



**ASSESSMENT OF INTESTINAL PARASITIC CONTAMINATION OF RAW
VEGETABLES COLLECTED FROM SELECTED MARKETS IN HAWASSA
CITY, ETHIOPIA**

M.Sc. THESIS

BY

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HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

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DECLARATION

I hereby declare that this MSc Specialty or equivalent thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been properly acknowledged.

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ACRONYMS/ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
DALYs	Disability-adjusted life-years
HIV	Human immune viruses
IPIs	Intestinal parasitic infections
ITS	Internal Transcribed Spacer
MZN	Modified Ziehl-Neelsen
NTDs	Neglected Tropical Diseases
PARF	Population Attributable Risk Fraction
PSAC	Preschool-Aged Children
SAC	School-Aged Children
SNPs	Single Nucleotide Polymorphisms
STH	Soil-Transmitted Helminths
WASH	Water, Sanitation, and Hygiene
WRA	Women of Reproductive Age

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ABSTRACT

Intestinal parasite infections are the most common cause of disease in humans and the main contributor to morbidity and mortality. Consumption of unwashed raw vegetables is one of the key entry routes for such illnesses. This study focuses on assessing the presence of intestinal parasites on raw vegetables sold in selected markets in Hawassa City. A cross-sectional study was conducted from February to April 2023 to determine the parasitic contamination of vegetables. The samples included 384 vegetables selected from 6 types of vegetable including lettuce, cabbage, carrot, tomato, green pepper, and potato. The samples were transported to the laboratory and analyzed using direct microscopy and staining methods. The data were analyzed using the Wilcoxon sign ranked test in SPSS software. Analyses revealed that out of the 384 samples collected, 196(51.04%) were contaminated with intestinal parasites. The identified parasite include Giardia lamblia 31.7%, Entamoeba histolytica 26.8%, Ascaris lumbricoides 21.3%, Cryptosporidium parvum 13.5%, Strongyloides stercoralis 12.2%, Enterobius vermicularis 6.7%, Balantidium coli 6.5%, Entamoeba coli 3.1%, hookworm 2.6%, Cyclospora caytanesis 1.8%, Isospora belli 1.8% and Trichuris trichiura 1.8%. The contamination varied among different vegetable types, with cabbage 75%, lettuce 70.3%, potato 56.3%, tomato 39.1%, carrot 37.5% and green pepper 28.1%. The samples were contaminated with eggs 22.6%, larvae 22.6%, cysts 61.7%, trophozoites 6.5% and oocysts 17.1%. The findings of this study highlight the presence of intestinal parasites on raw vegetables sold in selected local markets in Hawassa City, Ethiopia. The vendors and consumers should practice proper sanitation and hygiene practices, as well as wear appropriate footwear.

Keywords: Contamination, Hawassa City, intestinal parasite, raw vegetables.

1. INTRODUCTION

Intestinal parasites are organisms that live in the gastrointestinal tract of humans and animals, feeding off their hosts. These parasites can be worms, protozoa, or other microorganisms. Infections with intestinal parasites are very common and pose a threat to public health. Situations of poverty, contaminated food such as vegetables, crowded living situations, and a lack of sanitation or hygiene are all strongly associated with infections with medically significant parasites (intestinal helminths and protozoa) (Bekele Fitsum *et al.*, 2017).

Intestinal parasite infections have been linked to several physical and mental health issues, such as iron deficiency anemia and infant development retardation. It is estimated that more than three billion individuals are impacted and 350 million individuals have been exposed to these illnesses (WHO, 2020). Recent cases of foodborne illness have been associated with fresh vegetable eating, particularly in developing countries (Gargiulo *et al.*, 2022). Protozoa and helminth parasites are the primary causes of these foodborne illnesses, which have substantial negative health and economic effects (Khan *et al.*, 2021).

The presence of intestinal parasites in food has a detrimental impact on both the health and economic well-being of individuals in the world. The high cost associated with treating these infections, coupled with the subsequent need for hospitalization, poses a significant socioeconomic burden. Intestinal parasite infections (IPIs), comprising helminths and protozoa, remain highly prevalent worldwide. These infections continue to affect a significant portion of the global population. The largest and most prevalent helminth, *Ascaris lumbricoides*, infects around 819 million people worldwide. *Trichuris trichiura*, which infects approximately 464.6 million people, and hookworm, which infects approximately 438.9 million people worldwide, are the next most frequent helminths

(Pipikova *et al.*, 2017). The two most common protozoan illnesses, affecting 200 million and 500 million people, respectively, are giardiasis and amebiasis, brought on by *Giardia duodenalis* and pathogenic *Entamoeba spp.* respectively (Hailemeskel Elifaged *et al.*, 2018).

Soil-transmitted helminths (STH) infections are most prevalent in tropical and subtropical areas, including China, India, Southeast Asia, and sub-Saharan Africa. In sub-Saharan Africa, infections caused by disease-causing helminths and protozoan species are considered to be widespread. The spread of these infections is caused by geographical location, socioeconomic circumstances, lack of proper sanitation facilities, personal hygiene conditions, and poverty (Biniyam Sahiledengle *et al.*, 2020).

Interestingly, Ethiopia contributes to the highest burden of intestinal parasitic infections (IPIs) and accounts for 8% of the global STH (Chelkeba Legese *et al.*, 2020). In the country, IPIs are the second most predominant cause of outpatient morbidity among preschool-age and school-age children (Chelkeba Legese *et al.*, 2022). The global estimate showed that school-aged children were highly infected with Hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*, and other STH species (Biniyam Sahiledengle *et al.*, 2020).

In underdeveloped nations, contaminated vegetables that cause foodborne illness are still a hazard to public health, particularly when they are sold directly from gardens, marketplaces, and the streets. Over the past twenty years, human diseases caused by consuming raw vegetables have risen alarmingly (Obaji *et al.*, 2018). Both high-income and poor countries have documented intestinal parasite outbreaks linked to raw vegetables (Ojo, 2023).

There are several ways to get an intestinal parasite infection, including by consuming contaminated water, food, or vegetables. Vegetables can be easily contaminated by hygiene issues during planting, harvesting, packaging, transportation, and storage, where the sources of contamination are typically feces and fecal-infected soil or water (Bekele Fitsum *et al.*, 2017). In particular, raw and unpeeled vegetables can act as carriers of intestinal protozoan and helminth infection (Auta *et al.*, 2017).

Numerous studies have found a high correlation between raw vegetables in particular and parasite diseases. Furthermore, raw vegetables have been associated with numerous outbreaks of protozoan infections in humans (Daryani *et al.*, 2012). People who eat contaminated, undercooked, or improperly washed vegetables might contract intestinal parasites such *Ascaris lumbricoides*, *Cryptosporidium species*, *Entamoeba histolytica*, *Enterobius vermicularis*, *Giardia intestinalis*, hookworm species, *Taenia species*, and *Trichuris trichiura* (Eraky *et al.*, 2014).

Additionally, studies demonstrate that eating unclean, uncooked, and improperly prepared vegetables, such as cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa*), Eggplants (*Solanum melongena*), cucumber (*Cucumis sativus*), carrot (*Daucus carota*), water leaf (*Talinum fruticosum*), pumpkin (*Cucurbita pepo*), spinach (*Spinacia oleracea L.*), and tomato (*Solanum lycopersicum*), is considered a risk factor for human parasitic infections (Mohamed *et al.*, 2016). Understanding the prevalence and types of parasites present in these vegetables is crucial for implementing effective preventive measures and promoting food safety practices. Therefore, this study aimed to assess the intestinal parasitic contamination of raw vegetables collected from selected markets in Hawassa City, Ethiopia. The results might influence better methods for handling, cleaning, and preparing vegetables to safeguard customers from intestinal parasite diseases.

1.1 Statement of the Problems

Intestinal parasites, such as helminths and protozoa, pose a significant public health risk including giardiasis, amoebiasis, and helminth infections. They are commonly transmitted through the ingestion of contaminated food, especially raw vegetables. Raw vegetables, particularly if grown in areas with poor sanitation practices, can become contaminated with parasite eggs or cysts from fecal matter. This makes vegetables an important potential source of infection. It is commonly known that eating raw vegetables greatly increases the risk of contracting an intestinal parasite, especially for those parasites that can survive in unfavorable environments (Bezanson *et al.*, 2014).

Several surveys have been done in different parts of the world such as in our country Ethiopia (Bekele Fitsum *et al.*, 2017), (Endale Adugna *et al.*, 2017) (Hailemeskel Elifaged *et al.*, 2018), (Alemu Getaneh *et al.*, 2020); Syria (Alhabbal, 2015); Nigeria (Auta *et al.*, 2017); Egypt (Etewa *et al.*, 2017); Brazil (Rodrigues *et al.*, 2020); Pakistan (Umbreen *et al.*, 2014); Vietnam (Chau *et al.*, 2014); indicated that the vegetables can be a key source of protozoan cyst transmission (*Entamoeba histolytica*; *Giardia lamblia*; *Entamoeba coli*; *Balantidium coli*), oocysts (*Isospora belli*; *Cryptosporidium spp.*) and helminths eggs and larvae (*Strongyloides stercoralis*; *Trichuris trichiura*; *Enterobius vermicularis*; *Fasciola hepatica*; *Toxocara spp.*; *Taenia spp.*; *Ascaris lumbricoides*; *Hymenolepis nana*; *Hymenolepis diminuta*). These studies highlight the potential risk associated with the consumption of raw vegetables contaminated with intestinal parasites.

Hawassa City is a rapidly growing urban area with a significant population and an increasing demand for fresh food, including raw vegetables. However, there was a scarcity of information regarding the presence and prevalence of intestinal parasites on these raw vegetables within the city. This knowledge gap poses a potential health risk to the residents

of Hawassa City, as the consumption of contaminated vegetables can lead to the transmission of intestinal parasites and subsequent health complications.

The problem at hand is to assess the prevalence of intestinal parasites on raw vegetables in Hawassa City and identify the specific types of parasites. This assessment is crucial for understanding the magnitude of the problem, informing local health authorities, and implementing appropriate preventive measures to ensure the safety of raw vegetable consumption in Hawassa City. By addressing this problem, this study establishes evidence-based guidelines for vegetable producers, vendors, and consumers to minimize the risk of intestinal parasite infections. Ultimately, this will contribute to the improvement of public health in Hawassa City, promoting a healthier and safer environment for its residents. This finding could open a new avenue of research in vegetables and could also lead to better practices in handling vegetables to protect against intestinal parasitic infections.

1.2 Objectives of the Study

1.2.1 General objective

- ✚ To determine the magnitude of intestinal parasite contamination of raw vegetables in Hawassa City, Ethiopia.

1.2.2 Specific objectives

- ✚ To determine the most prevalent intestinal parasites on raw vegetables.
- ✚ To assess the most contaminated vegetables with intestinal parasites.
- ✚ To identify the types and stages of parasites that contaminate the vegetables.

1.3 Research Question

The study attempted to answer the following questions.

- ✚ What are the most prevalent intestinal parasites on raw vegetables?

- ✚ What are the most commonly contaminated vegetables with intestinal parasites?
- ✚ Which type and stages of the intestinal parasite contaminate the raw vegetables?

1.4 Significance of the Study

The information in this study is valuable for consumers in Hawassa as it allows them to identify the vegetables that are most likely to be contaminated. This information is crucial in raising awareness among the public about the transmission of intestinal parasitic infections through raw or slightly cooked vegetables. Consequently, people can improve their hygiene practices by thoroughly washing vegetables before consumption and ensuring clean hands before meals. The study's findings hold significant implications: they provide concrete information about intestinal parasitic infections on raw vegetables. Moreover, the study adds to the existing literature on foodborne diseases and contributes to public health knowledge. It also opens avenues for future research to address any gaps that this study may have overlooked.

1.5 Scope of the Study

The scope of the study for the assessment of intestinal parasitic contamination of raw vegetables in Hawassa City, Ethiopia, included the following components: the study area was focused specifically on selected local markets of Hawassa City such as Addis Gebeya and Aroge Gebeya. A diverse range of raw vegetables commonly consumed in Hawassa City was gathered, including leafy greens (such as lettuce, and cabbage), root vegetables (such as carrots, and potatoes), and other commonly consumed vegetables (such as tomato, and green pepper). The intestinal parasites present in the vegetable samples were included, such as protozoan parasites and helminths. The study was conducted by this scope, and it gained valuable insights into intestinal parasitic contamination on raw vegetables in

Hawassa City it also contributed towards enhancing food safety and public health in the local community.

1.6 Limitations of the Study

The findings of the study only apply to the specific context of Hawassa City and may not be representative of other cities or regions in Ethiopia. Indeed, the assessment of parasitic contamination of vegetables in Ethiopia has been conducted in specific regions, but there is still a need to address this issue in other regions as well. It is important to have a comprehensive understanding of the extent of parasitic contamination across the country to develop effective strategies for prevention and mitigation. To address this gap, future research and assessments should aim to cover a wider range of regions in Ethiopia. This would provide a more accurate representation of the overall situation and help identify regional variations in parasitic contamination.

2 LITERATURE REVIEW

2.1 Overview of vegetable

Vegetables are the edible portions of plants, specifically herbaceous plants, that are consumed as a nourishing food source. They can exhibit a range of flavors, including aromatic, bitter, or even tasteless characteristics. They are recognized for their high nutritional value, providing essential nutrients like polyphenols, vitamins, dietary fiber, proteins, carbohydrates, and minerals (Li *et al.*, 2017). Consuming fresh vegetables brings about several advantages, including the reduction of chronic disease risk, lowering cholesterol levels, and minimizing the chances of birth defects (Yemmireddy *et al.*, 2022). In general, including vegetables in a well-balanced diet is highly recommended by healthcare professionals. They emphasize the significance of vegetable consumption to patients due to the wide range of benefits associated with them (Archer *et al.*, 2015).

Furthermore, there is a growing preference among consumers for ready-to-eat foods such as fresh vegetables due to time constraints and the increasing demand for convenience. This has led to an increased emphasis on the importance of quick and easy meal options in response to the busy lifestyles of individuals (Abadias, 2018). Fresh vegetables offer an affordable means for most families to embrace the healthier standards that the world is progressing towards, requiring minimal effort. However, alongside the growing popularity and consumption of fresh vegetables, there has also been a rise in foodborne illnesses associated with it (Bennett *et al.*, 2018).

2.2 Intestinal Parasites

Parasites are defined as organisms that get food and shelter from other organisms or the host and often harm them. If the parasites have an intestinal life cycle stage it to be considered intestinal parasites. Additionally, it may have a life cycle stage in the heart,

circulation, lung, tissue, and other animals in the surroundings (Ayalew Fentahun *et al.*, 2019). The most common routes of entry of intestinal parasites into the human body are ingestion, skin penetration, and auto-infections. Ingestion (fecal-oral routes); contamination of water and food is generally the foremost style of transmitting protozoan infections (Baye Sitotawet *et al.*, 2019). IPIs comprise both helminths and protozoans which form the most common infections worldwide. IPIs, are a significant issue for low-income countries and are the main cause of high morbidity and death. The majority of infectious disorders brought on by intestinal parasites are classified as Neglected Tropical diseases (NTDs) and affect a large proportion of poor communities (Sitotaw Baye and Wakgari Shiferaw, 2020). Intestinal parasitic infections are widespread worldwide and cause significant public health, economic, physical, and cognitive development intimidation, particularly in children in developing countries such as Ethiopia. The poor personal hygiene, poor environmental hygiene, and poor health care systems commonly seen in developing countries make the prevalence highest among these populations. Nearly 450 million people have clinical morbidity from intestinal parasite infections, which affect over 3.5 billion people worldwide (Celestino *et al.*, 2021).

2.2.1 Intestinal protozoa

A wide variety of unicellular creatures called intestinal protozoa live inside the human gastrointestinal system. In the human body, protozoan parasites with just one cell can proliferate (Cama and Mathison, 2015). Most infections are brought on by drinking or consuming water or food that is contaminated and has cysts or oocysts in it (Torgerson *et al.*, 2014). Intestinal protozoa parasite infections, which are common throughout the world but are particularly prevalent in tropical and subtropical regions, cause millions of cases of diarrhea every year. The most significant causes of diarrhea are *E. histolytica*, *G.*

intestinalis, and *Cryptosporidium species*, which are all intestinal protozoa (Berhe Birhane *et al.*, 2018).

They can multiply rapidly in the body, leading to the development of a serious infection. Most protozoa infections are asymptomatic. However, common symptoms associated with it include abdominal discomfort, vomiting, and dysentery (Christy *et al.*, 2012). When the infection load is severe, it can lead to several complications, such as diarrhea, malaise, bloating, fatigue, upper abdominal discomfort, malnutrition, malabsorption, intestinal ulcers, gastroenteritis, weight loss, abscesses, intellectual disability, and even death. Protozoa infections in humans can also result in structural and functional abnormalities of the small intestine and be misdiagnosed as appendicitis or other inflammatory diseases of the gastrointestinal tract. Children are the main victims of gastrointestinal protozoa parasites. Therefore, disease control measures need to be focused on the pediatric group (Abdullah *et al.*, 2016).

2.2.1.1 Transmission of intestinal protozoa

The most frequent causes of intestinal parasites are ingestion of contaminated food, water, infected soil, and inadequate hygiene and sanitation (Ziegelbauer *et al.*, 2013). They are transmitted through the fecal-oral pathway. Except for *Dientamoeba fragilis* (*D. fragilis*), which lacks a cyst stage, they often display life cycles with oocysts and trophozoites stages. The mature cysts of protozoa are infectious forms of parasites and enter humans through contaminated food and drinking water. The cyst enters the alimentary canal, passes through the stomach unchanged (the cyst wall is not dissolved by the action of gastric juice), and then reaches the small intestine, *Giardia lamblia* (*G. lamblia*), *Balantidium coli* (*B. coli*), or the colon, *Entamoeba histolytica* (*E. histolytica*), where cysts release the active motile trophozoites. The trophozoites (vegetative form) cause distortion and necrosis of the

intestinal surface. Sometimes they invade the deeper layer, and some can reach the liver, as in the case of *E. histolytica* (Mills and Cumming, 2016).

2.2.1.2 Clinical presentation

The most frequent parasite intestinal ailment in the United States is giardiasis and among travelers with chronic diarrhea, but intestinal protozoa infections can range from asymptomatic to invasive disease (in the case of *E. histolytica* or *B. coli*) to severe and/or chronic and persistent diarrhea (in the case of giardiasis or individuals severely immunocompromised by spore-forming protozoa infections) (Abdullah *et al.*, 2016).

Clinical signs like dehydration, dysentery, and severe diarrhea could appear in people infected with *E. histolytica* (Nakada-Tsukui and Nozaki, 2016). However, having been infected with *G. lamblia* will lead to abdominal cramping, bloating, and diarrhea among the populations (Halliez and Buret, 2013). Cryptosporidiosis can cause self-limiting diarrhea in healthy people, but it can also cause persistent diarrhea in people with impaired immune systems. Weight loss and malnutrition can be seen in the communities as a result of the prevalence of diarrhea and dysentery in people with intestinal protozoa infection (Ferreira *et al.*, 2015).

2.2.1.3 Diagnoses

When patients present with gastroenteritis, doctors rarely order the proper tests, which makes it difficult to determine the incidence of protozoan infections, and there are no sensitive methods available to identify pathogenic protozoa in stool specimens (Fletcher *et al.*, 2012).

Giardiasis is a common gastrointestinal parasitic infection associated with diarrhea, stomach cramps, an upset stomach, and excessive gas. Although Giardia can be diagnosed using a variety of methods, its very recognizable appearance makes microscopic detection

easier. *Giardia* cysts can be observed in fresh smears, on formalin-ethyl acetate, or a permanent stained smear, although the latter is associated with a higher sensitivity for identification. Because encystation starts before passing through the colon, trophozoites are not always seen in stools. When *Giardia* is suspected but not present in feces, duodenal samples, such as those acquired by a string test, may be used for permanent stains and concentrated wet mounts (Feng and Xiao, 2011).

2.2.1.4 Treatment

Antibiotics, including metronidazole and tinidazole, are more commonly used in treating individuals infected with *G. lamblia*. However, albendazole and nitazoxanide may also be used as alternatives to treat giardiasis (Fletcher *et al.*, 2012). Moreover, the above-mentioned antibiotics are highly effective against *E. histolytica*, but the use of at least one luminal agent, including paromomycin and iodoquinol, is recommended in combination with antibiotics to eliminate the cysts from the intestines (Gonzales *et al.*, 2019). Nevertheless, the infection caused by *Cryptosporidium spp.* is usually self-limiting with little or no drug required, but nitazoxanide has proven effective for immunosuppressed individuals with *Cryptosporidium spp.* (Sparks *et al.*, 2015).

2.2.1.5 Epidemiology of intestinal protozoa

Intestinal parasitic infections occur worldwide, with the highest prevalence in developing countries, mainly due to a lack of sanitation facilities, an inappropriate human waste disposal system, a lack of a safe water supply, and a lack of legal socioeconomic status (Alyousefi *et al.*, 2011). The prevalence rate of intestinal protozoa is estimated at 104 million people infected with *E. histolytica*, 184 million with *G. lamblia*, and 64 million with *Cryptosporidium spp.* (Kirk *et al.*, 2015). In addition to being a substantial cause of opportunistic infections in HIV/AIDS and immunosuppressed patients in underdeveloped

nations who are already undernourished or have poor access to healthcare, intestinal protozoa are also a significant cause of morbidity in children (Agholi *et al.*, 2013).

Giardiasis, amoebiasis, and cryptosporidiosis are common diseases caused by *G. lamblia*, *E. histolytica*, and *Cryptosporidium spp.*, respectively (Tellevik *et al.*, 2015). They are mainly attributed to a lower level of education coupled with insufficient knowledge and awareness of infection transmission and prevention, as well as widespread food contamination (Yates *et al.*, 2017).

2.2.2 Intestinal helminths

One of the most widespread infections in humans is caused by helminthic parasites (WHO, 2015). They are worms with many cells and cause disease in humans. They include *E. vermicularis* (pinworm), *A. lumbricoides* (roundworm), *T. trichiura* (whipworm), *N. americanus* and *A. duodenal* (hookworm), and *S. stercoralis* (threadworm). Biologically, the highest health risk is for helminthic infections compared with other pathogens because helminths persist for longer periods in the environment and the infective dose is small (Sunil *et al.*, 2014). These parasites impact over 25% of the world's population and are a significant cause of human sickness and disability (Krolewiecki *et al.*, 2013).

2.2.2.1 Transmission of intestinal helminths

Helminths are transmitted to humans by many different routes. Humans become infected when they ingest infected eggs (*A. lumbricoides* and *T. trichiura*) or larvae (some hookworms). Other worms have larvae that actively penetrate the skin (Hookworms, Schistosomes, and Strongyloides) (Lustigman *et al.*, 2017). Worms can be present on the outer layers of food, such as on vegetables. Therefore, people who eat food without washing themselves are susceptible to intestinal worms. Transmission by humans

themselves (autoinfection) can occur with *E. vermicularis*, *S. stercoralis*, and *H. nana* infections (Adejumoke and Morenikeji, 2015).

A parasitic worm infection called helminthiasis can harm a host and make them ill. They are harmful to human health, infect both people and animals and create growth problems. As their eggs and larvae are constantly contaminating the environment, helminth infections are a serious problem in both developing and industrialized nations. The STH or intestinal nematodes, roundworms, schistosomes, and the onchocerciasis worm are the most prevalent (Nalule *et al.*, 2013).

2.2.2.2 Clinical presentation

The distribution of soil-borne helminth infections is scattered, i.e., relatively few heavily infected individuals harbor the majority of worms, and this may be due to both exposure and host susceptibility. Although most individuals with low and moderate-intensity infections often have limited or nonspecific symptoms, physicians should be aware that such infections can also present as acute and chronic cases. The severity of the morbidity correlates with the intensity of the infection. Moderate to high-intensity infections are associated with, anemia, diarrhea, malnutrition, and physical and cognitive impairment (Campbell *et al.*, 2016a). The largest worm loads for hookworms are often found in adults, while *Ascaris* and *Trichuris* are most frequently found in school-age children (SAC) (Turner *et al.*, 2015).

A. lumbricoides infections are generally asymptomatic or may cause mild, nonspecific symptoms. In patients who seek medical attention, symptoms depend on the stage of the parasite's life cycle and the intensity of the infection. Patients present with cough, dyspnea, and hemoptysis and may have abnormal breath sounds. In rare cases, the syndrome can lead to pleurisy or pleural effusion (Lal *et al.*, 2013). Infections with adult *A. lumbricoides*

may present as an acute abdomen, most commonly in children, with a high worm load, including upper gastrointestinal hemorrhage, small bowel obstruction, volvulus and intestinal invagination, peritonitis, and ascariasis of the stomach, even with perforation (Das, 2014).

2.2.2.3 Diagnosis

Besides microscopy which is widely used for stool diagnosis, antibody assays, although yet to be standardized, may in well-resourced settings, aid in the diagnosis of returning travelers with first-time exposure and/or in stool-negative cases (Lamberton and Jourdan, 2015). Also, polymerase chain reaction assays are being developed for Soil-transmitted helminths, both for clinical case management and for public health purposes; however, the tests have yet to be made broadly available. Co-infections with multiple parasites are a common finding in endemic areas and may make individual diagnoses challenging (Staudacher *et al.*, 2014).

2.2.2.4 Treatment

Drugs used to treat helminths include albendazole, mebendazole, levamisole, and pyrantel pamoate. The anthelmintic medicine albendazole is the most effective against *A. lumbricoides* and hookworms. This is because albendazole has increased egg loss rates for *A. lumbricoides* and hookworms, with a moderately low rate for *T. trichiura* (Moser *et al.*, 2017). As a result of its increased egg loss rates for *A. lumbricoides* and hookworm and somewhat high rates for *T. trichiura*, mebendazole is the second most commonly used medication for STHs. Levamisole and pyrantel pamoate can be used as alternatives, as they showed high egg reduction rates against *A. lumbricoides* in the same study but moderately low efficacy against hookworms and *T. trichiura*. In addition, ivermectin has proven effective in treating strongyloidiasis; however, the use of ivermectin is restricted in several

countries, so albendazole is used as an alternative against *S. stercoralis* (Mendes *et al.*, 2018).

2.2.2.5 Epidemiology of intestinal helminths

The most prevalent infectious diseases worldwide are still those caused by intestinal parasites. The burden of morbidity and mortality from parasitic infections brought on by intestinal helminths is one of the highest in poor nations (Houweling *et al.*, 2016). The high frequency of intestinal parasite diseases in underdeveloped nations is mostly caused by inadequate and unsafe water supplies, poor sanitation, harmful human excretions, waste disposal systems, and a low socioeconomic level. People with poor hygiene habits can not only contract intestinal worms but also spread infections to other people (Faria *et al.*, 2017).

T. trichiura infects an estimated 477 million people, with the highest prevalence and intensity of infection in children (Nejsum *et al.*, 2012). In addition, an estimated 819 million people were infected with *A. lumbricoides*, 439 million with hookworm, and about 100 million with *S. stercoralis* (Pullan *et al.*, 2014).

Soil-borne helminths mainly include hookworms, roundworms (*A. lumbricoides*), and whipworms (*T. trichiura*). Anaemia, malnutrition, and impeded physical and cognitive development are all linked to soil-borne helminthiasis, which has a cumulative worldwide impact of nearly 3.3 million disability-adjusted life years (Kyu *et al.*, 2018). The World Health Assembly urged regular anthelmintic treatment for three high-risk groups, including Preschool-Aged Children (PSAC), School-Aged Children (SAC), and Women of Childbearing Age (WRA), as the main recommendation for eradicating soil-borne helminthiasis as a public health issue (Tchuente, 2011).

A. lumbricoides (the roundworm) is thought to be present in 804 million people, mainly children and adolescents. The number of Ascaris-associated disability-adjusted life-years (DALYs) is currently equivalent to one-fourth of the illness burden from 1990 (De-Vlas *et al.*, 2016). Mortality accounts for about one-sixth of the burden of disease; severe morbidity is largely related to wasting (Pullan *et al.*, 2014).

When viable, larvae eggs are consumed (for example, via food, water, or feces), they hatch and release larvae that infiltrate the intestinal mucosa and subsequently migrate to the liver and lungs. The fourth-instar larvae are eaten after ascending the trachea, and they then establish themselves as adults in the small intestine. *A. lumbricoides* can also affect the gastrointestinal tract, leading to blockage, related issues, and occasionally even death (Jex *et al.*, 2011).

Hookworms (*A. duodenal* and *N. americanus*) infect an estimated 472 million people. Unlike ascariasis and trichuriasis, hookworm prevalence and infection intensity are highest in adults, although children are commonly infected. The burden of hookworm disease is largely due to anemia, and estimates suggest the burden could be as high as 4 million DALYs, with productivity losses of up to \$139 billion per year (Bartsch *et al.*, 2016).

Southeast Asia is home to three cestode species that are significant for medicine: Tapeworms such as *Taenia solium* (found in pork), *Taenia saginata* (found in cattle), and *Taenia asiatica* (found in Asia). Taeniasis, a disease that is still common and has been proven to be a reasonably severe public health issue not only in Southeast Asia but also globally, is caused by these parasites (Okamoto *et al.*, 2010). Human taeniasis caused by *T. saginata* and very rarely by *T. solium* has been reported as an imported case in Japan; *T. asiatica* is not generally considered an indigenous occurrence in Japan (Yamasaki, 2013).

People who do not eat undercooked beef are unlikely to get taeniasis. Many people who have taeniasis are unaware that they have the infection because symptoms are typically

weak or nonexistent. Due to consuming undercooked pork and undercooked vegetables, inadequate sanitation, and pigs roaming freely due to poor swine husbandry practices, taeniasis caused by *T. solium* is becoming increasingly prevalent, especially in poorer populations. As a result, it is common throughout Asia, including China, Indonesia, Nepal, India, South Korea, Thailand, Cambodia, Laos, and Vietnam (Trung *et al.*, 2013).

Solium teniasis can also result in cysticercosis, a parasitic tissue infection brought on by the tapeworm's larval cysts. One of the most significant zoonotic illnesses in the world, cysticercosis affects about 50 million individuals worldwide. Pork intake is dangerous because cysticercosis kills 50,000 affected people annually. It typically happens when there is poor sanitation, no or ineffective meat control, and improper pig husbandry (Del-Brutto and Garcia, 2012).

2.3 Intestinal Parasites which Contaminate Raw Vegetables

Humans may contract intestinal parasites like hookworms, *E. vermicularis*, *T. trichiura*, Hymenolepis, Taenia, and Fasciola species, as well as *G. lamblia*, *E. histolytica*, *A. lumbricoides*, and *Entamoeba spp.* (Alemu Getaneh *et al.*, 2019). They have been reported to be associated with the consumption of contaminated fresh vegetables. The consumption of raw or unpeeled vegetables is thought to increase the risk of parasite transmission to humans (Hassan *et al.*, 2012). *Cryptosporidium*, *Cyclospora*, *Giardia*, *Entamoeba histolytica*, *Entamoeba coli*, and *A. lumbricoides* are considered the most common parasitic contaminants of vegetables (Tefera Tamirat *et al.*, 2014).

It was reported that two billion people worldwide are infected with pathogenic and non-pathogenic intestinal parasites (Gelaw Aschalew *et al.*, 2013). Intestinal protozoa parasites can cause foodborne diseases, and some protozoa infections cause serious health and economic problems in many developing and developed countries. In the United States, 9.4

million foodborne illnesses are reported each year; of these, 0.2 million cases are caused by parasites (Scallan *et al.*, 2011).

2.3.1 Contamination of vegetables with intestinal protozoan parasites

Inadequate sanitation facilities and practices can contribute to the contamination of vegetables. Improper disposal of human and animal feces, lack of toilet facilities, and improper waste management can contaminate the soil and water used for vegetable cultivation. This can introduce protozoan parasites, such as *G. lamblia* and *C. paravium*, into the agricultural environment (Winkler *et al.*, 2017).

The use of contaminated water for irrigation can lead to the contamination of vegetables. Water sources such as rivers, ponds, or wells can be contaminated with protozoan parasites due to sewage or animal waste runoff. If the untreated or improperly treated water is used for irrigation, it can directly contaminate the vegetables (Ogunsola and Mehtar, 2020).

Animals, both domestic and wild, can carry protozoan parasites that can contaminate the agricultural environment. For example, livestock or wildlife feces can introduce parasites into the soil or water used for vegetable cultivation. Additionally, pests like flies can transfer parasites from fecal matter to vegetables during feeding (Khor *et al.*, 2019).

The global problem of human parasite infection by raw vegetables has only recently been recognized, despite the health advantages of these vegetables in non-pharmacological illness prevention. Several investigations have shown that vegetables can be contaminated with human infectious organisms (Rodrigues *et al.*, 2020).

The parasitic protozoa include *C. cayetanensis*, *Blastocystis spp.*, *G. duodenalis*, *B. coli*, *Entamoeba spp.*, *Toxoplasma gondii*, *Enterocytozoon bienersi*, and *Cystoisospora belli* (Eraky *et al.*, 2014).

2.3.1.1 *Cryptosporidium* contamination of vegetables

Cryptosporidium spp., are common protozoan parasites infecting humans and animals and are the second leading cause of diarrhea in children after rotavirus (Bouزيد *et al.*, 2018).

Cryptosporidium spp. is characterized by its extensive genetic variation, which leads to the existence of 38 species and more than 60 genotypes of this parasite (Fenget *et al.*, 2018).

At least 20 different species cause moderate or severe diseases in humans, of which *C. hominis* and *C. parvum* are the major causative agents (Khan *et al.*, 2018).

Contamination of vegetables with *Cryptosporidium spp.* is documented in many countries. Among the *Cryptosporidium species* *C. parvum*, *C. hominis*, and *C. ubiquitum* were detected in the contaminated vegetable samples (Duedu *et al.*, 2014). *Cryptosporidium spp.*, which uses food as a vector to threaten public health, are significant human infection and the primary cause of human cryptosporidiosis (Caradonna *et al.*, 2017).

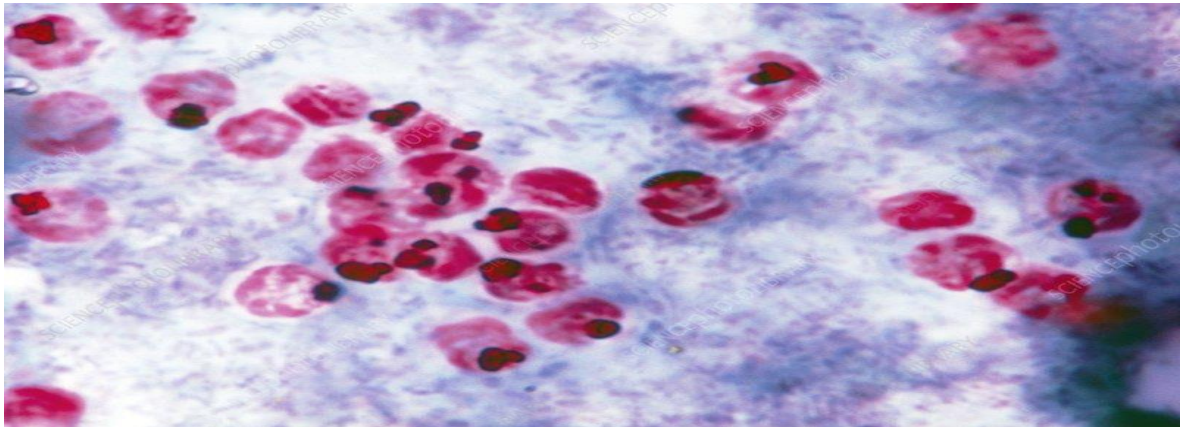


Figure 1: Oocyst of *Cryptosporidium parvum*

Source: <https://www.researchgate.net/publication/267815222>

2.3.1.2 *Giardia duodenales* contamination of vegetables

G. duodenal, also known as *G. intestinalis* or *G. lamblia* is a non-invasive protozoan parasite that attaches to and colonizes the upper small intestine and produces acute watery diarrhea in both people and animals (Einarsson *et al.*, 2016). Through the consumption of

infected foods, this parasite is thought to be responsible for 28.2 million instances of diarrhea every year. Giardiasis outbreaks have also been linked to numerous processed foods. Consuming tainted raw vegetables is frequently linked to human infections with *G. duodenal* (Colli *et al.*, 2015).

Transmission occurs through the ingestion of cysts in water and contaminated food or human-to-human contact (fecal-oral transmission), particularly in low-sanitary environments. Sometimes individuals with giardiasis are asymptomatic and act as the main disseminators of the disease. However, when symptomatic, it is characterized by diarrhea, weight loss, nausea, vomiting, bloating, malabsorption, and abdominal cramps (Strkolcova *et al.*, 2016).

In numerous nations, reports of *G. duodenal* cysts in vegetables have been made. Infected vegetable and fruit samples commonly contained assemblages of zoonosis A and B of *G. duodenal* (Caradonna *et al.*, 2017).



Figure 2: Cyst of *Giardia duodenales*

Source: <https://www.researchgate.net/figure/Contamination>.

2.3.1.3 Cyclospora cayentanensis contamination of vegetables

Another significant protozoan parasite that affects humans and typically spreads through food is *C. cayentanensis* (Giangaspero and Gasser 2019). *C. cayentanensis* is a significant

human protozoan that spreads through food on a global scale. The epidemics of cyclosporiasis associated with raw vegetable eating have been the subject of numerous reports (Ortega and Sanchez, 2010).

This protist causes the gastrointestinal disease cyclosporiasis in humans, which is typically characterized by clinical indications like nausea, stomach pains, and profuse, watery diarrhea. It spreads to humans by way of food that has been contaminated with infective oocysts. When a vulnerable person consumes food tainted with sporulated oocysts, infection results. The primary source is the consumption of fresh produce, such as vegetables, typically in underdeveloped nations or communities (Caradonna *et al.*, 2017).

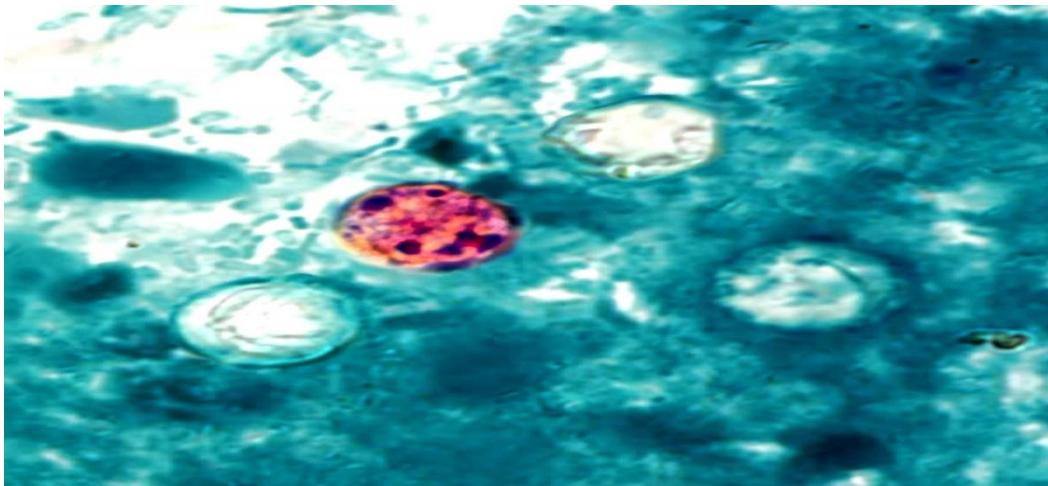


Figure 3: Oocyst of *Cyclospora cayetanensis*

Source: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10536660>

2.3.1.4 *Entamoeba* spp., contamination of vegetables

Among *Entamoeba* spp., *E. histolytica* is responsible for most cases of human amebiasis and remains one of the top three causes of parasitic deaths worldwide (Sitotaw Baye *et al.*, 2019). Although some *E. histolytica* infections are asymptomatic, many infections can result in severe amebic colitis and disseminated disease (Anuar *et al.*, 2012). *Entamoeba* spp. infections are strongly related to the consumption of contaminated vegetables (Gabre

and Shakir, 2016). The disease is still one of the main public health issues in subtropical and tropical regions (Oyibo *et al.*, 2013).

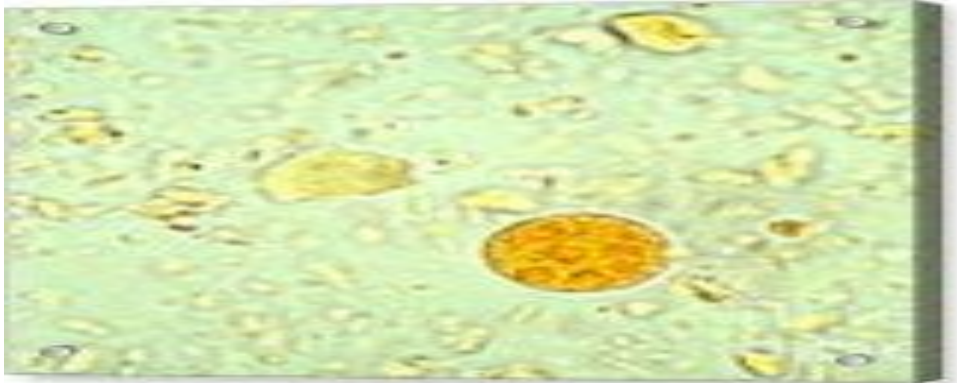


Figure 4: Cyst of *Entamoeba histolytica*

Source: <https://www.researchgate.net/figure/Molecular>

2.3.2 Other intestinal protozoan contaminations of vegetables

Fresh vegetables can infrequently contain additional intestinal protozoa such as *Enterocytozoon bieneusi*, *Blastocystis species*, *Cystoisospora belli*, and *Balantioides coli*. Vegetables have been reported to be contaminated with *B. coli* in several studies, posing issues for worldwide public health. Using light microscopy, *Balantioides coli* is typically seen on vegetables. In Bangladesh, Cameroon, Brazil, Ghana, and Ethiopia, it has been demonstrated that vegetables are contaminated with *Balantioides coli*; the incidence of infection is thought to be 9.3% globally (Alemu Getaneh *et al.*, 2019).

Tropical and subtropical regions of the world frequently report *Cystoisospora belli* infections (Legua and Seas, 2013). Consuming contaminated food might result in cystoisosporiasis. In Ethiopia and Ghana, *Cystoisospora belli* contamination of vegetables has been reported three times. According to estimates, contamination is present on average at 1.9% (Bekele Fitsum *et al.*, 2017). Human isosporosis is more prevalent in warm places with poor hygienic conditions. It can be contracted by consuming food that has been

contaminated with sporulated *I. belli* oocysts, which can cause fever, diarrhea, abdominal cramps, and vomiting. Children and people with some immunodeficiency are more likely to experience more severe forms of this disease, which can cause persistent diarrhea and even require hospitalization due to dehydration and weight loss (Rodrigues *et al.*, 2020).

Enterocytozoon bieneusi is a prominent species of microsporidia that affects individuals (Matos *et al.*, 2012). The investigation of single-nucleotide polymorphisms (SNPs), which resulted in more than 500 legitimate genotypes of the infectious agent, was used to infer the genetic variation of the pathogen. Eleven genetic subgroups (groups one to eleven) were discovered by phylogenetic examination of the valid genotypes, together with details on their host preference and potential for zoonotic transmission. *E. bieneusi* infection of vegetables has been recorded in China, Poland, and Costa Rica. Foodborne transmission of this pathogen has also been documented (Karim *et al.*, 2020).

2.3.3 Contamination of vegetables with intestinal helminths parasites

Parasites of intestinal helminths include *T. trichiura*, hookworms, *Taenia spp.*, *A. lumbricoides*, *E. vermicularis*, and *S. stercoralis*, which infect people who consume contaminated, undercooked, or improperly washed vegetables (Bekele Fitsum *et al.*, 2017). Eating raw, whole, or in dishes such as salads without adequate washing, sanitizing, or cooking is an important risk factor for the transmission of intestinal helminthiasis (Anuar *et al.*, 2014).

2.3.3.1 *Ascaris lumbricoides* contamination of vegetables

A. lumbricoides ubiquitous distribution is due to the high number of eggs produced by the fecund female parasite, which contributes to the parasite's ubiquitous distribution, and the strong and resistant nature of the eggs, which enables them to survive unfavorable conditions. The eggs can live at 5-10°C for two years and are unharmed by desiccation for two to three weeks while remaining oxygen-free. According to the previous study, *A.*

lumbricoides was a parasite that was frequently found on raw vegetables in the southern Ethiopian city of Arba Minch (Bekele Fitsum *et al.*, 2017). These roundworm eggs are "sticky" and can be transferred to the mouth by touching inanimate objects or foods (Sunil *et al.*, 2014).

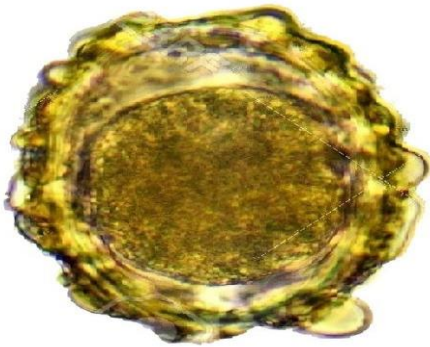


Figure 5: Egg of *Ascaris lumbricoides*

Source: *Ascaris lumbricoides* Egg: Morphology, Characteristics and Identification (laboratorytests.org).

2.3.3.2 *Trichuriasis trichiura* (whipworm) contamination of vegetables

T. trichiura, often known as the human whipworm, causes human trichuriasis. The intestinal parasite *T. trichiura* has been estimated to infect more than 400 million people (Hansen *et al.*, 2016). Eating or drinking tainted food or water that contains *T. trichiura* embryonated eggs can cause trichuriasis (Parija *et al.*, 2017).

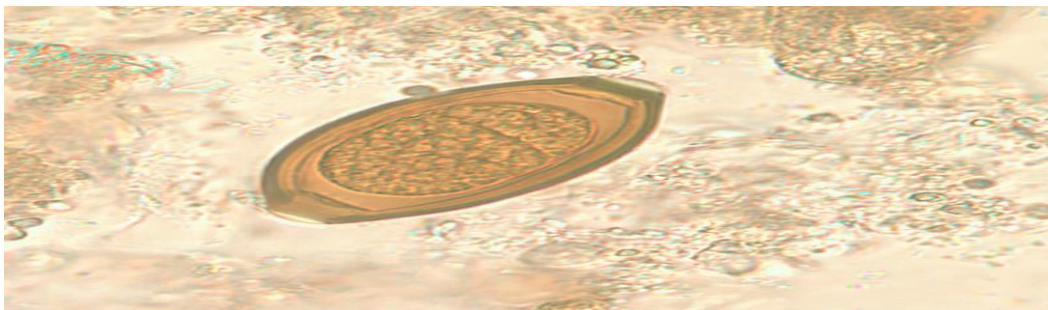


Figure 6: Egg of *Trichuriasis trichiura*

Source: CDC - DPDx - Trichuriasis

2.3.3.3 Hookworm contamination of vegetables

Poor sanitation and irrigation with water contaminated with human waste may be the reason for the high incidence of hookworm egg contamination of vegetables. The cause of hookworm infection may be due to improper footwear and skin contact with contaminated soil. Furthermore, changes in geographic location, climatic circumstances, and soil types may contribute to the high incidence of hookworm (Silver *et al.*, 2018).

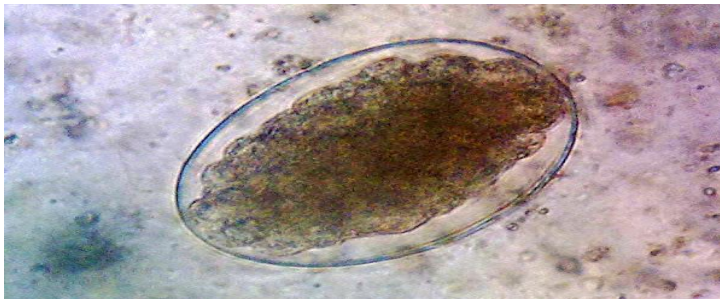


Figure 7: Egg of Hookworm

Source: Hookworm Egg | Project Noah

2.3.3.4 *Strongyloides stercoralis* (threadworm) contamination of vegetables

The parasite that was found most commonly was a *Strongyloides spp.*, larva. This might be because the parasite lives freely in the environment, where it is abundant and easily contaminates vegetables. Additionally, *Strongyloides spp.* that infect animal reservoirs like dogs may contaminate the environment (Schar *et al.*, 2013).

The *Strongyloides* parasite typically causes many infections in asymptomatic individuals. At the site where the skin has been penetrated, symptoms like dermatitis, edema, itching, and minor bleeding may be present. If the parasite enters the lungs, signs of pneumonia such as wheezing, coughing, and a burning sensation in the chest may appear. Eventually, the intestines may get infected, resulting in ulcers, sepsis, tissue destruction, and burning pain. In severe circumstances, edema may lead to intestinal blockage and peristaltic contraction loss (Adamu *et al.*, 2012).

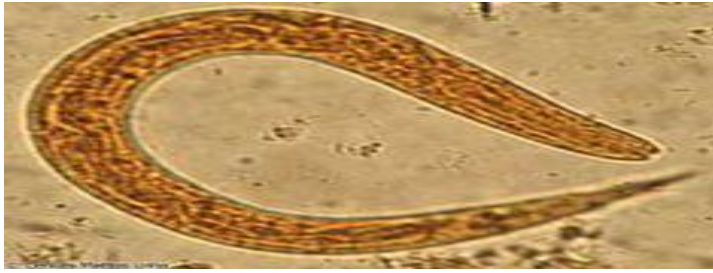


Figure 8: Larvae of *Strongyloides stercoralis*

Source: Verminoses - Estrongiloidíase (*Strongyloides stercoralis*) Medical laboratory.

2.4 Protective Strategies for Intestinal Parasitic Infection

To combat IPIs by lowering the risk of getting sick and transmitting infections, many hygiene-related measures have been widely used. One of the interventions is WASH (water, sanitation, and hygiene), which aims to increase the quantity and quality of water as well as enable sanitation and hygiene practices in low-income countries (Yates *et al.*, 2017). The likelihood of IPIs that cause STH can be effectively decreased in communities by 33-70% by implementing WASH treatments (Campbell *et al.*, 2016b).

To ensure proper disposal of human excreta, WASH programs may involve properly built sanitation infrastructure at home. Furthermore, having access to a clean and treated water supply will support the promotion of personal hygiene practices such as washing hands with soap before and after eating and defecating (Yates *et al.*, 2017). Additionally wearing protective footwear when walking through potentially contaminated fields, may help to prevent the transmission of the disease (Nery *et al.*, 2015).

To lower the occurrence of IPIs, the government should also provide adequate basic residential infrastructure. Therefore, traditionally constructed homes should be kept up properly with an emphasis on overall cleanliness and by offering the local populations necessities like a treated water supply, a water filtration system, and indoor toilets. Less open defecation may lessen the occurrence rate of STHs (Campbell *et al.*, 2016b).

Practicing proper food and personal hygiene can protect susceptible communities against intestinal protozoa (Al-Delaimy *et al.*, 2014). Washing raw vegetables before consumption plays an essential role in protecting against intestinal protozoa as consumption of unwashed raw vegetables and contaminated fruit is one of the associated risk factors (Anuar *et al.*, 2014). Moreover, practicing good standards of personal hygiene can reduce the number of intestinal parasite infections. This is based on one Malaysia study results of population attributable risk fraction (PARF) analysis, revealing that drinking safe water, properly using toilet facilities for defecation, wearing shoes outside the house, and washing hands can reduce the percentages of getting IPIs by 12.1, 22.1, 25.9, and 13.0%, respectively (Al-Delaimy *et al.*, 2014). Besides, human hands act as an important vector in carrying pathogens, thus, hand-washing with soap and weekly fingernail-clipping can effectively prevent transmission via the fecal-oral route and reduce the chances of getting infected with intestinal parasites (Mahmud *et al.*, 2015).

3 MATERIALS AND METHODS

3.1 Description of the Study Area

Hawassa, formerly known as Adare, is a City located in Ethiopia. The City serves as the administrative center and capital of the Sidama Region in Ethiopia. It is situated on the eastern shore of Lake Hawassa in the Great Rift Valley. It is located 273 kilometers south of Addis Abeba via Bishoftu, 130 kilometers east of Sodo, and 75 kilometers north of Dilla. Geographically, it lies between 7°3' latitude north and 38° 28' longitude east (Figure 1). The city is located at an altitude of 1697 m above sea level. The boundaries of Hawassa city are defined by the Oromia region to the north, Lake Hawassa to the west, Wondogenet wereda to the east, and Shembedino wereda to the south. There are 32 "kebele" and eight "sub-cities" inside the 157.2 square kilometers that make up the city administrator. The eight sub-cities are Misrak, Tabor, Bahil Adarash, Menaharia, Addis Ketema, Hawella-Tula, Haik dar, and Mehal (Mamuye Natnael and Bute Gotu, 2015). The city of Hawassa is known for its consumption of vegetables (Bekele *et al.*, 2021). As one of Ethiopia's major agricultural centers, Hawassa has a strong focus on farming and horticulture. The city's favorable climate and proximity to fertile lands make it an ideal location for vegetable production. The local populations, as well as visitors, have access to a wide variety of fresh and locally grown vegetables. The demand for vegetables in Hawassa is high, and the city is known for its vibrant markets and supermarkets that offer a diverse selection of vegetables to supply the needs and preferences of the residents (Chora, 2020).

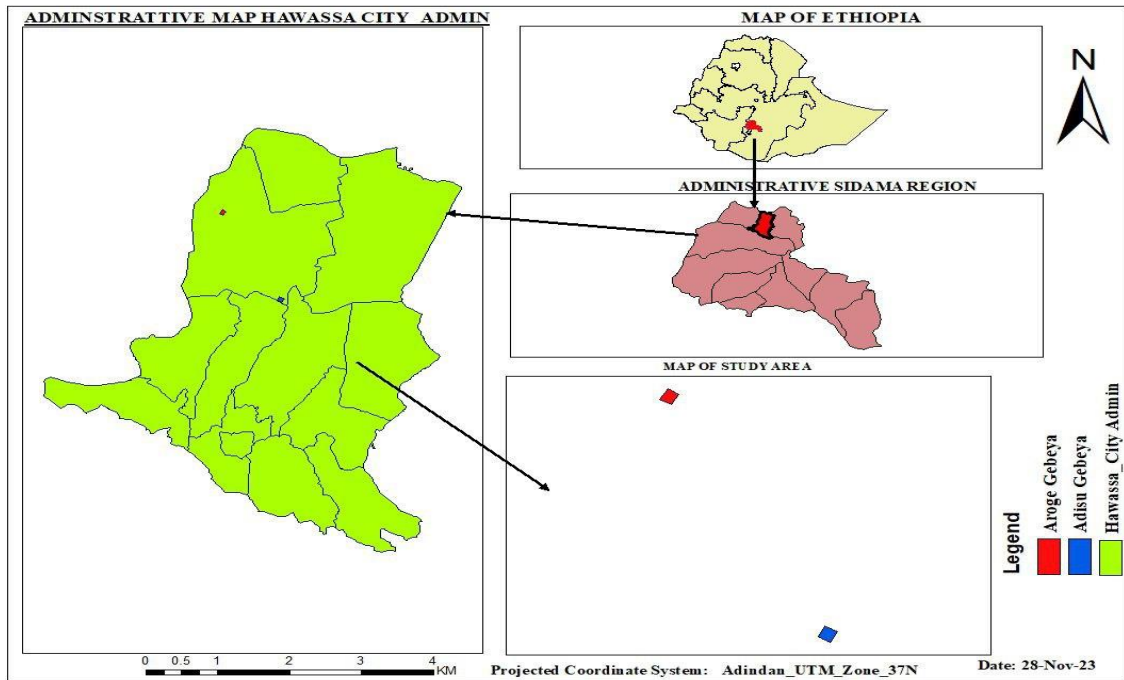


Figure 9: Map showing the location of the study area (source: ArcGIS)

3.2 Study Design and Period

A cross-sectional study was conducted from February to April 2023, to determine the parasitic contamination of vegetables sold in selected markets in Hawassa city. A purposive sampling technique was applied for the selection of the sampling sites, based on the availability of vegetables in the selected markets. The selected markets were visited to take the samples. Parasitic analysis was conducted in this study, and it was done in the parasitological laboratory of Hawassa University. The analysis involved techniques such as microscopy to identify and quantify the parasites.

3.3 Study Samples

The sample selection process was determined based on specific criteria, such as the types of vegetables commonly consumed, availability, and accessibility. The vegetables used in this study include lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*), carrot (*Daucus carota*), tomato (*Solanum lycopersicum*), green pepper (*Capsicum annum*), and potato

(*Solanum tuberosum*). Green peppers are commonly used in salads, which are typically consumed without cooking. Although tomatoes and carrots are often cooked, they can also be consumed raw in salads. While cabbage, lettuce, and potatoes are predominantly cooked, there are instances where they may be consumed raw or undercooked in certain dishes.

3.4 Sample Size Estimation

To estimate the sample size, a single population proportion formula was used (Daniel and Cross, 2018).

$$n = \frac{z^2(p)(1-p)}{d^2}$$

Where:

- ✚ **n** is the sample size
- ✚ **p** is the estimated proportion
- ✚ **1-p** is the estimate of variance
- ✚ **z** is the acceptable margin of error for the proportion being estimated.

Since no previous study had been conducted in the area to determine the prevalence of medically important parasites on vegetables, it was estimated to be 50%. The selected alpha level was 1.96 at 95% confidence intervals and the degree of precision was set at 5%, indicating the acceptable margin of error. Using these parameters, the sample size was calculated to be 384. This means that to obtain a reliable estimate of the prevalence of medically important parasites in vegetables, 384 vegetable samples needed to be collected. Six different types of vegetables were collected, with each type having sixty-four samples. The mass of the tomatoes was 4kg, the carrots weighed 3kg, the potatoes also weighed 4kg,

the green peppers were 1/4kg, and there were four bunches of lettuce and cabbage taken wholesale from the market.

3.5 Sample Collection

Six types of vegetables including lettuce, cabbage, carrot, tomato, green pepper and potato were purchased from two conveniently selected markets, namely, Addisu Gebeya, and Aroge Gebeya in Hawassa City. A total of 384 vegetable samples were collected, comprising 64 samples for each vegetables. To purchase the vegetable samples, vendors were chosen using a systematic random sampling method (Mishra and Alok, 2022). There were a total of 672 vendors in the market, determined by counting the number of vendor stalls or houses. The sampling interval was determined by dividing the total number of vendors, which was 672 from both markets, by the desired sample size for each vegetable type, which was 384. As a result, the sampling interval was calculated as 1.75 (rounded up to 2 for simplicity).

$$K = N/n$$

Where: K- Sampling interval

N- Total vendor size

N- Desired sample size

To start the sampling process, a random starting point was selected between the first vendor and the sampling interval. This starting point represented the first vendor's vegetable to be included in the sample. The starting point was chosen from the first vendor, and subsequently, every second vendor was selected, following the sampling interval of 2. This process continued until a total of 384 vegetable samples were collected. The samples were taken, with the following weights: tomatoes (4kg), carrots (3kg), potatoes (4kg),

green peppers (1/4kg). Additionally, four bunches of lettuce and cabbage were obtained wholesale from the market. The samples were collected under normal purchase conditions and were carefully placed in plastic bags for transportation to the Parasitology Laboratory of Veterinary Medicine at Hawassa University.

3.6 Processing of the Samples

3.6.1 Sample preparation and parasitological examination procedures

Samples were prepared for testing within three hours of collection to avoid contamination. The leafy vegetables, including lettuce and cabbage, along with commonly consumed vegetables like green peppers, were chopped. On the other hand, smooth vegetables such as tomatoes, potatoes, and carrots were peeled. Then after, approximately 50-200 grams each vegetable samples were soaked, and rinsed in a beaker with 1 L of normal saline (0.85% NaCl) to maintain isotonic condition, followed by stirring on a shaker for 15 minutes to facilitate proper washing (Ismail, 2016). The samples were then removed from the beaker using a sieve to remove unwanted substances, and the washing solution was allowed to stand for 24 hours (Tefera Tamirat *et al.*, 2014). The top part of the washing solution was removed and the remaining 15 ml of the sediment was transferred to a centrifuge tube. To sufficiently concentrate the parasitic stages, the contents were centrifuged at 3000 rpm for 5 minutes (Idahosa, 2011). The sediment was then gently agitated by hand to resuspend it after the supernatant was gently decanted, leaving roughly 5 ml of sediment behind. Part of the sediment was used for the preparation of direct and iodine-based wet mount smears and examined under a light microscope using 10X and 40X objectives for the identification of parasite stages (Bekele Fitsum *et al.*, 2017).

3.6.2 A modified Ziehl-Neelsen staining technique

It is crucial for laboratories to visually identify these coccidian due to the significance of *Cryptosporidium parvum*, *Cyclospora cayetanensis*, and *Isospora belli* as opportunistic parasites. It was used to identify oocysts of *Cryptosporidium*, *Isospora*, and *Cyclospora* species. The oocysts of the parasites were detected microscopically based on their morphological features (Alemu Getaneh *et al.*, 2019). The best sensitivity appears to be achieved by the modified Ziehl-Neelsen procedure, which colors oocysts pink to red (Eraky *et al.*, 2014). In this technique, a thin smear was prepared directly from the sediment and allowed to air dry. The slides were then fixed with absolute methanol for 3 minutes and stained with carbol fuchsin for 15 minutes. After that, the slides were cleaned using distilled water and decolorized with acidic alcohol (1% sulfuric acid in alcohol) for 20 seconds. After the slides were washed with distilled water, they were counterstained with 0.4% malachite green (or methylene blue) for one minute. After being cleaned with distilled water, the slides were allowed to air dry. The oil immersion was placed on the smeared slide and then observed under a light microscope at 100x magnification. *Cryptosporidium* oocysts appear as round, red-stained structures, while *Isospora* and *Cyclospora* oocysts may appear oval or ellipsoidal and stain red as well (Bekele Fitsum *et al.*, 2017).

3.7 Data Quality Assurance

In order to attain the desired results, the necessary method was implemented. This entailed using a sterile plastic bag to collect the samples, followed by processing them using sterilized laboratory equipment. A microscope was utilized to identify the stages of parasites (Kumar *et al.*, 2023). Additionally, modified Ziehl-Neelsen staining techniques were employed to identify coccidian parasites (El-Sayed *et al.*, 2023).

3.8 Data Analysis

Laboratory data were recorded on specially designed study record forms. This was then entered into an Excel worksheet. Frequencies were performed on all variables to check for errors and inconsistencies. These were corrected by checking against the original entry on the filing forms. The data were analyzed using descriptive prevalence and counting statistics. By dividing the number of positive samples by the total number of vegetables, and multiplying the result by 100, the proportion of vegetables with various parasite stages was calculated. The prevalence of vegetable contamination was evaluated using the one-sample proportion test. The results of the laboratory investigations were cleaned and entered into a computer, and statistical analysis was performed using SPSS for Windows version 26. The Wilcoxon sign ranked test was used to test the ranking of distribution of the parasites on the vegetables as well as their contamination. Significance was considered at $p < 0.05$.

4 RESULTS

4.1 The Contamination Rate of Vegetables

Out of the 384 vegetable samples that were examined microscopically, 196 (51.04%) were found to be contaminated with one or more human intestinal parasites. The distribution of intestinal parasitic contamination varied across different types of raw vegetables. Leafy greens such as cabbage and lettuce showed a higher prevalence of contamination compared to root vegetables like carrots, potatoes tomatoes, and green peppers. Upon further analysis of the positive samples, it was observed that cabbage had the highest contamination rate. Out of the 64 cabbage samples examined, 48 (75%) were found to be contaminated with human intestinal parasites. Lettuce showed a similar trend, with 45 (70.3%) testing positive for contamination. Among the smooth-surfaced vegetables, potatoes had 36 (56.3%) were contaminated with parasites. Tomato revealed 25 (39.1%) showing evidence of contamination. Similarly, carrots had 24 (37.5%) contaminated, while green peppers exhibited a lower contamination rate, with 18 (28.1%) contaminated (Table 1).

Of the 196 (51.04%) contaminated vegetable samples, 152 (39.5%) were found to be contaminated with a single species of parasite. In addition, two different parasite species were identified in 31 (8.07%) contaminated vegetable samples. Furthermore, three different parasite species were detected in 9 (2.3%) contaminated vegetable samples. Interestingly, four different parasite species were found in 3 (0.7%) contaminated vegetable samples. Lastly, only 1 (0.3%) contaminated cabbage sample contained five different parasite species (Table 1).

Among the contaminated cabbage samples, 30 (46.9%) were found to be contaminated with one type of parasite species. Additionally, two different parasite species were identified in 11 (17.2%) contaminated cabbage samples. Furthermore, three different

parasite species were detected in 4 (6.2%) contaminated cabbage samples. Interestingly, four different parasite species were found in 2 (3.1%) contaminated cabbage samples. Lastly, only 1(1.6%) contaminated cabbage samples contained five different parasite species (Table 1).

For lettuce samples, 45(70.3%) were found to be contaminated. Specifically, it was identified that 33 (51.6%) contaminated samples were harbored with a single type of parasite species. Moreover, in 9 (14.1%) contaminated lettuce samples, two different parasite species were identified. Furthermore, three distinct parasite species were detected in 2 (3.1%) contaminated lettuce samples. Lastly, in 1 (1.6%) contaminated lettuce samples, four different parasite species were found (Table 1).

Among the contaminated potato samples, it was observed that 33 (51.6%) were contaminated with a single type of parasite species. Moreover, in 2 (3.1%) contaminated potato samples; two different parasite species were identified. Additionally, in 1 (1.6%) contaminated potato samples, three distinct parasite species were detected (Table 1).

Further analysis of the contaminated tomato samples revealed interesting findings. Among the contaminated samples, 19 (29.6%) were contaminated with a single type of parasite species. Additionally, two different parasite species were identified in 5 (7.8%) contaminated tomato samples. Furthermore, three distinct parasite species were detected in 1 (1.6%) contaminated tomato samples (Table 1).

Further analysis of the contaminated carrot samples revealed interesting findings. Among the contaminated samples, it was observed that 21 (32.8%) were contaminated with a single parasite species. Additionally, two different parasite species were identified in 2 (3.1%) contaminated carrot samples. Furthermore, three distinct parasite species were detected in 1 (1.6%) contaminated carrot samples (Table 1).

Among the contaminated green pepper samples, it was observed that 16 (25%) were contaminated with a single parasite species. Additionally, in 2 (3.1%) contaminated green pepper samples, two different parasite species were identified (Table 1).

The Wilcoxon Signed Ranks Test was conducted on a subset of six vegetable samples. Based on the p-value of 0.028, there was evidence to suggest that there is a significant difference among the contaminated vegetable samples. Among the vegetables examined, cabbage was found to be the most contaminated, while green pepper had the least contamination (Table 1).

Table 1: Vegetable samples positive for parasitic structures with mono and multiple contaminants collected from local markets in Hawassa City.

Vegetable	Number examined	Number of positive (%)	Number of parasite species detected					P - value
			One (%)	Two (%)	Three (%)	Four (%)	Five (%)	
Cabbage	64	48(75)	30(46.8)	11(17.2)	4(6.2)	2(3.1)	1(1.6)	0.028
Lettuce	64	45(70.3)	33(51.6)	9(14.1)	2(3.1)	1(1.6)	0	
Potato	64	36(56.3)	33(51.5)	2(3.1)	1(1.6)	0	0	
Tomato	64	25(39.1)	19(29.6)	5(7.8)	1(1.6)	0	0	
Carrot	64	24(37.5)	21(32.8)	2(3.1)	1(1.6)	0	0	
Green pepper	64	18(28.1)	16(25)	2(3.1)	0	0	0	
Total	384	196(51.04)	152(39.5)	31(8.1)	9(2.3)	3(0.7)	1(0.3)	

4.1.1 The contamination rate of vegetables according to the markets

Samples were gathered from Addisu Gebeya and Aroge Gebeya in Hawassa city. Upon analyzing the findings concerning the prevalence of parasitic contamination on vegetables across these markets, it was identified that out of 384 samples, 106 (55.2%) from Addisu Gebeya, whereas 90 samples (46.8%) from Aroge Gebeya were contaminated with intestinal parasites (Figure 2).

The study findings indicate that both Addisu Gebeya and Aroge Gebeya markets in Hawassa had vegetable samples contaminated with intestinal parasites. Cabbage exhibited the highest contamination rate in Addisu Gebeya (27), while lettuce showed the highest contamination rate in Aroge Gebeya (23). On the other hand, Addisu Gebeya had comparatively lower contamination rates for carrots (12) and tomatoes (12). Interestingly, green pepper had the lowest contamination rate among the vegetable samples in Aroge Gebeya (4) (Figure 2).

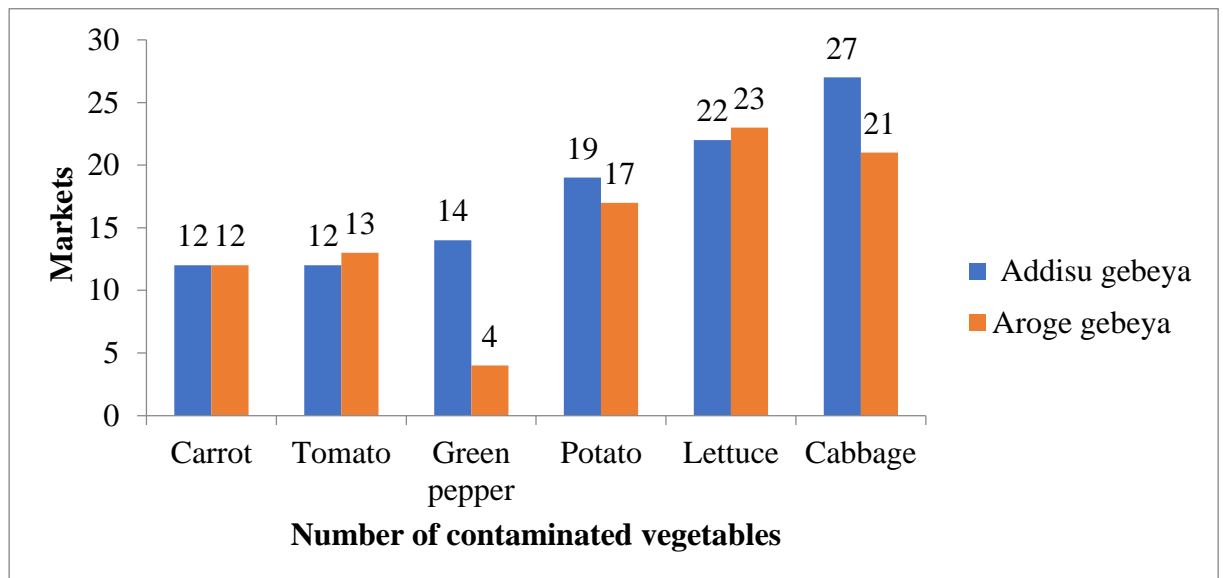


Figure 10: Intestinal parasites positivity distribution between two markets in Hawassa city.

4.2 Prevalence of Intestinal Parasites on Vegetable Samples

The total frequency of intestinal parasite stages were 500 across the different vegetable samples. Among these, 328 parasites stages were identified as protozoa, while 172 parasites stages were identified as helminths. Specifically, the tomato samples (25) contained 61 parasites, with 48 protozoa and 13 helminths. The potato samples (36) had 59 parasites, consisting of 48 protozoa and 11 helminths. The cabbage samples (48) exhibited the highest parasite count, with 169 parasites, including 90 protozoa and 79 helminths. The carrot samples (24) exclusively harbored 34 protozoa parasites, while no helminths were found. The green pepper samples (18) revealed a total of 22 parasites, with 16 protozoa and 6 helminths, while the lettuce samples (45) displayed 155 parasites, comprising 92 protozoa and 63 helminths (Table 3).

The study identified a total of twelve different parasites associated with various types of vegetables. These parasites include *Giardia lamblia*, *Entamoeba histolytica*, *Ascaris lumbricoid*, *Cryptosporidium parvum*, *Strongyloides stercoralis*, *Enterobius vermicularis*, *Balantidium coli*, *Entamoeba coli*, hookworm, *Cyclospora cayetanensis*, *Isospora belli*, and *Trichuris trichiura*. Among the detected parasites, *Giardia lamblia* was the most prevalent, accounting for 122 (31.7%) of the cases. This was followed by cysts of *Entamoeba histolytica* at 103 (26.8%), eggs/larvae of *Ascaris lumbricoides* at 82 (21.3%), and *Cryptosporidium parvum* at 52 (13.5%). *Trichuris trichiura*, *Isospora belli*, and *Cyclospora cayetanensis* had equal contamination rates of 7 (1.8%) and were less widespread (Table 3).

Among the six vegetable types included in the study, *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium parvum* were detected in all the vegetable samples. *Ascaris lumbricoid* and *Enterobius vermicularis* were detected on cabbage, lettuce, potato,

tomato, and green pepper. *Trichuris trichiura* egg was found only on cabbage samples. *Strongyloides stercoralis*, hookworm, *Cyclospora caytanesis*, and *Entamoeba coli* were found on two different types of vegetable samples, while *Balantidium coli* and *Isospora belli* were found on three different types of vegetable samples (Table 3).

The results of this study on cabbage samples revealed a highest prevalence of intestinal parasites. The most prevalent parasite found was *G. lamblia*, with a prevalence rate of 60.9%. *Ascaris lumbricoid* contamination was present in 53.1% of the cabbage samples, indicating a relatively high incidence. *Entamoeba histolytica* was identified in 42.2% of the cabbage samples, while *Strongyloides stercoralis* was detected in 35.9%, both indicating notable prevalence. *Cryptosporidium paravium* was found in 21.8% of the cabbage samples, suggesting a lower prevalence compared to the previously mentioned parasites. *Entrobium vermicularis* contamination was present in 17.2% of the cabbage samples, indicating a moderate presence. *Trichuris trichiura* was detected in 10.9% of the cabbage samples, while both *Entamoeba coli* and Hookworm were found in 10.9% and 6.2% of the cabbage samples, respectively. *Isospora belli* was detected in only 4.6% of the cabbage samples, suggesting a relatively lower prevalence. Notably, no occurrences of *Cyclospora caytanesis* and *Balantidium coli* contamination were found in any of the cabbage samples analyzed (Table 3).

The most common parasites found on the contaminated lettuce samples were *Giardia lamblia* and *Ascaris lumbricoid*, both detected in 53.1% of the samples. Following them, *Entamoeba histolytica* was detected in 43.7% of the samples, while *Strongyloides stercoralis* was found in 37.5%. *Cryptosporidium paravium* was identified in 28.1% of the samples, *Balantidium coli* in 18.7%, and *Entrobium vermicularis* in 7.8%. Notably, no

incidents of *Cyclospora caytanesis*, *Entamoeba coli*, Hookworms, *Trichuris trichuria*, and *Isospora belli* were found in any of the lettuce samples (Table 3).

The prevalence of parasites on potato samples was assessed, revealing the following findings. *Entamoeba histolytica* cysts were the most commonly detected parasites, with a prevalence rate of 29.6%. They were followed by *Giardia lamblia* contamination, which had a prevalence rate of 20.3%. *Ascaris lumbricoid* and *Cyclospora caytanesis* showed an equal prevalence rate of 9.4%. Additionally, *Entamoeba coli*, *Cryptosporidium paravium*, and *Entrobilus vermicularis* were found to have the same prevalence rate in potatoes, accounting for 7.8%. However, *Balantidium coli*, *Isospora belli*, hookworm, *Trichuris trichiura*, and *Strongyloides stercoralis* were not detected in the potato samples (Table 3).

The analysis of tomato-contaminated samples revealed the presence of various parasites. *Giardia lamblia* was the most prevalent, detected on 23.4% of the samples. Following closely was the presence of *Entamoeba histolytica* cysts, with a prevalence rate of 20.3%. *Cryptosporidium paravium* was found on 18.7% of the samples, while *Balantidium coli* were detected on 12.5%. Hookworm was present on 9.4% of the tomato samples, and *Ascaris lumbricoid* showed a prevalence rate of 6.2%. *Entrobilus vermicularis* was detected on 4.7% of the samples. However, *Cyclospora caytanesis*, *Entamoeba coli*, *Isospora belli*, *Trichuris trichiura*, and *Strongyloides stercoralis* were not detected in any of the tomato samples (Table 3).

Out of the total carrot samples analyzed, a contamination rate was found. The main contaminant observed was *Giardia lamblia*, with a prevalence rate of 29.6%. Following that, *Entamoeba histolytica* was detected in 18.7% of the samples. *Isospora belli* was found in 3.1% of the carrot samples, while *Cryptosporidium paravium* was present in 1.5% of the

samples. However, no traces of *Cyclospora caytanesis*, *Entamoeba coli*, or any helminth parasites were detected on the carrot samples (Table 3).

Among the green pepper samples analyzed, the highest contamination rate was observed for *Balantidium coli*, with a prevalence of 7.8%. The number of contaminated samples with *Entamoeba histolytica* and *Ascaris lumbricoid* was equal, both detected in 6.2% of the samples. *Giardia lamblia*, *Cryptosporidium paravium*, *Isospora belli*, and *Entrobilus vermicularis* were detected in 3.1% of the samples. *Cyclospora caytanesis* was found in 1.5% of the green pepper samples. Notably, *Entamoeba coli*, hookworm, *Trichuris trichiura*, and *Strongyloides stercoralis* were not detected in any of the green pepper samples (Table 3).

The Wilcoxon Signed Ranks Test was performed on twelve intestinal parasites found on vegetable samples. *G. lamblia* and *E. histolytica* had higher prevalence compared to other parasites. The statistical analysis supported this difference with a significance value of 0.042. This indicates a significant variation in the occurrence of intestinal parasites on vegetable samples (Table 3).

Table 2: Frequency of detected parasites depending on the type of vegetables sold at local markets of Hawassa City.

Detected Parasites	Tomato (n=64) (%)	Potato (n=64) (%)	Cabbage (n=64) (%)	Carrot (n=64) (%)	Green pepper (n=64) (%)	Lettuce (n=64) (%)	Total (N=384) (%)	P-value
<i>Giardia lamblia</i>	15(23.4)	13(20.3)	39(60.9)	19(29.6)	2 (3.1)	34 (53.1)	122(31.7)	0.042
<i>Entamoeba histolytica</i>	13(20.3)	19(29.6)	27(42.2)	12(18.7)	4 (6.2)	28 (43.7)	103(26.8)	0.042
<i>Ascaris lumbricoid</i>	4(6.2)	6 (9.4)	34(53.1)	0	4 (6.2)	34 (53.1)	82(21.3)	0.109
<i>Cryptosporidium parvium</i>	12(18.7)	5 (7.8)	14(21.8)	1 (1.5)	2 (3.1)	18 (28.1)	52(13.5)	0.102
<i>Strongyloides stercoralis</i>	0	0	23(35.9)	0	0	24 (37.5)	47(12.2)	0.18
<i>Entrobium vermicularis</i>	3 (4.7)	5 (7.8)	11(17.2)	0	2 (3.1)	5 (7.8)	26(6.7)	0.109
<i>Balantidium coli</i>	8(12.5)	0	0	0	5 (7.8)	12 (18.7)	25(6.5)	0.18
<i>Entamoeba coli</i>	0	5(7.8)	7 (10.9)	0	0	0	12(3.1)	0.317
Hookworm	6 (9.4)	0	4 (6.2)	0	0	0	10(2.6)	0.317
<i>Cyclospora caytanesis</i>	0	6 (9.3)	0	0	1 (1.5)	0	7(1.8)	1.00
<i>Isospora belli</i>	0	0	3 (4.6)	2 (3.1)	2 (3.1)	0	7(1.8)	0.317
<i>Trichuris trichiura</i>	0	0	7 (10.9)	0	0	0	7(1.8)	0.317
Total	61 (95.3)	59 (92.1)	169 (264.1)	34 (53.1)	22 (34.4)	155 (242.1)	500 (130.2)	

4.2.1 The number of vegetables positive for intestinal parasites

Out of the 384 vegetable samples analyzed, *Giardia lamblia* was present on 53 samples. The contamination rate varied among different types of vegetables, with cabbage and lettuce having the highest contamination rates (13 out of 64 and 14 out of 64 samples respectively). Potato and tomato samples had lower contamination rates (3 out of 64 and 5 out of 64 samples respectively), while carrot samples had a higher contamination rate (16 out of 64 samples). Green pepper samples had the lowest contamination rate (2 out of 64 samples). These findings suggest that *Giardia lamblia* contamination in vegetables is not uncommon and varies depending on the type of vegetable (Figure 3).

Balantidium coli trophozoites were present on 14 out of 384 vegetable samples analyzed. The contamination rate was higher on tomato, green pepper, and lettuce samples. No contamination was found on cabbage, carrot, and potato samples (Figure 3).

Entamoeba histolytica was identified on 54 out of 384 vegetable samples, contamination rates varied depending on the type of vegetable, with cabbage, lettuce, and potato having the highest rates of contamination (Figure 3).

Cryptosporidium parvium a type of parasitic contamination was present on 35 samples of vegetables. The contamination rates varied depending on the type of vegetable, with cabbage having the highest contamination rate (12 out of 64 samples) followed by tomato (10 out of 64 samples). The other vegetables had lower rates of contamination (Figure 3).

Cyclospora caytanesis a type of parasitic contamination was identified on 7 samples of vegetables. The study found that *Cyclospora caytanesis* was only detected on potato and green pepper samples, and not on other vegetables such as cabbage, carrot, tomato, or lettuce (Figure 3).

Entamoeba coli were detected in 8 out of 384 vegetable samples. Among the specific vegetables examined, *Entamoeba coli* were found on 5 out of 64 potato samples and 3 out of 64 cabbage samples. On the other hand, no *Entamoeba coli* cysts were detected in any of the carrot, tomato, lettuce, and green pepper samples (Figure 3).

Isoospora belli was identified on 6 samples, specifically with *Isoospora belli* oocysts found on 3 out of 64 cabbage samples, 1 out of 64 carrot samples, and 2 out of 64 green pepper samples. It is important to note that *Isoospora belli* was not detected in any of the lettuce, tomato, and potato samples (Figure 3).

Hookworm was detected on 6 out of the 384 vegetable samples analyzed. Specifically, Hookworm eggs and larvae were found on 4 out of the 64 cabbage samples. Similarly, Hookworm eggs were identified on 2 out of the 64 tomato samples. No Hookworm was detected in any of the lettuce, carrot, potato, and green pepper samples (Figure 3).

Ascaris lumbricoides were detected on 38 out of the 384 vegetable samples examined. Specifically, *Ascaris lumbricoides* eggs and larvae were identified on 12 out of the 64 cabbage samples. Similarly, 14 out of the 64 lettuce samples tested positive for *Ascaris lumbricoides* eggs and larvae. Among the potato samples, 6 out of the 64 were found to be contaminated with *Ascaris lumbricoides* eggs. Among the tomato samples, 2 out of the 64 were contaminated with *Ascaris lumbricoides* eggs. Lastly, 4 out of the 64 green pepper samples were contaminated with *Ascaris lumbricoides* eggs. No *Ascaris lumbricoides* were detected in any of the carrot samples (Figure 3).

Enterobius vermicularis, another parasitic worm, was found in 17 out of the 384 vegetable samples analyzed. Among the specific vegetables, *Enterobius vermicularis* eggs were identified on 6 out of the 64 cabbage samples. Similarly, *Enterobius vermicularis* eggs were detected on 4 out of the 64 lettuce samples. For potatoes, 2 out of the 64 samples

were contaminated with *Enterobius vermicularis* eggs. Among the tomato samples, 3 out of the 64 were contaminated with 3 *Enterobius vermicularis* eggs. Lastly, 2 out of the 64 green pepper samples were contaminated with *Enterobius vermicularis* eggs. No *Enterobius vermicularis* was detected in any of the carrot samples (Figure 3).

Trichuris trichiura, yet another parasitic worm, was observed on 2 out of 384 vegetable samples. Among the various vegetables tested, *Trichuris trichiura* eggs were identified on 2 out of 64 cabbage samples. No *Trichuris trichiura* eggs were detected on the lettuce, tomato, potato, carrot, and green pepper samples (Figure 3).

Finally, *Strongyloides stercoralis* was detected in 19 out of 384 vegetable samples. Among the specific vegetables examined, *Strongyloides stercoralis* larvae were found on 11 out of 64 cabbage samples and 8 out of 64 lettuce samples. No *Strongyloides stercoralis* larvae were detected on the potato, carrot, tomato, and green pepper samples (Figure 3).

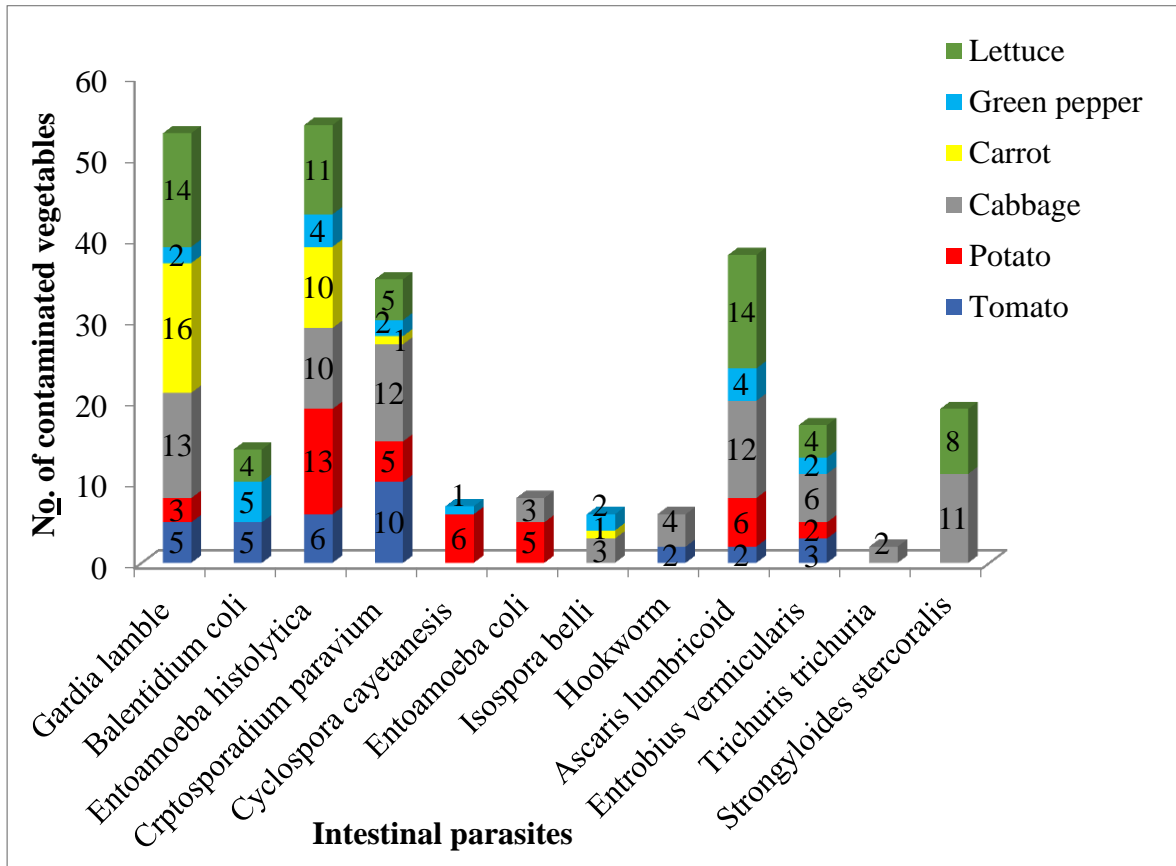


Figure 11: The number of vegetable samples contaminated with intestinal parasites.

4.2.2 The prevalence of intestinal parasites according to the markets

When the parasite types were analyzed for the market, it was noticed that *Giardia lamblia* was the most common parasite found in Addisu Gebeya (n = 82). On the contrary, *Entamoeba histolytica* was the most common in Aroge Gebeya (n = 60). It was also observed that *Entamoeba histolytica*, *Giardia lamblia*, *Ascaris lumbricoid*, *Entrobilus vermicularis*, *Balantidium coli*, *Isospora belli*, *Cryptosporidium parvum*, and *Cyclospora caytanensis* were prevalent in both markets included in this study. Hookworm and *Entamoeba coli* were prevalent in Aroge Gebeya whereas *Strongyloides stercoralis* and *Trichuris trichiura* were identified in Addisu Gebeya. Out of 500 parasites identified, 302 were found in Addisu Gebeya and 198 were in Aroge Gebeya. The type of parasite prevalent according to markets is depicted in below Table 4 and Table 5.

The study conducted at Aroge Gebeya found that a total of ten different parasites were present in various types of vegetables. The most common parasite detected was *Entamoeba histolytica*, accounting for 31.2% of the cases ($P = 0.04$). *Giardia lamblia* cysts were the second most prevalent, found in 20.8% of the samples. *Cryptosporidium parvum* was identified in 16.6% of the cases, while *Ascaris lumbricoid* was present in 10.4% of the samples. *Balantidium coli* were found in 6.7% of the vegetables, followed by *Entamoeba coli* in 6.25%. *Enterobius vermicularis* was detected in 3.6% of the samples, and *Isospora belli* was the least common, present in only 0.5% of the cases (Table 4).

A total of 198 intestinal parasites were detected on the vegetable samples, with 161 being protozoa and 37 being helminths. Specifically, the analysis of tomato samples identified 32 parasites, including 26 protozoa and 6 helminths. Potato samples contained 24 parasites, all of which were protozoa, while no helminths were found. The lettuce samples had the highest parasite count, with 66 parasites, consisting of 49 protozoa and 17 helminths. Carrot samples exclusively harbored 14 protozoa parasites, with no helminths detected. Green pepper samples revealed 4 parasites, all of which were protozoa, while cabbage samples exhibited 58 parasites, comprising 44 protozoa and 14 helminths (Table 4).

Table 3: The type of intestinal parasites prevalent on vegetable samples collected from Aroge Gebeya.

Parasite	Tomato (n=32) (%)	Potato (n=32) (%)	Cabbage (n=32) (%)	Carrot (n=32) (%)	Green pepper (n=32) (%)	Lettuce (n=32) (%)	Total (N=192) (%)	P- Value
<i>Entamoeba</i>								
<i>histolytica</i>	10(31.2)	14(43.7)	13(40.6)	5(15.6)	0	18(56.2)	60(31.2)	0.04
<i>Giardia</i>								
<i>lamblia</i>	3(9.3)	0	11(34.4)	9(28.1)	0	17(53.1)	40(20.8)	0.15
<i>Cryptosporid</i>								
<i>ium parvum</i>	5(15.6)	2(6.2)	13(40.6)	0	2(6.2)	10(31.2)	32(16.6)	0.11
<i>Ascaris</i>								
<i>lumbricoid</i>	0	0	7(21.8)	0	0	13(40.6)	20(10.4)	0.18
<i>Balantidium</i>								
<i>coli</i>	8(25)	0	0	0	1(3.1)	4(12.5)	13(6.7)	0.18
<i>Entamoeba</i>								
<i>coli</i>	0	5(15.6)	7(21.8)	0	0	0	12(6.25)	0.31
Hookworm	6(18.7)	0	4(12.5)	0	0	0	10(5.2)	0.31
<i>Entrobilus</i>								
<i>vermicularis</i>	0	0	3(9.3)	0	0	4(12.5)	7(3.6)	0.31
<i>Cyclospora</i>								
<i>caytanesis</i>	0	3(9.3)	0	0	0	0	3(1.5)	1.00
<i>Isospora belli</i>	0	0	0	0	1(3.1)	0	1(0.5)	1.00
	32	24	58	14	4	66	198	
Total	(100)	(75)	(181.2)	(43.7)	(12.5)	(206.6)	(103.1)	

The analysis of 192 vegetable samples from Aroge Gebeya revealed the presence of various parasitic contaminants. The highest number of samples were contaminated with *Entamoeba histolytica* cysts. Among these contaminated samples, cabbage and lettuce were the most contaminated. On the other hand, the lowest number of vegetable samples were contaminated with *Isospora belli* oocysts. The oocysts were only found on green pepper samples (Figure 4).

Out of 192 vegetable samples tested, 118 samples were contaminated with different intestinal parasites. Among the contaminated vegetable samples, *Giardia lamblia* was detected on 22 samples, *Balantidium coli* trophozoites were found on 9 samples, *Entamoeba histolytica* was identified on 32 samples, *Cryptosporidium parvium* was found on 21 samples, *Cyclospora caytanesis* was detected on 3 samples, *Entamoeba coli* was found on 8 samples, *Isospora belli* was identified on 1 sample, Hookworm was detected on 6 samples, *Ascaris lumbricoides* was detected on 9 samples, and *Enterobius vermicularis* was found on 7 samples (Figure 4).

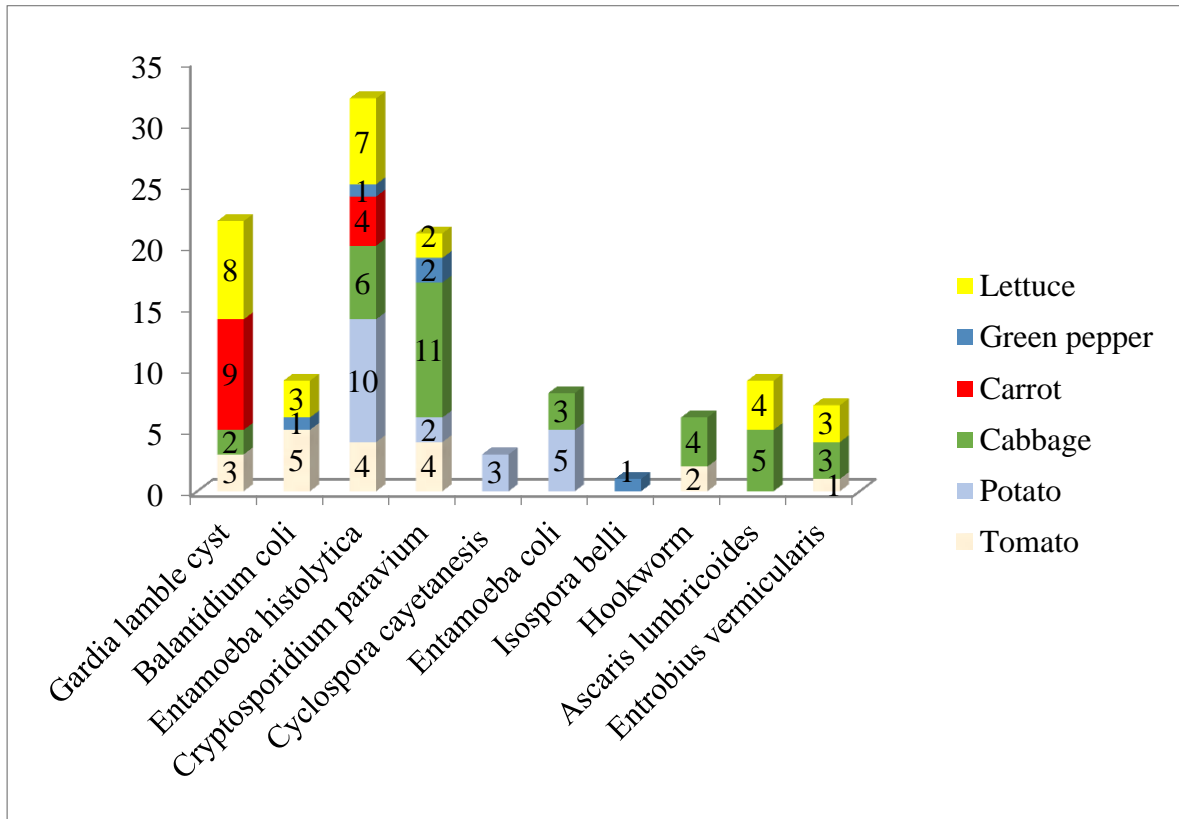


Figure 12: The number of vegetable samples contaminated with intestinal parasites collected from Aroge Gebeya.

A total of ten different parasites were found on various types of vegetables from Addisu Gebeya. These parasites include *Giardia lamblia*, *Entamoeba histolytica*, *Ascaris lumbricoid*, *Cryptosporidium parvum*, *Enterobius vermicularis*, *Balantidium coli*, *Cyclospora caytanensis*, *Isospora belli*, *Strongyloides stercoralis*, and *Trichuris trichiura*. Among these parasites, *Giardia lamblia* was the most common, accounting for 42.7% of the cases (P= 0.04). *Ascaris lumbricoid* cysts were the second most prevalent, found on 32.2% of the samples, followed by *Strongyloides stercoralis* at 24.4%. *Entamoeba histolytica* was detected on 22.3% of the cases, while *Cryptosporidium parvum* was present on 10.4% of the samples. *Enterobius vermicularis* was found on 9.8% of the vegetables, while *Balantidium coli* were identified on 6.2% of the cases. *Trichuris trichiura* was present

on 3.6% of the samples, while *Isospora belli* and *Cyclospora caytanesis* were less widespread, found in 3.1% and 2.1% of the cases, respectively (Table 5).

A total of 302 intestinal parasites were identified on the vegetable samples, with 167 being protozoa and 135 helminths. Specifically, the tomato samples contained 29 parasites, consisting of 22 protozoa and 7 helminths. The potato samples had 35 parasites, including 24 protozoa and 11 helminths. The cabbage samples exhibited the highest number of parasites, with 111 in total, comprising 46 protozoa and 65 helminths. Carrot samples exclusively harbored 20 protozoa parasites, with no helminths detected. Green pepper samples revealed 18 parasites, consisting of 12 protozoa and 6 helminths, while lettuce samples displayed 89 parasites, including 43 protozoa and 46 helminths (Table 5).

Table 4: The type of intestinal parasites prevalent on vegetable samples collected from Addisu Gebeya.

Parasite	Tomato (n=32) (%)	Potato (n=32) (%)	Cabbage (n=32) (%)	Carrot (n=32) (%)	Green pepper (n=32) (%)	Lettuce (n=32) (%)	Total (N=192) (%)	P - Value
<i>Giardia</i>								
<i>lamblia</i>	12(37.5)	13(40.6)	28(87.5)	10(31.2)	2(6.2)	17(53.1)	82(42.7)	0.04
<i>Ascaris</i>								
<i>lumbricoid</i>	4(12.5)	6(18.7)	27(84.3)	0	4(12.5)	21(65.6)	62(32.2)	0.06
<i>Strongyloides</i>								
<i>stercoralis</i>	0	0	23(71.8)	0	0	24(75)	47(24.4)	0.18
<i>Entamoeba</i>								
<i>histolytica</i>	3(9.3)	5(15.6)	14(43.7)	7(21.8)	4(12.5)	10(31.2)	43(22.3)	0.04
<i>Cryptosporidi</i>								
<i>um parvum</i>	7(21.8)	3(9.3)	1(3.1)	1(3.1)	0	8(25)	20(10.4)	0.18
<i>Entrobilus</i>								
<i>vermicularis</i>	3(9.3)	5(15.6)	8(25)	0	2(6.2)	1(3.1)	19(9.8)	0.11
<i>Balantidium</i>								
<i>coli</i>	0	0	0	0	4(12.5)	8(25)	12(6.2)	0.18
<i>Trichuris</i>								
<i>trichiura</i>	0	0	7(21.8)	0	0	0	7(3.6)	0.317
<i>Isospora belli</i>								
<i>belli</i>	0	0	3(9.3)	2(6.2)	1(3.1)	0	6(3.1)	0.31
<i>Cyclospora</i>								
<i>caytanesis</i>	0	3(9.3)	0	0	1(3.1)	0	4(2.1)	1.00
Total	29 (90.6)	35 (109.3)	111 (346.8)	20 (62.5)	18 (56.2)	89 (278.1)	302 (157.2)	

A total of 192 vegetable samples were collected from Addisu Gebeya and analyzed for parasitic contaminants. The highest number of samples were contaminated with *Giardia lamblia* cyst. Among these contaminated samples, cabbage was the most contaminated. On the other hand, the lowest number of vegetable samples were contaminated with *Trichuris trichiura* egg. The egg was only found on cabbage samples (Figure 4).

Out of 192 vegetable samples tested, 141 samples were contaminated with different intestinal parasites. Among the contaminated vegetable samples, *Giardia lamblia* was detected on 31 of the samples, *Balantidium coli* trophozoites were found on 6 samples, *Entamoeba histolytica* was identified on 22 samples, *Cryptosporidium parvium* was found on 14 samples, *Cyclospora caytanesis* was detected on 4 samples, *Isospora belli* was identified on 5 samples, *Ascaris lumbricoides* was detected on 29 samples, *Enterobius vermicularis* was found on 10 samples, *Trichuris trichiura* was found on 2 samples, and *Strongyloides stercoralis* was found on 19 samples (Figure 4).

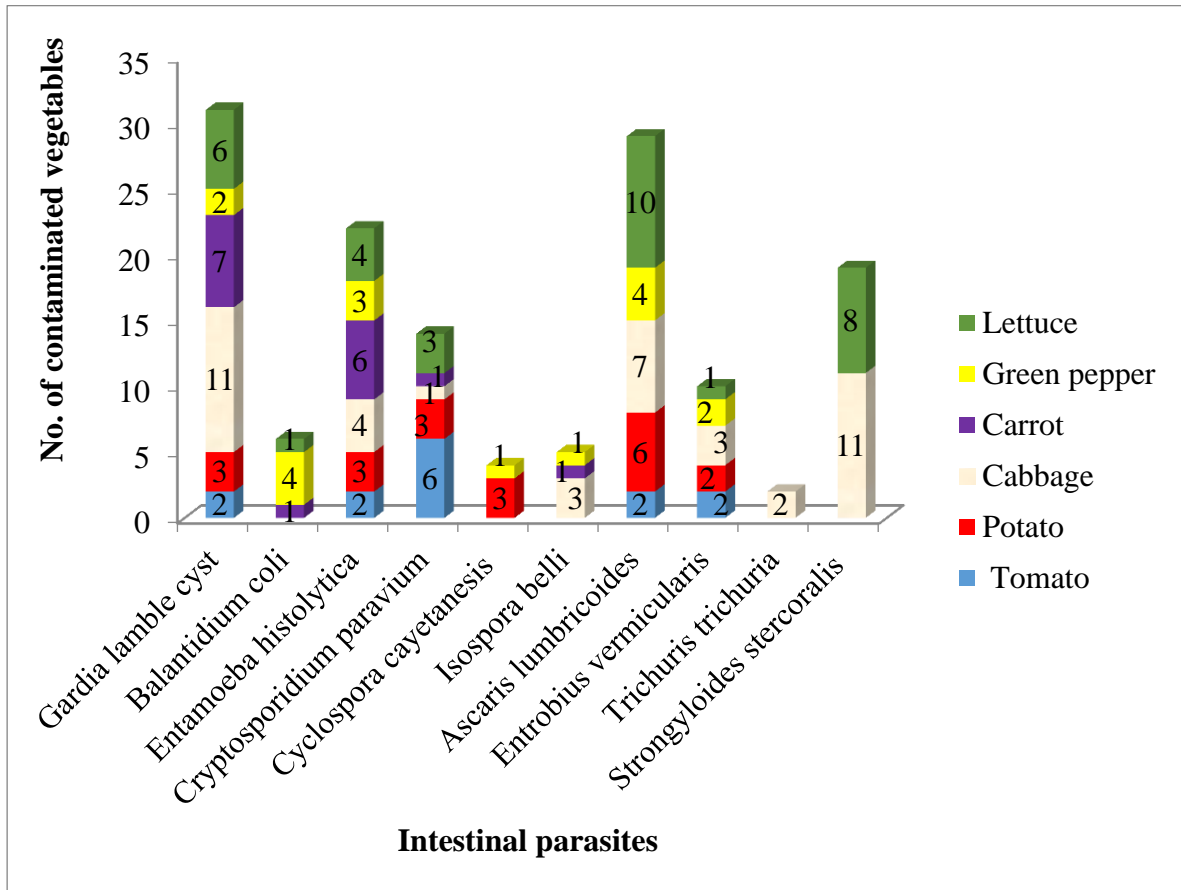


Figure 13: The number of vegetable samples contaminated with intestinal parasites collected from Addisu Gebeya.

4.3 The Stages of Parasites Detected on Vegetable Samples

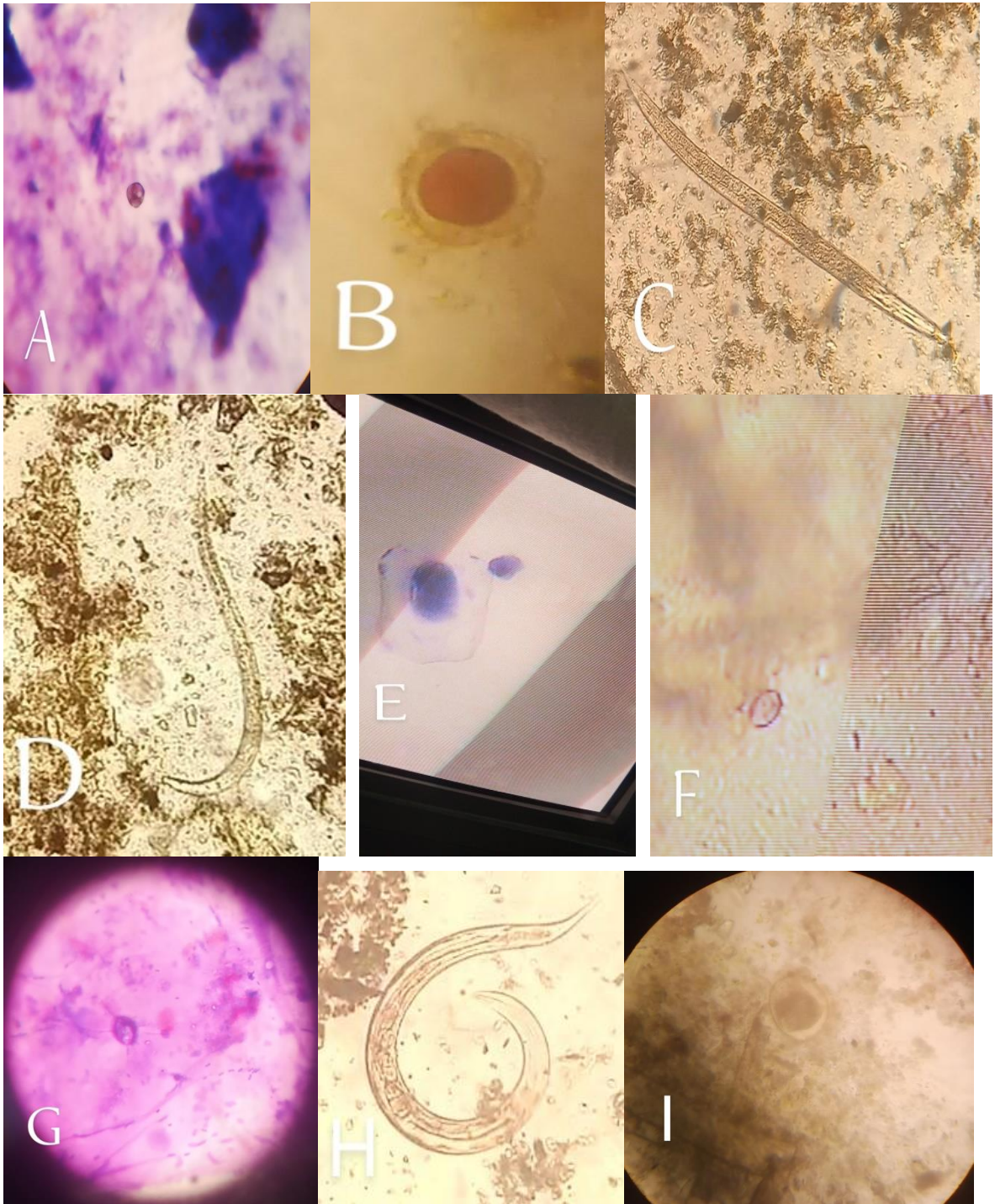
The result of the study revealed the stages of parasites observed through microscopic examination. The specific species and stages of parasites detected during the examination were as follows: cysts of *G. lamblia*, cysts of *Entamoeba histolytica*, egg/larvae of *Ascaris lumbricoid*, larvae of *Strongyloides stercoralis*, egg of *Entrobilus vermicularis*, trophozoites of *Balentidum coli*, cyst of *Entamoeba coli*, egg/larvae of Hookworm, and egg of *Trichuris trichiura* (Table 4).

Through the application of modified Ziehl-Neelsen staining on all samples, the presence of oocysts from coccidian parasites was successfully identified. The detected oocysts included *Cryptosporidium paravium*, *Cyclospora caytanesis*, and *Isospora belli* (as indicated in

Table 5). The identified stages of parasites included eggs, cysts, larvae, oocysts, and trophozoites, with a visual representation provided in Figure 5.

Table 5: The stages of parasites which contaminate vegetables

Parasite	Life-Stage Observed	Frequency (%)
Hookworm	Larvae	3(0.78)
Hookworm	Eggs	7(1.8)
<i>Ascaris lumbricoides</i>	Eggs	46 (11.9)
<i>Ascaris lumbricoides</i>	Larvae	36 (9.4)
<i>Strongyloides stercoralis</i>	Larvae	47 (12.2)
<i>Trichuris trichuria</i>	Eggs	7 (1.8)
<i>Enterobius vermicularis</i>	Eggs	26 (6.7)
<i>Giardia lamblia</i>	Cysts	122 (31.7)
<i>Entamoeba histolytica</i>	Cysts	103 (26.8)
<i>Entamoeba coli</i>	Cysts	12 (3.1)
<i>Balantidium coli</i>	Trophozoites	25 (6.5)
<i>Isospora belli</i>	Oocyst	7 (1.8)
<i>Cryptosporidium parvum</i>	Oocyst	52 (13.5)
<i>Cyclospora cayetanensis</i>	Oocyst	7 (1.8)



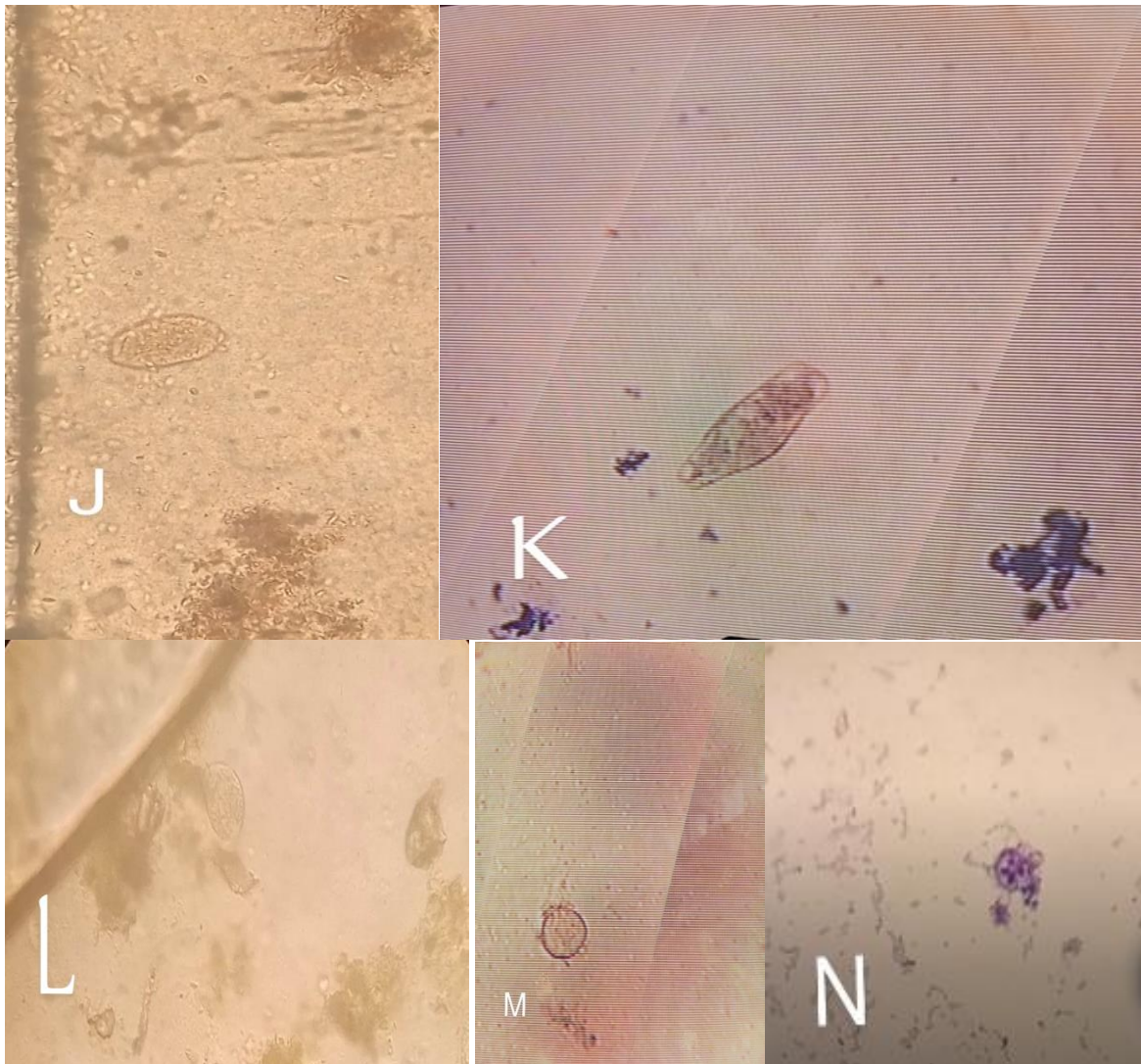


Figure 14: The identified stages of parasites on the microscope. (Source: from the laboratory report).

A. *cryptosporidium paravium* (on Modified Ziehl-Neelsen staining), B. ovum of *Ascaris lumbricoid*, C. *Strongyloides stercoralis* Rhabditiform larva, D. *Ascaris lumbricoides* larva, E. Oocysts of *Isospora belli* (on a Modified Ziehl-Neelsen staining), F. *Giardia lamblia* cyst, G. Oocysts of *Cyclospora cayetanensis* (on a Modified Ziehl-Neelsen staining), H. Hookworm filariform larva, I. Hookworm ovum, J. *Enterobius vermicularis* egg, k. *Trichuris trichiura* egg, L. *Balantidium coli* trophozoite, M. *Entamoeba coli* cyst, N. *Entamoeba histolytica* cyst.

5 DISCUSSIONS

The discussion section of this study focuses on the key findings related to the contamination and prevalence of intestinal parasites on vegetables sold at local markets in Hawassa, Ethiopia. The study revealed that vegetables in the market chain are at risk of being contaminated with parasite pathogens due to the multiple hands they pass through. This highlights the urgent need for improved hygiene practices and proper handling of vegetables throughout the market chain to minimize the risk of contamination. The identification of intestinal parasites on raw vegetables serves as evidence that vegetables can potentially act as carriers for the transmission of harmful parasites. This finding emphasizes the significance of promoting safe food handling and preparation practices, including through washing of vegetables before consumption. By adhering to these practices, the risk of transmitting harmful parasites through contaminated vegetables can be significantly reduced (Beiromvand *et al.*, 2013).

The findings showed that the overall incidence of intestinal parasites was 51.04% across all categories of vegetables sold in the targeted marketplaces in Hawassa City. This suggests that one in every two samples of the vegetables sold in the area were contaminated with intestinal parasites. Similar results have been seen in Ethiopia, Dire Dawa towns at 47.3% (Endale Adugna *et al.*, 2018), Brazil at 50.9% (Luz *et al.*, 2017), and Ghana, Cape Coast metropolis market at 52.4% (Al-Naggar *et al.*, 2018).

However, the present result was lower than the findings of similar studies in Jimma (57.8%) (Tefera Tamirat *et al.*, 2014), Dessie towns in Ethiopia (63.4%) (Hailemeskel Elifaged *et al.*, 2018), Asmara State of Eritrea (57.07%) (Ahmed *et al.*, 2018), Yemen (100%) (Al-Sanabani *et al.*, 2016) and Koforidua in the Eastern Region of Ghana (57.5%) (Kudah *et al.*, 2018). For instance, samples in the study from Ghana were completely

washed twice with saline solution (Kudah *et al.*, 2018), whereas samples in the current study were washed just once. The variances in sample sizes and methodologies may be the reason for the variation of results between this study and others.

Alternatively, the overall contamination rate in the present study was higher than findings in Arba Minch (25.1%) in Ethiopia (Alemu Getaneh *et al.*, 2019), Iran (8.4%) (Matini *et al.*, 2017), Ibadan city of Southwest Nigeria (11.6%) (Adejumoke and Morenikeji, 2015), Alexandria (31.7%) (Said, 2012) and Benha (29.6%) Egypt (Eraky *et al.*, 2014) and Sudan (10.6%) (Mukhtar, 2016). For instance, samples were cleaned with water for 6-7 minutes to remove dust and other particles before being submerged in physiological saline in the research from Egypt (Said, 2012). This may diminish the result, because some parasites may be eliminated by dirt and dust particles. Only 150 samples were tested in an investigation from Sudan, and only non-leafy products were screened. Intestinal coccidian oocysts were not examined since the items were not processed using modified acid-fast staining (Mukhtar, 2016). The current investigation used the same number of items, whereas Arba Minch's study screened different numbers of vegetable samples from each item. This could result in differences between the two investigations since various vegetables have varied susceptibilities to contamination (Alemu Getaneh *et al.*, 2019). Each of them resulted in a lower level of contamination than the current investigation. The components mentioned above might be the reason for the lower contamination of the current study compared to previous studies.

The six different vegetable types sold in particular Hawassa city marketplaces were studied in the current study. While samples of smooth-surfaced vegetables showed lower parasite contamination rates, leafy vegetables and vegetables with large surfaces showed higher contamination rates. Lettuce and cabbage, with their wider and uneven surfaces, are more

susceptible to parasite attachment compared to smooth-surfaced vegetables (Ismail, 2016). Additionally, due to the soft nature of lettuce and cabbage leaves, vendors tend to neglect thorough washing before displaying them for sale (Duedu *et al.*, 2014). On the contrary, vegetables with smooth surfaces like green peppers, tomatoes, potatoes, and carrots have a lower rate of contamination. The smooth surface of these vegetables is easier to wash and may decrease the attachment of parasites (Said, 2012). Cabbage was the vegetable with the highest parasite contamination rate, followed by lettuce. The least contaminated vegetables included potatoes, tomatoes, carrots, and green peppers. Statistical analysis indicated a significant difference in the contamination of vegetable samples ($P = 0.028$). This difference in contamination could be attributed to the variations in the surfaces of the vegetables.

In addition, the variation of contamination among examined vegetable samples maybe the variation of the waste disposal system, hand hygiene practices and the use of irrigation water (Alemu Getaneh *et al.*, 2019). Moreover, the variation of viability and survival of parasites in environment in which vegetable grown areas, maybe the factors for variations of contamination on vegetable samples. For instance, hookworms can survive long periods in the environment, if the conditions are favorable (Silver *et al.*, 2018).

Giardia lamblia cyst was found in 31.7% in this study with the highest frequency out of the twelve different parasite species that were identified. The prevalence was higher than in Dessie town (1.33%) (Hailemeskel Elifaged *et al.*, 2018) and the findings of Khartoum state of Sudan (22.9%) (Mohamed *et al.*, 2016), but it was lower than earlier studies conducted in Asmara state of Eritria (36.4%) (Ahmed *et al.*, 2018). These differences in prevalence rates may be attributed to variations in environmental conditions, water sources, sanitation practices, and levels of awareness about the disease (Abbas *et al.*, 2022).

The second most prevalent parasite identified in the current investigation was *E. histolytica* cysts, which were found in 26.8% of the samples. This percentage was greater than those from Arba Minch (8.4%) (Alemu Getaneh *et al.*, 2019) and Dessie Town (24%) (Hailemeskel Elifaged *et al.*, 2018), but lower than findings from Sudan (42.9%) (Mohamed *et al.*, 2016). Differences in the geographic spread of *E. histolytica* and the cyst's longer survival under cool, wet circumstances may be reasons for variations in the prevalence of the pathogen (Etewa *et al.*, 2017). Vegetables with an *Entamoeba histolytica* cyst present are harmful to consume because the cyst still poses a risk of developing acute amoebiasis. The cyst is resilient and can withstand its surroundings (Bishop and Inabo, 2015).

The third most common intestinal parasite found on vegetables in this investigation was *Ascaris lumbricoides*. They were identified in this study at a frequency of 21.4%, which is comparable to studies done in Arba Minch town, Southern Ethiopia (20.83%) (Bekele Fitsum *et al.*, 2017), but lower than in the Philippines (45.00%) (Su *et al.*, 2012). This dominance may be related to the widespread distribution of the parasite, the large number of eggs laid by the fecund female parasite, and the strong and resistant nature of the eggs, which make it possible to survive unfavorable conditions. The eggs can survive a lack of oxygen for two years at 5-10 °C and are unharmed by desiccation for two to three weeks. (Bekele Fitsum *et al.*, 2017).

In this study, the fourth most prevalent parasite was *Cryptosporidium parvium* oocysts (13.5%). The percentage of *Cryptosporidium parvium* oocysts (13.5%) was lower than that of a study done similarly in Alexandria (29.3%) (Said, 2012). However, lower rates were seen in Arba Minch town, South Ethiopia (4.72%) (Bekele Fitsum *et al.*, 2017) and Ghana (11.11%) (Kudah *et al.*, 2018). *Cryptosporidium* oocysts are one of the more than 150 intestinal illnesses that can be transmitted by contaminated vegetables. The diameter of

a *Cryptosporidium* oocyst ranges from 4 to 9 μm . Coccidian oocyst stages are extremely resistant to environmental stress and chemical disinfection because of their strong oocyst wall, a sophisticated protective barrier consisting of a double layer of a protein-lipid carbohydrate matrix, which is the reason for the spread of *Cryptosporidium* oocyst through vegetables. A reservoir of infection can be created by the ability of infectious *Cryptosporidium* oocysts to survive in the environment (particularly in colder climates) (Utaaker *et al.*, 2017).

There is relatively little information available on the *Strongyloides* spp. found on vegetables in Hawassa City. In this study, the prevalence of *Strongyloides stercoralis* larvae was 12.2%. This result was lower than those of earlier research conducted in Jimma Town, Ethiopia (21.9%) (Tefera Tamirat *et al.*, 2014) and Accra, Ghana (43%) (Duedu *et al.*, 2014). The presence of *Strongyloides stercoralis* on vegetables was observed in studies from Saudi Arabia (El Bakri *et al.*, 2020). The large documented numbers of *Strongyloides* species could be explained by this study. *Strongyloides* species have complicated life cycles, including free-living phases in the soil. This could potentially be the cause (Adamu *et al.*, 2012). Some fruits, vegetables, and herbs have been found to harbor parasite ova and act as reservoirs for infection when grown in soil with high humidity and good drainage. *Strongyloides stercoralis* ova develop into rhabditiform larvae in the soil. The presence of *Strongyloides stercoralis* rhabditiform larvae on vegetables does not endanger humans because infection only occurs when the filariform larval stage penetrates intact skin. However, given that human infection by this nematode is mostly obtained by skin penetration of filariform larval forms in barefoot humans rather than ingestion, the current observation may not pose any substantial risk to consumers. On the other hand, if *Strongyloides stercoralis* predominates, there will be a greater risk of infection for those who work on vegetable farms and touch produce (Zeehaida *et al.*, 2011).

Eggs of *Enterobius vermicularis* were found in samples of vegetables in this investigation (6.7%). The results were almost identical in southwestern Saudi Arabia (El Bakri *et al.*, 2020), where 6.3% of vegetables were contaminated by *Enterobius vermicularis*. In Bahir Dar City, northwestern Ethiopia (0.5%) (Alemu Getaneh *et al.*, 2020) and Turkey (0.9%) (Adanir and Tasci, 2013), lower contamination rates were found. In Dhamar, Yemen, the rates were higher (19.2%) (Al-Sanabani *et al.*, 2016). These variations in contamination rates could be attributed to differences in sanitary practices and diagnostic methods used across different studies. It is important to note that *Enterobius vermicularis* is one of the most prevalent helminth infections, and humans are the only natural hosts for this parasite. The small weight and size of the eggs (50-60 µm by 20-25 µm) make them easily spread in the surrounding environment. The eggs have a characteristic uneven surface and a smooth double-walled shell. The ability of *Enterobius vermicularis* eggs to survive in a moist environment for up to three weeks, depending on temperature, further highlights the potential risk of transmission through contaminated vegetables. Consumption of food contaminated with infected eggs can lead to human infection (Alemu Getaneh *et al.*, 2020). In the current study, *Balantidium coli* were also found (6.5%). A greater prevalence was found in Ghana (13.6%) (Kudah *et al.*, 2018) and Ilorin, Nigeria (8.19%) (Alade *et al.*, 2013). This variation may depend on the number of contaminated pigs in the area. *B. coli* is a commensal protozoan of the pig intestinal lumen that can parasitize humans when a lesion occurs in the mucosa of the colon and the cecum, causing secondary infection since it is not able to penetrate the intact mucosa alone (Alemu Getaneh *et al.*, 2019). In this investigation, 3.1% of the parasite contamination included *Entamoeba coli*. Similar outcomes (3.75%) were found in Metro Manila, Philippines (Su *et al.*, 2012). However, higher contamination of *E. coli* was found (6.8%) in Benha, Egypt (Eraky *et al.*, 2014) and (9.2%) in Shahrekord, Iran (Fallah *et al.*, 2012). *E. coli*, a commensal protozoan species

found in the human gut is important to notice since it serves as an accurate indicator that an area has been contaminated. Not less significant, despite being commensal, the presence of *E. coli* in samples is thought to be a sign of parasitic contamination. Diagnosing this condition is crucial to spread preventative measures to prevent other contaminations, such as those caused by pathogenic amoeba-like *E. histolytica* (Rodrigues *et al.*, 2020).

In the current investigation, samples of tomato and cabbage had hookworm, which had a prevalence of 2.6%. However, greater rates were found in research done in Sudan (5.7%) (Mohamed *et al.*, 2016), Ghana (13%) (Duedu *et al.*, 2014), Nakhon Si Thammarat province in southern Thailand (16.60%) (Punsawad *et al.*, 2019), and northern Iran (4.40%) (Rostami *et al.*, 2016). In contrast, no ova of hookworm species were found in several earlier investigations conducted in Arba Minch town, Southern Ethiopia (Bekele Fitsum *et al.*, 2017) and Benha, Egypt (Eraky *et al.*, 2014). This variation in hookworm egg contamination of vegetables may be due to variations in geographic location, climatic conditions, and soil types. Hookworm eggs can live in soil for several weeks or months in the right conditions. Ingestion of contaminated vegetables is the main route of transmission for hookworm infection (Silver *et al.*, 2018).

Trichuris trichiura, a common intestinal helminth in humans, was also detected in cabbage samples. The prevalence rate of *T. trichuria* in the current study (1.8%) was comparable to that in some other studies conducted in Khartoum state, Sudan (2.9%) (Mohamed *et al.*, 2016), Mazandaran province, northern Iran (2.2%) (Rostami *et al.*, 2016), and Accra, Ghana (2%) (Duedu *et al.*, 2014). However, Lusaka, Zambia, showed higher rates (5.3%) (Nyirenda *et al.*, 2021). In comparison, the outcome is higher than that of Nigeria, where the contamination rates were 0.5 percent (Adamu *et al.*, 2012). This variation of the result may be the variation of sanitary practices in the area. *Trichuris trichiura* is a helminth that

spreads through the soil, and its ova take some time to mature in the soil before they may spread infection. The *Trichuris trichiura* infection is caused by ingesting eggs. This can occur if dirty hands or fingers are placed in the mouth, or if someone eats uncooked, improperly cleaned, or unpeeled vegetables. As a result, the finding that cabbage was contaminated with this parasite was not good for consumers (Parija *et al.*, 2017).

Protozoan pathogens that are transmitted through food, like Cyclospora, have a significant impact on public health because they induce intestinal illnesses. In both industrialized and developing nations, Cyclospora especially impacts the very young, the elderly, and those with impaired immune systems or immunosuppression (Ortega *et al.*, 2010). In this study, Cyclospora oocysts were found in 1.8% of the vegetable samples that were tested, which is almost identical to earlier findings in Arba Minch town, southern Ethiopia (2%) (Alemu Getaneh *et al.*, 2019). Nevertheless, Alexandria (21.3%) had higher rates (Said, 2012). Cyclospora oocysts can spread through vegetables because of their small size (8–10µm), low specific gravity, and high infectiousness (Caradonna *et al.*, 2017). Depending on the temperature of the environment, such oocysts can survive for weeks to months in food and withstand standard chemical or sanitization disinfection processes used in irrigation systems, recreational waterways, or drinking water treatment facilities (Ortega *et al.*, 2010).

In the current investigation, *Isospora belli* oocysts were found in 1.8 percent of the samples that were tested, but this was lower than the earlier finding in Arba Minch town, southern Ethiopia (3.06%) (Bekele Fitsum *et al.*, 2017) and in Dire Dawa towns (4.3%) (Endale Adugna *et al.*, 2018). In Accra, Ghana (0.3%) (Duedu *et al.*, 2014), lower contamination rates were found. The variation may be the difference in geographical locations and

hygienic methods. Because human isosporiasis is more prevalent in warm places with poor hygienic conditions (Rodrigues *et al.*, 2020).

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the above results the following conclusions were given:

- *Giardia lamblia* is the most contaminant of the raw vegetable samples whereas *Trichuris trichiura*, *Cyclospora cayetanensis*, and *Isospora belli* show the lowest contamination at the same frequency.
- The cabbage samples show the highest parasitic contamination whereas green pepper samples show the lowest parasitic contamination.
- The isolation of parasites from vegetable samples shows the existence of *Giardia lamblia* cysts, *Entamoeba histolytica* cysts, *Ascaris lumbricoid* eggs/larvae, *Cryptosporidium parvum* oocysts, *Strongyloides stercoralis* larvae, *Enterobius vermicularis* eggs, *Balantidium coli* trophozoites, *Entamoeba coli* cysts, hookworm eggs/larvae, *Cyclospora cayetanensis* oocysts, *Isospora belli* oocysts, and *Trichuris trichiura* eggs.
- Vegetables purchased from Addisu Gebeya and Aroge Gebeya can be presumed unsafe for direct consumption without proper precautions.

6.1 Recommendations

The following recommendations were made in consideration of the results and conclusion of the study:

- Vegetables should be thoroughly washed and soaked in a solution of clean water mixed with a food-grade disinfectant or a few drops of vinegar for about 15-20 minutes.
- The vegetable should be cooked well before consumption to kill the parasites which are not removed through washing.
- In addition, vendors and consumers should practice proper sanitation and hygiene practices, as well as wear appropriate footwear to protect against skin penetration, especially when handling vegetables.
- The public health department should educate the public on the precautions to avoid and control foodborne illness.
- Future researchers should conduct a similar study in a different region in Ethiopia to discover new knowledge and add to the limited literature on the contamination of vegetables.

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7 APPENDICES

Appendix 1: Sample collection sites and forms of display



A. Potato, B. Tomato, C. Carrot, D. Green pepper, E. Cabbage, and F. Lettuce.

Source: Images were captured during sampling.

Appendix 2: Chopping and peeling of vegetables to rinse in the beaker



Appendix 3: Chemicals used to detect parasites in vegetables (methylene blue, iodine solution, absolute methanol, Carbon fuchsin solution, acidic alcohol).



Appendix 4: Staining of sediments to identify *Cryptosporidium*, *Isospora*, and *Cyclospora* oocysts



BIOGRAPHICAL SKETCH

Author, Debebe Dogiso Deboche, was born in January 1989 E.C in Wolaita Zone Bolosore Woreda at Mehal kifile ketema. He attended his elementary education (1-8) at Wadu Primary School. He attended his high school (9-12) in Areka secondary and preparatory school. He joined Wolaita Sodo University in 2010 E.C and was awarded a degree in Biology in 2013 E.C. In September 2014 E.C, joined the School of Postgraduate Studies of Hawassa University Department of Biology to pursue a Master of Science (MSc) degree in Biomedical science.