Addis Ababa University
School of Graduate studies
Environmental science program

**Determination, enumeration and viability test of *Giardia* cyst and *Cryptosporidium* oocyst from municipal drinking water in Addis Ababa**

This thesis submitted to school of graduate studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Environmental Science

By: Tesfalem Atnafu W/Gabriel

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<table>
<thead>
<tr>
<th>Acronyms</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(oo)cysts</td>
<td>cysts and oocysts</td>
</tr>
<tr>
<td>µL</td>
<td>microliter</td>
</tr>
<tr>
<td>AAWSA</td>
<td>Addis Ababa Water and Sewarage Authority</td>
</tr>
<tr>
<td>AAWT</td>
<td>Addis Ababa Working Team</td>
</tr>
<tr>
<td>CC</td>
<td>Charge Coupled</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
</tr>
<tr>
<td>Crypto</td>
<td>cryptosporidium</td>
</tr>
<tr>
<td>CSA</td>
<td>Central Statistics Authority</td>
</tr>
<tr>
<td>CWTF</td>
<td>Conventional Water Treatment Facilities</td>
</tr>
<tr>
<td>DAPI</td>
<td>4', 6-Diamidino-2-Phenyl indole</td>
</tr>
<tr>
<td>DIC</td>
<td>Differential Interference Contrast</td>
</tr>
<tr>
<td>DM</td>
<td>Dichroic Mirror</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immuno Sorbant Assay</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescent In-situ Hybridization</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein Isothiocyanate</td>
</tr>
<tr>
<td>FL</td>
<td>Fluorescein</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hanks Balanced Salt Solution</td>
</tr>
<tr>
<td>ICR</td>
<td>Information Collection Requirement</td>
</tr>
<tr>
<td>IFA</td>
<td>Immuno Fluorescent Assay</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon gamma</td>
</tr>
<tr>
<td>IMS</td>
<td>Immuno Magnetic Separation</td>
</tr>
<tr>
<td>mg/l</td>
<td>miligram per liter</td>
</tr>
<tr>
<td>mm³</td>
<td>milimeter cube</td>
</tr>
<tr>
<td>N.D</td>
<td>Not Detected</td>
</tr>
<tr>
<td>nm</td>
<td>nano meter</td>
</tr>
<tr>
<td>O&amp;P</td>
<td>Ovum &amp; Parasite</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymorhis Chain Reaction</td>
</tr>
<tr>
<td>PDS</td>
<td>Propidium Iodide</td>
</tr>
<tr>
<td>PI+</td>
<td>Potassium Dichromate Solution</td>
</tr>
<tr>
<td>RFLP</td>
<td>Revers Fragmented Lengh Polymorphism</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>SWTF</td>
<td>Surface Water Treatment Facilities</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>USEPA</td>
<td>United States of Environmental Protection Authority</td>
</tr>
<tr>
<td>WHO</td>
<td>World Human Organization</td>
</tr>
<tr>
<td>DPD</td>
<td>N,N-diethly-1, 4 phylenediamine</td>
</tr>
</tbody>
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Abstract

*Giardia* cysts and *Cryptosporidium* oocysts are (protozoan single cell) causative agent for most of enteric diarrhea and resistant to most of disinfectants. The main purpose of this research was to determine, enumerate and test viability of *Giardia* cyst and *Cryptosporidium* oocysts in Addis Ababa municipal drinking water. A total of 115 samples were collected from different sampling sites at source, reservoir, and public tap. A 100 liters of water collected from each sample and a total of over 10,000 liters of water filtered, concentrated and examined for identifying (oo)cysts. Detection of (oo)cysts in water sample was conducted using (USEPA Method 1623) Aqua-Glo™ G/C Direct Comprehensive Kit (Waterborne™, Inc.) fluorescein-labeled monoclonal antibody for *Simultaneous Direct Immunofluorescence* detection of *Giardia* cysts and *Cryptosporidium* oocysts. *Giardia* detected at source 33.3%, reservoirs 5.88%, and public tap 12.5%. Likewise *Crypto* was found at source, reservoirs, and public tap 55.6%, 8.82%, and 18.06% respectively. The mean concentration of *Giardia* and *Crypto* ranges from 5 to 0.15 and 9.89 to 0.5 respectively. The viability test demonstrated all the (oo)cysts collected was nonviable except at source (untreated water) (DAP-PI+). This can be due to the age of the (oo)cysts, long distance source of contamination, sporulation of the (oo)cyst before maturation with the combination of the disinfectants used in treatment results the non viability (or death) of (oo)cysts. The physico-chemical parameters (temperature and free chlorine residue) significantly correlated with the occurrence of (oo)cyst (p<0.05). In addition occurrence of *Giardia* cysts and *Crypto* oocysts was significantly correlated (r=0.55, p<0.05). *Giardia* cysts and *Cryptosporidium* oocyst were identified from the Addis Ababa municipal drinking water. Continuous monitoring of *Giardia* cyst and *Cryptosporidium* oocysts was necessary for public health concern.

**Key words:** *Giardia* cyst, *Cryptosporidium* oocyst, drinking water, viability, public health concern, DAPI, Fluorescein and PI
1. Introduction

*Cryptosporidium* and *Giardia* are single celled microscopic protozoan parasites that cause enteric disease in humans and other mammals (Thompson, 2004; Appelbee *et al*., 2005; Abe *et al*., 2005). *Cryptosporidium* is a ubiquitous protozoa discovered by Tyzzer in 1907 (Haigh, 1999), and *Giardia* was first described by Antoine Van Leeuwenhoek in 1681 (Adams, 2001; Dobell, 1920; Ford, 2005). The health importance of *Cryptosporidium* was not known till 1976, but currently becoming one of the most prevalent emerging waterborne and food borne disease in humans (Barerr and Wright, 1990; Xiao *et al*., 1998).

Environmental pollution (climate change) is becoming a global concern and issues like water contamination and lack of safe and sufficient drinking water are problems that can lead to serious public health and life treat consequences. Recently, there has been a dramatic incidence of waterborne disease outbreaks caused by the protozoan parasites, *Cryptosporidium* and *Giardia* spp. transmission is sustained both by zoonotic and anthropoanotic cycles (Thompson, 2000; Karanis *et al*., 2005; Appelbee *et al*., 2005).

These characteristics, together with the low dose (oo)cysts required for an infection make them among the most critical pathogens in the production of safe drinking-water from surface water. For instance, the infectivity dose of the *C. parvum* oocyst can be around 132 in health individuals and it can also be as low as 30 (Dupont *et al*., 1995; Haas and Rose, 1994). While the median dose of infection for *Giardia lamblia* in humans reaches around 50-100 cysts, but some individuals can be infected 10 or fewer cysts (Rendtorff, 1954; Adam, 1991).

*Cryptosporidium* and *Giardia* causes diarrhea in a wide range of vertebrate organisms including humans and this is especially significant in immune compromised individuals (Watanabe *et al*., 2005). *Cryptosporidium* is the main cause of diarrhea around the world (Clark, 1999). Especially in developing countries diarrhea is the main cause of mortality and morbidity (Kosek *et al*., 2003). *Cryptosporidium* accounts 1-10% of diarrheal disease in the world (Xian-Ming and Larusso, 1999). Studies indicate that *Cryptosporidium* seroprevalence in developed nations covers 25-35% where in developing nations the figure is higher ranging from 60-90% (Chen *et al*., 2002; Xian-Ming and Larusso, 1999). *Cryptosporidiosis* is one of
the main causes of mortality for infants and young children in developing countries. In children the prevalence of *Cryptosporidium* in developing nations is 1.3-22% while as in developed nations it is about 0.3-4.3% (Casemore, 1990). Similar to other developing countries in Ethiopia diarrhea and HIV/AIDS are a major cause of mortality (UNAIDS/WHO, 2005; Kosek *et al*., 2003). *Cryptosporidiosis* accounts about 12% and 7% of children disease respectively in developing and developed nations (Chen *et al*., 2002).

World wide around 1.1 billion peoples have no access for adequate water, 2.2 billion for proper sanitation, and 2.2 million dies every year because of these problems (WHO, 2002). In Ethiopia, where water supply and sanitation services are inadequate, only 32% of the total population has reasonable access to adequate water supply (MoWR/UNESCO/WWAP, 2004). In the capital city, Addis Ababa, nearly 80% of the population is dependent on public water points and in-house storage of water (Crampton, 2005). Addis Ababa possess health threats including diarrhea, typhoid, cholera, and intestinal worms due to contamination of water and food, poor waste collection, overcrowded housing and insufficient water for hygiene (AAWT, 2000). In AIDS patient’s *C. parvum* causes diarrhea 10-16% in developed nations and 30-50% of population in developing countries (Xian-Ming and Larusso, 1999). Previous studies conducted in Addis Ababa reported high prevalence of *Cryptosporidiosis* and *Giardiasis* among HIV/AIDS patients and diarrheic children (Fisseha *et al*., 1998).

In Ethiopia studies on *Giardia* cysts and *Cryptos* oocyst in humans is well documented (Halileeyesus Adamu *et al*., 2005; Seyoum *et al*., 1981; Eyasu Tigabu, 2007; Tekola Endeshaw *et al*., 2004; Tekola Endeshaw, 2005; Dawit Ayalew *et al*., 2008; Afework Kassu *et al*., 2007; Amare Deribew *et al*., 2007; Assefa *et al*., 1996) but on drinking water (Nigus Fikrie *et al*., 2008) it is not studied in detail. In the recent period *Giardia* and *Cryptos* are becoming an emerging pathogen (Guerrant, 1997; Karanis, 2006a; Shields, 2008; Thompson, 2002), as a result identification of these parasites in the surface drinking water samples is becoming an essential factor for public health particularly in reservoirs and public supply points (Carmena *et al*., 2007). Currently in 2009 an out break of acute waterborne diarrheal (AWD) disease was causes for over 11, 000 cases and 100 deaths in Addis Ababa including three regions (EHA, 2009; WHO, 2009).
Therefore, it is essential to evaluate the status of Cryptosporidium oocysts and Giardia cysts in drinking water systems of Addis Ababa. In order to determine the existing conditions of (oo)cysts in water their viability was tested.

2. Objectives

2.1 General objective
➢ To determine the status of Cryptosporidium oocysts and Giardia cysts in Addis Ababa municipal drinking water system.

2.2 Specific objectives
➢ To determine and enumerate the occurrence of Cryptosporidium oocyst and Giardia cyst in Addis Ababa municipal drinking water from the source, reservoirs to the distribution point.

➢ To determine the viability of Cryptosporidium oocyst and Giardia cyst; if the (oo)cysts detected in the water samples.

3. Literature review

3.1 Cryptosporidium

3.1.1 Classification and Taxonomy
Cryptosporidium classified to the phylum Apicomplexa and categorized under coccidea (Finch and Belosevic, 2002). Morphological characters and host specificity have been used up to 1990’s (WHO, 2006), even though it is not specific to differentiate between the species of Cryptosporidium (Fall et al., 2003). Classification of Cryptosporidium using vertebrate hosts was successful, but underestimates the variation among the parasite (Xiao et al., 1998).

The current molecular detection method improved from immuno histochemistry, morphological and host specification for categorization of the different species of
*Cryptosporidium* for the different host ranges (Morgan et al., 1999). The molecular classification based on several markers-18S rRNA gene, *Cryptosporidium* oocyst wall protein, and molecular genotyping methods (PCR and RFLP) provide a more complex and clear view in taxonomy (Appelbee et al., 2005; Morgan et al., 1999; Coup et al., 2005), besides *in vivo* and *in vitro* studies (Pérez-Cordón, 2006). Molecular identification ability further helps understanding of zoonotic, epidemiological and taxonomy of this parasite (Appelbee et al., 2005).

*Cryptosporidium* species identified as infectious to humans are two: *C. parvum* and *C. hominis*. The former *C. parvum* is considered as zoonotic while the later *C. hominis* is anthropologic source (Cacciò et al., 2002). Human infection cases other than *C. parvum* like *C. felis* are reported, indicating the importance of public health concern over the parasite (Cacciò et al., 2002). In immune compromised persons other species of *Cryptosporidium* can also infect such as *C. meleagris*, *C. felis*, *C. canis*, *C. suis* (also known as *C. pig* genotype), *C. baileyi* and *C. muris* (Finne, 2006) (Table 2).

### Table 1: Taxonomic classification of *Cryptosporidium*

<table>
<thead>
<tr>
<th>Classification</th>
<th>Taxonomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom</td>
<td>Protista</td>
</tr>
<tr>
<td>Phylum</td>
<td>Apicomplexa</td>
</tr>
<tr>
<td>Class</td>
<td>Sporozoasida</td>
</tr>
<tr>
<td>Sub class</td>
<td>Coccidiasina</td>
</tr>
<tr>
<td>Order</td>
<td>Eucoccidiorida</td>
</tr>
<tr>
<td>Sub order</td>
<td>Eimeriorina</td>
</tr>
<tr>
<td>Family</td>
<td>Cryptosporidiidae</td>
</tr>
</tbody>
</table>

Source: Finch and Belosevic, 2002

Previously eight species have been identified capable of infecting humans. But currently, two new *C. parvum* genotypes such as *Cryptosporidium cervine* have been detected in humans. *C. parvum* is further classified in two sub categories genotype 1 and genotype 2, which are
zoonotic and anthroponotic and zoonotic genotype respectively (Cacciò et al., 2002). Currently ten species of Cryptosporidium oocysts was identified based on host specificity, morphology of oocyst and further classification to species level (Xian-Ming and Larusso, 1999).

Morphology and host specificity of C. meleagridis determines the parasite resides in small intestine like C. parvum. Molecular studies on variable region of SSU rRNA gene of C. meleagridis from turkey revealed the parasite is distinct from Crypto species with a broad range of hosts (Sreter et al., 2000). Cryptosporidium saurophilum was identified in snake fecal through molecular studies (Plutzer and Karanis, 2007).

### Table 2: Different species of Cryptosporidium

<table>
<thead>
<tr>
<th>Species</th>
<th>Hosts</th>
<th>Original host</th>
<th>Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. hominis</td>
<td>Humans</td>
<td>Homo sapiens (human)</td>
<td>4.9x5.2</td>
</tr>
<tr>
<td>C. parvum</td>
<td>Mammals</td>
<td>Mus musculus (house mouse)</td>
<td>5.0x4.5</td>
</tr>
<tr>
<td>C. meleagridis</td>
<td>Turkey, humans</td>
<td>Meleagris gallopavo (turkey)</td>
<td>4.0x4.5</td>
</tr>
<tr>
<td>C. muris</td>
<td>Rodents, ruminants</td>
<td>Mus musculus (house mouse)</td>
<td>5.6x7.4 or 6.1 or 8.1</td>
</tr>
<tr>
<td>C. andersoni</td>
<td>Cattle, Camel</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. felis</td>
<td>Cats</td>
<td>Felis catis (cat)</td>
<td>4.0x4.6</td>
</tr>
<tr>
<td>C. canis</td>
<td>Dogs</td>
<td>Canis famillaris (dog)</td>
<td>4.7x5.0 or 4.2x4.8</td>
</tr>
<tr>
<td>C. wrairi</td>
<td>Guinea pigs</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. baileyi</td>
<td>Gallinaceous birds</td>
<td>Gallus gallus (chiken)</td>
<td>6.1±0.4</td>
</tr>
<tr>
<td>C. galli</td>
<td>Birds</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. serpentis</td>
<td>Snakes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. saurophilum</td>
<td>Lizards</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. molnari</td>
<td>Sea bass, sea bream</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Source: Sreter et al., 2000; WHO, 2006

#### 3.1.2 Morphology of Crypto oocyst

The oocyst measures around 5µm in diameter and it can vary and elongated depending on the species, it is the infective stage of the parasite (Haigh, 1999; Finch and Belosevic, 2002).
oocysts are spherical or ovoid in shape and contain four sporozoites surrounded by smooth oocyst wall (Finne, 2006). Oocyst wall is composed of different layers outer layer made of glycoprotein, central part consists of complex lipid material (essential in acid fast staining) and thick inner layer consisting glycoprotein (Bonnin et al., 1991). Ultra structure of Crypto oocyst indicates the presence of a single suture spanning the wall of oocyst which undergoes dissolution during excystation (Finne, 2006; Robertson et al., 1993).

3.1.3 Life cycle
The life cycle of Cryptosporidium can be categorized in to six stages: first oocyst excystation in the intestine of the host, replication within the host, gamete formation, fertilization, oocyst wall formation, and sporozoites formation (Finch and Belosevic, 2002) (Figure 1). Transmission of Crypto occurs directly through fecal-oral route and indirectly through water (Fahey, 2003).

Ingested oocyst opens the oocyst wall and initializes the development to infect the host (Clark, 1999). (Fayer and Ungar, 1986; CDC, 2009b) The merozoites released into lumen of intestine mature to gametocytes (sexual form) or infect epithelial cells (type I). The meroants (type II) undergo sexual reproduction (gamogony) contains micro (male) and macrogamete (female). Hence the microgamete fertilizes the macrogamete producing more oocyst. When the oocyst wall matures it breaks from the host cell and become thin or thick walled (Clark, 1999; USDA/APWL, 2004). Thin wall results auto infection and it can’t survive out side of the host, while thick wall is capable of surviving out of the host and having a potential to cause infection (Finch and Belosevic, 2002; Fayer and Ungar, 1986). C. parvum infects a broad range of animals’ small intestine including amphibians, fish, reptiles, and mammals (USDA/APWL, 2004).
3.1.4 Pathology

*Cryptosporidium* oocyst and other life stage surface proteins, glycoprotein, and phospholipids are immunogenic. The initial attachment to glycocalyx of intestinal mucosal assisted by glycoprotein and lectins in the surface of sporozoites (Langer and Riggs, 1999).

Infection of *Cryptosporidium* causes the secretion of prostaglandin E$_2$-mediated chloride in the small intestines which contributes to components of diarrhea (Fahey, 2003). At the first invasion of epithelial cells by *Crypto* causes secretion of inflammatory chemokines. Secretion of prostaglandin E$_2$, which causes secretion of mucin or antimicrobial β defensins, helps *Crypto* infected cells from being attacked by parasitic domination (Chen et al., 2002).
Human recombinants IFN-γ act as a therapeutic agent in response to inflammatory reactions induced by Cryptosporidium infection in bile duct (Stephens et al., 1999). Infection of Cryptosporidium parvum induces causes apoptosis of biliary epithelial by Fas/Fas ligand-dependent mechanism (Chen et al., 1999). Innate immunity humoral and cell mediated (T cell) involves to Cryptosporidiosis infection resistance. In addition interferon gamma and T cell CD4+ has also a protection role (Chen et al., 2002).

Cryptosporidium does not infect cells above the superficial surface of intestinal tissues, but interferes with intestinal functions. The main mechanism it causes is not clearly yet identified (Guerrant, 1997; Chen et al., 2002).

### 3.1.5 Diagnosis and treatment

The diagnosis of Cryptosporidium spp. is through microscopy, endoscopy, molecular techniques, and modified acid fast staining are a means to detect the parasite. Even though serological examination can be done, but most healthy persons are positive and lead to incorrect identification (Chen et al., 2002; Xian-Ming and Larusso, 1999). Fluorescent monoclonal antibody detection is ten times more sensitive than that of acid-fast staining. Compared to immuno fluorescent technique acid fast-staining is time consuming (15-20 min normally while 2-3second for IFA), cheap, difficult to determine sensitivity, and clarity of determination is based on smear thickness (Garcia et al., 1987).

Diagnosis effectiveness depends on targeting of treatment and reduction of morbidity. In recent years more sensitive methods (immunofluorescence) than morphological identification based on microscope developed (Karanis, 2006a). In environmental samples to identify the parasite source has been a great challenge through this method. However molecular technique is more accurate to identify and define different genotypes of the parasite (Xiao et al., 1998).

However the common therapeutic drugs for Cryptosporidium infection in humans and other animals includes Zoaquin, Clopidol, Lincomycin, Lasalocid, Ipronidazole, Ivermectin Cholestyramine, Cimetadine, Clindamycin, Clonidine, Clopidol, Cloxacillin, Colistin,
Cotrimoxazole, Decoquinate, Difluoromethyl-ornithine, Diloxanide furoate, Dimetridazole, Dinitolmide, and so on (Fayer and Ungar, 1986).

### 3.1.6 Control and prevention

In drinking water treatment most of the disinfectants used such as chlorine are not capable of impairing the Cryptosporidium oocysts infectivity (Xian-Ming and Larusso, 1999). The best mechanism to control Giardia and Cryptosporidium in water was the use of effective physical and chemical removal (Finch and Belosevic, 2002). In addition, prevention of Cryptosporidium oocysts involves avoiding contact of oocyst with humans through boiling, isolation of infected individuals, carefully handling and disposal of biohazards (Xiao et al., 1998). For instance USEPA (1999a) recommend the use of point filtration and boiling of drinking water especially for immuno compromised individuals and at the time of outbreaks.

### 3.1.7 Clinical manifestation

Symptoms in immuno compromised ones are diarrhea, fever, right-upper-quadrant pain, jaundice, weight loss, and vomiting (Chen et al., 2002; Karanis, 2006a). After incubation periods with 7 to 12 days acute diarrhea lasts for ninety percent of patients and followed by nausea, vomiting, and cramp like abdominal pain, thirty six percent have also fever (Fayer and Ungar, 1996). But incubation period in immunocompetent individuals is two weeks and two days up to life time (Chen et al., 2002). In addition, diarrhea lasts more than four months and for 55% of AIDS patients as a death cause recorded (Guerrant, 1997).

Diarrhea is the most common manifestation of Cryptosporidium. The complexity and type of symptoms depend on the immune status of the individuals infected (Xian-Ming and Larusso, 1999). In immune compromised individual (HIV/AIDS, cancer, diabetes, and other patients) clinical symptoms can be complicated (Kneen et al., 2004). Symptomatic infection is more common in infants than adults (WHO, 1997).

In histopathologic point of view disease signs were damage of intestinal mucosal cells, villous atrophy and inflammation of lamina. However human intestines may not show all this
features and it remains intact (Marcial and Madara, 1986). Incubation period is about 7 days and it is usually self limiting diarrhea (Dupont et al., 1995).

3.2 Giardia

3.2.1 Classification and Taxonomy

Giardia classified in to phylum Sarcomastigophora, class Zoomastigophorea, in the order Diplomonadida, and family Hexamitidae (USEPA, 1999b). Molecular investigation currently demonstrates there are seven distinctly Giardia species (Abe et al., 2005). However there is no universal agreement which criteria (host specificity, body size and shape, median body, biochemical generic and molecular techniques) to use to define species in the genus (USEPA, 1999b). For instance on host specificity more than 40 species, morphological criteria used to define Giardia into three species G. lamblia G. agilis and G. muris (Adam, 1991; Adam, 2001). G. muris, G. Lamblia (also named as intestinalis or duodenalis), G. ardeae, and G. agilis infect respectively rodents, different animals, herons, and amphibians (Adam, 2001) (Table 3).

Table 3: Classification of Giardia

<table>
<thead>
<tr>
<th>Species name</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. agilis</td>
<td>Amphibians</td>
</tr>
<tr>
<td>G. muris</td>
<td>Rodents</td>
</tr>
<tr>
<td>G. lamblia</td>
<td>Numerous mammals, including humans</td>
</tr>
<tr>
<td>G. ardae</td>
<td>Herons</td>
</tr>
<tr>
<td>G. psittaci</td>
<td>Psittacine birds</td>
</tr>
<tr>
<td>G. microti</td>
<td>Voles and muskrats</td>
</tr>
</tbody>
</table>


3.2.2 Morphology of Giardia cyst and trophozoite

Giardia lamblia cysts have ovoid shape and have a length of 8-12µm and a diameter of 7-10 µm depending on the species (Finch and Belosevic, 2002; Dobell, 1920). The cyst wall varies from 0.3 to 0.5µm in thickness, and electron microscope demonstrated it is composed of thin
fibrous elements interspersed with thin fine particles. The cyst was surrounded by a rigid cell wall (Filice, 1952).

The study conducted by Dutta (1964) through cytochemical and fluorescence microscopic revealed the presence of parabasal bodies, lipid bodies and mitochondria, in addition to two or four nuclei in the cyst of *Giardia intestinalis*. Cysts of *Giardia* commonly found in the large and small intestine including in fecal materials, while trophozoites found in duodenum (Finch and Belosevic, 2002).

The trophozoites of *Giardia* was a pear shape and measures around 12µm up to 15µm in length and 5 to 9µm wide (Adam, 1991), and thickness of 2-4µm (Dobell, 1920). Cytoskeleton structure revealed the presence of four pairs of flagella at the anterior, ventral, dorsal and posterior, which gives a total of eight flagella (Dobell, 1920). The trophozoite consists of two nuclei with out nucleoli; Golgi complex found in the cyst form of *Giardia* and not detected in the trophozoite of form of *Giardia* (Baron, 1996). Glycogen and ribosomal granules present in cytoplasm (Adam, 1991). Median bodies are not only one or two but it varies in number, shape and size. In addition it is found in mitotic and interphasic trophozoites (Piva and Benchimol, 2004).

### 3.2.3 Life cycle

The life cycle of *Giardia* is simpler than that of *Cryptosporidium* and includes developmental stages of encystations and excystation (Finne, 2006). *Giardia* cyst found in the soil, water, food, or surfaces contaminated from infected mammals or humans (CDC, 2008) (Figure 2). Then individuals become infected when swallowing the parasite cyst. Exposure of the cyst to low pH of the gastric acid and proximal slightly alkaline in small intestine induces leads to the excystation of the cyst (Dobell, 1920; Filice, 1952), then followed by the formation of four trophozoites after the division of the excozoite (Filice, 1952). Excystation occurs in the colon as the cyst transits towards intestine. Each cyst releases two trophozoites. Trophozoites besides cyst can pass through but it can not survive in the environment for long period of time (CDC, 2009b).
Transmission of *Giardiasis* occurs through the ingestion of resistant cyst from contaminated water and food (Baron, 1996). The *Giardia* cysts are the infective and diagnostic stage while the trophozoites are the diagnostic stage. Trophozoite attaches or remains free in duodenum and jejunum in which it undergoes binary division through asexual division (Dobell, 1920). In small intestine the pH, bile salt and fatty acid stimulates the encystations of *Giardia lamblia*, maximum encystations occurred at pH of 7.8 (Gillin et al., 1988).

Source: [http://www.dpd.cdc.gov/dpdx](http://www.dpd.cdc.gov/dpdx)

**Figure 2: Life cycle of Giardia**
3.2.4 Pathology
After excystation in the upper epithelia cells in the small intestine, adhesion of the trophozoites results the first step of pathogenesis (Adam, 2001). *Giardia* results damage to small intestine in which the effect continues to nutrient absorption, mainly on the absorption of disaccharides (Cheeramakara *et al.*, 2004). Experiment conducted by Roberts-Thomson *et al.* (1976) on mice indicates *Giardia muris* interfere with nutrient absorption due to injury to small intestine, brush border enzyme deficiency and villus atrophy detected in jejunum, and duodenum in which trophozoites most commonly found.

3.2.5 Diagnosis and treatment
Diagnosis of *Giardia* is commonly undertaken using microscopic methods (Adams, 2001). Enzyme immuno assay based on antibody antigen reaction for identification of *Giardia* cysts in stool samples was 97% and 82% sensitive for formalinized and unfixed stool samples respectively (Stibbs, 1989)

Currently recommended antigiardial treatments for *Giardiasis* are nitroheterocyclic drugs: tinidazole, metronidazole, furazolidin, the substituted acridine, quinacrine, benzimidazole and albendazole (William *et al.*, 2008). In addition to lack of complete eradication of this parasites (Chen *et al.*, 2002), with currently existing antigiardial drugs, treatment failures and resistance was reported (William *et al.*, 2008).

3.2.6 Control and prevention
Vaccination of *Giardiasis* is currently becoming commercially available means for prevention of *Giardiasis* and reduction of cysts shaded (Olson *et al.*, 2000). Because of lack of effective means of treatment for these parasites the most efficient mechanism to overcome *Giardiasis* infection was monitoring of *Giardia* cysts and *Cryptosporidium* oocyst in drinking water for both treated and untreated water source. According to USEPA (1999a) for immuno compromised individuals boiling of drinking water which is expected to be contaminated with cysts and oocysts. Boiling of water at rolling point for one minutes and 3 minutes for altitudes greater than 2, 000 meters (6,562 feet) can remove *Giardia* from drinking water (CDC, 2008).
Another mechanism to avoid *Giardiasis* is washing hands before and after meal, maintains good personal hygiene and properly disposing of fecal materials. In addition alternative means to prevent *Giardia* is point use of filter: reverse osmosis, absolute pore size of one micrometer or lesser (CDC, 2008).

### 3.2.7 Clinical features and symptoms

*Giardiasis* clinical features range from asymptomatic to severe acute and chronic diarrhea that includes malabsorption (Cheeramakara et al., 2004). Symptoms range from self-limiting diarrhea to life treating in immune competent individuals and severely immune compromised individuals respectively (Haigh, 1999). In the beginning of the infection weight loss and malaise observed in the majority of *Giardiasis* patients. In addition gastrointestinal symptoms like abdominal cramps, bloating, nausea, including lowering of appetites. Inflammatory retort revealed during resolution of infection (Adam, 1991). Diarrhea, Gas or flatulence, Greasy stools that tend to float, Stomach or abdominal cramps, Upset stomach or nausea are among various intestinal signs and symptoms (CDC, 2008).

### 3.3 Methods to detect *Giardia* and *Cryptosporidium* from water sample

Detection methods for the protozoan parasites was developed first after the first infection outbreaks in Aspen, CO, USA, in 1965 and *Cryptosporidiosis* in Braun satiation, USA, Texas in 1984 (Finch and Belosevic, 2002). Different standard methods currently developed to detect *Giardia* and *Cryptosporidium* from water sample USEPA method 1623 (USEPA, 2005), USEPA method 1622 (USEPA, 1999a), ICR (USEPA, 1995).

Various techniques are currently available to concentrate (oo)cyst from large volume of water cartridge filtration, membrane filtration, continuous flow centrifugation, and calcium carbonate flocculation (Musial et al., 1987). According to Shepherd and Wyn-jones (1996) cellulose acetate with 1.2µm gives best recovery for *Cryptosporidium* and, cellulose nitrate with 3.0µm for *Giardia* cyst. Detection method incorporates dye and immunological techniques. But due to lack of sensitivity in using dyes, immunological method is applicable for environmental sample. Immunological based ELISA can also be used to identify parasite.
Propylene cartridge filter can detect (oo)cyst in water samples less than one liter (Musial et al., 1987).

Enzyme Immuno Assay (EIA) method is more effective in detecting Giardia cysts and Cryptosporidium oocysts than standard microscope staining techniques. In which it is estimated around twice more sensitive than that of microscope staining techniques but more economical as well (Aziz et al., 2001). Most probable number (FDM-MPN) can also be used to enumerate the number of infectious oocyst in vitro (Slifko et al., 1999). Mouse monoclonal antibody IgG, which is made against wall of Giardia cyst, used as solid phase to bind cyst wall (Stibbs, 1989). Oocysts labeled with fluorescein appeared as green apple fluorescent when viewed under fluorescent microscope (USEPA, 2005).

The sensitivity and specificity for detecting Giardia by immuno assay was 96 and 100%, while for (Ovum & Parasite) O&P 74 and 100%. Immuno assay offers more sensitive and simple operation than did the conventional method. The main advantage of O&P examination was its ability to identify large number of intestinal parasites than more specific and single parasite detecting methods. However to achieve more than 90% sensitivity for diagnosis of Giardia, it is advisable to use both EIA and O&P (Hanson and Cartwright, 2001).

### 3.4 Viability examination methods for Giardia and Cryptosporidium

The detection and enumeration of Giardia cyst and Cryptosporidium oocyst in the water sample through USEPA method 1623 alone can not determine the viability of parasite (oo)cyst (USEPA, 2005). The viability of Giardia cyst and Cryptosporidium oocyst can be determined by several methods in vitro excystation (Campbell et al., 1992, Schupp and Erlandsen, 1987; Hoff et al., 1985), cell tissue culture (Slifko et al., 1997; ), cyst morphology by using light microscope (Schupp and Erlandsen, 1987), the inclusion (uptake) or exclusion of fluorogenic dyes (Jenkins et al., 1997; Campbell et al., 1992; Neumann et al., 2000), and animal infectivity models (Neumann, et al., 1999; Finch et al., 1993a).

Viability of Crypto oocysts was determined using fluorogenic dyes PI and DAPI (Campbell et al., 1992). PI does not cross intact cell membrane; hence damaged or disrupted cell membrane
intake PI (Campbell et al., 1992). Further the Propidium iodide (PI) binds to the double strand of DNA by intercalating between the bases with little or no preference on the basis of the DNA including the stochio-chemistry of one dye per 4-5 base pairs of DNA (Barni et al., 1981). Propidium iodide also binds to the RNA, giving an important identification method between the RNA and DNA staining. Once the dye binds to DNA or RNA it fluorescence 20 up to 30 fold, the fluorescence maximum shifted 30 to 40 nm to the red and the fluorescence excitation shifted to 15 nm to blue (Campbell, et al., 1992; Barni et al., 1981).

The nucleic acid stain DAPI selectively binds to dsDNA-AT clusters of the minor groove and major groves of poly [d(G-C)]2 in which the molecular plane is parallel to the double helix (Seog et al., 1993). Because of the displacement of water molecules from minor groove and DAPI, the binding of DAPI to dsDNA appears around 20 fold fluorescence enhancement (Campbell et al., 1992). In vivo excystation occurs after the passage through the acidic environment of human stomach (Adams, 2001). Excystation in vitro was first conducted by exposing the human cyst and animal cyst, to acidic pH (Bingham and Meyer, 1979). In vitro excystation of the Cryptosporidium using vital dyes, revealed the staining of the oocyst with Propidium iodide (PI) as indication of the nonviable oocyst (Campbell et al., 1992).

Hence Crypto oocysts which are PI+DAPI- are considered as nonviable while DAPI+PI-oocysts are taken as viable (Campbell et al., 1992). And Giardia cysts PI+ and PI- are considered as nonviable and viable respectively (Schupp and Erlandsen, 1987)

Determination of the viability of Cryptosporidium using Fluorescent Insitu Hybridization (FISH) was reported by using ribosomal directed RNA- probe (Smith et al., 2004). The other means of determining the viability of Cryptosporidium parvum was immunomagnetic capture PCR (Stinear et al., 1996) and reverse transcriptase PCR to detect mRNA and amyloglucosidase (Wagner-Wiening and Kimmig, 1995).

FISH and FITC conjugated monoclonal antibodies are most sensitive and specific method for determination of viable Giardia and Cryptosporidium. Despite cost and labor required in vivo method is more beneficial than in vitro excystation method of Giardia cyst (Isaac-Renton et al., 1992). In vitro excystation to determine viability is inefficient method because unexcysted
oocyst that is expected to be nonviable can cause infection (Neumann et al., 2000). In environmental samples Crypto viability can be determine using dye permeability assay (Jenkins et al., 1997). However, low number of oocyst in environmental samples soil, sediment and water is a challenge to determine the efficiency of dye permeability assay to investigate viability.

3.5 Giardiasis and Cryptosporidiosis in Ethiopia

In Addis Ababa hospitals among diarrheal patients Giardia lamblia (6.3%) and Cryptosporidium parvum (8.1%) were detected (Halileeyesus Adamu et al., 2005). Study conducted in Yekatit 12 (Addis Ababa) on pre-school children Giardia infection was found more prevalent (Assefa et al., 1996). Similarly in preschool children in Addis Ababa the prevalence of Giardia was reported 9.3% (Seyoum et al., 1981). In the central and northern high land of the country the prevalence of Giardia lamblia ranges 11% (Mcconnel and Armstrong, 1976).

The study conducted by Dawit et al. (2008) in Lege Dini (Ethiopia) demonstrated the prevalence of Cryptosporidium 80 (12.2%) and Giardia 231(35.3%). Benishangul, Gumuz Region in Pawi district among children examined 102 (26.6%) and 31 (8.1%) were found positive for G. lamblia and C. parvum infection, respectively (Eyasu Tigabu, 2007). In addition the overall co-infection with intestinal parasites found in 4.4% of the study participants, among these parasitic infection Giardia and C. parvum comprised the highest proportion. Among the recorded intestinal parasites higher proportion was covered by G. lamblia and C. parvum in the area.

In Ethiopia among HIV infected individuals Cryptosporidium infection was reported 28.6% and 7.1% in HIV negative individuals (Tekola Endeshaw, 2005). In south western Ethiopia similar study on Cryptosporidiosis among HIV patients revealed 11% (Awole et al., 2003). According to Afework Kassu et al. (2007) on HIV/AIDS patients in Gondar Giardia lamblia (5.7%) and Cryptosporidium parvum (1.9%) was detected including other intestinal parasites such as Ascaris lumbricoides (6.2%), Entamoeba histolytica (10.0%), and Schistosoma mansoni (4.3%).
In addition in urban dwellers of south western Ethiopia under-five years’ Crypto prevalence (one of diarrhea causing agent) of 5.6% reported (Amare Mengistu, 2007). Giardia trophozoites infection was significantly higher prevalence of infection in pre school children than other age groups.

4. Materials and methods

4.1 Description of the study area

4.1.1 Geographical location of the study area

This study was conducted in Addis Ababa Municipal drinking water. Addis Ababa, the capital city of Ethiopia, is located at 9°N and 38°E as shown in Figure 3. Currently the city of Addis Ababa is divided in to ten sub cities Kolfe, Gulele, Yeka, Bole, Nifas Silk/Lafto, Akaki/Kaliti, Lideta, Kirkos, Arada and Addis Ketema. In addition, Addis Ababa is a seat for different international institutions such as UNDP, UNEP, and AU, regional WHO and others. Addis Ababa was established in 1887 with the total surface area coverage of 540 km² of this 18 km² covered by the rural area. The altitude of the city ranges from 2000-2800 meter above the sea level (Mesfin et al., 1998).

The climate of Addis Ababa is mild afro alpine and warm, and categorized in to three main distinct seasons. The period of heavy rains (Kiremt) occurs between June and September, in which 70 percent of the mean annual rain fall 1240 mm is recorded. The dry period (Bega) is between October and January, the small rain (Belg) occur between March and May. The maximum temperature through out the year is 26°C, minimum and average temperature of 6°C and 16°C respectively (Mesfin et al., 1998).
Figure 3: Map of the study area locating Gefersa sub-system and the remaining Legedadi including reservoirs and major pipe lines in the city
4.1.2 Demography of the study area

The 2007 population census and housing survey showed that the population of Addis Ababa grows at an average annual rate of 2.1 (CSA, 2008). The population of Addis Ababa in three subsequent census year’s was 0.4 million (1961), 0.7 million (1967), 1.1 million (1978), 1.4 million (1984) (CSA, 1995; CSA, 2008; CSA, 1998). In the 2007 population census and housing survey the population of Addis Ababa was 2,738,248 out of which 1,304,518 (47.6%) was males and 1,433,730 (52.4%) females (CSA, 2008).

The highest number of population of Addis Ababa is found in Kolfe-Keranyo-Sub city with the population number of 428,654 followed by Yeka-Sub city (346,484). The smallest number of population of Addis Ababa was found at Akaki Kality-Sub city with the population number of 181,202 (CSA, 2008) (Table 4).

Table 4: The distribution of Addis Ababa population in each sub city

<table>
<thead>
<tr>
<th>Addis Ababa</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akaki kalit-Sub city</td>
<td>181,202</td>
</tr>
<tr>
<td>Nefas Silk Lafto-Sub city</td>
<td>316,108</td>
</tr>
<tr>
<td>Kolfe Keranyo-Sub city</td>
<td>428,654</td>
</tr>
<tr>
<td>Gulele- sub city</td>
<td>267,381</td>
</tr>
<tr>
<td>Lideta- Sub city</td>
<td>201,613</td>
</tr>
<tr>
<td>Kirkos-Sub city</td>
<td>220,991</td>
</tr>
<tr>
<td>Arada- Sub city</td>
<td>212,009</td>
</tr>
<tr>
<td>Addis Ketema-Sub city</td>
<td>255,092</td>
</tr>
<tr>
<td>Yeka-Sub city</td>
<td>346,484</td>
</tr>
<tr>
<td>Bole-sub city</td>
<td>308,714</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2,738,248</strong></td>
</tr>
</tbody>
</table>

Source: CSA, 2008
4.1.3 Sampling sites

Water samples were collected from source (untreated), reservoirs and public tap or household level. The selected raw water sources, which are not treated, were Legedadi, Gefersa dam Kaliti Gabriel Well (KGW). Legedadi treatment plant was located around 42 km to the North of Addis Ababa. Gafarsa dam was situated on the left of Addis Ababa approximately 25 km. It was a manmade lake which provides Addis Ababa with drinking water. Legedadi dam had the potential of providing 50,000 m$^3$ of water per day. And the Gafarsa dam’s production capacity was 30,000 m$^3$/day. Generally, a total of 256,000 m$^3$/day of water was available from the existing water sources but this was below the demand 479,000 m$^3$/day (AAWSA, 2007). In this investigation the selected source raw water are Gefersa, Legedadi, and Kaliti Garbiel Well (KGW).

The studied reservoirs in this investigation are seventeen R1, R1, R2, R4, R5, R6, R9, R12, R13, R15, R16, R17, S.3 (chlorinated Fanta spring), CT, GW1, GW2, and GW3. Collection Tank (CT) was located about 20 kilometers to the east of Addis Ababa. It provides treated water to Akaki Kaliti sub city population including other areas in the city of Addis Ababa. In addition the treated water from CT was stored in various reservoirs named as GW1, GW2, GW3 and GW4.

Fanta spring (FS) was located around 25 kilometers away from the city of Addis Ababa. The production capacity of FS was 1,068 $^3$/day and obtained from the natural spring water. Intern it provides services almost for half of Akaki population. Kaliti Gerbil Well (KGW) is one of none disinfected source (well water) located in the Akaki kaliti sub city which provides service directly to the population of Akaki Kaliti sub city. Its production capacity was 367,920 m$^3$/year.
4.2 Sample collection, concentration and Microscope examination

Water sample collection, filtration, elution, centrifugation, and microscopic observation were conducted based on USEPA method 1623 with certain modification for identification, determination and enumeration of *Giardia* cysts and *Cryptosporidium* oocysts (USEPA, 2005).

4.2.1 Sample collection

Water samples were collected using 20 liter sterilized white plastic containers. Before sample was taken from distribution and tap water, pipe tip was pre-sterilized using flame (ethanol) and marked from where it was collected including date, and time of sampling. Before the second sample was collected the plastic containers were pre-serialized using sodium hypochlorite (NaOCl), and rinsed using distilled water. Finally the collected water samples shipped to the laboratory for filtration, elution, centrifugation and examination.

Samples were collected at source, reservoirs and public tap at household level. The surface raw water at sources included in this investigation was Gefersa dam and Legedadi. In addition one source (untreated) well water (KGW) was also investigated. At each sampling point of surface source water three (n=3) raw water samples were taken at Gefersa, Legedadi dam and well (KGW). A total of nine samples (n=9) were taken at the source untreated water in the study area. Seventeen sites were selected from reservoirs and a total of 34 samples were collected in two rounds. At the distribution point (household level) thirty six (n=36) sampling sites were selected at different places throughout the city, a total of seventy two (n=72) samples collected at public tap or household level. A total of one hundred fifteen (N=115) samples was collected in Addis Ababa municipal drinking water.

The volume of sample water collected for each selected site except at sources was a hundred liter (100L/sample). At source ten liter (10L) of water sample was collected. The volume of sample collected decreases at source due to higher turbidity that causes logging of filter paper during filtration. Sample sites were selected based on purposive random sampling method. In which the sampling site was determined on purpose at the source, reservoirs and public tap or at household level. And the samples were taken randomly from the selected sites at household
level. Samples collected over the period of nine months (January, February, March, April and May, June, July, August, December). And the samples were taken twice at each selected site of investigation per two weeks time intervals.

4.2.2 Sample concentration

4.2.2.1 Sample filtration

Filter papers with the pore size more than 1µm can pass oocyst (Finch and Belosevic, 2002). A cellulose acetate filter paper with the pore size of 1µm and diameter of 47 mm, white in color filter paper was used (Pall filter paper). Vacuum pump (Thomas model number TA 1061, Monroe, USA and Edwards vacuum pump) was used to filter water sample through the filter paper with the negative pressure of -0.4 up to -1bar pressure. This is around 2-4L/minute. The filtration cap was Gellman science using a bottle and tin barrel for sucking the air form the flask. One hundred liters of water (100L) for each sample of treated water was filtered. While for source raw water ten liters (10L) of water was filtered using these apparatus for concentration.

4.2.2.2 Elution

The surface of the filter paper scraped using a smooth edge plastic loop and detergent of 0.01% Tween 80 and Phosphate Buffer Saline solution (PBS) (both PBS and Tween 80, Sigma Chemical Co., St. Louis, Mo.). The filter paper elute was transferred to 15ml conical polyethylene centrifuge tubes for centrifugation.

![Figure 4: Elution of cellulose acetate filter membrane](image)
4.2.2.3 Centrifugation of (oo)cysts

The eluted material was centrifuged at 1,500 x G for 15 minutes and marked with the respective sample site for the purpose of identification (USEPA, 2005). Supernatant discarded and pellets washed (Figure 5) with distilled water. Purified clear (oo)cysts transferred to sterile Ependorf tube. In which Potassium Buffered Saline solution (PBS, pH=7.2) and disinfectants, Procancillin penicillin G (100U/ml), were added to make the media sterile till observation and staining was done. Potassium dichromate solution (PDS) with 2.5% added to the final stored sample for preservation.

![Figure 5: Pellets formed after centrifugation](image)

4.2.2.4 Staining

Identification of *Giardia* cysts and *Cryptosporidium* oocysts was done using mixture of fluorescein-labeled mouse monoclonal antibody reagents for outer wall antigen sites (epitopes) of *Giardia* lamblia and *Cryptosporidium parvum* (Waterborne Inc., 2007).

Slide staining was conducted according to the manufacturer instruction (Waterborne Inc.), using Aqua-Glo™ G/C direct comprehensive kit (Waterborne™, Inc. Hurst Street, New Orleans, and L.A 70118 USA). In this method, filtered water sample pellets about ten microliters (10µl) of aliquot dried on well slide on the open air for 15 to 30 minutes. 50 µl of DAPI (working dilution) was added on each well for one minute. Excess DAPI rinsed by adding 50–
100 µL SureRinse™ washing buffer. Intern excess fluid was absorbed using absorbent materials (soft paper) placed at the long edge of the slide, with out touching the surface of the slide. A drop (approximately 45 µL) of Aqua-Glo™ G/C antibody reagent was added to each well’s of slide, and incubated for 25 minutes at 37°C. Following this 50 – 100 µL SureRinse™ wash buffer was added and left for one minute to make well slides free of excess antibody that is not conjugated; excess fluid was absorbed using absorbent materials placed at the edge of the well slides. BlockOut™ counter stain added at this step to minimize the non specific fluorescence and to enhance the reddish back ground contrast. After one minute 50 – 100 µL Sure Rinse™ wash buffer was added and left for one minute and excess fluid was absorbed using absorbent placed at the edge of the slide. Finally, slide well’s dried placing the slide cover for observing the slide.

4.2.3 Microscope examination of Giardia cyst and Crypto oocyst

4.2.3.1 Fluorescent microscope examination of Giardia and Crypto

The stained material for both the control and sample was observed through Olympus BX51 (BX51TF, Japan) fluorescent microscope for the determination of the existence of the Giardia cyst and Cryptosporidium oocyst. The Cryptosporidium Oocysts was identified as oval to round shape with the diameter of 8-13µm in length and 7-10µm in width and the Giardia cyst was identified as 3-5µm in diameter (Waterborne™, Inc.). Analysis Softimage software (windows version 3) coupled with CC-12 camera on Olympus BX51 fluorescent microscope was used to measure the size of the (oo)cysts and to capture images.

DAPI positive (DAPI+) oocyst fluoresce a blue color with distinct nuclei or diffused blue color. The fluorescence characteristics of DAPI for determination of Cryptosporidium oocyst and Giardia cyst was detected with BX51 fluorescent microscope using UV filter block (350nm excitation and 450 emission, Dichroic mirror DM400, excitation filter BP330-385, barrier filter BA420). DAPI observed at a minimum of 400X total magnification and further precise decision taken at 1000X total magnification. Cysts and oocyst recorded as DAPI positive (DAPI+) if four or less distinct nuclei observed or deep intense blue color is observed. DAPI negative (DAPI-) if light blue internal staining with no distinct nuclei was observed.
Fluorescein observed at peak Excitation 492nm and Emission of 516nm. The combination of the oocyst and cyst wall with the antibody conjugated Fluorescein observed as a green apple (sharp rim) color surrounding the (oo)cyst wall. Further the green appeal sharp rim fluorescing Giardia cysts and Crypto oocysts shape and size was also taken into consideration to identify from pseudo and naturally green fluorescing (certain algal) substances.

4.2.1 Viability examination of Cryptosporidium oocysts
Viability of the oocyst of Cryptosporidium was determined according to Campbell et al. (1992). Fluorogenic dyes DAPI (D101: Waterborne, Inc., New Orleans, LA) and PI (Sigma Chemical Co., St. Louis, Mo.) were used to observe the staining of the Crypto oocysts. One hundred microliters (100µl) of aliquots containing Cryptosporidium oocysts added to Eppendorf tube and centrifuged at 1,200 x G about four minutes. Re-suspended in acidified HBSS (pH=2.75) and incubated at 37°C for one hour. Then it was washed twice with non-acidified HBSS (pH=7.5) by centrifugation at 1,200 x G for four minutes. Ten microliters (10µl) working solutions of DAPI (2mg/l in absolute methanol) and PI (1 mg/ml in 1X PBS, pH 7.2) was added and incubated for two hours at 37°C.

A ten microliter (10µl) of sample examined through Olympus BX51 fluorescent microscope to determine inclusion and exclusion of PI and DAPI. PI observed under green filter block 500 nm excitation and 630 nm emission (Dichroic mirror DM565, excitation filter BP520-550, barrier filter BA580). DAPI observed using UV filter block at peak excitation of 350nm and 450nm emission.

4.2.2 Viability examination of Giardia cysts
Giardia cyst viability in this study was determined according to Schupp and Erlandsen (1998) by the fluorogenic dye exclusion and inclusion method and adopted by (DeRegnier et al., 1989). Inclusion of PI indicates the cyst was not viable, while exclusion of the PI demonstrate the cyst was viable after confirmed through DIC for the presence of internal structures (such as nucleus or median bodies). A working dilution of PI prepared by adding 0.5 mg of the
stock solution of PI (Sigma Chemical Co., St. Louis, Mo.) with 50 ml of Phosphate Buffered Saline solution (PBS) at neutral pH (pH=7).

Aliquots of 100µl of sample containing *Giardia* cyst was added with 0.03ml of PI stock solution and 0.05ml of DAPI working solution incubated for 10 minutes at room temperature. A 10 µl of solution containing both *Giardia* cyst and PI placed on the slide with cover slip on the specimen, finally viewed under the fluorescent microscope (Olympus BX51). PI observed using green filter block at excitation 500nm and emission of 630nm (excitation filter BP520-550, 565-nm chromatic beam splitter and 580-nm barrier filter). *Giardia* cysts PI positive (PI+) at the specified wave length appeared as bright red fluorescent.

Because PI (Propidium Iodide) and DAPI was not selective for staining only *Giardia* cyst and *Cryptosporidium* oocyst; over estimated viability identification from interferences of algae, bacteria and other microorganisms discriminated through applications of antibiotics such as Procancillin penicillin G (100U/ml), and oxidative like Potassium Dichromate Solution (PDS). And further confirmed using DIC for determining content’s of internal structures.

4.3 Measurements of free chlorine residue and temperature
Free chlorine in the treated water measured onsite of sampling point for treated water using DPD chlorine No.1 tablet. In addition temperature was measured for both treated and untreated water on site of sampling using hand-held thermometer. Free chlorine residue was expressed in terms of milligram per liter (mg/L) and the temperature was taken in degree Celsius (°C).

4.4 Data analysis
All analysis was conducted using SPSS windows version 17 (SPSS Inc., Chicago, IL, USA) software. Chi-Square ($\chi^2$) test was used and $p$-values less than 0.05 (P<0.05) considered as statistically significant. In addition, for determining the correlation between the occurrence of *Giardia* cyst and *Cryptosporidium* oocyst detected in the distribution system liner regression test was applied. Pearson correlation coefficient and non-parametric Spearman’s rho were used to determine the relationship of cyst and oocyst with residual chlorine and temperature.
4.5 Ethical considerations

Sample collection and selection of sampling site was performed after the consent of AAWSA and obtaining the necessary information. Sampling at household level also done with there consent after explaining the intention of the sampling was for the purpose of research, and making the necessary payment since the sample volume collected from household tap was larger (100 liters from households).

5. Results

This chapter discusses the occurrence, density and viability of Giardia and Crypto oocysts at the three experimental sites; sources, reservoirs, and distribution points as presented below. In addition, the residual free chlorine and temperature in relation to occurrence of Giardia cysts and Crypto oocysts are discussed.

5.1 Giardia and Cryptosporidium occurrence determination

The occurrence of Giardia cysts and Crypto oocyst was different for the same distribution system (Table 5). For instance the occurrence of Giardia and Crypto oocysts in the raw untreated surface water is found in 33.3% and 55.6% samples respectively.

<table>
<thead>
<tr>
<th>Diss. system</th>
<th>Giardia cysts</th>
<th>Crypto oocysts</th>
<th>Overall (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>%</td>
<td>Positive</td>
</tr>
<tr>
<td>Untreated Source</td>
<td>3</td>
<td>33.3</td>
<td>5</td>
</tr>
<tr>
<td>Treated Reservoirs</td>
<td>2</td>
<td>5.88</td>
<td>3</td>
</tr>
<tr>
<td>Public tap</td>
<td>9</td>
<td>12.5</td>
<td>10</td>
</tr>
</tbody>
</table>

Like wise for thirty four samples collected from reservoir showed 5.88 and 8.2 percent positive results for Giardia cysts and Crypto oocysts respectively. These results were obtained from two out of the seventeen reservoirs under study implying that 8.82% of the reservoirs were contaminated with Crypto oocysts. The Giardia cyst and Crypto oocyst detected at
public tap samples were 12.5% and 13.9% respectively. This shows that the cysts and oocysts per samples of water in public tap is lower than source (33.3% cysts and 55.6% oocysts) but higher than that of reservoirs (5.88% cysts and 8.82% oocysts). The percentage of *Giardia* and *Crypto* found in the raw water exceeds that of the reservoirs by 27.42% of *Giardia* and 37.54% of *Crypto* prevalence and at household level by 20.8% for *Giardia* and 37.54% for *Crypto*.

The laboratory result for *Giardia* cysts revealed that 66.7% of samples collected from Geferesa site were positive while 33.33% of samples collected from Legedadi were positive. This is to say that the occurrence of *Giardia* cysts was more prevalent in Gefersa dam than Legedadi. On the other hand no *Giardia* cyst was detected in the samples collected from underground well water (KGW raw water) sources.

**Table 6: *Giardia* cysts and *Crypto* oocysts determination at source (untreated) water**

<table>
<thead>
<tr>
<th>Source raw water</th>
<th><em>Giardia</em> cysts</th>
<th>Total <em>Giardia</em> Cyst (%)</th>
<th><em>Cryptosporidium</em> oocyst</th>
<th>Total <em>Crypto</em> Oocyst (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Total sample</td>
<td>Negative</td>
</tr>
<tr>
<td>Legedadi</td>
<td>2(66.7%)</td>
<td>1(33.3%)</td>
<td>3</td>
<td>1(33.3%)</td>
</tr>
<tr>
<td>Gefersa</td>
<td>1(33.3%)</td>
<td>2(66.7%)</td>
<td>3</td>
<td>1(33.3%)</td>
</tr>
<tr>
<td>KGW</td>
<td>3(100%)</td>
<td>0(0%)</td>
<td>3</td>
<td>2(66.7%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6(66.7%)</td>
<td>3(33.3%)</td>
<td>9</td>
<td>4(44.4%)</td>
</tr>
</tbody>
</table>

In like manner, Crypto oocysts were found in 66.7%, 66.7%, and 33.3% of samples collected from Legedadi, Gefersa and KGW respectively. The proportion of *Cryptosporidium* oocysts was relatively higher at the surface raw water than the ground water (KGW). These imply that the surface raw water was more exposed to *Giardia* cysts and *Crypto* oocysts than the well water (Table 6).
Table 7: Overall *Giardia* and *Cryptosporidium* oocysts concentrations in the distribution system

<table>
<thead>
<tr>
<th>Diss. system</th>
<th>Giardia cysts/liter</th>
<th>Crypto oocysts/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>SD&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Source</td>
<td>0&lt;sup&gt;1&lt;/sup&gt;-20</td>
<td>7.91</td>
</tr>
<tr>
<td>Reservoirs</td>
<td>0-3</td>
<td>0.61</td>
</tr>
<tr>
<td>Public tap</td>
<td>0-6</td>
<td>1.35</td>
</tr>
</tbody>
</table>

<sup>1</sup> samples which are negative and no (oo)cysts detected

<sup>2</sup> Geometric mean calculated only for the positive samples in the given distribution system because it calculates the<sup>n</sup>th root of a given values.

<sup>3</sup> Standard Deviation (SD) of *Giardia* cysts/liter or *Crypto* oocysts/liter in the Diss. system

Moreover, the concentrations of the oocysts were found to be higher in the source with the mean value of 9.89±4.02 oocysts/l while the lowest was 0.5±0.26 oocysts/l detected in the reservoir. The statistical analysis implicate this difference was significant (p=0.048, p<0.05).

It should be noted that the concentration of the public tap was closer to the reservoir however it appears to be lower.

Table 8: Concentration of (oo)cysts/liter (mean ± SE) at source (untreated) raw water

<table>
<thead>
<tr>
<th>Source (untreated) raw water</th>
<th>(oo)cysts/liter</th>
<th>Giardia cyst/liter</th>
<th>Crypto oocyst/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>SD&lt;sup&gt;2&lt;/sup&gt;</td>
<td>mean ± SE&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Legedadi</td>
<td>0-20</td>
<td>11.56</td>
<td>6.67±6.67</td>
</tr>
<tr>
<td>Gefersa dam</td>
<td>0-15</td>
<td>7.64</td>
<td>8.3±4.4</td>
</tr>
<tr>
<td>KGW (Well)</td>
<td>N.D&lt;sup&gt;3&lt;/sup&gt;</td>
<td>N.D&lt;sup&gt;3&lt;/sup&gt;</td>
<td>N.D&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>0-20</td>
<td>7.91</td>
<td>5±2.64</td>
</tr>
</tbody>
</table>

<sup>1</sup>N.D= Not Detected (*Giardia* cyst, *Cryptosporidium* oocyst, or 0 cysts or oocyst/liter)

<sup>2</sup>SD= Standard Deviation

<sup>3</sup>SE= Standard Error of the mean

The concentrations of *Giardia* and *Crypto* were different for different sources, where the highest 20 cysts/l of *Giardia* cyst and 25 oocysts/l of *Cryptosporidium* oocysts were collected.
from Legedadi water sources. In addition, the highest concentrations of *Giardia* were observed in Legedadi untreated raw water with the mean concentrations of 6.67±6.67 cyst/liter. The concentrations in the Gefersa dam were 8.3±4.4 cyst/liter on average.

On the other hand, the examination for the average concentrations of *Crypto* oocysts for the three experimental sources indicated 9.78±4.02 oocyst/l. A look at individual site shows the highest concentrations in Gefersa and Legedadi dam which was 25 oocyst/liter followed by KWG.

The average *Giardia* cysts per liter at Gefersa dam was 1.24 times the cysts found at Legedadi dam. In addition, the average *Crypto* oocyst detected at Gefersa dam was larger than that of Legedadi dam with 1.13 folds. In the source untreated raw water among the observed *Cryptosporidium* oocysts the lowest mean concentration was recorded at the ground well water with the mean value of 1.3±1.3 (SD: 1.73).

### 5.3 *Giardia* cysts and *Cryptosporidium* oocysts viability

*Giardia* and *Cryptosporidium* viability test for Propidium iodide (PI+) was positive in reservoirs and public tap water (treated) while negative for 4’, 6-diamidino-2-phenlyindole (DAPI-) (*Table 9*). Thus, both *Giardia* and *Crypto* detected in the treated water samples were nonviable.

**Table 9: Mean ± SE of (oo)cysts (PI+,PI-, DAPI-PI-, DAP-PI+, and DAPI+PI-)**

<table>
<thead>
<tr>
<th>Diss. system</th>
<th><em>Giardia</em> cysts</th>
<th><em>Cryptosporidium</em> oocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PI-</td>
<td>PI+</td>
</tr>
<tr>
<td>Source</td>
<td>4.4±2.43</td>
<td>3.89±2.19</td>
</tr>
<tr>
<td>Reservoirs</td>
<td>-</td>
<td>0.15±0.11</td>
</tr>
<tr>
<td>Public tap</td>
<td>-</td>
<td>0.54±0.18</td>
</tr>
</tbody>
</table>

Similar experimentation for the source indicated 53.33 percent of *Giardia* cysts excluded the fluorogenic dye PI (means cysts are PI-). Further investigation of PI- through DIC 38.67% showed the presence of internal structures and the remaining 14.67% the contents of the
internal structures was not observed (Table 10). This implies that of the 53.33% potentially viable samples 38.67% confirmed viable while 14.67% nonviable. The overall proportion of viable and nonviable *Giardia* cysts was 38.67% and 61.33% respectively (Table 12). The mean population of the viable *Giardia* of the source was 4.4 with SE of 2.43 (Table 9).

The test for *Crypto* oocyst viability demonstrated that 7.95 % was found to be DAP+ while 61.93% and 30.11% are DAPI-PI+ and DAPI-PI- respectively. The examination of PI- *Crypto* oocysts under DIC revealed that 18.75% were the contents of internal structure observed while the other 11.36% the contents of internal structure was not observed. The mean viable, nonviable and potentially viable *Crypto* oocysts were 2.33, 17.5 and 6.2 respectively.

### Table 10: Fluorogenic dyes (PI and DAPI) percentage of *Giardia* and *Crypto* oocysts

<table>
<thead>
<tr>
<th>Diss. system</th>
<th>Giardia cysts (%)</th>
<th>Cryptosporidium oocyst (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>PI-</td>
<td>PI+</td>
</tr>
<tr>
<td>Reservoirs</td>
<td>53.3</td>
<td>46.67</td>
</tr>
<tr>
<td>Public tap</td>
<td>No¹</td>
<td>100</td>
</tr>
<tr>
<td>No</td>
<td>100</td>
<td>No</td>
</tr>
</tbody>
</table>

¹No= samples which are Negative/excludes fluorogenic dyes in a given Diss. system
²CS= Content Seen through DIC observation for *Giardia* PI- or *Crypto* DAPI-PI-
³CN= Content Not seen through DIC observation for *Giardia* PI- or *Crypto* DAPI-PI-

The viability test for *Giardia* from samples collected at Legedadi dam showed 34.62% and 65.38% potentially viable (PI-) and nonviable (PI+) results respectively (Table 11). Further experimental investigation under DIC of this potentially viable sample indicated 26.92% viable and 7.69% nonviable results.

Likewise, 63.27% samples collected from Gefersa dam were potentially viable (PI-) while 36.73% found nonviable (PI+). The DIC test for potentially viable samples resulted in 44.90% viable and 18.73% nonviable. Viability test was not undertaken for samples collected from well water since there was no *Giardia* cysts detected (Table 11).
The tests for the viability of Crypto oocysts for the three sources were described below. The viability test result of samples from Legedadi dam depicted no viable at assay, 57.14% nonviable and 42.86% potentially viable. The extended investigation carried out for potentially viable samples under DIC illustrated 32.47% confirmed as viable whilst 10.39% nonviable (Table 11).

Similar test for Gefersa dam indicated that 15.73%, 68.54% and 16% were found viable, nonviable and potentially viable respectively. The subsequent investigation of potentially viable samples through DIC showed 8.99% confirmed viable and 6.74% nonviable.

The result of the well for viability test was appeared to be 0% viable at assay, 57.14% nonviable and 42.86% potentially viable. All this potentially viable samples found nonviable under the DIC test.

Table 11: Summary of viability (DAPI+PI-, DAPI-PI+, DAPI-PI-) results of Giardia and Cryptosporidium oocysts at sources (untreated) raw water

<table>
<thead>
<tr>
<th>Source (untreated) water</th>
<th>Giardia cysts (%)</th>
<th>Cryptosporidium oocyst (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PI-</td>
<td>PI+</td>
</tr>
<tr>
<td>Legedadi</td>
<td>34.62</td>
<td>65.38</td>
</tr>
<tr>
<td>Gefersa dam</td>
<td>63.27</td>
<td>36.73</td>
</tr>
<tr>
<td>Well</td>
<td>N.D¹</td>
<td>N.D</td>
</tr>
<tr>
<td>Total</td>
<td>53.33</td>
<td>46.67</td>
</tr>
</tbody>
</table>

¹N.D = (oo)cysts Not Detected
²CS = Content Seen through DIC observation for Giardia PI- or Crypto DAPI-PI-
³CN= Content Not seen through DIC observation for Giardia PI- or Crypto DAPI-PI-

The proportions of viable and nonviable Giardia cysts at source were 38.67% and 61.33% respectively. Giardia cysts and Crypto oocysts detected at reservoirs and public tap were nonviable, as a result the proportion of viable was null (Table 12). The overall proportion of (oo)cysts indicated that 9.24% and 90.76% of Giardia cysts were viable and nonviable.
respectively. Likewise 6.14%, 29.39% and 70.61% of Crypto oocyst were viable, potentially viable and nonviable.

The treatment plant efficiency for removal of Giardia was 96.60% to 98.31 and for Cryptosporidium oocyst was 94.54% to 95.91%.

Table 12: Proportion of Giardia cysts and Crypto oocysts viability result

<table>
<thead>
<tr>
<th>Diss. system</th>
<th>Proportion of viable, potentially viable and nonviable (%)</th>
<th>Giardia cysts (%)</th>
<th>Crypto oocysts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Viable</td>
<td>Potentially</td>
<td>Nonviable</td>
</tr>
<tr>
<td>Untreated</td>
<td>Source</td>
<td>38.67</td>
<td>61.33</td>
</tr>
<tr>
<td>Treated</td>
<td>Reservoirs</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Public tap</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

1Potentially = potentially viable indicates Crypto DAPI-PI- and DAPI+PI- further investigated through DIC to determine the content of internal structures
2Viable = Giardia cysts PI- and Crypto DAPI-PI- which are confirmed through DIC for the presence of internal structure’s
3Nonviable = Giardia PI+ and Crypto DAPI-PI+ confirmed under DIC the contents of internal structures not seen

5.4 Physico-chemical (temperature and free chlorine) analysis

In the same comparison, 8.82% and 58.3% of the samples collected from the reservoir and public tap falls under the range WHO acceptable range of temperature respectively. In addition the overall result for temperature though out the distribution system implicates only 6.95% was with in the acceptable range and the largest portion 93% were above the permissible limit set by WHO (Table 13).

The overall data on free chlorine residue disclosed that 23.6%, 30.19% and 46.23% were above, below and permitted range of free chlorine residue respectively (Table 13). The analysis of data from reservoir showed that the free chlorine residue public tap (48.61%) and reservoir (41.16%) were recorded during investigation.
Table 13: Temperature and free chlorine analysis result in terms of WHO guidelines in the study area

<table>
<thead>
<tr>
<th>Diss. system</th>
<th>Temperature</th>
<th>Free chlorine residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Above¹</td>
<td>Permitted¹</td>
</tr>
<tr>
<td>Source</td>
<td>8(88.9%)</td>
<td>1(11.1%)</td>
</tr>
<tr>
<td>Reservoirs</td>
<td>31(91.17%)</td>
<td>3(8.82%)</td>
</tr>
<tr>
<td>Public tap</td>
<td>68(94.4%)</td>
<td>4(58.3%)</td>
</tr>
<tr>
<td>Overall</td>
<td>107(93%)</td>
<td>8(6.95%)</td>
</tr>
</tbody>
</table>

¹Above = above the WHO allowable limit (>temperature 15°C and >chlorine 0.2-0.5mg/l)
²Below = parameters below the WHO set limit (<chlorine 0.2-0.5mg/l)
³Permitted = acceptable with WHO guidelines (≤temperature 15°C and = chlorine 0.2-0.5mg/l)

The highest mean of temperature was recorded at the source (19.5°C) and lowest mean of the temperature measured reservoirs (18.20°C). In the distribution system the range of temperature at source (12-25°C), reservoirs (14-24°C) and household level (12-25°C) was recorded (Figure 6). At reservoirs the maximum and minimum temperature recorded was respectively 14°C and 24°C respectively with an average temperature of 18.47°C.

Figure 6: Temperature (means and range) in the distribution system
The mean temperature recorded at source was 18.3°C, 19.7°C and 23°C for Gefersa dam, Legedadi and well water respectively. Likewise the overall average temperature of source water was 19.5°C, with the maximum and minimum of 25°C and 14°C respectively (Figure 7). The source water is un-chlorinated. The average free chlorine residue in reservoirs was 0.56 mg/l with the maximum and minimum of 1.3 and 0 mg/l respectively. At household level, the average temperature and free chlorine residue was 19.04°C and 0.28 mg/l respectively. The maximum and minimum temperature was 12°C and 25°C and free chlorine was 1 mg/l and 0 mg/l at household level.

**Figure 7: Free chlorine residue (mg/l) (mean and range) in the study area**

![Free chlorine residue graph](image)

- **Key:** The minimum value was zero so that it is not displayed

**Correlation of *Giardia* and *Cryptosporidium* with Physico-chemical parameters**

The mean Physico-chemical parameters, temperature and free chlorine residue, were 18.86±0.26°C and 0.37±0.03 mg/l respectively (Figure 8). The viability result demonstrates that all the oocysts and cysts detected in treated (chlorinated) water was Propidium iodide positive (PI+ cysts and PI+DAPI-oocysts).
As indicated in the Figure 8, the occurrence of *Giardia* and *Crypto* increases in the limited range of chlorine concentration and then decreases with an increase in chlorine concentration. The percentage analysis of the (oo)cysts in relation to free chlorine residue indicated that 71% of the detected *Giardia* cysts and 61.1% of *Crypto* oocysts were found in the samples that has a concentration below 0.2 mg/l. This implies that the (oo)cysts were more dense in the range of 0.00-0.25 mg/l free chlorine residue.

Similarly the occurrence of (oo)cysts increase the range of temperature between 15°C and 22°C. 85% of *Giardia* cysts and 83.3% *Crypto* oocysts were detected in this range of temperature, 15°C-22°C. This shows that the occurrence of *Giardia* cysts and *Crypto* oocysts were higher in this range of temperature.

**Figure 8: Relationship of (oo)cysts/liter with free chlorine residue and temperature**

Keys: A negative sign for free chlorine residue indicates the sample was un chlorinated or the samples were taken from the source untreated raw water.
There was statistically significant but negative correlation between free chlorine residue and (oo)cysts occurrence, (p<0.05), (Giardia $r = -0.410$; Crypto $r = -0.44$). Similarly statistically significant correlation between occurrence of Giardia cysts and Crypto oocysts with temperature was observed (p<0.05).

6. Discussion

Giardia and Cryptosporidium were identified in this study. In similar studies Giardia and Crypto were reported by different scholars in several part of the world. For instance Cryptosporidium oocyst and Giardia cyst were detected in surface or drinking water of Spain (Carmena et al., 2007), Varna (Stoyanovai et al., 2006), Russia and Bulgaria (Karanis et al., 2006b), South Africa (Jarmey-Swan et al., 2001; Kfir et al., 1995; Sigudu et al., 2008), Taiwan (Watanabe et al., 2005; Tai-Lee, 2002), Canada (Wallis et al., 1996; LeChevallier et al., 1991b), USA (LeChevallier et al., 1991b), Greece (Karanis et al., 2002), India (Anbazhagi et al., 2007), Malaysia (Lim et al., 2004), Norway (Robertson et al., 2002), Brazil (Nishi et al., 2008), including Addis Ababa (Nigus Fikrie et al., 2008).

From the total collected 115 samples, Giardia was detected in 33.3%, 5.8% and 12.5% and Cryptosporidium in 55.6%, 8.82% and 13.89% of the samples respectively at source, reservoirs, and public tap. This result was comparatively closer to similar studies conducted around the globe. For example a study conducted by Wallis et al. (1996) and his co-researchers detected Giardia in 18.2% of treated water samples. And a detection of Crypto in 18.2% in treated water samples in Southern Russia and Bulgaria was reported by Karanis et al. (2006b). Likewise, the investigation on a treated drinking water in Spain detected Giardia in 19.2% of SWTF samples and Cryptosporidium in 15.4% of CWTF samples (Carmena et al., 2007).

Contrary to these, there are studies that in which either or both Giardia cysts and Cryptosporidium were not detected in treated and untreated raw water (Bakir et al., 2003; Watanabe et al., 2005; Karanis et al., 2002).
**Giardia** and **Cryptosporidium** mean concentrations at source were 5 cysts/l and 9.8 oocysts/l, reservoirs 0.15 cysts/l and 0.5 oocysts/l and public tap 0.47 cyst/l and 0.72 oocysts/l detected. These findings were nearly similar to the study of LeChevallier et al. (1995) that reported the average concentration of 2.1 cysts/liter (range 0.4-6.3) and 2.0 oocysts/liter (range 0.3 - 9.8). The findings of this study were much lower that the finding of Sigudu et al. (2008) that reported the concentration of more than 1,400 oocysts/10 liter and 2,700 cysts/10 liter but higher than the mean concentration of 0.15 oocysts/l and 0.2 cysts/l recorded by Nishi et al. (2008). An investigation made by Stoyanovai et al. (2006) on drinking water supply contamination with **Giardia** and **Cryptosporidium** in Varna found positive with an average number of 5 cysts/liter.

**Giardia** (33.3%) and **Cryptosporidium** (55.6%) found at source is the highest percentage than reservoirs (**Giardia** 5.88% and **Cryptosporidium** 8.82%) and at public tap level (**Giardia** 12.5% and **Cryptosporidium** 13.9%) (Table 5). In addition the statistical analysis result demonstrates there is **significant difference** between the source untreated raw water and treated water (reservoirs and public tap) (p<0.05). Likewise, in the earlier study in Addis Ababa drinking water demonstrates significant difference in concentration of **Giardia** and **Cryptosporidium** between treated and untreated water (Nigus Fikrie et al., 2008).

**Giardia** and **Cryptosporidium** were detected in 33.3% and 55.6% of samples from source raw water respectively (Table 5). Similarly Carmena et al. (2007) detected **Giardia** in 45.2% samples from SWTF and 92.3% in samples from river and **Cryptosporidium** in 22.6% of samples from SWTF and in 63.5% samples from river. A study conducted in South Africa by Sigudu et al. in 2008 detected **Giardia** and **Cryptosporidium** in all (100%) raw water samples collected from selected catchments. **Giardia** cysts was found in 3/6 (50%) of samples from river water while no **Giardia** and **Cryptosporidium** were reported both in untreated dam raw water and municipal drinking water (Bakir et al., 2003). Robertson et al. (2001) study in Norway raw water demonstrates the presence of **Cryptosporidium** in 13.5%, **Giardia** in 9% and both parasites in 2.5% samples. Intern Nishi et al. (2007) found **Giardia**, **Cryptosporidium** and both in 6.66%, 26.66% and 13.33% samples from raw water respectively. Karanis et al. (2005) also detected **Giardia** and **Cryptosporidium** in 81.81% of
samples from river water. Wallis et al. (1996) detected *Giardia* in 21% of raw water samples. In addition Karanis et al. (2002) identified *Giardia* in 20% of samples from river water and 55.56% from lake water.

The overall status of *Giardia* cysts and *Cryptosporidium* oocysts found in ground well water were lower than that of surface water identified. This can be due to surface water was more exposed to various contaminants than that of ground water (USEPA, 2001). Even though ground water has lower possibilities for contamination by cysts or oocysts but it can be contaminated from surface activities through infiltration. For instance ground water (well) is usually free of *Giardia* and *Cryptosporidium* but it can be contaminated occasionally (LeChevallier et al., 1995). In likewise well water *Giardia* and *Cryptosporidium* were found in 11.1% and 16.7% samples respectively (Karanis et al., 2006b). In addition *Giardia* cysts were reported by Bakir et al. (2002) in 5.9% of samples from well water. And Watanabe et al. (2005) detected *Cryptosporidium* in underground well water.

In this investigation *Giardia* and *Cryptosporidium* were found in 5.88% and 8.82% of samples from reservoir respectively. Similar experiment conducted by Carmena et al. (2007) reported the presence of *Giardia* and *Cryptosporidium* in reservoirs. The researcher found *Giardia* and *Cryptosporidium* in 55.5% and 33.3% of sample water from reservoirs.

In public tap at household distribution point *Giardia* and *Cryptosporidium* was found in a percentage of 12.5% and 13.8% samples respectively. Similarly Karanis et al. (2006b) found *Giardia* and *Cryptosporidium* in 5.2% and 12.1% sampled tap water. In addition, in Greece Karanis et al. (2002) reported *Giardia* in drinking water (14.29%). Also in Addis Ababa various studies indicated improper waste disposal and poor sanitation were the major factors that contributes for the pollution of water, and pipe line also crosses through sewage lines that expose the treated water for contamination (Mengestayhu Birhanu, 2007; Solomon and Ruth et al., 2004; Mesfine Tilaye, 1998). In addition leakage and breakage of pipelines enhances the likelihood of drinking water contamination with these parasites. As quoted by Solomon and Ruth et al. (2004) 30-40% of water supplied by the agency was lost due to leakage reason.
The (oo)cysts detected in water samples was 55.6% at sources, 8.82% at reservoirs and 18.06% at public tap. This indicates that high prevalence of the parasites as compared to tap water and reservoir. The comparison made between the public tap and reservoir in terms of the level of *Giardia* cysts and *Crypto* oocyst prevalence showed closer result and there is no statistically significant difference (p=0.103, p>0.05).

This finding was also in agreement with the investigation conducted by Lim *et al.* (2004) on river water frequently used by the Temuan Orang Asli (aborigine) in Malaysia. They indicated that 66.7% of the river water samples were *Giardia* cyst positive and 5.6% were *Cryptosporidium* oocyst positive. In addition the study conducted by Clapham and Franklin (1998) from 15 drinking water supplies of private drinking water supplies in US nine of them have *Cryptosporidium* (60%) and eight sites were positive for *Giardia* (53.3%). But (oo)cysts proportion found in this investigation was much lower than similar study conducted on surface water that reported 97% positive for *Giardia* or *Cryptosporidium* and 81% for *Giardia* and 87% for *Cryptosporidium* individually (LeChevallier *et al.*, 1991a).

This may be due to source water was not treated and it had wide possibilities for contamination than that of reservoirs and tap water which are treated and confined in pipe lines. Source water, can be easily contaminated by grazing animals, animal farming and run off. This analysis can be supported by the study conducted on microbial pollution of major rivers in Greece that indicated human interference and lack of proper pollution monitoring activities as main factors for the contamination of rivers by *Giardia* and *Cryptosporidium* (Karanis *et al.*, 2005).

In the present investigation *Cryptosporidium* is more frequently found than *Giardia* with 2.63% increment. *Cryptosporidium* oocyst average density was 0.03 times more than that of *Giardia* cyst. This finding agrees with different scholars who reported that *Crypto* oocysts were more prevalent than *Giardia* cysts (LeChevallier *et al.*, 1991b; Stoyanovai *et al.*, 2006; Sigudu, *et al.*, 2008; Tai-Lee, 2002).
Another possibility could be due to the fact that *Crypto* is smaller in size, more resistant and has better capacity to penetrate most conventional treatment (chemical and physical) method than *Giardia*. For instance WHO (2006) stated that *Cryptosporidium* is more resistant to chemical disinfectants, small in size and persistent in the environment as compared to *Giardia, Cyslospora, Entamoeba, Toxoplasma, Balantidium and Cyclospora*.

Although *Crypto* was more frequently found than *Giardia*, the analysis for the relationship between cysts and oocysts revealed their coexistences. The statistical analysis for their relationship indicated that there was statistically significant correlation between the occurrence of *Giardia* cysts and *Crypto* oocyst (r = 0.55, p<0.05). This finding was consistent with LeChevallier et al. (1991b) that reported the occurrence of *Crypto* and *Giardia* was significantly correlated (r=0.59, p<0.01).

However, the physico-chemical parameters indicated, negative correlation between chlorine and (oo)cysts existences (p<0.05), while there is positive correlation with temperature (p<0.05) This finding is inconsistent with various studies that reported no significant correlation between (oo)cysts and free chlorine residue (Finne, 2006; Clapham and Franklin, 1998).

Fluorogenic dyes staining of *Giardia* cysts provides 38.67% PI-, and 61.33% PI+ at source (Table 12), meanwhile no cyst observed under PI- at reservoirs and public tap. That is to say all cysts found in reservoir and tap water samples were PI+. Viability examination of *Crypto* oocysts at source indicated 8.59%, 66.87% and 25.54% for DAPI+PI, DAPI-PI+ and DAPI-PI- respectively. In treated water, reservoirs and public tap, *Crypto* oocysts were found Propidium iodide positive (PI+). These indicated that the viability result of cysts and oocysts were viable only at source. The overall viability tests result at source indicated lower proportion of viable and potentially viable oocysts (8.59% and 33.1%) but higher number of nonviable oocysts (66.87%). The study undertaken by Wallis et al. 1996, in Canada confirms higher proportion of nonviable cysts.
The mean temperature recorded at source was 18.3°C and 19.7°C for Gefersa dam and Legedadi respectively. The overall average temperature of source water was 20°C, with the range of 17°C to 25°C. The average temperature in various distribution systems was 18.89°C with maximum and minimum of 12°C and 25°C respectively. *Giardia* cysts survival decreases with an increase in temperature. Survival in tap water at 8°C was 77 days but 4 days for 37°C and 54 days at 21°C (Bingham and Meyer *et al*., 1979).

According to WHO the concentration of free chlorine in piped water should be maintained at a level of 0.2-0.5 mg/l to reduce growth of microbes and the risk of being contaminated again (WHO, 2004). In support of this standard 71% of cysts and 61.1% of oocysts detected were below the acceptable limit of WHO, 0.2 mg/l free chlorine residue. Mean while the minimum time required for 90 percent inactivation of *Giardia* cysts was 25-30 minute with 1 mg/l (WHO, 2008). In such a way the amount of free chlorine obtained in this investigation 23.6%, 30.19% and 46.23% were above, below and at an acceptable limit of WHO. Similarly the study conducted on assessment of physico-chemical quality of Akaki (Addis Ababa) drinking water by Mengestayhu Birhanu, (2007) indicated, 48.6% was at an acceptable free chlorine range (0.2-0.5mg/l) while and 17.1% above the recommended level (0.5 mg/l). Further he reported at house hold level 14.3% samples were within the acceptable free chlorine range.

The average free chlorine residue in treated water at reservoirs was 0.5 mg/l (ranging from 0-1.3 mg/liter) and an average temperature was 18.5°C with a range of 14-24°C (Figure 8). The mean free chlorine in public tap at household level was 0.28 mg/l (range 0-1 mg/l) and temperature of 19.04°C (range 12-25°C). The common disinfectant chlorine at a concentration of 8 mg/l for 10 min at pH of 6 and 7, and for 30 minute at pH of 8 makes them all nonviable (Jarroll *et al*., 1981).

In this investigation treatment plant efficiency for removal of *Giardia* and *Crypto* was 96.60% to 98.31% and 94.54 to 95.91% respectively. In the previous study Nigus Fikrie *et al*., (2008) reported that the removal efficiencies of *Giardia* was 92.31% to 94.74% and that of *Crypto* oocysts was 96.3% to 98%. *Giardia* and *Crypto* removal efficiency is determined by the method employed. Slow sand filtration can remove 83% of heterotrophic bacteria, 100% of
Giardia cysts, 99.98% of Cryptosporidium oocysts and 50-90% of organic and inorganic substances (Palmateer et al., 1999). Sandy loam or higher soil bulk density constructed vegetative buffer has lower potential to remove Cryptosporidium than silty clay or loam, or at lower bulk density. However Addis Ababa drinking water treatment plant uses fast sand filtration to meet higher demand of the populations. As a result some (oo)cysts could infiltrate into drinking water.

The treatment plant of Addis Ababa uses chlorination. Even though application of UV light (Shin et al., 2001) and ozonation (Korich et al., 1990) can potentially affect the viability of Giardia and Cryptosporidium oocyst; it can not affect those parasites contaminating treated water through out the pipe after treatment in the reservoir. Hence, chlorination can be preferred method to use as disinfectant. But Giardia cysts and Crypto oocysts are resistance to chlorine inactivation (Rice et al., 1982). Chlorination alone can not totally affects the existence (or viability) of these parasites. Alternative ways to increase efficiency of sand filtration and minimize fecal contamination of treated water is through differentiating sewage pipe line from drinking water pipe, controlling breakage and leakage of pipes due to oldness and human activities.

Giardia and Crypto mean concentration at public tap level was higher by 3.13 cysts/l and 1.44 oocysts/l times than the average (oo)cysts detected at reservoirs. This implicates contamination of drinking water effluent after treated in reservoirs. Even though good water quality provided at source, fecal contamination of drinking water container could take place at house hold level (Wright et al., 2004). Drinking water quality at household level affected by various mechanisms such as handling and choice of storage containers, collection, during storage, hygiene, number of family members, education and socio-cultural affects drinking water quality at household level. In addition water storage using narrow necked material (difficulties in washing), water distribution with out treatment and inefficient chlorine, sewerage line and pipe arrangement, poor hygiene practices were main cause for microbial contamination at household level (Mengestayhu Birhanu, 2007; Crampton, 2005).
7. Conclusions

*Giardia* cyst and *Cryptosporidium* oocyst were detected in Addis Ababa municipal drinking water. The occurrence of *Cryptosporidium* was more frequent than that of *Giardia* cysts in the drinking water. The possible source of contamination of the treated drinking and source untreated water can be zoonotic and human activities such as agricultural and waste dumping around the water catchment area. This demonstrates the need for continuous monitoring of *Giardia* and *Cryptosporidium* in Addis Ababa municipal drinking water.

The concentrations of *Giardia* cysts and *Crypto* oocysts were highest at source (4.4 cysts/l and 9.8 oocysts/l) as compared to than reservoirs (0.15 cysts/l and 0.32 oocysts/l) and public tap (0.41 cysts/l and 0.72 oocysts/l). Accordingly, the concentrations were higher in tap water than reservoir. The statistical test showed significant differences between the concentrations of *Giardia* and *Crypto* in treated and untreated water (P<0.05).

The treatment plant removal efficiency for *Giardia* and *Crypto* was from 96.60% to 98.31% and 94.54% to 95.91% respectively. Hence, this treatment plant alone was not sufficient to completely remove *Giardia* cysts and *Cryptosporidium* oocysts.

Like the concentration of the occurrence of *Giardia* and *Crypto* were higher at source (33.3%; 55.6%) than both reservoirs (5.88%; 8.82%) and public tap (12.28%; 15.65%).

Viability test at the reservoirs and public tap for the *Giardia* cysts and *Cryptosporidium* oocyst revealed nonviable results. While *Giardia* cysts and *Crypto* oocysts found in raw surface water was viable, nonviable and potentially viable.

In this study the physico-chemical parameters (temperature and free chlorine residue) were significantly correlated to the occurrence of *Giardia* cysts and *Cryptosporidium* oocyst. In addition the occurrence of *Giardia* cysts and *Crypto* oocysts was significantly correlated (r = 0.55, p<0.05).
8. Recommendations
As described above Giardia cysts and Crypto oocysts were detected with considerable concentrations. The removal efficiency of treatment plants was not to the required capacity to eliminate the parasites. The following recommendations are drawn in light of these facts.

- Improving the removal efficiency of the treatment plant through better filtration methods such as slow sand and membrane filtrations.

- Periodical checking the status of the Giardia cyst and Cryptosporidium oocyst in the treated and untreated raw water particularly at household level. Because conducting studies at different times help to coup with seasonal variation.

- Minimize the risk of infection through point use of: filtration, treatment (aqua-tab) or boiling drinking water.

- Detailed studies on possible sources of Giardia cysts and Crypto oocysts contamination in recreational water and food items (milk, raw and vegetables and so on) for public health concern.

- Develop simultaneous method for identifying and conducting viability test for Giardia cysts and Cryptosporidium oocysts to reduce lengthy process and ease the monitoring of drinking water qualities.

9. References


http://www.healthunlimited.org/Programmes/Emergencies/Ethiopiadiseaseoutbreak


Mengestayhu Birhanu. (2007). Assessment of Physico-chemical and Microbiological Quality of Drinking Water at Sources and Household in Selected Communities of Akaki-kaliti sub city, Addis Ababa City Administration. M.sc thesis. Environmental science, Faculty of science, AAU. Pp: 30-64.


http://en.afrik.com/article16082.html


Annexes

Annex 1: Preparation of working dilution of DAPI
1. Prepare environmental sample(s) to be applied to well slide.
2. Dilute DAPI to a 1X working dilution.
   - Add 1 µL D101 to 5 mL of PBS (phosphate-buffered saline solution, pH 7.4).
   - Alternatively, 10 µµL may be diluted in 50 mL PBS. Mix by inversion. Prepare working dilution daily. Discard any unused 1X solution.

Annex 2: Preparation and components of HBSS
Step 1: Prepare one thousand (1000 ml) pre sterilized conical flask
Step 2: Measure each of the components based on the following amounts

<table>
<thead>
<tr>
<th>Components</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>8000</td>
</tr>
<tr>
<td>Potassium Chloride (KCl)</td>
<td>400</td>
</tr>
<tr>
<td>Potassium Phosphate, Monobasic (anhydrous) KH₂PO₄</td>
<td>60</td>
</tr>
<tr>
<td>Glucose</td>
<td>1000</td>
</tr>
<tr>
<td>Phenol Red, Na salt</td>
<td>10</td>
</tr>
<tr>
<td>Sodium Phosphate, Dibasic●7H₂O anhydrous.</td>
<td>48</td>
</tr>
<tr>
<td>Magnesium Sulfate, anhydrous. MgSO₄</td>
<td>98</td>
</tr>
<tr>
<td>Calcium Chloride, anhydrous.</td>
<td>140</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>350</td>
</tr>
</tbody>
</table>

Step 3: Mix the measured amounts using magnetic stirrer.
Annex 3: Sample collection containers and methods

a) White plastics containers (sterilized using sodium hypochlorite after each sampling)

b) Sterilization of tap using flame: holding ethanol wetted cotton using forceps

c) Collected samples shipment to laboratory

Annex 4: Calculation of Giardia cysts and Crypto oocyst per liter of water

a. Calculating the cysts and oocysts per liter:-

1st calculate the fraction of final concentrate examined “E”  \[ E = \frac{(Va \times Na)}{Vc} \]

Where:-
- Va= is volume of aliquots
- Na= is number of aliquots of equal volume Va placed on slide membrane
- Vc= is volume of final concentrate

2nd calculating the final fraction “F” of the initial concentrate used to prepare the final concentrate from  
\[ F = \frac{V_f}{V_t} \]

Where:-
- Vf= is volume of initial concentrate used for flotation
- Vt= is total volume of initial concentrate

NB: - if the initial concentrate is not further concentrated or subjected to flotation step, or if all the initial concentrate is concentrated, the volume of F is equal to 1

3rd calculating the number of oocysts per liter of the original sample
\[ O = \frac{C}{(V \times F \times E)} \]

Where:-
- C= is the total number of oocysts counted on the microscope slides
- V= is the volume (in liters) of water samples
- F= is the fraction of initial concentrate which is further concentrated
- E= is the fraction of final concentrate that is examined on the slide

b. Calculating proportion of viable oocysts and cysts

The proportion of viable oocysts and cysts calculated by dividing DAPI+/PI- by the total number of oocysts examined.

\[ \frac{DAPI+/PI-}{\text{Total observed}} \times 100 \]

c. Calculating proportion of potential viable oocysts and cysts

The proportion of potentially viable oocysts calculated by taking the summation of DAPI+/PI- and DAPI-/PI- and dividing by total observed oocysts and cysts.

\[ \frac{(DAPI+/PI-) + (DAPI-/PI-)}{\text{Total observed}} \times 100 \]

Annex 5: Treatment plant removal efficiency of *Giardia* and *Crypto* oocysts calculation
\[
\text{% of Removal efficiency} = \frac{C_{in} - C_{fin}}{C_{in}} \times 100
\]

Where, \( C_{in} \) = initial concentration of *Giardia* cysts and *Cryptosporidium* oocysts in raw water
\( C_{fin} \) = final concentration of *Giardia* cysts and *Cryptosporidium* oocysts in treated water
Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university and that all sources of materials used for the thesis have been correctly acknowledged.

Name: Tesfalem Atanfu WGabriel
Sign: ______________