PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG THE PATIENT VISITING, TERTIARY CARE HOSPITAL OF EASTERN NEPAL

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ABSTRACT

Intestinal parasitic infections still constitutes one of the major public health problems in Nepal. Present study was done to find out the prevalence of intestinal parasitosis in patients visiting Tertiary care hospital of eastern Nepal. A total of 480 stool samples were collected from March 2016 to June 2017. The samples were collected in clean, dry and screw capped plastic container and were subjected to macroscopic examination for adult parasites and/or segment of parasites. Samples fixed in 10% formal-saline and parasites were examined microscopically after concentration by formal ether sedimentation technique. Overall parasite positive rate was 10.625% with no significant difference in two genders (male: 5%, female: 5.625%) (p=0.07). The percentage of mono parasitism (98.4%) were higher than multiparasitism (1.96%). Altogether 6 species of parasites were detected of them. *Entamoeba histolytica* was most common followed by *Giardia lamblia*,.Patients (aged ≤ 15) had marginally higher positive rate (52.3%) than older (p=0.44)**.** Prevalence of parasitic infection rate was higher in family with low education (P=0.1).. The parasitic prevalence rate was higher among patients using normal tap water. Results showed that the patients having intestinal parasitosis and suggests periodic deworming as well as sanitary hygienic practices.

Key words: Intestinal parasites, *Entamoeba histolytica*, *Giardia lamblia.*

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LIST OF ABBREVATIONS

STH	Soil Transmitted Helminthes
A. lumbricoides	*Ascaris lumbricoides*
S. stercoralis	*Strongyloides stercoralis*
H. nana	*Hymenolepsis nana*
E. histolytica	*Entamoeba histolytica*
E. coli	*Entamoeba coli*
I. butschlii	*Iodamoeba butschlii*
C. mesnili	*Chilomastix mesnili*
E. nana	*Endolimax nana*
E. hartmani	*Entamoeba hartmani*
B. hominis	*Blastocystis hominis*
C. cayetanensis	*Cyclospora cayetanensis*
T. trichiura	*Trichuris trichiura*
N. americanus	*Necator americanus*
P. westermani	*Paragonimus westermani*
VDC	Village Development Committee
WHO	World Health Organisation
MoHP	Ministry of Health and Population
Total. no	Total number
Pos. n	Positive number

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CHAPTER I

INTRODUCTION

1.1. BACKGROUND

Human beings have been exposed to diverse group of intestinal parasite. Intestinal parasitosis, a major public health problem,particularly in the developing countries, affects 3.5 billionpeople globally. Moreover, WHO has estimated that *Ascaris lumbricoides*, hookworm and *Trichuristrichiura*infect 1.4billion, 1.3 billion and 1.0 billion people worldwide, espectively. Over 60 species ofprotozoan parasitescause diseases on people worldwide. *Entamoebahistolytica* and *Giardia lamblia* are estimated to infect about 60 million and 200 million people worldwide, respectively (Murray et al., 2002).It is generally estimated that at least 2.5 billion of the estimated world's 6.9 billion people are currently infected with intestinal protozoan parasites cutting across all continents and regions of the world (Morales-Espinoza et al., 2003).

The high prevalence rate is attributed to lack of education, lack of latrines, occurrence of diarrhea, lowersocio-economic status, inadequate disposal of humanexcreta and the level of sanitation in households. (Smith H). In Nepal, the prevalence ranges from 27.0% to 76.4% indifferent studies carried out among general population indifferent geographical areas(4-7 article); whereas, hospital recordsin Nepal showed the infection rate of 30.0-40.0%. (4). Additionally, a hospital based study conducted by Raietal (1995) over one decade in Kathmandu illustrated the intestinal parasitosis rate ranged from 29.1-44.2%, with astatic prevalence of A. lumbricoides, the most common parasite in Nepal.((Morales-Espinoza et al., 2003).

Amoebiasis is an infection caused by an intestinal protozoa *Entamoeba histolytica*, is the third most common cause of death from parasitic disease (after schistomiasis and malaria). Areas of highest incidence (due to inadequate sanitation and crowding) include most developing countries in the tropics, particularly Mexico, India and nation of central and South America, tropical Asia, and Africa. Upon ingestion the cysts pass through the stomach and excyst in the lower portion of the small intestine, and undergo repeated rounds of binary fission. Amoebas can also metastasize to other organs and produce an extra intestinal amoebiasis (Haque*et al*., 2003). The non-invasive disease is often asymptomatic, but can cause diarrhea or other gastro-intestinal symptoms such as abdominal pain or craps. This non-invasive infection can persist or progress to an invasive disease in which trophozoite penetrate the intestinal mucosa and kill the epithelial cells (Stanley, 2003). With regards to intestinal protozoan infections, giardiasis caused by *Giardia intestinalis*, is the most prevalent protozoa infection with estimated prevalence rates ranging from 2 to 7% in developed countries but 20 to 30% in most developing countries and affecting approximately 200 million people worldwide [1016/S0140-6736(09)61749-9]

Intestinal parasitic infections cause various intestinal symptoms including abdominal bloating, cramps, constipation, diarrhea, lack of appetite and vomiting [Gordon C]. Most of these symptoms are non-specific and are similar to those of other pathogens such as viruses, bacteria and other non-infectious conditions affecting the intestinal system including irritable bowel syndrome, ulcerative colitis, pancreatitis and peptic ulcer disease [Gordon C]. Diagnosis of parasites is laboratory based where stool is examined for ova, cysts or trophozoites.

Several community wide out-breaks of Giardiasis and Amoebiasis have been linked to drinking municipal water or other water sources contaminated with these parasites (Stevens and Adam, 2004). These and other intestinal protozoa infections are commonly associated to sanitary conditions and socio-economic factors. In addition there is also a marked seasonality in the onset of illness due to intestinal protozoan parasite infections (Gamboa et al., 2003).

Among the conditions influencing the development of intestinal protozoan parasitic infections are poor sanitary conditions, lack of clean water supply lowering resistance of the host, and lack of awareness of transmission of the parasite. This disease can affect children's development, educational achievement, reproductive health and social and economic development and some of these parasitic infections can cause morbidity and mortality.

Nevertheless, treatment is often neglected for economic reasons and because most patients have no symptoms (Guyatt, 2000).

Intestinal parasitic infestation continues to be of public health importance in many tropical and subtropical countries for their high prevalence and effects on the morbidity in the population. This analysis was aimed to find out the intestinal protozoal parasitic profile in pre-school and school-going children visiting the hospital with gastrointestinal illness in western Nepal. *G. lamblia* was the most prevalent pathogenic protozoan intestinal parasite (73.4%), followed by *E. histolytica*(24.4%). Interestingly, newer opportunistic pathogens like *C. cayetanensis* (1.0%) and *Cryptosporidium* sp. (1.0%) were detected from immunocompromised children below 2 years of age (Easow et al., 2005).LK Khanal et al; 2011, studies of intestinal parasites among school children in kathmandu shows prevalence of intestinal worm infestation wasfound to be 17.6% (Boys=22.0% vs girls=13.5%). Children aged 6-8 years were found to be highly infectedwith intestinal worms (21.4%) followed by 9-12 years old (18.6%). Those between 13-16 years of age weresignificantly less infected (10.7%) compared to others ($p<0.05$). Ova/ cysts of intestinal parasites detectedinclude Trichuristrichiura (32.0%), Ascarislumbricoides (20.0%), Hymenolepis nana (16.0%), Hookworm (8.0%) and 24.0% cases showed mixed parasitic infections.

A Report shows that almost 35%people, mainly children, in Nepal take medicine against worm infestations.[5]Developing countries in SoutheastAsian region spent 3.76% of total annual budget forhealth in year 2010.[6] As worm infestation appears asone of the major economic burden also to the country,Nepal government has initiated National Deworming Program in recent years to control it. However, accordingto W.H.O., 1100 million people were defecating in theopen resulting in high levels of environmental contamination and exposure to the risk of worminfestations in year 2008. So study on such mattersappears very much necessary even today.

This study aimed to estimate the prevalence of intestinalworm infestations among people of far western Region findings of which could be beneficial for healthplanning authority in Nepal to overcome the existinglimitations for achieving ultimate goal in the near future.

Stool examination for intestinal protozoan parasites is one of the most frequently performed examinations in laboratories. Most of protozoan parasites usually excrete through stool in both cyst and vegetative stages. The prime focus of the study is to explore frequency of infections caused by intestinal parasites in the patients visiting hospital. The study will also demonstrate the present scenario of Protozoal parasite in Sunsari district. This would be useful for the future planning and policy making in healthcare centers and hospitals in order to combat with the spreading infectious diseases.

1.2 OBJECTIVES

1.2.1 General objective

To determine the prevalence of intestinal parasites infections among patient visiting tertiary care hospital of Eastern Nepal.

1.2.2 Specific objectives

To determine the prevalence of intestinal protozoan parasites infections among patient visiting tertiary care hospital of Eastern Nepal.

a) To identify intestinal helminthic parasites among patient visiting tertiary care hospital of Eastern Nepal.

b) To detect protozoan and helminthic parasite species and determine their prevalence of occurrences in drinking water sources of the study area.

CHAPTER III

LITERATURE REVIEW

3.1 Global Scenario

According to WHO, 2000; most of the illness in the world is caused by inadequate sanitation, unsafe water and unavailability of water, intestinal parasitosis being one of them. Both the protozoa and helminthes are responsible for the intestinal infection leading to many cases and deaths, particularly in underdeveloped countries.

Samples of faeces from children and adults were collected and submitted to the techniques of direct wet mount, flotation in zinc sulphate solution, tube sedimentation; sedimentation in formalin-ether and staining by Kinyoun and iron hematoxylin methods (70.7%) showed protozoa and/or helminthes in stool samples. The most prevalent species were *E. nana* (33.7%), *B. hominis* (26.5%), *G. lamblia* (18.2%), *E. coli* (17.1%), *A. lumbricoides* (16.6%), *I. butschlii* (9.4%), and ancylostomatidae (7.7%) (Nascimento et al., 2005).

A national survey of the prevalence of intestinal parasitic infections in the Islamic Republic of Iran was made by Sayyari et al., 2005. Altogether 45,128 stool samples were analyzed by formalin-ether precipitation. Among them, intestinal parasitic infections were found (19.1%) in female. *G. lamblia* (10.9%), *A. lumbricoides* (1.5%), *E. histolyica* (1.0%) and *E. vermacularis*(0.5%) were common parasites. The infection rate was highest in the 2-14 years age group (25.5%).

Bitkowska et al., 2004 examined stool of group of 7 years old children of Poland for the presence of intestinal parasite. The study was based on the examination of single fecal specimen and a cellophane swab. The studies include 31,504 children. The most frequently encountered parasites in the examination included: *E. vermacularis* (15.0%), *A. lumbricoides* (0.83%), *G. intestinalis* (0.96%), *E. coli* (0.60%) and *T.trichuria* (0.12%). The overall percentage of the infected children was 15.4%. The number of infected among children inhabiting countryside (19%) was significantly higher than among those from the towns (10.4%).

The prevalence of intestinal parasite was determined for 1,370 children in Khan Younis Governorate, Gaza strip. The age of the children ranged from 6 to 11 years. For stool samples inspection, direct smear microscopy, flotation and sedimentation techniques were used. The general prevalence of intestinal parasites was 34.2%. Different types of intestinal parasites were detected during this survey. *A. lumbricoides* seemed to be the most common parasite (12.8%), whereas *G. lamblia* had a prevalence of (8.0%), *E. histolytica (*7.0%), *E. coli* (3.6%), *T. trichiura (*1.6%) and *H. nana* (1.0%). The prevalence of enterobiasis was determined using a scotch tape preparation. A total of 20.9% of the children examined were infected and there was sex variation in the prevalence of enterobiasis (Astal, 2004).

The study was conducted Oliveira et al., 2003 in a rural area of Uberlandia, State of minas Gerais, Brazil in which 65.5% of the total population were found to be infected with intestinal parasites among which 45.1% were children and 54.5% adults. Within this study group 66.7% were mono infected, 17.6% bi-infected and 15.7% polyinfected. 47.0% individual was infected with protozoa, 29.4% helminthes and 23.6% with both. According to sex, the positively rate for intestinal parasites was 41.0% for male and 24.4% for female. Regarding to age group, high positive rate (29.6%) was found in children of age 1-15 years followed by 16-30 years (20.6%), 31-45 years (5.1%), 46-60 years (6.4%) and above 60 (3.8%). Among the parasites, *H. nana* was the most frequent helminthes (14.1%) and *G. lamblia* (11.5%) the major protozoa. High positive rate of 6.4% was detected both for hookwormand *S. stercolaris. T. trichiura* was found in 5.1% of the study.

Miller et al., 2003 examined the presence of intestinal protozoa and helminthes infections and their association with clinical signs and symptoms in children in Trujillo, Venezuela. Conventional microscopic method (thick-smear, saline and iodine solution) was used to identify parasites in stool samples in of 301 children attending day care centre. A subgroup of 45 children was evaluated clinically and parasitological five times during a 1-month period using conventional method and the Kinyong acid fast for Cryptosporidium identification. The point prevalence of protozoa infection was 21.0% for *G. duodenalis*, 1.0% for *E.histoltica/dispar*, 4.0% for *E. coli,* 16.0% for *B. hominis*, and 89% for *C.parvum*. Prevalence of helminthes was 11% for *A. lumbricoides*, 10.0% for *T. trichuria,* 0.3% for *S. stercolaris,* and 1.3% for *H .nana*. Over a 1 month time frame, new infection were observed at a rate of 11.0% for *G. duodenalis* 4.0% for *E. histolytica* ,7.0% for *A. lumbricoides* , 11.0% for *T. trichuria,* 0% for *S. stercolaris,* and 2% for *H. nana* . Intestinal parasitic infections contribute significantly to the enteric disease burden experienced by this group of children.

Intestinal parasitosis alone affects almost 3.5 billion people worldwide and due to these infection 450 million are suffered from various kind of illness, the majority being children (WHO, 2000). According to WHO, 1997; globally *A. lumbricoides* infect 250 million, hookworm infect 151 million and *T. trichuria* infect 45 million people, respectively. Annually, each of these parasites has been responsible for the deaths of 65,000, 60,000 and 70,000 people, respectively (WHO, 2000).

Paul and Gnanmani et al., 1998 carried out a study to determine the prevalence and intensity of intestinal helminthes infections among the children belonging to lower socio-economic status. Stool samples collected were processed by modified formalin ethyl acetate sedimentation technique.177 children were infected with one or more of intestinal parasites as *A.lumbricoides*, *T. trichiura* andhookworm. The overall prevalence of infection was 82.0%. *A.lumbricoides* was the most common infection with prevalence of 75.0% followed by *T. trichiura* (66.0%) and hookworm (9.0%).

A study of prevalence and intensity of soil-transmitted helminthes among pre-school children aged 0 to 7 years from an Orang Asli village of Malasiya. The overall prevalence of soil transmitted helminthes (STH) infections was 56.0%. The predominant helminthes found was *A. lumbricoides* while the commonest type of infection was a mixed infection with *A. lumbricoides* and *T. trichiura.* The prevelace rates of *Ascaris,Trichuris* and hookworm infection were 47.5%, 33.9% and 6.2% respectively. The prevalence of helminthiasis (STH) shows an-age dependent relationship, with the lowest prevalence in 0-< 1year age group and highest in the 6-< 7 year age group (Zukifli et al., 1999).

Knightlinger et al., 1998 surveyed 633 children, within age group 4-10 years living in Southern Madagascar. The study revealed the maximum (93.0%) prevalence rate of *A. lumbricoides* followed by *T. trichiura* and hookworm by 55.0% and 27.0%, respectively.

Mafiana et al., 1998 conducted an investigation to determine the prevalence of STH parasites in children in Ogun state, Nigeria. Fecal examination of 1,060 children reveledthe prevalence of (64.0%) for *A. lumbricoides*, (21.9%) for *T. trichiura* and (14.5%) for hookworm.

Saito et al., 1996 surveyed for intestinal parasites by using thin smear and floating method for fecal examination in residents in Caazapa Department, Paraguay. Out of 608 samples of residents in Boqueron, a community of Caazap Department, 343 (56.55%) were found positive. The most prevalent parasite was *N. americanas* (27.0%) followed by *E. coli*.

Sugnanet al.,1996 carried out of survey among the rural and urban settler and two urban groups *viz*, Nicobares and Onges, of Andaman and Nicobar is island in 1996.The survey was conducted between preschool school aged children and adults. Among the preschool children, Nicobares showed highest overall prevalence rate (80.5%) followed by urban (46.7%) and rural (38.6%) preschool children. *A. lubricoides* was the commonest form of parasite encountered in all the groups of preschool children, followed by *T. trichiura*. The school age children among rural settles showed an overall prevalence rate of 61.1% which was significantly higher than that among the rural preschool children. The Nicobarese and Onage adults showed significantly higher overall prevalence rates (72.2%) and (71.1% vs. 48.6%) compared to rural adults. In all among trichuriasis and giardiasis were common than ascariasis.

Milano et al., 1995 have evaluated the importance of enteroparasitosis in young urban population. Serial stool sample were analyzed and was also studied. Environmental data were also collected via semi-structured surveys. Soil samples were tested to determine the degree of soil contamination. The following species were identifiedsuch as *B.hominis, E. vermicularis, cocidius*sp., *G. intestinalis,* hookworms, *S. stercolaris, T. trichiura, A. lumbricoides, E. coli, E. nana,* and *Taenia*sp. Children infection prevalence was 73.5%. The frequency of enteroparasitosis was largest in the population from 3 to 8 years. The homes of the children analyzed were brick houses with tin roof and access to tap water. Of these 79.5%, houses had bedrooms. The remaining used outdoor latrines. In 95.5% of these houses, the residents lived with one or more dogs and cats. The stool collected from nine houses was contaminated with infectious forms of *T. canis*and*Anlyomideous*. The relationship between parasitosis and latrines and overcrowding were verified. Five cases of malnutrition were detected (4.4%).

A community-based study was conducted among randomly selected 300 children aged less than five years selected from three camps of the police force in Khartom, Sudan. The commonest infestations were giardiasis (21.1%), taeniasis (10.45%) and enterobiasis (7.4%). Non pathogenic*E. coli, E. histolytica*and *T. saginata* were detected (2.7%), (0.7%) and (1.7%) of stools specimen, respectively. Children aged between 3 years and above were the most affected group and the infection rate was highest among the illiterate, overcrowded and large sized families. Malnourished children comprised (9.4%) of the study group was but there was no significant association between and malnutrition and the overall prevalence of intestinal infestations, although *G. lamblia* significantly affected the malnourished group (Karrar and Rahim, 1995).

Gbakima in 1994 assessed the prevalence of intestinal and urogenital parasites in Moyamba District, South-central Sierra Leone. Stool and urine sample were submitted by 1106 individuals and examined by the iron-haematoxylin staining and the formalin-ether concentration techniques for fecal sample and centrifugation method for the urine sample. The overall parasitic infection rate was 61.7% while 5.9% of the population had multiple infections. *E. histolytica* in infection rate was 12.3% and most infected individual passing cysts. *G. lamblia* and *Trichomonasvaginalis* infection rates were 10.0% and 0.4%, respectively. Among the helminthic infection, *A. lumbricoides* was the most commonly observed (13.7%), followed by hookworm (12.1%), *T. trichiura* (9.3%), *S. stercolaris*(7.7%) and tapeworm (2.6%). The high parasitic infection rate (61.7%) and the frequency of multiple infections indicate an interrelationship of environmental factors which support transmission rather than single factor.

Lee et al., 1994 examined fecal specimens of a total 52,522 patients by formalin-ether sedimentation or direct smear method. The overall egg positive rate of helminthes was 6.5%. The egg positive rate for each species of helminthes was *C. sinensis* (3.2%), *T. trichuria* (2.0%), *A. lumbricoides* (0.2%), *Trichostrongylousorientalis* (0.1%), *Taenia*sp. (0.05%), *H. nana* (0.03%) , hookworms (0.03%), *P. westermani* (0.02%), *E. chinostoma* sp.(0.03%), *E.vermacularis* (0.02%), *S. stercolaris* (0.1%) and *Diphyllobothriumlatum* (0.004%). In comparison to various data, *A. lumbricoides* and *T. trichuria* has been found in decreasing ratio.

Anderson et al., 1993 carried out the research on the distribution of intestinal helminth infections in a rural village in Guatemala. Fecal egg count scores were used to investigate the distribution and abundance of intestinal helminthes in the population of a rural village. Prevalence of the major helminthes was 41.0% with *A. lumbricoides*, 60.0% with *T.trichiura*and 50.0% with *N. americannus*. Infected females had higher burdens of *T. trichiura* than infected males in all age classes of the population; there were no other effects of the host gender. Analysis of associations between parasites within hosts reveled strong correlations between *A. lumbrcoides*and *T. trichiura*. Individuals with heavy infections with *A. lumbricoides* and *T. trichiura* showed highly significant aggregation within households. Association between a variety of household features and heavy infections with *A. lumbricoides* and *T. trichiura* were described.

A survey conducted by Marnell et al., 1992 among the refugee in Juba, Sudan, involving 241 fecal samples revealed 66.0% of the population harbored intestinal helminthes. The most commonly found infection was hookworm (36.0%), followed by *S. mansoni* (26.0%), *S. stercolaris* (20.0%), *H. nana* (11.0%), *A. lumbricoides* (1.2%), *T. trichiura* (0.8%) and *Taenia*sp. (0.4%).

Among examinee, 42.0% had single infection, 21.0% had double and 3.0% had multiple infections. Parasitic prevalence and intensities were analyzed in relation to age, sex, relation and occupation. Females were less infected (50.0%) than Christians (68.0%) and agriculturists (90.0%) were the most infected occupational group.

Obiamiwe and Nmorst, 1991 examined 862 fecal samples from male and female donors of nine age groups (1-90 years) in three geographical zones, after examination of concentration from formal-ether concentration technique. They found the overall percentage incidence of six parasites which were encountered were as follows, *E. coli* (19.9%), *E. histolytica* (3.9%), *G. lamblia*(1.4%), hookworm (29.4%), *A. lumbricoides*(38.2%), *T. trichuria*(7.3%).

Stool specimens from 1,282 children between the age of 5 and 13 years attending ten primary schools for boys in Saudi Arabia, were examined for the presence of intestinal parasites. Of these, 313 (24.4%) were found to be infected with one or more species of 11 intestinal protozoa and helminthes. The most common pathogenic protozoa being *G. lamblia* (10.9%) followed by *E. histolytica* (4.1%). The non pathogenic protozoa, *E. coli* had the highest prevalence rate (11.3%) in the children stools. *H. nana* was the commonest intestinal helminth (3.0%). Other intestinal helminthes, including *A. lumbricoides, T. trichiura, S. mansoni, Dicrocoeliumdendriticum* were detected to a lesser extent. The distributions of the common intestinal infections among the children surveyed were also analyzed according to age and multiplicity of infection. Prevalence of *E. histolytica* was found to increase with age whereas *Giardia* infections were less common among older children (Omar et al., 1991).

In cockle Province, Republic of Panama, Robertson et al.,1989 carried out a survey of intestinal helminthes in children by microscopic examination (modified Kato-katz technique) of stool samples from 661 children attending primary school. The overall prevalence of *A. lumbricoides*, hookworm and *T. trichiura* infection were found (18.2%), (12.0%) and (27.5%) respectively. There were significant differences between the infection prevalence values for children attending the different school, but with not respect to age or sex.

Positive association was detected between particular pairs of infection and there were most evident with *T. trichiura.*

Haswell et al., 1989 studied the distribution of *A. lumbricoides* within the community and found significant variation in the intensity of infection between household in the community. The number of family members living in the house strongly influenced the mean *Ascaris* burden and proportion of relatively heavy infection within adults and children. This finding suggest that the density of people in the house positively influences the frequency of exposure to infective stages of *Ascaris*, which in turn plays a major role in determining which individual will harbor heavy infection. A comparative examination of hypothetical treatment strategies suggest that for *Ascaris* infection in this community, targeting age groups with antihelminthic treatment would probably be more cost-effective in the long term in reducing the abundance of this parasite than selective treatment of individually identified heavy infection.

Fagberno-Beyioku and Oyerindo, 1987 examined microscopically the stool samples from 1,659 children, aged 15 years and below in metropolitan Lagos and showed a (71.9%) and (68.3%) infection with *T. trichuria* and *A. lumbricoides* respectively. While the infection rate with hookworm was 22.5%. Infection with more than one parasite was also very common. Those children whose household had no toilet facilities had the lowest infection frequency with the intestinal parasite, while those who used buckets as their means of fecal disposal had highest rates.

According to Wiesenthal et al., 1980 165 Meo Laotians stool sample were screened for intestinal parasites. One hundred thirty- nine had at least one parasite detected. Hookworm was detected most frequently, followed by *G. lamblia*, *T. trichiura*, and *A. lumbricoides*. Hookworm and overall infection were more frequent in persons 4 years of age and older, while giardiasis, ascariasis, and trichuriasis were the most common in the 4-14 years age group. Most infection were helminthes and of no public health consequences in the United States. However, giardiasis was seven times as prevalent in refugee children as in the general US population, posing a potential public health risk in child- care setting.

Study conducted at South Kalimantan Province of Indonesia; by Cross et al., 1975 to1998, different intestinal parasitic infections were detected (97.0%) of the people. Those parasites most frequently found were *T. trichiura*(83.0%), *A. lumbricoides* (79.0%), and hookworm (65.0%), followed by *E. coli* (37.0%), *E. nana* (12.0%), *E. histolytica* (12.0%), *G. lamblia* (5.0%), *E. hartmanni* (2.0.0%), *C. mesnili* (2.0%). Other parasites found were *E. vermicularis, S. stercoralis, Capillaria*sp.,*Echinostoma*sp., *H. diminuta and T. hominis*. *G. lamblia* was found more often in younger people. Hookworm occurred more frequently in males and *A.lumbricoides* in females.

3.2 SAARC countries scenario

Sayyari et al.,2005 in a national survey of intestinal parasitosis in Iran, intestinal parasitic infection rate was detected as 19.3% (19.7% male, 19.1% female). In the study, *G. lamblia*(10.9%), *A. lumbricoides*(1.5%), *E. histolytica*(1.0%) and *E. vermicularis*(0.5%) were the most common parasites. The infection rate was highest in the 2–14 years age group (25.5%) and in rural residents (23.7%). The prevalence rate of *T. saginata, T. colubriformis, T. trichiura* and *A. duodenale* were 0.2%, 0.2%, 0.1% and less than 0.1% respectively. The total prevalence of intestinal parasite among people of age group 40-69 was 15.0% and greater than 69 years was 11.6%. The prevalence of individual parasites in 40-69 years age group was *G. lamblia*(7.3%), *A. lumbricoides* (1.5%), *E. histolytica*(1.1%) and *E. vermicularis*(0.2%). The prevalence of individual parasites was *G. lamblia*(5.0%), *A. lumbricoides* (1.2%), *E. vermicularis*(0.1%) and *E. histolytica*(0.7%) in age group.

Study conducted in the union Territory, Chandigarh, India estimates the prevalence of intestinal parasitic infections in different population groups. The prevalence of intestinal parasitic infections was found to be 14.6% with highest prevalence of 19% from the slum area. Children were the most commonly affected group (18%) with those from slums showing the highest prevalence (24.6%). The most common parasite was Giardia (5.5%) followed by *H. nana* and *A. lumbricoides*(2.8% and 2.7%, respectively), (Khurana et al., 2005).

Singh et al., 2004 studied parasitic infection among primary school-going children between the age group of 5 to 10 in the urban and rural areas of Manipur. A total of 248 (24.5%) were positive for various helminthes. Among the positive cases, 110 (26.3%) were from the urban area (city) and 138 (23.4%) from the rural areas of Manipur. Maximum number of parasitic infection occurred in the age group of 5 to 6 years (27.0%) in both sexes. Among the parasites, *A. lumbricoides* was the commonest (19.6%) followed by *T.trichiura* (2.18%), *H. nana* (0.99%), tapeworm (0.19%), hookworm (0.09%), *S. stercoralis*(0.09%), *E. vermicularis*(0.09%).

Several reports of patients with cysticercosis from many countries in Asia such as India, China, Indonesia, Thailand, Korea, Taiwan and Nepal are a clear indicator of the wide prevalence of *T. solium,*cysticercosis and taeniosis in these and other Asian countries. It is also a major cause of epilepsy in Bali (Indonesia), Vietnam and possibly China and Nepal. Seroprevalence studies indicate high rates of exposure to the parasite in several countries (Vietnam, China, Korea and Bali (Indonesia)) with rates ranging from 0.02 to 12.6%. An astonishingly high rate of taeniosis of 50.0% was reported from an area in Nepal populated by pig rearing farmers. Undoubtedly, cysticercosis is a major public health problem in several Asian countries affecting several million people by not only causing neurological morbidity but also imposing economic hardship on impoverished populations. (Rajshekhar et al., 2003).

A. lumbricoides infestation (ALI) is one of the most common helmintic diseases of the gastrointestinal tract, and may cause severe surgical complications, especially in children. A case of a 5-years old Pakistan girl treated in Italy for acute abdomen in which Ali was detected during surgical exploration (Mosiello et al., 2003).

Astudy carried out at the northern part of Bangladesh to determine the impact of sanitary latrine use and of health education on intestinal parasites in school-aged children found to have the highest prevalence rate (36.2%) and hookworm the lowest (10.7%). Intestinal parasite infection was significantly lower ($P < 0.05$) among those who used a sanitary latrine and received health education (Hosain et al., 2003).

The study conducted in Konkor, Gadap, District East, Karachi to determine the prevalence of intestinal parasitic infection. Out of 263 residents 185 tested for intestinal parasites and 88 (47.5%) had pathogenic parasites. The distributions of parasites were *G. lamblia* (50%) and *E. histolytica* (48.86%). Statistically none of the socio demographic variables were associated except education and age group (Siddiqui et al., 2002).

Gilgen et al., 2001 conducted a randomized clinical intervention trial over 24 weeks on a tea estate in north-east Bangladesh to investigate the effect of iron supplementation and antihelmintic treatment on the labour productivity of adult female tea pluckers. A total of 553 full-time tea pluckers, not pregnant and not breastfeeding, were randomly assigned to one of the four intervention groups: group 1 received iron supplementation on a weekly basis, group 2 received antihelmintic treatment at the beginning and half-way through the trial (week 12), group 3 received both iron supplementation as group 1 and antihelmintic treatment as group 2, and group 4 was a control group and received placebos. However, there was a negative association for all three worms (*A. lumbricoides, T. trichiura* and hookworms) between the intensity of helminth infections (eggs/ faeces) and all measures of labour productivity. Lower haemoglobin values and anaemia (< 120 g/l Hb) were both associated with lower labour productivity and more days sick and absent. Taller women with greater arm circumference were able to pluck more green leaves, earn higher wages and were absent less often.

In developing countries many enteric infections are caused by acid-sensitive pathogens. Study was conducted in 185 Bangladeshi men admitted to hospital for the treatment of enteric infection. Patients with dysentery (amoebiasis, n = 24 and shigellosis, n = 19) and culture-negative diarrhoea (n = 69) had similar mean gastric acid levels (basal, 3-5 mmol/h; stimulated, 11-17 mmol/h), which remained stable in those patients studied throughout 12 weeks of convalescence. Gastric acid levels were not associated with *G. duodenalis* or *S. stercoralis* co-infection, fever, use of tobacco, or chewing betel nut. Factors which impair gastric acid secretion may predispose to diarrhoeal disease in developing countries (Evans etal., 1997).

A study conducted in Srilanka, showed examination of total 192 stool samples from the adult and the pre-school children) was done. *E. histolytica* was not seen in any of the samples; *Giardia* cysts and Cryptosporidiumoocysts were seen in 3 and 1 sample respectively from the pre-school children. The overall prevalence of geohelminth infections was 21.3% among the adults and 24.5% among the children. *A. lumbricoides* was the predominant species in both populations (De silva et al., 1994).

Stool survey was carried out in some of the villages of Dadraul and BhawalKhera PHC's of district Shahjahanpur (Uttar Pradesh). Among them, 29.2 % were found positive for one or the other intestinal parasite. *A. lumbricoides* superseded all the parasites by showing a positivity of 17.8%. Other parasites found were Hookworm, *H. nana,* Tapeworm, *T. trichiura, E. vermicularis*, *E. coli* and *G. lamblia*. Parasitic load was slightly higher in females (33.5%) than males (28.1%). The highest positivity was encountered in the age groups between 6 to 14 years (Virk et al., 1994).

Analysis of egg and worm counts of *Ascaris*recorded at various intervals following a mass antihelmintic treatment programme in a South Indian fishing community was done. Three indices of infection in the community are compared, namely the prevalence and intensity of egg output (at 2, 6 and 11 months following treatment) and the number of worms expelled following an 11 month period of reinfection. Detailed examination of these measurements revealed significant associations with patient sex and age. Although 85.0% of both males and females harbored *Ascaris*initially, the prevalence following 11 months reinfection was decreased, due to a significantly lower proportion of males being reinfected. By the 11th month of reinfection, the age-intensity profiles of egg output were similar to those observed at initial treatment in the older age groups (10 years and above) and in male children (less than 10 years) (Elkins et al., 1988).

3.3 National Scenario

Rai et al., 2007 was carried out aretrospective study to see the prevalence of intestinal parasitosis among the patients visiting a health care centre in

KathmanduValley. Of the total 1,316 subjects included, 395 (30.0%) showed some kind of parasites. Females had significantly higher positive rate (34.0%) compared with males (27.5%). Highest positive rate (32.3%) was found in the age group 16-30 years followed by the age group 31-45 (30.8%) and others ($p>0.05$). Most of the patients (89.9%) were infected with single parasites. Protozoan parasites were more common than helminthes. Of the helminth parasites detected, *A. lumbricoides* was the commonest one followed by others. Among the protozoa, *E. histolytica* topped the list.

The study was carried out among the elderly people in KathmanduValley to assess the prevalence of intestinal parasitosis in them. Stool samples were collected from 235 elderly people. The samples were examined by formal ether sedimentation and Sheather's sucrose floatation followed by Kinyoun's modified Ziehl-Neelsen staining. The overall prevalence of intestinal parasites was found to be 41.7% out of which 30.6% had multiple parasitisms. The government elderly home had significantly higher parasitic prevalence (50.8%) followed by the rural community (46.8%) and the private elderly home (21.2%). Males (43.8%) had slightly high infection rate than females (40.4%). There was equal infection rate with protozoa (25.8%) and helminths (27.0%). *T. trichiura* (39.4%) and *E. histolytica* (19.7%) were the commonest helminth and protozoa respectively (Shakya et al., 2006).

Adhikari et al., 2004 was found 34% prevalence of intestinal helminthic infection among school children in KathmanduValley. Such infection was found equally among males and female population. *T. trichiura* was the most common parasite among the study subjects followed by *A. lumbricoides*, hookworm, *H. nana* and *S. stercoralis*. It was observed that the rate of helminthic infection among *Dalits*,*Indo-Aryans* and *Tibeto-Burman* does not differ significantly.

Rai et al., 2005 studied the predisposing factors of enteric parasitic infections in school children in a rural area in Kathmandu Valley, Nepal. Fecal samples were examined by formal-ether concentration technique. A total of 71.2% children had parasite. Altogether nine types of parasite were recovered; *T.trichiura*, was the commonest one followed by hookworm and others. Among the protozoa, *E.*

coli was commonest followed by *E. histolytica,* and others. Boys had relatively higher prevalence compared with girls. Highest infection rate was observed in the children aged ≥ 15 years. Infection rate was found higher in *Dalit* children compared with *Tibeto-Burman* and *Indo-Aryan*. Prevalence of parasitic infection among children with history of talking antihelminthic drugs within past ix months was found significantly lower compared to those not taking drugs.

Kimura et al., 2005 studied diarrhoeal diseases associated with *C. cayetanensis* in Nepal and Lao PDR. *C. cayetanensis*was detected by direct microscopy using ultraviolet and differential interference contrast microscopy. The overall positive rate in Nepal was 9.2% (128/1397). A higher positive rate was observed in children aged 10 years and under (11.1%) and was lowest in the age group of 51-60 years (3.1%). A significantly higher positive rate was observed in the summer (rainy season) (12.6%) with the lowest prevalence in the spring (dry season) (1.8%) ($P<0.05$). The positive rate was closely associated with rainfall (ml/month). Only one of the total 686 samples (0.1%) from Lao PDR was found to be positive for *Cyclospora*oocysts.

The study was conducted to represents the status of intestinal parasitosis in public schoolchildren (1 to 10 classes) in a rural area of the Kathmandu Valley to their habits, including factors predisposing to parasitic infections. Stool samples from the children were examined. The overall prevalence of parasitosis was 66.6% (395/533). Altogether, nine types of parasites were recovered. The recovery rate of helminthes was higher (76.9%) than protozoa (23.1%). *T. trichiura* was the most common helminthes detected, followed by hookworm, *A. lumbricoides* and others. *E. coli* was the most common protozoan parasite, followed by *E. histolytica*, *G. lamblia* and others (Sharma et al., 2004).

Intestinal parasitological survey was conducted to clarify the distribution of intestinal parasites in Nepal and Lao Peoples' Democratic Republic (Lao PDR) from 2001 to 2003.The stool specimens were examined using the formalin-ether sedimentation (FES) and sucrose centrifugal flotation (SCF) techniques. Nine species (3 Nematoda, 1 Cestoda, and 5 Protozoa) of parasites were recovered from Nepal, whereas seven species (3 Nematoda, 1 Trematoda, and 3 Protozoa) from Lao PDR. Out of which (14.4%) was the most common in Nepal, and was *O. viverrini*(29.8%) in Lao PDR. Infection rates were markedly different among age groups in both countries; higher rates were observed in age groups of 10-29 years than in 0-9 years group (Takemasa et al., 2004).

Intestinal parasites were detected in diarrheal stool samples collected from individuals aged 1 to 68 years (males: 239 and females: 157) in Nepal. Parasites were detected by employing the formal-ether sedimentation technique. Of a total of 396 fecal samples investigated, 193 (49.0 %) were positive for some kind of parasite. Altogether, 15 species of parasites were detected. *G. intestinalis* topped the list of protozoa, whereas *T. trichiura* was the most frequently detected among helminth parasites. Of the 193 positive samples, 109 (56.0 %) had single parasite infections, whereas 84 (43.0 %) had multiple infections with a maximum of five species. Of the total positive, 45 (23.0%) had both protozoa and helminths whereas 37 (19.0%) had only protozoa. Females (52.0%) and children (15 years and under) (52%) had a marginally higher prevalence compared with males (46.0%) and adults (45.0%), respectively ($p > 0.05$) (Uga et al., 2004).

Ishiyama et al.,2001 had reported the parasite prevalence of 72.4% among school children in Kathmandu. *Indo-Aryans* were found to have marginally high rate of infection. Among which (46.9%) had multiple infections. *T. trichiura* (30.4%) was found to be the commonest helminth and *G. lamblia* (17.0%), the commonest protozoan. No bacterial enteropathogens were reported in the study population.

Rai et al.,2001 studied the intestinal parasitic infection in rural hilly area of Western Nepal, Achham district. The stool test revealed (76.4%) prevalence of intestinal parasites in the children of the district. A total of nine species of parasites were detected, of which, *A. lumbricoides*was the commonest one followed by hookworm and others.

Rai et al., 2000 intestinal helminth infection and its effect on vitamin A, retinol and B carotene, was studied in Okharpauwa Village Development Committee (VDC) (Nuwakot district) and 79 inhabitants (mainly adults) of Boya VDC

(Bhojpur District)] subjects living at an altitude of 2000 m. Most common helminth detected was *A. lumbricoides* followed by *T. trichiura* in Okharpauwa VDC and by Hookworm in Boya VDC, respectively. Mixed helminth infections are were relatively low (7.3% in Okharpauwa VDC and 11.1% in Boya VDC). The retinol and B-carotene were estimated by high performance liquid chromatography (HPLC). The retinol concentration in helminth eggs positive children significantly increased after one month of anti-helminthic (albendazole) treatment (P>0.05). No significant difference in serum retinol concentration was observed among helminth eggs positive and negative inhabitants of Boya VDC (P>0.05) but in B-carotene level (P<0.05).

Rai and Rai, 1999 a hospital-based study in Kathmandu showed ascariasis as major casuses of public health problem in Nepal though the extent of ascariasis-associated morbidity and mortality has not been investigated yet. In some rural areas, over (75.0 %) people are infected with this parasite.

The parasitic infection rate of (50.0%) has been reported by the studies conducted in the Nepalese communities from 1979-1995 by different organizations. *A. lumbricoides* and *G.lamblia*topped the list respectively among helminthes and protozoans. Similarly, the hospital records showed the infection rate of 30 to 40 % (Chhetri, 1997).The burden of parasitic infection including other infections also reflected in the list of top ten diseases of Nepal.

According to Rai et al.,1997 the health and sanitary status of Boya Village was studied. The number of households having latrine increased significantly (p<0.05) in one year period but without significant impact on the reduction of intestinal helminth infection (p>0.05). *A. lumbricoides* was the commonest intestinal parasite followed by hookworm and others. Public piped line water was provided to (32.6%) households while remaining (48.4%) and (19.0%) were using natural tap and*kuwa* water, respectively. No association between the type of water source and gastro-enteritis was observed. There was poor hygienic condition. Majority of complaints were gastrointestinal.

Sherchand et al., 1997 carried out a stool survey on intestinal parasites and its transmission factors in rural village of Dhanusha Districts, Southern Nepal. Out of 604 children aged between 0-9 years examined, 363 (60.1%) were found positive for one or more intestinal parasites. Hookworm infection superseded all the parasites by showing a positivity of (11.6%). Other parasites found were *A. lumbricoides*, *T. trichura,Oxyurisvermicularies,S. stercolaris,* tapeworm, *H. nana,E. histolytica, E. coli, G. lamblia, C.parvum*, *C. cayetanensis,I. belli*, *O. viverrini*, *S. mansoni* and *I. butschlii*. The parasitic load was found slightly higher in female children (58.1%) compared to male children (41.9%).

The study was conducted on intestinal parasites from the Kathmandu area of Nepal was done in children and adults. The total parasite load was 28.1% and 38.8%, respectively, whereas children and adults with abdominal discomfort had a load of 62.7% and 67.8%. The prevalence of nematodes in the 4 groups was significantly higher in those with abdominal discomfort, particularly of hookworm, *Enterobius*, and*Ascaris*. *H. nana* was the most common tapeworm, and with the highest incidence in patients with abdominal complaints. *T. solium* and *T. saginata* were only found in the two adult groups, but with low prevalence rates. The highest incidence of *Cryptosporidium* was found in both groups with abdominal discomfort, notably among children. The presence of *Giardia* was highest among the sick children, many "healthy" carriers among both children and adults were noted. *E. histolytica* and *E. dispar* had a surprisingly low prevalence in all 4 groups. *B. hominis* was most common among adults with abdominal complaints (2.8%). *Trichomonas* sp. was also more common in this group, in which of 34 positive specimen, 28 were from women (Sherchandet al., 1996).

Stool samples were investigated for the prevalence of intestinal parasitic infection inTribhuvan University Teaching Hospital, in Kathmandu. Among the various types of protozoan parasites, the most common was *G. lamblia* followed by *E. histolytica* (Rai etal., 1995).

A study conducted in Jiri revealed that the prevalence of whipworm, roundworm and hookworm among*Jirel* were 18.1%, 25.3% and 73.5%, respectively. Similarly, prevalence of whipworm, roundworm and hookworm among *Sherpa* were 11.2%, 23.6% and 46.1%, respectively. Similarly, the prevalence of whipworm, roundworm and hookworm among *Hindu* of age group 45 above were 7.1%, 26.2% and 59.5% respectively. The study found the increasement in multiple helminthic infections with increasement in age (Blangero et al., 1993).

3.4.Human Intestinal Protozoan Parasitic Infection

Protozoa are a diverse group of organisms that have evolved to occupy a variety of ecological niches. There are over 30 phyla of protozoa; Most of these have evolved a totally parasitic existence. The enteric protozoa that cause human illness are usually transmitted by the consumption of food and drink, or through environmental contamination and poor hygiene. Some of these can cause substantial illness, and have economic consequences (Buzby and Roberts, 1997).

Intestinal protozoal diseases are caused by unicellular microorganisms which invade the wall of the intestine such as Amebiasis, Giardiasis, and Cryptosporidiosis. Numerous protozoa inhabit the gastro-intestinal tract of humans. The majority of intestinal protozoa is non-pathogenic commensals, or only result in mild disease. Some of these organisms can cause severe disease under certain circumstances. Apicomplexa and microsporidia species, which normally do not evoke severe disease, can cause severe and life-threatening diarrhea in AIDS patients and other immunocompromised individuals (Adamu.2006).

Intestinal protozoan parasite infections are a significant problem with more than 58 million cases in children each year. Pathogenic intestinal protozoa are especially important in the developing world where they may cause death. Most intestinal protozoan parasites are spread by faecal–oral contact or contamination of water or food. Poor sanitation and poverty are contributory factors in many low income countries. Symptoms of intestinal protozoan parasite infections include diarrhea, abdominal pain, and nausea, vomiting and weight loss (Marshall et al, 1997).

3.5. Life Cycle of Intestinal Protozoan Parasites Infections

Several members of the genus *Entamoeba* infect humans. Among these only *E.histolytica* is considered pathogenic and the disease it causes is called amoebiasis or amebic dysentery. *E. dispar*is morphologically identical to *E. histolytica,* but is not pathogenic. The two species are found throughout the world, but like many other intestinal protozoa, they are more common in tropical countries or other areas with poor sanitary conditions. It is estimated that up to 10% of the world'spopulation may be infected with either*E. histolytica* or *E. dispar* and in many tropical countries the prevalence may approach 50%. It is also estimated that about 100,000 deaths and 50 million cases of amoebiasis occur per year in the world and humans are the only host of *E. histolytica* and there are no animal reservoirs (Haque et al., 2003).

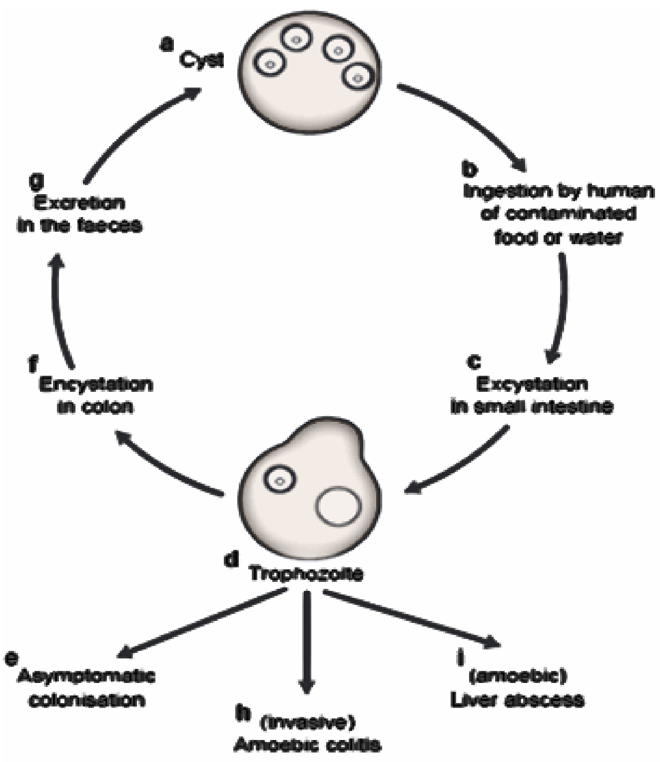


Figure 1: Lifecycle of Entamoeba

The life cycle of Entamoeba histolytica as showed in figure-1 includes the infective cyst and the invasive trophozoite forms. Infection is acquired by ingestion of infectious cyst through water or undercooked food contaminated by human feaces. After ingestion of the cyst, which is resistant to gastric acids and enzymes, excystation occurs in the ileoceacal area of the intestine to form trophozoites. The trophozoites are larger in size and actively motile organisms. According to the bind-lyse-eat model, the trophozoites bind to the large intestine and invade the wall releasing amoeba pores and phospholipases, causing ulceration of the mucous membrane (called flask shaped ulcers), and sometimes large vessels may be eroded and severe intestinal hemorrhage result (Petri and Singh,1999).

The parasite *Giardia lamblia* reproduces by binary fission which is a type of reproduction in which one cell divides into two new cells by mitosis. During the growth cycle the components of the *Giardia* cell multiply so that each daughter cell would be a complete copy of the original parent cell. The newly formed cells then pinch off from each other; in so doing a complete reproduction cycle would occur the infective stage of *Giardia lamblia*, the cyst, is elliptical in shape and its size ranges from 6 to 10 microns and contains two to four nuclei. The cyst possesses a structure that enables it to be resistant to most environmental factors and disinfection and make it successful in being the infective stage of the parasite. The cyst has a thin and protective wall that allows it to survive in feces for weeks or for about 3 months in water at 40^0C (Meyer and Jarrol, 1980).

Giardiasis could be contracted through drinking contaminated waters or ingestion of contaminated food stuffs. The cyst passes through the stomach and enters the small intestine. The acidic environment of the stomach could not harm the cyst because it has a thin protective wall to protect it until it reaches the alkaline environment, the small intestine (Ortega and Adam, 1997). This alkaline environment initiates excystment of the cyst (Erlandsen and Mayer, 1990). During excystation, the cyst wall ruptures at the pole opposite to the nuclei so that the flagella and other projections emerge from the rupture point. The cyst wall is then completely shed and the parasite will enter into its trophozoitestage (Erlandsen and Mayer, 1990).

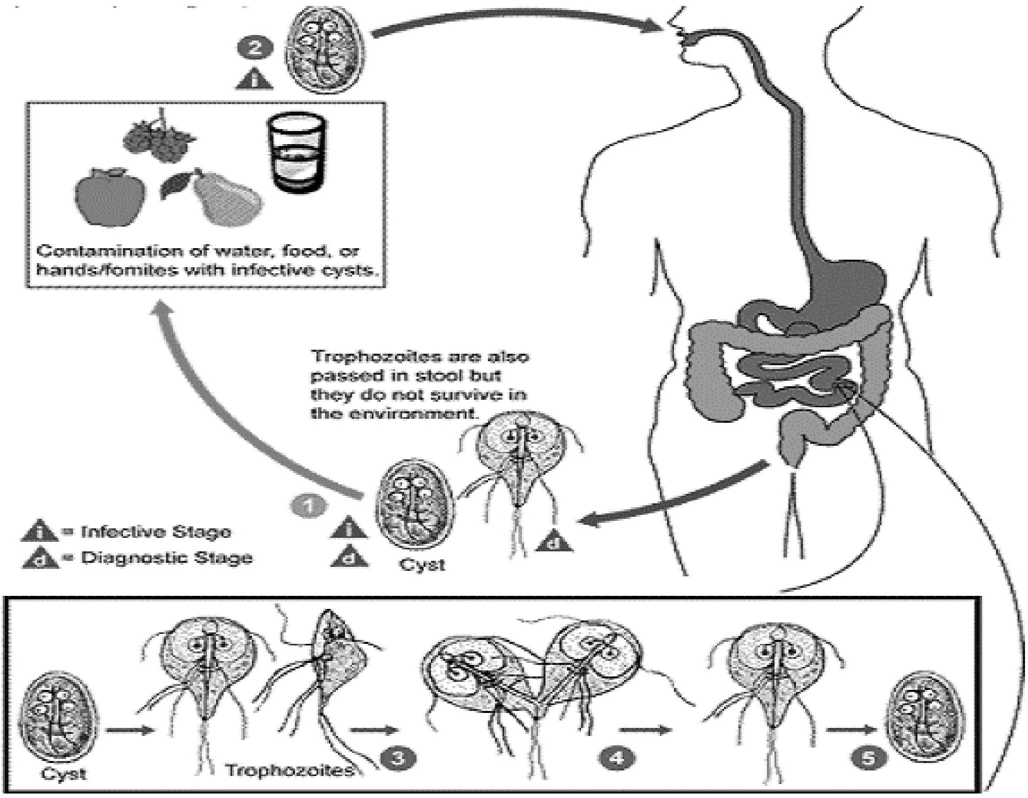


Figure 2. Lifecycle of *Giardia lamblia* (*Source*:http://www.dpd.cdc.gov/dpdx)

3.6. Water-Borne Intestinal Protozoan Parasites

Water-borne diseases are caused by pathogenic microorganisms that most commonly transmit by contaminated water infection commonly results during bathing, washing, drinking, in preparation of food and consumption of food. Various forms of water born diarrhoeal disease are the most prominent examples (Jump et al., 2006). Water-borne intestinal protozoan parasites include such as Amoeba, Cryptosporidium and Giardia have become a challenge to human health worldwide. These protozoans have several common characteristics biologically. Their major habitat is intestinal epithelial cells, and they are all intracellular parasites. In addition, they produce infectious spores that are excreted from the hosts in their stools and Giardia produces cyst (Akiyoshi et al., 2003).

The pathogenesis of diarrhea and malabsorption that can occur in Giardiasis is not fully understood; diarrhea may be a result of both intestinal malabsorption and hypersecretion. The small intestine is the site of the major structural and functional abnormalities associated with Giardiasis. Light microscopy may demonstrate no abnormalities, mild or moderate partial villous atrophy, or subtotal villous atrophy in severe cases. An increase in crypt depth may be seen, and microvilli shortening or disruption may occur. Deficiencies in epithelial brush border enzymes, such as lactase, may develop (Buret, 2011).

Pathogenesis of Amoebiasis is believed to be a multi-step, multifactorial process. Though a large number of studies have attempted to unravel the factors/molecules responsible for the pathogenesis of Amoebiasis, the processes involved in pathogenesis are poorly understood. The aspects of pathogenesis which have been investigated experimentally can be broadly categorized into mechanisms involving interactions with the intestinal flora, lysis of target cell by direct adherence, lysis of target cell by release of toxins and phagocytosis of target cells (Sehgal et al., 2010).

The life cycle of *C. parvum* begins following ingestion of the oocyst by a susceptible host. The oocyst is spherical in shape measuring 3-6 mm in diameter and it may be either thick- or thin-walled. Thin-walled oocysts mayexcyst within the same host and start a new life cycle (autoinfection). This can lead to heavily infected intestinal epithelia and result in malabsorptive or secretory diarrhoea. Thick-walled oocysts are excreted with the faeces and it is the resistant stage found in the environment (Fayer et al., 2000).

These parasites are intracellular, enclosed by a thin layer of host cell cytoplasm. Once the oocyst is ingested, the host body temperature, the interaction with stomach acid and bile salts triggers excystation and releasing infective sporozoites in the gastrointestinal tract (Li et al., 2005). After oocystexcystation in the intestinal lumen, sporozoites penetrate the host cell and develop into trophozoites within parasitophorous vacuoles located in the microvillous region of the mucosal epithelium. Trophozoites undergo asexual division (merogony) to form merozoites. After release from type I meronts, merozoites enter adjacent host cells and multiply to form additional type I meronts, or to form type II meronts. Type II meronts do not recycle but enter host cells to commence the sexual phase of the life cycle with the formation of microgamonts and macrogamonts. Most (approximately 80%) of the zygotes formed after fertilization develop into environmentally resistant, thick-walled oocysts that undergo sporogony to form sporulatedoocysts containing four sporozoites. A smaller percentage of zygotes (approximately 20%) form thin-walled oocysts surrounding the four sporozoites that represent the auto infective life cycle forms that can maintain the parasite within the host without repeated oral exposure to the thick-walled oocysts present in the environment. The presence of these autoinfectiveoocysts and recycling type I meronts are believed to be the means by which persistent chronic infections may develop in hosts without further exposure to exogenous oocysts(Carey et al., 2004).

3.7. Pathogenesis and Clinical Manifestation of Human Intestinal Protozoan Parasite Infections

Intestinal protozoan parasite infection can result in gastrointestinaldisease in humans. As a result of infection of the parasite more or less similar clinical sign and symptom can be observed. Forexampleinfections with *E. histolytica* have no symptoms in many individuals, and most canclear their infection without any signs of disease (Ravdin& Petri, 1995). For unexplainable reason, however, 4-10 % of asymptomatic individuals infected with *E. histolytica* develop disease over a year. In other words, different studies indicate that in upto 90 % of *E. histolytica* infections, the symptoms are absent or very mild .There is a wide spectrum of clinical presentations of *E. histolytica* infection symptomatic amoebiasis is primarily an intestinal disease, and when it becomes extraintestinal, it usually involves the liver. Pathogenesis of amoebiasis is believed to be a multi-step, multifactorial process. Though a large number of studies have attempted to unravel the factors/molecules responsible for the pathogenesis of amoebiasis, the processes involved in pathogenesis are poorly understood. The aspects of pathogenesis which have been investigated experimentally can be broadly categorized into mechanisms involving (i) interactions with the intestinal flora, (ii) lysis of target cell by direct adherence, (iii) lysis of target cell by release of toxins and (iv) phagocytosis of target cells (Sehgal et al., 1996).

Symptoms of amoebiasis could be acute (Frequent dysentery with necrotic mucosa and abdominal pain) and chronic (Recurrent episodes of dysentery with blood and mucus in the feces). There are intervening gastrointestinal disturbances and constipation. Cysts are found in the stool. The organism may invade the liver, lung and brain where it produces abscesses that result in liver dysfunction, pneumonitis, and encephalitis (WHO, 2002).*G. lamblia* is usually weakly pathogenic for humans. Cysts may be found in large numbers in the stools of entirely asymptomatic persons. In some persons, however, large numbers of parasites attached to the bowel wall may cause irritation and low-grade inflammation of the duodenal or jejunal mucosa, with consequent acute or chronic diarrhea associated with crypt hypertrophy, villous atrophy or flattening, and epithelial cell damage. The stools may be watery, semisolid, greasy, bulky, and foul-smelling at various times during the course of the infection. Malaise, weakness, weight loss, abdominal cramps, distention, and flatulence can be occur. Children are more liable to clinical Giardiasis than adults. Immunosuppressed individuals are especially liable to massive infection with severe clinical manifestations. Symptoms may continue for long periods (Butel and Stephen, 2007). The pathogenesis of Cryptosporidium are associated with diarrhoea, weight loss and mortality are not well understood but recent research in animal models have provided insight into the patho-physiology of the disease and understanding of the clinical signs. The complicated life cycle, the variety of parasitic forms within the host, the different Cryptosporidium species.

As in any parasitic infections, host parasite interaction is the initial steps in the pathogenesis of giardiasis. In this interaction, first the Giardia trophozoites attach to the cell surface of villi by means of a disk on their posterior or ventral surface. Lectin, a protein on the trophozoite lining, recognizes specific receptors on the intestinal cell and may be partly responsible for the tight attachment between the parasite and the villi following attachment of trophozoites, there will be major structural and functional abnormalities in the small intestine. Some of these abnormalities include mucosal damage as a result of mechanical obstruction or blockage of the intestine by a large number of parasites, the release of cytopathic substances such as thiol proteinases water intended for consumption, thoroughly washing hands before handling food, maintaining good personal cleanliness, properly disposing of fecal material and information dissemination through print media to educate the public regarding the dangers of giardiasis (Backer, 2000).

3.8. Pathogenesis and Clinical Manifestation of IntestinalHelminths

Nematode infections in humans include ascariasis, trichuriasis, hookworm, enterobiasis, strongyloidiasis, filariasis, trichinosis, and angiostrongyliasis (rat lungworm disease), among others. The phylum Nematoda, also known as the roundworms, is the second largest phylum in the animal kingdom, encompassing up to 500,000 species.

Many roundworm species are free living in nature. Recent data have demonstrated that approximately 60 species of roundworms parasitize humans. Intestinal roundworm infections constitute the largest group of helminthic diseases in humans. According to a 2005 report by the World Health Organization (WHO), approximately 0.807-1.221 billion humans have ascariasis, 604-795 million have trichuriasis, and 576-740 million have hookworm infections worldwide. [1]

3.9 Pathophysiology

The life cycle of parasitic nematodes is clinically important. Some nematode infections can be transmitted directly from infected to uninfected people; in others, the nematode eggs must undergo a process of maturation outside the host. In a third category, the parasites may spend a part of their life cycle in the soil before becoming infective to humans.

As with other parasitic infections, definitive diagnosis of nematode infections depends on demonstration of the stage of the life cycle in the host. Nematodes, as with most other worms infectious to humans, almost never complete their entire life cycle in the human host.

The life cycles of nematodes are complex and highly varied. Some species, including *Enterobiusvermicularis,* can be transmitted directly from person to person, while others, such as *Ascarislumbricoides, Necatoramericanus,* and *Ancylostomaduodenale,* require a soil phase for development. Because most helminthic parasites do not self-replicate, the acquisition of a heavy burden of adult worms requires repeated exposure to the parasite in its infectious stage, whether larva or egg. Hence, clinical disease, as opposed to asymptomatic infection, generally develops only with prolonged residence in an endemic region.

Unlike with protozoan infections, a casual or a low degree of exposure to infective stages of parasitic nematodes usually does not result in patent infection or pathologic findings. Repeated or intense exposure to a multitude of infective stage larvae is required for infection to be established and disease to arise. *Anisakis* species cause erosive and/or hemorrhagic lesions in or near the main lesion, forming a tunnel through the gastric mucosa to the submucosa.

Many infections are asymptomatic; pathologic manifestations depend on the size, activity, and metabolism of the worms. Immune and inflammatory responses also cause pathology

The most serious helminth infections are acquired in poor tropical and subtropical areas, but some also occur in the developed world; other, less serious, infections are worldwide in distribution. Exposure to infection is influenced by climate, hygiene, food preferences, and contact with vectors. Many potential infections are eliminated by host defenses; others become established and may persist for prolonged periods, even years. Although infections are often asymptomatic, severe pathology can occur. Because worms are large and often migrate through the body, they can damage the host's tissues directly by their activity or metabolism. Damage also occurs indirectly as a result of host defense mechanisms.

Helminths are transmitted to humans in many different ways. The simplest is by accidental ingestion of infective eggs (*Ascaris, Echinococcus, Enterobius, Trichuris*) or larvae (some hookworms). Other worms have larvae that actively penetrate the skin (hookworms, schistosomes, *Strongyloides*).

Human behavior is a major factor influencing susceptibility to infection. If the infective stages of helminths are present in the environment, then certain ways of behaving, particularly with regard to hygiene and food, will result in greater exposure.

Children are more susceptible to many helminths than are adults, and frequently are the most heavily infected members of a community. The waning of immune competence with age may also result in increased levels of infection. Individuals differ genetically in their ability to resist infection, and it is well known that in infected populations, some individuals are predisposed to heavier infections than others

Many helminths undertake extensive migrations through body tissues, which both damage tissues directly and initiate hypersensitivity reactions. The skin, lungs, liver, and intestines are the organs most affected. Petechial hemorrhages, pneumonitis, eosinophilia, urticaria and pruritus, organomegaly, and granulomatous lesions are among the signs and symptoms produced during these migratory phases.

CHAPTER IV

4. MATERIALS AND METHODS

4.1 Materials

A list of materials, chemicals, equipments, reagents for the study is presented in Appendix 1.

4.2 Methods

4.2.1 Study area

The laboratory investigation was carried out at Department of Microbiology Lab, of Sunsari Technical College, Dharan. The study period was from March 2017 to June 2017. The stool samples were collected from the Patients visiting the Hospital.

4.2.2 Samples collections

Each Patient suspected with intestinal discomfort was given the brief description about the importance of the examination of stool to detect the parasite. They were advised not to contaminate the stool with water and urine. The containers were labeled with patient's name, code number, date and time of collection. During the process of specimen collection from each patient, a questionnaire accompanying the queries about their clinical history, hygienic practice was filled. Labeled dry, clean disinfectant free wide mouthed plastic container was distributed and asked them to bring about 20 gms stool sample in same day or next morning.

4.2.3 Transportation of the samples

The collected stool samples were brought to the laboratory immediately .Then, processing was done at laboratory.

4.2.4 Laboratory processing of the samples (Rai et al., 1996)

Each stool sample was processed in 2 steps as macroscopic examination and microscopic examination.

4.2.4.1 Macroscopic Examination

The direct visualization of each sample was done for the color, consistency, presence of mucus, blood, and adult worm or worm segment.

Color

Based on the color, the stool specimen were categorized into groups i.e. normal color of stool (yellowish brown) and abnormal color of stool (muddy, black, pale etc.)

Consistency

Based on consistency stool specimen were classified as formed, semi-formed and loose.

Blood and mucus

The stool specimens were observed whether it contains blood and mucus or not.

Adult worms and segments

The stool specimens were observed whether it contains adult worms and segments or not. The adult worms of *A. lumbricoides* and *E. vermicularis* are often seen in the specimen. Tapeworm segments may be occasionally seen in stool specimen.

4.2.4.2 Microscopic examination

Microscopic examination was carried out for the detection and identification of Protozoal cysts, Oocysts, Trophozoites and Helminthic eggs or larva.

Microscopic examination was done by Saline and Iodine wet mount preparation. The slides were observed under low power (10x) followed by high power (40x) of the Microscope.

While performing wet mount, all the samples were subjected to concentration. There are various floatation and sedimentation techniques of concentration.

Formal-ether sedimentation method leading to saline/Iodine wet mount

The technique performed as follow:

1. Further 3-4 ml of 10% formal saline was added in a preserved sample and then shaken well.
2. The suspension was sieved through cotton gauge in a funnel into a 15 ml centrifuge tube.
3. 3-4 ml of ether was added and shaken vigorously for 5 minutes.
4. The tube was immediately centrifuged at 1000 rpm for 10 minutes
5. Four layers of suspension were obtained in the tube after centrifugation.
    a. A small amount of sediment at the bottom of the tube containing the parasite.
    b. A layer of formalin on the top of the sediment.
    c. A plug of fecal debris on the top of formalin layer.
    d. A layer of diethyl ether at the top.
    e. The plug of debris formed between diethyl ether and formalin was removed by rotating the tip of the applicator along the inner wall of the tube.
6. The supernatant layers of suspension were discarded and the sediment was examined by saline and iodine wet mount.

Saline wet mount

It was used to detect helminthes eggs, larvae and the protozoa cysts. A drop of normal saline was taken on a clean glass slide; a drop of sediment from the above process was mixed with it and observed under microscope after covering with a cover slip.

Iodine wet mount

This was mainly used for detecting protozoa cysts; however helminthes eggs were also stained and could be detected. Iodine stained cysts showed pale refractile nuclei, yellowish cytoplasm and brown glycogen material. A drop of 5 times diluted Lugol's iodine was taken on a slide and a drop of sediment from above process was mixed with it. The preparation was covered with a cover slip and observed microscope.

4.2.5 Recording of the results

After laboratory processing of the samples the result obtained was noted. Then it was recorded in computer.

4.4.4 Report distribution

The report distribution was done as the result was obtained after laboratory processing of the samples and given to the patient next day.

4.4.5 Statistical analysis

Chi-square test was applied for statistical analysis of results using Win Pepi software program (PEPI-for-Windows): computer programs for epidemiologists, 2004. Association of intestinal infections with different variables was tested. Results were considered significant if P values were less than 0.05.

CHAPTER V

5. RESULT

5.1 Study population

Out of 480 stool samples, the numbers of males and females were 251(52%) and 229(48%) respectively.

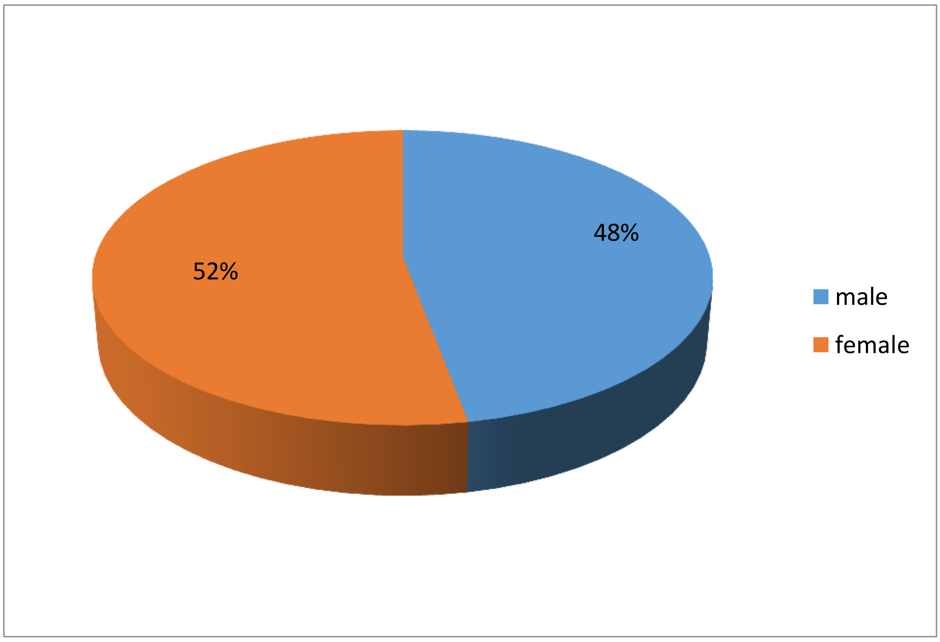
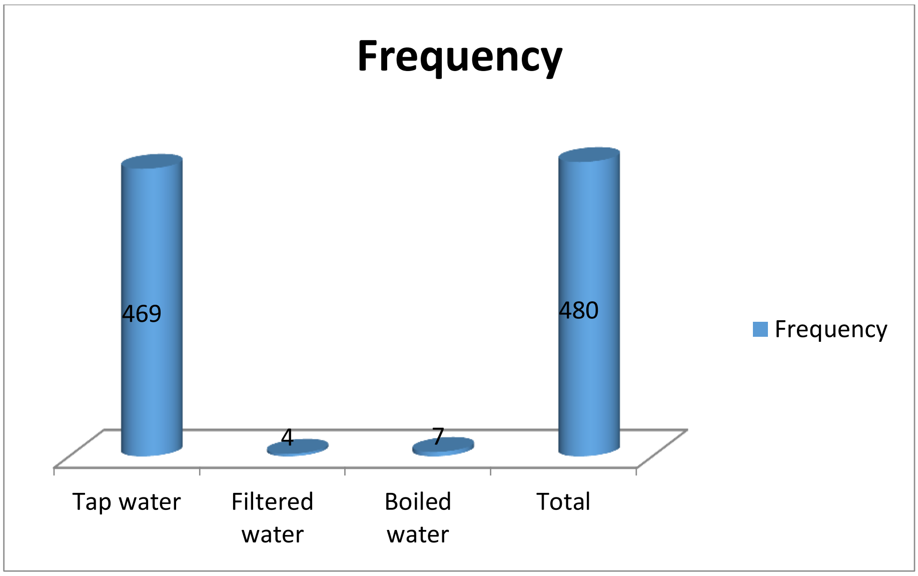


Fig: sex wise distribution of data

5.2 Sources of Drinking Water

Out of total 480 water samples, 469 was taken from tap water similarly 7 were taken from boiled tap water and 4 were taken from filtered tap water.



5.2 Demographic pattern of result

Out of 480 stool samples, the numbers of positive were 51 in which positive males and females were 24 and 27 respectively. Statistically, there is no significant difference between sex and occurrence of the parasitic infection (p=0.07**)**

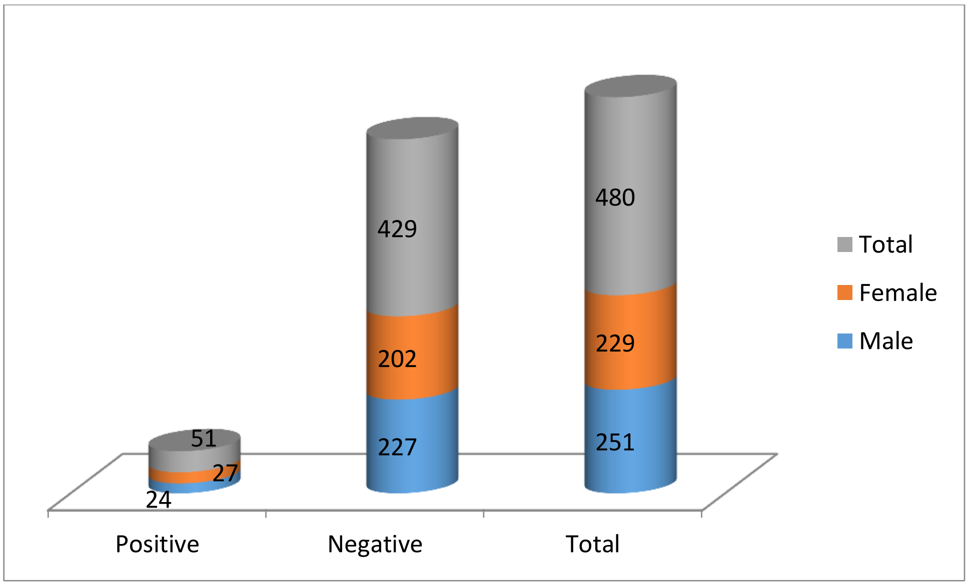
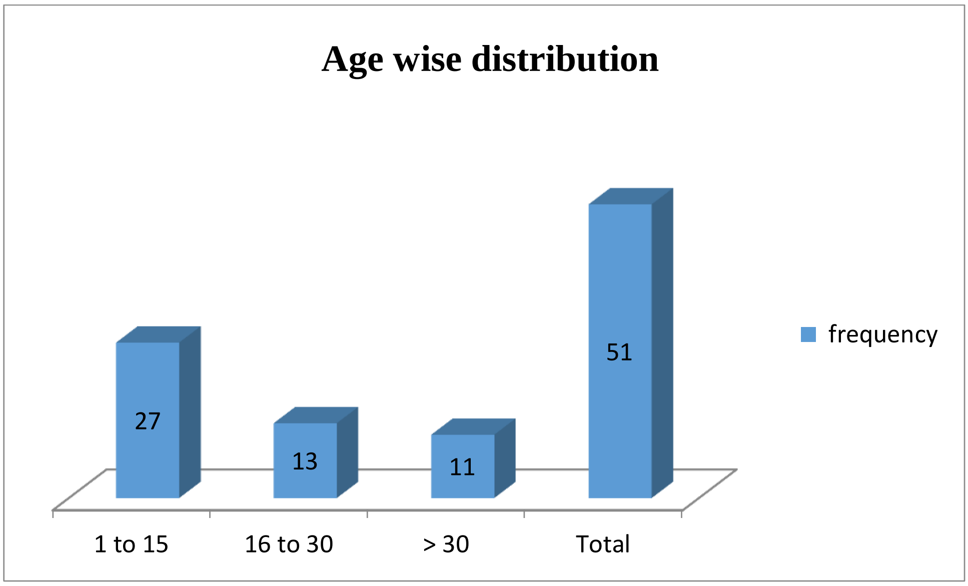


Fig: Gender wise distribution of positive sample

5.3 Age wise distribution of data



In age group, the highest positive cases were in ≤ 15 years with 27 positive results. Similarly 13 positive samples were found between age group 16 to 30 years and 11 positive samples were observed above 30nyears of age. The occurrence of parasitic infection with age group was statistically insignificant (p=0.44).

5.4 Frequency of protozoans isolates

Out of 52 isolates of protozoans' highest isolates was cyst of E. histolytica with frequency 20 followed by cyst of G. lamblia with 13. One positive result was found with the mixed protozoans of cyst of Giardia and Cyst of E. histilytica.

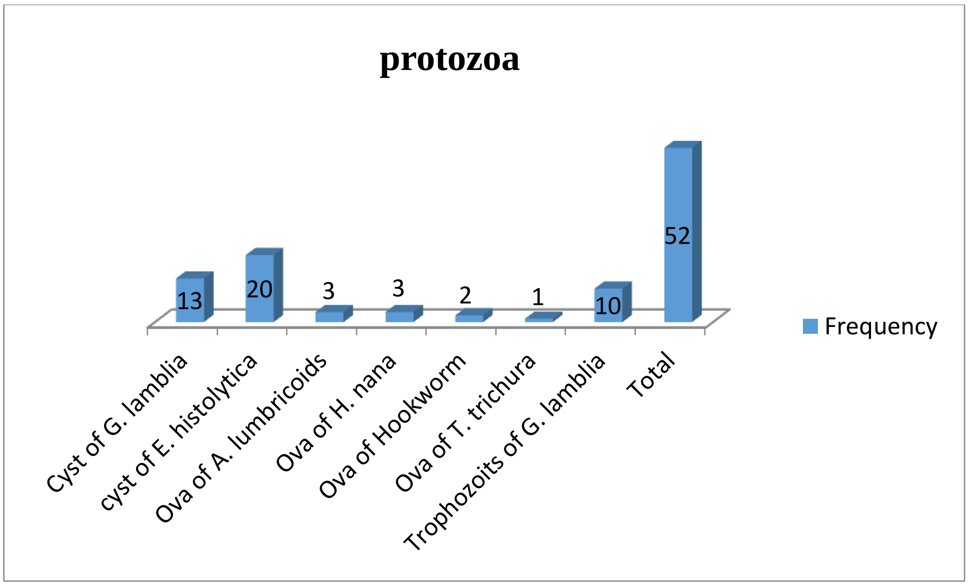


Fig: frequency of isolates protozoans

5.6 sex wise distribution of positive samples

Out of total 13 Cysts of Giardia isolates 6 were seen in male while 7 were seen in female samples. Similarly out of 20 cyst of E. histolytica 11 were seen in male and 9 were seen in female.

protozoans	**males**	**female**	**total**
Cyst of *Giardia lamblia*	6	7	13
Cyst of *E. histolytica*	11	9	20
Ova of *A. lumbricoids*	2	1	3
Ova of *H. nana*	2	1	3
Ova of *Hookworm*	0	2	2
Ova of *T. trichuria*	0	1	1
Trophozoit of *G. lamblia*	4	6	10
Total	**25**	**27**	**52**

CHAPTER-VI

6.1 DISCUSSION

Sanitation and proper waste disposal are a major problem in squatter community because of poor socio-economic conditions. They share a house with domesticated animals and pets which are a good indicator of the poverty and poor hygienic condition of the household. Although more thanone-third of the children were infected in this study with any one type of parasites, the result is found to be less prevalenton parasitic infection than on a research was done by Magar *et al*., 2011 at Kathmandu valley which was 43.3% and higher than observed by Khanal *et al*., on 2011 at Kathmandu (17.6%) and Chandrashekhar *et al*., at Kaski (21.3%) on 2005 and Gyawali *et al*., at 2009 at Dharan (22.5%). This investigation showed the report of the very high positive rate of intestinal parasitic infections according to previous research among children elsewhere in the country (Mukhiya *et al*., 2012; Jaiswal *et al*., 2013). In Nepal highest prevalence published till now is 81.94% on 2003 among healthy children of Lalitpur district by Shrestha and the overall prevalence of our study was less than this report. A marginally higher positive rate of infection among boys as compared to girls was observed in this study which might be due to more outdoor activities of male children. This result agrees with the data of study done by Shrestha *et al*.,on 2012. Helminthic infections were less prevalent as compared to the protozoal infections and this result was similar to other studies were done in Dharan and Kathmandu (Gyawali *et al*., 2009; Pradhan *et al*., 2014). The Periodic campaign of anti-helminthic drug administration to the children and nationwide bi-annual integrated deworming as well as Vitamin A supplementation could possibly explain the lower prevalence of helminthic infections seen in this study (DoHS, 2010/11). Giardia lamblia was the only and the most prevalent (73.41%) protozoan among intestinal parasites. The finding was consistent with other reports from Nepal (Pooja *et al*., 2014, Gyawali *et al*., 2009). Unhealthy lifestyle, poor hygiene and lack of potable water might be the cause of high frequency of G. lamblia among squatter community children. Furthermore, contamination of drinking water in Nepal by fecal matter and the resistance of *G. lamblia* cyst to the normal level of chlorination of drinking water aid in parasitic infection in such areas (Magar *et al*., 2011). The result of the most prevalent helminth *Ascaris lumbricoides* (18.98%) was in agreement with the previous study done in Nepal (Shrestha and Maharjan, 2013). *Hymenolepis nana* occurs worldwide, reportedly with highest prevalence rates under the condition of poor sanitation and overcrowding but this study showed a lower prevalence which matches with other studies done in Nepal (Malla *et al*., 2004; Shrestha and Maharjan, 2013; Pradhan *et al*., 2014). High prevalence among Dalits (63.88%) appeared which was in agreement with the reports (Agrawal *et al*., 2012; Ishiyama *et al*., 2003). This could be due to low literacy rate, health ignorance or low socioeconomic status. There was the significant association between higher intestinal infection and those children, who did not take antiparasitic drugs did not use soap water after defecation and did not have water on the toilet. It means the children who were not following hygiene practices were more infected which is also depicted by other studies in Nepal (Shakya *et al*., 2006, Sherchand *et al*., 2

CHAPTER-VII

CONCLUSION AND RECOMMENDATIONS

7.1 Conclusions

Parasitosis in Hospital Visiting Patientsis still prevalent as major public health problems This result concluded a poor hygiene and sanitary conditions, improper water supply and uneducated family. In order to prevent this infection, appropriate health education should be given to children and their parents concerning disease transmission, personal hygiene and safe drinking water. Efforts from the municipality to improve the quality of drinking water supply and the types of toilets being used will certainly lower the number of parasitic infections in such area.

7.2 Recommendations

1. The study recommends for the provision for intensive and habitual health education for behavioral changes related to personal hygiene and mass treatment for the effective control of intestinal parasitic infections in the concerned area.

2. Periodic administration of deworming programs with the inclusion of antiprotozoal drugs through effective scheduling should be undertaken as an integral part of school health services to break the transmission chain of these intestinal parasitic infestations.

3. The study suggests for a multi-sectorial approach including initiatives from public authorities as well local participation to reduce the morbidity and mortality associated with intestinal parasitic infections amongst young children

4. Further epidemiological studies focusing on control of parasitic diseases are required to be undertaken in a coordinated manner to monitor the situation along with the state of various underlying associated predisposing factors.

5. Improvement in domestic water supplies with the regular maintenance of piped and closed sewerage systems is likely to have the most marked impact in decreasing overall infection rates

6. Special programs should be launched especially to educate women in the community with regards to creating awareness to control the intestinal infections in the family.

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APPENDIX -I

Materials and Chemicals used

1 Chemicals and reagents

Sodium chloride	Qualigens, India
Basic Fuschin	Qualigens,India
Ethanol	Bengal, India
Diethyl ether	Qualigens, India
Formaldehyde	Qualigens, India
Iodine crystals	Loba Chemic, India
Sulphuric acid	Qualigens, India
Methanol	Loba Chemic, India
Malachite Green	Qualigens, India
Sucrose Crystals	Qualigens, India
2.5% Potassium Dichromate	Qualigens, India

2 Materials

Test tube	Borosil, India
Conical Flask	Borosil, India
Beaker	Borosil, India
Measuring cylinder	Borosil, India
Glass slide and cover slips	Borosil,India
Droppers	Borosil, India
Pipettes	Borosil, India
Glass rod	Borosil, India
Test tube stand	Borosil, India

3 Equipments

Microscope	Olympus (Japan)
Refrigerator	LG, Korea
Centrifuge	Remi, India

APPENDIX II

COMPOSITION AND PREPARATION OF REAGENTS

Lugol's Iodine

Stock solution

Ingredients	Amount
I_2 Crystals	5.0 gms
KI	10 gms
Distilled Water (DW)	100 ml

- KI was dissolved in DW and I_2 crystals were added slowly.
- The solution was filtered and kept in a stoppered amber-coloured bottle.
- The solution deteriorates quickly hence should be prepared every 2 weeks.

Working solution (5 times diluted)

2 ml stock solution was mixed with 8 ml DW.

Physiological saline

NaCl	0.85 gm
Distilled Water	100 ml

10% Formalin

Formaldehyde (40%)	100 ml
Normal saline (0.85%)	900 ml

Dilute formalin in 900 ml of normal saline (Distilled water may also be taken).
NOTE: Formaldehyde is available as 30-40 % preparation, but for all purposes it is taken as 100%.

0.3 % Tris buffer Saline

Tris base	3 gm
Sodium chloride	8 gm
Potassium chloride	0.2 gm
Distilled water	1000 ml

APPENDIX III

MICROBIOLOGICAL PROFILE

Serial No: **Date:**
Name: **Age:**
Gender: **Class:**

Patient's Clinical

History: ……………………………………………………………….
………………………………………………………………………………

Questionnaire:

1. How many members are there in your family? ....
2. What is your father's occupation? I. Agriculture II. Business III. Service IV. Abroad V. Others
3. Which is the source of water you use to drink? I. Tap II. Open Well III. Kuwa IV. Spring spout
4. Which type of water do you drink? I. Boiled II. Non-boiled III. Filtered
5. Do you wash your hands before meal? Yes / No
6. Do you have toilet in your house? Yes / No
7. Do you wash your hands after toilet? Yes / No
8. Did you suffer from diarrhea recently? Yes / No
9. Have you taken anthelmenthic drugs recently? Yes / No

Report of stool examination

Macroscopic examination Colour: Consistency: Blood and mucus: Treatment:	Microscopic examination Wet mount / Iodine mount / Concentration technique RBC: Protozoa: Cyst of …………Trophozoite of …………………. Helminthes: Ova of………………………………….

Authorized signature………………

Appendix IV

Statistical Package for the Social Sciences (SPSS)
Product

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.420	14	.350
Likelihood Ratio	16.262	14	.298
Linear-by-Linear Association	.074	1	.785
N of Valid Cases	51		

```
CROSSTABS
  /TABLES=Sex BY Seen
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ
  /CELLS=COUNT COLUMN
  /COUNT ROUND CELL.
```

Frequency Table

Age Group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1 to 15 Years	173	36.0	36.0	36.0
	15 to 30 Years	148	30.8	30.8	66.9
	More than 30 Years	159	33.1	33.1	100.0
	Total	480	100.0	100.0	