

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الآية

قال تعالى:

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

سورة البقرة الآية 32

# Dedication

*To my caring family which always had been there for me....*

*To my mother who always supported me...*

*To my father who always took care of me...*

*To my brothers and my sister who always inspired me...*

# Acknowledgement

*Special thanks and appreciations to my caring supervisor Dr. Mohammed Baha Eldin for his caring supervision and close guidance.*

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## Abstract

This study was conducted at Khartoum central market to detect the parasitic contamination of vegetables sold for consumption. Microscopic examination of the samples was carried out using wet preparation and sedimentation technique.

Total of 150 samples of vegetables were examined with five types of vegetables (watercress, onion, carrot, pepper and tomatoes). Among the 150 samples, 16 (10.6%) were positive for intestinal parasites. Among positive samples, onion and watercress were found to have the highest parasitic prevalence; onion positive samples were 8 (50%) , watercress positive samples were 6 (37.5%), pepper positive samples were 2 (12.5%), no parasite were detected in both tomatoes and carrot.

The parasite identified were *Schistosma mansoni* egg, *Schistosma haematobium* egg, *Hook worm*, *Strongyloides stercoralis* larvae, *Taenia spp* egg and *Balantidium coli* ciliate.

The most prevalent parasite encountered was *Strongyloides stercoralis* larvae which was detected ten times constituting 62% out of 16 positive samples, followed by *Schistosma spp* egg which was detected three times (19%) , then comes *Hook worm* egg, *Taenia spp* egg and *Balantidium coli* ciliate which were detected one time with the percentage of 6% for each.

## الخلاصة

أجريت هذه الدراسة في سوق الخرطوم المركزي للخضروات لتقييم تلوث الخضار المستهلكة يومياً بالطفيليات المعوية.

الفحص المجهرى للعينات تم بواسطة المسحة الرطبة و طريقة الترسيب.

تم فحص ما مجموعه 150 عينة تتضمن خمس أنواع من الخضار: الجرجير, البصل, الجزر, الفلفل و الطماطم. من ضمن 150 عينة, وجد أن 16 عينة (10.6%) كانت موجبة للطفيليات المعوية. ومن ضمن العينات الموجبة وجد أن أعلى معدلات الانتشار للطفيليات المعوية وجدت في البصل ثم الجرجير, حيث وجد ان العينات الموجبة في البصل كانت 8 (50%), و العينات الموجبة في الجرجير كانت 6 (37.5%), أما الموجبة في الفلفل 2 (12.5%), ولم يتم إيجاد طفيليات معوية في الطماطم و الجزر .

الطفيليات التي تم إيجادها كانت: بويضة البلهارسيا بنوعيهما, بويضة الدودة الاسطوانية البرازية,

بويضة الدودة الخطافية, بويضة دودة التينا الشريطية و القربية القولونية.

أكثر طفيل تم إيجاده كانت بويضة الدودة الاسطوانية البرازية, وتم إيجاده عشرة مرات بنسبة 62% من ضمن 16 عينة موجبة, و من بعدها بويضة طفيل البلهارسيا بنوعيهما تم إيجاده ثلاث مرات (19%), ثم بويضة طفيل الدودة الخطافية وبويضة دودة التينا الشريطية و القربية القولونية وجد كل منهم مرة واحدة بنسبة 6% لكل منهم على حده.

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## **Chapter one**

### **Introduction and literature review**

#### **1.1 About vegetables :**

Vegetables are essential for good health and they form a major component of human diet. They are vital energy contributors that are depended upon by all levels of human as food supplement or nutrients. They have high water content as seen in water leaf, lettuce and cabbage. Many vegetables and fruits are good sources of vitamin C, carotene and mineral elements such as iron (Duckworth, 1996).

Diets that include a sufficient amount of potassium from fruits and vegetables also help to reduce the chance of developing kidney stones and the effect of bone loss.

Fruits are generally rich sources of fibers and carbohydrates. They are very healthy, whole to be enjoyed in moderation because some fruits are high in calories, carbs and glycomic index.

An important nutritional value of fruits is its antioxidant contents; fruits such as orange, carrot; garden egg and tomato have the highest antioxidant value (Halvorsen, *et al.*, 2002).

The nutritional content of vegetables varies considerably, generally they contain little protein and fat, dietary minerals, carbohydrate and varying proportion of vitamins such as vitamin A, vitamin K and vitamin B6. Vegetables contain a great variety of other phytochemicals some of which have been claimed to have antioxidant, antibacterial, antiviral and arcinogenic properties (Gruda, 2005).

The cultivation of vegetables and fruits for commercial and domestic purposes in Nigeria is mostly carrsied out by peasant farmers who depend on irrigation and/ or natural rainfall (Lucas, *et al.*, 2000).

Since vegetables require a moist environment for growth, these conditions favor the development of transmissible form of entero-parasites such as cysts and eggs (Oliverra, 1992).

Vegetables and fruits particularly those eaten raw and without peeling can be agent of transmission of protozoa and helminthes (Porter, *et al.*, 1990).

The cultivation of vegetables in many parts of the world has been amplified with the application of fertilizer and or manure. In Africa, the transmission of intestinal parasitic infection has been considered to increase successfully due to the frequent use of untreated human or animal dung as manure in cultivation by the local farmers, which serves as a source of enhancement of zoonotic parasitic infection (Lucas, *et al.*, 2000).

Consumption of raw or unhygienically prepared vegetables such as cabbage (*Brassica eracea*), lettuce, okra, garden egg (*Solanum macrocarpum*), cucumber, carrot (*Daucus carota*), waterleaf (*Talinum triangulare*), pumpkin (*Cucurbita*), spinach, tomatoes (*Lycopersicon esculentum*), etc, is considered to be a risk factor for human parasitic infections (Chessbrough, 1991).

These vegetables though seasonal, are cultivated in the same piece of land every year. As a result of this continuous land usage, there is depletion of nutrient hence the need for fertilizer or manure. Most farmers use untreated animals and human feces as manure, which are known to contain various species of parasites that are of medical and veterinary importance (Okoronkwo, 1998).

Indiscriminate fecal disposition in bushes, farm lands and even in present farms with a belief of enriching the lands is also a common practice by farmers and unlearned citizens. Some of the water bodies used for irrigation is also polluted with parasites infected excreta that could lead to recycling of infection (Ayer, *et al.*, 1992).

Increasing water scarcity in dry climate regions with agriculture based economy forces people to use untreated wastewater for irrigation of crops.

Practice of using untreated municipal waste water for irrigation, raw manure as fertilizer and habit of eating vegetables raw or undercooked are reported to result in risk of infection with intestinal parasites in developing countries (Blumenthal, *et al.*, 2000).

Infection with vegetable-transmitted parasites and pathogenic bacteria can occur due to occupational exposure or through consumption of vegetables that are contaminated with human or animal excreta without proper washing and disinfection (Beuchat, 1998).

Unhygienic sewage disposal and absence of its treatment facilities pose potential health hazards through contaminating irrigated food crops with parasites in urban and suburban areas of African countries including Ethiopia (Srikanth and Naik, 2004).

Vegetables are reported to harbor intestinal parasites such as *Ascaris lumbricoides*, *Taenia* spp, *Fasciola hepatica*, *Hymenolepis nana*, *Echinococcus* spp, *Trichuris* spp, *Enterobius vermicularis*, *Trichostrongylus* spp, *Toxocara* spp, *Strongyloides stercoralis*, *Giardia lamblia*, *Entamoeba* spp, *Iodamoeba butschlii*, *Blastocystis hominis* and *Cryptosporidium parvum* (Gharavi, *et al.*, 2002).

The resistant nature of infective stages of the aforementioned parasites such as eggs, cysts or oocysts to adverse temperatures, desiccation, natural irradiation, chemicals and commonly used disinfectants results in high prevalence of vegetable-transmitted parasites in developing countries (Beuchat, 1998).

On the other hand, living under immunocompromised situation mainly due to HIV/AIDS exposes some percentage of the population across the world to opportunistic vegetable-transmitted parasites such as *Cryptosporidium* spp (Esrey, *et al.*, 1998).

Several studies documented prevalence of intestinal parasites in different parts of Ethiopia including Tigray region through microscopic examination of stool samples collected from suspected human population (Legesse and Erko, 2004).

## **1.2 About intestinal parasites:**

Parasites are living organisms that live off the host (a living organism) and utilize the host's nutrients completely, harming it in due process. Parasites enter the human body through many channels: air, water and the food we eat.

Once they get in, not only do they thrive on the food we eat, but also start eating us from within, to accelerate their multiplication they can thrive anywhere in the body but are mostly found in the gastro-intestinal tract. There are over a thousand types of parasites that can live in the human body; some are extremely microscopic while others are big enough for the human eye to see. In humans, intestinal parasites can cause serious health conditions by making the body weak and undernourished, increasing its vulnerability to viral, fungal, bacteria, chemical and metal poisoning as well as various diseases (Taylor, 2007).

### **1.2.1 Intestinal protozoa: -**

Protozoa are single-celled eukaryotes, some of them are free living but others are parasites for humans and other species (Steven, 2003).

The most common intestinal protozoan parasites are: *Giardia intestinalis*, *Entamoeba histolytica*, *Cyclospora cayentanensis*, and *Cryptosporidium* spp.

The diseases caused by these intestinal protozoan parasites are known as giardiasis, amoebiasis, cyclosporiasis, and cryptosporidiosis respectively, and they are associated with diarrhea (Guyatt, 2002).

*G. intestinalis* is the most prevalent parasitic cause of diarrhea in the developed world, and this infection is also very common in developing countries. Amoebiasis is the third leading cause of death from parasitic diseases worldwide, with its greatest impact on the people of developing countries. (WHO) estimates that approximately 50 million people worldwide suffer from invasive amoebic infection each year, resulting in 40-100 thousand deaths annually (WHO, 2006).

Cryptosporidiosis becoming most prevalent in both developed and developing countries among patients with AIDS and among children aged less than five years.

Several outbreaks of diarrheal diseases caused by *C.cayetanensis* have been reported during the last decade (Herwaldt, 2000).

Spread of these protozoan parasites in developing countries mostly occurs through fecal contamination as a result of poor sewage and poor quality of water.

Food and water-borne outbreaks of these protozoan parasites have occurred, and the infectious cyst form of the parasites is relatively resistant to chlorine (Okhuysen and White, 1999).

#### **1.2.1.1 Classification of intestinal protozoa:**

The protozoa contain unicellular organisms, which belong to the Kingdom, Protista. Protozoa are more primitive than animals, and no matter how complex their bodies may be, all the different structures are contained in a single cell.

Protozoa, like most organisms, are eukaryotic, in that their genetic information is stored in chromosomes contained in a nuclear envelope.

In this way they differ from bacteria which do not have a nucleus and whose single chromosome is coiled like a roll of wool in the cytoplasm.

This primitive arrangement, found only in bacteria, *rickettsia* and certain algae, is called prokaryotic and such organisms may be regarded as neither animal nor plant, but as a separate kingdom of prokaryotic organisms, called the Monera (Taylor, 2007).

Classification of the subkingdom protozoa (kingdom Protista) is extremely complex and the classification given below is intended to give outline of the basic differences in the structure and life cycles of the main groups.

To a large extent, the common characteristics of each group are reflected by similarities in the diseases they cause.

There are four phyla of protozoa of veterinary importance, the Sarcomastigophora, Apicomplexa, Microspora and Ciliophora.

#### **1.2.1.1 .1 Phylum Sarcomastigophora :-**

Protozoa with locomotion by pseudopodia and/or flagella.

##### **Has two subphylum's:-**

Subphylum Sarcodina:-

Members of the subphylum sarcodina move by means of pseudopods, which are also used for feeding, their cytoplasm is divided into endoplasm, containing food vacuoles and nucleus, and relatively clear ectoplasm.

Reproduction is asexual by binary fission. Only a few species of the sarcodina are pathogenic.

##### **Family Endamoebidae:-**

Members of this family are parasitic in the digestive tract of vertebrates and invertebrates.

Three genera contain parasites of animals and man (*Entamoeba*, *Iodamoeba*, *Endolimax*) but only *Entamoeba* contains pathogenic species of veterinary significance.

Genera are differentiated on the basis of their nuclear structure.

##### **Subphylum Mastigophora:-**

These are flagellate protozoa having one or more flagella, (like *giardia lamblia*). Multiplications mainly asexual by binary fission with some species producing cysts (Taylor, 2007).

#### **1.2.1.1 .2 Phylum Apicomplexa:-**

Class sporozoa and the subclass coccidia representative species within this group include *Cryptosporidium parvum*, *Cyclosporacayetansis*, *Isospra belli*, *Toxoplasma gondii* and *Sarcocystis spp.*

#### **1.2.1.1 .3 Phylum Cliliphora:-**



Class Kinetofragminophorea and the order is *Trichostomatida*. The only ciliate *Blantidium coli* contain two nuclei, a large macronucleus and a small micronucleus (Beaver, 1984).

#### **1.2.1.1 .4 Phylum Microsporidia:-**

It is a group of protozoa characterized by obligate intracellular replication and spore formation (Balbiani, 1882). *Enterocytozoon bienersi* and *Septata intestinalis* are two important *Microsporidia* species that cause severe, persistent and watery diarrhea in AIDS patients.

#### **1.2.1.2 Transmission and life cycle of intestinal protozoa:**

Most intestinal protozoa are transmitted by fecal-oral route, particularly in contaminated food, water or hands (Petri, *et al.*, 2006).

Also person to person transmission may occur through anal-oral sexual stimulation (Steven, *et al.*, 1981).

The life cycles of intestinal protozoa are very similar, with the exception of *Dientamoeba fragilis*, which lacks a cyst stage.

The mature cysts of protozoa are infective forms of the parasites and enter into man through contaminated food and drinking water, the cyst enter into the alimentary canal pass unaltered through the stomach (cyst wall is not dissolved by the action of gastric juice), and then reach the small intestine (*G.lambillia* and *B.coli*) or the large intestine (*E.histolytica*), where cysts liberates the active motile trophozoites. The trophozoites (vegetative form) cause distortion and necrosis of the intestinal surface (Gillespie and Pearson, 2001).

Sometimes enter into the deeper layer and some may reach the liver, as in the case of *Entamoeba histolytica*.

In spore forming protozoa (intestinal coccidia), spore ingestion begins a life cycle that is similar in all 4 of the intestinal spore-forming protozoa. The ingested spores release sporozoites that invade enterocytes, primarily in the small intestine. The enterocyte infection progresses through 2 stages: merogenic and sporogenic. The merogenic (or schizogonic) stage involves the

maturation and development of meronts to reproduce and multiply in the infected cell or to infect other enterocytes. This asexual stage allows the infection to spread into many enterocytes, even if the host is not exposed repeatedly to the organism. The sporogenic (i.e., gametogonic, sexual) stage involves the maturation and development of sporozoites enclosed in cysts or spore. As the infected enterocytes die, cyst or spore shedding occurs. The spores are then excreted in the stool (Gillespie and Pearson, 2001).

### **1.2.1.3 Epidemiology:**

Intestinal parasitic infection occurs worldwide with highest prevalence in developing countries, this mainly due to deficiency of sanitary facilities, unsafe human waste disposal system, inadequacy and lack of safe water supply, and low socioeconomic status (Ali, *et al.*, 2011).

Giardiasis is a global disease which is caused by *G.lambillia*. It infects nearly 2% of adults and 6% to 8% of children in developing countries worldwide. Nearly, 33% of people in developing countries have had *giardiasis*. It occurs worldwide but it's more prevalent in areas with inadequate sanitary conditions and where water treatment facilities and procedures are poor (Ortega, *et al.*, 1997).

*Giardiasis* is also found among people living in developed countries where sanitation is adequate and water supplies are piped and purified (Mank, 2001).

Amoebiasis is a worldwide disease which is caused by *E.histolytica*. It represents the third most common cause of death due to parasitic infection after malaria and *Schistosomiasis*, as estimated by the world health organization (Janyuksel, *et al.*, 2003).

It has been estimated that about 10% of those with *amoebiasis* develop *amoebic* disease and that annually probably 40000-110000 people die from invasive *amoebiasis* worldwide. *Amoebiasis* is responsible for approximately 10000 deaths per year, mainly in the central and South America, Africa and India (Steven, 2003).

Spore forming protozoa may be common worldwide, and their frequency may be related to the inadequacy of sanitation. In general, the infections are more commonly in developing countries. The prevalence rates among patients with AIDS are higher in developing countries than in the United States (Assefa, *et al.*, 2009).

*B.coli* infections are rare in humans; it is widely distributed among those who have contact with pigs, particularly in warm climates (Beaver, 1984).

In Sudan, the following data is available about *giardiasis* and *amoebiasis*:

- In Khartoum, among intestinal parasitic cases there are 18 cases (0.3%) of *amoebiasis* and 1215 cases (37.8%) of *giardiasis*.
- In Omdurman among intestinal parasitic cases there are 0 cases (0%) of *amoebiasis* and 764 cases (3.4%) of *giardiasis*.
- In Bahri among intestinal parasitic cases there are 141 cases (3.4%) of *amoebiasis* and 1998 case of *giardiasis* (Ministry of health, Khartoum state annual reports, and 2008).

#### **1.2.1.4 Clinical presentation:**

In *E.histolytica* infection, symptoms range from mild diarrhea to dysentery with blood and mucus (Hague, *et al.*, 2006).

Invasive *E. histolytica* occur in two forms: dysentery and amoebic colitis (Adams and Mc loeod, 1977).

If parasite reaches blood stream, it can spread through the body, mostly ending in the liver causing *amoebic* liver abscess. (Maltzand and Knauer, 1991).

*Giardiasis* is the most frequently diagnosed intestinal parasitic disease in United States and among travelers with chronic diarrhea (Huang, *et al.*, 2006).

Spore forming protozoa causes watery diarrhea, especially immunocompromised patients, e.g.; AIDS.

After infection of intestinal epithelium, *T. Gnodaii* and some *Sarcocysts spp.* spreads to other organs (brain, lungs, liver and eyes) (Garcia, 2001).

## **1.2.2 Intestinal helminthes:-**

### **1.2.2.1 Definition:**

It's a multi-cellular, bilaterally symmetrical animals; helminthes of importance to humans are divided into three main groups: cestodes (tape worms), trematodes (flukes) and nematodes (round worms).

Intestinal helminthes are those who parasitize gastrointestinal tract. (Peter, 2005).

### **1.2.2.2 Classification:-**

Belong to subkingdom Metazoa, which is subdivided into two phyla:

Nematoda and Platyhelminthes.

#### **1.2.2.2 .1 Phylum Nematoda:-**

It's the second largest class among animal kingdom, it has up to 500.000 species, and many of them are free living.

These worms are cylindrical, bilaterally symmetrical, round in cross sections, has well developed digestive system, they are dioecious with the exception of *Strongyloides stercoralis* in which the female may be parthenogenetic (Grassi, 1876).

Their bodies are unsegmented, containing body cavity, not lined with mesothelium.

Males generally are smaller than females, the specialized structures at the anterior end of the body serves for attachment, penetration, abrasion and sensory purposes; it may be equipped with hooks, teeth, plates, stylet, and papillae (Beaver, *et al.*, 1984).

#### **1.2.2.2 .2 Phylum Platy helminthes:-**

**Sub divided into two classes:**

##### **1. Class cestoda:**

Adult cestodes are long, flat, segmented worms that lack body cavity and digestive tract, they absorb nutrients from the host through their integument, and there is no respiratory tract or blood vascular system.

It has three recognizable portions; scolex (head), which acts as a holdfast organ located at the anterior end by means of suckers which are usually four in number and bear hooks, the head is followed by the neck which is a segmented region, it gives rise to the rest of the worm which consists of a numerous segments. (called strobilia).

Mature proglottides contain both sex organs in the same segment (monoecious).

The important intestinal cestodes of human beings are placed in two orders:

Pseudophyllidea and cyclophyllidea (Beaver, *et al.*, 1984).

## **2. Class trematoda:**

Trematodes also called flukes, their body is flattened and leaf like with the exception of *schistosomes*, which is elongated.

They have one muscular sucker which is always present at the anterior or ventral surface, their elementary canal consists of a short median anterior portion which bifurcates and blindly ends.

Trematodes are monoecious with the exception of *schistosomes*, they vary in size (Beaver, *et al.*, 1984).

### **1.2.2.3 Transmission and life cycle of intestinal helminthes:**

The common route is through any kind of contact with the feces of an infected person.

Also it happens when human feces are used as fertilizers in farms, or when clothes of an infected person and soiled diapers of children are washed.

People who are in contact with the soil or water that contain human feces stand a high chance of getting infected with intestinal worms (Gharavi, *et al.* 2002).

Eating food that is contaminated with the worms is another common way of transmission.

Worms can exist on the outer layers of the food as in fruits and vegetables; hence people consuming foods without washing thoroughly are prone to intestinal worms (Ali, 2011).

Similar infection is caused by consuming contaminated water (Okorokwo, 1998).

Raw meats, milk and eggs can contain worms that can cause intestinal infections.

Meat like beef, pork and fish that is under cooked can contain live worms (*Taenia spp*, and *Fasciola spp*).

Worms like *S.stercolaris* enter the human body through feet skin (Grove, 1989).

Hence people who move around on the open ground with bare feet have a very high degree of getting infected with hook worms, also through house flies (Umeche, *et al.*, 1989).

Transmission by person himself (auto-infection) may occur in *E.vermicularis*, *S. stercolaris* and *H. nana* infections (Gillespie and Pearson, 2001).

Worms are transferred during sexual contact; Cunnilingus and fellatio are very common modes of transmitting worms from infected person to the other (Steven, *et al.*, 1981).

Although the three classes of intestinal parasitic helminthes may share common modes of transmissions, they are differing in biological life cycles.

#### **1.2.2.3 .1 Life cycle of cestodes:**

Cestodes require one or more intermediate hosts in their life cycle; eggs are passed in the environment from primary host, they are ingested by intermediate host in which they hatch, the larvae enter the tissues of intermediate host and encyst, and the primary host ingests the cysts in the flesh of the intermediate host.

When humans are the primary hosts, the adult cestode is limited to the intestinal tract, and when humans are the intermediate host, the larvae are within the tissues, migrating through the different organ systems. In most Cestodes infestations (*i.e. T.solium*, *T. saginata*, *Diphyllobothrium* species and *Hymenolepis* species) humans are the primary hosts. Adult worm survive inside

their human hosts, where they are limited to the intestinal tract. Human fecal contamination of the environment is needed to sustain these life cycles. In the remaining cestodes (i.e. *Echinococcus species* and *T. multiceps*) humans function as the intermediate hosts. The larvae exist within the tissues and migrate through different organ systems.

*Hymenolepis* species and *T. solium* are the only cestodes for which humans can function as both primary hosts and intermediate hosts. *Hymenolepis diminuta* is primarily a cestode of rodents, although humans can be rare and accidental hosts in the life cycle. Humans are infected by swallowing insects that contain cysticercoid larvae, most often by ingesting mealworms or grain beetles that infest dried grains, cereals, flour and dried fruit (Beaver, *et al.*, 1984).

#### **1.2.2.3 .2 Life cycle of trematodes:**

Food-borne illness from trematodes has been associated with the ingestion of many different types of potentially infected foods, such as different types of both freshwater and brackish-water fish and snails, reptiles (amphibians and certain snakes), aquatic plants and insects (Chai, *et al.*, 2009).

Trematodes have a rather complicated life cycle in two or more hosts consisting of three or more generations constituting different larval stages to reach adult stage. The first larva is called miracidium, the next three stages named sporocyst, radia and cercaria respectively, occurs in the snails and the final infective stage, the metacercaria in trematodes other than schistosomes where cercaria is the infective stage, is found in mollusk, on vegetation (*F. buski* and *Fasciola spp.*), in fish (*H. heterophyes*, *M. yokogawai* and *Opisthorchis spp.*) or crustacean (*Paragonimus spp.*) (Beaver, *et al.*, 1984).

#### **1.2.2.3 .3 Life cycle of intestinal nematodes:**

Intestinal nematodes share similar life-cycles that have been involved in response to new ecological niches. The core of life-cycle involves development from an egg through five stages of growth. The first four stages are known as

larval stages and refer to as L1, L2, L3 and L4. The fifth and final stage is the sexually mature adult worm (Gillespie and Pearson, 2001).

There are usually two sites of entry for the intestinal nematodes infecting humans: the mouth and the skin. The ingestion of mature eggs or in some cases, L3 larvae, results in infections. Eggs may be, for example, inadequately washed, uncooked vegetables (Uneke, 2007).

The L3 larvae of hookworms, notably *Ancylostomaduodenale* and *Necatoramericanus*, have the capacity of penetrating intact skin. After skin penetration they pass through the blood vessels and are circulated via the liver to the lungs from there they follow a path similar to that set out below for other intestinal nematodes, finally developing into adults in the small intestine (Smith, 1990).

Parasitic females of *S.stercolaris* are found in the epithelium of the duodenum or upper jejunum and reproduce by parthenogenesis.

Embryonated eggs are laid in the mucosa; the eggs mature rapidly and hatch in the mucosa.

First stage rhabditiform larvae pass in the feces and develop in the soil into infective filariform larvae and those penetrate the skin of humans, other cutaneous blood vessels, migrate throughout the body specially lungs and finally mature in the small intestine.

Females begin to lay eggs after about 1 month from the time it infected the host.

In the indirect life cycle, larvae passed in the feces develop on the soil into free living adult, males and females which then mates then the laid eggs gives arise a generation of infective larvae, which can penetrate the skin and develops as in direct life cycle (Grove, 1989).

*Enterobius vermicularis* L3 larvae mature in the large intestine.

When a gravid female migrates out of the anus at night, the anal sphincter is relaxed and lay eggs that adhere to the perianal skin.



The female essentially ruptures, releasing as many as 10.000 eggs.

The eggs embryonates and becomes infective within few hours after being laid.

Infection is transmitted hand - to - mouth, then ingested eggs hatch in the small intestine, each releases infective stage larvae.

The parasite moves to the caecum and matures into adults from 2 to 4 weeks after infecting the host (Garcia, 2001).

#### **1.2.2.4 Epidemiology:**

The high prevalence of intestinal parasitic infections in developing countries is mainly due to deficiency of sanitary facilities, unsafe human waste disposal system, inadequacy and lack of safe water supply, and low socioeconomic status (Ali, *et al.*, 1999).

Epidemiological surveys have revealed that poor sanitation and inappropriate environmental conditions, coupled with indiscriminate defecation, geophagy, and contamination of water are the most important predisposing factors for intestinal worms, but they can also pass the infection to other people (Kang, *et al.*, 1998).

##### **1.2.2.4 .1 Epidemiology of nematodes:**

Intestinal nematodes are widespread; it's prevalence is higher in tropical and developing countries.

In 2002, an estimated 1.5 billion, 1.3 billion and 1.1 billion people were infected with *Ascaris*, hookworms and *Trichuristrichura* respectively (WHO, 2002).

Prevalence of *E. vermicularis* worldwide with highest rates in temperate regions.

It's estimated that more than 200 million persons are infected.

Strongyloidiasis is the most common in tropical and subtropical areas, but is much less prevalent than hookworm infection (Grove, 1989).

##### **1.2.2.4 .2 Epidemiology of cestodes:**

Cestodes infestations occur worldwide, *Taenia spp*; have high endemicity in Latin America, Africa, Middle East, and central Asia with moderate endemicity in Europe, South Asia, Japan and Philippines.

Cysticercosis, caused by infection with larval stage of tapeworm. *Diphyllobothrium* infection is prevalent in northern Europe, Canada, Africa, Japan, Taiwan, Manchuria, Siberia, Papua new Guinea, and Australia.

*H.nana* infections is the most common cestode of humans, it is prevalent in areas of poor hygiene and sanitation, especially in warm and arid countries of the Mediterranean's, India and South America.

The prevalence in children in these areas may reach 20%. Infection rates are higher among children.

#### **1.2.2.4 .3 Epidemiology of liver and intestinal flukes:**

More than 40 million people are infected with flukes, approximately, 21 million with lung flukes, 20 million with liver flukes and unknown millions with intestinal flukes (WHO, 1995).

They are world widely distributed; the highest prevalence is in East and Southeast Asia.

Prevalence is related to eating habits and the presence of fresh water snails, crustaceans, fish, or edible aquatic plant intermediate plant (WHO, 1995).

#### **1.2.2.5 Clinical presentation:**

It varies according to the type of parasite, common nematodes (such as round worms, hook worms and whip worms, etc. Infections may result in symptoms such as; abdominal discomfort, diarrhea, vomiting, malnutrition or weight loss. If infected with large number of parasites, blockage, or other inflammatory symptoms may occur (Bethony, *et al.*, 2006).

Pinworm infections often lead to symptoms like; loss of appetite, amnesia, irritation and itching around the anal area, extra intestinal invasion may occur particularly in females (Kacker, 1973).

Tape worms produce symptoms such as; abdominal cramps, loss of appetite, nausea, vomiting, weakness, malnutrition and weight loss.

*D. latum* rarely causes pernicious anemia due to interference of vitamin B12 absorption in jejunum.

Tape worm eggs in pork hatches in the host's small intestine, and then migrates to other organs and tissues, leading to cysticercosis.

Cysticercosis commonly occurs under the skin and between muscle layers with no apparent symptoms; however, if cysticercosis occurs in the brain, eyes or spine, the complications will be more severe (Gillespie and Pearson, 2001).

Mild infections of humans with liver flukes do not show apparent symptoms.

Symptoms such as loss of appetite, weakness, discomfort in the upper abdomen, diarrhea, indigestion, pain in the upper right abdomen, and hepatomegaly will start to appear.

If the infection is heavy; bile duct blockage, bile duct inflammation, or jaundice may occur.

Further long term infections may induce symptoms such gall stones or even liver cirrhosis or cancer (Assefa, *et al.*, 2009).

The intestinal flukes attach to the duodenal and jejunal mucosa and produces focal inflammations, ulcerations and small abscesses at the site of attachment (Palut, *et al.*, 1969).

Early symptoms include; epigastric pain, mimicking peptic ulcer disease, and diarrhea, anorexia, nausea and vomiting may occur.

In heavy infections, edema of the face, abdominal wall, and legs, ascites, and severe prostration have been described (Sadun and Maiphoom, 1953).

After nine days on average following ingestion of metacercaria, dyspepsia, colicky abdominal pain, diarrhea and eosinophilia may occur (Cho, *et al.*, 1984).

A mild focal inflammatory reaction and superficial erosions are produced at the site of attachment. The flukes appear to live for less than a year. The fluke may

penetrate the mucosa, and eggs may transmit from these intra mucosal sites via lymphatic to the systemic vascular system (Tantachamrun, *et al.*, 1978).

### **1.3 Role of vegetables in the transmission of parasites:**

Previous studies were performed to detect the contamination among fresh and raw vegetables:-

A study performed at Akure Metropolis, Ondo State, Nigeria, microscopic examinations of six hundred (600) samples of fruits and vegetables were carried out using standard floatation techniques. High levels of contamination were recorded in both fruits (92.5%) and vegetables (76.5%) examined. The Protozoan's and *Helminthes* identified were *Ascarislumbricoides* as the most prevalent parasite (25.7%), *Balantidium coli*, *Entamoebahistolytica*, *Giardia lamblia*, *Hymenolepis nana* and *Ancylostomaduodenale* as the least parasite found (8.5%).The study indicated high rate of protozoan and helminthes contamination of fruits and vegetables in the study area. The implications of the findings and preventive measures were discussed. (Simon-Oke, *et al.*, 2014).

Another study was performed on raw vegetables in Mannuthy, Kerala state, India. The study aimed to detect the parasitic contamination of raw vegetables retailed at Mannuthy in Thrissur district of Kerala state, India.

Helminthic eggs were detected in three (2.7%) of 112 samples. Two samples of cabbage (1.8%) and one sample of onion (0.9%) was contaminated with ova of *Ascaris spp.*

The study emphasizes the need for proper washing of vegetables before they are consumed or cooked, because vegetables can act as potential source of gastrointestinal parasitic infections (Sunil, *et al.*, 2014).

Another study was conducted to determine the level of parasitological contamination of markets and farms vegetables consumed in southern Iran, there a number of 270 markets and farms vegetable samples from 32 farms in 11 cities in Fras province, southern Iran (Shiraz, Kazeroon, Fasa, Jahrom,

Noorabadmamassani, Firoozabad, Farrashband, Arsenjan, Zarghan, Marvdasht). In the period between 2011-2012, market vegetables were examined; the samples were washed with water and tested microscopically after performance of sedimentation method for detection of metazoan and protozoan parasitic contaminations. Fifty two out of 135 market vegetables (38.5%) and sixty six out of 135 farm vegetables (48.9%) were found to have parasitic contamination, of helminth eggs such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Trichostrongylus* spp. *Toxocara cati*, *Toxocara canis*, *Toxascaris leonina*, *Taenia* sp., *Enterobius vermicularis*, *Hymenolepis nana*, *Dicrocoelium*, *fasciola* and Larvae of Nematodes, protozoa cysts like *Giardia lamblia* was seen, the highest rate of contamination was found in Lettuce and Cress ( Olyaei and Hajivandi , 2003).

In a study performed in Mekelle City and Its suburb, Tigray at Ethiopia. The objective of this study was to calculate the rate of contamination of pre-harvest vegetables in Mekelle city and its suburban village, ‘Mariam Dahan’.

Pre-harvest vegetables collected from the ground during the dry season, and then it is washed using physiological saline, left to sediment overnight, centrifuged and examined for infective stages of intestinal parasites.

Total of tested samples, 32.41% in Mekelle city and 30.49% in ‘Mariam Dahan’, contained at least one parasitic contaminant. *Strongyloides stercoralis* was most encountered parasitic contaminant in the study areas, followed by, *Taenia* and *Entamoeba* spp. Highest rate of parasitic contamination was detected on Swiss chard, which was 45.5 and 41.67% in Mekelle city and ‘Mariam Dahan’, respectively.

Presence of infective stages of intestinal parasites on wastewater- irrigated vegetables may pose public health hazards to farming communities in the study areas.

Therefore, evaluation and surveillance of parasitological quality of vegetables is crucial in attempt to control vegetable-transmitted parasitic infections.

(Zewdneh, 2012).

In Norway, a study was conducted on 475 vegetable samples; those samples were examined for *cryptosporidium* and *Giardia* cysts.

29 samples (6%) were found to be positive, and no sample was found to contain both of the parasites at the same time.

Among the 19 *cryptosporidium* positive samples, 5 (26%) were detected in lettuce, and 14 (74%) in mung bean sprouts.

Of the 10 *Giardia* positive samples, 2 (20%) were detected in dill, 2 (20%) in lettuce, 3 (30%) were detected in mung bean sprouts, 1 (10%) was detected in radish sprouts and 2 (20%) in strawberries (Robertson, *et al.*, 2001).

Another study on helminth egg contamination of vegetables purchased at suburban market in Hanoi, Vietnam was performed, A total of 317 vegetables were examined and 82 (26%) were revealed to be positive for parasite eggs. Of the 15 varieties, 13 were positive except for horseradish and cucumber. Contamination was highest in leafy vegetables (31%), followed by root vegetables (17%) and fruit vegetables (3%). Throughout the survey, five species of parasite eggs were found: *Ascaris spp.*, *Trichuris sp.*, *Toxocara sp.*, *Taenia sp.* And *Ascaridi agalli*.

In the interview with the villagers, 121 (81%) of 149 adult villagers stated that they usually use not only animal feces but also human feces as a fertilizer. Throughout the survey, a total of 453 eggs were recovered.

Number of eggs recovered from vegetables was higher in the dry season (355 eggs) than in the rainy season (98 eggs).

Considering the eating habits of the Vietnamese and the 17% embryonation rate of detected parasites, vegetables seem to play an important role in soil-transmitted helminth infection in this country (Uga, *et al.*, 2009).

A study done in Rio de Janeiro and Niteroi, where 128 samples of lettuce and watercress from supermarkets in both of the cities, greengrocer shops and self service restaurants, only 6.2% of the samples were positive for animal

parasites, *Ascaris* eggs, insects, nematode larvae and ciliated protozoa (Mesquita, *et al.*, 1999).

Another study, also from Nigeria in Jos Markets on Common vegetables brought for sale in market within Jos South Local Government area of Plateau State was studied for human parasites. Four hundred (400) samples were obtained in five different markets of the Local Government Area and screened using centrifugation method. Cysts, ova and larvae of intestinal protozoa, cestodes and nematodes were recovered. 225 (56.25%) of the samples were positive for different species of parasites, 5 (2.0%) were cysts of *Entamoeba coli*, 10 (4.0%) were *Entamoeba histolytica*, 2 (0.8%) were *Hymenolepis nana*, 5 (2 %) were *Trichuris trichiura*, 6 (2.4%) were *Ascaris lumbricoides*, 70 (28.2%) were *Hookworm* species and 150 (60.4%) were *Strongyloides stercoralis*. *S. stercoralis* with 60.4% of the positive cases has the highest occurrence, while *H. nana* with 0.8% has the least occurrence. The study also showed that water-leaf with 90% infection rates has the highest parasitic load, while garden egg with 15% has the least load of parasites. Lettuce was found to have the highest multiple parasitic contamination of six (6), while carrot and garden egg had the least multiple parasites of two (2). None of the vegetables had single parasitic contamination (Ojemudia, *et al.*, 2011).

**Rationale:**

Raw vegetables used in most Sudanese houses and food markets as a component of green salad increases the risk of intestinal parasitic infection when they are insufficiently washed. Information on intestinal parasite prevalence in fresh vegetable sold in Sudanese market is not available, this information is important to increase the hygienic awareness in the population and to assist the local and health authorities to take the proper action to improve the quality of such food. All of these have urged the undertaking of this study to determine the prevalence of parasitic contamination of vegetable sold in Khartoum state markets.



**Objectives:****General objective:**

To study the role of vegetables in the transmission of intestinal parasites in Khartoum central market.

**Specific objectives:**

- 1- To detect different parasitic species carried by those vegetables.
- 2- To find out the most prevalent parasite in this market.
- 3- To find out the most contaminated type of vegetable.

## **Chapter two**

### **Materials and Methods**

#### **2.1 Study design:**

This is a cross sectional study.

#### **2.2 Study area and study period:**

The study was conducted in Khartoum central market state during the period from June to December 2015. Vegetable samples were collected from the central vegetable market in Khartoum town.

#### **2.3 Study samples:**

The vegetable used in this study were tomato (*Solanum lycopersium*), carrots (*Daucuscarota l*), water cress (*ErucaSaveta*), Onion (*Allium cepa*) and pepper (*Capsicumannuum*).

#### **2.4 Sample size:**

A total of 150 samples were collected, vegetables were picked randomly from the market to obtain qualitative estimation of parasitic contamination of these vegetables.

#### **2.5 Sample processing:-**

##### **2.5.1 Sample collection:**

Samples were randomly collected from the market during 2 months in tow groups (group A and group B); each group includes 75 samples, group (A) prepared and examined before collecting group (B). Each sample was placed in a labeled plastic bag. The label contained the sample type and date of collection.

##### **2.5.2 Procedure:**

The samples were washed in 10% formal saline, each sample was soaked and washed in 30 ml of the washing saline which then was allowed to stand for 24 hours, then 15 ml of the sediment was centrifuged at 3000 rpm for 5 minutes using 15 ml falcon tubes, then the sediment was prepared for microscopical examination. The samples were examined under light microscope (10 x and 40

x) for parasite stages (oocyst, cyst, egg and larva), the parasitic stage was identified according to Soulsby (1982).

## **2.6 Data analysis:-**

Data were analyzed using Statistical Package for the Social Sciences (SPSS program). Frequencies and percentages tests were used, and then the data were presented in tables and figures.

## Chapter Three

### Results

Five different types of fresh unwashed, untreated vegetables were tested from major central Khartoum market.

A total of 150 samples were tested for intestinal parasites microscopically, 16 (10.6%) of the 150 samples were positive for intestinal parasites.

Among positive samples, onion and watercress were found to have the highest parasitic prevalence, onion positive samples were 8 (50%) , watercress positive samples were 6 (37.5%), pepper positive samples were 2 (12.5%), no parasite were detected in both tomatoes and carrot (Table 1, Figure1).

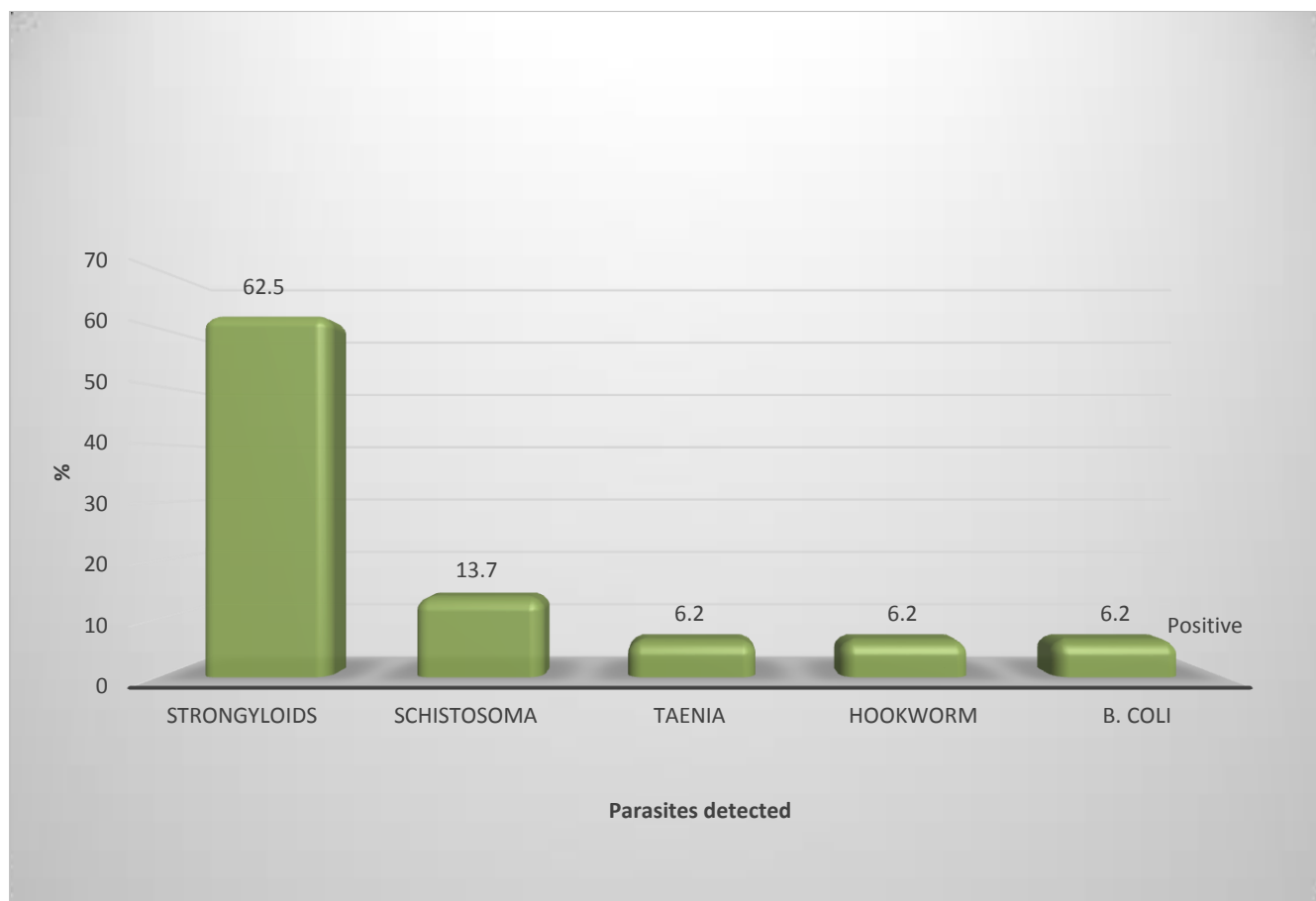
The results showed that *S.stercoralis* was the most prevalent parasites as it was detected in 10 positive samples. *B.coli* was seen in only one case, *Schistosomes* were detected in 3 cases, *Taenia spp.* and *Hook worms* were detected in only one case. The results revealed that high mixed infection was observed with watercress (4 parasites) followed by pepper (2 parasites). (Table 2, Figures, 2, 3, 4, 5, 6).

**Table 3.1:** Distribution of intestinal parasites among vegetables.

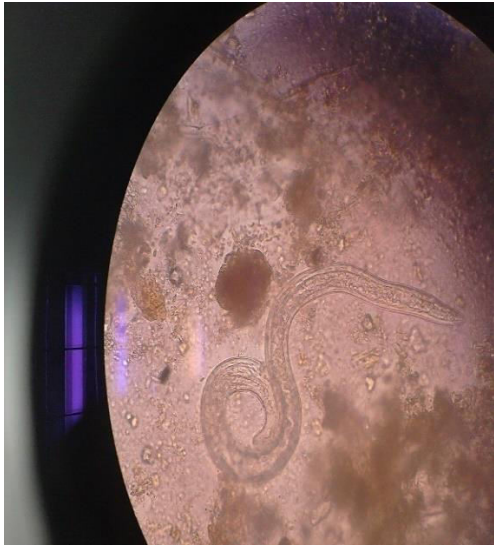
<b>Vegetable type</b>	<b>Group 1</b>		<b>Group 2</b>		<b>Total</b>	
	Number of Examined	Number of Positives (%)	Number of Examined	Number of Positives (%)	Number of Examined	Number of Positives (%)
<b>Tomatoes</b>	15	0.0 %	15	0.0 %	30	0.0 %
<b>Onion</b>	15	46.6 %	15	6.6 %	30	26.6 %
<b>Carrot</b>	15	0.0 %	15	0.0 %	30	0.0 %
<b>Pepper</b>	15	13.3 %	15	0.0 %	30	6.6 %
<b>Watercress</b>	15	26.6 %	15	13.3 %	30	20.0 %
<b>Total</b>	75	17.3 %	75	4.0 %	150	10.6 %

**Table 3.2:** Distribution of intestinal parasites with relation to the type of vegetables.

<b>Vegetables</b>	<b><i>B. Coli</i></b>	<b><i>S. Stercolaris</i></b>	<b><i>Schistosoma Spp.</i></b>	<b><i>Taenia Spp.</i></b>	<b><i>Hookworms</i></b>	<b><i>Total</i></b>
<b>Tomatoes</b>	0	0	0	0	0	0
<b>Onion</b>	0	8	0	0	0	8
<b>Carrot</b>	0	0	0	0	0	0
<b>Pepper</b>	1	0	1	0	0	2
<b>Watercress</b>	0	2	2	1	1	6
<b>Total</b>	1	10	3	1	1	16



**Figure 3.1:** Distribution of intestinal parasites among positive samples.



**Figure 3.2:**

*Strongyloides stercoralis* larvae



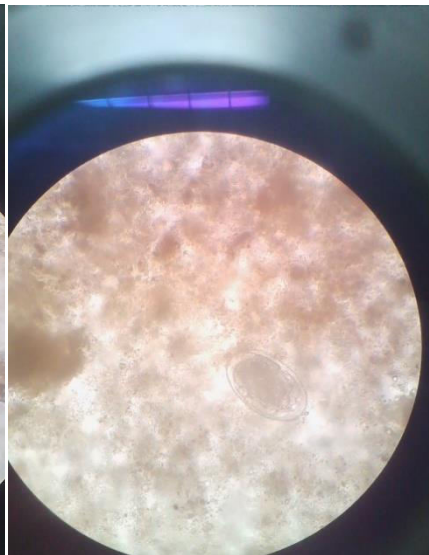
**Figure 3.3:**

*Schistosoma haematobium* egg



**Figure 3.4:**

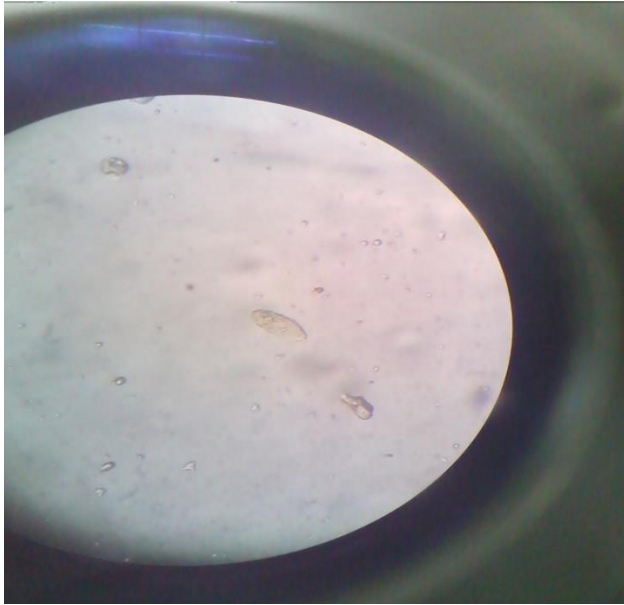
*Schistosoma mansoni* egg



**Figure3.5:**

*Hook worm* egg





**Figure 3.6:**

*Blantidium coli* trophozoite

## Chapter four

### Discussion

The present study investigated 5 of the most daily vegetables used for human consumption in Sudan, in which several types of parasites were detected, indicating that the consumption of raw vegetables play an important role in the transmission of parasites.

Transmission of parasitic diseases through food was shown to be a serious problem which needs a further highlight by scientists and health care authorities, as food becomes a potential source of human infections.

It was noticed that a number of reported cases of food borne illness related to vegetables consumption has increased (Kang, *et al.*, 1998).

It has been proved that poorly washed vegetables are considered to be a major way for transmission of parasitic infections (Shahonazi, *et al.*, 2010).

Pre harvest fecal contamination may occur due polluted water from irrigation water canal, this is supported by the findings of our study, as *Schistosoma haematobium* eggs were isolated indicating water pollution.

Soil seems to be a very important source of contamination as in this study a number of nematode larvae were detected from leafy vegetables. This may be because leafy plants grow inside or near the soil which exposes it to contamination especially fecal contamination, those nematodes may be animal or human parasites, with variation in size and shape.

Some larvae have nematode features (rhabditifoid eosaphagus), but it was too small or too long to be defined as *S.stercolaris* or hook worm larvae.

So another type of none nematodes larvae was detected, especially on leafy vegetables.

*Strongyloides stercolaris* larvae were the most frequent parasite isolated in this study indicating soil pollution.

It was detected in rhabditiform and filariform shape, reflecting fecal pollution of the soil, also it is known that *Strongyloid stercolaris* can undergo a free

living existence, because of that and because it is able to withstand a wide variety of adverse environmental conditions it may be an annoying health problem specially to the farmers who obviously seem to be exposed to larval skin penetration. Also the presence of *Taenia* eggs shows the role of raised cattle in transmission of parasites such as *T. saginata* (transmitted by cows) and *T. solium* (transmitted by pigs)

Also the use of organic fertilizers may serve as an effective way of transmission, fecal matter may contain parasitic organisms, it was indicated by this study as *Schistosoma mansoni* ova, *Hook worm* ova and *Blantidium coli* ciliate were detected.

Finally the presence of soil transmitted helminthes is a good indicator of a poor socio-economic condition as well as poor environmental and sanitation practice.

Also reporting of parasitic stages from vegetables may be helpful in indicating the prevalence of intestinal parasites among a given population.

Several surveys that have been conducted from different parts of the world showed that the vegetables can be effective agents for transmission of protozoa and helminthes (Idahosa, 2011).

Compared with a previous study, our study showed a difference in prevalence rates, probably due to geographical and socio-economic differences, type and number of samples tested, methods used for detection, type of water used in irrigation and post harvesting handling methods of such vegetables and even the type of water used to clean vegetables like the use of sewage water for irrigation which plays an important role in the epidemiology of transmission of the parasitic diseases (Gupta, *et al.*, 2009).

As revealed in our study, one sample may contain more than one parasitic species, this indicate and reflects the poly fecal contamination of vegetables which may result in multiple parasitic infection.

Also the study results highlighted the high rate of parasitic contamination of onion 8 (50%) and watercress 6 (37.5%) compared to the other tested vegetable types. This might probably reflect the excess use of onion and watercress in our food.

The highest parasitic prevalence was *S. stercorarius* (62.5%) and *Schistosoma spp* (18.75%).

In this study, it was observed that the degree of contamination vary according to the type of vegetables, this may be due to that, watercress, onion, pepper, carrot and tomatoes have unequal surface on which parasites may attach more easily on one type of vegetables than another and also due to different conditions of growing and environment exposure.

## **Chapter five**

### **Conclusion and Recommendations**

#### **5.1 Conclusion:**

- This study concluded the fact that fresh vegetables play an important role in human parasitic disease transmission, and that daily sold vegetables represents source of parasitic contamination if not treated and washed.
- It has been shown in this study that fresh vegetables represents a source of parasitic contamination and concluded that the possible reasons for this contamination are: irrigation water, organic fertilizers, domestic animals and poor hygienic conditions.

#### **5.2 Recommendations:**

- It is recommended then to adopt strict strategies and guidelines to control the process of cultivation especially during irrigation and addition of fertilizers to the soil, and also to regularly check the domestic animals for parasitic illnesses and control its health state to reduce the risk of disease transmission through food to humans.
- Also, it is important to avoid the use of untreated fertilizers as it has been shown to be a source of transmission.
- People's awareness about risks associated to food borne diseases should be increased, in addition to the importance of strengthening people's awareness about personal hygiene, safety and proper washing procedures should be considered to ensure safe daily food consumption.

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