PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG THE PATIENT VISITING SUB REGIONAL HOSPITAL OF DADELDHURA,FAR WESTERN REGION,NEPAL

A Dissertation Submitted to Central Campus of Technology, Hattisar Dharan, Tribhuvan University In Partial Fulfillment of the Requirement for the Award of the Degree of Master of Science in Microbiology

(**Medical**)

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This is to certify that **Mr. Sohan Jha** has completed this dissertation work entitled **"PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG THE PATIENT VISITING SUB-REGIONAL HOSPITAL OF DADELDHURA, FAR WESTERN REAGION, NEPAL"** as a partial fulfillment of Master of Science Degree in Microbiology under our supervision. To the best of our knowledge, this work has not been submitted for any other degree.

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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 Sub Regional Hospital, Dadeldhura, 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 prevalence was 10.63% with no significant difference between male (5%) and female (5.63%). The percentage of single parasitosis (98.4%) was 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 ≤ 10 years) had marginally higher positive rate (52.3%) than older patients**.** Prevalence of parasitic infection rate was higher in family with low education. The parasitic prevalence rate was higher among patients using normal tap water. Results showed that majority of patients had intestinal parasitosis and thus suggests periodic deworming as well as sanitary hygienic practices.

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

TABLE OF CONTENTS

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
CDC	Centers for disease control and prevention

LIST OF TABLE

LIST OF FIGURES

LIST OF APPENDICES

LIST OF PHOTOGRAPHS

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 billion people globally. Moreover, WHO has estimated that *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* infect 1.4 billion, 1.3 billion and 1.0 billion people worldwide respectively. Over 60 species of protozoan parasites cause diseases on people worldwide. *Entamoeba histolytica* and *Giardia lamblia* are estimated to infect about 60 million and 200 million people worldwide, respectively (Luka et al., 2000).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 (Mukhopadhyay *et al*, 2007).

The high prevalence rate of intestinal parasitosis is attributed to lack of education, lack of latrines, occurrence of diarrhea, lower socio-economic status, inadequate disposal of human excreta and the level of sanitation in households. (Stanley, 2003). 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 records in Nepal showed the infection rate of 30.0-40.0% (Shakya et al., 2006). Additionally, a hospital based study conducted by (Rai et al., 2004) 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 (Malla *et al*, 2008).

Amoebiasis is an infection caused by an intestinal protozoa *Entamoeba histolytica*, is the third most common cause of death from parasitic disease (after schistosomiasis 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 exist 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 (Harhay *et al*, 2010). 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 (Workneh *et al*, 2014).

Intestinal parasitic infections cause various intestinal symptoms including abdominal bloating, cramps, constipation, diarrhea, lack of appetite and vomiting (Luka *et al,* 2000). 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 (Ngrenngrmlert *et al*, 2007)

Several community wide out-breaks of Giardiasis, Amoebiasis, Ascariasis, Trichuriasis and Enterobiasis have been linked to drinking municipal water or other water sources contaminated with these parasites in an urban slum of Karachi (Mehraj *et al*, 2008). 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 (Garcia, 2009).

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 (Gelaw *et al*, 2013).

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 approx 3/4th followed by *E. histolytica* 1/3rd. Interestingly, newer opportunistic pathogens like *C. cayetanensis* (1.0%) and *Cryptosporidium* sp. (1.0%) were detected from immunocompromised children below 2 years of age (Eckardt *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 were significantly less infected (10.7%) compared to others ($p<0.05$). Ova/ cysts of intestinal parasites detected include *Trichuris trichiura* (32.0%), *Ascaris lumbricoides* (20.0%), *Hymenolepis nana* (16.0%), Hookworm (8.0%) and 24.0% cases showed mixed parasitic infections (Khanal *et al*, 2011).

A Report shows that almost 35% people, mainly children, in Nepal take medicine against worm infestations. Developing countries in Southeast Asian region spent 3.76% of total annual budget for health in year 2010. As worm infestation appears as one of the major economic burden also to the country, Nepal government has initiated National Deworming Program in recent years to control it. However, according to (WHO, 2014) 1100 million people were defecating in the open resulting in high levels of environmental contamination and exposure to the risk of worm infestations in year 2008. So study on such matters appears very much necessary even today.

This study aimed to estimate the prevalence of intestinal worm infestations among people of far western Region findings of which could be beneficial for health planning authority in Nepal to overcome the existing limitations 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 Dadeldhura 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 Sub-Regional Hospital, Dadeldhura, Nepal.

1.2.2 Specific objectives

a) To identify intestinal helminthic parasites among patient visiting Sub-Regional Hospital, Dadeldhura.

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 Intestinal Parasites

A parasite (an organism that lives in or on and takes its nourishment from another organism) in the intestinal tract. Intestinal parasites include both helminths and protozoa. Helminths are worms such as tapeworms, pinworms, and roundworms. All of these worms can live, but typically not reproduce, inside the human intestine. In contrast to worms, which are composed of many cells, protozoa are single-celled organisms that can multiply inside the body. Examples of protozoa that can live in the intestinal tract are Giardia and Cryptosporidium (Hotez *et al*, 2009).

An intestinal parasite lives in the intestines (guts). Intestinal parasites are usually protozoa (such as Giardia) or a worm (such as pinworms or tapeworms) that get into your child's body and uses the intestine as shelter. The parasite will live in the intestine or other parts of the body and often reproduce. The parasite may or may not cause symptoms or infection (Ostan *et al*, 2007).

3.2 Infection

Parasites are organisms that live off other organisms, or hosts, to survive. Some parasites don't noticeably affect their hosts. Others grow, reproduce, or invade organ systems that make their hosts sick, resulting in a parasitic infection (Al-Mohammed *et al*, 2010).

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 (Bakr *et al*, 2009).

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 (Awolaju *et al*, 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 (Ahsan-ul-Wadood *et al*, 2005).

Parasitic infections are a big problem in tropical and subtropical regions of the world. Malaria is one of the deadliest parasitic diseases. Parasitic infections can also occur in the United States. Common parasitic infections found in the United States include: Trichomoniasis Giardiasis Cryptosporidiosis Toxoplasmosis (Chandrashekhar *et al*, 2005).

Infection by intestinal parasitic worms (geohelminths) is widespread throughout the world, affecting hundreds of millions of people. Children are particularly susceptible and typically have the largest number of worms. Three of the most common kinds of worms are roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and hookworm (*Ancylostoma duodenale*and *Necator americanus*). These worms live in the intestines and their numbers build up through repeated infection. It is possible to be infected with more than one kind of worm (Latham *et al*, 2003).

3.3 Epidemiology

Parasitic diseases are cosmopolitan and may affect all the world population. They kill several million people every year. The migrations and tourism make that even tropical diseases can be frequently met outside their geographical distribution area. Except the arthropod-borne infections, the great majority of these diseases are in relation with the faecal contamination of soil, the general level of hygiene and the food practices. Malaria remains the first world parasitic disease in term of mortality. The strong fall of the amoebic endemicity is due only to the improvement of the methods of molecular diagnosis. The socio-political and climatic upheavals may result in a creeping extension of the geographical limits of many parasites (Pradhan *et al*, 2005)

3.3.1 National scenario

Nepal is a small underdeveloped nation located in South Asia and has full of ancient glories rich in tradition, culture and civilization which exhibits social, ethnical, linguistic and cultural diversity with various infectious diseases including intestinal parasitosis. It is alone one of the most common public health problems in all over Nepal. The distribution and prevalence of the various intestinal parasites species depend on social, geographical, economical and inhabitant customs Intestinal worm infestation is one of the major childhood health problems in Nepal. Most common intestinal parasites reported from school going children in Nepal are *Ascaris lumbricoides, Hymenolepis nana, Hookworm, Trichuris trichiura, Giardia lamblia* and *Entamoeba histolytica*. Of the protozoal infections, amoebiasis and giardiasis are most frequently reported. Ascaris lumbricoides, Trichuris trichiura and Hookworms, collectively referred to as soil-transmitted helminths (STHs) which are the most common intestinal parasites. These parasites are associated with diverse clinical manifestations such as malnutrition, iron deficiency anemia, malabsorption syndrome, intestinal obstruction, mental and physical growth retardation (Mehraj *et al*, 2008).

Rai, 2005 had carried out a retrospective study to see the prevalence of intestinal parasitosis among the patients visiting a health care centre in Kathmandu Valley. 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 (Shrestha *et al*, 2016).

The study was carried out among the elderly people in Kathmandu Valley 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).

Ghimire *et al*, 2014 was found 34% prevalence of intestinal helminthic infection among school children in Kathmandu Valley. 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.

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 (Tandukar *et al*, 2013).

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 (Thaisom *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).

3.3.2 SAARC countries scenario

In a national survey of intestinal parasitosis in Iran, intestinal parasitic infection rate was detected as 19.3% where 19.7% were male, and 19.1% were female (Sherchand *et al*, 2010). 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 (Kamalu *et al*, 2013).

Sah *et al*, 2013 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. (Hussein, 2010).

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 girl treated in Thailand for acute abdomen in which Ali was detected during surgical exploration (Ngrenngaemlert *et al*., 2007).

A study conducted in Iran , 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 (Nematian *et al*, 2004).

3.3.3 Global Scenario

According to (WHO, 2010) 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. (Ishiyama *et al*, 2003) 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%) (Chhin *et al*, 2006).

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 (Greenwood *et al*, 2007).

According to (Loukopoulos *et al*, 2007) 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.

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, 2015).

3.4 Types of Intestinal parasites

Major groups of parasites include protozoans (organisms having only one cell) and parasitic worms of these, protozoans, including cryptosporidium, microsporidia, and isospora, are most common in HIV-infected persons. Each of these parasites can infect the digestive tract, and sometimes two or more can cause infection at the same time.

There are many parasites that infect people and cause a number of disorders and diseases. They can invade your body through water and/or food sources, by inhalation, by direct contact with them, or via carriers like mosquitoes. As the parasites multiply, the infection gradually spreads, and can even turn into an epidemic (Harhay *et al*, 2010).

3.4.1 Protozoa: They are single celled parasites that were first discovered by Antony van Leeuwenhoek, a Dutch scientist (also known as the "father of microbiology"). There are approximately 45,000 species of Protozoa that exhibit approximately the same physiological functions as other more complex organisms. They can infest the blood, nervous system, and digestive tracts of most humans. This single celled organism has two essential parts, the nucleus and the cytoplasm. Covered with a non-rigid membrane, the protozoa have its own specific form of locomotion like cilia or flagella. Some of the common types include: *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium*, *Toxoplasma gondii* (Husseint *et al*. 2010).

3.4.1.1 *Entamoeba histolytica*

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's population 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 (Ishiyama *et al*, 2003).

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 (Quihui *et al*, 2006).

3.4.1.2 *Giardia lamblia*

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 ^{0}C (Ortega *et al*, 2010).

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 (Okyay *et al*, 2004). This alkaline environment initiates excystment of the cyst (Kidane *et al*, 2014). 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 trophozoite stage (Kightlinger *et al*, 1995).

3.4.2 Helminths: Worm-like parasites are classified as helminths. While roundworms, tapeworms, flukes, and hookworms, in humans, are endoparasites, that is, they live inside the host's body; leeches are ectoparasites that attach themselves to the outer parts of the host. One of the famous nematodes or roundworm is *Trichinella spiralis*, which lives in the muscle tissues of animals like pigs, and often gets transferred to the human intestinal system, when they happen to ingest infected, undercooked pork. Some of the most common types of helminths include: Pinworms, Hookworms, Roundworms, Tapeworms, Liver Fluke(Kurup *et al*, 2010).

3.5 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 (Li *et al*, 2009).

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 (Shrestha *et al*, 2012).

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 (Tandukar *et al*., 2013).

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 (Tiwari *et al*., 2013).

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*, 2009). 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 (Rai *et al*, 2004).

3.6 Pathogenesis and Clinical Manifestation of Human Intestinal Protozoan Parasite Infections

Intestinal protozoan parasite infection can result in gastrointestinal disease in humans. As a result of infection of the parasite more or less similar clinical sign and symptom can be observed. For example infections with *E. histolytica* have no symptoms in many individuals, and most canclear their infection without any signs of disease (Regmi *et al*, 2014). 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 (Yadav *et al*., 2016).

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, 2010). *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 (Shrestha *et al*, 2013). 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 (Shrestha *et al*, 2007).

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 (Rai *et al*, 2000).

3.7 Pathogenesis and Clinical Manifestation of Intestinal Helminths

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 (WHO, 2015).

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 (Gyawali *et al*, 2009).

3.8 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 (Latham *et al*, 2003).

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 (Hussein, 2010).

The life cycles of nematodes are complex and highly varied. Some species, including *Enterobius vermicularis,* can be transmitted directly from person to person, while others, such as *Ascaris lumbricoides, Necator americanus,* and *Ancylostoma duodenale,* 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 (Gyawali *et al*, 2009).

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 (*Yoder et al*, 2008)

Many infections are asymptomatic; pathologic manifestations depend on the size, activity, and metabolism of the worms. Immune and inflammatory responses also cause pathology (Yong *et al*, 2000).

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 (Brooker *et al*, 2006).

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*) (Osten *et al*, 2007).

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 (Bakr *et al*, 2009).

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 (Kidane *et al*, 2014).

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 (Ortega *et al*, 2010).

3.10 Diagnosis

Many kinds of lab tests are available to diagnose parasitic diseases. The kind of test(s) health care provider will order will be based on the signs and symptoms, any other medical conditions, and travel history. Diagnosis may be difficult, so more than one kind of test (Garcia, 2009).

3.10.1 Microscopic

Despite recent advances in diagnostic technology, microscopic examination of stool specimens remains central to the diagnosis of most pathogenic intestinal protozoa. Microscopy is, however, labor-intensive and requires a skilled technologist (Chhin *et al*, 2006).

3.10.2 A fecal (stool) exam, also called an ova and parasite test (O&P)

This test is used to find parasites that cause diarrhea, loose or watery stools, cramping, flatulence (gas) and other abdominal illness. CDC recommends that three or more stool samples, collected on separate days, be examined. This test looks for ova (eggs) or the parasite.

Your health care provider may instruct you to put your stool specimens into special containers with preservative fluid. Specimens not collected in a preservative fluid should be refrigerated, but not frozen, until delivered to the lab or the health care provider's office.

Your health care provider may request that the lab use special stains or that special tests be performed to look for parasites not routinely screened for (Mehraj *et al*, 2008).

3.10.3 Endoscopy/Colonoscopy

Endoscopy is used to find parasites that cause diarrhea, loose or watery stools, cramping, flatulence (gas) and other abdominal illness.

This test is used when stool exams do not reveal the cause of your diarrhea.

This test is a procedure in which a tube is inserted into the mouth (endoscopy) or rectum (colonoscopy) so that the doctor, usually a gastroenterologist, can examine the intestine. This test looks for the parasite or other abnormalities that may shows signs and symptoms (Mehraj *et al*, 2008).

3.10.4 Blood tests

Some, but not all, parasitic infections can be detected by testing your blood. Blood tests look for a specific parasite infection; there is no blood test that will look for all parasitic infections. There are two general blood test are done (Luka *et al*. 2000).

3.10.4.1 Serology

This test is used to diagnosis for antibodies or for parasite antigens produced when the body is infected with a parasite and the immune system is trying to fight off the invader. This test is done by health care provider taking a blood sample and sending it to a lab (Nematin *et al*, 2004).

3.10.4.2 Blood smear

This test is used to look for parasites that are found in the blood. By looking at a blood smear under a microscope, parasitic diseases such as filariasis, malaria, or babesiosis, can be diagnosed. This test is done by placing a drop of blood on a microscope slide. The slide is then stained and examined under a microscope (Malla *et al*, 2008).

3.10.5 X-ray, Magnetic Resonance Imaging (MRI) scan, Computerized Axial Tomography scan (CAT)

These tests are used to look for some parasitic diseases that may cause lesions in the organs (Rai, 2005).

3.11 Prevention

Good hygiene is the best defense against intestinal parasites. This includes frequent and thorough hand washing, especially after changing diapers, after going to the bathroom, and before handling food. Doctors advise that travelers to undeveloped countries drink and brush their teeth with bottled water and avoid eating raw fruits and vegetables, food from street vendors, and unpasteurized dairy products. In addition, cooking all food until it is steaming hot kills parasites. Always wearing shoes and avoiding swimming in bodies of fresh water such as ponds, rivers, and lakes can minimize the risk of contact with contaminated soil and water (Regmi *et al*, 2014).

3.12 Treatment

The main treatment is prescription antiparasitic medication. This family of drugs can kill parasites and help pass them through your system. In most cases, you'll have to take the medication for several weeks. You shouldn't stop taking it, even if you feel better.In very severe cases in which parasites have invaded other parts of the body, additional treatments like surgery may be necessary.

Parasitic infections can usually be treated with antiparasitic drugs. Albendazole and mebendazole have been the treatments administered to entire populations to control hookworm infection. However, it is a costly option and both children and adultsbecome reinfected within a few months after deparasitation occurs raising concerns because the treatment has to repeatedly be administered and drug resistance may occur. Another medication administered to kill worm infections has been pyrantel pamoate. For some parasitic diseases, there is no treatment and, in the case of serious symptoms, medication intended to kill the parasite is administered, whereas, in other cases, symptom relief options are used. Recent papers have also proposed the use of viruses to treat infections caused by protozoa (Okyay *et al*, 2004).

CHAPTER IV

4. MATERIALS AND METHODS

4.1 Materials

A list of materials, chemicals, equipment, 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, Sub-Regional Hospital, Dadeldhura Nepal. 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 (Shakya *et al.* 2012).

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*, 2000)

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

Blood and mucus

The stool specimens were observed for detecting blood and mucus.

Adult worms and ova

The stool specimens were observed whether it contains adult worms and ova of *H. nana*. 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 either by Saline or Iodine mount preparation or both wet n. 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. (Sah *et al*, 2013)

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 (Pradhan *et al*, 2005)

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 (Malla *et al*, 2008).

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

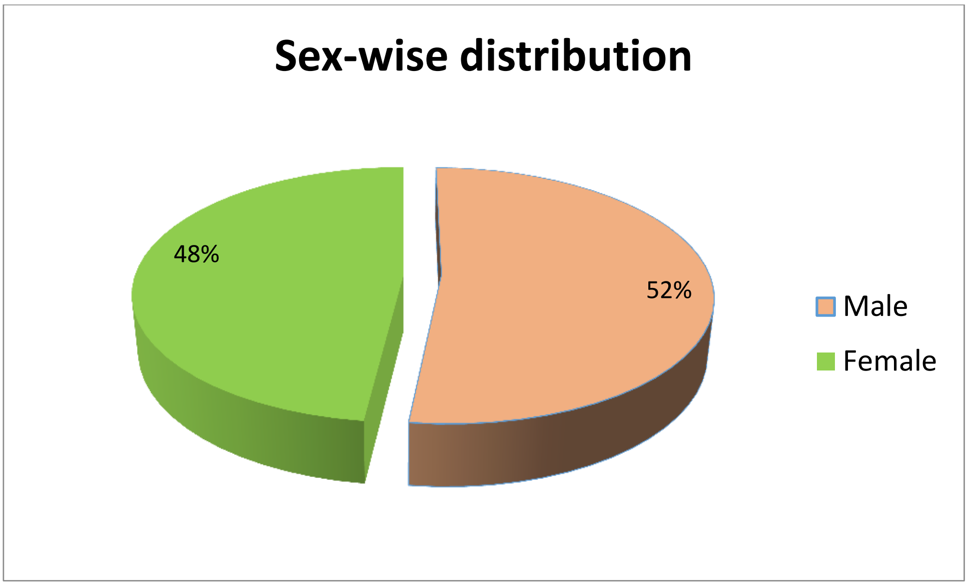
Chi-square test was applied for statistical analysis of results using Win Pepi software program (PEPI-for-Windows): computer programs for epidemiologists, 2004. Association of intestinal infections with different variables was tested. Results were considered significant if P values were less than 0.05.

CHAPTER V

5. RESULT

5.1 Study population

Patients attending sub-regional hospital of Dadeldhura, Nepal (Province No. 7), suspected of parasitic infection were included in this study. Stool samples were collected for test of parasitic infection. Of total 480 stool samples, 251 were from male (52%) and 229 were from female (48%) patients.



5.2 Study population according to age group

Out of total 480 samples according to age group, it was observed that number of subjects in below10 year age group was 142 subjects (male= 91 and female = 51).Number of male patients was more than their female counterparts in less than 10 years age group and 30-40 year age group. Female subjects were more than their male counterparts in age group of 20-30 years and 40-50 years.

Table 5.2 Study population according to age group

Age Group	Sex		Total
	F	M	
Below 10 Years	51	91	142
10-20 Years	49	43	92
20-30 Years	50	37	87
30-40 Years	24	29	53
40-50 Years	20	15	35
50 Years and Above	35	36	71
Total	229	251	480

5.3 Sex wise distribution of parasitosis

Out of total 480 samples, 51 samples were positive and 429 were negative for parasitic infection. *E. histolytica* was major cause of infection in 19 patients (male = 10 and female = 9). Ova of *T. trichiura*was minor cause of infection in 1 patient respectively. Co-infection with cyst of *E. histolytica* and *G. lamblia* was seen in only one male patient. Infection with trophozoite stage of *G.lamblia* was seen in 10 patients (male = 4, female = 6). Distribution of different parasitic infection did not show any sex related preponderance. On analyzing observation it was found that infection with protozoal parasites was more prevalent than helminthic parasites and the result was statistically insignificant (p-value: 0.657).

Table 5.3: Sex wise distribution of parasitosis

Name of parasites	Sex		Total	P-value
	F	M		
Cyst of *E. histolytica*	9	10	19	0.657
Cyst of *E. histolytica* and *G. lamblia*	0	1	1	
Cyst of *G.lamblia*	7	5	12	
Trophozoite of *G.lamblia*	6	4	10	
Ova of *T.trichiura*	1	0	1	
Ova of *A. lumbricoides*	1	2	3	
Ova of *H.nana*	1	2	3	
Ova of hookworm	2	0	2	
Not Seen	202	227	429	
Total	229	251	480	

5.4 Age-wise distribution of parasitosis

No significant difference (p-value= 0.758) in distribution of different types of parasitosis was found when patients were classified according to age groups mentioned in methodology section (Table 5.4). Maximum number of patients with cyst of *E.histolytica* infection and *G. lamblia*was in below ten year age group with 9 and 5 subjects respectively the result was statistically insignificant (p-value: 0.758).

Table 5.4: Age-wise distribution of parasitosis

Name of parasites	Age- wise category						Total	P-value
	Below 10 Years	10-20 Years	20-30 Years	30-40 Years	40-50 Years	50 Years and Above		
Cyst of *E.histolytica*	9	6	1	1	1	1	19	0.758
Cyst of *E.histolytica* and *G.lamblia*	1	0	0	0	0	0	1	
Cyst of *G.lamblia*	5	3	2	1	0	1	12	
Trophozoite of *G.lamblia*	5	1	2	1	1	0	10	
Ova of *A. lumbricoides*	0	0	1	1	0	1	3	
Ova of *H. nana*	1	2	0	0	0	0	3	
Ova of hookworm	0	0	0	1	0	1	2	
Ova of *T. trichiura*	1	0	0	0	0	0	1	
Not Seen	120	80	81	48	33	67	429	
Total	142	92	87	53	35	71	480	

5.5 Source and type of drinking water

Among tap water users maximum cases of parasitosis were found to be infected with *E .histolytica* (N=18), followed by cyst of *G. lamblia* (N=12) and trophozoite of *G. lamblia* (N=8). Infection with ova of *A. lumbricoides*, *H. nana* was found in 3, ova of hookworm in 2 and ova of *T. trichiura* in 1 patient. No significant observation in distribution of different types of parasitosis was found in boiled (tap water) and filtered (tap water). No significant difference was observed in distribution of different parasitosis in users with direct tap water, tap water – boiled and tap water – filtered the result was statistically insignificant (p-value: 0.163)..

Table 5.5: Source and type of drinking water

Name of parasites	Source for drinking			Total	P-value
	Tap Water	Tap Water (boiled)	Tap Water(filtered)		
Cyst of *E. histolytica*	18	0	1	19	
Cyst of *E. histolytica* and *G. lamblia*	1	0	0	1	
Cyst of *G. lamblia*	12	0	0	12	
Trophozoite of *G. lamblia*	8	1	1	10	0.163
Ova of *A. lumbricoides*	3	0	0	3	
Ova of *H. nana*	3	0	0	3	
Ova of hookworm	2	0	0	2	
Ova of *T. trichiura*	1	0	0	1	
Not seen	421	6	2	429	
Total	469	7	4	480	

5.6 Educational status in patients with different parasitic infection

Distribution of educational qualification in patients with different parasitic infection has been shown in Table 5.6. None of the patients with parasitic infection had university level educational qualification. Maximum infected patients were of primary level educational qualification (N= 30). Twenty-one infected patients were of secondary level educational qualification. Maximum patients were of primary level educational qualification that might be due to lack of knowledge regarding hygiene in daily life the result was statistically insignificant (p-value: 0.253).

Table 5.6: Educational status in patients with different parasitic infection

Name of parasites	Education			Total	p- value
	University Level	Secondary Level	Primary Level		
Cyst of *E. histolytica*	0	9	10	19	0.253
Cyst of *E. histolytica* and *G. lamblia*	0	0	1	1	
Cyst of *G. lamblia*	0	4	8	12	
Ova of *A. lumbricoides*	0	2	1	3	
Ova of *H.nana*	0	1	2	3	
Ova of hookworm	0	0	2	2	
Ova of *T.trichiura*	0	1	0	1	
Trophozoite of *G.lamblia*	0	4	6	10	
Not Seen	21	148	260	429	
Total	21	169	290	480	

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 prevalent on parasitic infection than on a research was done by Malla *et al*., 2008 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 (Shrestha *et al*, 2012). 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). 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 (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 (Regmi *et al*, 2014). The result of the most prevalent helminth *Ascaris lumbricoides* (18.98%) was in agreement with the previous study done in Nepal (Shrestha *et al*, 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*., 2008; Shrestha *et al*, 2013). High prevalence among Dalits (63.88%) appeared which was in agreement with the reports (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*, 2010).

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 specimens, 28 were from women (Sherchand *et al*., 2010).The prevalence of parasitic infection was found to be more in female than male. And the difference is statically significant. The finding of the present study was in agreement with the finding of Sherchand et *al*, 2010 & Rai *et al*, 2002. More prevalence of parasitic infection in female in this area may be due to uneducated lower cast parents who give more priorities to the health of their sons than their daughters. And daughters are only one who works daily on field and house. Females are the ones who take care for cattle, goats, cut grass for house animals, cooking and wash dishes regularly so female are more susceptible to parasitic infection than male. However the males were dominant in different parts of Nepal (Rai *et al*, 2005 and Ishiyama *et al*, 2001) in Lahore (Ahsan-ul-wadood *et al*, 2005) in Madhya prades (Maharjan *et al*, 2013 in Gombak, Malaysia (Hussein, 1994) in Northwest Ethiopia (Gelaw *et al*, 2013) in Saudi Arabia (Al-Mohammed *et al*, 2010). This variation could be due to males regularly gone to landfield to work and in field contaminated water supply makes man more infecting than females (Pradhan *et al*., 2005).

The prevalence of parasitic infection was also different countrywide as reported 31.4% in Saudi Arabia (Al-Mohammed *et al*, 2010), 18.4% Tehran (Nematian *et al*, 2004) 80.4% Thailand (Thaisom *et al*, 2004), 88% United states (Yoder *et al*, 2008) 47.5% in Karachi, Pakistan (Al-Mohammed *et al*, 2010), 47% Tanzania (Latam *et al*, 2003), 15.19% Karachi (Mehraj *et al*, 2008), and 12% in Thailand (Ngrermgarmlert *et al*, 2007).

Infection is acquired by ingestion of infectious cyst through water or undercooked food contaminated by human feces. After ingestion of the cyst, which is resistant to gastric acids and enzymes, excystation occurs in the ileocecalarea of the intestine to form trophozoites. The trophozoites are larger in size and actively motile organisms. According to thebind-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 sometimeslarge vesselsmay be eroded and severe intestinal hemorrhage result (Li *et al*, 2009).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 acomplete 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 athin and protective wall that allows it to survive in feces for weeks or for about 3 months in water at 40^0C (Thaisom *et al*, 2004).

CHAPTER-VII

CONCLUSION AND RECOMMENDATIONS

7.1 Conclusions

Parasitosis in Hospital Visiting Patients 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 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.
3. The study suggests for a multi-sectorial approach to reduce the morbidity and mortality associated with intestinal parasitic infections amongst young children.
4. 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
5. Special programs should be launched in the community with regards to creating awareness to control the intestinal infections in the family.

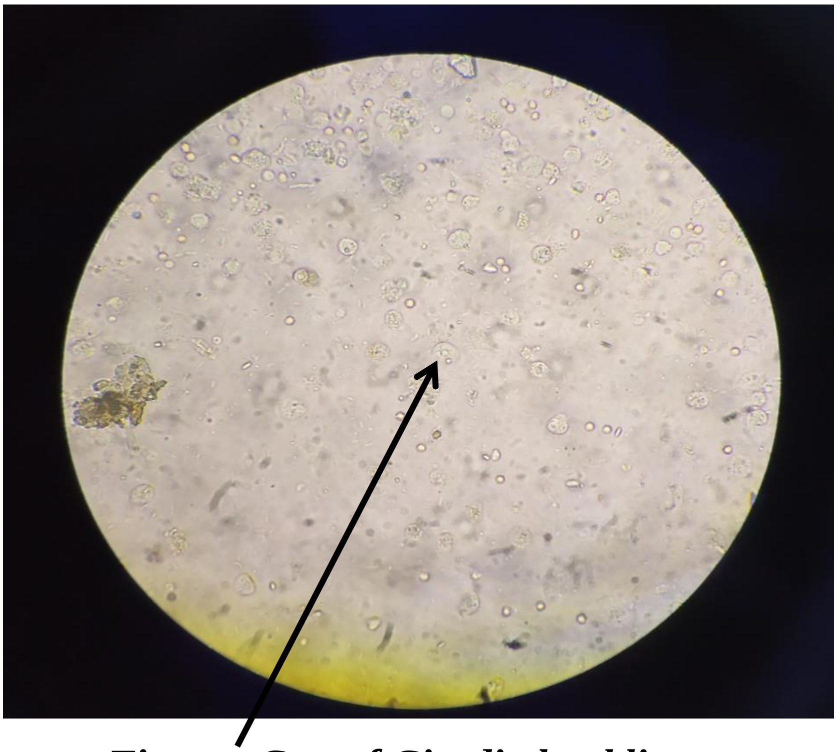


Figure: ***Cyst of Giardia lamblia.***

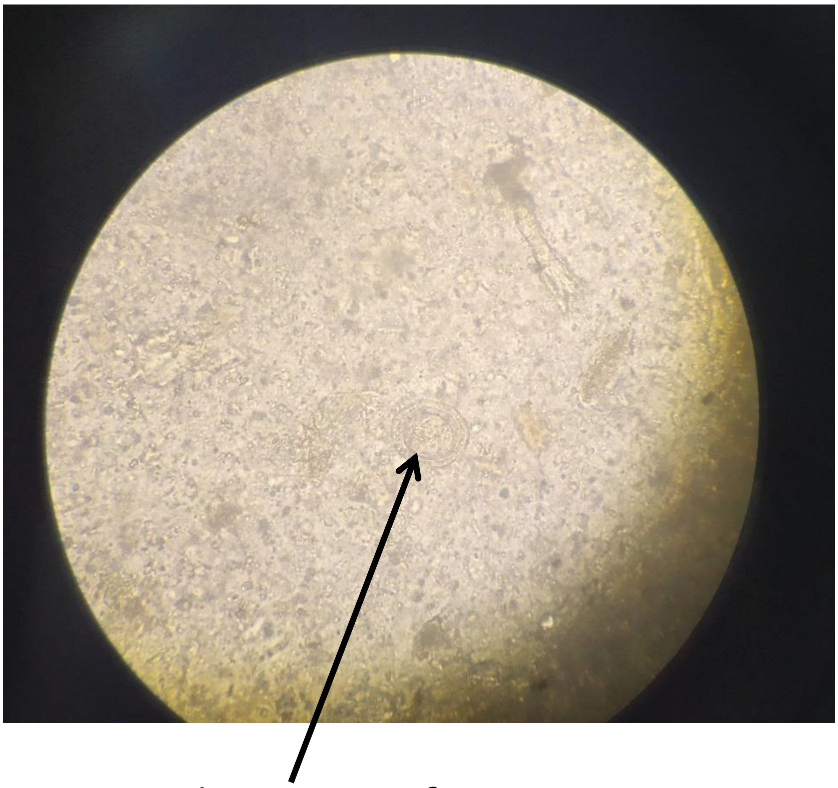


Figure: ***Ova of H. nana.***

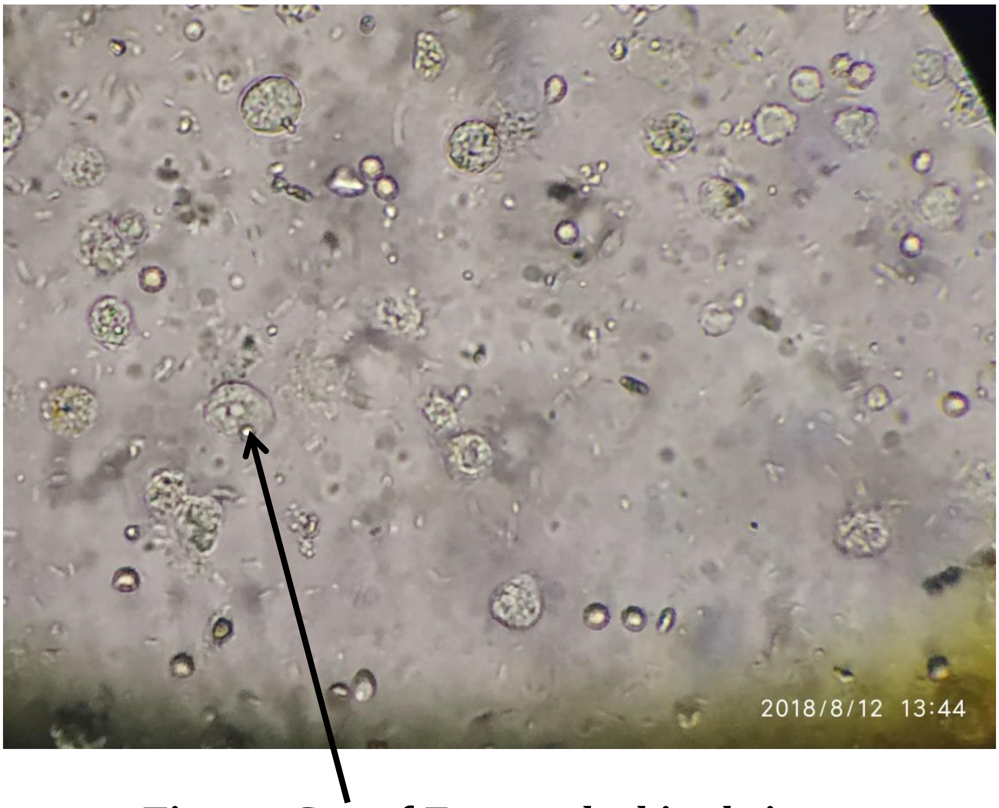


Figure: ***Cyst of Entamoeba histolytica.***

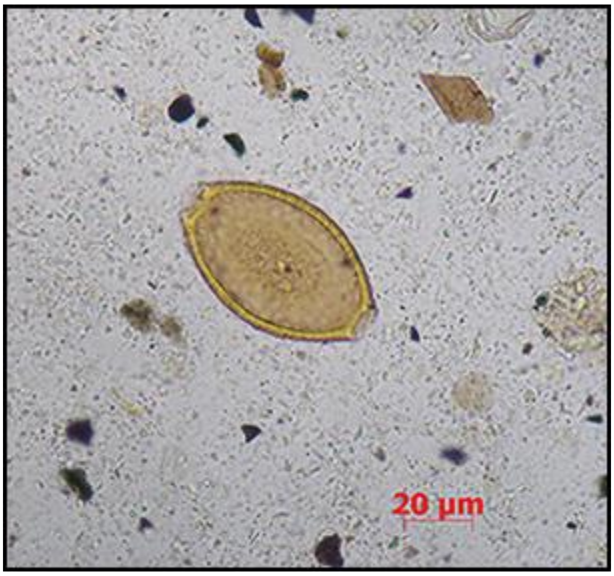


Figure: Ova of *Trichuris trichiura*

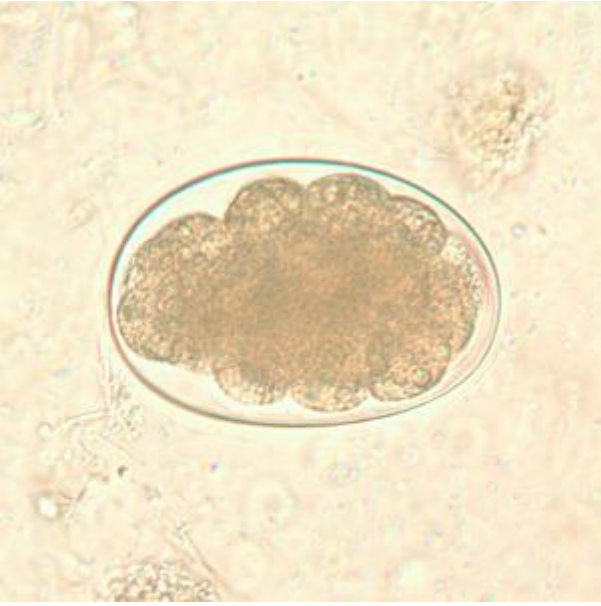


Figure: Ova of Hookworm

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