Chapter 1

Introduction

Water is a precondition for life, including that of all parasites and other organisms that infect humans. Indeed, many infectious diseases are water-related, i.e., they directly depend on water bodies for their spread and transmission or as a habitat for intermediate or final hosts (Zhou, 2012). Water-borne diseases are caused by pathogenic microorganisms that most commonly are transmitted in contaminated fresh water. Infection commonly results during bathing, washing, drinking and in the preparation of food or the consumption of food that is infected. Various forms of water-borne diarrheal disease probably are the most prominent examples and affect mainly children in developing countries; according to the World Health Organization, such diseases cause about 1.5 million human deaths annually. The World Health Organization estimates that 58% of that burden, or 842,000 deaths per year, is attributable to unsafe water supply, sanitation and hygiene (WHO, 2014). Water-related parasites can be categorized into three groups according to their transmission route. The first group is associated with drinking water, which may be contaminated with cysts or oocysts, larvae, or eggs from various parasites. The second group is transmitted via penetration of the human skin during water contact. The parasites in this group may swim freely in water until they find a human host. The transmission of the third group of parasites depends on the consumption of uncooked freshwater products, e.g., plants, fish, snails or crustaceans. Obviously, the first two groups are closely related to water contact, while the key element of transmission of the third group is not water but hosts and vectors living in the water (Huntington, 2012).

The most important members of water-related parasitic diseases are amoebiasis, giardiasis, cryptosporidiosis, cyclosporiasis, blastocystosis, schistosomiasis, fascioliasis and paragonimiasis (Shan *et al.*, 2013).

Rationale

Unfortunately, water quality problems and massive fecal contamination remain unsolved. Therefore, studies of water quality and sanitation should be continually performed. Contaminated water by parasites and other microorganism lead to serious health problems and affects academic performance of the students. The first step to reduce the gastrointestinal diseases and outbreaks of diarrhea is by ensuring the safe drinking water since the contaminated water is a major source of parasitic disease transmission, therefore this study was conducted to detect parasitic infections and their associated risk factors in drinking water at basic schools in Khartoum state.

Objectives

General objective:

To detect parasitic infections and their associated risk factors in drinking water at basic schools in Khartoum state.

Specific objectives:

- To determine frequency of parasitic infections in drinking water and determine species involve.
- To assess the possible associated factors with drinking water contamination.

Chapter 2

literature review

2.1 Definition of parasite:

The word parasite originates from two Greek words “para” which means “beside” and “sitos” which means food. A parasite is an organism that is entirely dependent on another organism, referred to as its host, for all or part of its life cycle and metabolic requirements. In a strict sense the term parasite can simply be said to be referred to any infectious agent, but mostly, it is generally restricted to infection caused by protozoa and helminthes (Suleiman, 2005).

2.2 Water-borne protozoa:

2.2.1 Definition:

Protozoa are microscopic, one-celled organisms that can pass to humans through contaminated food or insect bites. Among the protozoa that can infect and cause disease in man are those associated with excreta and found in the intestinal track (WHO, 1994, Sulaiman, 2005).

2.2.2 Transmission:

Most protozoa are transmitted by fecal-oral route, particularly in contaminated food, water or hands (Petri *et al.*, 2006). Water-borne protozoa most commonly are transmitted in contaminated fresh water (WHO, 2016).

2.2.3 Life cycle of water borne protozoa:

2.2.3.1 Life cycle of *Entamoeba histolytica:*

Cysts and trophozoites are passed in feces. Cysts are typically found in formed stool, whereas trophozoites are typically found in diarrheal stool. Infection by *Entamoeba histolytica* occurs by ingestion of mature cysts in fecally contaminated food, water, or hands. Excystation occurs in the small intestine and trophozoites are released, which migrate to the large intestine.

The trophozoites multiply by binary fission and produce cysts, and both stages are passed in the feces. Because of the protection conferred by their walls, the cysts can survive days to weeks in the external environment and are responsible for transmission (CDC, 2016) (figure 2.1).

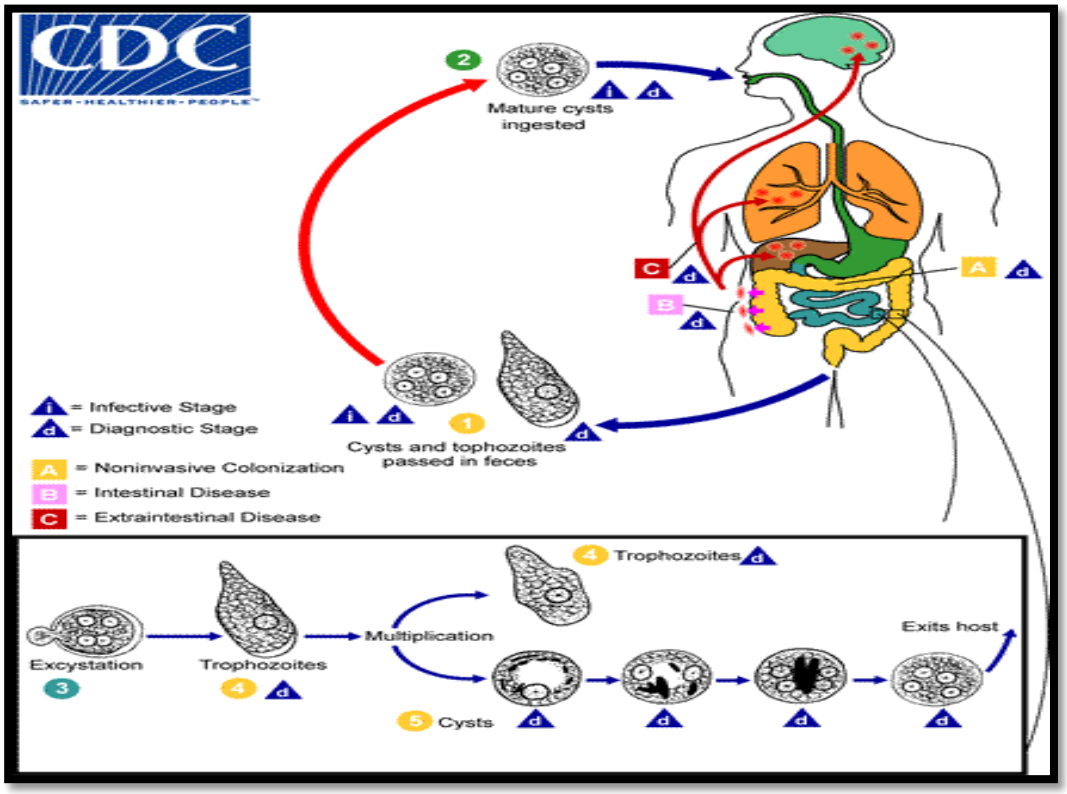

Figure (2.1): *Entamoeba histolytica* life cycle (CDC, 2016)

2.2.3.2 Life cycle of *Giardia lamblia:*

The life cycle begins with a noninfective cyst being excreted with the faeces of an infected individual. The cyst is hardy, providing protection from various degrees of heat and cold. Once ingested by a host, the trophozoite emerges to an active state of feeding and motility. After the feeding stage, the trophozoite undergoes asexual replication through longitudinal binary fission. The resulting trophozoites and cysts then pass through the digestive system in the faeces. While the trophozoites may be found in the faeces, only

the cysts are capable of surviving outside of the host (Tovar *et al.*, 2003) (figure 2.2).

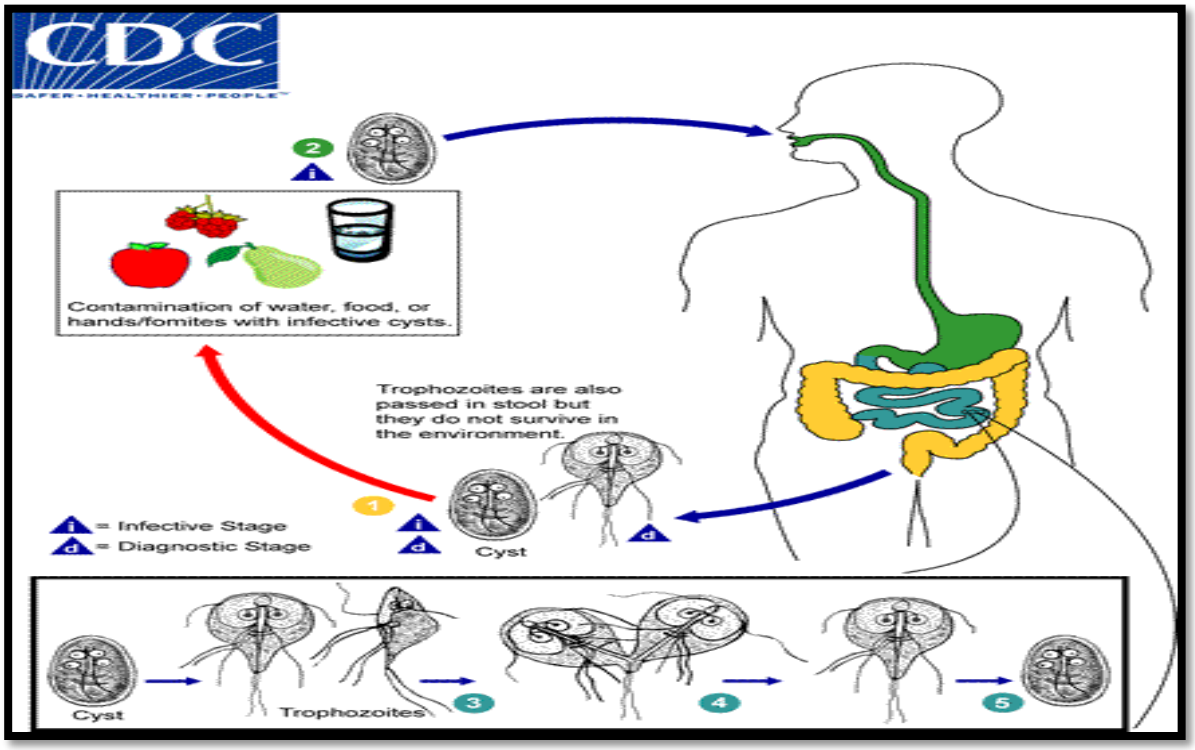

Figure (2.2): *Giardia lamblia* life cycle (CDC, 2017)

2.2.3.3 Life cycle of *Cyclospora cayetanensis:*

When freshly passed in stools, the oocyst is not infective (thus, direct fecal-oral transmission cannot occur; this differentiates *Cyclospora* from another important coccidian parasite, *Cryptosporidium*). In the environment, sporulation occurs after days or weeks at temperatures between 22°C to 32°C, resulting in division of the sporont into two sporocysts, each containing two elongate sporozoites. Fresh produce and water can serve as vehicles for transmission and the sporulated oocysts are ingested (in contaminated food or water). The oocysts excyst in the gastrointestinal tract, freeing the sporozoites which invade the epithelial cells of the small intestine. Inside the cells they undergo asexual multiplication and sexual

development to mature into oocysts, which will be shed in stools (Herwaldt, 1990, Ortega *et al.*, 1994) (figure 2.3).

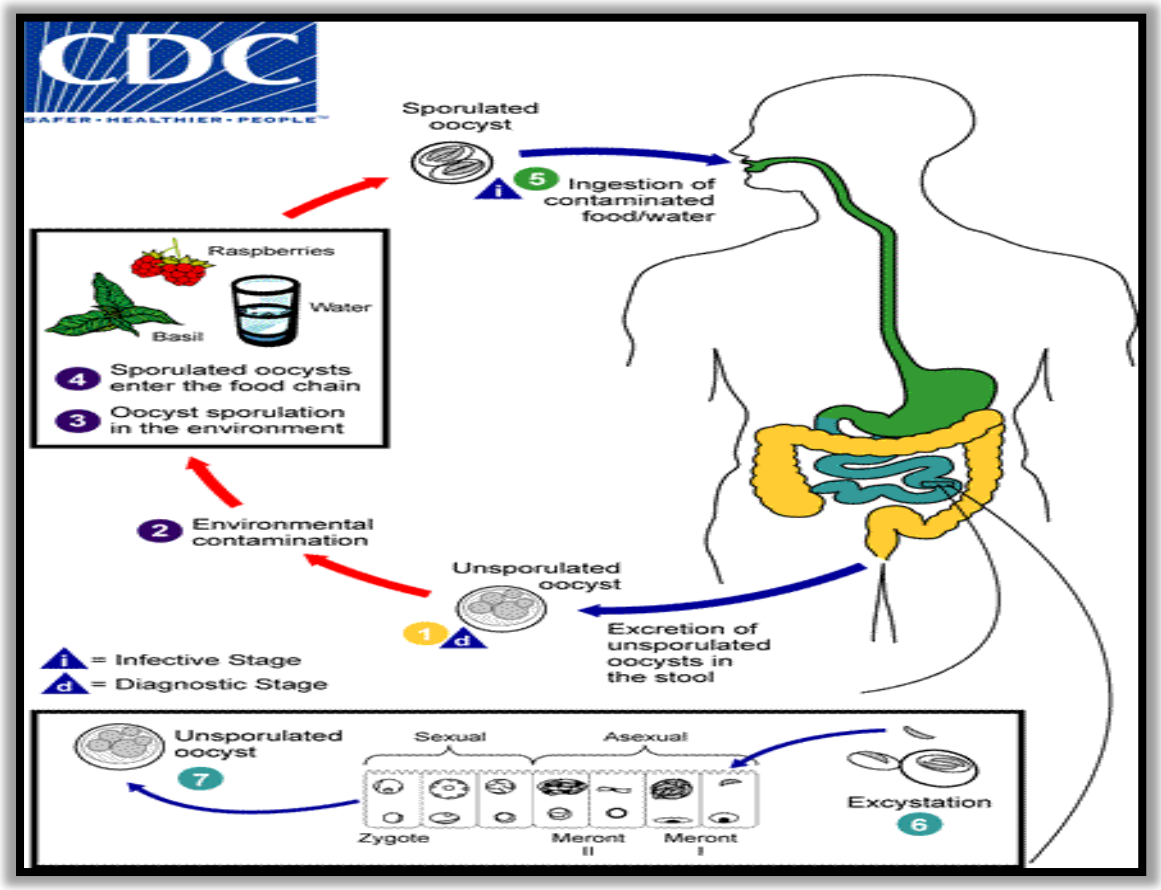

Figure (2.3): Life cycle of *Cyclospora cayetanensis* (CDC, 2015)

2.2.4 Clinical features of water-borne protozoa:

2.2.4.1 Amoebiasis:

Amoebiasis, also known amoebic dysentery, is an infection caused by any of the amoebas of the *Entamoeba* group. Symptoms are most common during infection by *Entamoeba histolytica*. Amoebiasis can present with no, mild, or severe symptoms. Symptoms may include abdominal pain, diarrhea, or bloody diarrhea. Complications may include inflammation of the colon with tissue death or perforation which may result in peritonitis. People affected may develop anemia due to loss of blood (Farrar *et al.*, 2013).

2.2.4.2 Giardiasis:

Giardiasis, popularly known as beaver fever (Minetti *et al*., 2016) is a parasitic disease caused by *Giardia lamblia*. About 10% of those infected have no symptoms. When symptoms occur they may include diarrhea, abdominal pain, and weight loss, vomiting, blood in the stool, and fever are less common. Symptoms usually begin 1 to 3 weeks after exposure and without treatment may last up to six weeks (Esch and Peteresen, 2013).

2.2.4.3 Cyclosporiasis:

C. cayentanensis causes gastroenteritis, with the extent of the illness varying based on age, condition of the host, and size of the infectious dose. Symptoms include "watery diarrhea, loss of appetite, weight loss, abdominal bloating and cramping, increased flatulence, nausea, fatigue, and low-grade fever", though this can be augmented in more severe cases by vomiting, substantial weight loss, excessive diarrhea, and muscle aches. Typically, patients with a persistent watery diarrhea lasting over several days may be suspected of harboring the disease, especially if they have traveled to a region where the protozoan is endemic. The incubation period in the host is typically around a week, and illness can last six weeks before self-limiting. Unless treated, illness may relapse (Global Health, 2013). Consuming food or water while visiting developing countries is a well-documented way of developing traveler's diarrhea. Travelers are often warned against such actions, but over 70 percent of certain produce items consumed in the United States is imported from developing countries, making "traveler's diarrhea" possible without international travel (Rabold *et al*., 1994, Brown and Rotschafer, 1999, Osterholm, 1997). The more severe forms of the disease can occur in immunocompromised patients (Global Health, 2013).

2.2.5 Epidemiology of water borne protozoa:

At least 325 water-associated outbreaks of parasitic protozoan disease have been reported (Karanis *et al*., 2007). The nature of water-borne outbreaks has changed during the past 100 years. This changing scenario of epidemiology and etiology has included: a decrease in individual cases and average number of cases per outbreak, an increase in the number of total outbreaks and of those in smaller community water systems, a change of etiology and a change in the effectiveness of predicting outbreaks by conventional testing methods (Stephen, 1989). North America and Europe accounted for 93% of all reports, and nearly two thirds occurred in the United States. *G lamblia* and *C.parvum* account for most outbreaks (40.6% and 50.8%, respectively), followed by *E.histolytica* and *C.cayetanensis* (1-3% each), and, less frequently (≤ 1%) by *Isospora belli* and *B.coli* (Lim *et al*., 2007). Over 30% of all outbreaks were documented from Europe, with the United Kingdom accounting for 24% of outbreaks, worldwide. *Giardia duodenalis* and *Cryptosporidium parvum* account for the majority of outbreaks 132 (40.6%) and 165 (50.8%) respectively. *Entamoeba histolytica* and *Cyclospora cayetanensis* have been the etiological agents in nine (2.8%) and six (1.8%) outbreaks, respectively, while *Toxoplasma gondii* and *Isospora belli* have been responsible for three outbreaks each (0.9%) and *Blastocystis hominis* for two outbreaks (0.6%). *Balantidium coli*, the microsporidia, *Acanthamoeba* and *Naegleria fowleri* were responsible for one outbreak, each (0.3%). Their presence in aquatic ecosystems makes it imperative to develop prevention strategies for water and food safety. Human incidence and prevalence-based studies provide baseline data against which risk factors associated with water-borne and food-borne transmission can be identified. (Karanis *et al.,* 2007). Amebiasis is responsible for approximately 100,000

deaths per year, mainly in Central and South America, Africa, and India, as well as for considerable morbidity manifested as invasive intestinal or extra-intestinal clinical features Worldwide, amebiasis is the third most common cause of death due to parasitic infection after malaria and schistosomiasis, as estimated by the World Health Organization. Amebiasis infections are endemic in most temperate and tropical climates in the developing world. In some tropical countries, antibody prevalence rates (reflecting past or recent infection) exceed 50%. The prevalence of amebiasis varies with the population of individuals affected, differing between countries and between areas with different socioeconomic conditions. Sometimes up to 50% of the population is affected in regions with poor sanitary conditions. It is thought that amebiasis directly affects over 50 million people, causing loss of manpower and subsequent economic damage (Mehmet and William, 2003). Prevalence of water borne protozoa disease is not associated with racial background (Arellano *et al*., 1996).

2.2.6 Laboratory diagnosis of water-borne protozoa:

2.2.6.1 Laboratory diagnosis of *Entamoeba histolytica:*

Microscopy cannot distinguish *E. histolytica* from the more common parasites *E. dispar* and *E. moshkovskii*. It is therefore an obsolete approach to the diagnosis of amebiasis, but still conducted in most parts of the world where modern diagnostic approaches have failed to take hold. For microscopy each sample should be divided into two portions. Direct microscopy should be done by mixing a small amount of the specimen in 0.9% sodium chloride solution (wet mount) or Lugol's iodine solution. This allows the detection of motile trophozoites of *Entamoeba histolytica/dispar* and can also provide information on the contents of the stool, that is, the presence of leucocytes and red blood cells. The second portion of the sample

is then stained with trichrome and/or iodine to identify trophozoites and cysts. Three negative stool samples are required before it can be accepted to report that there is no amoebic infection (Li and Stanley, 1996). Trophozoites containing ingested RBCs are more common with *E. histolytica* than *E. dipar* (González *et al.*, 1994, Haque *et al*., 1995). The sensitivity of microscopy is as less as 60% and confounded with misleading results due to misidentification of macrophages as trophozoites, polymorphonuclear leukocytes (PMNs) as cysts particularly when lobed nuclei of PMNs break apart, and other *Entamoeba* species (Tanyuksel and Petri, 2003). The combination of serology and stool antigen assays is more sensitive and specific than microscopy for the diagnosis of *Entamoeba histolytica*, infection. The tests of choice for serology are indirect fluorescent antibody test (IFAT), counter immunoelectrophoresis (CIEP), or enzyme linked immunosorbent assay (ELISA). Serologic tests are positive at the time of clinical presentation of amebiasis in 60–90% of cases, with positive serology seen in the overall population of endemic areas of 5–10% raising the issue of both false positive and false negative results with serology (Pillai *et al.*, 1999).

2.2.6.2 Laboratory diagnosis of *Giardia lamblia:*

Microscopy of direct fecal smears or smears prepared following formol-ether concentration and iodine staining has been reported to reach 97% sensitivity if three stool samples are examined (Wolfe, 1979). Sodium acetate-acetic acid-formalin (SAF) preservation in diagnosing intestinal protozoans and found both an increased yield with the concentration of preserved samples and a further increase of about 20% with permanent staining (Yang and Scholten, 1977). However, false negatives can occur, with reasons including intermittent excretion of cysts, use of antidiarrheal

medication and barium use for diagnostic imaging (Flanagan, 1992). The string test, duodenal aspirate, intestinal impression smear and intestinal biopsy have all been proposed as techniques to improve microscopic diagnosis (Koneman, 1992). Results have been conflicting, with some reports finding microscopy of direct smears without preservation as low as 50% sensitive (Kamath and Murugasu, 1974). While others suggest that there is little diagnostic gain from more invasive and expensive testing (Thornton *et al*., 1983). Serology has also been used to detect *Giardia* infection (Visvesvara *et al.,* 1980). Several studies using ELISA to detect a serological response suggest that, compared with immunoglobulin G (IgG), the immunoglobulin M (IgM) antibody response is shorter and more indicative of active infection (Sullivan *et al.,* 1987). Reported sensitivities and specificities for IgM-ELISA range from 63% to 99% and 79% to 96%, respectively. Another approach to serodiagnosis is Western blot analysis. The molecular weights of the antigenic determinants to serum IgG, IgM and immunoglobulin A (IgA) responses in patients with giardiasis have been determined using sodium-dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting (Char *et al.,* 1991).

2.2.6.3 Laboratory diagnosis of *Cyclospora cayetanensis:*

Diagnosis can be difficult in part because even persons who are symptomatic might not shed enough oocysts in their stool to be readily detectable by laboratory examinations. Therefore, patients might need to provide several specimens collected on different days. In addition, the laboratory should use sensitive recovery methods (concentration procedures) and detection methods that highlight *Cyclospora* oocysts. The oocysts can be stained with modified acid-fast or modified hot safranin techniques. *Cyclospora* oocysts also are auto fluorescent, meaning that when stool containing the parasite is

viewed under an ultraviolet (UV) fluorescence microscope the oocysts appear blue or green against a black background (CDC, 2015).

2.2.7 Treatment of water-borne protozoa:

2.2.7.1 Treatment of *Entamoeba histolytica:*

There are a number of effective medications to *E. histolytica*. Intestinal infection: usually nitroimidazole derivatives are used because they are highly effective against the trophozoite form of the *Amoeba*. Since they have little effect on *Amoeba* cysts, usually this treatment is followed by an agent (such as paromomycin or diloxanide furoate) that acts on the organism in the lumen. Liver abscess: in addition to targeting organisms in solid tissue, primarily with drugs like metronidazole and chloroquine, treatment of liver abscess must include agents that act in the lumen of the intestine (as in the preceding paragraph) to avoid re-invasion. Surgical drainage is usually not necessary except when rupture is imminent. People without symptoms: for people without symptoms (otherwise known as carriers, with no symptoms), non-endemic areas should be treated by paromomycin, and other treatments include diloxanide furoate and iodoquinol. There have been problems with the use of iodoquinol and iodochlorhydroxyquin, so their use is not recommended. Diloxanide furoate can also be used by mildly symptomatic persons who are just passing cysts (Kucik *et al*., 2004).

2.2.7.2 Treatment of *Giardia lamblia:*

Treatment is not always necessary as the infection usually resolves on its own. However, if the illness is acute or symptoms persist and medications are needed to treat it, a nitroimidazole medication is used such as metronidazole, tinidazole, secnidazole or ornidazole (Barry *et al.*, 2015).

2.2.7.3 Treatment of *Cyclospora cayetanensis:*

Trimethoprim/sulfamethoxazole (TMP/SMX), sold under the trade names Bactrim, Septra, and Cotrim, is the usual therapy for *Cyclospora* infection. No highly effective alternative antibiotic regimen has been identified yet for patients who do not respond to the standard treatment or have a sulfa allergy (CDC, 2015).

2.2.8 Control of water-borne protozoa:

Unlike most water-borne pathogens, such as bacteria and enteric viruses, *Cryptosporidium* and *Giardia* oocysts and cysts are very resistant to chlorine disinfection, which is commonly used to treat surface and ground waters. Because infected individuals shed oocysts for approximately one week after symptoms resolve, there is an increased risk of future outbreaks. Lack of awareness and failure to reduce transmission of these protozoan parasites using alternative water-sanitizing technologies make future *Cryptosporidium* and *Giardia* outbreaks inevitable. While substantial regulatory efforts have been directed at drinking water, there has not been a corresponding effort to regulate public recreational water facilities. Alternative water-sanitizing techniques that have proven successful for the disinfection of protozoa include the use of ozone and ultra-violet (UV) light as disinfectants (Extension, 2010).

2.2.9 Water-borne protozoa in Sudan:

Unsafe water and poor sanitation and hygiene have been reported to rank third among the 20 leading risk factors for health burden in developing countries, including Sudan (Shanan *et al.,* 2015). In Sudan, *Cryptosporidium* species are an important cause of diarrhea in children, and it is suggested that interfamilial spread occurs (Adam *et al.,* 1994). The highest prevalence of diarrheal diseases was recorded among Port Sudan children (15.5%),

followed by children living in areas where people draw water from unrectified hafirs (13.5%) and finally children living in areas where water is drawn from rectified hafirs (6.0%) (Awad *et al.*, 1985).

2.3 Water-borne helminths:

2.3.1 Definition:

Helminthes are large multicellular organisms, which when mature can generally be seen with the naked eye. They are often referred to as intestinal worms even though not all helminthes reside in the intestines, for example schistosomes are not intestinal worms, but rather reside in blood vessels (Samuel, 1996).

2.3.2 Transmission:

Helminthes are transmitted to human in many different ways. fecal-oral transmission of eggs or larvae passed in the faeces of one host and ingested with food or water by another (e.g. the ingestion of *Ascaris* eggs and *Strongyloides* larvae leads to a pulmonary migration phase before gut infection in humans). Transdermal transmission of infective larvae in the soil (geo-helminths) or water actively penetrating the skin and migrating through the tissues to the gut where adults develop and produce eggs that are voided in host faeces (e.g. larval hook worms penetrating the skin, undergoing pulmonary migration and infecting the gut where they feed on blood causing iron-deficient anaemia in humans) (Peter, 2010).

2.3.3 Life cycle of helminthes:

2.3.3.1 Life cycle of *Ascaris lumbricoides*:

The eggs pass on to the ground via the faeces. Fertilized eggs require 10 to 40 days in the outside world to mature before they become infectious. Direct self-infection is thus ruled out. Once they are mature the eggs are taken up once more (faeco-oral transmission) via infected food, drink, dirty hands or

fingernails. In the intestine small larvae emerge from the eggs, and these bore through the intestinal wall. In this way they reach the blood (portal vein system). They are carried with the blood, through the liver to the lungs (lung passage occurs 3 to 14 days after ingestion). In the lungs the larvae make their way to the bronchial lumen and climb via the respiratory branches into the throat. They are swallowed, and in this way, they again reach the intestine. They grow into adult worms in the jejunum. They do not damage the intestinal wall. Egg-laying begins two months after infection. The adult worm survives on average for 1 year. The creatures reach 15 to 40 cm. There is no animal reservoir. Occasionally infections with *Ascaris* suum (parasite of pigs) occur, this worm resembles *Ascaris lumbricoides* very closely and some think the parasites are identical (ITG, 2009) (figure 2.4).

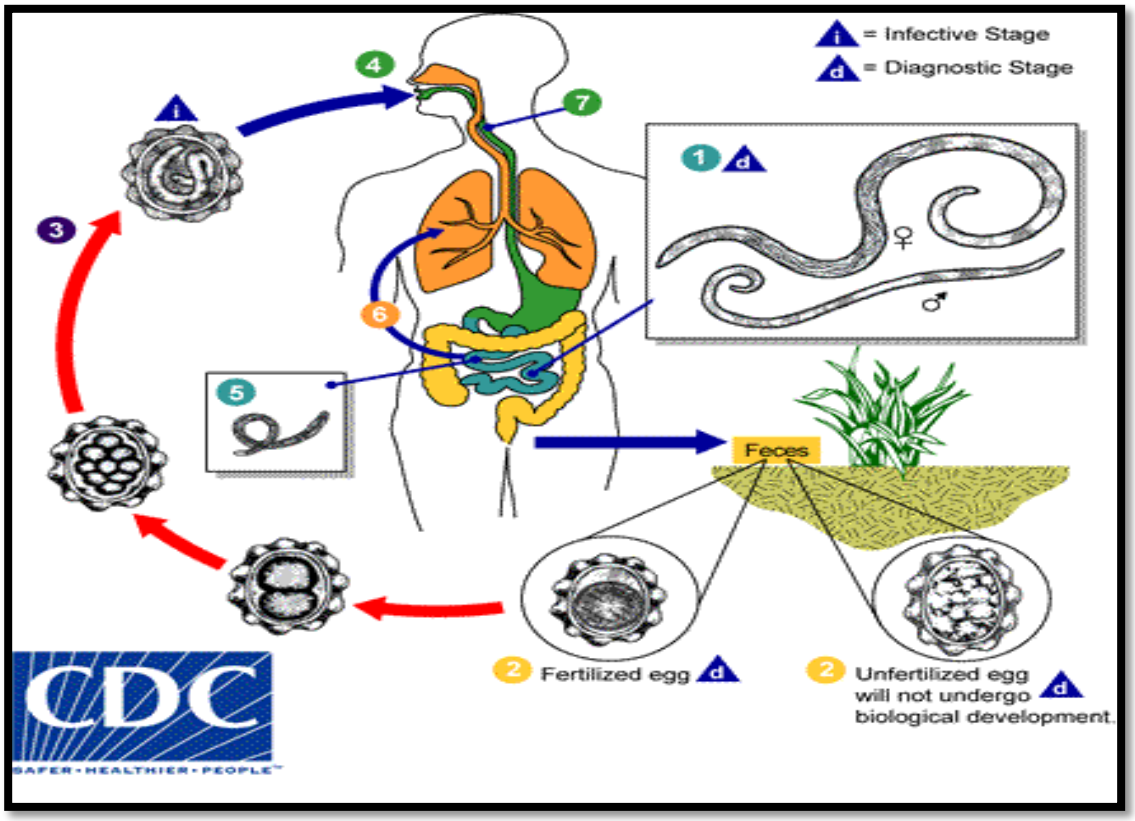

Figure (2.4): life cycle of *Ascaris lumbricoides* (CDC, 2016)

2.3.3.2 Life cycle of hook worms:

The adult worms are found in the small intestine. They measure approximately 1 cm. Adult hook worms survive for several years, *Necator* longer than *Ancylostoma*. A few weeks or months after infection eggs can be found in the faeces. Once the eggs arrive in the outside world with the faeces, they take one week to mature to infectious larvae. At first, they are rod-shaped (rhabditiform), later thread-shaped (filariform). They may survive for weeks or months (at an optimal temperature and humidity for as much as 2 years). A soil with neutral pH is optimal for their development, as is shade and a sufficiently high temperature (23°C to 30°C is ideal). Infection occurs via the mouth (*A. duodenale*) or via the skin (*A. duodenale* and *N. americanus*). If they enter through the skin, the young parasites have to pass through the lungs. The adult hook worms bore a hole in the mucosa of the duodenum and the small intestine and suck blood. They adhere with hooked teeth in their mouth (*Ancylostoma*) or with two buccal cutting plates (*Necator*). *A. duodenale* sucks 5 to 10 times more blood than *N. americanus* (approximately 30 µl per day for *Necator* and 260 µl for *Ancylostoma*). It is estimated that the life span of adult worms is 5 to 15 years (ITG, 2009, Olushola *et al*., 2010) (figure 2.5).

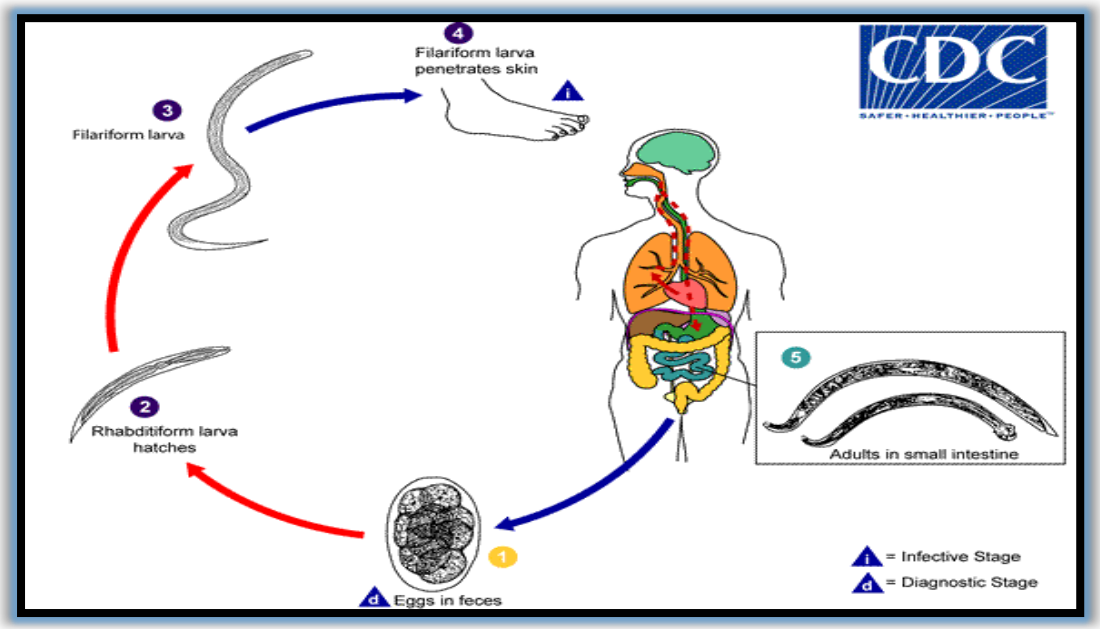

Figure (2.5): Life cycle of hook worms (CDC, 2013)

2.3.3.3 Life cycle of *Strongyloides stercoralis*:

The adult female worm, (average 2.7 mm) is found in the mucosa of the small intestine. Males cannot penetrate the intestinal mucosa and perish. Reproduction is asexual via parthenogenesis. The females lay eggs after 2-3 weeks, from which larvae are quickly produced. Initially the larvae are described as rhabditiform. These quickly develop into filariform (infectious) larvae. These larvae may either penetrate back into the intestinal mucosa or pass to the perianal skin and from there again penetrate the body (auto re-infection). In auto re-infection there is always another lung passage. In this way an infection with *Strongyloides* may persist for a very long time (more than 30 years) or pass to the outside world with the faeces. From there, after molting, they may go in either of two directions. The larvae either again penetrate the skin of a human (sometimes even via the mouth) or they develop to adult worms in the outside world. They may then via sexual

reproduction in their turn lay eggs, from which new larvae develop. The worm can thus survive without a host (Olushola *et al*., 2010) (figure 2.6).

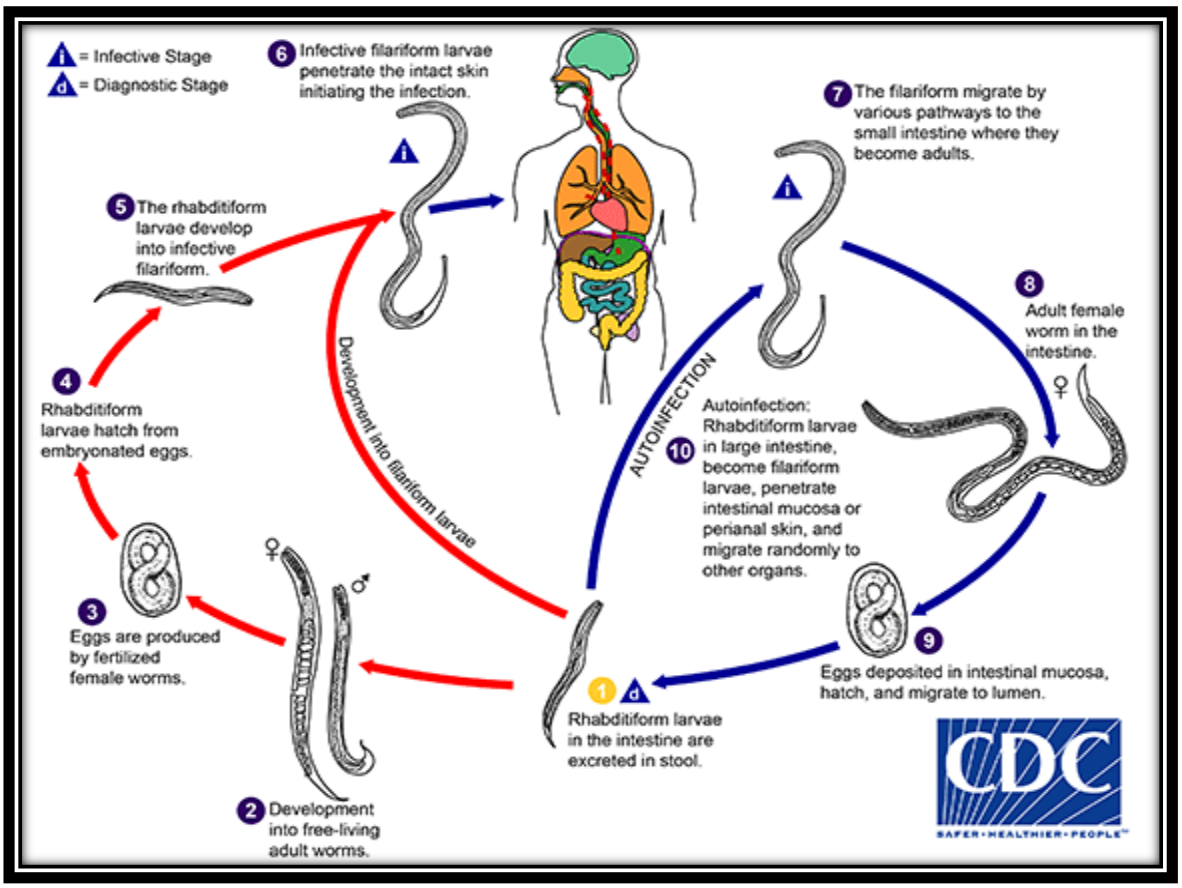

Figure (2.6): Life cycle of *Strongyloides stercoralis* (CDC, 2015)

2.3.4 Clinical presentation:

2.3.4.1 Ascariasis:

Clinical features of helminthiasis vary a lot depending on the helminth species, intensity of infection, and host age. Pulmonary symptoms occur in a small percentage of patients when round worm larvae pass through the lungs. Round worm can also cause intestinal discomfort, obstruction, and impaired nutritional status. Several *Ascaris* larvae migrating from the intestine to the other organs are destroyed in the liver and lungs. The remains of the disintegrating larvae induce most of the eosinophilia seen in ascariasis. Pulmonary manifestation is the usual symptom. This presents as a slight

cough for a few days or, in areas where transmission is seasonal, as a severe seasonal Loffler's pneumonia-like syndrome (Arfaa, 1984). Adult worms residing in the intestine may induce mild occasional abdominal pains. When the worm load is heavy, and especially in children, the abdominal pain is severe, and the patient may be restless with loss of appetite, occasional vomiting and intermittent loose stools, constipation, passing of worms from the rectum or the mouth, colicky, abdominal distension and abnormal abdominal sounds (Arfaa, 1984, Gelpi and Mustafa, 1967).

2.3.4.2 Hook worm infection:

Hook worm infection can lead to anemia due to blood loss and chronic protein deficiency. However, travelers are rarely at risk because these more severe manifestations are generally associated with high worm burdens seen in indigenous populations. Early symptoms include: epigastric pain, mimicking peptic ulcer disease, diarrhea, and anorexia and vomiting may occur. In heavy infections, edema of face, abdominal wall, legs, ascites, and severe prostration have been described (Sadun and Mainphoom, 1953).

2.3.4.3 Strongyloidiasis:

Many people infected are asymptomatic at first. Symptoms include dermatitis: swelling, itching, larva currens, and mild hemorrhage at the site where the skin has been penetrated. Spontaneous scratch-like lesions may be seen on the face or elsewhere. If the parasite reaches the lungs, the chest may feel as if it is burning, and wheezing and coughing may result, along with pneumonia-like symptoms (Löffler's syndrome). The intestines could eventually be invaded, leading to burning pain, tissue damage, sepsis, and ulcers. Stools may have yellow mucus with a recognizable smell. Chronic diarrhea can be a symptom (Thamwiwat *et al.*, 2014). In severe cases, edema may result in obstruction of the intestinal tract, as well as loss of peristaltic

contractions (Roberts and Janovy, 2005). Strongyloidiasis in immunocompetent individuals is usually an indolent disease. However, in immunocompromised individuals, it can cause a hyperinfective syndrome (also called disseminated strongyloidiasis) due to the reproductive capacity of the parasite inside the host. This hyperinfective syndrome can have a mortality rate close to 90% if disseminated (Igra *et al.*, 1981).

2.3.5 Epidemiology:

Areas with the highest prevalence of helminthiasis are tropical and subtropical areas including sub-Saharan Africa, central and east Asia, and the Americas. Some types of helminthiasis are classified as neglected tropical diseases. The soil-transmitted helminths, *A. lumbricoides, N. americanus, A. duodenale*, and schistosomes, collectively infect more than a quarter of the human population worldwide at any one time, far surpassing human immunodeficiency virus (HIV) (Crompton and Savioli, 2007, Lustigman *et al*., 2012). Another source estimated a much higher figure of 3.5 billion infected with one or more soil-transmitted helminths (Ojha *et al*., 2014). Because of their high mobility and lower standards of hygiene, school-age children are particularly vulnerable to helminthiasis. Most children from developing nations will have at least one infestation. Multi-species infections are very common (Bethony *et al.*, 2006). Even in areas of high prevalence, the frequency and severity of infection is not uniform within communities or families (Magill *et al*., 2013). A small proportion of community members harbor most worms, and this depends on age. The maximum worm burden is at five to ten years of age, declining rapidly thereafter. Individual predisposition to helminthiasis for people with the same sanitation infrastructure and hygiene behavior is thought to result from differing immunocompetence, nutritional status, and genetic factors (Magill

et al., 2013). Because individuals are predisposed to a high or a low worm burden, the burden reacquired after successful treatment is proportional to that before treatment (Magill *et al*., 2013). It is estimated that intestinal nematode infections cause 5 millions disability-adjusted life years (DALYS) to be lost, of which hook worm infections account for more than 3 millions DALYS and *Ascaris* infections more than 1 million (Silva and Hall, 2010). There are also signs of progress: The Global Burden of Disease Study published in 2015 estimates a 46 percent (59 percent when age standardized) reduction in years lived with disability (YLD) for the 13-year time period from 1990 to 2003 for all intestinal/nematode infections, and even a 74 percent (80 percent when age standardized) reduction in YLD from ascariasis (Vos and Barber, 2015). As many as 135,000 dies annually from soil and water transmitted helminthiasis (Lustigman *et al*., 2012). Another 20 millions have severe consequences from the disease. It is the most deadly of the neglected tropical diseases (Kheir *et al*., 1999).

2.3.6 Laboratory diagnosis:

2.3.6.1 Laboratory diagnosis of *Ascaris lumbricoides*:

Microscopic identification of eggs in the stool is the common method for diagnosing ascariasis and simple smears are often adequate because of the high output of eggs produced daily by gravid female worms. Stool concentration procedures such as Kato-Katz thick smear or formalin-ethyl acetate sedimentation can also be done. *Ascaris* larvae can also be found in sputum or gastric aspirates during pulmonary migration before eggs are present in feces. Adult worms are occasionally passed in the stool or through the mouth or nose are easily recognizable by their macroscopic characteristics. Polymerase chain reaction (PCR) based asssays can identify and quantify the DNA of *Ascaris*. Serology is more useful for epidemiologic

purposes than for individual diagnosis because the IgG antibodies developed by patients cross react with the antigens from other helminths. Eosinophilia is a non-specific finding that is not used solely for diagnosis. Eosinophilia is usually more prominent during early infection but often subsides in established adult worm infestation in the intestines. Eosinophilia is often in the 5-10% range but can rise as high as 50% (Ferri, 2017, Durand, 2015).

2.3.6.2 Laboratory diagnosis of Hook worm:

Diagnosis depends on finding characteristic worm eggs on microscopic examination of the stools, although this is not possible in early infection. The eggs are oval or elliptical, measuring 60 µm by 40 µm, colorless, not bile stained and with a thin transparent hyaline shell membrane. When released by the worm in the intestine, the egg contains an unsegmented ovum. During its passage down the intestine, the ovum develops and thus the eggs passed in feces have a segmented ovum, usually with 4 to 8 blastomeres. As the eggs of both *Ancylostoma* and *Necator* (and most other hook worm species) are indistinguishable. Adult worms are rarely seen (except via endoscopy, surgery or autopsy), but if found, would allow definitive identification of the species. Classification can be performed based on the length of the buccal cavity, the space between the oral opening and the esophagus: hook worm rhabditoform larvae have long buccal cavities whereas *Strongyloides* rhabditoform larvae have short buccal cavities. Recent research has focused on the development of DNA-based tools for diagnosis of infection, specific identification of hookworm, and analysis of genetic variability within hook worm populations (Gasser *et al.,* 2009). Because hookworm eggs are often indistinguishable from other parasitic eggs, PCR assays could serve as a molecular approach for accurate diagnosis of hook worm in the feces (Gasser *et al.,* 2009, Yong *et al.,* 2007).

2.3.6.3 Laboratory diagnosis of *Strongyloides stercoralis:*

Locating juvenile larvae, either rhabditiform or filariform, in recent stool samples will confirm the presence of this parasite. Other techniques used include direct fecal smears, culturing fecal samples on agar plates, serodiagnosis through ELISA, and duodenal fumigation. Still, diagnosis can be difficult because of the day-to-day variation in juvenile parasite load (Roberts and Janovy, 2005).

2.3.7 Treatment:

2.3.7.1 Treatment of *Ascaris lumbricoides*:

In endemic areas without an organised control programme, individuals may take antihelminthic drugs periodically to prevent infection. Identification and treatment of these individuals is carried out mainly at the hospital level when such persons present with symptomatic complaints. This approach is most useful for identifying and treating individuals predisposed to infection with heavy worm burden in a community because it is only severe symptomatic manifestations often associated with very heavy infections that encourage individuals to go to hospitals (Hlaing, 1987). Hospital based individual treatments have therefore been suggested as a possible approach to reducing morbidity in a community (Asaolu *et al.,* 1991). There are several drug formulations available for the treatment of ascariasis. Levamisole, the drug of choice, is highly effective and well tolerated. It acts on the worm's nerve ganglia paralysing the musculature within minutes of contact resulting in the immediate ejection of the worms by normal peristaltic movement in less than 24 hours. Levamisole is available as 40 mg levamisole tablets and 40 mg/5 ml drinkable suspensions (Stephenson *et al.,* 1983). Mebendazole, a benzimidazole derivative, interferes with the cellular tubulin formation in worms, disturbs glucose

uptake and the normal digestive functions leading to instant autolytic process and death. The worms are expelled within 24 hours of drug administration, sometimes by mouth. Mebendazole is available as oral tablets, each containing 100 mg mebendazole and drinkable suspension containing 20mg mebendazole/ml. Both the tablet and the suspension are given in 6-regimens of 1 tablet (adults) or 5ml suspension (children below the age of 5 years) twice daily (morning and evening) for 3 consecutive days. An alternative dose of 500 mg single dose has also been found to be equally effective (Albonico *et al.,* 1996). Nitazoxanide, a nitrothiazole benzamide has been reported to be effective against a broad range of parasites including *Ascaris* and other nematodes. The drug is administered at a dose of 1 tablet (500 mg) twice daily for 7 consecutive days. At this dose, it is very effective among all age groups (Dombo *et al.*, 1997).

2.3.7.1 Treatment of Hook worm:

The most common treatment for hook worm are benzimidazoles, specifically albendazole and mebendazole. BZAs kill adult worms by binding to the nematode's β-tubulin and subsequently inhibiting microtubule polymerization within the parasite (Bethony *et al.,* 2006). In certain circumstances, levamisole and pyrantel pamoate may be used. A review in 2008 found that the efficacy of single-dose treatments for hook worm infections were as follows: 72% for albendazole, 15% for mebendazole, and 31% for pyrantel pamoate (Keiser and Utzinger, 2008). This substantiates prior claims that albendazole is much more effective than mebendazole for hookworm infections. Also, of note is that the World Health Organization does recommend antihelminthic treatment in pregnant women after the first trimester. It is also recommended that if the patient also suffers from anemia that ferrous sulfate (200 mg) be administered three times daily at the same

time as antihelminthic treatment; this should be continued until hemoglobin values return to normal which could take up to 3 months (Bethony *et al.*, 2006).

2.3.7.1 Treatment of *Strongyloides stercoralis*:

Ivermectin is the drug of first choice for treatment because of higher tolerance in patients (Johnston *et al.*, 2005). Thiabendazole was used previously, but, owing to its high prevalence of side effects (dizziness, vomiting, nausea, malaise) and lower efficacy, it has been superseded by ivermectin and as second-line albendazole. However, these drugs have little effect on the majority of these auto-infective larvae during their migration through the body. Hence, repeated treatments with ivermectin must be administered to kill adult parasites that develop from the auto-infective larvae. This means at least two weeks treatment, then a week's pause, then again treatment. Follow-up treatment and blood tests are necessary for decades following infection. In the UK, mebendazole and piperazine are currently preferred. Mebendazole has a much higher failure rate in clinical practice than albendazole, thiabendazole, or ivermectin (Boulware *et al.*, 2007).

2.3.8 Control of water-borne helminthes:

Water-transmitted helminth infections are caused by different species of parasitic worms. They are transmitted by eggs present in human faeces, which contaminate the water in areas where sanitation is poor. Infected children are physically, nutritionally and cognitively impaired. Control is based on: periodical deworming to eliminate infecting worms, health education to prevent re-infection, improved sanitation to reduce water contamination with infective eggs and Safe and effective medicines are available to control infection (WHO, 2017).

2.3.9 Drinking water problems in Sudan:

One of the biggest necessities in the world today is water, but that does not mean everyone is given the clean water that is needed to survive. Especially in rural villages of Sudan, people are deprived of fresh drinking water on a daily basis. They then have to resort to unsanitary means of water, which later leads to water-borne and water-based disease. These diseases are the primary cause of preventable illness and premature deaths in these villages, with children being particularly vulnerable. Purified water should always be attainable, no matter the circumstances at hand. Sudan's current water situation is a very serious issue. 12.3 million People have access to only contaminated water. 30% of the population have clean drinking water. Out of 10 people only 4 have access to clean water. In 2008, nearly 800,000 people living in Darfur got a water-borne disease due to contaminated drinking water. The problem is these desperate people have no access to clean drinking water hence they are forced to drink from contaminated water and furthermore receive severe illnesses. Access to clean drinking water has a direct link to health. Many water-borne diseases affect Sudan's population. The conflicts in Sudan have intensified the need for water projects. The problem is not just that the water is not clean but that the process of making clean water more available is not simple. Water is not easily reachable and water projects are very expensive, especially for poor countries like Sudan (Kaitlyn, 2011).

Chapter 3

Materials and Methods

3.1 Study design:

It is cross- sectional study.

3.2 Study area:

The study was taken place in the basic schools in Khartoum state which include three regions: Khartoum, Bahri and Omdurman.

3.3 Study population:

All water drinking sources available in the schools under study.

3.4 Study duration:

This study was carried out in the period of June to September in 2017.

3.5 Sample size:

Since there was an available data on the prevalence of parasites in drinking water in Sudan as described by Shanan *et al.* (2015) (9.5%). To calculate the sample size, the following equation was used:

$$n = z2pq / d2$$

Where n = Sample size

z = Standard normal deviation at 1.96 (which corresponds to 95% confidence interval).

p = Prevalence rate from previous studies on parasites in drinking water 9.5% in other words probability of contaminated drinking water is 0.095.

q = 1-p; 1-.095= 0.905 the probability of not having contamination in drinking water is 0.905.

d =degree of accuracy/precision expected (allowable error) 5% = 0.05.

Thus, n = 1.96*1.96*0.095*0.905/0.05*0.05.

=0.330/0.0025 = 132.

According to the above finding, the study was conducted on 132 samples of drinking water of basic schools under the study.

3.6 Sampling:

Two-liters water samples were collected from all sources of drinking water of basic schools under the study. Collection were taken randomly by using simple random sampling method in which was have equal chance of drawing each unit. Each school was chosen randomly and entirely by chance such that each school had the same probability of being chosen at any stage during the sampling process. Water samples were transported to the laboratory of parasitology department of the college of medical laboratory science and examined by sedimentation technique for general parasite cysts, trophozoites and helminthes eggs. Also, samples stained with modified Ziehl-Neelsen acid fast stain, a special stain for detection of coccidian parasites.

3.7 Data collection:

Designed questionnaire (appendix) contained the following variables: type of schools, water source, cleanness of toilet, type of toilet, presence of water tank, presence of hand washbasin, distance between toilet and source of drinking water, source of drinking water supply and method of purification, was used to obtain information that helped in the study.

3.8 Methods:

3.8.1 Sedimentation technique:

Two liters of water were collected in clean sterile bottle and left to stay undisturbed for 24 hours at room temperature. The supernatant was sucked and removed. Then each sample was washed in 10% formal saline, 15 ml of washed samples were centrifuged at 5000 rpm for 3 minutes using 15 ml falcon tubes, then the drop of sediment was examined under microscope using x10 and x40 objectives.

3.8.2 Ziehl-Neelsen acid fast stain:

Water smears were made directly from the concentration deposit. Allowed to air dry. Then fixed in absolute methanol for 3 minutes. The smears were stained with strong carbol fuchsin for 15-20 minutes. Then rinsed thoroughly in tap water and decolorized in acid alcohol (1% HCl in methanol) for 15-20 seconds. Then rinsed thoroughly in tap water. Counterstained with 0.4% malachite green (or methylene blue) for 30-60 seconds. Then rinsed again thoroughly and air dried and then examined using x100 objectives.

3.9 Data analysis:

The data obtained were analyzed using the Statistical Package for Social Sciences (SPSS) version 18. Frequencies, crosstabulation and Chi-squ=ire test were used. Then data were presented in tables and figures.

3.10 Ethical consideration:

The study adopted was approved by College of Medical Laboratory Science-Sudan University of Science and Technology and permission was taken from the authorities of the schools included in the study.

Chapter 4

Results

4.1 General characteristic of studied drinking water samples:

This is study was conducted on 132 drinking water samples from basic schools. Out of them, 61 were girl's schools, 57 were boy's schools and 14 were mixed (table 4.1). The samples were divided into 3 regions of Khartoum state (Omdurman, Bahri, Khartoum) 44 samples for each one (table 4.1).

Table (4.1): Frequency of type of schools among Khartoum state

Type of schools	Frequency			Total
	Omdurman	Bahri	Khartoum	
Girls	17	16	28	61
Boys	21	20	16	57
Mixed	6	8	0	14
Total	44	44	44	132

4.2 Overall frequency of water borne parasites in the drinking water:

A total of 132 samples were examined for water borne parasites. 56 (42.4%) were positive, while 76(57.6%) were negative (table 4.2).

Table (4.2): Overall frequency of water borne parasites in the drinking water

No. of samples examined	No. of positive	Prevalence
132	56	42.4%

4.3 Distribution of study sample according to presence of pathogenic parasites:

From 56 contaminated samples, 28 (50 %) contained pathogenic parasite, 10 (17.9 %) samples were nonpathogenic, and 18 (32.1%) of positive samples contained more than one pathogen (figure 4.1).

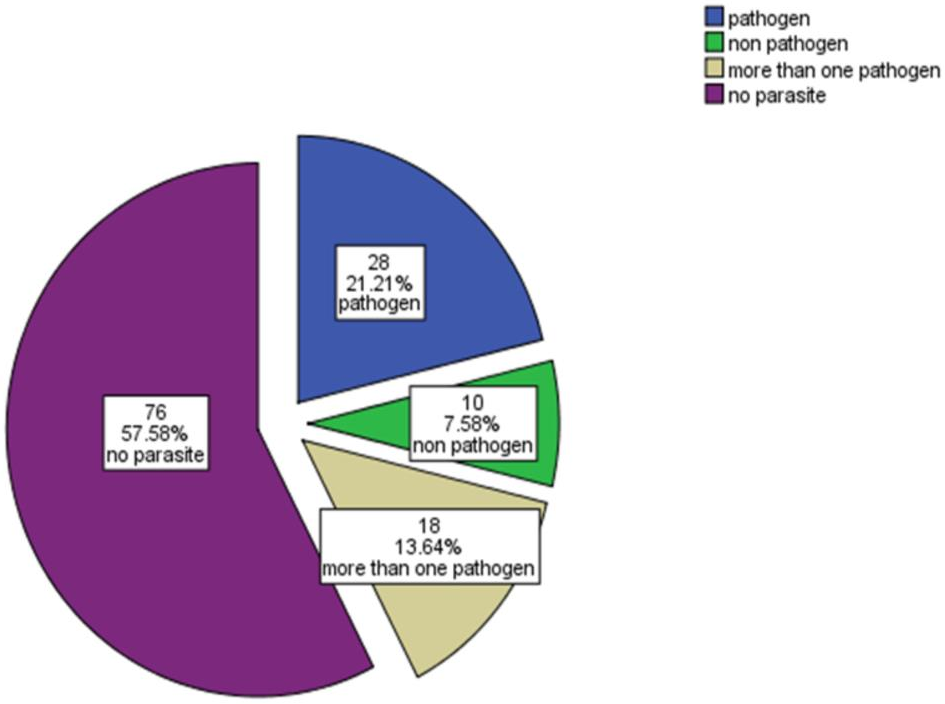

Figure (4.1): Distribution of study sample according to presence of pathogenic parasites

4.4 Distribution of water-borne parasites species among contaminated samples:

Nine species of water borne parasites were observed, *Entamoeba histolytica* 21 (15.9%) being the most predominant followed by *Giardia lamblia* 19 (14.4%). Total number in the following table is not equal to the number of positive samples because there were some positive samples 18 (32.1%) which included more than one parasitic species (table 4.3).

Table (4.3): Distribution of water-borne parasites species among contaminated samples

	Number of positive	Percentage
Entamoeba histolytica cyst	21	15.9 %
Giardia lamblia cyst	19	14.4 %
Strongyloide developing Rhabditiform larva	22	16.7 %
Miracidum of Schistosoma	10	7.6 %
Ascaris lumbricoides	4	3 %
Hook worm egg	4	3 %
Cyclospora	1	0.8 %
Entamoeba coli	14	10.6%

4.5 Distribution of contaminated samples with relation to region in Khartoum state (Khartoum, Bahri and Omdurman):

Forty-four samples were collected from each region of Khartoum state (Khartoum, Bahri, Omdurman), Omdurman was the most frequent as it contained 26 (59.1%) contaminated samples. The difference in rate was found to be statistically significant at the p. value = 0.010 (table 4.4).

Table (4.4): Distribution of contaminated samples with relation to region in Khartoum state

Region	No. of sample examined	No. of positive	Percentage (%)	P. value
Khartoum	44	18	40.9%	0.010
Bahri	44	12	27.3 %	
Omdurman	44	26	59.1%	
Total	132	56	42.4%	

4.6 Distribution of contaminated samples with relation to type of schools (girls, boys, mixed schools):

Girls' schools have more frequent of contamination of drinking water than boy's schools but the differences in rate was found to be statistically insignificant at the p. value= 0.724 (table 4.5).

Table (4.5): Distribution of contaminated samples with relation to type of schools

	No. of sample examined	No. of positive	Percentage (%)	P. value
Girls	61	28	45.9 %	
Boys	57	22	38.6%	
Mixed	14	6	42.9%	0.724
Total	132	56	42.4%	

4.7 Distribution of contaminated samples among different water sources:

The distribution of water borne parasites differs according to different water sources, water from the Zeer had the highest degree of contamination (69.7%) followed by water container (50%). The difference in rate was found to be statistically significant at p. value= 0.012 (table 4.6)

Table (4.6): Distribution of contaminated samples among different water sources

	No. of sample examined	No. of positive	Percentage (%)	P. value
Tap water	37	13	35.1%	
Zeer	33	23	69.7%	
Thermal bricks	30	9	30.0%	0.012
Cooler	21	6	28.6%	
Water container	6	3	50 %	
Hose	5	2	40 %	
Total	132	56	42.4 %	

4.8 Distribution of intestinal parasites in the 6 different water sources:

Only the Zeer harbored all the nine species of intestinal parasite. The distribution of miracidium and non-pathogen *Ameaba* in different water sources have significant statistical difference at p. value =.044 and p. value = 0.001 respectively (table 4.7)

Table (4.7): Distribution of intestinal parasites in the 6 different water sources

	Cooler	Zeer	Thermal bricks	Tap water	Water container	Hose	Total	P.value
Giardia lamblia cyst	2 (10.5%)	6 (31.6%)	5(26.3%)	4 (21.1%)	1(5.3%)	1 (5.3%)	19	.918
Entamoeba histolytica cyst	4(19%)	8 (38.1%)	4(19%)	4(19%)	1(4.8%)	0	21	.596
Strongyloide developing Rhabditiform larva	3 (13.6%)	9(40.9%)	2(9%)	6(27.3%)	2(9%)	0	22	.189
Ascaris egg	0	3(75%)	1(25%)	0	0	0	4	.297
Hook worm	0	3(75%)	0	1(25%)	0	0	4	.299
Miracidum of Schistosoma	0	6(60%)	3(30%)	0	1(10%)	0	10	.044
Cyclospora cyst	0	1(100%)	0	0	0	0	1	.696
Entamoeba coli	1(7.1%)	10 (71.4%)	0	2 (14.3%)	0	1 (7.1%)	14	.001

4.9 Association between toilet characteristics and the contamination of drinking water in basic schools at Khartoum state:

The presence of traditional toilets at schools and toilets without roofs were associated with more contamination of drinking water (57.9%) and (61.5%) respectively with significant statistical difference at p. value= 0.022 and p. value =0.028 respectively. Other factors like cleanness of toilet, presence of hand washbasin near the toilet and the distance between the toilet and source of drinking water (above 18 meters or less than 18 meters) have no significant statistical difference. The presence of water tank in the schools also have no effect in contamination of drinking water (table 4.8).

Table (4.8): Association between toilet characteristics and the contamination of drinking water in basic schools at Khartoum state

		Positive	Negative	Total	P. value
Cleanness of toilet	clean	21(47.7%)	23(52.3%)	44	0.383
	Not clean	35(39.8%)	53(60.2%)	88	
Type of toilet	Siphon	34(36.2%)	60(63.8%)	94	0.022
	Traditional Toilet	22(57.9%)	16(42.1%)	38	
Presence of roof	Yes	40(37.7%)	66(62.3%)	106	0.028
	No	16(61.5%)	10(38.5%)	26	
Presence of water tank	Yes	37(37.8%)	61(62.2%)	98	0.065
	No	19(55.9%)	15(44.1%)	34	
Presence of hand washbasin	Yes	24(42.9%)	32(57.1%)	56	0.931
	No	32(42.1%)	44(57.9%)	76	
Distance between the toilet and source of drinking water	Above 18 meters	20(37.7%)	33(62.3%)	53	0.372
	Less than 18 meters	36(45.6%)	43(54.4%)	79	

4.10 The relation between drinking water supply and contamination of water:

Drinking water from ground water supplies was more contaminated than water coming from Nile river supplies as appears in table (4.9).

Table (4.9): The relation between drinking water supply and contamination of water

Source of drinking water supply	No. of sample examined	No. positive	No. negative	p. value
Nile water	96	36(37.5%)	60(62.5%)	
Ground water	36	20(55.6%)	16(44.4%)	0.062

4.11 The relation between purification method used and contamination of water by water borne parasite:

Only 3 schools used a method of purification of drinking water by filtration technique (table 4.10).

Table (4.10): The relation between purification method used and contamination of water

purification	No. of sample examined	No. positive	No. negative	P. value
Nothing	129	56(43.4%)	73(56.6%)	0.133
Filtration	3	0	3(100%)	

Chapter 5

Discussion, conclusion and recommendations

5.1 Discussion:

This study showed that 56 (42.4%) of studied samples were contaminated with parasitic agents, of which 46 (34.8%) were pathogenic. This result was much higher than what was found by Shanan *et al.* (2015) in his study on drinking and environmental water sources in Sudan, which showed that out of 600 drinking water samples, 57 (9.5%) were positive. The justification of this big difference may be that Shanan study was searching for only protozoa and did not include helminthes. The other justification may be that water sources in Shannan study included lakes and streams.

Current study result was similar to what showed by the study of Abera (2014) on prevalence of intestinal parasite among primary school children and drinking water source in Ethiopia, which revealed that out of 105 water samples, 39 (37.14%) were positive.

Other study in Iran (Rafiei *et al.,* 2014) showed that out of 44 water sample 28 (63.6%) were positive, and another study in Pakistan on the prevalence of parasites in drinking water resulting in an overall prevalence of contamination of water sources was (65.5%) (Ayaz *et al.,* 2011). The current study showed less frequency than Rafiei study and this may be due to different circumstances; the later study was carried out after an outbreak of gastrointestinal parasitic disease which was reported by a local health organization at that time.

The species of intestinal parasites detected were *Entamoeba histolytica, Giardia lamblia,* rhabditiform larva, miracidium, *Ascaris lumbricoides,* hook worm eggs and *Cyclospora*. *Entamoeba histolytica* was detected in 21 samples (37.5%) being the most predominant. This result was similar to the

result reported by Rafiei *et al.* (2014) who showed that 22 (50%) of water samples was *Entamoeba spp*. Other studies done by Leiva *et al.* (2008) and Tsvetkova *et al.* (2004) reported that the *Amoebae* were found in 43% and 61% of the water samples respectively. In a recent study in Turkey, only three species of free-living amoeba, *Acanthamoemba castellanii*, *A.polyphaga*, and *Hartmannella vermiformis*, were identified from tap water (Coskun *et al.*, 2013). In addition, *Cryptosporidium, Giardia,* and *Acanthamoeba* were isolated from stations of recreational lakes in Malaysia (Onichandran *et al.,* 2013). In contrast, result in Sudan detected a limited number of *Amoebae, Cryptosporidium,* and *Giardia* (Shanan *et al.*, 2015).

From 56 positive samples in the present study, 18 (32.1%) contained more than one pathogens. This percentage is much less than what was found in an Egyptian study conducted in tap and tank water by Sakran *et al.* (2017) who found that multiple contamination was (72.4%) and (76.7%) respectively.

The study revealed that Omdurman had the highest prevalence of parasitic agents as it contained 26 (59.1%) contaminated samples (P. value =0.010) especially in Al-Salha area and the possible cause for this may be that the water supplement to this area was ground water and most of the schools use the zeer as main source of drinking water to their students. There are no available studies comparing the presence of parasitic agents in drinking water among these three parts.

The study showed that girls' schools (45.9 %) had more frequency of contamination of drinking water than boys schools (38.6%) but the differences in rate was found to be statistically insignificant at p. value = 0.724, however, another study in sudanese schools found that intestinal parasites were more prevalent among the males (80%) than the females (60%) (Siddig *et al.*, 2017).

Water from the zeer had the highest degree of contamination (69.7%) followed by water container (50%) and then the other water sources, with significant statistical difference (P = 0.012). There was lack of studies on zeer water to be used in comparison but the most relevant findings to this result were reported by Sakran *et al.* (2017) in theis study in drinking water in Egypt; They found more contamination in tank water (100%) compared with tap water (90.8%). Most of the zeers were uncovered and filled with hose, and students use cups to scoop water. Dirty hands of children and dirty cups are possible causes of water contamination. Hands and fingers of students might be easily contaminated with soil that contains cyst and eggs of parasitic organisms that leads to intestinal infection (Tamirat, 2017). Most of zeers were not washed regularly and water stays for long period and this may allow parasites multiplication especially water borne and free-living parasites. This justifies that only zeers harbored all the nine species of intestinal parasites as follows, *Giardia lamblia* (13), *Entamoeba histolytica* (17.4%), rhabditiform larva (13%), *Ascaris* egg (6.5%), hook worm (6.5%), miracidium (13%), *Cyclosporidium* (2.2%) and nonpathogenic larva (21.7%).

Only 3 schools used filtration technique as method of purification of drinking water, therefore, different distribution of parasites in different drinking water sources in this study may be resulted due to lack of adequate purification of water and unhygienic practices around the water source.

Regarding the possible associated factors with water contamination, the study revealed that the presence of traditional toilets at schools and toilets without roofs were associated with more contamination of drinking water with significant statistical differences; (P=0.022) (P=0.028) respectively. All traditional toilets were not containing washing water supplies inside them,

and all of them were not clean. Toilets without roofs may allow insects and house fly to transmit parasites.

The current study did not prove a significant association between the presence of hand washbasin near the toilet and water contamination with parasites (p=0.931), which may suggest that the presence of hand washbasin was not enough to decrease water contamination unless it was combined with good personal hygiene and actual attitude of hand washing. Lansdown *et al.* (2002) reported the health education and promotion of healthy behaviors to students play a key role in reduction the incidence of contamination of water. Students who have not thoroughly washed their hands after using the bathroom may pose a risk.

The distance between the toilet and source of drinking water (above 18 meters or less than 18 meter) have no significant statistical difference. This was in contrast to the result obtained by Yousefi *et al.* (2009) who showed that the distance between water and sources of contamination (above 18 meters or less than 18 meters) have significant statistical difference (p=0.001).

5.2 Conclusion:

The study concluded that the frequency of water borne parasite in drinking water in basic schools at Khartoum state was 42.4%.

5.3 Recommendations:

- Further analysis of the drinking water sources for the presence of cysts and oocysts should be done.
- Advanced technique should be used such as Polymerase Chain Reaction (PCR) to confirm the result.
- There should be a need to provide a well-protected and treated drinking water to the schools.
- Health authorities should collaborate with school health program for delivering health education to increase the knowledge and attitude of basic schools children about personal hygiene, environmental sanitation, toilet facilities and proper waste disposal.

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Sudan University of Science and Technology

College of Graduate Studies

M.Sc. in Medical Laboratory Science (Parasitology and Medical Entomology)

Questionnaire

-**School number**……

-**Type of school**: Girls ☐ Boys ☐ Mixed ☐

-**Region**: Khartoum ☐ Bahri ☐ Omdurman ☐

-**Type of water source:** Tap water ☐ Thermal bricks ☐ Zeer ☐

Cooler ☐ Hose ☐ water container ☐

Zeer – covered: Yes ☐ No ☐

Filled of zeer: With hose ☐ Direct from tap water ☐

How many time washed the zeer……

Water source supply: Nile water ☐ Ground water ☐

Method for drinking water purification:

Nothing ☐ Boiling water ☐ Solar water ☐ Disinfection ☐ Filtration ☐

Toilet: Cleanness of toilet: Clean ☐ Not clean ☐

-**Type:** Traditional ☐ Siphon ☐

-**Presence of roof**: Yes ☐ No ☐

-**Presence of hand washbasin:** Yes ☐ No ☐

-**Presence of water tank:** Yes ☐ No ☐

-**Distance between toilet and drinking water source:** Above 18 ☐ Less than 18m ☐

Laboratory result:

- Sedimentation result: **…….**
- ZN stain result**: ……..**