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DRINKING WATER SOURCE AND THE PREVALENCE OF *Giardia lamblia* AND *Cryptosporidium parvum* AMONG CHILDREN IN SELECTED VILLAGES OF PAWI SPECIAL DISTRICT, BENISHANGUL_GUMUZ REGION.

BY

EYASU TIGABU

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (BIOMEDICAL SCIENCE)

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Drinking Water Source and The Prevalence of ***Giardia lamblia*** and ***Cryptosporidium parvum*** Among Children in Selected Villages of Pawi Special District, Benishangul-Gumuz Region.

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Biomedical Science)

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July, 2007

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List of abbreviations

a.s.l.	Above sea level
AAU	Addis Ababa University
AIDS	Acquired immunodeficiency Syndrome
CD	Cluster of Differentiation
DNA	Deoxyribo Nucleic Acid
EHNRI	Ethiopian Health and Nutrition Research Institute
ELISA	Enzyme linked immunosorbent Assay
ERCP	Endoscopic Retrograde Cholangiopancreatography
Gal-GalNAc	Galactose-Nacetylgalactoseamine
GI	Gastrointestinal
HAART	Highly Active Antiretroviral Therapy
HCl	Hydrochloric acid
HIV	Human Immunodeficiency virus
IF	Immunofluorscence
IFA	Immunofluorscence Assay
PCR	Polymerase Chain Reaction
Prev.	Prevalence
PVA	Polyvinyl alcohol
Rpm	Rounds per minute
SAF	Sodiumacetate-Acetic acid-Formaldehyde
WHO	World Health Organization

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Abstract

Giardia lamblia and *Cryptosporidium parvum* are parasitic protozoans that infect humans as well as domestic and wild animals all over the world. These parasites are implicated in many water borne disease outbreaks in different parts of the world. The present study was conducted to assess the prevalence of these two parasites among children below 14 years old that drink water from unprotected water sources-Ali-spring, Diga dam, and hand dug wells and "protected" wells with hand pumps in selected villages (Almu, K2V24 and K2V23/45) in Pawi Special District Benishangul-Gumuz Region. Single stool specimens were collected from a total of 384 children from the three villages. For identification of *Cryptosporidium parvum* the modified Ziehl-Neelsen staining method was used. *Giardia lamblia* was detected using direct microscopy based on wet mount and formalin-ether concentration techniques. Out of the 384 children examined, **102 (26.6%)** and **31 (8.1%)** were found positive for *G.lamblia* and *C.parvum* infection, respectively. Overall co-infection with intestinal parasites was detected in **4.4%** of the study participants, in which *G.lamblia* and *C.parvum* comprised the highest proportion. Prevalence of giardiasis in female children was significantly higher than in the males ($p<0.05$), suggesting a higher risk of exposure of females to contaminated water. However, no significant association was observed for infection of cryptosporidiosis between the two sexes ($p>0.05$). *G.lamblia* and *C.parvum* infection prevalence was not significantly different among the different age groups ($p>0.05$). On the other hand, the prevalence of *G.lamblia* and *C.parvum* was associated with the source of drinking water with more cases of giardiasis detected in study participants using water from unprotected water sources than those using the "protected" water ($p<0.05$). Contrary to this, more cases of cryptosporidiosis ($P<0.05$) were detected in those using "protected" water sources (water wells with hand pumps). This could be an indication of the possibility of water well contamination through seepage from domestic latrines as these wells are constructed close to residential houses. Children from relatively high monthly income families were less ($P<0.05$) affected by giardiasis than from low monthly income families; however, no such association was detected in the case of cryptosporidiosis. Furthermore, breast feeding and its duration was negatively associated ($P<0.05$) with *G.lamblia* prevalence suggesting a protective role for breast milk against giardiasis. Thus from the finding of the study one can conclude that provision of well protected and treated drinking water must be considered a priority to reduce the existing high prevalence of giardiasis and cryptosporidiosis in Pawi Special District.

1. Introduction

Parasitic diseases are incriminated in causing more than 33% of global deaths of which intestinal parasitic infections are believed to take the major share (WHO, 1991; 1998). Lack of safe drinking water and environmental sanitation are largely responsible for more than 800 million expected cases of diarrheal diseases and 4.5 million associated deaths in many developing countries every year (Esery *et al.*, 1990). Morbidity and mortality due to diarrheal diseases in developing countries remain to be the main public health problems that need due attention. Although there could be many other causes of diarrhea, the enteric protozoa *Cryptosporidium parvum* and *Giardia lamblia* have been recognized as important causes of both out-break-related and sporadic diarrhea in humans. Both immunocompetent and immunocompromised individuals could be the victims of diarrheal diseases due to these parasites (Esery *et al.*, 1990).

The etiologic agent of giardiasis is *Giardia lamblia* and that of cryptosporidiosis is *Cryptosporidium parvum*. Globally, around 200 million people are chronically infected with *Giardia lamblia* and 500 000 new cases are reported annually (WHO, 1998).

Giardia lamblia was first described by Antoine Van Leeuwenhoek (1632-1723). Although it was the first protozoan parasite that was discovered earlier, its importance in medicine did not get emphasis until 1970s. The organism was believed to be of no medical importance by being considered as part of the commensal fauna of the intestine. However, later on *Giardia lamblia* got due attention of many scientists as it was found to be involved in many endemic and epidemic diarrheal diseases through out the world (Adams, 1991).

Cryptosporidium parvum is an increasingly recognized causative agent of intestinal infection in immunocompetent and immunocompromised humans and many other animals. Persistent diarrhea, especially in children, is caused by this pathogen in developing countries (Griffith, 1998). It also causes a self-limited diarrheal disease in

immunocompetent individuals. Human-to-human fecal-oral transmission, animal to human transmission and water borne transmission are the most routinely known causes of cryptosporidiosis (Current and Garcia, 1991). Worldwide 1 to 10% of acute self-limiting diarrheal diseases in immunocompetent individuals are attributable to cryptosporidiosis (Xian-Ming and LaRusso, 1999).

Cryptosporidium species was first described and named in 1907. However, important information regarding its biology, human infection and epidemiology has been well understood only within the past two decades. Although the first case of human cryptosporidiosis was reported in 1976, more information and awareness about this organism came to the fore in the 1980s because of the association of the infection with the pandemic disease, HIV/ AIDS (Meisel *et al.*, 1976; James, 1988).

In the 1990s, *Cryptosporidium parvum* was found as one of the most important pathogenic contaminants detected in drinking waters. This is mostly attributed to its low infective dose and high resistance to the common water disinfectants such as Chlorine and against environmental factors such as low temperature (Fayer *et al.*, 1998; Payment, 1999). In Nordic countries, recent data reveal that the parasite was detected in surface water sources in rivers and lakes and can pose a potential biothreat for drinking water supplies (Robertson and Gjerde, 2001; Rimhanen-Finne *et al.*, 2002).

Cryptosporidium parvum and *Giardia lamblia* have been implicated in many water born diarrheal out-breaks both in adults and children that are exposed to contaminated drinking water and swimming in lakes and rivers (Hojlying *et al.*, 1987; Isacc *et al.*, 1987; Flanagan, 1992; Serpil *et al.*, 2005). There is also a marked seasonality in the onset of illness due to these intestinal protozoan infections (Soriano *et al.*, 2001; Gamboa *et al.*, 2003). Giardiasis and cryptosporidiosis in children are more common and the transmission may be effected through close contact with infected individuals; for example, children in daycare centers are at great risk of having giardiasis and

cryptosporidiosis when compared with the general population (Hlavsa *et al.*, 2005; Addis *et al.*, 1991).

1.1. The organisms

Cryptosporidium species

The genus C*ryptosporidium* is comprised of a group of coccidian parasites of the phylum protozoa. On the basis of the difference in their host specificity, oocyst morphology and site of infection, thirteen species of *Cryptosporidium* are recognized. Most of these species are known to infect only one or a few group of animals (Table-1).

Table-1. Valid names of *Cryptosporidium* species that infect mammals and other animals (Coupe *et al.*, 2005).

Species	**Host Animal**	**Infection site**
C.andersoni	Cattle	Abomasum
C. baileyi	Chicken	Cloaca/respiratory tract/ kidney
C.felis	Domestic cat	Intestine
C.meleagridis	Turkey	Intestine/respiratory tract
C.muris	Rodent	Stomach
C.nasorum	Fish	Stomach/intestine
C.parvum	Mammals/humans	Intestine (predominantly)
C.saurophilum	-	-
C.serpentis	Snakes	Stomach
C.wrairi	Guinea pigs	Intestine
C.hominis	Mammals	Intestine
C.canis	Mammals	Intestine
C.gallis	Birds	Cloaca/respiratory tract

Cryptosporidium parvum isolates are separated into two genetically distinct sub groups, designated as genotype 1 and 2. Genotype 1 is anthroponotic and has so far been associated with human and primate infection. Genotype 2 is zoonotic and is known to infect a wide range of mammals, including humans (Spano *et al.*, 1998; Patel *et al.*, 1998).

Giardia species

Giardia lamblia is a unicellular flagellated protozoon that is recognized as one of the ten top parasites of man. Two major subtypes of *G.lamblia* (Assemblage A and B) are known to occur (Guy, *et al.*, 2004). More than 40 animal species has been known to act as a host for this parasite (Meyer, 1994). Different animal species are parasitized by five known species of the genus *Giardia*. *Giardia lamblia* can infect mammals including man, rodents, birds and reptiles. *Giardia muris* infects rodents, birds and reptiles. *Giardia agilis* infects amphibians (Filice, 1952); *Giardia ardae* infect the great blue heron (Erlandsen *et al.*, 1990) and *Giardia psittasci* infects the budgerigar (Erlandsen and Bemrick, 1987).

1.2. Biology and life cycle

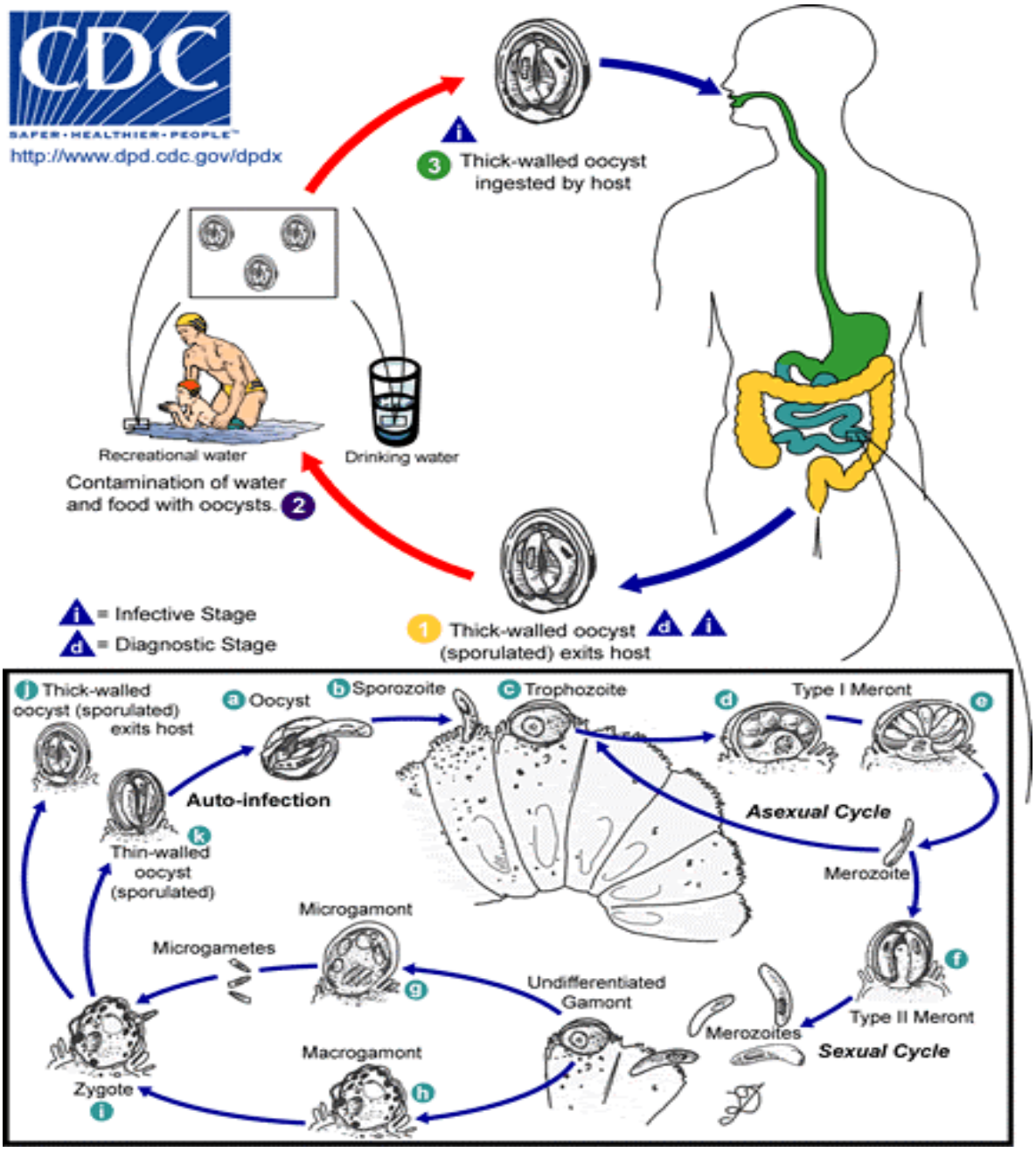
Cryptosporidium parvum

The life cycle of *Cryptosporidium parvum* commence after ingestion of the infective stage, the oocyst, by a susceptible host (Figure-1). The oocyst is spherical in shape measuring 3-6µm in diameter and it may be either thick or thin walled (Ramirez *et al.*, 2004). The resistant stage that is found usually in the environment is the thick walled oocyst excreted together with feces (Fayer and Ungar, 1986). Each oocyst has 4 infective sporozoites that come out from the oocyst using the suture at one side of the oocyst. The ileum is the preferable site of infection and the sporozoites penetrate epithelial cells of the ileum. *Cryptosporidium parvum* resides on the luminal surface of epithelial cells and it is used to be thought to reside extracellularly. However, ultra

structural observations have revealed that it is intracellular but extracytoplasmic, enclosed by a thin layer of host cell cytoplasm. Infection could possibly occur with ingestion of as few as 30 oocysts; some infection has also occurred with just a single oocyst (Fayer, 1995).

Cryptosporidium parvum can complete its life cycle in as short as 2 days and the infection may be short lived or may be persistent for months. Excystation of the oocyst is initiated by the body temperature, interaction with stomach acid and bile salt. The released sporozoites attach to epithelial cell and become enclosed within parasitophorous vacuoles. The trophozoite stage then undergo asexual proliferation by merogony and two types of meronts are produced, Type I meronts and Type II meronts (Fayer and Ungar, 1986; O'donoghue, 1995). Type I meronts form 8 merozoites that are released from the parasitophorous vacuole when they mature. The merozoites then enter another brush border surface epithelium where they undergo another cycle of type I merogony (multiple fission or schizogony) or else they may develop into type II meronts. The type II meronts give rise to 4 merozoites which do not undergo further merogony but produce gamonts, the sexual reproductive stages which fuse and form the only diploid stage in the life cycle, the zygote. A resistant oocyst wall is then formed around the zygote. The zygote undergoes asexual development (sporogony) and gives rise to sporulated oocyst that contains 4 sporozoites. Two possible auto-infective cycles occur in *Cryptosporidium parvum*. The first is by the continuous recycling of Type I meronts and the second through sporozoites rupturing from thin-walled oocyst.

Experimentally infected animals have shown a prepatent period of 4 days but sometimes it could be 3 days in heavy infection. In humans when lower numbers of oocysts are probably ingested, the prepatent period is typically 4 to 6 days. The length of time in which oocysts are shed in feaces generally lasts 6 to 18 days (4 to 10 days of diarrhea) in immunocompetent individuals but it may be prolonged in immunocompromised individuals. Some patients may discharge oocyst yet they appear asymptomatic (Fayer and Ungar, 1986).



Source:- http://www.dpd.cdc.gov/dpdx

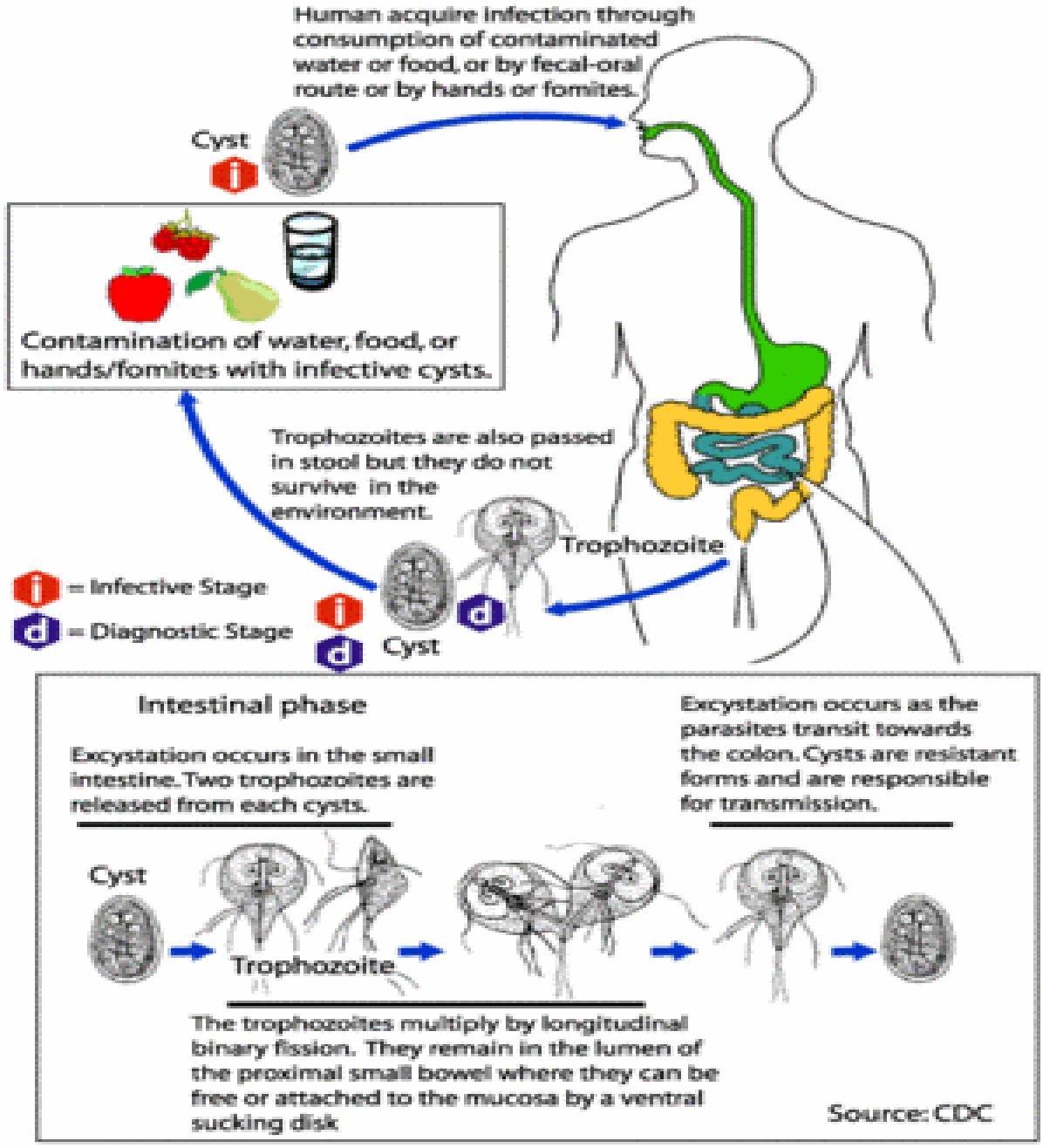
Fig-1 Diagrammatic representation of life cycle of *Cryptosporidium parvum*.

Giardia lamblia

The parasite reproduces by binary fission which is a type of reproduction in which one cell divides into two new cells by mitosis. During the growth cycle the components of the *Giardia* cell multiply so that each daughter cell would be a complete copy of the original parent cell. The newly formed cells then pinch off from each other; in so doing a complete reproduction cycle would occur (Figure- 2).

As it has been explained by Heresi and Cleary (1997), the infective stage of *Giardia lamblia*, the cyst, is elliptical in shape and its size ranges from 6 to 10 microns and contains two to four nuclei. The cyst possesses a structure that enables it to be resistant to most environmental factors and disinfection and make it successful in being the infective stage of the parasite. The cyst has a thin and protective wall that allows it to survive in feces for weeks or for about 3 months in water at 4^0C (Meyer and Jarrol, 1980).

Giardiasis could be contracted through drinking contaminated waters or ingestion of contaminated food stuffs. The cyst passes through the stomach and enters the small intestine. The acidic environment of the stomach could not harm the cyst because it has a thin protective wall to protect it until it reaches the alkaline environment, the small intestine (Ortega and Adam, 1997). This alkaline environment initiates excystment of the cyst (Erlandsen and Mayer, 1984). During excystation, the cyst wall raptures at the pole opposite to the nuclei so that the flagella and other projections emerge from the rupture point. The cyst wall is then completely shed and the parasite will enter into its trophozoite stage (Erlandsen and Mayer, 1984).



Source:- http://www.dpd.cdc.gov/dpdx

Fig-2 Life cycle of *Giardia lamblia*

1.3. Epidemiology and mode of transmission

Cryptosporidium parvum

Cryptosporidium parvum has been reported from patients of ages of 3 days to 95 years. It has been implicated in many epidemics as well as endemic level intestinal infections (Rose, 1997). Cryptosporidiosis is most frequently spread by direct person-to-person transmission via feacal-oral route. It could also be transmitted by sputum and vomits. Cattle and sheep may also be important source of infection. *Cryptosporidium parvum* can spread to the environment chiefly through water (Hojlyng *et al.*, 1987).

Oocyst of *Cryptosporidium parvum* could remain viable for several months, especially under moist conditions (Fayer *et al.,* 1998). Prevalence studies have shown that oocyst excretion rates are known to vary between 1 to 3% in industrialized world and 10% in the less industrialized world. Geldreich, (1989) estimated that 0.6 to 4.3% of humans are infected by *Cryptosporidium parvum* in the world. *Cryptosporidium parvum* infection has been reported in 10 to 15% of children with diarrhea and 30 to 50% of AIDS patients with chronic diarrhea in the developing world (Sanchez-Mejorada, 1994; Colford *et al*, 1996). Infection principally occurs in the intestine of both immuno-competent and immuno-compromised individuals, however, biliary cryptosporidiosis has been reported in HIV infected patients, with 20-65% of the patients presenting with the so called AIDS cholangiopathy (Vakil, 1996). In contrast when samples from asymptomatic individuals were examined, the prevalence ranged from 0 to 2% in developed nations compared with 0 to 9.8% in case of developing countries (O'Donoghue, 1995).

Giardia lamblia

According to Thompson *et al*. (1993), 5% of acute diarrhea and 20% of chronic diarrheal illness in the world are attributable to *Giardia lamblia.* The incidence of diarrhea associated with *Giardia lamblia* is generally higher in developing countries such as Africa, Asia, South and Central America, where access to clean water and basic sanitation is lacking. The prevalence rate of *Giardia lamblia* infection in developed nations is around 2 to 5% but in developing nations, it is reported to be 20 to 30% (Thielman and Guerrant, 1998). Nearly all children in developing countries will acquire *Giardia lamblia* at some point in their childhood. In developed countries, such as Western Europe and the United States, *Giardia lamblia* infection is highly associated with contaminated drinking water, person to person contact, recent foreign travel and recreational swimming in contaminated lakes, rivers and swimming pools (NATHNAC, 2004).

1.4. Pathogenesis

Cryptosporidium parvum

The pathogenic mechanisms by which *Cryptosporidium parvum* causes diarrhea, malabsorption and wasting are poorly understood. Whatever these mechanisms may be, the initial host-parasite interactions (attachments and invasion) are the critical primary events in the pathogenesis. Epithelial cells of the small intestine are damaged by *Cryptosporidium parvum* through:-

i/ Cell death as a result of parasite invasion, multiplication and extrusion

ii/ Cell damage that could occur through T-cell mediated inflammation, producing villus atrophy and hyperplasia of the crypt(Goodgame, 1996).

The initial host parasite interactions of attachment, invasion and parasitophorous vacuole formation are complex processes that involve multiple parasite ligands and host receptors. Invasive 'zoite' stage of apicomplexans, including *Cryptosporidium*

parvum, have specialized secretory organelles collectively known as apical complex. During initial host-parasite interactions, these organelles secrete and successively exocytose proteins such as CSL, GP900, GP15, and CP15...etc, that are helpful in facilitating attachment, invasion and parasitophorous vacuole formation (Tzipori and Ward, 2002).

Two sequential steps are known to occur when *C.parvum* sporozoites infect epithelial cells. These are attachment and invasion and parasitophorous vacuole formation (Goodgame, 1996). Attachment of Sporozoites to the plasma membrane of epithelial cells is a primary event in the initial host-parasite interaction and a prerequisite for the pathopysiological consequence. In *C.parvum* sporozoite there is a surface lectin galactose-N acetylgalactoseamine (Gal-GalNAc), which mediates the attachment of sporozoites to host epithelial cells (Thea *et al.*, 1992; Joe *et al.*, 1998). This surface lectin also secretes a phospholipase that appears to be essential in the attachment process (Pollok *et al.*, 2003). *Cryptosporidium parvum* adheres intimately to the epithelium, taking up a unique location within epithelial cells but in an extracellular domain (Bird and Smith, 1980; Marcial and Madara, 1986).

Invasion of the sporozoites into host cells involves invagination of the host cell plasma membrane which engulfs and eventually surrounds the sporozoite to form a parasitophorous vacuole in which the organism remains intracellular but extracytoplasmic (Marcial and Madara, 1986; Rosales *et al.*, 1993). The parasite has a unique 'attachment 'or 'feeder' organelle which is prominent at the bases of each parasitophorous vacuole. This organelle is thought to aid in the uptake of nutrients by the parasite from host cell. After sporozoite attachment, it has been hypothesized that the epithelial mucosal cells release cytokines that activate resident phagocytes (Goodgame, 1996). These activated cells release soluble factors that increase intestinal secretion of water and chloride and also inhibit absorption. These soluble factors include histamine, serotonin, adenosine, prostaglandins, leukoteriens and platelet-activating cells. During cryptospoidial and coccidial infection, the host T-cell response

with resultant production of pro-inflammatory cytokines in the mucosa, is most likely to be the significant factor in the mechanism of pathogenesis.

The production of pro-inflammatory cytokines by intestinal epithelial cells in response to *C.parvum* infection could also result in the arrival of large number of inflammatory cells such as neutrophiles, monocytes and other immune cells to the gut. This again results in tissue damage and diarrhea due to increasing permeability of the epithelial cells membrane either directly or indirectly by inducing production of secrtagogoues such as prostaglandins neural peptides, reactive oxygen intermediates and byproducts of nitric oxide (McDonald, 2000). Histological changes associated with intestinal cryptosporidiosis are relatively non-specific and include blunting of villi, hyperplasia of intestinal crypt cells and infiltration of inflammatory cells into the luminal propria (French *et al.*, 1995; Teare *et al.*, 1997).

Giardia lamblia

The extent of pathogenesis caused by *G.lamblia* seems to be dependent on the type of isolates of the pathogen that infected patients. Assemblage A isolates were shown to be associated with mild intermittent diarrhea whereas infection with isolates belonging to Assemblage B were shown to be associated with profound diarrhea with weight loss and fatigue (ESCMID, 2005). Host parasite interaction is the initial phase in the pathogenesis of giardiasis. First the trophozoite of *G.lamblia* attaches to the cell surface of the villi by using its attachment disk located on its posterior or ventral surface. A protein called lectine, found on the surface of the trophozoite lining, recognizes a specific receptor on the membrane of the intestinal cells. This protein is believed to be partly involved in the tight attachment between the parasite and the villi. Following the attachment of trophozoite, there will be major structural and functional abnormalities in the small intestine. Some of these abnormalities include mucosal damage as a result of mechanical obstruction or blockage of the intestine by large number of parasites, the release of cytopahtic substances like thiol proteinase and lectines from *Giardia* trophozoites, the stimulation of host immune response that

result in the release of cytokines and mucosal inflammation and deconjugation of bile salt (Heyworth, 1992; Chavez *et al.*, 1995; Djamiatun and Faubert, 1998; Farthing, 1997).

The histopathological changes that may occur at the mucosal sites range from minimal to severe enough to cause enteropathy with entrocyte damage, villous atrophy and crypt hyperplasia. This damage in turn will bring about the impairment of the process of absorption of digested foods and essential nutrients (Magne *et al.*, 1991), deficiencies in enzymes (eg. Lactase) of the brush border may also be observed. These alterations in epithelial structure and function probably play important role in the pathogenesis (Ferguson *et al.*, 1990). The penetration of the epithelium by the trophozoite stage of *Giardia* is not commonly observed, but it may sometimes invade extra-intestinal tissues like the gallbladder and the urinary tract when conditions are favorable (Goldstein *et al.*, 1978).

1.5. Symptoms and clinical manifestations.

Cryptosporidium parvum

Immunocompetent and immunocompromised individuals differ greatly in their clinical symptoms. The most frequently observed clinical manifestations of cryptosporidiosis are profuse and watery diarrhea, often containing mucus but rarely blood or leucocytes and the symptoms include abdominal cramp, low grade fever, nausea and vomiting (Fayer and Ungar, 1986; Chen *et al.*, 2002). In immunocompetent individuals the disease is an acute self-limiting diarrheal illness lasting 1-2 weeks. However, in immunocompromised patients the disease is much more severe and symptoms include watery diarrhea with stool frequency of upto 10 times a day with a mean volume of one liter (Pitlik *et al.*, 1983; Juranek, 1995). *Cryptosporidium parvum* has no predilection to specific tissue and has been found to infect the biliary tract, respiratory system, middle ear, pancreas and even the stomach (Clark, 1999).

Giardia lamblia

Broad spectrum of clinical manifestations has been shown in symptomatic *Giardia* infection. However, in the majority of the cases giardiasis results in asymptomatic carrier state. The asymptomatic infections are most common in children and people with prior exposure to a source of infection (Ortega and Adam, 1997).

When *Giardia* infection starts, it could results in occasional days of acute watery diarrhea with abdominal pain or patients may experience a prolonged, intermittent often debilitating disease, which is characterized by passage of foul-smelling stool associated with flatulence, abdominal distention and anorexia. Although the symptoms may resolve spontaneously, young children and immunocompromised individuals may have chronic diarrhea, anorexia combined with malabsorption of fat, protein and carbohydrate as a result there could be a significant weight loss, failure to thrive and anemia (Vesy and Peterson, 1999; Farthing, 2003). Extra-intestinal manifestations like mucopopular, urticaria and atopic dermatitis have been rarely associated with giardiasis and treatment of patients with proper anti-giardiasis drugs resolve the above symptoms (Canonne *et al.*, 2000).

1.6. Diagnosis

Cryptosporidium parvum

Traditionally, cryptosporidiosis is diagnosed by microscopic observation of developmental stages of the organism in an intestinal biopsy specimen. These days, most *cryptosporidial* infections are diagnosed by the microscopic examination of host feacal material for the presence of *C.parvum* oocysts. *C.parvum* oocysts are much smaller than those of other intestinal coccidian parasites, and they differ in many of their staining and buoyancy characteristic (Clark, 1999). However, a variety of diagnostic options and staining procedures are available for the detection of

Cryptosporidium in stool samples. Auramine-rhodamine screening of stool sediment smears followed by modified Ziehl-Neelsen acid-fast staining techniques are the techniques of choice for many diagnostic laboratories and are a sensitive and specific approach for the identification of *Cryptosporidium* oocysts in stool (McPherson and McQueen, 1993; Clark, 1999).

Immunologically, anti-*cryptosporidial* IgM, IgG and IgA can be detected by the Enzyme Linked Immunosorbent Assay (ELISA) or by the Immunofluorescence Assay (IFA), but neither of these assays gives a direct diagnosis of *Cryptosporidium* infection (Heyworth, 1992). Nowadays new genetic methods of detecting *Cryptosporidium* infection have been devised using polymerase chain reaction (PCR) (Johnson *et al.*, 1995; Leav *et al.*, 2003).

When extra intestinal infection of *Cryptosporidium* occurs, biliary disease is commonly suspected in which case ultrasonography could be the best initial diagnostic method. In addition computerized tomography might also be helpful. In HIV infected patients the most sensitive method to diagnose biliary tract disease is endoscopic retrograde cholangiopancreatography (ERCP). In AIDS cholangiopathy, the biliary tree appears irregular and distorted with focal dilation and narrowing in the intra-hepatic and /or extra hepatic biliary tree (Xian-Ming and LaRusso, 1999).

Giardia lamblia

Diagnosis of *Giardia* infection has been carried out using microscopic identification of cysts or trophozoites in a single or multiple stool specimens. The standard methods used to increase the sensitivity of *Giardia* detection includes iodine stained wet smear, trichrome-stained cyst concentrates prepared by Formalin ether acetate centrifugation or by zinc sulfate flotation, and trichrome-stained polyvinyl alcohol (PVA) preserved stools (Broke, 1977; Healy,1979). Microscopic examination of duodenal aspirate and jejunal biopsies are sometimes necessary. Since these two methods are invasive they

are rarely employed in children (Wolfe, 1990). Microscopic examination of stool samples most usually detects cysts, while duodenal aspiration and biopsy helps to identify trophozoites and with this technique 50 to 70% sensitivity has been reported by Broke (1977). The low sensitivity of these techniques could be attributable to lack of regular excretion of cysts or trophozoites and examination of stool samples by unskilled personnel (Danciger and Lopez, 1975).

Currently, Immunofluorescence (IF) and Enzyme-linked Immunosorbent Assay (ELISA) are developed for detection of *Giardia* antigens in the stool. These techniques work on the bases of *Giardia* specific polyclonal or monoclonal antibodies. The sensitivity and specificity of these tests are very good and are much easier and require less experience when compared with microscopy. Moreover, it can permit a large number of stool samples to be tested rapidly and may reduce technicians' time and the bias among observers (Addis *et al.*, 1991). The Immunofluorescence (IF) test showed 100% sensitivity and specificity whereas ELISA showed sensitivity of 87%-92% and specificity of 87%-91% (Heresi and Cleary, 1997).

1.7. Control and prevention

There is no effective and specific therapy against cryptosporidiosis but effective treatment for giardiasis is present. Given this situation, still preventive measures are of great importance. Identification of the most common routes of transmission and a better understanding of the species risk factor for exposure that lead to infection would greatly facilitate development of a more reliable prevention strategy. Since most infections of *Cryptosporidium* and *Giardia* are initiated through ingestion of oocysts and cysts, respectively, controlling these stages limits the spread of the disease. Strategies for prevention of *Cryptosporidium* and *Giardia* infections are those usually recommended for avoiding any pathogen transmitted by the faecal-oral route (NSTC, 1995). As in most diarrhea-causing agents, disease outbreaks due to giardiasis and cryptosporidiosis could be prevented by: testing of purified and unpurified water to

check for the presence of oocysts and cysts of the parasites, boiling water intended for consumption and information dissemination through print media to educate the public regarding the dangers of cryptosporidiosis and giardiasis (Backer, 2000).

1.8. Treatment

Cryptosporidium parvum

One of the most biologically fascinating and clinically provoking features of *Cryptosporidium parvum* is its resistance to antimicrobial drugs, probably because it establishes a unique compartment (parasitophorous vacuole) within the host cell. This vacuole may shelter the parasite from the action of antimicrobial drugs (Griffiths, 1998). Treatment option of cryptosporidiosis depends largely on the immune status of the host (Griffiths, 1998). Since the disease is self-limiting in immunocompetent individuals there is no need of specific therapy. However, supportive care with oral fluid and electrolyte replacement is beneficial in alleviating the dehydration. In immunocompromised hosts, particularly AIDS patients with CD4 cell counts below 200/mm^3, cryptosporidiosis could be life threatening and must be treated properly. In people with AIDS the ideal treatment involves partial restoration of immune function with HAART (Highly Active Anti Retroviral Therapy). Several case reports have demonstrated that the resolution of *Cryptosporidial* diarrhea coincides with a rise in CD4 cell count upon initiation of antiretroviral therapy (Carr *et al.*, 1998; Xian-Ming and LaRusso, 1999). In AIDS patients, in addition to HAART therapy, a number of antibiotics such as paromomycine, nitazoxanide, azithromycin that have partial efficacy against cryptosporidiosis are available on clinical trial (Fichtenbaum *et al.*, 1993; Xian-Ming and LaRusso, 1999). It is also possible to use a combination of different anti-cryptospoidial drugs. For instance, a combination of paromomycin and azithromycin has been proposed for the treatment of cryptosporidiosis (Smith *et al.*, 1998).

Giardia lamblia

The most routinely used classes of anti *Giardia* drugs include Nitroimidazoles (such as metronidazole, tinidazole, ornidazole and senidazole), Quinacrine, Furazolidone, Paromomycin and Benzimidazoles. Metronidazole is the most common drug used for treatment of giardiasis world wide. As it is explained by Gardner and Hill (2001), unlike other drugs Metronidazole is absorbed in body tissue quickly and completely. It has also a capacity to penetrate body tissue and be found in secretions like saliva, breast milk, semen and vaginal secretions. Furazolidone (Furoxone) is one of the nitrofuran compounds. It is the only drug that is available in a liquid suspension in the United States and remains an important therapeutic agent world wide. It has been widely used in pediatric population (Lerman and Walker, 1982). Its efficacy is slightly lower than those of metronidazole and quinacrine (Gardner and Hill, 2001). According to Kerutner *et al.*, (1981), another group of drug, paromomycin (Humatin), has been proposed as a treatment for giardiasis in resistant infection and during pregnancy.

1.9. Current situation of giardiasis and cryptosporidiosis in Ethiopia

According to Abebe (1986), over 60% of the communicable diseases occurring in Ethiopia are due to poor environmental health conditions as a result of unsafe and inadequate water supply and poor hygiene and sanitation practice. Approximately, 80% of the rural and 20% of urban population have no access to safe water. Three fourth of the heath problems of children in Ethiopia are communicable diseases encountered from water and sanitation. Very surprisingly, 46% of the mortality of children less than five years is due to diarrhea in which water related diseases occupy a high proportion (MOH, 1997).

As it is reported by McConnell and Armstrong (1976), many intestinal parasites including *G.lamblia* and *C.parvum* are widely distributed in the country. Different parts

of the country have different prevalence rates of giardiasis and cryptosporidiosis. The prevalence of cryptosporidiosis in children presenting with diarrhea ranged from 3.3% in Jimma, 5.6% in Addis Ababa to 9% in North- western Ethiopia and from eastern part of the country, 38% of giardiasis and 11.9% cryptosporidiosis was also reported (Mersha and Tiruneh, 1992; Assefa, *et al.*, 1996; Gebru and Girma, 2000 and Ayalew, 2006). *Cryptosporidiosis* nowadays is being considered as an AIDS defining illness that involves chronic diarrhea and the most common cause of enteric disease in HIV/ AIDS patients (Martins and Guerrant, 1995; Chen *et al.*, 2003). In Ethiopia the prevalence of *cryptosporidiosis* in HIV / AIDS patients reaches up to 25.9% (Fisseha, *et al.,* 1998).

McConnel and Armstrong (1976) reported an overall *Giardiasis* prevalence of about 11.4% in a study conducted on the central plateau of Ethiopia. Seyoum, *et al.* (1981) have also reported varying degree of prevalence rate in different communities. According to Hailu and Berhanu (1995) based on a country wide survey of *giardiasis,* the overall prevalence among schoolchildren and residents were 8.9% and 3.1%, respectively and that of the non- school children were 4.4%. Recently it was reported that the prevalence of *C.parvum* and *G.lamblia* among diarrhea patients referred to EHNRI (Ethiopian Health and Nutrition Research Institute) in Ethiopia were 20.8% and 8.6%, respectively (Endeshaw *et al.*, 2004).

1.10. Justification of the study

Stevens and Adam (2004) indicated that several outbreaks of giardiasis and cryptosporidiosis, occurring in a community, have been linked to drinking municipal waters or other water sources contaminated with these parasites. In many parts of Ethiopia, people are known to consume unprotected water from different sources. More than 80% of diseases in the world are attributed to unsafe drinking water or to inadequate sanitation practice (Raza, 2003). In this respect in many villages of rural parts of Ethiopia the community is forced to use unprotected water from rivers, streams, irrigation cannels, ponds, shallow wells, water harvesting ponds etc. In such areas where people use water from different sources, the possibility of infection with water born diseases such as cryptosporidiosis and giardiasis is expected to be extremely high. Although these infections could occur at all age levels, they are most common among young children (Current and Garcia, 1991). These and other intestinal protozoan infections are commonly associated with climatic factors, sanitary conditions and socioeconomic factors.

Although a number of studies have been conducted on the distribution and prevalence of intestinal parasites in different parts of Ethiopia (Tedla and Ayele, 1986; McConnel and Armstrong, 1976), there are still many localities where more epidemiological information is not available. Hence, the present study was conducted to fill the existing gap and enable decision makers to focus on improving water quality, sanitation and hygiene in Pawi Special District. Although water quality data was not available in Pawi Special District, the clinical records in clinics have shown diarrhea to be dominant and on the whole its prevalence to be next to malaria (Table-2). Furthermore water from Diga dam and Ali-spring (the two main drinking water sources), have not had proper treatment, neither chemically nor physically, for the last 14 years. As a result the drinking water sources are expected to be contaminated with *G.lamblia* and *C.parvum* contributing to the prevalence of high diarrheal infections in the population.

Table-2 Ten top diseases, reported from 13 clinics found in Pawi Special District, Benishangul-Gumuz Regional State, (2003-2005).

S.no	Ten top diseases	years		
		2003	2004	2005
1.	Malaria	40,473 (80%)	41,671(73.8%)	26,957(60.2%)
2.	Bronchopneumonia	2490 (4.9%)	4480 (7.9%)	5640(12.6%)
3.	**Diarrhea**	**2801 (5.5%)**	**3369 (5.9%)**	**3541(7.9%)**
4.	Parasitic helminths	1552 (3.1%)	2136 (3.8%)	2716 (6.1%)
5.	Muscular rheumatics	1449 (2.9%)	1680 (2.8%)	1464(3.3%)
6.	**Gastritis**	**852 (1.7%)**	**1266 (2.2%)**	**903 (2.0%)**
7.	Anemia	693 (1.4%)	975 (1.7%)	619 (1.4%)
8.	Unidentified fever	270 (0.5%)	820 (0.5%)	611(1.4%)
9.	**Other abdominal diseases**	**-**	**-**	**1690(3.8%)**
10.	Other unidentified diseases	-	-	646 (1.4%)
Total		50,580 (100%)	56,397(100%)	44,787(100%)

2. Objectives

General objective:

To assess the possible association of drinking water source with *Giardia lamblia* and *Cryptosporidium parvum* infection prevalence among children in three selected villages of Pawi Special District.

Specific objectives:

1. To determine the prevalence of Cryptosporidiosis and Giardiasis in children using protected and unprotected water in different villages.
2. To compare the prevalence of Cryptosporidiosis and Giardiasis between different sites (villages).
3. To determine the prevalence of Cryptosporidiosis and Giardiasis among children at different age groups and sex.
4. To compare the prevalence of Giardiasis and Cryptosporidiosis among children in relation to breast feeding.
5. To explore the impact of family economy on the prevalence of Giardiasis and Cryptosporidiosis
6. To determine the presence of other intestinal parasites in the study communities.

3. Materials and methods

3.1 The study area

Pawi Special District is one of the 20 districts found in Benishangul-Gumuz Regional state. It is located at $11^0$19'59.47"N latitude and $36^0$25'00.66" E longitude with an altitude of 1500-1769 m a.s.l. in western parts of Ethiopia that is 556 km away from Addis Ababa (Figure-3). The district receives an average monthly rainfall of 107.1mm and have uni-modal pattern, occurring from May to November. The monthly average maximum and minimum temperatures are 24.1^{o}c and 12.0 ^{o}c, respectively and the mean annual relative humidity is 40.5% (source from the National Meteorological Service Agency). Pawi was established as a result of settlement program in 1985. More than 45,000 people are estimated to live in the district. Most of the people are poor farmers who were formerly dependent on food aid. The main crops that grow in the area are sorghum, sesame, ground nut and maize.

The district has 20 villages. These all villages get water mainly from three sources, '*Ali-spring*', '*Diga'* dam and other sources like hand pump and hand dug wells. The present study was conducted in December 2006 in three selected villages of the district (*Almu, K2V24 and K2V23/45*). Almu is one of the villages of the district that get water from Diga dam, K2V24 gets from Ali-spring and K2V23/45 from none of these two sources but they get from "protected" hand pumps and unprotected hand dug wells.

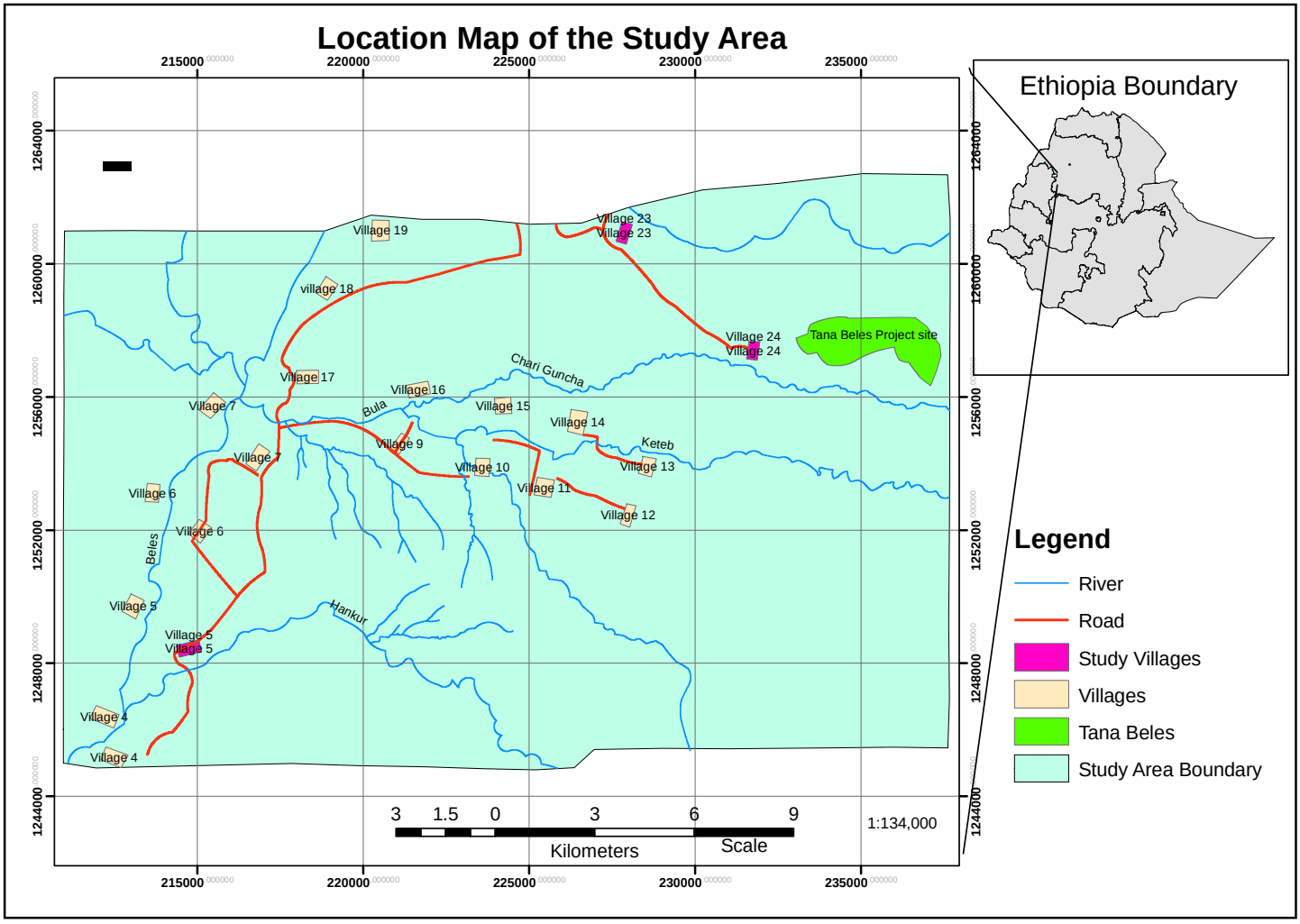


Figure-3. Location map of Pawi Special District, Benishangul-Gumuz Regional state (Source the Ethiopian Map Works Agency)

3.2. The study population

A total of 384 children of age between 2 months and 14 years from the three selected villages were examined at one season (December, 2006). *'Almu'* was selected among all villages that get water from *'Diga'* dam, *'K2V24'* was selected as a representative of all village that get water from *'Ali-spring'* and *'K2V23/45'* was selected among those villages that could not get water from these two main sources rather they get water from "protected" hand pump and unprotected hand dug water. All children were selected relatively from different educational back ground and from different water source.

The sample size was calculated using the formula for crossectional survey, $n = Z^2 xpxq/d^2 = 1.96^2x0.50x0.50/.05^2 = 384$

Z=1.96 at 95% confidence interval, p is the proportion of positive individuals. Since there were no studies conducted concerning the present topic in the area, p was taken as 50% to achieve the maximum sample size. d is the absolute precision and is taken as 0.05.

3.3. Stool collection and processing

A single fresh stool sample was collected with labeled stool cup from consulted study subjects (n=384) (December, 2006). The questionnaires concerning the prevalence study were filled by all the study participants during sample collection. A portion of the stool was preserved with SAF (15g Sodium acetate, 20ml glacial acetic acid, 40ml formalin and 925ml distilled water) in a proportion of 1g of stool in 3ml of SAF. The remaining part was processed using the following methods (Ortega and Adam, 1997):

3.3.1 Direct wet mount method

A direct wet mount with normal saline (0.85% NaCl solution) was prepared at the field and observed for the presence of motile intestinal parasites and trophozoite under light microscope at 10X and 40X magnification. Lugol's iodine staining was used to observe cysts of intestinal parasites.

3.3.2 Formalin ether concentration.

Using an applicator stick, about 1 g of preserved stool sample was placed in a clean 15 ml conical centrifuge tube containing 7 ml formalin. The sample was dissolved and mixed thoroughly with applicator stick. The resulting suspension was filtered through a sieve (cotton gauze) into a beaker and the filtrate was poured back into the same tube. The debris trapped on the sieve was discarded. After adding 3 ml of diethyl ether to the mixture and hand shaken, the content was centrifuged at 2000 rpm for 3 minutes. The supernatant was poured away and the tube was replaced in its rack. Iodine stain preparation was made from the sediments. Finally the entire area under the cover slip was systematically examined using ×10 and ×40 objective lenses (Lindo, 1998).

3.3.3. Modified Zeihl-Neelsen Method

For detection of *Cryptosporidium* oocyst, direct and concentration smears were prepared. Fresh faecal sample was collected from children and thin smears were prepared, dried, fixed with methanol for 5 minutes in the field and stained by Zeihl-Neelsen techniques at EHNRI and the same procedure was used for smears prepared after concentration. In this technique, the slides were stained with carbol fuschine for 30 minutes and there after, they were washed with tap water. The slides were

decolorized in acid alcohol for 1 minute and were counter stained with methlyene blue for another 1 minute. Finally, the stained smears were microscopically observed using 100x magnifications (Endeshaw, *et al.,* 2004; Assefa, *et al.*, 1996 and Garcia *et al.,* 1993).

3.4. Ethical clearance

The study was reviewed and approved by the ethical committee of Biology Department, Addis Ababa University. Ethical considerations were addressed by treating positive intestinal parasites using the standard drug. Individuals with positive result were treated with the appropriate treatment and the drugs were administered by clinicians working in the study areas. Written consent was sought from parents or guardians of the selected children. Besides, parents or care givers were asked to fill the questionnaire and assist during stool sample collection.

3.5. Data analysis

Statistical analysis was performed with SPSS software version 13. Chi square was used to verify possible association between infection and exposure with different factors. Values were considered to be statistically significant when the p-value obtained was less than 0.05.

4. Result

4.1. Giardiasis and Cryptosporidiosis among children of different sexes

Out of the 384 study subjects, 197 were males and 187 were females. Among the males (197) **21.3%** were positive for Giardiasis and among the females (187) **32.1%** were positive for Giardiasis. The prevalence of Cryptosporidiosis was **9.1%** and **6.9%** in the male and female children, respectively. The difference in the prevalence of Giardiasis was significant ($p<0.05$) between males and females but the difference was not statistically significant ($p>0.05$) in the case of Cryptosporidiosis (Table-3).

Table-3 Prevalence of Giardiasis and Cryptosporidiosis among children by sex in Almu, K2V24 and K2V23/45 villages in Pawi, 2006/07.

Parasite identified	sex	No examined	No positive	Prev.(%)	p-value
Giardia lamblia	male	197	42	**21.3**	*0.017
	female	187	60	**32.1**	
Cryptosporidium parvum	male	197	18	**9.1**	NS0.432
	female	187	13	**6.9**	
Total		384	133	**34.6**	

* Significant difference at $P<0.05$
NS Non-significant difference at $P>0.05$

4.2. Giardiasis and Cryptosporidiosis in the three study villages.

In village "K2V24" 140 stool samples were examined and out of these **32.1%** and **5%** were found positive for Giardiasis and Cryptosporidiosis, respectively. In "Almu" village a total of 153 stool samples were examined and out of these **18.9%** were found positive for Giardiasis and **7.8%** for Cryptosporidiosis. In village K2V23/45, 91 stool samples were examined and out of these **30.8%** and **13.2%** were found positive for Giardiasis and Cryptosporidiosis, respectively. Infection prevalence of Giardiasis and Cryptosporidiosis has shown a significant difference among the three study sites ($p<0.05$) (Table-4). There were much lower infections for Giardiasis in Almu while there were much higher infections at K2V23/45 for cryptosporidiosis.

Table-4 Prevalence of Giardiasis and Cryptosporidiosis among children in Almu, K2V24 and K2V23/45 villages in Pawi, 2006/07.

Study sites	Number of children examined	Parasite identified					
		G.lamblia			*C.parvum*		
		No positive	Prev.(%)	P-value	No positive	Prev.(%)	P-value
Almu	153	29	**18.9**		12	**7.8**	
K2V24	140	45	**32.1**	*0.000	7	**5**	*0.006
K2V23/45	91	28	**30.8**		12	**13.2**	
Total	384	102	**26.6**		31	**8.1**	

* Significant difference at $P<0.05$
NS Non-significant difference at $P>0.05$

4.3. Giardiasis and Cryptosporidiosis among children of different age groups

Out of the 384 study subjects, 35 of them were between 2 and 12 months old, 116 were 1 to 5 years and 233 were 6 to 14 years. Among the 35 children aged 2-12 months, **11.4%** and **11.4%** were positive for Giardiasis and Cryptosporidiosis, respectively. Among the 116 children in the 1 to 5 years age category, **26.7%** were found positive for Giardiasis and **9.5%** for Cryptosporidiosis. Within the 233 children of age between 6 to 14 years, **28.8%** and **6.9%** were found positive for Giardiasis and Cryptosporidiosis, respectively. The difference in the prevalence of Giardiasis and Cryptosporidiosis among the different age groups was not significant (Table-5).

Table-5. Prevalence of Giardiasis and Cryptosporidiosis among children of different age groups in Almu, K2V24 and K2V23/45 villages in Pawi, 2006/07.

Parasite identified	Age groups	No examined	No positive	Prev.(%)	P-value
Giardia lamblia	2-12 months	35	4	**11.4**	^{NS}0.096
	1-5 years	116	31	**26.7**	
	6-14 years	233	67	**28.8**	
Cryptosporidium parvum	2-12 months	35	4	**11.4**	^{NS}0.522
	1-5 years	116	11	**9.5**	
	6-14 years	233	16	**6.9**	
Total		384	133	**34.6**	

* Significant difference at P<0.05
^{NS} Non-significant difference at P>0.05

4.4. Giardiasis and Cryptosporidiosis among children that use different drinking water sources.

With regard to source of drinking water, all the community in K2V24 use Ali-spring water which is unprotected and with no proper treatment. Community in Almu use water from Diga dam which is also unprotected and with no treatment at all.

Community in K2V23/45 use water from two main sources "protected" hand pump and unprotected hand-dug well.

Among the 140 stool samples collected from the children using water from Ali-spring, **32.1 %** were positive for Giardiasis and **5%** were found positive for Cryptosporidiosis. Among the 153 study subjects that use water from Diga dam, **18.9%** were found positive for Giardiasis and **7.84%** for Cryptosporidiosis. Among the 27 Study subjects that use "protected" water (hand pump), **3.7%** were found positive for Giardiasis and **25.9%** for Cryptosporidiosis. From the 64 study subjects that use unprotected hand-dug well, **42.2%** were found positive for Giardiasis and **7.8%** for Cryptosporidiosis (Table-6).

Analysis of the prevalence of Giardiasis and Cryptosporidiosis in relation to water source revealed that there was a statistically significant difference ($p<0.05$) among children that use the different types of water sources (Table-6).

Table-6 Prevalence of Giardiasis and Cryptosporidiosis among children using different water sources in Almu, K2V24 and K2V23/45 villages in Pawi, 2006/07.

Water source	Number of children examined	Parasite identified					
		Giardia lamblia			*Cryptosporidium parvum*		
		No positive	Prev.(%)	P-value	No positive	Prev.(%)	P-value
Ali-spring (unProtected)	140	45	**32.1**	*0.000	7	**5**	*0.006
Diga dam(unprotected)	153	29	**18.9**		12	**7.84**	
Hand pump("protected")	27	1	**3.7**		7	**25.9**	
Manually-dug well(unprotected)	64	27	**42.2**		5	**7.8**	
Total	384	102	**26.6**		31	**8.1**	

* Significant difference at $P<0.05$
NS Non-significant difference at $P>0.05$

4.5. Giardiasis and Cryptosporidiosis in relation to water usage practices.

Three types of water usage practices were obtained from the questionnaire distributed during stool sample collection: directly, boiled and filtered water. A single case of Giardiasis and no case of Cryptosporidiosis was found among the **seven** study subjects who said they filter water for drinking and among the **seven** study subjects who said they boil water for drinking, no cases of Giardiasis and Cryptosporidiosis were found positive. Out of the **370** study subjects who said they use water directly, **27.3%** were found positive for Giardiasis and **8.4%** were positive for Cryptosporidiosis (Table-7).

Table-7. Prevalence of Giardiasis and Cryptosporidiosis in relation to water usage practice in Almu, K2V24 and K2V23/45 villages in Pawi, 2006/07.

Parasite identified	Water usage practice	No Respondents	No positive	Prev.(%)
Giardia lamblia	Filtered	7	1	**14.3**
	Boiled	7	0	**0**
	Direct	370	101	**27.3**
Cryptosporidium parvum	Filtered	7	0	**0**
	Boiled	7	0	**0**
	Direct	370	31	**8.4**
Total		384	133	**34.6**

4.6. Giardiasis and Cryptosporidiosis among breast feeding and non-breast feeding children.

Among the 384 study subjects, 54 were found to be breast feeding and 330 were non-feeding during stool sample collection. Within the breast feeding groups (54), **9.3%** and **11.1%** were found positive for Giardiasis and Cryptosporidiosis, respectively and within the non-feeding groups (330), **29.4%** and **7.6%** were found positive for Giardiasis and Cryptosporidiosis, respectively. Analysis of the prevalence of Giardiasis in relation to breast feeding showed significant difference between breast feeding **(9.3%)** and non-feeding **(29.4%)**. However, there were no statistical difference between breast feeding and non-feeding in the case of cryptosporidiosis (Table-8).

Table-8. Prevalence of Giardiasis and Cryptosporidiosis among breast feeding and non-feeding children in Almu, K2V24 and K2V23/45 villages in Pawi, 2006/07.

Parasite identified	Breast feeding status	No examined	No positive	Prev.(%)	p-value
Giardia lamblia	feeding	54	5	**9.3**	*0.02
	Non-feeding	330	97	**29.4**	
Cryptosporidium parvum	feeding	54	6	**11.1**	^{NS}0.377
	Non-feeding	330	25	**7.6**	
Total		384	133	**34.6**	

* Significant difference at P< 0.05
^{NS} Non-significant difference at P>0.05

4.7. Giardiasis and Cryptosporidiosis in relation to duration of breast feeding.

Three groups of study subjects were found with regard to breast feeding duration. These are <1 years, 1 to 2 years and 3 to 5 years (Table-9). Within the 72 study subjects that had breast feeding duration <1 years, **36.1%** and **9.7%** were found positive for Giardiasis and Cryptosporidiosis, respectively. Within the 145 study subjects that had breast feeding duration ranging between 1 to 2 years, **38.6%** and **9.6%** were found positive for Giardiasis and Cryptosporidiosis, respectively. Within the 167 study subjects that had breast feeding duration 3 to 5 years, **11.9%** and **5.9%** were found positive for Giardiasis and Cryptosporidiosis, respectively. Analysis of the prevalence of Giardiasis in relation to breast feeding duration has shown a statistically significant difference ($p<0.05$) among the different range of breast feeding durations. However, no significant difference was seen among the groups with respect to the prevalence of Cryptosporidiosis (Table-9).

Table-9. Prevalence of Giardiasis and Cryptosporidiosis among children with different breast feeding durations in Almu, K2V24 and K2V23/45 villages in Pawi, 2006/07.

Parasite identified	Breast feeding duration	No examined	No positive	Prev.(%)	p-value
Giardia lamblia	<1 year	72	26	**36.1**	*0.000
	1-2 years	145	56	**38.6**	
	3-5 years	167	20	**11.9**	
Cryptosporidium parvum	<1 year	72	7	**9.7**	NS0.619
	1-2 years	145	14	**9.6**	
	3-5 years	167	10	**5.9**	
Total		384	133	**34.6**	

* Significant difference at P<0.05
NS Non-significant difference at P>0.05

4.8. Giardiasis and Cryptosporidiosis among children from families with different monthly incomes.

Out of the 384 study subjects 80 of them were from families having monthly income less than 200 Birr, 179 having monthly income 200 to 300 Birr and 125 of them having monthly income greater than 300 Birr. Within the 80 study subjects having monthly income <200 Birr, **70%** and **8.8%** were found positive for Giardiasis and Cryptosporidiosis, respectively. Within the 179 study subjects having monthly income 200 to 300 Birr, **20.7%** and **8.4%** were found positive for giardiasis and Cryptosporidiosis, respectively. Within the 125 study subjects having monthly income greater than 300 birr, **7.2%** were found positive for giardiasis and **7.2%** for cryptosporidiosis (Table-10). The prevalence of Giardiasis has shown significant difference ($p<0.05$) among the children from different monthly income families but the prevalence of Cryptosporidiosis has not shown a significant difference ($p>0.05$).

Table-10. Prevalence of Giardiasis and Cryptosporidiosis among children from different monthly income families in Almu, K2V24 and K2V23/45 villages in Pawi, 2006/07.

Parasite identified	Monthly income	No examined	No positive	Prev.(%)	p-value
Giardia lamblia	<200 Birr	80	56	**70**	*0.000
	200-300 Birr	179	37	**20.7**	
	>300 Birr	125	9	**7.2**	
Cryptosporidium parvum	<200 Birr	80	7	**8.8**	NS0.905
	200-300 Birr	179	15	**8.4**	
	>300 Birr	125	9	**7.2**	

* Significant difference at $P<0.05$
NS Non-significant difference at $P>0.05$

4.9. Intestinal parasites other than *G.lamblia* and *C.parvum* and drinking water source.

Based on parasitological examination of stool specimens by direct and formalin-ether concentration techniques, different types of intestinal parasites (both pathogenic and non-pathogenic) other than *Cryptosporidium parvum* and *Giardia lamblia* were identified (Table-11). The study has shown that the prevalence of Hookworm spp. was 18.3%, *Ascraris lumbricoides* was 8.3%, *S.mansoni* was 6.3%, *Entameba histolytica/dispar* was 6.3, *Blastocystis hominis* was 3.4% and that of *Hymenolepies nana* was 1%. Other non-pathogenic intestinal parasites like *Entameoba coli* and *Iodoamoeba butschilii* were also encountered in the study. Single parasite infection had the highest prevalence followed by double and triple. Overall co-infection was detected in 4.4% of the study subjects. Among the double parasitic infection, *G. lamblia* and *C. parvum* comprised the highest proportion.

Table-11. Pathogenic and non pathogenic intestinal parasites other than *G.lamblia* and *C.parvum* in Almu, K2V24 and K2V23/45 villages in Pawi, 2006/07.

Intestinal parasites	**No observed (%)**			
	K2V24(n=140)	**Almu(n=153)**	**K 2V23/45(n=91)**	**Total(N=384)**
Hookworm spp.	34(24.2)	15(9.8)	21(23.1)	70(18.3)
Ascaris lumbricoides	13(9.3)	13(8.5)	6(6.6)	32(8.3)
Entameoba histolytica/dispar	14(10)	8(5.2	2(2.3)	24(6.3)
Schistosoma mansoni	1(0.7)	18(11.8)	5(5.5)	24(6.3)
Blastocystis hominis	7(5)	1(0.7)	5(5.5)	13(3.4)
Hymenolepis nana	1(0.7)	2(1.3)	1(1.1)	4(1.0)
Hymenolopis diminuta	2(1.4)	1(0.7)	0(0)	3(0.8)
Enterobious vermicularis	3(2.1)	2(1.3)	0(0)	5(1.3)
Taenia species	3(2.1)	4(2.6)	1(1.1)	8(2.1)
Entameoba coli	2(1.4)	0(0)	1(1.1)	3(0.8)
Iodoamoeba butschilii	0(0)	1(0.7)	0(0)	1(0.3)

5. Discussion

In the present study many of the children in the three villages participating in the study were found harboring many intestinal parasites. Although the study subjects were living in different villages of the district, the infection prevalence of *G.lamblia* and *C.parvum* on the average was similar to what was reported by Iqbal *et al.*, (1999) in which the prevalence of *C.parvum* infection among children in developing countries was shown to range between 5% and 10%. This is also in agreement with the report of Gebru and Girma (2000) in which the prevalence of *C.parvum* in diarrheal children in Jimma was found to be 3.3% and Assefa *et al*, (1996) in which the prevalence of *C.parvum* in diarrhoeal children in Addis Ababa was reported to be 5.3%. The higher prevalence of *Cryptosporidium parvum* infection observed in children would not only be the result of contaminated water and food but their contact with domestic animals could also contribute to the high prevalence as the community is involved in rearing cattle (Goh *et al.*, 2004).

Mersha and Tiruneh (1992) had reported higher rate of *C. parvum* detected in North-western Ethiopia than the present study. In addition, *C.parvum* infection prevalence found in the present study was much lower than what was reported from adult diarrhoeal AIDS patients (25.9%) from Addis Ababa hospitals (Fisseha *et al.*, 1999). Since the study participants included children below the age 14 years, which is not a high risk group for HIV infection, it can be assumed that the cryptosporidiosis detected in the present study, by and large, is not opportunistic. Thus, the lower prevalence of *C.parvum* is to be expected (Adamu, 2004).

Similarly, higher level of giardiasis among children of age between 2 months and 14 years is an indicator of the development of Giardiasis at early life and high carrier rate in all age groups and the possibility of re-infection in the community. Prevalence of giardiasis in the present study (26.6%) was higher than what was reported by Seyoum *et al* (1981), who reported a much lower prevalence in preschool children (9.3%). In

Central and Northern highlands of Ethiopia 3 to 23% prevalence was reported by McConnel and Armstrong (1976) and Endeshaw *et al.* (2004) reported a prevalence of 20.8% among diarrheal patients referred to EHNRI. However, Ayalew (2006) reported a prevalence of 38% among children from eastern Ethiopia (Dire-Dawa), which is higher than the current study. According to Gilman *et al.* (1988) unless prior infections were protective for giardiasis, one would expect to see a higher rate of giardiasis in developing countries where the living standard of the society is very low. However, usually there are asymptomatic *Giardia* infections serving as unidentified carriers and may be responsible for transmission of infection (US. EPA, 1989).

An overall difference in the prevalence of Giardiasis and Cryptosporidiosis has been shown in many studies during the dry and wet season samplings (Ayalew, 2006). In the current study it was not possible to investigate seasonality in the prevalence of giardiasis and cryptosporidiosis because of financial constraints. The prevalence of intestinal protozoan parasites has been reported to be associated with the amount of rain fall (Adegbola *et al.,* 1994; Enriquez *et al.*, 1997). Studies in Central America, South Africa, Kuwait and India have also revealed a high peak incidence of these parasites in the rainy seasons (Casemore *et al.*, 1997; Iqbal *et al.*, 2001; Leach *et al.*, 2000). The same trend was observed in eastern Ethiopia (Ayalew, 2006). With regard to this phenomenon, the two water sources, Ali-spring and Diga dam, are highly exposed to runoff in the rainy season. Diga dam, especially, is totally changed into runoff water as a result of which the district Water Resource Office is forced to close the dam and stops providing service to the community during the rainy season. This shows that contamination of drinking water sources by runoff containing feces of infected humans and animals is an inevitable phenomenon.

In the present study the prevalence of *G.lamblia* infection was significantly different between males and females ($p<0.05$). The females were more infected than males. This is opposite to the report by Mahmud *et al* (1995) where a higher prevalence of *G.lamblia* infection among males than females in Egypt was recorded. Similar finding

to that reported from Egypt also comes from northern Jordan (Nimric, 1994). The possible explanation for our finding could be because of the increased chance of exposure of females to contaminated waters as they are usually engaged in fetching water for the family in Ethiopia.

C.parvum infection was not significantly associated in the present study with sex which may indicate that both sexes have equal chance of being infected. The current finding is in agreement with a study conducted in Mexico City where the gender of children did not influence the rate at which *C.parvum* infections were detected (Enriquez *et al.*, 1997). Similarly male and female individuals tested for cryptosporidiosis in Kwa-Zulu-Natal population were found to have similar prevalence (Jarmey-Swan *et al.*, 2001). However, an opposite observation was reported from Guinea Bissau by Molbak *et al,* (1994a) and by Frasere *et al*, (1997) among Bedouin infants in Israel where the prevalence of *C.parvum* infection in males was higher than in females. In their study they have suggested that there might have been an unmeasured intra-familial factor functioning to expose infant boys or to protect infant girls in their study population, which necessitates the need for further investigation as to why there is such discrepancy among male and female children. Likewise, a study conducted in hospitalized children in Delhi showed that *C.parvum* infection was predominant in males than in females (Mahajan *et al.*, 1992) whereas an increased prevalence was found in females in Nigeria (Okafor and Okunji, 1994). In Ethiopia, Adamu (2004) reported that the prevalence of *C.parvum* in males was detected to be higher than in females.

Although difference in prevalence between the age groups was not statistically significant, there was higher prevalence of *Giardia lamblia* infection among 6 to 14 years and 1 to 5 years but less prevalent in the <1 year groups. The lower prevalence observed in the < 1 year group may be explained by the fact that the community has a culture of prolonged breast feeding of children. The established trend of giardiasis is that the infection prevalence of giardiasis increases with age and with development of

immunity in older individuals, prevalence of giardiasis becomes reduced (Lindo *et al.*, 1998). The report of Lindo *et al.*, (1998) which states that giardiasis increases with age of children and is not clustered in a particular age group strengthens the above explanation. The overall non-significant difference among different age groups in our case may indicate that Giardiasis is not limited to some age groups so it can infect individuals of any age groups.

The pattern of age difference in infection with *C.parvum* appears not significant in the present study ($p>0.05$) showing that there was no variation in *Cryptosporidium parvum* infection between infants < 1 year, whose immune status is not well established and in older children. This finding is in agreement with what was reported by Ayalew (2006). The non-significant variation among the three age groups may be attributable to the poor personal and environmental hygiene, poor water quality and close contact of all age groups to domestic animals. On the contrary difference in infection prevalence of *C.parvum* between age groups was reported by Lindo *et al.*, (1998) in Jamaica, Adegbola *et al.* (1994) and Tumwine *et al.* (2003) in Sub-Saharan African countries including Uganda and Gambia and Assefa *et al.*(1996) in Ethiopia. In general, the prevalence of *C.parvum* infection in the present study is more or less in agreement with what was reported by Assefa *et al.*(1996) and Gebru and Girma (2000) in Addis Ababa and southwest Ethiopia among children presented with diarrhea and Endeshaw *et al.*(2004), where they report the prevalence of cryptosporidiosis among diarrheal patients referred to EHNRI.

Prevalence of *C.parvum* and *G.lamblia* infection has shown a significant difference between the three villages. As the living standard of the communities in the three villages is more or less comparable, the existing difference in the prevalence of both parasites in the three villages seems to be associated with the type of water source each village uses. More than 50% of the demand for water supply in Pawi is met from the two water sources (Ali-spring and Diga dam). The prevalence of giardiasis and cryptosporidiosis in other villages which were not included in the current study is

expected to be as high as that was observed in the selected villages, because they use the same water source for domestic purposes and also have more or less the same socioeconomic status.

In village K2V23/45, children who use water from "protected" hand pump have shown significantly reduced infection prevalence of giardiasis when compared with those who use water from unprotected hand dug wells ($p<0.05$), which implies the need for strengthening water development to intervene in the transmission of giardiasis. However, children that use the "protected" hand pump water source have shown significantly increased prevalence of *C.parvum* infection when compared with those who use unprotected water source. Isaac-Renton, *et al*. (1999) had reported that there was lower risk of exposure to *Cryptosporidium* infection in people that use protected water sources as they were not able to detect oocyst in protected deep wells. The possible explanation for the observed increased prevalence of *Cryptosporidium parvum*, in the study participants that drink water from "protected" hand pumps, could be due to seepage from latrines because these hand pumps are mostly constructed near houses so these water sources might have been inadequately protected. This is in agreement with the report of Craun *et al*, (1998) in USA where they explain that inadequately protected ground water sources cause twice as many water borne parasite outbreaks than would un-protected surface water. The other possible explanation could be due to the unhygienic practice of children immersing their contaminated hands into stored water in the house (Jensen *et al.*, 2004).

Questionnaires were used to assess the kind of water usage practices that exist in the community. In relation to the way the community uses water for drinking purpose, the prevalence of both parasites was found to be high in those groups of study subjects who use water directly without any treatment like boiling and filtering. Boiling and filtering water for drinking could be a one method to reduce the existing increased prevalence of giardiasis and cryptosporidiosis (wwwn.cdc.gov/travel/yellowBookCh2-FoodWaterRisks.aspx). Boiling drinking water is believed to be the surest method to

make water safe to drink and also kill the infective stages of *G.lamblia* and *C.parvum* (www.epa.gov/safewater/faq/emerg.).

Reports regarding the importance of breast feeding in protecting children from diarrhoeal diseases, including giardiasis, are well documented. Children borne to mothers that have no immunity against Giardiasis have shown a significantly higher risk of acquiring *Giardia* infection and developing Giardiasis with more severe symptoms compared with those borne from immune mothers (Tellez *et al.*, 2003).

Breast feeding seems to be important for children by protecting them from acquisition of the parasite and reduce symptoms (Tellez *et al.*, 2003). The beneficial effect of breast feeding appears not restricted to infancy. Incidence of diarrhea was found to be higher in weaned children than in partially breast fed children both at their first year of life and at their second year of life (Molbak *et al.*, 1994b). A study conducted in Peru has also shown that children that were weaned during the second 6 month of life were found to be at increased risk of diarrhea incidence and prevalence (Kenneth *et al.,* 1989). Similarly, in the current study we observed that children who were still breast feeding during stool sample collection were significantly less infected with *G.lamblia* compared with those who were not. An association between breast feeding duration and giardiasis was also observed indicating the importance of prolonged breast feeding. The study community has a culture to breast feed children even up to five years and this may also partially explain why we were not able to find higher prevalence of giardiasis in children under five years of age.

In the present study children from families with relatively high monthly income (>300 birr) have shown significant reduction in prevalence of giardiasis which is in agreement with the report of Nimri (1994). This is associated with nutritional condition of the children. That is children from families with higher monthly income are relatively provided with a better hygienic environment and in the event of infection can obtain medication. On the other hand, the absence of any association between *C.parvum*

infection prevalence and family income could be explained by the fact that since there is no effective treatment for cryptosporidiosis, income would have no influence in the prevalence of cryptosporidiosis.

The level of prevalence of intestinal helminths and protozoa other than *G.lamblia* and *C.parvum* in the present study is more or less in agreement with what was reported by others (Ali *et al.*, 1999; Seyoum *et al.*, 1981; McConnell and Armstrong, 1976 and Tedla and Ayele, 1986) for other parts of Ethiopia. However, the prevalence of Hookworm spp. infection was not much higher (18.3%) than the national average (22%) (Tedla and Jemaneh, 1985). This high prevalence of Hookworm spp. together with the existing high malarial disease situation in the district would undoubtedly exacerbate the public health situation in the area, especially in association with anemia. The relatively high prevalence of *Ascaris lumbricoides* and *Entamoeba histolytica/dispar* would reflect the unhygienic situation in the study area.

The prevalence of *S.mansoni* was found to be high (6.3%), compared to what was reported (0.7%) by the Aklilu Lemma Institute of Pathobiology (1986) in Pawi area. Schistosomiasis in the study area is underestimated by clinicians and at the same time there is little awareness about the disease by most of the population in the area. The possible reason for this could be the less reliability of the routinely used diagnostic technique (simple wet mount). The fact that in this study we were only able to get a single case of schistosomiasis in the field by wet mount but in laboratory we got additional 23 cases by formalin ether concentration tecquniqes may strengthen the above statement.

6. Conclusions and recommendations

Higher prevalence rate of *Giardia lamblia* (26.6%) and *Cryptosporidium parvum* (8.1%) is detected in the present study. The higher prevalence was associated with the type of water source that the study participants were using.

The finding of the study provides partial explanation to the etiology of the highly prevalent diarrhea, gastritis and abdominal diseases that are frequently reported as among the ten top diseases by health institutions in the District. The finding may reflect that *G.lamblia* and *C.parvum* would be implicated in any of the above mentioned diseases.

Reduced infection prevalence of *G.lamblia* observed among children using water from "protected" source (hand pump) when compared with those drinking water from unprotected sources (Ali-spring, Diga dam and hand-dug wells), support the need for provision of protected and treated safe drinking water for the community to combat water borne parasites.

In the current study we also observed that there was a protective association between breast feeding and *Giardia lamblia* infection.

Based on the study the following recommendations can be made:-

- There is an urgent need to provide a well protected and treated drinking water to the community.
- Further analysis of the drinking water sources for the presence of cysts and oocysts should be done.
- It is important to investigate further the importance of breast feeding in controlling diarrhreal diseases in general and giardiasis in particular.

- There is a need to investigate the real impact of schistosomiasis and hookworm infection in the overall health of the community.

- The role of *Entamoeba histolytica/ dispar* as an etiology of diarrhea in the area needs to be confirmed by using more precise techniques such as the Polymerase Chain Reaction method.

- Boiling and filtering water for drinking could be a recommended method to reduce the existing increased prevalence of giardiasis and cryptosporidiosis.

- To differentiate between the virulent and the less virulent genotypes, genetic characterization of *G.lamblia* should be made by PCR method.

References

Abebe , L. (1986). Hygienic water quality: its relation to health and the testing aspects in tropical conditions, Department of Civil Engineering, Tempeere university, Finland.

Adams, R.D. (1991). The biology of *Giardia spp*. *Microbiol. Rev*. **55**: 706-732.

Adamu, H. (2004). *Opportunistic intestinal parasitic infections in pediatric patients with diarrhea in relation to some demographic features in Addis Ababa hospitals.* Master thesis, Dept. Biology, Addis Ababa University. Pp 68.

Addiss, D.G., Mathews, H.M., Stewart, J.M., *et al*. (1991). Evaluation of commercially available enzyme linked immunosorbent assay for *Giardia lamblia* antigen in stool. *J.Clin. Microbiol*. **29(6)**: 1137-1142.

Adegbola, R.A., Demba, E., Deveer, G. and Todd, F. (1994). *Cryptosporidium* infection in Gambian children less than 5 years age. *J. Trop. Med. Hyg.* **97**: 103-107.

Ali, I, Mekete, G. and Wodajo, N. (1999). Intestinal parasitism and related risk factors among students of Asendabo Elementary and Junior Secondary School, South western Ethiopia. *Ethiop. J. Health Dev*. **13(2)**: 1-5.

Assefa, T., Mohamed, H., Abebe, A., Abebe, S. and Tafesse, B. (1996). Cryptosporidiosis in children seen at children's clinic of Yekatit 12 hospital, Addis Ababa. *Ethiop. Med. J.* **34**: 43-45.

Ayalew, D. (2006). *Assessment of the association of Cryptosporidium parvum, Giardia lamblia and Entamoeba histolytica/dispar infection prevalence with drinking water source among children in Legedini, Adada and Legfbira, Dre-Dawa, Eastern Ethiopia*. Master thesis, Dept. Biology, Addis Ababa University. Pp.68.

Backer, D.H. (2000). Giardiasis. An elusive cause of gastrointestinal distress. *The Physician and Sports medicine*.**28**: 7.

Bird, R.G. and Smith, M.D. (1980). Cryptosporidiosis enteritis in man: parasite life cycle and fine structural pathology. *J.Pathol*. **132**:217-233.

Broke, J.A. (1977). The clinical and laboratory diagnosis of giardiasis. *Crit.Rev. Clin.Lab. Sci*. **7**: 373-391.

Canonne, D.D., Brama, A., Segard, M., Pitte, F. and Delaporte, E. (2000). Well's syndrome associated with recurrent giardiasis. *Bri. J. Derma*. **143**: 425-427.

Carr, A., Marriott, D., Field, A., Vasak, E. and Cooper, D.A. (1998). Treatment of HIV-1 associated microsporidiosis and cryptosporidiosis with combination antiretroviral therapy. *Lancet*. **351**: 256-261.

Casemore, D.P., Wright, S.E. and Coop, R.L. (1997). Cryptosporidiosis human and animal epidemiology. In: Fayer, R. (ed.) *Cryptosporidium and cryptosporidiosis*. CRC, New York.

Chen, X.M., Keithly, J.S., Paya, C.V. and Laruson, N.F. (2002). Cryptosporidiosis. *N.Engl. J. Med.* **346**: 1723-1731.

Chen, C., Gulati, P. and French, S.W. (2003). Pathologic quiz case: A patient with Acquired Immunodeficiency Syndrome and an unusual biliary Infection. *Archives of Pathology and Laboratory Medicine*. **127(2)**: 243-244.

Chavez, B., Gonzalez-Mariscal, L., Cedillo-Rivera, R. and Martinez-Palomo, A. (1995). *Giardia lamblia: in vitro* cytopathic effect of human isolates. *Exp. Parasitol*. **80**: 133-138.

Clark, P.D. (1999). New insights into human cryptosporidiosis. *Clin. Microbiol. Rev*. **12(4)**: 554-563.

Coupe, S., Sarfati, C., Hamane, S. and Derouin, F. (2005). Detection of *Cryptosporidium* and identification to the species level by Nested PCR and Restriction Fragment Length Polymorphism. *J. Clin. Microbiol*. **43(3)**: 1017-1023.

Colford, J.M., et al., (1996). Cryptosporidiosis among patients infected with human immunodeficiency virus. Factors related to symptomatic infection and survival. *Am. J. Epidemiol.* **144**: 807-816.

Craun, G.F., Hubbs, S., Frost., F,J., Calderon, R.L. and Via, S. (1998). Epidemiology of waterborne cryptosporidiosis in the United States, United Kingdom and Canada. *J. Am. Water Work Assoc. (sep).*

Current, L.W. and Garcia, S.L. (1991). Cryptosporidiosis. *Clin. Microbiol. Rev*. **4(3)**: 325-358.

Danciger, M. and Lopez, M. (1975). Number of Giardia in the faeces of infected children. *Am. J. Trop. Med. Hyg*. **7:** 237-242.

Djamiatun, K. and Faubert, G.M. (1998). Exogenous cytokines released by spleen and peyer's patch cells removed from mice infected with *Giardia muris*. *Parasite Immunol.* **20**: 27-36.

Enriquez, F.J., Avila, C.R., Santos, J.L., Tanaka-Kido, J., Vallejo, O. and Sterling, C.R. (1997). *Cryptosporidium* infections in Mexican children: clinical, nutritional, enteropathogenic and diagnostic evaluations. *Am. J. Trop. Med. Hyg.* **56**: 254-257.

Endeshaw, T. Mohamod, H.M. and Tilahun, W. (2004). *Cryptosporidium parvum* and other intestinal parasites Among Diarrhoeal Patients Referred to EHNRI in Ethiopia. *Ethiop.Med. J.* **42**: 195-198.

Erlandsen, S.L. and Meyer, E.A. (1984). *Giardia* and Giardiasis: biology, pathogenesis and Epidemiology, Pp. 7-27. *Plenum Press.* New York.

Erlandsen, SL. and Bemrick, W.J.(1987). S.E.M. evidence for a new species, *Giardia psittaci*. *J. of Parasitol.* **73**: 623-629.

Erlandsen, S.L. (1990). Axenic culture and characterization of *Giardia ardae* from the great blue hereon (*Ardae herodias*). *J. of Parasitol.* **76**: 717-724.

Esrey, S.A., Potash, J., Robert, S.L. and Shiff, C. (1990). Health benefits from improvement in water supply and sanitation: survey and analysis of the literature on selected diseases. WASH Report # **66.**

Farthing, M. (1997). The molecular pathogenesis of giardiasis. *J. Pediatr. Gastroenterol. Nutr.* **24**: 79-88.

Farthing, M.J.G. (2003). Immune response-mediated pathology in human intestinal infection. *Parasite immunol.* **25**: 247-257.

Fayer, R. and Ungar, B.L.P. (1986). *Cryptosporidium spp.* and cryptosporidiosis. *Microbiological Reviews.* **50**: 458-483.

Fayer, R. (1995). Effect of Sodium hypochlorite exposure on infectivity of *C.parvum* oocyst for neonatal BALBlc mice. *Appl. Environ. Microbiol.* **61**: 844-46

Fayer, R., Trout, J.M. and Jenkins, M.C.(1998) .Infectivity of *Cryptosporidium parvum* oocyst stored in water at environmental temperature. *J.Parasitol.* **80**: 1165-1169.

Ferguson, A., Gillon, J. and Munro, G. (1990). Pathology and pathogenesis of the intestinal mucosal damage in giardiasis, pp. 155-173. In : Meyer, E.A. (ed.,) *Giardiasis. Elsevier Publishing Co.* New York.

Fichtenbaum, C.J., Ritchie, D.J., and Powderly, W.G. (1993). Use of Paromomycin for treatment of cryptosporidiosis in patients with ADIS. *Clin. Infect. Dis*. **16**: 298-300.

Filice, F.P. (1952). Studies on the cytology and life history of *Giardia* from the laboratory rat. University of California Publications on Zoology. **57**: 53-143.

Fisseha, B., Petros, B. and Wodemichale, T. (1998). *Cryptosporidium* and other parasites in Ethiopian AIDS patients with chronic diarrhea. *E. Afr. Med. J.* **75**: 100-101.

Fisseha, B. Petros, B. , Woldemicheal, T, and Mohamed, H. (1999). Diarrhea associated parasitic infectious agents in AIDS patients within selected Addis Ababa Hospitals. *Ethiop. J Health Dev*. **13(3)**: 169-173.

Flanagan P.A. (1992). *Giardia*-diagnosis, clinical course and epidemiology, A review, *Epidemiol.Infect*. **102:** 1-22.

Fraser, D. Dagan, R., Naggan, L., Greene, V., El-On, J. Abu-Rbiah, Y. and Dekelbaum, R. J. (1997). Natural history of *Giardia lamblia* and *Cryptosporidium* infections in a cohort of Israel Bedouin infants: a study of a population in transition. *Am. J. Trop. Med. Hyg.* **57**: 544-549.

French, A.L., Beaudet, L.M., Benator, D.A., Levt, C.S., Kass, M. and Orenstein, J.M.(1995). Cholecystectomy in patients with AIDS: Clinicopathologic Correlations in 107 cases. *Clin. Infect. Dis*. **21**: 852-858.

Garcia., L.S., Bruckener, D.A., Brewer., T.C. and Scimizu, R.Y. (1993). Techniques for the recovery and identification of *Cryptosporidium* oocyst from stool specimen. *J. of Clinical Microbiol*. **33(2)**: 416-418.

Gamboa, M.I., Basualdo, J.A., Cordoba, M.A., Pezzani, B.P., Minvielle, M.C. and Lahitte, H.B. (2003). Distribution of intestinal parasitoses in relation to Environmental and Sociocultural Parameters in La Plata, Argentina. *J. Helmithol*. **77**: 15-20.

Gardner, T.B. and Hill, D.R. (2001). Treatment of Giardiasis. *Clin. Microbiol. Reviews*, **14(1)**: 114-128.

Geldreich, E.E. (1989). Drinking water microbiology-new directions toward water quality enhancement. *Int. J. Food Microbiol*. **9:** 295-312.

Gebru, K. and Girma, M. (2000). Prevalence of *Cryptosporidium* infection in children at the pediatric clinic of Jimma Hospital, South west Ethiopia, *Ethio. J. Health Sci.* **10**: 123-127.

Gilman, R.H., Miranda, E., Marquis, G.S. *et al.*, (1988). Rapid re-infection by *Giardia lamblia* after treatment in a hyperendemic third world community. *Lancet*. **1**: 343-345.

Goodgame, R.W. (1996). Understanding intestinal spore- forming protozoa: *Cryptosporidia, Microsporidia, Isospora* and *Cyclospora. Ann. Intern, Med.* **124(4)**: 429-441.

Goldstein, F., Thornton, J. and Szyldlowski, T. (1978). Biliary tract dysfunction in giardiasis. *Am. J. Dig. Dis*. **23**: 559-560.

Goh, S., Reacher, M., Casemore, D.P., *et al*. (2004). Sporadic cryptosporidiosis. North Cambria, England 1996-2000. *Emerging Infect. Dis*. **10**: 1007-15.

Griffith, J.K. (1998). Human cryptosporidiosis: Epidemiology, transmission, clinical disease, treatment and diagnosis. *Adv. Parasitol*. **40**: 37-85.

Guy, R.A., Xiao, C. and Horgen, P.A. (2004). Real-Time PCR Assay for Detection and Genotyping Differentiation of *G.lamblia* in stool specimen. *J. Clin. Microbiol.* **42**(7): 3317-3320.

Hailu, B., Berhanu, E., (1995). Giardiasis in Ethiopia. Ethiop. *J. Health Dev.* **9(1)**: 77-80.

Heyworth, M.F. (1992). Immunology of *Giardia* and *Cryptosporidium* Infections. *J. Infect. Dis*. **166(30**): 465-472.

Healy, G.R., (1979). The presence and absence of *Giardia lamblia* in studies on parasite prevalence in the U.S.A. , pp. 92-103. In: Jakubowski, W. and Hoff, J.C. (eds), *Water borne transmission of Giardiasis*. U.S. Environmental Protection Agency, Cincinnati.

Heresi, G. and Cleary, T.G. (1997). *Giardia. Pediatrics in Review*. **18(7)**: 243-247.

Hlavsa, M.C., Watson, J.C. and Beach, M.J. (2005). Cryptosporidiosis surveillance—United States 1999-2002. *MMWR Surveill Summ.* **54**(**1**): 1-8.

Hojlyng, N., Holten-Andersen, W. and Jespen, S. (1987). Cryptosporidiosis: A case of airborne transmission. *The Lancet*, **2:** 271-272.

http://www.dpd.cdc.gov/dpdx/HTML/Giardiasis.htm. (Accessed on 19 May, 2007 14:37:06).

http://www.dpd.cdc.gov/dpdx/HTML/Frames/A-F/cryptosporidiosis/body_cryptosporidiosis_Lifecycle.lrg.htm. (

Accessed on 4 May 2007 10:48:52).

http://wwwn.cdc.gov/travel/yellowBookCh2-FoodWaterRisks.aspx (Accessed on 9 Jul 2007 09:25:32).

http://www.epa.gov/safewater/faq/emerg.html (Accessed on 13 Jul 2007 07:58:54).

Institute of Pathobiology (1986). Expanded programme of applied research and training towards prevention and control of schistosomiasis in Ethiopia (project/MPD/003). Progress Report, No 4, 107pp.

Iqbal, J., Munir, M.A. and Kahan, M.A. (1999). *Cryptosporidium* infection in young children with diarrhea in Rawalpinidi, Pakistan. *AJTMH,* **60(5)**: 868-870.

Iqbal, J. *et al.* (2000). Cryptosporidiosis in Kuwait children: seasonality and endemicity. *Clinical Microbiology and Infection.* **7(5):** 261-266.

Isaac-Renton, J.L., Fogel, D., Stibbs, H.H.and Ongerth, J.E., (1987). *Giardia* and *Cryptosporidium* in drinking water. *Lancet*, **1:** 973-974.

Isaac-Renton, J.L. and Philion, J.J. (1999). Factors associating with acquiring giardiasis in British Columbia residents, *Can. J. Public Health.* **83(2)**: 155-158.

James, S,J., (1988). (ATN) Treatment leads on cryptosporidiosis: preliminary report on opportunistic infection. *AIDS Treatment News* No.049.

Jarmey-Swan, C., Bailey, I.W. and Howgrave-Graham. A.R. (2001). Ubiquity of water-borne pathogens, *Cryptosporidium* and *Giardia*, in Kwa-Zulu-Natal populations. *Water SA.* **27(1)**: 57-64.

Jensen, P.K., Jayasinghe, G., van der Hoek, W., Carncross, S. and Dalsgaard, A. (2004). Is there an association between bacteriological drinking water quality and childhood diarrhea in developing countries? *Tropical Medicine and International Health.* **9***:* 1210-1215.

Joe, A., Verdon, R., Tzipori, S., Keusch, G.T. and Ward, H.D. (1988). Attachment of *Cryptosporidium parvum* sporozoites to human intestinal epithelial cells. *Infect.Immun.* **66**: 3429-3432.

Johnson, D.W., Pieniazek, N.J., Griffin, D.W., Misener, L. and Rose, J.B. (1995). Development of a PCR protocol for sensitive detection of *Cryptosporidium* oocyst in water samples. *Appl.Environ. Microbiol.* **61(11)**: 3849-55.

Juranek, D.D. (1995). Cryptosporidiosis: source of infection and guideline for

presentation. *Clin. Infect. Dis*. **21(supp I)**: 557-561.

Kenneth, H.B., Robert, E.B., Guillermo, D.L. and Hilary, C.K. (1989). Infant-feeding practices and their relationship with diarrheal and other diseases in Huascar (Lima), Peru. *Pediatrics*. **83(1)**: 31-40.

Kerutner, A.K., DelBene, V.E. and Amstey, M.S. (1981). Giardiasis in pregnancy. *Am. J. Obstet. Gynecol.* **140**: 895-901.

Leav, B.A., Mackay, M. and Ward, H.D. (2003). *Cryptosporidium* species: new insights and old challenges. *Clin. Infect. Dis*. **36**: 903-908.

Leach, C.T., *et al*, (2000). Prevalence of *Cryptosporidium parvum* infection in children along the Texas-Mexico border and associated risk factors. *Am. J. Trop. Med. Hyg* .**62(5)**: 656-661.

Lerman, S.J. and Walker, R.A. (1982). Treatment of Giardiasis: Literature reviews and recommendations. *Clin. Pediatr*. **21**: 409-414.

Lindo, F.J., Levy, A.V., Baum, K.M. and Palmer, J.C. (1998). Epidemiology of giardiasis and cryptosporidiosis in Jamaica. *Am. J. Trop. Med. Hyg.* **59(5)**: 717-721.

Marcial, M.A., and Madara, J.L. (1986). *Cryptosporidium*: Cellular Localization, Structural analysis of absorptive cell-parasite interactions in Guinea pigs and suggestion of protozoan transport by M-cells. *Gastroenterology*. **90**: 583-594.

Mahmud, M.A., Chappll, C., Hossain, M.M., Habib, M. and Dupont, H.L. (1995). Risk factors for development of first symptomatic *Giardia* infection among infants of a birth cohort in rural Egypt. *Am. J. Trop. Med. Hyg.* **53**: 84-88

Mahajan, M., Mathur, M. and Talwar, C. (1992). Cryptosporidiosis in east Delhi children. *J. Commun. Dis*. **24**: 133

Magne, D., Favennec, L., Chochillon, C., et al., (1991). Role of cytoskeleton and surface lectins in *Giardia duodenalis* attachment to Caco2 cells. *Parasitol. Res*. **77**: 659-662.

Martin, C.A. and Guerrant, R.L. (1995). *Cryptosporidium* and cryptosporidiosis. *Parasitol. Today.* **11(11**): 434-436.

MacPherson, D.W. and McQueen, R. (1993). Cryptosporidiosis: multiattribute evaluation of six diagnostic methods. *J. Clin. Microbiol*. **31**: 198-202.

McDonald, V. (2000). Host cell-mediated response to infection with *Cryptosporidium*. *Parasite immunology*, **22**: 597-604.

McConnel, E. and Armstrong, J.C. (1976). Intestinal Parasitism in Fifty communities on the Central Plateau of Ethiopia. *Ethiop. Med. J.* **14**: 159-169.

Meyer, E.A. (1994). *Giardia* as an organism. In: Thompson, R.C.A., Reynoldson, J.A., Lambery, A.J., and Eds.. *Giardia*: from molecules to disease. Wallingford,England, CAB International: 3-14.

Meyer, E.A. and Jarrol, E.L. (1980). *Giardia* and Giardiasis. *Am. J. Epidemol.* **111**: 1-12

Mersha, D. and Tiruneh, M. (1992). Frequency of *Cryptosporidium* oocyst in Ethiopian children with diarrheal disease. *East Afr. Med. J.* **69**: 314-315.

Meisel, J.L., Perera, D.R., Meligro, C. and Rubin, C.E. (1976). Overwhelming watery diarrhea associated with a *Cryptosporidium* in an immunosuppressed patients. *Gastroenterology.* **70**: 1156-1160.

MOH (1997). Knowledge, attitude and practice of water supply. Environmental sanitation and hygiene practice in selected woredas of Ethiopia.

Molbak, K., Aaby, P., Hojlying, N. and Da-Silva , A.P. (1994a). Risk factors for *Cryptosporidium* diarrhea in early childhood: a case control study from Guinea Bissau, West Africa. *Am. J. Epidemol.* **139**: 734-740.

Molbak, K., Gottschau, A., Aaby, P., Hojlying, N. and Da-Silva , A.P. (1994b). Prolonged breast feeding, diarrheal disease, and survival of children in Guinea-Bissau. *BMJ.* **308**: 1403-1406.

National Science and Technology Council (NSTC). Infectious disease- A global health threat, September 1995.

NATHNAC (2004). http:// www.nathnac.org/travel/factsheet/pdf/giardiasis.pdf (Accessed on 2 July 2007 16:14:39).

Nimri, L.F. (1994). Prevalence of giardiasis among primary school children. *Child Care Health Dev.* **20**: 231-237.

O'donoghue, P.J. (1995). *Cryptosporidium* and cryptosporidiosis in man and animals. *Int. J. Parasitol.* **25**: 139-195.

Okafor, J.L. and Okunji, P.O. (1994). Cryptosporidiosis in patient with diarrhea in five hospitals in Nigeria. *J. Commun. Dis.* **26**: 75.

Ortega, Y. and Adam, R. (1997). *Giardia*: an overview and update. *Clin. Infect. Dis.* **25(3)**: 545-549.

Payment, P. (1999). Poor efficacy of residual chlorine disinfection in drinking water to inactivate water borne pathogens in distribution systems. *Can. J. Microbiol.* **45(80):** 709-715.

Patel., S., Pendraza-Diaz, S., McLauchlin, J. and Casemore, D.P. (1998). Molecular characterization of *Cryptosporidium parvum* from two large suspected water borne outbreaks. *Commun. Dis. Public Health*. **1**: 231-233.

Pawi special district Health office report from 13 clinics (2003-2005).

Pitlik, S.D., Fainstein, V. and Garza, D. (1983). Human cryptosporidiosis: spectrum of disease. Report of six cases and review of the literature. *Arch. Interna. Med.* **143**: 2269-2275.

Pollok, R.C.G., McDonald, V., Kelly, M.P. and Farthing, M.J.G. (2003). The role of *Cryptosporidium parvum*-derived phospholipase in intestinal epithelial cell invasion. *Parasitol. Res*. **90**: 181-186.

Ramirez, N.E., Ward, L.A. and Sreevatsan, S. (2004). A review of the biology and epidemiology of cryptosporidiosis in humans and animals. *Microbes and infection.* **6**: 773-785.

Raza, H. (2003). Urban environment. Northern areas strategy for sustainable development. Pp.1-38. IUCN, Northern Areas Program. Pakistan.

Rimhanen-Finne, R., Horman, A., Ronkainen, P., Hanninen, M.L. (2002). An IC-PCR method for detection of *cryptosporidium* and *Giardia* in natural surface waters in Finland. *J. Microbiol. Methods*, **50**: 299-303.

Robertson, L.J. and Gjerde, B. (2001). Occurrence of *Cryptosporidium* oocyst and *Giardia* cyst in raw waters in Norway. Scand. *J. Public. Health*. **29**: 200-207.

Rose, J.(1997). Environmental ecology of *Cryptosporidium* and public health implications. *Annu. Rev. Public Health,* **18**: 135-161.

Rosales, M.J., Mascaro, C. and Osuna, A. (1993). Ultrastructural study of *Cryptosporidium* development in Madin-Darby canine kidney cells, *Vet. Parasitol.* **45:** 267-273.

Sanchez-Mejorada, G., Ponce-de-Leon, S. (1994). Clinical patterns of diarrhea in AIDS Etiology and prognosis. *Rev. Invest. Clin*. **46**: 187-196.

Serpil, D.E., Ali, C.K., Kader, K. and Semra, O. (2005). Prevalence of *Cryptosporidium* spp and *Giardia* spp in cows and calves in Sivas. Turk. *J. Vet. Anim. Sci.* **29:** 995-999

Seyoum, T., Abdulahi, Y. and Haile-Meskel, F. (1981). Intestinal parasitic infection in pre-school Children in Addis Ababa. *Eth. Med. J.* **19**: 35-40.

Smith, N.H., Cron, S., Valdez, L.M., Chappell, C.L. and White, A.C. (1988). Combination drug therapy for cryptosporidiosis in AIDS. *J. Infect. Dis.* **178**: 900-903.

Soriano, S.V., Barbieri, L.M., Pierangeli, N.B., Giayetto, A.L., Manacorda, A.M., Castronovo, L., Pezzani, B.C., Minvielle, M.C. and Basualdo, J.A. (2001). Intestinal Parasites and the Environment: Frequency of Intestinal Parasites in children of Nequen, Patagonia, Argentina. *Rev. Latinoam Microbiol.* **43**: 96-101.

Spano, F., Putigni, L., Cristianti, A., *et al.*(1998). Multilocus genotypic analysis of *Cryptosporidium parvum* isolates from different hosts and geographical origins. *J. Clin. Microbiol.* **36**: 3255-3259.

Stevens, D.M. and Adam, H.M. (2004). Giardiasis and Cryptosporidiosis. *Pediatrics in reviews.* **25(7**): 260-261.

Tedla, S. and Jemaneh, L. (1985). Distribution of *Ancylostoma dudenales* and *Necator americanus* in Ethiopia. *Ethiop. Med. J.* **23(4**): 149-58.

Tedla, S. and Ayele, T. (1986). Ascariasis distribution in Ethiopia. *Ethiop. Med. J.* **24**: 79-80.

Tellez, A., Winiecka-Krusnell, J., Paniagua, M. and Linder, E. (2003). Antibodies in mother milk protect children against giardiasis. *Scand. J. Infect. Dis.* **35(5**): 322-325.

Teare, J.P., Daly, C.A., Rodger, C., Padly, S.P., Coker, R.J. Main, J., Harris, J.R., Scullion, D., Bray, G.P. and Summerfield, J.A. (1997). Pancreatic abnormalities and ADIS, related sclerosing cholangitis. *Genitourin. Med.* **73**: 271-273.

The World Health Report. (1998). Life in the 21st century: A vision for all. Geneva.

The 15th European Congress of Clinical Microbiology and Infectious Diseases. Copenhagen/Denmark, April 2-5, 2005

Thielman, N.M. and Guerrant, R.L. (1998). Persistent diarrhea in the returned traveler. *Inf. Dis. Clin. N. Amer.* **12(2)**: 489-501.

Thompson, R.C., Reynoldson, J.A. and Mendis, A.H.W. (1993). *Giardia* and Giardiasis. *Adv. Parasitol.* **32**: 71-160.

Thea, D.M., Pareira, M.E.A., Kotler, D., Sterling, C.R. and Keusch, G.T. (1992). Identification and partial purification of a lectin on the surface of the sporozoite of *Cryptosporidium parvum. J. Parasitol.* **78**: 886-893.

Tumwine, J.K., Kekitiinwa, A., Nabukeera, N., Akiyoshi, D.E., Rich, S.M., Widmer, G., Feng, X. and Tzipori, S. (2003). *Cryptosporidium parvum* in children with diarrhea in Mulago Hospital, Kampala, Uganda. *Am. J. Trop. Med. Hyg.* **68**: 710-715.

Tzipori, S. and Ward, H. (2002). Cryptosporidiosis: Biology, Pathology and Disease. *Microbes and Infection,* **4**: 1047-1058.

U.S. EPA. (1989). Giardia: risk for infants and children. United States Environmental Protection Agency, (EPA-823-R-99-011), Washington, D.C.

Vakil, N.B., et al., (1996). Biliary cryptosporidiosis in HIV infected people after the water borne outbreak of cryptosporidiosis in Milwaukee. *New Engl. J. Med.* **334**: 19-23.

Vesy, C.J. and Petersen, W.L. (1999). The management of Giardiasis. *Aliment. Pharamacol. Ther.* **13**: 843-850.

World Health Organization. (1991). Basic laboratory methods in medical parasitology, Geneva: 25-26.

World Health Organization. (1998). Control of tropical diseases. WHO, Geneva.

Wolf, M.S. (1990). Clinical symptoms and diagnosis by traditional methods. In: Mayer, E.A. giardiasis. Vol. 3**,** *Human Parasitic Diseases*. Elsevier Science Publisher Biomedical Division, Amsterdam. pp. 175-185.

Xian-Ming, C. and LaRusso, F.N. (1999). Human intestinal and biliary cryptosporidiosis. *World J. Gastroenterol.* **5(5)**: 424-429.

Appendix I: CONSENT FORM

Code No----------

Name of the study participant ---------- Age -------- Sex --------
Name of Physician ---------- Site/Health center ----------

I have been informed about a study that plans to investigate the “Drinking water and the prevalence of Giardiasis and Cryptosporidiosis in children of age 2 months -14 years in Pawi District, Benishangul-Gumuz Region” which helps in understanding the possible source of infection to the different types of water sources and at the same time it enables concerned bodies in designing a better control measures of water borne parasitic diseases in the study area.

For this study I was requested to give a stool sample for Cryptosporidium and Giardia identification. I was informed that I will get proper therapy if I found to be positive for any of intestinal parasites. The investigator has also briefed me that there would no major risks associated with the sampling procedure. He also informed me that all laboratory results would be kept in secret. Moreover I was clearly informed that I have a right to withdraw from participating in this study and in so doing there will be no impact on the overall management of my conditions. I was given enough time to think over before I signed this informed consent. It is therefore; with full understanding of the situation that I gave informed consent and cooperate at my will in the course of the conduct of the study.

Name (participant) ---------- Signature ---------- Date ----------
Name (investigator) ---------- Signature ---------- Date ----------
Name (Witness) ----------Signature ---------- Date ----------

☛⑦⑦☙⑤ፚ⓪❹▤❜❜ ☒❶☙⑩❶⓪⑥⑤⑤ፚ⓪⑨☙
☛ ☒❶☙⑩❶⓪⑥⑤⑤ፚ⓪⑨☙ ✓⑨☙⑦ፚ⑨☙ፚ ❶⑥ ☞⑥③③☙ክ❶ ☚ፚ❶ፚ ፋ⑥⑨ ❶ᕒ☙ ⑩❶❶ፚ❺ ⑥ፋ ☚⑨⓪⑤②⓪⑤ሎ ❸ፚ❶☙⑨ ⑩⑥❶⑨ክ☙ ፚ⑤ፚ ❶ᕒ☙ ✓⑨ ☙❷ፚ③☙⑤ክ☙ ⑥ፋ ☞⑨❺⑦❶⑥⑩⑦⑥⑨⓪ፚ⓪❶④ ⑦ፚ⑨❷❶④ ፚ⑤ፚ ❜⓪ፚ⑨ፚ⓪ፚ ③ፚ④ጠ③⓪ፚ ❜⑤ፋ☙ክ❶⓪⑥⑤⑩ ፚ④⑥⑤ሎ ☞ᕒ⓪③ፚ⑨☙⑤▤ ✓ፚ❸⓪ ☒⑦☙ክ⓪ ፚ③ ⃠⑥⑨ፚፚ▤ ☜☙⑤⓪⑩ᕒፚ⑤ሎ❶③ •❜❶④❶❻ ☑☙ሎ⓪⑥⑤▥

1. የቦታ ስም---------- ቀበሌ ----------
2. የቤት ቁጥር----------የቤተሰብ ብዛት----------
3. የገቢ ሁኔታ----------
   3.1 አርሶ አደር----------
   3.2 የመንግስት ሰራተኛ----------
   3.3 ነጋዴ----3.4 የቀን ሠራተኛ___3.5 የቤት ሠራተኛ___3.6 ሥራ አጥ___3.7 ሌላ__
4. የቤተሰቡ ወርሃዊ ገቢ----------
5. የቤተሰብ አባል (ከ15 አመት በታች)

ተ.ቁ	ስም	እድሜ	ጾታ	የት.ደረጃ	የበሽታ ምልክት 1.የሆድ ቁርጠት 2.ተቅማጥ 3. ትኩሳት	የሰገራው ዓይነት 1. ደም 2. ልሩጭ 3.ተቅማጥ 4.የለሰለሰ	Slide NO.	Result	
								Giardia	Others
1									
2									
3									
4									

6. ከዚህ በፊት ለሆድ ሁከት መድኃኒት ወስዶ/ ወስዳ ነበር? አዎ---------- አይ----------
7. ለመጠጥ አገልግሎት የሚያውሉት የውሃ አይነት
   7.1 ዲጋ----------
   7.2 አለ----------
   7.3 የእጅ ጉድጓድ------
   7.4 የእጅ ፓንፕ------
   7.5 ምንጭ (ያልጎለበተ) -------
   7.6 ሌሎች----------
8. ውሃን ለመጠጥ አገልግሎት ሲያውሉት
   8.1 ተጣርቶ ----------
   8.2 ተፈልቶ----------
   8.3 በቀጥታ---------
   8.4 ሌላ ካለ ይገለፅ------
9. የሰገራ አወጋገድ ባህል
   9.1 ሽንት ቤት --------
   9.2 ሜዳ ላይ----------
10. ሕክምና የምታገኙት የት ነው?
   10.1 ሆስፒታል
   10.2 ጤና ጣቢያ
   10.3 ሌሎችም (የግል ክሊኒክ/ፋርማሲ)
11. ህፃኑ ጡት ይጠባል
   11.1 ይጠባል_____ከመቼ ጀምሮ_____
   11.2 አይ አይጠባም_____ከመቼ ጀምሮ_____

☛🙢🙦③🙤⑨🙤⓿⓪⑥⑤

✍🗎 ⓿🙡🙢 ❶⑤🙥🙢⑨⑩⓪🙠⑤🙢🙥🗎 🙥🙢🙦③🙤⑨🙢 ⓿🙡🙤⓿ ⓿🙡⓪⑩ ⓿🙡🙢⑩⓪⑩ ⓪⑩ ④❺ ⑥⑨⓪🙠⓪⑤🙤③ ❸⑥⑨② 🙤⑤🙥 🙡🙤⑩ ⑤⑥⓿ 🙧🙢🙢⑤ ⑦⑨🙢⑩🙢⑤⓿🙢 🙥 🙣⑥⑨ 🙤 🙥🙢🙠⑨🙢🙢 ⓪⑤ 🙤⑤❺ ⑥⓿🙡🙢⑨ ❶⑤⓪❷🙢⑨⑩⓪⓿❺ 🙤⑤🙥 ⓿🙡 🙤⓿ 🙤③③ ⑩⑥❶⑨🙦🙢 ⑥🙣 ④🙤⓿🙢⑨⓪🙤③⑩ ❶⑩🙢🙥 🙣⑥⑨ ⓿🙡🙢 ⓿🙡🙢⑩⓪ ⑩ 🙡🙤❷🙢 🙧🙢🙢⑤ 🙦⑥⑨⑨🙢🙦⓿③❺ 🙤🙦②⑤⑥❸③🙢🙥🙠🙢🙥🗐

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