CHAPTER I
INTRODUCTION

1.1 Background

The global prevalence of intestinal parasites is high with the soil transmitted helminthes alone being implicated in infecting over two billion people. They manifest chronic and insidious health consequences rather than mortality. South East Asian region is heavily infected region and Nepal has 3% children requiring preventive chemotherapy in this region (WHO, 2012).

Among enteric pathogens, intestinal parasites are the major manifestor of diarrhea accounting for a varied range from 27.9% - 85.9% (Chand, 2000; Khadka, 2005; Piya, 2000). Diarrheal diseases are the seventh leading cause of deaths in the world and the fifth leading cause of disease burden. Due to diarrheal diseases, at least 5 million deaths per year occur in developing countries (Shakya *et al.,* 2007). In Nepal alone 1,817,498, 2,034,892 and 1,735,844 diarrheal cases were reported in fiscal years 08/09, 09/10 and 10/11 respectively in children less than five years of age. The national incidence of diarrhea in children less than five years of age is 500 per 1000 (DoHS, 2010/11).

Intestinal parasitosis is endemic in most tropical and subtropical countries, particularly in developing (Shakya *et al.,* 2007). It is a threat in impoverished settings with filthy environment, concentrated particularly in rural areas or urban slums and shantytowns (WHO, 2010). Among intestinal parasites, soil transmitted helminthes are highly prevalent in Nepal (Ghimire and Mishra, 2005; Ishiyama *et al.,* 2001, 2003; Khanal *et al.,* 2011a; Ono *et al.,* 2001; Rai *et al.,* 2008; Sharma *et al.,* 2004; Thapa Magar *et al.,* 2011; Uga *et al.,* 2004). The major contributing factors for this are poor sanitation, poverty and lack of health education (Ishiyama *et al.,* 2001; Matsumura *et al.,* 1998; Rai *et al.,* 2000a, 2001a). Similarly *Entamoeba histolytica* and *Giardia lamblia* infections are the most common among protozoans in Nepal (Chandrashekhar *et al.,* 2005; Khanal *et al.,* 2011b; Okyay *et al.*, 2004; Rai *et al.*, 2002, 2005).

Globally, contaminated water is a serious problem that can cause severe pain, disability and even death among the vulnerable group. Common global water-related diseases caused by parasites include amoebiasis, cryptosporidiosis, giardiasis, guinea worm and schistosomiasis (CDC, 2010a). Globally, around 1.1 billion people do not have access to improved water supply sources. Unsafe water and poor hygiene is implicated to cause about two million deaths per year, most of whom are toddlers (WHO, 2013a).

Different kinds of foods including raw vegetables, undercooked fish, meat etc play vital role in the transmission of the parasites. The symptoms vary greatly from simple diarrhea to different kinds of gastrointestinal manifestations (CDC, 2010b). Similarly, soil has become one of the prominent sources to harbor and transmit intestinal parasitosis (Quihui *et al.*, 2006; Rai *et al.*, 2000b). In Nepal 15 million people still practice open defecation making soil reservoir of intestinal parasites especially, soil transmitted helminthes (WHO and UNICEF, 2012).

Though considered a benign disease; intestinal worm infections rank fourth among the top ten diseases of Nepal (Shrestha *et al.*, 2009). Although they target all age groups equally, children are commonly affected and suffer from malnutrition associated morbidity and mortality. Intestinal parasites present even in low or moderate number can cause persistent and poor nutritional status in children by causing subtle reduction in appetite, digestion, absorption and acute phase status and increasing intestinal nutrient losses. They are also the notorious cause of impaired cognitive function and learning ability in children (Stephenson *et al.*, 1998; WHO, 2010).

Though majority of pre-school age children (PSAC) are covered by MDA, many school age children (SAC) are still not covered (DoHS, 2010/11) with the national coverage rate of 42.4% in SAC (WHO, 2012). Provisions of safe water, sanitation and hygiene have displaced intestinal parasitosis to no more an important disease in the developed world (WHO, 2010). However, in developing countries like Nepal, population using improved drinking water sources and improved sanitation facilities are limited accounting only 89% and 31% respectively as on 2010 (WHO, 2013b) thus leading people fragile to intestinal parasitosis.

In Nepal majority of the population live on agricultural subsistence so, majority of the population are highly vulnerable to soil transmitted helminthes (Rai and Gurung, 1986; Rai *et al*, 1994a, 1995). Though the prevalence varies considerably from one study to another, the average prevalence is 60% (Shrestha *et al.*, 2009). Due to poor hygienic practice and lack of basic health information, very high numbers of people are implicated by intestinal parasites.

The easy access of the parasites is probably due to the carelessness of uneducated people and health personnels along with the concerned persons. The most prominent mode of their transmission is the feco-oral route and contaminated foods, water and hands the major vector. These infections could be reduced drastically through our small initiation. For this, educating people about the prevalence of intestinal parasites on major sources is foremost. However, limited information is available on integrated study of the contamination of soil, drinking water and vegetables with the parasites. The present study is thus conducted to explore the parasitic contamination status of the soil along with the drinking water and the vegetables being consumed in the community that will help to correlate the intestinal parasitic infection in the community. This will certainly assist to understand the prevailing health status and vicious parasitic circle undergoing these days in the communities of Nepal.

1.2 Objectives

1.2.1 General objective

To determine the prevalence of intestinal parasites in the Rai community of Nuwakot district.

1.2.2. Specific objectives

i. To determine the prevalence of intestinal parasites on drinking water, vegetables and soil samples from the Rai community.
ii. To determine the prevalence of intestinal parasites in school children of the Rai community.
iii. To describe the relation between the prevalence of intestinal parasites and different demographic variables considered in the study.

CHAPTER II
LITERATURE REVIEW

2.1 Intestinal parasitosis

Intestinal parasitosis is one of the major public health problems in the world particularly in the developing countries approaching the prevalence of 100% in some areas (Ishiyama *et al.,* 2003). About 80% of all illness and disease in the world is caused by inadequate sanitation, unsafe water and unavailability of water; intestinal parasitosis being one of them (WHO, 2002). Intestinal parasitosis is spread throughout the world affecting about 3.5 billion people (WHO, 2000).

Intestinal parasitosis implicates the disease caused by wide varieties of intestinal protozoans and helminthes singly or in combination. These intestinal parasites are responsible for the majority of the morbidity in the areas with poor environmental sanitation, hot and humid climate, and poor personal hygienic practices and overcrowding. Their prevalence differs not only according to the geographical and other environmental factors but also according to individual health status. The infecting species are determined by the immune and health status of the individual. Most of the intestinal parasites are more common and their manifestations are more severe in children and other immune-compromised individuals. Infection in children is associated with outcomes like malnutrition, stunting and poor cognitive development. Besides the health impact, intestinal parasites have significant socioeconomic impact in terms of absence from work, diagnostic and treatment expenses (Montresor *et al*., 1998; Shakya *et al.,* 2007; WHO, 2010).

2.2 Intestinal parasites

The intestinal parasitic infections of global public health concerns are *Entamoeba histolytica* (*E. histolytica*) and *Giardaia lamblia* (*G. lamblia*) among protozoal species and *Ascaris lumbricoides (A.* lumbricoides*), Trichuris trichiura (T.*

trichiura) and hookworm (*Ancyolstoma duodenale* and *Necator americanus*) among the helminthes (WHO, 2000).

2.2.1 *Entamoeba histolytica*

i. Introduction

E. histolytica infection is common in most developing countries (Petri and Singh, 1999). The two species *E. histolytica* and *Entamoeba dispar* are morphologically identical but pathologically distinct (WHO, 1997; Petri and Singh, 1999). Only *E. histolytica* is capable of causing disease (WHO, 1997). *E. histolytica* is reported to be responsible for approximately 50 million cases of invasive amoebiasis. The majority of the cases are reported from Asia, Africa, south and Central America. These are often associated with poor water and food hygiene and sanitation practices. The life cycle includes the infective cyst and invasive trophozoite forms of which cyst forms present in fecally contaminated water and food is primarily responsible to infection. It can be also transmitted from person to person contact (Petri and Singh, 1999).

ii. Clinical manifestations

The incubation period may vary from few days to months; generally one to four months, depending upon the area of endemicity. *E. histolytica*, besides being an intestinal pathogen, is able to invade tissues and the presentation of disease may range from an asymptomatic infection to a disseminated fatal disease. The four major intestinal syndromes caused by the infection are asymptomatic colonization; acute amoebic colitis (which usually presents with lower abdominal pain, frequent bloody stools over a period of several weeks, and fever); fulminant colitis (which occurs most often in children who present with diffuse abdominal pain, profuse bloody diarrhea, and fever) and ameboma (which presents as a completely asymptomatic lesion or as a tender mass accompanied by symptomatic dysentery). Approximately 90% of all intestinal infections are asymptomatic **(**Petri and Singh, 1999; Reed, 1992).

iii. Epidemiology

Infections with *E. histolytica* occur worldwide and it has been suggested that 12% of the world's population is infected with this organism. About 10% of those infected every year have clinical symptoms, 80 to 90% of these patients have symptoms related to the intestinal mucosa and in the remaining 2 to 20% the amoebae invade beyond the intestinal mucosa (WHO, 2003).

Food and water contaminated by human feces that contain cysts are the main sources of reservoirs of the infections. Carriers are also the principal reservoir. The transmission is either via feco-oral route or person to person spread, by vectors like flies and by sexual contact especially in homosexuals. Infection is common in community with poor personal hygiene, unsafe drinking water, poor sanitation, and where human feces are used as fertilizers (Bakir *et al*., 2003; Bdir and Adwan, 2010; Kaur *et al*., 2002; Yousefi *et al*., 2009) Moreover, it is an important cause of diarrhea in AIDS patients.

2.2.2 *Giardia lamblia*

i. Introduction

Giardia lamblia/ intestanilis/ duodenalis is considered as the commonest human intestinal protozoan to cause infection. The transmission of the parasite is mainly by the feco-oral route and also by person to person transmission (Gracia, 1999). The life cycle includes the infective cyst and non invasive trophozoite form (Brooks *et al*., 2010).

ii. Clinical manifestation

The majority of infected persons in the endemic area are asymptomatic. The acute giardiasis is characterized by the sudden unset of anorexia, nausea, abdominal distension, discomfort, diarrhoea, etc. Steatorrhoea is frequently accompanied by epigastric and abdominal cramp. Stool is voluminous, foul smelling and greasy in appearance. Typically no mucus or blood is present in feces. Giardiasis is also accompanied by wasting and impaired absorption of folate and vitamin $B_{12.}$ The acute phase is usually nonspecific resembling traveller's diarrhoea (Brooks *et al*., 2010; Farthing and Keusch, 1989; Solomons, 1982).

iii. Epidemiology

G. lamblia infection occurs worldwide with an incidence varying from 1.5-2.5 %. Giardiasis shows two distinct epidemiological patterns; endemic and epidemic with a high prevalence rate 15-20 % occurring in children less than 10 years of age in developing countries while all age groups are equally susceptible in developed world (Aksoy *et al*., 2007; Mengistu *et al*., 2007; Quihui *et al*., 2006; Taheri *et al*., 2011). The transmission is mainly by feco-oral route, the vehicles for which are usually contaminated water and food (Al-Megrin, 2010; Basualdo *et al*., 2007). It can also spread by direct person to person contact in person with poor sanitation and poor feco-oral hygiene and sexually transmitted in homosexuals (Garcia and Bruckner, 1993).

2.2.3 Soil Transmitted Helminthes (STH)

i. Introduction

STH are the commonest neglected tropical diseases transmitted through contact with and/or ingestion of soil contaminated with eggs or immature larval stages of the parasites. *A. lumbricoides* and *T. trichiura* are usually transmitted when a person ingests infective eggs in contaminated food or water and from hands that have been fecally contaminated. Though hookworms are also spread via contaminated soil but their common mode of transmission is through skin penetration rather than ingestion (Cheesbrough, 1992). Common round worm *A. lumbricoides*, whipworm *T. trichiura* and hookworms (*A. duodenalae* and *N. americanus*) are categorized as STH. STH are more prevalent among school age children though they infect all age groups which is probably due to their playing habit in fecally contaminated ground (Hotez *et al*., 2008).

ii. Clinical manifestations

Diseases caused by these worms are referred to as soil transmitted helminthiasis. Soil transmitted helminthiasis are usually either asymptomatic or nonspecific such that infected people usually exhibit mild or even no overt symptoms (Savioli and Albonico, 2004). Nonspecific manifestations could be abdominal pain, nausea, anorexia, vomiting, weight loss, skin rash, mild respiratory symptoms and diarrhea. However, in case of heavy infections various symptoms like malnutrition, obstruction of intestinal tract, chronic diarrhea, intestinal ulceration, anemia, poor developmental rate, weight loss and rectal prolapse may be present. The children are more liable to have chronic infections rather than other age groups (Hotez *et al*., 2003; Wasilewaska *et al*., 2011).

Chronic infections may lead to several health impairments in the infected individuals like anemia, impairments in physical, intellectual and cognitive development, malnutrition and poor school performance (Casapia *et al*., 2006; Ezeamama *et al*., 2005; Mupfasoni *et al*., 2009; Sorensen *et al*., 2011). *A. lumbricoides* causes ascariasis which manifests impaired childhood nutrition, surgical complications, allergic reactions and pneumonitis. Similarly *T. trichiura* causes trichiuriasis and its manifestations include impaired childhood nutrition, rectal prolapse and dysentery. Hookworm infections are generally manifested as impaired iron status and iron deficiency anemia (Crompton and Neisheim, 2002).

iii. Epidemiology

Soil transmitted helminthiases are endemic in all the six WHO regions, with the highest number of children requiring preventive chemotherapy residing in South East Asian Region (SEAR) followed by African Region (AFR). The proportion of children requiring preventive chemotherapy for soil transmitted helminthes by regions were 42%, 32%, 11%, 9%, 5% and 1% in SEAR, AFR, Western Pacific Region (WPR), Eastern Mediterranean Region (EMR), American Region (AMR) and European Region (EUR) respectively. There are around one billion children throughout the world requiring treatment. Among them, 883 million children throughout the world were in need of preventive chemotherapy out of which approximately 31% were covered in 2009. The highest coverage, 46% and 39% was achieved by AMR and SEAR respectively. The preventive chemotherapy coverage on rest regions; AFR, WPR, EUR and EMR were 32%, 14%, 9% and 3% respectively (WHO, 2012).

STH are known to infect over two billion people throughout the world. Developing regions of Asia, Africa and Latin America are the regions with the highest prevalence of ascariasis (Cauyan *et al*., 2008; Cook *et al*., 2009; Gyoten *et al*., 2010; Jiraamonnint *et al*., 2006; Ulukanligil and Seyrek, 2003). High prevalence of trichuriasis is found in central Africa, southern India and Southeast Asia (Ahmed *et al*., 2011; Al-Mekhlafi *et al*., 2007; Standley *et al*., 2009). Prevalence of hookworm infection is common in Sub-Saharan Africa, South China and Southeast Asia (Aini *et al*., 2007; Anantaphruti *et al*., 2004; Rayapu *et al*., 2012; Verle *et al*., 2003). Approximately 807-1,221 million people in the world are infected with ascariasis. Similarly trichuriasis and hookworm infections infect about 604-795 million and 576-740 million people respectively (CDC, 2013). The people from tropical and subtropical areas with hot and humid climate, inefficient health services, poverty, inadequate water supply, poor environmental and personal hygiene are at high risk (Rai *et al*., 2000a,2001a; Shakya *et al*., 2007; WHO, 2010). The disease accounts for almost 40% of the global morbidity from the infectious diseases (Hotez *et al*., 2003).

2.3 Intestinal parasitic infections in Nepal

The total population of Nepal according to recent census of 2011 is 26,494,504 of which 10,546,863 are children below 16 years of age. Among the total population, nearly one-fourth lives below the poverty line. Agriculture is still the major occupation with 76% of the households involved in agricultural activities. Sources of drinking water and its sanitation have crucial role in transmitting the intestinal parasites especially protozoal parasites. The main source of drinking water is tap/piped water for 47.8% of the total households. Similarly tube well/hand pumps, spout, uncovered well/kuwa and covered well/kuwa is the main source of drinking water for about 35%, 5.7%, 4.7% and 2.5% of the total households respectively. On the other hand, 38.2% of the households still do not have toilet in their houses. The average number of members in each household was found to be 4.9. The present literacy rate was reported to be 65.9% with male and female literacy rate of 75.1% and 57.4% respectively (Central Bureau of Statistics, 2011).

The BMI indicators assessed for children below 5 years of age showed that 41% were stunted, 29% were underweight and 11% were wasted. Despite the different programs implemented by government to address under nutrition like infant and young child feeding, community management of acute malnutrition and hospital based nutrition management and rehabilitation, malnutrition seems to be highly prevalent. The consequences like malnutrition, iron deficiency disorder, anemia are debilitating the health status of the children (DoHS, 2010/11).

2.3.1 Soil samples

Contamination of soil with helminth eggs were studied by Rai *et al.* (2000b) which showed the overall prevalence rate of 36.5%. There was seasonal variation however the prevalence was uniform in Kathmandu valley (36.9%) and outside the valley (35.3%). *A. lumbricoides* was the predominating species in Kathmandu valley and *Trichostrongylus* in outside the valley samples.

A study conducted in Kathmandu valley showed the soil contamination rate of 28.5%. *A. lumbricoides* was the predominant species in the soil samples. At the same time, stool samples tested of the school children and patients visiting health care centers in Kathmandu valley showed the prevalence rate of 42.5% and 2.8% respectively. *T. trichiura* and *A. lumbricoides* were the commonest infecting helminthes in the school children and patients with the prevalence rate of 35.2% and 1.1% respectively (Shrestha, 2006).

2.3.2 Vegetable, water and stool samples

A study conducted targeting cyclosporiasis indicated the prevalence rate of 7.9% in fecal samples. Of 1669 cyclosporiasis negative samples, 31.1% were suffering from different intestinal parasitosis. *G. lamblia* (39.1%) and *T. trichiura* (8.1%) were the common protozoan and helminth respectively among the intestinal parasites. Green leafy vegetables, water from different sources were also found to be contaminated with *Cyclospora* species. It was also found prevalent in the animal excreta (Sherchan *et al.*, 2010).

2.3.3 Stool samples

The study assessing the parasitic infection in local school children in Birgunj showed the prevalence rate of 13.9%. The infection rate was found to be low among boys (10.3%) than girls (19.1%). *E. histolytica* (36%) and *A. lumbricoides* (28%) were the commonest protozoal and helminthic infecting agents. Larger family size, age group of five years and less, illiterate parents and agriculture as parents occupation were found to be the promoting factors for increasing the probability of intestinal parasitic infections (Shakya *et al.*, 2012).

Parasitic study conducted in sukumbasi basti (people living without land ownership) in Kathmandu valley revealed the prevalence rate of 43.3%. Children receiving antihelminthic drug within last six months had significantly low prevalence (25.4%) than the missed groups (48.2%). Protozoal infection was more common to helminthic infection. *T. trichiura* and *G. lamblia* were the commonest among helminth and protozoan respectively (Thapa Magar *et al.*, 2011).

Healthy school children from a public high school in Kathmandu valley had the prevalence rate of 17.6%. Younger aged children were more infected by intestinal parasites in comparison to the older ones. Helminthic infections were common than the protozoal infections. *T. trichiura*, *A. lumbricoides*, *H. nana* and hookworm were the common helminthes with the prevalence rate of 32%, 20%, 16% and 8% respectively (Khanal *et al.*, 2011a).

A retrospective study conducted among patients visiting hospital within two years period in Birgunj, Nepal for the detection of intestinal parasitosis exhibited the prevalence rate of 20.7% (Shakya *et al.*, 2009). The prevalence rate was slightly higher in first year (23.9%) than in second year (15.8%). The old aged people (≥60 years) were found to be the most infected group among the study population. In overall, the helminthic infection dominated the protozoal infection. However, the single leading causative agent was found to be *E. histolytica* (38%) which was followed by *A. lumbricoides* (31.8%).

A community based investigation conducted in Dharan municipality; Nepal revealed the parasitic prevalence rate of 22.5%. Protozoal infection dominated the helminthic infection. The predominant protozoan was *G. lamblia* (11.5%) which was followed by *E. histolytica* (4.4%). *A. lumbricoides* was the common one among the helminthic agent with the prevalence rate of 3.3%. Socio-economic status, type of toilet and hand washing practice were identified to have strong correlation with the incidence of parasitic infection (Gyawali *et al.*, 2009).

A two years study conducted in pediatric group in Pokhara showed the prevalence rate of 38.8%. Protozoal infections were more to helminthic infections. *G. lamblia* and *E. histolytica* were the commonest infecting agents. Children using toilets were significantly less infected compared to those not using toilets. Parasite positivity rate was higher among rural children compared to urban children (Das *et al.*, 2006).

A comparison study conducted between the urban and rural school children for parasitic infestation showed the overall prevalence of 21.3%. The infestation rate was 18.7% and 24.1% respectively among urban and rural school children respectively. *G. lamblia* was the predominant infecting agent which infected much more school children than by the rest of all other combined agents. The prevalence rate of *G. lamblia* was 13.2% which was followed by *A. lumbricoides* (2.1%) (Chandrashekhar *et al.,* 2005).

The intestinal parasitic infection prevalence rate of 66.6% was observed in public school children in rural setting of Kathmandu valley. Helminthic infection (76.9%) superseded the protozoal infection. *T. trichiura* (34.6%) was the commonest helminth and *E. coli* (6.4%) was the commonest among protozoan. Multiple parasitic infestation was common with more than half (53.8%) of the children being implicated (Sharma *et al.,* 2004).

Of the 396 diarrheal stool samples collected at different medical centers located in Kathmandu and two public schools in a village setting in Kathmandu valley and outside, 193 (49%) were positive for certain type of parasites. The proportion of helminthic isolates was much higher than the protozoal isolates. *T. trichura* (26%) was the commonest infecting species which was followed by hookworm, *A. lumbricoides* and *G. lamblia*. Population with polyparasitic infections was also found to be higher (43%) in the study (Uga *et al.,* 2004).

Irrespective of the deworming of the children of age 1-5 years in the biannual vitamin A supplementation program, deworming of all pregnant women with a single dose of albendazole tablet after first trimester of pregnancy and biannual deworming of government school students throughout the country under school health and nutrition; worm infestation is highly prevalent. Moreover regular evaluations of the programs are lacking so that success of these interventions in terms of decreasing prevalences or intensities, improving health status, nutritional status and cognitive abilities of children that receive deworming treatment are not known. It seems very difficult to achieve the Millennium Development Goal to reduce the worm infestation rate in children (preschool) to less than 15% and national target to reduce the worm infestation among children and pregnant women to less than 10% by 2017(DoHS, 2010/11).

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

A complete list of materials, equipments, chemicals and reagents used in this study are given in the Appendix B.

3.2 Methods

A descriptive cross-sectional study was conducted in the Microbiology Laboratory of Central Department of Microbiology, Tribhuvan University from March to October 2012. The different samples viz. stool, soil, vegetables and water samples were collected from a Rai community in Nuwakot district. The community is on a distance of around 80Km west from the capital, Kathmandu. It is linked by pitched road with Kathmandu valley. Almost all members of the community are involved in agriculture and the community is forwarding towards the commercial vegetable farming.

3.3 Sample collection and processing

3.3.1 Soil samples

Sites for soil samples collection were chosen on the basis of maximum dwelling of the children. The area around the houses, school playgrounds, areas adjacent to toilets, vegetable field and areas around the water sources were the soil sample collection sites. All the soil samples were collected during the spring season. About 200 grams of soil sample from a depth of about 2cm was collected using a small potable shovel. The soil samples were collected on sterile transparent plastics, labeled and taken to the laboratory. The overall procedure from collection, processing and microscopic examination of soil samples were carried out following the standard protocol (Uga *et al*., 1989).

As soon as the soil samples were transported to the laboratory, they were spread over aluminium foil and dried overnight at room temperature. Next day, the soil samples were grinded and sieved through a 150µm mesh sieve. Two gram of powdery soil was taken in a test tube and suspended in 8ml of 0.05% Tween-20 solution. It was then centrifuged at 1000rpm for 10 minutes. The supernatant was discarded. Then the tube was then filled with 8ml sucrose solution of specific gravity 1.2 and again centrifuged at 2000rpm for 10 minutes. Lastly the surface layer was taken out with the help of a loop and kept in the glass slide and observed under 10X and 40X.

3.3.2 Drinking water samples

Drinking water samples were collected from the community as well as from the schools where the higher number of children from the community were enrolled. Piped line untreated water and the springs were the source of drinking water in the community. Water from irrigation channel was also collected since it was used for household purposes like cleaning the utensils, for feeding domesticated animals and above all, the children used to spend the maximum time by swimming in the channel water. The overall procedure from collection to processing and examination of drinking water samples were carried out following standard protocol (Bakir *et al*., 2003).

One and half liters of the water samples were collected from each source on each time on a clean water bottle. The bottles were labeled with the site and date of collection and transported to the laboratory. After transportation, one and half liter of the collected water sample was filtered through membrane filter of dimension 47mm and 0.45±0.02 µm. The filtration was carried out using filtration apparatus and the filtration was enhanced by using suction pump. The filter paper was then eluted with 0.9% normal saline. The elute was centrifuged for 20 minutes at 1164×g. The supernatant thus obtained was discarded. Finally saline and iodine mount were prepared from the pellet and observed under 10X and 40X.

3.3.3 Vegetable samples

Seasonal vegetable samples cultivated in the community including leafy vegetables (mustard leaves, onion leaves, garlic leaves, cabbage, coriander, radish leaves), root vegetable (radish) and fruit vegetables (brinjal, cauliflower, pumpkin, bean, sweet peepers, chilly, tomato, gourd) were collected avoiding contamination during harvesting. All the vegetable samples were collected during the spring season. Each vegetable were packed in separate clean plastic bags to avoid cross contamination and were marked with the site and date of collection. Then the samples were transported to the laboratory for further processing. The samples were processed according the standard protocol of Uga *et al.* (2009) for the detection of intestinal parasitic contamination.

After arrival of vegetable samples to the laboratory, about 100 grams of vegetable (leafy vegetables or skin of root and fruit vegetables) was chopped into small pieces. Then the chopped pieces were soaked in 500ml of 0.5% Tween-20 solution. It was allowed to sediment for overnight. Next day, the bits of leaves or skins of other vegetables were removed with a tweezers. After that about 300ml of the supernatant was discarded. Then remaining solution was centrifuged at 2000 rpm for 10 minutes. Thus obtained supernatant was discarded and the sediment was examined under low and high power objective after preparing the slides on saline and iodine for the detection of parasites.

3.3.4 Stool samples

Samples were collected from door to door visit in the community. Before the distribution of the collection bottles and bamboo sticks, children were given brief description about the purpose of the study. Ethical consent was taken from the parents and honored members of the society, who helped to collect the samples throughout the study period. First of all, the questionnaire was filled with assistance of guardians then the height and weight were taken. Wherever available digital weighing machine was used to take weight. However, in its absence bathroom scale was used. After that children were informed on how to collect the samples. They were provided with sterile, clean, dry and leak proof plastic container along with bamboo stick requesting to collect about half the container of stool. They were strictly advised not to contaminate the stool sample with the urine or any other contaminants. The containers were labeled with the subject's name, code and date of distribution and the samples were collected the other day from door to door visit and brought to laboratory as soon as possible.

3.3.4.1 Macroscopic examination

The macroscopic examination was conducted on the day of sample collection. Macroscopic examination involved direct visualization of each sample for color, consistency, presence of mucus, blood and adult worm or worm segments. The protocol of Cheesbrough (1999) was followed for the examination. Then 10% formalin solution was added to the samples as preservative and transported to Kathmandu after collection of all samples for laboratory processing in Central Department of Microbiology.

3.3.4.2 Microscopic examination

Microscopic examination was carried out to examine the presence of ova, oocysts and larvae of the parasites. Samples were concentrated to enhance the detection of the intestinal parasites by formal-ether concentration technique following the protocol of Cheesbrough (1999). The slides were then examined under low power (10X) and high power (40X) by saline wet mount and iodine wet mount methods.

a. Formal-ether sedimentation method (Cheesbrough, 1999)

About 1 gm of feces was emulsified in about 4 ml of 10% formal-saline, shaken well and allowed to stand for about 30 minutes for fixation. Then about 3-4 ml of 10% formal-saline was added and the tube was capped and shaken well. After shaking, the suspension was sieved through double gauze in a funnel into 15 ml centrifuge tube. About 3-4 ml of ether was added and the tube was shaken vigorously for 5 minutes. Then the content of the tube was centrifuged at 1000 rpm for 10 minutes. Four layered suspension was obtained after centrifugation.

The layer of fecal debris formed between ether and formalin was removed along the side of the tube with a stick. The supernatant layers of liquid were poured off. The smears were prepared using the collected sediments in glass slides. Finally the smear was examined by iodine and saline wet mounts under the microscope at 10X and 40X objective.

b. Saline wet mount

A drop of normal saline was taken on a clean glass slide and a drop of sediment from formal-ether sedimentation was mixed with it, covered with cover slip and observed under microscope.

c. Iodine wet mount

A drop of Dobell's iodine was taken on a slide to which a drop of processed stool sediment obtained from formal-ether sedimentation was mixed and observed under microscope after covering with a cover slip for the observation of protozoal cysts.

3.4 Statistical analysis

The data were entered and analyzed using SPSS version 16.0. Statistical significance was analyzed by using Chi-square test. Significance was determined on a 5% significance level (P-value <0.05).

CHAPTER IV
RESULTS

4.1 Soil samples

During the study period from March to October 2012, a total of 68 soil samples were collected. Of the total samples, 26.5% were found to be contaminated with parasites. Area around the toilet was the most vulnerable site for the acquisition of parasitic infection. Vegetable fields were ranked second as the most vulnerable site for the acquisition of parasitic infection (Table 1).

Table 1: Site wise distribution of intestinal parasites observed in soil samples

Sample collection site	Number of samples included	Number of contaminated sites	Percentage
Vegetable field	29	11	37.9
Adjacent to toilet	2	2	100
In front of houses	7	1	14.3
Adjacent to water bodies	15	3	20.0
School playground	5	0	0
Adjacent to animal rearing	4	0	0
Behind houses	6	1	16.7
Total	**68**	**18**	**26.5**

Eight different species of intestinal parasites were detected in the soil samples. Soil transmitted helminthes were most frequently observed among which *A. lumbricoides* was recovered from the highest number of soil samples which was followed by *T. trichiura*. Of the protozoans, *Giardia lamblia* and *E. coli* were isolated (Table 2). Of the 18 contaminated samples, monoparasitic contamination was detected from 15 samples and polyparasitic contamination with the involvement of two species was recorded from three samples.

Table 2: Observation of parasites on soil samples of different sites

Parasite detected	**Number**	**Percentage**
A. lumbricoides	8	36.4
T. trichiura	5	22.7
Hookworm	3	13.7
Trichostrongylus	1	4.5
Strongyloides stercoralis	1	4.5
Taenia	1	4.5
Giardia lamblia	2	9.1
Entamoeba coli	1	4.5
Total	**22**	**100**

4.2 Water samples

A total of 14 water samples from the community were tested and the overall contamination rate was found to be 14.3%. None of the water samples from tap and spring were contaminated with intestinal parasites.

Table 3: Observation of intestinal parasites on different sources of water

Source of water	**Number of samples included**	**Number of contaminated sources**	**Percentage**
Tap	6	0	0
Spring	4	0	0
Irrigation channel	4	2	50
Total	**14**	**2**	**14.3**

Intestinal parasites were detected only from irrigation channel water and the species isolated were *Giardia lamblia* cyst among protozoa and ova of *A. lumbricoides* and *T. trichiura* among helminthes (Table 3). Mixed polyparasitic contamination was noticed from both the positive cases.

4.3 Vegetable samples

A total of 59 seasonal vegetables were collected during the study period, of which 39% were contaminated with intestinal parasites. Leafy vegetables were found to be the commonest vehicle to transmit intestinal parasites which were followed by root vegetable (Table 4). Intestinal parasites were fairly observed among rest fruit vegetables. None of the tomato and the gourd samples was found contaminated.

Table 4: Parasite observance rate from different vegetable samples

Types of vegetable	**Number of samples observed**	**Number of contaminated samples**	**Percentage**
Cabbage	9	1	11.1
Cauliflower	4	2	50.0
Radish (root vegetable)	5	3	60.0
Brinjal	6	2	33.3
Pumpkin	4	2	50.0
Leafy vegetables	12	8	66.7
Sweet peepers	4	2	50.0
Chilly	4	1	25.0
Bean	3	2	66.7
Tomato	6	0	0
Gourd	2	0	0
Total	**59**	**23**	**39**

The recovery pattern of the parasites revealed *A. lumbricoides* and hookworm as the predominant intestinal parasitic species to be transmitted from vegetables (Table 5). Polyparasitic contamination was noted from three samples and rest positive samples were contaminated with single type of parasite.

Table 5: Types of parasite detected from vegetable samples

Parasite detected	**Number**	**Percentage**
A. lumbricoides	10	37.0
Hookworm	9	33.3
Trichostrongylus	2	7.4
Strongyloides	1	3.7
Taenia	1	3.7
Giardia	3	11.1
Entamoeba coli	1	3.7
Total	**27**	**100**

4.4 Stool samples

The questionnaire was filled for 197 students out of which only 141 provided the stool samples. Intestinal parasitosis was detected from 80 (56.7%) school children and it was common in male to female (Table 6). The difference was statistically significant.

Table 6: Gender wise recovery pattern of parasitic infection in school children

Gender	**Total children**	**Number of parasite positive cases**	**Percentage**	**P-value**
Male	71	47	66.2	P<0.05
Female	70	33	47.1	
Total	**141**	**80**	**56.7**	

The intestinal parasitosis was found higher in students studying in private schools in comparison to those studying in public school (Table 7). However, the difference was statistically insignificant.

Table 7: Prevalence of parasites according to the type of school

Type of school	**Total students**	**Number of parasite positive cases**	**Percentage**	**P-value**
Private	76	45	59.2	
Public	65	35	53.8	P>0.05
Total	**141**	**80**	**56.7**	

The infection trend was found higher in school children of age group 4-8 which was followed by age group 8-12, which in turn was closely followed by age group 12-16. There was higher enrollment in private school than in public school. Teen age students were confined to public schools only (Table 8)

Table 8: Age and school type wise distribution pattern of intestinal parasites

Age group	**Public school**			**Private school**			**Total**		
	Total children	Positive cases	%	Total children	Positive cases	%	Total children	Positive cases	%
<4	1	0	0	10	5	50.0	11	5	45.5
4-8	7	4	57.1	27	17	63.0	34	21	61.8
8-12	24	14	58.3	39	23	59.0	63	37	58.7
12-16	28	16	57.1	0	0	0	28	16	57.1
>16	5	1	20.0	0	0	0	5	1	20.0
Total	**65**	**35**	**53.8**	**76**	**45**	**59.2**	**141**	**80**	**56.7**

The children obliged to practice open defecation were highly infected than those having toilet facility at their homes. The difference was statistically significant (Table 9).

Table 9: Prevalence of parasites with respect to the availability of toilet facility in the house

Toilet facility	**Total children**	**Number of parasite infected children**	**Percentage**	**P-value**
Yes	90	45	50.0	P<0.05
No	51	35	68.6	
Total	**141**	**80**	**56.7**	

Children receiving antihelminthic drugs within last six months were found less implicated with intestinal parasitosis in comparison to those children who were not receiving the drugs (Table 10). However, the difference was statistically insignificant.

Table 10: Prevalence of intestinal parasitosis in school children according to antihelminthic drug uptake within last 6 months

Antihelminthic drug uptake	**Total children**	**Number of parasite infected children**	**Percentage**	**P-value**
Yes	73	39	53.4	P>0.05
No	68	41	60.3	
Total	**141**	**80**	**56.7**	

Intestinal parasitic prevalence rate was found higher in those without reporting to have gastrointestinal disorder than those who reported to feel so. Complaints viz. abdominal pains, loss of appetite, nausea/vomiting and perianal itching were considered as gastrointestinal disorderHowever, the difference was statistically insignificant (Table 11).

Table 11: Association of gastrointestinal disorder with the parasitic prevalence

Gastrointestinal disorder	Total children	Number of parasite infected children	Percentage	P-value
Yes	118	62	52.5	P>0.05
No	23	18	78.3	
Total	**141**	**80**	**56.7**	

Children habituated to irregular footwear use were found to have marginally higher infection rate. However, the difference was statistically insignificant (Table 12).

Table 12: Footwear using habit and its impact on parasitic infection

Using foot wear	Total children	Number of parasite infected children	Percentage	P-value
Yes	24	13	54.2	P>0.05
No	117	67	57.3	
Total	**141**	**80**	**56.7**	

Table 13: Prevalence of intestinal parasites with respect to BMI class of children

BMI class	Total children	Number of parasite infected children	Percentage	P-value
<18.5	112	64	57.1	P>0.05
18.5-24.9	16	10	62.5	
>24.9	0	0	0	
Total	**128**	**74**	**57.8**	

Out of 141 school children who provided sufficient amount of stool sample, BMI were recorded only of 128 school children. Majority (87.5%) of school children were underweight. The intestinal parasites were fairly recorded among the underweight and optimum weight children (Table 13).

Hygienic habit as proper hand washing showed certain difference in intestinal parasitic infection rate; however, the difference was statistically insignificant (Table 14).

Table 14: Hand washing behavior and its impact on parasitic infection

Hand washing	**Total children**	**Number of parasite infected children**	**Percentage**	**P-value**
Yes	46	24	52.2	P>0.05
No	95	56	58.9	
Total	**141**	**80**	**56.7**	

The parasitic infection was higher in children from joint family in comparison to those from nuclear family though the result was statistically insignificant (Table15).

Table 15: Prevalence of infection with respect to family type of children

Family type	**Total children**	**Number of parasite infected children**	**Percentage**	**P-value**
Nuclear	73	38	52.1	P>0.05
Joint	68	42	61.8	
Total	**141**	**80**	**56.7**	

The prevalence of parasitic infection was found higher in children of crowded family with family members more than five (Table 16). The number of family members ranged from 4-14 in the community.

Table 16: Prevalence of infection in accordance to family size

Family size	Total children	Number of parasite infected children	Percentage	P-value
≤5	57	31	54.4	
>5	84	49	58.3	P>0.05
Total	**141**	**80**	**56.7**	

Monoparasitic infection was common in children. Only helminthes were implicated in all cases of monoparasitism. Protozoans were involved only in polyparasitism (Table 17). Among 24 cases of polyparasitism, 21 cases had combination of two parasities and rest 3 cases had three parasites in combination.

Table 17: Pattern of infection in the children

Type	Frequency of isolated parasites	Percentage
Monoparasitism	**56**	**70.0**
T. trichiura	51	
A. lumbricoides	4	
Taenia	1	
Polyparasitism	**24**	**30.0**
Helminthes	19	
Helminth and protozoan	5	
Total	**80**	**100**

Helminthic parasites overwhelmed the protozoans in the school children. Out of 107 parasites obtained, 95.3% were helminthes and the rest 4.7% were protozoans. *T. trichiura* was most frequently observed in the study population. *A. lumbricoides* was next to follow *T. trichiura*. Only two species of protozoans were detected from the infected individuals of which *G. lamblia* was the common one (Table 18).

Table 18: Types of parasites detected in school children

Type of parasites	Number	Percentage
T. trichiura	73	68.2
A. lumbricoides	24	22.4
Hookoworm	3	2.8
Taenia	1	0.93
Hymenolepis nana	1	0.93
Total helminthes	**102**	**95.3**
E. coli	2	1.7
G. lamblia	3	2.8
Total protozoans	**5**	**4.7**
Total parasites	**107**	**100**

In the study, among the family members involved in different profession, almost all mothers were involved in agriculture with the exception of three mothers. The prevalence rate of intestinal parasites was higher in children of mothers involved in service (Table 19).

Table 19: Prevalence of intestinal parasites with respect to mother's occupation

Mother's occupation	Total children	Number of parasite infected children	Percentage	P-value
Agriculture	136	77	56.6	P>0.05
Service	3	2	66.7	
Total	**139**	**79**	**56.8**	

Infection was higher among the children coming from the families in which the father's were involved in agriculture. The infection was less on other group which included the father's involved in other occupations like driving, government service or working abroad. The difference was statistically significant (Table 20).

Table 20: Prevalence of parasitic infection with reference to father's occupation

Father's occupation	**Total children**	**Number of parasite positive children**	**Percentage**	**P-value**
Agriculture	104	67	64.4	P<0.05
Other (driving, working abroad or government service)	37	13	35.1	
Total	**141**	**80**	**56.7**	

CHAPTER V
DISCUSSION

5.1 Soil samples

The soil contamination rate was found to be 26.5%. Similar prevalence was reported by Shrestha (2006) on soil of Kathmandu valley. This contamination rate was lower than that reported earlier (Rai *et al.*, 2000b). This variation in contamination rate might be due to difference on parasite recovery technique. Geographical condition, seasonal variation and other environmental factors may be implicated in such variation. Soils samples adjacent to shoots of vegetables were collected as sample from the vegetable fields. These are the sites where chemical manure is added directly. The direct contact with such chemicals might have been implicated in the lower prevalence of parasites in the soil.

The most common soil contaminant in this study was found to be *Ascaris* eggs. This finding was in agreement with other studies (Chhetri, 1997; Rai *et al.*, 2000b) from Nepal and elsewhere (Toan, 1997). The *Ascaris* eggs are able to survive in adverse environmental conditions due to the presence of chitin protein in their shells (Rai and Rai, 1999; WHO, 1987). Eggs of *A. lumbricoides* may survive in the external environment and maintain their invasiveness for up to 6 years (Strauch, 1993). Dominance of *Ascaris* eggs might be due to the dispersion of *Ascaris* eggs through stool of infected individual in the soil (Rai and Rai, 1999). A single female *Ascaris* can lays about 240,000 eggs daily so, even a single infected individual practicing open defecation can contaminate a wide area. Their spread in the environment has been enhanced by the indiscriminate open defecation, poor and non-existence sanitation and poverty.

The low prevalence of *Trichuris* eggs observed in this study was in agreement with the earlier reports from Nepal (Rai *et al*, 2000b) and Vietnam (Toan, 1993). Despite topping the list of intestinal parasites in the stool samples, its prevalence was relatively low in soil sample. Surprisingly it was not detected from any of the vegetable samples. This could be due to easy destruction of *Trichuris* eggs in dry condition. The study was conducted in spring and summer season and most of the samples were collected during the spring season.

5.2 Water samples

Despite the improper sanitary conditions prevailing in the society, the parasitic prevalence detected in the water sample was lower. Only two samples out of 14 were contaminated. Both the samples were from the irrigation channel which was used for cleaning utensils, swimming, to feed domesticated animals and for irrigation purpose. The irrational defecation habit of the children, lack of sanitary practice, use of the contaminated water for irrigation of vegetable field and other prevailing haphazard creates and runs the chain of contamination cycle in the community. This stabilizes the intestinal parasitiosis circle in the community. Similar level of contamination of irrigation water was reported by Bakir *et al.* (2003) in Turkey. Higher parasitic contamination was reported in a study conducted in Iran by Yousefi *et al.* (2009).

5.3 Vegetable samples

In the present study, almost all the vegetable samples studied (garlic leaves, onion leaves, radish leaves, cabbage, cauliflower, radish, brinjal, pumpkin, chilly, and sweet peepers) were found to be contaminated. Similar results were reported in Nepal (Sherchand and Cross, 2004; 2007) and elsewhere in the world (Al-Binali *et al.*, 2006; Uga *et al.*, 2009; Yodmani *et al.*, 1983). The highest prevalence was observed among the leafy vegetables. In contrast lower prevalence of intestinal parasites was also reported in the leafy vegetables by Al-Megrin (2010) in Saudi Arabia. Higher prevalence than the present study was noted on both the vegetables collected directly from vegetable fields as well as those sold on markets in a study conducted in Iran (Daryani *et al.*, 2008). Similar higher contamination of vegetables was reported on a study conducted in Tripooli-Libya by Abougrain *et al.* (2009). Relatively lower prevalence in the current study might be due to avoidance of use of human manure as fertilizer which practice has been reported from above journal and Uga *et al.* (2009). Open defecation trend, contaminated soil, use of animal dung as fertilizer and the contaminated water from irrigation channels might be the contributing factor for the higher contamination of vegetables.

Helminthes were the predominant contaminants of vegetables in comparison to the protozoans. Similar predominance of the helminthes was reported by Abougrain *et al.* (2009); Klapec and Borecka (2012) and Uga *et al.* (2009). Protozoans predominance on vegetables was reported by Al-Megrin (2010). *Ascaris* eggs were the commonest contaminants of vegetables which were followed by hookworm and *Trichuris* eggs in this study. Predominance of *Ascaris* eggs was also reported by Abougrain *et al.* (2009) and Uga *et al.* (2009). Use of animal manure and the open defecation trend might be the potential source of soil transmitted helminthes contamination of soil (Al-Megrin, 2010; Clavo *et al.*, 2004; Vuong *et al.*, 2007) which in turn serves as vector to transmit them to the vegetables. Considering the raw vegetable eating habit of the community people and the contamination level, vegetables play an important role in transmitting intestinal parasitic infection.

5.4 Stool samples

Initestinal parasitosis was detected in 56.7% of the study population. Similar prevalence, 56.8% was obtained in study conducted in Slovakian children (Ruhdohradska *et al.*, 2012); 50% during baseline survey in Nigeria (Kirwan *et al.*, 2009); 52.8% among children in India (Mehraj *et al.*, 2008); and 54.7% in Nigeria (Andy and Palmer, 2005). The cumulative egg positive rate for intestinal helminthes was 61.9% in a national scale survey conducted among primary school children in Laos (Rim *et al.*, 2003).

Higher prevalence of intestinal parasites than present study was reported by Mengistu *et al.* (2007) Ethopia and Shakya *et al.* (2004) Nepal. Lower prevalence than that obtained in the present study was reported by Khanal *et al.* (2011a);

Rayapu *et al.* (2012); Shakya *et al.* (2009) and Uga *et al.* (2004) from Nepal and Taheri *et al.* (2011) from Iran. This fluctuation in the prevalence rate can be due to the variation in climatic and geographic conditions (Aksoy *et al*., 2007, Rim *et al*., 2003; Taheri *et al*., 2011). Further hygienic care and environmental sanitation plays important role in the fluctuation of parasitic prevalence (Al-Mohammed *et al*., 2010; WHO, 2010). The hot and humid climate in the geographical area may have aggravated the prevalence of intestinal parasitiosis.

In the present study, seven species of intestinal parasites were detected in stool samples which included five helminth and two protozoal species. Among them *T. trichiura* (68.2%) was observed most frequently among all parasites. Highest prevalence of *T. trichiura* have also been reported from Nepal (Ishiyama *et al.*, 2001; Khanal *et al.*, 2011a; Rai *et al.*, 2005; Sharma *et al.*, 2004; Sherchand and Cross, 2007; Uga *et al*., 2004) and elsewhere (Mengitsu *et al.*, 2007 from Ethopia; Sagin *et al.*, 2002 from Malaysia). Predominance of trichuriasis was also reported from Malaysia with the prevalence rate of 84.6% (Ahmed *et al.*, 2011). In Nepal the prevalence of *T. trichiura* ranges from 5.0% (Houston and Schwartz, 1990) to as high as 94.5% in a backward community in Bhaktapur District (Sahu *et al.*, 1983).

The higher prevalence of *T. trichiura* may be due to its special mode of attachment to cecal mucosa, longer lifespan of parasites as well as its refractory reaction to most anthelminthics. It might be partially due to low efficacy of single dose of albendazole against *T. trichiura* (Knopp *et al.*, 2008). In addition, blood sucking nature and difficulty in deworming during heavy infections could be associated with a higher prevalence of this parasite (Rai *et al.*, 2001a). The higher prevalence of *T. trichiura* in this study and other studies indicated it as the most common intestinal helminth. Therefore, effective deworming of the parasites should be done and may be its omen to reanalyze the single dose regimen to treat intestinal helminthic infections.

In present study, *Ascaris* prevalence rate (22.4%) in children was lower than that of *T. trichiura*. Similar prevalence rate (20%) of *A. lumbricoides* was reported by Khanal *et al.* (2011a) among school children of Kathmandu valley. Lower prevalence rate to present study were reported by Gyawali *et al.* (2009), Sharma *et al.* (2004), Thapa Magar *et al.* (2011) and Uga *et al.* (2004). Higher prevalence (31.8%) and (35%) of ascariasis than the present study was reported by Shakya *et al.* (2009) and Shakya *et al.* (2004). Neither the children from public schools nor those from private schools were facilitated with the regular antihelminthics. They only reported that they received drug during the drug distribution for lymphatic filariasis and many of them refused to take the drug due to the rumors spread about the side effects of the drug for filariasis treatment. Such prevailing deprivation of drug and drug intake might have enhanced the parastitic prevalence.

In this study, among the school children very low prevalence of hookworm was detected (2.8%). This was lower than the year-to-year incidence ranging from 3.8% to 10.7% as reported earlier (Rai *et al.*, 1997). Similar prevalence was reported by Thapa Magar *et al.* (2011), Gyawali *et al.* (2009) and Ghimire and Mishra (2005). Higher prevalence ranging from 7.8% - 33.6% was reported by Khanal *et al.* (2011a,b), Rai *et al.* (2008), Shakya *et al.* (2009), Shakya *et al.* (2004), Sharma *et al.* (2004), Uga *et al.* (2004), and Yong *et al.* (2000). Lower prevalence of hookworm could be due to the use of shoes/slippers by school children even in rural areas, which prevents skin penetration by larvae. Besides, the hot and less humid climatic condition and the season might be responsible for the lower prevalence of hookworm.

Present study showed the very low percentage of *Taenia* spp. and *H. nana* (one for each). Similar low prevalence, 1.6% and 3.3% of *Taenia* spp. and *H. nana* respectively were detected in fecal samples collected in southern Nepal (Sherchand, 1997). A slightly higher (4%) prevalence of *Taenia* was reported by Singh *et al.* (2010) in school children of Kashmir. Also a ten years (1985-1994) study conducted in hospital population has shown an incidence of less than 5% throughout the study period except in the first year (Rai *et al.*, 1994b; 1995). Similarly eggs of *H. nana* (2.6%) have been found in soil samples studied in Kathmandu (Rai *et al.*, 2000b). Higher prevalence (4.4%) of *H. nana* was reported in school children of Andhra Pradesh by Rayapu *et al.* (2012). The low prevalence of *Taenia* infection can be attributed to no consumption of pork meat in the community. Further there was only a single house throughout the community rearing pig.

Among protozoa, *G. lamblia* was the commonest parasite. Most of the studies from Nepal have shown *G. lamblia* as the commonest intestinal parasite (Chandrashekhar *et al.*, 2005; Gyawali *et al.*, 2009; Rai *et al.*, 2002; Sherchand, 1997; Shrestha *et al.*, 2009; Thapa Magar *et al.*, 2011) and similar findings have also been reported from elsewhere (Kaur *et al.*, 2002; Okyay *et al.*, 2004; Saksirisampant *et al.*, 2004; Sheikh *et al.*, 2009). The high prevalence of *G. lamblia* could be due to its easy excess through drinking water (Ishiyama *et al.*, 2001). Also the cysts of *G. lamblia* are more resistant to osmotic lysis compared to *E. histolytica* (Rai *et al.*, 1994a). In contrast, lowest prevalence of *G. lamblia* was reported by Ishiyama *et al.* (2003) from Nepal and Virk *et al.* (1994) from India. Lower prevalence of *G. lamblia* in the present study could be attributed to the availability of relatively safe drinking water sources in the community. Similarly the soil and vegetables from the community were found to be less contaminated with the protozoan.

More than two-third of the children were infected with single parasite, *T. trichiura*. Helminthes overwhelmed the protozoans in the study population. Helminthic preponderance was observed in the studies conducted by Ghimire and Mishra (2005), Rai *et al.* (2008), and Sharma *et al.* (2004) from Nepal with one or the other helminthes being implicated in every study. Protozoal preponderance was also reported in contrast by and Gyawali *et al.* (2009) and Thapa Magar *et al.* (2011). In the present study, helminthes were involved in both monoparasitism and polyparasitism; however protozoans were detected only from polyparasitic infection. Helminthic pervasion in the community is likely due to its preponderance in probable sources, soil and vegetables.

The present study revealed preponderance of monoparasitism over polyparasitism. This was in agreement with the previous reports (Rai *et al.*, 2008; Khanal *et al.*, 2011a; Sharma *et al.*, 2004; Thapa Magar *et al.*, 2011; Yong *et al.*, 2000) from Nepal. Similar findings were reported from the studies conducted in Turkey (Aksoy *et al.*, 2007) and Nigeria (Andy and Palmer, 2005). In contrast Rai *et al*. (2001a) and Sharma *et al.* (2004) have reported the preponderance of polyparasitism over monoparasitism in Nepal. Polyparasitic infection may be due to filthy environment, overcrowded community, very poor health hygiene and sanitary practices.

The prevalence of parasites was highest among the age group 4-8 and 8-12 years. Similar finding was reported by Khanal *et al.* (2011a) showing the highest prevalence of intestinal parasites among age group 6-8 years followed by 9-12 years children. Similarly highest prevalence among children of age group 8-11 years was also reported in India by Singh *et al.* (2010). The irrational dwelling habit, lack of knowledge about hygienic condition and improper sanitary practices may be implicated to highest parasitic infection in this age group.

Higher prevalence of parasitic infection was detected in male to female and this was found to be statistically significant. Higher parasitic prevalence among male to female was reported by different studies conducted in Nepal (Chandrashekhar *et al*., 2005; Khanal *et al.*, 2011a, b; Thapa Magar *et al.*, 2011) and in Thailand by Saksirisampant *et al.* (2004). Slightly high prevalence was noted among female to male children from studies conducted in Nepal by Rai *et al.* (2008); Shakya *et al.* (2009); Yong *et al.* (2000) and studies conducted in India (Rayapu *et al.*, 2012). In contrast, higher prevalence among female to male was reported by Ghimire and Mishra (2005); Shrestha (2006) and Uga *et al.* (2004). Although intestinal parasitosis is gender independent, the difference obtained in the present study might be related to personal hygiene and exposure level to filthy environment.

In general, it is presumed that children of larger and socio-economically under privileged family are infected with various infective pathogens including intestinal parasites. The children from families with members more than five were more infected (58.3%) with intestinal parasites than those with family size less than five (54.4%). The prevalence of intestinal parasitosis was more common in the children from joint families (61.8%) than those from nuclear families (52.1%). This result was in accordance to the previous reports by Aksoy *et al*. (2007) Turkey; Al-Mekhlafi *et al*. (2007) Malaysia and Al-Mohammed *et al*. (2010) Saudi Arabia. This is likely due to relatively less individual care, crowded living, availability of low nutrient diet and burden to take care of younger children by elder ones living them prone to parasitic infections in the larger families.

The children who had not taken anthelminthic drugs within past 6 months had higher (60.3%) parasitic infection than those who had taken anthelminthic drugs. Similar findings, lower prevalence in dewormed individuals was also reported by (Kirwan *et al.*, 2009; Rai *et al.*, 2005; Shrestha, 2006; Thapa Magar *et al.*, 2011). This clearly indicates the importance of deworming. The prevalence of helminthic infection was reduced remarkably and there was dramatic reduction in infection intensity after deworming program (WHO, 2002). However the difference was not significant which might be due to exposure of both the population to the same filthy environment and their unhygienic habits. The vicious cycle of intestinal parasites ongoing in the community is likely to make mass drug administration less effective. The drug was distributed during the campaign for filariasis and many complained about the rumor of its side effects and refusal to take the drugs. It indicates the utmost need to implement the government plan of including all schools under mass drug administration for intestinal parasitosis.

Hygienic habits are critical in maintaining sound health. Proper hand washing habit is mandatory to avoid infectious diseases like soil transmitted helminthes and protozoal infections along with other diarrheal diseases. Children with proper hand washing habit were less infected with intestinal parasites than those who neglects. Similar finding was reported by Gyawali *et al.* (2009). Children of lower age carrying out self cleaning after defecation, improper cleaning procedure, absence or infrequent hand washing after defecation and before eating were reported to be responsible to enhance parasitic infection (Al-Mohammed *et al.*, 2010).

In present study, the children from private schools had higher prevalence. It was in contrast with the general assumption. However the underlying fact behind this must be the age group, the exposure to the same environment and the similar socioeconomic status of the family. Private schools were crowded with the children of age group less than 12 and they were the most vulnerable population to the parasitic infections. The highest prevalence of intestinal parasites in the study was noted in the age group 4-8 and 8-12 and the students from private school superseded those from the public in these age groups. Previous studies from Nepal (Khanal *et al.*, 2011a); India (Singh *et al.*, 2010); Saudi Arabia (Al-Mohammed *et al.*, 2010) and Turkey (Aksoy *et al.*, 2007) have retrieved similar results. Age, irrational dwelling, high outdoor activities, lack of basic knowledge about the sanitary practices, environmental contamination with parasites and open defecation trend prevailing among these age groups might be the underlying cause for the highest prevalence of intestinal parasites in these age groups.

Barefoot provide excess to the soil transmitted helminthes, especially hookworm. In the present study, the prevalence of intestinal parasites was higher in the children with irregular footwear using habit or those who usually do not use foot ware except the time they spend in school. Al-Mekhlafi *et al.* (2007) reported unhygienic habits and barefoot playing as important risk factors for the infection of children and toddlers.

Children deprived of toilet facilities in their home and practicing open defecation were highly infected with intestinal parasites than those who had toilets in their home. Al-Mekhlafi *et al.* (2007) from Malaysia has also reported the absence of toilet as the contributing factor for ascariasis. Gyawali *et al.* (2009) has stated a strong correlation between the type of toilet facility and the prevalence of parasitic infection. Similarly, the defecation in open areas was reported as the significant factor related to parasitic infection in Mexico by Quihui *et al.* (2006). Open defecation trend serves as an active mode to transmit the parasites to soil, water, vegetables etc from which the population gets the infection in return. Besides, open defecation trend provides excess of the intestinal parasites to the environment which serve as either source, vector or the reservoir to the intestinal parasites, thus completing the vicious cycle. Similar result was reported by Singh *et al.* (2010).

CHAPTER VI
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The intestinal parasitosis was found to be endemic in the community with high prevalence rate. The vegetables and the soil of the community were found to be highly contaminated imposing high risk of infection. The water samples from the irrigation channel were also detected to be contaminated. The empirical and the observational inspection both lead to the conclusion that the environment prevailing in the community is filthy and a vicious intestinal parasitic cycle is prevailing in the community. Of the intestinal parasites, soil transmitted helminthes superseded all other parasites; *T. trichiura* being the robust of all. Polyparasitism was high in children with *A. lumbricoides* and *T. trichiura* coinfection being the commonest. This indicates the poor sanitary condition. Even the students of secondary level were unaware of the intestinal parasites which indicate the lagging of the health education and information in the community. This clearly states the gap between the implementation and plan of the government. The findings state that the age, open defecation trend, contaminated environment, lack of health information and improper sanitary habits as the risk factors for the intestinal parasitosis.

6.2 Recommendations

i. Higher prevalence of intestinal parasitic infection with the predominance of helminthic infections urges the implementation of proper health hygiene and sanitary practices.

ii. Education level of neither parents nor children themselves made significant difference in the prevalence of intestinal parasitosis indicating the dire need to aware the community with the causative agents, their mode of transmission and the risk behaviors to be avoided.

iii. Similar studies including composite samples are more precise to draw conclusion and hence such study should be continued in the future in order to explore the true condition prevailing in the society.

REFERENCES

Abougrain AK, Nahaisi MH, Madi NS, Saied MM and Ghenghesh KS (2009). Parasitological contamination in salad vegetables in Tripoli-Libiya. *Food Control*. 21: 760-762.

Ahmed A, Al-Mekhlafi HM, Choy SH, Ithoi I, Al-Adhroey AH, Abdulsalam AM and Surin J (2011). The burden of moderate-to-heavy soil transmitted helminth infections among rural Malaysian aborigines: an urgent need for an integrated control programme. *Parasites and Vectors*. 4: 242-247.

Aini UN, Al-Mekhlafi MSH, Azlin M, Shaik A, Saiah A, Fatmah MS, Ismail MG, Firdaus A, Aisah MY, Rozlida AR and Norhayati M (2007). Serum iron status in Orang Asli children living in endemic areas of soil-transmitted helminthes. *Asia Pac J Clin Nutr*. 16: 724-730.

Aksoy U, Akisu C, Bayram-Delibas S, Ozkoc S, Sahin S and Usluca S (2007). Demographic status and prevalence of intestinal parasitic infections in schoolchildren in Izmir, Tureky. *The Turkish Journal of Pediatrics.* 49: 278-282.

Al-Binali AM, Bello CS, El-Shewy K and Abdulla SE (2006). The prevalence of parasites in commonly used leafy vegetables in south western, Saudi Arabia. *Saudi Med J.* 27: 613-616.

Al-Megrin WAI (2010). Prevalence intestinal parasites in leafy vegetables in Riyadh, Saudi Arabia. *International Journal of Tropical Medicine*. 5: 20-23.

Al-Mekhlafi MSH, Atiya AS, Lim YAL, Mahdy AKM, Ariffin WAW, Abdullah HC and Surin J (2007). An unceasing problem: soil-transmitted helminthiases in rural Malaysian communities. *Southeast Asian J Trop Med Public Health*. 38: 998-1007.

Al-Mohammed HI, Amin TT, Aboulmagd, Hablus RH and Zaza BO (2010). Prevalence of intestinal parasitic infections and its relationship with socio-demographics and hygienic habits among male primary school children in Al-Ahsa, Saudi Arabia. *Asia Pacific Journal of Tropical Medicine.* 3: 906-912.

Anantaphruti MT, Waikagul J, Maipanich W, Nuamtanong S and Pubampen S (2004). Soil-transmitted helminthiases and health behaviors among school children and community members in a west-central border area of Thailand. *Southeast Asian J Trop Med Public Health.* 35: 260-266.

Andy EO and Palmer AD (2005). Soil transmitted helminthiasis among school age children in Ethiope east local government area, Delta state, Nigeria. *African Journal of Biotechnology*. 4: 938-941.

Bakir B, Tanyuksel M, Saylam F, Tanriverdi S, Arazl RE, Hacim AK and Hasde M (2003). Investigation of waterborne parasites in drinking water sources of Ankara, Turkey. *The Journal of Microbiology*. 41: 148-151.

Basualdo JA, Cordoba MA, Deluca MM, Ciarmela ML, Pezzani BC, Grenovero MS and Minvielle MC (2007). Intestinal parasitoses and environmental factors in a rural population of Argentina, 2002-2003. *Rev Ins Med Trop S Paulo.* 49: 251-255.

Bdir A and Adwan G (2010). Prevalence of intestinal parasitic infections in Jenin Governorate, Palestine: a 10-year retrospective study. *Asian Pacific Journal of Tropical Medicine*. 3: 745-747.

Brooks GF, Carroll KC, Butel JS, Morse SA and Mietzner TA (2010). Jawetz, Melnick and Adelberg’s Medical Microbiology, Twenty-fifth edition. Mc Graw-Hill Companies, America. Pp. 669-670.

Casapia M, Joseph SA, Nunez C, Rahme E and Gyorkos TW (2006). Parasite risk factors for stunting in grade 5 students in a community of extreme poverty in Peru. *Int J Parasitol*. 36: 741-747.

Cauyan GA, Andaman DE, Cotaco M, Mari SA, Sabado A and Sahagun C (2008). Prevalence of intestinal parasites among school children in a coastal rural area of Maragondon, Cavite, Southern Luzon, Philippines. *Acta Manilana*. 56: 43-49.

Center for disease control and prevention (CDC) (2010a) – Parsites; food. (http://www.cdc.gov/parasites/food.html,2010). Accessed 12 March 2013.

Center for disease control and prevention (CDC) (2010b) – Parsites; water. (http://www.cdc.gov/parasites/water.html,2010). Accessed 12 March 2013.

Center for disease control and prevention (CDC) (2013) – Parsites; Soil Trasmitted Helminths. (http://www.cdc.gov/parasites/water.html,2010). Accessed 18 June 2013.

Central Bureau of Statistics (2011). National Population and Housing Census 2011 (National Report).

Chand AB (2000). A Prospective study on aetiological agents of diarrhoeal disease in children in relation to parasites and to determine the antibiotic sensitivity pattern of bacterial isolates. M. Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University. Pp. 30-39.

Chandrashekhar TS, Josh, HS, Gurung M, Subba SH, Rana MS and Shivananda PG (2005). Prevalence and distribution of intestinal parasitic infestations among school children in Kaski district, western Nepal. *Journal of Biomedical Sciences.* 4: 78-82.

Cheesbrough M (1992). Medical Laboratory Manual for Tropical Countries. Vol 1, second edition. ELBS Butterworth-Heinemann Ltd, Cambridge, Uk. Pp 605.

Cheesbrough M (1999). Microbiological tests. District Laboratory Practice in Tropical Countries. Part 1: Cambridge University press. Pp. 97-234.

Chhetri MK (1997). Status of intestinal parasite infection among Kathmandu (IP and Non IP Areas) and Sunsari (IP and Non IP Areas) school children in Nepal. In: *collected papers on control of soil transmitted helminthiasis Asian Parasite Control Organ*. 6: 111-121.

Clavo M, Carazo M, Arias ML, Chaves C, Monge R and Chinchilla M (2004). Prevalence of *Cyclospora sp.*, *Cryptosporidium* sp., *Microsporidia* and fecal coliform determination in fresh fruit and vegetables consumed in Costa Rica. *Arch. Latinoam. Nutr*. 54: 428-432.

Cook DM, Swanson RC, Eggett DL and Booth GM (2009). A retrospective analysis of prevalence of gastrointestinal parasites among school children in the Palajunoj valley of Guatemala. *J Health Popul Nutr*. 27: 31-40.

Cooper PJ, Ayre G, Martin C, Rizzo JA, Ponte EV and Cruz AA (2008). Geohelminth infections: a review of the role of IgE and assessment of potential risks of anti-IgE treatment. *Allergy*. 63: 409-417.

Crompton DW and Nesheim MC (2002). Nutrirional impact of intestinal helminthiasis during the human life cycle. *Annu Rev Nutr*. 22: 35-59.

Daryani A, Ettehad GH, Sharif M, Ghorbani L and Ziaei H (2008). Prevalence of intestinal parasites in vegetables consumed in Ardabil, Iran. *Food Control*. 19: 790-794.

Das R, Pradipta S, Kumar and Biswas R (2006). Prevalence of intestinal parasites and its association with sociodemographic, environmental and behavioral factors in children in Pokhara valley, Nepal. *Afr J CLN Exper Microbiol.* 7: 106-115.

Department of Health Services (DoHS) (2010/11). Annual Report: Government of Nepal; Ministry of Health and population Kathmandu, Nepal.

Ezeamama AE, Friedman JF, Acosta LP, Bellinger DC, Langdon GC, Manalo DL, Olveda RM, Kurtis JD and McGarvey ST (2005). Helminth infection and cognitive impairment among Filipino children. *Am J Trop Med Hyg*. 72: 540-548.

Farthing MJG and Keusch GT (1989). Global impact and patterns of intestinal infection. In: *Enteric Infection; Mechanisms, Manifestations and Management.* Chapman and Hall, London. 1: 3-12.

Garcia LS and Bruckner DA (1993). Diagnostic Medical Parasitology. American Society for Microbiology, Washington D.C. Pp. 237-245.

Ghimire TR and Mishra PN (2005). Intestinal parasites and haemoglobin concentration in the people of two different areas of Nepal. *Journal of Nepal Health Research Council*. 3: 1-7.

Gyawali N, Amatya R and Nepal HP (2009). Intestinal prasitosis in school going children of Dharan municipality, Nepal. *Tropical Gastroenterology*. 30: 145-147.

Gyoten J, Hoa NTV, Fujimaki Y, Tanaka K, Uga S and Noda S (2010). The correlation between contamination of soil with *Ascaris* species eggs in school yards and ascariasis among primary school children in Mai Trung Commune, Northern Vietnam. *Tropical Medicine and Health*. 38: 35-38.

Hotez P (2008). Forgotten people and forgetten diseases, the neglected tropical diseases and their impact on global health and development. American Society for Microbiology, Washington DC. Pp. 215.

Hotez PJ, De Silva N, Brooker S and Bethony J (2003). Soil transmitted helminth infections: the nature, causes and burden of the condition. Disease Control Priorities Project: working paper number 3. World Bank, WHO, Fogarty International Centre, National Institutes of Health, US Department of health and Human Services.

Houston R and Schwarz E (1990). Helminthic infections among peace corps volunteers in Nepal. *J Amer Med Assoc*. 263: 373-374.

Ishiyama S, Ono k, Rai CK, Rai G, Tsuji H, Sharma AP,Chaudhary DR and Rai SK (2001). Study of enteropathogens and its pre-disposing factors in a sub-urban public school children in Kathmandu. *Nepal Med Coll J.* 3: 5-9.

Ishiyama S, Rai SK, Ono K and Uga S (2003). Small-scale study on intestinal parasitosis in a remote hilly village in Nepal. *Nepal Med Coll J.* 5: 28-30.

Jiraamonninit C, Wongkamchai S, Santabutr W, Loymek S, Monkong N, Nochot H, Wankhom S and Choochote W (2006). The prevalence of intestinal parasitic infections among school children with annual anthelminthic treatment in Narathiwat Province, Thailand. *J Trop Med Parasitol*. 29: 45-50.

Kaur R, Rawat D, Kakkar M, Uppal B and Sharma VK (2002). Intestinal parasites in children with diarrhea in Delhi, India. *Southeast Asian J Trop Med Public Health.* 33: 725-729.

Khadka D (2005). Detection of enteropathogens (*Salmonella* species, *Shigella* species and parasites) in the stool specimen of children suffering from diarrhea and admitted at Kanti children hospital. M. Sc. Dissertation submitted to the Central Department of Microbiology, Tribhuvan University. Pp. 32-45.

Khanal LK, Choudhury DR, Rai SK, Sapkota J, Barakoti A, Amatya R and Hada S (2011a). Prevalence of intestinal worm infestations among school children in Kathmandu, Nepal. *Nepal Med Coll J.* 13: 272-274.

Khanal Lk, Rai SK, Khanal PR and Ghimire G (2011b). Status of intestinal parasitosis among hospital visiting patients in Deukhury valley, Dang, Nepal. *Nepal Med Coll J.* 13: 100-102.

Kirwan P, Asaolu SO, Molloy SF, Abiona TC, Jackson AL and Holland CV (2009). Patterns of soil-transmitted helminth infection and impact of four-monthly albendzole treatments in preschool children from semi-urban communities in Nigeria: a double-blind placebo-controlled randomized trial. *BMC Infectious Diseases*. 9: 20-33.

Klapec T and Borecka A (2012). Contamination of vegetables, fruits and soil with geohelminths eggs on organic farms in Poland. *Annals of Agricultural and Environmental Medicine*. 19: 421-425.

Knopp S, Mohammed KA, Khamis IS, Mgeni AF, Stothard JS, Rollinson D, Marti H and Utzinger J (2008). Spatial distribution of soil-transmitted helminthes, including *Strongyloides stercoralis*, among children in Zanzibar. *Geospatial Health*.3: 47-56.

Matsumura T, Rai SK and Ono K (1998). Study on environmental and health conditions in an "unknown disease outbreak" hit area in Far-Western Region, Nepal. *Kobe Univ Sch Med Sinryokukai Zassi Japan*. 14: 145-148.

Mehraj V, Hatcher J, Akhtar S, Rafique G and Beg MA (2008). Prevalence and factors associated with intestinal parasitic infection among children in an urban slum of Karachi. *PLoS ONE*. 3.

Mengistu A, Gebre-Selassie S and Kassa T (2007). Prevalence of intestinal parasitic infections among urban dwellers in southwest Ethopia. *Ethiop. J. Health Dev*. 21: 12-17.

Montresor A, Crompton DWT, Hall A, Bundy DAP and Savioli L (1998). Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. World Health Organization: Geneva, Switzerland. WHO/CTD/SIP/98.1.

Mupfasoni D, Karibushi B, Koukounari A, Ruberanziza E, Kaberuka T, Kramer MH, Mukabayire O, Kabera M, Nizeyimana V, Deville MA, Ruxin J, Webster JP and Fenwick A (2009). Polyparasite helminth infections and their association to anemia and undernutrition in Northern Rwanda. Plos Negl Trop Dis. 3: e517.

Okyay P, Ertug S, Gultekin B, Onen O and Beser E (2004). Intestinal parasites prevalence and related factors in school children, a western city sample-Turkey. *BMC Public Health.* 4: 458-464.

Ono K, Rai SK, Chikahira M, Fujimoto T, Shibata H, Wada Y, Tsuji H, Oda Y, Rai G, Kawamura T and Uga S (2001). Seasonal distribution of enteropathogens detected from diarrheal stool and water samples collected in Kathmandu, Nepal. *Southeast Asian J Trop Med Public Health.* 32: 520-526.

Petri WA and Singh U (1999). Diagnosis and management of amebiasis. Clinical infectious diseases. 29:1117-1125.

Piya S (2000). A study on diarrhea in children in relation to behavioral and environment factors. M. Sc. Dissertation submitted to the Central Department of Microbiology, Tribhuvan University. Pp. 39-52.

Quihui L, Valencia ME, Crompton WT, Phillips S, Hagan P, Morales G and Diaz-Camacho SP (2006). Role of the employment status and education of mothers in the prevalence of intestinal parasitic infections in Mexican rural school children. *BMC Public Health*. 6: 225-233.

Rai DR, Rai SK, Sharma BK, Ghimire P and Bhatta DR (2005). Study on enteropathogens and its predisposing factors in gastroenteritis suspected children attending Kanti Children Hospital, Kathmandu, Nepal. *J Nepal Assoc Med Lab Sci.* 6: 48-53.

Rai SK and Gurung CK (1986). Intestinal parasitic infection in high school level students of Birgunj City. *J Inst Med (Nepal).* 8: 33-38.

Rai SK and Rai G (1999). *Ascaris,* ascariasis and its present scenario in Nepal. *J Inst Med (Nepal).* 21: 243-250.

Rai SK, Bajracharya K, Budhathoki S, Khadka JB, Rai KK, Shrestha MK, Sharma CM, Nakanishi M, Kubo T and Shrestha HG (1995). Status of intestinal parasitosis at TU Teaching Hospital. *J Inst Med (Nepal).* 17: 134-142.

Rai SK, Bajracharya K, Budhathoki S, Sharma CM, Shrestha MK, Shrestha HG, Prasai BR, Sibata H, Sumi K, Nakanishi M, Kubo T and Matuoka A (1994a). Prevalence of intestinal protozoan parasitic infection in Nepal. *Hyogo J Med Tech (Japan).* 15: 39-44.

Rai SK, Hirai K, Abe Y, Ishiyama S, Rai G, Ono K and Uga S (2002). Intestinal Parasitosis among school children in a rural hilly area of Dhading district, Nepal. *Nepal Med Coll J.* 4: 54-58.

Rai SK, Kubo T, Nakanishi M, Sumi K, Shibata H, Matsuoka A and Shrestha HG (1994b). Status of soil-transmitted helminthic infection in Nepal. *Kansenshogaku Zasshi.* 68: 625-630.

Rai SK, Matsumura T and Ono K (2001a). Intestinal parasitosis in an “unknown disease outbreak” hit rural hilly area in western Nepal. *Nepal Med Coll J*. 3: 68-73.

Rai SK, Nakanishi M, Upadhay MP, Hirai K, Ohno Y, Ono K, Uga S, Shrestha HG and Matsumura T (2000a). Effect of intestinal helminth infection on retinol and β-carotene status among rural Nepalese. *Nutr Res.* 20: 15-23.

Rai SK, Nakanishi M, Upadhyay MP, Rai CK, Hirai K, Ohno Y, Shrestha HG, Ono K, Uga S and Matsumura T (1998). Effect of Intestinal helminth infection on some nutritional parameters among rural villagers in Nepal. *Kobe J Med Sci (Japan).* 44: 91-98.

Rai SK, Rai G, Hirari K, Abe A and Ohno Y (2001b). The Health System in Nepal: An Introduction. *Envt’l Health Prev Med*. 6: 1-8.

Rai Sk, Shrestha HG, Nakanishi M, Kubo T, Ono K, Uga S and Matsumura T (1997). Hookworm infection recorded at an University Teaching Hospital in Kathmandu, Nepal over one decade period. *Jpn J Trop Med Hyg*. 25: 81-84.

Rai SK, Uga S, Ono K, Rai G and Matsumura T (2000b). Contamination of soil with helminth parasite eggs in Nepal. *Southeast Asian J Trop med Public Health.* 31: 388-393.

Rayapu V, Dhandapanyl SP, Shakerl IA and Kasukurthy S (2012). Prevalence of intestinal helminthic parasities in school going children in rural area of Kuppam, Andhra Pradesh. *International Journal of Basic Medical Sciences and Pharmacy (IJBMSP).* 2: 76-79.

Reed SL (1992). Amoebiasis: An update. *Clin Infect Dis*.14:385-393.

Rim HJ, Chai JY, Min DY, Cho SY, Eom KS, Hong SJ, Sohn WM, Yong TS, Deodato G, Standgaard H, Phommasack B, Yun CH and Hoang EH (2003). Prevalence of intestinal parasite infections on a national scale among primary school children in Laos. *Parasitol Res*. 91: 267-272.

Rudohradska P, Halanova M, Ravaszova P, Goldova M, Valencakova A, Halan M, Papajova I, Pohopencova A, Valko J Cislakova L and Juris P (2012). Prevalence of intestinal parasites in children from minority group with low hygienic standards in Slovakia. *Helminthologia.* 49: 63-66.

Sagin DD, Mohamed M, Ismail G, Jok JJ, Lim LH and Pui JNF (2002). Intestinal parasitic infection among five interior communities at upper Rejang river, Sarawak, Malaysia. *Southeast Asian J Trop Med Public Health*. 33: 18-22.

Sahu RB, Gurbacharya VL and Upadhaya DP (1983). Effect of indigenous drug, "*Butea monosperma*" in parasite infection in Nepal. In: *collected papers on the control of soil transmitted helminthiasis. Asian Parasite Control Organ*. 2: 138-144.

Saksirisampant W, Prownebon J, Kanmamee P, Thaisom S, Yenthakam S and Nuchprayoon S (2004). Prevalence of parasitism among students of the Karen Hill-Tribe in Mae Chame District, Chiang Mai Province, Thailand. *J Med Assoc Thai*. 87: 278- 283.

Savioli L and Albonico M (2004). Soil-transmitted helminthiasis. *Nat Rav Microbiol* 2: 618-619.

Shakya B, Bhargava D, Shrestha S and Rijal BP (2009). Intestinal parasitosis. *Journal of Institute of Medicine*. 31: 3-6.

Shakya B, Rai SK, Rai G, Shrestha A and Singh A (2007). Study on infections by parasite and some bacteria among elderly people of Kathmandu valley. *Nepal Med Coll J*. 8: 243- 247.

Shakya B, Shrestha S, Madhikarmi NL and Adhikari R (2012). Intestinal parasitic infection among school children. *J Nepal Health Res Counc*. 10: 20-23.

Shakya SR, Bhandary S and Pokharel PK (2004). Nutritional status and morbidity pattern among governmental primary school children in the eastern Nepal. *Kathmandu University Medical Journal*. 2: 307-314.

Sharma BK, Rai SK, Rai DR and Roy Choudhury D (2004). Prevalence of intestinal parasitic infestation in school children in the northeastern part of Kathmandu valley, Nepal. *Southeast Asian J Trop Med Public Health*. 35: 501-505.

Sherchan JB, Sherpa K, Tandukar S, Cross JH, Gajadhar A and Shrechand JB (2010). Infection of Cyclospora cayetanensis in diarrhoeal children of Nepal. *Journal of Nepal Paediatric Society*. 30: 23-30.

Sherchand JB (1997). Intestinal Parasitic Infections in Rural Areas of Southern Nepal. *Journal of Institute of Medicine.*19: 115-121.

Sherchand JB and Cross JH (2004). Parasitic epidemiological studies of *Cyclospora cayetanensis* infection in Nepal. *Southeast Asian J Trop Med Public Health*. 35: 1-8.

Sherchand JB and Cross JH (2007). An epidemiological study of Cyclospora cayetanensis in Nepalese people. *J Inst Med (Nepal)*. 29: 8-13.

Shrestha A (2006). A study on soil transmitted helminthiasis in Kathmandu valley. M. Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University. Pp. 27-36.

Shrestha A (2007). Prevalence of soil transmitted parasites in raw vegetables of Kathmandu valley and stool samples of school children. M. Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University. Pp.51.

Shrestha A, Rai Sk, Vitrakoti R and Pokharel P (2009). Parasitic infection in school children in Thimi area, Kathmandu valley. *J Nepal Assoc Med Lab Sci*. 10: 31-33.

Singh C, Zargar SA, Masoodi I, Shoukat A and Ahmad B (2010). Predictors of intestinal parasitosis in school children in Kashmir: A prospective study. *Tropical Gastroenterology*. 31: 105-107.

Solomons NW (1982). Giardiasis: Nutrirional implications. Reviews on Infectious Diseases. 4: 859-869.

Sorensen WC, Cappello M, Bell D, Difedele LM and Brown MA (2011). Polyhelminth infection in east guatemelan school children. *J Glob Infect Dis*. 3: 25-31.

Standley CJ, Adriko M, Alinaitwe M, Kazibwe F, Kabatereine N and Stothard JR (2009). Intestinal schistosomiasis and soil-transmitted helminthiasis in Ugandan school children: a rapid mapping assessment. *Geospatial Health*. 4: 39-53.

Stephenson LS, Latham MC, Kinoti SN, Kurz KM and Brigham H (1998). Improvement in physical fitness in Kenya school boys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole. *Trans R Soc Trop Med Hyg*. 84: 277-282.

Strauch D (1993). Survival of pathogens and parasites in excreta, manure and sewage sludge. *Part I. Med Wet*. 49: 59-65.

Taheri F, Namakin K, Zarban A and Sharifzadeh G (2011). Intestinal parasitic infection among school children in south Khorsan province, Iran. *Jouarnal of Research in Health Science (JRHS).* 11: 45-50.

Thapa Magar D, Rai SK, Lekhak B and Rai KR (2011). Study of parasitic infection among children of sukumbasi basti in Kathmandu valley. *Nepal Med Coll J*. 13: 7-10.

Toan ND (1993). Prevalence of soil transmitted helminthiasis among children aged 4-15 in Vietnam. In: *collected papers on control of soil transmitted helminthiasis. Asian Parasite Control Organ*. 6: 87-91.

Toan ND (1997). Contamination of soil with *Ascaris* and *Trichuris* eggs in rural areas of Vietnam. In: *collected papers on control of soil transmitted helminthiasis*. *Asian Control Organ*. 6: 87-91.

Uga S, Hoa NTV, Noda S, Moji K, Cong L, Y Aoki, Rai SK and Fujimaki Y (2009). Parasite egg contamination of vegetables from a suburban market in Hanoi, Vietnam. *Nepal Med Coll J*. 11: 75-78.

Uga S, Matsumura T, Aoki N and Kataoka N (1989). Prevalence of *Toxocara* eggs in the sandpits of public parks in Hyogo Prefecture, Japan. *Jpn J Parasitol*. 38: 280-284.

Uga S, Rai SK, Kimura K, Rai G, Kimura D, Wakasugi M, Miyake Y, Ishiyama S and Rajbhandari TP (2004). Parasites detected from diarrheal stool samples collected in Nepal. *Southeast Asian J Trop Med Public Health*. 35: 19-23.

Ulukanligil M and Seyrek A (2003). Demographic and parasitic infection status of schoolchildren and sanitary conditions of schools in Sanliurfa, Turkey. *BMC Public Health*. 3: 29-35.

Verle P, kongs A, De NV, Thieu NQ, Depraetere K, Kim HT and Dorny P (2003). Prevalence of intestinal parasitic infections in northern Vietnam. *Tropical Medicine and International Health.* 8: 961-964.

Virk KJ, Prasad RN and Prasad H (1994). Prevalence of intestinal parasites in rural areas of district Shahjahanpur, Uttar Pradesh. *J Commun Dis*. 26: 103-108.

Vuong TA, Nguyen TT, Klank LT, Phung DC and Dalsgaard A (2007). Faecal and protozoan parasite contamination of water spinach (*Ipomoea aquatica*) cultivate in urban wastewater in Phnom Penh, Cambodia. *Trop. Med. Int. Health.* 12: 73-81.

Wasilewska J, Kaczmarski MG, Sawicka-Zukowska M, Tomaszewska B, Majewska A, Plewal K, Oldak E and Debkowska K (2011). Analysis of clinical symptoms and selected hematological indices in hospitalized children with *Ascaris lumbricoides* infection from the northeastern region of Poland. *Wiadomooeci parazytologiczne*. 57: 43-51.

WHO (1987). Prevention and control of intestinal parasitic infections. Technical report series 749.

WHO (1997). Report of a consultation of experts on amoebiasis. WHO weekly epidemiological record number 14.

WHO (2000). World Health Report, conquering suffering enriching humanity.

WHO (2002). Prevention and control of schistosomiasis and soil transmitted helminthes. Technical report series 912.

WHO (2003). Quantifying selected major risks to health. The World Health Report, 2002.

WHO (2010). Working to overcome the global impact of neglected tropical diseases. First WHO report on neglected tropical diseases.

WHO (2012). Soil transmitted helminthiases. Eliminating soil transmitted helminthiases as a public health problem in children: Progress report 2001-2010 and strategic plan 2011-2020.

WHO (2013a). Water supply, sanitation and hygiene development. Accessed on 12 March 2013.

WHO (2013b). MDG7: Environment sustainability; water and sanitation indicators by country.

WHO and UNICEF (2012). Progress on drinking water and sanitation: 2012 update. WHO/UNICEF Joint Monitoring Program for Water Supply and Sanitation.

Yodamani B, Sornmani S, Phatihatakorn W and Harinasuta C (1983). Re-infection of ascariasis after treatment with pyrantel pamoate and the factors relating to its active transmission in a slum in Bangkok. In: *collected papers on the control of soil transmitted helminthiasis. Asian parasite control organ*. 2: 89-103.

Yong TS, Sim S, Lee J, Ohrr H and Kim H (2000). A small scale survey on the status of intestinal parasite infections in rural villages in Nepal. *Korean J Parasitol*. 38: 275-277.

Yousefi Z, Hezarjaribi HZ, Enayati AA and Mohammadpoor RA (2009). Parasitic contamination of wells drinking water in Mazandaran Porvince. *Iran J. Environ. Health. Sci. Eng*. 6: 241-246.

APPENDIX –A

Questionnaire

1. Serial No: ……………………… **2. Date:** ……………..

3. Name: …………………………. **4. Sex:** Female/Male

5. Class: …………………………. **6. Age:** ……………...

7. Height ……. **8. Weight** ……….

9. School type ……………………

10. Number of family members …….. **11. Type of family**- Nuclear/Joint

12. Mother's Education: i) Illiterate ii) Below S.L.C iii) Above S.L.C

13. Mother's occupation: i) Agriculture ii) Service iii) Business iv) Others

14. Father's education: i) Illiterate ii) Below S.L.C iii) Above S.L.C

15. Father's occupation: i) Agriculture ii) Service iii) Business iv) Others

16. Water source: i)Tap ii)Spring iii)River

17. Drinking water outside home (not mineral water)**:** Yes/No

18. Drinking water quality: i) Untreated ii) Boiled iii)Chemical treated

19. Eating street food: Yes/No

20. Washing hand: Before eating anything/ Only before eating meal

21. Washing hand: With only water/ With soap water

22.Which water is used to wash the vegetables? i) Tap ii) Spring iii) River

23. Raw vegetables consumed in your family: i) Lettuce ii) Radish iii) Carrot iv) Cucumber v) Coriander

24. Do you wash your hands after going to toilet? Yes/ NO

25. What do you wash your hands with? ……….

26. Dietary habit: Vegeterian/non-vegeterian

27. Do you have following symptoms:

i) Abdominal pain ii) Nausea/ vomiting

iii) Loss of appetite iv) Perianal itching

28. Have you taken anthilmenthic drugs recently (within 6 months)? Yes / No

29. Do you use footwear while playing or working in field? Yes/No

30. Do you have toilet in your home? Yes /No

31. Do you swim? Yes/No

APPENDIX-B

Materials and chemicals used

1 Chemicals and reagents

Diethyl ether	Qualigens, India
Distilled water	
Formaldehyde	Qualigens, India
Iodine crystals	Loba Chemic, India
Potassium iodide	Qualigens, India
Sodium chloride	Qualigens, India
Sucrose Crystals	Qualigens, India
Tween-20	Qualigens, India

2 Materials

Beaker	Borosil, India
Centrifuge tubes	
Conical Flask	Borosil, India
Droppers	Borosil, India
Glass rod	Borosil, India
Glass slide and cover slips	Borosil,India
Gloves	
Knife	Kiwi, India
Measuring cylinder	Borosil, India
Measuring tape	
Pipettes	Borosil, India
Plastic bag	
Plastic bottles	
Scale	
Spatula	Borosil, India
Test tube stand	Borosil, India
Test tubes	Borosil, India
Whatmann filter paper	

3 Equipments

Camera (Cybershot DSC-TX1)	Sony, Korea
Centrifuge	Remi, India
Microscope	Olympus (Japan)
Refrigerator	LG, Korea
Weighing machine	

APPENDIX – C

Composition and preparation of reagents

Dobell's Iodine

I_2 Crystals	2.0 gms
Potassium iodide	4.0 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.

Physiological saline

Sodium chloride	0.85 gm
Distilled Water	100 ml

Physiological saline (0.9%)

Sodium chloride	0.9gm
Distilled water	100ml

10% Formalin

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

Dilute formaldehyde 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%.

0.05% Tween -20

Tween-20	0.05ml
Distilled water	99.95ml

0.5% Tween-20

Tween-20	0.5ml
Distilled water	99.5ml

Sucrose solution (specific gravity- 1.2)

Sucrose crystals	500gm
Distilled water	625ml