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Prevalence of Intestinal Parasitic Infections and Its Association with Anaemia and other Risk Factors among Pregnant Women Attending Antenatal Care in Yifag Health Centre

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BY

MINICHIL LIYIH

A THESIS SUBMITTED TO THE DEPARTMENT OF BIOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS DEGREE IN BIOLOGY (BIOMEDICAL SCIENCES) OF BAHIR DAR UNIVERSITY .

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AUGUST 2020

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I hereby certify that I have supervised, read, and evaluated this thesis entitled Prevalence of Intestinal Parasitic Infections and Its Association with Anaemia and other Risk Factors among Pregnant Women Attending Antenatal Care in Yifag Health Center by Minichil Liyih prepared under my guidance. I recommend the thesis be submitted for oral defense.

Destaw Damtie (PhD)	____________	August,2020
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APPROVAL OF THESIS FOR DEFENSE RESULT

We here by certify that we have examined this thesis entitled €Prevalence of Intestinal Parasitic Infections and Its Association with Anaemia and other Risk Factors among Pregnant Women Attending Antenatal Care in Yifag Health Centre• by Minichil Liyih. We recommend that the thesis is approved for the degree of €Master of Science in Biomedical Sciences.•

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DECLARATION OF AUTHOR

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/ ACRONYMS

ANC	Antenatal Care
CDC	Centers for Disease Control and Prevention
DHS	Demographic and Health Survey
g/dl	Gram per decilitre
Hgb	Haemoglobin
ICF	International Classification of Functioning
IPIs	Intestinal Parasitic Infections
L1	Larval stage one
NTDs	Neglected Tropical Diseases
SPSS	Statistical package for social science
STHs	Soil Transmitted Helminths
UNICEF	United Nations International Children,s Educational Fund
WHO	World Health Organization
YHC	Yifag Health Centre
ƒm	Micrometer
„^{2}	Chi-square

ABSTRACT

Intestinal parasites are distributed worldwide and are widely prevalent in tropical and subtropical countries. Intestinal parasitic infections (IPIs) sometimes result in anaemia. In Ethiopia, anaemia in pregnant women is the main concern. The objective of this study was to determine the prevalence of intestinal parasitic infections, their associated risk factors and its association with anaemia among pregnant women attending antenatal care at Yifag Health Center. A cross-sectional study was conducted from November 2019 to March 2020. The data were collected by questionnaire interview technique, collecting the stool samples, and blood samples from each pregnant woman. Wet mount and formol ether concentration techniques were applied to identify the IPIs. Data were analysed using SPSS version 25 and p value <0.05 was taken as statistically significant. A total of 280 pregnant women were selected using convenient random sampling technique a response rate of 99%. The prevalence of IPIs among pregnant women was 53.4% (95% CI: 47.37, 59.42). Taenia species (18.1%) was the predominant followed by Giardia lamblia 12.6%, Entamoeba histolytica (9.4%), hookworms (9%), Ascaris lumbricoides (4%), Schistosoma mansoni (3.2%), Hymenolepsis nana (0.7%), Strongyloides stercoralis (0.4%), and Enterobius vermicularis (0.4%). Eating raw meat (AOR = 1.779; 95% CI: 1.070, 2.959; p = 0.026) was the only associated risk factor for the overall prevalence of IPIs. However, eating raw vegetables (AOR= 2.72; 95% CI: 1.27, 5.85; P=0.010) and poor personal hygiene (AOR= 402; 95% CI: 1.46, 11.07; P=0.007), were associated risk factor of G. lamblia. Eating raw meat ((AOR = 2.477; 1.252, 4.902; P=0.009) was associated risk factors for Taenia species. Anaemia was determined by hematocrit using heparinized hematocrit tube. The result was read by using hematocrit reader and the result is divided by three to get the haemoglobin concentration. The prevalence of anaemia among the study participants was 10.1% (95% CI: 6.8, 14.3%). The majority of them (8.7%) were mild anaemic and the rest 1.4% moderately anaemic. The prevalence of anaemia was not significantly associated with IPIs in this study. The prevalence of IPIs was high which indicating; still health burdens on pregnant women. The high prevalence of anaemia needs intervention. Avoiding eating raw meat, strengthening sanitation and hygiene programs, routine deworming of pregnant mothers, and iron supplementation are recommended to reduce the burden of IPIs and anaemia among pregnant women.

Keywords: Anaemia, Associated Risk Factors, Intestinal Parasites, Pregnancy, Prevalence, Yifag Health Center

1. INTRODUCTION

1.1 Background of the study

Intestinal parasites infections (IPIs) are described as a group of diseases caused by one or more species of protozoa and helminths. *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), *Schistosoma mansoni*, *Taenia species*, *Hymenolepsis nana*, *Enterobius vermicularis*, *Strongyloides stercoralis*, *Entamoeba histolytica*, and *Giardia lamblia* are the common IPIs species reported globally (WHO, 2010). Several infectious diseases caused by some members of these organisms are taken as Neglected Tropical Diseases (NTDs) (WHO, 2010).

The global burden of IPIs reaches 3.5 billion with over 450 million and 200,000 annual morbidities and mortalities respectively (Saki *et al*., 2016) Globally, about 300 million people suffer from severe helminth infections, leading to morbidity and over 150,000 deaths annually as cited in (Aranzales *et al*., 2018). Amoebiasis caused by *E. histolytica* is the cause of 40,000 to 100,000 deaths per year, whereas giardiasis is the main cause of parasitic diarrhea worldwide and an important cause of waterborne disease outbreaks as cited in (Aranzales *et al*., 2018).

Intestinal parasitic infection (IPIs) is very common in Ethiopia and the magnitude of the infections varies from place to place (Hylemariam Mihiretie *et al*., 2017; Amelo Bolka and Samson Gebremedhin, 2019). The most frequent IPIs in Ethiopia are *A. lumbricoides*, hookworm, *S. mansoni*, *H. nana*, *T. trichiura*, and *Tania* species (Agumas Ayalew *et al*., 2019), *E. histolytica*, and *G. lamblia* (Getaneh Alemu *et al*., 2019). The prevalence of IPIs among pregnant women ranges from 12.0% (Alemayehu Bekele *et al*., 2016) to 70.6% (Berhanu Elfu and Tadesse Hailu, 2018). Consequences of these infections are; compromised growth, cognitive impairment, malnutrition, anaemia, poor immunity in infants, mucosal loss and lymphatic leakage, and local hemorrhages (Aranzales *et al*., 2018; Abiye Tigabu *et al*., 2019).

Factors associated with IPIs are poor personal hygienic practices, lack of shoe wearing habit, lack of clean and safe water, high population density, poor waste disposal, noncompliance with health standards, poor post defecation hand washing, incorrect fingernail trimming, eating raw food, poor knowledge about IPIs, and lack of hand wash before and after feeding (Dawit Jember et al., 2015; Esmael Besufikad et al., 2017; Berhanu Elfu and Tadesse Hailu, 2018). Pregnant women are also at high risk of parasitic infection due to their close relationship with children (Berhanu Elfu and Tadesse Hailu, 2018).

Anaemia in pregnancy defined as the haemoglobin (Hb) concentration of less than 11g/dl in the body, which reduces oxygen-carrying capacity of the red blood cells to tissues (WHO, 2001; WHO, 2011). Anaemia could be classified as mild, moderate, and severe. The Hb levels for each class of anaemia in pregnancy are 10.0-10.9g/d1 (mild), 7-9.9g/dl (moderate), and <7g/dl (severe) (WHO, 2011). If the prevalence of anaemia above 5.0%, it considered as significance public health problem (WHO, 2008). The causes of anaemia during pregnancy are micronutrient deficiencies of iron, foliate, and vitamins A and B12 and anaemia due to parasitic infections such as malaria and hookworm or chronic infections like TB and HIV (Brooker et al., 2008; Okube et al., 2016). Globally anaemia affected more than 1.62 billion people of which 56 million are pregnant women (WHO/CDC, 2008). Sub-Saharan Africa is the most affected region, with anaemia estimated prevalence of 17.2 million among pregnant women, which makes up 30% of total global cases (Susan and Blackburn, 2007). Anaemia accounts 20% of maternal death worldwide (Driskell, 2008). In Ethiopia, 29% of pregnant women are anaemic (Central Statistical Agency of Ethiopia and ICF (2017). Anaemia causes for sever shock on pregnant women and associated with stillbirth, prematurity and low birth weight (Elliott et al., 2011).

In the present study area, lack of personal hygiene, low latrine system, lack of clean water source for hygiene, poor environmental sanitation, open defecation, and walking on barefoot were common among the local people. Mostly these problems are associated with IPIs (Adane Derso et al., 2016). IPIs, mostly, hookworm infection causes anaemia especially in children and pregnant women (Hylemariam Mihiretie et al., 2017). To reduce the consequence of parasitic infection during pregnancy, studying the prevalence of intestinal parasitic infections among pregnant women is very important. Therefore, the present study was intended to determine the prevalence of IPIs, their associated risk factors and its association with anaemia among pregnant women attending antenatal care in YIFAG health centre.

1.2 Statement of the problem

Intestinal parasitic infections caused by pathogenic helminth and protozoan species are endemic throughout the world especially in developing countries (Sah and Maharjan, 2017; Daniel Gebretsadik et al., 2018). About one-fourth of the world's population is infected with intestinal parasites (WHO, 2018). The distribution of IPIs depends on many factors such as low socio-economic status, poor sanitation and personal hygiene, and lack of clean water (Satoskar et al., 2009). In Ethiopia, the prevalence of intestinal parasites varies from area to area among pregnant women. About 38.7% of pregnant women from Wondo Genet area, southern Ethiopia (Amelo Bolka and Samson Gebremedhin, 2019), 41% of Gilgel Gibe dam area, Southwest Ethiopia (Million Getachew et al., 2012), and 43.8% from Lalo Kile district, Oromia, Western Ethiopia (Dejene Abraham et al., 2019) were positive for IPIs. In Amhara region the prevalence of IPs were high in pregnant women. For instance, in Mecha district, Northwest Ethiopia, IPIs prevalence was 70.6% (Berhanu Elfu and Tadesse Hailu, 2018), in Bahir Dar, northwest Ethiopia 31.5% (Adane Derso et al., 2016) and at Anbesame health center, Northwest Ethiopia 21.1% (Melashu Balew et al., 2017). IPIs affect haemoglobin level in pregnant women (Berhanu Elfu and Tadesse Hailu, 2018). This has indirect effect on the growth of the fetus (Tadesse Hailu et al., 2019). Studies by Hylemariam Mihiretie et al., (2017) and Meaza Lebso et al., (2017) in Ethiopia show that IPIs are associated with anaemia. In the present study area, open field defecation, open field waste disposal, poor environmental and personal hygiene, bathing in river water, and lack of shoe wearing were common. And also there was not study conducted on pregnant women about prevalence of intestinal parasitic infections and its association with anaemia in the present study area. Therefore, the purpose of this study was to determine the prevalence of intestinal parasites, associated risk factors, and its association with anaemia among pregnant women who were attending antenatal care at Yifag Health Center.

1.3 Objectives

1.3.1 General objective

To determine the prevalence of intestinal parasitic infections and their associated risk factors as well as the association of IPIs with anaemia among pregnant women attending antenatal care at Yifag Health Center.

1.3.2 Specific objectives

- To determine prevalence of intestinal parasitic infections among pregnant women attending antenatal care at Yifag Health Center
- To determine the associated risk factors of intestinal parasitic infections among pregnant women attending antenatal care at Yifag Health Center.
- To determine the prevalence of anemia among pregnant women visiting Yifag Health Center for antenatal care.
- To determine the association of the prevalence of intestinal parasitic infections with anaemia among pregnant women taking antenatal care at Yifag Health Center.

1.4 Significance of the study

The findings from this study would provide information about the status of IPIs and anaemia among pregnant women in Amhara region, would serve as a spring board for Libo Kemkem District health officers and regional health officers to take actions to save pregnant women and their children, and will provide baseline information for further studies

1.5 Limitation of the Study

Use of single season data, use of only wet mount and formol ether concentration techniques to identify IPIs, taking stool samples only in the day time which may affect E. vermicularis load, and use of only hematocrit haemoglobin concentration to determine haemoglobin (not CBC and haemcue) were the limitations of this study

2. REVIEW LITERATURE

2.1 Human Intestinal Parasites

Globally, over 3.5 billion people are affected by IPIs. These infections contribute to high global health burden and are causes of 450 million clinical morbidities, the majority of whom are from developing countries (Alum et al., 2010).

Intestinal helminths are classified into three major groups; nematodes such as A. lumbricoides and hookworms, S. stercoralis, T. trichiura and E. vermicularis; Trematodes (S. mansoni), and cestodes (Taenia solium and Taenia saginata) (Ridley, 2012). Helminthic infections, hookworm, trichuriasis, and schistosomiasis contribute to severe anemia in patients through blood loss and micronutrient deficiencies (Berhanu Elfu and Tadesse Hailu, 2018).

A lot of protozoa live in the gastrointestinal tract of humans and are causes of human infections. Protozoa are unicellular organisms, under Kingdom Protista (Ridley, 2012). E. histolytica and G. lamblia are recognized as the two most common parasitic protozoa of major public health concern (Dadi Marami et al., 2018; Habtamu Sewunet and Daniel Tekelia, 2019).

2.1.2 Intestinal Protozoan Parasites

2.1.2.1. Amoebiasis

Amoebiasis is caused by E. histolytica/dispar. Amoebae are simple protozoan without fixed shape. Pseudopodium (locomotary structure) is formed by thrusting out its cytoplasm. Besides locomotion, pseudopodia are used to engulf food by phagocytosis (Satoskar et al., 2009).

Epidemiology and risk factors of Amoebiasis

Amoebiasis occurs worldwide and the highest incidence and prevalence is in areas with poor sanitation (Piort et al., 2015). This parasite infects more than 500 million people worldwide and results 75,000-100,000 deaths per year (Mehlhorn, 2016). The prevalence of E. histolytica is relatively high in Ethiopia. For example, it was 8% in Southern Ethiopia (Amelo Bolka and Samson Gebremedhin, 2019) and 40.9% in Northwest Ethiopia (Tadesse Hailu et al., 2019).

The risk factors for amoebiasis are, drinking contaminated water, eating contaminated foods, association with food handlers whose hands are contaminated, anal sexual practices, contaminated medical devices such as colonic irrigation devices, malnourishment, corticosteroid medications, pregnancy, young age, and travelling to endemic areas (Ridley, 2012).

Life cycle of *E. histolytica*

There are only two stages to the life cycle of *E. histolytica*, the infectious cyst stage and the multiplying, disease-causing trophozoite stage (Figure 1). Cysts typically contain four nuclei (Satoskar *et al*., 2009). Cysts are carried to the lower ileum, and excyst and trophozoites reach the lumen of the colon, multiply, invade and destroy the tissue of the colon wall. Occasionally if trophozoites enter the circulatory systems, they invade and multiply in other organs such as the lungs, the liver, and the brain (Despommier *et al*., 2017).

Figure 1: Life cycle of *E. histolytica*

(Source: paniker and Ghosh, 2018)

Signs and symptoms of amoebiasis

Diarrhoea, flatulence, and cramping are complaints of symptomatic patients. The severe disease shows the passing of numerous bloody stools in a day. Systemic signs of infection such as fever, leukocytosis, and rigors are present in patients with extra-intestinal amebiasis. Pain over the liver with haepatomegaly and elevation of the diaphragm is also observed (Dawit Assafa *et al.*, 2006).

Prevention and control of amoebiasis

The prevention mechanisms of amoebiasis are sanitation measures, education about its routes of transmission, and avoiding eating raw vegetables especially grown by sewerage irrigation and night soil (Dawit Assafa *et al.*, 2006). Metronidazole is the choice drug for symptomatic amebiasis and Iodoquinol for asymptomatic infection (Despommier *et al.*, 2017)

2.1.1.2. Giardiasis

Giardiasis in humans is caused by a single-celled flagellate called *G. lamblia*. *G. lamblia* lives in the duodenum and upper jejunum and is the only protozoan parasite found in the lumen of the human small intestine which can cause diarrhoea (Paniker and Ghosh, 2013).

Epidemiology and risk factors of *G. lamblia*

It is the most common protozoan pathogen worldwide (Paniker and Ghosh, 2013). *G. lamblia* infects over 200 million people worldwide with 50,000 annual incidences (Barda *et al.*, 2013). It is very high in areas with low sanitation, especially in the tropics and subtropics (Fitsum Mardu *et al.*, 2019). Visitors to such places frequently develop traveler's diarrhea caused by giardiasis through contaminated water (Paniker and Ghosh, 2018). The prevalence of *G. lamblia* was 14.1% in Venezuela (Rodr†‡guez-Morales *et al.*, 2006), 39% in Papua New Guinea (Phuanukoonnon *et al.*, 2013), 19.2% in northwest, Ethiopia (Tadesse Hailu *et al.*, 2019), 12.6% in southern Ethiopia (Fekede Weldekidan *et al.*, 2018), and 13.3% in Bahir Dar, northwest Ethiopia (Adane Derso *et al.*, 2016).

Humans acquire infection by ingestion of cysts in contaminated water and food. Ingestion of as far as 10 cysts is sufficient to cause infection in a man. Direct person-to-person transmission may also occur in children, male homosexuals, and mentally ill people (Ridley, 2012). Enhanced susceptibility to giardiasis is associated with blood group A, achlorhydria, use of cannabis, chronic pancreatitis, and malnutrition (Paniker and Ghosh, 2018)

Life cycle of *G. lamblia*

G. lamblia has a simple life cycle consisting of two stages; trophozoite and cyst (Figure 2). The cyst released from the host through faeces into the environment which infects other hosts (Satoskar *et al.*, 2009). After ingestion, the cyst hatches out into two trophozoites, which multiply successively by binary fission and colonize in the duodenum. The trophozoites live in the duodenum and upper part of jejunum, feeding by pinocytosis (Paniker and Ghosh, 2018).

Figure 2: Life cycle of *G. lamblia*.

(Source: Despommier *et al.*, 2017).

Signs and symptoms of giardiasis

Symptomatic giardiasis ranges from mild diarrhoea to intensive malabsorption syndrome. Usually, the onset of the disease is sudden and consists of foul smelling, watery diarrhoea, abdominal cramps, flatulence, and streatorrhoea. Sometimes blood and pus are present in stool specimens, a feature consistent with the absence of tissue destruction (Ridley, 2012).

Diagnosis of giardiasis

Giardiasis can be detected by identification of cysts of *G. lamblia* in the formed stools and the trophozoites and cysts of the parasite in diarrheal stools. On microscopic examination, faecal specimens containing *G. lamblia* may have an offensive odour, are pale coloured and fatty, and float in water. Cysts and trophozoites can be found in diarrheal stools by saline and iodine wet preparations. Often multiple specimens need to be examined and a concentration technique like formol-ether is used for the detection of cyst. In asymptomatic carriers, only the cysts are seen (Paniker and Ghosh, 2018). Other like enterotest (string test) is useful method for obtaining duodenal specimen. And serodiagnosisis antigen detection enzyme-linked immunosorbent assay, immunochromatographic strip tests and indirect immunofluorescence tests using monoclonal antibodies have been developed for detection of Giardia antigens in faeces (Paniker and Ghosh, 2018)

Prevention and control of Giardiasis

The prevention mechanism of giardiasis are avoiding contaminated food and water, proper waste disposal, use of latrine, and practice of good personal hygiene (Dawit Assafa et al., 2006; Paniker and Ghosh, 2018). Boiling of water is required because chlorination of water is ineffective for inactivating cysts (Paniker and Ghosh, 2018). The choice of treatment is metronidazole but forbidden during pregnancy unless symptomatic (Dawit Assafa et al., 2006). Paromomycin can be given to symptomatic pregnant women (Paniker and Ghosh, 2018).

2.1.2 Intestinal Nematode Parasites

Nematodes are non-segmented roundworms, and are among the most abundant life forms on earth. Most parasitic nematodes are highly host specific and cannot survive in any other host

(Despommier *et al.*, 2017). According to Jeevitha *et al.*, (2014) over two billion people are infected with soil-transmitted helminthes (STHs) globally.

2.1.2.1 Ascariasis

Ascariasis, a soil-transmitted infection, is caused by *A. lumbricoides*. The adult Ascaris worms reside in the lumen of the small intestine where they feed on predigested food. Their life span ranges from 10 to 24 months. The adult worms are covered with a shell composed of collagens and lipids. This outer covering helps protect them from being digested by intestinal hydrolases. They also produce protease inhibitors that help them to prevent digestion by the hosts, enzymes (De Silva *et al.*, 2003).

Epidemiology and risk factors of ascariasis

Globally at least 819 million people were infected with *A. lumbricoides* (Pullan *et al.*, 2014). The prevalence of *A. lumbricoides* high in countries of Africa and Asia (40%) and Latin America (32%) (Bogitsh *et al.*, 2013). The prevalence of *A. lumbricoides* is different from countries to countries. For instance, its prevalence in Nigeria was 52.2% (Ivoke *et al.*, 2017), Venezuela 57.0% (Rodr†‡guez-Morales *et al.*, 2006), Bahir Dar, northwest Ethiopia 2.9% (Adane Derso *et al.*, 2016), East Wollega, Oromia, Ethiopia 6.5% (Hylemariam Mihiretie *et al.*, 2017), Gilgel Gibe Dam area, southwest Ethiopia 15% (Million Getachew *et al.*, 2013), Maytsebri primary hospital, north Ethiopia the prevalence 12.7% (Menasbo *et al.*, 2019), Wondo Genet district, Southern Ethiopia 24.9% (Amelo Bolka and Samson Gebremedhin, 2019), and Mecha district, Northwest Ethiopia 32.2% (Berhanu Elfu and Tadesse Hailu, 2018). Contaminated soil, keeping hands in the mouth, and eating contaminated vegetables, are common source of infection (Bogitsh *et al.*, 2013).

Life cycle of *A. lumbricoides*

An adult female can lay up to 200,000 unebryonted fertilized eggs. A freshly laid egg contains only the egg cell (Mehlhorn, 2016). Embryonation takes place after exist from the host through faeces in the soil. The first stage larvae (L1) which are the infective stage develop into the second stage larvae (L2) in the embryonated egg. The L2 larvae are stimulated to hatch by a combination of alkaline conditions in the small intestine, and the solubilisation of certain outer layers of the egg shell, facilitated by bile salts (Despommier *et al.*, 2017). The hatched parasite lives in the lumen of the intestine penetrates the intestinal wall and is transferred to the liver through the portal circulation. Then it migrates to the heart via the bloodstream and to pulmonary circulation. The larva moults twice, enlarges and breaks into the alveoli of the lung. Then they pass up through the bronchi and into the trachea, are swallowed and reach the small intestine once again. Within the small intestine, the parasites moult twice more and mature into adult worms. The adult worms mate, although egg production may precede mating (Satoskar *et al.*, 2009)(Figure 3).

Figure 3: Life cycle of *A. lumbricoides*

(Source: Despommier *et al.*, 2017)

Signs and symptoms of ascariasis

The pathogenesis of *A. lumbricoides* adult worms in the intestine cause abdominal pain and intestinal obstruction especially in children. Larvae in the lungs may cause inflammation of the lungs and pneumonia-like symptoms (Dawit Assafa et al., 2006). Eosinophilia is often concurrently observed with a set of signs that may include coughing, dyspnea, and a mild fever. The intestinal phase includes abdominal discomfort and bloating along with nausea, vomiting, pains, and diarrhea may occur (Paniker and Ghosh, 2018)

Diagnosis of ascariasis

Adult worm can be detected in stool or sputum of patient by the naked eyes. Barium meal may reveal the presence of adult worm in the small intestine. Definitive diagnosis of ascariasis is made by demonstration of eggs in faeces (Dawit Assafa et al., 2006). A single female may account for about three eggs per mg of faeces. At this concentration, the eggs can be readily seen by microscopic examination of a saline emulsion of faeces. Both fertilized and unfertilized eggs are usually present. Occasionally, only one type is seen. The fertilized eggs may sometimes appear decorticated (Paniker and Ghosh, 2018).

Prevention and control of ascariasis

Ascariasis can be eliminated by preventing faecal contamination of the soil. The A. lumbricoides egg is highly resistant so, night soil destruction of the eggs is ensured by proper composting. Avoid eating raw vegetables, good personal hygiene, and treatment of infected persons are advisable (Paniker and Ghosh, 2018). The choices of drugs for treatment are albendazole, mebendazole, or ivermectin. These medications are contraindicated in pregnancy; however, pyrantel pamoate is safe in pregnancy (Despommier et al., 2017).

2.1.2.2 Hookworm Infection

Hookworm infection in humans is caused by an infection with the helminth nematode parasites N. americanus and A. duodenale. The hookworm thrives in warm soil where temperatures are over 18°c and average rainfall is more than 1000mm a year. They exist primarily in sandy or loamy soils and cannot live in clay or muck (Hotez et al., 2005).

Epidemiology and risk factors of hookworm infection

About 1.2 billion people infected by hookworm worldwide (Bala, 2010) and 3000 to 6500 deaths occur from hookworm related diseases annually worldwide (Hotez, 2011). It common in poverty areas and the most affected regions are Asia and sub-Saharan Africa (Hotez, 2008; Walana et al., 2014).

Hookworm transmission occurs when third-stage infective filariform larvae come into contact with skin; usually during walking with barefoot (CDC, 2010).

The prevalence of hookworm varies from country to country. For instance, its prevalence in Venezuela 8.1% (Rodr†‡guez-Morales et al., 2006), Kenya 3.92% (Wekesa et al., 2014), Uganda 40.5% (Chami et al., 2015), and Nigeria it was 44.4% (Ivoke et al., 2017). In Ethiopia, in Wondo Genet district, southern, Ethiopia was 11.2% (Amelo Bolka and Samson Gebremedhin, 2019), Azezo Health Center Gondar town, Northwest Ethiopia hookworm 4.7% (Meseret Alem et al., 2013), Mecha district, northwest, Ethiopia was 14.2% (Berhanu Elfu and Tadesse Hailu, 2018), Bahir Dar, Norwest, Ethiopia was 5.5% (Adane Derso et al., 2016), Lalo Kile district, Oromia, Western Ethiopia 33.7%, and Northwest Ethiopia was 50% (Tadesse Hailu et al., 2019).

Life cycle of hookworm

The filariform larva (L3) (infective) and adult worm inhabiting the small intestine of man attach themselves to the mucous membrane by means of mouth parts (Paniker and Ghosh, 2018). The female worm lays eggs which freshly pass in faeces and deposit in the soil. The embryo develops inside the eggs optimally in sandy loamy soils, with decaying vegetation under a warm, moist, and shady environment (Paniker and Ghosh, 2018). In about two days, a rhabditiform larva hatches out of the egg which feeds on bacteria and other organic matter in the soil and grows in size (Figures 7). It moults twice, on the third and fifth days after hatching to become the L3 which can live in the soil for 5-6 weeks (Figure 7) (Despommier et al., 2017). When a person walks barefooted on the soil containing the filariform larvae, they penetrate the skin and enter the subcutaneous tissue. The larva may penetrate the buccal mucosa to reach the venous circulation and complete their migration via the lungs (Mehlhorn, 2016). Inside the human body, the larvae are carried along the venous circulation to the right side of the heart and to the lungs, (Hotez 2008). Here, they escape from the pulmonary capillaries into the alveoli, migrate up the respiratory tract to the pharynx, and are swallowed, reaching the small intestine. During migration or on reaching the esophagus, they undergo third moulting. They grow in size, and undergo a fourth and final moulting in the small intestine and develop the buccal capsule, attach themselves to the small intestine, and grow into adults (Paniker and Ghosh, 2018)

Figure 4: Life cycle of hookworm

(Source: Paniker and Ghosh, 2018)

Signs and symptoms of hookworm infection

Larvae may give rise to severe itching at the site of penetration. Creeping eruption formed due to subcutaneous migration of filariform larvae, mild transient pneumonitis, or bronchitis occurs when larvae break out of pulmonary capillaries into alveoli (Paniker and Ghosh, 2018) Epigastric pain, dyspepsia, nausea, vomiting, and diarrhea, iron deficiency anemia and protein energy malnutrition are common (Paniker and Ghosh, 2018).

Diagnosis of hookworm infection

Demonstration of oval segmented hookworm eggs in faeces by direct wet microscopy or by concentration methods is the best method of diagnosis (Satoskar *et al*., 2009). Adult hookworms may sometimes be seen in faeces (Paniker and Ghosh, 2018). Eggs of *A. duodenale* and *N. americanus* cannot be differentiated by morphology. Thus, specific diagnosis can only be made by studying morphology of adult worms. Duodenal contents may reveal eggs or adult worms; Harada-Mori method of stool culture is carried out to demonstrate L3 larvae which help in distinguishing *A. duodenale* and *N. americanus* (Satoskar *et al*., 2009; Paniker and Ghosh, 2018)

Prevention and control of hookworm infection

Preventing soil pollution by faeces; proper disposal of night soil; use of sanitary latrines; not to walk barefoot and environmental sanitation are the preventing mechanisms (CDC, 2013). The choices of drugs are albendazole or mebendazole, pyrantel pamoate is also effective and can be used during pregnancy (Paniker and Ghosh, 2018)

2.1.2.4 Strongyloidiasis

It is caused by *S. stercoralis* that has a widespread distribution throughout the tropics and subtropics (Satoskar *et al*., 2009). Mainly found in the warm moist tropics, but may also occur in the temperate regions. It is common in Brazil, Columbia, and in the Far East Myanmar, Thailand, Vietnam, Malaysia, and Philippines (Paniker and Ghosh, 2018). The number of people infected with this nematode is unknown, but estimates range from 30 million to 100 million (Satoskar *et al*., 2009). Its prevalence varies from country to country and from region to region. For example, among pregnant women the prevalence was 1.3% in Nigeria (Ivoke *et al*., 2017), 3% Papua New Guinea (Phuanukoonnon *et al*., 2013), and 3.3% Venezuela (Rodr†‡guez-Morales *et al*., 2006). In Ethiopia it showed lower pattern among pregnant women. For instance, in the study conducted in East Wollega, Oromia, Ethiopia 0.3% (Hylemariam Mihiretie *et al*., 2017), in Bahir Dar, northwest Ethiopia 1.6% (Adane Derso *et al*., 2016), and in Mecha district, Northwest Ethiopia (6.4%) (Berhanu Elfu and Tadesse Hailu, 2018).

Morphology of S. stercoralis

The female is thin, transparent, 2.5 mm long and 0.05 mm wide opening through the anus situated ventrally (Figure 9A). The reproductive system contains paired uteri, vagina, and vulva.

In gravid female, uteri contain thin walled transparent ovoid eggs (Paniker and Ghosh, 2018). The male is shorter and broader measuring 1.6 mm in length and 40-50 µm in width with copulatory spicules that penetrate the female during copulation (Figure 9B). The male cannot be seen in human infection because it has not penetrating power (Paniker and Ghosh, 2018).

Eggs are oval and clearly visible within the uterus of gravid female. 10 eggs arranged anteroposteriorly in a single row each uterus (Fig. 9A and 9C) (Paniker and Ghosh, 2013). The first stage Rhabditiform Larva measures 0.25 mm in length, with short muscular double bulb esophagus (Figure 9D). Filariform larva is long and slender and measures 0.55 mm in length with a long esophagus of uniform width and notched tail (Figure 9E) (Paniker and Ghosh, 2018).

Figure 5: Adult female (A), and male (B), Egg of S. Stercoralis (C), Rhabditiform Larva (D) and Filariform larva (E)

(Source: Paniker and Ghosh, 2018)

Life cycle of S. stercoralis

The life cycle of S. stercoralis is complex because of the multiplicity of pathways through which it can develop (Figure 10). Man is its natural host but dogs and cats are found infected with morphologically indistinguishable strains. Filariform larva is the infective form that enters by penetration of the skin and passes into the circulation (Paniker and Ghosh, 2018). In the blood stream, the larvae are transported to the right heart, lungs, trachea, and pharynx where they are swallowed and mature into adult worms in only two weeks (Ridley, 2012). The female produces embryonated eggs that upon hatching release rhabditiform larvae into the intestine. Rhabditiform larvae moult twice to develop into filariform larvae (infective stage) (Ridley, 2012). The embryonated eggs laid in the mucosa hatch immediately to rhabditiform larvae which migrate into the lumen of the intestine and passes down the gut to be released in faeces (Paniker and Ghosh, 2018). The rhabditiform larvae passed in stools develop in moist soil into free living males and females and they mate in the soil and produce eggs and non infective larvae that then develop into infective larvae upon incubation in the soil (Ridley, 2012). The individual worm has a lifespan of 3 or 4 months, but because it can cause autoinfection, the infection may persist for years (Paniker and Ghosh, 2018)

Figure 6: Life cycle of S. stercoralis

(Source: Paniker and Ghosh, 2018)

Symptoms of strongyloidosis

Generally strongyloidosis infection is benign and asymptomatic. Blood eosinophilia and larvae in stools are being the only indications of infection (Ridley, 2012). It may cause severe and even fatal clinical manifestations, particularly in those with defective immune responses. The clinical disease may have cutaneous at the site of penetration. Pruritis and urticaria, particularly around the perineal skin and buttocks, are symptoms of chronic strongyloidiasis (Satoskar *et al*., 2009)
In the intestinal manifestations mucus diarrhea is often present. In heavy infection, the mucosa may be honey- combed with the worm and there may be extensive sloughing, causing dysenteric stools (Paniker and Ghosh, 2018)

Laboratory diagnosis

Direct wet mount of stool by demonstration of rhabditiform larvae in freshly passed stools is the most important method of specific diagnosis (Paniker and Ghosh, 2018). Concentration methods of stool by formol ether or Baermann,s funnel gauze method may be employed to examine for larvae more efficiently (Satoskar *et al*., 2009).

Prevention and treatment of strongyloidiasis

It can be prevented by prevention of contamination of soil with faeces, avoiding barefooted walking, avoiding contact with infective soil and contaminated surface waters, and treatment of all cases (Paniker and Ghosh, 2018). The drug of choice for treatment of strongyloidiasis is ivermectin, albendazole, and thiabendazole (Satoskar *et al*., 2009; Paniker and Ghosh, 2018)

2.1.2.5. Enterobiasis

Enterobiasis is caused by *E. vermicularis* also called pinworm which has the largest geographical distribution of any helminth (Satoskar *et al*., 2009). Unlike the usual situation, where helminthic infections are more prevalent in the poor people of the tropics; it is common in the affluent nations in the cold and temperate regions (Satoskar *et al*., 2009). The prevalence *E. vermicularis* in different countries show patchy pattern. For example its prevalence in Gilgel Gibe Dam area, southwest Ethiopia was 0.3% (Million Getachew *et al*., 2013), Nigeria 2% (Egwunyenga *et al*., 2001) and 3.5% (Alli *et al*., 2011), Kenya 4.8% (Wekesa *et al*., 2014), Venezuela 6.3% (Rodr†‡guez-Morales *et al*., 2006), and Iraq 32.9% (Al-Hamairy *et al*., 2013).

Morphology

The female is large may reach a length of 7 to 13 mm, making the adult females easily visible in the stools (Figure 11A) (Ridley, 2012). The male worm is 2-5 mm long and 0.1-0.2 mm thick; posterior end is tightly curved ventrally, sharply truncated and carries a prominent copulatory spicule (Figure 11B) (Paniker and Ghosh, 2018). The egg is oval but flattened on one side and has a thick and colorless shell (Figure 11C). Larvae may be visible inside the egg due to the colorless shell of the embryonated eggs (Figure 11D) (Ridley, 2012; Paniker and Ghosh, 2018).

Figure 7: Adult female (A), male (B), egg (C), and embryo (D) of *E. vermicularis* (Source: Paniker and Ghosh, 2013)

Life cycle and transmission of *E. vermicularis*

E. vermicularis is passing its entire life cycle in the human host (natural host) which has no intermediate host and does not undergo any systemic migration. Most infections originate from soiled fingers or embryonated eggs containing larva ingesting by means of contaminated fingers or autoinfection (Ridley, 2012). But it may occur through contaminated food, bedding, clothing, or other personal items. Eggs laid on perineal skin containing infective larvae are swallowed and hatch out in the intestine. They moult in the ileum and enter the caecum and mature into adults. Within two weeks to two months, the ingested eggs develop into gravid females which are ready to lay eggs (Paniker and Ghosh, 2018). Then gravid females migrate down the colon to the rectum. At night in bed, the worm comes out through the anus and crawls on the perineal to lay their sticky eggs (Figure 12) (Satoskar et al., 2009). After all the eggs are laid, the female worm dies or gets crushed by the host during scratching (Paniker and Ghosh, 2018). These Patients have eggs of *E. vermicularis* on fingers and under nails leading to autoinfection and occurs from anus to mouth. In retro infection process, the eggs laid on the perineal skin immediately hatch into the infective stage larva and migrate through the anus to develop into worms in the colon. This mode of infection occurs from anus to colon (Paniker and Ghosh, 2018)

Figure 8: Life cycle of *E. vermicularis*

(Source: Paniker and Ghosh, 2018)

Laboratory Diagnosis

The €Scotch tape test• is the easiest and effective means of diagnosing enterobiasis infection. Applying a piece of transparent cellophane tape over a tongue blade into the perineal folds late at night or before daylight affords the best opportunity of recovering evidence of infection and examined microscopically (Ridley, 2012). The adult worm will also be found on the slide along with the characteristic ova (Paniker and Ghosh, 2018)

Symptoms

Symptoms range from asymptomatic to the following signs and symptoms. An itchy anus due to an inflammatory response to the adult worm, especially at night is the most frequent symptom (Ridley, 2012). In addition to this irritability in children with nervousness and disruptive behavior, severe scratching of anus, female children may also have an itchy and inflamed vagina. Adult worms may be seen in the stool, eggs may be seen with the naked eye, clinging to the skin around the anus but, mostly do not contribute to abdominal pain (Paniker and Ghosh, 2018)

Prevention and treatment

Maintenance of hygiene is the main prevention mechanisms of enterobiasis (Paniker and Ghosh, 2018). The choices of drugs are pyrantel pamoate which can be used in pregnancy, albendazole can be used for single dose therapy, and piperazine has to be given daily for one week. It is necessary to repeat the treatment after two weeks to take care of autochthonous infections and ensure elimination of all worms. As pinworm infection usually affects a group, it is advisable to treat the whole family or group of children, as the case may be (Paniker and Ghosh, 2018)

2.1.3 Intestinal cestodes (tapeworm) parasites

The most important cestodes of clinical interest and that pathogenic to man are *T. solium* and *T. saginata*. Cestodes have a worldwide distribution regardless of climate and environmental conditions. The infection occurred by tapeworms is called taeniasis (Ridley, 2012). The incidence is higher in developing countries due to lack of sanitation and food safety laws. The prevalence of *Taenia* species among pregnant women is low in different studies. For example in Iran 0.014% (Saki et al., 2016), Nigeria 2.1%, Azezo Health Center Gondar town, Northwest Ethiopia 1% (Meseret Alem et al., 2013), Bahir Dar, northwest Ethiopia 0.8% (Adane Derso et al., 2016), and in East Wollega, Oromia, Ethiopia 1.3% (Hylemariam Mihiretie et al., 2017).

Life cycle *Taenia* species

Larval cysts of the tapeworm are ingested with poorly cooked infected meat that leads to a disease called cysticercosis. Upon ingestion, the larvae escape the cysts and pass to the small intestine where they attach to the mucosa. The proglottids develop as the worm matures in the intestines over a period of three to four months. Eggs produced by the proglottids can persist on vegetation for several days and may be consumed by cattle or pigs, in which they hatch and form cysticerci, which may then be passed to other animals (Figure 13) (Ridley, 2012).

Figure 9: Life cycle of *Taenia* species
(Source: Ridley, 2012).

Signs and symptoms of taeniasis

People with these infections may complain of epigastric pain, abdominal discomfort, diarrhea, weight loss, hunger sensation, and vomiting (Dawit Assafa *et al*., 2006).

Diagnosis of *Taenia* species

Recovery of the gravid segments is the most reliable diagnostic method of *Taenia* species 15-20 lateral branches in *T. saginata*, and less than 13 branches in *T. solium* (Ridley, 2012). Eggs of *Taenia* species can be detected by microscopic examination, formol-ether concentration method of stool, and cellophane swab method in patients are also used (Paniker and Ghosh, 2018)

Prevention and control of taeniasis

Avoiding contact with potential accumulations of human faeces close to latrines, parking places and wild camping grounds are the main prevention mechanisms. Cooking of meat above 57^{0}C and proper disposal of human excrete are advisable. The choices of drugs are Niclosamide and Mebendazole (Dawit Assafa et al., 2006).

2.1.4 Trematodes (flatworms)

All trematodes are parasitic but not all species are found in humans; mammals are hosts for infections by these flatworms (Ridley, 2012).

2.1.4.1 Schistosomiasis

Intestinal schistosomiasis is caused by *S. mansoni* in human. The male size is between 1-1.4 cm in length and which is covered by coarse tubercles which have 6-9 testes and the female is 1.5-2.0 cm in length. The ovary is present in the anterior third and Vitelline glands occupy the posterior two-thirds. It lays about 100-300 eggs daily, and the uterus is short containing few ova. Adult worms reside in pairs: the female laying in gynecophoral canal of the male (Ridley, 2012).

Epidemiology and risk factors of schistosomiasis

Globally about 2 billion people are affected in the topics and subtropics, 779 million people are at risk and 200 million are infected (WHO, 2006). The prevalence of *S. mansoni* in Ethiopia shows patchy pattern. For example, in Bahir Dar, northwest Ethiopia it was 2.9% (Adane Derso et al., 2016), Anbesame health centre, northwest, Ethiopia 2.2% (Melashu Balew et al., 2017), wondo Genet, southern Ethiopia 2.3% (Amelo Bolka and Samson Gebremedhin, 2019), and Mecha district, northwest, Ethiopia 17.4% (Berhanu Elfu and Tadesse Hailu, 2018).

People who live or travel in areas where schistosomiasis is endemic and who are exposed to or swim in standing or running freshwater wherever the appropriate type of Biomphalaria snails are present, are at risk for infection. Transmission is intermittent, but under the right circumstances only a brief (<2 minute) exposure can result in infection (Satoskar et al., 2009).

Life cycle of schistosomes

The life cycle of the schistosomes requires fresh water in order to infect a host. Man is infected by cercaria in fresh water by skin penetration (Ridley, 2012). After entering, the cercaria travel through the venous vessels of their human hosts to the heart, lungs, and portal routes of circulation. In three weeks, these forms mature and reach the mesenteric vessels where they live and ovulate for the duration of the host's life (Satoskar *et al.*, 2009). Eggs germinate as they pass through the vessel wall to the intestine and are excreted in faeces. In fresh water, the larval miracidia hatch out of the egg and swim until they find an appropriate snail (Figure 14). After two generations of multiplication in the snail, the fork-tailed cercariae emerge into the water and infect another human (Ridley, 2012).

Figure 10: Life cycle of schistosomes
(Source: Ridley, 2012)

Signs and symptoms of schistosomiasis

Patients infected with *S. mansoni* suffer from cercarial dermatitis, dysentery, blood in stool with tenesmus, enlargements of the spleen and liver (Dawit Assafa *et al.*, 2006). Signs and symptoms include high fever, rash, arthralgia, and eosinophilia (Paniker and Ghosh, 2018).

Diagnosis of schistosomiasis

Stool microscopy of eggs with lateral spines may be demonstrated in stools (Dawit Assafa et al., 2006). Concentration methods may be required when infection is light. Kato-Katz thick smear provides quantitative data on the intensity of infection (Paniker and Ghosh, 2018). Proctoscopic biopsy of rectal mucosa may reveal eggs when examined as fresh squash preparation between two slides (Paniker and Ghosh, 2018)

Prevention and control of schistosomiasis

Prevention mechanisms are safe water supply, avoid urination and defecation in canals, avoid contact with canal water, periodic clearance of canals from vegetation, manual removal of snails and their destruction, use of natural enemies to the snails to kill the snails (Dawit Assafa et al., 2006). The choice of treatment is Praziquantel (Paniker and Ghosh, 2018)

2.2 Epidemiology of Human Intestinal Parasitic Infections

2.2.2 Global epidemiology of human IPIs

Today, intestinal parasites are among the major contributors to the global disease load and death. Globally 800–1000 million cases of *A. lumbricoides*, 700–900 million cases of *N. americanus* and *A. duodenale*, and 500 million cases of *T. trichiura* (Wekesa et al., 2014), 200 million cases of *G. lamblia*, and 500 million cases of *E. histolytica* (Pullan et al., 2014) were reported. Pregnant women in different parts of the world face diverse parasitic challenges of intestinal parasites. For example, in Venezuela, the overall prevalence of IPIs was 73.9% of which *A. lumbricoides* prevalence was 57%, *T. trichiura* 36%, *G. lamblia* 14.1%, *E. hystolitica* 12%, *N. americanus* 8.1%, and *E. vermicularis* 6.3% (Rodríguez-Morales et al., 2006). In Bogotá, Colombia, the overall prevalence of intestinal parasitism in pregnant women were 41% with 9% polyparasitism (Aranzales et al., 2018), whereas, in southwest Iran, the overall prevalence was low 4.8% of which *G. lamblia* was more prevalent 3.16% followed by *E. histolytica* 0.7%, *Taenia* species 0.014%, and *E. vermicularis* (0.007%) (Saki et al., 2016). Infectious and parasitic diseases represented the sixth biggest causes of morbidity in Brazil in 2014, corresponding to 7.28% of hospital morbidity in the period (Santos et al., 2017), however, in Nepal the prevalence of intestinal parasitic infection among pregnant women was found to be 35% out of which *A. lumbricoides* was 30.5%, *E. histolytica* 2.5% and *T. trichiura* 1 % (Sapkota and Maharjan, 2017); in Kenya the prevalence of IPIs was 13.8% of which Ascariasis was the most prevalent 6.5%, followed by hookworm 3.9%, and trichuriasis 1.3% (Wekesa *et al*., 2014)

2.2.3 Epidemiology of Human Intestinal Parasitic Infections in Ethiopia

Intestinal parasitic infection is very common in Ethiopia and the magnitude of infection varies from area to area. For instance, in Lalo Kile district, Oromia, western Ethiopia the overall prevalence of intestinal parasitic infection among pregnant women was 43.8% with the predominance of hookworm 33.7% followed by *A. lumbricoides* 7.3% (Dejene Abraham *et al*., 2019), in Wondo Genet district, Southern Ethiopia the prevalence of IPIs was 38.7% which *A. lumbricoides* was the predominant infection (24.9%), followed by hookworms (11.2%), *G. lamblia* (5.4%), *E. histolytica* (3.4%), *T. trichiura* (2.9%), and *S. mansoni* 2.3% (Amelo Bolka and Samson Gebremedhin (2019). Furthermore, in Anbesame health centre, northwest Ethiopia, 21.1% pregnant women were IPIs positive (Melashu Balew *et al*., 2017); in Bahir Dar town, northwest Ethiopia it was 31.5% of which the most predominant intestinal parasites were *G. lamblia* 13.3% followed by *E. histolytica* 7.8%, hookworm 5.5%, *A. lumbricoides* 2.9%, *S. mansoni* 2.9%, *S. stercolaris* 1.6%, *Taenia* spp. 0.8% and *Hymenolepis nana* 0.3% in descending order (Adane Derso *et al*., 2016). However, IPIs prevalence was higher in Maytsebri primary hospital, North Ethiopia (51.5%), of which the most common prevalent parasites were hookworm (40.09%) followed by *A. lumbricoides* (12.7%) and *T. trichiura* (1.12%) (Menasbo *et al*., 2019). Moreover, in Mecha district, Northwest Ethiopia, prevalence of IPIs were 70.6% where *A. lumbricoides* was the most prevalent 32.7% followed by *S. mnsoni* 17.4%, hookworm 14.2%, and *S. stercolaris* 6.4% (Berhanu Elfu and Tadesse Hailu, 2018). Furthermore, the prevalence was 15.3% in Urban Area of Eastern Ethiopia (Kefyalew Addis and Abdulahi Mohamed, 2014), 17.8% in Adigrat General Hospital, Tigrai, northern Ethiopia (Brhane Berhe *et al*., 2019), 19% in Wolayita Sodo Town, Southern Ethiopia (Lealem Gedefaw *et al*., 2015).

2.3 Anaemia

Globally, 41.8% of the pregnant women and close to one third of pregnant women (30.2%) are anaemic (Ndegwa, 2019). Hgb level below 11g/dl in pregnancy is considered as anaemic (WHO, 2011). Anaemia could be classified as mild, moderate, and severe. The Hgb levels for each class of Anaemia in pregnancy are 10-10.9g/d1 (mild), 7-9.9g/dl (moderate), and <7g/dl (severe)(WHO, 2011). IPIs have devastating effects on the level of Hgb and cause anaemia since they affect iron absorption by the intestine and consume the red blood cells (Tadesse Hailu *et al*., 2019). The study by Wubet Worku *et al*. (2018) showed that most of the anaemic pregnant women were described as a moderate anaemic and a study done by Tadesse Hailu *et al*. (2019) showed mild anaemia, and the finding in Gilgel Gibe dam area, Southwest Ethiopia showed 55% mild, 42.1% moderate, and 2.9% severe anaemia (Million Getachew *et al*., 2012). Furthermore, prevalence of anaemia was 7.9% in Adigrat, northern Ethiopia (Brhane Berhe *et al*., 2019), 11.6% at St. Paul,s Hospital, Addis Ababa, Ethiopia (Angesom Gebreweld and Aster Tsegaye, 2018), 15% at Adama Hospital Medical Collage, Adama, Ethiopia (Bizuneh Ayano and Befekadu Amentie, 2018). Moreover, the prevalence was 1.3% in Tikur Anbessa Specialized Hospital, Addis Ababa Ethiopia (Alemayehu Hailu and Tewabech Zewde, 2013), 23.2% in Southern Ethiopia (Meaza Lebso *et al*., 2017), 30.5% in Dera District, northwest Ethiopia (Terefe Derso *et al*., 2017), 31.5% in Ilu Abba Bora Zone, South West Ethiopia (Adamu Kenea *et al*., 2018), 56.8% in Urban Area of Eastern Ethiopia (Kefyalew Addis and Abdulahi Mohamed, 2014), 63% in Papua New Guinea (Phuanukoonnon *et al*., 2013).

2.4 Associations between intestinal parasites and anaemia

There are so many factors that cause anaemia during pregnancy. IPIs are the major factor in developing countries (Lealem Gedefaw *et al*., 2015; Sapkota and Maharjan, 2017). The causes of anaemia during pregnancy include; Iron, Folate, Vitamin B12, and Vitamin A deficiencies. During pregnancy women might suffer with anaemia during the first trimester, second trimester, as well as third trimester (Lealem Gedefaw *et al*., 2015). Studies showed that hookworm infection is highly associated with anaemia (Sapkota and Maharjan, 2017; Amelo Bolka and Samson Gebremedhin, 2019). But parasites such as *A. lumbricoides*, *H. nana*, *T. trichiura*, *E. histolytica*, *T. trichiura*, and *S. stercoralis* are also prevalent among the anaemic pregnant women (Sapkota and Maharjan, 2017). Studies in Wolayita Sodo Town, southern Ethiopia (Lealem Gedefaw *et al*., 2015) and Gondar town (Meseret Alem *et al*., 2013) also showed association of IPIs with anaemia among pregnant women.

3. MATERIALS AND METHODS

3.1 Study area

The study was conducted among pregnant women attending ANC in Yifag Health Centre. YHC is found in Libo Kemkem district, South Gondar Zone, North West Ethiopia (Figure 15). The geographical location of Yifag town is at 12° 5' 0'' latitude north and 37° 44'0'' longitude east with an elevation between 1829 and 1868 metres above sea level (Figure 15). It is about 77 kms east of Bahir Dar, the capital city of Amhara region and 645 kms northwest of the capital city of Ethiopia, Addis Ababa. The mean rainfall is 1330 ml and the mean temperature ranges from 22-30°C. There are five Kebeles (lowest administrative units) whose residents are getting service from the health centre namely; Yifag, Bura, Ginaza, Shina and Yifag Akababi. The estimated total population of these Kebeles is 37031 of whom 18515 were females and 18516 were males (CSA, 2007). Around 8732 of the females were between the ages 15-49 (CSA, 2007). Most of the residents of the study area are farmers who use mixed agriculture. The main cultivated crops are rice, maize, chickpea, oats (rye), and little irrigation practice of onion and garlic. The sources of drinking water in the study area are pump and pipe water but most people use river water for hygienic purposes. The sanitary facilities in the study area are poor, open latrine systems is common. There are six health posts and one health center in the study area.

Figure 11: Location map of the study Yifag town.

3.2 Study design and period

Health center based cross sectional study was conducted from November 30, 2019 to March 07, 2020.

3.3 Source population

All the women visiting Yifag Health Center were considered as source population

3.4. Study population

The study population were all pregnant women who came to Yifag Health Centre for antenatal care during the study period.

3.5. Inclusion and exclusion criteria

Inclusion criteria

Women who showed willingness and ability to give informed consent and had no anti-parasitic treatment for the last three weeks were included as respondents in this study.

Exclusion criteria

Women who were not voluntary to be part of this study, who were taking antiparasitic drugs during the study period, or women who took medication within three weeks, period were excluded from the study.

3.6 Sampling technique and sampl determination

Sample size was calculated using single population proportion formula considering 95% CI and P 0.21 (Melashu Balew et al., 2017) and adding 10% assumption of non response rate as follows;

$n = Z_{(\alpha/2)}^2 P(1-P)/d^2 = 280$; where n is sample size, $Z\alpha/2 = 1.96$ for the standard scale of 95% level of confidence, d is the marginal error which is 0.05. Finally, 280 pregnant women were enrolled by convenient.

3.7. Method of data collection

Stool samples, blood samples, and structured questionnaires were used for data collection.

3.7.1 Collection of stool samples

The pregnant women were advised properly and given clean labeled collection cups along with applicator sticks, and from each mother about four gram of fresh stool was collected. At the time of collection, date of sampling, the name of the participant, and age was recorded for each subject on a recording format. A portion of each of the stool samples was processed and examined microscopically using direct wet mount and formal-ether concentration techniques following the procedures in WHO guidelines (WHO, 2000) in the YHC laboratory class for parasitological examination.

3.7.2 Blood sample collection

About four drops of capillary blood samples were collected via finger prink with disposable lancets and haemoglobin concentration was measured using a portable hematocrit apparatus by health professionals from Yifag Health Center.

3.7.3 Questionnaires

A structured questionnaire based on socio-demographic data, risk factors that may contribute to parasitic infections like water source, personal hygiene, availability of latrine in the close vicinity of their homes, residence of households, attitudes on parasitic infections, basic knowledge on common signs and symptoms of intestinal parasitic infections, knowledge about anemia, pregnancy month and inter pregnancy was developed in English and translated into local language (Amharic). The questionnaires were pre-tested among threety mothers before the actual data collection. Necessary corrections were made for the actual questionnaires. The study participates were interviewed during collection of stool and blood samples. Then, the responses were translated back into English.

3.8 Laboratory examination procedures

3.8.1 Direct microscopy (wet mount)

In the wet mount, fresh stool sample of each participant (about 2mg) was placed on a glass slide with a wooden applicator, emulsified with a drop of physiological saline (0.85%) for diarrheic and semi-solid samples. For formed stools, iodine was used. Then, covered with cover slide and examined for the presence of motile intestinal parasites and trophozoites under microscope using first X10 objectives and then X40 objectives (Abiye Tigabu et al., 2019)

3.8.2 Formol-ether concentration technique

A portion of each stool sample was used for detection of parasitic ova and protozoan cysts using the formol-ether concentration technique. About 2 gram of each stool sample was first emulsified with three to four ml of 10% formol saline. This was mixed thoroughly and passed through gauze (Abiye Tigabu et al., 2019). Three to four ml of diethyl ether was added and mixed by inverting and intermittent shaking for one minute, and centrifuged at 3,000 rpm for five minutes.

After centrifugation, the supernatant (layers of ether, debris, and formol saline) was discarded by pipette and the sediment (containing the parasites at the bottom of the test tube) was re-suspended in formol saline. The sediment was examined microscopically under 10X by 10X and 10X by 40X magnifications for the presence of any parasitic organisms (Abiye Tigabu et al., 2019). To maintain the reliability of the study findings, the specimen was reexamined at the end by experienced laboratory technologist who was blind for the first examination result.

3.8.3 Determination of haemoglobin concentration

The blood for hematocrit measurement was taken using heparinized hematocrit tube and three-fourth was filled. The capillary tube after being sealed at one end was centrifuged in the microhematocrit centrifuge at 10,000g for 5 minutes. Then, the result was read using hematocrit reader and the result is divided by three to get the haemoglobin concentration (USAID, 1997; WHO, 2001).

For accuracy the centrifuge was checked by running blood samples with known haemoglobin values from haemoglobin ranges (5-10.0 g/dl and 10.0-15.0 g/dl).

3.9 Variables

The prevalence of the parasitic infections was dependant variables, while associated risk factors, Socio-demographic factors (e.g. residence, educational level, family size, and religion), socioeconomic factors (occupation, access of clean water, access of toilet, knowledge about IPIs), environmental factors (source of water), behavioural factors (hand wash before food and after toilet, eating raw food, personal hygiene, shoe wearing habit, waste discharge habit, fingernail status) were independent variables. In the case of anaemia, parasitic infections were independent, and anaemia was dependant variables.

3.10 Data analysis

Statistical package for social sciences (SPSS) version 25 was used to analyse the collected data. Descriptive statistics such as frequency, percentage, mean and range were determined for each intestinal parasite. Two categorical variables were carried out using Pearson chi-square (χ^2) test to verify the relationship between independent factors and the outcome variables. Binary logistic regression was used to measure the strength of association between the prevalence of infection and the risk factors using odds ratio. In the model process, a univariate analysis was first done with less than a 0.25 level of significance to select the candidate variables for multivariate analysis. The variables, significant at P value < 0.25 in the univariate analysis, were then included in the multivariate analysis (Lemeshow et al., 2013). Values were considered significant at $P < 0.05$.

3.11 Ethical considerations

Ethical clearance was obtained from the ethical review committee of Science College, Bahir Dar University before data collection. A letter describing the objective of the study was written to Libokemkem health office and to Yifag Health Center. The researcher obtained consent from the study participants after explaining the purposes and the procedures of the study. The laboratorial test and the questioners were conducted with strict privacy and confidentiality. The pregnant women whose test results are positive were given standard drugs free of charge.

4. RESULTS

4.1. Socio-demographic characteristics of the study participants

From a total of 280 sample size for this study 277 (99%) pregnant women gave stool for intestinal parasitic examination and filled questionnaire. The mean age of the study subjects was 26.6 years between 18 and 45 years. The majority of the pregnant women 95 (34.3%) live in Yifag kebele followed by Bura 92 (33.2%), Around Yifag 43 (15.5%), Shina 28 (10.1%), and Ginza 19 (6.9%) kebeles. Two hundred four (73.6%) were farmers, 22 (7.9%) house wives, 9 (3.3%) government employed, and 42 (15.2%) of them were merchants. Two hundred thirteen (76.9%) and 64 (23.1%) of the participants were lived with the family sizes of less than or equal to five and above five persons per house, respectively. With regard to education status, 175 (63.2%), 58 (20.9%), 29 (10.5%), and 15 (5.4%) of the participants were illiterate, primary school, secondary school, and diploma and above, respectively (Table 1).

Six (2.2%) of the mothers were first trimesters, 127 (45.8%) second trimesters, and 144 (52.0%) were on the third trimester. About 28.9% of the mothers were pregnant for the first to 3^{rd}, 55.6% were pregnant for the 4^{th} to 6^{th}, and 15.5% became pregnant for the 7^{th} times and above. Lastly, 144 (52%) of the mothers were supplemented with iron folic and 133 (48.0%) didn,t (Table 2).

Two hundred fifty eight (93.1%) and 19 (6.9%) of the participants had access to protected water supply and latrine, respectively. Ninety-two (33.2%) and 185 (66.8%) of them were with good and poor personal hygiene, respectively. About two hundred twenty-three (80.5%) and 54 (19.5%) were with poor and good awareness to parasitic infection, respectively. Almost all (99.6%) the participants washed their hands before feeding; 163 (58.8%) of the participants washed their hands after defecation, and the remaining 114 (41.2%) had not hand washing habits after toilet. One hundred fifty (54.2%) had habit of wearing shoes regularly and 127 (45.8%) of them didn,t wear shoes. Based on the waste disposal mechanism only 25 (9.0%) of them used to burry/burn wastes while the majority of them 252 (91.0%) discharged wastes into the open field. One hundred sixty two (58.5%) were with trimmed fingernails and the 115 (41.5%) with untrimmed fingernails. One hundred eleven (40.1%) and 127 (45.8%) of the mothers had the habits of eating raw meat and raw vegetables, respectively (Table 3)

Table 1: Socio-demographic profile of pregnant women at Yifag Health Center, Northwest Ethiopia (N=277), November 2019 to March 2020

Variable	Categories	Frequency	Percent
Age group	18-30	224	80.9
	31-45	53	19.1
Residence	Around Yifag	43	15.5
	Bura	92	33.2
	Ginaza	19	6.9
	Shina	28	10.1
	Yifag	95	34.3
Religion	Muslim	17	6.1
	Orthodox	260	93.9
Occupation	Agriculture	204	73.6
	Government employed	9	3.2
	House wives	22	7.9
	Merchant	42	15.2
Educational level	Diploma and above	15	5.4
	Illiterate	175	63.2
	Primary	58	20.9
	Secondary	29	10.5
Family no.	‰ 5	213	76.9
	>5	64	23.1

Table 2: Profile of pregnant women related to anaemia and pregnancy at Yifag Health Center, Northwest Ethiopia (N=277), November 2019 to March 2020

Pregnancy month	1-3	6	2.2
	4-6	127	45.8
	7-9	144	52.0
No. pregnancy	1-3	80	28.9
	4-6	154	55.6
	Š7	43	15.5
Year between pregnancy	0	83	30
	1-4	28	10.1

	Š5	166	59.9
Knowledge anaemia	No	225	81.2
	Yes	52	18.8
Iron foliate supplementation	No	133	48.0
	Yes	144	52.0

Table 3: Practice of pregnant women related to personal and environmental hygiene taking ANC at Yifag Health Center, Northwest Ethiopia (N=277), November 2019 to March 2020

Waste disposal	Burring or burning	25	9.0
	Open field	252	91.0
Have toilet	No	206	74.4
	Yes	71	25.6
Type of toilet	Private	31	11.2
	open fie	209	75.5
	Public	37	13.4
source of water	Pipe	258	93.1
	Unprotected	19	6.9
Hand washing before feed	No	1	0.4
	Yes	276	99.6
Hand washing after toilet	No	163	58.8
	Yes	114	41.2
Fingernail status	Trimmed	162	58.5
	Untrimmed	115	41.5
Eating raw meat	No	166	59.9
	Yes	111	40.1
Eating raw vegetables	No	150	54.2
	Yes	127	45.8
Personal hygiene	Good	92	33.2
	Poor	185	66.8
Shoe wearing	No	127	45.8
	Yes	150	54.2
Transmission and prevention	No	223	80.5
Knowledge of IPI	Yes	54	19.5

4.2. Prevalence of IPIs in the study population

Two species of protozoa and seven species of intestinal helminths were identified from the stool samples of the study participants. Out of the 277 pregnant women examined for IPIs, 148 (53.4%) were positive for one or more intestinal parasites. The prevalence of *Taenia* species was the highest (50, 18.1%), followed by *G. lamblia* (35, 12.6%), and *E. vermicularis* and *S. stercoralis* were least prevalent (1, 0.4%) each. Prevalence of protozoa was 61 (22.0%), and helminths 99 (35.8%) in this study. Single, double and triple infections were identified at the rate of 137 (49.5%), 10 (3.6%) and 1 (0.4%), respectively (Table 4). Most of the double infection was occurred between *Tania* species with others and triple infection occurred among *E. histolitica*, *G. lamblia* and hookworm.

Table 4: Prevalence of IPIs among pregnant women (N=277) attending ANC in Yifag Health Center, Northwest Ethiopia, November 2019 to March 2020

Parasite	Number women infected	Percent
Protozoa		
E. histolytica/dispar	26	9.4
G. lamblia	35	12.6
Total	61	22
Helminthes		
A. lumbricoides	11	4.0
Hookworm	25	9.0
H. nana	2	0.7
S. mansoni	9	3.2
Taenia spp	50	18.1
E. vermicularis	1	0.4
S. stercoralis	1	0.4
Total	99	35.8
Single and multiple infections		
Single infections	137	49.5
Double infections	10	3.6
Triple infections	1	0.4
Over all prevalence of IPIs	148	53.4

4.3 Prevalence of anaemia among pregnant women

The average hemoglobin concentration was 12.53 g/dl and ranged from 9.6 to 15g/dl. The prevalence of anaemia in this study was 10.1% among pregnant women. The prevalence of moderate and mild anaemia was 1.4% and 8.7%, respectively. None of the women had severe anaemia (Table 5).

Table 5: Prevalence of anaemia among pregnant women N = 277 attending ANC in YHC, Northwest Ethiopia, November 2019 to March 2020

Hemoglobin g/dl	Number of women	Percent
Severe anaemic (below	0	0
Moderate anaemic (7-9.9)	4	1.4
Mild anaemic (10-10.9)	24	8.7
Non-anaemic (11-15)	249	89.9
Total	277	100

4.4 Factors Associated with IPIs among pregnant women visiting YHC for ANC

4.4.1 Chi-square analysis of the different risk factors associated with IPIs

Chi-square analysis of the different risk factors associated with IPIs is presented in Table 6. Mothers who used to walk barefoot (76, 51.4%) were highly associated with IPIs compared to those who used to wear shoes (72, 48.6%). Mothers who had poor personal hygiene (108, 73.0%) were more affected with IPIs than their counterparts (40, 27.0%). Whereas, age, residence, occupation, family size, toilet access, source of water, hand washing habit, eating raw meat, eating raw vegetables, fingernail trimming status, method of waste disposal, and knowledge about IPIs were not associated with IPIs (P>0.05).

Table 6: Chi-square analyses of socio-demographic, socio-economic, and behavioral risk factors associated with intestinal parasitic infection among pregnant women attending ANC at YHC, Northwest Ethiopia, November 2019 to March 2020.

Risk factors	IPIs Total No. (%)	IPIs Negative No. (%)	IPIs Positive No. (%)	χ^2	P-value
Age					
18-30	224 (80.9)	107 (82.9)	117 (79.1)	0.675	0.411
31-45	53 (19.1)	22 (17.1)	31 (20.9)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Residence					
Around Yifag	43 (15.5)	18 (14)	25 (16.9)	3.317	0.506
Bura	92 (33.2)	42 (32.6)	50 (33.8)		
Ginaza	19 (6.9)	11 (8.5)	8 (5.4)		
Shina	28 (10.1)	10 (7.8)	18 (12.2)		
Yifag	95 (34.3)	48 (37.2)	47 (31.8)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Religion					
Muslim	17 (6.1)	8 (6.2)	9 (6.1)	0.002	0.967
Orthodox	260 (93.9)	121 (93.8)	139 (93.9)		
Total	277 (100)	129 (46.6)	148 (53.4)		

	IPIs				
	Total	Negative	Positive		
Risk factors	No. (%)	No. (%)	No. (%)	€^{2}	P-value
Occupation					
Agriculture	204 (73.6)	95 (73.6)	109 (73.6)	0.843	0.839
House wives	22 (7.9)	10 (7.8)	12 (8.1)		
Merchant	42 (15.2)	21 (16.3)	21 (14.2)		
Government employed	9 (3.2)	3 (2.3)	6 (4.1)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Educational level					
Diploma and above	15 (5.4)	7 (5.4)	8 (5.4)	2.453	0.484
Illiterate	175 (63.2)	76 (58.9)	99 (66.9)		
Primary	58 (209)	32 (24.8)	26 (17.6)		
Secondary	29 (10.5)	14 (10.9)	15 (10.1)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Number of family					
1-5	213 (76.9)	98 (76)	115 (77.7)	0.117	0.733
6-10	64 (23.1)	31 (24)	33 (22.3)		
Total	277 (100)	129 (46.6)	148 (53.4)		

	IPIs				
	Total	Negative	Positive		
Risk factors	No. (%)	No. (%)	No. (%)	€^{2}	P-value
Hand wash after toilet					
No	163 (58.8)	73 (56.6)	90 (60.8)	0.507	0.476
Yes	114(41.2)	56 (43.4)	58 (39.2)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Shoes wearing habit					
No	127 (45.8)	51 (39.5)	76 (51.4)	3.876	0.049*
Yes	150 (54.2)	78 (60.5)	72 (48.6)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Have a toilet					
No	206 (74.4)	96 (74.4)	110 (74.3)	0.000	0.986
Yes	71 (25.6)	33 (25.6)	38 (25.7)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Type of toilet					
Private	31 (11.2)	16 (12.4)	15 (10.1)	0.484	0.785
Open field	209 (75.5)	97 (75.2)	112 (75.7)		
Public	37 (13.4)	16 (12.4)	21 (14.2)		
Total	277 (100)	129 (46.6)	148 (53.4)		

	IPIs				
	Total	Negative	Positive		
Risk factors	No. (%)	No. (%)	No. (%)	€[2]	P-value
Source					
Protected	258 (93.1)	122 (94.6)	136 (91.9)	0.776	0.378
Unprotected	19 (6.9)	7 (5.4)	12 (8.1)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Waste disposal					
Burial or burning	25 (9.0)	15 (11.6)	10 (6.8)	1.992	0.158
Open filed	252 (91.0)	114 (88.4)	138 (93.2)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Fingernail status					
Trimmed	162 (58.8)	81 (62.8)	81 (54.7)	1.845	0.174
Untrimmed	115 (41.5)	48 (37.2)	67 (45.3)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Eating raw meat					
No	166 (59.9)	85 (65.9)	81 (54.7)	3.576	0.059
Yes	111 (40.1)	44 (34.1)	67 (45.3)		
Total	277 (100)	129 (46.6)	148 (53.4)		

	IPIs				
	Total	Negative	Positive		
Risk factors	No. (%)	No. (%)	No. (%)	€^{2}	P-value
Eating raw vegetables					
No	150 (54.2)	68 (52.7)	82 (55.4)	0.201	0.654
Yes	127 (45.8)	61 (47.3)	66 (44.6)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Personal hygiene					
Good	92 (33.2)	52 (40.3)	40 (27.0)	5.482	0.019*
Poor	185 (66.8)	77 (59.7)	108 (73.0)		
Total	277 (100)	129 (46.6)	148 (53.4)		
Knowledge about IPIs					
No	223 (80.5)	106 (82.2)	117 (79.1)	0.427	0.514
Yes	54 (19.5)	23 (17.8)	31 (20.9)		
Total	277 (100)	129 (46.6)	148 (53.4)		

*= Statically significant $P< 0.05$

4.4.2. Logistic Regression Analysis (LRA) of the Risk Factors for IPIs

The important risk factors for IPIs among pregnant women in Yifag Health Center were identified using Univariate and Multivariate Logistic Regression Analyses (MLRA) (Table 7). Mothers who were not wearing shoes were 1.614 fold (COR= 1.614; 95% CI: 1.001, 2.604; p = 0.05) more infected with IPIs than their counterparts. The odds of IPIs among pregnant women who had poor personal hygiene were 1.823 fold (COR = 1.823; 95% CI: 1.10, 3.022; p = 0.020) prone to IPIs than those who had good personal hygiene (Table 7). In the current study, the multivariate regression showed that eating raw meat was the only identified risk factors for IPIs. Accordingly, mothers who had the habit of eating raw meat were 1.779 times increased odds of having IPIs as compared to their counterparts (AOR= 1.779; 95% CI: 1.075, 2.959; p = 0.026) (Table 7).

Table 7: Assessment of risk factors associated with PIs among pregnant women attending ANC YHC, Northwest Ethiopia from November 2019 to March 2020

Risk factors	Parasitic infection		Univariate Logistic Regression		Multivariate Logistic Regression	
	Negative N (%)	Positive N (%)	COR (95% CI: Lower, Upper)	p-value	AOR (95% CI: Lower, Upper)	p-value
Age						
18-30	107 (82.9)	117 (79.1)	0.776 (0.423, 1.423)	0.412		
31-45	22 (17.1)	31 (20.9)	1			
Total	129	148				
Residence						
Around Yifag	18 (14)	25 (16.9)	1.418 (0.685, 2.935)	0.513		
Bura	42 (32.6)	50 (33.8)	1.216 (0.684, 2.160)			
Ginaza	11 (8.5)	8 (5.4)	0.743 (0.274, 2.010)			
Shina	10 (7.8)	18 (12.2)	1.838 (0.769, 4.394)			
Yifag	48 (37.2)	47 (31.8)	1			
Total	129	148				
Religion						
Muslim	8 (6.2)	9 (6.1)	1			
Orthodox	121 (93.8)	139 (93.9)	1.021 (0.382, 2.729)	0.967		
Total	129	148				

Risk factors	Parasitic infection		Univariate Logistic Regression		Multivariate Logistic Regression	
	Negative N (%)	Positive N (%)	COR (95% CI: Lower, Upper)	p-value	AOR (95% CI: Lower, Upper)	p-value
Occupation						
Agriculture	95 (73.6)	109 (73.6)	0.574 (0.140, 2.357)	0.843		
House wives	10 (7.8)	12 (8.1)	0.600 (0.119, 3.032)			
Merchant	21 (16.3)	21 (14.2)	0.500 (0.110, 2.268)			
Government employed	3 (2.3)	6 (4.1)	1			
Total	129	148				
Educational level						
Diploma and above	7 (5.4)	8 (5.4)	1			
Illiterate	76 (58.9)	99 (66.9)	1.140 (0.396, 3.282)	0.487		
Primary	32 (24.8)	26 (17.6)	0.711 (0.228, 2.220)			
Secondary	14 (10.9)	15 (10.1)	0.937 (0.269, 3.268)			
Total	129	148				
Number of family						
1-5	98 (76)	115 (77.7)	1			
6-10	31 (24)	33 (22.3)	0.907 (0.518, 1.587)	0.733		
Total	129	148				

Risk factors	Parasitic infection		Univariate Logistic Regression		Multivariate Logistic Regression	
	Negative N (%)	Positive N (%)	COR (95% CI: Lower, Upper)	p-value	AOR (95% CI: Lower, Upper)	p-value
Hand wash after toilet						
No	73 (56.6)	90 (60.8)	1.190 (0.737, 1.923)	0.476		
Yes	56 (43.4)	58 (39.2)	1			
Total						
Shoe wearing habit						
No	51 (39.5)	76 (51.4)	1.614 (1.001, 2.604)	0.050*	1.341 (.789, 2.281)	0.278
Yes	78 (60.5)	72 (48.6)	1		1	
Total	129	148				
Have a toilet						
No	96 (74.4)	110 (74.3)	0.995 (0.579, 1.709)	0.986		
Yes	33 (25.6)	38 (25.7)	1			
Total	129	148				
Type of toilet						
Private	16 (12.4)	15 (10.1)	1	0.786		
Open field	97 (75.2)	112 (75.7)	1.232 (0.579, 2.621)			
Public	16 (12.4)	21 (14.2)	1.400 (0.537, 3.652)			
Total						

Risk factors	Parasitic infection		Univariate Logistic Regression		Multivariate Logistic Regression	
	Negative N (%)	Positive N (%)	COR (95% CI: Lower, Upper)	p-value	AOR (95% CI: Lower, Upper)	p-value
Source of water						
Protected	122 (94.6)	136 (91.9)	1			
Unprotected	7 (5.4)	12 (8.1)	1.538 (0.587, 4.031)	0.381		
Total	129	148				
Waste disposal						
Burial or burning	15 (11.6)	10 (6.8)	1			
Open filed	114 (88.4)	138 (93.2)	1.816 (0.786, 4.196)	0.163	1.309 (0.540, 3.171)	0.551
Total	129	148				
Fingernail trimming status						
Trimmed	81 (62.8)	81 (54.7)	1		1	0.182
Untrimmed	48 (37.2)	67 (45.3)	1.396 (0.862, 2.260)	0.175	1.412 (0.851, 2.341)	
Total	129	148				
Eating raw meat						
No	85 (65.9)	81 (54.7)	1		1	0.026*
Yes	44 (34.1)	67 (45.3)	1.598 (0.982, 2.601)	0.059	1.779 (1.070, 2.959)	
Total	129	148				

Risk factors	Parasitic infection		Univariate Logistic Regression		Multivariate Logistic Regression	
	Negative N (%)	Positive N (%)	COR (95% CI: Lower, Upper)	p-value	AOR (95% CI: Lower, Upper)	p-value
Eating raw vegetables						
No	68 (52.7)	82 (55.4)	1			
Yes	61 (47.3)	66 (44.6)	0.897 (0.559, 1.441)	0.654		
Total	129	148				
Personal hygiene						
Good	52 (40.3)	40 (27)	1		1	
Poor	77 (59.7)	108 (73)	1.823 (1.10, 3.022)	0.020*	1.555 (0.883, 2.738)	0.126
Total	129	148				
Knowledge about IPIs						
No	106 (82.2)	117 (79.1)	0.819 (0.449, 1.492)	0.514		
Yes	23 (17.8)	31 (20.9)	1			
Total	129	148				

*= Statically significant P< 0.05

4.4.3 Risk factors associated with *E. histolytica*, *G. lamblia*, *A. lumbricoids*, *S. mansoni*, Hookworm and *Taenia* species

Risk factors associated with *E. histolytica* infection

In the univariate logistic regression, the type of toilet was the only associated risk factor for *E. histolytica* infection for further analyses in multivariate, but not significantly associated risk factor (P> 0.05) (Table 8).

Risk factors associated with *G. lamblia* infection

In the univariate logistic regression, fingernail status, eating raw vegetables and personal hygiene were identified as significant for further analysis in multivariate logistic regression (Table 8).

In the multivariate analysis eating raw vegetables and poor personal hygiene were predictors of *G. lamblia* (P < 0.05). However, untrimmed fingernail status was not associated with *G. lamblia* (P > 0.05). Mothers who had habit of eating raw vegetables were more infected by *G. lamblia* (AOR= 2.721; 95% CI: 1.266, 5.849; P= 0.010) than those who had not habit of eating raw vegetables, and mothers who had poor personal hygiene four times more likely infected (AOR= 4.015; 1.456, 11.07; P= 0.007) compare to their counterparts (Table 8).

Risk factors associated with *A. lumbricoides* infection

In the univariate analysis not hand wash habit after toilet, open field waste disposal, untrimmed fingernail, and knowledge about IPIs were selected for multivariate analysis. In univariate analysis open field waste disposal (COR= 0.240; 95% CI: 0.059, 0.972; P= 0.046) and knowledge about IPIs (COR= 0.183; 95% CI: 0.054, 0.626; P= 0.007) were less likely associated with *A. lumbricoides*. There were not statistically significant associated risk factors for *A. lumbricoides* in multivariate analysis (P > 0.05) (Table 8).

Risk factors associated with hookworm infection

The univariate regression analysis showed that variations in age, habit of eating raw meat and knowledge about IPIs were statistically significant differences in the occurrence of hookworm infection for further multivariate analysis. The multivariate logistic regression analysis showed that there were not predictors of hookworm infection (P> 0.05) (Table 8).

Risk factors associated with *S. mansoni* infection

Statistically significant differences in *S. mansoni* infection occurred from variation of occupation, hand wash after toilet, shoe wearing, and access of toilet, type of toilet, fingernail status and personal hygiene in the selection process of variables for multivariate analysis. However, there were not statistically significant associated risk factors for *S. mansoni* both in univariate and multivariate logistic regression (Table 8).

Risk factors associated with *Taenia* species infection

Personal hygiene, residence, religion, occupation, hand wash habit after toilet, shoe wearing habit, type of toilet access, eating raw meat, and eating habit of raw vegetables were statistically significant for *Taenia* species infection in univariate logistic regression analysis for further multivariate analysis. Mothers lived in Around Yifag, Bura, and Shina, mothers who did not have habit of hand wash after toil, barefooted, and eating raw meat were associated risk factors for *Taenia* species infection (P < 0.05). Taeniasis was high among mothers who lived in Around Yifag (COR= 3.285; 95% CI: 1.245, 8.664) followed by mothers who lived in Shina (COR= 3.185; 95%CI: 1.064, 9.539), and mothers who lived in Bura (COR= 3.003; 95% CI: 1.300, 6.937) compared with mothers who lived in Yifag. However, mothers who lived in Ginaza were less likely infected (COR= 0.531; 95% CI: 0.063, 4.456) by *Taenia* species than mothers who lived in Yifag (P= 0.033). Furthermore, the odds of having taeniasis higher among mothers who had not hand wash habit after toilet (COR= 2.025; 95% CI: 1.035, 3.959; P= 0.039) than who did, among barefooted mothers (COR= 2.226; 1.187, 4.176; P=0.013) than mother who had habit of shoe wearing. The rate of getting *Taenia* species infection higher among women who had habit of eating raw meat (COR= 2.208; 95% CI: 1.187, 4.106; P= 0.012) than those who had not habit of eating raw meat. In addition, mothers had not access of toilet 2.4 times (COR= 2.412; 95% CI: 1.031, 5.640; P= 0.042) more likely infected than who had got toilet access. The only associated risk factor in the multivariate logistic regression analysis was the habit of eating raw meat. Mothers who had habit of eating raw meat were 2.5 times (AOR = 2.477; 1.252, 4.902; P= 0.009) more likely to be infected by *Taenia* species than mothers who had not habit of eating raw meat (Table 8)

Table 8: Uivariate and Multivariate logistic regression analysis of potential risk factors associated with IPIs among pregnant women in Yifag Health Center, Northwest Ethiopia, November 2019 to March 2020

			Negative	Positive	Univariate Logistic Regression		Multivariate Logistic Regression	
Risk factors	Categories	Total	No. (%)	No. (%)	COR (95% CI)	P-value	AOR (95% CI)	P-value
				E. histolytica/dispar				
Type of toilet	Private	31	25 (80.6)	6 (19.4)	1	0.149	1	0.149
	Open field	209	192 (91.9)	17 (8.1)	0.369 (0.133, 1.023)		0.369 (0.133, 1.023)	
	Public	37	34 (91.9)	3 (8.1)	0.368 (0.084, 1.613)		0.368 (0.084, 1.613)	
				G. lamblia				
Fingernail status	Trimmed	162	146 (90.1)	16 (9.9)	1	0.104		0.111
	Untrimmed	115	96 (83.5)	19 (16.5)	1.806 (0.885, 3.685)		1.831 (0.870, 3.853)	
Eating raw vegetables	No	150	136 (90.7)	14 (9.3)	1	0.076	1	0.010*
	Yes	127	106 (83.5)	21 (16.5)	1.925 (0.935, 3.963)		2.721 (1.266, 5.849)	
Personal hygiene	Good	92	87 (94.6)	5 (5.4)	1	0.015*	1	0.007*
	Poor	185	155 (83,8)	30 (16.2)	3.368 (1.261, 8.996)		4.015 (1.456, 11.07)	
				A. lumbricoides				
Hand wash after toilet	No	163	159 (97.5)	4 (2.5)	0.385 (0.110, 1.346)	0.135	0.827(0.186, 3.676)	0.803
	Yes	114	107 (93.9)	7 (6.1)	1		1	
Waste disposal	Burial/burning	25	22 (8.3)	3 (27.3)	1	0.046*	1	0.372
	Open field	252	244 (91.7)	8 (72.7)	0.240 (0.059, .972)		0.487 (0.100, 2.362)	
Fingernail status	Trimmed	162	158 (97.5)	4 (2.5)	1	0.141	1	0.140
	Untrimmed	115	108 (93.9)	7 (6.1)	2.560 (0.732, 8.959)		2.618 (0.729, 9.409)	
Knowledge about IPIs	No	223	218 (97.8)	5 (2.2)	0.183 (0.054,0.626)	0.007*	0.240 (0.057, 1.005)	0.051
	Yes	54	48 (88.9)	6 (11.1)	1		1	

Risk factors	Categories	Total	Negative No. (%)	Positive No. (%)	Univariate Logistic Regression		Multivariate Logistic Regression	
					COR (95% CI)	P-value	AOR (95% CI)	P-value
				Hookworm				
Age group	18-30	224	206 (92)	18 (8)	1	0.242	1	0.383
	31-45	53	46 (86.8)	7(13.2)	1.742 (0.687, 4.413)		1.520 (0.593, 3.896)	
Eating raw meat	No	166	154 (92.8)	12 (7.2)	1	0.206	1	0.204
	Yes	111	98 (88.3)	13 (11.7)	1.702 (0.746, 3.883)		1.713 (0.746, 3.932)	
Knowledge about IPIs	No	223	200 (89.7)	23 (10.3)	2.990 (0.683, 13.092)	0.146	2.871 (0.648, 12.727)	0.165
	Yes	54	52 (96.3)	2 (3.7)	1		1	
				S. mansoni				
Occupation	Agriculture	204	200 (98.0)	4 (2.0)	0.400 (0.071, 2.259)	0.083	-	0.518
	House wives	22	19 (86.4)	3 (13.6)	3.158 (0.486, 20.503)		-	
	Merchant	42	40 (95.2)	2 (4.8)	-		-	
	Government	9	9 (100.0)	0 (0.0)	1		1	
Hand wash after toilet	No	163	160 (98.2)	3 (1.8)	0.338 (0.083, 1.379)	0.130	0.919 (0.132, 6.389)	0.932
	Yes	114	108 (94.7)	6 (5.3)	1		1	
Shoe wearing	No	127	125 (98.4)	2 (1.6)	0.327 (0.067, 1.602)	0.168	0.709 (0.104, 4.841)	0.725
	Yes	150	143 (95.3)	7 (4.7)	1		1	
Toilet	Absent	206	201 (97.6)	5 (2.6)	0.417 (0.109, 1.597)	0.202	-	0.999
	Present	71	67 (94.4)	4 (5.6)	1		1	
Type of toilet	Private	31	30 (96.8)	1 (3.2)	1	0.233		0.745
	Open	209	204 (97.6)	5 (2.4)	0.735 (0.083, 6.511)		-	
	Public	37	34 (91.9)	3 (8.1)	2.647 (0.261, 26.823)		2.614 (0.225, 30.400)	
Fingernail status	Trimmed	162	155 (95.7)	7 (4.3)	1	0.248	1	0.468
	Untrimmed	115	113 (98.3)	2 (1.7)	0.392 (0.080, 1.922)		0.537 (0.100, 2.879)	

Risk factors	Categories	Total	Negative No. (%)	Positive No. (%)	Univariate Logistic Regression COR (95% CI)	P-value	Multivariate Logistic Regression AOR (95% CI)	P-value
	Good	92	86 (93.5)	6 (6.5)	1	0.045*	1	0.231
Personal hygiene	Poor	185	182 (98.4)	3 (1.6)	0.24 (0.058, 0.967)		0.341 (0.059, 1.98)	
Taeniaspecies								
Residence	Around Yifag	43	32 (74.4)	11 (25.6)	3.285 (1.245, 8.664)	0.033*	2.051 (0.485, 8.668)	0.510
	Bura	92	70 (76.1)	22 (23.9)	3.003 (1.300, 6.937)		1.616 (0.410, 6.366)	
	Ginaza	19	18 (94.7)	1 (5.3)	0.531 (0.063, 4.456)		0.332 (0.030, 3.710)	
	Shina	28	21 (75.0)	7 (25.0)	3.185 (1.064, 9.539)		1.691 (0.344, 8.309)	
	Yifag	95	86 (90.5)	9 (9.5)	1		1	
Religion	Muslim	17	16 (94.1)	1 (5.9)	1	0.208	1	0.718
	Orthodox	260	211(81.2)	49 (18.8)	3.716 (0.481, 28.692)		0.616 (0.044, 8.542)	
Occupation	Agriculture	204	161 (78.9)	43 (21.1)	0.534 (0.128, 2.224	0.057	0.108 (0.013, 0.904)	0.063
	House wives	9	6 (66.7)	3 (33.3)	0.200 (0.027, 1.490)		0.117 (0.013, 1.045)	
	Merchant	22	20 (90.9)	2 (9.1)	0.100 (0.014, 0.727)		0.054 (0.006, 0.489)	
	Government	42	40 (95.2)	2 (4.8)	1		1	
Hand wash after toilet	No	163	127 (77.9)	36 (22.1)	2.025 (1.035, 3.959)	0.039*	1.442 (0.613, 3.395)	.402
	Yes	114	100 (87.7)	14 (12.3)	1		1	
Shoes wearing	No	127	96 (75.6)	31 (24.4)	2.226 (1.187, 4.176)	0.013*	1.595 (0.741, 3.435)	0.233
	Yes	150	131 (87.3)	19 (12.7)	1		1	
Toilet	Absent	206	163 (79.1)	43 (20.8)	2.412 (1.031, 5.640)	0.042*	0.513 (0.040, 6.549)	0.608
	Present	71	64 (90.1)	7 (9.8)	1		1	
Type of toilet	Private	31	29 (93.5)	2 (6.5)	1	0.085	1	0.613
	Open field	209	165 (78.9)	44 (21.1)	3.867 (0.888, 16.834)		4.795 (0.212, 108.62)	
	Public	37	33 (89.2)	4 (10.8)	1.758 (0.300, 10.310)		1.675 (0.223, 12.550)	

Risk factors	Categories	Total	Negative No. (%)	Positive No. (%)	Univariate Logistic Regression COR (95% CI)	P-value	Multivariate Logistic Regression AOR (95% CI)	P-value
Eating raw meat	No	166	144 (86.7)	22 (13.3)	1	0.012*	1	0.009*
	Yes	111	83 (74.8)	28 (25.2)	2.208 (1.187, 4.106)		2.477 (1.252, 4.902)	
Eating raw vegetables	No	150	119 (79.3)	31 (20.7)	1	0.220	1	0.355
	Yes	127	108 (85)	19 (15)	0.675 (0.361, 1.265)		0.708 (0.341, 1.471)	
Personal hygiene	Good	92	81 (88)	11 (12)	1	0.066	1	0.595
	Poor	185	146 (78.9)	39 (21.1)	1.967 (0.955, 4.050)		1.295 (0.499, 3.358)	

*= Statically significant P< 0.05

4.5 Chi-square analysis of the IPIs and iron supplementation associated with anaemia

G. lamblia, Hookworm, overall IPI, Helminths and Iron supplementation were significantly associated with anaemia (P < 0.05). The prevalence of anaemia among pregnant women infected with intestinal parasite (24, 16.2%) was significantly higher than the prevalence among women who were not infected (4, 3.1%) (P < 0.001). Mothers who were infected by helminths (16, 16.7%) were significantly associated with anameia compared to those who were not infected (12, 6.6%). G. lamblia positive mothers (20.0%) were more affected with anaemia than G. lamblia negative mothers (8.7%). Further the prevalence of anemia was high among mothers diagnosed with hookworm (32%) compared with their counterparts (7.9%). Whereas, there was not significance association between anaemia and E. histolytica, A lumbricoides, S mansoni, Taenia species, H. nana, E. vermicularis, and S. stercoralis (P>0.05). Mothers who are supplemented iron folic were more anaemic (13.9%) than mothers who were not (6.0%) (Table 9).

Table 9: Chi-square analyses of IPIs, and iron supplementation associated with anaemia among pregnant women attending YH, Northwest Ethiopia, November 2019 to March 2020.

Parasitic infections	Total No.	Anaemia: Anaemic No. (%)	Anaemia: Non-anaemic No. (%)	χ^2	P-value
E. histolytica					
Negative	251	25 (10.0)	226 (90.0)	0.65	0.799
Positive	26	3 (11.5)	23 (88.5)		
Total	277	28 (10.1)	249 (89.9)		
G. lamblia					
Negative	242	21 (8.7)	221 (91.3)	4.314	0.038*
Positive	35	7 (20.0)	28 (80.0)		
Total	277	28 (10.1)	249 (89.9)		
A. lumbricoides					
Negative	266	27 (10.2)	239 (89.8)	0.013	0.909

	Anaemia				
Parasitic infections	Total No.	Anaemic No. (%)	Non-anaemic No. (%)	€^{2}	P-value
Positive	11	1 (9.1)	10 (90.9)		
Total	277	28 (10.1)	249 (89.9)		
Hookworm					
Negative	252	20 (7.9)	232 (92.1)	14.494	0.000*
Positive	25	8 (32.0)	17 (68.0)		
Total	277	28 (10.1)	249 (89.9)		
H. nana					
Negative	275	28 (10.2)	247 (89,8)	0.227	0.634
Positive	2	0 (0.0)	2 (100.0)		
Total	277	28 (10.1)	249 (89.9)		
S. mansoni					
Negative	268	27 (10.1)	241 (89.9)	0.010	0.919
Positive	9	1 (11.1)	8 (88.9)		
Total	277	28 (10.1)	249 (89.9)		
Taeniaspp					
Negative	227	21 (9.3)	206 (90.7)	1.017	0.313
Positive	50	7 (14.0)	43 (86.0)		
Total	277	28 (10.1)	249 (89.9)		
E. vermicularis					
Negative	276	28 (10.1)	248 (89.9)	0.113	0.737
Positive	1	0 (0.0)	1 (100.0)		
Total	277	28 (10.1)	249 (89.9)		

S. stercoralis					
Negative	276	28 (10.1)	248 (89.9)	0.113	0.737

		Anaemia			
Parasitic infections	Total No.	Anaemic No. (%)	Non-anaemic No. (%)	χ^2	P-value
Positive	1	0 (0.0)	1 (100.0)		
Total	277	28 (10.1)	249 (89.9)		
Any IPI					
Negative	129	4 (3.1)	125 (96.9)	13.048	0.000*
Positive	148	24 (16.2)	124 (83.8)		
Total	277	28 (10.1)	249 (89.9)		
Helminths					
Negative	181	12 (6.6)	169 (93.4)	6.954	0.008*
Positive	96	16 (16.7)	80 (83.3)		
Total	277	28 (10.1)	249 (89.9)		
Protozoa					
Negative	217	18 (8.3)	199 (91.7)	3.625	0.057
Positive	60	10 (16.7)	50 (83.3)		
Total	277	28 (10.1)	249 (89.9)		
Iron supplementation					
No	133	8 (6.0)	125 (94.0)	4.717	0.030*
Yes	144	20 (13.9)	124 (86.1)		
Total	277	28 (10.1)	249 (89.9)		

*= Statically significant P< 0.05

4.6 Association between intestinal parasitic infection and anaemia

The occurrence of anaemia had weak association with IPIs (Table 10). Based on the univariate regression analysis result, the contribution of being infected with intestinal parasites for anaemia was 16.5% (COR= 0.165; 95% CI: 0.056, 0.490; p = 0.001). The contribution of infection with helminthes was 35.5% (COR= 0.355; 95% CI: 0.160, 0.786; p = 0.011). Infection by *G. lamblia* and hookworm were associated with anaemia (p < 0.05). The contribution of *G. lamblia* for anaemia was 38.0% (COR= 0.380; 95% CI: 0.148, 0.974; P = 0.044) and that of hookworm was 18.3% (COR= 0.183; 95% CI: 0.070, 0.477; P= 0.001). Furthermore, iron foliate supplementation had association with anaemia ($p < 0.05$). Pregnant women who did not take iron supplementation were three times more susceptible to anaemia (COR= 2.52; 95% CI: 1.07, 5.936; P = 0.034) than women who were supplemented with iron foliate. Based on the results from multiple regression; however, only hookworm infection was associated with anaemia ($p < 0.05$). Hookworm infection had 27% contribution for anaemia (AOR= 0.270; 95% CI: 0.080, 0.913; p = 0.032).

Table 10: Association between intestinal parasitic infection, taking iron and anaemia among pregnant women from YHC, Northwest Ethiopia, November 2019 to March 2020

Parasites	Anaemia: Anaemic (%)	Anaemia: Non-anaemic (%)	Univariate Logistic Regression: COR 95% CI	Univariate Logistic Regression: P- value	Multivariate Logistic Regression: AOR 95% CI	Multivariate Logistic Regression: P- value
Any IPI						
Positive	24 (85.7)	124 (49.8)	0.165 (0.056, 0.490)	0.001*	0.286 (0.030, 2.736)	0.277
Negative	4 (14.3)	125 (50.2)	1		1	
Protozoa						
Positive	10 (35.7)	51 (20.5)	0.452 (0.197, 0.040)	0.062	0.863 (0.098, 7.586)	0.894
Negative	18 (64.3)	198 (79.5)	1		1	
Helminths						
Positive	16 (57.1)	80 (32.1)	0.355 (0.160, 0.786)	0.011*	0.866 (0.114, 6.576)	0.890
Negative	12 (42.9)	169 (67.9)	1			
G. lamblia						
Positive	7 (25.0)	28 (11.2)	0.380 (0.148, 0.974)	0.044*	0.548 (0.116, 2.953)	0.449
Negative	21 (75.0)	221 (88.8)	1			
Hookworm						
Positive	8 (28.6)	17 (6.8)	0.183 (0.070, 0.477)	0.001*	0.270 (0.080, 0.913)	0.032*
Negative	20 (71.4)	232 (93.2)	1			
Iron foliate supplementation						
Yes	20 (71.4)	124 (49.8)	1	0.034*		0.408
No	8 (28.6)	125 (50.2)	2.52 (1.07, 5.936)		0.584 (0.163, 2.089)	

*= Statically significant P< 0.05

5. Discussion

The prevalence of intestinal parasitic infection in this study was 53.4% (95% CI: 47.4, 59.4). This result was comparable with previous studies conduct in North Ethiopia (51.5%) (Menasbo Gebru *et al*., 2019) and Nigeria (56.8%) (Amuta *et al*., 2010) and higher than previous studies conducted in Gilgel Gibe dam Area, Southwest Ethiopia (11.6%) (Million Getachew *et al*., 2013), Lalo Kile district, Oromia, Western Ethiopia (43.8%) (Dejene Abraham *et al*., 2019), and rural Dembiya, northwest Ethiopia (25.8%) (Zemichael Gizaw *et al*., 2018) in Colombia (41%) (Aranzales *et al*., 2018), Nepal (35%) (Sapkota and Maharjan, 2017), Kenya (13.8%) (Wekesa *et al*., 2014). However, it was lower than the studies conduct in Mecha district, Northwest Ethiopia (70.6%) (Berhanu Elfu and Tadesse Hailu, 2018), Papua New Guinea (81.0%) (Phuanukoonnon *et al*., 2013), Venezuela (73.9%) (Rodr†‡guez-Morales *et al*., 2006). This might be due to the differences in the socio-demographic factors and lack of awareness on the prevention of parasitic infections, personal hygiene, eating raw food, incorrect fingernail trimming, open field waste disposal, bare footedness, lack of clean water source, and environmental factors may also contribute for the variation.

The most prevalent intestinal parasitic infection in this study was *Taenia* species (18.1%). In contrast to the current finding, far lower prevalence of *Taenia* species was reported from Arba Minch Town, Gamo Gofa Zone, Ethiopia (0.6%) (Alemayehu Bekele *et al*., 2016), Bahir Dar, northwest Ethiopia (0.8%) (Adane Derso *et al*., 2016), East Wollega, Oromia, Ethiopia (1.3%) (Hylemariam Mihiretie *et al*., 2017), Iran (0.014%) (Saki, *et al*., 2016) and Nigeria (2.1%). This difference may be due to the differences in the habit of eating raw/improperly cooked meat, altitude, open defecation, and poor awareness in the prevention of this parasite (WHO, 2019).

G. lamblia was the second most prevalent parasite (12.6%) among the study participants. This fining was in line with previous studies conduct in southern Ethiopia (12.6%) (Fekede Weldekidan *et al*., 2018), Bahir Dar, northwest Ethiopia (13.3%) (Adane Derso *et al*., 2016), and Venezuela (14.1%) (Rodr†‡guez-Morales *et al*., 2006). It was lower than the prevalence in studies conducted in Northwest Ethiopia (19.2%) (Tadesse Hailu *et al*., 2019) and Papua New Guinea (39%) (Phuanukoonnon *et al*., 2013). But, it was higher than studies conducted in Wolayita Sodo Town, Southern Ethiopia (3.6%) (Lealem Gedefaw *et al*., 2015), Lalo Kile district, Oromia, Western Ethiopia (0.9%) (Dejene Abraham *et al.*, 2019), in Wondo Genet district, Southern Ethiopia (5.4%) (Amelo Bolka and Samson Gebremedhin, 2019). Accessibility of safe water, open field defecation, and variations in hand wash implementation might bring such differences (Espelage *et al.*, 2010).

E. histolytica infection among the study participants was 9.4%. This finding was consistent with other studies conducted in Gamo area Southern Ethiopia (11.4%) (Teklu Wegayehu *et al.*, 2013), Bahir Dar, northwest Ethiopia (7.8%) (Adane Derso *et al.*, 2016) and Venezuela (12.0) (Rodr†‡guez Morales *et al.*, 2006). But it was lower than the reports from Nigeria (18.9%) (Amuta *et al.*, 2010) and Papua New Guinea (43%) (Phuanukoonnon *et al.*, 2013) and higher than in the findings from southwest Ethiopia (5.5%) (Ayalew Jejaw *et al.*, 2014), in Wondo Genet district, Southern Ethiopia (3.4%) (Amelo Bolka and Samson Gebremedhin, 2019), and Tanzania (0.7%) (Mahande and Mahande, 2016). Accessibility of safe water, open field defecation, variations in hand washing habit before food and after toilet, eating raw vegetables, contact with night soil, sanitation problems, and environmental and climate factor might bring such differences (Pereira *et al.*, 2007)

The level of hookworm in this study was 9.0%. This finding was in agreement with the reports from southern Ethiopia (9.9%) (Feleke Weldekidan *et al.*, 2018), Wondo Genet district, Southern Ethiopia (11.2%) (Amelo Bolka and Samson Gebremedhin, 2019) and Venezuela (8.1%) (Rodr†‡guez Morales *et al.*, 2006). However, this prevalence was lower than that of the study conducted in northwest Ethiopia (20%) (Melashu Balew *et al.*, 2017), Gilgel Gibe Dam area, southwest Ethiopia (29.4%) (Million Getachew *et al.*, 2013), Lalo Kile district, Oromia, Western Ethiopia (33.7%) (Dejene Abraham *et al.*, 2019), Maytsebri primary hospital, north Ethiopia (39.96%) (Menasbo Gebru *et al.*, 2019), Uganda (40.5%) (Chami *et al.*, 2015), and Nigeria (44.4%) (Ivoke *et al.*, 2017) and was higher than the reports from Kenya (3.92%) (Wekesa *et al.*, 2014) and Bahir Dar, northwest Ethiopia (5.5%) (Adane Derso *et al.*, 2016). This difference might be due to the differences in geography, shoes wearing habit, level of income, and agricultural practice (Dejene Abraham *et al.*, 2019)

The prevalence of *A. Lumbricoides* in the present study was 4%. It was comparable with results of the studies in Wolayita Sodo Town, Southern Ethiopia (5.5%) (Lealem Gedefaw *et al.*, 2015), Bahir Dar, northwest Ethiopia (2.9%) (Adane Derso *et al.*, 2016) and Kenya (6.5%) (Wekesa *et* al., 2014), But it was higher than the findings in Arba Minch Town, Gamo Gofa Zone, Ethiopia (0.3%) (Alemayehu Bekele *et al*., 2016), in Ghana (0.9%) (Baidoo *et al*., 2010), and lower than results from Lalo Kile district, Oromia, Western Ethiopia (7.3%) (Dejene Abrham *et al*., 2019), Gilgel Gibe Dam area, southwest Ethiopia (15%) (Million Getachew *et al*., 2013), Maytsebri primary hospital, north Ethiopia (12.7%) (Menasbo Gebru *et al*., 2019), Wondo Genet district, Southern Ethiopia (24.9%) (Amelo Bolka and Samson Gebremedhin, 2019), Mecha district, Northwest Ethiopia (32.2%) (Berhanu Elfu and Tadesse Hailu, 2018), Nigeria (52.2%) (Ivoke et al., 2017), and Venezuela (57.0) (Rodr†‡guez-Morales *et al*., 2006). The observed difference might be due to environmental conditions and environmental sanitation problems, differences in eating raw vegetables, lack of hand washing, agricultural practices, waste disposal habit, lack of clean waters, and open defecations (Berhanu Elfu and Tadesse Hailu, 2018; Yang *et al*., 2018)

In the current study the prevalence of *S. mansoni* was 3.2%. It was comparable with studies conducted in Bahir Dar, northwest Ethiopia (2.9%) (Adane Derso *et al*., 2016) and Jimma, southwest Ethiopia (3.6%) (Ayalew Jejaw *et al*., 2014). However, it was higher than the finding from northwest Ethiopia (2.2%) (Melashu Balew *et al*., 2017), Wondo Genet district, Southern Ethiopia (2.3%) Amelo Bolka and Samson Gebremedhin, 2019), and Wolayita Sodo Town, Southern Ethiopia (0.6%) (Lealem Gedefaw *et al*., 2015), and lower than that of the studies conducted in Mecha district, Northwest Ethiopia (17.4%) (Berhanu Elfu and Tadesse Hailu, 2018), Waja-Timuga, District of Alamata, northern Ethiopia (73.9%) (Nigus Abebe *et al*., 2014) Cameroon (28.01%) (Tonga *et al*., 2019), and Uganda (36.4%) (Chami *et al*., 2015). This could be due to the differences in the geographical areas, environmental pollution with urine and faeces, the habit of crossing and bathing in river water, and eating raw or improperly cooked vegetables and foods, and cercariae infested water sources (Nigus Abebe *et al*., 2014).

The prevalence of *H. nana* in this study was 0.7%. This prevalence agreed with the findings from Gondar town, northwest Ethiopia (0.5%) (Meseret Alem *et al*., 2013) and Bahir Dar, northwest Ethiopia (0.3%) (Adane Derso *et al*., 2016). Studies reported from Gilgel Gibe Dam area, southwest Ethiopia (1.6%) (Million Getachew *et al*., 2013), East Wollega, Oromia, Ethiopia (1.6%) (Hylemariam Mihiretie *et al*., 2017), and in Baghdad/Iraq (6.67%) (Al-Marsome, 2012) were higher than the current finding. These variations might be due to the variations in maintenance of good personal hygiene and sanitary improvements, consumption of contaminated food and water, and rodent control (Paniker and Ghosh, 2018)

The prevalence of *S. stercoralis* in the current finding was 0.4% which was in line with the study conducted in East Wollega, Oromia, Ethiopia (0.3%) (Hylemariam Mihret *et al*., 2017), Lalo Kile district, Oromia, Western Ethiopia (0.3%) (Dejene Abraham *et al*., 2019). But the current study showed a lower prevalence of *S. stercoralis* as compared to those studies conducted in Bahir Dar, northwest Ethiopia (1.6%) (Adane Derso *et al*., 2016); Mecha district, Northwest Ethiopia (6.4%) (Berhanu Elfu and Tadesse Hailu, 2018); Nigeria (1.3%) (Itoke *et al*., 2017); Papua New Guinea (3%) (Phuanukoonnon *et al*., 2013); and Venezuela (3.3%) (Rodr†‡guez Morales *et al*., 2006). This difference might be due to the differences in contamination of soil with faeces, waking with barefoot, open latrine system, and environmental sanitations (Yitagele Terefe *et al*., 2019)

In this study the prevalence of *E. vermicularis* was 0.4%. It was consistent with the study conducted in Gilgel Gibe Dam area, southwest Ethiopia (0.3%) (Million Getachew *et al*., 2013). However, it was lower than other studies reported from Nigeria (3.5%) (Ali *et al*., 2011); Kenya (4.8%) (Wekesa *et al*., 2014); Venezuela (6.3%) (Rodr†‡guez Morales *et al*., 2006) and far lower than Iraq (32.9%) (Al-Hamairy *et al*., 2013). These variations in prevalence among studies could be due to the differences in environmental sanitation, parasitological methods used during the study, and maintenance of personal and community hygiene such as frequent hand washing, finger nail cleaning and regular bathing, and washing of night clothes and bed linen (Paniker and Ghosh, 2018)

Studies reported that high family size, unsafe and inadequate provision of water, unhygienic living conditions, the absence of proper utilization of latrine, absence of proper hand washing after latrine, and habit of walking with a bare foot were significantly associated with IPIs (Dawit Jember *et al*., 2015; Berhanu Elfu and Tadesse Hailu, 2018; Abiye Tigabu *et al*., 2019 and Dejene Abraham *et al*., 2019), but these were not statistically associated with IPIs in the present finding.

In the current finding, intestinal parasitic infection was statistically associated with eating raw meat with odds of 1.66 (AOR= 1.66; 95% CI: 1.03-2.67, P=0.036). This finding is congruent with the study finding around Lake Zwai, Ethiopia (Ayalew Sisay and Brook Lemma, 2019).

G. lamblia was significantly strongly associated with eating raw vegetables with odds of 2.72 (AOR= 2.72; 95% CI: 1.27, 5.85; P=0.010) which is supported by the study in East Wollega, Oromia, Ethiopia (Hylemariam Mihiretie *et al*., 2017). The other associated risk factor was poor personal hygiene. Mothers whose personal hygiene was poor were 4.02 times (AOR= 402; 95% CI: 1.46, 11.07; P=0.007) more likely to be infected than whose personal hygiene were good. This is in agreement with the finding in Jawi town, northwest Ethiopia (Baye Sitotaw *et al*., 2019), and Goiânia, Goiás State, Brazil (Pereira *et al*., 2007).

Taenia species was significantly associated with eating raw meat. Mothers who ate raw meat were 2.26 times more infected than their counterparts (AOR= 2.26; 95% CI: 1.13, 4.55, P=0.02). This finding was supported by the finding from around Lake Ziwai, Ethiopia (Ayalew Sisay and Brook Lemma, 2019). However, other identified IPIs were not significantly associated with any of the potential risk factors.

The overall prevalence of anaemia among pregnant women attending ANC in current study was found to be 10.1%. This prevalence is in agreement with the study conducted in Adigrat, northern Ethiopia (7.9%) (Brhane Berhe *et al*., 2019); St. Paul,s Hospital, Addis Ababa, Ethiopia (11.6%) (Angesom Gebreweld and Aster Tsegaye, 2018); Adama, Ethiopia (14.9%) (Bizuneh Ayano and Befekadu Amentie, 2018). The prevalence of anaemia in this study is lower compared to in Tikur Anbessa Specialized Hospital, Addis Ababa Ethiopia 21.3% (Alemayehu Hailu and Tewabech Zewde, 2014); in Southern Ethiopia (23.2%) (Meaza Lebso *et al*., 2017); Dera District, northwest Ethiopia (30.5%) (Terefe Derso *et al*., 2017); Ilu Abba Bora Zone, South West Ethiopia (31.5%) (Adamu Kenea *et al*., 2018); Wondo Genet district, Southern Ethiopia (31.5%) (Amelo Bolka and Samson Gebremedhin, 2019); Wolayita Sodo Town, Southern Ethiopia 39.94% (Lealem Gedefaw *et al*., 2015) Urban Area of Eastern Ethiopia 56.8%) (Kefyalew Addis and Abdulahi Mohamed, 2014); Papua New Guinea (63%) (Phuanukoonnon *et al*., 2013). This difference could be due to differences in study area and administration of iron supplements in the health centres which is helpful in combating anaemia during pregnancy (Hylemariam Mihiretie *et al*., 2017).

In this study, the majority of anemic cases, 8.7% were mild and 1.4% was moderate cases of anaemia. Similar studies in Ethiopia reported in which majority of the cases mild anaemia followed by moderate anaemia (Alemayehu Bekele *et al.*, 2016; Bizuneh Ayano and Befekadu Amentie, 2018; Brhane Berhe *et al.*, 2019).

After adjusting for potential confounders, there is no positive association between overall intestinal parasitic infection and anaemia. However, a study by Fikir Asrie (2017) showed anaemia significantly associated with IPIs. The comparatively low prevalence of blood-sucking nematodes such as hookworm (9.0%) and *S. stercoralis* (0.4) may explain the lack of relationship between intestinal parasitic infection and anaemia in this study. However, anaemia was associated with hookworm infection (AOR= 0.270; 95% CI: 0.080, 0.913; p = 0.032). This parasite contributed for 27% of the anaemia cases. However, other findings in Ethiopia (Meseret Alem et al., 2013; Hylemariam Mihiretie et al., 2017; Meaza Lebso et al., 2017; Amelo Bolka and Samson Gebremedhin, 2019) revealed high significant association of hookworm with anaemia.

6. Conclusion and Recommendation

This study indicated that there was high prevalence of IPIs and anaemia among the pregnant women in the selected area. The most common detected intestinal parasites were *Taenia* species followed by *G. lamblia*, *E. histolytica* and hookworms. Eating raw meat was associated risk factor for IPIs. Eating raw vegetables, and poor personal hygiene were predictor of *G. lamblia* and eating raw meat was associated risk factor for *Taenia* species. Additionally there was interaction between hookworm infection and the prevalence of anaemia. So, avoiding eating raw meat and vegetables, making and strengthening sanitation and hygiene programs, creating awareness about IPIs and anaemia, making closed toilet, routine deworming of mothers before pregnancy and on second and third trimesters and iron supplementation are recommended.

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8. Appendices

Appendix A: English Version of the Questionnaires

Questionnaire format for collection of information on the Prevalence of intestinal parasites associated risk factors and its association with anemia among pregnant women attending antenatal care in Yifag Health Center South Gondar, Ethiopia, 2019

Code no.___________

Part I. Participant identification

1. Area of residence_________ kebele _______________ Ethnicity_________

Part II. Personal profile of household

2. Age ___________. Religious Christianity (Orthodox) ______ Muslim______ other______

3. Occupation, agriculture_____, House wives____ Government employed___ Trading ____

4. Education status: illiterate ____, Primary_____, Secondary______, Above secondary

5. Number of family ______

Part III. Information on Risk Factors

1. Do you wash your hands always before meal? Yes ______. No______
2. Do you wash your hands always after latrine use? Yes ______. No______
3. Do you wear shoes always? Yes ______ No______
4. Finger nail A. trimmed _______ B. untrimmed______
5. Do you have toilet? Yes ______ No______
6. What types of latrine you use during defecation? Private___ Public____ Open field
7. Where do you get drinking water? Pond ___, spring ______River ______, Pipe______.

8. How do you use drinking water? Boiling___, Filtering__, Direct, Chlorine treated__.

9. How do you dispose house hold wastes? Burry underground/Incinerate______ open Field______, in to the river______.

10. Have you ever eaten raw meat? Yes_________No___.

11. What about uncooked vegetables? Yes_____ No _________.

12. What about your personal hygiene and life skill practice? Good______, Poor______.

13. Your level of knowledge on parasitic infection? Good______, Poor______.

14. Do you take any anti-parasitic drug for the last three weeks? Yes______, No______.

15. Concerning alcohol drink and related problems

    a. Did you drink alcohol? Yes_______ No_________

    b. if your answer is yes in how much frequency

       day to day ______Three to four days per week _____sometimes ______

16. About pregnancy

    a. On what trimester are you: first________second___________ third_________

    b. for what times your pregnancy one____ two____ three____ four______

    c. What is the year between your pregnancies: _______

17. Information on Anemia

    a. Did you know about anemia? Yes______ NO_____

    b. Did you know about causes of anemia? Yes______ NO______

    c. Did you know about prevention mechanism of anemia? Yes______ NO______

    d. Have eaten fruits and vegetables specially vegetables Yes_______ No______

    e. If you are not eaten why? I cannot get the access ____ I am not interested ____ I don,t know its function ______

    f. Have you taken iron tablet? Yes_______ No________ if your answer is Yes how many months you take ______

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Appendix C: English version of consent form

I will conduct a study to assess public health importance of intestinal parasites in order to determine Prevalence of intestinal parasites associated risk factors and its association with anemia among pregnant women attending antenatal care in Yifag Health Centre

You are being to participate in this study. If you agree, I would like to obtain stool specimen, in plastic sheet and blood from your finger to respectively, from you, which would be used only to detect the presence of intestinal parasites and level of your hemoglobin. You will not get any risk in participating. When you are found positive for the above parasites, you will receive standard drugs free of charge. The information in your records is strictly confidential.

Your participation in this study is completely voluntary and you can refuse to participate or free to yourself from the study. Refusal to participate will not result in loss of medical care provided or any other benefits. Do you understand what has been said to you? If you have questions, you have the right to get proper explanation.

I am informed to my satisfaction about the purpose of this study nature of laboratory investigation. I am also aware of my right to out of the study without having to give reasons for doing so. This consent form has been readout to me in my own language (Amharic language) and I understand the content and I am voluntarily consent to participate in the study.

Study participant code no. ________ Signature ________ Date ______________

Thanks to your participation in this study

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Appendex D. ethical clearance

Appendex E.photo during data collection

During tool examination	Preparation of formol-ether concentration
During re-examined by lab technician	Formol-ether concentration by lab technician
During capillary tube adjustment in hematocrit Machine	During haemoglobin reading