



**FACULTY OF LIFE SCIENCES, DEPARTMENT OF BIOLOGY, MICROBIAL,
CELLULAR AND MOLECULAR PROGRAM UNIT**

**INTESTINAL PARASITE CONTAMINATION OF RAW VEGETABLES FROM
SELECTED FARMS AND MARKETS IN ADDIS ABABA, ETHIOPIA**

**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS
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**BY
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LIST OF ABBRIVATIONS AND ACRONYMS

DALYs	Disability Adjusted Life Years
DFA	Direct fluorescence Antibody
EHNRI	Ethiopian Health and Nutrition Research Institute
ELISA	Enzyme linked immunosorbent assays
PCR	Polymerase chain reaction
rpm	revolution per minute
RFLP	Restriction fragment length polymerase
SAF	Sodium Acetate-acetic acid Formalin
Spp	Species
STH	Soil Transmitted Helminths

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Abstract

Raw vegetables can be agents of transmission of intestinal parasites. This study aimed to determine the prevalence of parasite contamination of selected raw vegetables (lettuce, cabbage, tomato and carrot) from farms and sold in major Addis Ababa markets. Sampling locations (Kaliti, Kera, Peacock, Goffa) were selected based on their accessibility. Merkato and Atikelt tera represented the vegetable markets. Out of 384 vegetable samples examined, 148(38.5%) were contaminated with parasite eggs and /or cysts. The parasites detected were *Ascaris lumbricoides* (20.3%), *Cryptosporidium* spp (8.6%), *Entamoeba histolytica/dispar* (7.6%), hookworm species (6.8%), *Giardia lamblia* (5.5%), *Enterobius vermicularis* (4.2%), *Trichuris trichiura* (1.3%) and *Taenia* spp (1.0%). Overall, the highest parasite contamination was detected in lettuce (58.3%), followed by cabbage (37.5%), tomato (33.3%) and carrot (25%). Lettuce from the farms were more contaminated (64.1%) than those from markets (46.9%) and the difference was statistically significant ($P<0.05$). The relatively higher parasite contamination of lettuce and cabbage may be attributed to their larger leafy surface areas. The wastewater used for irrigation was also contaminated with pathogenic parasites, which is an indication of the source of contamination for vegetables grown in Addis Ababa. Similarly, vegetables, whose irrigation status is not known, and sold in the markets, were also contaminated. In either case, the routine practice of freshening vegetables at the markets, by splashing with water that may have been contaminated with parasites could be an additional source of contamination. This study showed the high potential for human infection with parasites through consumption of raw vegetables from farms and markets in Addis Ababa. Therefore, there is an acute need for environmental health education and institution of sanitary standards for wastewater disposal and use in Addis Ababa. Furthermore, given the fact that urban vegetable farming makes use of polluted rivers for irrigation, national guidelines on disinfecting raw vegetables for human consumption must also be provided.

Key words: vegetables, parasites, contamination, irrigation, wastewater, Addis Ababa

1. INTRODUCTION

Parasites are among the main public health problems around the world especially in tropical and sub-tropical countries. The prevalence is highest among the inhabitants of towns in developing countries, where there is improper disposal of garbage and untreated sewage into streams and rivers, poor health systems and overcrowding. Globally, it is estimated that some 3.5 billion people are affected, and that 450 million are sick from intestinal parasite infections, with an estimated 200,000 deaths annually (Wakid, 2009). Parasite infections such as *Ascaris lumbricoides* (1.2 billion), *Trichuris trichiura* (795 million), hookworms (*Ancylostoma duodenale* and *Necator americanus*) (740 million) and so many others affect people all over the world (De Silva *et al.*, 2003; Bethony *et al.*, 2006).

While mortality from helminths and protozoa is relatively low, morbidity and the indirect effects have a substantial impact on health and quality of life. These include stunting of linear growth, physical weakness and low educational achievement in schoolchildren (WHO, 2005). Furthermore, chronic intestinal parasite infections have become the subject of speculation and investigation in relation to the spreading and severity of other infectious diseases like AIDS, tuberculosis and malaria.

Intestinal parasite infections are caused either by protozoa or helminths or both. Protozoa are able to replicate in the intestines of infected hosts and are excreted in the feces, helminths produce ova and larvae aiding in their survival and dispersion in the environment, both have a variety of human and non human animal hosts and transmitted primarily by the fecal-oral route (Jaykus, 1997).

The transmission of parasite infections to humans are facilitated through poor personal hygiene (Okuyay *et al.*, 2004), environmental conditions like contamination of food and water sources with human feces, sewage contaminated irrigation water, use of night soil as fertilizers and improper human handling of food (Daryani *et al.*, 2008; Erdogrul and Sener, 2005; Sinski, 2003).

Vegetables are an important source of nourishment containing carbohydrates, proteins, vitamins, minerals as well as trace elements (Itanna, 2002; Farooq *et al.*, 2008; Gharavi *et al.*, 2002). They are grown mostly throughout the year using rain during wet season and irrigation during dry season. Vegetables may get exposed to parasite contaminants pre-harvest (cultivation, irrigation, livestock manure etc...), post-harvest handling- storage, transportation, or while processing for consumption (Erkan and Vural 2008) (Figure 1).

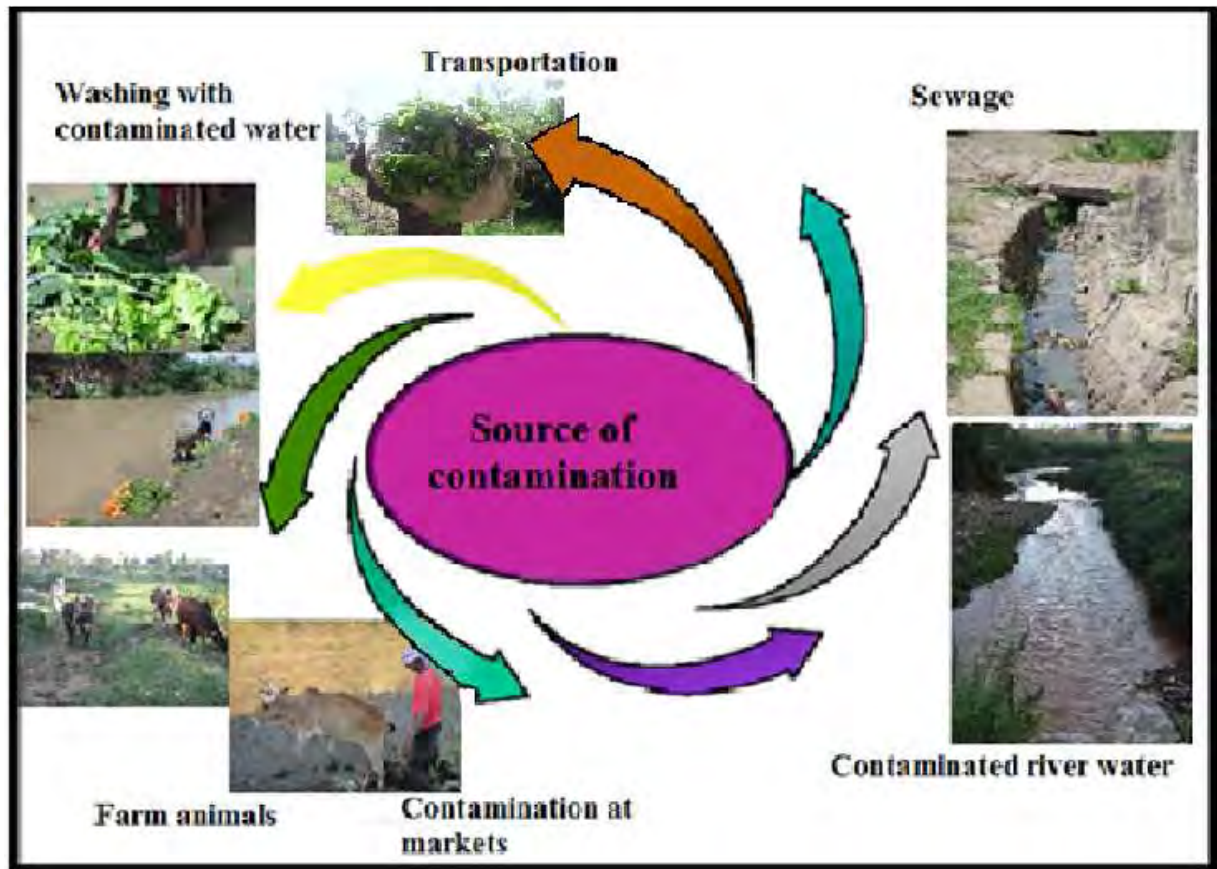


Figure 1: Possible sources of contamination of farm vegetables with pathogenic organisms

The direct application of night soil, animal manure and wastewater as an agricultural fertilizer has also been practiced for centuries in many parts of the world, and has been gaining prominence, in developing countries (Damen *et al.*, 2008; Kozan *et al.*, 2005 and Feenestar *et al.*, 2000). The practice is getting prominence due to the growing scarcity of water, population growth and urbanization. It is well established that the use of excreta-polluted irrigation water to grow vegetables is a health risk to the farmer and consumers (Zavadil, 2009). In spite of this, the use of wastewater has been used for farming purposes for many types of vegetables (Amahmid *et al.*, 1999).

Wastewater frequently contains high numbers of protozoan and helminth parasites, which are of primary public health concern for wastewater reuse. An important characteristic of these organisms is the production of highly resistant cyst and ova that can survive for very long in the wastewater (Erdogrul and Sener, 2005).

Parasites such as *Cryptosporidium* spp, *Gardia lamblia*, *Entamoeba histolytica*, *Ascaris lumbricoides*, hookworms, *Enterobius vermicularis*, *Trichuris trichiura*, *Toxocara* spp., *Hymenolepis* spp, *Taenia* spp, *Fasciola* spp and members of the family Trichostrongylidae could infect humans as a result of consumption of contaminated, uncooked or improperly washed vegetables (Kozan *et al.*, 2005). Thus, the uncontrolled use of wastewater from sources contaminated with human and animal feces without the proper disinfection and treatment to irrigate and wash vegetables has been reported to be responsible for their high rates of contamination. Outbreaks of intestinal parasite infections epidemiologically associated with raw vegetables have been reported from developed and developing countries (Robertson and Gjerde 2001a; Ortega *et al.*, 1997 and Abougrain *et al.*, 2010). Epidemiological studies have also indicated that in areas of the world where parasitic diseases are endemic in the population and where wastewater is used to irrigate vegetables which are eaten raw; the consumption of wastewater irrigated vegetables without proper washing may lead to parasite infection (Feenestra *et al.*, 2000; Erdogrul and Sener, 2005 and Damen *et al.*, 2008). Furthermore, an increased risk of diarrheal and non diarrheal diseases associated with the consumption of uncooked vegetables contaminated with parasites is also a matter common perception.

The risk of parasite infections has been reported to be higher among the inhabitants of towns of developing countries like Ethiopia, where there is poor disposal of garbage, poor health systems, illiteracy and overcrowding (Nyarango *et al.*, 2008). Over 60% of the communicable diseases in

Ethiopia are due to poor environmental health conditions arising from unsafe and inadequate water supply and poor hygienic and sanitation practices (Abebe, 1986). As a result, parasite infections are very high in Ethiopia.

However, there is little information available on the risks of parasite infections associated with the consumption of contaminated vegetables in Ethiopia. (Erko *et al.*, 1995) have shown vegetable contamination with amoeba cyst and *Ascaris* eggs on vegetables grown in faecally contaminated gardens. As these parasites are highly resistant and able to withstand harsh conditions, it is important to assess the risk of human infections associated with the consumption of raw vegetables that might be contaminated with these parasites during cultivation, transport, washing or storage.

1.1 Protozoan parasites frequently associated with vegetable consumption

Protozoan parasites, including *Giardia lamblia*, *Cryptosporidium* spp and *Entamoeba histolytica* are ubiquitous organism that have been recognized as the most common parasitic protozoa of major public health concern (Amahmid *et al.*, 1999). They are transmitted by the fecal-oral route and tend to exhibit similar life cycles consisting of cystic and trophozoite stages. These protozoa have highly resistant cysts which are suited to food and water borne transmission and are major causes of diarrheal disease in humans, worldwide.

Their life cycles are completed within an individual host, the transmissive stage the cysts, are produced in large numbers and are infectious when excreted. This problem is exacerbated by the fact that the number of cysts required to induce infection is small. Infectious doses as low as 10 cysts have been reported for *Cryptosporidium* spp and 19-50 cysts for *Giardia lamblia* (Adam, 2001; Chappell, *et al.*, 2006). The cystic stages have marked resistance to environmental and water treatment stresses, which is a decisive factor in their dissemination.

Giardia, *Cryptosporidium* and amoeba are among the common protozoan parasites, which are wide spread in Ethiopia (Assefa *et al.*, 1996). Reports from different parts of Ethiopia present different prevalence rate of cryptosporidiosis, giardiasis and amebiasis. Even though there might be an understatement and over statement of reports due to the diagnostic methods used. Endeshaw *et al.*, (2004) has shown the prevalence of *C. parvum* and *G. lamblia* among diarrheal patients referred to Ethiopian Health and Nutrition Research Institute (EHNRI) to be 20.6% and 8.6%, respectively. (Gebbru and Girma, 2000) have also reported prevalence of *Cryptosporidium* infection in children with diarrhea to be 3.3%. Ayalew *et al.*, (2008) had reported a prevalence of 12.2% and 35.5% for *Cryptosporidium* and *Giardia* respectively. Other studies conducted in Addis Ababa on the prevalence of *Cryptosporidium* spp infection have also reported 5.6%

(Assefa *et al.*, 1996), 8.1% (Adamu *et al.*, 2006). A more recent study by (Adamu *et al.*, 2010) reported a prevalence of (7.6%) for *Cryptosporidium* spp among the general population from different part of the country. High prevalence of intestinal amoebiasis reported based on microscopy has been determined to be an over diagnosis (Kebede *et al.*, 2004).

Epidemiological surveys have indicated that the most important source for human parasite diseases is unsafe and contaminated drinking water (Fikrie *et al.*, 2008; Abebe, 1986). Food, animals and infected people under poor hygienic and sanitary practices could also serve as sources of transmission and the use wastewater to irrigate vegetables is another important contributory factor (Dillingham *et al.*, 2002), in addition to the fecal-oral route of transmission.

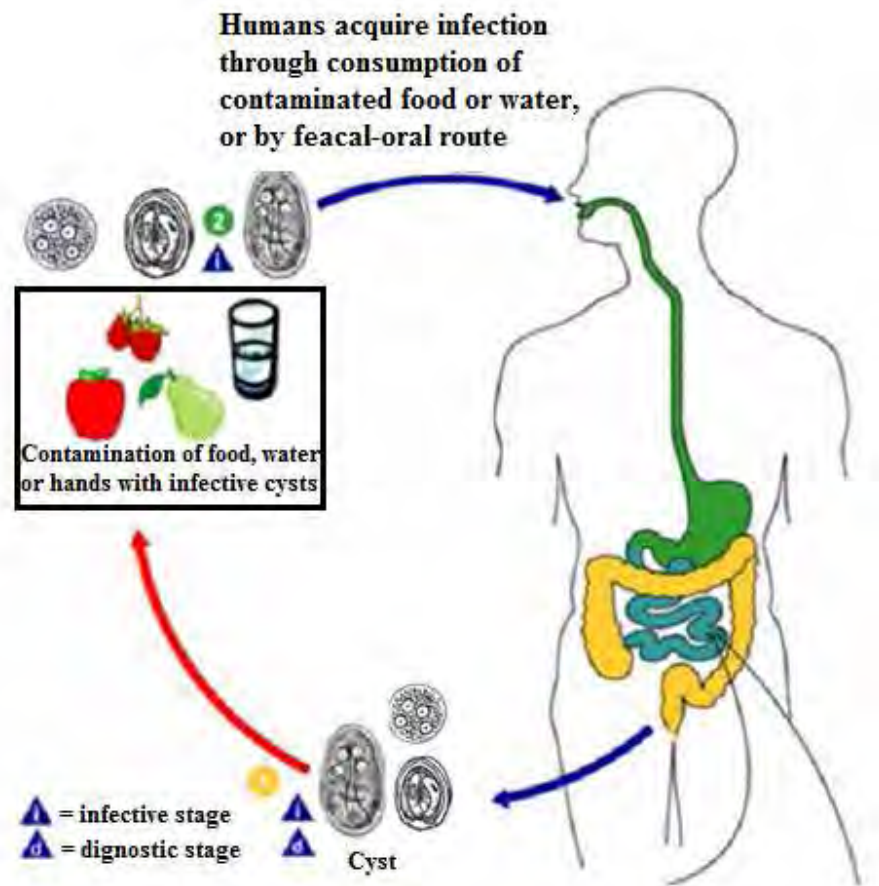


Figure 2: A typical life cycle of a cystic protozoan parasites-*Giardia lamblia*

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

Entamoeba histolytica

Entamoeba histolytica parasitizes the large intestine of the host. Although several members of the genus *Entamoeba* reside in humans intestine, *E. histolytica* is the cause of invasive amoebiasis and hence the only species of public health importance (Diamond and Clark, 1993).

Epidemiological studies have shown that low socioeconomic status low standards of hygiene and sanitation, in particular those related to crowding, contamination of food and water, and inadequate disposal of faeces, are all significant risk factors for infection with *E. histolytica*.

E. histolytica cysts are resistant to acidification, chlorination and desiccation, and capable of surviving in a moist environment for several weeks (Singh, *et al.*, 2009). This characteristic is responsible for survival of the cyst in sewage effluents that pollute water bodies. They survive the acidic pH of the stomach and pass into the intestine.

Traditionally *E. histolytica* is diagnosed by microscopy through identification of either cysts or motile trophozoites in the stool, but this method does not allow differentiation among pathogenic and non pathogenic *Entamoeba* spp with similar morphological features particularly *E. dispar*. Recent advances have made new diagnostic tools available including serologic tests and the commercial availability of stool *E. histolytica*-specific antigen testing (Stauffera and Ravdinb 2003).

***Cryptosporidium* spp**

Cryptosporidium spp are coccidian parasites and there are at least 16 established *Cryptosporidium* spp and a further more than 33 genotypes are also described (Smith *et al.*, 2007). At least 8 of the defined genotypes have been reported in humans. These include *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, and *C. suis*, and the *Cryptosporidium cervine* genotype (Cama *et al.*, 2008).

Human infection, however, is predominantly caused by the two main species of *Cryptosporidium*- *Cryptosporidium hominis* and *C. parvum* which cause the disease cryptosporidiosis in humans (Hunter *et al.*, 2007; Kosek *et al.*, 2001).

This protozoan parasite is a serious concern for the water supply sector; it is also a concern for the fresh produce industry, since contamination via contaminated irrigation water may occur. Water may be polluted with sewage and agricultural run-off from adjacent farms (Millary *et al.*, 2002; Kniel and Jenkins, 2005). *Cryptosporidium* oocysts have been isolated from several foodstuffs and these have mainly been associated with fruits and vegetables (Robertson *et al.*, 2002; Cook *et al.*, 2006). Studies have identified *C. parvum* oocysts on more than 14% of randomly sampled vegetables in Peru, Costa Rica and Norway (Ortega *et al.*, 1997; Monge and Arias, 1996; Robertson and Gjerde, 2001a).

Cryptosporidium spp produce robust oocysts in the faeces of infected animals, transmitted directly through faecal-oral contact from infected persons or animals or indirectly by ingesting oocyst contaminated water or food (Moore *et al.*, 2007; Caccio *et al.*, 2005).

At present, most cryptosporidial infections are diagnosed by the microscopic examination of host fecal material for the presence of *C. parvum* oocysts. A differential staining technique choice for

many diagnostic laboratories is acid-fast staining (Clark, 1999). Further, several immunolabelling techniques have also been developed to detect oocysts, antigen detection via immunofluorescence, enzyme linked and agglutination immuno-assays. Polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and other DNA-based detection methods are also present (Chen and Larusso, 1999).

Giardia lamblia

Giardia, flagellated protozoan, is the most common causative agent of persistent diarrhea worldwide (Adam, 2001). It is one of the most common protozoa that infect humans and a wide range of domestic and wild animals. *Giardia duodenalis* (also referred as *G. intestinalis* or *G. lamblia*) that is hosted by mammals is the only species found in humans today (Thompson and Monis, 2004; Smith *et al.*, 2007).

According to Thompson *et al.* (1993), 5% of acute diarrhea and 20% of chronic diarrheal illness in the world are attributable to *G. lamblia*. The incidence of diarrhoea associated with *G. lamblia* is generally higher in developing countries such as Asia, South and Central America and Africa where prevalence of human giardiasis is about 20% (4-43%), where access to clean water and basic sanitation is lacking. compared with 5% (3-7%) in developed countries (Farthing, 1993).

Transmission is sustained by both a zoonotic and an anthroponotic cycle (Fayer *et al.*, 2000; Thompson *et al.*, 2000). *Giardia* can be transmitted to a person through contaminated water (Karanisa *et al.*, 2006). It can also be transmitted from the environment; the use of contaminated water to irrigate food crops is a potential source of *Giardia* cysts which may play a significant role in giardiasis transmission (Amahmid, *et al.*, 1999). Cysts have been detected on produce in

several countries (Amahmid, *et al.*, 2000; Robertson and Gjerde, 2000; Robertson and Gjerde, 2001b; Robertson, *et al.*, 2002).

The traditional diagnosis of giardiasis has been carried out using direct detection of trophozoites in stool samples (Broke, 1977). In recent years, rapid diagnostic tests that use antigen detection methods have been widely employed. Two of the most common antigen detection assays are direct fluorescence antibody (DFA) tests that detect intact organisms, and enzyme immunoassays (EIAs) that detect soluble stool antigens (Garcia and Shimizu, 1997).

1.2 Helminth parasites frequently associated with raw vegetable consumption

Helminths are endemic in many areas and are also associated with poor hygienic practices. Host immunity is usually low to non-existent and the infective dose is small. An important characteristic of these organisms is the production of ova and larvae aiding their survival and dispersion in the environment (Gupta *et al.*, 2009; Amhamid *et al.*, 1999) causing human infection through contact with parasite eggs or larvae (Bethony *et al* 2006). The most common helminths are the soil transmitted helminths (STHs) which include roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and the hookworms (*Necator americanus* and *Ancylostoma duodenale*). However, others like *H. nana*, *Taenia* spp and *Enterobius vermicularis* also parasitize human beings.

Globally, millions of people suffer from parasite infections such as *Ascaris lumbricoides* (1.2 billion), *Trichuris trichiura* (795 million), hookworms (*Ancylostoma duodenale* and *Necator americanus*) (740 million). This infection is distributed virtually throughout the world and has been causing morbidity most commonly associated with infections of heavy intensity in communities where poor environmental sanitation and poor personal hygiene are prevalent (WHO, 2008).

The most important helminth parasites predominantly distributed in Ethiopia include *A.lumbricoides*, hookworm, *H. nana*, *T. trichiura* and *E. vermicularis*; with varying prevalence in different areas. They are also the second most predominant cause of outpatient morbidity (Erko and Medhin, 2003; Mengistu *et al.*, 2007).

Humans acquire infection through consumption of contaminated food or water, or by fecal-oral route

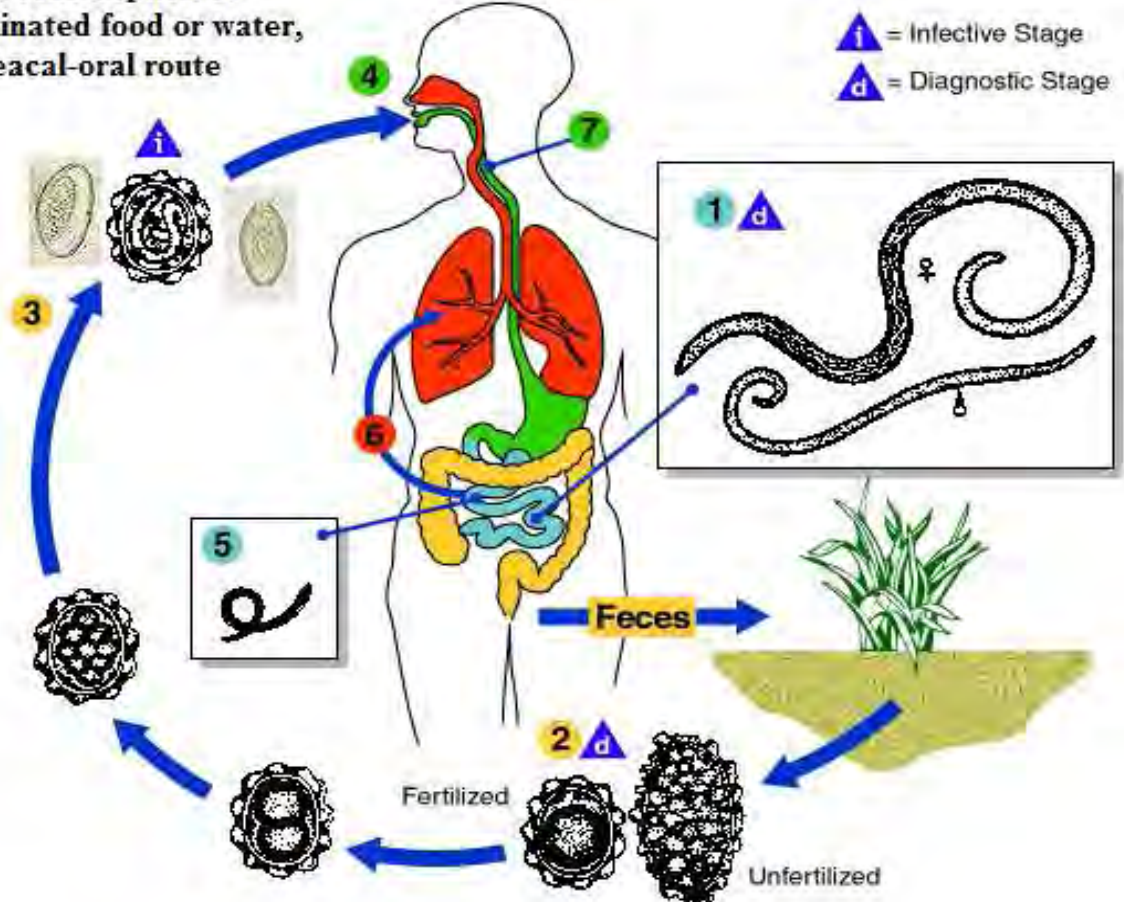


Figure 3: A typical life cycle of a geo-helminth parasite-*Ascaris lumbricoides*

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

Hookworms

Hookworm infection in humans is caused by an infection with the helminth nematode parasites *Necator americanus* and *Ancylostoma duodenale* (Jemaneh and Tedla, 1985). The infection is acquired through contact with contaminated soil with third-stage infective larvae, which either penetrate the skin as do both *N. americanus* and *A. duodenale* or when they are ingested *A. duodenale* only (Hawdon and Hotez, 1996). The infection occurs in areas where the standard of living of the population is low. These conditions favor the development of filariform larvae and infection of hosts (Jemaneh and Tedla, 1985).

The infection is very intense in the tropics and sub-tropics with an estimated 740 million cases per annum (De Silva *et al.*, 2003), and the global DALYs for hookworm is estimated to be 22.1 million life years lost (Chan, 1997). The examination of human stool samples for the presence of hookworm eggs remains the most reliable means of diagnosis although the species of hookworm cannot usually be identified.

Ascaris lumbricoides

1.2 million People in the world are infected with *Ascaris lumbricoides* and it is more prevalent in the developing world (De Silvia *et al.*, 2003). The fecal oral route is significant in the transmission of parasitic infections to humans via poor personal hygiene, as a result is very common in people who live in areas whose environment is contaminated with contaminated soil and water sources with human faeces or indirectly by fecal contaminated irrigation water. When the soil or water becomes contaminated, the eggs can be transferred onto vegetables then onto the hands and transferred directly into the mouth or ingested by eating raw vegetables

Ascaris (roundworms) is one of the most resistant of the enteric pathogens and it is often used as a parasitological indicator of fecal contamination (Watson *et al.*, 1983). Eggs of *Ascaris* are highly resistant to hostile environmental factors (Amhamid *et al.*, 1999). *A. lumbricoides* is a well-known cause of malnutrition, intestinal obstruction, biliary colic and pancreatitis estimated to infect a quarter of the world's population (Hall, 2000).

Infection is acquired through the ingestion of infective eggs from fecally contaminated food or water. Infections can be diagnosed by direct microscopy of faeces (Hall, 2000).

Trichuris trichiura

Trichuriasis or whipworm infection is caused by *Trichuris trichiura*. It is common in the warm moist tropical and sub tropical countries. The infection is estimated to be around 795 million persons worldwide (De Silva, 2003). Humans are the primary host for infections caused by *Trichuris trichiura*.

Infection with *Trichuris trichiura* occurs through the oral-fecal route caused by ingestion of infective eggs from contaminated foods, hands or water. The most severe manifestation, include chronic dysentery, rectal prolapse, anemia, and growth stunting (Stephenson *et al.*, 2000). Intellectual and cognitive impairments and delays are also associated with chronic heavy infections (Partovi *et al.*, 2007).

Enterobius vermicularis

Enterobius vermicularis or pinworm is an intestinal nematode of humans which mainly causes itching in the anogenital area and affects more than 200 million people worldwide (Elston, 2003).

Their normal lifecycle is as follows: gravid females migrate down the colon to the ano-genital region where they lay their eggs and then die. The resultant itching evokes scratching, so that eggs are frequently transferred to the fingers and thence to the mouth where they are swallowed.

The presence of pinworms can be confirmed in one of two ways. The first is direct observation of the adult worms around the anus, perineum, or entrance to the vagina. A second test is to observe the eggs, which are about the size of the head of a pin, under a microscope.

Taenia spp

Taeniasis in man is caused by *Taenia solium*, *Taenia saginata* and *T. saginata asiatica*. Man is the final host while cattle (*T. saginata*) and pigs (*T. solium* and *T. s. asiatica*) are intermediate hosts. *Taenia solium*-taeniasis and cysticercosis are diseases present in developing countries, which are associated with social and environmental conditions such as lack of latrines, poor hygiene and free roaming pigs with access to human feces (WHO, 2002).

Worldwide, taeniasis and cysticercosis are common parasitic infections: 2-3 million people are thought to be infected with adult *T. solium*, 45 million with adult *T. saginata*, and 50 million with *T. solium* cysticerci every year. Man, the obligatory final host, acquires the infection with the adult parasite by eating raw or undercooked pork or beef contaminated with viable cysticerci (WHO, 2002).

Taeniasis can be diagnosed by demonstrating *Taenia* proglottids or eggs in the feces (Allan *et al.*, 1996). *Taenia* spp. eggs cannot be distinguished from each other by their morphology; however, enzyme-linked immunosorbent assays (ELISAs) and polymerase chain reaction (PCR) test can differentiate the eggs of *T. solium* from *T. saginata*, and morphology can be used to distinguish between these two proglottids (Allan *et al.*, 1996).

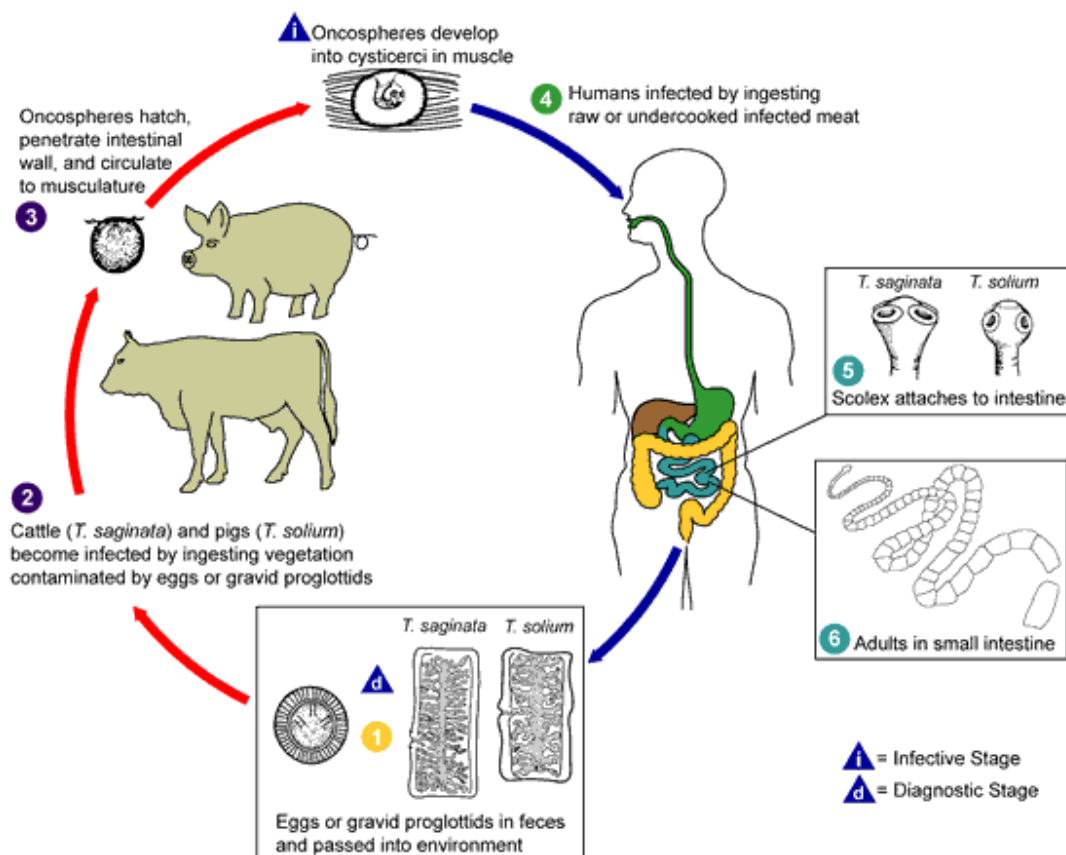


Figure 4: A typical life cycle of *Taenia* spp

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

1.2 Statement of the problem

Vegetables during cultivation, harvest, transportation and further processing can be contaminated with pathogens from human or animal sources. As a result, they have been implicated in a number of documented outbreaks of food-borne parasitic illnesses. The use of sewage effluent for irrigation exposes the public to the dangers of infection with a variety of pathogens such as bacteria, viruses, protozoa and helminths.

In addition, vegetables are washed after harvesting by producers and at markets and water used for this purpose may not be clean. Furthermore, secondary contamination of vegetables may occur during transportation, ware housing and contamination by market venders. In such conditions where irrigation water is contaminated with human and animal wastes and where vegetables are washed with wastewater and where secondary contamination is possible, the risk of getting parasitic diseases by workers who come in contact with or consume these products is high.

Although a number of studies have been conducted on the distribution and prevalence of parasites in Addis Ababa, there is a limited study addressing the importance of vegetables as contributing source for the high prevalence. Therefore, this study, attempts to determine the extent of raw vegetable contamination with parasites that could be transmitted to humans.

1.4. Hypothesis

The prevalence of parasite contamination is high in vegetables irrigated with water polluted with domestic sewage and sold in markets in Addis Ababa.

2. OBJECTIVES

2.1 General objective

- Determine the prevalence of human parasite contamination in raw vegetables from farms and markets in Addis Ababa.

2.2 Specific objectives

- To assess the potential of raw vegetables as source of human parasitic infections in Addis Ababa.
- To assess the health risk of the use of water from rivers contaminated with sewage for vegetable irrigation.
- To compare the prevalence of parasite contamination in farm and market lettuce samples in Addis Ababa.

3. MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Addis Ababa, the capital city of Ethiopia, which has a total population of 2,979,086 according to the 2009 population census ([http:// www. csa. gov.et/doc/ cen 2010](http://www.csa.gov.et/doc/cen2010)). There are agricultural farms in and around the city, which are irrigated with river water. These rivers receive numerous discharges of raw sewage, community refuse and urban wastewater. The main vegetables grown include cabbage (*Brassica oleracea L. Var. capitata*), potato (*Solanum tuberosum L.*), swiss chard (*Beta vulgaris L.var.cicla*), carrot (*Daucus carota L.*), lettuce (*Lactuca sativa L.*), cauliflower (*Brassica oleracea L.Var.botrytis*), red beet (*Beta Vulgaris L.var.Vulgaris*) and tomato (*Lycopersicum sativus L*) (Itanna, 2002) which are sold in the nearby markets in the city vegetables sold in the major markets in Addis Ababa Merkato and Atkilt tera were also studied.

3.2 Sampling locations

Raw salad vegetable, lettuce, which is cultivated on the embankments along the major rivers within Addis Ababa were picked from four major farms (Kera, Peacock, Kaliti and Goffa) (Figure 5) which were selected after a preliminary survey of finding farms in and around Addis Ababa out of these, four irrigated farms were selected based on their accessibility. These farms use water from rivers which receive contaminated raw sewage, waste refuse and polluted water from the community (Itanna, 2002). Raw vegetables, lettuce, cabbage, carrot and tomato were also picked from two major markets, Merkato and Atekilt tera, to determine possible parasite contamination of market vegetables as well.



Figure 5: Pictures of selected vegetable farms-Goffa, Kaliti, Kera and Peackok, in Addis Ababa, where the study was conducted, (December 2009- October 2010).

3.3 Collection of samples for the study

In estimating the sample size (n), 50% prevalence, 95% confidence interval for Z^2 , conventionally 1.96, e is the desired level of precision (taken to be 5%, $e=0.05$) and P is the estimated proportion of an attribute that is present in the population 0.5. A representative sample for proportions was thus calculated using the following statistical formula (Cochran 1963 cited in Kasiulevičius *et al.*, 2006).

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

Vegetables were picked six times during the study period, 96 samples of each vegetable type were taken. One sample was taken as a portion of vegetable weighing 250 grams. A total of 384 vegetable samples were taken 320 from market and 64 lettuce samples from farms. 120 liter of irrigation water sample, 30 liters from each farm was collected as the farmers were watering the vegetables from the rivers using pumps (Figure 6). All samples were collected on clean polyethylene bags while water samples were collected on clean 30 liters plastic containers. Samples were then brought to Biomedical Laboratory, Addis Ababa University for analysis.



Figure 6: Pump used to draw water from rivers to the farms in Addis Ababa (Akaki river), (December 2009- October 2010).

3.4 Vegetable analysis

Parasites from vegetable were analyzed by taking a portion of vegetables weighing (250 g) and washed with 1000ml physiological saline solution (0.85% NaCl) wearing gloves in a bucket. The washing was left for 24 hours for sedimentation to take place (Figure 7). The top physiological saline solution was then discarded carefully without shaking and the remaining 5 ml washing physiological saline solution was centrifuged (Gallenkamp Angle head centrifuge Cat.No CFB 700 0100 HZ50) at 2000 g for 5 min. The supernatant discarded and the residue were agitated gently by hand in physiological saline solution for further distribution of the cysts and eggs in the residue, then examined in Lugol's iodine (10.5% in distilled water) by using light microscope (Bailenger,1962 cited in Daryani *et al.*,2008). The remaining was preserved in sodium acetate-acetic acid formalin (SAF) (15g sodium acetate, 20ml glacial acetic acid, 40ml formalin in 925ml distilled water).



Figure 7: Sedimentation of vegetable washings with physiological saline solution in the laboratory.

3.5 Water examination

Filtration: The water samples were filtered as soon as they were brought to the laboratory by passing through a sieve to remove big debris. It was then filtered using (Whatman 3030-917 Grade No. 3MM Cellulose, Chr Chromatography Paper, 46 x 57 cm Sheets) with the aid of a vacuum pump (AC motor, Type Bs 2406) (Figure 8A) and a Buckner filter flask. The paper was cut into circles and was put in the funnel in layers to enhance an effective trapping of parasite eggs and cysts (Figure 8B). When the pores were blocked during the filtration process as indicated by the slow flow rate of filtrate into the Buckner filter flask, the top most layer of the paper was removed and another added at the bottom. All the filters, which had trapped minute particles, were collected into labeled polythene bags and stored in the refrigerator to keep them moist until further processing.



A

B

Figure 8: Filtration set up (A), and Whatman 3030-917 Grade No. 3MM Cellulose, Chr Chromatography Paper, 46 x 57 cm Sheets (B) used for filtration.

Backwashing: The filters were eluted by several backwashing with 200 ml of distilled water in which 10% wash solution (0.1% Tween 20 in distilled water) was included to enhance the recovery of oocysts (Uneke and Uneke, 2008).

Concentration: The sucrose gradient sedimentation technique was used for the concentration of oocysts. After the backwashing, 9 ml of the wash sample was placed in a conical centrifuge tube and 2 ml of Sheather's sugar solution (454 grams sugar, dissolved in 355 ml water and 6ml formalin) was added. This was stirred and centrifuged (Gallenkamp Angle head centrifuge Cat.No CFB 700 0100 HZ50) at 3000 rpm for 10 minutes. The supernatant was carefully removed until about 2 ml pellet was left in the centrifuge tube (Uneke and Uneke, 2008). Pellets from water were stained by the modified Ziehl – Neelsen method. The remaining backwashed water was left for 24 hours for sedimentation to take place. The top water was then discarded carefully without shaking and the remaining 5 ml was centrifuged (Gallenkamp Angle head centrifuge Cat.No CFB 700 0100 HZ50) at 2000 g for 5 min. The supernatant discarded and the residue were agitated gently by hand in physiological saline solution for further distribution of the cysts and eggs in the residue, then examined in Lugol's iodine (10.5% in distilled water) by using light microscope according to Bailenger, (1962 cited in Daryani *et al.*, 2008) for observation of helminth eggs.

3.6 Modified Ziehl – Neelsen staining method

Smears were prepared from the SAF preserved fractions and the samples for detection of *Cryptosporidium* cysts. After the smears have air-dried they were fixed with 70% methanol for 3 minutes. This was then stained with Carbol-fuchsin (0.34% fuchsine and 4% w/v phenol) for 30 minutes which was then washed off with tap water. The smears were then decolorized using acid alcohol (1% hydrochloric acid–ethanol 95%) for 1minute and were counter-stained with 1% methylene blue for another 1minute. The stain was again washed off with tap water and the smears were microscopically examined by using 1000x magnification (Henriksen and Pholenz, 1981).

3.7 Ethical consideration

Informed consent was obtained from the vegetable farmers as the samples were collected.

4. DATA ANALYSIS

Data were entered into Microsoft Excel and analyzed using SPSS version 17. P-values were calculated using Chi-square test appropriate. A P-value <0.05 was considered statistically significant.

5. RESULTS

5.1 Prevalence of intestinal parasites on four vegetables

Out of the 384 vegetable samples from four farms and two markets comprising 96 samples of each vegetable- carrot, cabbage, tomato and lettuce, 148(38.5%) vegetable samples were contaminated with one or more parasites. Of the 4 vegetable types examined, lettuce was the most contaminated, 58.3%, followed by cabbages 37.5%, tomato 33.3% and carrot 25.0% (Table 1).

Three species of parasitic protozoa, including *Cryptosporidium* spp, *Entamoeba histolytica/dispar*, *Giardia lamblia* and the commensal *Entamoeba coli* which was present on almost all the samples and five species of helminths - hookworm, *Enterobius vermicularis*, *Ascaris lumbricoides*, *Trichuris trichiura* and *Taenia* spp., were detected (Table 1).

On helminth contamination of lettuce samples, *A. lumbricoides* was the most common (34.4%) followed by hookworm (16.7%). Among the protozoa, *Cryptosporidium* spp (17.7%) was the most common followed by *E. histolytica/dispar* (10.4%) and *Giardia lamblia* (10.4%). The study also indicated that the most prevalent parasite on the four vegetable types examined was *A. lumbricoides* (20.3%) (Table 1).

Further analysis of vegetable contamination with parasites showed that 25.5% of the vegetables were contaminated with only one species of parasite while 9.6% were contaminated with two species and 3.1% were contaminated with three parasites and only a few 0.3% with quadruple contaminants. Single contaminations were high on the cabbage samples while multiple contaminations were common on lettuce samples (Table 2).

Table 1: Comparison of the level of parasite contamination of four vegetables- carrot, lettuce, tomato and cabbage in Addis Ababa, Ethiopia (December 2009- October 2010).

	Vegetables				Total n =384 (%)
	Carrot	Lettuce	Tomato	Cabbage	
Parasites detected	n=96 (%)	n =96 (%)	n =96 (%)	n =96 (%)	
<i>Cryptosporidium spp</i>	6(6.3)	17(17.7)	3(3.1)	7(7.3)	33(8.6)
<i>Entamoeba histolytica/dispar</i>	7(7.3)	10(10.4)	6(6.3)	6(6.3)	29(7.6)
<i>Giardia lamblia</i>	2(2.1)	10(10.4)	3(3.1)	6(6.3)	21(5.5)
<i>Enterobius vermicularis</i>	4(4.2)	4(4.2)	6(6.3)	2(2.1)	16(4.2)
Hookworm	2(2.1)	16(16.7)	3(3.1)	5(5.2)	26(6.8)
<i>Ascaris lumbricoides</i>	12(12.5)	33(34.4)	16(16.7)	17(17.7)	78(20.3)
<i>Taenia spp</i>	0	3(3.1)	1(1.0)	0	4(1.0)
<i>Trichuris trichiura</i>	1(1.0)	4(4.2)	0	0	5(1.3)
Total parasite contaminated vegetables	24(25.0)	56(58.3)	32(33.3)	36(37.5)	148(38.5)
Frequency of parasites detection*	34(35.4)	97(101)	38(39.6)	43(44.8)	212(55.2)

*One vegetable sample might be contaminated by more than one parasite at the same time

Table 2: Frequency of single and multiple parasite contamination of the four vegetables-carrot, lettuce, tomato and cabbage in Addis Ababa, Ethiopia (December 2009- October 2010).

Vegetables					
Parasites detected	Carrot	Lettuce	Tomato	Cabbage	Total
	n=96 (%)	n=96 (%)	n=96 (%)	n=96 (%)	n=384 (%)
Single parasite contamination	16(16.7)	27(28.1)	26(27.1)	29(30.2)	98(25.5)
Double parasite contamination	6(6.3)	18(18.8)	6(6.3)	7(7.3)	37(9.6)
<i>A.lumbricoides</i> / <i>Cryptosporidium</i> spp	2	2	1	3	8
<i>A.lumbricoides</i> / <i>E. histolytica/dispar</i>	2	1	1	1	5
<i>Cryptosporidium</i> spp / <i>E. histolytica/dispar</i>	1	3			4
<i>Cryptosporidium</i> spp / Hookworm	1	1			2
<i>A.lumbricoides</i> / <i>Taenia</i> spp		2			2
<i>A.lumbricoides</i> / Hookworm		3	1	1	5
<i>A.lumbricoides</i> / <i>Giardia lamblia</i>		2	1	1	4
<i>A.lumbricoides</i> / <i>Enterobius vermicularis</i>		1	1	1	3
Hookworm / <i>Giardia lamblia</i>		3			3
<i>Cryptosporidium</i> spp / <i>Giardia lamblia</i>			1		1
Triple parasite contamination	2(2.1)	10(10.4)	0	0	12(3.1)
<i>A.lumbricoides</i> / <i>Cryptosporidium</i> spp / <i>Giardia lamblia</i>	1	1			2
<i>A.lumbricoides</i> / Hookworm / <i>Enterobius vermicularis</i>	1				1
<i>A.lumbricoides</i> / <i>Cryptosporidium</i> spp / <i>E. histolytica/dis</i>		1			1
<i>A.lumbricoides</i> / <i>Enterobius vermicularis</i> / <i>Trichuris trichiui</i>		1			1
<i>A.lumbricoides</i> / <i>Enterobius vermicularis</i> / <i>E.histolytica/dispar</i>		1			1
<i>Cryptosporidium</i> spp / Hookworm / <i>Taenia</i> spp		1			1
<i>A.lumbricoides</i> / Hookworm / <i>Cryptosporidium</i> spp		2			2
<i>A.lumbricoides</i> / Hookworm / <i>E. histolytica/dispar</i>		1			1
<i>A.lumbricoides</i> / <i>Trichuris trichiura</i> / Hookworm		2			2
Quadruple parasite contamination	0	1(1.0)	0	0	1(0.3)
<i>A.lumbricoides</i> / <i>Cryptosporidium</i> spp / <i>E.histolytica/dispar</i> / <i>Enterobius vermicularis</i>		1			

5.2 Pictures of parasites taken during the study

The parasite isolation method used revealed the typical cysts of *Entamoeba histolytica/dispar*, *Giardia lamblia* and the commensal *Entamoeba coli*, the oocyst of *Cryptosporidium* spp and eggs of helminths - hookworm, *Enterobius vermicularis*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Taenia* sp and *Fasciola hepatica* from the vegetables and/or irrigation water samples (Figure 9).



(a)

(b)

(c)

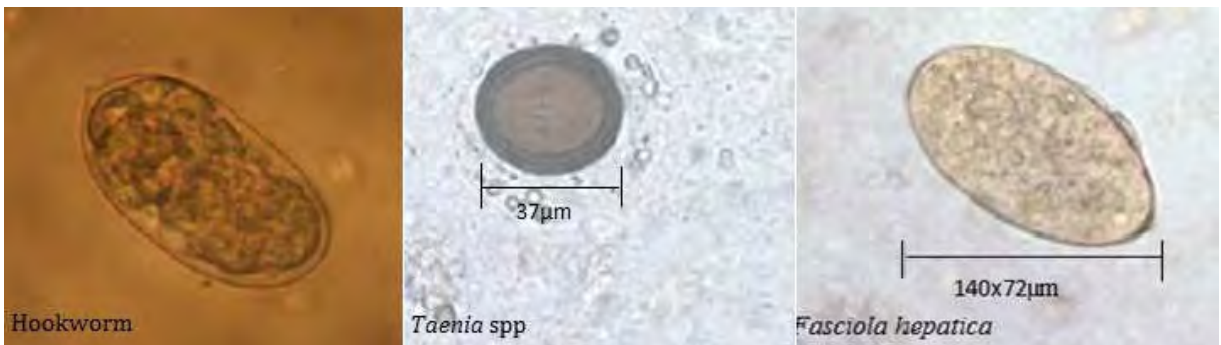
(d)



(e)

(f)

(g)



(h)

(i)

(j)

Figure 9: Light microscopic pictures of cysts (a-c), eggs (e-j) of parasites in wet mount magnification 400x and oocyst (d) in modified Ziehl-Neelsen stain, magnification 1000x, isolated from vegetable and water samples in Addis Ababa, (December 2009-October 2010).

5.3 Parasite contamination of the four vegetable types in the two market sites

When the parasite contamination rates of market vegetable samples were compared, lettuce samples showed statistically significant higher rates than carrot and tomato ($p < 0.05$). Likewise, Cabbage was more contaminated than tomato ($P < 0.034$) where as the contamination rate of the non leafy vegetables were more or less similar (Table 3). On the other hand comparison of parasite contamination between the two markets-Merkato and Atkilttera showed equal contamination (37.5%) for cabbage while carrot and tomato contamination showed significant difference between the two sites ($P < 0.05$) and lettuce was the highly contaminated in both markets (Figure 10).

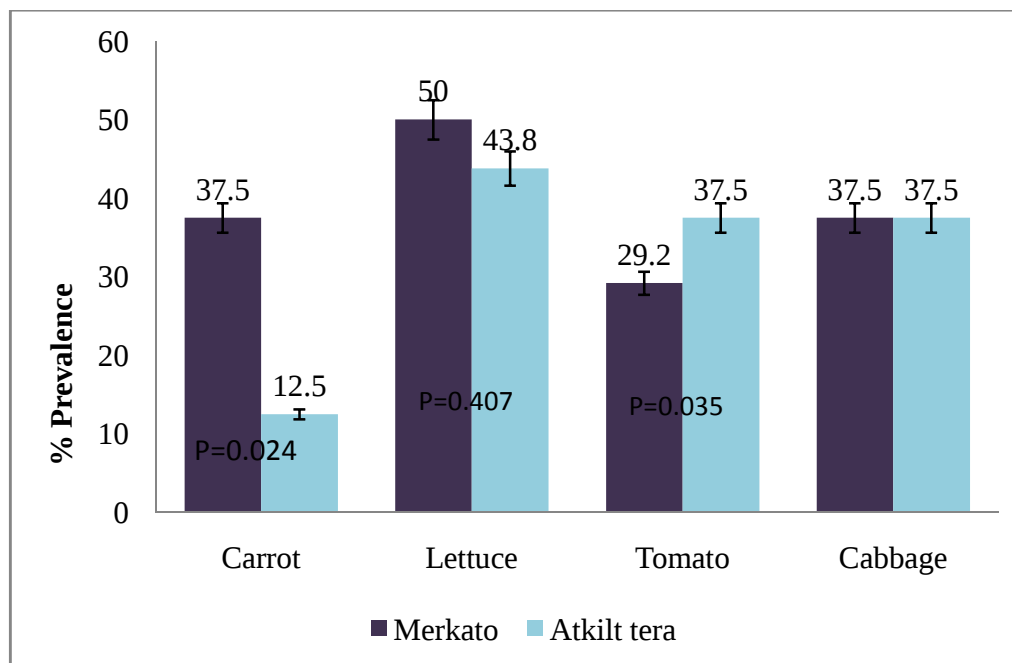


Figure 10: Comparison of overall prevalence of parasite contamination of four vegetables-carrot, lettuce, tomato and cabbage- between markets- Merkato and Atkilt tera- in Addis Ababa, (December 2009- October 2010).

Table 3: Comparison of total parasite contamination between vegetables from markets in Addis Ababa, Ethiopia (December 2009- October 2010).

Vegetables compared n (%)	Total parasite contamination n(%)	X²	P-Value
Lettuce (n=32) Carrot (n=96)	15(46.9) 24(25.0)	4.095	0.043 ^a
Lettuce (n=32) Tomato (n=96)	15(46.9) 32(33.3)	5.935	0.015 ^a
Lettuce(n=32) Cabbage (n=96)	15(46.9) 36(37.5)	6.981	0.08
Carrot (n=96) Tomato (n=96)	24(25.0) 32(33.3)	10.351	0.090
Carrot (n=96) Cabbage (n=96)	24(25.0) 36(37.5)	3.289	0.070
Cabbage (n=96) Tomato(n=96)	36(37.5) 32(33.3)	4.507	0.034 ^a

^a- Represents statistically significant difference (P<0.05)

5.4 Parasite contamination of lettuce among the different farm sites

The rate of parasitic contamination of vegetables collected from different farms showed different levels of contamination. That is, at Kaliti (29.3%), Goffa and Peacock the same level (24.4%) and at Kera (21.9%) contamination were detected. The parasites detected were *Ascaris lumbricoides* (39.1%) was the dominant, followed by Hookworm (20.3%) and *Cryptosporidium* spp (18.8%) in farm lettuce (Table 4).

Table 4: Prevalence of intestinal parasites on lettuce among the different farms in Addis Ababa, Ethiopia (December 2009- October 2010).

Parasites	Lettuce				
	Kaliti(n=16)	Kera(n=16)	Peacock(n=16)	Goffa(n=16)	Total(n=64)
	No	No	No.	No	No
	positive(%)	positive(%)	positive(%)	positive(%)	positive(%)
<i>Cryptosporidium</i> spp	4(25.0)	3(18.8)	3(18.8)	2(12.5)	12(18.8)
<i>Entamoeba histolytica/dispar</i>	1(6.3)	3(18.8)	1(6.3)	3(18.8)	8(12.5)
<i>Giardia lamblia</i>	4(25.0)	1(6.3)	2(12.5)	1(6.3)	8(12.5)
<i>Enterobius vermicularis</i>	2(12.5)	1(6.3)	0	1(6.3)	4(6.3)
Hookworm	0	4(25.0)	6(37.5)	3(18.8)	13(20.3)
<i>Ascaris lumbricoides</i>	8(50.0)	4(25.0)	6(37.5)	7(43.8)	25(39.1)
<i>Taenia</i> spp	0	1(6.3)	1(6.3)	0	2(3.1)
<i>Trichuris trichiura</i>	1(6.3)	0	0	3(18.8)	4(6.3)
Positive samples	12(29.3)	9(21.9)	10(24.4)	10(24.4)	41(64.1)

n (%) - number positive and percentage prevalence; N-number of observation

5.5 Relative prevalence of parasites between farm and market lettuce samples

Out of the 96 samples of lettuce collected from the two Markets, 46.9% and from the four farms 64.1% tested positive for parasites. Considering the contaminating parasite species, out of the parasite positive market lettuce samples, *Ascaris lumbricoides* was the most frequent contaminant (25.0%) followed by *Cryptosporidium* spp (15.6%). Similarly, in farm lettuce samples the parasite with the highest prevalence was *Ascaris lumbricoides* (39.1%) followed by hookworm (20.0%) and *Cryptosporidium* spp (18.8%). And, overall, parasite contamination of farm lettuce was significantly ($P<0.000$) more contaminated than the market lettuce (Table 5).

Table 5: Prevalence of intestinal parasite contamination of lettuce samples from farms and markets in Addis Ababa, Ethiopia (December 2009- October2010).

Parasites	Lettuce			X ²	P –value
	Markets n=32(%)	Farms n=64(%)	Total n=96(%)		
<i>Cryptosporidium</i> spp	5(15.6)	12(18.8)	17(17.7)	3.367	0.067
<i>Entamoeba histolytica/dispar</i>	2(6.3)	8(12.5)	10(10.4)	0.818	0.366
<i>Giardia lamblia</i>	2(6.3)	8(12.5)	10(10.4)	1.360	0.243
<i>Enterobius vermicularis</i>	0	4(9.4)	4(4.2)	----	----
Hookworm	3(9.4)	13(20.3)	16(16.7)	3.391	0.066
<i>Ascaris lumbricoides</i>	8(25.0)	25(39.1)	33(34.4)	4.205	0.040 ^a
<i>Taenia</i> spp	1(3.1)	2(3.1)	3(3.1)	1.333	0.248
<i>Trichuris trichiura</i>	0	4(6.3)	4(4.2)	-----	-----
Overall positive samples	15(46.9)	41(64.1)	56(58.3)	13.253	0.000 ^a

^a- Represent statistically significant difference (P<0.05)

5.6 Parasite prevalence from water examination

Examination of irrigation waters used for vegetable cultivation showed contamination of water samples by protozoan parasites *Entamoeba histolytica/dispar*, *Giardia lamblia* and *Cryptosporidium* spp and eggs of *Ascaris lumbricoides*, *Trichuris trichiura*, *Fasciola* spp and *Hymenolopis nana* were detected. Water samples from all study sites were contaminated with commensal *Entamoeba coli*, *Giardia lamblia* and *Ascaris lumbricoides*. *Cryptosporidium* spp detected in all except Kera and *Entamoeba histolytica/dispar* and *Fasciola hepatica* detected only in kaliti (Table 6).

Table 6: Occurrence of parasite cysts, oocysts and eggs in 30 liters irrigation water samples from each farm site in Addis Ababa, Ethiopia (December 2009- October 2010).

Parasites detected	Farm site			
	Kaliti	Kera	Peacock	Goffa
<i>Hymenolopis nana</i>	-	+	-	-
<i>Cryptosporidium</i> species	+	-	+	+
<i>Entamoeba histolytica/dispar</i>	+	-	-	-
<i>Giardia lamblia</i>	+	+	+	+
<i>Ascaris lumbricoides</i>	+	+	+	+
<i>Trichuris trichiura</i>	+	+	+	-
<i>Fasciola</i> spp	+	-	-	-

+ represents presence of parasite; - represents absence of parasite

6. DISCUSSION

This study has shown that vegetables can get contaminated with eggs of *Ascaris*, hookworms, *Taenia* spp, *E. vermicularis* and *T. trichiura* and oocysts of *Cryptosporidium*, and cysts of *Giardia* and *E. histolytica/dispar* that cause infections in humans. The presence of parasites on the vegetables is an indication that contamination may occur in a variety of ways such as contact with the soil, the water used for irrigation and to freshen up vegetables, non-hygienic conditions in markets and from contaminated hands of market retailers, each of which were reported from cities in west Africa by (Amoah *et al.*, 2007) as a potential source of contamination with pathogenic organisms.

The association of parasite contamination of vegetables with sewage polluted waters used for irrigation has been demonstrated even from developed countries such as Norway (Robertson and Gjerde 2001a). Therefore, contamination of vegetables from the use of irrigation water contaminated with human-borne and animal-borne parasites such as *Cryptosporidium* spp, *Giardia lamblia* and *Fasciola* spp or just human borne parasites such as *E. histolytica/dispar*, *A. lumbricoides*, hookworms, *Taenia* spp, *E. vermicularis* and *T. trichiura* from Addis Ababa, is to be expected.

It is well established that acidification, chlorination and desiccation cannot destroy the resistant stages of intestinal protozoan parasites (Fayer *et al.*, 2000; Hunter and Thompson 2005; Singh *et al.*, 2009). As a result, they have been implicated in many water borne and food borne diarrheal out-breaks (Karanis *et al.*, 2007). A massive waterborne *Cryptosporidium* outbreak in the city of Milwaukee, USA, involving an estimated 403,000 persons (MacKenzie *et al.*, 1994) and a large waterborne giardiasis outbreak described to date occurred in Norway between October and December 2004, affecting more than 1500 cases (Robertson *et al.*, 2006).

The finding of this study that the irrigation water contained cysts and eggs, that are known to be highly resistant to environmental stress and hazard, is an indicator of the source of vegetable contamination. Other epidemiological studies have also revealed parasitic contamination of vegetables. These include the study by Abougrain *et al.* (2010) who recorded helminth eggs and *Giardia* cysts in 58% of salad vegetables in Tripoli, Libya and a study from Kisii municipality in Kenya that reported 65.5 % parasite contamination of raw vegetables (Nyrango *et al.*, 2008). Similar studies from other parts of the world have shown vegetable contamination with *Cryptosporidium* spp and related coccidian parasites to be common. These include a 14.5% prevalence of *C. parvum* and *Cyclospora cayetanensis* from Peru (Ortega *et al.* 1997) and 8% prevalence in seed sprouts from Norway (Robertson *et al.* 2002).

Furthermore, variation in contamination between vegetable types has been observed both in the developed countries such as Norway, whereby lettuce was 45% contaminated with *Cryptosporidium* spp and 17.7% in the present study. A relatively high prevalence of *Cryptosporidium* and *Giardia* seen in lettuce in relation to the other vegetables, could be attributed to its greater surface area and leafy foliage which better accommodate these parasites.

Similar reports for *Giardia lamblia* vegetable contamination that support the findings of the present study also exist. These include a study by Abougrian *et al* (2010), in Tripoli, Libya, who reported *Giardia* prevalence of 10% from total vegetables; another study that reported 7% *Giardia* prevalence on vegetables consumed in Ardabi, Iran Daryani (2008) and the reports of Monge and Arias (1996) in Costa Rica (5%).

As zoonotic parasites, the origin of *Giardia lamblia* and *Cryptosporidium* spp cysts/oocysts that contaminate the vegetables in Addis Ababa could be both human and animal fecal materials known to be released untreated into the water bodies used for irrigation. The possible zoonotic involvement was substantiated by the finding of Adamu *et al.*, (2010) that reported majority of human cryptosporidiosis in Ethiopia to be zoonosis.

The finding that vegetable contamination by *Cryptosporidium* spp (25%) and *Giardia lamblia* (25%) in Kaliti was higher compared to the other farms, which could be attributed to the location of the farms, where cattle and other farm animals graze around, drink and wade through the streams used for irrigation. *Cryptosporidium* oocysts, and *Giardia* cysts are highly resistant and excreted in the feces of infected hosts by which irrigation water sources can be polluted.

Similar studies from other parts of the world have also shown vegetable contamination with *E. histolytica/dispar*, which has been the best documented food and water borne parasite (Jackson, 1990). These include Turkey and Nigeria (Erdogrul and Sener, 2005; Damen *et al.*, 2008). A substantial amount of vegetables have also been contaminated with the abundant *E. coli*, non-pathogenic protozoa, which is a well established indicator of faecal contamination (Daryani, 2008), indicating the likelihood of vegetable contamination in Addis Ababa.

Helminths such as *A. lumbricoides*, *T. trichiura* and *E. vermicularis* can be transmitted to humans through consumption of vegetables irrigated with water contaminated with human faeces and animal faeces as helminth eggs persist for long periods in the environment (Amhamid *et al.*, 1999). As a result epidemics of Ascariasis associated with food contaminated with wastewater have been reported (Bryan 1977). The actual risk of infection for people exposed to wastewater is also the highest for *A. lumbricoidis*, *T. trichiura* and hookworms (WHO 1989).

Ascaris lumbricoides is known to have the most environmentally resistant eggs among the enteric pathogens (Watson *et al.*, 1983). Hence it is often used as a parasitological indicator of fecal contamination of sewage sludge. Among the different intestinal helminth parasites identified in the study, *A. lumbricoides* was the most predominant species observed in the four vegetable samples and recorded as the highest vegetable contaminant. Similar studies from other parts of the world have shown vegetable contamination with *A. lumbricoides* to be most dominant (Amahmid *et al.*, 1999; Gharavi *et al.*, 2002 and Erdogrul and Sener, 2005).

Hookworm was the second most prevalent helminth, which could be attributed to direct contact of the vegetables with wastewater contaminated soil, similar to that found in the city of Jos, Nigeria (Damen *et al.*, 2008). The fact that hookworm eggs are fragile indicates that sewage contamination of the irrigation water may have been continuous and fresh. Furthermore, variation in hookworm egg contamination between vegetable types has been observed, whereby lettuce was (16.7%) contaminated with hookworm and when hookworm prevalence was compared between market and farm lettuce samples, farms with 20.3% were more contaminated than markets (9.4%). This could be attributed to its greater surface area and leafy foliage which are in direct contact with the wastewater contaminated soil surface (Gupta *et al.*, 2009). Moreover, the presence of hookworm eggs indicates the potential of infectious L3larva stage. Therefore, as hookworms are major causes of chronic anemia, this is cause for serious concern for the wellbeing of the population that works in vegetable farms in Addis Ababa.

Vegetables examined in the study were also contaminated with *T. trichiura*. Similar studies from other part of the world have shown vegetable contamination with *T. trichiura*. These include 3% prevalence on lettuce and 2% on cabbage in Ghana (Amoah *et al.*, 2007). The detection of *E. vermicularis* as a vegetable contaminant has been reported from Turkey where it was isolated

from a high percentage of vegetables (Erdogrul and Sener 2005). *Taenia* spp contamination of vegetables has also been reported from Iran (Gharavi *et al.*, 2002). Since some vegetable leftovers are also fed to milk cows raised in Addis Ababa, *Taenia saginata* life cycle can be easily maintained, since the cows are ultimately slaughtered for consumption. If *Taenia* eggs found on vegetables belong to *Echinococcus granulosus*, eating raw vegetables contaminated with these eggs may increase the risk of infection with hydatid cysts, which is a much severe health hazard to humans.

Variation in contamination between vegetable types has been observed, whereby lettuce was (58.3%) and cabbage (37.5%) contaminated with pathogenic parasites, which is a relatively higher prevalence compared to carrot and tomato. This finding is in line with that of Uga *et al.* (2009) in Hanoi, Vietnam who explained the observation as a factor of increased surface area to volume ratio, which will lead to increased exposure to the environmental contaminants such as parasite cysts and eggs. This foliage would also protect cysts and eggs against hostile environmental factors. Carrots recorded the least parasitic contamination which also is similar to the study in Turkey (Kozan *et al.*, 2005). Even if carrots have direct contact with soil, their smoother surface may not allow the accumulation of parasites as much as the larger surfaced vegetables.

Furthermore, the variation in the prevalence of contamination between lettuce from the markets and farms was similar to the report by Daryani *et al.* (2008) in Ardabil, Iran, who reported the detection of intestinal parasites in 50% of market and 71% of garden vegetables. The difference in the contamination between farm and market lettuce samples may be explained by the possibility that market lettuces may have been washed before sampling with clean water at the markets. It is possible such treatment would remove some, if not all, parasite contaminants.

The presence of parasites in irrigation water and contamination of vegetables from the farms and markets with parasites increases the risk of parasite infection to consumers who come in contact with and consume the products in Addis Ababa. Furthermore, the contact with livestock manure (Kaliti vegetable farm) and the washing of vegetables in contaminated river water can be important sources of vegetable contamination. *Faciola hepatica* eggs were also observed from Kaliti river water samples which may indicate the presence of metacercaria on vegetables. *Faciola hepatica* life cycle can also be maintained, since farm animals feed vegetable leftovers. It was also observed that vegetables may also get contaminated post-harvest by splashing with water that may not be clean, which the vendors use over and over again from the same bucket to keep vegetables moist and fresh in the markets. In addition to irrigation with wastewater, vegetables may also get contaminated during handling- storage, transport, or direct contamination from hands of food handlers who may be infected or from flies that land on food and faeces, hence increasing risks of transmission of intestinal parasites to consumers and farm workers.

7. CONCLUSION

- ✓ Contamination of vegetables in Addis Ababa markets and farms, with *Ascaris lumbricoides*, *Entamoeba coli*, *Cryptosporidium* spp, *Entamoeba histolytica/dispar*, hookworm, *Giardia lamblia*, *Enterobius vermicularis*, *Trichuris trichiura* and *Taenia* spp, therefore is a potential source of infection to humans.
- ✓ *Ascaris lumbricoides* was the most prevalent contaminant in the four vegetables considered in the study.
- ✓ The higher risk is on lettuce and cabbage that because of their larger surface and dense foliage bear more parasites.
- ✓ Parasite prevalence was also higher in farm lettuce samples when compared to market lettuce samples.
- ✓ Irrigation water examination from Addis Ababa detected parasite cysts and ova, providing evidence for domestic waste polluted rivers as the possible source of fecal contamination of vegetables.
- ✓ It is possible that vegetable contamination with parasites in Addis Ababa markets could result from the practice of splashing with water that may have been contaminated with parasites, to freshen up vegetables.
- ✓ Contamination of raw vegetables from farms and markets in Addis Ababa with pathogenic parasites would increase the risk of disease to the population that consumes or works with these products.

8. RECOMENDATIONS

- Reduce vegetable contamination with parasites by proper wastewater treatment before use for irrigation.
- Proper municipal wastewater and sewerage disposal systems must be implemented in Addis Ababa.
- A comprehensive health education must be provided to the farming community, market venders, food handlers and the general population to create awareness of the risk involved in the use of polluted water bodies for irrigation of vegetables.
- Thorough washing and disinfection of vegetables are highly recommended prior to consumption.
- Conduct study on farm workers, market venders and food handlers to better establish the epidemiology of these parasites in Addis Ababa.
- Training of market venders and food handlers on the proper washing and handling of vegetables.

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