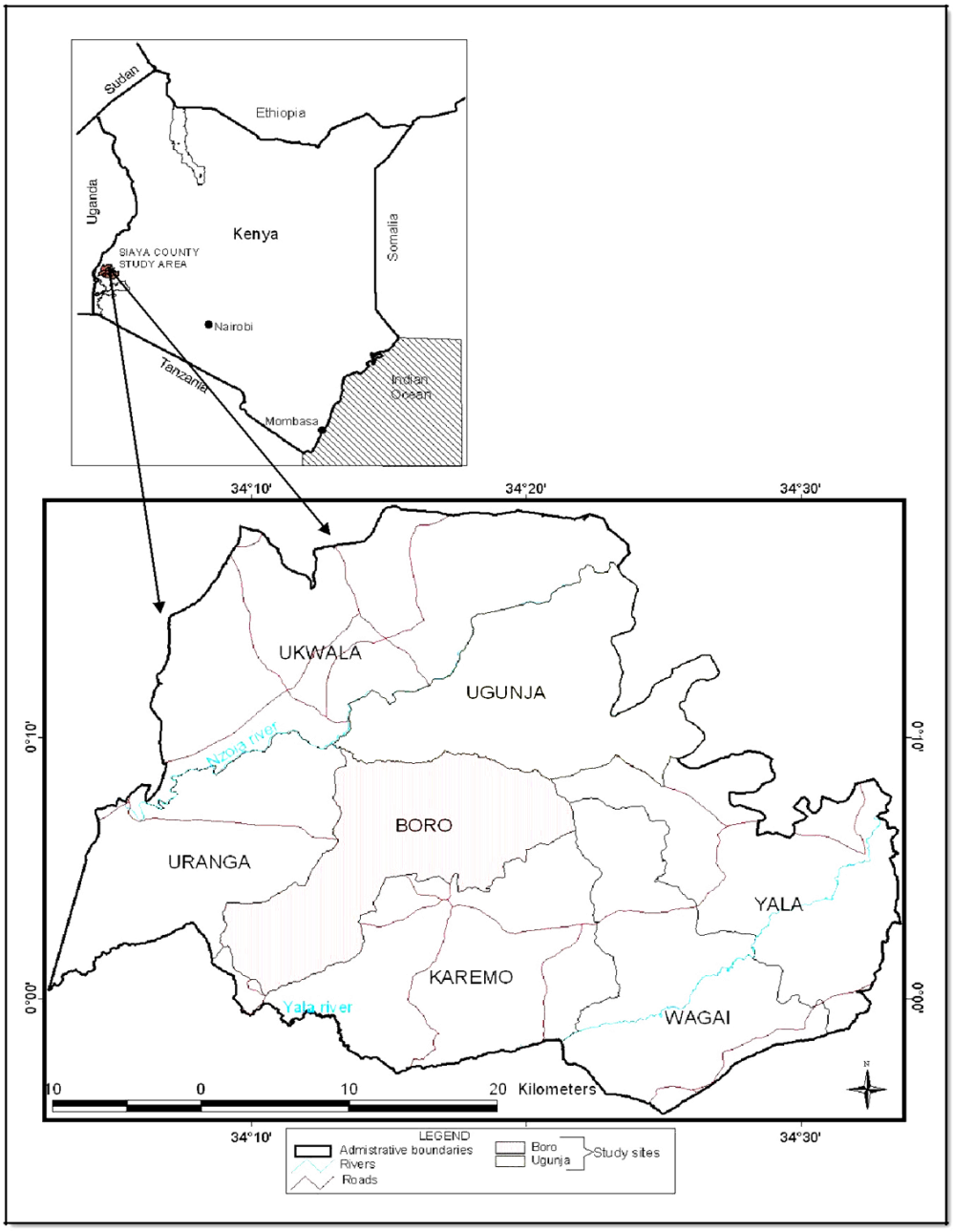

Figure 3: Map of Siaya County

3.2. Ethical clearance

Permission to conduct research in the study area was given by Egerton University Research Ethical Review Committee (Appendix D). Written consent was sought from patients and parents or care takers of the children (Appendix B). The inclusion of participants in the study was strictly voluntary and based on their informed consent after being informed of the objectives of the study. Consequently the patients enrolled in the project made an informed decision hence signed a consent form give in English. Respondents who could not read or write in English were interviewed in dholuo by an assistant. Participants were asked to fill the questionnaire (Appendix C) and care takers were asked to assist during stool sample collection in case participants were minors.

3.3. Sample size

A total of 384 patients referred to Siaya district hospital laboratory were examined. The laboratory received 200 patients presenting with diarrhoea each month. The sample size was calculated using the formula for cross-sectional survey (Fisher, 1998).

$$n = \frac{Z^2 p (1- p)}{d^2}$$

$$= \frac{1.96^2 x 0.50 (1-0.50)}{0.05^2} = 384$$

Z = 1.96 at 95% confidence interval.

P = is the proportion of positive individuals. Since there were no studies conducted concerning the present topic in the area, p is taken as 50% to achieve the maximum sample size.

d = is the absolute precision and is taken as 0.05

3.4. Inclusion and Exclusion Criteria

3.4.1. Inclusion criteria

The study involved all patients presenting with diarrhoea sent to the laboratory for stool examination.

3.4.2. Exclusion criteria

Patients sent to the laboratory for stool examination who were not having diarrhoea were not included in the study.

3.5. Study population

The study population were patients of all ages who were referred to the hospital laboratory with diarrhoea by the clinicians at the out-patient department and wards. A total of 384 patients participated in the study. Diarrhoea was defined as passage of loose or watery stool in the previous 24 hours and still present when the faecal specimen was collected at the hospital.

3.6. Study Design

This was a hospital based prospective cross-sectional study, which was conducted between the period of January 2013 and March 2013. The study involved microscopic examination of single fresh diarrhoeic stool for presence of trophozoites and cysts of *G. lamblia* and oocysts of *cryptosporidium* and also immunological tests for presence of antigens.

3.7. Sampling procedure

Convenience sampling procedure was used. Convenience sampling is a non-probability sampling method that relies on data collection from population members who are conveniently available to participate in the study.

3.8. Sample collection

A single fresh stool sample was collected from each patient and in the case of children collection was done by guardians. The specimen was placed into a clean disposable stool container with tight fittings. The consistency of the stool was directly observed and classified and recorded as either loose, semi-formed, formed, mucoid or watery. In this study, each sample was tested by laboratory methods, namely, microscopy and immunoassay tests for Giardia and Cryptosporidium. Blood sample was also collected from each patient and $CD4^+$ T-lymphocytes count was estimated using flow cytometry method.

3.8.1. Microscopic examination of stool

Each stool sample was processed by direct smear slide preparations and formal-ether concentration procedures for *G. lamblia* cysts, as well as the modified Ziehl-Neelsen technique for *Cryptosporidium* oocysts, for observation under a microscope.

3.8.2. Detection of *G. lamblia* cysts and trophozoites

A drop of diarrhoeal stool sample (approximately 2 ml stool sample) was separately added to iodine and saline drops placed at the two ends of a microscope slide, and mixed thoroughly using an applicator stick. They were covered with cover slips and examined under light microscope with power 10 objective (Olympus microscope, model CX21FS1) for either motile trophozoites (saline preparation) or cysts of *G. lamblia* (iodine preparation)(WHO, 1991).

To perform the formal ether concentration technique, 10 ml of 10% formal saline solution was added to approximately 1gm of stool sample (or 2ml if stool is watery) and stirred using an applicator stick until a slightly cloudy suspension was obtained. The stool suspension was filtered through four layer gauze placed in a funnel, into a centrifuge tube until the 7 ml mark was reached. The debris trapped on the sieve was discarded. After 3ml of diethyl ether was added to the mixture and hand shaken, the content was centrifuged at 2000 rpm for 3 minutes. The supernatant poured away and the tube replaced in its rack. The sediment was well mixed and a drop transferred onto a slide for examination. Each preparation was stained with iodine before observation. Finally the entire area under the cover slip was systematically examined using power 10 objective lenses for cysts of *G. lamblia* (WHO, 1991)

3.8.3. Detection of *Cryptosporidium* oocysts

The modified Ziehl-Neelsen method was used to detect *Cryptosporidium* species microscopically. A faecal smear was made on a slide, and left to dry, after which it was then fixed in methanol for 2 minutes. The smear was stained with cold carbol-fuchsin for 10 minutes, and the preparation was the differentiated in 1% hydrochloric acid-ethanol until colour ceased to flow out. It was rinsed in tap water, and counter stained with methylene blue for 1 minute, drained dry, and examined, first with high power objective of an optical microscope, and the morphology confirmed using oil immersion. The oocysts appeared as bright rose-pink spherules against a pale green background (figure 4).

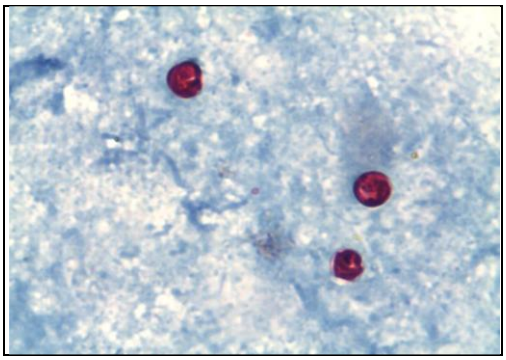

Figure 4: Oocyts of *Cryptosporidium* species (red coloured)

3.8.4. Enzyme linked immunosorbent assey test

The enzyme immunosorbent assey test was performed on faecal sample by use of *Giardia* II/*Cryptosporidium* II ELISA kit (TechLab Inc. Blacksburg VA) for the two parasites. The test involves a qualitative detection of *Cryptosporidium* and *Giardia* using monoclonal antibodies to the *Cryptosporidium* oocysts and *Giardia* cysts antigens in human faecal samples. The components of the kits are as follows:

i) Conjugate (rabbit polyclonal antibody to a *Cryptosporidium* oocysts and Giardia cysts antigen coupled to horseradish peroxidise in a protein buffered solution containing 0.02% thimerosal,
ii) Diluent (buffered protein solution containing 0.02% thimerosal),
iii) Stop solution (0.6 N sulphuric acid),
iv) Positive control (heat-inactivated bovine faecal material containing *Cryptosporidium* and *Giardia* antigen in a protein buffered solution with 0.02% thimerosal),
v) Negative control (Tris buffer saline solution),
vi) Substrate (solution containing tetramethylbenzidine and peroxide,
vii) Wash buffer concentrate (phosphate buffered saline, detergent and 0.02% thimerosal) and
viii) Microassay plate coated with monoclonal antibody.

The microassay plate in the kit contains an immobilized monoclonal antibody against *Cryptosporidium* oocyst and *Giardia* cyst antigen. In the assay, an aliquot of a diluted faecal sample is transferred to a microassay well. If *Cryptosporidium* oocyst or *Giardia* cyst antigen is present, it binds to the immobilized monoclonal antibody. Upon addition, the conjugate then binds to the antigen/antibody complex. Any unbound materials are removed during the washing

steps. Following the addition of a substrate, a colour is detected due to the enzyme-antibody-antigen complexes that form in the presence of *Cryptosporidium* oocyst or *Giardia* cyst antigen.

The Enzyme immunoassay test was performed by following a number of steps, which were the same for *Cryptosporidium* and *Giardia* detection in the manufacturer's instructions. For each sample, a 400 µL volume of diluent was added to a microcentrifuge tube, and 100 µL of the diarrhoeal stool sample (or approximately 0.1 gm of formed or semiformed sample, about the size of a small pea) added. The resulting solution was the well mixed. To run the test, two wells were selected on the microassay plate, to be used as controls, one as positive control and the other as negative control. The rest of the wells were considered as test wells.

A 100µL volume of diluent was transferred into each test well, after which 50µL of prepared stool sample was added and gently tapped to mix. After filling all the test wells with test sample, 50µL of positive control and 100 µL of negative control were added to their respective wells. The plate was well sealed with a plate sealer and incubated for 1 hour at room temperature. The contents of the wells were discarded after the period of incubation and the wells were washed thoroughly, and slapped on a dry towel to completely remove any traces of wash solution. The washing process was carried out four times.

In the next step, 50µL of the conjugate was added to each well and gently tapped to mix and then sealed with a plastic adhesive sheet. It was incubated for 30 minutes at room temperature, after which the contents of the plate was discarded and washed as described previously. After washing and thorough removal of all traces of solution, 100 µL of substrate was added to each well and gently tapped to mix. There was a final incubation of wells at room temperature for 10 minutes. In the final step, 50 µL of stop solution was added to each well and gently tapped to mix contents. The stop solution changes the blue colour formed to yellow colour. Results were read both visually by assessing the colour formed in each well, and quantified by measuring the absorbance at 450 nm on a microplate ELISA reader.

Any sample that was colourless or resembles the negative control well in intensity of colour (i.e. clear to slight yellow) was interpreted as negative. It implied that if the well contained any antigen at all it was below the detectable limits of the assay. The absorbance value of the negative control, positive control and test samples were determined. The recommended values (cut-off points) were <0.150 OD_{450}, for the negative control, and ≥ 0.150 for the positive

control. In the test wells, any value greater than, or equal to 0.150 (≥0.150) was considered positive (TECH LAB, 2006).

3.8.5. $CD4^+$ T-lymphocyte count for determination of immune status

Five millilitres of whole blood was collected in a vacutainer containing potassium ethylene diethyl tetraceticacid (K_3EDTA) for enumeration of the $CD4^+$ T-lymphocytes using flow cytometry method(model BD FACScalibur).

3.8.6. Demographic information

The questionnaire (Appendix C) was used to collect demographic data that included individual information and also risk factors associated with *Cryptosporidium* and *G. Lamblia* infection. It captured information on gender, age, and risk factors that included source of water used for cooking and drinking, level of education, hand washing practice with soap before handling food and mode of faecal disposal. Pre-testing of the questionnaire was conducted in 38 randomly selected outpatients (10% of the sample size) in Siaya district hospital to assess clarity, and flow of the questions as well as time taken to fill the questionnaire.

3.9. Data Analysis

The data collected by use of questionnaire, microscopy and immunoassay (ELISA method) were stored and maintained in MS Excel spreadsheet software. Data analysis was done using statistical package for social sciences (SPSS version 17).The prevalence of these parasite infections was calculated in percentage. Chi-square was used to determine the degree of association between level of education, water source, human waste disposal method and hand washing before handling food and *G, lamblia* and *Cryptosporidium* infection. Student t- test was used to test association between *Cryptosporidium* and *G. lamblia* infection and CD4 cell count. A value less than 0.05 was considered to be statistically significant.

CHAPTER FOUR

RESULTS

4.1. Prevalence

4.1.1.Prevalence of *Cryptosporidium* species and *G. lamblia*

A total of 384 faecal samples from patients presenting with diarrhoea were examined for *Cryptosporidium* and *G. lamblia* infection. $CD4^+$ T-lymphocyte cells enumeration for each patient was also performed. Out of these number, protozoa etiologic agents of diarrhoea was identified in 107(27.9%). A total of 78 (20.3%) were positive for *G. lamblia* infection while 29 (7.6%) had *Cryptosporidium* species (Table 4).

Table 4: Prevalence of *Giardia lamblia* and *Cryptosporidium* species infection

Parasite	**Frequency**	**Prevalence (%)**
G. lamblia	78	20.3
Cryptosporidium species	29	7.6
Total	**107**	**27.9**

4.1.2.Prevalence of *Cryptosporidium* species and *G. lamblia* in patients by gender

Out of the 384 study subjects, 107 males and females tested positive for *G. lamblia* and *Cryptosporidium*. The prevalence of *G. lamblia* infection in female subjects was higher 46 (22.7%) than males 32 (17.7%). Male subjects showed higher prevalence in *Cryptosporidium* species infection numbering 19 (10.5%) while females recorded 10 (4.9%) (Table 5). In order to determine if the high prevalence of *G. lamblia* infection in females and the high prevalence of *Cryptosporidium* species infection in males was significant, a Chi-Square test was performed (Appendix I table A). The Chi-Square test so performed was to determine if there was any dependence (independence) between *G.lamblia* and *Cryptosporidium* species infection and gender. If the dependence between the two categories (Gender and *G. lamblia* and *Cryptosporidium* species infection) is established, then it would suffice to conclude that the high prevalence of *G. lamblia* infection in females and the high prevalence of *Cryptosporidium* species infection in males was significant.

Table 5: Cross Tabulation by Gender of *Cryptosporidium* and *G. lamblia* species infection

Parasite	**Gender**		**Total**
	Male	**Female**	
G. lamblia	32	46	78
Cryptosporidium	19	10	29
Total	**51**	**56**	**107**

The first row of Appendix I (Table A) shows the Pearson chi-square value (5.083) with a p-value of 0.024. Clearly, $p < 0.05$. Since the p-value is less than 0.05, the null hypothesis of the independence is rejected an indication that there is dependence between gender and *G. lamblia* and *Cryptosporidium* species infection. This means, therefore, there is association between gender and *Cryptosporidium* and *G. lamblia* infection. The high prevalence of *G. lamblia* infection in females and the high prevalence of *Cryptosporidium* species infection in males was significant.

4.1.3. Prevalence of *G. lamblia* in patients segregated by age

The results of the study show that infection occurred in all the age groups. Among the 80 patients aged 0-9 years, 23 (28.8%) were positive for *G. lamblia* while 57 (71.2%) tested negative. Among the 118 patients in the 10-19year category examined for *G. lamblia* 28 (23.7%) tested positive for the parasite against 90 (76.3%) who tested negative. Those aged 20-29 years who numbered 75 had 13 (17.3%) individuals testing positive and 62 (82.7%) testing negative for *G. lamblia*. Among the 46 patients aged 30-39 years, 5 (10.9%) and 41 (89.1%) were found to be positive and negative respectively. Those between 40 and 49 years old who numbered 35 had 5 (14.3%) individuals testing positive and 30 (85.7%) testing negative for the infection. 4 (13.3%) out of the 30 patients aged 50 years and above tested positive for *G. lamblia* while 26 (86.7%) tested negative. The prevalence of *G. lamblia* infection among different age groups was determined by considering the frequency of those who tested positive for the infection in each age category. Table 6 shows the distribution of *G. lamblia* by age groups. From the table, it is clear that the prevalence of *G. lamblia* was high among the individuals aged 10-19 years. To determine if the high prevalence in the 10-19 years age category was significant, a Chi-Square test was performed (Appendix A table I).

Table 6: Prevalence of *G. lamblia* infection according to age

Age Range (Years)	Prevalence		Total
	Positive	Negative	
0-9	23	57	80
10-19	28	90	118
20-29	13	62	75
30-39	5	41	46
40-49	5	30	35
>50	4	26	30
Total	**78**	**306**	**384**

The first row of Table II (Appendix A) shows The Pearson Chi-Square value (9.003) with p-value of 0.109. Clearly, $p > 0.05$. Since the p-value is greater than $\alpha = 0.05$, the null hypothesis of independence is not rejected. This means that there is no association between age range and *G. lamblia* infection. Therefore, the high prevalence of *G. lamblia* infection among the individuals aged 10-19 years was not significant. Similarly, the difference in the prevalence of *G. lamblia* infection among different age groups was not statistically significant ($p > 0.05$).

4.1.4.Prevalence of *Cryptosporidium* species in patients segregated by age

The analysis of the study showed that infection of *Cryptosporidium* occurred in all the age groups (categories). Among the 80 patients aged 0-9 years, 11 (13.8%) were positive for *Cryptosporidium* while 69 (86.2%) tested negative. Among the 118 patients in the 10-19 years category examined for *Cryptosporidium*, 10 (8.5%) tested positive for the parasite against 108 (91.5%) who tested negative. Those aged 20-29 years who numbered 75 had 3 (4.0%) individuals testing positive and 72 (96%) testing negative for *Cryptosporidium*. Among the 46 patients aged 30-39 years, 2 (4.3%) and 44 (95.7%) were found to be positive and negative respectively. Those between 40 and 49 years old who numbered 35 had only 1 (2.9%) individual testing positive and 34 (97.1%) testing negative for the infection. 2 (6.7%) out of the 30 patients aged 50 years and above tested positive for *Cryptosporidium* while 28 (93.3%) tested negative.

The prevalence of *Cryptosporidium* infection among different age groups was determined by considering the frequency of those who tested positive for the infection in each age category. Table 7 shows the distribution of *Cryptosporidium* by age groups. From the table, it is clear that the prevalence of *Cryptosporidium* was high among the individuals aged 0-9 years.

Table 7: Prevalence of *Cryptosporidium* infection according to age.

Age Range (Years)	Prevalence		Total
	No. Positive	No. Negative	
0-9	11	69	80
10-19	10	108	118
20-29	3	72	75
30-39	2	44	46
40-49	1	34	35
>50	2	28	30
Total	**29**	**355**	**384**

To determine whether the high prevalence of *Cryptosporidium* species infection in the 0-9 years age category and whether the difference in the distribution of the parasites among the different age categories was significant, a Chi-Square test was performed (Appendix A Table III). The first row of Table 10 shows The Pearson Chi-Square value (7.716) with p-value of 0.173. Clearly, $p > 0.05$. Since the p-value is greater than $\alpha = 0.05$, the null hypothesis of independence is not rejected. This means that there is no association between age range and *Cryptosporidium* infection. Therefore, the high prevalence of *Cryptosporidium* infection among individuals aged 0-9 years was not significant. Similarly, the difference in the prevalence of *Cryptosporidium* species infection among different age groups was not statistically significant (since $p > 0.05$).

4.2. Association between $CD4^+$ T-cell count and *Cryptosporidium* and *Giardia* infection

The study population consisted of 384 respondents. $CD4^+$ count for each respondent was recorded. Among the 16 patients with $CD4^+$ count less than 200 cells/µl, *Cryptosporidium* was identified in 8 patients and no *G. lamblia* was identified in this group. Of the 164 patients with $CD4^+$ count 200-499 cells/µl, parasites were detected in 14 cases, in which *G. lamblia* was found to be prevalent parasite and was identified in 10 cases while *Cryptosporidium* accounted for the remaining 4 cases. Of the 204 patients with $CD4^+$ count > 500 cells/µl parasites were detected in 85 cases, of which *Cryptosporidium* was detected in 17 cases while *G. lamblia* was seen in 68 cases.

The proportion of parasites in patients with $CD4^+$ count <200 cells/µl was lower, 8, than the other groups with $CD4^+$ cell count 200-499 who numbered 14 and $CD4^+$> 500 cells/µl who numbered 85. Table 8 shows the distribution of the individuals infected with *Cryptosporidium* and *G. lamblia* among the various WHO categories of $CD4^+$ T-cell count.

Table 8: Cross tabulation between CD4 count categories and *Cryptosporidium* and *G. lamblia* species infection

Parasite	**CD4 count Categories**			**Total**
	CD4+ <200 cells/µl (n=16)	**CD4+ 200-499 cells/µl (n=164)**	**CD4 + >500 cells/µl (n=204)**	
G. Lamblia	0	10	68	78
Cryptosporidium	8	14	7	29
Total	**8**	**24**	**75**	**107**

To determine whether the sampled respondents were at risk, one sample t-test was conducted. The test was carried out to establish if the mean (average) $CD4^+$ counts of the 384 sampled respondents was greater than 200. The following hypothesis was tested: $H_0 : \mu = 200$ versus $H_1 : \mu > 200$ (µ is the population mean). The test criteria is that if p-value < 0.05, then the null hypothesis is rejected at 5% level of significance. The last two columns of Table IV (Appendix A) show the calculated t-statistic (24.37) and p-value of 0.000. it is clearly seen that p < 0.05. Hence the null hypothesis is rejected. This implies that the alternative hypothesis ($H_1 : \mu > 200$) is true. Hence, the average CD4 count of the sampled individuals is greater than 200. The sampled individuals are therefore not at risk.

4.3. Risk factors associated with *G. lamblia* and *Cryptosporidium species* infection

4.3.1. Association between *G. lamblia* and *Cryptosporidium* species and water sources

Table 9 shows the different water source for the respondents who tested positive for *G. lamblia* and *Cryptosporidium* species. Majority of patients who tested positive for both *Cryptosporidium* species and *G. lamblia* who numbered 48 (12.5%) used well/boreholes water for drinking and cooking followed by those using rivers or streams 26 (6.8%), dams/ ponds 16

(4.2%), and lake 15 (3.9%). The lowest prevalence 2 (0.5%) was observed in patients where tap water was used.

Table 9: Association between *G. lamblia* and *Cryptosporidium* species and water sources

Parasite	**Water source**					
	Tap water	**Rivers/Streams**	**Lakes**	**Ponds/Dams**	**Wells/ Boreholes**	**Total**
G. lamblia	1	18	11	12	36	78
Cryptosporidium	1	9	3	5	11	29
Total	2	27	14	17	47	107

A Chi-Square test was carried out to determine if there is any degree of association between water source and parasites *G. lamblia* and *Cryptosporidium*. Table V (Appendix A) gives the various Chi-Square statistic values, degree of freedom and the corresponding p-values respectively. Our interest is in the Pearson Chi-Square statistic value (1.388) with p-value of 0.846 as is in the first row of Table V. Comparing this p-value (0.846) with the conventional significance level ($\alpha = 0.05$), we see that $p > 0.05$. We therefore fail to reject the null hypothesis and conclude that there was no association between the water source and the parasites (*G. lamblia* and *Cryptosporidium*).

4.3.2. Level of education

Table 10 shows the cross tabulation between *Cryptosporidium* species and *G. lamblia* infection with level of education. Prevalence of *G. lamblia* and *Cryptosporidium* species was higher 80 (74.8%) in those who have primary education, followed by secondary 15 (14.0%) and patients with no formal education 9 (8.4%). The least was those with post-secondary education 3(2.8%).

Table 10: Cross tabulation between Level of Education and *Cryptosporidium* species and *G. lamblia* infection

Parasite	Level of Education				Total
	Illiterate	Primary	Secondary	College/University	
G. Lamblia	6	59	11	2	78
Cryptosporidium	3	21	4	1	29
Total	**9**	**80**	**15**	**3**	**107**

However, there was no association between level of education and *Cryptosporidium* species and *G. lamblia* infection as it is seen from Table VI (Appendix A) that the Pearson Chi-Square p-value is 0.966 (i.e. p=0.966 > 0.05). A p-value that is greater than $\alpha = 0.05$ leads to the failure to reject the null hypothesis which assumes independence or lack of association between level of education and the parasites (*Cryptosporidium* species and *G. lamblia*).

4.3.3.Hand washing practice with soap before handling food

The epidemiological data revealed that out of the 384 who participated in this exercise 107 were positive for the two parasites. Table 11 shows that 78 (72.9%) who did not wash their hands with soap before handling food suffered from *G. lamblia* infection while 13(12.2%) suffered from *Cryptosporidium* infection. It was therefore concluded that washing hands before handling food leads to decrease in *G. lamblia* and *Cryptosporidium* infection.

Table 11: Cross Tabulation between Hand Washing before Handling Food and *Cryptosporidium* and *G. lamblia* infection

Parasite	Hand washing Before Handling Food		Total
	Yes	No	
G. Lamblia	0	78	78
Cryptosporidium	16	13	29
Total	16	91	107

Chi-Square test of independence between hand washing before handling food and *Cryptosporidium* species and *G. lamblia* infection was conducted (Appendix A table VII). Since the zero (0) observation on those who did not wash hands before handling food and suffered from *G. lamblia* infection was observed (Table 11), correction for continuity was used in

calculating the Chi-Square statistic value. The second row of table VII was therefore considered where it is seen that Chi-Square value (continuity correction) is 46.356. The corresponding p-value for this Chi-Square statistic is 0.000 (p = 0.000). It is clearly seen that p < 0.05. The null hypothesis that assumes independence between Hand Washing before Handling Food and *Cryptosporidium* species and *G. lamblia* infection is therefore rejected. This implies that there was an association (dependence) between hand washing practice and *G. lamblia* and *Cryptosporidium* species infection in this study.

Table VIII (Appendix A) shows the symmetric measures between Hand washing Practice before handling food and *Cryptosporidium* species and *G. lamblia* infection. The Phi and Cramer's V values are the symmetric measure that shows the strength of association. From Table 19, it is seen that Phi and Crammer's V value (0.688) are very high with very low p-values (p = 0.00 < 0.05). We can therefore conclude with 95% confidence that there was a very strong association between Hand Washing Practice before handling food and *Cryptosporidium* species and *G. lamblia* infection.

4.3.4. Cryptosporidiosis and giardiasis and human waste disposal methods

A total of 384 patients requested to participate in this study responded to this question. The respondents mainly used bush, pit latrines and flush toilets methods for disposing human waste. Table 12 shows the proportions using the different human waste disposal methods. Majority of the respondents 56(52.3%) used bush while 39 (36.5%) used pit latrines. Respondents who used flush toilets were the least 12 (11.2%).

Table 12: Cross Tabulation between Human waste disposal and *Cryptosporidium* and *G. lamblia* infection

Parasite	**Human Waste Disposal**			**Total**
	Pit Latrine	**Flash Toilet**	**Bush**	
G. Lamblia	28	9	42	79
Cryptosporidium	11	3	14	28
Total	**39**	**12**	**56**	**107**

From Table IX (Appendix A), it is seen that Pearson Chi-Square statistic has p-value of 0.936. This p-value is greater than $\alpha = 0.05$ (i.e. p = 0.936 > 0.05). Thus the null hypothesis which assumes independence is accepted. This shows that there was no significant association between mode of human waste disposal and *G. lamblia* and *Cryptosporidium* species infection (since p > 0.05).

CHAPTER FIVE

DISCUSSION

Cryptosporidium and *Giardia lamblia* infection have been rated the most predominant infections in developing countries, causing considerable amount of morbidity and mortality. The two protozoan species are medically important enteric parasites which are both associated with diarrhoea especially in communities without proper sanitation and piped water. This has prompted several research studies into these intestinal parasitic infections and their associated risk factors in different parts of the world with great enthusiasm.

In the present study, the prevalence of both parasites among patients presenting with diarrhoea at Siaya county referral hospital was studied. The study revealed that *G. lamblia* and *Cryptosporidium species* are prevalent among patients presenting with diarrhoea in the hospital. This finding was higher than those observed by Chunge *et al.,* (1992), which was 2.7% for *Cryptosporidium* species and 3.8% for *G.lamblia*. The reason could be due to the fact that studies cited above used microscopy to demonstrate cysts of *G. lamblia* and oocyst of *Cryptosporidium* species in stool samples. The prevalence was also higher than what was reported by Gatei *et al.,* (2006) which was 4% for *Cryptosporidium* species and 16% for *G. lamblia* (Mbae *et al*., 2013). However, prevalence of *Cryptosporidium* was lower than the study reported in Mbagathi hospital (Mbae *et al*, 2013) at 30.5%. The prevalence recorded in this study was similar to those observed in Ethiopia (Tigabu *et al*., 2010) which was 8.5%.

This is an indication that both parasites are associated with causing diarrhoea among patients. In this study, there were more infections of *G. lamblia* than *Cryptosporidium* which seems to suggest that presently in Siaya, diarrhoea caused by *G. lamblia* appears to be more common than cryptosporidial diarrhoea. Similar findings were reported in Kaduna state, Nigeria where *G. lamblia* infection (3.2%) was higher than *Cryptosporidium* infection (1.9%) (Maikai *et al*., 2012). The study also agrees with Eyasu *et al.* (2010) who reported a prevalence of 26.6% for *G. lamblia* and 8.1% for Cryptosporidium infections respectively among Ethiopian children.

In the present study, age and gender of patients were studied to ascertain whether a particular gender or age group has significantly more infection than others. All the age groups were infected with the protozoan as revealed in the study. The prevalence decreased with increase in age but as patients advanced in age the prevalence also increased. This could be due

to the fact that as people age, and in this case those above the age of 65 years, immunity tend to decrease. Age specific prevalence of *Cryptosporidium* species and *G. lamblia* in the present study showed that those aged 0-9 years had higher prevalence than what was reported in Ethiopia by Tagabu *et al.,* (2010) with prevalence of 26.6% but was lower than what Ayalew (2006) observed (38%) in Ethiopia. The difference in infection prevalence of the two protozoa parasites between age groups in the present study is in agreement with what was observed in Uganda by Tumwine *et al.,* (2003).

In the present study the prevalence of *G. lamblia* infection was higher in females than in males. The females were more infected than males. This is in agreement with what was observed by Tigabu (2010) in Ethiopia. Similar observations were made among Uganda children who were hospitalized for diarrhoea at Mulago hospital in Uganda (Tumwine *et al*., 2003). This is opposite to the report by Mahmud *et al.,* (1995). This finding therefore suggests that *Cryptosporidium* species and *G. lamblia* detection rate can vary depending on the gender of the patient. The possible explanation could be because of increased chance of exposure of females to contaminated water as they are engaged in fetching water for use at home as is the case in most family set up in Kenya.

$CD4^+$ counts play an incredibly important role in the presentation of diarrhoea as well as in the control of protozoa in immunocompromised individuals. The impairment of the immune system in immunocompromised patients makes them extremely vulnerable to the specific opportunistic infections which are unable to establish in immunocompetent host. In the current study *Cryptosporidium* and *G. lamblia* infection was higher in patients with $CD4^+$count >200cells/µl than those with $CD4^+$<200 cells/µl. The study is in agreement with the report in India by Raytekar *et al* (2012). This variation may be pertaining to the infectivity of these parasites at different level of immunosuppression. For example, infections by *Cryptosporidium* parasites are mainly seen in the lower immunity (<200 T-cells/µl), whereas other protozoan like *Entamoeba histolytica* and *G. lamblia* are seen in patients with higher $CD4^+$ T-cell level in diarrhoeal patients.

One important objective of the present study was to identify any associated risk factors for *G. lamblia* and *Cryptosporidium* infections among patients attending Siaya county referral hospital presenting with diarrhoea. Knowledge of associated risk factors with *G. lamblia* and *Cryptosporidium* infections will contribute towards measures that can be taken to avoid

infection. Access to clean water, good human waste disposal practice, high level of education, and good hand washing practice before meals are factors that could play a major role in control and prevention of giardiasis and cryptosporidiosis.

With regards to source of drinking water for patients, five main categories were identified. These were patients who depended on water from wells/boreholes, rivers/streams, dams/ponds, lakes, and tap water. In the present study *G. lamblia* and *Cryptosporidium* infection was not significant with patient source of drinking water. However, patients whose source of water for drinking was borehole or well had high *G. lamblia* and *Cryptosporidium* infection compared to those who relied on piped water. This could be attributed to low level of contamination of piped water by the infective oocysts and cysts of the parasites. This study is in agreement study in Ghana by Walana *et al* (2014) and Youn *et al* (2009). The results from this study however differed with study in Ethiopia by Tigabu (2010).

The general notion held by many people worldwide is that people who are highly educated are likely to practice personal hygiene better than those who have only low level of education. Since level of sanitation and hygiene both affect transmission of these parasites, it is expected that the rate of infections among patients or children whose caretakers are illiterate will be higher than those patients /caretakers with higher education. In the present study, there was no correlation of educational background with infection rate of both parasites. The current study is in agreement with observations made in a study conducted in Ethiopia by Adamu (2006).

The prevalence was also higher among those who did not wash hands with soap before handling food than those who did. There was strong association between handwashing practice before handling food and the protozoa infection ($p< 0.05$). This study is in agreement with what was reported by Hoque *et al* (1995) and the study done in Malawi by Morse *et al* (2008) and Haque (2007). Several observational studies have indicated the impact of hand washing on the prevention of intestinal parasitic infection. A case control study conducted in Viet Nam demonstrated a significantly reduced risk of intestinal parasitic infection among individuals who frequently washed hands with soap (Mahmud *et al.,* 2015). The use of soap facilitates detachment of pathogens from hand surface and therefore this findings support the promotion of proper handwashing as a public health measure to reduce this intestinal parasitic infection.

Subjects who used bush as mode of human waste disposal were higher than those who used pit latrine, and flush toilets. However, the present study showed no association between the

above mentioned risk factor and the acquisition of cryptosporidiosis and giardiasis. This study is in agreement with the report in India by Walana *et al* (2014) who observed that infection could be due to *Cryptosporidium* and *Giardia* being present in low level concentration in water for human consumption.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This study was performed to determine the prevalence of parasites causing diarrhoea in Siaya county referral hospital. It established that *Cryptosporidium* species was prevalent and presence of this parasite in patients with diarrhoea is an indication that cryptosporidiosis exists in this area. It was established that the prevalence of this parasitic infection was higher in males than in females. The study also established that patients aged 0-9 years had higher prevalence of *Cryptosporidium* species infection. Prevalence of *G. lamblia* was also found to be high in females was higher than in males.

The current study established that *Cryptosporidium* and *G. lamblia* infection was higher in patients with $CD4^+$ count of >200cells/µl than those who had $CD4^+$ count of <200 cells/µl. This therefore indicates that patients were not at risk of prolonged diarrhoea.

Patients who used well/borehole water for drinking and cooking showed high prevalence *G. lamblia* and *Cryptosporidium* species. Prevalence was also higher among those who had primary level of education.

The study also revealed that there was strong association between hand washing practice and infection. There was a strong link between hand washing practice using soap and reduced *G. lamblia* and *Cryptosporidium* species infection. This shows that washing hands with soap can reduce *Cryptosporidium species* and *G. lamblia* infection.

Respondents who used bush as a way of faecal disposal showed high prevalence of infection by the two parasites.

6.2. Recommendations

From the results obtained the following recommendations should be carried out:

1) To address the high prevalence of *G. lamblia* and *Cryptosporidium* species, patients found to be infected should be treated so as to reduce contamination of environment including water with cysts and oocysts.
2) Health education should also be conducted in barazas, particularly on environmental and personal hygiene, emphasizing on the need for washing hands with soap before handling food and building toilets. Public health programmes should also be sustained to further

promote better understanding of the sources and adverse impact of these infections among people and in particular children

3) Stool samples should be examined for oocysts of *Cryptosporidium* species as a routine procedure among diarrhoeic patients in health facilities. Method of choice for the diagnosis of this parasite should be Ziehl Neelsen because the procedure is easy to perform and is not expensive.

   There is also need to include immunoassay tests in addition to microscopy in the diagnosis of giardiasis and cryptosporidiosis especially on patients presenting to health facilities with diarrhoea.

4) Other agents of diarrhoeal illness (bacterial and viral agents) other than parasitic agents should be examined to confirm the high incidence of diarrhoeal in the area

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APPENDICES

APPENDIX A

STATISTICAL OUTPUT TABLES

Table I: Chi-Square test of independence between Gender and *Cryptosporidium* and *G. lamblia* species infection

	Value	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	5.083	1	.024
Continuity Correction	4.149	1	.042
Likelihood Ratio	5.133	1	.023
Linear-by-Linear Association	5.036	1	.025
N of Valid Cases	107		

Table II: Chi-Square test of independence between G. *lamblia* infections and age categories

	Value	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	9.003	5	.109
Likelihood Ratio	9.251	5	.099
Linear-by-Linear Association	7.335	1	.007
N of Valid Cases	384		

Table III: Chi-Square Test of independence for *Cryptosporidium* species infection and age categories

	Value	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	7.716	5	.173
Likelihood Ratio	7.610	5	.179
Linear-by-Linear Association	4.451	1	.035
N of Valid Cases	384		

Table IV: One Sample t-test of the Average CD4 count ($H_0 : \mu = 200$ versus $H_1 : \mu > 200$)

Variable	N	Mean	Std	SE of Mean	95% Lower Bound	T value	p-value
CD4 Count	385	437.61	191.30	9.75	421.53	24.37	0.000

Table V: Chi-Square Tests for water sources and the Parasites

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.388	4	.846
Likelihood Ratio	1.336	4	.855
Linear-by-Linear Association	1.058	1	.304
N of Valid Cases	108		

Table VI: Chi-Square Test of Independence between Level of Education and Parasites

	Value	df	Asymp. Sig.(2-sided)
Pearson Chi-Square	.267	3	.966
Likelihood Ratio	.257	3	.968
Linear-by-Linear Association	.009	1	.924
N of Valid Cases	107		

Table VII: Chi-Square Tests of independence between Hand Washing before Handling Food and *Cryptosporidium* and *G. lamblia* species infection

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	50.601	1	.000		
Continuity Correction	46.356	1	.000		
Likelihood Ratio	50.394	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	50.128	1	.000		
N of Valid Cases	107				

Table VIII: Symmetric measures between Hand Washing Practice before handling food and *Cryptosporidium* species and *G. lamblia* infection

		Value	Approx. Sig.
Nominal by Nominal	Phi	.688	.000
	Cramer's V	.688	.000
N of Valid Cases		107	

Table IX: Chi-Square Tests of independence between Human waste disposal and *Cryptosporidium* and *G. lamblia* species

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.132	2	.936
Likelihood Ratio	.131	2	.937
Linear-by-Linear Association	.117	1	.733
N of Valid Cases	107		

APPENDIX B
CONSENT FORM

My name is James Koskei,a post-graduate student from Egerton University, Department of Biological Sciences. I intend to interview project participants to find out social background and medical situation that would help in identifying possible new dimension in the management of such patients as well as for further planning and study purpose. I kindly request you to participate in the interview. Your kind participation will be of very much help for better understanding of the disease in Siaya district as well as for the entire region.

I am going to ask you some personal questions pertaining to the above issues in which all your answers will be kept confidential. Your name will not be written in this form, and will never be used in connection with any of the information you tell me. You do not have to answer any question that you do not want to answer. You may end this interview at any time you want to. However, your honest answers to these questions will help us understand the social background in relation to the diseases mentioned above.

I, the undersigned, confirm that the objective of the study has been explained to me and that I have given my consent to participate in the study.

Name of participant (or guardian)..... Signature..... Date.....

Signature (investigator)..... Date.....

APPENDIX C

QUESTIONNAIRE

This is a postgraduate research on Prevalence of Cryptosporidium species and *G. lamblia* in patients attending Siaya district hospital. Kindly fill the questionnaire as honest as possible. Each information you give will be treated confidentially and used for the purpose of this research work only. Thank you for your co-operation.

A) Socio-demographic information

1. Patient code number _____
2. Date of interview _____
3. Name of the interviewer _____
4. Gender of the respondent

   a) Male [ ] b) Female [ ]
5. Age (how old are you?)

   a) _____ years b) I don't know [ ] c) No response [ ]
6. Educational status: what is your highest level of education?

   a) No formal education [ ] b) Primary level [ ] c) Secondary level [ ]
   d) Post-secondary [ ] e) No response [ ]

B) Risk factors associated with giardiasis and cryptosporidiosis.

7. How do you dispose human waste?

   a) Flush toilets [ ] b) In the bush [ ] c) Pit latrines [ ]
   d) Others (specify) [ ]

8. Do you normally wash your hands with soap before handling food?

   a) Yes [ ] b) No [ ]

9. Where do you fetch your water for drinking, washing and cooking from?

   a) Dams/Ponds [ ] b) Rivers / Streams [ ] c) Wells/boreholes [ ]

   d) Tap (piped) [ ] e) Lakes [ ] f) others (specify) [ ]

APPENDIX D

ETHICAL APROVAL

EGERTON UNIVERSITY
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P. O. BOX 536
EGERTON, KENYA

OFFICE OF THE DEPUTY VICE-CHANCELLOR
DIVISION OF RESEARCH & EXTENSION

RESEARCH ETHICS COMMITTEE

Ref: EU/DVRE/028 **December 6, 2012**

James K. Kosgei
P.O. Box 536,
EGERTON

RE: APPLICATION FOR ETHICAL APPROVAL OF RESEARCH PROJECT

Reference is made to your application for ethical clearance of your research project entitled "***Prevalence of Cryptosporidium Species and Giardia Lamblia Infection in Patients Attending Siaya District Hospital.***

This is to inform you that the Egerton University Research Ethics Review Committee met on 4th Dec 2012 and discussed your application. The Committee observed that due consideration was given to the following ethical issues that would arise from the conduct of the study:

i) That participation is based on informed consent from participants.
ii) That the specimen collection and testing is a routine activity done in many hospitals and therefore the participants are not exposed to risks.
iii) That participants are over 16 years of age and therefore were not a vulnerable group.
iv) That the identity of the participants would not be exposed.
v) That the study is beneficial as participants found to be positive with parasites would be treated.

The committee therefore gave ethical clearance to your research project.
Please further note that the Standard Operating Procedures (SOPs) requires that you submit a copy of the final report of your study to the Committee.

Prof. M. K. Limo
CHAIRMAN – RESEARCH ETHICS COMMITTEE

c.c. DVC (R&E)
Director Research

MKL/pao

Egerton University is ISO 9001 : 2008 Certified