ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF ZOOLOGICAL SCIENCES

PREVALENCE OF GIARDIA AND AMOEBIA INFECTIONS AMONG DIARRHOEAL PATIENTS ATTENDING NEBELET HEALTH CENTER IN TIGRAY ,ETHIOPIA

BY

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A Thesis Presented to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the Requirements for the Degree of MSc in Biology (Summer-in Service)

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September 2019

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ACKNOWLEGEMENTS

I would like to express my deepest gratitude to my advisor Dr. Araya Gebresilassie for his willingness to advise me to conduct my thesis research and provide-me valuable Information an d reference materials as well as continuous follow up of my work to enrich my knowledge and skill in conducting my thesis.

It is also my pleasure to express my gratitude to Nebelet Health Center staff, particularly data recorder and Nebelet Health Center Director for their unreserved support they pledge during data collection. I also forward my special thanks to Hailay G/Kidan and GetachewWeldu preparatory school Teachers who supported my thesis work directly or indirectly. My special thanks also extend to Addis Ababa University for accepting me as a Master's student and providing financial and material provisions for this study. The Federal Ministry of Education is also acknowledged for financial support and the sponsorship provided to me to pursue my post-graduate study.

Finally, to my beloved family members, in particular to my brother Biniam G/slassie, my sister Mebrhit Miruts and my daughter Mahlet Leul are thanked for their moral support and encouragement throughout the study.

LIST OF ACRONYMS

AIDS	Acquire Immunodeficiency syndrome
CDC	Centers for Disease Control and Prevention
CSA	Child Support Agency
DNA	Deoxyribonucleic acid
EPHI	Ethiopian Health Institute
ELISA	Enzyme-linked Immunosorbent Assay
FDA	Food and Drug Administration
IgA	Immunoglobulin Antibody A
NTHC	Nebelet Town Health Center
PCR	Polymerase Chain Reaction
WHO	World Health Organization

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ABSTRACT

Parasitic infections including intestinal parasites are considered as the most common communic able diseases Worldwide, especially in developing countries Ethiopia, like other developing count ries, intestinal parasitic infections are the major public health problems affecting millions annually. With this in mind, this study was designed with the objective of identifying the prevalence of protozoan infections among diarrheal patients attending Nebelet Health Center in Tigray Regional State, northern Ethiopia. Retrospective analysis was conducted to determine the prevalence of intestinal parasitic protozoan infection in patients attending Nebelet Health center from September 2013 to August 2018 from records of stool examination. Stool samples collected from the patients were examined using direct wet mount and formal Ether concentrati on techniques by experienced laboratory technologists of the health center. All cases intestinal protozoan parasitic infection reported between 2013 and 2018 were reviewed and analyzed. In the past five years, a total 2,928 stools sample was examined for various intestinal parasitic infections. Out of these, 489 (16.7%) were positive to intestinal protozoan parasitic infections. The only protozoan parasites detected in the examined stool samples in the health center were Entamoeba histolytica/-dispar and Giardia lamblia. In particular, G lamblia was the most prevalent parasite (56.4%) followed by E. histolytica with an infection of (43.6%). The result also showed that there was difference in the overall prevalence rate of the parasitic infection in diffe rent age groups: <5yr 172 (35.17%), 5-14yr 127 (25.97%), 15-29yr 91 (18.60%), 30-44yr, 68 (13.9 0%),>44yr, 31 (6.36%). Higher infection was observed among males (55.2%) than females (44.8 %). In overall, the study revealed that protozoan parasitic infections represent a major public health problem amongst patients attending the health center in Nebelet Town. Therefore, in order to minimize the risk and exposure of the community to these intestinal parasites all the concerned government and non- government sectors should design appropriate and cost-effective control methods that improve the quality of personal hygiene and environmental sanit ation in the study area.

1. INTRODUCTION

1.1 Background of the study

Intestinal protozoa are single-celled eukaryotic microorganisms that reside in the gastrointestinal tract of humans and other animals. They are known to affect all vertebrate and many invertebrate species and are able to life in virtually all body sites of their hosts. Most of the time, protozoans are microscopic and due to their minute sizes, protozoans can easily enter the human body, thus making them a good candidate for parasitic organisms. Some protozoans are harmless; while some can cause disease that ranges from benign to sever.

Intestinal parasitic infections are considered as one of the main socioeconomic and health problems around the World; mostly in tropical and sub-tropical regions (Hotez*, et al*., 2009: Pullan *et al*, 2014). According to the World Health Organization (WHO) reports, approximately two-thirds of the world population is infected with a wide range of parasitic worms and protozoa,and yearly, 450 million people demonstrate clinical manifestations (WHO, 2014).These reports also showed that, nearly 16million of the total deaths occurring in developing countries, especially school age children, have the maximum rate of morbidity to intestinal parasites in comparison with other ages (WHO, 2007). Intestinal parasites are neglected tropical diseases widely distributed in Sub-Saharan Africa, including Ethiopia largely due to the lower socio-economic status, poor environmental and personal hygiene, poor nutrition, low literacy rate, overcrowding and climatic conditions that favor the development and survival of these parasites are some of the factors contributing to the high prevalence of intestinal parasitic infection (WHO, 2012; Hotez*et al*., 2014).

The main intestinal parasitic agents that are considered as major causes of morbidity and mortality in developing countries are *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium species* (Davis *et al.,* 2002). These parasites cause gastrointestinal disorders such as diarrhea, dysentery, vomiting, lack of appetite, hematuria, and abdominal distension and in some cases mental disorders. The Global Burden of Disease Study (GBDS) found that *amebiasis* caused by

E. *histolytica* was responsible for more than 55, 000 deaths and 2.2 million Disability-adjusted life years, (DALYs) and *cryptosporidiosis* accounted for about 100,000 deaths and 8.4 million DALYs in 2010 (Hotez, 2014). Also, *giardiasis* caused by *G. lamblia* produced 171,1oo DALYs in 2010 (Toreerson*et al*., 2015). Both *G. lamblia* and *E. histolytica* are frequently transmitted by contaminated food and/or drinking water, as well as potentially spread from person to person through fecal-oral contact (Stanley, 2003). Intestinal parasitic protozoans affect people of all ages, but children are more prone to heavy infection because of their low development of immune system and routine play with focally contaminated soil.

Intestinal parasites are widely distributed largely in Ethiopia due to lack of environmental and personal sanitation, contamination of food and drinking water resulted from open defection around the settlement and lack of awareness of simple health promotion practices (Asrat*et al.,* 2011; Teklu*et al*., 2013). According to the Ethiopian Ministry of Health (more than half a million annual visits of the outpatient services of the health institutions are due to intestinal parasitic infections. However, this report may be an underestimate as most of the health institutions lack appropriate diagnostic method to detect low levels of parasite burden. In addition, some of the diagnostic methods for specific intestinal parasites; especially for newly emerging opportunistic intestinal parasites are not available to peripheral health institutions. The prevalence of intestinal parasite infection among various groups of the community ranges from 14.5 to 44.1% out of which *G. lamblia, E. histolytica, Schistosomiasis, hookworm* and *Ascariasis* infections are the most frequent in Ethiopia (Asrat*et al*., 2011; Teklu*et al*., 2013; Dejen*et al*.,2017).

A number of studies have been conducted on the distribution and prevalence of intestinal parasites in different parts of Ethiopia (Abraraw *et al*., 2013; Teklu *et al*., 2013; Gebremichael, 2016; Dejen*et al*., 2017; Aschale*et al*., 2019). The findings of these studies showed that the distribution and prevalence of various species of intestinal parasites differ among localities and age groups because of several environmental, social, economic and geographical factors.

1.2 Statement of the problem

In most parts of Ethiopia, people consume unprotected food and water from different sources, increasing the possibility of infection with intestinal parasitic protozoans. More than 80% of diseases in the world are attributed to unsafe drinking water or to inadequate sanitation practice (Raza, 2003). In many villages of rural parts of Ethiopia, the community is forced to use unprotected water from rivers, streams ponds, shallow wells, water harvesting ponds etc. In such area where people use water from different sources, the probability of contamination by water borne diseases such as *giardiasis* and *amebiasis* is expected to be high. However epidemiological data on the prevalence infections in Nebelet town and its surroundings remains unknown. Therefore, the current study was initiated with the following objectives.

1.3 Significance of the study

The finding of the study will help all the concerned stakeholders such as Governmental and non-Governmental organizations to design evidence-based control and prevention strategies that could overcome the widespread public health challenges associated with the mortality and morbidity of intestinal parasitic protozoan infections in northern Ethiopia in general and Nebelet town and its surrounding in particular. Similarly, partners of the project will benefit from the experience and scientific knowledge obtained in the project activities. Research findings will also be distributed to different bodies through publications of books, manuals, research articles, and leaflets**.**

2. LITERATURE REVIEW

2.1 Amoebiasis

Amoebiasis caused by *E. histolytica* it is a protozoan parasite of phylum Sarcomastigophora that colonizes and reproduces in large intestines of humans. The disease may remain restricted to intestinal lumen or invade intestinal lining causing amebic dysentery. It is not only causes severe diarrhea but also result in extra intestinal manifestations including rectal bleeding, amoeboma, toxic megacolon, pneumatosis coli, peritonitis and abscesses in the intestine, liver, lung and other organs. *Entamoeba histolytica* is reported to be responsible for deaths of approximately 1, 00,000 persons per year, secondly to another protozoan infection, malaria (WHO, 1997).

Amebiasis is widely spread parasitic disease and caused by the protozoa *E. histolytica***.** *E. Histolytica* is morphologically identical with the apathogenic *Entamoeba dispar***.** E. histolytica cyst is spherical, usually 10 to 16µm in diameter and is surrounded by a retractile chitin-containing wall. It contains four nuclei when mature, one nucleus when immature with glycogen in a vacuole and often with chromatoid bodies. The trophozoites, sized ranged from 20 to 40µm in diameter, are as they were protected by the wall, and the survival time could be longer especially in highly Movable (WHO, 1997; Stantly, 2003). The cysts can remain alive outside the host for weeks or months in moist conditions such as water, soil and on foods. They can be identified by means of zymodem and DNA analysis with monoclonal antibodies (Kayser *et al*., 2005). *E. histolytica* is estimated to inflict severe disease in 48 million people and 100 000 Death per year (Petri and Singh, 1999). *E. histolytica* is the causative agents of amebic Dysentery and amebic liver abscess it is one of the leading causes of death from parasitic Diseases (Davis *et al*., 2009). The most common manifestation of amebic infection is dysentery and liver abscess, but infections of the lung, heart and brain also occur (Haque*et al*., 2003). In low-income Countries amebic infection depends largely on cultural habits, ages, and levels of sanitation, Crowding and socio economic status (Petri *et al*., 2000). However, in confirmatory diagnostic analysis using PCR solution hybridization enzyme linked immunoassay E. dispar was identified in only 21 of 232 cases while no *E. histolytica* DNA was detected (Kebede*et al*., 2003).

Three genera of amoeba may inhabit the intestinal tract of humans: *Entamoeba, Iodamoeba* and *Endolimax*. These members of the genera considered non-pathogenic include *Entamoebah artmani*, *Entamoeba gingivalis*, *Entamoeba coli*, *Endolimax nana*, and *Indamoeba butschlii* (Mah on and Manuselis 2000, (Washingtan Winn *et al.,* 2006). Amebiasis is caused by protozoa *Entam oeba histolytica*. This infection is one of the leading causes worldwide of diarrhea.Approximatel y 480-500 million people are infected worldwide, although only 10% are symptomatic (Eddlesto n *et al*., 2005; Nagata *et al.,* 2012). Infection can progress from watery to bloody diarrhea and, if untreated, may be fatal. The parasite is passed from person to person by fecal oral transmissi on when food or water become contaminated with human feces containing cysts and can also pass during sexual contact (CDC, 2010). In addition to gastro intestinal infection, amoebas can spread to extra-intestinal sites, such as the liver, lungs, and brain, leading to serious disease (Bogitsh*et al.,* 2013; Nagata *et al.,* 2012; Rey, 1980).

2.1.1 Multiplication and life cycle of *Entamoeba histolytica*/dispar

Amoebae multiply in their host by simple binary fission. Most multiplication occurs in the host, and survival outside the host depends on the desiccation-resistant cyst form. Encystment occurs apparently in response to desiccation as the amoeba is carried through the colon. After encystment, the nucleus divides twice to produce four nucleate mature cysts. Mature cysts are ingested via contaminated water or food. Excystment occurs after ingestion and is followed by rapid cell division to produce four amoebae which undergo a second division in the small intestine. As (Enrique and Douglas**,** 2007), described trophozoites inhabit the large intestine and can either invade the tissue (pathogenic amoeba) or eliminated in the stools. Trophozoites do not survive outside the body. Each cyst yields eight tiny amoebae (Mahon and Manuselis, 2000; Washington *et al.*, 2006).

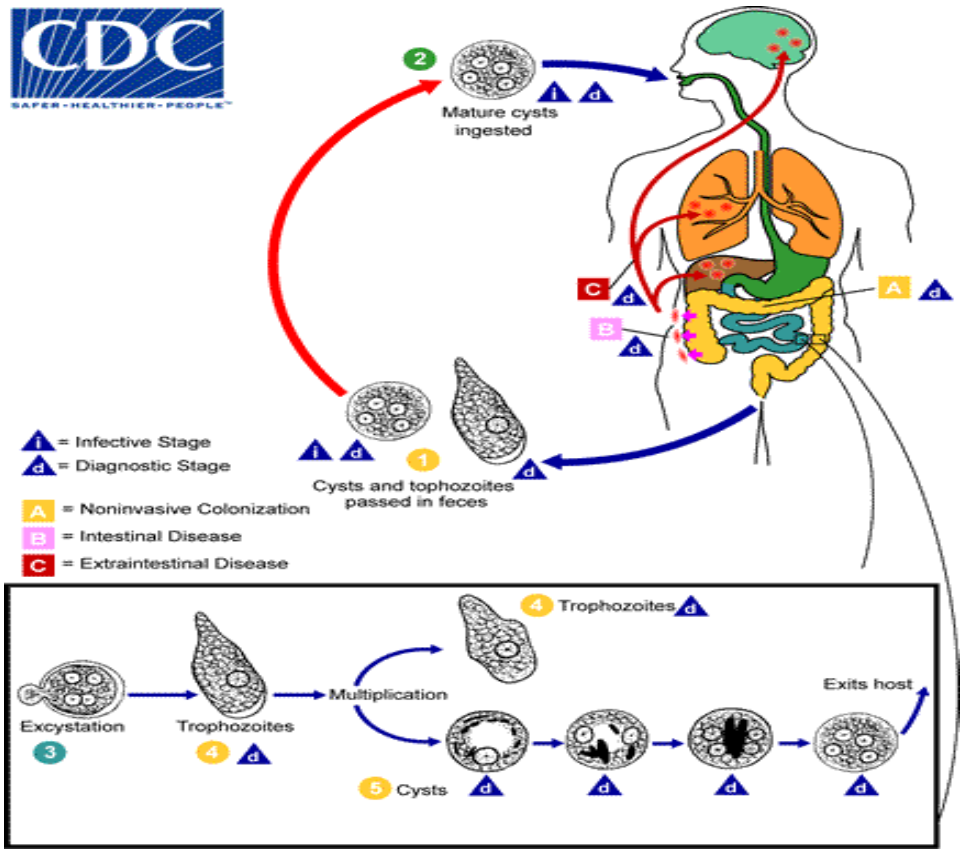

Figure 1. *Life cycle of E. histolytica* (Source https://www.cdc.gov> parasites > pathogen20/2015)

2.1.2 Epidemiology of Amoebiasis

Amoebiasis around the world is complicated by the existence of three different forms that are morphological identical but genetically distinct and include *E. histolytica* which is a known pathogen, *E. dispar* and *E. moshkovskii*which are non-pathogens (Ali *et al*., 2008). This is particularly relevant to the African continent as well as many other developing countries in the world, including Latin American and Asian countries, where there is lack of specific diagnostic tools. According to some studies conducted in some African countries (Alonzo *et al.,* 1993; Molback *et al*., 1994; Njoya *et al*., 1999; Roche *et al.,* 1999); from 6% to 75% of the population c arry the parasite.These studies were conducted using microscopic examination giving a general idea on the distribution of the disease in the population. Such results require confirmation by te chniques that clearly differentiate *E. histolytica* from *E. dispar*, which is not pathogenic.

Countries in Central and Latin America where the parasite displays endemic behavior include Mexico, Brazil, and Ecuador. In Mexico for example, the incidence rate of intestinal Amoebiasis from 1995 to 2000 was reported to be between 1000 and 5000 cases/100,000 inhabitants annually. Incidence values from 2002 to 2006 were 1128.8 to 615.85/100,000 inhabitants per year. As in other developing countries, those under 15 years of age were the most frequently affected group, with a notable increase in children aged 5–9 (Ximenez, 2009).

In Aracaju and Brazil, Lawson *et al*., (2004) demonstrated *E. histolytica* in 1% of cases whereas *E. dispar* was found in 13% of the cases. Whilst in Pernambuco state, northeastern Brazil *E. dispar* was found in 74.19% of culture positive samples using the PCR method, no *E. histolytica* was reported (Pinheiro*et al*., 2004). In a remote area of Ecuador, Gatti*et al* (2002) using isoenzyme analysis reported an 18.9% infection rate with *E. histolytica* while 70.3% were infected with *E. dispar*. In the Indian subcontinent, the prevalence of intestinal Amebiasis among hospitalized patients was found to be around 11.7% using microscopy. However, using molecular biology tools such as PCR, *E. histolytica* was shown to be in 3.5% of those infected (Khairnar*et al*., 2007). In another endemic country such as Bangladesh and using ELISA antigen detection kits, *E. histolytica* prevalence was found to be 4.2% among children living in the urban slums of Dhaka (Hague *et al.,* 2006). Many studies have been conducted in different parts of the world, (Ghosh *et al.,* 2000). But the region most concerned by this problem (Africa), remains unexplored. Thus, the epidemiology of Amoebiasis still remains very uncertain particularly in this part of the world.

2.1.3 Diagnosis of Amoebiasis

Laboratory diagnosis of intestinal Amoebiasis is based on examination of a fresh diarrheic or dysenteric fecal specimen or rectal scraping for motile amoebae using saline. Examination of formed or semi-formed feces for cyst stages is also done. Such stool can be examined by direct saline and/or iodine smear, and zinc sulphate floatation or centrifugal floatation method. Specimens must be examined without delay otherwise identification of the trophozoites becomes impossible because the amoeba lose their motility, extrude vacuoles containing red cells, round up with the recognition that *E.histolytica* is morphologically identical but genetically

distinct from *E.dispar*, cysts,formerly reported as *E.histolytica* should be now reported as *E.hist orlytica/dispar/moshkovskii*.Since the above method relies on microscopic examination by morphology of protozoa, it is difficult to identify among protozoan with similar morphology. The only way to differentiate these organisms is using zymogen and DNA analysis with monoclonal antibodies.

2.1.4 Mode of Transmission of Amoebiasis

The parasite which causes Amoebiasis is passed from person to person by fecal oral transmissio n when food or water become contaminated with human feces containing cysts and can also be passed during sexual contact (CDC, 2010).*E. histolytica* cysts and trophozoites are passed in feces and infection occurs when mature cysts are ingested from contaminated food, water, and hands (CDC, 2010). Trophozoites can remain in the intestinal lumen without causing disease resulting in asymptomatic carriers or they invade the intestinal mucosa, enter the bloodstream and cause extra intestinal disease (CDC, 2010). Cysts shed in feces may remain viable and infective for up to two months outside the human host in cool, moist conditions (Eddleston *et al*., 2005).

2.1.5 Control and Prevention of Amoebic dysentery

To prevent the spread of amebiasis around the home: wash hands thoroughly with soap and hot running water for at least 10 seconds after using the toilet or changing a baby's diaper, and before handling food ,clean bathrooms and toilets often; pay particular attention to toilet seats and taps, avoid sharing towels or face washers. To help prevent infections: avoid raw vegetables when in endemic areas, as they may have been fertilized using human feces, Boil water or treat with iodine tablets, avoid eating street foods especially in public places where others are sharing sauces in one container, good sanitary practice, as well as responsible sewage disposal or treatment are necessary for the prevention of *E. histolytica* infection on an endemic level. *E. histolytica* cysts are usually resistant to chlorination; therefore, sedimentation and filtration of water supplies are necessary to reduce the incidence of infection.

Entamoeba histolytica cysts may be recovered from contaminated food by methods similar to those used for recovering *Giardia lamblia* cysts from feces. Filtration is probably the most practical method for recovery from drinking water and liquid foods. *E. histolytica* cysts must be distinguished from cysts of other parasitic (but nonpathogenic) protozoa and from cysts of free-living protozoa as discussed above. Recovery procedures are not very accurate; cysts are easily lost or damaged beyond recognition, which leads to many falsely negative results in recovery tests.

Prevention of amebiasis at present requires interruption of the fecal oral spread of the infectious cyst stage of the parasite. Because cysts are resistant to chlorine or iodine, in developing countries water must be boiled before it is safe to drink, and raw vegetables must be washed with soap and then soaked in vinegar for 15 min before they can be eaten. Since amebiasis often spreads within a household, it is prudent to screen family members of an index case for intestinal *E. histolytica* infection (Petri and Singh, 1999).

Amoebic dysentery is a food and waterborne disease that can be found worldwide. Effective prevention and control rely on (a) maintaining good environmental sanitation, especially in controlling quality of drinking water; (b)prompt investigation of cases and implementation of control measures to prevent spread of the disease; (c) health education to the general public and food trade on observance of good personal, environmental and food hygiene. The existing prevention and control measures are examined and some potential areas are discussed for further improvement.

2.1.6 Treatment of Amoebiasis

Symptomatic patients with bloody stools containing motile trophozoites with ingested erythroc ytes should be treated according to the severity of the disease. Therapy for invasive infection differs from therapy for noninvasive infection. Noninvasive infections may be treated with paromomycin whereas invasive amebiasis (e.g., colitis, liver abscess) should be treated particula rly with metronidazole (Powell *et al*., 1966). There is no vaccine; there are two treatment options depending on the infection, amebiasis in tissues is treated with metronidazole,

tinisazole, nitazoxanide, dehydroemetine or chloroquine, while luminal infection is treated with diloxanidefuroate or iodquinoline (Madigan, *et al*, 2003). Amoebicide: *E. histolytica* infections occur in both the intestine and (in people with symptoms) in tissue of the intestine and/or liver. (*Ryan, et al., 2004);* those with symptoms require treatment with two medications, an amoebici dal tissue-active agent and a luminal cysticidal agent.

2.2. Giardiasis

Giardiasis is a disease caused by *Giardia lamblia* it is a flagellated protozoan parasite of phylum Sarcomastigophora that colonize and reproduce in small intestines of humans. *Giardia* is most frequently reported as a cause of diarrhea worldwide. Giardiasis can be responsible for severe malabsorption syndrome causing malabsorption of fat, proteins, folic acid, Vitamin A and vitamin B12 and these nutritional deficiencies in turn may lead to serious organ damage [Ali SA, Hill DR (2003). *Giardia intestinalis* results in stunted growth and poor psychomotor develop ment of children (Monajemzadeh SM, Monajemzadeh (2008).

The most common human protozoan pathogen of the gastrointestinal tract is *Giardia intestinali s* (Bogitsh*et al.,* 2013; Eddleston *et al*., 2005). It affects approximately 2% of adults and 7% of children worldwide (CDC, 2011). A person infected with *G. intestinalis* may shed 1 to 10 billion infective cysts daily in their feces and as few as 10 cysts can lead to disease in another person (CDC, 2011). Once a human ingests a cyst, excystation occurs in the small intestine as two trophozoites are produced. The trophozoites multiply by longitudinal binary fission and can attach to intestinal mucosa by their ventral discs (CDC, 2011). While both cysts and trophozoites pass in feces, only cysts can live outside the host and may be viable for months in cold water (Bogitsh*et al*., 2013; CDC, 2011; Desponmmier*et al.,* 2005).

For the majority of *Giardiasis* cases, the disease is self-limiting, but reoccurrence does happen (Bogitsh*et al*., 2013). For those who develop the disease, the most common symptom is diarrhea (Desponmmier*et al.,* 2005; Eddleston *et al.,* 2005). Other symptoms include gas, chronic malabsorption syndrome and failure to thrive, steatorrhea or fatty diarrhea, abdominal cramping, gas, nausea and vomiting, dehydration, and gas. Diarrhea may last for months if untreated, varying in intensity (Bogitsh *et al*., 2013; CDC, 2011; Desponmmier *et al*., 2005; Gide

on, 2007a; Rey, 1980). Other physiologic responses to *Giardiasis* include IgA immune productio n and gluten sensitivities The severity and duration of the infection depends on both the immun e and non immune defenses of the host and the parasite's ability to evade them (Bogitsh *et al.,* 2013; CDC, 2011; Desponmmier *et al.,* 2005; Rey, 1980).Diagnosis is confirmed by microscopic i dentification of *G. intestinalis* cysts in feces and multiple specimens may be required to increase the sensitivity as the cysts can be excreted intermittently (Bogitsh*et al*., 2013; CDC, 2011). Fecal immunoassays are the most sensitive and specific (CDC, 2011). Rehydration and symptomatic relief are usually used for treatment, as well as anti-giardia drugs such as metronidazole, imidazole, and nitazoxanide.

2.2.1 Multiplication and life cycle of Giardia lamblia

Trophozoites are actively metabolizing, motile form, live in the duodenum and jejunum and multiply by binary fission. Trophozoites that are swept into the fecal stream lose their motility, round up, and are excreted as dormant; resistant cysts. Excreted trophozoites disintegrate the cyst is sufficiently hardy to survive host-to-host transfer. The exposure of cysts to host stomach acidity and body temperature triggers encystation, with the emergence of trophozoites which rapidly multiply and attach to the host small intestinal villi by means of a disk on their posterior or ventral surface (Meyer, 1990). *Giardia* infection is acquired by ingestion of mature cysts (infective dose varies from 10-100 cysts) via contaminated water or food. The life cycle of Giardia lamblia was summarized below:

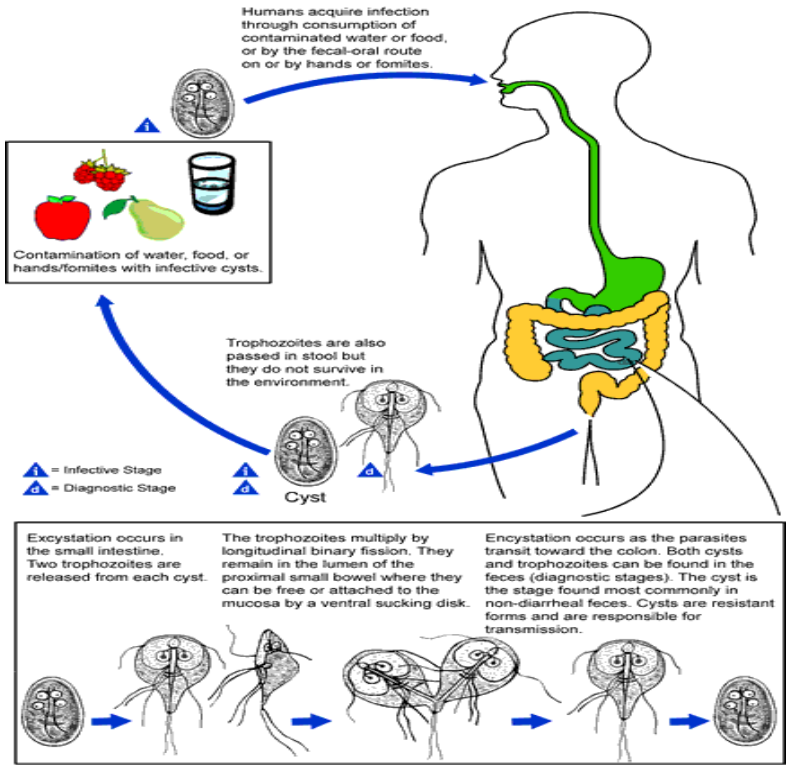

Figure 2.Life cycle of Giardia lamblia (Sources https://www.researchgatte.net Publication: Mar22, 2018)

2.2.2 Epidemiology of *Giardiasis*

According to Thompson *et al*. (1993), 5% of acute diarrhea and 20% of chronic diarrheal illness in the world are attributable to *Giardia lamblia.* The incidence of diarrhea associated with *Giardia lamblia* is generally higher in developing countries such as Africa, Asia, South and Central America, where access to clean water and basic sanitation is lacking. The prevalence rate of *Giardia lamblia* infection in developed nations is around 2 to 5% but in developing nations, it is reported to be 20 to 30% (Thielman and Guerrant, 1998). Nearly all children in developing countries will acquire *Giardia lamblia* at some point in their childhood. In developed countries, such as Western Europe and the United States, *Giardia lamblia* infection is highly associated with contaminated drinking water, person to person contact, recent foreign travel and recreational swimming in contaminated lakes, rivers and swimming pools (NATHNAC, 2004)

2.2.3 Diagnosis of Giardiasis

Diagnosis is confirmed by microscopic identification of *G. intestinal* iscysts in feces and multiple specimens may be required to increase the sensitivity as the cysts can be excreted intermittently (Bogitsh*et al.,* 2013; CDC, 2011). Fecal immunoassays are the most sensitive and specific (CDC, 2011). Rehydration and symptomatic relief are usually used for treatment, as well as anti-giardia drugs such as metronidazole, imidazole, and nitazoxanide. Prevention is similar to that of *E. histolytica* and includes hand-washing after using the bathroom and before eating or preparing food as well as proper water treatment (Bogitsh*et al.,* 2013; CDC, 2011; Desponmmier*et al.,* 2005; Eddleston *et al*., 2005).

2.2.4 Mode of Transmission of Giardiasis

Giardiasis transmits from person to person by contaminated food and water, fecal-oral transmission by contaminated hand. The parasite transmitted indirectly by contaminated water. *G. lamblia*is frequently identified as the etiologic agent in waterborne diarrhea out breaks from contaminated surface water that has been ineffectively filtered or pretreated. Thus contaminated water source include surface waters, shallow wells and household water from either of these sources.

2.2.5 Control and Prevention of Giardiasis

Watershed protection is one of the controls and prevention of protozoan infection .Which is the recognition of local sources of contamination with *Cryptosporidium* and *Giardia,* control of t hat contamination by diversion or treatment of discharges and reduction of direct input of farces, especially in otherwise pristine waters, by people, farm animals, and wildlife or from manure storage. Treatment of sewage in activated sludge systems or waste stabilization ponds is an important barrier against environmental transmission: both processes remove 90–99.7% of the cysts and oocytes (Sykora*et al.,* 1991; Grimason*et al.,* 1992). Treatment of agricultural wastes before their application to the land also reduces the number and viability of *Cryptosporidium* oocytes: aerobic treatment of cattle slurry at increased temperatures and

ammonia concentrations rapidly inactivates oocyte (Svoboda *et al.,* 1997) and composting of bedding reduces the Viability of oocyte.

Storm run-off and snowmelt from unprotected watersheds have been implicated as sources of peak contamination of source water (Stewart *et al*., 1997; Atherholt*et al.,* 1998), and may result in treatment overload and the contamination of drinking-water with oocytes. Knowledge of the characteristics of the plume of contamination from watershed sources can be used to locate and design abstraction points. An illustration of the importance of this is provided by the intake of the southern plant of Milwaukee in Lake Michigan, which proved to be exactly in the plume of the Milwaukee River. The turbidity in the raw water peaked and this coincided with treatment failure, resulting in the breakthrough of turbidity and oocyte into Milwaukee drinking-water and a consequent massive outbreak of disease (MacKenzie*et al*., 1994). Installation of pretreatment storage reservoirs flattens peak contaminations (Ketelaars*et al*., 1995) and the storage capacity makes it possible to stop the intake of surface water temporarily during high contamination events. Since the protozoa are typically related to faecal contaminati on of surface water, several studies have investigated the use of indicator bacteria to predict high levels of protozoa. No consistent relationship has been observed, however, between indica tor bacteria (thermo tolerant coliform) levels and concentrations of *Giardia* or *Cryptosporidium.* The low and varying recovery rates of the protozoa detection methods may be an important confounder in detecting these relationships. Since oocytes are much more persistent than coliforms and enterococci in water, it is likely that these bacteria are not valid indicators, especially if the contamination source is distant. More persistent bacterial indicators (spores of *Clostridium perfringens*) may prove useful indicators for these protozoa (Payment & Franco, 1993; Hijnen*et al.,* 1997). In the absence of valid surrogates, watershed assessment to determine local sources of contamination and define the amount of treatment necessary should include monitoring for protozoa. Development of transport and fate models f or predicting (oo) cyst concentrations based on data on the sources may help in identifying important sources.

2.2.6 Treatment of Giardiasis

The most routinely used classes of anti *Giardia* drugs include Nitroimidazoles (such as Metronid azole, tinidazole, ornidazole and senidazole), Quinacrine, Furazolidone, Paromomycin and Benzi midazoles. Metronidazole is the most common drug used for treatment of giardiasis worldwide. As it is explained by Gardner and Hill (2001), unlike other drugs Metronidazole is absorbed in body tissue quickly and completely. It has also a capacity to penetrate body tissue and be found in secretions like saliva, breast milk, semen and vaginal secretions. Furazolidone (Furoxone) is one of the nitrofuran compounds. It is the only drug that is available in a liquid suspension in the United States and remains an important therapeutic agent worldwide. It has been widely used in pediatric population (Lerman and Walker, 1982). Its efficacy is slightly lower than those of metronidazole and quinacrine (Gardner and Hill, 2001). According to Kerutner*et al*., (1981), another group of drug, paromomycin (Humatin), has been proposed as a treatment for giardiasis in resistant infection and during pregnancy.

2.3 Prevalence of Amoebiasis and Giardiasis Worldwide

Intestinal amoebiasis caused by the protozoan *Entamoeba histolytica* is the third greatest parasi tic disease responsible for death in the world after malaria and schistosomiasis. (Voigt *etal.*, 1999). It affects approximately 180 million people, of whom 40,000 to 110,000 die each year (WHO, 1997). Among important enteric protozoan, *Giardia lamblia*; is worldwide distributi on and it is common in warm moist climatic conditions (Keating, *et al.,* 1998). *Giardiasis* caused by *Giardia lamblia*, is a frequent cause of diarrhea (Faye, *et al*., 1997). That can have a negative impact on growth and development of children (Siimsek*et al.,* 2004). It also affects approximately 200 million people worldwide (Mineno, *et al*., 2003).

2.4 Giardiasis and Amebiasis in Ethiopia

Over 60% of the communicable diseases in Ethiopia are due to poor environmental health condi tions arising from unsafe and inadequate water supply and poor hygienic and sanitation practices (Abebe, 1986). According to The Ministry of Health (1997), nearly 80% of the rural and 20% of urban population have no access to safe water. Three-fourth of the health problems of

children in Ethiopia are communicable diseases arising from the environment, especially water and sanitation. A lot of mortality in less than five years is due to diarrhea in which water related diseases occupy a high proportion. As a result of low level standards of living, poor environment al sanitations and ignorance of simple health promoting factors, intestinal parasitism is very high. Even though the prevalence of individual parasites varies in different parts of the country, *Ascaris lumbricoides*is the most prevalent intestinal parasites (Tedla and Ayele, 1986).

In a study conducted in southwestern Ethiopia, the prevalence of *giardiasis* was 13.7% though the rate is much lower than *Ascaris lumbricoides* (Ali *et al.,* 1999).Intestinal parasites including *Giardia, Cryptosporidium* and *amoeba* are widely distributed in the country (McConnel and Armstrong, 1976). Reports from different parts of Ethiopia showed different prevalence rate of *cryptosporidiosis*, *giardiasis* and *Amebiasis*. The prevalence of *Cryptosporidium* infection in children with diarrhea ranged from 3.3 percent in Jimma, 5.6 percent in Addis Ababa to 9 percent in Northwestern Ethiopia (Mersha and Tiruneh, 1992; Assefa*et al*., 1996; Gebru and Girma, 2000).In Ethiopia the prevalence of *cryptosporidiosis* in HIV / AIDS patients reached up to 25.9% (Fisseha, *et al.,* 1998, Endeshaw*et al*., 2004). McConnel and Armstrong (1976); reported an overall *giardiasis* prevalence of about 11.4% in a study conducted on the central plateau of Ethiopia. Seyoum*et al*., (1981) have also reported varying degree of prevalence rate in different communities. According to Birrie and Erko (1995), based on a countrywide survey of giardiasis*,* the overall prevalence among school children and residents were 8.9% and 3.1%, respectively and that of the non- school children were 4.4%. A number of survey and routine diagnosis in Ethiopia indicate that *amebiasis* is one of the most widely distributed diseases (Kloos and Tesfayohanis, 1993)**.** In a country wide survey of amebiasis in 97 communities, the overall prevalence of *Entamoeba histolytica* infections, as measured by rate of cyst-passers, in schoolchildren and non-school communities were 15.0% and 3.5%, respectively (Erko*et al*., 1995).

The prevalence of *amebiasis* as high as 55% was reported in a survey conducted among Saysay population, in Blue Nile gorge (Torrey, 1965). In another survey of 50 communities of the central plateau of Northern Ethiopia, the parasite was reported in 94% of the communities,

with prevalence rate ranging from 3% to 55% (McConnel and Armstrong, 1976). Recent report indicate that the prevalence of *Cryptosporidium parvum* and *Giardia lamblia* among diarrhea patients referred to EHNRI (Ethiopian Health and Nutrition Research Institute) were 20.8% and 8.6% respectively (Endeshaw*et al*., 2004).

2.5 Prevalence of Amoebiasis and Giardiasis in Tigrai

In a study undertaken among inmates of Mekelle prison, Tigrai Region to determine the prevalence of intestinal protozoan parasites and associated risk factors, *E. histolytica*/dispar was the predominant parasite, accounting for (23.3%) of the infections followed by *G. lamblia* (10.3%) (Mardu*et al*., 2017). Another study in Wukro town showed that he overall prevalence of intestinal parasitic infections among all age groups of the pupils in two schools was 60.7% (Eleni *et al*., 2014). Of these, the prevalence of any intestinal parasitic infections for males and females was 58.2% and 60.8%, respectively.

3. OBJECTIVE

3.1 General objective

The overall objective of this study was to determine the prevalence of *Giardiasis* and *Amoebiasis* infections among diarrheal patients attending Nebelet Health Center in Central Tigrai Northern Ethiopia.

3.2 Specific objectives

i. To identify the major protozoan parasites occurring among diarrheal patients attending Nebelet Health Center.

ii. To compare the prevalence of Giardia and Amoeba infections among diarrheal patiet ns attending in the study area.

iii. To compare the prevalence of different protozoan infection among patients at different age group and between male and female patients

4. MATERIALS AND METHODS

4.1 Description of the study area

The study was conducted in Nebelet Health Center in Werie Leke wereda in the Central Tigrai Northern Ethiopia (Figure 3.1). The town of Nebelet is located around 918 and 135km north of Addis Ababa and Mekelle cities, respectively. Geographically, the town-is situated with latitude and longitude of $14^0$5'48" N and $39^0$16' 5"E, respectively. Nebelet lies on ragged topography with an altitude ranging from 2150-2270 meter above sea level. It has a total area of 154. 45km^2.The study area has Kola agro ecological zone with annual temperature ranging 12 to 25^0c and annual rainfall ranging from 759-1500 in mm.

The town has in habitants of 4750, out of these, 2430 are females and 2320 are males. In Nebelet town the major economic activities are mainly related to petty trade, daily labor and urban agriculture such as dairy farming, irrigation, and poultry. Selling '*Tella*' is among the major sources of incomeand is the main means of income for women to sustain their life and educate their child. The residents of the town mainly depend on tap water that is pumped from Chiemit (Gidey and Zeleke, 2015).

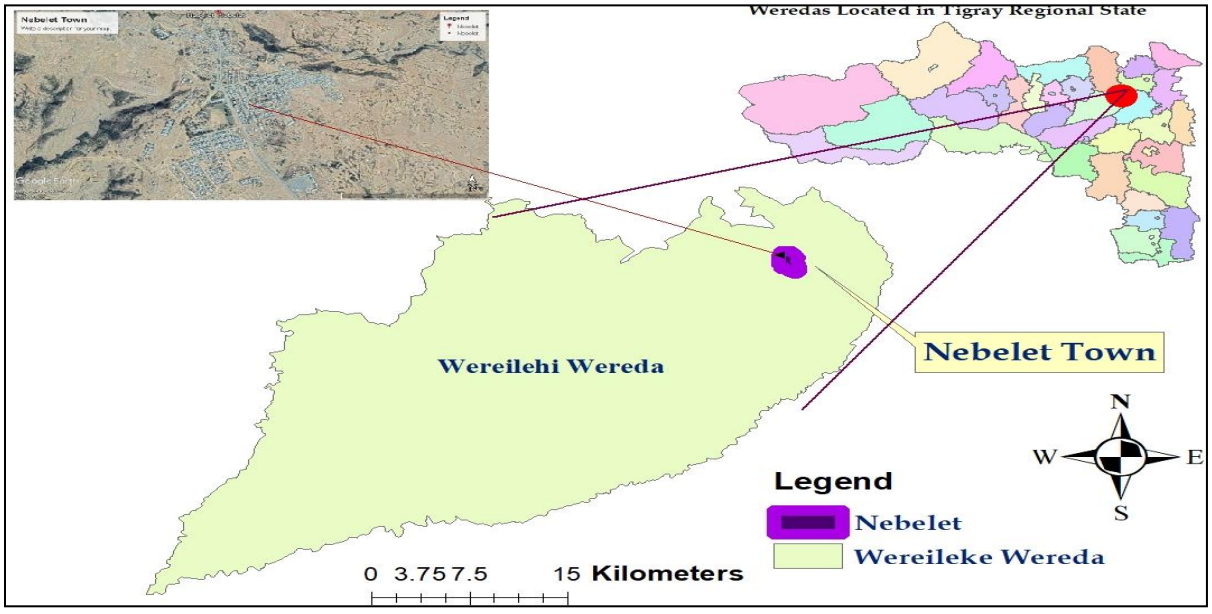

Figure 3. Map of Nebelet town

4.2 Study design and population

A retrospective study design was implemented to describe and analyze the prevalence of intestinal protozoa among patients in Nebelet Health Center from September 2013 to August 2018. The study participants were all diarrheal patients with intestinal protozoan infection who attended care and treatment in the health center during the past five-year Stool specimens from the outpatients and inpatients during the study period were included in the analysis. Stool specimen provided by admitted patients for follow-up purpose and incomplete records were excluded from the analysis.

4.3. Data collection tools and procedures

Secondary data were collected from laboratory records of the patient's registration book of Nebelet health center. In the Nebelet health center, all stool specimens were collected using clean wide mounted stool sample container and examined by experienced laboratory technolog ists within 2 hours of collection. Microscopic stool examination foe the presence of whole or part of adult parasite and microscopic examination for the detection of parasite egg, cyst and trophozoites were performed. Saline wet mount was prepared and examined for microscopic detection of trophozoites of protozoa and helminthes egg and larva. Protozoan cysts were detected using iodine wet mount. Formal-Ether concentration technique was performed for patients who have strong suggestive clinical manifestations but no parasites detected in wet mount examination.

4.4. Data analysis

The patient analysis in wet mount examination manifestation summarized and analyzed using descriptive statistical tools such as frequencies and percentages. SPSS version 20 software package crosstab was used for the frequency distribution of both dependent and independent variables. The organized data were presented in the form of tables, graphs and percentages.

5. RESULTS

5.1. Overall distribution of Giardiasis & Amoebiasis parasitic infection

A total of 2928 outpatients were examined for intestinal parasitic infection in Nebelet Health Center from September 2013 to August 2018 (Table 5.1.). Out of these, 1222 (41.73%) and 1706 (58.27%) were males and females, respectively. Of 2928 patients examined, 489 (16.7%) were positive to one of intestinal parasitic protozoans. Moreover, the overall prevalence of intestinal protozoan infection showed variations across the five years of retrospective data, where there was an increment from 2013/14 to 2015, and a gradual decline in prevalence from 2015 to 2018 (Table 5.1.).

Table 5.1.Overall prevalence of Giardiasis & Amoebiasis parasitic infection (Sep. 2013 to August, 2018)

Year	No- examined people %	No- positive %	No-of negative %
2013/14	585(29.06%)	108(18.46%)	477(81.54)
2015	564(24.94%)	120(21.28%)	444(78.72)
2016	508(17.03%)	107(21.06%)	401(78.94)
2017	603(15.10%)	83(43.76%)	520(56.24)
2018	668(13.87%)	71(10.63%)	597(89.37)
Total	2,928(100%)	489(16.7%)	2,439(83.3%)

5.2. Prevalence of Giardiasis & Amoebiasis infections among diarrheal patients

The prevalence of different intestinal protozoan parasite infections among diarrheal patients is Table 5.2. Two different species of intestinal parasites, including *Giardia lamblia* and *E. histolytica/dispar* were identified. The most prevalent parasite was *G. lamblia* (56.4%), followed by *E. histolytica/dispar* (43.6%). The five years retrospective study showed that the prevalence rate of *G. lamblia* and *E. histolytica/dispar* varied in different seasons (Table 5.2). A higher rate of *G. lamblia* was detected during 2016 while the lowest was in 2015.Similarly; prevalence *E. histolytica/dispar* was higher in the year 2015 while the lowest was in 2018.

Table 5. 2. Prevalence of Giardiasis &Amoebiasis among diarrheal patients

Year	Types of intestinal protozoan		Total
	G. lamblia	*E. histolytica/dispar*	
2013/14	56 (51.86%)	52 (48.14%)	108 (22.08%)
2015	62 (51.66%)	58 (48.33%)	120 (24.54%)
2016	67 (62.61%)	40 (37.38%)	107 (21.88%)
2017	47 (56.62%)	36 (43.39%)	83 (16.97%)
2018	44 (61.97%)	27 (38.02%)	71 (14.53%)
Total	276 (56.44%)	213 (43.56%)	489 (16.7%)

5.3. Age associated distribution of Giardiasis & Amoebiasis infection

The overall age associated distribution of Giardiasis & Amoebiasis parasite is indicated in Table 5.3. The prevalence of different protozoan parasites in the age groups <5 year, 172 (35.17%), 5-14 years old 127 (25.97%), 15-29 years old 91 (18.6%), 30-44 years old 68(13.9%), >44 years old (6.36%). The prevalence of intestinal protozoan infection was higher in age group < 5 years with the prevalence rate of 35.17% while the lowest prevalence rate was observed in the adolescent , >44 years (6.36%). *G. lamblia* was prevalent in children under the age of five (39.9%) while the least infection rate was detected in >44 years (5.07%). However, the rate of *E. histolytica/dispa r* infection was high in children under 5 years (29.6), followed by the least affected age group > 44 years old (7.9%).

Table 5.3. Age associated distribution of Giardiasis & Amoebiasis (Sep. 2013 to August 2018).

Total number of positive cases Percentage (%)		Parasite species	
		G. lamblia	*E. histolytica/dispar*
Age categorical	<5 (35.17%)	109 (39.5%)	63 (29.6%)
	5-14 (25.97%)	80 (28.98%)	47 (22.06%)
	15-29 (18.60%)	50 (18.11%)	41 (19.24%)
	30-44 (13.90%)	23 (8.33%)	45 (21.12%)
	>44 (6.36%)	14 (5.07%)	17 (7.98%)
	489 (16.7%)l	276 (56.4%)	213 (43.6%)

5.4 Overall sex related prevalence of Giardiasis & Amoebiasis infection

Fig. 4 shows the overall prevalence of Giardiasis & Amoebiasis infection by sex. In the five years retrospective study, the prevalence of Giardiasis and Amoebiasis was higher in males (55.2%) than females (44.8%). The prevalence of *G. lamblia* was higher in males (56.9%) than females (43.1%). Similarly, *E. histolytica/dispar* was more prevalent in males than females, with the prevalence rate of (53.1%) and (46.9%), respectively.

Figure 4.Sex related distribution of Giardiasis & Amoebiasis

5.5 Prevalence of Giardiasis & Amoebiasis within five years

The prevalence of intestinal protozoan infection showed fluctuation from year to year, in which there was increment from 2014 to 2015 followed by decreased prevalence from 2015 to 2018 (Table 5.4.) In general, from 2013-2018 higher rate of infection was detected in males (55.2%) than females (44.8%). The distribution of intestinal protozoan infections from 2013-2014, in both males and females showed an increment from (22.0%) to (24.53%). However, the protozoan infection in both sexes showed decrement from 2014-2018.

Table 5- 4 Overall Prevalent rate of Giardiasis & Amoebiasis from 2014-2018

Year	Number of Positive (%)		
	Male	Female	Total
2013/14	58	50	108 (22.0)
2015	68	52	120 (24.5)
2016	72	35	107 (21.9)
2017	33	50	83 (16.9)
2018	39	32	71 (14.7)
Total	270	219	489

6. DISCUSSION

Epidemiological study on the prevalence of infection of intestinal parasites in different regions/localities is a primary objective to identify high-risk communities and formulate appropriate intervention. In view of this, the present study attempted to assess the prevalence of different intestinal protozoan parasitic infections in Nebelet Town Health Center in central Tigrai Regional State, northern Ethiopia. The results of the study indicated the occurrence of two types of protozoan intestinal parasites of public health importance among diarrheal outpatients and inpatients attending the health center in the past five years.

In the present study, the overall prevalence of Giardiasis & Amoebiasis was 16.7% among diarrh eal outpatients and inpatients attending the Nebelet Health center. Similar studies from different parts of Ethiopia and outside Ethiopia,69.4% prevalence among Delgi schoolchildren North Gondar reported by Ayalew *et al.,* (2011),16.9 in selected primary school children in Wukro town Tigray Region North Ethiopia reported by Eleni *et al*., (2014), (Onyango and Angien da, 2010) reported a prevalence of diarrheal disease In Kenya was (16.7%). The most possible reasons by fecal contaminated food and water, limited health education, open field elimination of stool practices, improper preparation of raw vegetables and poor knowledge of parasite transmission and awareness of the disease, However, this prevalence is smaller than 26% rate reported by Mengste (2014) in Debre Markos town among HIV positives and (46.61%) in Hawassa city administration Millennium health center (Dobo (2018).

The most prevalent parasites in this study area from the number of positive to intestinal protozoan infection, (56.4%) were infected by *Giardia lamblia* which is higher than 4.4% in Anambra state reported by Emmy-Egbi *et al*.(2012),6.4% in Nigeria reported by Auta*et al*.(2013), 6.7% in Babile town Eastern Ethiopia reported by Tadesse (2005), 6.6% in Teda health center north Gondar, 4.9% in Gorgora and chuahit health center North Gondar reported by Abate *etal*., (2013). One of the possible reasons might be the presence of poor hand wash habit, open field stool elimination practice, contamination of food and water. Nevertheless, the current *giardiasis* prevalence is lower than 69.4% among Delgi School children North Gondar reported by Ayalew *et al*., (2011). The prevalence rate of *E. histolytica* in this study was 43.6%, which is higher than the findings by Teklehymanot (2009) in southwestern Ethiopia, who

reported 16% prevalence rate. However, the present study was lower when compared with the prevalence of Entamoeba in Nigeria (65.7%) (Ogunlesi *et al*., 2006) in Hawassa city (58.25%) reported by (Dobo. (2018).

The prevalence of intestinal protozoan parasitic infection in the current study was higher in males than females. Similar findings also reported from Tigray in northern Ethiopia (Meles and Bekele, 2017; Feleke *et al*., 2017), Hawassa City (Dobo. 2018), and elsewhere. This may be due to the fact that males are more engaged in playing outdoors. And also, majority of males were move from place to place in order to work, contaminated with soil which contain intestinal parasite protozoa during farming, playing football more frequently than females. However, Mazigo *et al*. (Mazigo *et al*., 2010) from Tanzania have reported a higher prevalence of intestinal protozoan parasitic infection in females. This might be due to the difference in the lifestyles and environmental factors between the study areas.

According to the result of this study, high prevalence of intestinal protozoan infection observes among children less than five years old (35.17%). This may be due to low immunity in individuals, less awareness of washing hands and other personal hygiene. Generally, this study showed that during the last five years, there was trend in the decrease of intestinal protozoan parasitic infection cases observed at Nebelet town health center. The highest peak was in 2014 which show some increment from 2013 to 2014, but in the other years it showed decrement from 2014 to 2018 from 21.28% to 10.63%.

7. CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

In conclusion, the following findings of this study were showed as follows:

The overall prevalence of intestinal Giardia and Amoeba infection in Nebelet town Health Center was 16.70%. The most common intestinal protozoan parasitic infection was *G. lamblia* followed by *E. histolytica.* An overall age respective study of <5 years old was highly affected group (35.17%) and greater than 44 years old were the least affected group (6.36%).From the result, males were highly affected than females. The study also showed that there was consequent reduction shift within five years.

The prevalence of Giardia and Amoeba infections from 2013/14 to 2018 (21.28% to 10.63%) the reason for reduction may be due to development of awareness about the effect of intestinal protozoan parasitic infection in the society from time to time In the Present study, a relatively high prevalence of intestinal protozoan infection was recorde d in children <5 years of age.

7.2 Recommendation

Based on the present findings, the following recommendations have been forward. To minimize or stop the spread and impact of intestinal parasitic infections, in particular amebiasis and giardiasis, the following actions should be performed:

- ✓ Governmental and non-Governmental organization, religious leaders and cultural coordinators and other members of society should participate effectively to minimize the spread and impact of intestinal protozoans by providing enough health services to infected individuals.
- ✓ Providing Proper education on sanitation, hygienic practice and potential risk factors to the community would help the reduction of higher prevalence of diarrhea causing intestinal protozoans.

- ✓ Generally by giving awareness to the people who do not know about the means of transmission and its impact and by giving vaccine against intestinal protozoan parasite and educate people to build and use enough toilette properly, treat drinking water by boiling prior to drinking and prepare food free of contamination with feces will be minimize the prevalence of intestinal protozoan parasitic infection in the study area.

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9. APPENDICES

Appendix 1.Sources of data for Prevalence of Giardiasis &Amoebiasis

	Males							Females					
Year	Type of disease	<5yr	5-14 yr	15-29 yr	30-44 yr	>44 yr	Total	<5yr	5-14 yr	15-29yr	30-44yr	>44yr	Total
2013 /14	Amoeba	12	6	4	7	-	29	10	4	3	4	2	23
	Giardia	9	10	5	3	2	29	9	12	6	-	-	27
2015	Amoeba	6	11	7	6	3	33	7	5	7	4	2	25
	Giardia	15	9	5	2	4	35	10	8	5	3	1	27
2016	Amoeba	10	2	1	7	3	23	3	5	2	4	3	17
	Giardia	26	12	5	4	2	49	6	5	5	2	-	18
2017	Amoeba	5	3	3	4	1	16	6	2	7	3	2	20
	Giardia	8	6	3	-	-	17	12	8	7	2	1	30
2018	Amoeba	2	3	4	3	-	12	2	6	3	3	1	15
	Giardia	10	5	6	4	2	27	4	5	3	3	2	17
	Total	193	67	43	40	17	**270**	69	60	48	28	14	**219**

Appendix 2. Sex & age related data sources for prevalence of Giardiasis & Amoebiasis

	Sex			*E. histolytica*			*G. lamblia*		
Age	M	F	T	M	F	T	M	F	T
<5yr	103	69	172	35	28	63	68	41	109
5-14yr	67	60	127	25	22	47	42	38	80
15-29yr	43	48	91	19	22	41	24	26	50
30-44yr	40	28	68	27	18	45	13	10	23
>44yr	17	14	31	7	10	17	10	4	14
Total	270	219	**489**	113	100	**213**	157	119	**276**

ADDIS ABABA UNIVERSITY

GRADUATE PROGRAMMES

DECLARATION

This is to certify that the thesis prepared by Mulu Hailu, entitled: Prevalence of intestinal protozoan infections among diarrheal patients attending Nebelet town health center in Tigrai Region Northern Ethiopia and submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee.

Name	Signature	Date
Examiner _____	_____	_____
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Chair of the Department: Dr. BezaworkAfework____	_____	_____