بسم الله الرحمن الرحيم

Sudan University of Science and Technology
College of Graduate Studies

# of Intestinal Parasitic Infections and Their A[illegible]

Risk Factors Among Children in Shendi City, River Nile State- Sudan

انتشار العدوى الطفيلية المعوية وعوامل الخطر المرتبطة بها وسط الأطفال في مدينة شندي، ولاية نهر النيل- السودان

A dissertation submitted in partial fulfillment for the requirements of the degree of M.Sc. in Medical Laboratory Science (Parasitology and Medical Entomology)

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الآيـــة

بسم الله الرحمن الرحيم

قال تعـــالى:

﴿ وَاصْبِرْ لِحُكْمِ رَبِّكَ فَإِنَّكَ بِأَعْيُنِنَا وَسَبِّحْ بِحَمْدِ رَبِّكَ حِينَ تَقُومُ ﴾

صدق الله العظيم

سورة الطور- الآية 48

Dedication

To my beloved wonderful mother for her support, encouragement and love over the years.

To my precious father, whose wise guidance has made a good person.

To my sister for her support.

To every one who always hope to see me the best in everything.

I dedicate this work.

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Abstract

This cross-sectional study was conducted in Shendi city, River Nile State- Sudan to determine the prevalence of intestinal parasitic infection and their associated risk factors among children during the period from March 2018 to March 2019. The study was conducted on 272 children, 137 (50.4%) were males and 135 (49.6%) were females. Fecal samples were taken from all children included in the study, in addition to the epidemiological and parasitological data were obtained and recorded. All samples were examined to detect intestinal parasite species by using wet preparation, floatation technique and formal ether concentration technique. The study showed that the prevalence rate of intestinal parasites was (27.5%) and intestinal protozoa were more prevalent (26.8%) than intestinal helminthes (0.7%). The prevalence rate of intestinal parasitic infections by using wet preparation, formal ether concentration technique and zinc sulphate floatation technique were (21.6%), (27.5%) and (20%) respectively (p. value=0.000). The highest prevalence rate (18.8%) of intestinal parasites in the study area was reported with *Giardia lamblia*. The study revealed that the highest prevalence rate (53.3%) was reported among males while females reported (46.7%) prevalence rate. The highest prevalence rate (37.3%) was reported among the ≤ 4 and 5-9 years old. The results showed that the difference in prevalence rates of intestinal parasitic infections according to symptoms was found to be statistically insignificant except with the abdominal pain (p. value=0.015). The results showed that the difference in prevalence rates of intestinal parasitic infections according to their associated risk factors was found to be statistically insignificant except with the hands washing, vegetables and fruits washing (p. value=0.0001 and 0.0003 respectively). The study indicated that the prevalence rate of intestinal parasitic infections among children in the study area was high (27.5%).

مستخلص الدراسة

أجريت هذه الدراسة المستعرضة في مدينة شندي، ولاية نهر النيل– السودان لتحديد انتشار العدوى الطفيلية المعوية وعوامل الخطر المرتبطة بها وسط الاطفال في الفترة من مارس 2018 إلى مارس 2019م. أجريت الدراسة على 272 طفل، 137 (50.4%) كانوا ذكوراً و 135(49.6%) كانوا أناثاً. عينات البراز تمّ أخذها من جميع الاطفال المتضمنين في الدراسة, بالإضافة للبيانات الوبائية والطفيلية تمّ اخذها وتسجيلها. كل العينات تمّ فحصها للتعرف على أنواع الطفيليات المعوية باستخدام التحضير الرطب, تقنية الطفو و تقنية تركيز الفورمال إيثر. أظهرت الدراسة أن معدل انتشار الطفيليات المعوية كان (27.5%) وكانت الاوليات المعوية أكثر انتشاراً (26.8%) من الديدان المعوية (0.7%). معدل انتشار الطفيليات المعوية باستخدام التحضير الرطب، تقنية تركيز الفورمال إيثر و تقنية الطفو كان (21.6 %) و (27.5 %) و (20%) على التوالي (القيمة المعنوية= 0.000). كان أعلى معدل انتشار (18.8%) للطفيليات المعوية في منطقة الدراسة سُجل مع *Giardia lamblia* . كشفت الدراسة أن أعلى معدل انتشار (53.3 %) سُجل وسط الذكور بينما سجلت الإناث (46.7%) معدل انتشار. كان أعلى معدل انتشار (37.3%) سُجل وسط الفئة العمرية ≥ 4 و5–9عاماً. أظهرت النتائج أنه لا يوجد فارق مقدر إحصائياً بين معدلات انتشار العدوى الطفيلية المعوية وفقاً للأعراض ماعدا مع ألم البطن (القيمة المعنوية= 0.015). أظهرت النتائج أنه لا يوجد فارق مقدر إحصائياً بين معدلات انتشار العدوى الطفيلية المعوية وفقاً لعوامل الخطر المرتبطة بها ماعدا مع غسيل الأيدي و غسيل الخضروات و الفواكه (القيمة المعنوية= 0.0001 و 0.0003 على التوالي). خلصت الدراسة إلى أن معدل انتشار العدوى الطفيلية المعوية وسط الاطفال في منطقة الدراسة كان عالياً بنسبة (27.5%).

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CHAPTER ONE

Introduction, Rationale and Objectives

Chapter 1

Introduction, rationale and objectives

1.1 Introduction:

Intestinal parasitic infections (protozoa and helminthes) are globally endemic and constitute one of the greatest causes of illness in developing countries (Forson *et al*., 2018). School children are particularly vulnerable to intestinal parasitic infections because of their poor hygiene, hand-mouth activity and immature immune system (Choi and Kim, 2017). Intestinal parasitic infections (IPIs) can be transmitted directly (hand-to-hand contact) or indirectly (contact with contaminated food or water and from environmental surfaces) (Forson *et al*., 2018). Many protozoan parasites live in human gastrointestinal tract, some of those are pathogenic, like *Giardia lamblia* and *Entamoeba histolytica*, and some are nonpathogenic forms, living in gastrointestinal tract as commensals. *E.histolytica* is causative agent of amoebiasis with considerable worldwide morbidity and mortality. *G. lamblia* is causative agent of giardiasis which is the most common infection in temperate and tropical countries. The prevalence of *G.lamblia* has been estimated that 2- 3% in the developed countries and 20-30% in developing countries. *Cryptosporidium* species are found worldwide with a prevalence ranging from 4 to 31% in some developing countries (Quihui-Cota *et al.*, 2017). *Blastocystis hominis* is a common intestinal protozoan and its pathogenicity is still controversial. The prevalence of *B. hominis* has been reported to be higher in developing countries (30-50%) than developed countries (1.5-10%) (Mahni *et al.*, 2016). *Entamoeba coli* and *Endolimax nana* are not known to be pathogenic but can be markers of environmental fecal contamination (Aiemjoy *et al*., 2017). The most common helminthes enteric parasites in humans are the soil-transmitted nematodes. *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma*

duodenale, *Necator americanus* and *Strongyloides stercoralis* and cestodes of the family Taeniidae, namely *Taenia saginata* and *T.solium*. Soil-transmitted helminthes (STHs) affect almost one-sixth of the global population and result in a broad spectrum of asymptomatic to symptomatic (Seguí *et al*., 2018). The World Health Organization (WHO) has stated that school-aged children are particularly at risk for STHs infection (Punsawad *et al*., 2018). Intestinal parasite infection may be asymptomatic or may present with clinical features such as abdominal pain, cramps, nausea, vomiting, diarrhea, anemia, weight loss, and lack of appetite. Eosinophilia may occur secondary to parasitosis and is generally due to the intra-tissue biological cycle of some helminthes (Almeida *et al*., 2017). Diagnosis of intestinal parasitic infections is routinely based on microscopy. Preparation of stool samples for microscopy performed by the direct wet mount method or the concentration methods (sedimentation and floatation). The concentration technique has become a routine procedure in the ova and parasite examination and allows the detection of small numbers of organisms that may be missed by using only a direct wet mount (Kardaman *et al*., 2016).

1.2 Rationale:

Intestinal parasitic infections constitute a global heath burden causing clinical morbidity particularly in developing countries. Elevated intestinal parasitic infections have been recorded in developing countries because of poverty, lack of safe drinking water, poor hygiene, malnutrition and hot humid tropical climate. The most susceptible age for contamination and infections are children. Many studies were conducted to determine the distribution of intestinal parasites in different localities in Sudan but there was missing of information about intestinal parasites that spread in Shendi city. Therefore, this study was conducted to detect the prevalence of intestinal parasitic infections and their associated risk factors among children in Shendi city, River Nile State- Sudan.

1.3 Objectives:

1.3.1 General objective:

To determine the prevalence of intestinal parasitic infections and their associated risk factors among children in Shendi city, River Nile State- Sudan.

1.3.2 Specific objectives:

- To determine the prevalence of intestinal parasitic infections in study subjects according to age groups.
- To determine the prevalence of intestinal parasitic infections in study subjects according to gender.
- To identify types of parasitic agents that are present in Shendi city.
- To assess the possible associated factors with the prevalence of intestinal parasitic infection among children in the study area.
- To compare between wet preparation, formal ether concentration technique and zinc sulphate floatation technique in detection of intestinal parasitic infections.

CHAPTER TWO

Literature Review

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 Intestinal protozoa:

2.2.1 Classification:

Four major categories or assemblages, the categories generally recognized are: the amoeboid forms (the sarcodina, in a broad sense); the flagellated forms (the mastigophora, including groups of autotrophic or photosynthetic as well as heterotrophic species); the ciliated forms (the ciliophora, the most stable and perhaps most circumscribed of all protozoan assemblages); and the various totally symbiotic or parasitic forms (primarily spore forming species that are typically endo- parasites, some highly pathogenic to their hosts, once assigned to a very broad group called the sporozoa, a high-level taxon that subsequently became divided into the sporozoa and the cnidosporidia) (Corliss, 2001).

2.2.2 Epidemiology:

Parasitic infections, and in particular those caused by protozoa, are a major public health problem worldwide. They are among the most widespread human infections in developing countries, with children being the most vulnerable population (Osman *et al*., 2016). A wide range of protozoan species can infect or colonies the gastrointestinal tract of humans and animals (Seguí *et al*., 2018). In particular

intestinal protozoan, such as *Cryptosporidium spp.* and *Giardia lamblia*, are major causes of diarrhea in children (Osman *et al*., 2016).

2.2.3 Transmission of intestinal protozoa:

Intestinal protozoa are typically transmitted through the fecal-oral route indirectly by ingestion of contaminated food, water, soil, or fomites. Direct transmission via person-to-person or animal-to-person contact is also possible for several species (Seguí *et al*., 2018).

2.2.4 Non-pathogenic intestinal protozoa:

Non-pathogenic intestinal protozoa are single-celled parasites commonly found in the intestinal tract but never associated with illness. They do not harm the body even in people with weak immune systems. Symptomatic people who are found to have these protozoa in their stool should be examined for other causes of their symptoms (CDC, 2012). The non-pathogenic protozoa can be divided into two groups: amoebae and flagellates, commonly range in length between 10 to 52 micrometers (Issa, 2014). The non-pathogenic intestinal protozoa include: *Chilomastix mesnili*, *Endolimax nana*, *Entamoeba coli*, *Entamoeba dispar*, *Entamoeba hartmanni*, *Entamoeba polecki*, *Iodamoeba buetschlii* (CDC, 2012).

2.2.4.1 Life cycle:

Entamoeba coli, *E. hartmanni*, *E. polecki*, *Endolimax nana*, and *Iodamoeba buetschlii* are generally considered non-pathogenic and reside in the large intestine of the human host. Both cysts and trophozoites of these species are passed in stool and considered diagnostic. Cysts are typically found in formed stool, whereas trophozoites are typically found in diarrheal stool. Colonization of the non-pathogenic amoebae occurs after ingestion of mature cysts in fecally-contaminated food, water, or fomites. 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 cell walls, the cysts can survive days to weeks in the external environment and are responsible for transmission. Trophozoites passed in the stool are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment (CDC, 2015) (figure 2.1).

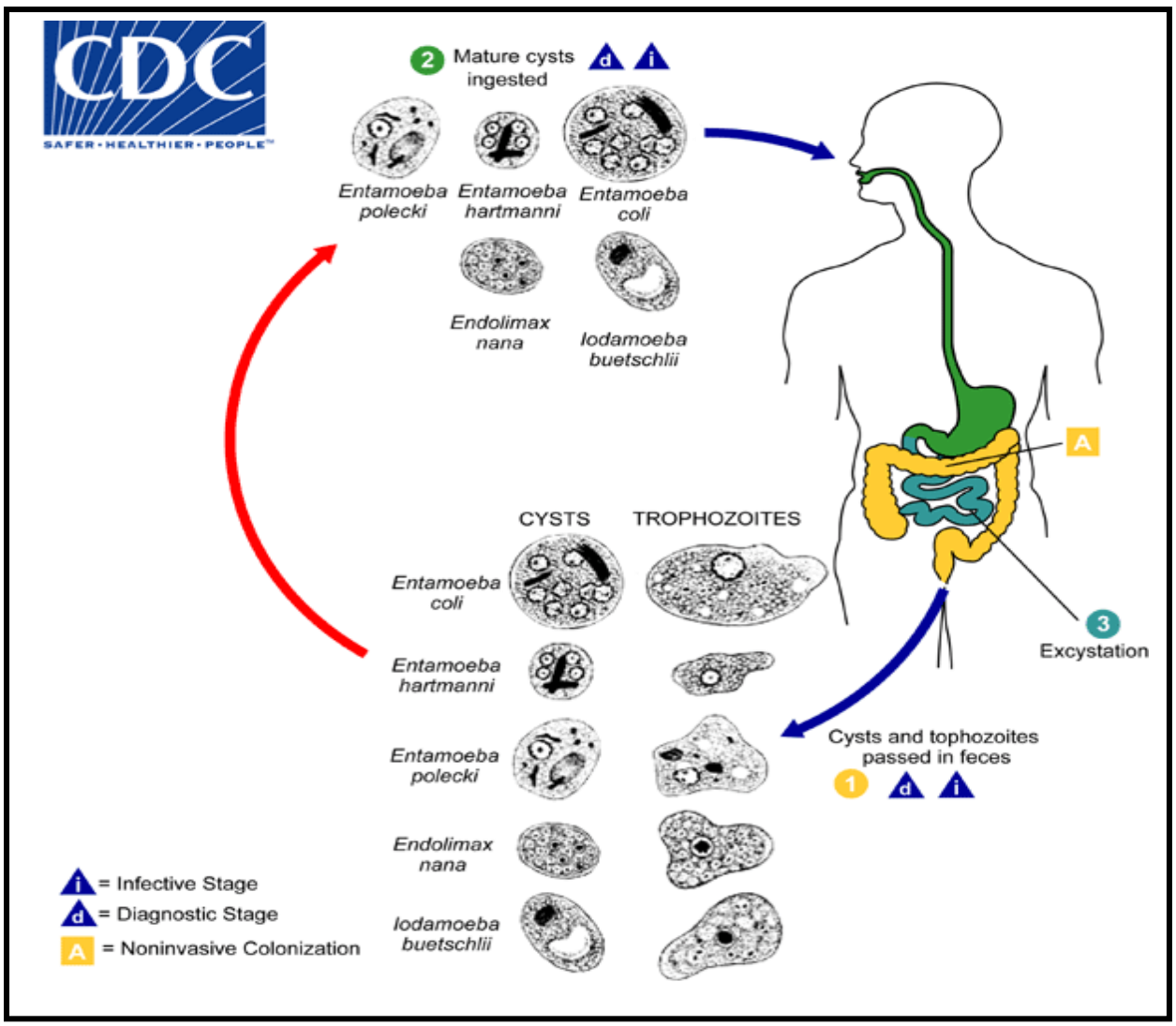

Figure (2.1): Life cycle of non pathogenic protozoa (CDC, 2015)

2.2.5 *Blastocystis hominis* infection:

Blastocystis is a unicellular, anaerobic, eukaryotic protest which lives in the intestinal tract of diverse hosts including humans. The organism exists in different

morphological forms with each form showing considerable variations in size. It is one of the frequently encountered parasites in human fecal samples in the developing countries. The organism was initially considered to be a commensal but later observations and studies strongly suggest it to be a pathogen (Parija and Jeremiah, 2013). The recognized forms of *Blastocystis spp*. are vacuolar, granular multi vacuolar, amoeboid and cystic forms. As other intestinal parasites transmission occurs by fecal oral route, although this has not been confirmed experimentally. Direct microscopic examination of fecal material, with or without addition of Lugol's iodine solution, had been suggested for diagnostic purposes. Permanent smear stained with trichrome had also been recommended for the diagnosis of *Blastocystis* spp. infection. Techniques for concentration using formalin-ether may be suitable because preservative liquids are used for storage and dilution of the feces (Elghareeb *et al*., 2015).

2.2.6 Pathogenic intestinal protozoa:

Infection with pathogenic intestinal protozoa (e.g *Entamoeba histolytica* and *Giardia intestinalis*) result in considerable gastrointestinal morbidity, malnutrition and mortality worldwide, particularly among young children in developing countries (Speich *et al.*, 2013).

2.2.6.1 Some species of pathogenic intestinal protozoa:

2.2.6.1.1 *Entamoeba histolytica:*

2.2.6.1.1.1 Epidemiology:

Entamoeba histolytica is a widely distributed parasitic protozoa and the major cause of morbidity and mortality in developing countries. Amoebiasis is a disease caused by *E. histolytica* (Arredondo *et al*., 2014). The prevalence of amoebiasis in underdeveloped countries reflects the lack of adequate sanitary systems, amoebas are found in all climates, arctic to tropical. Symptomatic infections (amoebic disease) are far more prevalent in certain geographic foci, and this uneven

prevalence of disease, as opposed to infection, is now explained by the variable geographic predominance of pathogenic zymodemes. Similar environments thus are likely to have a comparable infection rate but may have a widely different disease prevalence (Sodeman, 1996).

2.2.6.1.1.2 Transmission:

The fecal-oral transmission of the amoeba usually involves contaminated food or water. The parasite can also be transmitted directly by ano-genital or oro-anal sexual contact. Fecal-oral transmission occurs when food preparation is not sanitary or when drinking water is contaminated. Contamination may come directly from infected food handlers or indirectly from faulty sewage disposal (Sodeman, 1996).

2.2.6.1.1.3 Life cycle:

The infection is acquired when cysts are ingested. The factors contributing to infection are the similar to other organisms transmitted by the fecal oral-route. Excystation takes place in the intestines after passing through the stomach. A trophozoite emerges through the disrupted cyst wall and begins to replicate by binary fission. This trophic period occurs on the mucosa of the large intestine. Some of the trophozoites will not replicate and undergo encystation leading to the production of cysts. Up to 45 million cysts can be passed per day in the feces of an infected person (Brooks *et al*., 2006) (figure 2.2).

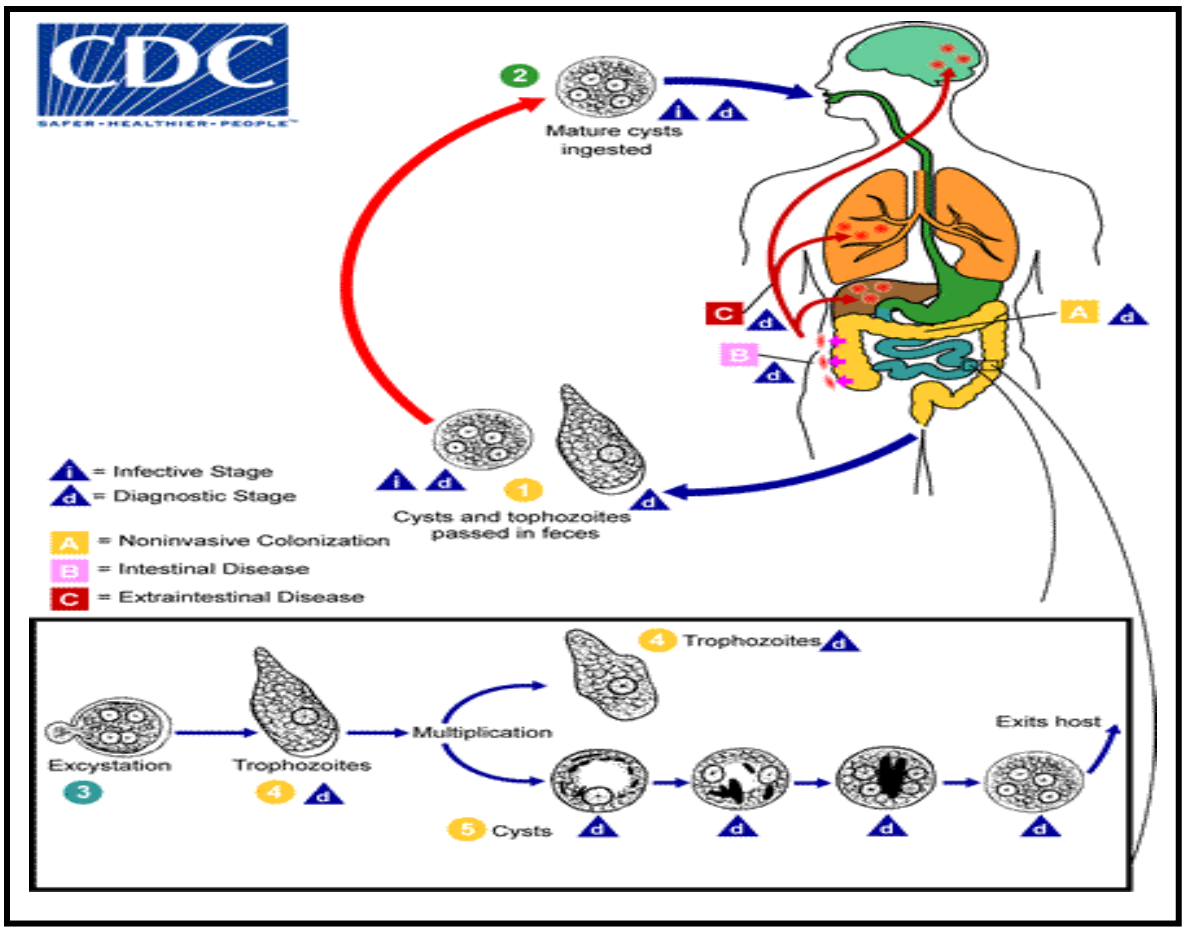

Figure (2.2): Life cycle of *Entamoeba histolytica* (CDC, 2017a)

2.2.6.1.1.4 Pathogenesis:

E. histolytica is a facultative pathogen. Normally *E. histolytica* lives in the human large intestine and feeds on the bacterial fauna. During this stage persons are often asymptomatic or exhibit symptoms such as a non-dysenteric diarrhea, cramps, or abdominal discomfort. Many individuals will clear the infection spontaneously in 6-12 months. The parasite can also penetrate the intestinal mucosa and epithelial cells and cause severe disease (Brooks *et al*., 2006). The initial stage of invasive disease is an ulceration of the colon. The trophozoites begin to ingest host cells instead of bacteria. The ingestion of host cells is indicated by the presence of trophozoites containing erythrocytes, or hematophagous amoeba. During this phase

the patient may exhibit dysentery and the feces may contain hematophagous trophozoites. The trophozoites destroy and ingest host cells leading to ulcer enlargement below the epithelial layer producing a characteristic flask-shaped ulcer. Peritonitis will result if the ulcer spans the colon wall (Brooks *et al*., 2006). Occasionally a tumor-like mass, known as an amoeboma, will form in the intestinal wall. This severe pathogenesis is not advantageous for the parasite, since cysts are no longer produced after the amoeba becomes invasive. The amoeba can also become extra-intestinal and metastasize to other tissues with the liver being the most commonly affect organ. The lesions in the intestines and liver can also expand by a direct extension to the skin or lungs. Extra-intestinal amoebiasis is a relentless and progressive disease which will result in death if untreated (Brooks *et al*., 2006).

2.2.6.1.1.5 Laboratory diagnosis:

Definitive diagnosis of amoebiasis requires the demonstration of *E.histolytica* cysts or trophozoites in feces or tissues. Stool specimens should be preserved and stained and microscopically examined. Cysts will tend to predominate in formed stools and trophozoites in diarrheic stools (Brooks *et al*., 2006). Fresh stools can also be immediately examined for motile trophozoites which exhibit a progressive motility. Sigmoidoscopy may reveal the characteristic ulcers, especially in more severe disease. Aspirates or biopsies can also be examined microscopically for trophozoites. *E.histolytica* and *E. dispar* cannot be distinguished on morphological criteria. Antigen detection kits are available for the positive identification of these species. Serology is especially useful for the diagnosis of extra-intestinal amoebiasis (Brooks *et al*., 2006). Greater than 90% of patients with invasive colitis and liver abscesses exhibit serum antibodies against *E. histolytica*. However, the antibodies can persist and distinguishing past and current infections may pose problems in endemic areas. Non-invasive imaging techniques (e.g. ultrasound) can

be used to detect hepatic abscesses (Brooks *et al*., 2006). Amoebas may be cultured from the stool. However, because the techniques involved are somewhat more cumbersome than those routinely used for bacterial organisms, culturing is not widely used as a diagnostic tool. It is essential for virulence testing (Sodeman, 1996).

2.2.6.1.1.6 Treatment:

Several drugs are available for treatment of amoebiasis and the choice of drug (s) depends on the clinical stage of the infection. The prognosis following treatment is generally good in uncomplicated cases. In cases where *E. histolytica* is confirmed or the species (i.e. *dispar* or *histolytica*) are unknown, asymptomatic cyst passers should be treated to prevent the progression to severe disease and to control the spread of the disease. The standard practice is to only treat symptomatic cases. Metronidazole, or tinidazole (if available), is recommended for all symptomatic infections. This treatment should be followed by or combined with luminal anti-amoebic drugs, such as iodoquinol, paromomycin, or diloxanide furoate, to eliminate the cysts (Brooks *et al*., 2006).

2.2.6.1.1.7 Prevention and control:

The basic approach to preventing amoebic infection is by improvement of living conditions and education in countries where invasive amoebiasis is prevalent. Specifically, methods of attack are aimed at improved environmental sanitation including water supply and food safety. Fecal-oral transmission via hands or food is very common (Davis and Pawlowski, 1985). Therefore, sanitation and personal hygiene have priority in the prevention and control of amoebiasis. The availability of sufficient water for washing hands and food may be more important than the quality of the water alone. Health education on amoebiasis should form part of the general education programmers for controlling infections transmitted by the fecal

oral route, which should be addressed to mothers, school children, and persons with influence in the community (Davis and Pawlowski, 1985).

2.2.6.1.2 *Giardia lamblia:*

2.2.6.1.2.1 Epidemiology:

Giardia lamblia is a protozoan parasite of the small intestine. It has a worldwide distribution. However, the prevalence is higher in tropical and developing countries. It is the most common protozoan isolated from human stools. The incidence is estimated at 200 million cases per year. *Giardia* is non-invasive and often results in an asymptomatic infection. Symptomatic giardiasis is typically characterized by acute diarrhea (Brooks *et al*., 2006).

2.2.6.1.2.2 Routes of transmission:

Fecal-oral transfer of *Giardia* cysts is the major route of transmission of giardiasis as indicated by the high prevalence in developing countries with poor standards of hygiene and sanitation, in day-care centers and nurseries and by secondary spread within the household of those who attend day-care centers. Food borne outbreaks are the result of contamination of food by infected workers or household members (Fricker *et al*., 2002).

2.2.6.1.2.3 Life cycle:

Giardia exhibits a typical fecal-oral transmission cycle and infection is acquired by ingesting cysts. Following passage through the stomach, the trophozoite emerges from the cyst. Trophozoites reside in the upper portions of the small intestine and reproduce by binary fission. On the ventral side of the trophozoite is a concave structure called the adhesive disc. The adhesive disc functions in attachment of the trophozoite to the intestinal epithelial cells. Some of the trophozoites will undergo an encystations process which results in the detachment from the intestinal epithelial and the maturation into cysts which are passed in the feces (Brooks *et al*., 2006) (figure 2.3).

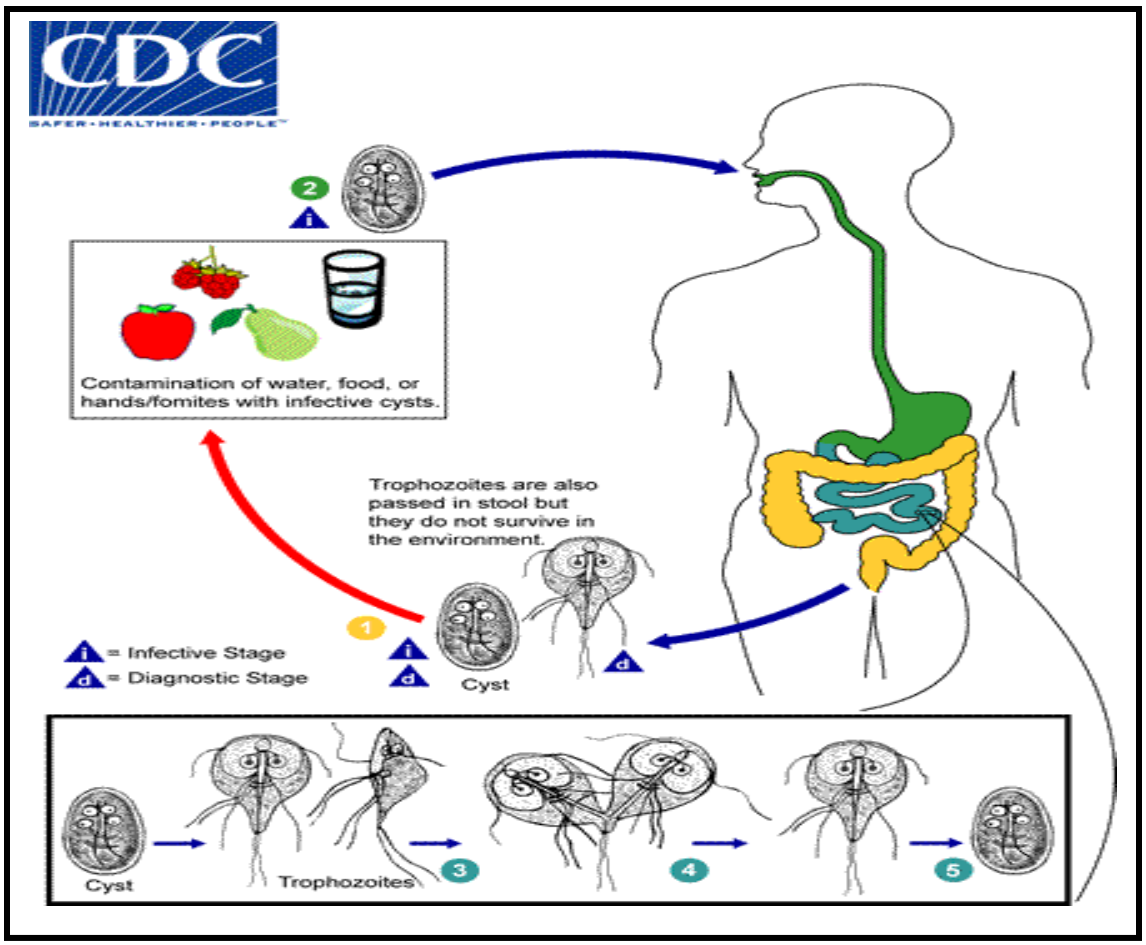

Figure (2.3): Life cycle of *Giardia lamblia* (CDC, 2017b)

2.2.6.1.2.4 Pathogenesis:

Symptoms associated with giardiasis range from asymptomatic to acute gastrointestinal manifestations. Generally the symptoms are more severe the first time a person experiences giardiasis and children are at the greatest risk for contracting clinical giardiasis. In the majority of untreated patients the infection resolves spontaneously but it can become chronic and last for several months or even years in rare cases (Brooks *et al*., 2006). The acute stage usually resolves spontaneously in 3-4 days and is often not recognized as giardiasis. Occasionally an acute infection will persists leading to mal-absorption, steatorrhea, debility and weight loss. Acute infections can also develop into long-standing sub-acute or chronic infections characterized by recurrent brief episodes of gastrointestinal

symptoms. Anorexia accompanied by marked weight loss is sometimes associated with chronic infections (Brooks *et al*., 2006). The disease manifestations appear to be related to mal-absorption particularly of fat and carbohydrates. *Giardia* infection can also lead to lactase deficiency, as well as other enzyme deficiencies in the microvilli. This reduce digestion and absorption of solutes may contribute to an osmotic diarrhea (Brooks *et al*., 2006). The mechanism by which *Giardia* causes diarrhoea and mal-absorption is still unclear (Fricker *et al*., 2002).

2.2.6.1.2.5 Laboratory diagnosis:

Giardiasis can be diagnosed by direct observation of the trophozoites or cysts in the feces. Either stained preparations (e.g. preserved with polyvinyl alcohol or 10% formalin) or unstained wet mounts can be used. Because they are small and can resemble other fecal components, *Giardia* cysts and trophozoites can sometimes be difficult to identify by morphology alone (Fever, 2012). Diagnosis is confirmed by finding cysts or trophozoites in feces or in duodenojejunal aspirates or biopsies. Diagnosis can also be made by examining duodenal fluid for trophozoites. Duodenal fluid is obtained by either intubation or the entero test (also called string test). The entero test consists of a gelatin capsule containing a nylon string of the appropriate length. The free end of the string is taped to the patient's face and the capsule is swallowed. After four hours the string is retrieved and the bile-stained mucus on the distal portion of the string is scraped off and examined by both wet mount and permanent staining (Brooks *et al*., 2006). Also, giardiasis can be diagnosed by enzyme-linked immunosorbent assays (ELISAs) and immune chromatographic tests to detect *G. duodenalis* antigens in the feces, as well as by direct-immunofluorescence. Polymerase chain reaction (PCR) assays can detect *Giardia* in clinical samples. Genetic characterization of isolates at the assemblage level is usually employed only in epidemiological studies and research. *Giardia* can be cultured in vitro, but this technique is used only in research (Fever, 2012).

Microscopy of direct fecal smears or smears prepared following formal ether concentration and iodine staining has been reported to reach 97% sensitivity if three stool samples are examined. The string test, duodenal aspirate, intestinal impression smear and intestinal biopsy have all been proposed as techniques to improve microscopic diagnosis (Behr *et al*., 1997).

2.2.6.1.2.6 Treatment:

Giardiasis can be treated with a number of drugs, such as nitroimidazole derivatives, benzimidazole compounds or acridine dyes. Metronidazole or tinidazole are used most often in humans, but other drugs (e.g. furazolidone or paromomycin) may be recommended in some cases. Supportive care, such as fluid and electrolyte management, may also be necessary. Symptoms can recur for a variety of reasons, such as drug resistant organisms, re-infection or post-*Giardia* lactose intolerance. In some cases, a lactose-free diet may be needed for several months. Asymptomatic carriers do not usually need treatment, but they may be treated to reduce transmission of the organism (Fever, 2012).

2.2.6.1.2.7 Prevention and control:

Good hygiene, such as hand washing, reduces the risk of acquiring giardiasis or transmitting it to others. Improve personal hygiene as well as treatment of infected patients to avoid transmission to family member. Vegetables and fruits should be washed before eating them. Drinking water treatment reduce the number of *Giardia* using conventional water treatment processes (e.g. filtration), followed by chemical or physical disinfection. *Giardia* cysts are very resistant to chlorine disinfection, which is commonly used to treat surface and ground waters. Alternative water-sanitizing techniques that have proven successful for the disinfection of *Giardia* include the use of ozone and ultra-violet (UV) light as disinfectants (Fever, 2012).

2.3 Intestinal helminthes:

2.3.1 Classification:

The helminthes are worm-like parasites. The clinically relevant groups are separated according to their general external shape and the host organ they inhabit. There are both hermaphroditic and bi-sexual species. The definitive classification is based on the external and internal morphology of egg, larval, and adult stages (Castro, 1996).

2.3.1.1 Tape worms (cestodes):

Adult tape worms are elongated, segmented, hermaphroditic flat worms that inhabit the intestinal lumen. Larval forms, which are cystic or solid, inhabit extra-intestinal tissues (Castro, 1996).

2.3.1.2 Flukes (trematodes):

Adult flukes are leaf-shaped flat worms. Prominent oral and ventral suckers help maintain position in situ. Flukes are hermaphroditic except for blood flukes, which are bi-sexual. The life-cycle includes a snail intermediate host (Castro, 1996).

2.3.1.3 Round worms (nematodes):

Adult and larval round worms are bi-sexual, cylindrical worms. They inhabit intestinal and extra-intestinal sites (Castro, 1996).

2.3.2 Epidemiology:

Intestinal helminthes infections are among the most common infections occurring throughout the developing world (Ekpenyong and Eyo, 2017). Soil-transmitted helminthes infections (STH) and schistosomiasis are among the most common infections worldwide (Abdi *et al*., 2017).

2.3.3 Routes of transmission:

Helminthes are transmitted to humans in many different ways. The mode of transmission varies with the type of worm; the simplest is by accidental ingestion of infective eggs (*Ascaris*, *Echinococcus*, *Enterobius*, *Trichuris*) or larvae (some

hook worms). Other worms have larvae that actively penetrate the skin (hook worms, *Schistosoma*, *Strongyloides*). In several cases infection requires an intermediate host vector and ingestion of infective stages in the meat of intermediate hosts (Wakelin, 1996).

2.3.4 Some species of intestinal helminthes:

2.3.4.1 *Hymenolepis nana:*

2.3.4.1.1 Epidemiology:

Hymenolepis nana is a cestode parasite commonly known as dwarf tape worm. It is found throughout the world, more frequently in warm climate and temperate zones commonly infects both rodents and human beings with school-aged children being more frequently infected. Light *H. nana* infections are usually asymptomatic (Alruzug *et al*., 2016).

2.3.4.1.2 Transmission:

The parasite is transmitted from person to person mainly by the fecal-oral route without an intermediate host. The tape worm form has a life span of 4-6 weeks. However, the parasites' eggs are infectious when shed and can re-infect the host leading to long-lasting infections (Cabada *et al*., 2016).

2.3.4.1.3 Life cycle:

Eggs of *Hymenolepis nana* are at once infective when passed with the stool and cannot survive more than 10 days in the external environment. When eggs are ingested by an arthropod intermediate host, they develop into cysticercoids, which can infect humans or rodents upon ingestion and develop into adults in the small intestine. When eggs are ingested (in contaminated food or water or from hands contaminated with feces), the oncosphere contained in the eggs are released (Sadaf *et al*., 2013). The oncosphere (hexacanth larvae) penetrate the intestinal villous and develop into cysticercoids larvae. Upon rupture of the villous, the cysticercoids return to the intestinal lumen, evaginate their scoleces, attach to the intestinal

mucosa and develop into adults that reside in the ilea portion of the small intestine producing gravid proglottids. Eggs are passed in the stool when released from proglottids through its genital atrium or when proglottids disintegrate in the small intestine (Sadaf *et al.*, 2013). An alternate mode of infection consists of internal autoinfection, where the eggs release their hexacanth embryo, which penetrates the villous continuing the infective cycle without passage through the external environment. The life span of adult worms is 4 to 6 weeks, but internal autoinfection allows the infection to persist for years (Sadaf *et al.*, 2013) (figure 2.4).

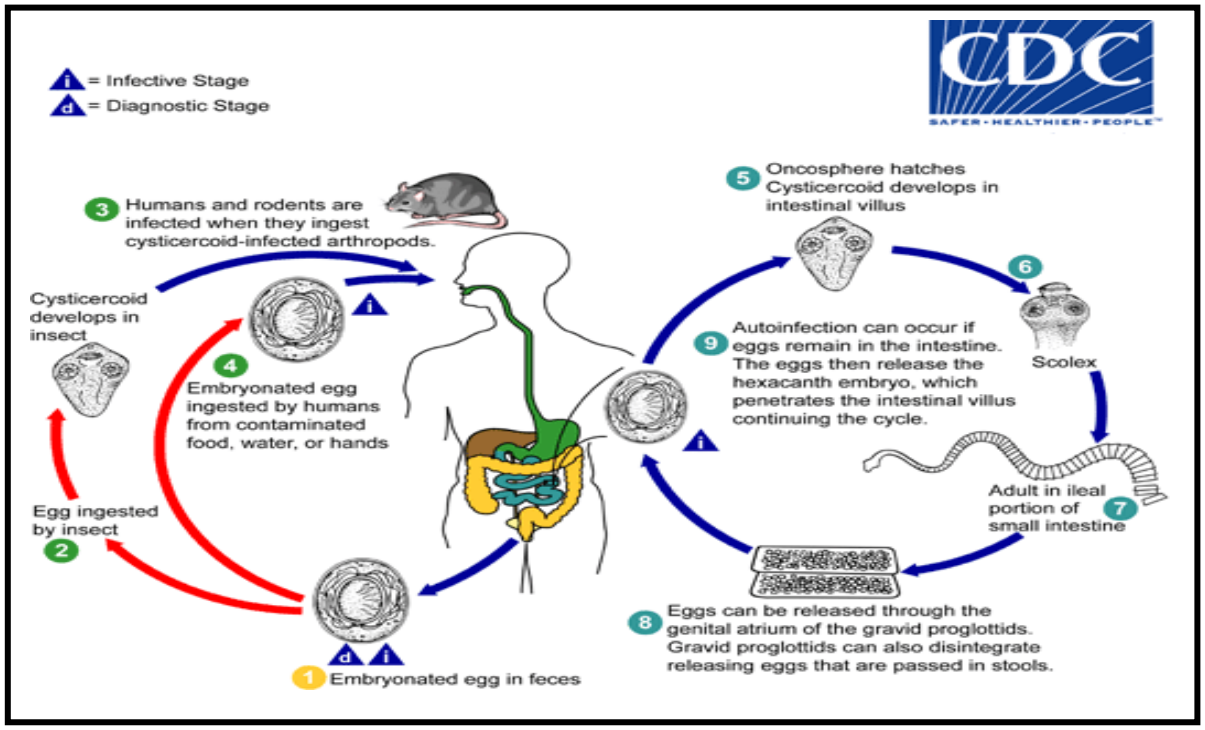

Figure (2.4): Life cycle of *Hymenolepis nana* (CDC, 2017c)

2.3.4.1.4 Pathogenesis:

Hymenolepis nana infection is most often asymptomatic. Heavy infections with *H. nana* can cause weakness, headaches, anorexia, irritability, abdominal pain, itching around the anus and diarrhea. Hymenolepiasis is usually asymptomatic in adults.

But prolonged infection or multiple tape worms especially in children can cause more severe symptoms. In symptomatic patients, the symptoms were mild and non-specific such as pruritus ani, abdominal pain, diarrhea, anorexia, headache and dizziness (Sadaf *et al*., 2013).

2.3.4.1.5 Laboratory diagnosis:

The diagnosis of *H. nana* depends on recovery and identification of the characteristic eggs in stool specimens. Concentration techniques and repeated examinations will increase the probability of detecting light infections. *H. nana* eggs are frequently spherical or ovoid with a thin hyaline shell and measure 30-47 μm in diameter. The oncosphere with its 3 pairs of hooklets lies in the center of the egg and is separated from the outer shell by sizeable space. The oncosphere has an internal membrane with polar thickenings from which arise 4 to 8 filaments. The oncosphere has six hooks. Adult worms and proglottids are rarely seen in stool samples (Sadaf *et al*., 2013).

2.3.4.1.6 Treatment:

Praziquantel or niclosamide are the drugs most frequently used to treat *H. nana* infection. *H. nana* cysticercoids are not as susceptible praziquantel in a single oral dose of 25 mg/kg body weight was effective and well tolerated in *H. nana* infected individuals. Niclosamide or albendazole has also been used (Sadaf *et al*., 2013).

2.3.4.1.7 Prevention and control:

To prevent getting infection, good hygienic condition must be applied, wash, peel or cook all fruits and vegetables. Wash hands with water and soap after using the toilet and before preparing food or eating. Quit the habit of putting fingers in your nose and mouth (Sadaf *et al*., 2013).

2.3.4.2 *Enterobius vermicularis*:

2.3.4.2.1 Epidemiology:

Enterobiasis (thread worm, or pin worm disease) is caused by the small nematode *Enterobius vermicularis*. It is probably the most common helminthes to infect humans. Infection is most common in young school children (5-10 years) living in overcrowded conditions (Cook, 1994).

2.3.4.2.2 Transmission:

Direct infection from the anal and peri-anal regions by fingernail contamination (autoinfection), and soiled night clothes, exposure to viable eggs on soiled bed and other contaminated environmental objects, by contaminated dust containing embryonated eggs and retro-infection after hatching on the anal mucosa, larvae migrate into the sigmoid colon and caecum (Cook, 1994).

2.3.4.2.3 Life cycle:

The life cycle takes place within the lumen of the gastrointestinal tract no visceral component exists as with hook worm and *Ascaris lumbricoides*. After ingestion, eggs hatch in the stomach and upper small intestine; larvae migrate to the ileum, caecum, and appendix. After moulting twice en route they become adults. Infected patients harbor few to several hundred adults. Adult females settle in the lower ileum (where copulation occurs), caecum, appendix or ascending colon; minute ulcerations form at the site (s) of attachment. Females survive for 37-93 days and males about 50 days, oviposition begins at five weeks. When the uteri are loaded with eggs, the gravid worm migrates from the colon, through the anus; while traversing the peri-anal skin, eggs are expelled by uterine contraction (Cook, 1994) (figure 2.5).

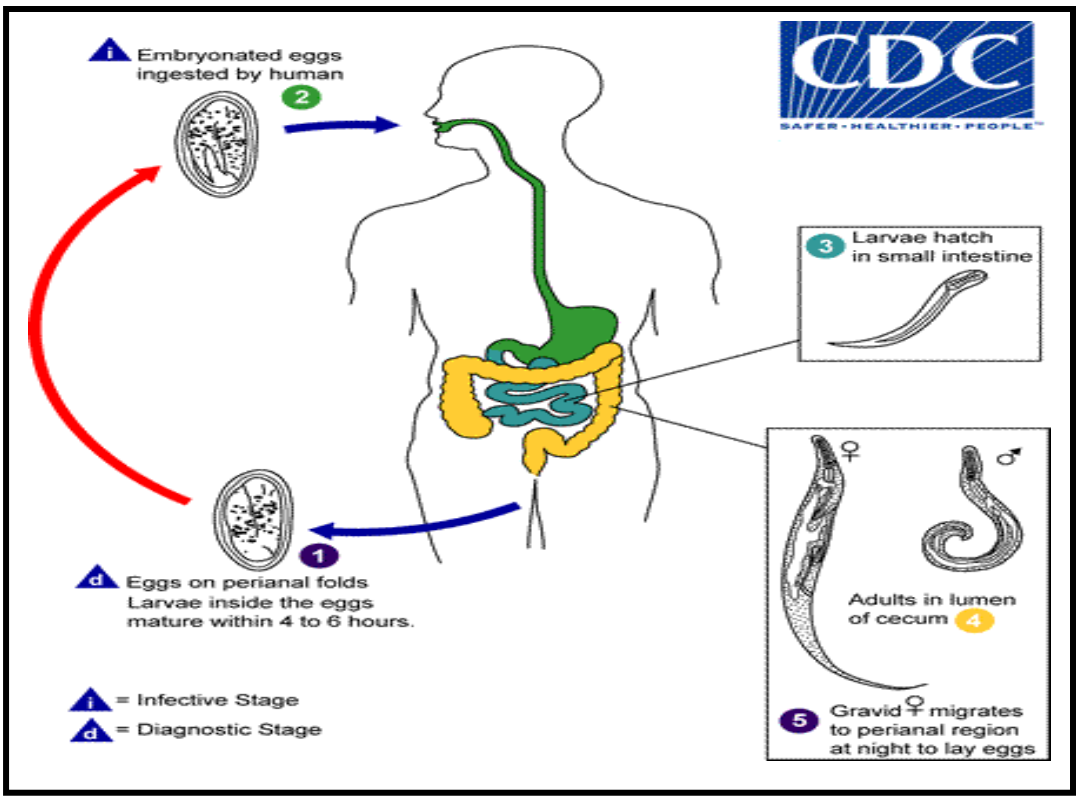

Figure (2.5): Life cycle of *Enterobius vermicularis* (CDC, 2017d)

2.3.4.2.4 Pathogenesis:

Adult worms migrate from the female genital tract to the peritoneum; they can also enter the peritoneal cavity through a perforated bowel wall, for example appendicitis, diverticulitis, or intestinal malignancy. Haematogenous spread is unproved. Dead worms and eggs surrounded by a granulomatous reaction (lymphocytes and a few eosinophils predominate) have been shown in high vagina cervix, endometrium, fallopian tubes, ovary, and peritoneum. Direct migration has been recorded after abdominal operation. Eosinophilic granulomas of the colon and omentum have been described. It has also been detected in a peri-anal abscess. Sites of ectopic infection include: the liver, spleen, kidney and lung (Cook, 1994).

2.3.4.2.5 Laboratory diagnosis:

Diagnosis is dependent on accurate identification of adult worms or eggs, or both which can be visualized in the peri-anal region (or less often vagina), usually at night. Application of adhesive tape to the anus is of value; when adherent (sticky side downwards) to a microscope slide, visualization of worms and eggs is straight forward (debris is cleared with a drop of toluene). The procedure is best carried out shortly after waking before defecation or bathing (Cook, 1994). Eggs may also be detected in peri-anal scrapings or swabs, or from beneath the fingernails. Routine examination of a fecal sample gives a positive diagnosis in 5-15% of infected subjects. Fecal obtained at rectal examination occasionally gives a positive result after mixing with normal saline, the specimen is examined under a cover slip. In a heavy infection, female worms may be adherent to a fecal bolus. Adult worms are occasionally visualized during colonoscopy (Cook, 1994).

2.3.4.2.6 Treatment:

The benzimidazole compounds (which inhibit microtubule function in the adult and cause glycogen depletion) are most effective. Single dose mebendazole is usually effective a repeat dose one week later is often recommended (Cook, 1994).

2.3.4.2.7 Prevention and control:

Re-infection must be prevented during treatment. Careful hand washes and finger (nails must be kept short) scrubbing after defecation and before meals is essential. Ideally, bed covers, sleeping garments, and hand towels should be changed daily and the bedroom floor kept clean. Children should wear gloves while asleep. Food should be covered to limit contamination with dust borne eggs (Cook, 1994).

2.4 Immunity to intestinal parasites:

Two major subsets of T cells, the helper (Th) phenotype and the cytotoxic/ suppressor cell (T) have been identified in most mammals, when activated; secrete a battery of regulatory glycoprotein known as lymphokines or cytokines (Miller,

1990). These regulate both the immune response and the inflammatory process cytokines, when bound to specific cell surface receptors, modulate the growth differentiation or function of the receptor-bearing cells, many of which are derived from bone marrow and serve inflammatory functions. Studies in rats have shown that Th-cell derived interluekine-3 (IL-3) regulates the growth and differentiation of intestinal mucosal mast cells (Miller, 1990). Mucosal mast cells are activated during the spontaneous expulsion of nematode parasites from the intestine. Eosinophil differentiation and recruitment are also regulated by Th-cells and are consistently associated with helminthes infection. There are many *in vitro* studies to show that eosinophils secrete granule products which are highly toxic to helminthes parasites (Miller, 1990). The type of inflammation operating against helminthes depends on the tissue localization of the parasite. The parasite itself can develop mechanisms of avoiding or suppressing the immunological response of the host. Evasion of the immune response by nematodes may be related to the ability of the parasite to produce suppress or molecules which down-regulate the inflammatory response (Miller, 1990).

2.5 Intestinal parasitic infections in Sudan:

Various studies were conducted to determine the prevalence and associated risk factors of intestinal parasitic infection. Previous epidemiological studies focused on the distributions of intestinal parasites in different community groups, such as school children, or different areas (Muhajir *et al*., 2017). A study conducted among school students in Malakal City, Upper Nile State, South Sudan by Kardaman *et al*. (2016) who concluded that *G.intestinalis* and *H.nana* were the most predominant parasites observed in school children in Malakal city and formalin-ethyl acetate concentration method was recommended for the diagnosis. In addition to a study conducted in Al-Kalakla, Khartoum State by Muhajir *et al*. (2017) who reported that the overall prevalence rate of intestinal parasitic infections among children

was 30% and various types of parasites were detected included *Entamoeba histolytica* (15.5%), *Giardia lamblia* (12.5%), *Hymenolepis nana* (1.5%) and *Shistosoma mansoni* (0.5%). Other study in Mayo area, Khartoum State conducted by Elfaki *et al*. (2015) who showed that the prevalence rate of intestinal parasitic infections was (24%) and (59%) when using wet preparation and formal ether concentration technique respectively.

CHAPTER THREE

Materials and Methods

Chapter 3

Materials and methods

3.1 Study design:

It is a cross-sectional study.

3.2 Study area:

The study was conducted in Shendi city in Northern Sudan- River Nile state, which is situated on the east bank of the River Nile, 150 kilometers north east of Khartoum and 45 kilometers south west of the ancient city of Meroe (Wikipedia, 2019).

3.3 Study duration:

The study was carried out in the period from March 2018 to March 2019.

3.4 Study population:

The study was carried out on children who were categorized according to gender and age groups.

3.5 Sample size:

The sample size was obtained according to the equation described by Open Epi (2003) as follow:-

$$Ss = \frac{Z^2 * (p) * (1-p)}{C^2}$$

Ss= Sample size.

Z= Z value (1.65 for 90% confidence level).

P= the prevalence rate of occurrence of intestinal parasitic infections (50%).

C= degree of precision (0.05).

According to the above formula, the study was conducted on 272 children in Shendi city.

3.6 Sampling:

Two hundred and seventy two fecal samples were collected from all children under the study in Shendi residential neighborhoods. Collections were taken randomly by using simple random sampling method. Fecal samples were transported to the laboratory of parasitology department- College of Medical Laboratory Sciences and examined by wet preparation, formal ether concentration technique and zinc sulphate floatation technique for general parasite cyst, trophozoites and helminthes eggs.

3.7 Data collection:

The primary data were collected by using self-administrated per-coded questionnaire (appendix) which was specifically designed to obtain information that helped in the study.

3.8 Methods:

3.8.1 Wet preparation:

A drop of normal saline was placed on the middle of the slide by using Pasteur pipette. With wooden stick, small portion of fecal sample was emulsified in the saline drop, then covered with cover glass and examined under microscope by using x10 objective for detection and x40 objective for identification (Elfaki *et al*., 2015).

3.8.2 Formal ether concentration technique (FECT):

About 1 gram of fecal sample was estimated and emulsified in 4 ml of 10% formal saline in a screw-cap bottle or tube. Further 3-4 ml of 10% formal saline were added. Mixed and shacked well then sieved in a beaker. The suspension was transferred to a conical (centrifuge) tube and 3-4 ml of diethyl ether were added. Mixed and centrifuged at 3000 rpm for 1 minute. By using a plastic bulb pipette the layer of fecal debris was loosened from the site of the tube and the tube was inverted to discharge the supernatant. The sediment was transferred to a slide then

covered with a cover glass and examined under microscope using x10 and x40 objectives (Cheesbrough, 2006).

3.8.3 Zinc sulphate floatation technique:

Zinc sulphate solution was added up to one quarter of tube which was placed in vertical position. About 0.5g of feces were added using applicator stick and emulsified in solution and then the tube was filled with the same solution until convex shape was formed. The tube covered by clean cover glass, and left to stand for about 30-45 minutes so as to leave cyst and egg to float. After that, the cover glass was taken and placed in a clean slide and examined under microscope using x10 and x40 objectives (Cheesbrough, 2006).

3.9 Data analysis:

The data obtained were analyzed using the computerized program of statistical package for social sciences (SPSS) version 18. Frequencies mean and Chi-squire test were used. Then data were presented in tables.

3.10 Sensitivity and specificity:

Sensitivity and specificity were calculated by Zhu *et al*. (2010) as follow:-

Sensitivity= TP/ (TP+FN) ×100%

Specificity=TN/ (TN+FP) ×100%

TP= True positive

TN= False negative

3.11 Ethical consideration:

Approval of the study was taken from the College of Medical Laboratory Science – Sudan University of Science and Technology. Permission for the samples collection from the children was taken from their guardian before being included in the study.

CHAPTER FOUR

Results

Chapter 4

Results

4.1 General characteristics of study population:

The study was conducted on 272 children in Shendi city, 137 (50.4%) were males and 135 (49.6%) were females (table 4.1). The age ranged between <1-14 years old with a mean age was 6 ± 3 years old. The age was divided into 3 groups as follow: ≤ 4, 5-9 and 10-14 years old. The frequency of each age group was 119 (43.8%), 100 (36.7%), 53 (19.5%) respectively (table 4.2).

Table (4.1): Frequency of study subjects according to gender

Gender	Frequency	Percentage (%)
Males	137	50.4%
Females	135	49.6%
Total	272	100.0%

Table (4.2): Frequency of study subjects according to age groups

Age groups (years)	Frequency	Percentage (%)
≤ 4	119	43.8%
5-9	100	36.7%
10-14	53	19.5%
Total	272	100.0%

4.2 Parasitological results:

4.2.1 The overall prevalence of intestinal parasitic infections by using wet preparation, formal ether concentration technique and zinc sulphate floatation technique:

Out of 272 fecal samples, 59 (21.6%), 75 (27.5%) and 54 (20.0%) were positive for intestinal parasitic infections by using wet preparation; formal ether concentration technique and zinc sulphate floatation technique respectively (table 4.3).

Table (4.3): The overall prevalence of intestinal parasitic infections by using wet preparation, formal ether concentration technique and zinc sulphate floatation technique

Technique	No. of sample examined	Positive (%)
Wet preparation	272	59 (21.6%)
Formal ether concentration technique	272	75 (27.5%)
Zinc sulphate floatation technique	272	54 (20.0%)

4.2.2 Distribution of intestinal parasitic infections among children according to the parasite species by using formal ether concentration technique:

Out of 272 fecal samples, 73 (26.8%) were protozoa and 2 (0.7%) were helminthes. The study found that, highest prevalence rate (18.8%) of single parasitic infection was *Giardia lamblia* followed by *Entamoeba histolytica* (1.1%). While the lowest prevalence rate (0.4%) for *Enterobius vermicularis* and the 0.4% rate for *Hymenolepis nana*. Prevalence rate of single commensals parasites was (5.5%). The highest prevalence rate (0.7%) of multiple parasitic infections (co-infection) was *E. histolytica*+ *E.coli*+ *Entamoeba hartmanni*

followed by the 0.4% rate for G. *lamblia+ E.coli+ Iodamoeba butschlii* and the 0.4% rate for *G. lamblia+ E. histolytica* (table 4.4).

Table (4.4): Distribution of intestinal parasitic infections among children according to the parasite species by using formal ether concentration technique

Single infection	No. of sample examined	Positive (%)
G. lamblia	272	51 (18.8%)
E. histolytica	272	3 (1.1%)
H. nana	272	1 (0.4%)
E. vermicularis	272	1 (0.4%)
Commensals parasites	272	15 (5.5%)
Multiple infections		
G. lamblia+ E. histolytica	272	1 (0.4%)
G.lamblia+ E.coli+ Iodamoeba butschlii	272	1 (0.4%)
E.histolytica+ E.coli+ Entamoeba hartmanni	272	2 (0.7%)

4.2.3 Distribution of intestinal parasitic infections among children according to the commensals species by using formal ether concentration technique:

Out of 18 commensals species, the study found that the highest prevalence rate (33.3%) of single commensals was *Entamoeba coli* followed by the rate (22.2%) for *Blastocystis hominis* and (16.6%) for *Entamoeba hartmanni*. The lowest prevalence rate was (5.6%) for *Endolimax nana* and *Iodamoeba butschlii*. Prevalence rate of multiple commensals was (11.1%) for *Entamoeba coli+ Entamoeba hartmanni* and was (5.6%) for *Entamoeba coli+ Iodamoeba butschlii* (table 4.5).

Table (4.5): Distribution of intestinal parasitic infections among children according to the commensals species by using formal ether concentration technique

Commensals	No. of sample examined	Positive (%)
Single		
Blastocystis hominis	18	4 (22.2%)
Entamoeba coli	18	6 (33.3%)
Entamoeba hartmanni	18	3 (16.6%)
Endolimax nana	18	1 (5.6 %)
Iodamoeba butschlii	18	1 (5.6 %)
Multiple		
Entamoeba coli+ Entamoeba hartmanni	18	2 (11.1%)
Entamoeba coli+ Iodamoeba butschlii	18	1 (5.6%)

4.2.4 Prevalence of intestinal parasitic infections among children according to gender by using formal ether concentration technique:

Out of 272 fecal samples, 75 were positive for intestinal parasitic infections, from them 40 (53.3%) were males and 35 (46.7%) were females. *Giardia lamblia* was the most prevalent 29 (38.7%) among males than females 22 (29.4%), while *Entamoeba histolytica* was 3 (4.0%) in females. *Enterobius vermicularis* and *Hymenolepis nana* were 1 (1.3%) in males. Commensals species were 8 (10.7%) in females and 7 (9.4%) in males. Prevalence rate of multiple parasitic infections was (1.3%) in females for *G. lamblia+ E.histolytica* and (1.3%) in males for G. *lamblia+ E.coli+ Iodamoeba butschlii. E.histolytica+ E.coli+ Entamoeba hartmanni* were (1.3%) in males and females. The difference in rate was found to be statistically insignificant at p. value= 0.357 (table 4.6).

Table (4.6): Prevalence of intestinal parasitic infections among children according to gender by using formal ether concentration technique

	Gender	
Parasite species	Males (Positive %)	Females (Positive %)
G. lamblia	29 (38.7%)	22 (29.4%)
E. histolytica	0 (0%)	3 (4.0 %)
H. nana	1 (1.3%)	0 (0%)
E. vermicularis	1 (1.3%)	0 (0%)
Commensals	7 (9.4%)	8 (10.7%)
G. lamblia+ E.histolytica	0 (0%)	1 (1.3%)
G. lamblia+ E.coli+ Iodamoeba butschlii	1 (1.3%)	0 (0%)
E.histolytica+ E.coli+ Entamoeba hartmanni	1 (1.3%)	1 (1.3%)
Total	40 (53.3%)	35 (46.7%)

P. value= 0.357

4.2.5 Prevalence of intestinal parasitic infections among children according to age groups by using formal ether concentration technique:

Out of 75 positive fecal samples, 28 (37.3%) were positive for age groups ≤ 4 and 5-9 years old, 19 (25.3%) were positive among age group 10-14 years old. *Giardia lamblia* was most prevalent 23 (30.7%) among age group ≤ 4 years old, followed by 19 (25.3%) among age group 5-9 years old and was lower 9 (12%) among age group 10-14 years old. *E. histolytica* was 1 (1.3%) in the age group 5-9 years old and 2 (2.7%) in the age group 10-14 years old. *H.nana* was 1 (1.3%) in the age group 10-14 years old, while *E.vermicularis* was 1 (1.3%) in the age group ≤ 4 years old. Commensals species were most prevalent 7 (9.3%) among age group 10-14 years old followed by 5 (6.8%) in age group 5-9 years old and 3 (4.0%) in age

group ≤ 4 years old. *G. lamblia+ E.histolytica* and *G. lamblia+ E.coli+ Iodamoeba butschlii* were found at rate (1.3%) in age group 5-9 years old, *E.histolytica+ E.coli + Entamoeba hartmanni* were found at rate (1.3%) in the age groups ≤ 4 and 5-9 years old. The difference in rate was found to be statistically insignificant at p. value= 0.216 (table 4.7).

Table (4.7): Prevalence of intestinal parasitic infections among children according to age groups by using formal ether concentration technique

	Age groups (years)		
Parasites species	≤ 4	5-9	10-14
G. lamblia	23 (30.7%)	19 (25.3%)	9 (12%)
E. histolytica	0 (0%)	1 (1.3%)	2 (2.7%)
H. nana	0 (0%)	0 (0%)	1 (1.3%)
E. vermicularis	1 (1.3%)	0 (0%)	0 (0%)
Commensals	3 (4.0%)	5 (6.8%)	7 (9.3%)
G. lamblia+ E.histolytica	0 (0%)	1 (1.3%)	0 (0%)
G. lamblia+ E.coli+ Iodamoeba butschlii	0 (0%)	1 (1.3%)	0 (0%)
E.histolytica+ E.coli+ Entamoeba hartmanni	1 (1.3%)	1 (1.3%)	0 (0%)
Total	28 (37.3%)	28 (37.3%)	19 (25.3%)

P. value= 0.216

4.2.6 Comparison between the three different methods used in detection of intestinal parasitic infections:

When formal ether concentration technique compared with wet preparation, 59 (78.6%) fecal samples were positive by two methods, while 16 (21.3%) were positive by formal ether concentration technique and negative by wet preparation. No fecal sample was positive by wet preparation and negative by formal ether concentration technique. The difference in rate was found to be statistically highly significant at p. value= 0.000 (table 4.8). Also when formal ether concentration technique compared with zinc sulphate floatation technique, 54 (72%) fecal

samples were positive by two methods, while 21 (28%) were positive by formal ether concentration technique and negative by zinc sulphate floatation technique. No fecal sample was positive by zinc sulphate and negative by formal ether concentration technique. The difference in rate was found to be statistically highly significant at p. value= 0.000 (table 4.8).

Table (4.8): Comparison between the three different methods used in detection of intestinal parasitic infections

		Formal ether concentration technique		Total	P. value
		Positive	Negative		
Wet preparation	Positive	59	0	59	P= 0.000
	Negative	16	197	213	
Total		75	197	272	
Zinc sulphate technique	Positive	54	0	54	P= 0.000
	Negative	21	197	218	
Total		75	197	272	

4.2.7 Sensitivity and specificity of wet preparation and zinc sulphate floatation technique by assuming the formal ether concentration technique as the gold standard:

Sensitivity and specificity of wet preparation according to formula mentioned in materials and methods were 78.6% and 100% respectively (table 4.9). Zinc sulphate floatation technique sensitivity and specificity were 72% and 100% respectively (table 4.10).

Table (4.9): Sensitivity and specificity of wet preparation

		Formal ether concentration technique	
		Positive	Negative
Wet preparation	Positive	59	0
	Negative	16	197

Table (4.10): Sensitivity and specificity of zinc sulphate floatation technique

		Formal ether concentration technique	
		Positive	Negative
Zinc sulphate technique	Positive	54	0
	Negative	21	197

4.2.8 Prevalence of intestinal parasitic infections according to abdominal pain by using formal ether concentration technique:

Out of 75 positive fecal samples, 23 (30.6%) had abdominal pain and 52 (69.3%) had no abdominal pain. The difference in rate was found to be statistically significant at p. value= 0.015 (table 4.11).

Table (4.11): Prevalence of intestinal parasitic infections according to abdominal pain by using formal ether concentration technique

Abdominal pain	Formal ether concentration technique		Total
	Negative	Positive	
Yes	37	23	60
No	160	52	212
Total	197	75	272

P. value= 0.015

4.2.9 Prevalence of intestinal parasitic infections according to constipation by using formal ether concentration technique:

Out of 75 positive cases, 4 (5.3%) had constipation and 71 (94.6%) had no constipation. The difference in rate was found to be statistically insignificant at p. value= 0.118 (table 4.12).

Table (4.12): Prevalence of intestinal parasitic infections according to constipation by using formal ether concentration technique

Constipation	Formal ether concentration technique		Total
	Negative	Positive	
Yes	4	4	8
No	193	71	264
Total	197	75	272

P. value= 0.118

4.2.10 Prevalence of intestinal parasitic infections according to diarrhea by using formal ether concentration technique:

Out of 75 positive cases, no one had diarrhea in fecal samples. The difference in rate was found to be statistically insignificant at p. value= 0.070 (table 4.13).

Table (4.13): Prevalence of intestinal parasitic infections according to diarrhea by using formal ether concentration technique

Diarrhea	Formal ether concentration technique		Total
	Negative	Positive	
Yes	9	0	9
No	188	75	263
Total	197	75	272

P. value= 0.070

4.2.11 Prevalence of intestinal parasitic infections according to presence of mucus in the fecal samples by using formal ether concentration technique:

Out of 75 positive cases, no one had mucus in fecal samples. The difference in rate was found to be statistically insignificant at p. value = 0.399 (table 4.14).

Table (4.14): Prevalence of intestinal parasitic infections according to presence of mucus in the fecal samples by using formal ether concentration technique

Mucus in fecal sample	Formal ether concentration technique		Total
	Negative	Positive	
Yes	2	0	2
No	195	75	270
Total	197	75	272

P. value= 0.399

4.2.12 Prevalence of intestinal parasitic infections in children according to education level of their mothers by using formal ether concentration technique:

Out of 75 positive cases**,** 20 their mothers were illiterate, 28 their mothers were secondary education and 27 their mothers were university education. The difference in rate was found to be statistically insignificant at p. value= 0.379 (table 4.15).

Table (4.15): Prevalence of intestinal parasitic infections in children according to education level of their mothers by using formal ether concentration technique

Education level of their mothers	Formal ether concentration technique		Total
	Negative	Positive	
Illiterate	30	20	50
Secondary education	97	28	125
University education	70	27	97
Total	197	75	272

P. value = 0.379

4.2.13 Prevalence of intestinal parasitic infections in children according to socioeconomic level of their parents by using formal ether concentration technique:

Out of 75 positive cases**,** 21 their parents were poor, 48 their parents were moderate and 6 their parents were good. The difference in rate was found to be statistically insignificant at p. value= 0.167 (table 4.16).

Table (4.16): Prevalence of intestinal parasitic infections in children according to socioeconomic level of their parents by using formal ether concentration technique

Socioeconomic level of their parents	Formal ether concentration technique		Total
	Negative	Positive	
Poor	48	21	69
Moderate	115	48	163
Good	34	6	40
Total	197	75	272

P. value= 0.167

4.2.14 Prevalence of intestinal parasitic infections according to hands washing before the meals and after use of toilet by using formal ether concentration technique:

Out of 75 positive cases, 49 were sometimes washing their hands before the meals and after use of toilet, 26 were always washing their hands before the meals and after use of toilet. The difference in rate was found to be statistically significant at p. value= 0.0001 (table 4.17).

Table (4.17): Prevalence of intestinal parasitic infections according to hands washing before the meals and after use of toilet by using formal ether concentration technique

Hands washing before the meals and after use of toilet	Formal ether concentration technique		Total
	Negative	Positive	
Sometimes	92	49	141
Always	105	26	131
Total	197	75	272

P. value= 0.0001

4.2.15 Prevalence of intestinal parasitic infections according to vegetables and fruits washing by using formal ether concentration technique:

Out of 75 positive cases, 47 were sometimes washing vegetables and fruits and 28 were always washing vegetables and fruits. The difference in rate was found to be statistically significant at p. value= 0.0003 (table 4.19).

Table (4.18): Prevalence of intestinal parasitic infections according to vegetables and fruits washing by using formal ether concentration technique

Vegetables and fruits washing	Formal ether concentration technique		Total
	Negative	Positive	
Sometimes	92	47	139
Always	105	28	133
Total	197	75	272

P. value= 0.0003

CHAPTER FIVE

Discussion, Conclusion and Recommendations

Chapter 5

Discussion, conclusion and recommendations

5.1 Discussion:

From the results, it was obvious that the overall prevalence rate of intestinal parasitic infections among children was 27.5%. This rate was found to be lower than the 30% and 59% rate reported by Muhajir *et al.* (2017) and Elfaki *et al*. (2015) respectively. Protozoa were more prevalent (26.8%) than helminthes (0.7%). These findings were in agreement with the findings of Ahmed *et al*. (2017) who reported that the prevalence rate of protozoa was 64.4%, while the rate of helminthes was 24.4%. The current study showed that the overall prevalence rate of intestinal parasitic infections by using wet preparation, formal ether concentration technique and zinc sulphate floatation technique were 21.6%, 27.5% and 20% respectively, and difference in prevalence rates was found to be statistically significant at p. value=0.000. These findings were in disagreement with the findings of Ahmed *et al.* (2017) who reported that the difference in rates was found to be statistically insignificant at p. value= 0.848. The results obtained from the present study showed that sensitivity of wet preparation was 78.6% when assuming formal ether concentration technique as gold standard for the detection of a variety of parasites. This finding was in disagreement with the finding of Hailu and Abera (2015) who reported that sensitivity of wet preparation was 61%. The results showed that the highest prevalence rate (18.8%) of single parasitic infection was reported with *G. lamblia* while the lower rate (0.4%) was reported with *E. vermicularis* and *H. nana*. These findings were in agreement with the findings of Siddig *et al*. (2017) who showed that *G. lamblia* was the most prevalent parasite (46.4%) and in disagreement with the findings of Muhajir *et al.* (2017) who showed that *E.histolytica* was the most prevalent parasite (15.5%). The highest prevalence rate (0.7%) of multiple parasitic infections was reported with

E.histolytica+ E.coli + E. hartmanni and the lower rate (0.4%) was reported with *G.lamblia+ E.histolytica*. The present study revealed that the most commensals parasites were *E. coli* with a rate of 2.2% followed by *Blastocystis hominis* with a rate of 1.4% and *Entamoeba hartmanni* with a rate of 1.1%, while the lowest rate (0.4%) was reported with *Endolimax nana* and *Iodamoeba butschlii* respectively. These rates disagreed with the rates reported by Quihui-Cota *et al.* (2017) who reported a 33% rate for *Endolimax nana* followed by a 17% rate for *Entamoeba coli* and a 0.6% rate for *Iodamoeba butschlii*. These variable results may be due to difference in environmental conditions, seasonableness and personal hygiene. From the investigation, the highest rate (37.3%) was reported with the age groups ≤ 4 and 5-9 years old, while the lower rate (25.3%) was reported with the age group10-14 years old. These may be due to the low hygienic measurements of the children under 10 years. As far as gender was concerned, the results showed that males reported the highest rate (53.3%), while females reported a 46.7% rate. These rates were in agreement with the rates reported by Siddig *et al*. (2017) for males and females (80% and 60% respectively). From the results obtained from the current study, it was clear that the difference in prevalence rates of intestinal parasitic infections according to symptoms was found to be statistically insignificant except with the abdominal pain at p. value=0.015. Regarding the possible associated factors with the infections, the study revealed that the difference in rates was found to be statistically insignificant with the education level of their mothers, socioeconomic level of their parents, while the difference in rates according to hands washing, vegetables and fruits washing was found to be statistically significant at p. value=0.0001 and 0.0003 respectively. These findings were in agreement with the findings of Punsawad *et al*. (2018) who reported that the difference in rates according to hands washing, vegetables and fruits washing was found to be statistically significant at p. value=0.037.

5.2 Conclusion:

The present study concluded that the overall prevalence rate of intestinal parasitic infections among children in Shendi city was 27.5%. The prevalence rate of intestinal protozoa was higher than intestinal helminthes. Also, the prevalence rate of single infection was higher than co-infections. *Giardia lamblia* was the most common parasites detected in children. Formal-ether concentration technique was the best method for the diagnosis of intestinal parasites. Intestinal parasites were more prevalent among the age groups ≤ 4 and 5-9 years old and more prevalent among the males than the females.

5.3 Recommendations:

- The burden of parasitic infections may be reduced by promoting awareness on the prevention and application of supportive programs to improvement of hygiene practices.
- Formal-ether concentration technique should be recommended for the routine diagnosis of intestinal parasites.
- More studies should be done to investigate intestinal parasites in all Shendi localities

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APPENDICES

Appendix

Questionnaire form

Sudan University of Science and Technology

College of Graduate Studies

M.Sc. in Parasitology and Medical Entomology

- Date: …….. ID: ……..

- Gender: Male ( ) Female ( )

- Age: …….

- Suffering from:

- Abdominal pain Yes ( ) No ( )
- Constipation Yes ( ) No ( )
- Diarrhea Yes ( ) No ( )
- Blood in stool Yes ( ) No ( )
- Mucus in stool Yes ( ) No ( )

- Vegetables and fruits washing:

Sometimes ( ) Always ( ) No washing ( )

- Hands washing before the meals and after use of toilet:

Sometimes ( ) Always ( ) No washing ( )

- Socioeconomic level of parents:

Poor ( ) Moderate ( ) Good ( )

- Education level of mother:

University education ( ) Secondary education ( ) Illiterate ( )

Laboratory results:

- Wet preparation result:

.....

- Formal ether concentration technique result:

……....

- Zinc sulphate floatation technique result:

……