PREVALENCE AND INTENSITY OF INTESTINAL PROTOZOAN AND HELMINTH INFECTIONS AND THEIR ASSOCIATIONS WITH ANTHROPOMETRIC MEASUREMENTS OF PRIMARY SCHOOL CHILDREN IN CHAGNI TOWN, AMHARA REGIONAL STATE, NORTH-WEST ETHIOPIA

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Prevalence and Intensity of Intestinal Protozoan and Helminth Infections and Their Associations with Anthropometric Measurements of Primary School Children in Chagni Town, Amhara Regional State, North -West Ethiopia

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As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by Teshager Worku, entitled **"Prevalence and Intensity of Intestinal Protozoan and Helminth Infections and their Associations with Anthropometric Measurements of Primary School Children in Chagni Town, Amhara Regional State, North-West Ethiopia".** We recommend that it can be submitted as fulfilling all the thesis requirements.

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DEDICATION

I dedicate this thesis to my lovely Family and Friends for their continual and unbound love, patience and strength that helped me to complete this work.

STATEMENT OF AUTHOR

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BIOGRAPHICAL SKETCH

The author, Teshager Worku, was born from his father Ato Worku Beyene and his mother Wozero Amommta Adel on January 12, 1992 G.C in Zigem Woreda, Awi Zone, Amhara Regional state, Ethiopia. He attended his primary school in Wingi Primary School and his secondary school in Zigem General Secondary School. After the completion of grade 9-10 from 2009- 2010 G.C, he attended his preparatory school education in Chagni Preparatory School from 2011-2012 G.C in Chagni town. In 2013 G.C, he joined Mekelle University and graduated with B.Sc. degree in Biology in 2015. He joined the postgraduate program directorate of Haramaya University in 2016 to pursue his M.Sc. studies in Applied Biology.

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LIST OF ABBREVIATIONS AND ACRONOMYS

AMRF	African Medical Research Foundation
BMI	Body Mass Index
CDC	Center for Disease Control
CSA	Central Statistical Authority
EPG	Eggs Per Gram of Feces
FMOH	Federal Ministry of Health
HAZ	Height for Age Z Score
IPI	Intestinal Parasitic Infection
KAP	Knowledge, Attitude and Practice
NCCLS	National Committee on Clinical Laboratory Standards
NCHS	National Center for Health Statistics
RPM	Rotation per Minute
SPSS	Statistical Package for Social Sciences
STHs	Soil Transmitted Helminthes
WAZ	Weight for Age Z Score
WHO	World Health Organization
WHZ	Weight for Height Z Score

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ABSTRACT

Intestinal protozoan and helminth infections are the major public health problems in many developing countries including Ethiopia. *This study was aimed to determine the prevalence and intensity of intestinal protozoan and helminth infections in school children and their associations with anthropometric measurement. A cross-sectional study was carried out and 393 school children were chosen using stratified random sampling technique and enrolled in the present study. Structured and pre-tested questionnaires were administered in both English and Amharic to gather relevant information on demographic and risk factors that predispose for intestinal parasitic infections. Stool samples were collected for microscopic examinations using direct wet mount, Formol-ether concentration method, Kato-Katz and modified Ziehl Nielsen staining technique. Anthropometry calculating software, anthroplus version 3.1 was employed to evaluate anthropometric parameters (age, sex, height and weight). Data were analyzed using SPSS statistical software version 20.0. The overall prevalence of intestinal protozoan and helimnth infections in the study area was 47.6% and 20.9%, respectively. The prevalence of Ascaris lumbricoides, Schistosoma mansoni, Taenia species, Cryptosporidium species, Hymenolepsis nana ,Hookworm, Entameoba histolytica/dispar and Giardia lamblia were 5.3%, 8.7%, 1.8%, 2.3%, 2.5%,2.3%,25.9% and 19.2%, respectively. Intensity of Ascaris lumbricoides, Schistosoma mansoni and hook worm ranged from 0-2880, 0-792 and 0-720 per gram of feces, respectively. Mean egg count for Ascaris lumbricoides, Schistosoma mansoni and hookworm infections were 744±346.5, 252±64.4, and 270±70.4, respectively. Malnutrition in terms of underweight, stunting and wasting was seen in 38.7%, 36.9% and 30.9% of the samples, respectively. The prevalence of intestinal parasitic infections was lower in underweighted (38.7%) students than Wasted (30.9%) and stunted (36.9%) students. Intestinal helminth and protozoan parasitic infections were the major problem in Chagni town due to prevailing risk factors. Coordinated work between health officers and school community was required on the prevention of helminth and protozoan infections.*

Keywords: Anthropometry, Chagni, Intensity, Helminth, Malnutrition, Prevalence, Protozoan

1. INTRODUCTION

Parasitic infections caused by helminths and protozoa are the major causes of human diseases in most countries of the tropical region. It is estimated that about 3.5 billion people in the world are infected with intestinal parasites, of whom 450 million are ill. They are among the most prevalent infections of humans living in areas of poverty in the developing world. Two billion individuals were reported to be parasitized with helminthic worms, majority of them living in resource-poor settings, 80% of these live in sub-Saharan Africa (Keiser and Utzinger, 2010). The African medical research foundation in 2007 (AMRF, 2007) indicated that the young children particularly affected by intestinal helminths are from poor background with failure to use latrines as a major reason for the increase in the spread of parasitic helminthic worms.

Epidemiological surveys have revealed that poor sanitary conditions such as open field defecation and faecal contamination of water bodies as well as poor personal hygiene are the most important factors leading to intestinal worm infections (Van eijk *et al*., 2009). In Ethiopia, helminthic infections are the second most predominant causes of outpatient morbidity (Alemu *et al*., 2011). This is due to the fact that Ethiopia has one of the lowest qualities of drinking water and sewage coverage in the world (Kumie and Ali, 2005). Children of pre-school and school age (0 – 15 years) are the groups at risk of getting infected with intestinal parasites (Fentiman *et al*., 2001).

Intestinal parasitic infections have detrimental effects on the survival, appetite, growth and physical conditions of children. Helminths can be found in a great variety of tissue niches, and although they cause very high morbidity, direct mortality of the host species remains low (Brooker, 2010). *Entamoeba histolytica*, *Cryptosporidium parvum*, *Giardia lamblia*, *Ascaris lumbricoides* and human hookworm infections are common infections that are caused by the protozoan and helminth parasite species. 50 million people worldwide suffer from invasive amoebic infection each year, resulting in 40-100 thousands of deaths annually (Petri *et al*., 2000).

The high prevalence of infection in children is attributed to the economic and social situation of the individuals which is the important cause of the prevalence of intestinal parasites (Wadood *et al*., 2005). Public health specialists are concerned that these infections impair children's growth and development (Rashid *et al*., 2011). Younger children are predisposed to heavy infections with intestinal parasites since their immune systems are not yet fully developed (Rao *et al*., 2006), and they are also habitually play in fecally contaminated soil. The high prevalence of these infections is closely correlated with poverty, poor environmental hygiene and impoverished health services (Alyousefi *et al*., 2011).

The prevalence of intestinal parasitic infections among pre-school children was also reported from different parts of Ethiopia at different times by a number of researchers. Some of these researchers include Mengistu and Berhanu (2004); Abraham (2005); Mengistu *et al*. (2007) and Asrat *et al*. (2011). In Ethiopia high prevalence of intestinal parasite infections are attributable to factors associated with low socio-economic status. Such factors include poor personal hygiene, environmental sanitation, low household income, overcrowding and lack of clean water supplies. For instance, Ethiopia has one of the lowest quality drinking water supply and latrine coverage (Mengistu *et al*., 2007).

The study was selected on the basis of lack of empirical sources on intestinal parasites among school children in Chagni town, North -Western Ethiopia and observation of different factors. These factors were poor waste disposal or avoiding habits, no safe toilet facility for this reason; these people have been defecating on open field in any open space, especially, at the riverbanks. Domestic waste products were released into the surrounding area without any care, population depends on open markets as a source of foodstuffs, such as meat, vegetables and fruits etc. food stuffs (meat, vegetables and fruits) may be contaminated by intestinal parasites because of houseflies and low personal hygiene of food handlers. In addition to this, it is believed to serve as a baseline data for the this study. Therefore, the general objective of this study was to determine prevalence and magnitude of intestinal parasitic infections and their associations with anthropometric measurements of school children, in Chagni town, Amhara regional state, North-West Ethiopia.

Specific objectives were:

1. To identify the major intestinal parasitic species from stool samples of school children in the study area
2. To determine the prevalence of intestinal protozoan parasitic infections of school children in Chagni town.
3. To determine the prevalence of intestinal helminth parasites of school children in Chagni town.
4. To determine the intensity of major intestinal helminth parasites in the study area.
5. To determine the associations of intestinal protozoan and helminth infections with anthropometric indices of school children.

2. LITRATURE REVIEW

2.1. Protozoan Parasites

Numerous protozoa inhibit the gastro-intestinal tract of humans. This list includes representatives from many diverse protozoan groups. Intestinal protozoan infections are a significant problem with more than 58 million cases in children each year. Pathogenic intestinal protozoa are especially important in the developing world, where they may cause death. Most intestinal parasites are spread by faecal-oral contact or contamination of water or food such as *Entamoeba histolytica/dispar, Cryptosporidium parvum* and *Giardia lamblia* are the main protozoan of concern in developing countries. The majority of these organisms can cause severe disease under certain circumstances (Stenzel and Boreham, 2004).

G. lamblia is a pear-shaped, flagellated protozoan that causes a wide variety of gastrointestinal complaints. Giardia is arguably the most common parasite infection of humans worldwide, and the second most common in the United States after pin-worm. Because giardiasis is spread by fecal-oral contamination, the prevalence is higher in populations with poor sanitation and close contact with contaminated people. The disease is commonly water-borne because *Giardia* is resistant to the chlorine levels in normal tap water and survives well in cold mountain streams. Food-borne transmission is rare but can occur with ingestion of raw or undercooked foods (Glaser *et al*., 2000).

Cryptosporidiosis is a parasitic disease caused by Cryptosporidium, a protozoan parasite in the phylum Apicomplexa. It affects the intestines and is typically an acute short-term infection. It is spread through the fecal-oral route, often through contaminated water (CDC, 2009). *Cryptosporidium parvum* is the organism most commonly isolated in HIV positive patients presenting with diarrhea. Treatment is symptomatic, with fluid rehydration, electrolyte correction and management of any pain. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exist in the small intestine and result in an infection of intestinal epithelial tissue. Cryptosporidiosis is typically an acute short-term infection but can become severe and non-resolving in children and immunocompromised individuals.

The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exist in the small intestine and result in an infection of intestinal epithelial tissue. Infection is through contaminated material such as earth, water, uncooked or cross-contaminated food that has been in contact with the feces of an infected individual or animal. Contact must then be transferred to the mouth and swallowed (CDC, 2009). It is especially prevalent amongst those in regular contact with bodies of fresh water including recreational water such as swimming pools. Other potential sources include insufficiently treated water supplies, contaminated food, or exposure to feces (CDC, 2009). The high resistance of Cryptosporidium oocysts to disinfectants such as chlorine bleach enables them to survive for long periods and still remain infective (Carpenter *et al*., 1999).

2.2. Helminth Parasites

Parasitic worms, often referred to as helminthes are a division of eukaryotic parasites (Maizels and Yazdanbakhsh, 2003). The intestinal helminth parasite infects humans and shares a common source of infection soil contaminated by faecal matters. The main species are Ascaris lumbricoides, Trichuris trichiura and the hookworms (Necator americanus and Ancylostoma duodenale) (Bethony *et al*., 2006). They are considered together because it is common, especially in children living in developing countries, to be chronically infected with the worms. Such children have malnutrition, growth stunting, intellectual retardation, cognitive and educational deficits (Gibson, 2005). They are worm-like organisms that live and feed off living hosts, receiving nourishment and protection while disrupting their hosts' nutrient absorption, causing weakness and disease. Ascariasis is infection with the parasitic roundworm, *Ascaris lumbricoides*. Ascariasis is caused by consuming food or drink contaminated with roundworm eggs. Ascariasis is the most common intestinal worm infection. It is found in association with poor personal hygiene, poor sanitation, and in places where human feces are used as fertilizer (Maguire, 2009). Hookworms (a type of roundworm) are another common intestinal parasite. The United States Centers for Disease Control (CDC) and Prevention estimates that 1 billion people worldwide have hookworm infestations, although improved sanitation has reduced the number of cases.

Ancylostoma duodenale and *Necator americanus*: Two species of hookworm, *A. duodenale* and *N. americanus*, are found exclusively in humans. *A. duodenale*, or "Old World" hookworm, is found in Europe, Africa, China, Japan, India, and the Pacific islands. *N. americanus*, the "New World" hookworm, is found in the America and the Caribbean, and has recently been reported in Africa, Asia, and the Pacific. Until the early 1900s, *N. americanus* infestation was endemic in the southern United States and was only controlled after the wide spread use of modern plumbing and foot wear (Hotez *et al*., 2004).

Pinworm infection should be suspected in children who exhibit nocturnal restlessness. Direct visualization of the adult worm or microscopic detection of eggs confirms the diagnosis, but only 5 percent of infected persons have eggs in their stool. The "cellophane tape test" can serve as a quick way to clinch the diagnosis (Procop, 2001).

2.3. Life Cycle of Intestinal Protozoan Parasites

Entamoeba histolytica /dispar

The life cycle of *Entamoeba histolytica/dispar* consists of two stages (Figure 1). The protected cysts and active trophozoites. Cysts measure 10-15 mm in diameter and typically contain four nuclei. Usually cysts, or occasionally trophozoites are ingested by man, ordinarily in contaminated water or food. Trophozoites ingested was killed by stomach acids, since they have no protective covering. Cysts are carried to the lower ileum, where the amebae excyst. The resultant eight trophozoites reach the lumen of the colon, multiply, and may invade and destroy the tissue of the colon wall (by secreting an enzyme) or invade and multiply in other organs (lungs, liver, brain, etc.) by way of the circulatory systems (Gill and Beeching, 2004).

Trophozoites may multiply in the lumen of the colon, the colon wall, and other organs they invade. Trophozoites in the lumen may either encyst or remain trophozoites and in either form pass in faeces; trophozoites are most common in dysenteric stools. Most trophozoites disintegrate soon after passage; cysts may be ingested and the life cycle continued (Gill and Beeching, 2004).

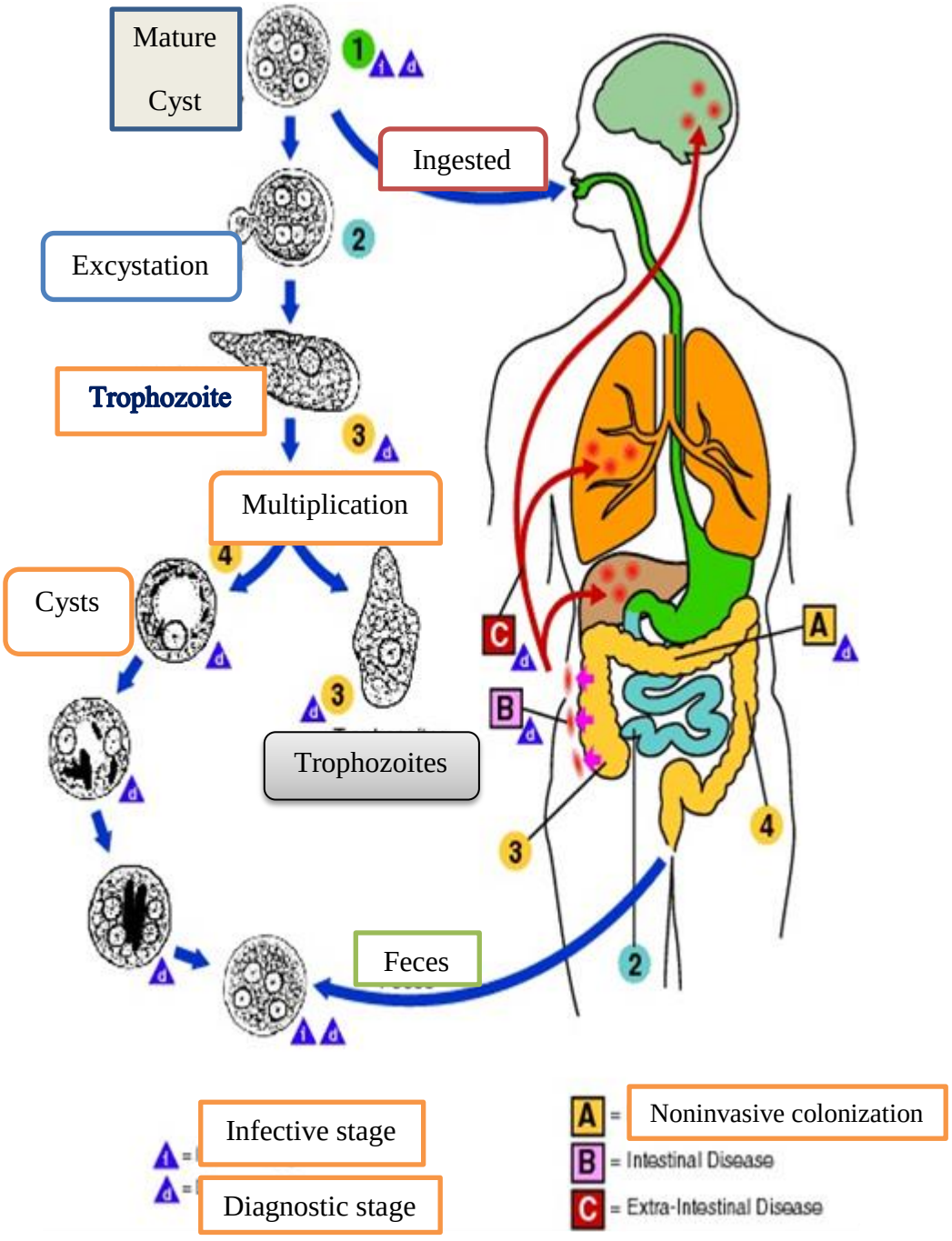

Figure 1. Life cycle of *Entameoba histolytica/dispar* (Source: http://www.dpd.cdc.gov/dpdx*).*

Giardia lamblia

Giardia species has two major stages in the life cycle; the cyst and vegetative tropozoite (Figure. 2). Giardia exhibits a simple and direct life cycle meaning that no intermediate host is required in the life cycle (Svärd *et al*., 2003). The trophozoite a vegetative (actively feeding and metabolizing) stage, is pear shaped, measuring from 7 to 10μm at its widest part, and 10-15μm long. The cyst, trophozoites encased in a multilayered cyst wall is oval, measuring from 10 to 15μm in length. Cysts are excreted in the host's faeces and are transmitted to the next host when cysts contaminate food and water. Ingested, viable cysts excyst after passage through the stomach and exposure to an acid environment of stomach, after excystation, trophozoites colonize and reproduce by binary fission in the host's small intestine where, in the presence of bile, they form cysts (Svärd *et al.,* 2003). Giardia exhibits a typical fecal-oral transmission cycle and infection is acquired by ingesting cysts. Factors leading to contamination of food or water with fecal material are usually associated with transmission (Bernander *et al*., 2001).

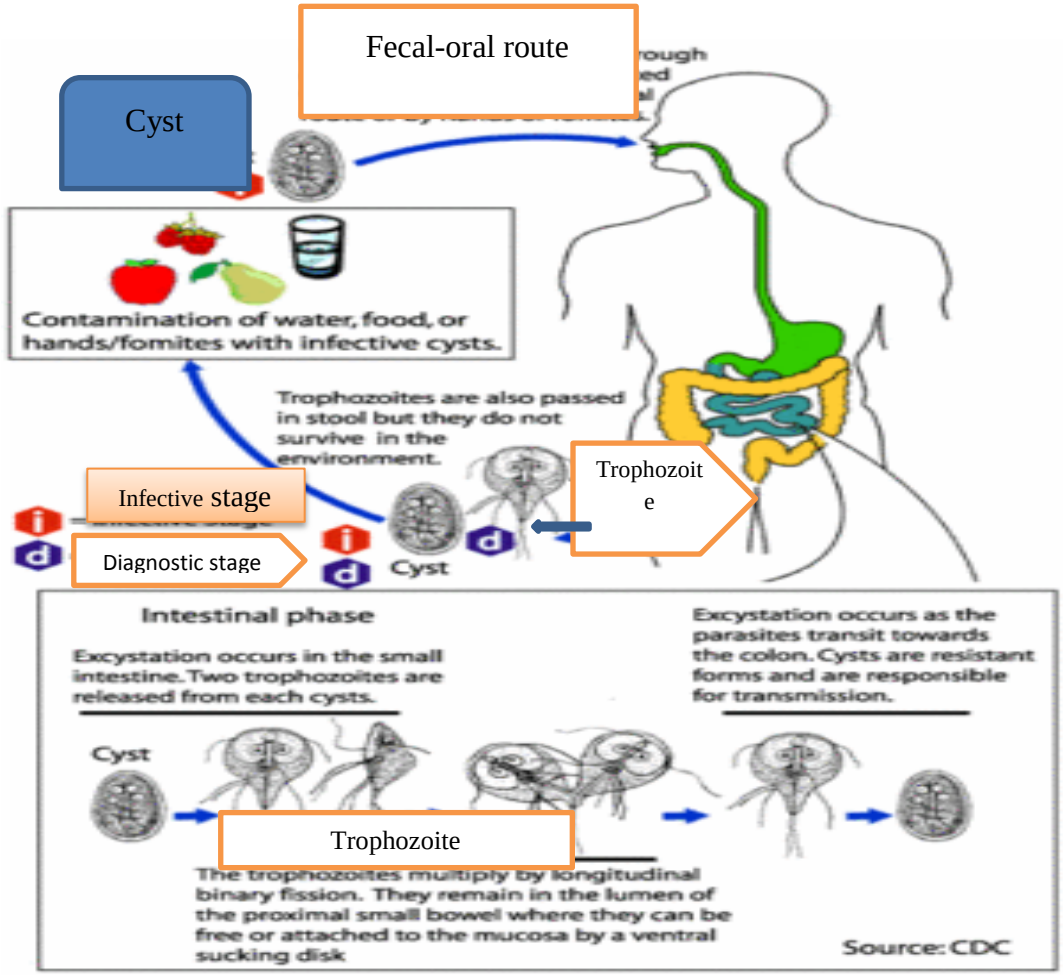

Figure 2. Life cycle of *Giardia lamblia* (Source: http://www.dpd.cdc.gov/dpdx)

Cryptosporidium parvum

The life cycle of *Cryptosporidium parvum* begin after ingestion of the infective stage, the oocyst, by a susceptible host. 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 faeces. Each oocyst has four infective sporozoites that come out from the oocyst using the suture at one side of the oocyst. 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 oocysts are initiated by the body temperature, interaction with stomach acid and bile salt. The released sporozoites attach to epithelial cell and become enclosed with in 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). Type I meronts form eight merozoites that are released from the parasitophorous vacuole when they mature (Huang *et al*., 2006). 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 four 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 (Ramirez *et al*., 2004).

2.4. Life Cycle of Intestinal Helminth Parasites

The life cycles of most helminths follow the same pattern.

Adult hookworms of the genera Necator and Ancylostoma parasitize the upper part of the human small intestine, whereas *Ascaris lumbricoides* parasitize the entire small intestine. Tape-worms inhabit the intestinal tracts of vertebrates, and the larvae inhabit the tissues of vertebrates and invertebrates (Robert and Tolan, 2009). *N. americanus* and *A. duodenale* hookworm eggs hatch in soil. The larvae molt twice to become infective third-stage larvae, which are non-feeding but motile organisms that seek out higher ground to improve the chance of contact with human skin. After skin penetration, they enter subcutaneous venules and lymphatic vessels to access the host's afferent circulation. Ultimately, the larvae become trapped in pulmonary capillaries, enter the lungs, pass over the epiglottis and migrate into the gastrointestinal tract. About 5–9 weeks are needed from skin penetration until development of egg-laying adults (Hotez *et al*., 2004). *A. duodenale* larvae are also orally infective and lactogenic transmission during breast feeding has been postulated. This feature is crucial for understanding of the epidemiology and clinical features of intestinal helminth infections, as well as the approaches to their control (Hotez. *et al.,* 2004).

Ascaris lumbricoides infections in humans occur when an ingested infective egg releases a larval worm that penetrates the wall of the duodenum and enters the blood stream. The parasites can live for several years in the human gastrointestinal tract. Human beings are regarded as the only major definitive host for these parasites, although in some cases *A.lumbricoides* infections can also be acquired from pigs (Crompton, 2011). From here, it is carried to the liver and heart, and enters pulmonary circulation to break free in the alveoli, where it grows and molts. In three weeks, the larvae pass from the respiratory system to be coughed up, swallowed and thus returned to the small intestine, where they mature to adult male and female worms. Fertilization can now occur and the female produces as many as 200,000 eggs per day for a year (Stephenson *et al.,* 2011)

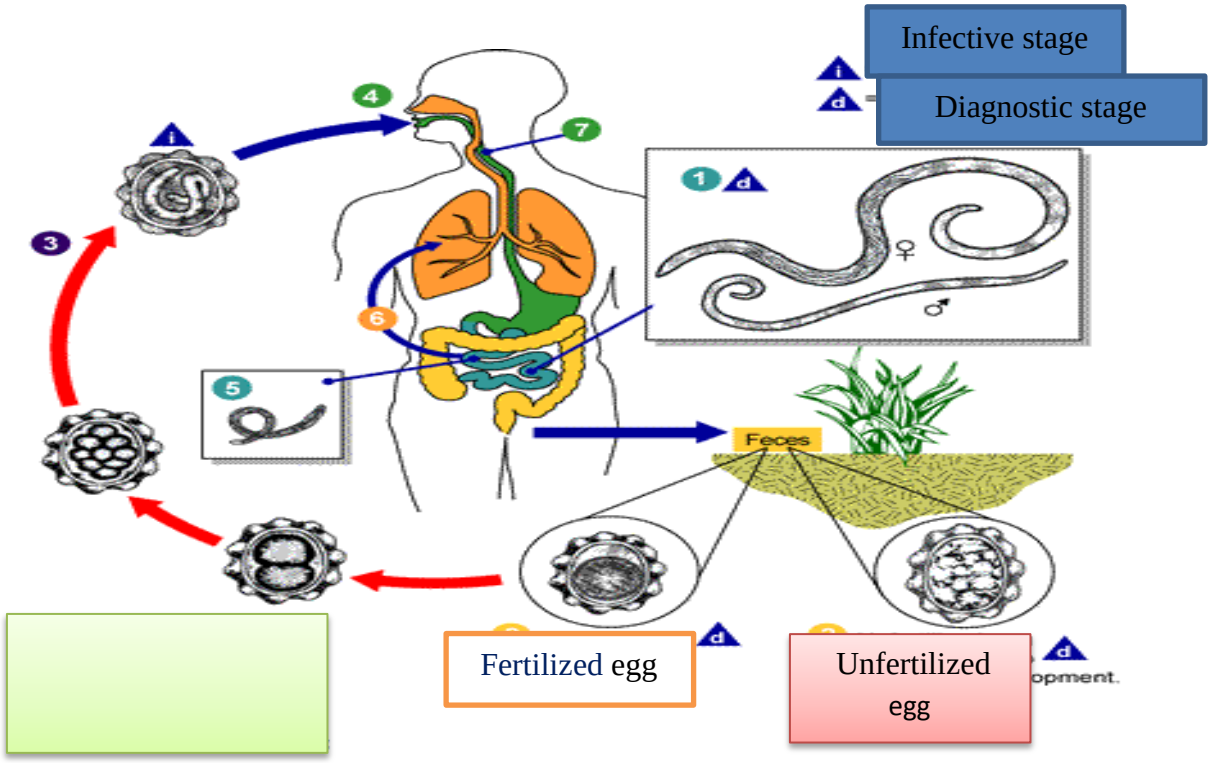

Figure 3. The life cycles of *Ascaris lumbricoides (*CDC, 2012*).*

Infection with *Trichuris trichiura* occurs via the oral-fecal route by the ingestion of infective eggs from contaminated food, hand or water. These then pass through the stomach to the small intestine where they hatch. The larvae penetrate the cell of the small intestine coming to lie above the lumina to undergo four molts. The immature adults emerge and are passively transported to the large intestine where they mature and embed their thin whip-like anterior into columnar cell. Taenia species parasites live in the intestine of human definitive host where it produces eggs that are excreted in the feces. Then the eggs are ingested by intermediate host, cattle where the eggs hatches into a larval form called cysticercus that lodges in the animal's muscles. When human consumes raw or undercooked beef containing cysticerci, the human develops taeniasis (Tortora *et al*., 2009). The adult species lives in small intestine of man and fully developed and reproductively mature as early as 10-12 weeks after infection of the host. Once mature, the species regularly shed its most posterior segments or proglottids which contain thousands of infective eggs. On average, a single Taenia parasite releases six to nine proglottids daily (Pawlowski and Murrell, 2007). One infected person can pass 100,000 eggs per segment each day in his or her feces as individual eggs or in intact segments (Kennedy, 2005).

2.5. **Diagnosis of Intestinal Parasitic Infections**

Diagnosis of intestinal parasites is confirmed by the recovery of protozoan trophozoites and cysts and helminthes eggs in the clinical parasitology laboratory. Microscopic examination of feces is essential for the recognition and identification of intestinal parasites. Due to the low density of the parasites in the feces, direct microscopy is useful for the observation of motile protozoan trophozoites and the examination of cellular exudates, is not recommended solely for the routine examination of suspected parasitic infections. It is essential to increase the probability of finding the parasites in fecal samples to allow for an accurate diagnosis. Therefore, a concentration method is employed (Lindo, 1998).

Direct wet mount examination should not be entirely excluded as the trophozoites are usually destroyed during the concentration procedure and therefore, microscopic examination of wet mounts should be performed. The concentration procedure requires the use of ether or ethyl acetate as a lipid removing agent and formalin as a fixative. Oocysts of the intestinal coccidians can be seen in a fecal smear by using modified Ziehl-Neelsen method (Arcari *et al*., 2000).

In protozoan cysts, the number of nuclei and the presence of inclusions are the aid identification of protozoa. In trophozoites, the presence of red cells in amoebae is diagnostic of Entamoeba histolytica and flagella also aid identification of some protozoan trophozoites. In helminthes eggs, the shape of the egg, the thickness of the shell, the color of the ovum and the presence or absence of features such as an operculum, spine or hook lets are diagnostic pointers to the identity of the parasite (Arcari *et al*., 2000).

Trophozoites and cysts of the intestinal amoebae, flagellates and ciliates can be found and identified best in permanently stained fecal smears. Trophozoite stages are most often found in watery or diarrheic fecal specimens and usually cysts are not seen in such specimens. On the other hand, cysts are the stage typically found in formed fecal specimens. A mixture of trophozoites and cysts may occur in softer and semi-formed feces. In direct smears of feces in saline, motile trophozoites may be found (WHO, 2004).

Antigen detection tests are now commercially available for the diagnosis of all three major intestinal protozoan parasites to resolve the problem of morphological similarity such as the pathogenic *E. histolytica* and the non-pathogenic *E. dispar*. However, the diagnosis and treatment of intestinal helminthic infections have not been changed much and the traditional microscopic method can be used for their diagnosis (Haque, 2007).

2.6. Pathogenesis and Clinical Manifestations of Intestinal Parasitic Infections

The term "pathogenesis," defined as the mechanisms Involved in the initiation, evolution, and ultimate outcome of a disease process, relates to host and parasite factors, this review focuses mainly on the parasitic mechanisms that may be related to invasive intestinal infections. Protozoan and helminth parasites have a global distribution and an especially high prevalence in countries where poor socioeconomic and sanitary conditions predominate (Stanley, 2003). In developing countries, the infection occurs primarily among travelers to endemic regions, recent immigrants from endemic regions, immuno-compromised persons and institutionalized individuals (Swords and Canytey, 2002).

Amoebiasis is the third common parasitic infection in about 10% of the world's total population causing death after Schistosomiasis (Ramasubrahamanian, 2006). 90% of *E. histolytica/dispar* infections, the symptoms are absent or very mild (Reed, 2000). Although people can be asymptomatically colonized with *E. histolytica/dispar*, they should be treated. Otherwise, the cyst carriers may be dangerous environmentally or may develop amoebic colitis (dysentery) after a period of months. It was thought that signs and symptoms of invasive amebiasis develop in approximately 10% of the infected population. Symptoms commonly attributed to *E. histolytica* colitis or dysentery is abdominal pain or tenderness and diarrhea; watery, bloody, or mucous (Reed, 2000).

trichuris parasitic worm's slender forelimb insert intestinal submucosa, intake of nutrition from tissue and blood, together with the secretions stimulate intestinal mucosa showed mild symptoms of inflammation or bleeding, can see that epithelial cells degeneration and necrosis(Tortora *et al*., 2009).

Hook worm, which is an allergic reaction at the site of parasitic penetration and entry, is common in patients infected with N. americanus. Additionally, cough and pneumonitis may result as the larvae begin to break into the alveoli and travel up the trachea. Then once the larvae reach the small intestine of the host and begin to mature, the infected individual suffer from diarrhea and other gastrointestinal discomfort. Ascaris eggs swallowed, hatch and release larvae in the intestine. Then, each larva migrates through the wall of the small intestine and is carried through the lymphatic vessels and bloodstream to the lungs. In the lungs, the larva passes into the air sacs. It then moves up to the respiratory tract and into the throat and is swallowed. Finally, the larva matures in the small intestine, where it remains as an adult worm. They might be huge enough to form a bolus and obstruct the intestine lumen or they might migrate to other places of the body due to some stimulation (Markell *et al*., 2006).

2.7. Epidemiology of Intestinal Parasitic Infections

2.7.1. Global Epidemiology of Human Intestinal Parasitic Infections

Today, intestinal parasites are among the major contributors to the global disease load. Populations in different parts of the world face diverse parasitic challenges. For example, *Enterobius vermicularis* is more prevalent in temperate areas (Vermund, 2000). The highest rates of *Ascaris lumbricoides* infection have been reported in China, Southeast Asia, coastal regions of West Africa, and Central Africa. *Trichuris* infestation is at its highest rate in Central Africa, southern India, and Southeast Asia. Hookworm infections are most common in sub-Saharan Africa, South China, and South-east Asia (Brooker *et al*., 2004).

Taenia infections are estimated to affect 100 million people worldwide, with major endemic areas located primarily in the developing countries of South America, Africa, India, China and South-east Asia (Carpio, 2002). Taenia infections are less common in North America; however neurocysticercosis has been recognized as an important health problem in California (Carpio, 2002), Although this disease is mainly seen in migrant workers from Latin American, it has also been reported in united state residents who have not traveled to endemic countries (Hoberg, 2006).

Hymenolepis nana is widely distributed in countries with warm climates including those of Africa, South America, Mediterranean Region, and South East Asia (Willms and Sotelo, 2001).

The intestinal trematodes are estimated to account for almost 1.3 million of the 40-50 million food-borne trematodes infections worldwide, particularly within endemic foci in Southeast Asia and the Western Pacific region where they are significant causes of pediatric malnutrition. *Schistosoma mansoni* is known to occur in 52 countries, including sub-Saharan Africa (where around 85% of the global burden is concentrated), North African and Eastern Mediterranean countries, South American countries (Brazil, Venezuela, Surinam) as well as several Caribbean countries (Saint Lucia, Montserrat, Martnique, Guadeloupe, Dominican Republic and Puerto Rico) (Carod *et al*.,2004).

2.7.2. Epidemiology of Human Intestinal Parasitic Infections in Ethiopia

Intestinal parasitic infection has cosmopolitan in distribution. There has been wide distribution of intestinal parasites in Ethiopia (Mengistu *et al*., 2007). Similar to other developing countries, wide distribution of intestinal parasites in Ethiopia could be due to low level of environmental sanitation, personal hygiene, food and water contamination with human excreta and un aware of simple health promotion practices such as personal hygiene, food hygiene, the effect of altitude, urbanization, irrigation, and resettlement with in the country (Endeshaw *et al*., 2004). The most important intestinal parasites predominantly distributed in the county include *Ascaries lumbricoides*, *Giardia lamblia*, hookworm, *Hymenolypsis nana*, and *Entamoeba histolytica/dispar* with varying prevalence in different areas. Heliminthic infection (both geohelminthiasis and schistosomiasis) are common in the country and are the second most predominant cause of outpatient morbidity in the country (Erko and Medhin, 2003; Mengistu *et al*. 2007).

It was shown that the prevalence of intestinal parasitic infection in Ethiopia is different in different parts of the country. For example, Jemaneh (2000) reported that the distribution of the three common helminths; *A. lumbricoides*, *T. trichiura* and the hookworm in school children in several communities of three altitudinal regions in Ethiopia have been shown to be

different. That is, the prevalence of *A. lumbricoides* infection was 29% in the highlands, 35% in the temperate areas and 38% in the lowlands.

The prevalence of hookworm infection was highest in the lowlands (24%) followed by the temperate (15%) and highland (7%) areas, while *T. trichiura* infection exhibited similar prevalence in all altitudinal regions (13% on the average). In addition, Legesse and Erko (2004) reported that 83.8%, of 259 surveyed students, had one or more intestinal parasites which include hookworm (60.2%), *S. mansoni* (21.2%), *T. trichuria* (14.7%), *Taenia* species (13.9%), *E. histolytica/dispar* (12.7%), *A. lumbricoides* (6.2%), *G. duodenalis* (6.2%) and *S. stercoralis* (5.8%) from rural area close to the southeast of Lake Langano, Ethiopia. Moreover, Mengistu *et al*. (2007) showed that *T. trichiura, A. lumbricoides, E. histolytica/dispar, G. lamblia, S. stercoralis, H. nana, Schistosoma mansoni, T. saginata, E. vermicularis* and hookworm with prevalence of 60.9%, 40.9%, 17.1% 13.9%, 17.5%, 2.1% 5.0%, 2.3%, 14.8% and 1.1% respectively were diagnosed from study groups in Jimma, south-western Ethiopia.

2.8. Factors Affecting the Epidemiology and Transmissions of Intestinal Parasites

2.8.1. Behavior, household clustering and occupation

Evidence for household clustering of infected individuals exists for most diseases caused by infections with helminth and protozoan, including *ascariasis, trichuriasis, strongyloidiasis giardiasis, and cryptosporidiosis.* This clustering can persist through time, as shown by familial predisposition to heavy infection with Ascaris lumbricoides and Trichuris trichiura in Mexico. Household aggregation of lymphatic filarial infection has been described in India and Polynesia. In one study of schistosomiasis, shared household accounted for 22% of the variance in *S. mansoni* egg counts (Bethony *et al*., 2001).

2.8.2. Poverty, sanitation and urbanization

Parasitic infections depend for transmission on environments contaminated with egg-carrying feces. Consequently, intestinal parasites are intimately associated with poverty, poor sanitation, and lack of clean water. The provision of safe water and improved sanitation are essential for the control of parasitic infections. Most of the populations in urban and rural areas do not have access to safe and adequate water supplies and sanitation facilities. Regarding food, water and personal hygiene, only few households show sufficient understanding of environmental sanitation or hygienic practices. As a result, three–fourths of the health problems in Ethiopia are due to communicable diseases attributable to unsafe, inadequate water supply and unhygienic/unsanitary waste management. The populations in developing countries live in conditions that are highly conducive to the acquisition of parasitic infestation. Poor hygiene, crowded household conditions, dietary habits, education level of the community and deficient Sanitation mark their day-to-day life (Culha *et al*., 2007).

2.8.3. Climate, water and season

Water, Climate and topography are crucial determinants of the distribution of helminth and protozoan infections (Brooker, 2007). Helminths transmitted by vectors are limited to landscapes in which host and vector come together in the same habitat, resulting highly focal distribution. The distribution of schistosomiasis reflects the biotic and abiotic features (i.e., climatic, physical, and chemical factors) that affect the survival and development of the snail vector. Soil-transmitted helminthes are highly affected by surface temperature (Brooker, 2003).

Rainfall and surface runoff have been a concern for different water-borne intestinal protozoan infection outbreaks. In this outbreak a period of heavy rainfall and runoff followed by a high turbidity load affected the potency of local drinking water treatment plant. Surface water may be contaminated by rain and wind that carrying cysts of various parasites from fields containing faeces of infected humans, livestock, or wild animals to nearby rivers, streams and other water source. As a result it increases the spread of parasitic infections (Baker, 2002).

2.8.4. Age dependency

The high prevalence rate of intestinal protozoan infection in children is attributed to many factors, particularly environmental and personal hygiene. For reasons not well understood, school aged children (including adolescents) and pre-school children tend to harbor the greatest number of intestinal worm (Bethony, 2002).

Much epidemiologic research has focused on heterogeneity in the intensity of helminth infection by age. Changes with age in the average intensity of infection tend to be convex, rising in childhood and declining in adulthood. For *Ascaris lumbricoides* and *Trichuris trichiura*, the heaviest and most frequent infections are in children aged 5–15 years, with a decline in intensity and frequency in adulthood. Similarly, for all the major schistosomes, the heaviest and most frequent infections are in older children aged 10–15 years (Tadesse, 2005). In contrast, hookworm frequently exhibits a steady rise in intensity of infection with age, peaking in adulthood (Bethony, 2002).

2.9. Prevention and Control of Intestinal Parasitic Infection

The prevention of intestinal parasitic infection depends upon the erection of barriers to the spread of parasites through the practical application of biologic and epidemiological knowledge. Almost every parasite at some time in its life cycle is susceptible to several special exterminate measures. Thus, for human barriers such as sanitary excreta deposal may be established to break the link in the life cycle (WHO, 2004). The infections can be controlled and prevented by improvement in environmental sanitation such as safe methods of faeces and waste disposal and provision of safe water supplies and health education on health promotion of personal and food hygiene (Trainer *et al*., 2003). Such measures are usually slow to take effect, require considerable investment and need to be accompanied by social, economic and educational development. In recent years, the availability of single-dose broad-spectrum anthelminthics has helped in reducing the worm burden in endemic communities. Studies have shown that periodic chemotherapy strategy has successfully lowered the intensity of protozoan and helminth infections (Che, 2010).

3. MATERIALS AND METHODS

3.1. Description of Study Area

This study was conducted in chagni town found in Guangua woreda, Awi zone of Amhara Region, North-west Ethiopia (Figure 4). It is the administrative center of Guangua woreda (Asnake, 2009). According to the CSA (2007) report the total population living in Chagni town was 30,938, of whom 16,035(51.8 %) and 14,903(48.2 %) were men and women, respectively. According to growth rates Ethiopian population in 2013 i.e. (2.58%) total population of Chagni town were estimated to be 37,675, of whom 18,757(49.9%) and 17,433(46.3%) were men and women, respectively. Town has a latitude and longitude of 10°57′N and 36°30′E, respectively and the altitude ranges from 1400-2200 meter above sea level; and rain fall ranging from 1300-1800mm/year, temperature of minimum 22c^{o} and maximum of 37c°. Chagni town is bordered on the west by Benishangul-Gumuz Region, Dangela in the east, Zigem in the south, Jawi in the North and 505 kilo meter far away from Addis Ababa. The town has one hospital and one clinic. There is one governmental and two private primary schools in the town. The study was carried out in the primary schools children of chagni town. Agriculture is the source of income in the area, where the farming system is small scale production of mixed crops and livestock, and trade is the little Source of income (Chagni town Agricultural Station, 2016).

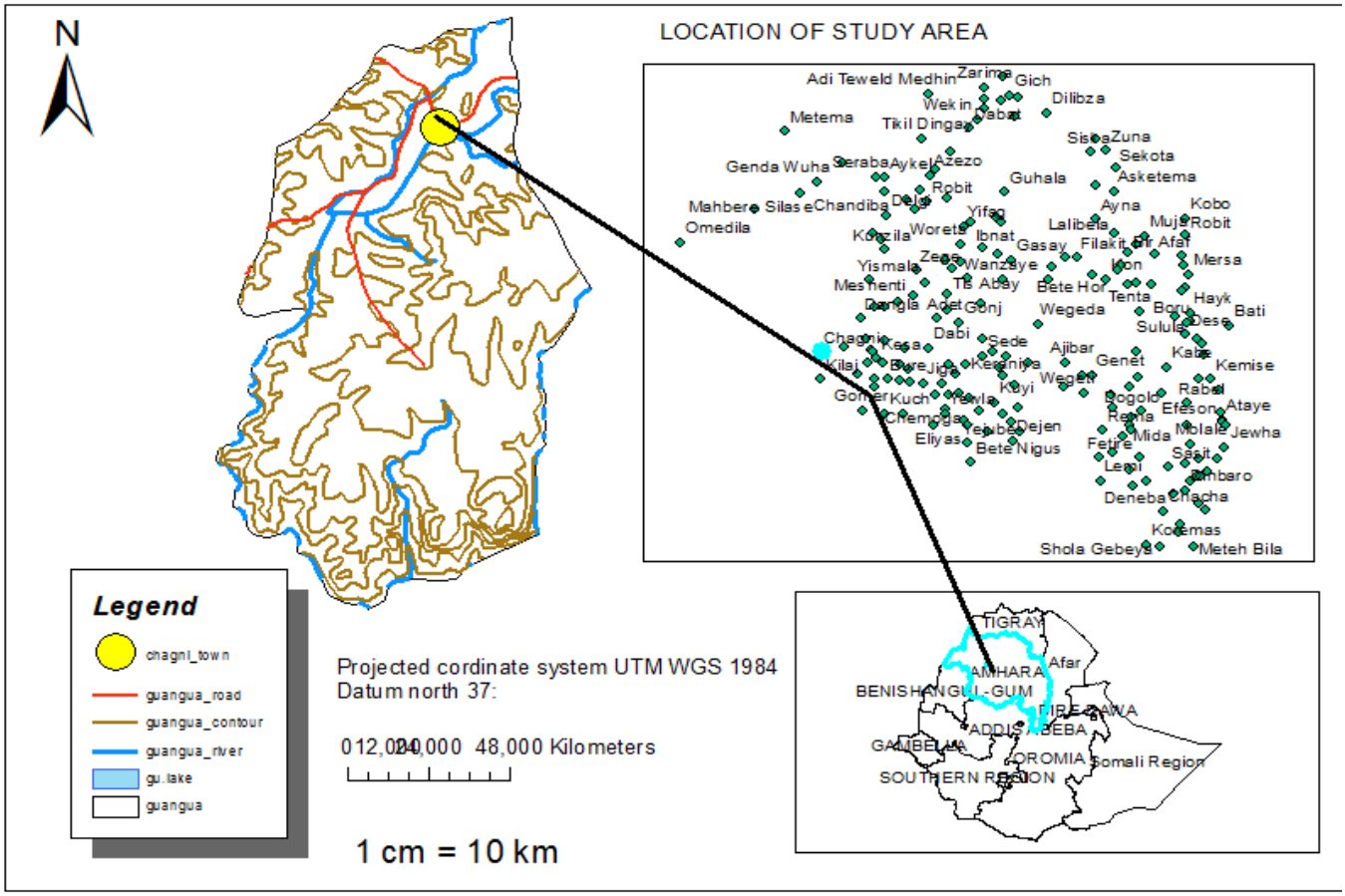

Figure 4. Map of study area

3.2. Study Design

The study design was a cross-sectional parasitological survey of intestinal protozoan and helminth parasites, while Prevalence and intensity of helminth and prevalence of intestinal protozoan infections of school children in the study area as well as their associations with anthropometric indices, additionally, major risk factors of school children were determined. It was conducted from September-December, 2016.

3.3. Study Population and Sampling Technique

The total student population of grade 1-8 children enrolled during the 2016/17 academic year in Chagni Primary Schools of town was 4110. Of these, 2144 were females and 1966 were males (Table 1).

To select the study participants, the students were first stratified according to their grade levels (1 to 8). Proportionate sample size was allocated for each grade and each section. Finally, the sample population was selected using random sampling method. To minimize an error from the likelihood of non-compliance and non-responsive of the study participants, 5% of the sample size was added on normal sample. Therefore, four hundred three (403) students were chosen to participate in the present study. However, 10 participants were excluded because of incomplete data and as a result total of 393 Childern were enrolled in the present study.

Table 1. Number of students by grade level and sex enrolled in Chagni primary schools during 2016/17 Academic year.

Grade	**Total population**		**Sample population**		
Level	Male number	Female number	Male number	Female number	Both sex
1	400	450	38	43	81
2	355	380	33	36	69
3	233	303	22	29	51
4	100	125	10	15	25
5	311	290	29	27	56
6	238	281	23	26	49
7	161	173	16	16	32
8	168	142	17	13	30
Total	1966	2144	188	205	393

3.4. Exclusion criteria

According to the information obtained from the students themselves, those that had been treated for any intestinal parasitic infections with in the past 3 months at the time of survey were not included in the study. The stool sample contaminated with the soil and urine were rejected.

3.5. Sample Size Determination

The sample size was determined based on the 95% confidence limits and 5% sampling error, calculated using the following formula for single population proportion (Naing *et al*., 2006).

$n = (Z\alpha/2)^2 \, p \, (1-p)/d^2$, $n = (1.96)2 \, 0.5 \, (1-0.5)/ \, (0.05)^2$, then

$n = 384$

Where: n = sample size

P = is the proportion of positive individuals.

d = marginal error b/n the samples and population (0.05).

$Z\alpha/2$ = critical value at 95% certainty (1.96), considering 5% sampling error.

Since the overall prevalence rates (p) of intestinal parasites were not known for the present study area, p was taken as 50%. For the calculation, 95% confidence level (z) and 5% sampling error (d) were used. Therefore, three hundred eighty four (384) school children from grade 1 to 8 were selected to participate in the present study; stratified random sampling technique was employed to select students from each grade and section, using class rosters as the sample frame.

3.6. Questionnaire Survey

Data related to socio-demographic characteristics (variables) of the study subjects such as risk factors (drinking water source, personal hygiene and life skill practice, residence of children, swimming habit, washing hand after toilet, eating raw meat and uncooked vegetable, basic knowledge on parasitic infections, availability of safe latrine in the close vicinity of their home) that predispose school children to parasitic infections were gathered using pre-tested and structured questionnaire prepared both in English and Amharic.

3.7. Anthropometric Measurements

Body weight and height was measured according to the standardized procedures mentioned in Gibson (2005) and body mass index (BMI) was calculated using the formula: BMI = weight in kg/ height in meter square. Weight was taken without shoes and minimum clothing using scale and was recorded to the nearest 0.1 Kg. The height was measured to the nearest 0.1 cm using a measuring tape. All the data were transformed and expressed in Z-scores and calculated using anthropometry calculating software program, AnthroPlus (WHO, 2007).

Under- nutrition was defined for a child who had less than -2 Z-scores (-2SD) from the National Center for Health Statistics (NCHS) median reference population values. Z-scores of less than -3SD was used to define severe under- nutrition. This was used as cut-off point to determine malnutrition.

Since wasting for those children with age above 9 years cannot be evaluated through WHO AnthroPlus, body mass index(BMI) (weight/height in meter square) was calculated and a BMI -for - age value less than 5^{th} percentile of reference data was considered as thinness or underweight (WHO, 2007). WHO anthropometric classification was used for the assessment of malnutrition. Based on the age, body weight and height, a number of indices such as weight for-age, height-for-age and BMI-for-age. Data were excluded if a child's height-for-age z- scores (HAZ) was below -6 or above +6, weight- for- age Z- score (WAZ) was below -6 or above +5, weight- for- height Z- score (WHZ) was below -5 or above +5, because these extreme values were most likely a result of errors in measurement or data entry (WHO,2006). Underweight is defined as low weight-for-age and it reflects past (chronic) and present (acute) under nutrition. Children with z-scores < -2.00 are said to be underweight. Stunting is defined as a low height-for-age for children, and it measures the past (chronic) child under nutrition. Children with z-scores < -2.00 are said to be stunted. Thinning is defined as low BMI-for-age for children, and it is a measure of current or acute under nutrition. Children with z-scores < -2.00 are said to be thinned (WHO, 2006).

$$\text{BMI}=\frac{Weight}{Height\ in\ m\text{eter square}}$$

Applying the classification of the World Health Organization (2000) the BMI scores can be categorized into four main groups:

Underweight group (< 20.0 kg/m^2); Normal (≥ 20.0 and < 25 kg/m^2); Overweight (≥ 25 and < 30 kg/m^2); Obese (≥30 kg/ m^2).

3.8. Stool Sample Collection

During stool collection, disposable plastic cups and spoon were distributed for each study subject of the selected children. They were also advised to bring their own 3 g of stool sample without contaminating with the soil and urine.

The unique codes of the students were labeled on the container. The stool samples were carried to laboratory room of chagni Hospital on the same day of collection for parasitological examination and enumeration.

3.9. Parasitological Examination Procedures

Stool sample collected from each study participant was examined using the following techniques.

3.9.1. Direct wet mount technique

Wet mounting was the simplest and easiest technique for the examination of feces. It assesses the overall prevalence of intestinal parasitic infections in the study area. About half gram of stool samples was emulsified with drop of normal saline, and then emulsified sample placed on a clean microscopic glass slide. The presence of intestinal Parasites ova and cyst was observed under the microscope (Lindo *et al*., 1998). The direct wet mount processed using iodine to identify the presence of motile intestinal parasites, cysts and egg under light microscope at 10X and 40 x magnifications. Direct smear was used only for watery stool to detect some intestinal parasites using 0.85% saline solution and the 10x and 40x objectives under microscopes (Enk, 2008; Bogoch *et al.,* 2006).

3.9.2. Formol ether Concentration

A portion of each stool samples were used for detection of parasitic ova and protozoan cysts using the formol-ether sedimentation or concentration technique. One gram (1 g) of each stool sample was first emulsified with drop of 3-4 ml of 10% formol saline. An additional 3-4 ml of 10% formol saline was added; mixed thoroughly and passed through gauze. Three to four (3-4) ml drop of diethyl ether was added and mixed, inverting and with intermittent shaking for 1 minute, and centrifuged at 3,000 rpm (rotation per minute) for 5 minutes. After centrifugation, the supernatant (layers of ether, debris, and formol saline) was discarded and the sediment (containing the parasites at the bottom of the test tube) was re-suspended in formol saline. The sediment was examined microscopically under 10X and 40X magnifications, for the presence of any parasitic organisms.

3.9.3. Kato-Katz method

Kato-Katz technique was used to assess the intensity of helminth parasite infections in the study area. This technique was applied to determine the parasitic load of helminth infection. Briefly, portion of the fecal specimen was taken by clean wooden/plastic spatula and forced through the nylon screen to separate fecal materials from the large debris.

The screened fecal material was transferred to the template which was laid flat centrally on microscope slide.

The template hole was completely filled with screened fecal material and leveled to the surface of the template. Cellophane square which was soaked in malachite green-glycerin solution was placed over the specimen. The specimen was made to spread evenly under the cellophane tape by pressing it with a glass slide (prepared for this purpose) (Bogoch *et al.*, 2006). The prepared Kato-Katz slides were examined under the microscope for helminth ova by principal investigator with assistance of experienced laboratory technician at Chagni Hospital. The number of eggs of each species was recorded and converted in to the number of eggs per gram of stool (EPG) in order to analyze intensity of infection. The eggs per gram of stool (EPG) was obtained by multiplying the number of eggs by 24 (Kato slides delivering 41.7 mg of stool plug).

3.9.4. Modified Ziehl Nielsen Staining Technique

A thin smear of sediment from the concentration technique was prepared, air-dried and fixed in methanol for 2-3 minutes. The slides were stained with cold carbol fuchsine for 30 minutes. The slides were washed with tap water and decolorized with 1% hydrochloric acid-ethanol solution (acid - alcohol) for 2 minutes. The slides were rinsed in distilled water and then counterstain with 1 % methylene-blue for 2 minutes. These then rinsed in tap water, air-dry, and examine microscopically under a 100x objective oil-immersion lens for Cryptosporidium Oocyst (Arcari *et al*., 2000).

3.10. Data Analysis

Data was analyzed with Statistical Package for Social Science (SPSS), Windows version 20. Descriptive statistics was applied to indicate the prevalence of intestinal parasitic infections and nutritional status as percentages and proportions.

Descriptive statistics such as frequency, percentage, and range were determined for each intestinal parasite. In order to analyze intensity of infection for intestinal parasites, the number of eggs were converted into the number of Eggs per Gram of stool and transformed to log scales for analysis of geometric mean. To test the null hypothesis, inferential statistical analyses of comparisons between two categorical variables were carried out using Pearson chi-square (x^2) test to verify the relationship between independent factors and the outcome variables. Logistic regression analysis (Odds ratios, OR) was used to determine the association of risk factors with disease. The 95% CI was used to show the accuracy of data analysis. Probabilities less than 5% ($P < 0.05$) for null hypothesis testing were considered statistically significant. Anthropometry indices were computed using the calculator mode of anthropometry calculating software program AnthroPlus (WHO, 2007). Wasting, stunting and underweight were defined as Z-score values of less than –2 SD (Standard Deviation), which was below expect on the basis of the international growth reference scale (WHO, 2007).

3.11. Quality control

To ensure quality all the laboratory procedures including collection and handling of Specimens were carried out in accordance with standard protocols. To ensure general safety, disposable gloves were wore and universal bio-safety Precautions (NCCLS, 2002) also followed at all times. The calibration factors for the 10x and 40x objectives was posted on the microscope for easy access; and the weight scales was checked at the beginning of each working day.

*3.*12. Ethical Clearance

The consent or permission was obtained from chagni Hospital (Appendix VIII). Prior to stool collection, the objectives of the study and procedure of sample collection was explained to school principals, teachers, students and other concerned authorities (Appendix V). Laboratory parasitological examination was done by the researcher with the assistance of experienced laboratory technicians.

4. RESULTS AND DISCUSION

4.1. Major Intestinal Protozoan Parasitic Species Identified from Examined Pupils

As the result shown in Table 2, three species of intestinal protozoan parasites were identified in examined school children. The predominant protozoan parasites species identified in the study were *Entameoba histolytica/dispar* (25.9%), *Giardia lamblia* (19.3%) and Cryptosporidium species (2.3%). This finding was in agreement with the report made by Ngonjo *et al*. (2012) in Thika District, Kenya, of which prevalence of amoebiasis and giardiasis was 14.6% and 6.9%, respectively. Also higher than report made in South-west Ethiopia by Amare *et al*. (2007), 3.1% and 3.6%, in amoebiasis and giardiasis, respectively. This finding was in agreement with report made by Endeshaw (2005). The observed differences in the rate of infection could be due to variations in geography and types of soil, socio-economic conditions, hygienic practices of the students, the methods employed for stool examination and the time of study, and sample size used (Albonico *et al*., 1999).

The higher prevalence of *E.histolytica* infection in current study might be attributed to the fact that most children in the study area were exposed to low level of environmental sanitation, lack of awareness to parasitic infection and water contamination with human excreta and lack of awareness in simple health promotion practices such as personal hygiene and food hygiene. This finding was almost similar with report made by William *et al*. (2014) in Gahana school children showed that the identification of intestinal protozoan parasitic infections was 42.9 %.

Table 2. Major intestinal protozoan parasitic species identified from examined stools samples of school children of Chagni primary school by age and sex from September-December, 2016

Age group(years) and sex	No. Examined	protozoan parasites		
		Eh/Ed	Gl	Cs
		No. pos. (%)	No.pos. (%)	No.pos. (%)
6-9				
Male	81	37(45.7)	20(24.7)	3(4)
Female	87	40(45.9)	22(25.3)	4(6)
10-15				
Male	78	7(8.9)	14(17.9)	0
Female	86	10(11.6)	20(23.3)	0
16-18				
Male	29	3(10.3)	0	2(7)
Female	32	5(15.6)	0	0
All age Groups				
Male	188	47(25)	34(18.1)	5(3)
Female	205	55(26.8)	42(20.5)	4(2)
Total	393	102(25.9)	76(19.3)	9(2.3)

Eh/Ed=Entameoba histolytica/dispar,Gl=Giardia lamblia and Cs=Cryptosporidium species

4.2. Prevalence of Intestinal Protozoan Parasitic Infections among pupils

The distribution of intestinal protozoan parasite infections by different age groups and sex shown in Tables 3, a total of 393 school children were involved in the present study. Among these students, 188 were males and 205 were females. The overall prevalence of protozoan infections among all age groups of the students in the selected school was 47.6%. Of these, the prevalence of intestinal protozoan parasitic infections for males and females was 46.3% and 48.8%, respectively, (Table 3).

The prevalence of intestinal protozoan parasite infection for the age group 6- 9 was 74.1% and 75.9% in males and females, respectively, (Table 3). While, for the age group 10-15 years old was 26.9% for males and 34.9% for females. The prevalence of protozoan parasitic infections for the age group of 16-18 year old was 20.7% and 12.5% for males and females; respectively, (Table 3).

This finding was lower than the report made by Eleni *et al. (*2014*),* in Wukro town, North Ethiopia. There was not statistically significant difference ($\chi 2_{=}0.033$, p-value$=0.061_{)}$ in prevalence of protozoan parasitic infections among male and female children with in the age group of 6-9 years old, also with in the rest age groups the prevalence of protozoan parasitic infections was not statistically significant (x^2=0.87,p-value=0.53)and(x^2=2.13,p-value =0.085) respectively. The prevalence of intestinal protozoan parasite infections was higher in age group 6-9 (75%) compared to age group 10- 15 (31.1%) and the age group of 16-18 (16.4%) (Table 3).

These findings were in agreement with that of report made by Eleni *et al. (*2014), Tadesse and Beyene (2009) from Tigray. The observed higher prevalence of intestinal protozoan infections in the age group 6-9 (75%) was higher than in other age groups may suggest the fact that the age group is higher risk in terms of acquiring parasitic infections. The reason could be lack of awareness about the need of washing hands and maintaining other personal hygiene and life skills in this age group. Higher prevalence of parasitic infections among school children may also be due to the poor sanitary conditions of the schools. This agrees with the study by Vivas *et al*. (2011) where students with a higher level of adequate knowledge on how to wash hands had a lower risk of intestinal parasitic infections.

Children usually do not take care of their personal hygiene. For instance, they play in contaminated outdoor environments, in and around waste disposal sites which can certainly cause serious health problems. They also face difficulties in proper use of latrine and lack of basic life skills, such as washing hands after toilet use and before and after meals. These findings agree with a similar study in Ethiopia by Abdulkader *et al*. (2015), those children who washed hands with soap and water was less likely to contact intestinal parasitic infections.

In current study, females had higher prevalence rate in age group 6-9 and 10-15 years old, which was 75.9% and 34.9%, respectively (Table 3). However, the difference was not statistically significant (χ^2=0.58 P=0.201**).** This may be due to the fact that females were more often involved in house hold activities than males. Preparing contaminated food is one of the most common modes of transmission (WHO, 1997). This gender associated infection difference was agreement with observation made by Odikamnoro and Ikeh (2004).

Table 3 Prevalence of intestinal protozoan parasitic infections by age and sex among school children of Chagni primary School, 2016.

Age group (in years)	Male		Female		Both Sexes		χ^2	P-value
	No. Exam	No.Pos (%)	No. Exam.	No.Pos (%)	No. Exam.	No.Pos (%)		
6-9	81	60(74.1)	87	66(75.9)	168	126(75)	0.033	0.061
10-15	78	21(26.9)	86	30(34.9)	164	51(31.1)	0.87	0.53
16-18	29	6(20.7)	32	4(12.5)	61	10(16.4)	2.13	0.085
Total	188	87(46.3)	205	100(48.8)	393	187(47.6)	0.58	0.201
X^2	-	2.36		4.823		4.143		
P-value	-	0.260		0.090		0.080		

No pos.= Number of students positive for intestinal parasitic infections. No. Exam.= Number of examined children.

The distribution of intestinal helminths parasitic infections by different age groups and sex presented table 4, infections with various intestinal helminths were common in the school children. The total of 393 children examined, about 20.6% (81/393) children were found positive for intestinal helminth parasitic infections (Table 4). This finding was higher than report made by Dinesh *et al*. (2016), in primary school children Kiwangwa ward, Bagamoyo district, Tanzania, but prevalence of helminth parasitic infections was 3.8 %. In the current study, the prevalence of helminth infections among younger children (6-9 years old) (29.2%) was higher than the other age groups, and statistically significant difference was observed between male and female children with in this age group (p=0.001) (Table 4). This indicated that younger children were more exposed to infections, which could be happen due to their higher exposure to contamination of the environment, especially the soil where the children usually play and ate foods without washing their hands. This could also be explained by the fact that, in this age group their immunity to parasitic infections has not been fully developed. This finding was similar to the Ethiopia (Angolela) study where pupils with young age had the highest levels of intestinal parasitic infections (Gelaw *et al*., 2013). In this study male children (25.5%) were infected more than the female (16.1%) (Table 4).

The observed higher prevalence of helminth infection among male children in present study might be due to the special male activities such as swimming in stream, fishing, playing football, bathing with river water contaminated of snail host, walking with bare foot, playing with soil as well as molding of houses using moist soil, which predisposes them to parasitic infections. This could have accounted for the high prevalence of intestinal helminth infections in males (25.5%) than in females (19.2%) (Table 4). The possible reason for this difference could be explained by the fact that males are more involved in outdoor activities than females. The overall prevalence of intestinal helminth parasite infection among male children in the present study (25.5%) was lower to the results of the studies conducted in school-aged children in Babile town, 27.2 % by Girum (2005), around Lake Zway, 43.7% by Gezahegn (2008), and study reported from south Gonder, 49%, by Leykun (2000). These differences in prevalence might be a reflection of the difference in local sanitary standard, environmental conditions, time and seasonal differences in the design of the survey work and personal hygiene (Albonico *et al*. 1999).

Regarding gender, this study has shown that, males were at higher risk for helminth infections than that of female children. However, the difference was statistically significant ($p<0.05$) (Table 4). Of these, the prevalence of helminths infections for the age group 6-9, 10-15 and 16-18 years old was 29.2%, 15.9%, 9.8%, respectively, (Table 4). The findings among different studies can be explained by variations in geography (land escaping), socio-economic conditions, category of the study population, the methods employed for stool examination, and the time of study may also have contributed to the differences (Albonico *et al*., 1999).

Generally, in the present study, the prevalence of helminth infections among the age group in the 16-18 years old (9.8%) was lower than 10-15 years old (15.9%), statistically significant difference was not observed in age group of 16-18 years old ($p=0.44$),(Table 4).

Table 4. Prevalence of intestinal helminth parasitic infections by age and sex among school children of Chagni primary school, 2016.

Age group (in years)	Male		Female		Both Sexes		χ^2	P-value
	No. Exam	No.Pos (%)	No. Exam	No.Pos (%)	No. Exam	No.Pos (%)		
6-9	81	29(35.8)	87	20(22.9)	168	49(29.2)	31.74	0.001*
10-15	78	15(19.2)	86	11(12.8)	164	26(15.9)	6.295	0.012*
16-18	29	4(13.8)	32	2(6.3)	61	6(9.8)	4.046	0.44
Total	188	48(25.5)	205	33(16.1)	393	81(20.6)	4.958	0.026*
X^2	-	6.86		9.25		7.49		
p-value	-	0.03*		0.041*		0.04*		

Key:
No.pos= Number of students positive for intestinal parasitic infections. No. Exam= Number of examined children, $\chi 2$= chi-square

* - Shows significant difference between intestinal helminth parasitic infections and age, sex

4.3. Major Intestinal Helminth Parasite Species Identified From Examined School Children

As the result shown in Table 5, five species of intestinal helminth parasites were identified in examined school children. The predominant helminth parasites species identified in the study were *Schistosoma mansoni* (8.7%), *Ascaris lumbricoides* (5.3%), *Hymenolepsis nana* (2.5%), hookworm (2.3%) and Taneia species *(1.8%)* were the predominant parasites in the school children (Table 5).

The Prevalence of *H. nana* infection in the present study was lower than the study conducted in Asia, 25.8% (Matthys *et al*., 2011). It was the most frequent parasite compared to the report from the study conducted in Babile town, eastern Ethiopia (Girum, 2005). This finding was contradicted by Abate *et al*. (2013) in Ethiopia. The medium prevalence rate of *H. nana* infection observed in this study indicates that hygienic practices of the school children in the study area were poor compared by Grang and Zumla (2003).

The overall prevalence of *S. mansoni* infection was 8.7% (Table 5), revealing that schistosomiasis public health problem among the school-age children in the study area, comparing with other helimnth species, because most of students swimming in stream that was contaminated with snail host species. This finding was lower than report made by Samuel *et al*. (2016), school-aged children in Ilemela district, north-western Tanzania. Similar studies have reported with higher prevalence of *S. mansoni* infection among boys than girls (Mazigo *et al*., 2010), most likely attributed to varied social-gender related reasons.

In the present study, *A. lumbricoides* was the second most prevalent parasite species next to *S. mansoni* (Table 5). Its prevalence (5.3%) was higher than that reported from in Babile town (3.9%) by Tadesse (2005). Conversely, the result of the present study was lower than the prevalence reported in north-west Ethiopia, Chilga district, (42.9%) by Leykun (2001) and different parts of Ethiopia, (37%) by Gezahegn (2008). This variation in the prevalence of *A. lumbricoides* could be due to differences in diverse environmental conditions such as surface temperature, altitude, soil type and rainfall, this factors affect prevalence rate of ascariasis. The finding was similar with report made by Brooker *et al.* (2003) and Gezahegn (2008).

The observed differences could also be explained by the fact that the prevalence and distribution of helminth parasitic infections varies by place and with age in study area as reported by Yeshambel *et al*. (2010). Age specific prevalence of *A. lumbricoides* and *H. nana* infection in the present study showed similar pattern of infection with the highest prevalence seen among children in the age group between 6-9 years and then a further decline in older age groups (Table 5). This was in agreement with previous studies (Girum, 2005; Erko *et al*., 2003), who reported higher prevalence of parasitic infections among younger children. This phenomenon probably reflects age related change in exposure to intestinal parasitic infections. A similar study in Brazil was also reported high prevalence of *A. lumbricoides* in the 6–9 years age group; this could be because of high level of soil contact activity and low personal hygiene in the youngest age group.

Table 5. Major intestinal helminth parasites species identified by age and sex from school children of Chagni primary school, September-December, 2016.

Age groups (years) and Sex	No. Examined	Helminth Parasite Species				
		Al	Sm	Hn	Tspp	Hw
		No. pos.(%)	No. pos. (%)	No. pos. (%)	No. pos. (%)	No. pos. (%)
6 -9						
Male	81	10(12.3)	5(6.2)	4(4.9)	4(4.9)	1(1.2)
Female	87	3(3.4)	5(5.7)	4(4.6)	2(2.3)	0
10 – 15						
Male	78	6(7.7)	13(16.7)	0	1(1.3)	4(5.3)
Female	86	2(2.3)	8(9.3)	2(2.3)	0	1(1.2)
16 – 18						
Male	29	0	2(6.9)	0	0	3(10.3)
Female	32	0	1(3.1)	0	0	0
All age groups						
Male	188	16(8.5)	20(10.6)	4(2.1)	5(2.7)	8(4.3)
Female	205	5(2.4)	14(6.8)	6(2.9)	2(0.1)	1(0.5)
Total	393	21(5.3)	34(8.7)	10(2.5)	7(1.8)	9(2.3)

Al=*Ascar*is *lumbricoides*, Sm=*Schistosoma mansoni*, Hn=*Hymenolepis nana*,Hw=Hookworm and T.spp= *Taenia species*, pos.=positive**,**

As result shown in table 6. 26(6.6%) of the school children had multiple intestinal parasitic infections of any two or more helminth and protozoan parasite species. The most frequent combinations of intestinal parasites diagnosed were multiple infections with *Giardia lamblia* and *Entamoeba histolytica/dispar*,*Entamoeba histolytica/dispar* and *Schis itosoma mansoni*, *Entameoba histolytica* /dispar and hookworm, *Entameoba Histolytica/ds par* and *Hymanolypsis nana*, *Giardia lamblia* and *Ascaris lumbricoides* ,*Giardia lamblia* n d *Schistosoma mansoni*, Hookworm, *E.histolytica* and *G.lamblia* , *G.lamblia,E.histolytica and A.lumbricoides* 1.5%, 1%, 0.5%, 1.3%, 0.5%, 0.3%, 0.3% and 0.5%, Respectively. Therefore, in this study multiple infections were relatively common (6.6 %) as compared to similar study that reported 7.5% of multiple infections from Wukro town by Eleni *et al*. (2014) and 13.3% multiple infections reported from south Gonder by Leykun (2000).

Multiple infections detected in the present study were a greater to that reported by Tadesse (2005) (3.0%), the study done on school children in Babile town, eastern Ethiopia. These differences in multiple parasitic infections in the different studies may be due to variation in sampling, nature of population and method employed for stool processing (Mengistu *et al*., 2007).

Table 6. Frequency and Percent of multiple infections of Helminths and Protozoan in the study area

multiple infection	Frequency	Percent (%)
G.lamblia and E.histolytica	6	1.5
E.histolytica/dispar and S.mansoni	4	1
E.histolytica/dispar and hookworm	2	0.5
E.histolytica/dispar and H.nana	5	1.3
G.lamblia and A.lumbricoides	2	0.5
G.lamblia and S.mansoni	1	0.3
Hook worm, *E.histolytica/dispar and G.lamblia*	1	0.3
G.lamblia, E.histolytica/dispar and A.lumbricoides	2	0.5
Total	26	6.6

4.4. Intensity of Major Helminth Parasitic Infections among School Children

Categorization of intensity of intestinal helminth infections with *Ascaries lumbricoides*, *Schistosoma mansoni* and hookworm was done according to WHO threshold (Montresor *et al*., 2002). Accordingly in this study the intensity of infection for helminth species has been determined and categorized as light, moderate and heavy. For *A. lumbricoides* infection light, include from 1- 4999 epg, moderate from 5000 - 49,999 epg and heavy infection ≥ 50,000 epg. The intensity of *S.mansoni* infection classified in to three levels; light infection (1-99 epg), moderate infection (100-399 epg) and heavy infection (> 400 epg),for hookworm infections to be light include from 1-1999 epg, moderate from 2000 – 3999 epg, and heavy infection ≥ 4000 epg (WHO, 1993).

In the present study of helminth infection were with in the range of light and heavy infections. Egg count for *A. lumbricoides*, *S. mansoni* and hookworm ranged from 0- 2880, 0 -792, and 0 -720 per gram of stool, respectively. Mean egg count for *A. lumbricoides*, *S. mansoni* and hookworm infections were 744±346.5, 252±64.4, and 270±70.4, respectively. This finding was contradictory with Mbuh *et al*. (2012), parasite intensity was highest in hookworm (938 epg), followed by *Ascaris lumbricoides* (721 epg). This was may be due to environmental condition such as season, soil type, temperature and rain fall content differ one area to other area.

Changes with age in the average intensity of infection tend to be convex, rising in childhood infection with hookworm species frequently exhibits a steady rise in intensity of infection with age, peaking in adulthood (Bethony *et al*., 2002). Similarly, in the present study, the highest mean egg counts, 744±346.5 was recorded in the youngest age group (6-9 years old) for *A.lumbricoides* (Table 7). This trend was also observed in other studies (Brooker *et al*., 2004). This could be because of high level of soil contact activity and low personal hygiene in the youngest age group. In the present study, less hookworm infection was observed in age group 6-9 years old, however, higher, intensity of hookworm infection was observed in age group 16-18 years old with mean egg count of 1200±108 for male and 400±169.3 for female, compared with the age group 6-9 and 10-15 years old (Table 7).

In Brooker *et al*. (2004), who had reported that age dependent intensity profile for hookworm species infection considerably increased with age until adulthood and formed plateaus.

In this study, the intensity of each helminth parasitic infections as measured by egg per gram of faeces was generally high and low. This was comparable with report from Abosa around Lake Zway, south Ethiopia (Gezahegn, 2008). The low intensity level of helminth infection in the present study might be due to low humidity, unfavorable soil formation, and chance difference in exposure to infection. In the present study the egg count for *Ascaris lumbricoides, Shistosoma mansoni* and hookworm in male and female ranged from (48-336 and 768-2880), (24-792 and 24-720), (72-720 and 24-720), respectively (Table 7). The mean egg count for *A. lumbricoides, S.mansoni* and hookworm infections in male was 172.8±46.4, 242.7±76.4, and 252±81.1, respectively and female was 1696±622.9, 231±108.8 and 306 ± 152.2, respectively. The highest mean egg count of *A. lumbricoides*, *1696* ± 622.9 was observed in female whereas the highest mean egg count 242 ±76.4 for *S. mansoni* was observed in male children. Intensity of *S. mansoni* infection in relation to sex showed that higher heavy infections were in males than females. This is similar to finding from previous studies by Tadesse *et al*. (2009) from Waja.

Frequency of water contact was identified as risk factor for *S.mansoni* infection in which children with frequent water contact were more infected than those who have less frequent water contact. This agrees with other studies (Matthys *et al*., 2007; Enk *et al*., 2010). This could be due to children who have frequent water contact activities may have high rate of vulnerability to *S.mansoni* infection.

Table 7. Mean±SEM and range of egg counts of intestinal helminth parasites per gram of faeces by age of examined children in Chagni primary school from September-December, 2016

Age and Sex	No of examined	Helminth parasite							
		A.lumbricoides		S.mansoni		H.worm		Total egg Load	
		Mean±SEM	Range	Mean±SEM	Range	Mean±SEM	Range	Mean±SEM	Range
6-9								137.1±44	24-336
Male	81	184±83.5	48-336	104±68.4	24-240	96± -	96-96	2880±-	0-2880
Female	87	2880± -	2880-2880	-	0	-	0		
10-15									
Male	78	-	0	298.7±116	24-792	204±93.2	72-480	296±82.4	0-792
Female	86	1104±336	768-1440	464±220.9	24-720	24±-	24-24	604±217.1	24-1440
16-18								148.4±39.7	24-720
Male	29	156±12	144-168	100.9±47	24-288	1200±108	144-720	253±86	0-384
Female	32	-	0	144±48.9	24-264	400±169.3	144-384		
All Age								202.8±37.7	24-792
Male	168	172.8±46.4	(48-336)	242±76.4	(24-792)	252±81.1	(72- 720)	306±152	24-720
Female	205	1696±622.9	(768-2880)	231±108.8	(24-720)	306±152.2	(24-720)		
Total	393	744±346.5	(0-2880)	252±64.4	(0-792)	270±70.4	(0-720)	309.7±76.9	0-2880

Key:

A.lumbricoides=Ascaris lumbricoides, S.mansoni=Schistosoma mansoni, SEM=Standard error of mean

4.5. Associated Risk Factors for Intestinal Parasitic Infections among School Children

The results of the questionnaire survey for risk factors and their associations with the intestinal parasitic infections in the school children were shown in Table 8, the majority of the study subjects were living in urban areas 276(70.2%) as compared to rural areas 117 (29.8%). However residence area in this study was significantly associated with intestinal parasitic infections among school children ($\chi 2$=6.488, p=0.011, OR (95%CI) 0.252(0.088-0.728). In the majority 227(57.8%) of participants their family size was greater than five persons per house hold while, 166 (42.2%) had a family size of less than five persons per house hold. There was no statistically significant association between family size with intestinal parasitic infections ($\chi 2$=6.065, p=0.418, OR (95%CI) 0.836 (0.54-1.291).)

Regarding source of drinking water 224 (57%) of them replied that they had been using protected water for drinking purpose. Whereas the other 169(43%) replied that they had unprotected water sources for drinking purpose (Table 8). Out of the children that obtain water from unprotected source, 137(81.1%) were positive for intestinal parasitic infections. significant association was found between infection rates of intestinal parasites and the use of unprotected water (table 8), ($\chi 2$= 4.324,P= 0.038,OR (95% CI)0.393(0.163-0.195).

Regarding the status of personal hygiene, 213(54.2%) were poor personal hygiene, but 180(45.8%) of them were good personal hygiene, 152 (71.4%) and 21 (11.7%) were positive for intestinal parasitic infections, respectively (Table 8).

Children with poor awareness for intestinal parasitic infections, with good awareness intestinal parasitic infections and with respect to positive for intestinal parasitic infections were 140(63.1%) and 33(19.3%), respectively. It was major risk factor and significantly association was found between infection rates of intestinal parasites and lack of awareness for intestinal parasitic infections (P=0.001) because, p<0.05), OR (95% CI) 5.625(2.352-13.46)).

Regarding habit of swimming, 358(91.1%) were swimming in stream, but 35 (8.9%) of them were not swimming in stream, of course with respect to intestinal parasitic infections 160 (44.7%), and 13 (37%) of the students had positive intestinal parasitic infections, respectively. significant association was not found between infection rates of intestinal parasites and swimming habit, p=0.062 and x^2=3.47(Table 8).

Regarding the status of shoes wearing habit, 368(93.6%) were wearing shoes, but 25(6.4%) of the study participants did not have the habit of wearing shoes, with respect to intestinal parasitic infections 53 (14.4%), and 20 (11.6%) of the students had positive intestinal parasitic infections, respectively. significant association was not found between infection rates of intestinal parasites and shoes wearing habit, p=0.151 and x^2=2.066 (Table 8).

Regarding habit of consuming of raw meat and uncooked vegetable, 339(86.3%) of the study participants replied that they had the habit of consuming raw meat and uncooked vegetable, but 54(13.7%) did not have such habit ,with respect to intestinal parasitic infections 159 (46.9%), and 54 (25.9%) of the students who had positive intestinal parasitic infections, respectively. significant association was not found between infection rates of intestinal parasites and eating raw meat and uncooked vegetable, p=0.884 and x^2=9.743, (Table 8).This finding was similar to a study by Liza *et al*. (2014). High prevalence of intestinal parasitic infections was found among children who had no safe latrine facility in home vicinity 340 (86.5%) compared to those who had safe latrine facility in their home 53(13.5%) (Table 8). More over statistically significant associations (p=0.044) were observed between intestinal parasitic infections and availability of safe latrine facilities (OR (0.149), 95% CI (0.023-0.95)) Table 8.This finding was similar with previous report by Narain (2000). The test for possible association between risk factors like swimming habit , shoes wearing habit, family size, level of life skill, Habit of eating raw meat and un cooked vegetable and intestinal parasitic infections did not show statistically significant associations (p>0.05). but, significant associations was found between intestinal parasitic infections and parents occupation, parents education level, availability of safe latrine, awareness of parasitic infection, personal hygiene, residence and habit of washing hand after toilet.

Table 8. Major socio-demographic factors associated with intestinal parasitic infections among children of Chagni primary school from September-December, 2016.

Risk factors	**Frequency** (%)	**Intestinal parasite**		**OR(95.0% Cl)**	**X^2**	**P-value**
		No positive (%)	No Negative (%)			
Family size						
≤5	166(42.2)	46(27.7)	120(72.3)	0.836(0.54-1.291)	6.065	0.418
≥5	227(57.8)	127(55.9)	100(44.1)			
Parent occupation						
Governmental worker	85(21.6)	16(18.8)	69(81.2)		0 .656	0.014*
Farming	96(24.4)	77(80.2)	19(19.7)	2.574(1.23-6.167		
Trade	139(35.4)	50(36)	89(64)			
worker	73 (18.6)	30(41.1)	43(58.9)			
PEL						
Illitrate	49 (12.5)	37 (75.5)	12(5.5)			
Read and write	230 (58.5)	27 (11.7)	203(88.3)	0.433(0.223-0.841)	6.107	0.013*
Primary education	54 (11.5)	18 (33.3)	36(66.7)			
Diploma and above	69(17.6)	8 (11.5)	61(88.4)			
ASL						
Absent	340(86.5)	123(36.2)	217(63.8)	0.149(0.023-0.95)	4.060	0.044*
Present	53(13.5)	20(37.7)	33(62.3)			
A P I						
Poor	222(56.5)	140(63.1)	82(36.9)	0.625(2.352-3.46)	15.065	0.001*
Good	171(43.5)	33(19.3)	138(80)			
Personal hygiene						
Poor	213(54.2)	152(71.4)	61(26.8)	1.157(3.397-4.29)	19.156	0.001*
Good	180(45.8)	21(11.7)	159(88.3)			
water handling						
Protected	224(57.0)	36(16.1)	188(85.5)	0.393(0.163-095)	4.324	0.038*
Unprotected	169(43)	137(81.1)	32(18.9)			

Continued……..

Level of life skill Poor Good	162(41.2) 231(58.8)	133(76.9) 40(23.1)	29(13.2) 191(86.8)	2.021.(0.831 - 4.92)	2.405	0.121
Shoes wearing habit Yes No	368(93.6) 25(6.4)	53(14.4) 20(11.6)	315(85.6) 5(2.3)	0.255(0.039-1.644)	2.066	0.151
Residence Urban Rural	276(70.2) 117(29.8)	87(31.5) 86(73.5)	189(68.5) 31(26.5)	0.252(0.088-0.728)	6.488	0.011*
Swimming habit Yes No	358(91.1) 35(8.9)	160(44.7) 13(37)	198(55.3) 22(62.9)	3.722(0.934-4.832)	3.470	0.062
HMV Yes No	339(86.3) 54(13.7)	159(46.9) 14(25.9)	180(53.1) 40(74.1)	0.029(1.951-3.63)	9.743	0.884
Hwt Yes No	379(96.4) 14(3.6)	164(43.3) 9(64.3)	215(56.7) 5(35.7)	0.873(0.141-5.40)	0.021	0.002*

Key;

Hwt=habit of washing hand after toilet, **HMV**=habit of eating raw meat and uncooked vegetable

API=awareness to parasitic infection, **ASL**=availability of safe latrine and **PEL**=parents education level.

***-** shows significant association between risk factors and intestinal parasitic infection

4.6. Effect of Intestinal Parasitic Infections on Physical Growth of Children

As recommended by WHO (2007), the anthropometric measurements of children in the survey were compared with an international reference population defined by the U.S. National Centre for Health Statistics (NCHS) and Centers for Disease Control and Prevention (CDC). Each of the three nutritional status indicators described below were expressed in standard deviation units (Z-scores) from the median of the reference population .Each of these indicators, height-for-age, weight-for-height, and weight-for-age provides different information about growth and body composition, which was used to assess nutritional status.

The height-for-age index is an indicator of linear growth retardation and cumulative growth deficits. Children whose height-for-age Z-score is below minus two standard deviations (-2 SD) from the median of the reference population are considered short for their age (stunted) and are chronically malnourished. Children who are below minus three standard deviations (-3 SD) from the median of the reference population are considered severely stunted. Stunting reflects failure to receive adequate nutrition over a long period of time and is also affected by recurrent and chronic illness. Height-for-age, therefore, represents the long-term effects of malnutrition in a population and does not vary according to recent dietary intake (CSA, 2005).

As shown in Table 9, 38.7%, 30.9% and 36.9% the study children aged 6-9 years old showed underweight, wasting and stunting, respectively. Of these, 37% and 36.7% of male and female stunting, 54.3% were males and 24.1% were females for Underweight, 37% were males and 25.3% were females for wasting. This finding was similar with report made by Mekonnen (2013) that the overall prevalence of stunting, underweight and thinness were (30.7%), (59.7%) and (37.2%), respectively, among Rural Primary School Children of Fogera district, North-west Ethiopia. Also, higher than study done on school-children of Babile town by Girum (2005) showed that Wasting (WHZ) was the predominant manifestation of malnutrition (11.6%) followed by stunting (HAZ) (5.4%) and underweight (WAZ) (5.2%). This finding was almost similar report made by Eleni *et al.* (2014), of which 44.3%, 28.6% and 27% were underweight, wasting and stunting respectively.

This finding was also similar with the prevalence of stunted and underweight individuals found to be higher among boys than girls in these age groups (6-9 years), but prevalence of wasting was less in girls than in boys. However, the observed differences was not statistically significant for the anthropometry indices (wasting, stunting and underweight, (X^2= 0.157, p=0.185), (X^2= 0.058, p=0.810) and(X^2= 0.170, p=0.68), respectively, Table 9). Generally, in the present study, the prevalence rate of malnutrition computed from the anthropometric indices of the study participants were not very critical compared to the national figure, here the rate was still high and requires immediate intervention of concerned body. The current finding was lower than the study conducted by Singh *et al*. (2014), showed that 41.00% children were underweight, 23.88% children were stunted and 36.18% were thinned.

Table 9. Prevalence of stunting, underweight and wasting status among male and female children aged 6-9 years in Chagni primary school, September-December, 2016.

Sex	Number Examined	Nutritional indicators		
		WAZ (Underweight) No. (%)	HAZ (Stunting) No. (%)	WHZ (Wasting) No.(%)
Female	87(%)	21(24.1)	32(36.7)	22(25.3)
Male	81(%)	44(54.3)	30(37%)	30(37)
Total	168(%)	65(38.7)	62(36.9)	52(30.9)
X^2	-	0.170	0.058	1.757
p-value	-	0.680	0.810	0.185

Key:

WAZ= z-score of weight for age, HAZ=z-score of height for age, WHZ=z-score of weight for height.

In older children, i.e. above 10 years, weight-for-age is not a good indicator as it cannot distinguish between height and body mass in an age period, where many children are experiencing the pubertal growth spurt and may appear as having excess weight (by weight-for-age) when in fact they are just tall. BMI-for-age is the recommended indicator for assessing thinness, overweight and obesity in children 10-19 years (WHO, 2009) .Therefore, in the current study, the prevalence of BMI-for-age which was an indication of being underweight for 10-18 years of age was 28.9% (65/225). Of which, 41.1 %(44/107) was for males and 17.8 % (21/118) was for females (Table 10).

In addition, BMI-for-age of normal weight was calculated for analyzing the status of normal growth for 10-18 years of age 71.1% (160/225), of which, 58.9% (63/107) was males and 82.2 % (97/118) was females. The Prevalence of underweight was found to be 28.9% which was lower than prevalence of a study done in Wukro town by Eleni *et al.* (2014). However, there was no any risk for overweight among the study school children (Table 10). But higher than the prevalence reported for Malaysian school children by Zulkifli *et al*. (2000), which was 25.7% of underweight and lower than the prevalence of report made by Gezahegn (2008) ,from Debub Achefer district, northwest Ethiopia, which was 30.7%. These variations may be probably due to differences in nutrition and types of staple food the communities used and poor life style of the population.

Table 10 Body mass index of underweight and/or thinness in the age group 10-18 years old by sex among school children from September-December, 2016.

Sex	Number Examined	Underweight	Healthy weight (Normal)
		Frequency (%)	Frequency (%)
Male	107	44(41.1%)	63(58.9%)
Female	118	21(17.8%)	97(82.2%)
Total	225	65(28.9%)	160(71.1%)

- WHO (2009), growth reference of BMI- for- age

-BMI-for-age under 5^{th} percentiles in age of 10-18 years = Underweight/thinness

-BMI-for-age 5^{th} – 85^{th} percentiles in age of 10-18 years = Healthy weight

Generally, in the present study, the prevalence of wasting (30.9 %) and underweight (38.7%) among age group 6-9 years were found to be higher than those of both the regional and the national rates, where wasting was 6.5% both nationally and regionally, and underweight was 29.7% regionally, but 20.8% nationally (FMOH, 2005). However, the prevalence of stunting (36.9 %) in the present study was found to be lower in comparison with the Ethiopian Demographic and Health survey report which was 51.3% (CSO, 2005).

4.7. Association of Intestinal Parasitic Infections with Anthropometric indices of School Children

This study has also analyzed relationship between anthropometric indices of the school children and the prevalence of intestinal parasitic infections. The overall prevalence of each intestinal parasite species diagnosed via school childern employed in the study and the proportion of different anthropometric measurements was presented in (Table 11).

The prevalence of intestinal parasitic infections was lower in underweighted (38.7%) students than Wasted (30.9%) and stunted (36.9%) students (Table 11). A significant association was found between intestinal parasitic infections and underweighted and wasted students ($p=0.007$, $x^2=7.261$ and $x^2=6.492$, $p=0.011$), odd ratio (OR) at 95% confidence interval, 2.878(1.309-6.324) and odd ratio (OR) at 95% confidence interval, 2.696(1.234-5.891), respectively, in the age group 6-9 years old. A significant association was not found between intestinal parasitic infections and stunted ($p=0.095$, $x^2=2.791$ and odd ratio (OR) at 95% confidence interval, 1.933(0.886-4.22)).

The comparison of the three anthropometric indices showed that underweighted (38.7%) school children had a lower prevalence of parasitic infection than the other indices. Wasted and stunted school-children showed a prevalence of 30.9% and 36.9%, respectively, (Table 11). The prevalence of intestinal parasitic infections had also a relationship with the body mass index for age of the school children with 84% of the infected pupils. A significant association was found between intestinal parasitic infections and body mass index for age ($p=0.035$, $x^2=4.468$ and odd ratio (OR) at 95% confidence interval, 3.022(1.052-8.679), because of $p\leq 0.05$ (Table 11).This could suggest that other factors such as poverty, poor health and sanitary conditions, limited knowledge of nutritional matters among certain households and fluctuations in incomes which affect the nutritional status are may be predominant among the study subjects as reported by Andrade *et al*. (2001) (Table 11).

Table 11. Relationship between intestinal parasitic infections and categorical nutritional indicators among school children aged 6-9 years and 10-18 years in Chagni primary school, 2016.

Nutritional indicators	Number Examined	Intestinal parasitic infections		OR (95% CI)	X^2	P-Value
		Negative (%)	Positive (%)			
Height- for- age						
Not stunted	106(%)	61 (57.5)	45(34.1)	1.933 (0.886-4.22)	2.791	0.095
Stunted	62(%)	19(30.6)	43(69)			
Weight- for- age						
Not underweight	103(%)	62(60.2)	41(39)	2.878(1.309-6.324)	7.261	0.007*
Underweight	65(%)	25(38.5)	40(61)			
Weight-for height						
Not wasted	116(%)	62(53.4%)	54(46)	2.696(1.234-5.891)	6.492	0.011*
Wasted	52(%)	10(19.2%)	42(80)			
BMI -for age						
<5thpercentile	65(%)	10(15.4%)	55(84)	3.022 (1.052-8.679)	4.468	0.04*
>5th percentile	160(%)	115(71.8	25(15)			

Key;

-BMI-Body mass index, *- and nutritional indicators shows significant association between intestinal parasitic Infections

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

The objective of the present study was to determine the prevalence and intensity of Helimnth infections and prevalence of intestinal protozoan infections and their associations with anthropometric measurements among school-children of chagni town. The design of the study was a cross-sectional parasitological survey involving a sample population of 393 school-children from grade one to grade eight in Chagni primary school from the September to December, 2016.

A total of 393 stool samples were collected and examined using Direct wet mount, Kato-Katoz, Modified Ziehl Nielsen staining and Formol ether concentration techniques on the fresh collected faeces. After screening of 393 stool samples, the overall prevalence of intestinal protozoan and helminth infections were 47.6% (46.3% of males and 48.8% of females), 20.6% (25.5% of males and 16.1% of females), respectively. In the current study, the prevalence of most common intestinal parasitic infections detected,such as *Entamoeba histolytica,Giardia lamblia*,*Schistosoma mansoni*,*Ascaris lumbricoides*,*Hymanolypis nana* ,Hook worm, Cryptosporidium species and Taenia species were 25.9%, 19.3%, 8.7%, 5.3%, 2.5%, 2.3%, 2.3% and 1.8%, respectively. 6.6% of School children had multiple intestinal parasitic infections of any two or more helminth and protozoan parasite species. The mean egg count and ranges of *Ascaris lumbricoides*, *Schistosoma mansoni* and Hookworm were 744±346.5 (0-2880), 252±64.4 (0-792) and 270±70.4 (0-720), respectively.

The prevalence of malnutrition in terms of underweight, stunting and wasting was 38.7%, 36.9% and 30.9%, respectively. Anthropometric indices of the study subjects were also measured and their associations with the intestinal parasitic infections were analyzed. However, statistically significant association was found between intestinal parasitic infections and anthropometric indices, although statistically significant association was not found between intestinal parasitic infections and height for age (p= 0.095). The observed problems might be due to other multifactorial problems such as shortage of a balanced diet. Risk factors like habit of personal hygiene and life skill practice, drinking water source, eating raw meat and uncooked vegetable, level of knowledge on mode of transmission of parasitic infection and latrine availability associated with intestinal parasitic infections.

5.2. Conclusions

Major intestinal helminths and protozoan parasites diagnosed in the school-children of Chagni Primary Schools were *Ascaris lumbricoides, Schistosoma mansoni, Hymanolypsis nana,*Taenia species,Cryptosporidium species,*Entameoba histolytica/dispar*, *Giardia lamblia* and hook worm. The findings in the present study showed that intestinal parasitic protozoan infections were the major public health problems in the school-children of Chagni town. *S.mansoni* infections were also major problem for the school children. *A.lumbricoides* and *H. nana* were found as dominant species of intestinal helminth parasites diagnosed in the stool samples of the school-children. The result of this study was showed that high prevalence of malnutrition among school children. Significant association was observed between intestinal parasite infections and malnutrition, However Significant association was not observed between intestinal parasitic infections and height for age.

5.3. Recommendations

Based on the present findings, the following recommendation can be forwarded:

- This study was not identified *Entameoba histolytica and Entameoba dispar*; thus, molecular level should be carried out to get detailed morphology of *Entameoba histolytica/ Entameoba dispar.*
- The school directors specifically assigned for school health should liaise (communicate) with the County Public Health Officer in charge of school health to ensure regular deworming program for school children and delivering health education to increase the knowledge, attitude and practice of school children as to how intestinal protozoan and Helminth infections are transmitted and prevented.
- This study involved only intestinal protozoan and helminth infections in primary school childern. Thus further study involving as many orphans as should be carried out so as to get full information.

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7. APPENDIX

Appendix I

Laboratory Data Collecting Format

Code	Sex	Age	Date/Month /Year	Presence/Absence	Parasites identified in the stool examination						Single infection	Double infections	Multiple infection	Others	Remark
					E. histolytica	G.lamblia	C. parvu	A.lumbricoides	T.trichura	H.nana					

Key: E. histolitica = Entamoeba histolitica, G. lamblia = Giardia lamblia, A. lumbricoides = Ascaris lumbricoides , T. trichura = Trichuris trichura

Appendix II

ID (optional)	Name (optional)	Sex	Date of birth	Date of measurement	Height		Weight	BMI	BMI %ile
					m	centimeter	kg		

BMI for age growth chart (BMI calculator Children and Teens)

Appendix III

Questionnaire to be completed during sample collection

Dear students use (✓) mark for your choice

Student name_____Sex_____Age_____

1. Do you wash your hands always before meal and after latrine use? Yes----.No----
2. Do you wear shoes always? Yes------ No------
3. Do you have safe toilet? Yes------ No-----
4. What is your parent's occupation? Farming------Trading----government worker---daily worker-------
5. What is your parents education level? Ilitrate-----read and write----primary education---- diploma and above--------
6. How do you use drinking water? Protected----, un Protected------
7. What is your personal hygiene and life skill? Good------poor------
8. What is number of your family? >5----- < 5----------
9. Have you ever eaten raw meat and uncooked vegetables Yes------, No----------
10. What about your personal hygiene and life skill practice? Good-----, Poor---------
11. Your level of knowledge on parasitic infection? Good-------- Poor---------
12. Do you swim in stream? Yes---- No-------
13. Do you take any antiparasitic drug for the last 4 months? Yes--------, No----------

Appendix IV

Questionnaire (Amharic Version)

ክፍል 1. ለተሳታፊዎች የሚሰጥ መለያ **መ□□ቅ-1**
በሐሮማያ ዩኒቨርሲቲ ስድሕረ ምረቃ ትምህርት ማሟያ ይህ መጠይቅ በሰነ-ሕይወት ትምህርት ክፍል የተዘጋጀ ጥናት ሲሆን አሳማው በአንጀት ጥገኛ የበሽተ አይነቶች ስርጭትን ዙሪያ መረጃ ለማሰባሰብ ነ□።ትክክለኛ ሐሳብ–ን ለመግለፅ ይህን "√" ምልክት በክፍት ቦታ□ □□ቀሙ

ቀን፡----------ቀበሌ፡----------ወረዳ፡----------የቤት ቁጥር፡----------

ክፍል 2. የቤተሰብ ኃላፊ መረጃ

1. ፆታ፡- ወንድ---- ሴት ------- 2. ዕድሜ፡--------- 3, የስራ ዐይነት ---------4. የትምህርት ደረጀ -----

5. የቤተሰብ ብዛት >5--------- < 5---------

ክፍል3. በሽታ መተላለፊያ መንስኤዎች

1. ከምግብ በፊት ና ሽንት ቤት ከተጠቀሙ በኃላ ሁልጊዜ እጅዎትን ይታጠባሉ? አዎ------- የለም-------

2. ሁል ጊዜ ጫማ ያደርጋሉ? አዎ-------፣የለም-------

3. ሽንት ቤት አልዎት? አዎ-------፣የለም-------

4. መልስዎት አዎ ከሆነ ምን አይነት? የግል-------፣የጋራ--------

5. ለመጠጥ የሚሆን ውሃ ከየት ነው የሚያገኙት? ከወንዝ------፣ከኩሬ--------፣ከምንጭ---- ፣ከቧንቧ--------

6. ለመጠጥ የሚገለገሉበትን ውሃ በምን ዘዴ ነው የሚጠቀሙበት?

በማፍላት------፣በማጥለል----፣በቀጥታበመጠቀም------፣በክሎሪንየታከመውሃ-------

7. ከቤት የሚወጣን ቆሻሻ በምን መልኩ ነው የሚያስወግዱት? ጉድጓድ ውስጥ በመቅበር-

፣በማቃጠል-------፣ሜዳላይ በመጣል-------፣ወንዝ ውስጥ በመጣል-------

8. የከተማ ግብርና ይጠቀማሉ? አዎ-------፣የለም------

9. ጥሬ ስጋ ይመጋባሉ? አዎ-------፣የለም-------

10. ጥሬ አትክልት ይመጋባሉ? አዎ-------፣የለም-------

11. የግል ንጽህና አጠባበቅዎት ና የህይወት ልምድዎት እንዴት ነው? ጥንቁቅ----- ፣ቸልተኛ------

12. በጥገኛ ተዋስያን አማካኝነት ስለ ሚከሰቱ በሽታዎች ግንዛቤ አለዎት? አዎ-------፣የለም---

13. ባለፉት ስድስት ወራት ውስጥ የፀረ-ጥገኛ ተዋሰወያን መድሃኒት ተጠቅመው ያውቃሉ? አዎ-------፣የለም---

14. የሥራ አይነት፡- የቀን ሠራተኛ _ ቋሚ ሰራተኛ _ የቤት ዕመቤት _ ልሳ _
15. የትምህርት ደረጃ ፡- የቀለም ትምህርት ያልወሰደ _ ማንበብና መፃሐፍ የሚችሉ _
1ኛ □ረ□ □ተማረ _ ሁለተኛ □ረ□ □ተማረ _ከሁለተኛ □ረ□ በላ

Appendix V

Written consent form

I have been informed and understand that the purpose of this particular research study is to find out the Prevalence and intensity of intestinal protozoan and Helminth infections and their Association with Anthropometric Measurements of Children, Chagni primary schools. I am requesting your children to participate in this study which would require his /her response to obtain stool sample. You are being to participate in this study. If you agree, I would like to detect the presence of intestinal parasites. When you or your children are found positive for either both of the above parasites, you will receive standard drugs free of charge. There is no any health related risk in participating. I have asked questions relevant to the study and got satisfied answers with clarifications. Information and data in the survey questionnaire will be handled with strictly confidential and used only for the specified study.

I have the right not to give any information, not cooperate and resign from this study and this will not affect my right from diagnosis. So, I understand, agreed and signed this consent form.

Study code no. _____

Name _____Signature of the participant: _____ Date _____

Name_____Signature_____Date_____Investigator

የስምምነት ቅፅ (በአማርኛ)

ኮድ----------

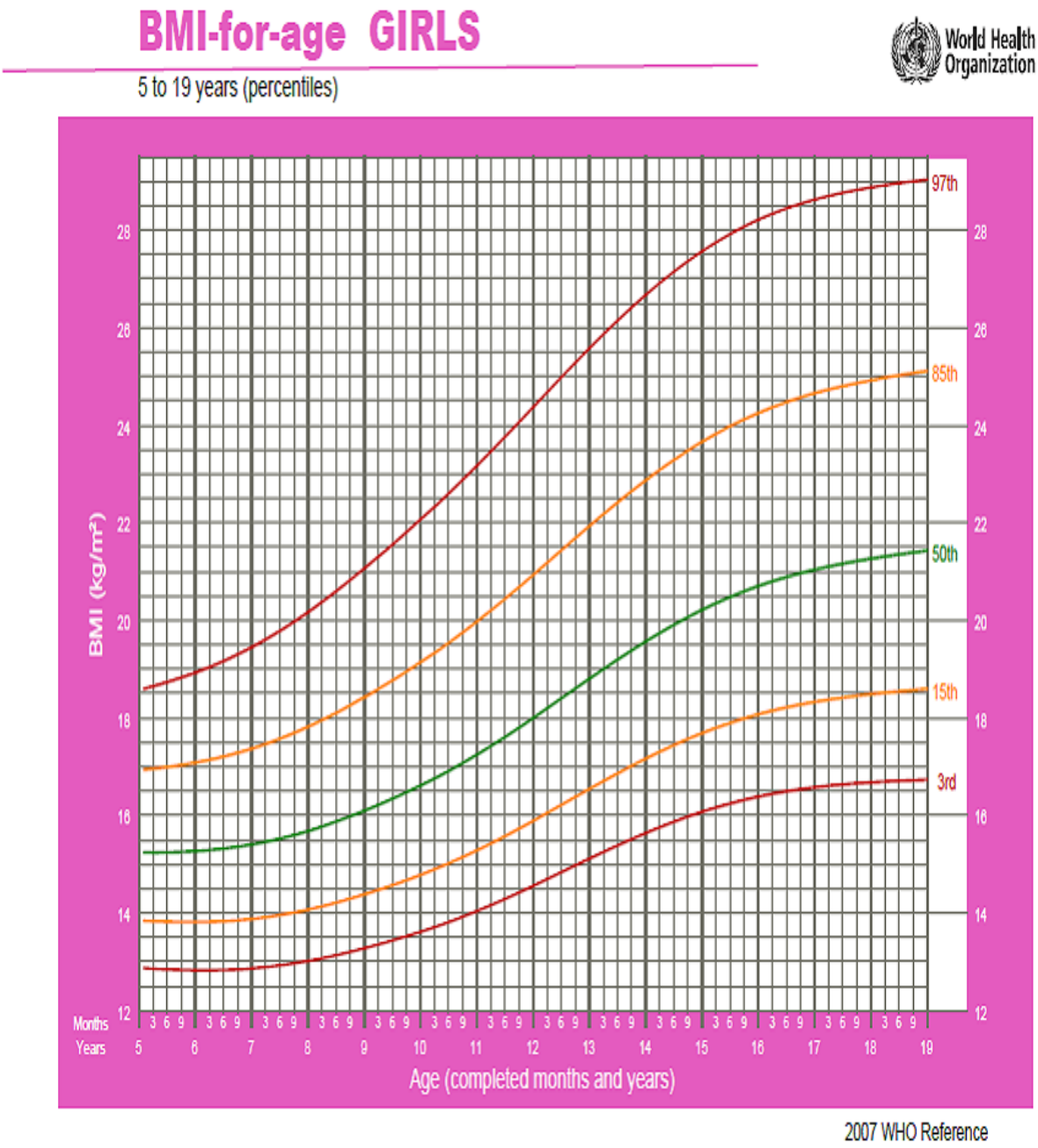
የተሳታፊው ሙሉ ስም----------

እኔ በሀረማያ ዩንቨርስቲይ በስነ-ህይወት የትምህርት ክፍል የሁለተኛ ድግሪ ተማሪ ነኝ። በአሁኑ ሰአት በአንጀት ጥገኛ ህዋስያን ትል ላይ የመስክ ጥናት እያደረግሁ ነው። የዚህ ጥናት አላማ ለአንጀት ጥገኛ ህዋስያን ትል ሊያጋልጡን የሚችሉ ነገሮችን ለመረዳትና ለሚመለከተው ክፍል ለማሳየት ነው። እናም ይህን የአንጀት ህዋስያን ትል ለመከላከል ወይም ለመቀነስ በሚያስችል እቅድ በማውጣት ወደ ስራ ለመተርጎም ነው። በመስኩ ጥናት ላይ ለመተባበር ፍላጎት የሌላችሁ ልጆች ወይም ወላጆች የመተው መብት አላችሁ። ነገር ግን በመስኩ ጥናት ላይ ለመተባበር ፍላጎት ያላችሁ ሁሉ ትብብራችሁን ታደርጉልኝ ዘንድ በአክብሮት እጠይቃለሁ። ከተማሪዎች ወይም ከቤተሰብ የተሰጠኝን መልስ በምስጥር እጠብቃለሁ። በተጨማሪም በማንኛውም ጊዜ በፍላጎታቸው በዚህ የመስክ ጥናት ውስጥ የተሳተፍትን እደግፋለሁ።

የተሳታፊው ስም ----------ፊርማ----------

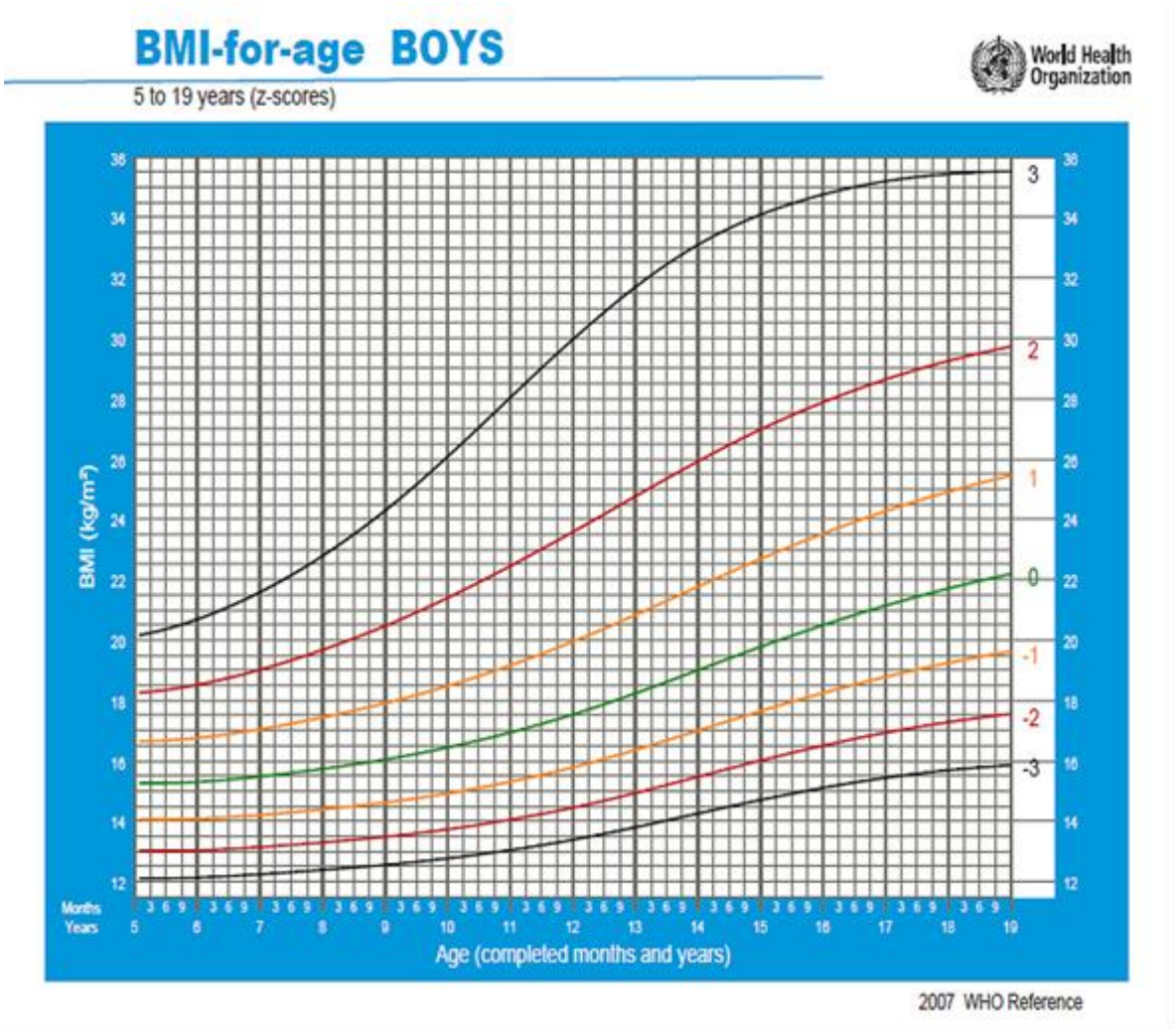
ቀን----------

Appendix VI

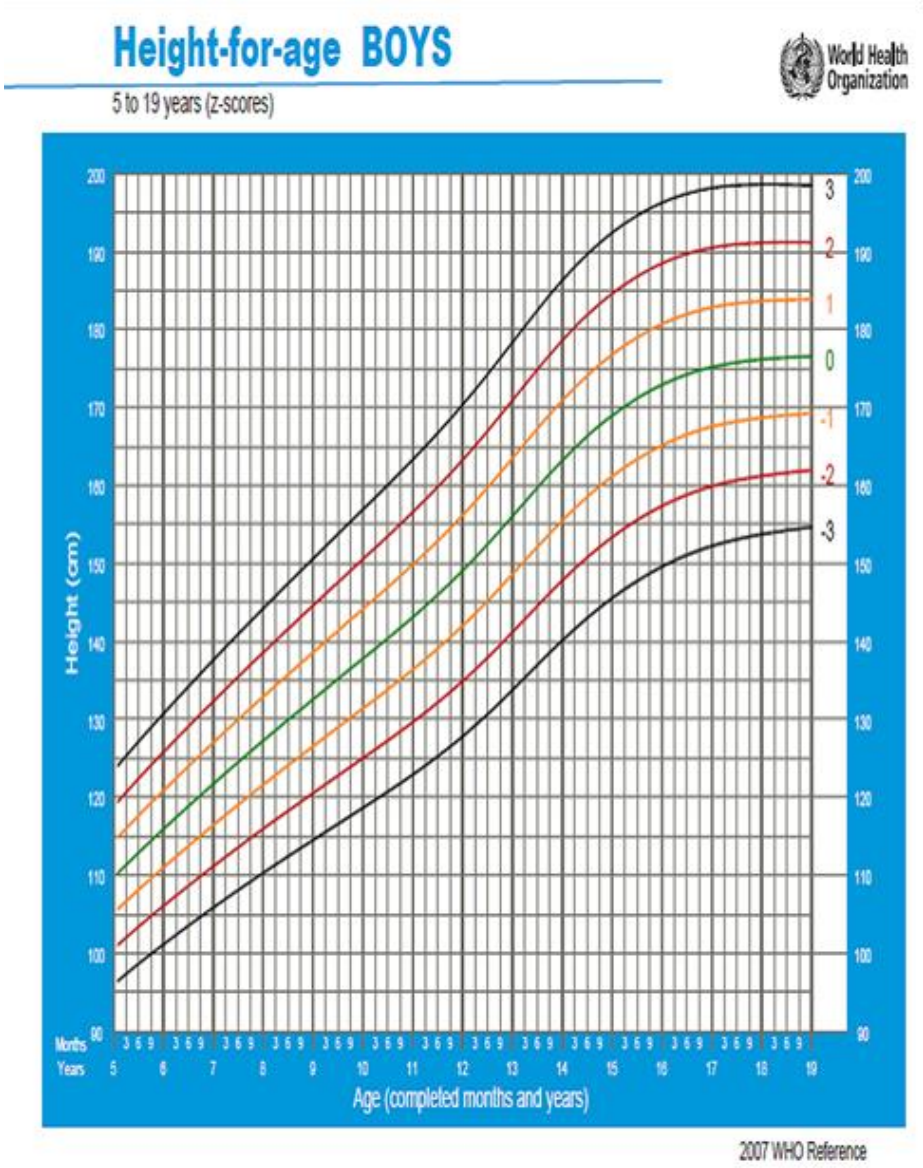


Apendix Figure 1. BMI-for- age boys aged from 5-19 years on the basis of growth referencedata(WHO, 2007)

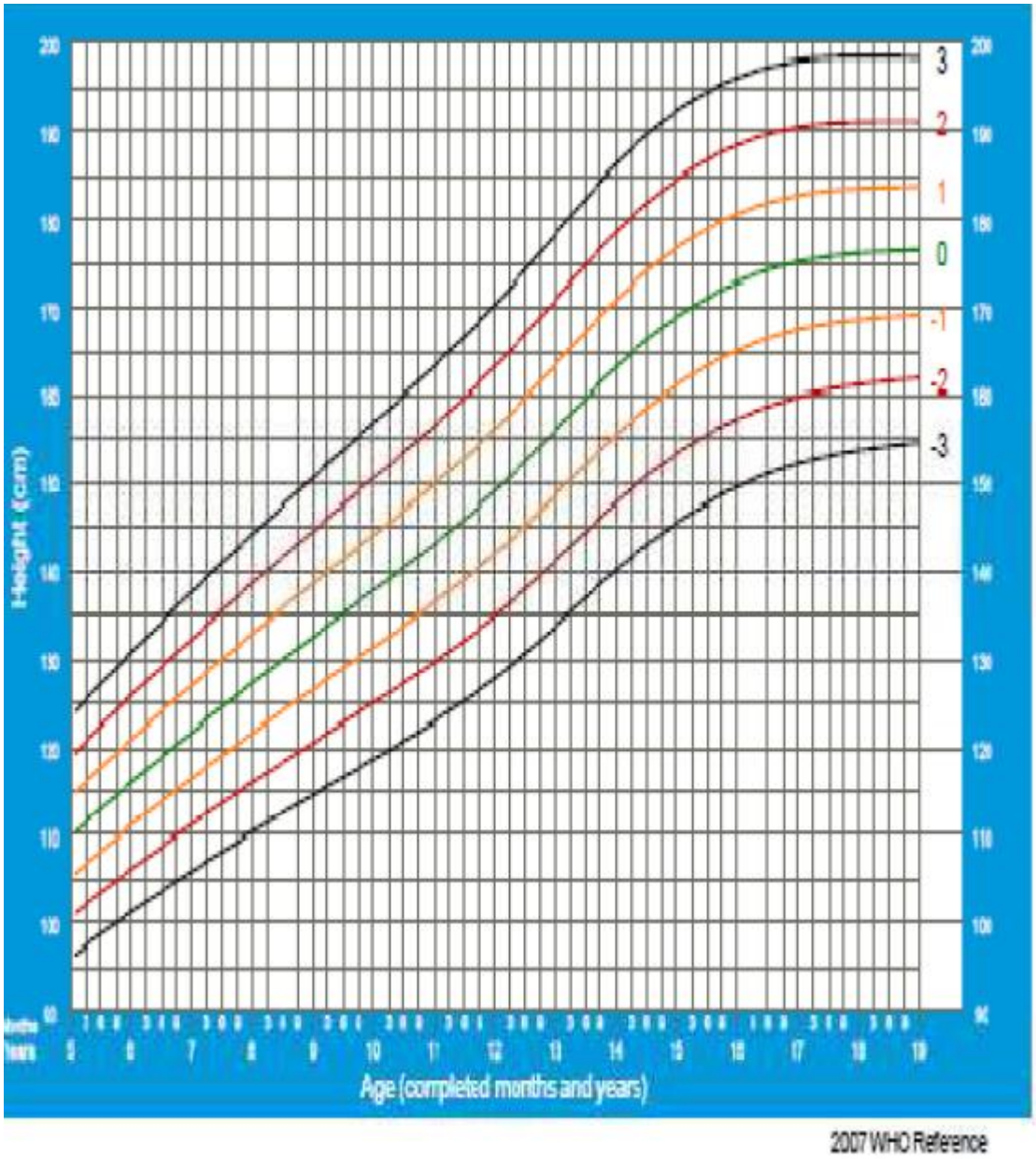
Appendix VII



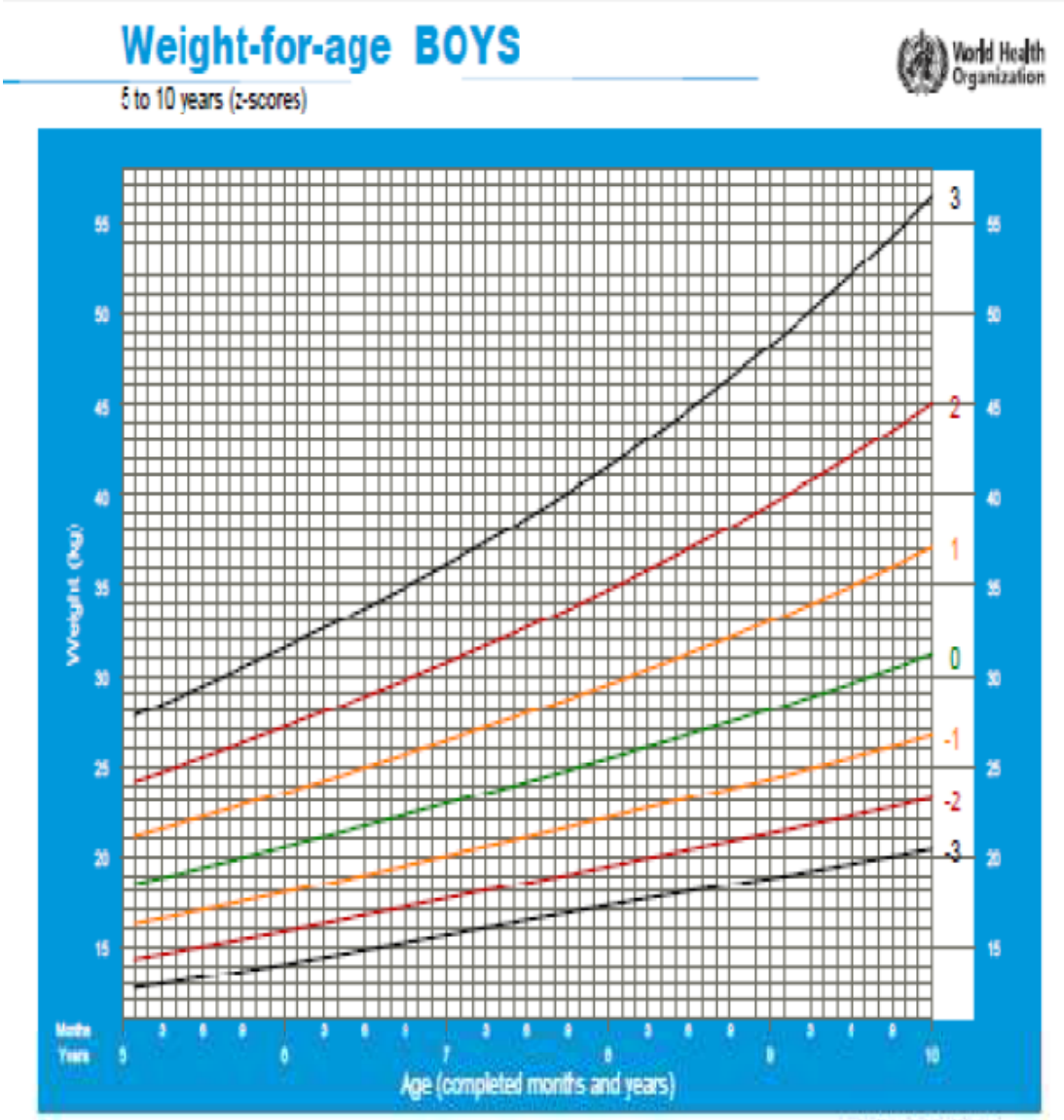
Apendix Figure 2. BMI-for- age boys aged from 5-19 years on the basis of growth reference data (WHO, 2007)



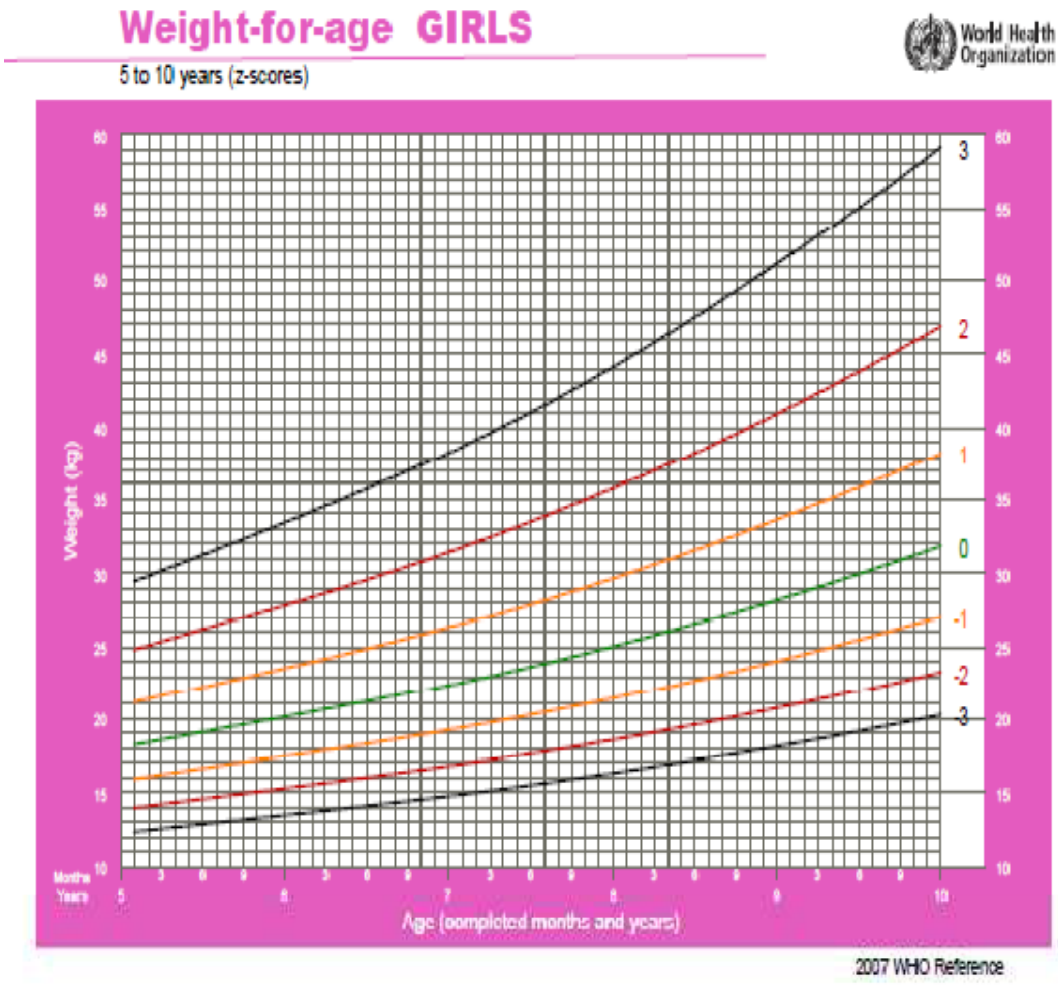
Apendix Figure 3. Height-for- age girls aged from 5-19 years on the basis of growth reference data (WHO, 2007)



Apendix Figure 4. Height-for- age boys aged from 5-19 years on the basis of growth reference data(WHO, 2007)



Apendix Figure 5. Weight-for-age of boys aged from 5-10 years on the basis of growth reference data (WHO, 2007)



Appendix Figure 6. Weight-for-age of girls aged from 5-10 years on the basis of growth reference data (WHO, 2007)

CHAGNI HOSPITAL

REVIEW COMMITTEE OFFICE (RC)

Ref.No 255/A-3103

Date 24/05/09

To: **Ms Teshager worku**

Chagni

Subject: **Ethical clearance**

Study Title :

"Prevalence and Intensity intestinal protozoan and helminth infection and association with anthropometric measurement of school children in chagni town Amhara region, North West Ethiopia."

We have received the research proposal to be under taken in the title mentioned above to took study September to December for data collection in chagni Hospital and has discussed over issue.we are pleased to inform you that the above research proposal has been approved after scrutinizing the research document and ethicaly cleared for implementation.therefore,we declare you the proposal is etaicaly cleared for implementation and to carry out research under taking as per document of proposal submitted. Further audit will be made due course when ever necessary.this ethical clearance issued for 12 months.

Regards

D/r YIBESRA DESTA

Chair person, RC, Chagni hospital

Chagni

CCO;

OFFICE of MIDICAL DIRECTORATE

CHIEF EXCUTIVE OFFICER

Head of laberatory technician