ROLE OF CRYPTOSPORIDIUM AS A CAUSE OF DIARRHOEA AMONG CHILDREN ATTENDING MATHARE NORTH HEALTH CENTRE, NAIROBI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (INFECTION DISEASES DIAGNOSIS) OF KENYATTA UNIVERSITY

AUGUST 2008

Kamwati, Stanley
Role of cryptosporidium as a...
DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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To my beloved wife, Nyokabi and my children, Kamwati, Muthoni and Mutiga. You make life worth living.
ACKNOWLEDGEMENT

This thesis is as a result of a cast of various people who made invaluable contributions to make sure that this work was a success. First, I am greatly indebted to my supervisors, Dr. Joseph Makumi, Prof. Eucharia Kenya and Dr. Njeri Wamae for their guidance, motivation and dedication. Many thanks also to Staff members of Mathare North Health Centre, parents and guardians of all the children who participated in this study, for their understanding and cooperation during sample collection. Danish Odera who assisted in sample collection from Mathare Health Centre for my study. I owe much success of this study to Dr. Wangeci Gatei for providing reagents and materials for genotyping work and the diligence and hard work of the laboratory personnel of the KEMRI, CMR Parasitology Section. They include Tabitha Irungu, Njeri Kariuki, Jonathan, Bett, Ian Waweru and Cecilia Mbae. I'm also grateful to Mukiri Wagema who helped in data analysis and Isaac Muthotho for helping in editing. The molecular analysis in this study was as a result of the help of Erastus Mulinge. The children of Mathare hospital also deserve special thanks. Lastly to my mother, brothers and sisters. May God bless you all abundantly.
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<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>bp</td>
<td>Base Pair</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CMR</td>
<td>Centre for Microbiology Research</td>
</tr>
<tr>
<td>df</td>
<td>Degrees of freedom</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTPs</td>
<td>2’-deoxynucleotide 5’triphosphates</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra acetate</td>
</tr>
<tr>
<td>F1</td>
<td>Forward primary primer</td>
</tr>
<tr>
<td>F2</td>
<td>Forward secondary primer</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>Human Immunodeficiency Virus Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
</tr>
<tr>
<td>mM</td>
<td>millimolar</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>Magnesium Chloride</td>
</tr>
<tr>
<td>Mw</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>RFPL</td>
<td>Restriction Fragment Length Polymorphism</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td>Polymerase Chain Reaction Restriction Fragment Length Polymorphism</td>
</tr>
<tr>
<td>R1</td>
<td>Reverse secondary primer</td>
</tr>
<tr>
<td>R2</td>
<td>Secondary reverse primer</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>RT</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>SAF</td>
<td>Sodium acetate formalin</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Scientists</td>
</tr>
<tr>
<td>SSU</td>
<td>Small subunit</td>
</tr>
<tr>
<td>Ssp1</td>
<td><em>Sphaerotilus</em> derived endonuclease</td>
</tr>
<tr>
<td>Taq</td>
<td><em>Thermus aquaticus</em> derived polymerase</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris-borate EDTA</td>
</tr>
<tr>
<td>TRAP-C1</td>
<td>Thrombospondin-related adhesive protein 1 of <em>Cryptosporidium</em></td>
</tr>
<tr>
<td>TRAP-C2</td>
<td>Thrombospondin-related adhesive protein 2 of <em>Cryptosporidium</em></td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet light</td>
</tr>
<tr>
<td>VIP</td>
<td>Ventilated Improved Pit latrine</td>
</tr>
<tr>
<td>Vsp1</td>
<td><em>Arthobacter</em> derived endonuclease</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>ZN</td>
<td>Zeihl Neelsen</td>
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Diarrhoea is a leading cause of morbidity and mortality in children. Although a number of enteric pathogens have been identified in recent years, the causes of many episodes of diarrhea remain undetermined. Cryptosporidium has emerged as an important enteric pathogen but its role in paedriatic diarrhoea among poor communities in Kenya is not clearly understood. Consequently a prospective survey of children aged below 12 years was conducted in Mathare Health Centre from January to May 2006. Stool samples from 401 children were examined by modified acid fast staining. DNA extraction and genotyping were also carried out to confirm Cryptosporidium species. A pretested questionnaire was administered to the respondents to establish the risk factors and symptoms associated with cryptosporidiosis. Results showed an overall Cryptosporidium prevalence rate of 3.2%. The prevalence was highest among the 1-2 year age group with 7.1% and closely followed by the 0-1 year age group, which had a prevalence rate of 6.7%. The other age groups had a prevalence rate of less than 2%. There were no sex differences in the prevalence of Cryptosporidium. Investigation into the nature of enteric diseases prompting ova and cyst examination showed that 86% had recurrent diarrhoea, 75.1% had acute diarrhoea and 17% had chronic diarrhoea. The main symptoms were vomiting (51.1 %) and abdominal pain (87.3%). There were no sex differences in the occurrence of type of diarrhoea or symptoms. However there were statistically significant differences between age and the occurrence of recurrent diarrhoea, acute diarrhoea, vomiting, abdominal pain and abdominal swelling. Cryptosporidiosis was significantly associated with vomiting ($\chi^2 = 6.032$, df = 1, $p = 0.014$). Genotype analysis based on the polymerase chain reaction-restriction fragment length polymorphism of the 18S rRNA gene fragment showed that 85% of the Cryptosporidium isolates were C. hominis while C. parvum, and C. felis were also identified from the isolates. Other parasites identified in the stool samples included Entamoeba coli (2.2%), Giardia intestinalis (5.2%), Entamoeba histolytica (1.5%), Trichuris trichiura (1%) Ascaris lumbricoides (3%) hookworm (0.5%) and Hymenolepis nana (0.5%). There was only one co-infection between Cryptosporidium and Giardia intestinalis. A majority of the respondents for the sampled children indicated that tap water was their main source of water. 71.3% of whom indicated that they treat their drinking water through boiling while only 28.7% of the respondents’ use chemicals to treat their water. 87% of the respondents for the sampled children indicated that they lived in a single room with two family members. Ninety of the respondents for the sampled children indicated that they use pit latrines as their main feacal waste disposal method while 14 indicated that they use VIP latrine. One hundred and forty seven of the respondents for the sampled children indicated that they kept pets at home. However none of these socioeconomic factors were statistically significant for Cryptosporidium. These results show that Cryptosporidium is an important etiological agent of childhood diarrhoea in the study population and site. These results also suggest that anthroponotic transmission plays an important role in the epidemiology of cryptosporidiosis in the studied participants. It is recommended that Cryptosporidium should be identified in routine parasitologic tests of diarrhoeal stool samples.
CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

There are numerous protozoa in the human gut, some of which live either as commensals, obligate parasites or opportunistic pathogens. Some of the protozoa are known aetiologic agents for acute diarrhoea and sometimes dysentery (such as Amoeba and Giardia) while others are associated with prolonged and sometimes chronic diarrhoea in children. While there are numerous causes of diarrhoea such as bacterial, viral or nutritional, approximately 30% of the diarrhoea is still classified as idiopathic. (Arcari et al., 2000) In most cases this may be due to limited diagnostic abilities of many clinical laboratories in developing countries while others may be due to misdiagnosis of some less common parasites (Marshall et al., 1997).

Protozoa parasites have been shown to be most common in developing world with prevalence of about 80% and generally lower in children than adults and higher in females than males (Chunge et al., 1991). However, when other parasites such as nematodes (Ascaris lumbricoides and Trichuris trichiura) are included, a higher prevalence among children aged 4-10 years is noted. (Ashford et al., 1992)

Spore forming enteric protozoa such as Cyclospora, Cryptosporidium and Isospora that are commonly associated with chronic diarrhoea are not routinely reported due to lack of specialized diagnostic techniques in most health laboratories (Chen et al., 2002 The first case of human cryptosporidiosis was reported in 1976 in the United States of America (Nime et al., 1976) and in Kenya in 1985 (Kangethe et al., 1985).
1.2 Literature Review

1.2.1 Historical Perspective of Gastrointestinal Parasites

Protozoa remained undetected due to their small size until the development of the microscope in the 17th Century by Anthony van Leeuwenhoek. By mid 18th century other parasitic protozoa were being reported at a rapid rate and new discoveries have continued to date. At least 45,000 species of protozoa have been described to date many of which are parasitic (Schmidt and Roberts, 2000). Parasitic protozoa still kill, mutilate and debilitate more people in the world than any other group of disease causing organisms. Protozoa therefore occupy a prominent place in the history of parasitology (Okhuysen et al., 1999; Schmidt and Roberts, 2000).

Gastrointestinal Tract (GIT) illnesses are caused by parasites that are cosmopolitan in distribution. Intestinal protozoa include species which can live in the human intestines and others which additionally live and reproduce in the cells of the intestinal wall. Parasites found in the intestines can be categorized into two groups: protozoa and helminths. Protozoa are differentiated by their method of motility, nuclear morphology, cystic and trophozoite stages (WHO, 2004) and include *Entamoeba*, *Giardia*, *Trichomonas*, *Cryptosporidium*, *Isospora*, *Pneumocytis* and *Balantidium*. 
1.2.2 Cryptosporidiosis

The genus *Cryptosporidium* was first described by Tyzzer (1907) in the gastric mucosa of laboratory mice and the first case in humans was described by Nime et al., (1976). During the last 30 years cryptosporidiosis has changed from a rare, largely asymptomatic infection to an important cause of gastrointestinal disease worldwide and is currently potentially fatal in complications of AIDS (Mosier and Oberst., 2000). It is a frequent cause of diarrhea and has gained notoriety because of its lethal consequences in the immunocompromised persons. Mwachari et al. (1998) indicated that the presence of *Cryptosporidium* oocysts was the single most important predictor of death in HIV-infected persons.

1.2.3 Public Health Importance of *Cryptosporidium* spp

*Cryptosporidium* is of public health importance due to its ability to cause infection as a result of several virulence factors. Firstly the hardy oocysts which are able to resist light chlorination and even full strength household bleach (Soave, 1992; Guerrant, 1997). Secondly, the small size oocysts which enables them to escape filtration from processed drinking water supplies, even when filtration is working optimally (Keusch et al., 1995) and a low infectious dose of less than 30 oocysts (Guerrant, 1997). They are fully infectious when shed and have zoonotic potential. These factors make it a real threat in drinking and recreational water, contaminated food, day care centres, hospitals and in persons exposed to animals such as veterinarians and dairy farm cattle workers (Keusch et al., 1995; Guerrant., 1997; Cordel et al., 1997).
1.3.2 Taxonomy of *Cryptosporidium*

*Cryptosporidium* is a genus in the phylum Apicomplexa. This phylum includes a large group of sexually reproducing spore forming protozoa with apical complex at some stage in their life cycle. *Cryptosporidium* is currently classified under the Class Sporozoasida, Subclass Coccidiasina, Order Euccoccidiorida, Suborder Eimeriorina, Family Cryptosporidiidae (Gatei, 2002).

As illustrated in Table 1, *Cryptosporidium* consists of a number of species capable of infecting broad range mammals and other vertebrates (Tzipori and Griffiths, 1998). Currently, 16 species are recognized, of which seven infect susceptible immunocompetent and immunocompromised individuals. (Sunnotel et al., 2006) *C. parvum* and *C. hominis* are the species predominantly found in humans, but others such as *C. meleagridis*, *C. felis*, *C. muris*, *C. canis*, and *C. suis* have also been occasionally identified (Xiao et al., 2004; Xiao and Ryan, 2004). In Kenya *C. parvum* and *C. hominis* (Gatei et al., 2006), *C. meleagridis* (Morgan et al., 2000) *C. muris* (Gatei et al., 2002) and *C. canis* (Gatei et al., 2006) have been reported. Despite this understanding, the taxonomy of *Cryptosporidium* remains unclear. Marked differences in the host ranges of different *Cryptosporidium* species and genotypes, and the growing evidence in differences in parasite development, growth rates, drug sensitivity and disease presentation in humans may explain this uncleanness (Hunter and Nichols, 2004). The in-depth understanding of the population structure of *Cryptosporidium* will therefore be invaluable in the public health investigations of cryptosporidiosis including the targeting of the most appropriate interventions.
Table 1: *Cryptosporidium* species.

<table>
<thead>
<tr>
<th><em>Cryptosporidium</em> spp</th>
<th>Size(µm)</th>
<th>Host</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. andersoni</em></td>
<td>5.5 x 7.4</td>
<td>Bovines</td>
<td>Abomasum</td>
</tr>
<tr>
<td><em>C. baileyi</em></td>
<td>4.6 x 6.2</td>
<td>Birds</td>
<td>Cloaca, bursa, Respiratory tract</td>
</tr>
<tr>
<td><em>C. canis</em></td>
<td>5.0 x 4.7</td>
<td>Canids, human</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. felis</em></td>
<td>4.5 x 5.0</td>
<td>Felids, human</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. galli</em></td>
<td>8.5 x 6.4</td>
<td>Birds</td>
<td>Proventriculus</td>
</tr>
<tr>
<td><em>C. hominis</em></td>
<td>4.5 x 5.5</td>
<td>Human</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. meleagridis</em></td>
<td>5.0 x 5.2</td>
<td>Birds, human</td>
<td>Intestine</td>
</tr>
<tr>
<td><em>C. molnari</em></td>
<td>4.7 x 4.5</td>
<td>Fish</td>
<td>Stomach</td>
</tr>
<tr>
<td><em>C. muris</em></td>
<td>5.6 x 7.4</td>
<td>Rodents, human</td>
<td>Stomach</td>
</tr>
<tr>
<td><em>C. parvum</em></td>
<td>4.5 x 5.5</td>
<td>Ruminants, human</td>
<td>Intestine</td>
</tr>
<tr>
<td><em>C. saurophilum</em></td>
<td>5.2 x 5.6</td>
<td>Lizards, snake</td>
<td>Intestinal and Cloacal mucosa</td>
</tr>
<tr>
<td><em>C. serpentis</em></td>
<td>5.6 x 6.6</td>
<td>Lizards, snake</td>
<td>Stomach</td>
</tr>
<tr>
<td><em>C. suis</em></td>
<td>5.1 x 4.4</td>
<td>Pigs, human</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. wrairi</em></td>
<td>5.0 x 5.6</td>
<td>Guinea pigs</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. bovis</em></td>
<td>4.8 x 5.4</td>
<td>Ruminants</td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>C. scopithalmi</em></td>
<td>4.7 x 5.0</td>
<td>Fish</td>
<td>Intestine</td>
</tr>
</tbody>
</table>

Source: Sunnotel *et al.*, 2006

1.2.3 Pathogenesis

Infection with *Cryptosporidium* begins when the ingested oocysts release sporozoites, which subsequently attach to and invade the intestinal epithelial cell. The parasite has a particular predilection for the jejunum and terminal ileum. This point deserves emphasis, because diagnostic evaluations, such as endoscopy, may miss the site of infection. In patients with AIDS, other sites within the gastrointestinal tract, including the stomach, duodenum, and colon, as well as the biliary tract may be involved (Hunter *et al.*, 2002). The histopathological features of cryptosporidiosis include a minimal inflammatory infiltrate and blunting of the villus. More-pronounced inflammatory changes, such as disruption of the epithelial cell barrier and more-extensive infiltration of the lamina propria with inflammatory cells, are seen in immunodeficient patients (Lumadue *et al.*, 1998).
The mechanism by which Cryptosporidium infection causes diarrhea remains elusive. The diarrhea is typically noninflammatory and is often profuse. The parasite does elicit a local inflammatory response, and increased production of prostaglandins and several cytokines, particularly IFN-γ, has been described (Chen et al., 2002). It is possible that these inflammatory mediators may consequently alter solute transportation in the intestinal epithelial cell, leading to osmotic diarrhea. Cryptosporidium infection has also been shown to inhibit apoptosis in infected epithelial cells as well as promote it in adjacent epithelial cells in vitro (Chen et al., 2002). This could theoretically prolong parasite survival and impair absorption in the intestinal mucosa.

The presence of an enterotoxin has also been hypothesized but never conclusively (Chen et al., 2002). Whatever the mechanisms by which Cryptosporidium infection causes disease may be, attachment to and invasion of host cells are crucial primary events in pathogenesis. However, little is known about specific parasite and host molecules involved in these processes. Knowledge of such molecules is essential for understanding the pathogenic mechanisms used by this parasite. Increasing recognition of Cryptosporidium species as emerging pathogens in humans has led to the identification of proteins, including CSL, GP900, p23/27, TRAP C1, gp40/45, cp47, and gp15/Cp17, which are implicated in mediating these interactions (Tzipori and Ward, 2002). However, progress in establishing the functional role of these proteins has been hindered by the inability to propagate Cryptosporidium in vitro and the lack of suitable systems for genetic manipulation of the parasite.
1. 2. 4 Life Cycle

Figure 1 shows the life cycle of *Cryptosporidium* sporulated oocysts containing four sporozoites are excreted by the infected host through faeces. Following ingestion by a suitable host, excystation occurs. The sporozoites are released and parasitize the epithelial cells. In these cells the parasites undergo asexual multiplication followed by sexual multiplication. Upon fertilization of the macrogamete (females) by the microgamete (males), oocysts develop after which they sporulate in the infected host and are excreted. Oocysts are infective upon excretion thus permitting direct and immediate faecal-oral transmission (Tzipori and Ward, 2000).

Figure 1: Life cycle of Cryptosporidia. Source: Laboratory Identification of Parasites of Public Health Concern, CDC. (http://www.dpd.cdc.gov/dpdx/HTML/Cryptosporidiosis.htm).
1.2.5 Routes of Transmission of Cryptosporidium

The oocyst is the stage transmitted from infected host to a susceptible host by the faecal oral route. Routes of transmission can be animal to human, person to person and waterborne. Transmission patterns vary from region to region perhaps due to a multiplicity of factors and interactions between the parasites, hosts and environments (Casemore 1990; Fayer et al., 1997). Until recently, many laboratories did not routinely check for Cryptosporidium organisms in cases of diarrhoea, thus it is often under reported and misdiagnosed (Skeels et al., 1990). Generally transmission is highest at the start of rainy season when survival of the oocysts and dissemination is easier. In United Kingdom (UK), peak incidences are in spring and late autumn to early Winter coinciding with peak rainfall and increased farm activities (Casemore 1990; Clavel et al., 1996; Hart 1999). Outbreaks or sporadic cases, however, continue to appear in different parts of UK., mostly around the same time each year but reasons for this pattern are not clear. Different parts of the country tend to have infections from same strains with the southern more densely populated area mainly recording human to human strains (genotype 1) while the northern parts of UK records more zoonotic strains (Genotype 2) (Furtado et al., 1998; McLaunchlin et al., 1998; Hart 1999).

1.4.1 Zoonotic transmission

Initial studies in human infections traced the source of cryptosporidiosis to calves (Current, 1994; Fayer et al., 1997). Since then; cross studies using this strain, between humans and a variety of other animals have been demonstrated. The zoonotic C. parvum ‘bovine’ genotype has been identified in different wildlife where a sylvatic cycle is well maintained (Fayer et al., 1997; Perz and Le Blancq, 2001). Lately, there
have been reports of other zoonotic species such as *C. muris*, *C. meleagridis*, *C. felis* and *C. canis* infecting human beings (Morgan *et al.*, 2000; Xiao *et al.*, 2001a; Pedraza-Diaz *et al.*, 2001a; Gatei *et al.*, 2002; Ong *et al.*, 2002). While it is clear these species are of animal origin, their transmission routes are not yet documented.

*Cryptosporidium* is suspected to be a major cause of transient diarrhoea among animal handlers including veterinarians ((Moon and Woomanse, 1986; Rahman *et al.*, 1996), Anderson and Donndelinger *et al.*, 1982). Infected animals excrete large numbers of oocysts sometimes up to $10^{10}$ daily for up to two weeks after the disappearance of symptoms (Tzipori and Griffiths, 1998; Orihel and Ash, 1995). However despite the continuous presence of pets in the households, rarely are they implicated as a source of infection. The presence of oocysts in the pets may imply that both owner and pet have a common reservoir source (Casemore *et al.*, 1997). Genotype analysis enables identification of strains in circulation and to determine possible transmission routes. (Morgan *et al.*, 1999d).

1. 4. 2 Person to Person Transmission

Cryptosporidiosis is a disease of public health concern due to person-to-person transmission via the fecal-oral contamination. Person to person transmission has been proposed in outbreaks occurring in day care centres (CDC, 1994), homosexual transmission (Ma and Soave, 1983; Goodgame *et al.*, 1993), nosocomial infections (Koch *et al.*, 1985) and household cross-infections (Fayer *et al.*, 1997). In endemic areas person to person transmission through poor hygiene standards perpetuates the disease in the communities (Reese *et al.*, 1982; Hunt *et al.*, 1984; Casemore, 1990; Rahman *et al.*, 1990). In the U.S.A, *Cryptosporidium* has been incriminated in many
day care centres outbreaks ans was isolated in 65% of the case from an outbreak in Pennsylvania, 55% in Michigan, and 17% in Georgia (CDC, 1994). A survey in Georgia day care centres showed cryptosporidiosis is nearly endemic (Walters et al., 1988). Other outbreaks have been reported in Spain, Portugal and South Africa (Melo et al., 1988; Clavel et al., 1996). Sporadic cases are often associated with recent diarrhoea or contacts with asymptomatic carriers (Current et al., 1983; Current, 1994; Rahman et al., 1996).

1.4.3 Waterborne transmission of Cryptosporidium

The biggest impact of Cryptosporidium as a public health concern has been on the water supply and resource industry (Moore et al., 1994). Surveys in the USA showed 65-97% of all surface water contained Cryptosporidium oocysts (USA Government Environmental Protection Agency - EPA 2001). While Cryptosporidium organism has been implicated in water borne outbreaks, few studies have characterized the precise species isolated (Kramer et al., 1996). So far, ‘human’ and ‘bovine’ and ‘cervine’ genotypes of C. Parvum and C. meleagridis have been identified contaminating water for human consumption (Xiao et al., 2000a; Glaberman et al., 2002).

Important sources of Cryptosporidium oocysts are discharges of untreated and treated domestic sewage and agricultural run-off (Medema and Schijven, 2001). Contamination occurs in surface water from catchment areas or boreholes through ground seepage. Major waterborne outbreaks are thought to be of zoonotic origin. However molecular analysis confirmed that the largest waterborne outbreak recorded in Milwaukee was due to ‘human’ type of C. parvum probably due to sewer contamination of the municipal water sources (Cicirello et al., 1997).
Since 1984, waterborne outbreaks have been reported in USA, UK, Japan and Australia, France, Sweden, Zambia and South Africa among other areas (Fayer et al., 1997). In USA, all outbreaks of waterborne cryptosporidiosis detected in 1984 to 1993 occurred in communities where water utilities met state and federal quality standards for acceptable drinking water. In all incidences, all surface water supplies implicated had been filtered. These outbreaks indicate that water treatment standards did not adequately protect against waterborne cryptosporidiosis (CDC, 1984). Similar outbreaks in UK have been linked to inadequately treated drinking water as well as contaminated swimming pools due to faecal accidents in recreational waters (Hunt et al., 1984; Hunter et al., 2007).

Recently, major resources have been allocated to ensure that all water for human consumption is free of Cryptosporidium and other protozoa such as Giardia in UK and USA (Smith and Rose, 1998; DWI Website 2001), USA-Environmental Protection Agency-EPA-website; 2001). In UK, current guidelines require water supply organizations ensure less than one oocyst per ten litres of water. Control strategies are aimed at removing or killing Cryptosporidium oocysts and Giardia cysts.

Multiple barriers are designed to remove or inactivate the pathogens. Water treatment methods such as sweep flocculation engulfment, interaction of oocysts with alum, ultraviolet and other radiations have been developed to rid water of Cryptosporidium (Bustamante et al., 2001; Craik et al., 2001). Water decontamination through parasite inactivation using ozone and monochloramine is applied especially where
contamination is with faecal material such as swimming pools (Driedger et al. 2001; Hirata et al., 2001). Stringent measures including flow cytometry, PCR, electrorotations, ELISA, turbidity correlation, colorimetric methods and immunofluorescence are undertaken to sample and analyse water quality and ensure compliance with these standards (Bustamante et al., 2001), however these processes are expensive and may not always ensure safe water.

A recent cost benefit assessment estimated that prevention of one reported case of Cryptosporidiosis costs roughly UK £ 1000 (Fairley et al., 1999a). While stringent measures might ensure drinking and utility water is largely free of Cryptosporidium at the point of supply, they do not prevent sporadic cases of cryptosporidiosis from post water treatment contamination, nor can they guarantee complete removal of oocysts. These measures are therefore set as regulation and not based on quantified health risks (DWI, 2001). In other countries including some states in USA and Canada, treatment and monitoring of Cryptosporidiosis is less stringent (Wallis et al., 1996; Payment et al., 2000). Monitoring the water supply may not predict an outbreak or rule out source of infection as distribution of oocysts is not homogenous and varies from source, treatment and distribution points (Casemore et al., 1997) Waterborne cryptosporidiosis has been linked to treated borehole and ground water (Bridgman et al., 1995; Wilcocks et al., 1998). Very low doses detected at any point may miss out non-homologous clusters of oocysts, the greater the chance of significant number of consumers being exposed to infectious doses (Casemore et al., 1997). Water related factors include abnormal weather conditions like flooding that increase surface water
runoff, contaminating water sources while high turbidity reduces water treatment efficiency. Other factors include ground water seepage into reservoirs, or burst underground water and sewer pipes (Casemore et al., 1997).

Although reports on waterborne outbreaks in developing countries are few, this is more likely due to under-reporting, misdiagnosis or complete absence of conclusive investigations of such outbreaks (Fayer et al., 1997). Water borne outbreaks have been documented in Lusaka, Zambia where 500 people presented with diarrhoea lasting two week (Kelly et al., 1997). Sustainable control measures to prevent to prevent waterborne cryptosporidiosis will need to be based on local conditions and needs.

The largest outbreak of infectious diarrhoea occurred in the greater Milwaukee, Wisconsin area, following contamination of the city's water supply by Cryptosporidium parvum after a spring run off from grazing lands. An estimated 403,000 residents experienced gastro-intestinal illness (Morris et al., 1998). In 1987, 130,000 case of C. parvum enteritis were reported in Georgia despite supply with filtered and chlorinated water. This outbreak was controlled through improved filtration (Cicirello et al., 1997; Morris et al., 1998).

Contamination can occur in surface water from catchments areas or borehole water through ground seepage. Limited social provisions in urban areas has led to careless dumping of garbage, blockage of drainage systems leading to contamination of water supplies with overflowing sewers and surface run off, not to mention complete absence of treated water in many urban slums (Hayes et al., 1989; Moore et al., 1994;
Thompson and Lymbery, 1996). This has led to the emergence of waterborne diseases such as cryptosporidiosis that are associated with low hygiene and poor sanitation (CDC, 1994).

1.5 Prevalence of Cryptosporidium

*Cryptosporidium* has a worldwide distribution and most surveys indicate that it is among the four major pathogens causing diarrhoeal diseases in children. Prevalence rates of cryptosporidiosis in diarrhoeal illness range from 0.1-2 % in cooler, more developed countries to 0.5 -10% in warmer less developed countries (Newman *et al*., 1999; Griffiths, 1998). Sporadic reports however show high prevalence rates of over 30% (Gatei, 2002). Furthermore prevalence rates in immunocompromised individuals tend to be higher. For example, a recent study in Uganda showed that 73.6% of HIV-infected children had cryptosporidiosis while 5.9% of HIV-negative children were infected (Tumwine *et al*., 2005).

The peak incidence of cryptosporidiosis is in children aged 1-5 years (Nacro *et al*., 1998; Nwabusi, 2001; Leelanyoova *et al*., 2001). Initial infection is early in developing countries (Sulaiman *et al*., 2005) but the peak prevalence is similar in both developed and developing countries (Griffiths, 1998). Day care centers are thought to contribute to the onset of cryptosporidiosis in developed countries (Clavel *et al*., 1996).

In Argentina Saredi and Bava. (1998) found that 3.8% of paediatric patients were infected with *Cryptosporidium parvum* with the majority of infections occurring in children less than 18 months who had diarrhea and from low socioeconomic background and living in poor sanitary conditions with no portable running water. A
Cuban study found 11.5% of children with diarrhoea to harbour oocysts of cryptosporidiosis while none of those without diarrhoea had oocysts (Núñez et al., 2003). In India, Kaur et al. (2002) reported 18.9% prevalence of Cryptosporidium oocysts.

In a study in Burkina Faso children aged less than 36 months admitted in hospital had a 5.2% prevalence of *C. parvum* (Nacro et al., 1998). Similarly, in Kwara State in Nigeria, children aged 0-14 years with diarrhoea examined and a prevalence rate of 15.1% with 86.7% of oocysts secretion occurring in the 0-2 year age group (Nwabusi, 2001). In Enugu State, stool samples of 373 primary school children comprising 38 watery and 335 formed stools were examined and oocysts found in 15% watery stool and 24% in the formed stool samples (Okofar and Okunji., 1996). In Gabon, samples of children aged 0-2 years gave a prevalence of 28% in those with acute diarrhoea and 14.8% in those without diarrhoea (Duong et al., 1995).

In Zambia, examination of children from four crowded townships gave a prevalence of 18% as reported by Nchito et al. (1998) while in Addis Ababa Assefa et al. (1996) sampled children less than 5 years with diarrhoea attending a clinic and on examination found 56% positive for Cryptosporidium. Tumwine et al. (2003) reported a 25% prevalence of cryptosporidiosis at Mulago hospital in Uganda by children aged 0-60 months of whom 72.7% had diarrhoea while the other 27.3% had no diarrhoea. A study among young children conducted in Egypt found a prevalence rate of 17% (Abdel-Messeh et al., 2005).
Most of the above reviewed reports have been based on hospital or laboratory based data, and thus detection, reporting and sampling biases cannot be ruled out. However, prospective population based studies have in general been consistent with the overall data available from laboratory surveys (Griffiths, 1998). Nevertheless it is difficult to ascertain accurately the worldwide prevalence or incidence of cryptosporidiosis.

There is no apparent sex-related disposition to infection. However a study in Guatemala reported a prevalence of 32% in children with a significant variation between females (44%) and male (17%) children (Laubach et al., 2004). Seasonal variations are also thought to explain the incidence of cryptosporidiosis. The incidence is highest in warm or wet seasons; for example Duong et al., (1995) found the incidence in children under 2 years old was greatest in the rainy season in Gabon. In Spain cryptosporidiosis among children was shown to be highest in the autumn-winter periods (Clavel et al., 1996). Local transmission is however more likely to be transmitted by rainfall, farming practices such as the use of manure and human behaviour.

Studies in Kenya show prevalence rates of about 4% (Gatei et al., 2006 and 3.8% Simwa et al., 1989) in children. In stool samples submitted for routine ova and parasites examination in Kenyatta National Hospital a prevalence of 3.8% was reported (Estambale et al., 1989). A prevalence rate of 17% in HIV infected adults has also been documented in Nairobi (Mwachari et al., 1998). There are however geographical differences in the prevalence of cryptosporidiosis in Kenya. In a recent study in Kenya Gatei et al., (2006) indicated that 46% of children infected with cryptosporidiosis came from peri-urban areas, 14.2% came from towns and 40% were
from rural areas. Thus residency may be a risk factor in contracting cryptosporidiosis. Furthermore this study indicated that the peak prevalence of cryptosporidiosis is among children aged 13 and 24 months. There were no sex differences in the infection rates. The study also showed that Cryptosporidium infections had a bimodal transmission with the highest infection rates of 6.1-8.2% occurring between November and February, this is a typical dry season that follows the short rains. A lower peak was observed in June-July with infection rates of 3-4.4%, in Kenya this is the dry and cold season that follows the long rainy period of April-May. Differences in the disease prevalence in Kenya could be due to geographic, social and economic variations. Such variations should be considered when conducting studies on cryptosporidiosis in Kenya.

1.6 Clinical Manifestation of Cryptosporidiosis

Clinical manifestations of cryptosporidiosis vary with age and immunological status. In children the most prominent symptoms include diarrhoea, vomiting and abdominal swelling (Gatei, et al., 2006; Adjei et al., 2004; Tuminwe et al., 2003). Other symptoms include bloody stool, mucus, and dehydration (Abdel-Messeh, et al., 2005). There are also indications that cryptosporidiosis can occur in cases where children have no diarrhoea (Adjei et al., 2004; Newman et al., 1999). A study in India showed presence of asymptomatic cryptosporidiosis in children (Palit et al., 2005), however opinion about these symptoms is mixed for example Gatei et al. (2006) found chronic diarrhoea, vomiting, and abdominal swelling to be statistically associated with cryptosporidiosis. In contrast Adjei et al. (2004) reported cases of vomiting among children with cryptosporidiosis but the association was not statistically significant.
Such mixed opinions render the clinical diagnosis of cryptosporidiosis difficult. Thus
need exists to identify the most prominent clinical manifestations of cryptosporidiosis.

In outbreak settings immunocompetent adults may have voluminous but self limiting
diarrhoea, with or without abdominal cramps, fatigue, vomiting and other symptoms
(Xiao and Ryan, 2004). However in immunodeficient humans, cryptosporidiosis can
be associated with chronic and potentially life-threatening diarrhoea (Cama et al.,
2003). A number of studies in Kenya have demonstrated the presence of
Cryptosporidium among immunocompromised individuals but are silent on the
clinical manifestations of cryptosporidiosis (Gatei et al., 2003; 2002) and Morgan et
al. (1999d). This is a study gap that needs further exploration.

1.7 Diagnosis and Molecular Tools for Detecting Cryptosporidiosis

Diagnosis of cryptosporidiosis is usually required for all patients with acute or
persistent diarrhea especially if they are immunocompromised. Definitive diagnosis
requires microscopic detection of parasite in body tissues or fluids (Chen et al., 2002),
even though clinical, endoscopic, immunologic and molecular techniques have a place
in the diagnosis and clinical assessment of cryptosporidiosis (Farthing, 2000).
Modified acid-fast staining of oocysts is the simplest method of detecting
Cryptosporidium. This method was utilized to identify Cryptosporidium positive
cases. The sensitivity and the specificity of this test in diagnostic laboratories can be
improved by newer tools such as immunofluorescent assays (IFA) and antigen-capture
enzyme-linked immunosorbent assays (ELISA).
In research settings polymerase-chain-reaction-based (PCR) techniques are available. PCR may target structural or housekeeping genes such as the 18S rRNA gene or Cryptosporidium-specific genes such as the Cryptosporidium oocyst cell wall protein (COWP), thrombospondin-related adhesive proteins 1 and 2 (TRAP-C1 and 2) and 60kDa glycoprotein (Jiang and Xiao, 2003).

Currently, most detection and genotyping methods utilize the 18S rRNA sequence of Cryptosporidium, since extensive sequence data are available for most of Cryptosporidium species and the gene has high (five) copy number (Sunnotel et al., 2006). Unlike PCR tools based on other genes, genotyping tools targeting the 18S rRNA gene can detect Cryptosporidium species that are genetically distant from C. parvum and C. hominis, but nevertheless can infect humans, such as C. canis, C. felis, C. muris and C. suis (Jiang and Xiao, 2003).

Two widely used genotyping methods in studies in Kenya are nested PCR and restriction fragment length polymorphism (RFLP) (Gatei et al., 2006, 2004, 2002; Morgan et al., 2000; 1999). Nested PCR is a two step technique that uses two types of primers. PCR-RFLP is the restriction analysis of PCR products after amplification of genomic DNA. Similar methods were used in this study.

1.8 Problem Statement

In Kenya, Cryptosporidium is not routinely investigated in clinical laboratories since its occurrence is considered insignificant. The prevalence of Cryptosporidium in the country among children has been established to be around 4% (Gatei, et al., 2006; Estambale et al., 1989; Simwa et al., 1989; Kangethe et al., 1985). A few studies such
as Gatei et al. (2006; 2002) and Morgan et al. (2000) have also examined Cryptosporidium species and strains infecting humans and their epidemiologic implications.

The studies failed to control for the residency of study subjects, a factor that has been shown to influence contracting Cryptosporidiosis (Gatei, et al., 2006), therefore there is still lack of in-depth understanding of the population structure of Cryptosporidium in the country which limits public health knowledge of cryptosporidiosis including the targeting of the most appropriate interventions. To address this problem this study identified and genotyped Cryptosporidium species among children attending Mathare North Health Centre.

1.9 Justification

The study of enteric protozoa has received little attention in Kenya, perhaps due to the relatively higher occurrence of bacteria and/or viral etiologic agents causing diarrhoea. Routine faecal examination does not include identification of spore cyst forming protozoa and no local data on the correlation of cryptosporidiosis with other parasitic aetiologic agents of diarrhoea is available.

Since there is no specific treatment of cryptosporidiosis currently available in the country, its prevention is of primary importance. This would involve more stringent quality control of water treatment plants, protection of water sources from human and animal contamination and early detection of primary cases by routine examination of stool in symptomatic children. Such stringent interventional measures can only be implemented if the magnitude and socio-demographic data of cryptosporidiosis is known. Molecular analysis of Cryptosporidium to characterize the current strains
Routine ova and parasite examination methods of faeces are not sufficient for the detection of Cryptosporidium cysts. Results from the current study will increase clinicians' awareness of Cryptosporidium. The information will facilitate policy guideline formulation and enhance the level of capacity in our laboratories. The genotypic analysis will lead to more conclusive diagnosis and speculation on possible sources of infection.

1.10 Research Questions

The research questions addressed in the present study are:

i. What is the role of Cryptosporidium in the aetiology of diarrhea among children in Mathare?

ii. What are the circulating Cryptosporidium species among children with diarrhoea in Mathare?

iii. What are the major risk factors associated with cryptosporidial infection among children in Mathare?

1.11 Hypothesis

Cryptosporidium has no significant role in paediatric diarrhea in Mathare.

1.12 Objectives of the Study

1.12.1 General Objective

To determine the role of cryptosporidia as a cause of diarrhoea in children in Mathare North in Nairobi.
1. 12. 2 Specific Objectives

i. To determine the prevalence of *Cryptosporidium* species in children aged 12 years and below in Mathare North Health Centre.

ii. To establish if there is any correlation between infection with *Cryptosporidium* and other intestinal pathogenic protozoa such as *Giardia*, *Entamoeba* and *Isospora*.

iii. To characterize pathogenic strains of *Cryptosporidium* infecting children.

iv. To establish the correlation between *Cryptosporidium* infection with social-economic and demographic factors.
2.1 Study Design

The study was a cross-sectional survey carried out at Mathare North Health Centre (MNHC), the only public health facility serving the residents of Mathare North most of whom are low income slum dwellers. Mathare North Health Centre was selected due to lack of data on Cryptosporidium among its target population. Laboratory services are available, affordable and accessible to the communities but there is no facility for stool examination thus, no study has been carried out in the area on Cryptosporidium infections. Parasitological and genetic investigations were done in the parasitology laboratory of Centre for Microbiology Research (KEMRI).

2.2 Sample Size Determination

Sample size calculation was based on the prevalence values of Cryptosporidium from other studies especially Gatei et al. (2006) and Mwachari et al. (1998) in Kenya. These studies indicate that the prevalence of Cryptosporidium is unlikely to exceed 15% in Kenya. The minimum number of samples was estimated using the formula by Lwanga and Lemeshow (1991).

\[ n = \frac{D z^2 p (1-p)}{d^2} \]

Where; \( n \) = minimum sample size

\( p \) = prevalence of Cryptosporidium from other studies (15%).

\( d \) = degree of precision ± 5%

\( z \) = 1.96 (from tables of standard normal distribution) corresponds to 95% confidence interval

\( D \) is the design effect (2 in this case)
Therefore n = 391 children. To allow for a no-response expectation, the sample size was increased to 401 children.

2.3 Inclusion and Exclusion Criteria

Children aged 12 years and below for whom informed consent by parent or guardian had been obtained were included while children aged 12 years and below for whom informed consent by parent or guardian not been obtained were excluded.

2.4 Ethical Consideration

(i) Consent ing parent or guardian was briefed about the study and signed a written consent form.

(ii) All information and results remained confidential and were only used for the purposes of the study.

(iii) Results useful in the treatment of the children were communicated to the attending doctors for management.

(iv) Ethical clearance was obtained from the KEMRI/ National Ethical Review Committee (Appendix 4).

2.5 Sample Collection and Processing

Fresh stool samples were collected in polypot containers from each patient, labeled and taken to the examination laboratory within two hours of collection. During sample collection questionnaires were administered. The specimens were subjected to gross examination for colour and consistency. Direct and concentrated microscopic examination for ova and parasites were carried out.
2.6 Parasitological Examination of Faecal Samples

Parasite identification was based on morphology and size of cysts and trophozoite in direct wet mounts and formal ethyl acetate concentration preparations. A drop of saline was placed on the centre of the left half of a labeled slide and a drop of iodine placed in the centre of the right half of the slide. With an applicator stick, a small portion of faeces (approximately 2mg which is about the size of a matchstick head) picked and added it to the drop of saline. The same was repeated on the drop of iodine. The two were mixed separately to form a suspension. Each drop was covered with a cover slip by holding the cover slip at an angle touching the edge of the drop and gently lowering the cover slip onto the slide so that air bubbles were not trapped under the cover slip. Examination of the preparation was done with the X10 objective. If needed for identification arose, higher power objectives were used so that the entire area was observed. When organisms or suspicious objects were seen, observation was done using X400 magnifications to see a more detailed morphology of the object in question (Forbes et al., 1998).

The Modified Acid Fast staining technique was used to detect and identify Cryptosporidium and related coccidian parasites. Each slide was labeled with a specific laboratory number. A pea size stool amount was used to make wet smears on the labelled slides using a drop of normal saline. This was left to dry completely and fixed by dipping them 6 times in Absolute alcohol and left to dry. Slides were placed in Carbol fuschin for about 7 minutes and washed in running tap water. Smears were then decolorized in 3% Acid alcohol, until no more colour came out and
subsequently washed in running tap water. They were counterstained in Malachite green for 7-10 minutes, washed, left to dry observed under X1000. All samples positive for *Cryptosporidium* by staining were aliquoated in 2.5% potassium dichromate (stored at 4°C) and 75% ethanol (stored at room temperature) for genomic DNA extraction.

2.7 Molecular Characterization of *Cryptosporidium* Strains

2.7.1 DNA Extraction from stool samples.

Genomic DNA was extracted from frozen stool using a modified QIAamp® DNA stool kit (Qiagen). Approximately 200 mg (200 µl) of stool sample was added to 2 ml microcentrifuge tubes. One ml of distilled water was added, mixed thoroughly with an applicator stick and vortexed for 15 sec. The mixture was centrifuged for 5 min at 13,000 rpm in a microcentrifuge and the supernatant poured out. A volume of 1 ml of distilled water was added to the stool and repeated four times. After the final wash, 1.4 ml of ASL (stool lysis buffer) was added and vortexed for 1 min, then placed in a water bath at 75°C for 15 min. The mixture was vortexed for 15 seconds and frozen at 80°C for 30 min; the heating and freezing steps were repeated 4 times. Freezing and thawing step in addition to lysis buffer raptured the oocysts. The sample was vortexed for 15 sec and centrifuged at 13,000 r.p.m for 1 min to pellet stool particles. One Inhibitor-EX tablet was added into a 2 ml microcentrifuge tube into which 1.2 ml of the supernatant was added and the pellet discarded. This helped in removal of DNA damage-substances and inhibitors of downstream manipulations of DNA (PCR, restriction digestions). The tube was vortexed continuously until the tablet was completely dissolved, incubated for 1 min at room temperature and then centrifuged at 13,000 r.p.m for 3 min.
All the supernatant was transferred into a new 1.5 ml microcentrifuge tube by
decanting, the pellet was discarded and sample centrifuged at 13,000 rpm for 3 min.
15 μl of Proteinase K was added into a new 1.5 ml centrifuge tube. A volume of 200
μl of the supernatant from the previous step and 200 μl of buffer AL were added to
the tube containing Proteinase K to help degraded cellular proteins and peptides. The
mixture was vortexed for 15s and incubated at 70°C for 10 min, after which 200 μl of
ethanol (96-100%) was added to the lysate and mixed by vortexing. The ethanol
helped precipitate the DNA. Complete lysate was transferred into labelled QIAmp
spin column inserted into a 2 ml collecting tube, the cap was closed and centrifuged at
13,000 rpm for 1 min. The spin column was placed into a new collecting tube, the
tube containing the filtrate was discarded and 500 μl of buffer AW1 was added into
the spin column and centrifuged for 1 min. The spin was transferred into a new
collecting tube, discarded and the tube containing the filtrate and 500 μl of buffer
AW2 added. The tube was centrifuged for 3 min, the collecting tube discarded and the
spin column transferred into a new 1.5 ml microcentrifuge tube. To elute the DNA, 60
μl of buffer AE was added directly into the spin column membrane, incubated for 1
min at RT and centrifuged for 1 min. The eluted DNA was transferred back to the
spin column, centrifuged for 1 min and kept -20°C.

2.7.2. Nested Polymerase Chain Reaction (PCR)
A preparation of 50μl of master mix for a primary PCR reaction was made. 25.75μl of
distilled water was put in a sterile 2ml eppendorf tube. 5μl of the 10X Perking Elmer
PCR buffer was added .A further 8μl of the 1.5mM dNTPs was added.1.25μl of each
10nM primer (forward & Reverse) was added. To the master mix 6μl of 25mM
MgCl₂,2μl of BSA was added .0.25μl of Taq polymerase was added and the master
mix vortexed briefly obtain homogeneous mixing. 49.5μl of the master mix was aliquoted into labeled 0.2ml tubes. 0.5μl of the template DNA was added into 0.2ml containing the master mix, vortexed briefly and placed into a thermocycler.

A total of 35 cycles each consisting of 94°C for 45 seconds for de-naturation, 58°C for 45 seconds for annealing and 72°C for 60 seconds for extension/polymerization with initial hot start at 94°C for 3 minutes and a final extension step at 72°C for 7 minutes. The products were stored at 4°C.

A master mix for a 50μl secondary PCR was prepared as follows. Distilled water (24.75μl) was put in a sterile 1.5ml or 2ml eppendorf tube. 5μl of the 10X Perking Elmer PCR buffer was added. An additional 8μl of the 1.5mM dNTPs was put followed by 2.5μl of each 10nM primer (forward and reverse). To the master mix 6μl of 25mM MgCl₂ added. 0.25μl of Taq polymerase was added and the master mix vortexed briefly for homogeneous mixing. 49μl of the master mix was aliquoted into labeled 0.2ml tubes. 1μl of the primary PCR product was added into 0.2ml containing the master mix, vortexed briefly and placed into a thermocycler. The same program is used for the secondary PCR.

The entire 18S SSU rRNA gene (1325 bp) was amplified from genomic DNA by primers F1 and R1 (Table 2a) by standard PCR conditions consisting of initial denaturation at 94°C for 3 min, 35 cycles each consisting of 94°C for 45 s, 58°C for 45 s and 72°C for 1 min and final extension at 72°C for 7 min. The second pair of primers F2 and R2 (Table 2b) amplified a short fragment of the gene ~ 830 to 860, the same cycling parameters were used for the secondary PCR but using primary PCR
product as DNA template. The secondary PCR product (10 μl) was separated on 1.5 % (w/v) agarose gel after adding 2 μl of 6 × loading dye.

Table 2a: PCR components for primary amplification of 18S rRNA gene

<table>
<thead>
<tr>
<th>PCR components</th>
<th>Volume (50 μl)</th>
<th>Master mix 18 reactions (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 × Perking Elmer PCR buffer</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>1.5 mM dNTP</td>
<td>8</td>
<td>144</td>
</tr>
<tr>
<td>10 μM 18S primer (F1)</td>
<td>1.25</td>
<td>22.5</td>
</tr>
<tr>
<td>10 μM 18S primer (R1)</td>
<td>1.25</td>
<td>22.5</td>
</tr>
<tr>
<td>25 mM MgCl₂</td>
<td>6</td>
<td>108</td>
</tr>
<tr>
<td>10 mg/ml Bovine serum albumin</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>Distilled water</td>
<td>25.75</td>
<td>463.5</td>
</tr>
<tr>
<td>Taq DNA polymerase</td>
<td>0.25</td>
<td>4.5</td>
</tr>
</tbody>
</table>

To 49.5 μl of primary master mix 0.5 μl of specific genomic DNA was added.

Table 2b: PCR components for secondary amplification of 18S rRNA gene

<table>
<thead>
<tr>
<th>PCR components</th>
<th>Volume (50 μl)</th>
<th>Master mix 18 reactions (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 × Perking Elmer PCR buffer</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>1.5 mM dNTP</td>
<td>8</td>
<td>144</td>
</tr>
<tr>
<td>10 μM 18S primer (F2)</td>
<td>2.5</td>
<td>45</td>
</tr>
<tr>
<td>10 μM 18S primer (R2)</td>
<td>2.5</td>
<td>45</td>
</tr>
<tr>
<td>25 mM MgCl₂</td>
<td>6</td>
<td>108</td>
</tr>
<tr>
<td>Distilled water</td>
<td>24.75</td>
<td>444.5</td>
</tr>
<tr>
<td>Taq DNA polymerase</td>
<td>0.25</td>
<td>4.5</td>
</tr>
</tbody>
</table>

To 49 μl of secondary PCR master mix 1 μl of primary PCR product was added.

Table 2c: 18S rRNA gene primers (primary and secondary)

<table>
<thead>
<tr>
<th>Name of primer</th>
<th>Sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>TTC TAG AGC TAA TAC ATG CG</td>
</tr>
<tr>
<td>R1</td>
<td>CCC ATT TCC TTC GAA ACA GGA</td>
</tr>
<tr>
<td>F2</td>
<td>GGA AGG GTT GTA TTT ATT AGA TAA AG</td>
</tr>
<tr>
<td>R2</td>
<td>CTC ATA AGG TGC TGA AGG AGT A</td>
</tr>
</tbody>
</table>
Agarose 1.5g was weighed into a clean conical flask and 100ml of 1X TBE buffer added. It was melted in a micro-wave and placed onto a stirrer to cool it. To the cooled gel 2.5μl of Ethidium bromide (10mg/ml) was added. The casting tray was assembled and positioned on a level surface bench designated for DNA electrophoresis. After placing the sample comb on one end of the tray, 1.5% (w/v) agarose gel was cooled to approximately 55°C (RT), and poured on the assembled casting tray. The combs were carefully removed upon setting of the gel; the casting tray was transferred into electrode chamber containing 1 × TBE buffer submerging the gel. The DNA samples were mixed with loading dye and loaded into the well, molecular weight marker was also loaded alongside. The samples were separated at 80 V for 1-2 h, until the orange dye of the 6X loading comes out of the gel. The gel was visualised by transilluminator under UV light and photographed using Polaroid camera.

2.7.4 Restriction Fragment Length Polymorphism (RFLP)

Molecular characterisations of Cryptosporidium, secondary PCR products of 18S rRNA gene were digested using two restriction enzymes (SspI and VspI). This region of the gene is very polymorphic and is used to differentiate various Cryptosporidium species.

The secondary PCR product (10μl) was placed into a sterile 0.2ml tube in two sets one for Ssp I and another Vsp I. A restriction buffer (4μl) was added respectively followed by addition of 4μl of Ssp I and 2μl Vsp I into the respective set of tubes. To the two tubes, 22μl & 24μl of distilled water was added into the Ssp I and Vsp I reactions respectively. They were vortexed briefly and incubated at 37°C overnight. Analysis of 20μl of the digest was done on 2% agarose gel with the Ssp I reaction on
the upper lane and \( Vsp\, I \) reaction on the lower lane of the gel for comparison.

Electrophoresis was carried out for 1 hour at 100V, then photographed on UV trans-illuminator using a Polaroid camera.

Table 3: Composition of reaction mixture for digestion of secondary PCR product with \( Ssp1 \) and \( Vsp1 \)

<table>
<thead>
<tr>
<th>Components</th>
<th>( Ssp1 ) reaction (40( \mu l ))</th>
<th>( Vsp1 ) reaction (40( \mu l ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary PCR product</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>( 10 \times ) buffers ( E ) and ( D ) (Promega)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Enzyme (Promega)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Distilled water</td>
<td>25</td>
<td>24</td>
</tr>
</tbody>
</table>

2.8 Data Management and Analysis

Data was summarized and presented using frequency tables, charts and graphs. Photomicrographs of \( Cryptosporidium \) oocysts were also used to present data.

Differences in various variables between positive and negative cases of cryptosporidiosis were performed using Pearson’s \( \chi^2 \). Fisher Exact tests were used when the expected frequencies were less than five. \( P \) values of less than 0.05 were considered as statistically significant. All the statistical tests for this study were performed using the Statistical Package for Social Sciences (SPSS) Version 13.0.
CHAPTER THREE
RESULTS

3.1 Prevalence of Cryptosporidium

A total of 401 samples were examined between January-May, 2006. The prevalence rate for Cryptosporidium spp. was 3.2 % (n =13) (Figure 2).

![Pie chart showing prevalence of Cryptosporidium](image)

Figure 2: Prevalence of Cryptosporidium in diarrhea samples

3.1.1 Age Distribution and Prevalence of Cryptosporidium

The age distribution of the sampled children is shown in Figure 3. Children examined in this study ranged from one month to 12 years old. Most of the children were in the 2-3 years age group (23.7%) followed by the 1-2 years age group (21.2%). Each of the remaining age groups constituted less than 20% of the study population.
Figure 3: Age distribution of sampled children

The distribution of Cryptosporidium among the age groups is shown in Table 4. The 1-2 years age group had the highest prevalence rate at 7.1%, followed by the 0-1 year age group with a prevalence of 6.7%. The 2-3 year and 3-4 year age groups had a prevalence rate than 2%. There were no positive cases in children above four years.

Table 4: Distribution of Cryptosporidium among the different age groups

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Number of stool samples</th>
<th>Number of positive samples</th>
<th>Percentage positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>73</td>
<td>5</td>
<td>6.7</td>
</tr>
<tr>
<td>1 &lt; 2</td>
<td>85</td>
<td>6</td>
<td>7.1</td>
</tr>
<tr>
<td>2 &lt; 3</td>
<td>95</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>3 &lt; 4</td>
<td>66</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>4 &lt; 5</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 &lt; 12</td>
<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>401</td>
<td>13</td>
<td>3.2</td>
</tr>
</tbody>
</table>
3.1.2 Distribution of *Cryptosporidium* among the sexes

Males accounted for 50.6% of the study sample while the females constituted 49.4%.

Most of the children aged below one year were females (52.1%). Likewise, the age groups of 4-5 years and 5-12 years had more females than males (Table 5).

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Sex</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>35</td>
<td>38</td>
</tr>
<tr>
<td>1 &lt; 2</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>2 &lt; 3</td>
<td>48</td>
<td>47</td>
</tr>
<tr>
<td>3 &lt; 4</td>
<td>36</td>
<td>30</td>
</tr>
<tr>
<td>4 &lt; 5</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>5 &lt; 12</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>203</td>
<td>198</td>
</tr>
</tbody>
</table>

Six males (2.96 %) and seven females (3.54 %) tested positive for *Cryptosporidium*. However, the differences in infection rates among the sexes was not statistically significant ($\chi^2 = 0.107$, df = 1, $p = 0.743$).

3.1.3 Diarrhoeal Symptoms

The different symptoms exhibited by the sampled children are shown in Table 6. A majority of the sampled children (87.3 %) presented with complaints of abdominal pain. The next most common symptom was recurrent diarrhoea which was observed in 86% of the children. Acute diarrhoea was observed in 75.1% of the children, while vomiting accounted for 51.1% of the cases. Chronic diarrhoea, abdominal swelling and other symptoms such as rashes and sweating were observed in less that 50% of the cases.
Table 6: Number of individuals exhibiting different symptoms

<table>
<thead>
<tr>
<th>Clinical Symptoms</th>
<th>Present</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Diarrhoea</td>
<td>301</td>
<td>75.1</td>
</tr>
<tr>
<td>Chronic Diarrhoea</td>
<td>68</td>
<td>17</td>
</tr>
<tr>
<td>Recurrent Diarrhoea</td>
<td>345</td>
<td>86</td>
</tr>
<tr>
<td>Vomiting</td>
<td>205</td>
<td>51.1</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>350</td>
<td>87.3</td>
</tr>
<tr>
<td>Abdominal Swelling</td>
<td>161</td>
<td>40.1</td>
</tr>
<tr>
<td>Other Symptoms</td>
<td>67</td>
<td>16.7</td>
</tr>
</tbody>
</table>

Table 7 shows the number of individuals, 75% of the children had acute diarrhea with children aged between 2 and 3 years had the highest incidences of acute diarrhoea (27%). Children aged between 1 and 2 years had the second highest incidences of acute diarrhoea (21%) while those aged between 1 and 2 years had 20% reported cases of acute diarrhoea. The remaining age groups had fewer than fifty cases of acute diarrhoea. The relationship between the incidence of acute diarrhoea and age group was statistically significant ($\chi^2 = 14.405, \text{df} = 1, p = 0.013$).

Fifty one percent of the sampled children had episodes of vomiting. Children aged between 2 and 3 years had the highest incidences (24%) of vomiting followed by those aged between 0 and 1 year (23.4%). Forty one cases and thirty four cases of vomiting were reported in children aged between 1 and 2 years and 3 and 4 years respectively. Children above four years had the least incidences of vomiting. The
relationship between the incidence of vomiting and age group was statistically significant ($\chi^2 = 13.490$, df = 1, $p = 0.019$).

Table 7: Age Group and clinical symptoms

<table>
<thead>
<tr>
<th>Clinical Symptoms</th>
<th>0 &lt; 1</th>
<th>1 &lt; 2</th>
<th>2 &lt; 3</th>
<th>3 &lt; 4</th>
<th>4 &lt; 5</th>
<th>5 &lt; 12</th>
<th>Total</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Diarrhoea</td>
<td>59</td>
<td>63</td>
<td>81</td>
<td>47</td>
<td>22</td>
<td>29</td>
<td>301</td>
<td>$p = 0.013$</td>
</tr>
<tr>
<td>Chronic Diarrhoea</td>
<td>11</td>
<td>13</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>68</td>
<td>$p = 0.055$</td>
</tr>
<tr>
<td>Recurrent Diarrhoea</td>
<td>48</td>
<td>77</td>
<td>86</td>
<td>54</td>
<td>35</td>
<td>45</td>
<td>345</td>
<td>$p = 0.000$</td>
</tr>
<tr>
<td>Vomiting</td>
<td>48</td>
<td>41</td>
<td>49</td>
<td>34</td>
<td>18</td>
<td>15</td>
<td>205</td>
<td>$p = 0.019$</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>57</td>
<td>70</td>
<td>86</td>
<td>60</td>
<td>34</td>
<td>43</td>
<td>350</td>
<td>$p = 0.024$</td>
</tr>
<tr>
<td>Abdominal Swelling</td>
<td>28</td>
<td>38</td>
<td>34</td>
<td>20</td>
<td>25</td>
<td>16</td>
<td>161</td>
<td>$p = 0.002$</td>
</tr>
<tr>
<td>Other Symptoms</td>
<td>11</td>
<td>19</td>
<td>18</td>
<td>10</td>
<td>0</td>
<td>9</td>
<td>47</td>
<td>$p = 0.082$</td>
</tr>
</tbody>
</table>

Forty percent of the sampled children had signs of abdominal swelling. Sampled children aged between 1 and 2 had the highest case of abdominal swelling (23.6%) followed by those aged between 2 and 3 years with 21% of the cases. The age group of less than 1 year had 15.5% cases of abdominal swelling. The other age groups had below 12.3% of the cases of abdominal swelling. Abdominal swelling was statistically associated with age distribution of the sampled children ($\chi^2 = 19.226$, df = 1, $p = 0.002$).

Eighty seven percent of the sampled children complained of abdominal pain. The age group 2 < 3 years had the highest case of abdominal pain (24.6%) followed by the 1 < 2 year age group (20%). The 3 < 4 year age group had 17.1% cases of abdominal pain while that of between less than one year had 16.3% of the cases. The other age
groups had below 14.3% of the cases of abdominal pain. The relationship between age distribution and abdominal pain was statistically significant ($\chi^2 = 12.924$, df = 1, $\rho = 0.024$).

A total of 345 children out of the 401 (85%) had incidences of recurrent diarrhoea. Slightly more males (50.1%) than females (49.9%) had recurrent diarrhoea. The relationship between sex and presence of recurrent diarrhoea was not statistically significant ($\chi^2 = 0.226$, df = 1, $\rho = 0.634$).

Seventeen percent of the sampled children had chronic diarrhoea. More females (57.4%) than males (42.6%) had incidences of chronic diarrhoea. However the relationship between sex distribution and presence of chronic diarrhoea was not statistically significant ($\chi^2 = 2.084$, df = 1, $\rho = 0.149$).

The distribution of presence of chronic diarrhoea among the different age groups was even. The age group of 1-2 had the highest number of cases at 19.1% followed by the age group of between 5 and 12 years with 17.6% of the cases. All the other age groups had equal number of chronic diarrhea cases at 31.4%. The association between age group and chronic diarrhoea was not statistically significant ($\chi^2 = 10.805$, df = 5, $\rho = 0.055$).

Children aged between 2 and 3 years had the most case of recurrent diarrhoea (25%). The age group 1-2 years had the next most frequent cases (22.3%) followed by the age group of 3-4 years which had 15.7% cases. All the children in the age group 4-5 had recurrent diarrhoea 10.1% of the cases. The relationship between age group and
the presence of recurrent diarrhoea was statistically significant ($\chi^2 = 38.4$, df = 5, $p = 0.000$).

Seventeen percent of the sampled children had signs of other symptoms such as fever, sweating and rashes. The age group of 1-2 years had the highest number of cases of other symptoms (28.4%) followed by the age group of 2-3 years. The less than one year age group had 16.7% cases of other symptoms. All the other age groups had less than ten cases of other symptoms. The age group of 4-5 years did not have any cases of other symptoms.

One hundred and fifty seven males had acute diarrhoea compared to 144 cases females. (Table 8). The relationship between sexes and the incidence of acute diarrhoea was not significant ($\chi^2 = 1.139$, df = 1, $p = 0.286$). More males (52.2%) reported cases of vomiting than the females (47.8%). There was no significant relationship between the sex of sampled children and the incidence of vomiting ($\chi^2 = .414$, df= 1, $p = 0.520$).

More males (51.6%) than females (48.4%) had cases of abdominal swelling but there was no significant association between sex and the incidence of abdominal swelling ($\chi^2 = 0.93$, df = 1, $p = 0.760$). Slightly more females (50.3%) than males (49.7%) had abdominal pain complaints. Sex distribution and the presence of abdominal pain was not statistically significant ($\chi^2 = .910$, df= 1, $p = 0.34$).
Eleven out of the thirteen positive cases of Cryptosporidium had incidences of vomiting, which was significantly associated with Cryptosporidium ($\chi^2 = 6.032, \text{df} = 1, \rho = 0.014$). Table 9 shows the relationships between Cryptosporidium and clinical symptoms. Twelve out of the thirteen positive cases of Cryptosporidium had incidences of acute diarrhoea. There was no significant association between the presence of Cryptosporidium and acute diarrhoea (Fishers Exact Test, $\rho = 0.200$). Five positive cases of Cryptosporidium had incidences of abdominal swelling. Abdominal swelling was not significantly associated with the presence of Cryptosporidium. Twelve out of the thirteen positive cases of Cryptosporidium had abdominal pain. However abdominal pain was not statistically associated with the presence of Cryptosporidium (Fishers Exact Test, $\rho = 1.000$). Ten of the thirteen positive cases of Cryptosporidium had recurrent diarrhoea. The presence of recurrent diarrhoea was not significantly associated with presence of Cryptosporidium (Fisher's Exact Test, $\rho = 0.405$). All the 68 cases of chronic diarrhoea did not have any
positive cases of *Cryptosporidium*. Thus the association between chronic diarrhoea and the presence of *Cryptosporidium* was not significant (Fisher’s Exact Test, $\rho = 0.137$). Three of the thirteen positive cases of *Cryptosporidium* had cases of other symptoms. There was no significant association between the presence of other symptoms and the presence of *Cryptosporidium* (Fisher’s Exact Test, $\rho = 0.463$).

Table 9: Association of clinical symptoms to *Cryptosporidium* infection and clinical symptoms

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Positive (n = 13)</th>
<th>Negative (n = 388)</th>
<th>$\rho$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Diarrhoea</td>
<td>12</td>
<td>289</td>
<td>0.200</td>
</tr>
<tr>
<td>Vomiting</td>
<td>11</td>
<td>194</td>
<td>0.014*</td>
</tr>
<tr>
<td>Abdominal swelling</td>
<td>5</td>
<td>156</td>
<td>0.900</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>12</td>
<td>338</td>
<td>1.000</td>
</tr>
<tr>
<td>Recurrent diarrhoea</td>
<td>10</td>
<td>335</td>
<td>0.405</td>
</tr>
<tr>
<td>Chronic Diarrhoea</td>
<td>0</td>
<td>68</td>
<td>0.137</td>
</tr>
<tr>
<td>Other Symptoms</td>
<td>3</td>
<td>64</td>
<td>0.463</td>
</tr>
</tbody>
</table>

Level of significance: * indicates $\rho < 0.05$

3.2 Co-infection

Table 10 shows protozoan parasites isolated from stool samples. *Giardia intestinalis* was the most common protozoa and was observed in 21 cases in the stool samples giving an infection rate of 5.2%. The second most common protozoa identified was *Cryptosporidium* with 13 cases (3.2%) followed by *Entamoeba coli* with 9 cases (2.2%). The least was *Entamoeba histolytica/dispar* with six cases (1.5%).

Table 10: Number of protozoan parasites isolated from the stool samples

<table>
<thead>
<tr>
<th></th>
<th>Frequency (n = 401)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptosporidium</em></td>
<td>13</td>
<td>3.2</td>
</tr>
<tr>
<td><em>Entamoeba coli</em></td>
<td>9</td>
<td>2.2</td>
</tr>
<tr>
<td><em>Giardia intestinalis</em></td>
<td>21</td>
<td>5.2</td>
</tr>
<tr>
<td><em>Entamoeba histolytica/dispar</em></td>
<td>6</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Table 11 presents prevalence of nematodes identified, four nematodes were identified in the stool samples. *Ascaris lumbricoides* was the most frequent with 12 cases out of the 401 stool samples. This is a prevalence rate of 3%. *Trichuris trichiura* with cases was the second most frequent nematode (1% prevalence) identified. *Hookworm* and *Hymenolepis nana* were the least identified nematodes with 2/401 cases each (0.5% prevalence).

There was only one co-infection of *Cryptosporidium* with *Giardia intestinalis*. This data is presented in Table 12. The other parasites did not have any co-infections with *Cryptosporidium*.

<table>
<thead>
<tr>
<th></th>
<th>Frequency (n = 401)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichuris trichiura</em></td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td><em>Hookworm</em></td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Hymenolepis nana</em></td>
<td>2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

3.3 Characterization of *Cryptosporidium* Species

The secondary PCR yielded a single product that corresponds to the expected size of between 820-860 base pair (bp) in the figure below.
Figure 4: Nested PCR on *Cryptosporidium* based on 18S rRNA gene. PCR products were separated on 1.5% (w/v) agarose gel and stained with ethidium bromide.
Lane M: molecular weight markers, lane 1- MNHC 04, lane 2- MNHC 011, Lane 3 MNHC 017, lane 4-MNHC 075, lane 5-MNHC 122, lane 6-MNHC 134, lane 7-MNHC 135, lane 8- MNHC 178 and lane 9-MNHC 1707.

RFLP analysis using SspI and VspI digestions were used to differentiate *Cryptosporidium* into various species. The SspI digestion of nested PCR products yielded three bands of approximately 449 bp, 254 bp and 111 bp for all samples except one, which showed two bands at about 390 bp and 426 bp.

The digestion of nested PCR products with VspI yielded two distinct bands of approximately 561 bp and 104 bp for lane 1-7, while lane 8 showed bands of about 630 bp and 104 bp and lane 9 showed three bands of about 476 bp, 182 bp and 104 bp. From the results of the two digestions and in reference to an identification tool
developed by Jiang and Xiao (2003), that lanes 1-7 was identified as *C. hominis*, lane 8 *C. parvum* and lane 9 *C. felis*.

Figures 5A and B: Restriction digests of secondary PCR products using *SspI* and *VspI* respectively.

Digests were separated on 2% (w/v) agarose gel and stained with ethidium bromide (section). Lane M:molecular weight markers, lane 1-MNHC 04, lane 2-MNHC 011, Lane 3- MNHC 017, lane 4- MNHC 075, lane 5 -MNHC 122, lane 6-MNHC 134, lane 7-MNHC 135, lane 8-MNHC 178 and lane 9-MNHC 1707.
3.4 Socio-demographic Factors Affecting the Prevalence of *Cryptosporidium*

3.4.1 Source of Water

A majority of the sampled children (99.3%) indicated that tap water was their main source of water. All the *Cryptosporidium* positive cases were isolated from children responding to the usage of tap water. However there was no significant association between usage of this source and the presence of *Cryptosporidium* (Fisher’s Exact Test, \( p = 1.000 \)).

3.4.2 Water Treatment

Two hundred and forty eight of the 401 respondents for the sampled children indicated that they normally treat their drinking water. Eight of the children whose respondents indicated that they normally treat their water tested positive for *Cryptosporidium*. There was no significant association between treatment of water and the presence of *Cryptosporidium* (Fisher’s Exact Test, \( p = 1.000 \)). Most of the respondents (71.3%) indicated that they treated their drinking water through boiling. The remaining 28.7% indicated that they did not treat their drinking water through boiling; nine (7.8%) of those had cryptosporidiosis. There was no significant association between boiling of water and the presence of *Cryptosporidium* (Fisher’s Exact Test, \( p = 1.000 \)). This data is presented in Table 13 below.

Table 12: Association of *Cryptosporidium* infection to water treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cryptosporidium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n = 13)</td>
<td>248</td>
<td>1.000</td>
</tr>
<tr>
<td>Negative (n = 388)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>401</td>
<td></td>
</tr>
<tr>
<td><strong>Treats Water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (62%)</td>
<td>240</td>
<td>1.000</td>
</tr>
<tr>
<td>Boils Water</td>
<td>4 (31%)</td>
<td>111</td>
</tr>
<tr>
<td>115</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Adds chemicals to water</td>
<td>1 (8%)</td>
<td>37</td>
</tr>
<tr>
<td>38 (9.5%)</td>
<td></td>
<td>1.000</td>
</tr>
</tbody>
</table>
As shown in Table 13 a few (9.2%) respondents indicated that they treat their water chemically. Twelve of those who do not treat their water chemically tested positive for Cryptosporidium. Only one case of Cryptosporidium was tested in the category of respondents using chemicals to treat their drinking water. There was no significant association between the chemical treatment of water and the presence of Cryptosporidium (Fisher’s Exact Test, $p = 1.000$).

### 3.4.3 Number of Rooms at Residence

Most of the respondents indicated that they lived in a single room (87%), while 48 respondents who indicated that they lived in two roomed houses only three respondents indicated that they lived in a three roomed house. A majority (76.9%) of the Cryptosporidium positive cases lived in a one roomed house. Three cases of cryptosporidiosis lived in two roomed houses while none of them lived in three roomed houses. This data is presented in Table 14.

<table>
<thead>
<tr>
<th>Number of Rooms</th>
<th>Cryptosporidium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One</td>
</tr>
<tr>
<td>Negative</td>
<td>339</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>349</td>
</tr>
</tbody>
</table>

### 3.4.4 Household Members

Most of the respondents for the sampled children indicated that they had two family members (53.9%). A hundred and forty eight respondents indicated that they had three family members while fifteen respondents indicated that they had more than four family members. A majority (69.2%) of the Cryptosporidium positive cases came
from a two member family setup. Three cases of cryptosporidiosis came from a three family member set up while came from a family with more than four family members. This data is presented in Table 15.

Table 14: Association of *Cryptosporidium* infection to size of family at residence

<table>
<thead>
<tr>
<th>Household Members</th>
<th>Two</th>
<th>Three</th>
<th>Four</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptosporidium</em></td>
<td>Negative</td>
<td>Positive</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>229</td>
<td>9</td>
<td>238</td>
</tr>
<tr>
<td></td>
<td>145</td>
<td>3</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1</td>
<td>15</td>
</tr>
</tbody>
</table>

3.4.5 Feecal Waste Disposal Methods

Ninety of the respondents for the children indicated that they use pit latrine as their main faecal waste disposal method. Six *Cryptosporidium* positive cases were isolated from children with respondents indicating usage of pit latrines. However, there was no significant association between usage of pit latrines and the presence of *Cryptosporidium* (Fisher’s Exact Test, \( p = 1.000 \)). Table 16.

Table 15: Association of *Cryptosporidium* infection to faecal waste disposal methods

<table>
<thead>
<tr>
<th></th>
<th><em>Cryptosporidium</em></th>
<th>Total</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n = 13)</td>
<td>Pit latrine</td>
<td>6</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Negative (n = 388)</td>
<td>90</td>
<td>388</td>
</tr>
<tr>
<td></td>
<td>Ventilated Improved Pit Latrine</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Flush Toilet</td>
<td>7</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96</td>
<td>289</td>
</tr>
</tbody>
</table>

Fourteen of the respondents used VIP latrines as their in faecal waste disposal method All the *Cryptosporidium* positive cases were isolated from children who do not use VIP latrines. There was no significant association between usage of pit latrines and the presence of *Cryptosporidium* (Fisher’s Exact Test, \( p = 1.000 \)).
As indicated in Table 16, a majority of the respondents for the sampled children indicated that they use flush toilet as their main fecal waste disposal method. Seven Cryptosporidium positive cases were isolated from children with respondents where use of flush toilets was indicated. However, there was no significant association between usage of flush toilets and the presence of Cryptosporidium (Fisher’s Exact Test, $p = 1.000$).

### 3.4.6 Pets

One hundred and forty seven of the respondents indicated that they kept pets at home. Six Cryptosporidium positive cases were isolated from children with respondents indicating that they kept pets. However, there was no significant association between rearing of pets and the presence of Cryptosporidium (Fisher’s Exact Test, $p = 1.000$).
CHAPTER FOUR
DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

*Cryptosporidium* has been confirmed as an emerging pathogen in children. In this study *Cryptosporidium* was shown to be a common enteric parasite associated with diarrhea. However tests for this parasite are not routinely performed as part of ova and parasite diagnosis in Health Centres. The overall prevalence of 3.2% reported here is similar to other observations in Kenya (Gatei *et al.*, 2006; Gatei, 2002; Estambale *et al.*, 1989; Simwa *et al.*, 1989; Kangethe *et al.*, 1985). There is some emerging evidence that the prevalence of *Cryptosporidium* in Kenya does not significantly differ regionally. Gatei *et al.* (2006) reported that the prevalence at Gertrude Gardens Children, Kenyatta National and Aga Khan Hospitals in Nairobi were 2%, 4% and 3.5%, respectively. Likewise the prevalence of *Cryptosporidium* in Narok, Thika and Machakos District Hospitals were 7%, 4% and 4% (Gatei *et al.*, 2006) respectively. Despite studies in diverse periods and locations in Kenya, the prevalence of *Cryptosporidium* appears to be the same. This suggests that Kenya has a reservoir of *Cryptosporidium* that exists in children with the potential to contaminate the environment, thus posing a risk to healthy as well as immunocompromised people. Indeed studies focusing on HIV/AIDS cohorts in Kenya show high prevalence rates of cryptosporidiosis (Gatei, 2002; Mwachari *et al.*, 1998).

The prevalence rate reported in this study falls within the universal prevalence rates which are estimated to be about 3-10% (Gatei, 2002; Saredi and Bava, 1998). Higher prevalence rates for *Cryptosporidium* have been identified in other cross sectional studies such as 17% in Egypt (Abdel-Messeh, 2005), 13% in Tanzania (Cegielski *et
al., 1999) 34% in Uganda (Tumwine et al., 2005) and 12.9% in Ghana (Addy and Aikins-Bekoe, 1986). The differences in these studies can be explained by the methods used to detect Cryptosporidium. Studies showing higher prevalence rates used commercial kits based on direct-fluorescent monoclonal antibody tests, immunofluorescence microscopy or Cryptosporidium enzyme based immunosorbent assay. There is also evidence that immunocompromised children have higher prevalence rates of Cryptosporidium (Tumwine et al., 2003).

The lack of association between Cryptosporidium and sex is similar to previous reports from Kenya (Gatei et al., 2006), Ghana (Adjei et al., 2004) and Peru (Xiao et al., 2001) but differs with that of Laubach et al. (2004) working in Guatemala who found significant differences in the presence of Cryptosporidium in children on sex. These differences can be explained by the varied lifestyle risk factors in different countries. In Mathare, young children are likely to be treated in the same way by their parents irrespective of sex since household chores practices in the urban set up tend to differ from the practices in the rural set up, thus making the risk factors among the sexes very similar.

The higher prevalence rate in the 1-2 year age group (7.1%) is consistent with other studies in Kenya (Gatei et al., 2006), Guatemala (Laubach et al., 2004) and Ghana (Adjei et al., 2004) but contrasts with observations from other developing countries such as Egypt (Abdel-Messeh, 2005; Sallon et al., 1991) and Ghana (Addy et al., 1986) which showed that the highest prevalence rates occur in children below one year. A study in the United Kingdom reported Cryptosporidium prevalence rate of
29.2% in children 19 months of age (Baxby et al., 1984). It is not yet clear why there are differences in susceptibility by age in children.

This study has shown that a relatively high percentage of children exhibited abdominal pain, acute diarrhea, recurrent diarrhoea, abdominal swelling and vomiting. Chronic diarrhoea and other symptoms such as fever, rashes and sweating were not frequently reported. However significant age differences were observed with the following symptoms: acute diarrhoea, recurrent diarrhoea, vomiting, abdominal pain and abdominal swelling. The profile of clinical symptoms suggested in this study agrees with that reported in Kenya (Gatei et al., 2006) and differs slightly with that reported in Ghana (Adjei et al., 2004). Differences in the profiles of symptoms may be related to the emphasis given to nausea, fever and blood in stool in the later study.

Results also indicated that Cryptosporidium is significantly associated with vomiting similar to results reported in other studies in Kenya (Gatei et al., 2006), Tanzania (Cegielski et al., 1999) and Egypt (Abdel-Messeh, 2005). The strong association of Cryptosporidium with vomiting as the sole symptom is unusual in this study. Previous studies suggest that Cryptosporidium is usually associated with vomiting together with other symptoms such as persistent diarrheoa (Gatei et al., 2006; Tumwine et al., 2003) and abdominal pain (Tumwine et al., 2005). The reasons for the observed differences in these results are unclear and render the clinical diagnosis of cryptosporidiosis difficult.

Three protozoa and four nematodes were identified. Giardia intestinalis was the most common protozoa identified (5.2%) which was slightly higher than the 2% prevalence
reported in another study in Kenya (Gatei et al., 2006). The prevalence rate obtained in this study is similar to that reported in Cuba (Núñez et al., 2003) but is however lower than those reported in other developing countries such as 7.1% in Nigeria (Ogunlesi et al., 2006), 7.7% in Ethiopia (Asfaw and Goitom, 2000) and 13.2% in Bangladesh (Hasan et al., 2006).

Other agents identified in this study include Cryptosporidium (3.2%), Ascaris lumbricoides (3%), Entamoeba coli (2.2%), Entamoeba histolytica (1.5%), Trichuris trichiura (1%), Hookworm and Hymenolepis nana (each 0.5%). Similar parasites are reported in previous studies in developing countries (Gatei et al., 2006; Hasan et al., 2006 Ogulesi et al., 2006; Tadesse 2005; Núñez et al., 2003; Chacin-Bonilla and Sanchez-Chavez, 2000; Isaac-Renton et al., 1999). However the profiles differ in the order of prevalence among various parasites and the presence of additional agents. Variability in these results could be explained by differences in socio economic and environmental factors. This underscores the need for continuous surveillance of diarrhoeal aetiologic agents in children in different parts of the world.

Generally Cryptosporidium occurred alone in the sampled children. There was only one co-infection of Cryptosporidium and Giardia intestinalis in this study. This sole coinfection is unusual in this study. Previous studies suggest that Cryptosporidium is usually associated with Giardia intestinalis together with other agents such as Entamoeba coli and Entamoeba histolytica (Gatei et al., 2006). The reasons for the observed differences in these results are unclear at this point since these agents are waterborne.
Socio-economic characteristics of different populations are thought to influence the occurrence of Cryptosporidium. Existing literature has shown that Cryptosporidium occurs in children from low socio-economic background and who live in poor sanitary conditions with no source of clean water (Palit et al., 2005).

This study demonstrated that tap water from the City Council of Nairobi was the main source of water for the study respondents. However there was no significant association between usage of tap water and the presence of Cryptosporidium. This indicates that effective dosages for the elimination of Cryptosporidium oocyst in the treatment of water are not met.

There was no significant association between treatment of water and the presence of Cryptosporidium, with the respondents indicating that they either treat their drinking water by boiling or the use of chemicals. There was no significant association between both boiling or chemical treatment of water and the presence of Cryptosporidium. This result agrees with findings reported by Adjei et al., (2004) and Goncalves et al., (2006). Lack of association between treatment of water and presence of Cryptosporidium suggests that the methods used for treatment of water are not effective. It appears that the amount of time used in boiling of water is inadequate to eliminate Cryptosporidium oocyst. Likewise, the chemical treatment of water in Kenya is generally chlorine based, and the concentrations recommended for treatment for human consumption may not be sufficient enough to kill Cryptosporidium oocyst. Recommendations for the correct management of water resources are available (Bloomfield and Jones, 2002).
Waterborne transmission is considered the major route in the epidemiology of cryptosporidiosis in tropical countries (Sulaiman et al., 2005; Tumwine et al., 2003). Results of this study do not support the common observation that waterborne transmission is the major route in the epidemiology of cryptosporidiosis in tropical countries.

Basic sanitary measures for feacal waste disposal are considered necessary for the control of cryptosporidiosis. The respondents indicated that they broadly use three methods of feacal waste disposal, that is, pit latrines, Ventilated Improved Pit Latrine (VIP) and flush toilets. There was no significant association between usage of any of these three feacal waste disposal methods and the presence of Cryptosporidium. The explanation for this observation is not obvious.

Contact and presence of pets has been associated with the presence of Cryptosporidium in previous studies (Sulaiman et al., 2005). However in this study no significant association was noted between the presence of pets and the presence of Cryptosporidium. This result agrees with a previous report (Adjei et al., 2004) and suggests that pets play an insignificant role in the transmission of Cryptosporidium in the study area.

This study indicated that 77% of Cryptosporidium positive cases lived in a one roomed house. Likewise 69% of the Cryptosporidium positive cases came from a two member family setup. However the association between both size of family and the size of the house was not statistically significant. The high proportion of the presence of Cryptosporidium in children coming from one roomed house is an indicator of
overcrowding. Overcrowding is considered a risk factor in Cryptosporidium (Solórzano-Santos et al., 2000). This suggests that anthroponotic transmission of Cryptosporidium cannot be ruled out.

The recognition and diagnosis of Cryptosporidium is of utmost importance for prompt management of cryptosporidiosis. Results indicate the potential use of simple staining techniques for the identification of parasites. Modified acid staining in this study was found to be an economical, easily applicable and sensitive method in the study site. The molecular analysis confirmed the Cryptosporidium species that were found positive using the staining method, thus ascertaining its usefulness as a molecular epidemiologic tool.

In addition results indicate the potential use of molecular tools in studying the transmissions of Cryptosporidium species. The PCR-RFLP analysis demonstrated the existence of at least three species of Cryptosporidium in humans in the study site: C. hominis, C. parvum and C. felis. C. hominis is almost exclusively a human parasite (Hunter et al., 2007). In contrast C. parvum are responsible for cryptosporidiosis in ruminants especially cattle, sheep and goats (Goncalves et al., 2006) while C. felis is associated with cats (Sulaiman et al., 2005). This profile of Cryptosporidium species in Kenya is similar to that reported in India (Das et al., 2006) but slightly differs with a previous study in Kenya (Gatei et al., 2006). Variations in the profile of the isolated Cryptosporidium species may be due to environmental and socioeconomic differences in study sites.
The identified *Cryptosporidium* species in this study are most likely associated with children from marginalized environments. The children came from an urban slum in Nairobi, where they live in overcrowded rooms and belong to a low socio-economic background characterized by poor hygiene but do not have direct contact with animals. As a result direct person to person transmission probably plays an important role in the epidemiology of cryptosporidiosis in the area. In addition most of the affected children utilize tap water supplied by the City Council of Nairobi. A common observation in the study site is that drinking water is fetched and stored in plastic vessels of unknown hygiene standards. Quite often children and adults dip their hands in these vessels to collect water, a practice that predisposes the stored water to contamination. This study also suggests that zoonotic transmission of *Cryptosporidium* is may not occur in the studied population.

### 4.2 Conclusion

1. This study demonstrated the frequent occurrence of *Cryptosporidium* among children in Mathare with prevalence rate of 3.2%. *Cryptosporidium* spp were more frequent in children younger than two years from deprived socioeconomic environments.

2. Three species of *Cryptosporidium* namely *C. hominis*, *C. parvum* and *C. felis* were identified in this study using PCR-RFLP technique suggesting that anthropogenic transmission plays an important role in the epidemiology of cryptosporidiosis in the area.

3. There was no significant association between both boiling or chemical treatment of water and the presence of *Cryptosporidium*. 
4. Generally, Cryptosporidium occurred alone, and only one co-infection of Cryptosporidium and Giardia intestinalis was found in this study.

5. There was no association between family size or size of the house occupied and infection with Cryptosporidium.

6. There was no significant association between presence of Cryptosporidium and feacal waste disposal method.

7. Cryptosporidiosis was significantly associated with vomiting in this study area.

In this study it was hypothesized that “Cryptosporidium has no significant role in paediatric diarrhea in Mathare”. From the conclusions above the hypothesis was therefore rejected and concluded that Cryptosporidium has a role in paediatric diarrhea in this study area.

Because there was no significant correlation between the incidence of Cryptosporidiosis and water treatment by boiling and chemical means, it is likely that post harvest storage and unhygienic handling may be important predisposition factors to transmission of Cryptosporidium. Poor environmental management, congestion as well as inadequacy of faecal and other solid waste disposal mechanisms may contribute to high incidence of Cryptosporidiosis in the study population. There is need for development of sound environmental policies to address protection of water
catchment and distribution channels, quality control of water treatment plants, storage, hygienic practices and solid waste management in poorly planned overcrowded urban settlement.

4.3 Recommendations

1. More comprehensive epidemiological studies are needed to elucidate accurately the source of *Cryptosporidium* infection. There is need to conduct detailed surveys in different geographical regions to obtain the epidemiology of *Cryptosporidium* in Kenya. Further baseline studies in hospitals should be conducted. Specifically, further sub typing of *Cryptosporidium* species using highly polymorphic makers is needed to improve our understanding of circulating genotypes and transmission pathways in Kenya. Further studies of the risk factors are needed to identify the transmission patterns of cryptosporidiosis.

2. The controversies in the clinical manifestations of cryptosporidiosis should be resolved through more thorough and a wider study. One way will be through the rigorous definition and measurement of the controversial manifestations such as vomiting.

3. Diagnosis of *Cryptosporidium* oocysts should be considered as a standard routine in clinical microbiological diagnosis of diarrhoeal cases in the study site.

4. Cryptosporidiosis should be addressed as a health policy issue. There is need to enhance awareness of health workers on *Cryptosporidium* as a causative agent of idiopathic parasitic diarrhea. Capacity building and sensitization measures on the correct way of boiling water should be initiated. Further there is need to
target cryptosporidiosis interventions on the profiles of children who are predisposed to acquiring cryptosporidiosis that is, children younger than two years of age and who come from deprived socio-economic environments.

REFERENCES


Xiao, L., Escalante, L., Sulaiman, I., Escalante, A. A., Montali, R. J., Fayer, R.

APPENDICES

APPENDIX I: INFORMED CONSENT FORM.

Project title: Role of Cryptosporidium in pediatric diarrhoea.

Patient's name: 
Guardian's name: 
Relationship: 
Date of birth: Age: Sex: 
Clinic Number: 

Purpose of the study. To determine the prevalence of cryptosporidiosis and other protozoan agents of diarrhoea in children aged 12 years and below attending Mathare North Health Centre and their role in paediatric diarrhoea.

Procedure to be followed. In this study you will give a stool sample for parasitological examination and molecular analysis. As a participant in this study you will be interviewed and requested to complete a questionnaire.

Risks. The procedure for collection of the stool sample does not pose any risk to you.

Benefits: Results useful for your treatment obtained from this study will be communicated to your doctor.

Confidentiality of the records. Information gathered from you will be coded for purposes of confidentiality and that your name will not be identified from these records. The code numbers will also be used in reports and publications.

Basis of participation. It is important for you to know that you have choice to decline from participating in this study. Should you have any question or clarification to be made, you can ask the Principal Investigator, Kang'ethe Stanley Kamwati of Tel No. 2720038 or Mobile No 0721218159.

Signature. I have read the above information and had an opportunity to ask questions have been answered. I consent to my child taking part in the study. I fully understand there are no risks associated with the collection of the stool samples.

Signature __________________ Date __________________
(parent/guardian)

I, the undersigned, have fully explained the relevant details of this study and the person authorized to consent for the patient.

Signature __________________ Date __________________
(Investigator)

Signature __________________ Date __________________
(Witness)

Address of witness.
APPENDIX II: QUESTIONNAIRE

ROLE OF CRYPTOSPORIDIUM IN PAEDIATRIC DIARRHOEA.

Centre ___________________ Care No._____________________

Date of collection ___________________

Study No_________ Date of birth______________ Sex M [ ] F [ ]

Specimen taken for microbiological survey

a) Stool [ ]

Do you have any of the following symptoms? (Tick as appropriate)

Acute diarrhoea. (Less than two weeks)
Chronic diarrhoea. (Over two weeks)
Recurrent/intermittent diarrhoea.
Vomiting.
Abdominal swelling.
Others.

Where do you live?

City (state neighbourhood)

Town

Peri-urban

Rural urban

3. Do you have any domestic animals/pets? Yes. [ ] No. [ ]

If yes, which ones? ____________________________

What is the source of your water?

Borehole [ ] Stream [ ] Tap [ ] Rainwater [ ]

5. Do you treat your drinking water? Yes. [ ] No. [ ]

If yes

Boiling. [ ] Filtering. [ ] Chemical additives [ ] other. [ ]

6. What type of toilet facility do you have?

Pit latrine [ ] IP latrine [ ] Sh toilet [ ]

Of specify [ ]

7. How many are you in the household

Alone [ ] 2-4 Members [ ] 5-7 Members [ ] Over 5 [ ]

8. How many rooms do you have? Single [ ] 2-4 [ ] Over 5 [ ]
Appendix III

Materials
Cryptosporidium positive stool samples
QIAmp® DNA stool kit (Qiagen).
Absolute ethanol
Genomic DNA samples
AmpliTaq® DNA polymerase PCR kit
dNTPs [1.5 mM]
18S rRNA gene primers (F1 and R1)
18S rRNA gene primers (F2 and R2)
Bovine serum albumin [10 mg/ml]

20 × Tris-boric acid EDTA buffer (TBE).
Tris base (216 g) boric acid (110 g) EDTA (18.6 g) were suspended in 800 ml of distilled water and made up to 1000 ml with distilled water.

1 × TBE working buffer solution.
20 × TBE (50 ml) was diluted in 1000 ml of distilled water and ethidium bromide was added to a final concentration of 0.2 µg/ml.

1.5 % (w/v) Agarose.
Agarose (1.5) was suspended in 100 ml of 1 × TBE buffer and heated in a microwave oven until the agarose was completely dissolved, 0.2 µg/ml of ethidium bromide was added to the dissolved agarose.

18S rRNA gene secondary PCR product
Ssp1 and Vsp1 restriction enzymes
10 × buffers E and D
Appendix IV: Ethical Review

Dear Sir,

Re: SSC Protocol No. 1099 (Revised) – The role of cryptosporidium and other intestinal protozoa in paediatric diarrhoea, by KS Kamwati et al

During the 137th Meeting of the KEMRI/National Ethical Review Committee held on the 19th of September 2006 the above protocol was discussed.

Your letter of 30th August 2006 refers.

The issues raised by the committee have been adequately addressed and the study is granted approval. You may proceed with the study. You are responsible for reporting to the Ethical Review Committee any changes to the protocol or in the Informed Consent Document. This includes changes to research design or procedures that could introduce new or more than minimum risk to human subjects.

Yours faithfully,

R. C. Kithinji
FOR: Secretary,
KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE
KANGETHE STANLEY KAMWATI  
KENYA MEDICAL RESEARCH INSTITUTE  
P.O. BOX 19464 – 00202  
NAIROBI

Dear Sir,

RE: AUTHORITY TO CONDUCT RESEARCH AT MATHARE NORTH CITY COUNCIL HEALTH CENTRE.

In reference of your letter dated 15th February 2006 ref no. CMR-ADMIN 2006 on the above matter.

This is to inform you that we have no objection to your request to conduct research on the role of Cryptosporidium and other Intestinal Protozoa in Pediatric Diarrhoea, on condition that a quality report is availed in this office.

By a copy of this letter the District Medical Officer of Health Kassarani District and the In-charge Mathare North Health Centre are requested to accord you the necessary assistance.

DR. D. M. NGUKU  
MEDICAL OFFICER OF HEALTH  
CC.
DMOH - KASSARANI  
INCHARGE – MATHARE NORTH
Appendix VI: Modified Ziehl Neelsen (ZN) acid fast stain—*Cryptosporidium*

Magnification ×1000