بسم الله الرحمن الرحيم

udan University of Science and Technology

College of Graduate Studies

Evaluation of Efficiency Formal Gasoline Concentration Technique in Detection of Intestinal Infections in Al-Rajhe Hospital in Dar Al-Salam Area, Ombada Locality, Khartoum State- Sudan

تقويم كفاءة تقنية الفورمال جازولين في التعرف على العدوى الطفيلية المعوية في مستشفى الراجحي في منطقة دار السلام، محلية أمبدة، ولاية الخرطوم- السودان

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

By

Ebtehal Sulum Ahmed Mahmoud

B.Sc. in Medical Laboratory Sciences- Karary University- 2014

Supervisor

Dr. Tayseer Elamin Mohamed Elfaki

Associate Professor of Parasitology and Medical Entomology, Sudan University of Science and Technology

January, 2020

الآيـــــــــــــة

بسم الله الرحمن الرحيم

قال تعـــــــــــالى:

﴿اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ ۖ الْمِصْبَاحُ فِي زُجَاجَةٍ ۖ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِن شَجَرَةٍ مُّبَارَكَةٍ زَيْتُونَةٍ لَّا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ ۚ نُّورٌ عَلَىٰ نُورٍ ۗ يَهْدِي اللَّهُ لِنُورِهِ مَن يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ﴾

صـــدق الله العظيـــم

سورة النـــور- الآية 35

Dedication

To whom I prefer to myself, and why not; I sacrificed for me.

(my mother's love).

To the perfumed biographer, enlightened thought, he had the first credit for reaching higher education (my beloved father).

To my uncle who had a big role in my support.

To my brothers who have had a great impact in many obstacles and difficulties.

To my friends, and all those who stood next to me and helped me with everything they had, and in many ways.

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

I dedicate this work.

Acknowledgement

First of all i would like to be grateful to Allah who gave me strength to complete this work.

I would like to express my immense gratitude and appreciation to my wonderful supervisor Dr. Tayseer Elamin Mohamed Elfaki for her close supervision, assistance and continuous support during this work.

My gratitude is extended to all patients included in the study for their cooperation.

Deep appreciation and thanks also go to my colleagues and the family of Medical Laboratory Science at Al-Rajhe hospital.

I am also, grateful to my colleagues Ibrahim arabi, Malaz Bakheet and Mujtaba Ali for their help and support.

Deep thanks to Omila Fath Alaleem, for sparing no effort helping me to overcome difficulties and for their continuous guidance.

Finally all my thanks are extended to everyone who supported and helped me to accomplish this study.

Abstract

This cross-sectional study was conducted in Al-Rajhe hospital in Dar Al-Salam area, Ombada locality, Khartoum State- Sudan to evaluate formal gasoline concentration technique in detection of intestinal parasitic infections during the period from October 2018 to October 2019. The study was conducted on 272 patients, 109 (40.1%) were males and 163 (59.9%) were females. Fecal samples were taken from all patients included in the study, in addition , the epidemiological and parasitological data were obtained and recorded. All samples were examined to detect intestinal parasite species by using wet preparation, floatation technique, formal ether concentration technique and formal gasoline concentration technique. The study showed that the prevalence rate of intestinal parasites was (45.5%) and intestinal protozoa were more prevalent (41.5%) than intestinal helminthes (4.0%). The prevalence rate of intestinal parasitic infections by using wet preparation, formal ether concentration technique, formal gasoline concentration technique and zinc sulphate floatation technique were (34.2%), (45.5%), (42.6%) and (28.3%) respectively (p. value=0.000). The highest prevalence rate (18.0%) of intestinal parasites in the study area was reported with *Giardia lamblia*. The study revealed that the highest prevalence rate (57.3%) was reported among females while males reported (42.7%) prevalence rate. The highest prevalence rate (67.7%) was reported among the ≤ 15 years old. The results showed that the difference in prevalence rates of intestinal parasitic infections according to symptoms was found to be statistically significant (p. value=0.000). The results showed that the difference in prevalence rates of intestinal parasitic infections according to their associated risk factors was found to be statistically significant except with the source of drinking water which was found to be statistically insignificant (p. value=0.991). The study indicated

that the prevalence rate of intestinal parasitic infections in the study area was high (45.5%). In addition to, the gasoline proved to be good in concentrating parasite eggs and cysts, as well as in maintaining characteristic morphology.

مستخلص الدراسة

أجريت هذه الدراسة المستعرضة في مستشفى الراجحي في منطقة دار السلام، محلية أمبدة، ولاية الخرطوم- السودان لتقييم تقنية تركيز الفورمال جازولين في التعرف على العدوى الطفيلية المعوية في الفترة من أكتوبر 2018 إلى أكتوبر 2019م. أجريت الدراسة على 272 مريض، 109 (40,1%) كانوا ذكوراً و 163(59,9%) كانوا أناثاً. عينات البراز تمّ أخذها من جميع المرضى المتضمنين في الدراسة، بالإضافة للبيانات الوبائية والطفيلية تمّ اخذها وتسجيلها. كل العينات تمّ فحصها للتعرف على أنواع الطفيليات المعوية باستخدام التحضير الرطب، تقنية الطفو، تقنية تركيز الفورمال إيثر و تقنية تركيز الفورمال جازولين. أظهرت الدراسة أن معدل انتشار الطفيليات المعوية كان (45,5%) وكانت الاوليات المعوية أكثر انتشاراً (41,5%) من الديدان المعوية (4,0%). معدل انتشار الطفيليات المعوية باستخدام التحضير الرطب، تقنية تركيز الفورمال إيثر، تقنية الفورمال جازولين و تقنية الطفو كان (34,2%)، (45,5%)، (42,6%) و (28,3%) على التوالي (القيمة المعنوية= 0,000). كان أعلى معدل انتشار (18,0%) للطفيليات المعوية في منطقة الدراسة سُجل مع *Giardia lamblia* . كشفت الدراسة أن أعلى معدل انتشار (57,3%) سُجل وسط الإناث بينما سجل الذكور (42,7%) معدل انتشار. كان أعلى معدل انتشار (67,7%) سُجل وسط الفئة العمرية $\geq$ 15عاماً. أظهرت النتائج أنه يوجد فارق مقدر إحصائياً بين معدلات انتشار العدوى الطفيلية المعوية وفقاً للأعراض (القيمة المعنوية= 0,000). أظهرت النتائج أنه يوجد فارق مقدر إحصائياً بين معدلات انتشار العدوى الطفيلية المعوية وفقاً لعوامل الخطر المرتبطة بها ماعدا مع مصدر مياه الشرب (القيمة المعنوية= 0,991). خلصت الدراسة إلى أن معدل انتشار العدوى الطفيلية

المعوية في منطقة الدراسة كان عالياً بنسبة (45,5%). بالإضافة إلى أن الجازولين أُثبت أنه جيد في تركيز بيض و أكياس الطفيليات، وكذلك في الحفاظ على الشكل المميز.

Table of contents

List of tables

List of figures

Chapter 1

Introduction, rationale and objectives

1.1 Introduction:

Parasitic diseases caused by intestinal parasites are a major public health problem in developing countries of Africa. Recent estimates of helminth infections indicate the existence of more than one billion infected individuals in underdeveloped areas of Africa, Asia and in Central and South America (Oguoma and Ekwunife, 2008). The most common intestinal helminthes leading to digestive disorders include: *Taenia saginata*, *Hymenolepis nana*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Enterobius vermicularis* and hook worm, and are usually transmitted from contaminated food or water or from the environment. The most common protozoans reported to lead to digestive disorders include: *Giardia lamblia* and *Entamoeba histolytica* (Babiker *et al.*, 2009). According to the World Health Organization (WHO), approximately 500 million people worldwide suffer from amoebiasis, with an annual mortality between 40,000 and 110,000. Man is undoubtedly the most important reservoir of *E. histolytica*, passing virulent cysts that are transmitted chiefly by ingestion of contaminated food or water or through direct contact. Generally, the prevalence rate of gastrointestinal parasites varies from one area to another depending on the degree of personal and community hygiene, sanitation and climatic factors, and different diagnostic techniques may also influence their detection. Young children and adolescents in developing countries display highest prevalence of intestinal parasites and burden morbidity. Intestinal parasites are linked to diarrhea, dysentery, weight loss, malnutrition, anemia, abdominal pain and other gastrointestinal ailments. Also, chronic parasitism impairs physical development and cognitive functions of growing children. Gastrointestinal parasites can cause infection in both humans and animals. Some of these

are potentially zoonotic. Moreover, intestinal opportunistic parasitic infections are also a serious public health problem, which has been increasing in recent years in developing countries (Mergani *et al*., 2014). 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. 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 Al-Rajhe hospital in Dar Al-Salam area. Different intestinal parasite species can co-occur in the same population. However, classic diagnostic tools can only frame a particular group of intestinal species. Hence, one or two tests do not suffice to provide a complete picture of infecting parasite species in a given population. Therefore, this study was conducted to evaluate formalin gasoline concentration technique in comparison with three other methods in detection of intestinal parasites and to detect the prevalence of intestinal parasitic infections and their associated risk factors in Al-Rajhe hospital in Dar Al-Salam area, Ombada locality, Khartoum state- Sudan.

1.3 Objectives:

1.3.1 General objective:

To evaluate formal gasoline concentration technique in detection of intestinal parasitic infections in Al-Rajhe hospital in Dar Al-Salam area, Ombada locality, Khartoum state- Sudan.

1.3.2 Specific objectives:

- To determine the overall prevalence of intestinal parasitic infections in the study area.
- To determine the prevalence of intestinal parasitic infections in the 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 the study area.
- To assess the possible associated factors with the prevalence of intestinal parasitic infection in the study area.
- To compare between the sensitivities and specificities of wet preparation, formal ether concentration technique, formal gasoline concentration technique and zinc sulphate floatation technique in detection of intestinal parasitic infections.

Chapter 2

Literature review

2.1 Definition of parasite:

A parasite is an organism that lives on or in a host organism and gets its food from or at the expense of its host (CDC, 2016).

2.2 Classification of parasite:

There are three main classes of parasite that can cause disease in humans: protozoa, helminthes and ectoparasites (CDC, 2016).

2.3 Intestinal protozoa:

2.3.1 Classification:

Human intestinal protozoan parasites are divided into non-pathogenic and pathogenic parasites (Mahmoudvand *et al*., 2018).

2.3.2 Epidemiology:

Intestinal parasitic infections are considered as one of the main socio-economic and health problems around the world, mostly in tropical and sub-tropical regions (Mahmoudvand *et al*., 2018). The protozoan parasites are the more common cause of gastrointestinal disorders compared to helminthes especially in developing countries. A number of intestinal protozoan parasites are reported in different parts of the world like *Giardia lamblia*, *Dientamoeba fragilis*, *Entamoeba histolytica*, *Blastocystis hominis*, *Isospora belli*, *Cyclospora cayetanensis* and *Microsporidia.* Among them *Entamoeba*, *Giardia* and *Cryptosporidium* are the major protozoan parasites of global public health concern (Abdullah *et al*., 2016).

2.3.3 Transmission of intestinal protozoa:

Infections usually occur through ingestion of cysts/oocysts contaminating raw food or drinking water (Hawash *et al*., 2015).

2.3.4 Non-pathogenic intestinal protozoa:

Non-pathogenic protozoa are important because their presence indicates fecal-oral

transmission in infected individuals. Contamination with non-pathogenic protozoa is an indicator of hygienic and health situation of people under study (Mahmoudvand *et al.*, 2018). The non-pathogenic protozoa can be divided into two groups: amoebae and flagellates.

2.3.4.1 The non-pathogenic flagellates:

The non-pathogenic flagellates include: *Trichomonas hominis, Chilomastix mesnili* and *Trichomonas tenax* (Issa, 2014).

2.3.4.2 The non-pathogenic amoebae:

Human beings can be parasitized by various species of intestinal amoebae. *Entamoeba histolytica* is the only intestinal amoeba recognized to be pathogenic, while other amoeba species, *E. dispar*, *E. moshkovskii*, *E. hartmanni*, *E. coli*, *E. polecki*, *Endolimax nana* and *Iodamoeba buetschlii* are considered to be non-pathogenic (Sard *et al*., 2011).

2.3.4.3 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, 2015a) (figure 2.1).

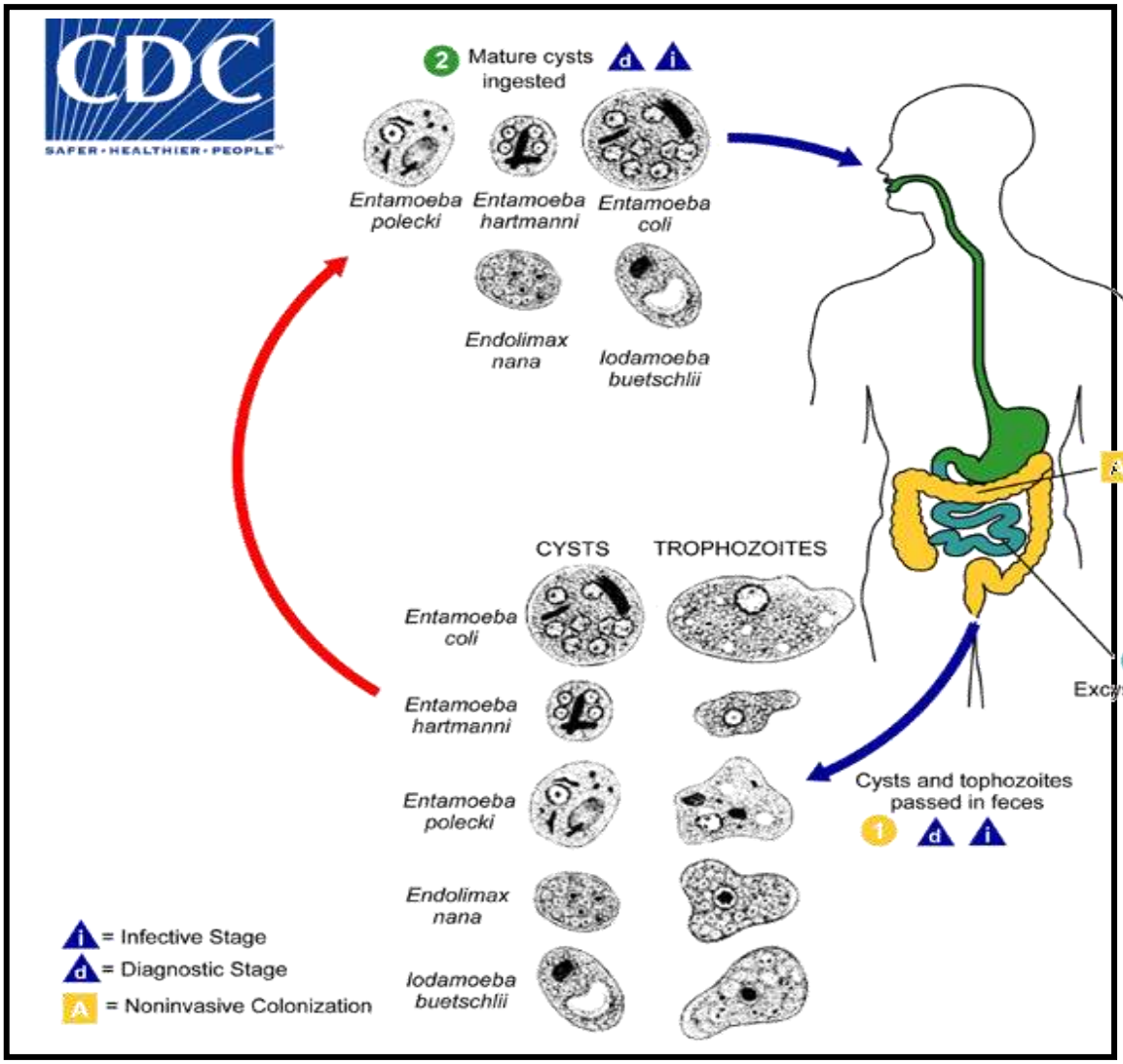

Figure (2.1): Life cycle of non-pathogenic protozoa (CDC, 2015a)

2.3.5 Pathogenic intestinal protozoa:

Intestinal parasites are organisms that live in the hosts' intestine and take up the nutrition from the host, and cause abdominal discomfort, dysentery, mechanical irritation of intestinal mucosa, malabsorption syndromes and obstruction. They can be transmitted by fecal-oral route (Noor Azian *et al*., 2007).

2.3.5.1 Some species of pathogenic intestinal protozoa:

2.3.5.1.1 *Entamoeba histolytica:*

2.3.5.1.1.1 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). Amoebiasis is responsible for around 100,000 deaths/year, mainly in Central and South America, Africa and India, as well as for a significant rate of morbidity manifested as invasive intestinal or extra intestinal disease. Infection with *E. histolytica* has been estimated to be as high as 50% in some developing countries as South and Central America, Africa and Asia. Factors as illiteracy, poverty, low socio-economic standards including bad sanitation, improper water supply, and overcrowding contribute positively to the increased rates of transmission of the parasite and disease. Infection is commonly detected in tropical and subtropical countries; however, in developed countries, infection is seen among travelers, immigrants, homosexual males, and cases in institutions. The infection usually prevails in two extremes of age: the children and the old individuals. One of the youngest reported cases is a 4-month-old baby boy in Iran (El-Dib, 2017).

2.3.5.1.1.2 Transmission:

Amoeba usually transmitted by fecal-oral contact. Transmission may occur directly from person to person or through contaminated food and water. Ingestion of less than 100 organisms is adequate to cause amoebiasis. Contamination may come directly from infected food handlers or indirectly from faulty sewage disposal (Sodeman, 1996).

2.3.5.1.1.3 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).

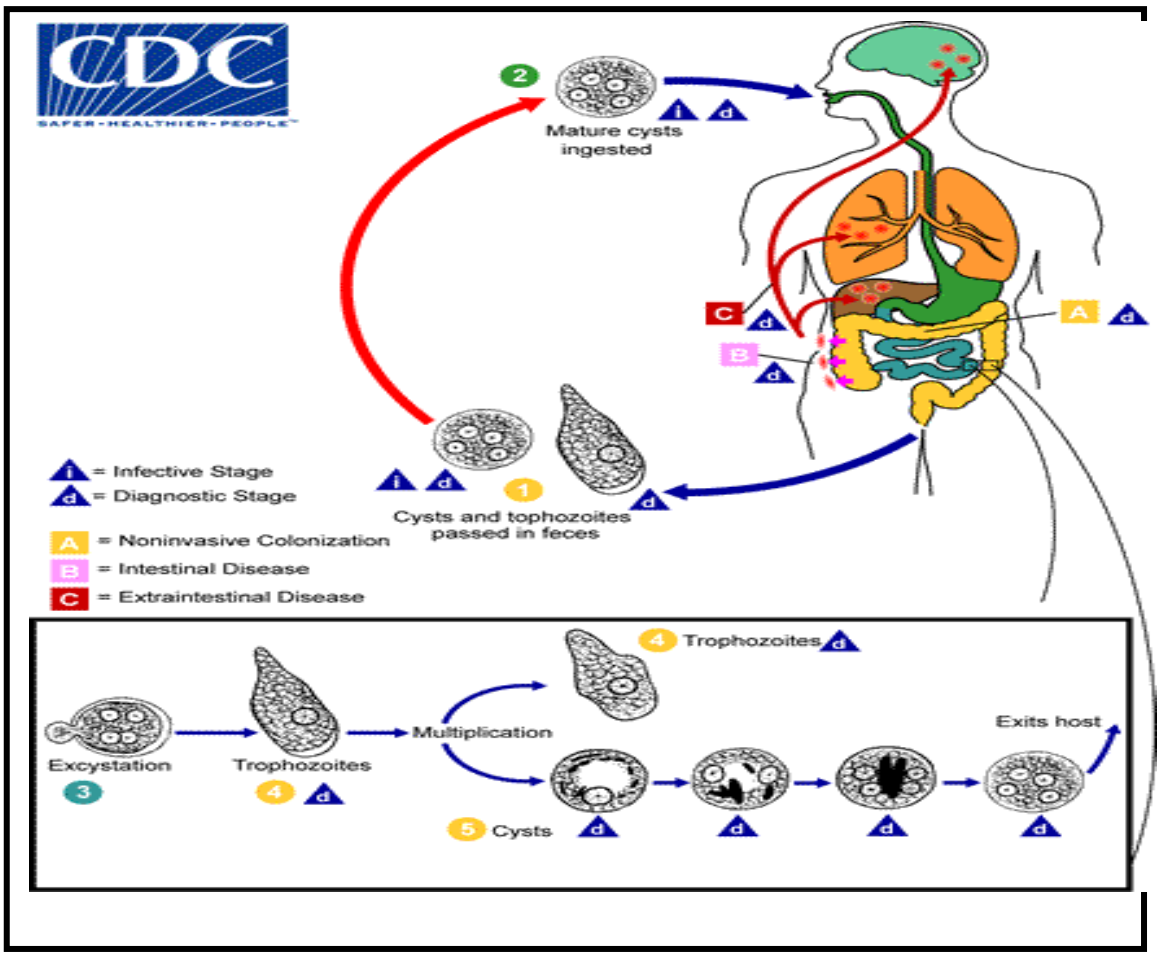

Figure (2.2): Life cycle of *Entamoeba histolytica* (CDC, 2017a)

2.3.5.1.1.4 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. 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).

2.3.5.1.1.5 Laboratory diagnosis:

We should ask ourselves about the extent to which improvement could be made in the performance of conventional or traditional diagnostic techniques. For several years, researchers have been searching for methods that will allow an accurate and reliable assessment of amoebiasis. Laboratory diagnosis of amoebiasis is usually based on microscopy and serological methods (Tanyuksel and Petri, 2003). Stool specimens should be preserved and stained and microscopically examined. Cysts will tend to predominate in formed stools and trophozoites in diarrheic stools. 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 (Brooks *et al.*, 2006). Serological methods including enzyme linked immunosorbent assay (ELISA), indirect hemagglutination assay (IHA) and latex agglutination test (LAT). During the last decade, there has been remarkable development in molecular biology-based diagnostic procedures to detect *E. histolytica*, to the point where today they are the preferred approach (Tanyuksel and Petri, 2003).

2.3.5.1.1.6 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).

2.3.5.1.1.7 Prevention and control:

Prevention of amoebiasis at present requires interruption of the fecal-oral spread of the infectious cyst stage of the parasite. Because cysts are resistant to low doses of chlorine or iodine, in developing countries water must be boiled before it is safe to drink, and raw vegetables must be washed with soap and then soaked in vinegar for 15 min before they can be eaten. 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. Since amoebiasis often spreads through a household, it is prudent to screen family members of an index case for intestinal *E. histolytica* infection. On the horizon is the development of a vaccine to prevent disease in residents of and travelers to the developing world. Both the amebic adherence lectin and serine-rich antigen have proven effective in the prevention of liver abscess. The lectin is a particularly attractive candidate antigen because it is required to initiate contact-dependent cytolysis, mediates evasion of the complement membrane attack complex, and is antigenically conserved among geographically distinct isolates of *E. histolytica* (Petri and Singh, 1999).

2.3.5.1.2 *Giardia lamblia:*

2.3.5.1.2.1 Epidemiology:

The epidemiology of giardiasis still is a matter of great discussion. From the original debates around its pathogenicity to the later ones about its speciation and biology, *G. lamblia* has proven to be an enigmatic and interesting organism. Although giardiasis is currently recognized as one of the main causes of diarrheal disease and a leading cause of death and illness among children under 5 years old in developing countries, the long-term impact of pediatric giardiasis remains unclear. Recent cohort studies have confirmed a high prevalence of persistent, subclinical giardiasis and its association with growth shortfalls, but such evidence has not been consistently reported in the literature. Commonly, giardiasis prevalence among poor populations is reported as very high, and when the infection became chronic, it has been associated also with malnutrition and cognitive deficits. In developed countries, giardiasis represents the leading cause of

traveler's diarrhea and is frequently reported among citizens that traveled to developing countries and expose themselves to untreated water from lakes, streams, and swimming pools (Marty, 2017).

2.3.5.1.2.2 Routes of transmission:

This parasite transmits via fecal-oral route through direct or indirect ingestion of infectious cysts 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. The incubation period varies from 9 to 15 days after ingestion of cysts (Hooshyar *et al*., 2019).

2.3.5.1.2.3 Life cycle:

Life cycle begins with the infection by the ingestion of the cyst. Then the excystation continues, which starts at the stomach triggered by the exposure of the cyst to the gastric acid, the presence of bile and trypsin in the duodenum and/or the alkaline, protease-rich milieu. Excystation ends at the proximal small intestine where the emerging parasites (excyzoites) quickly transform into trophozoites that attach to the intestinal epithelial cells using the adhesive disc. The adhesive disc is essential for attachment and appears to play a major role in the virulence of *Giardia*. Several disc-associated proteins have been identified using proteomics, and it is clear that the disc is an advanced cytoskeletal structure. At the jejunum, the trophozoites start to encyst forming the wall that enables the parasite to survive outside the host for several weeks in cold water. This process is triggered by a particular composition of biliary secretions; possibly by a deprivation of cholesterol regulatory factors are encystation-specific transcription factors, chromatin remodeling enzymes and posttranslational modifications, which vary their expression in correlation with the variation

of antigens on the parasite surface. Finally, trophozoites and cysts are released with the stool, with cysts continuing the transmission of the disease when ingested by another host (Marty, 2017) (figure 2.3).

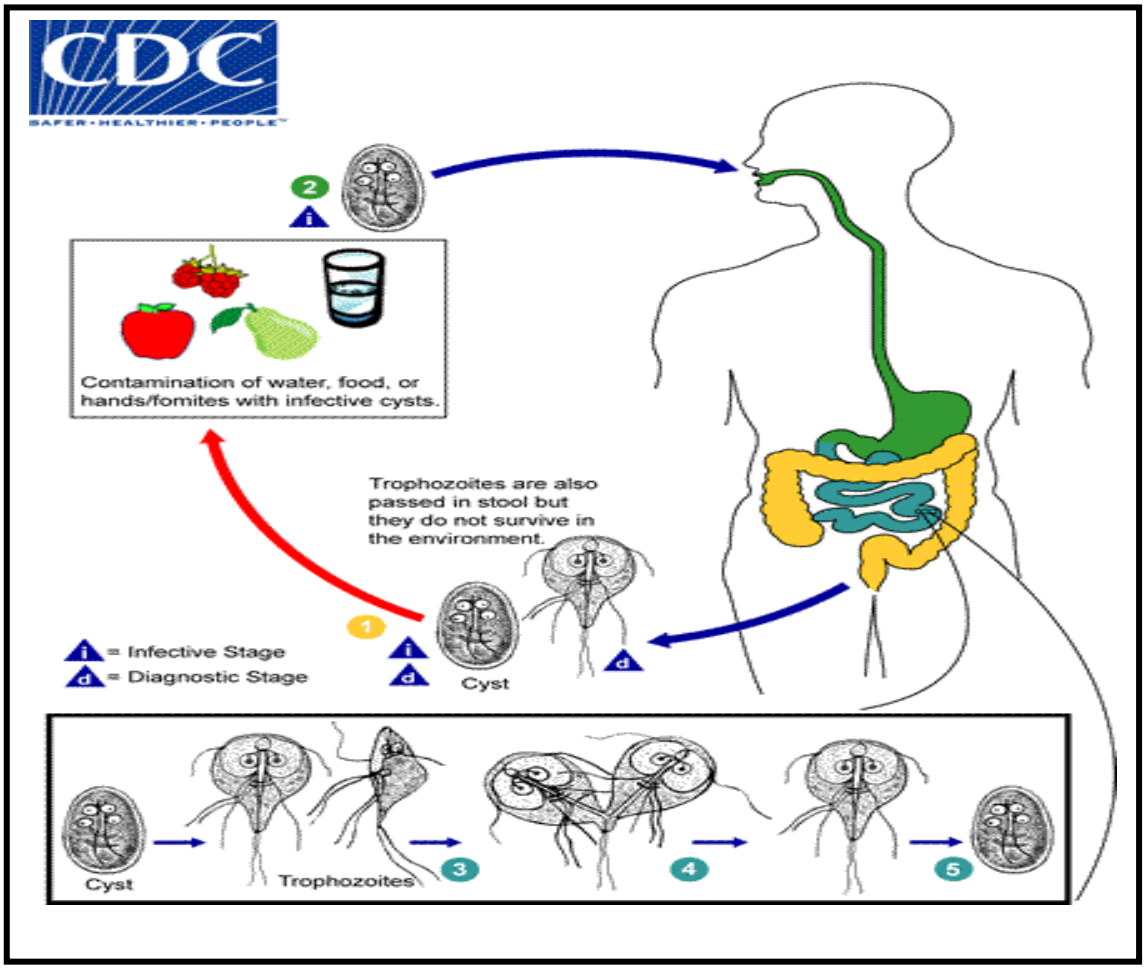

Figure (2.3): Life cycle of *Giardia lamblia* (CDC, 2017b)

2.3.5.1.2.4 Pathogenesis:

Giardiasis is the most common small intestinal protozoal infection and is found worldwide. The mechanisms by which *Giardia duodenalis* (*G. lamblia*) produces chronic diarrhoea and malabsorption have still not been clearly defined. Many infections are associated with mild to moderate mucosal damage which in animal models of infection have functional correlates. Possible mechanisms include direct physical injury, release of parasite products such as proteinases or lectin and mucosal inflammation associated with T cell activation and cytokine release. Other possible

mechanisms of mal-absorption include associated bacterial overgrowth and bile salt deconjugation, bile salt uptake by the parasite with depletion of intra-luminal bile salts and inhibition of pancreatic hydrolytic enzymes. Thus, there is no single mechanism to explain the diarrhoea and mal-absorption caused by *Giardia* which currently should be regarded as a multi-factorial process (Farthing, 1993).

2.3.5.1.2.5 Laboratory diagnosis:

Because *Giardia* cysts can be excreted intermittently, multiple stool collections (i.e., three stool specimens collected on separate days) increase test sensitivity. The use of concentration methods and trichrome staining might not be sufficient to identify *Giardia* because variability in the concentration of organisms in the stool can make this infection difficult to diagnose. For this reason, fecal immunoassays that are more sensitive and specific should be used. Rapid immune-chromatographic cartridge assays also are available but should not take the place of routine examination. Only molecular testing (e.g., polymerase chain reaction) can be used to identify the subtypes of *Giardia* (CDC, 2015b).

2.3.5.1.2.6 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).

2.3.5.1.2.7 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).

2.4 Intestinal helminthes:

2.4.1 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).

2.4.1.1 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).

2.4.1.2 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).

2.4.1.3 Round worms (nematodes):

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

2.4.2 Epidemiology:

Helminth infections are widely distributed in tropical and subtropical areas and, since they are linked to a lack of sanitation, occur wherever there is poverty. Soil-transmitted helminthes infections (STH) and schistosomiasis are among the most common infections worldwide. Estimates indicate that more than 880 million children are in need of treatment for these parasites (WHO, 2019).

2.4.3 Routes of transmission:

Helminthes are transmitted to humans in many different ways. 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. In some cases the intermediate vector transmits infective stages when it bites the host to take a blood meal (the arthropod vectors of filarial worms); in other cases, the larvae are contained in the tissues of the intermediate host and are taken in when a human eats that host (*Clonorchis* in fish, tape worms in meat and fish, *Trichinella* in meat). The levels of infection in humans therefore depend on standards of hygiene (as eggs and larvae are often passed in urine or feces), on the climate (which may favor survival of infective stages), on the ways in which food is prepared and on the degree of exposure to insect vectors (Wakelin, 1996).

2.4.4 Some species of intestinal helminthes:

2.4.4.1 *Hymenolepis nana*:

2.4.4.1.1 Epidemiology:

Hymenolepis nana, the dwarf tape worm, is the smallest and a common tape worm in humans worldwide. *H. nana* infection occurs more frequently in warm climates and temperate zones such as Asia, Central and South America and Eastern Europe. Light *H. nana* infections are usually asymptomatic, whereas heavy infections with more than 2,000 worms can induce a wide range of gastrointestinal symptoms and allergic responses (Kim *et al.*, 2014).

2.4.4.1.2 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).

2.4.4.1.3 Life cycle:

The life cycle of *H. nana* is of particular biological significance: it represents a modification of the typical cyclophyllidean life cycle pattern in that the parasite requires only one host to complete its development. Natural definitive hosts, in addition to humans, are rodents, particularly mice and rats. Gravid proglottids from adult worms rupture, releasing oncosphere-containing eggs into the host’s intestine to be eliminated with feces. The morphology of the egg, infective upon release, is characteristic of hymenolepid eggs. The oncosphere is enclosed in a thin shell and an embryophore with two polar thickenings, from each of which extend four to eight filaments. Upon being ingested by a new host, the oncosphere, freed in the small intestine from its encapsulating membranes, penetrates a

villus into the lamina propria. There, about 4 days later, it becomes a modified cysticercoid larva known as a cercocystis. The cercocystis erupts from the villus into the lumen of the small intestine, attaches itself to the mucosal lining, and develops into a sexually mature adult in about 30 days. In the case of rodents, an insect, such as the flour beetle, may serve as an intermediate host. In this case, when the insect host is ingested by a rodent, or even accidentally by a human, the cysticercoid attaches to the intestinal wall and develops to sexual maturity. Autoinfection can exacerbate the condition by increasing the number of worms; eggs released from gravid proglottids, instead of passing to the exterior to infect new hosts, hatch in the small intestine and re-infect the same host. The freed oncospheres penetrate villi and repeat the cycle (Bogitsh *et al*., 2012) (figure 2.4).

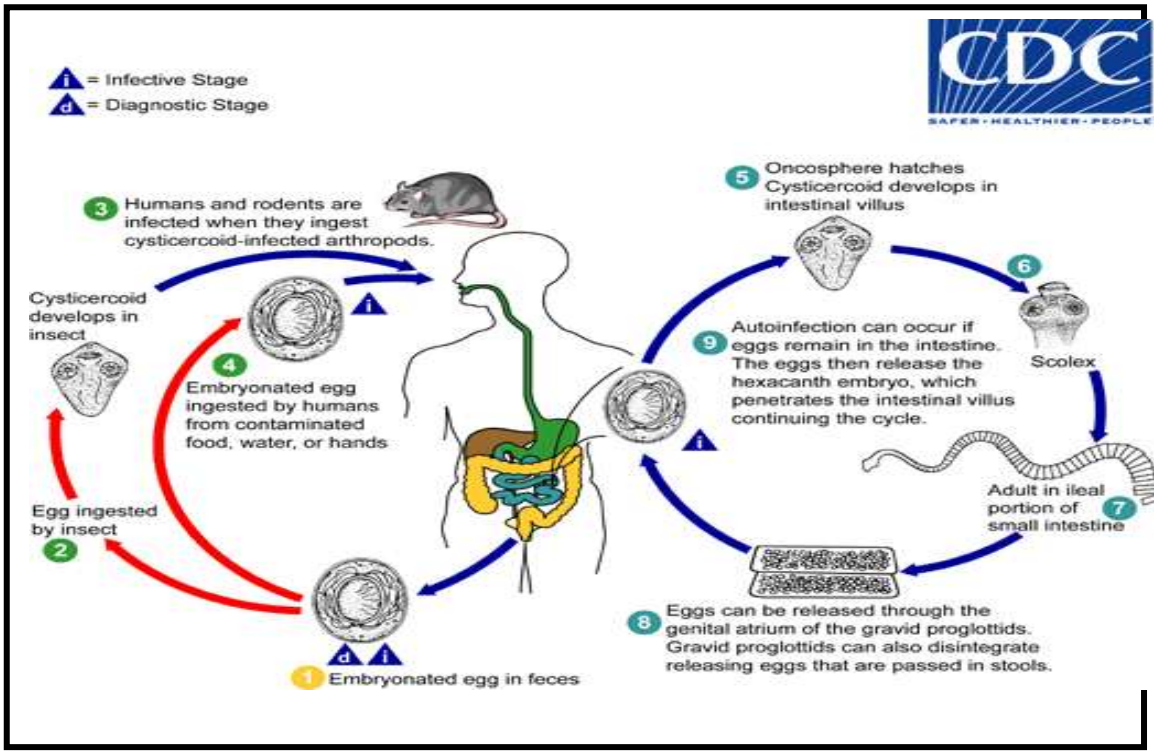

Figure (2.4): Life cycle of *Hymenolepis nana* (CDC, 2017c)

2.4.4.1.4 Pathogenesis:

Since it is possible for a human victim to harbor massive numbers of these parasites, damage to the intestinal mucosa may be sufficient to produce enteritis. Most infections, however, are light and virtually symptomless,

although autoinfection can lead to heavy worm burdens, particularly in children and immunosuppressed patients. In adult patients, the infection is usually self-limiting. In children with a moderate parasite burden, there may be loss of appetite, diarrhea, some abdominal pain, and dizziness. In many patients, a low humoral immune response is detectable (Bogitsh *et al*., 2012).

2.4.4.1.5 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).

2.4.4.1.6 Treatment:

The treatment of choice for *H. nana* infection is praziquantel. *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 (Pearson, 2018).

2.4.4.1.7 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).

2.4.4.2 *Enterobius vermicularis:*

2.4.4.2.1 Epidemiology:

The male to female infection frequency is 2 to 1. However, a female predominance of infection is seen in those between the ages of 5 and 14 years. It most commonly affects children younger than 18 years of age. It is also commonly seen in adults who take care of children, institutionalized children. Center for disease control and prevention data indicates that there about 40 million people estimated to have been infected in the United States (Rawla and Sharma, 2019).

2.4.4.2.2 Transmission:

Transmission can occur via contact with contaminated clothes, bedding, personal care products and furniture. Fecal-oral is the most common mode of transmission. Rarely, transmission can occur via inhalation mode when eggs are inhaled and then subsequently swallowed (Rawla and Sharma, 2019).

2.4.4.2.3 Life cycle:

Gravid adult female *Enterobius vermicularis* deposit eggs on peri-anal folds. Infection occurs via self-inoculation (transferring eggs to the mouth with hands that have scratched the peri-anal area) or through exposure to eggs in the environment (e.g. contaminated surfaces, clothes, bed linens, etc.). Following ingestion of infective eggs, the larvae hatch in the small intestine and the adults establish themselves in the colon, usually in the cecum. The time interval from ingestion of infective eggs to ovi-position by the adult females is about one month. At full maturity adult females measure 8 to 13 mm, and adult males 2 to 5 mm; the adult life span is about two months. Gravid females migrate nocturnally outside the anus and ovi-posit while crawling on the skin of the peri-anal area. The larvae contained inside the eggs develop (the eggs become infective) in 4 to 6 hours under optimal conditions. Rarely, eggs may become airborne and be

inhaled and swallowed. Retro-infection, or the migration of newly hatched larvae from the anal skin back into the rectum, may occur but the frequency with which this happens is unknown (CDC, 2019) (figure 2.5).

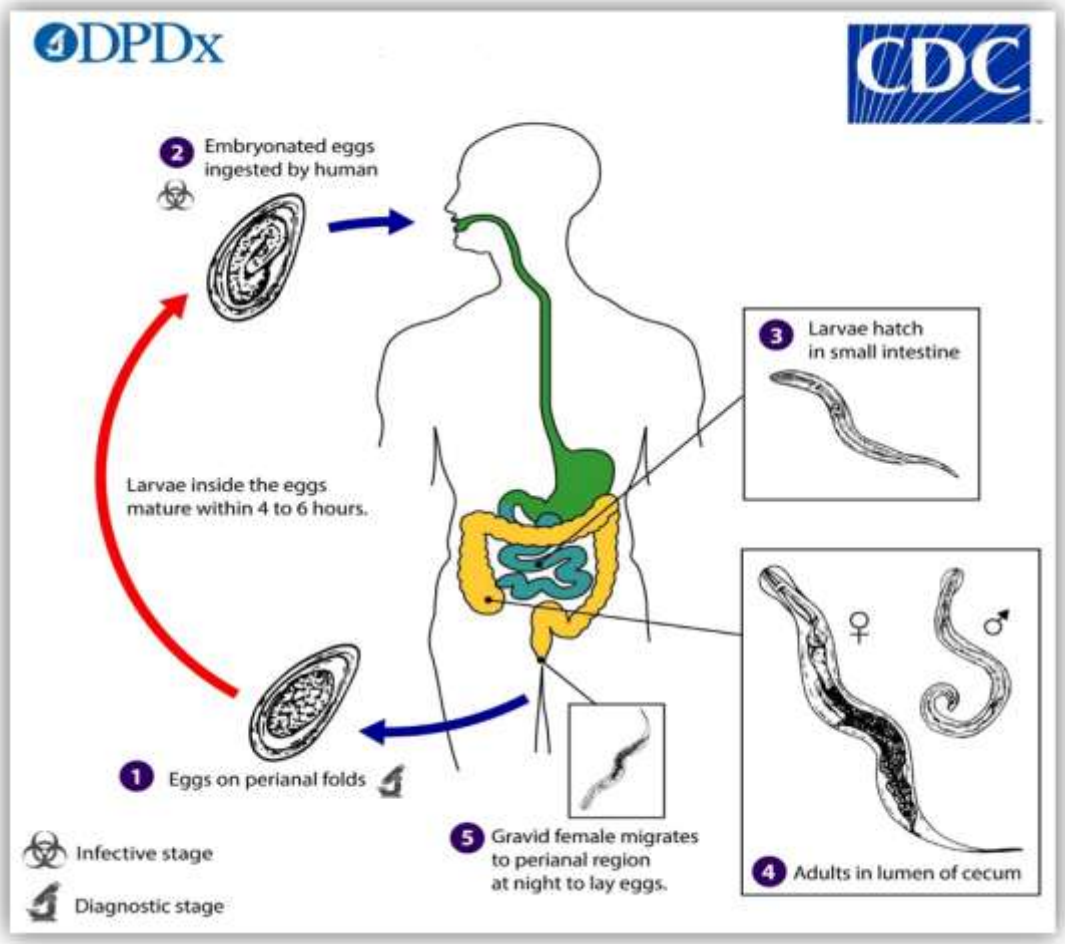

Figure (2.5): Life cycle of *Enterobius vermicularis* (CDC, 2019)

2.4.4.2.4 Pathogenesis:

Enterobius vermicularis is an organism that primarily lives in ileum and cecum. Once *E. vermicularis* eggs are ingested, they take about 1 to 2 months to develop into adult worms which happen in the small intestine. These do not usually cause any symptoms when confined to the ileocecal area. The female adult worms and ova migrate to the anal area mostly at night time and deposit thousands of eggs in the peri-anal area. This migration causes a lot of itching and pruritus. Eggs hatch near the anal area

causing itching, scratching and this causes peri-anal pruritus. 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. 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 (Rawla and Sharma, 2019).

2.4.4.2.5 Laboratory diagnosis:

In addition to the typical patient history involving the cardinal symptom, the worm eggs that are invisible to the naked eye can be swabbed using commercially available adhesive cellulose tape (scotch tape) in the morning prior to defecation and before washing the genital area (scotch tape test). Microscopic detection of the characteristic worm eggs confirms infection. Microbiological laboratories and pharmacies now offer readymade diagnostic kits. Alternatively, one can swab the anal region with a cotton swab and then place this in physiological saline solution (Wendt *et al*., 2019).

2.4.4.2.6 Treatment:

Treatment consists of the following anti-helminthic medications: albendazole: given on an empty stomach, a 400 mg, one-time dose followed by a repeat dose in 2 weeks, mebendazole: a 100 mg, one-time dose followed by a repeat dose in two weeks and pyrantel pamoate: available over the counter in the United States; dose of 11 mg/kg up to a maximum 1 gm given 2 weeks apart. Other medications which have been used to treat enterobiasis are ivermectin and piperazine, although the latter has lower efficacy and higher toxicity (Rawla and Sharma, 2019).

2.4.4.2.7 Prevention and control:

Preventive strategies recommended are: washing hands regularly, particularly before eating, after changing diapers. Taking a bath early in the

morning to prevent egg contamination should be encouraged in at-risk patients. Trimming of fingernails should be encouraged. Children should be advised to avoid sucking their fingers and touching their peri-anal area (Rawla and Sharma, 2019).

2.5 Immunity to intestinal parasites:

Adult worms are spontaneously expelled from the intestine at the end of the second week of infection. Expulsion from the jejunum requires the presence of immune T lymphocytes and immunoglobulin G (IgG) antibodies. Mucosal mast cells (MMCs) are a prominent part of the jejunal inflammatory response. They are derived from a hematopoietic stem cell, possibly the same precursor as basophils. Their differentiation is not absolutely T dependent but their accumulation at the site of infection. The possible involvement of immunoglobulin E (IgE) antibodies and intestinal MMCs through a "leak lesion" is still uncertain. Increased mucus secretion from epithelial goblet cells is also a prominent feature of the inflammatory reaction at the site of infection. Goblet cell numbers increase two to four times at the onset of worm expulsion; this increase is regulated by T lymphocytes and possibly immune serum. The mechanism of mucus secretion in these infections is not clear; it may be a response to mast cell mediators. Together with anti-worm antibodies, intestinal mucus may trap worms and prevent them from surviving in the inter-villous spaces of the jejunum. Thus, expulsion of this intestinal parasite may occur through a non-specific process that is induced by specific immune mechanisms (Levy and Frondoza, 1983). Th2 responses are associated with protection against intestinal helminthes through the activation of several effector mechanisms at the host-parasite interface. Novel functions for epithelial cells and mucosal innate immune cells have been shown to be crucial for initiating and regulating type 2 immunity. The generation of Th1 responses associated with susceptibility to infection is underestimated, though

mechanisms leading to Th1 activation are keys for a better understanding of the immune regulation of these infections. An understanding of the early mechanisms determining the development of protective type 2 immunity or Th1 responses, associated with chronicity, is key for the development of control tools against intestinal helminthes. Protective immunity against intestinal helminthes is associated with development of type 2 responses. Nevertheless, in some host-intestinal helminth combinations, local Th1 responses are initiated, inducing chronicity (Cortés *et al.*, 2017). Host immune reaction against helminths may control the infection; it can also be responsible for tissue lesions and symptoms which are often the primary cause of disease during worm infection. Immunopathologic phenomena have been thoroughly investigated in infections with *Schistosoma* spp., acute schistosomosis is associated with Th1-like responses against adult parasites. The Th2-like responses, induced as a result of egg antigens secretion, downregulate the production and effector functions of Th1-like mediators. When Th2-like responses against the eggs were blocked experimentally, an exacerbated granuloma driven by Th1 and Th17 cells resulted in hepatic damage and death. Granulomatous responses evolve from an early Th1 to a sustained and dominant Th2-like response. Whereas tissue fibrosis stimulated by Th2-like cytokine (interleukin-13 (IL-13)) promotes tissue healing, excessive fibrosis may become pathogenic with loss of hepatic functions and portal hypertension (Cortés *et al.*, 2017). It seems that during trematode infections Th1-like responses are more protective than Th2-like responses against which these parasites have developed many escape mechanisms. Although Th1-like responses are closely associated with immunopathogenesis, Th2-like responses may also contribute to inflammatory damage. T-regulatory (T-reg) cells seem to regulate this detrimental immune response by suppressing the Th1-like response and by down-regulating any excessive Th2-like response during

granuloma formation. Protection against gastrointestinal nematodes and against tissue-dwelling trematodes is controlled by Th2- and Th1-like responses, the immune mechanisms, particularly those regulated by Th1-like cytokines, are responsible for considerable immunopathological damage and for the clinical signs observed during a helminthic disease. Even if the immune responses against most of helminthes are orchestrated by Th2-like cytokines, the worms are still able to persist in the host for a long time. Indeed, the immune response during the chronic phase of infection was recently reported to be a modified Th2-like response, that is, a Th2-like response associated with T-reg activity and the production of anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). The induction of immunomodulatory Th2/T-reg responses would allow the survival of both partners, by downregulating the host's inflammatory response and the immunopathological lesions observed during helminth infection and also the protective immune mechanisms directed against the parasite (Moreau and Chauvin, 2010).

2.6 Intestinal parasitic infections in Sudan:

Intestinal parasites are widely distributed in Sudan. Different studies were performed to determine the prevalence and related risk factors of intestinal parasitic infection and the resultant, prevalence of these infections significantly changes in terms of infection rate or risk factors (Bayoumi *et al*., 2018). 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 White Nile State by Suliman *et al.* (2019) who reported that the overall prevalence rate of intestinal parasitic infections among children was 56.9% and the commonest intestinal parasites were detected included *E. histolytica*, *G. lamblia* and *H. nana*. The most infected age group was 10-

13 years old. Females were more infected than males. In addition to a study conducted in Abugota Province- Gezira State by Bayoumi *et al.* (2018) who reported the highest prevalence rate (50.1%) was seen with *Giardia lamblia* followed by *Entamoeba histolytica/dispar* (39.8%), *Cryptosporidium* species (20.4%), *Hymenolepis nana* (14.8%), *Entrobius vermicularis* (1.2%) and *Schistosoma mansoni* (0.6%) respectively. The highest prevalence (36.3%) of parasites was seen in age under 12 years old. Other study conducted by Gamar *et al.* (2018) who showed that the overall prevalence rate of protozoan infections among food handlers in Khartoum State- Sudan was 20.26% while helminthic infections was 5.97%. Formal ether concentration technique was better for detection of intestinal parasites than direct fecal smear technique.

Chapter 3

Materials and methods

3.1 Study design:

It is a cross-sectional hospital based study.

3.2 Study area:

The study was conducted in Al-Rajhe hospital in Dar Al-Salm area, Ombada locality- Khartoum state.

3.3 Study duration:

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

3.4 Study population:

The study was carried out on the patients who were attended to Al-Rajhe hospital. The patients 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 patients.

3.6 Sampling:

Two hundred and seventy two fecal samples were collected from all patients. Collections were taken randomly by using simple random

sampling method. Fecal samples were examined by wet preparation, formal ether concentration technique, formal gasoline 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 Formal gasoline concentration technique (FGCT):

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 gasoline 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 (Ahmadi and Damraj, 2009).

3.8.4 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 according Zhu *et al.* (2010) as follow:-

Sensitivity= TP/ (TP+FN) ×100%

Specificity= TN/ (TN+FP) ×100%

TP= True positive

FN= False negative

TN= True negative

FP= False positive

3.11 Ethical consideration:

Approval of the study was taken from the College of Medical Laboratory Science- Sudan University of Science and Technology. Permission was taken from all patients or their guardians before being included in the study. Each patient was informed on the nature of the study.

Chapter 4

Results

4.1 General characteristics of study population:

The study was conducted on 272 study subjects, 109 (40.1%) were males and 163 (59.9%) were females (table 4.1). The age ranged between 1-75 years old with a mean age was 19± 15 years old. The age was divided into 5 groups as follow: ≤15, 16-30, 31-45, 46-60 and 61-75 years old. The frequency of each age group was 142 (52.2%), 82 (30.1%), 32 (11.8%), 7 (2.6%) and 9 (3.3%) respectively (table 4.2).

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

Gender	Frequency	Percentage (%)
Males	109	40.1%
Females	163	59.9%
Total	272	100.0%

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

Age groups (years)	Frequency	Percentage (%)
≤15	142	52.2%
16-30	82	30.1%
31-45	32	11.8%
46-60	7	2.6%
Over 61	9	3.3%
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, formal gasoline concentration technique and zinc sulphate floatation technique:

Out of 272 fecal samples, 93 (34.2%), 124 (45.5%), 116 (42.6%) and 77 (28.3%) were positive for intestinal parasitic infections by using wet preparation, formal ether concentration technique, formal gasoline 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, formal gasoline concentration technique and zinc sulphate floatation technique

Technique	No. of sample examined	Positive (%)
Wet preparation	272	93 (34.2%)
Formal ether concentration technique	272	124 (45.5%)
Formal gasoline concentration technique	272	116 (42.6%)
Zinc sulphate floatation technique	272	77(28.3%)

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

Out of 272 fecal samples, 113 (41.5%) were protozoa and 11 (4.0%) were helminthes. The study found that, highest prevalence rate (18.0%) of single parasitic infection was *Giardia lamblia* followed by *Entamoeba histolytica* (17.3%). While the lowest prevalence rate (2.2%) for *Hymenolepis nana*.

Prevalence rate of single commensals parasites was (3.7%). The highest prevalence rate (1.5%) of multiple parasitic infections (co-infections) was *G. lamblia+ H. nana* followed by the 1.1% rate for *G. lamblia+ E. histolytica* and the 1.1% rate for *G. lamblia+ E. coli*. The lowest prevalence rate (0.4%) of multiple parasitic infections was *E. histolytica+ E. coli* and the 0.4% rate for *E. histolytica+ H. nana* (table 4.4).

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

Single infection	No. of sample examined	Positive (%)
G. lamblia	272	49 (18.0%)
E. histolytica	272	47 (17.3%)
H. nana	272	6 (2.2%)
Commensals parasites (*E. coli*)	272	10 (3.7%)
Multiple infections		
G. lamblia+ E. histolytica	272	3 (1.1%)
G. lamblia+ E. coli	272	3 (1.1%)
E. histolytica+ E. coli	272	1 (0.4%)
G. lamblia+ H. nana	272	4 (1.5%)
E. histolytica+ H. nana	272	1(0.4%)

4.2.3 Distribution of intestinal parasitic infections according to the parasite species by using formal gasoline concentration technique:

Out of 272 fecal samples, 100 (36.8%) were protozoa and 16 (5.8%) were helminthes. The study found that, highest prevalence rate (16.5%) of single parasitic infection was *E. histolytica* followed by *Giardia lamblia* (14.3%). While the lowest prevalence rate (2.6%) for *Hymenolepis nana*. Prevalence rate of single commensals parasites was (3.7%). The highest prevalence rate (2.6%) of multiple parasitic infections (co-infections) was *G. lamblia+ H. nana* followed by the 0.7% rate for *G. lamblia+ E. histolytica*, the 0.7%

rate for *E. histolytica+ E. coli* and the 0.7% rate for *G. lamblia+ E. coli*. The lowest prevalence rate (0.4%) of multiple parasitic infections was *G. lamblia+ E. vermicularis* and the 0.4% rate for *E. histolytica+ H. nana* (table 4.5).

Table (4.5): Distribution of intestinal parasitic infections according to the parasite species by using formal gasoline concentration technique

Single infection	No. of sample examined	Positive (%)
G. lamblia	272	39 (14.3%)
E. histolytica	272	45 (16.5%)
H. nana	272	7 (2.6%)
Commensals parasites (*E. coli*)	272	10 (3.7%)
Multiple infections		
G. lamblia+ E. histolytica	272	2 (0.7%)
G. lamblia+ E. coli	272	2 (0.7%)
E. histolytica+ E. coli	272	2 (0.7%)
G. lamblia+ E. vermicularis	272	1 (0.4%)
G. lamblia+ H. nana	272	7 (2.6%)
E. histolytica+ H. nana	272	1 (0.4%)

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

Out of 272 fecal samples, 124 were positive for intestinal parasitic infections, from them 53 (42.7%) were males and 71 (57.3%) were females. *Giardia lamblia* was the most prevalent 31 (25.0%) among females than males 18 (14.5%), while *Entamoeba histolytica* was 27 (21.8 %) in females. *Hymenolepis nana* was 4 (3.2%) in females. Commensals species were *E. coli*, 5 (4.0%) in females and 5 (4.0%) in males. Prevalence rate of multiple parasitic infections was (2.4%) in males for *G. lamblia+ H.nana* and (1.6%) in males for *G. lamblia+ E. histolytica* and *G.*

lamblia+ E. coli. The difference in rate was found to be statistically insignificant at p. value= 0.609 (table 4.6).

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

	Gender	
Parasite species	Males (Positive %)	Females (Positive %)
G. lamblia	18 (14.5%)	31 (25.0%)
E. histolytica	20 (16.1%)	27 (21.8 %)
H. nana	2 (1.6%)	4 (3.2%)
Commensals (*E. coli*)	5 (4.0%)	5 (4.0%)
G. lamblia+ H. nana	3 (2.4 %)	1 (0.8%)
G. lamblia+ E. histolytica	2 (1.6%)	1 (0.8%)
G. lamblia+ E. coli	2 (1.6%)	1 (0.8%)
E. histolytica+ E. coli	0 (0%)	1 (0.8%)
E. histolytica+ H. nana	1 (0.8%)	0 (0%)
Total	53 (42.7%)	71(57.3%)

P. value= 0.609

4.2.5 Prevalence of intestinal parasitic infections according to gender by using formal gasoline concentration technique:

Out of 272 fecal samples, 116 were positive for intestinal parasitic infections, from them 51 (44.0%) were males and 65 (56.0%) were females. *Giardia lamblia* and *Entamoeba histolytica* were the most prevalent 25 (21.6%) among females than males 14 (12.1%) for *Giardia lamblia* and 20 (17.2%) for *Entamoeba histolytica. Hymenolepis nana* was 4 (3.4%) in females. Commensals species were 5 (4.3%) in females and males. Prevalence rate of multiple parasitic infections was (5.2%) in males for *G. lamblia+ H. nana*. *E. histolytica+ E.coli* were (1.7%) in females.

The difference in rate was found to be statistically insignificant at p. value= 0.365 (table 4.7).

Table (4.7): Prevalence of intestinal parasitic infections according to gender by using formal gasoline concentration technique

	Gender	
Parasite species	Males (Positive %)	Females (Positive %)
G. lamblia	14 (12.1%)	25 (21.6%)
E. histolytica	20 (17.2%)	25 (21.6 %)
H. nana	3 (2.6%)	4 (3.4%)
Commensals (*E. coli*)	5 (4.3%)	5 (4.3%)
G. lamblia+ H. nana	6 (5.2 %)	1 (0.9%)
G. lamblia+ E. vermicularis	0 (0%)	1 (0.9%)
G. lamblia+ E. histolytica	1 (0.9%)	1 (0.9%)
G. lamblia+ E. coli	1 (0.9)	1 (0.9%)
E. histolytica+ E. coli	0 (0%)	2 (1.7%)
E. histolytica+ H. nana	1 (0.9%)	0 (0%)
Total	51 (44.0%)	65 (56.0%)

P. value= 0.365

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

Out of 124 positive fecal samples, 84 (67.7%) were positive for age groups ≤ 15 years old, 22 (17.7%) were positive among age group 16-30 years old, 13 (10.5%) were positive among age group 31-45 years old, 5 (4.0%) were positive among age group 61-75 years old, there was no positive result in age group 46-60 years old. *Giardia lamblia* was most prevalent 33 (26.6%) among age group ≤ 15 years old, followed by 9 (7.3%) among age group 16-30, 5 (4.0%) among 31-45 years old and was lower 2 (1.6%) among age group 61-75 years old. *E. histolytica* was 31 (25.0%) in the age group ≤ 15 years old, 8 (6.5%) in the age group 16-30 years old, 6 (4.8%) in the age group 31-45 years old and was lower 2 (1.6%) among age group 61-75

years old. *H. nana* was 6 (4.8%) in the age group ≤ 15 years old. Commensals species were most prevalent 5 (4.0%) among age group ≤ 15 years old followed by 3 (2.4%) in the age group 16-30 years old and 1 (0.8%) in the age group 31-45 and 61-75 years old. *G. lamblia+ H. nana* were most prevalent 4 (3.2%) among age group ≤ 15 years old followed by *G. lamblia+ E. histolytica* were 3 (2.4%) among age group ≤ 15 years old. *G. lamblia+ E. coli* and *E. histolytica+ H. nana* were found at rate (0.8%) in age group ≤ 15 years old while (1.6%) for *G. lamblia+ E. coli* among age group 16-30 years old. *E. histolytica+ E. coli* were found at rate (0.8%) in age group 31-45 years old. The difference in rate was found to statistically insignificant at p. value= 0.538 (table 4.8).

Table (4.8): Prevalence of intestinal parasitic infections according to age groups by using formal ether concentration technique

	Age groups (years)			
Parasites species	≤15	16-30	31-45	61-75
G. lamblia	33 (26.6%)	9 (7.3%)	5 (4.0%)	2 (1.6%)
E. histolytica	31 (25.0%)	8 (6.5%)	6 (4.8%)	2 (1.6%)
H. nana	6 (4.8%)	0 (0%)	0 (0%)	0 (0%)
Commensals (*E. coli*)	5 (4.0%)	3 (2.4%)	1 (0.8)	1 (0.8%)
G. lamblia+ E. histolytica	3 (2.4%)	0 (0%)	0 (0%)	0 (0%)
G. lamblia+ E. coli	1 (0.8%)	2 (1.6%)	0 (0%)	0 (0%)
E. histolytica+ E. coli	0 (0%)	0 (0%)	1 (0.8%)	0 (0%)
G. lamblia+ H. nana	4 (3.2%)	0 (0%)	0 (0%)	0 (0%)
E. histolytica+ H. nana	1 (0.8%)	0 (0%)	0 (0%)	0 (0%)
Total	84 (67.7%)	22 (17.7%)	13 (10.5%)	5 (4.0%)

P. value= 0.538

4.2.7 Prevalence of intestinal parasitic infections according to age groups by using formal gasoline concentration technique:

Out of 116 positive fecal samples, 79 (68.1%) were positive for age groups ≤ 15 years old, 18 (15.5%) were positive among age group 16-30 years old,

14 (12.1%) were positive among age group 31-45 years old, 1 (0.9%) were positive among age group 46-60 years old and 4 (3.4%) were positive among age group 61-75 years old. *Giardia lamblia* was most prevalent 28 (24.1%) among age group ≤ 15 years old, followed by 5 (4.3%) among age group 31-45 years old, 4 (3.4%) among age group 16-30 years old and was lower 1 (0.9%) among age group 46-60 and 61-75 years old. *E. histolytica* was 28 (24.1%) in the age group ≤ 15 years old followed by 8 (6.9%) in the age group 31-45 years old, 7 (6.0%) in the age group 16-30 years old and was lower 2 (1.7%) among age group 61-75 years old. *H. nana* was 7 (6.0%) in the age group ≤ 15 years old. Commensals species were most prevalent 5 (4.3%) among age group 16-30 years old followed by 4 (3.4%) in the age group ≤ 15 years old and 1 (0.9%) in the age group 61-75 years old. *G. lamblia+ H. nana* were most prevalent 6 (5.2%) among age group ≤ 15 years old followed by *G. lamblia+ E. histolytica* were 2 (1.7%) in the age group ≤15 years old. *G. lamblia+ E. coli* were found at rate (0.9%) in age group ≤ 15 and 16-30 years old. *E. histolytica+ E. coli* were found at rate (0.9%) in age group ≤15 and 31-45 years old. *E. histolytica+ H. nana* were found at rate 1 (0.9%) in age group ≤15 years old. *G. lamblia+ H. nana* were found at rate (0.9%) in the age group 16-30 years old, while *E. vermicularis+ E. coli* were (0.9%) in the age group ≤ 15 years old. The difference in rate was found to statistically insignificant at p. value= 0.862 (table 4.9).

Table (4.9): Prevalence of intestinal parasitic infections according to age groups by using formal gasoline concentration technique

	Age groups (years)				
Parasites species	≤15	16-30	31-45	46-60	61-75
G. lamblia	28(24.1%)	4(3.4%)	5(4.3)	1(0.9%)	1(0.9%)
E. histolytica	28(24.1%)	7(6.0%)	8(6.9%)	0(0.0%)	2(1.7%)
H. nana	7(6.0%)	0(0)%	0(0)%	0(0)%	0(0)%
Commensals (*E. coli*)	4(3.4%)	5(4.3%)	0(0)%	0(0)%	1(0.9%)
G. lamblia+E. histolytica	2(1.7%)	0(0%)	0(0%)	0(0%)	0(0%)
G. lamblia+ E. coli	1(0.9%)	1(0.9%)	0(0%)	0(0%)	0(0%)
E. histolytica+ E. coli	1(0.9%)	0(0%)	1(0.9%)	0(0%)	0(0%)
G. lamblia+ H. nana	6(5.2%)	1(0.9%)	0(0%)	0(0%)	0(0%)
E. histolytica+ H. nana	1(0.9%)	0(0%)	0(0%)	0(0%)	0(0%)
E. vermicularis+ E. coli	1(0.9%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	79(68.1%)	18(15.5%)	14(12.1)	1(0.9%)	4(3.4%)

P. value= 0.862

4.2.8 Comparison between the four different methods used in detection of intestinal parasitic infections:

When formal ether concentration technique compared with wet preparation, 93 (34.2%) fecal samples were positive by two methods, while 31 (11.4%) 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. Also when formal ether concentration technique compared with formal gasoline concentration technique, 108 (39.7%) fecal samples were positive by two methods, while 16 (5.9%) were positive by formal ether concentration technique and negative by formal gasoline

concentration technique, 8 (2.9 %) were positive by formal gasoline concentration technique and negative by formal ether concentration technique. The difference in rate was found to be statistically highly significant at p. value= 0.000. Also when formal ether concentration technique compared with zinc sulphate floatation technique, 73 (26.8%) fecal samples were positive by two methods, while 51 (18.8%) were positive by formal ether concentration technique and negative by zinc sulphate floatation technique. 4 fecal samples were 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 4.10).

Table (4.10): Comparison between the four different methods used in detection of intestinal parasitic infections

		Formal ether concentration technique		Total	P. value
		Positive	Negative		
Wet preparation	Positive	93	0	93	P= 0.000
	Negative	31	148	179	
Total		124	148	272	
Formal gasoline concentration technique	Positive	108	8	116	P= 0.000
	Negative	16	139	155	
Total		124	148	272	
Zinc sulphate technique	Positive	73	4	77	P= 0.000
	Negative	51	144	195	
Total		124	148	272	

4.2.9 Sensitivity and specificity of wet preparation, formal gasoline 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 75.0% and 100% respectively (table 4.11). Formal gasoline concentration technique sensitivity and specificity were 87.1% and 94.6% respectively (table 4.12). Zinc sulphate floatation technique sensitivity and specificity were 58.9% and 97.3% respectively (table 4.13).

Table (4.11): Sensitivity and specificity of wet preparation

		Formal ether concentration technique	
		Positive	Negative
Wet preparation	Positive	93	0
	Negative	31	148

Table (4.12): Sensitivity and specificity of formal gasoline concentration technique

		Formal ether concentration technique	
		Positive	Negative
Formal gasoline concentration technique	Positive	108	8
	Negative	16	140

Table (4.13): Sensitivity and specificity of zinc sulphate floatation technique

		Formal ether concentration technique	
		Positive	Negative
Zinc sulphate technique	Positive	73	4
	Negative	51	144

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

Out of 124 positive fecal samples, 118 (95.2%) had abdominal pain and 6 (4.8%) had no abdominal pain. The difference in rate was found to be statistically significant at p. value= 0.000 (table 4.14).

Table (4.14): Prevalence of intestinal parasitic infections according to abdominal pain by using formal ether concentration technique

Abdominal pain	Formal ether concentration technique		Total
	Positive	Negative	
Yes	118	111	229
No	6	37	43
Total	124	148	272

P. value= 0.000

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

Out of 124 positive cases, 19 (15.3%) had constipation and 105 (84.7%) had no constipation. The difference in rate was found to be statistically significant at p. value= 0.000 (table 4.15).

Table (4.15): Prevalence of intestinal parasitic infections according to constipation by using formal ether concentration technique

Constipation	Formal ether concentration technique		Total
	Positive	Negative	
Yes	19	53	72
No	105	95	200
Total	124	148	272

P. value= 0.000

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

Out of 124 positive cases, 81 (65.3%) had diarrhea in fecal samples and 43 (34.7%) had no diarrhea. The difference in rate was found to be statistically significant at p. value= 0.000 (table 4.16).

Table (4.16): Prevalence of intestinal parasitic infections according to diarrhea by using formal ether concentration technique

Diarrhea	Formal ether concentration technique		Total
	Positive	Negative	
Yes	81	58	139
No	43	90	133
Total	124	148	272

P. value= 0.000

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

Out of 124 positive cases, 79 (63.7%) had mucus in fecal samples and 45 (36.3%) had no mucus in fecal samples. The difference in rate was found to be statistically significant at p. value= 0.000 (table 4.17).

Table (4.17): 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
	Positive	Negative	
Yes	79	23	102
No	45	125	170
Total	124	148	272

P. value= 0.000

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

Out of 124 positive cases, 47 (37.9%) had blood in fecal samples and 77 (62.1%) had no blood in fecal samples. The difference in rate was found to be statistically significant at p. value = 0.000 (table 4.18).

Table (4.18): Prevalence of intestinal parasitic infections according to presence of blood in the fecal samples by using formal ether concentration technique

Blood in fecal sample	Formal ether concentration technique		Total
	Positive	Negative	
Yes	47	2	49
No	77	146	223
Total	124	148	272

P. value= 0.000

4.2.15 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 124 positive cases, 57 (46.0%) were sometimes washing their hands before the meals and after use of toilet, 54 (43.5%) were always washing their hands before the meals and after use of toilet and 13 (10.5%) were not washing their hands before the meals and after use of toilet. The difference in rate was found to be statistically significant at p. value= 0.000 (table 4.19).

Table (4.19): 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
	Positive	Negative	
Sometimes	57	27	84
Always	54	99	153
No washing	13	22	35
Total	124	148	272

P. value= 0.000

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

Out of 124 positive cases, 93 (75.0%) were sometimes washing vegetables and fruits and 31 (25.0%) were always washing vegetables and fruits. The difference in rate was found to be statistically significant at p. value= 0.000 (table 4.20).

Table (4.20): 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
	Positive	Negative	
Sometimes	93	69	162
Always	31	78	109
No washing	0	1	1
Total	124	148	272

P. value= 0.000

4.2.17 Prevalence of intestinal parasitic infections according to eat and drink from handlers by using formal ether concentration technique:

Out of 124 positive cases, 50 (40.3%) were eat and drink from handlers and 74 (59.7%) were not eat and drink from handlers. The difference in rate was found to be statistically significant at p. value= 0.005 (table 4.21).

Table (4.21): Prevalence of intestinal parasitic infections according to eat and drink from handlers by using formal ether concentration technique

Eat and drink from handlers	Formal ether concentration technique		Total
	Positive	Negative	
Yes	50	36	86
No	74	112	186
Total	124	148	272

P. value= 0.000

4.2.18 Prevalence of intestinal parasitic infections according to source of drinking water by using formal ether concentration technique:

Out of 124 positive cases, 78 (62.9%) were drink from tap water and 46 (37.1%) were drink from tank water. The difference in rate was found to be statistically insignificant at p. value= 0.991 (table 4.22).

Table (4.22): Prevalence of intestinal parasitic infections according to source of drinking water by using formal ether concentration technique

Source of drinking water	Formal ether concentration technique		Total
	Positive	Negative	
Tap water	78	93	171
Tank water	46	55	101
Total	124	148	272

P. value= 0.991

Chapter 5

Discussion, conclusion and recommendations

5.1 Discussion:

From the results, it was obvious that the overall prevalence rate of intestinal parasitic infections was 45.5%. This rate was found to be lower than the 56.9% and 64.3% rate reported by Suliman *et al.* (2019) and Bayoumi *et al*. (2018) respectively. Protozoa were more prevalent (41.5%) than helminthes (4.0%). These findings were in agreement with the findings of Ahmed *et al*. (2017) who reported that 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, formal gasoline concentration technique and zinc sulphate floatation technique were 34.2%, 45.5%, 42.6% and 28.3% 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 the difference in rates was found to be statistically insignificant at p. value= 0.848. The present study showed that the formal gasoline and the formal ether sedimentation techniques detected 116 and 124 positive of total specimen, these findings were in disagreement with the findings of Ahmadi and Damraj (2009), who reported the formal gasoline and the formal ether sedimentation techniques detected 165 and 156 positive of total specimens respectively and gasoline proved to be as good as ether in concentrating parasite eggs and cysts, as well as in maintaining characteristic morphology. The results obtained from the present study showed that sensitivity of wet preparation was 75.0 % 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 sensitivity of wet

preparation was 61%. Formal gasoline concentration technique sensitivity and specificity were 87.1 % and 94.6% respectively, these findings were in disagreement with the findings of Ahmadi and Damraj (2009), who reported the formal gasoline concentration technique sensitivity and specificity to be 100.0% and 97.1% respectively. The results showed that the highest prevalence rate (18%) of single parasitic infection was reported with *G. lamblia* while the lower rate (2.2%) was reported with *H. nana* by using formal ether concentration technique. 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%). When using formal gasoline concentration technique, the results showed that the highest prevalence rate (16.5%) of single parasitic infection was reported with *E. histolytica* while the lower rate (2.6%) was reported with *H. nana*. These findings were in disagreement with the findings of Siddig *et al.* (2017) who showed that *G. lamblia* was the most prevalent parasite (46.4%) and in agreement with the findings of Muhajir *et al.* (2017) who showed that *E. histolytica* was the most prevalent parasite (15.5%). The highest prevalence rate (1.5%) of multiple parasitic infections was reported with *G. lamblia+ H. nana* by using formal ether concentration technique and (2.6%) by using formal gasoline concentration technique and the lower rate (0.4%) was reported with *E. histolytica+ E. coli* and *E. histolytica+ H. nana* by using formal ether concentration technique while by formal gasoline concentration technique, the lower rate (0.4%) was reported with *G. lamblia+ E. vermicularis* and *E. histolytica+ H. nana*. The present study revealed that the most commensals parasites were *E. coli* with a rate of 3.7% by the two methods. This rate was in disagreement with the rate reported by Quihui-Cota *et al.* (2017) who reported a 33% rate for *Endolimax nana* followed

by a 17% rate for *E. coli*. These variable results may be due to difference in environmental conditions, seasonableness and personal hygiene. From the investigation, the highest rate (67.7%) was reported with the age groups ≤ 15 years old, while the lower rate (4.0%) was reported with the age group 61-75 years old by using formal ether concentration technique. When using formal gasoline concentration technique, the highest rate (68.1%) was reported with the age groups ≤ 15 years old, while the lower rate (0.9%) was reported with the age group 46-60 years old. As far as gender was concerned, the results showed that females reported the highest rate (57.3%), while males reported a 42.7% rate. These rates were in agreement with the rates reported by Suliman *et al.* (2019) for females and males (35.6% and 21.3% 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 significant (p. value=0.000). Regarding the possible associated factors (the hands washing before the meals and after use of toilet, vegetables and fruits washing, eat and drink from handlers, the source of drinking water) with the infections, the study revealed that the difference in rates was found to be statistically significant except with the source of drinking water at p. value=0.991. 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 in Al-Rajhe hospital in Dar Al-Salam area was 45.5 %. The prevalence rate of intestinal protozoa was higher than intestinal helminthes. Also, the prevalence rate of single infection was higher than co-infections. *G. lamblia* and *E. histolytica* were the most common parasites detected in the study area. Formal ether concentration technique was the best method for the diagnosis of intestinal parasites and gasoline proved to be good in concentrating parasite eggs and cysts, as well as in maintaining characteristic morphology. Intestinal parasites were more prevalent among the age groups ≤ 15 years old and more prevalent among the females than the males.

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.
- In the absence of formal ether, the formal gasoline concentration technique should be recommended for the routine diagnosis of intestinal parasites.
- More studies should be done to investigate intestinal parasites in all Ombada localities.

References

Abdullah, I., Tak, H., Ahmad, F., Gul, N., Nabi, S. and Sofi, T. A. (2016). Predominance of gastrointestinal protozoan parasites in children: A brief review. *Journal of Health Education Research and Development*, **4** (4):194.

Ahmadi, N. A. and Damraj, F. (2009). A field evaluation of formalin-gasoline technique in the concentration of stool for detection of intestinal parasites. *Parasitology Research*, **104** (3):553-557.

Ahmed, M. H., Mohammed, A. M., Terkawy, I. Y., Anglo, A. H., Ismail, D. A., Mohammed, A. H., Karrar, T. I., Sid Ahmed, A. M. and Zarroug, K. S. M. (2017). Prevalence of gastro intestinal parasite among primary school sudanese children in Banat and Alhalanga villages in Kassala Sudan. *International Journal of Development Research*, **7** (12):17868-17871.

Arredondo, J. L., González, M. B., Coria, A. L., Ortega, J. E., Vargas, J., Villarreal, J. L. and Vallarta, M. M. (2014). *Entamoeba histolytica*: trophozoite, precyst and cyst studied by atomic force microscopy. *Advances in Scientific Research and Education*, **66** (451):153-160.

Babiker, M. A., Ali, M. S. M. and Ahmed, E. S. (2009). Frequency of intestinal parasites among food-handlers in Khartoum, Sudan. *Eastern Mediterranean Health Journal*, **4** (15):1098-1104.

Bayoumi, M., Abd, H., Kardaman, M., Talab, H. A. S., Alhidai, S. A., Ali, F. M. N., Mohamed, R. Y. H., Eissa, M. E. M. and Saeed, A. (2018). Prevalence of intestinal parasitic infections in Abugota province, Gezira state, Sudan. *European Academic Research*, **6** (6):2902-2916.

Bogitsh, B. J., Carter, C. E. and Oeltmann, T. N. (2012). Human Parasitology. 4th edition. 225 Wyman Street, Waltham, MA 02451, USA: Elsevier. pp. 245-247.

Brooks, G. F., Butel, J. S. and Morse, S. A. (2006). Medical Microbiology. 25th edition, United States. pp. 199-207.

Cabada, M. M., Morales, M. L., Lopez, M., Reynolds, S. T., Vilchez, E. C., Lescano, A. G., Gotuzzo, E., Garcia, H. H. and White, A. C. (2016). *Hymenolepis nana* impact among children in the highlands of Cusco, Peru: an emerging neglected parasite infection. *The American Journal of Tropical Medicine and Hygiene*, **95** (5):1031-1036.

Castro, G. A. (1996). Helminths: structure, classification, growth and development. **In Baron, S. (ed.**). Medical Microbiology. 4th edition, Galveston (TX): University of Texas Medical Branch at Galveston. Chapter 86. Available at: https://www.ncbi.nlm.nih.gov/books/NBK 8282/.

Centers for Disease Control and Prevention (CDC) (2015a). Biology. Available at: https://www.cdc.gov/parasites/nonpathprotozoa/biology.html.

Centers for Disease Control and Prevention (CDC) (2015b). Diagnosis and Detection. Available at: https://www.cdc.gov/parasites/giardia/index.html.

Centers for Disease Control and Prevention (CDC) (2016). About parasites. Available at: https://www.cdc.gov/parasites/index.html.

Centers for Disease Control and Prevention (CDC) (2017a). Amoebiasis. Available at: https://www.cdc.gov/dpdx/amebiasis/index.html.

Centers for Disease Control and Prevention (CDC) (2017b). Giardiasis. Avaliable at: https://www.cdc.gov/dpdx/giardiasis/index.html.

Centers for Disease Control and Prevention (CDC) (2017c). Hymenolepiasis. Avaliable at: https://www.cdc.gov/dpdx/hymenolepiasis/index.html.

Centers for Disease Control and Prevention (CDC) (2019). Biology. Available at: https://www.cdc.gov/parasites/pinworm/biology.html.

Cheesbrough, M. (2006). District laboratory practice in tropical countries. 2nd edition, United States: Cambridge University Press. pp. 195-199.

Cortés, A., Antoli. C. M., Esteban, J. G. and Toledo, R. (2017). Th2 and Th1 responses: Clear and hidden sides of immunity against intestinal helminthes. *Trends in Parasitology*, **33** (9):678-693.

El-Dib, N. A. (2017). *Entamoeba histolytica*: an overview. *Tropical Medicine in the Mediterranean Region.* DOI 10.1007/s40475-017-0100-z.

Elfaki, T. E. M., Osman, S. A. E. and AbdAlla, A. B. (2015). Prevalence rate of intestinal parasites in Mayo Area, Khartoum State, Sudan. *European Academic Research*, **3** (8):9346-9357.

Farthing, M. J. G. (1993). Pathogenesis of giardiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**(3):17-21.

Fever, B. (2012). Giardiasis. *The Center for Food Security and Public Health*, **50** (11):1-13.

Gamar, T. A., Musa, H. H. and Altayb, H. N. (2018). Prevalence of intestinal parasites among food handlers attending public health laboratories in Khartoum State, Sudan. *F1000Research*. **7**:687. Available at: https://doi.org/10.12688/f1000research.14681.1.

Hailu, T. and Abera, B. (2015). Performance evaluation of direct saline stool microscopy, formal ether concentration and Kato Katz diagnostic methods for intestinal parasitosis in the absence of gold standard methods. *Tropical Doctor*, **45** (3):178-182.

Hawash, Y. A., Dorgham, L. S., Amir, E. M. and Sharaf, O. F. (2015). Prevalence of intestinal protozoa among Saudi patients with chronic renal failure: A case-control study. *Journal of Tropical Medicine*, **2015**:1-9. Available at: https://doi.org/10.1155/2015/563478.

Hooshyar, H., Rostamkhani, P., Arbabi, M. and Delavari, M. (2019). *Giardia lamblia* infection: review of current diagnostic strategies. *Gastroenterology Hepatology Bed Bench*, **12**(1):3-12.

Issa, R. (2014). Non-pathogenic protozoa. *International Journal of Pharmacy and Pharmaceutical Sciences*, **6** (3):30-40.

Kardaman, M., Bayoumi, M., Nykwac, O., Mans, U., Alshammari, E. Sandström, G., Saeed, A. and Abd, H. (2016). Intestinal parasitic infections in school students in Malakal City, Upper Nile State- South Sudan. *SOJ Microbiology and Infectious Diseases*, **4** (1):1-5.

Kim, B. J., Song, K. S., Kong, H. H., Cha, H. J. and Meesunock, M. (2014). Heavy *Hymenolepis nana* infection possibly through organic foods. *Korean Journal of Parasitolog*, **52**:85-87.

Levy, D. A. and Frondoza, C. (**1983).** Immunity to intestinal parasites: role of mast cells and goblet cells. *Federation Proceedings Journal*s. **42**(6):1750-5.

Mahmoudvan, H., Taee, N., Faraji, G. M. and Ebrahimzadeh, F. (2018). Prevalence and risk factors of intestinal protozoan infections in children (2-15 years old) from Lorestan Province, Western Iran. *Tropical Biomedicine*, **35** (1):259-266.

Marty, G. (2017). Giardiasis. Rijeka: Intech Open. Available at: http://dx.doi.org/10.5772/intechopen.70338.

Mergani, M. H., Mohammed, M. A., Khan, N., Bano, M. and Khan, A. (2014). Detection of intestinal protozoa by using different methods. *Dentistry and Medical Research*, **2** (2):197-202.

Moreau, E. and Chauvin, A. (2010). Immunity against helminthes: interactions with the host and the inter-current infections. *Journal of Biomedicine and Biotechnology*, **2010**:1-9. Available at: https://doi.org/10.1155/2010/428593.

Muhajir, A. M. A., Hajissa, K., Mohamed, Z. and AbdelAal, A. A. (2017). Prevalence of intestnal parasitic infection among children in Al-Kalakla, Khartoum-Sudan.*World Applied Sciences Journal*, **35** (2):219-222.

Noor-Azian, M. Y., San, Y. M., Gan, C. C., Yusri, M. Y., Nurulsyamzawaty, Y., Zuhaizam, A. H., Maslawaty, M. N., Norparina, I. and Vythilingam, I. (2007). Prevalence of intestinal protozoa in an Aborigine community in Pahang, Malaysia. *Tropical Biomedicine*, **24** (1):55-62.

Oguoma, V. M. and Ekwunife, C. A. (2008). The need for a better method: comparison of direct smear and formal-ether concentration technique in diagnosing intestinal parasites. *The Internet Journal of Tropical Medicine*, **3** (2). Available at: http://www.ispub.com/ostia/index.php?xmlFilePath=journals/ijtm/vol3n2/formol.xml.

Open Epi (2003). Documentation for sample size for a proportion. Avaliable at: https://www.openepi.com/PDFDocs/SSProporDoc.pdf.

Pearson, R. D. (2018). *Hymenolepis nana* (dwarf tape worm) infection. Merck Manual. Available at: https://www.merckmanuals.com/en-ca/professional/infectious-diseases/cestodes-tapeworms.

Petri, W. A. and Singh, U. (1999). Diagnosis and management of amoebiasis. *Clinical Infectious Diseases*, **29**(5):1117-1125.

Punsawad, C., Phasuk, N., Bunratsami, S., Thongtup, K., Viriyavejakul, P., Palipoch, S., Koomhin, P. and Nongnaul, S. (2018). Prevalence of intestinal parasitic infections and associated risk factors for

hook worm infections among primary school children in rural areas of Nakhon Si Thammarat, southern Thailand. *BMC Public Health*, **18** (1):1118.

Quihui-Cota, L., Morales-Figueroa, G. G., Javalera-Duarte, A., Ponce-Martínez, J. A., Valbuena-Gregorio, E. and López-Mata, M. A. (2017). Prevalence and associated risk factors for *Giardia* and *Cryptosporidium* infections among children of northwest Mexico: a cross-sectional study. *BMC Public Health*, **17** (1):852.

Rawla, P. and Sharma, S. (2019). *Enterobius vermicularis* (pin worm). In: Stat Pearls. Treasure Island (FL): Stat Pearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK536974/.

Sadaf, H., Khan, S., Kanwal, N., Tasawer, B. and Ajmal, S. (2013). A review on diarrhoea causing *Hymenolepis nana*-dwarf tapeworm. *International Research Journal of Pharmacy*, **4** (2):32-35.

Sard, B. G., Navarro, R. T. and Sanchis, J. G. E. (2011). Amebas intestinales no patógenas: una visión clinicoanalítica. *Enfermedades Infecciosasy Microbiología Clínica*, **29**(3):20-28.

Siddig, H. S., Mohammed, I. A., Mohammed, M. N. and Bashir, A. M. (2017). Prevalence of intestinal parasites among selected group of primary school children in Alhag Yousif Area, Khartoum, Sudan. *Health Sciences*, **6** (8):125-131.

Sodeman, W. A. Jr. (1996). Intestinal protozoa: amebas. **In Baron, S. (ed.).** Medical Microbiology. 4th edition, Galveston (TX): University of Texas Medical Branch at Galveston. Chapter 79. Available at: https://www.ncbi.nlm.nih.gov/books/NBK7742.

Suliman, M. A., Magboul, A. M., Mohammed, H. Y., Tamomh, A. G., Bakhit, H. A., Altoum, S. A. and Ahmed, S. M. (2019). Prevalence of intestinal parasitic infections and associated risk factors among school

children in White Nile State, Sudan. *Journal of Infectious Diseases and Diagnosis*, **4**:100-125.

Tanyuksel, M. and Petri, W. A. (2003). Laboratory diagnosis of amebiasis.*Clinical Microbiology Rev*iews, **16** (4):713-29.

Wakelin, D. (**1996).** Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston. pp. 81-91.

Wendt, S., Trawinski, H., Schubert, S., Rodloff, A. C., Mössner, J. and Lübbert, C. (2019). The diagnosis and treatment of pin worm infection. *Deutsches Arzteblatt International*, **116** (13):213-219.

World Health Organization (WHO) (2019). Epidemiology. Available at: https://www.who.int/intestinal_worms/epidemiology/en/.

Zhu, W., Zeng, N. and Wang, N. (2010). Sensitivity, specificity, accuracy, associated confidence interval and ROC analysis with practical SAS implementations. *Health care and Life Sciences*, **19**:67.

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 ( )

- **Eat and drink from handlers:**

Yes ( ) No ( )

- **Vegetables and fruits washing:**

Sometimes ( ) Always ( ) No washing ( )

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

Sometimes ( ) Always ( ) No washing ( )

- **Source of drinking water:**

Tap water ( ) Tank water ( )

Laboratory results:

- Wet preparation result:

.....

.....

- Formal ether concentration technique result:

……

……....

- Formal gasoline concentration technique result:

……

……....

- Zinc sulphate floatation technique result:

……

……