CHAPTER I

1. Introduction

Intestinal parasites cause major health problem in developing countries. Parasitic diseases still wreak a swathe of destruction, disproportionately plaguing the poor, especially impoverished communities in low-income countries. Highly debilitating rather than deadly, worm-induced diseases and soil-transmitted helminthiases (STH) are particularly pernicious and remain a major health hazard in developing countries. Intestinal parasitic infections are among the ten most common infections in the world (WHO, 1987). It is assumed, however, that this morbidity rate is an underestimate, since many cases may be underreported, and new cases occur continually in conjunction with increasing urbanization in most developing countries (Crompton and Savioli, 1993).

Intestinal parasitosis are endemic in most tropical and subtropical countries, particularly in developing countries and are one of the important causes of diarrhoeal diseases. Due to diarrhoeal diseases, at least 5 million deaths per year occur in developing countries (Shakya *et al*, 2007). Gastroenteritis is a major killer disease in Nepal. Every year 30-40 thousand people die of gastroenteritis (Bista *et al*, 1993). *Cyclospora cayetanensis* and *Cryptosporidium parvum* an emerging parasitic enteropathogen of human being increasingly recognized throughout the world.

About 3.5 billion people in the world are infected from intestinal parasitoses and 450 million are ill as the result of these infections, the majority being children (WHO, 2000). *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* have been estimated to infect 250 million, 151 million and 45 million people respectively (WHO, 1997). Each of these parasites has been responsible for the death of 65000, 60000 and 70000 people respectively (WHO, 2000). However, it is prevalent nearly one hundred percent in the rural areas of developing countries (Reily, 1980; Estevez *et al*, 1983; Rai and Gurung, 1986).

Among the helminth parasites infection, STH infection remain as the important public health problem. It is estimated that more than one billion people in the world are infected by STH, mainly *A. lumbricoides*, hookworms and *T. trichiura* (Crompton, 1999). Annually more than 2 million people die of STH infection in the world. Soil-transmitted helminthiasis remains as an important cause of morbidity and sometimes mortality in developing tropical countries, particularly among pediatric age group (WHO, 1987).

At least 750 million episodes of diarrhea occur per year in developing countries resulting in five million deaths. Globally, an estimated 1.8 billion episodes of diarrhoea occur each year leading to the death of 3 million children below age of 5 years (WHO, 2000). The protozoan parasites although being less common are associated with the highest number of mortalities (Chan *et al*, 1994). Intestinal parasitic infections are distributed virtually through out the world, with high prevalence rate in many regions. Amoebiasis, ascariasis, hookworm infection and trichuriasis are common among the top ten infections in the world (Warren *et al*, 1984).

Children are more commonly infected with parasites than adult in Nepal (Rai *et al*, 1986). There was strong association between giardiasis and malnutrition of many school children (Chaudhary *et al*, 2000). Malnutrition is more common among children aged less than five years and it is associated with child mortality (Rai *et al*, 2002 b).

Although STHs affect all age groups, the problem is predominant among the worlds' estimated 400 million school children, and is often associated with poor growth, reduced physical activity, impaired cognitive function and learning ability (Stephenson *et al*, 1998). The main cause of distribution of STH in soil is indiscriminate defecation. Thus, studies on soil contamination and probable sources are needed to gain better understanding of infection in the population.

According to WHO it has been stated that intestinal helminthes rank first in causing disease in children. Children are most potential group to get infection with parasite. However, most such studies were based on stool examinations and therefore they cannot directly indicate the extent to which residents are at risk of parasitic disease but simply demonstrate the parasite distribution in a population at a given time. Examination has to be made on intestinal parasite eggs in soil (Uga *et al*, 1995).

Effective control of STH infections depends on improvement in sanitation and living conditions, awareness in health and hygiene but implementation is usually hampered by lack of resources and political will. In the short term, school based deworming has been recommended as a highly cost-effective public health measure in less developed countries (Yokogawa *et al*, 1983). In addition, baseline surveys provide basis for development of control programmes at national, regional and district levels. Preventive measures include avoiding contacts with soil that may be contaminated with human faeces, proper management of disposing faeces, wastes and sewage, good personal hygiene and food or water sanitation. When traveling to countries where sanitation and hygiene are poor, avoid water or food that may be contaminated, wash, peel or cook all raw vegetables and fruits before eating.

Despite increasing commitment to the health and learning of schoolchildren, progress on these fronts can be seriously threatened by helminth infections. Studies have shown clearly the detrimental effects of infection on educational performance and school attendance, as well as the significant improvements in language and memory development that can be realized following treatment. Helminth infections are also associated with nutritional deficiencies, particularly of iron and vitamin A, with improvements in iron status and increases in vitamin A absorption after deworming. Adolescent girls are particularly at risk of anemia aggravated by helminth infection and iron stress (WHO, 2002).

Nepal is a small and impoverished country located in South Asia where intestinal parasites are prevalent. Among enteric parasites, STH are highly prevalent in Nepal (Rai and Gurung, 1986; Rai *et al*, 1994a, 1994b, 1995, 1997c, 1998, 2000a, 2000b; Sherchand *et al*, 1996, 1997; Ishiyama *et al*, 2001, 2003; Ono *et al*, 2001). The main factors responsible for this are poor sanitation, poverty and lack of health education (Matsumura *et al*, 1998; Rai *et al*, 2000b, 2001b; Ishiyama *et al*, 2001).

Nepal, majority of population living on agricultural subsistence, has diverse geo-topography with diverse climatic condition. The population densities and lifestyles vary according to the region and ethnic groups, respectively (Rai *et al*, 2000a). Approximately 70% of the health problems in Nepal are infectious diseases (Rai *et al*, 2001b). Of them, STH infection alone is most important (Rai and Gurung, 1986; Rai *et al*, 1994a, 1995) and has been found significantly affect on the nutritional status of

Nepalese (Rai *et al*, 1998). However, the vegetables contamination with STH eggs of both human and animal origin has not been well investigated. The present study was done to explore the condition of STH in vegetables used daily as well as the health of the selected population. The study was based on the detection of STH from vegetables and stool samples. The present study imparts the importance of the study of STH through vegetables along with the intestinal parasites. The prevalence of STH in vegetables of Kathmandu Valley will help to know the sanitary status and public awareness about the intestinal parasites.

CHAPTER II

2 OBJECTIVES

2.1 General objective

To determine the prevalence of intestinal parasites in raw vegetable and stool samples of school children of Kathmandu Valley, Nepal.

2.2 Specific objectives

a. To assess the most prevalent parasites in raw vegetables available in local vegetable markets.
b. To find out the parasitic prevalence in the school children.
c. To assess the pattern of parasitic infections in school children.
d. To correlate the parasitic infections among the school going children with their socioeconomic conditions.
e. To know the sanitary status as well as the public health awareness in school going children.

CHAPTER III

3. LITERATURE REVIEW

About 80.0% of all illness in the world is caused by inadequate sanitation, unsafe water and unavailability of water (WHO, 2000), intestinal parasitosis is being one of them. Both protozoa and helminthes are responsible for the intestinal infections leading to high mortality and morbidity, particularly in developing countries.

Soil transmitted helminth infections are endemic in the communities where poor environmental sanitation and poor personal hygiene are prevalent, as occurs in majority of developing countries (Yodmani *et al*, 1982). Yu *et al* (1993) showed that environmental pollution, sanitary condition and human behaviour play an important role in transmission of STH. Yodmani *et al* (1982) indicated that many sources of Ascariasis from the host and in the environment such as soil in the shantytowns and vegetables sold in the market resulted in the continuous active transmission of Ascariasis in the particular area.

Soil pollution with fecal materials is instrumental in transmission of STH infection. Fertilized eggs deposited in the soil develop rapidly and, depending on environmental conditions, may reach the infective stage within a matter of weeks. Thereafter, eggs are transferred from soil to the vegetables then onto the hands and finally to the mouth.

Global Views

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

Imai *et al* (1985) carried out a survey on soil transmitted helminthiasis in North Sumatra, Indonesia and found extremely high prevalence, average (97.0%) in three villages and more than (70.0%) harbored three or more helminthes, especially *A. lumbricoides*, hookworm and *T. trichiura*.

The survey of the stool samples from 1,659 children aged 15 years and below in metropolitan Lagos showed 71.9% and 68.3% infection with *T. trichiura* and *A. lumbricoides*, respectively while the infection rate with hookworm was (22.5%). Multiple infections were very common (Fagberno-Beyioku *et al*, 1987).

In 1994, Lee *et al* examined fecal specimens of a total of 52,552 patients by formalin-ether sedimentation and direct smear method. The overall egg positive rate of helminthes was (6.5%). The egg positive rate for each species of helminthes was *Clonorchis sinensis* (3.2%), *T. trichiura* (2.0%), *A. lumbricoides* (0.2%), *Trichostrogylous orientalis* (0.1%), *Taenia* spp. (0.05%), *H. nana* (0.03%), hookworms (0.03%), *Enterobious vermicularis* (0.02%), *S. stercoralis* (0.1%) and *Diphyllobothrium latum* (0.004%). By analysis of 9 years data, they stated that the prevalence of STH such as *A. lumbricoides* and *T. trichiura* has been decreasing remarkably, while that of snail-transmitted helminthes such as *C. sinensis* has not.

In 1994, Hassan *et al* surveyed 4 primary and 2 secondary schools at Imbaba, Egypt. Stool specimens of 791 students were examined. The results revealed *E. histolytica* (22.4%), *H. nana* (6.2%), *A. duodenale* (5.7%), *A. lumbricoides* (1.5%) and *E. vermicularis* (1.1%). The statistical difference in rate of infection between primary and secondary school students was not observed.

A study on parasitological examinations in stool specimens from 407 residences of Sao Paulo, Southeastern Brazil was performed. Intestinal parasites were detected in (45.7%) of the stool collected. The most prevalent parasites in this population were *A. lumbricoides* (23.8%) followed by *T. trichiura* (17.2%). The prevalence rates of infection by *A. lumbricoides* and *T. trichiura* were the highest among children aged 2-12 years. The mean intensity of infection of *A. lumbricoides* was highest among children aged 6-12 years and young adults under 25 years, while in *T. trichiura* infected subjects the highest egg counts were observed in children aged 2-12 years (Ferreira *et al*, 1994).

Chandiwana *et al* (1989) surveyed to access the prevalence and intensities of hookworm and roundworm infections in 15 farm-worker communities in Zimbabwe with poor living conditions. Examinations of 1,635 fecal samples showed that hookworms were the commonest helminthes (61.7%). They were followed by *A. lumbricoides* and *T. trichiura*. Age prevalence and age intensity profiles for hookworms showed that infections increased with age, with a peak in the adult age groups. *A. lumbricoides* prevalence was relatively high in children but declined in adults.

In Nigeria, an epidemiological survey of intestinal helminthiasis on 766 primary school children aged 5-16 years was conducted and found a prevalence of *A. lumbricoides* (88.5%), *T. trichiura* (84.5%), hookworm (33.1%) and *S. stercoralis* (3.0%). The influence of host age and sex on infection level was assessed. Evidence was obtained for pre-disposition of individuals to heavy and light infection with *A. lumbricoides* (Holland *et al*, 1989).

Examination of stool samples from primary school children of North Thailand was carried out by Kasuya *et al* (1989) in 491 students and revealed overall prevalence of 48.7%. The most common type of parasite was found to be STH such as hookworm (26.3%) and *S. stercoralis* (11.2%), while *A. lumbricoides* was not so prevalent (1.2%). Ophisthorchiasis is another parasitic disease with a relatively high prevalence rate of (7.5%). This disease rate was found to be increasing with age. The most common protozoan was *G. lamblia* (7.7 %).

In Cockle Province, Republic of Panama, Robertson *et al* (1989) carried out a survey of intestinal helminthes in children by microscopic examinations (modified Kato-Katz technique) of stool samples from 661 children attending primary schools. The overall prevalence of *A. lumbricoides*, hookworm and *T. trichiura* infections were found (18.2%), (12.0%) and (27.5%) respectively. There were significant differences between the infection prevalence values for children attending the different schools, but not with respect to age or sex. Positive association was detected between particular pairs of infections and there were most evident with *T. trichiura.*

In Brazil, Goncalves *et al* (1990) carried out parasitological examinations on 485 inhabitants of four villages. Approximately (99.6%) of the inhabitants were infected with at least some species of intestinal parasites. A high prevalence of *S. mansoni* (82.1%), hookworm (80.2%), *T. trichiura* (69.9%), *A. lumbricoides* (61.9%) and *E. coli* (36.7%) infections were demonstrated.

Hesham *et al* (2006), studied on 281 Orang Asli children in eight Orang Asli villages in Selangor. 26.3% of the children were infected with single parasitic infection either *A. lumbricoides*, or *T. trichiura* or hookworm and 72.6% with mixed infection. The most prevalent parasite was *T. trichiura* (98.2%) followed by *A. lumbricoides* (61.9%) and hookworm (37.0%). Jamaiah and Rohela (2005) conducted a survey in public of Malaysia. The overall infection rate was 6.9% with *T. trichiura* being the commonest parasite followed by *A. lumbricoides*, *C. sinensis*, hookworm and *E. histolytica*. The highest infection rate was among Chinese followed by Malays and Indians.

An investigation for the prevalence and intensity of STH was undertaken in four villages in Oyo state, Nigeria by Asaolu *et al* (1992). Diagnosis was based on examination of stool samples for the presence of helminthes ova in all age groups. The prevalence *A. lumbricoides* ranged from (61.5%) to (72.2%), *T. trichiura* from (65.0%) to (74.0%) and hookworm from (52.4%) to (63.0%) depending on the village concerned.

Uga *et al* (2005) examined the stool samples of school children, in Vietnam. Of 217 children examined, 166 (76.0%) were positive for at least one of the nine species of parasites. Among helminth parasites, *T. trichiura* (67.0%), *A. lumbricoides* (34.0%) and hookworm (3.0%) were detected. In case of protozoan parasites, *E. coli* (8.0%) and *E. histolytica* (2.0%) were detected.

In a survey in Indonesia, 419 stool samples were examined by using direct smear, flotation and formalin ether concentration techniques. Five nematodes and seven protozoan parasites were detected while trematodes and cestodes infections were not observed. STH infections were predominant. Among the younger inhabitants aged less than 15, positive rates of *A. lumbricoides*, *T. trichiura* and hookworm infections were almost same (45.7%), (45.3%) and (47.7%), respectively. Among the people aged 15 or more, positive rate of hookworm infection (89.4%) was much higher than *A. lumbricoides* and *T. trichiura* infections (19.3%) and (26.1%), respectively (Hasegawa *et al*, 1992).

A survey conducted by Marnell *et al* (1992) among the refugee in Juba, Sudan, involving 241 fecal samples revealed (66.0%) of the population harbored intestinal helminthes. The most commonly found infection was hookworm (36.0%), followed by *S. mansoni* (26.0%), S. *stercoralis* (20.0%), *H. nana* (11.0%), *A. lumbricoides* (1.2%), *T. trichiura* (0.8%) and *Taenia* spp., (0.4%). Among examinee 42.0% had single infection, 21.0% had double and (3.0%) had multiple infections. Parasitic prevalence and intensities were analyzed in relation to age, sex, religion and occupation. Females were more infected (70.0%) than males (64.0%). Muslims were less infected (50.0%) than Christians (68.0%) and agriculturalists (90.0%) were the most infected occupational group.

Andrade *et al* (2001) studied stool samples in Ecuador. The prevalence of STH was 65%, *A. lumbricoides* being the commonest (63.0%) followed by *T. trichiura* (10.0%) and hookworm (1.4%). A significant relationship was found between the worm burden and the degree of stunting. This study suggests that the periodic administration of an anthelminthic drug should be targeted to preschool and school children to allow a normal growth spurt and prevent stunting.

A community based prospective study was conducted by Karrar and Rahim (1995) in Khartoum. The commonest infestation was giardiasis (21.1%) followed by taeniasis (10.4%) and enterobiasis (7.4%). Non pathogenic *E. coli*, *E. histolytica* and *T. saginata* were detected in (2.7%), (0.7%) and (1.7%) of stools specimens, respectively.

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

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

Paul and Gnanamani (1999) carried out a study to determine the prevalence and intensity of intestinal helminthes infections among the children belonging to lower socio-economic status. Stool samples collected were processed by modified formalin ethyl acetate sedimentation technique. 177 children were infected with one or more of intestinal parasites as *A. lumbricoides*, *T. trichiura* and hookworm. The overall prevalence of infection was (82.0%). *A. lumbricoides* was the most common infection with prevalence of (75.0%) followed by *T. trichiura* (66.0%) and hookworm (9.0%).

Another study was conducted by Lee *et al* (2000) in a small scale survey of intestinal parasites infection among school children and adolescents in Philippines. The overall prevalence rate was (78.1%) with *T. trichiura* topping the list (51.0%) followed by *A. lumbricoides (*40.0%), hookworm (23.4%), *Iodamoeba butschlii* (15.6%), *Endolimax nana* (14.1%), *E. coli* (9.4%) and *G. lamblia* (7.8%). The infection rate of primary school children, preschool children and adolescent were (95.5%), (64.7%) and (87.7%), respectively. The infection rate in urban area was (56.0%) and (92.3%) in rural areas.

Ascariasis was the most common parasite in China. According to nation wide survey in 1988-1992 the average infection rate was 47.0% that is around 531 million people were infected (Feng *et al*, 2001). Another study by Chai *et al* (2001a) in Jiangxi province of China has reported 72.5% prevalence rate. The positive rate of different parasite was *A. lumbricoides* (50.9%), *T. trichiura* (33.4%), hookworm (11.4%), *G. lamblia* (2.4%), *E. coli* (1.2%) and *E. histolytica* (0.8%).

A national survey of the prevalence of intestinal parasitic infections in the Islamic Republic of Iran was made by Sayyari *et al* (2004). 45,128 stool samples were analyzed by formalin-ether precipitation. Among them, intestinal parasitic infections were found in 19.3% of the study population (19.7% male, 19.1% female). *G. lamblia* (10.9%), *A. lumbricoides* (1.5%), *E. histolytica* (1.0%) and *E. vermicularis* (0.5%) were common parasites. The infection rate was highest in the 2-14 years age group (25.5%).

A survey of the prevalence of intestinal parasites in primary school children was carried out by Enekwechi and Azubike (1994) in Nigeria. Stool samples were taken from children belonging to eight primary schools. Of the 1,536 stool samples examined using light microscopy after formal-ether centrifugation, 922 (60%)

harboured one or more parasites. The prevalence of the parasites was as follows: *A. lumbricoides* (20.8%); *T. trichiura*, (15.3%); *N. americanus* (13.0%); *E. histolytica* (6.8%); *T. saginata* (2.3%); *S. stercoralis* (1.3%); *Schistosoma mansoni* (0.3%); *G. lamblia* (0.1%). The overall infection was 31.9% for males and 27.5% for females. Infection was more prevalent in male and female children of (10-12 years). Mixed infections were also observed.

In 2005, Araujo *et al* studied 413 stool samples. The over all prevalence of infection was 64.4%. The most prevalent infection was caused by *A. lumbricoides* (35.6%) followed by *T. trichuria* (18.6%) hookworm (9.9%), *S. stercoralis* (1.0%), *E. vermicularis* (0.5%), *E. coli* (24.9%), *E. histolytica* (13.3%) and *G. lamblia* (1.0%).

In 2005, Dada Adegbola *et al* surveyed 170 stool samples including 88 male and 82 female. The diagnosis of intestinal helminthes was made by Kato-Katz thick smear examination technique. Intestinal helminthes was detected in (68.2%) of the sample collected. *A. lumbricoides* were the most predominant parasite among all the children, with the prevalence of 81.2%, 63.3%, and 52.4% among children aged 12-17 years, 6-11 years and 0-5 years, respectively.

Stool specimens from 1,282 children between the age of 5 and 13 years attending 10 primary schools for boys in Saudi Arabia, were examined for the presence of intestinal parasites. Of these, 313, (24.4%) were found to be infected with one or more species of 11 intestinal protozoa and helminths. The most common pathogenic protozoa being *G. lamblia* (10.9%) followed by *E. histolytica* (4.1%). The nonpathogenic protozoan, *E. coli* had the highest prevalence rate (11.3%) in the children's stools. *H. nana* was the commonest intestinal helminth (3.0%). Other intestinal helminths, including *A. lumbricoides, T. trichiura. S. mansoni*, *Dicrocoelium dendriticum* were detected to a lesser extent. The distribution of the common intestinal infections among the children surveyed were also analysed according to age and multiplicity of infection. Prevalence of *E. histolytica* was found to increase with age whereas *Giardia* infections were less common among older children (Omar *et al*, 1991).

A study was carried out of sewage farms, streams and vegetables to determine the sources and routes of STH infection in Sanliurfa, Turkey. Stool samples from farmhouse inhabitants as well as soil and vegetable samples from the gardens were collected and examined. In addition, water samples from streams and vegetable samples from the city market were collected and examined. One hundred and eighty-seven (59.5%) of a total samples (n= 314) were positive. Among them, 88.4% of the stool samples, 60.8% of the water samples, 84.4% of the soil samples and 14% of the vegetable samples were found to be positive for STH eggs. These results indicate that the water, soil and vegetables are heavily contaminated, and suggest a vicious circle between humans and the environment (Ulukanligil *et al*, 2001).

Between August 1999 and January 2001, samples of various fruits and vegetables obtained within Norway were analyzed by published methods for parasite contamination. Neither *Cyclospora* oocysts nor *Ascaris* (or other helminth) eggs were detected on any of the samples examined for these parasites. However, of the 475 samples examined for *Cryptosporidium* oocysts and *Giardia* cysts, 29 (6.0%) were found to be positive. No samples were positive for both parasites. Of the 19 *Cryptosporidium*-positive samples, 5 (26.0%) were in lettuce, and 14 (74.0%) in mung bean sprouts. Of the 10 Giardia-positive samples, 2 (20.0%) were in dill, 2 (20.0%) in lettuce, 3 (30.0%) in mung bean sprouts, 1 (10.0%) in radish sprouts, and 2 (20.0%) in strawberries. Mung bean sprouts were significantly more likely to be contaminated with *Cryptosporidium* oocysts or *Giardia* cysts than the other fruits and vegetables (Robertson and Gjerde, 2001).

Bitkowska (2004) examined stool of group of 7-year-old children of Poland for the presence of intestinal parasites. The study was based on the examination of a single faecal specimen and a cellophane swab. The studies included 31,504 children. The most frequently encountered parasites in the examinations included: *E. vermicularis* (15.0%), *A. lumbricoides* (0.83%), *G. intestinalis* (0.69%), *E. coli* (0.60%) and *T. trichiura* (0.12%) The overall percentage of the infected children was 15.4%. The number of infected among children inhabiting countryside (19%) was significantly higher than among those from the towns (10.4%).

The prevalence of intestinal parasites was determined for 1,370 children in Khan Younis Governorate, Gaza Strip. The age of the children ranged from 6 to 11 years. For stool samples inspection, direct smear microscopy, flotation and sedimentation techniques were used. The general prevalence of intestinal parasites was 34.2%.

Different types of intestinal parasites were detected during this survey: *A. lumbricoides* seemed to be the most common parasite (12.8%), whereas *G. lamblia* had a prevalence of 8.0%, *E. histolytica* 7.0%, *E. coli* 3.6%, *T. trichiura* 1.6% and *H. nana* 1.0%. The prevalence of enterobiasis was determined using a scotch tape preparation. A total of 20.9% of the children examined were infected and there was sex variation in the prevalence of enterobiasis (Astal, 2004).

Quadros *et al* (2004) found two hundred children from nursery schools in Lages, southern Brazil, to be infected with parasites. The overall prevalence of helminths and protozoa was 70.5%, affecting 61.4% of male and 74.5% of female children. The most prevalent parasites were *A. lumbricoides* (35.0%), *G. lamblia* (14.0%) and *T. trichiura* (13.0%).

Food-borne trematodes (FBT) are important causes of parasitic infections in many Asian countries. Parasitological surveys in Xai Udom, a small fishing community on the Nam Ngum reservoir, Lao PDR, revealed an overall parasitic infection rate in May 1999 of 68.8% (n = 173) and in December 1999 of 65.9% (n = 261). The liver fluke, *Opisthorchis viverrini* accounted for most of the infections (prevalence of 53.8% and 42.1%, during the first and second surveys, respectively). The prevalence and intensity showed increasing trends with age. Minute intestinal flukes were also present but with relatively low infection rates (3.8-10.9%). The second common group of parasites comprised soil-transmitted nematodes, *T. trichiura A. lumbricoides*, hookworm and *S. stercoralis*, with prevalences of 22.4% and 17.6%, 20.8 and 8.0%, 16.8% and 13.4%, and 4.0% and 15.3% (first and second surveys, respectively). Most people had no or only light infections, with a few people having heavy infections. Coexisting intestinal protozoa were *G. lamblia* (5.2% and 4.9%) and *E. coli* (6.9% and 6.5%). Concurrent tapeworm infections were *Taenia* (1.7 and 1.1%) and *H. nana* (0.7 and 0.6%) (First and second surveys, respectively) (Sithithaworn *et al*, 2005).

Displacement and refugee camps provide ideal grounds for the transmission of parasites and increase the risk of acute respiratory infections, diarrhoeal diseases, and intestinal parasitic infection. *C. parvum, G. lamblia, E. histolytica, A. lumbricoides*, hookworm infection, *S. haematobium, S. mansoni* and *S. stercoralis* are important cosmopolitan intestinal parasites that are common among children, the immunocompromised and displaced populations. The total 518 stool specimens were used for the detection of *Cryptosporidium* specific and *Giardia* specific antigens by the DMSO modified Acid-Fast and Trichrome-PLUS stain for *C. parvum* and *G. lamblia* and *E. histoyltica* respectively. Stool specimens for the demonstration of helminth eggs and larvae were prepared by the modified Kato technique. One hundred and seventy eight (31%) samples were collected from the children below 10 years of age and were selected because they were screened for various forms of malnutrition. However, the data on *C. parvum* and *G. lamblia* were included in the analysis for all parasites. More children were positive for *G. lamblia* (29%) than for *C. parvum* (10%) and 5% had double infection with both parasites. The antigen positive rate decreased with age for *C. parvum* and *G. lamblia* infections. Adult samples were also examined for the intestinal parasites. Hookworm had the highest pevalence rate of 18% among the 581 IDP residents followed by *S. mansoni* (16.7%) and *A. lumbricoides* (15%). The overall prevalence of *E. histolytica* among the study population was 9.0%. The results of this study indicate that intestinal protozoan and helminth parasites are highly prevalent among camp residents in Sierra Leone with five different helminth parasites demonstrated in the stool specimens of residents in the five IDP camps (Gbakima *et al*, 2007).

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

Chacin-Bonilla *et al* (1992) assessed the prevalence of *E. histolytica* and other intestinal parasites in a suburban community of Maracaibo, Venezuela, by examination of a stool specimen from each of 342 individuals, using iron-haematoxylin stained faecal smears and formalin-ether concentration. The overall parasitic infection rate was 80.4%, and 65.8% of the population had multiple infections. The overall amoebic infection rate, which was highest in female adults, averaged 39.7%. The *E. histolytica* infection rate was 8.7% and most of those infected were passing cysts. *E. polecki* was observed in two samples. Amongst the protozoa, *E. coli* was observed most frequently (24.8%) and *G. lamblia* was the predominant pathogen (13.0%). *T. trichiura* (71.9%) and *A. lumbricoides* (54%) were the most common parasites, particularly in school-children. The high rates of parasitic and multiple infections reflect the low socio-economic status of the community studied.

A study was conducted in southern Sudan to determine the prevalence of intestinal parasites among school children. A total of 275 stool samples which were examined using formol-ether concentration techniques yielded 15 different species of parasites. Hookworm with a prevalence of 13.1% was the predominant nematode followed by *S. stercoralis* (3.3%), *Trichostrongylus* (2.5%), *S. mansoni* (2.2%) and *T. trichiura* (1.8%). *A. lumbricoides* and cestodes were not detected in this population. Common intestinal protozoans found were *E. coli* (37.8%), *E. histolytica* (28.4%) and *G. lamblia* (9.8%). Children in the age group 6-10 years old were the most affected followed by the 11 to 15- year-old age group. The infection rate was slightly higher in males than females (Magambo *et al*, 1998).

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

Soil-transmitted helminthiases are a public health problem in rural communities. A cross-sectional study of the prevalence and distribution of *A. lumbricoides, T. trichiura* and hookworm was conducted in 281 Orang Asli children (aborigines) aged between 2 and 15 years, from eight Orang Asli villages in Selangor, Malaysia. All the children were infected with STH, with 26.3% of the children infected either with *A. lumbricoides, T. trichiura* or hookworm and 72.6% having mixed infection. The overall prevalences of *A. lumbricoides, T. trichiura* and hookworm were 61.9%, 98.2% and 37.0%, respectively. Approximately 19.0%, 26.0% and 3.0% of the children had severe infection of ascariasis, trichuriasis and hookworm infection, respectively. Severe ascariasis and trichuriasis may lead to other health and medical problems (Al-Mekhlafi *et al*, 2006).

In a study conducted by Aimpun and Hshieh (2004) in Belize, Central America 66.0% of the population was found to have one or more intestinal parasites. The most common infection was hookworm (55.0%) followed by *A. lumbricoides* (30.0%), *E. coli* (21.0%), *T. trichiura* (19.0%), *G. lamblia* (12.0%), *E. histolytica/dispar* (6.0%) and *I. butschlii* (9.0%).Other parasites found were *E. hartmani, S. stercoralis, E. nana, I. belli* and *C. mesnili*. Children were more often infected than adults and more females had hookworm infection.

In a study conducted in Bendal state of Nigeria, the overall percentage incidence of six parasites encountered was as follows: *E. coli* (19.7%), *E. histolytica* (3.9%), *G. lamblia* (1.4%), hookworm (29.4%), *A. lumbricoides* (38.2%), and *T. trichiura* (7.3%). Males had higher protozoan and helminthes than females (Obiamiwe and Nmorsi, 1991).

In Guatemala, Anderson *et al* (1993) conducted a study. Among the studied population 41.0% was infected with *A. lumbricoides* and 60.0% with *T. trichiura*. In a study among 100 residents of Central America, 48.0% helminth infection was assessed which consisted of hookworm, *T. trichiura*, and *Ascaris* infection. The prevalence of each of the individual parasites was considered light to moderate and the intensity of infection was generally low in this population (Elias *et al*, 1997).

The study was conducted by Oliveira *et al* in 2003 in a rural area of Uberlandia, State of minas Gerais, Brazil in which 65.4% of the total population were found to be infected with intestinal parasites among which 45.1% were children and 54.9% adults. Within this study group 66.7% were mono infected, 17.6% bi-infected and 15.7% polyinfected. 47.0% individuals were infected with protozoa, 29.4% with helminthes and 23.6% with both. According to sex, the positivity rate for the intestinal parasites was 41.0% for male and 24.4% for female. Regarding to the age groups, high positive rate (29.5%) was found in children of age 1-15 years followed by 16-30 years (20.6%), 31-45 years (5.1%), 46-60 years (6.4%) and above 60 (3.8%). Among the parasites, *H. nana* was the most frequent helminth (14.1%) and *G. lamblia* (11.5%) the major protozoa. High positive rate of 6.4% was detected both for hookworm and *S. stercoralis. T. trichiura* was found in 5.1% of the study.

A community based study by Raso *et al* (2005) in rural Cote d'Ivoire found the prevalence of hookworm, *E. histolytica/E. dispar* and S. *mansoni* to be 45.0%, 42.2% and 39.8%, respectively. Three-quarters of the population harboured multiple parasites.

In a study conducted by Pratdesaba *et al* (2001), *C. cayatanensis* was observed in samples of 7 out of 474 (1.5%) subjects, distributed as follows: 6 of HIV or AIDS patients (3.8%) and 1 of the 111 malnourished children (0.9%). No *C. cayetanensis* oocysts were observed in any of the samples from the raspberry farm workers. For the 474 subjects based on wet preparation only and the Modified AF stains for coccidia, the most commonly observed parasites were *E. coli* (19.6%), *A. lumbricoides* (14.8%), *E. nana* (13.3%), *T. trichiura* (12.0%) and *B. hominis* (11.4%). *C. parvum* was observed in samples from 25 (3.2%) subjects.

Burstein (2005) had reported the prevalence of *C. cayentanensis* among apparently healthy persons in Peru. One group included those consulting private physician and next included people in marginal area. They had the common complain of the abdominal pain and diarrohea. The incidence rate had found to be 41.6% and 7.3%, respectively. He reported higher rate among young and elder adults up to 60 years old.

Miller *et al* (2002) examined the presence of intestinal protozoan and helminth infections and their association with clinical signs and symptoms in children in Trujillo, Venezuela. Conventional microscopic methods (thick-smear, saline and iodine solutions) were used to identify parasites in stool samples of 301 children attending day care centres. A subgroup of 45 children was evaluated clinically and parasitologically five times during a 1-month period using conventional methods and the Kinyoun acid-fast stain for *Cryptosporidium* identification. The point prevalence of protozoan infections was 21.0% for *G. duodenalis*, 1.0% for *E. histolytica/dispar*, 4.0% for *E. coli,* 16.0% for *B. hominis*, and 89% for *C. parvum*. Prevalence of helminth infection was 11% for *A. lumbricoides*, 10.0% for *T. trichiura*, 0.3% for *S. stercoralis,* and 1.3% for *H. nana*. Over a 1-month time frame, new infections were observed at a rate of 11.0% for *G. duodenalis*, 4.0% for *E. histolytica/dispar*, 7.0% for *A. lumbricoides*, 11.0% for *T. trichiura*, 0% for *S. stercoralis,* and 2% for *H. nana*. Intestinal symptoms (diarrhoea, vomiting, gas, stomach pain, and loss of appetite) were associated with presence of one or more of *C. parvum* or *B. hominis* organisms in stool samples. Intestinal parasitic infections contribute significantly to the enteric disease burden experienced by this group of children.

van der Hoek *et al* (2003) surveyed on soil-transmitted helminthes in Vietnam between 1990 and 2001. From this study, it was found that 33.9 million people in Vietnam were infected with *Ascaris* (prevalence 44.4%), 17.6 million with *Trichuris* (prevalence 23.1%), and 21.8 million with hookworm (prevalence 28.6%). Prevalence of *Ascaris* and *Trichuris* showed a declining trend from the north to the south of the country. Vegetable cultivation in which nightsoil is used as fertilizer was a risk factor for hookworm infection.

In April 2004, an outbreak of acute diarrheal illness occurred among the Orang Asli (aborigine) in the Cameron Highlands, Pahang State, Peninsular Malaysia. In the course of the epidemic investigation, stool samples were collected and examined for infectious agents including parasites. Soil transmitted helminthes (STH), namely *A. lumbricoides* (25.7%), *T. trichiura* (31.1%) and hookworm (8.1%), and intestinal protozoa, which include *G. lamblia* (17.6%), *E. histolytica/E. dispar* (9.4%), *B. hominis* (8.1%) and *C. parvum* (2.7%), were detected. Forty-four subjects (59.5%) were infected with at least one parasite, 24 (32.4%), 12 (16.2%) and 8 (10.8%) had single, double and triple parasitic infections, respectively. *G. lamblia*, though the most commonly found parasite in samples from symptomatic subjects, was within the normally reported rate of giardiasis among the various communities in Malaysia, and was an unlikely cause of the outbreak. However, heavy pre-existing parasitic infections could have contributed to the severity of the rotavirus diarrheal outbreak (Hakim *et al*, 2007).

In a study by Feng *et al* (2001b), the infection rate of STH in Fujian Province was found to be 55.5%. Among them 55.1% were *E. vermicularis*, 36.5% were *A. lumbricoides*, 19.2% *T. trichiura* and 11.3% hookworm. In the general population, the infection rates were found to be 74.4% and 83.2% for male and female, respectively in 1998. Similarly, the infection rates were found to be 56.2% and 54.7% for male and female, respectively. In 1998 the single, double and multiple infection rates were 44.2%, 47.0% and 8.8% respectively. In 1999 the single, double and multiple infection rates were 76.7%, 22.2% and 1.5%, respectively.

A study of helminthic infection in Vientiance Province of Lao PDR found the positive rate 63.3%. The single, double, triple and multiple infection rates were 64.0%, 28.0%, 7.0% and 1.0% respectively. *A. lumbricoides* (40.0%) was the commonest helminth followed by *T. trichiura* (28.0%) and hookworm (8.0%). The study had found that the women have 1.25 times more chance of being infected with *A. lumbricoides* than others ($p<0.005$). The monoinfection rate of the *T. trichiura* was found to be only 8.6% while its infection along with *A. lumbricoides* was 23.5% (Phetsouvannh *et al*, 2001).

Dong and Lee in 1972 performed a parasitic survey on vegetables collected from markets and vegetable gardens in Korea. Three species of vegetables, lettuce, radish and Chinese cabbage were selected. Five species of parasitic eggs (ascarid, trichurid, *Trichostrongylus*, *Clonorhis* and hookworm) and two larvae (filariform and rhabditiform) were found. Of the parasites found, ascarid egg was found to be highest (49.0%), followed by *Trichostrongylus* eggs (18.0%), as well as filariform larva of hookworms (19.7%), and the least often observed was rabditiform larva of hookworm.

de Oliveira and Germano (1992) analysed the vegetables, commercially traded in the metropolitan area of Sau Paulo, Brazil, by means of the appropriate methodology with a view to discover and identify protozoan cysts of medical interest. The vegetables under study consisted of 50 samples of each of the varieties listed below: lettuce - oily leaves and crisp-head varieties, endive and watercress. Results showed high rates of contamination in all the varieties of vegetable analysed. However, the watercress was the one which presented the highest frequencies of enteroparasites. Both the oily leaves and crisp-head varieties of lettuce presented the lowest rates of contamination, whereas endive presented values ranking, in general, between those of the lettuce and those of the watercress. A great variety of those protozoans which occur frequently in the population resident in the metropolitan area of S. Paulo were observed in the samples, the most frequent being *Entamoeba* spp., and *Giardia* spp., cysts of *Iodamoeba* spp., *Endolimax* spp. and *Chilomastix* spp. were also recovered from the samples, thus corroborating the occurrence of high rates of fecal contamination.

In Costa Rica, a total of 640 samples of eight different vegetables used for raw consumption, were analyzed for the presence of intestinal parasites and fecal coliforms. Eighty samples of each vegetable were analyzed, forty during the dry season and forty in the rainy. A greater, but insignificant ($p > 0.05$) level of fecal coliforms was found during the dry season. Levels of *Escherichia coli,* were higher ($p < 0.05$) during the dry season in lettuce and cilantro leaves. Cysts of *E. nana, E. coli, E. histolytica*, *G. intestinalis* and *Cryptosporidium* spp. were found in all vegetables. The greater percentage of positive samples was found during the dry season, although this relation was only corroborated ($p < 0.05$) in radish and cilantro leaves. Only lettuce and cilantro levels showed a positive linear correlation ($p < 0.05$) between occurrences of intestinal parasites and fecal coliforms(Monge *et al*, 1996).

Parasitological examinations were performed in material derived from 220 vegetables to detect cysts and eggs of intestinal parasites. This material was collected from supermarkets in northern and southern areas of the Rio de Janeiro city. Vegetables proceeding from the northern areas presented a greater degree of contamination mostly of helminth eggs; lettuce was the most contaminated vegetable. The authors suggest that differences found between the northern and southern areas of the city may be due to either packing or manipulation by shop assistants and consumers (da Silva *et al*, 1995).

This study reveals the occurrence of some pathogenic microorganisms in vegetable consumed on a daily basis by Costa Ricans. *Cryptosporidium* spp. oocysts were found in 5.2% (4/80) of cilantro leaves, in 8.7% (7/80) of cilantro roots and 2.5% of lettuce samples. A 1.2% (1/80) incidence was found in other vegetables samples (carrot, cucumber, radish and tomato). Oocysts of this parasite were absent in cabbage. *G. intestinalis* was only detected in 5.2% (4/80) of cilantro leaves and in 2.5% (2/80) of cilantro roots. *E. histolytica* cysts were found in 6.2% (5/80) of cilantro leaves, in

2.5% (2/80) cilantro roots, in 3.8% (3/80) lettuce and in 2.5% (2/80) radish samples. At least a 2% incidence of this amoeba was found in other vegetable samples (carrot, cucumber, cabbage and tomato) (Monge and Arias, 1996).

Mesquita *et al* in (1999) evaluated the parasitological contamination of vegetables to be consumed raw and commercialized in Niterói and Rio de Janeiro cities using 128 samples of vegetables. The samples including lettuce and watercress were collected from supermarkets, greengrocer shops and self-service restaurants. Only 6.2% of the samples were positive for parasitic structures with morphological aspects similar to those of animal parasites. The results revealed the acarids, acarid eggs, insects, nematode larvae and ciliated protozoa in most of the samples (96.1%), including those from restaurants. This high percentage suggests a risk of human infection since parasite structures capable of infecting man may exist in association with these agents.

The presence of *Cyclospora* spp., *Cryptosporidium* spp., and microsporidia and the levels of fecal coliforms were determined in lettuce, parsley, cilantro, strawberries and blackberries obtained in local agricultural markets of the Central Valley of Costa Rica. During the second semester of 2001 and the first of 2002, 50 different samples of each product (25 taken in the dry season and 25 in the rainy season) were taken for the study. The fecal coliforms count was also done. The parasite determination was done using Zielh Nielsen and Weber staining techniques from a sediment obtained through the rinse of the mentioned products, using sterilized peptone water 0.1% and centrifuging at 900 G for 15 min. One hundred per cent of vegetable samples had fecal coliforms and the greatest prevalence was obtained during the rainy season. Although all vegetables presented fecal coliforms in high concentrations, lettuce and cilantro presented statistical difference between rainy and dry season, being greater during the rainy season. Fecal coliforms were not detected in strawberries and blackberries probably due to its low pH. All products examined showed, at least once, *Cryptosporidium* spp., *Cyclospora* spp. and Microsporidia, revealing the risk to Public Health. *Cryptosporidium* was present in all products. *Microsporidia* was present in all products except blackberries and *Cyclospora* was only isolated from lettuce during the dry season. These results show the importance of introducing in the country Good

Agricultural Practices, especially due to the resistance of *Cryptosporidium* and *Cyclospora* to disinfecting agents (Calvo *et al*, 2004).

Al-Binali *et al* (2006) surveyed the commonly used green leafy vegetables for the contamination by parasites from September 2004 to May 2005. Five commonly used leafy vegetables, namely, green onion, radish, watercress, lettuce and leek were studied. The prevalence of parasites was 28.0% in green onion, 25.0% in radish, 17.0% in watercress, 17.0% in lettuce, and 13.0% in leek. The parasites were most common in the months of September to December. Hookworm, *E. coli*, *A. lumbricoides* and *B. hominis* were the most common isolated parasites.

Yodmani *et al* (1983) collected various vegetables sold in the market of Bangkok and examined every month during August 1980- July 1981. The samples namely lettuce, spring onions, coriander, white greens, Chinese celery, mint, cucumber, cabbage, morning glory and water cress. A total of 120 examinations were made in the study. The results revealed that 4.2% were positive for *Ascaris* eggs. The contaminated vegetables were lettuce (16.7%), white greens (8.3%), cucumber (8.3%), and water cress (8.3%). But remainings were negative for *Ascaris* eggs.

In the SAARC Countries

A study was done among the children of age below 4 years suffering from gastrointestinal problem in rural Bengal. Most common parasite was *G. lamblia* (17.2%), followed by *E. vermicularis* (12.2%) and *E. histolytica* and *A. lumbricoides* both (8.1%). A significantly lower infection rate was observed in children below one year (24.4%) than older age groups (66.4%) (Saha *et al*, 1995).

Socio-economic and behavioral factors affecting the prevalence of geo-helminthes in preschool children in Sri Lanka was studied by de Silva *et al* (1996). They examined relationship between the prevalence of geo-helminthes infection in pre-school children living in the urban slum area and parental education, socio-economic status, the use of anthelminthics and beliefs regarding these helminthes. Stool sample from 307 children were examined by direct smear and concentration method. Overall prevalence was found (26.4%) and more interestingly *A. lumbricoides* (90.1%) predominated all of helminthes. The parental education level and socio-economic conditions influenced geo-helminthes infections in children.

Virk *et al* (1994) examined the stool samples in some of the villages of Utterpradesh, India. 29.2% were positive for parasitic infection. *A. lumbricoides* topping the list followed hookworm, *H. nana*, tapeworm, *T. trichiura*, *E. vermicularis*, *E. histolytica*, *E. coli* and *G. lamblia*. The highest positivity was encountered in the age groups between 6-14years. The high prevalence of intestinal parasites may be due to lack of awareness about personal cleanliness and hygiene and literacy among rural women. Majority of them had helminthes infections.

A study was conducted by Awasthi and Pandey (1997) in prevalence of intestinal parasite in pre-school slum children in Lucknow. The samples were examined by direct smear technique. 17.5% children were found to be infected with parasites. *A. lumbricoides* was found in 68.1% and *G. lamblia* in 32.9% children. There was no association between weight or height and parasite positivity.

The parasitic causes of diarrhea in children in Delhi were determined by the direct smear technique; stool specimens of 127 children were examined for intestinal parasites. In 59 cases (46.5%) intestinal helminths and protozoa were demonstrated. *A. lumbricoides* was observed in 1 (0.8%) case, while *T. trichiura* was the finding in 3 (2.4%). Protozoal parasites included *G. intestinalis* and *E. histolytica* in 14 (11.0%) cases each, *Balantidium coli* in 3 (2.4%) cases and *Cryptosporidium* spp., in 24 (18.9%) patients. Mixed infection was not seen in any of the cases (Kaur *et al*, 2002).

A comparative analysis of the various intestinal parasites detected among children attending schools was carried out in a rural and urban location in and around Chennai. A total of 324 stool samples were examined by routine microscopy. All suspicious samples were subjected to zinc sulphate concentration technique as well as modified Ziehl Neelson stain and Trichrome stains to identify the other uncommon intestinal parasites. Out of 125 specimens tested from the rural location, the overall prevalence of intestinal parasites was 91.0%. *A. lumbricoides* was the most common helminthic parasite detected (52.8%) followed by *T. trichura* (45.6%), *A. duodenale* (37.6%), *S. stercoralis* (3.2%) and *H. nana* (1.6%). *G. lamblia* was the most common protozoan parasite detected (16.0%), followed by *E. histolytica* (4.0%). In contrast under urban settings, out of the 199 stool specimens tested the positivity rate was 33.0%. *Giardia* was the most common parasite detected (22.6%) followed by *E. histolytica* (10.6%). All other intestinal parasites such as *T. trichura* (2.0%), *H. nana* (1.0%) and *A. lumbricoides* (0.5%) were found to have much lower prevalence in comparison to the rural area tested. *E. vermicularis* (0.5%) was also detected. *A. duodenale* and *S. stercoralis* were not encountered at all in the urban setting studied (Fernandez *et al*, 2002).

The present study was undertaken among 258 subjects in Kottoor and Achankovil areas in Kerala, South India. Out of the total 258 stool samples examined, 60 showed ova of one or more intestinal helminthes, showing the overall prevalence of 23.3%. Among the tribal populations of two areas studied, Achankovil area showed an increased overall prevalence rate (26.1%) as compared to Kottoor area (22.3%). The difference in prevalence rates of the two areas is found to be statistically significant. Hookworm infestation was found to be predominant (58.82%) in Achankovil and the remaining (41.1%) was due to only roundworm. Whereas in Kottoor area, roundworm infestation predominated (74.4%) followed by hookworm (18.6%) and other types (6.9%). Analysis of haemoglobin (Hb) level of the 190 study population showed that 66.32% were anaemic (<11 gm/dl) from both the areas. The proportions of subjects either moderately anaemic (7 gm/dl-11 gm/dl) or severely anaemic (<7 gm/dl) were almost same in the two study groups, showing a similar pattern in both the areas with no statistically significant difference. Haemoglobin level in relation to helminthic infestation revealed that in the worm infested group, 81.1% (43/53) was anaemic, as compared to only 60% (83/137) of non-worm infested group, showing significant difference (p value=0.01). Hb level in relation to helminthic infestation also seemed to differ in both the areas. Among the environmental factors studied in relation to helminthic infestation, the practice of hand wash alone was found out to be statistically significant; showing that habit of proper hand wash considerably reduces the risk of helminthic infestation (Farrok *et al*, 2002).

In May 2003, a survey was conducted in the western region of Bhutan to assess the prevalence and intensity of soil-transmitted helminth (STH) infections after 15 years of school deworming in the country. Five schools were randomly selected in the region and 266 school children were examined. Stool samples were collected from each child as well as nutritional indicators and general information on each school.

The survey found a cumulative prevalence of 16.5% STH (4.8% in schools treated in the last three months and 24.0% in the untreated schools). An unexpected finding was that the tapeworm infection rate of 6.7%. WHO recommends a 50.0% prevalence as the threshold for the establishment of community intervention (Allen *et al*, 2004).

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

National scenario

In Nepal due to lower socio-economic condition, lack of proper education and poor hygienic conditions of the people intestinal parasitosis is very much prevalent (Nepal and Palfy, 1980; Estevez *et al*, 1983; Rai and Gurung, 1986; Sherchand *et al*, 1996, 1997; Rai *et al*, 1994a, 1994b, 1995, 1997, 2000, 2001; Ishiyama *et al*, 2001; Ono *et al*, 2001). Therefore, it can be assumed that intestinal parasites are important causative agents of the major public health problems of the country. In some rural areas of Nepal parasitic infection rate approach one hundred percent with significant portion of parasitic infection (Reily, 1980; Estevez *et al*, 1983).

A random sample study of patients in Bhaktapur was conducted to ascertain the incidence of roundworm infection (Sharma, 1965). A total 976 stool samples were collected over a 5 years period. Among them 430 samples were from adult males, 326 were from adult females and 220 were from children of both sexes under 12 years of age. The result showed that (32.0%) of adult males, (44.0%) of adult females and (49.0%) of children were infected giving an overall incidence of (40.0%).

Sharma and Tuladhar (1971) carried out a microscopical examination of 80 students studying in Auxiliary Health Worker's school. They found (87.4%) students were infected by different parasites like protozoa and helminthes. *A. lumbricoides* was the commonest parasites followed by hookworm, *T. trichiura*, *G. lamblia* and *E. vermicularis*.

The survey was carried out in which a total of 225 stools samples were examined to find the prevalence of intestinal parasites. Only (4.4%) of the samples showed negative result. The most common helminth was *A. lumbricoides* (63.5%) followed by hookworm (55.9%) and *T. trichiura* (37.6%). Among protozoa *E. histolytica* (28.8%) and *G. lamblia* (28.8%) were the most common followed by and *E. coli* (24.4%). 55.1% samples showed the presence of more than one parasite (Nepal and Palfy, 1980).

Khetan (1980) examined 2,073 stool samples in the pathology laboratory of Narayani Zonal Hospital during 1977 to 1980. Among them 1,592 stool samples were found positive for parasitic infestation. (29.0%) were infected by hookworm followed by *A. lumbricoides* (21.9%), *T. trichiura* (9.9%), *G. lamblia* (8.5%) and *E. histolytica* (4.0%).

Estevez *et al* (1983) collected and examined 40 specimens of stool samples in a remote area of Western Nepal. Among them, (90.0%) were found positive for parasites as determined by direct wet mount and trichome smears. 83.3% of individuals were infected with hookworm, 52.8% with roundworm and 5.5% with whipworm. All of the positive samples contained several parasite species, averaging 4 spp. per specimen.

Shrestha in 1983 conducted a study in Bhaktapur district. The result showed (99.0%) stools were positive for the eggs of STH, (94.0%) positive for *A. lumbricoides*, (42.0%) for *T. trichiura* and (11.0%) for hookworm. Similarly, from the Panchkhal area in Kavre District (41.0%) stools were positive for the eggs of helminthes. Of these (75.0%) were of *T. trichiura*, (37.0%) were of hookworm, (19.0%) were of *A. lumbricoides*.

A retrospective study for the evaluation of the status of STH infection in Nepal was conducted by Rai *et al* (1994a) and found that the annual prevalence for STH during the period of 1985 to 1992 range from 18.0% to 36.6% with marginal decrease in successive years. The incidence however, showed an increase rate after the year 1993.

The positive rate for intestinal parasitosis was 29.1% to 44.2%. The most common helminthes was *A. lumbricoides* followed by hookworm. The incidence of *A. lumbricoides* remained constant through out the study period; while the incidence of hookworm and *T. trichiura* decreased remarkably till year 1992 followed by an increasing trend on the year 1993 and 1994.

The survey was conducted on the prevalence of helminthes infection for three Ethnic groups (*Jirels*, *Sherpas* and high caste *Hindus*) in Jiri region of Eastern Nepal. There was the significant difference between these groups for prevalence of hookworm with *Jirels* having the overall highest infection rate (Blangero *et al*, 1993).

Another retrospective study conducted by Rai *et al* (1994b) on the prevalence of intestinal protozoan parasitic infection in Nepal showed the annual rate of protozoan parasitosis range from 3.3% in 1985 to 13.6% in 1990. The overall annual incidences of these parasites found increasing every successive calendar year. The majority of protozoan parasite found was *G. lamblia* followed by *E. histolytica*. Both of these parasites showed an increasing tendency in every successive year except for the year 1989 during which *G. lamblia* showed relatively low infection.

In the study of intestinal parasitosis in rural area of southern part of Nepal, the prevalence rate of parasites recorded was 63.1%. Hookworm was the commonest parasite (11.6%) followed by *A. lumbricoides*, *E. vermicularis*, tapeworms, *H. nana*, *E. histolytica*, *G. lamblia*, *C. parvum*, *C. cayetanesis*, *Opisthorchis* spp., *Schistosoma* spp. and *I. butschlii.* The incidence rate was higher in female children (58.1%) compared to male children (41.0%). High prevalence of intestinal parasitosis is an indication of human behaviour like walking bare foot, poor sanitary condition, illiteracy and lack of awareness (Sherchand *et al*, 1997).

Rai *et al* (1997b) conducted the serological study of amoebiasis among adults and found a significantly low seropositivity rate among the *Rai/Limbus* (5.0%) compared with each of the *Tamangs/Sherpas* (40.0%), *Newars* (35.0%) and *Brahmins/Chhetries* (40.0%). In the study conducted in eastern hilly region of Nepal, *A. lumbricoides* was found to be most common parasite followed by hookworm and others (Rai *et al*, 1997c). In another study conducted in a rural hilly area of Western Nepal, the overall prevalence rate of parasite was 76.4%. Parasites were almost uniformly distributed in both male and female and both in adult and children. *A. lumbricoides* was most predominant parasite (47.7%) followed by hookworm and others. Amongst the protozoan parasites, *E. coli* was the most common (Rai *et al*, 2001a).

Yong *et al* (2000) carried out a research on the status of intestinal parasite infections in two rural villages of Chitwan District in Nepal. The examination of 300 stool samples of school children were performed by formalin-ether sedimentation technique and the prevalence rate of intestinal parasite infections in female was slightly higher than that in male without statistical significance. Among the protozoan parasites, *E. coli* was the commonest parasite (21.0%) followed by *G. lamblia* (13.7%) and others (5.3%). Hookworm was the most prevalent intestinal helminth (13.0%) followed by *T. trichiura* (3.0%) and others (5.0%). Forty-three specimens (14.3%) showed mixed infections.

Among the school children of Kathmandu Valley, the parasitic prevalence of 72.4% has been reported by Ishiyama *et al* (2001). Helminthes parasites dominated the protozoan parasites with 46.9% multiple infections. *T. trichiura* (30.4%) was most frequently found helminth followed by *A. lumbricoides* (21.7%) and *G. lamblia* (17.0%) was most common protozoan followed by *B. hominis* (7.9%).

A study conducted by Rai *et al* (2002b) in rural hilly areas of Dhading District reported the overall prevalence rate of 60.0%. *A. lumbricoides* (69.6%) was most common parasite which is followed by hookworm and others. *G. lamblia* (5.2%) was only protozoa detected. Ethnically, *Dalits*, lower caste and so called untouchable people with low socio-economic status, (NPC, 2002) had significantly higher (74.1%) prevalence rate followed by *Tibeto-Burmans* (65.7%) and *Indo-Aryans* (38.5%). A marginally low prevalence was found in children having toilet compared to children who had not. Taking anthelminthic drugs in last six months had no effect on parasitic infection.

A study conducted in remote hilly village in Western Nepal revealed the highest prevalence of *A. lumbricoides* followed by hookworm and *T. trichiura*. The incidence rate of parasitosis was (27.0%) with marginally higher prevalence rate in male. The higher prevalence of intestinal parasitosis has been reported among *Dalits* as compared to *Tibeto-Burman* and *Indo-Aryans*. Majority of the subjects has single parasitic infection (Ishiyama *et al*, 2003).

In 1997, Shrestha studied nail, fingers, house dusts and stool samples for the presence of STH. In the stool samples, roundworm infection was the commonest followed by whipworm and hookworm. However, there was low prevalence of STH eggs in the soil of kitchen garden and around toilet in a village located 12 km away from Kathmandu City. About 3.0% of house dust showed the contamination with *A. lumbricoides* eggs whereas no any helminthes ova were detected in nails. Females were infected slightly higher than males.

Sharma *et al* (2004) studied the prevalence of intestinal parasitic infestation in the children in Northeastern part Kathmandu Valley. 66.6% of the children had some kind of parasitic infections. *T. trichiura* was the commonest parasite (34.6%) among the helminthes followed by hookworm (23.7%) and *A. lumbricoides* (13.8%). Among protozoa, *E. coli* (6.45%) topped the list followed by *E. histolytica* and others. More than half of the children had mixed parasitic infections.

The study was done to identify an effective intervention group for the control of the intestinal helminth infection among school-age children in rural Nepal. A total of 1677 stool samples of school-age children from 25 schools and 1014 samples from 25 communities in rural Nepal were examined. Formalin-ether sedimentation technique was used for the microscopic examination of the stool samples and identified three major intestinal helminths: *A. lumbricoides*, hookworm and *T. trichiura*. The results revealed that under-6-year-old children at the schools were more widely infected with at least one of the three major helminths than those in the communities. The significant difference was found in the prevalence of *A. lumbricoides*, hookworm and *T.trichiura* between under-6-year-old children at the schools and those at the communities. The study results suggest that an appropriate deworming programme is needed for the newly enrolled under-6-year-old children in the primary schools in Nepal (Poudyal *et al*, 2006).

Mukhopadhya *et al* (2005) surveyed 253 diarroheal stool samples of children in western Nepal. Of 253 children with persistent diarrhoe, 90 (35.5%) had protozoal infections, 63(24.9%) helmithes, 32 (12.6%) had bacterial infections and 16(6.3%) had mixed infections. The common helminth was *A. lumbricoides* (39.6%) followed by *T. trichiura* (28.5%), hookworm (26.9%) and *S. stercoralis* (4.7%). Among the protozoa, *G. lamblia* (67.7%) was the most predominant, *E. histolytica* (27.7%), *Cryptosporidium* spp. and *C. cyatenensis* each 2.2%.

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

A Health care based study by Sherchand *et al* (1999) showed that the 632 (29.8%) among the total 2,123 patients were infected with *C. cayetanensis*. Different other parasites were also detected from 434 (68.7%) along with the infection of *C. cayetanensis*. The predominant protozoa was *G. lamblia* 133(21.0%), followed by *E. coli* 102 (16.1%), *E. nana* 52 (8.2%), *Chilomastix mesnili* 21 (3.3%), *B. hominis* 22 (3.5%), *I. belli* 14(2.2%), *E.histolytica* 9(1.4%). Among the helminthes *A. lumbricoides* 78(12.3%) was most prevalent followed by hookworm 53(8.2%), *T. trichiura* 43(6.8%), *H. nana* 31(4.9%), *S. stercoralis* 19(3.0%) and *E. vermicularis* 16(2.5%). Similarly the study was carried out in green vegetables including cabbage, lettuce, cauliflower, spinach, green onions, radishes, green leafy vegetables, mustard leaves and carrot. Among the studied samples cabbage, lettuce and mustard leaves were found to be contaminated with *C. cayetanensis*. Likewise, in the study, drinking water, sewage water collected from the same endemic areas of the Nepal were found to be contaminated with *Cyclospora* in June, July, August and November.

Cyclospora is an increasingly recognized cause of Traveler's diarrhoea, causing upto 11% to 20% of diarrhoea in the studies of expatriates in Nepal (Shilm *et al*, 1991; Hoge *et al,* 1995). The infection occurs most commonly via contaminated water (Huang *et al,* 1995; Rabold *et al*, 1994). Contaminated food has long been proposed as a possible route for transmission of *Cyclospora* (Connor and Shilm, 1995).

Similarly Shercand and Cross carried out a longitudinal study on Cyclosporiasis in different parts of Nepal from April, 1995 until November, 2000. The study found that 1,619 (24.6%) were positive for *C. cayetanensis*. Among these infected, one or more parasites were detected. *G. lamblia* and *A. lumbricoides* being most common protozoa and helminthes, respectively. Likewise, green vegetables, collected from 14 vegetables markets consisted of cabbage, lettuce, cauliflower, spinach, green onions, radishes, green leafy vegetables, mustard leaves and carrot. Among these studied samples, cabbage lettuce and mustard leaves were found to be contaminated with *Cyclospora*. In the contrary, drinking water, sewage water collected from different areas of the Kathmandu valley were found to be contaminated with *Cyclospora* in June, July and August.

A study of total 1842 stool specimens from gastroenteritis patients was carried out from March 2005 to February 2006 in different areas of Nepal. Similarly samples of water, green leafy vegetables and faecal specimens from animals were also examined. Among the total, 146 (7.9%) were positive for *Cyclospora*. Green vegetables collected from 14 community and vegetable markets consisted of cabbage, lettuce, cauliflower, spinach, green onions, radishes, green leafy vegetables, and carrot in which lettuce, spinach and mustard leaves were found to be contaminated with *Cyclospora. Cyclospora* contamination in irrigation canal and Pond water was found high in June and July. Several other parasites: *G. lamblia*, amoeba, unidentified tropozoites, ova of *Ascaris*, larva of helminthes and many small insects, worms were detected. In two sources of water samples (pond and irrigation canal) *C. parvum* oocysts were also identified (Sherchand *et al,* 2007).

CHAPTER IV

4. MATERIALS AND METHODS

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

4.1 Methods

4.1.1 Vegetables

4.1.1.1 Subjects and study area

Vegetable samples were collected from June 2006 to July 2007 in different vegetable markets in Kathmandu Valley. These include Ason, Machhindrabahal, Tukucha, Bhimsensthan, Kalimati and Lagankhel. The samples were collected in different seasons i.e dry season (November to April) and wet season (June to September). Vegetable samples viz, *Chinese sag*, Garlic, Leafy vegetables, Radish, Carrot, Onion, Coriander and Salad were collected and taken to the laboratory. The samples were processed and examined in the laboratory of National Institute of Tropical Medicine and Public Health Research, Narayan Gopal Chowk Shanka Marga, Kathmandu, Nepal.

4.1.1.2 Sample processing

The samples were processed according the following method.

1. Vegetables samples were collected and taken to laboratory.
2. The samples were washed by shaking them in a glass cylinder filled with one liter of Tris buffer saline.
3. It was allowed to sediment for overnight.
4. The supernatant was discarded.
5. The precipitated strain of washed vegetables were poured to centrifuge tube (10 ml)
6. It was centrifuged at 2000 rpm for 10 minutes.
7. The supernatant was discarded.

8. The deposit was transferred to wide mouthed glass bottle filled with saturated brine solution.
9. The mouth of the bottle was covered with cover slip not allowing to trap the air bubbles.
10. The bottle was left undisturbed over night.
11. Next day cover slip was removed carefully and placed on the glass slide.
12. Then, microscopy was done.

4.1.2 Stool specimens of school children

4.1.2.1 Subject and study area

The study was carried out in the laboratory of National Institute of Tropical Medicine and Public Health Research, Maharajgunj, Nepal. The stool samples were collected from the school children of Thimi. Thimi is situated at the distance of 4 km south east of capital city Kathmandu. It is inhabited by *Newars*, *Brahmins* and *Chettries*, majority being *Newars.*

4.1.2.2 Sample collection

Before the distribution of containers, the school children were given the brief description about health and hygiene, source of parasitic infection, modes and effects of infection and preventive measures as well as the importance of the stool examination. Then, the questionnaire (Appendix no. III) was filled after the verbal consent with teachers and students.

The clean, dry and leak proof plastic container was distributed and students were advised to collect about 20 gm or 20 ml stool sample avoiding contamination with urine or other substances. The container was labeled with subjects name/code, class, date and time of sample collection.

Next day the samples were collected and taken to Institute of Tropical Medicine and Public Health Research, Maharajgunj, Nepal.

4.1.2.3 Sample processing

Each stool sample was processed in 2 steps as:

A. Macroscopic examination

B. Microscopic examination

A. Macroscopic examination

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

a. Colour

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

b. Consistency

Based on consistency stool specimen were classified as formed, semi-formed and loose. The trophozoites are usually found in the soft or loose stools whereas the protozoal cysts are found in formed and semi-formed stool. Heminthic eggs and larva can be found in any type of stool specimen.

c. Blood and mucus

The stool specimens were observed whether it contains blood and mucus or not as this indicates the pathological condition. Blood and mucus may be found in stool from patients with amoebic dysentery, intestinal taeniasis, intestinal schistosomiasis, invasive balantidiasis and in severe *T. trichiura* infections. Other non parasitic conditions in which blood and mucus may be found include bacillary dysentery, *Campylobacter enteritis*, ulcerative colitis, intestinal tumor and haemorrhoids.

d. Adult worms and segments

The stool specimens were observed whether it contains adult worms and segments or not.

B. Microscopic examination

Microscopic examination was carried out to know the presence of parasites. Likewise it also results in the presence or absence of RBCs and WBCs in the stool. To detect parasites Saline wet mount, Iodine wet mount method and Modified Acid- Fast (AF) staining technique were performed followed by concentration method. This concentration method concentrates the eggs, larvae and cysts if they are present in small number increasing the sensitivity of the microscopic examination. The mounted slides were examined under low power (10 X) followed by (40X) and the AF stained smears were examined in oil immersion (100X). By microscopy protozoal cysts, oocysts, trophozoites and helminthic eggs or larvae were detected based on their morphology, motility and staining characteristics.

I. Concentration technique

i. Formal-ether sedimentation method leading to saline/ iodine wet mount

This is the most sensitive method of concentrating cysts, eggs and larva without distortion of their morphology. It takes short time and the chances of error are minimum. The technique was conducted as follow:

a. About 1 gm of faeces was emulsified in about 4 ml of 10% formal-saline shaken well.
b. The suspension was allowed to stand for 30 minutes for fixation.
c. About 3-4 ml of 10% formal-saline was added and the tube was capped and shaken well.
d. The suspension was sieved through double gauze in a funnel into 15 ml centrifuge tube.
e. 3-4 ml of ether was added and the tube was shaken vigorously for 5 minutes.
f. The tube was then centrifuged at 1000 rpm for 10 minutes.
g. The layer of faecal debris formed between ether and formalin was removed along the side of the tube with a stick.
h. The supernatant layers of liquid were poured off.
i. The sediments were tapped and smear was made in glass slide.
j. The smear was examined by iodine and saline wet mounts under the microscope.

ii. Saline wet mount

It was used to detect protozoan cysts and helminth ova and larvae. A drop of normal saline was taken on a clean glass slide and a drop of sediment from above process was mixed with it, covered with cover slip and observed under microscope.

iii. Iodine wet mount

A drop of 5 times diluted Lugol's Iodine was taken on a slide. A drop processed stool was mixed with it and observed under microscope after covering with a cover slip. It was mainly used to observe the protozoal cyst. Iodine stained cysts showed pale reflectile nuclei, yellowish cytoplasm and brown glycerol material.

II. Sheather's sucrose flotation technique

Sheather's sucrose floatation technique was applied for the diarrhoeal stool samples followed by modified acid fast staining for the dectection of the oocysts of *C. parvum, I .belli, C. cayetanensis.*

1) About one gram of stool was mixed with 5 ml of normal saline in test tube.
2) The suspension was filtered through 3 layers of cotton gauze to obtain 2 ml in a test tube.
3) The tube was filled up to its brim with the sucrose solution. The tube was centrifuged at 1000 rpm for 10 minutes. With the help of a bent inoculating loop of shorter length the particles floating on the top surface of the sucrose solution were picked up and were used to prepare smears on the glass slide.
4) The smear was stained with Modified Acid Fast stain.

Modified AF staining (Ziehl-Neelson)

It is required for the accurate identification of *C. parvum, I. belli, C. cayetanensis* and the spores of *Microsporidia* spp. The oocysts are acid-fast and stained red or pink against background stained with Malachite green. The Cold Kinyoun method was followed in this study.

1) The smear was made with the particles obtained from Sheather's sucrose flotation

method and dried on air.

2) The smear was fixed in absolute methanol for 3 minutes.
3) The slide was flooded with Carbol Fuschin for 15-20 minutes and washed with tap water.
4) The smear was decolourised with 1% acid alcohol for 10-15 seconds.
5) The smear was washed with tap water and then counter stained with 0.5% Malachite Green for 30 seconds.
6) The slide was washed with tap water, air dried and examined under 40x followed by oil immersion (100x).

Sporulation of *C. cayetanensis* oocysts for the identification

C. cayetanensis oocysts are excreted unsporulated in the feaces. Specific identification of this coccidian parasite can be established by stimulating its sporulation and subsequent finding of two sporocysts within each oocysts of the parasites. For the enhancement of sporulation, about 2gm of stool sample was mixed with about 5ml of 2.5% Potassium dichromate solution and incubated at room temperature for 15 days. Assessment of sporulation was confirmed in light microscopy by observing two sporocysts in each oocysts.

Deworming of students.

Those students who had parasitic infections were given a full course of anti-parasitic drugs. The complete dose of antiparasitic drugs distributed were albendazole, metronidazole, niclosamide and trimethoprim sulfamethoxazole according to the parasites detected. The respective antiparasitic drugs were distributed with the help of local health worker (CMA).

Statistical analysis

Statistical significance was analyzed by using Chi-Square (χ^2) test. The results were considered significant if the P-values were less than 0.05.

CHAPTER V

5. RESULTS

5.1 Vegetable samples

Within the study period of June 2006 to July 2007, a total of 261 vegetable samples were collected from different vegetable markets in Kathmandu Valley. These include

Ason, Machhindrabahal, Tukucha, Bhimsensthan, Kalimati and Lagankhel. Most of the vegetables were grown in Bhaktapur district one of the main supplier of vegetables in the valley.

Out of 261 vegetable samples, 77 (29.5 %) were found to be positive for parasites. Out of 77 positive samples, 74 (28.4%) were positive for *Cyclospora* spp. and 3 (1.2%) with hookworm eggs.

Table 1 Parasitic recovery rate from different vegetables

S.N	Type of vegetables	Samples n	Positive	%
1	*Chinese sag*	55	27	49.0
2	Garlic	9	3	33.4
3	Leafy vegetable	50	15	30.0
4	Radish	18	5	27.8
5	Carrot	20	5	25.0
6	Onion	58	14	24.1
7	Salad	14	3	21.4
8	Coriander	37	5	13.5
Total		**261**	**77**	**29.5**

Table 2 Parasitic recovery rate from vegetables in two different seasons

Seasons	Samples n	Positive	%	P-value
Dry	107	27	25.2	P>0.05
Wet	154	50	32.2	
Total	**261**	**77**	**29.5**	

Out of 261 vegetable samples, 107 samples were collected in dry season (November to April) and 154 in wet season (June to September). Among 107 samples collected in dry season, 27 (25.2%) were contaminated with some kinds parasites. Similarly, out of 154 samples collected in wet season, 50 (32.5%) were positive for parasites. But the difference was not statistically significant (Table 2).

5.2 Stool samples of school children

As the most of the vegetable samples were from Bhaktapur (Thimi) area, we also collected stool samples from school children in Thimi area to see the prevalence of intestinal parasites.

Table 3 Pattern of Parasitic positive rate in school children

Gender	Total n	Positive	%	P-value
Male	137	84	61.3	**P>0.05**
Female	178	122	68.5	
Total	**315**	**206**	**65.4**	

Of the total 315 stool samples collected from school children, 206 (64.5%) were found to be positive for some kinds of parasites. The positive infection rate in boys and girls were 61.3% (84/137) and 68.5% (122/178), respectively. The difference was not statistically significant (P>0.05) (Table 3).

Table 4 Types of intestinal parasitic infection in school children

Types of infection	Total n	%
Single	**79**	**38.4**
Protozoa	9	11.4
Helminths	70	88.6
Multiple	**127**	**61.6**
Protozoa	50	39.4
Helminths	50	39.4
Protozoa + Helminthes	27	21.2
Total	**206**	**100.0**

Out of 206 positive cases, 38.4% school children were infected with single type of infection either protozoa or helminthes. Of single parasitic infection, 11.4% children were infected with protozoa and 88.6% were infected with helminth parasites. 61.6% students had multiple infection out of which 39.4% had multiple protozoal infection, 39.4% had mixed helminthic infection and 21.2% had both protozoal and helminthic infection (Table 4).

Table 5 Frequency of parasites detected in school children

Types of parasites	Total n (n=392)	%
T. trichiura	116	29.2
A. lumbricoides	62	15.8
Hookworm	32	8.2
H. nana	11	2.8
Taenia spp.	1	0.3
E. vermicularis	1	0.3
Total helminthes	**223**	**56.9**
E. histolytica	55	14.0
G. lamblia	36	9.2
E. coli	26	6.6
B. hominis	25	6.2
E. nana	15	3.8
Cyclospora spp.	9	2.3
E. hartmani	3	0.8
Total protozoa	**169**	**42.8**

Out of 392 parasites obtained from 315 stool samples (206 positive samples), 56.9% were helminthes and 42.8% were protozoan parasites. Among the helminthes, *T. trichiura* was the most common (29.6%) followed by *A. lumbricoides* (15.8%), hookworm (8.2%), *H. nana* (2.8%), *Taenia* spp. (0.3%) and *E. vermicularis* (0.3%). Among protozoa, *E. histolytica* (14.0%) was the most common followed by *G. lamblia* (9.2%) and *E. coli* (6.6%) (Table 5).

Table 6 Age wise distribution of parasitic infection in children

Age	Total n	Positive	%	P-value
≤ 10	169	110	65.1	**P>0.05**
> 10	146	96	65.7	
Total	**315**	**206**	**65.4**	

Of total 315 study population, 146 were of age group above 10 years and 169 were age group below 10 years. Among age group above 10 years, 96 (65.7%) were found to be infected with parasites and among age group equal or below 10 years, 110 (65.1%) were found to be infected with parasites, however, statistically insignificant (Table 6).

Table 7 Prevalence of parasites in different ethnic groups

Ethnic group	Total n	Positive	%	P-value
Dalit	36	22	61.1	**P>0.05**
Tibeto-Burman	178	117	65.7	
Indo-Aryan	101	67	66.3	
Total	**315**	**206**	**65.4**	

Of different ethnic groups, 36 were *Dalits*, 178 were *Tibeto-Burmans* and 101 were *Indo-Aryans*. Among them 22 (61.1%) *Dalits*, 117 (65.7%) *Tibeto-Burmans* and 67 (66.3%) *Indo-Aryans* were found to be infected with parasites. The difference was statistically insignificant (P>0.05) (Table 7).

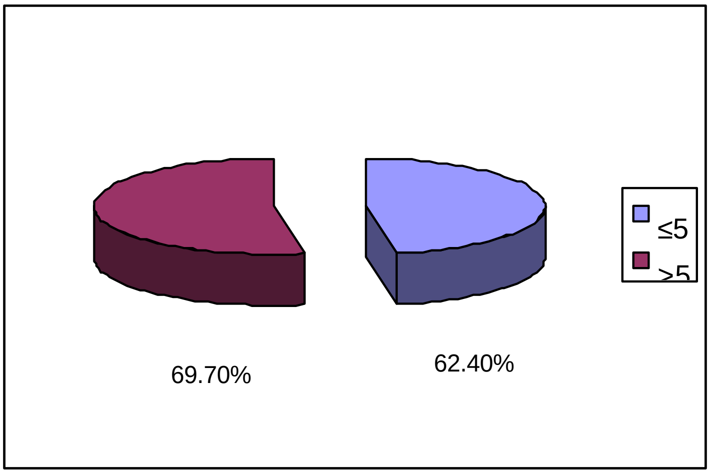


Fig 1. Prevalence of parasites according to family size

The higher (69.7%) prevalence of parasitic infection was observed in family size > 5 than in family size ≤ 5(62.4%) with no such significant difference (P>0.05) (Fig 1).

Table 8 Parasitic prevalence in relation to presence of toilet at their houses

Toilet	Total n	Positive	%	P-value
Yes	242	148	61.2	**P<0.05**
No	73	58	79.5	
Total	**315**	**206**	**65.4**	

The children without toilet at their home were found to be significantly more infected than those having toilet (P<0.05). Out of 242 children having toilet, 148 (61.2%) were infected whereas out of 73 children not having toilet, 58 (79.5%) were infected with parasitic infection (Table 8).

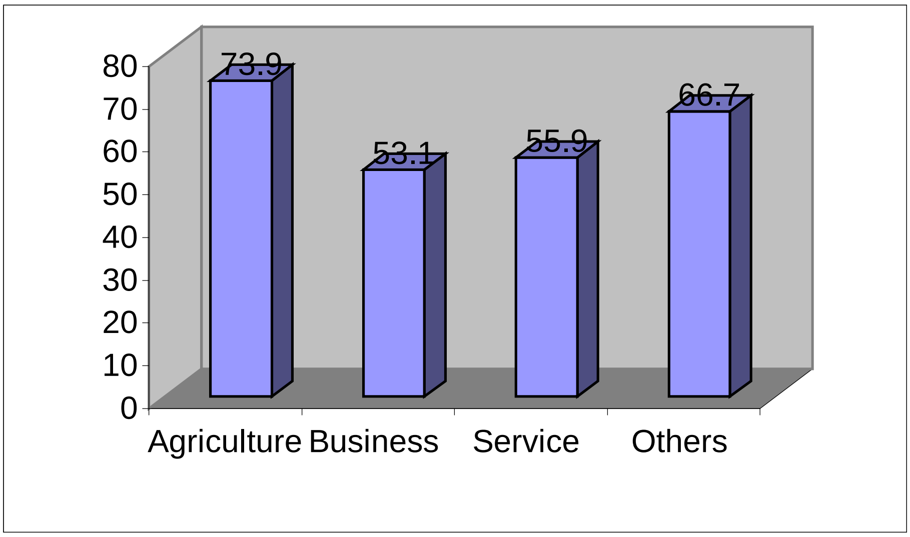


Fig 2 Prevalence of parasites according to parent's occupation

The highest prevalence rate of parasitic infection was found in children from parent with agriculture as occupation (73.9%), followed by parent occupation other than mentioned (66.7%), business (53.1%), and service (55.9%) . The difference was statistically significant (P<0.05) (Fig 2).

Table 9 Prevalence of parasitic infection in relation to anthelminthic drug intake in the past six months

IA*	Total n	Positive	%	P-value
Yes	15	5	33.3	
No	300	201	67.0	**P<0.05**
Total	**315**	**206**	**65.4**	

***I** = Intake **A** = Anthelminthic drug

In relation to the use of anthelminthic drug in past six months, out of 15 students who had intake anthelminthic drug, 5 (33.3%) were found to be infected with parasites where as out of 300 students who had not taken anthelminthic 201 (67.0%) were found to be infected with statistically significant (P<0.05) (Table 9).

Table 10 Parasitic infection among the children according to nail cutting habit

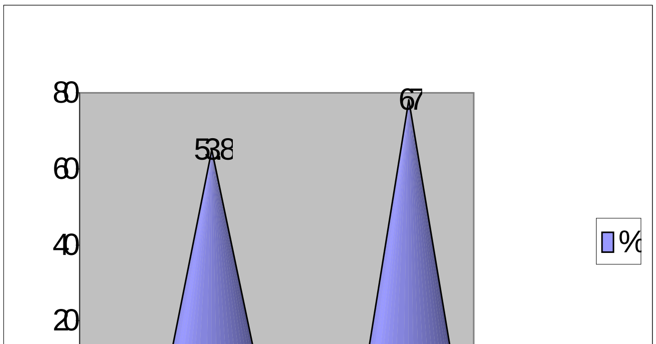
Nail cut	Total n	Positive	%	P-value
Yes	214	131	61.2	**P<0.05**
No	101	75	74.2	
Total	**315**	**206**	**65.4**	

Regarding to nail cut habit of students, the higher prevalence was found in those without nail cut. Among 214 students with nail cut, 131 (61.2%) were found to be positive while among 101 students who had not nail cut, 75 (74.2%) were found to be infected with different types of parasites with statistically significant (Table 10).

Table 11 Parasitic infection according to the source of drinking water

Source of water	Total n	Positive	%	P-value
Tap	238	154	64.7	**P>0.05**
Others	77	52	67.5	
Total	**315**	**206**	**65.4**	

According to the source of water used more prevalence was found in those who used water source other than tap (67.2%). These include spring spout, *Kuwa,* pump water, open well and tap (piped) water users (64.7%) with statistically insignificant (Table 11).



*Boiled water and filtered water

Fig 3 Prevalence of parasitic infection according to the type of water used for drinking purpose

With regard to the type of water used, more prevalence of parasitic infection was found in those students who use untreated (non-boiled) water. Out of 276 untreated water users, 185 (67.0%) were found to positive with some kind of parasites. This was marginally higher than treated water consumers (53.8%) with statistically insignificant. (Fig 3)

Table 12 Parasitic prevalence according to symptom of GI infection

GI symptoms	Total n	Positive	%	P-value
Yes	142	94	66.2	**P>0.05**
No	173	112	64.7	
Total	**315**	**206**	**65.4**	

Among the 142 students having complain of abdominal pain, 94 (66.2%) were found to positive for parasites. On the other hand, 173 have no complain among whom 112 (64.7%) were found to infected with some kind of parasites. This was not significant statistically (Table 12).

Table 13 Parasitic prevalence rate according to eating habit.

Types of food	Total n	Positive	%	P-value
Non vegetarian	295	195	66.1	

Vegetarian	20	11	55.0	**P>0.05**
Total	**315**	**206**	**65.4**	

Of the total population 295 were non vegetarian and 20 were vegetarian. Among them 195 (66.1%) of non vegetarian and 11 (55.0%) of vegetarian were found positive for parasitic infection. The difference was statistically insignificant (P>0.05) (Table 13).

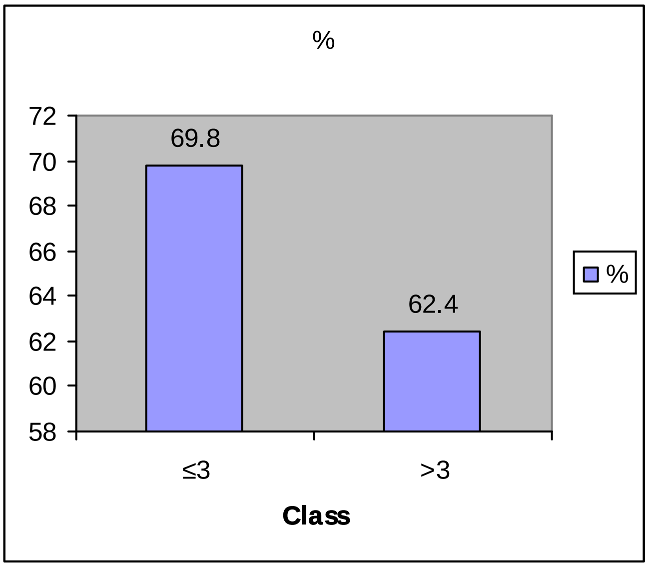


Fig 4 Prevalence of parasitic infection according to education level

The highest prevalence of parasitic infection was found in the children of class ≤3 (69.8%) whereas in the class >3 the positive rate was 62.4% but it was not statistically significant (Fig 4).

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

Nepal is a small and impoverished country with various parasites and bacterial infections, (Rai *et al,* 2001, 2002a) consisting one of the important causes of morbidity and mortality (Rai *et al*, 2001, 2002a).

In stool samples, thirteen species of intestinal parasites were detected, six helminths and seven protozoa. Among them, *T. trichiura* (29.2%) was found to be the most common parasite. *T. trichiura* topping the list of parasites have also been reported from Nepal (Ishiyama *et al*, 2001; Uga *et al*, 2004; Sharma *et al*, 2004; Rai *et al*, 2005; Sherchand *et al*, 2007) and elsewhere (Kan 1983; Rajeswari *et al*, 1994; Kabatereine *et al*, 1997; Lee *et al*, 2000; Uga *et al*, 2005; Jamaiah and Rohela, 2005). It could be due to ineffective deworming with single dose of anthelminthic drug particularly in case of heavy infections. 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 high prevalence of *Trichuris* is because of its special mode of attachment to cecal mucosa, longer lifespan of parasites as well as its refractory reaction to most anthelminthic and remains in intestine causing chronic infection. The higher prevalence of *Trichuris* in this study and other studies showed that *Trichuris* is the most common intestinal helminth. Therefore, effective deworming of the parasites should be done.

In present study, *Ascaris* prevalence rate in children was marginally reduced than that of *Trichuris*. This was not consistent with the previous reports from Nepal (Rai *et al*, 1998; Sherchand *et al*, 1999; Sherchand *et al*, 2001). However, this might be due to the twice-yearly single dose deworming program. Furthermore, *Ascaris* eggs can survive in environment for longer period due to the presence of chitin protein layer in their shell (Rai and Rai, 1999).

In this study, among the school children very low prevalence of hookworm was detected (8.2%). This was consistent to the year-to-year incidence ranging from 3.8%

to 10.7% as reported earlier (Rai *et al*, 1997). However, the higher rate of finding of hookworm reported (Rai *et al*, 1997) could be due to difference in the study population in which they have included hospital-attending people having some kinds of abdominal ailments. On the other hand, in part, this also could be due to the use of shoes/slippers by school children even in rural areas, which prevent skin penetration by larvae. In one study from Nepal hookworm topping the list has also been reported (Sherchand *et al*, 1997). The geographical variation may be another factor for lower prevalence of *A. lumbricoides* (Adhikari *et al*, 2006).

In this study, *H. nana* (2.8 %) was detected from the stool specimen of school children. *H. nana* is most common tapeworm infecting man in Nepal. This was in agreement with the report given previously by other researchers (Sherchand *et al*, 2001; 2007). It was reported to be 3.3% in southern Nepal (Sherchand *et al*, 1997) and 4.9% in Kathmandu Valley (Sharma *et al*, 2004). *H. nana* was also reported as the commonest tapeworm in the Kathmandu Valley (Sherchand *et al*, 1996). Also a ten years (1985-1994) study conducted in hospital population has shown an incidence of less than 5% through the study period except in the first year (Rai *et al*, 1994; 1995). Eggs of *H. nana* (2.6%) have also been found in soil samples studied in Kathmandu (Rai *et al*, 2000b). This seemed to be due to co-existence of people with rodents inside their home. Infection takes place by ingestion of fleas containing infective larva. Therefore, it is associated with the poor sanitation and low socio-economic status in the community. This prevalence in present study may be due to faeco-oral route by ingesting of eggs from contaminated hands, poor personal hygiene and poor environmental hygiene that contribute to result in such prevalence among the children. Rarely the transmission occurs from the ingestion of food contaminated with fleas harbouring the cysticercoid larvae.

Present study showed the very low percentage of *Taenia* spp. and *E. vermicularis* (one for each). Similar low prevalence of *E. vermicularis* and *Taenia* spp. in faecal samples collected in southern Nepal was found in 2.2% and 1.6%, respectively (Sherchand *et al*, 1997). This low prevalence of *E. vermicularis* might be due to the detection technique. Had scotch tape (NIH swab) method been used, the actual prevalence would have obtained. Similarly, the low prevalence of *Taenia* infection appears to be attributed to low raw pork eating in Nepal. Though this is very uncommon case, it is worthwhile to investigate in this regard in future.

Among protozoa, *E. histolytica* was found to be the commonest parasite in this study. This was in agreement with the report from Nepal (Nepal and Palfy, 1980) and elsewhere (Hassan *et al*, 1994; Ali *et al*, 2005; Hesham *et al*, 2006). However, this finding was inconsistent with some of the reports from Nepal (Yong *et al*, 2000; Rai *et al*, 2001a; Sharma *et al*, 2004; Rai *et al*, 2005) and other countries in Asia (Uga *et al*, 2005). Some reports have shown *E. coli* as the commonest parasite. This might be due to the commensal nature of this protozoan parasite. As reviewed by Rai (2005), the incidence of *E. histolytica* infections ranges from 3% to 28.8% in Nepal. Nepal and Palfy (1980) reported the highest incidence rate of *E. histolytica* (28.8%) from Nepal. The decrease in the prevalence rate might be due to the difference in the study population as well as the site of study. Infection with *E. histolytica* is common in people of developing countries; it predominantly affects people with poor socioeconomic conditions, non-hygienic practices and malnutrition (Braga *et al*, 1998). Amoebiasis may be more severe during pregnancy and lactation and in people with immunodeficiency, homosexuals, immigrants from certain tropical countries, and travelers. Urban migration, the deterioration of the economics of certain developing countries and the increasing size of urban slums with crowded, unhygienic conditions may accelerate the spread of amoebiasis and so result in greater morbidity and mortality from this infection in future (WHO, 1987).

Most of the studies from Nepal have shown *G. lamblia* as the commonest intestinal parasite (Khetan 1980; Rai *et al*, 1995; Rai *et al*, 2002b; Sherchand *et al*, 1997) and similar findings have also been reported by other investigators (Saha *et al*, 1995; Okyay *et al*, 2004). In contrast, the prevalence of *G. lamblia* was lowest in this study. This was in agreement with the previous findings of Ishiyama *et al* (2003) from Nepal and Virk *et al* (1995) from India. In part, this might be due to improvement of water quality and awareness among the people during recent years. Further, the secretary IgA and IgM might have played some role in these findings as these two antibodies have been reported to play a role in the clearance of intestinal parasites (Oda *et al*, 2002).

In the study, the prevalence rate of *E. coli* was found to be lower than that of *E.*

histolytica. The high *E. coli* prevalence rate has been reported by Oberst and Alquiza (1987) in Phillipines, Cabrera *et al* (2000) in Peru and Saito *et al* (1996) in Paraguay. In this study, prevalence rate of *B. homonis* was found to be only 6.2%. *B. hominis* has been reported earlier in people with abdominal complaints (Sherchand *et al*, 1996) and in general population (Gianotti, 1990; Rai *et al*, 2001a). In contrast, Ishiyama *et al* (2001) reported that the prevalence of 7.8% in school children of Kathmandu Valley. However, other workers in similar types of study did not report *B. hominis*. It may be due to the autolysis of *B. hominis* cysts during the time lapsed between sample collection and examination (Uga *et al*, 2004).

This study showed the low prevalence rate of *E. nana* (3.8%). Similar low prevalence rate was reported by Uga *et al* in 2004. Likewise, Ishiyama *et al* in 2001 found less than 1.0% of *E. nana* which was lower than this study. In this study very low rate (0.8%) of *E. hartmani* was found.

Similarly, the prevalence of C*yclospora* was found to be 2.3%. This was in agreement with the report of Mukhopadhya *et al* (2007). However, this was not consistent with the previous reports (Sherchand *et al*, 1999, 2001; Sherchand *et al*, 2007). It may be due to utilization of contaminated drinking water with fecal materials. Contaminated food has long been proposed as a possible route for transmission of *Cyclospora* (Cannor and Shlim, 1995). Vegetables are easily contaminated and provide organisms with an optional environment for survival prior to host ingestion. Cabbage, lettuce and mustard leaves were found to be contaminated with *Cyclospora,* which confirmed that food borne transmission, is also feasible (Sherchand and Cross, 2001).

Other possible sources of infection are birds, rodents and insects due to which food and rippen fruits become unfit for human consumption. However, more studies are needed to determine the correlation between parasitic infection and other sources.

Almost two third (68.5%) of the female school children were infected by the intestinal parasites. Similar findings have also been reported from Nepal (Sherchand *et al*, 1997; Yong *et al*, 2000; Uga *et al*, 2004) and elsewhere (Kightlinger *et al*, 1995; Rajeswari *et al*, 1994; Jamaiah and Rohela, 2005). Marnell *et al*, 1992 has also reported the high prevalence of infection (70.0%) among female. But these appeared to be in contrast to the earlier reports from Nepal (Rai and Gurung, 1986; Ishiyama *et al*, 2001). This indicated that both male and female children are equally exposed to infections in this area.

Almost one fifth (38.4%) of the children were infected with single parasite and the majority of which (88.6%) were infected with the helminth parasite. Helminthes dominating the protozoan parasite were in agreement with the earlier reports from Nepal (Nepal and Palfy 1980; Estevez *et al*, 1983; Rai and Gurung 1986; Rai *et al*, 1995, 2000a, 2001a; Sherchand *et al*, 1996; Uga *et al* 2004) and else where in the world (Kasuya *et al*, 1989; Hasegawa *et al*, 1992). It may be due to the consumption of the vegetables washed in contaminated river water and raw vegetables being sold in the market. Besides, unhygienic and unmanaged street vegetable market may be the cause behind it. This was also in the agreement with the considerably high soil contamination rate in Kathmandu Valley with helminth parasite eggs (Rai *et al*, 2000b). Inspite of the regular twice-yearly deworming under the National Vitamin A Program, helminth parasitic infection was higher (56%) among the children. This result indicated that deworming alone is not effective to eliminate the helminth parasites and effort should be placed in the improvement of sanitary condition.

Regarding to the age of the children, no significance difference was found in distribution of the parasites. Almost all the age group had the same prevalence of parasites. This appeared prevalence might be associated with their unhygienic habit and age. It was not in agreement with the findings from Nepal (Rai *et al*, 1994; Rai *et al*, 2002b; Sharma *et al*, 2004; Rai *et al*, 2005). This could be due to the increment of their childish activity with their age and move around frequently increasing the possibility of acquiring infections from sleazy environment.

In this study, the significantly higher incidence of parasite was found in *Indo-Aryans* followed by *Tibeto-Burmans* and *Dalits*. Similar type of very low prevalence in *Dalits* has been found by Sharma *et al* (2004) and Rai *et al* (2003; 2005). This was dissimilar to the result that had been reported earlier (Rai *et al*, 2002b; Ishiyama *et al*, 2003). Although relatively poor hygienic, cultural and behavioral practice, low socioeconomic status, illiteracy and lack of health awareness in *Dalits* compared to their counter social groups, no significant differences in parasite rates was found. This also supports the wide distribution of parasites in the study area. This could also be due to the small sampling size as has been explained by other investigations (Rai *et al*, 2002b; NPC, 2002).

In general, it is presumed that children of larger and socio-economically under privileged family are infected with various infective pathogens including intestinal parasites. Almost two third of the children living in large (>5) family size compared with those children coming from smaller family (<5) size were found to be positive for some kinds of parasites. This finding, however, was consistent with the finding of others (Karrar and Rahim, 1995). However, this was not consistent to the finding of Rai *et al* (2005).

However, parasitic infection rate can also be correlated well with the unavailability of toilet at their houses and the difference was statistically significant. This finding was consistent with the previous findings from Nepal (Rai *et al*, 2002b; Rai *et al* 2005) and elsewhere (Sorensen *et al*, 1994; Toma *et al*, 1999). Lack of or inadequate toilet affects the environmental sanitation on prevalence of STH. Due to lack of proper toilet, indiscriminate defecation around the house, fields, road, playgrounds increase the chance of parasitic infection.

The significantly higher infection rate (73.9%) has been observed in children belonging to farming family. Farmers and their family members are more prone to infection with the soil contaminated with parasites as they are more exposed to soil and environment. They usually eat raw and unwashed vegetables. Further while working in the field, they are likely to be infected by the infective hookworm larvae. Elsewhere, similar trends of infection have been reported (Marnell *et al*, 1992; Habbari *et al*, 1999; Ishiyama *et al*, 2003) but without significance. Rai *et al* (2005) reported the lowest rate of infection in children with father's occupation as business. Similar report was found in this study. However, the education levels of parents influence the prevalence of parasitic infection in children as reported by other researchers (de Silva *et al*, 1996; Ishiyama *et al*, 2003). High parental literacy directly results in better employment potential, which leads to higher family income and therefore, better life standard and in turn lowers parasitic infection.

More than half (67.0%) of the children who had not taken anthelminthic drug in past 6 months had significantly higher parasitic infection than those who had taken anthelminthic drug. Similar findings were also reported by (Bundy *et al*, 1987; Albonico *et al*, 1999; Rai *et al*, 2005). This clearly indicates the importance of deworming. The prevalence of helminth infection was reduced remarkably and there was dramatic reduction in infection intensity after deworming program (WHO 2002). However, insignificant difference in the prevalence has also been reported earlier (Rai *et al*, 2002b).

In this study, higher parasitic prevalence rate was found in those children who had not cut their nails (74.2%) regularly with statistical difference. The finger nail is one of the many sources of infection and may be the important one in Nepal. The children are more commonly infected due to their unsanitary habit and poor knowledge of health as the children suck their fingers and play anywhere in the house and kitchen garden. Shrestha in 1998 reported the similar finding in those adult populations who did not cut nail regularly. Likewise, high positive rate was found in children too (65.1%). Similar parasitic positive reports from the nail dust were given in elsewhere in the world (Yodmani *et al*, 1983; Ismid and Rukmono, 1983).

No significant difference was observed in the infection rate of the children concerning with the use of treated water or untreated water. In this study, high prevalence (67.0%) was observed in untreated water consumers. This was in agreement with the previous reports given (Ishiyama *et al*, 2001; Oda *et al*, 2002).This may be due to heavily contaminated drinking water source of Kathmandu Valley (Adhikari *et al*, 1986; Ono *et al*, 2001). In the contrary, no significant difference was found in the sources of water. High prevalence was found to be in other than tap water users in which the sources include spring spout, open well, pump (tube well) and *Kuwa.* This might be due to the poor sanitary conditions, lack of awareness, unhygienic condition and lack of sanitary practices.

In this study, relatively low parasitic prevalence (29.5%) was found in the vegetable samples. Similar reports of the positive rate were given elsewhere (Ulukanligil *et al*, 2001; Robertson and Gjerda, 2001). This was not in agreement with the report given earlier (Monge *et al*, 1996). This might be due to the technique used to recover the parasites. Likewise, the season of sample collection may also affect the positive rate.

Protozoan parasites were found in high prevalence in this study (28.4%). This was in agreement with the findings elsewhere in the world (de Oliveira and Germano, 1992; Monge *et al*, 1996). This was not consistent with the report of Dong and Lee (1972). This might be due to the use of contaminated water for the washing purpose of the vegetables. Likewise, use of the night soil as the fertilizer during the cultivation of the vegetables attribute to contamination. In this study the helminthic prevalence was very low. Since the main source of contamination of helminthes is soil, the different parameters of the soil contribute the chance of contamination. Climatic factors, soil factors and biology of parasite in relation to transmission influence the transmission of the parasites. The parasitic positive rate was high in wet season. This was in agreement with the previous reports in Nepal (Sherchand *et al*, 1999, 2001, 2004; Sherchand *et al*, 2007).

Cyclospora infection occurs most commonly by the contaminated water and the oocysts are resistant to the chlorination and not readily detected (Huang *et al*, 1995; Sturbaum *et al*, 1998). It has been proposed that contaminated food is the probable route for the transmission of *Cyclospora* (Connor and Shlim, 1995). Vegetables are mainly suspicious since they are often ingested raw or undercooked. They are easily contaminated and provide organisms with an optimal environment for the survival prior to host ingestion. Use of the human waste as the fertilizer in crops and indirectly via the contaminated water used for crop irrigation and to freshen produce could lead the contamination of the vegetables. A combination of soil contamination and the adhesive nature of the soil-bearing eggs probably results in the gradual contamination of most of the objects in the houses and the public places as well as of the food, particularly vegetables grown in faeces –fertilized soil.

In the present study, almost all the samples studied (Chinese sag, Lettuce, Mustard leaves, Coriander, Green onion, Radish, Garlic and Carrot) were found to be contaminated. Similar results were reported in Nepal (Sherchand *et al*, 1999, 2001, 2004; Sherchand *et al*, 2007) and elsewhere in the world (Yodmani *et al*, 1983; Monge and Arias, 1996; Al-Binali *et al*, 2006). This may be due to use of feacally contaminated water in vegetables as irrigation water or directly contaminated hands of food handlers. Moreover, in Nepal vegetables available in the markets are rinsed into highly contaminated water of ponds or rivers in order to wash and clean the soil, but actually it becomes contaminated once again with STH. In cities of Nepal, the water supply is contaminated through the seepage into water pipes from sewage. The water sources themselves are polluted with contaminated deposits.

Present findings along with the previous reports indicated that the health status of Nepalese school children is still very poor. Approximately 2/3 of the health problems in Nepal are infectious disease (Rai *et al,* 2001b). Frequently epidemic occurs with high rate of morbidity and mortality. Among the various types of infectious disease, intestinal parasitosis (mainly soil transmitted helminthiasis) alone constitutes major health problem in Nepal (Rai and Gurung 1986; Rai *et al,* 1994a; 1995; 1997; 1998).

Many factors are involved in the failure of the parasitic control programme such as human behavior (eating habits, occupation) their beliefs (religion and culture), natural phenomenon (climate, rain, flooding) and the most serious problems was partial cooperation of the people in mass treatment. However, intestinal parasites have been decreased sharply in the developed countries like Japan (Yokogawa *et al,* 1983), Korea (Chai *et al,* 2001b) and Taiwan (Chen *et al,* 1993).

Soil transmitted helminthes infections are endemic in the communities where poor environmental sanitation and poor personnel hygiene, as occurs in majority of developing countries. Yodmani *et al* (1982) and Yu *et al* (1993) showed that environmental pollution, sanitary condition and human behavior play an important role in transmission of STH infections. STH (*A. lumbricoides, T. Trichiura* and hookworms) cause morbidity in humans in different ways by affecting nutritional equilibrium, inducing intestinal bleeding, inducing malabsorption of micronutrients, reducing growth, reducing food intake, causing complications such as obstruction rectal prolapsed and abscess and affecting congenital development.

Public health education regarding the improved personal and environmental hygiene are cardinal in the control, additionally, routine periodic screening of children and possibly, the caregivers will guarantee early detection, prompt therapy and interruption of transmission of the pathogens. Changing behavior like wearing slippers, avoiding raw vegetables and meat also help in declining the parasitic ratio. Development of proper sewage system and use of treated piped water are the main elements in controlling the parasite infection. These factors may help mitigate the impact of community based intestinal parasitosis.

Inspite of increase in the health awareness and knowledge of sanitation in people, there is still the presence of different kinds of parasites. This reveals that the health status of Nepalese people is still poor. It shows the poor hygiene and sanitary conditions, wide dispersion of parasites, influx of parasitic infected people from rural area, unplanned urbanization, direct disposal of sewage into stream, use of sewage water in irrigating vegetable fields, improper water supply etc in the valley. As health of citizens' and environment play the crucial role in development of socio-economic status of the country, these problems must be solved as soon as possible. The maintenance of health and hygiene and sound environment are the needs of human beings. For this, health education should be made accessible to the community either through the education program, media, information or else. Moreover, the parasitic infections can also be controlled when the socio-economic status of community is improved like better living status, proper sanitation, accessibility towards deworming, improvement in nutritional status etc. Further, for this, it requires not only the financial change but also the conceptual change in both individual and community.

The government of Nepal and private sector are also taking part in the promotion of health services to every citizen. In spite of these efforts, no significant progress has been made in controlling the intestinal parasitic infection in Nepal. Therefore, more effort is expected towards this and more practicable and reasonable policy has to be implemented without ambivalently.

6.2 Conclusion

Hence, it can be concluded that the intestinal parasitoses is still prevalent causing the major public health problems. *T. trichiura* was the most common helminth parasite in the school children reflecting the need of proper and effective management of the deworming programs. Similarly in vegetables also proper cleansing and cooking is necessary. Effective knowledge and proper hygenic education should be provided to the agriculturists and public.

CHAPTER VII

7. SUMMARY AND RECOMMENDATIONS

7.1 Summary

- A total of 261 vegetable samples were collected from the Kathmandu Valley. Among them 29.5% were found to be positive for the parasites.
- The oocysts of *Cyclospora* spp.was found most prevalence (28.4%) in vegetables followed by the hookworm larvae (1.2%). Parasitic contamination rate in vegetables was 32.2% in wet season and 25.2% in dry season.
- Among the total of 315 stool samples of school children, 64.5% had intestinal parasitic infection. Helminth parasites were the most common than the protozoa in children. *T. trichiura* (29.2%) was the commonest helminth and *E. histolytica* (14.0%) was the commonest protozoa. Other parasites detected were *A. lumbricoides*, hookworm, *H. nana*, *Taenia* spp., *E. vermicularis*, *G. lamblia*, *E. coli*, *B. hominis*, *E. nana*, *Cyclospora spp*., and *E. hartmani*.
- The study showed the higher prevalence in female (68.5%) children than male children (61.3%). Among the total children, 61.6% had the multiple parasitism while 38.4% had the single parasitic infection.
- Almost equal parasitic infection was found in the children of age equal to or less than 10 years and above 10 years (65.1% and 65.7% respectively).
- Of different ethnic groups, *Indo-Aryans* had the high parasitic infection rate (66.3%) followed by the *Tibeto-Burmans* (65.7%) and *Dalits* (61.1%).
- Higher prevalence (69.7%) of parasitic infection was observed in family size above 5 than the family size equals to or below 5 (62.4%).
- The children without toilet in their house were found to be more infected (79.5%) than those having toilet (61.2%).
- In this study, the highest prevalence rate of infection was in children from farming family (73.9%) and lowest (55.9%) in those whose parents were in service.

- The children who had who had not taken anthelminthic drug within past six months had significantly higher prevalence rate (67.0%) than those who had used (33.3%).
- The parasitic infection rate was higher (74.2%) in those children who had not cut their nail during the stool samples collection than those who had cut their nails (61.2%).
- Marginally low prevalence of parasitic infection rate (64.7%) was found in those children who use tap (piped) as the source of drinking water than other sources (67.5%).
- Regarding the type of water used for drinking purpose, more prevalence of parasitic infection was found in those students who used untreated water (67.0%) while that of treated water had lower prevalence (53.8%).

7.2 Recommendations

- The parasitic contamination had been found in almost all types of raw vegetables used in this study. However, this may not reflect the condition of different parts of the country. So this type of study should be carried out through out the country to know the exact figure. Likewise, more other vegetable samples should be studied to access the real picture of the situation of vegetables.
- Proper washing of vegetables with clean water is highly recommended. Washing of fruits, scrapping off the wax substances on the outer surface of fruits and vegetables are also the suggestible factor.
- High prevalence of STH has been observed in school children in the study conducted in sub urban area of the valley. So this type of study should be conducted throughout the country to obtain the real picture helminth parasites infection.
- Periodic as well as mass deworming pogramme in both urban and rural region of the nation must be continued to reduce the dispersion of the parasite in the community. Among the STH, *T. trichiura* was found to be most prevalent parasite in children. So single dose of anthelminth drug is insufficient and proper course of drugs should be administered.
- Periodic administration of antiparasitic drugs is highly recommended and awareness creating activities with regard to controlling intestinal parasitic infection should be launched.
- Since the transmission and persistence of intestinal parasitic infections are influenced by human behavior and culture, appropriate health education measures should be applied at all levels of program implementation.
- Though no significance impact of various predisposing factor studied was observed, use of toilet, maintaining hygienic conditions, not walking bare foot, proper washing of hands before and after meal, drinking of treated (boiled or filtered) water and others are highly recommended.

CHAPTER VIII

8. REFERENCES

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APPENDIX -I

Materials and Chemicals used

1 Chemicals and reagents

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

2 Materials

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

3 Equipments

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

APPENDIX II

COMPOSITION AND PREPARATION OF REAGENTS

Lugol's Iodine

Stock solution

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

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

Working solution (5 times diluted)
2 ml stock solution was mixed with 8 ml DW.

Physiological saline

NaCl	0.85 gm
Distilled Water	100 ml

10% Formalin

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

Dilute 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%.

0.3 % Tris buffer Saline

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

APPENDIX III

MICROBIOLOGICAL PROFILE

Serial No: **Date:**
Name: **Age:**
Gender: **Class:** **Vegetarian/Non vegetarian**

Patient's Clinical History: ……………………………………………………………….

…………………………………………………………………………………………

Questionnaire:

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

Report of stool examination

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

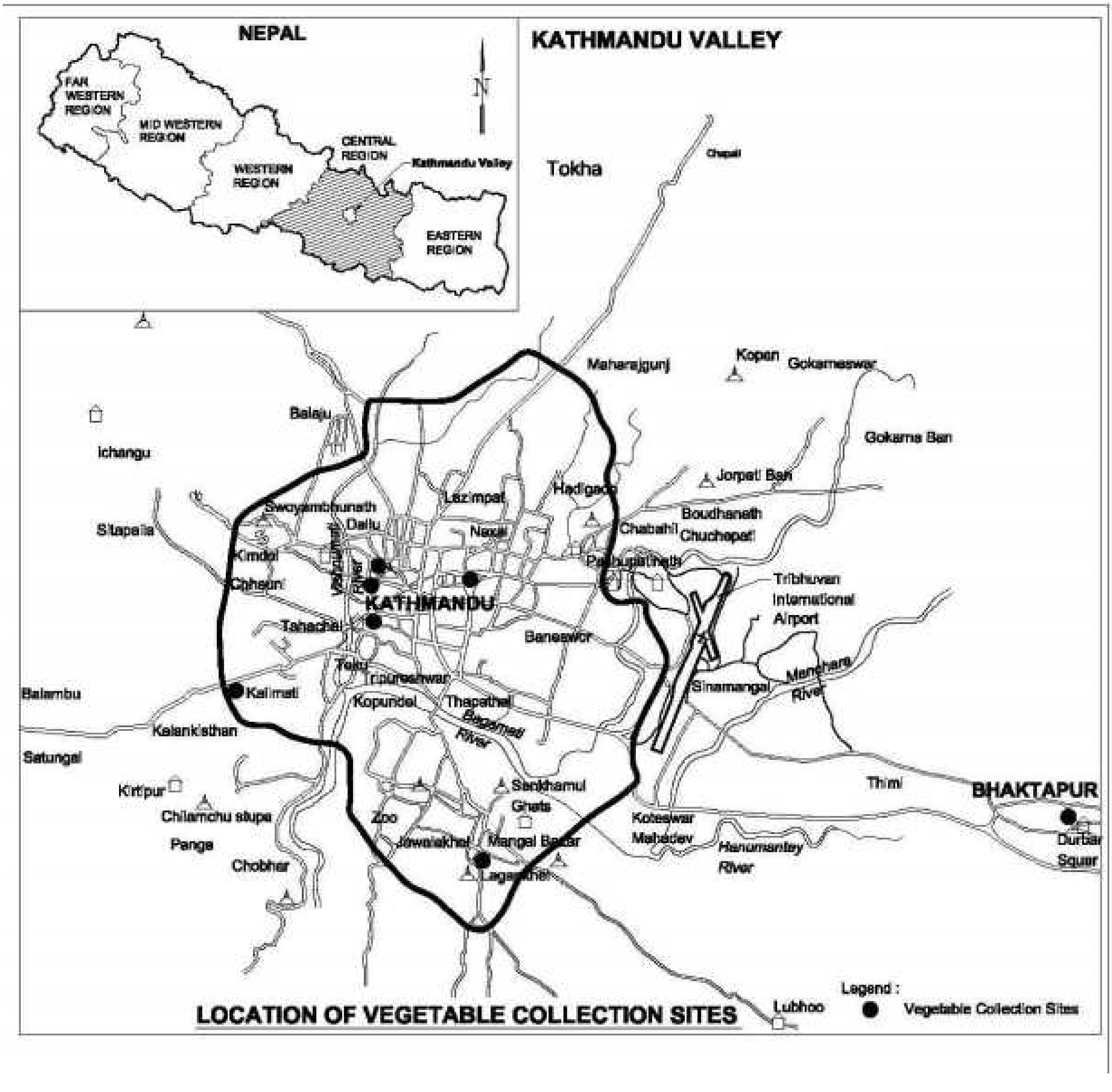
Authorized signature………………

Report of stool examination:

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

Authorized Signature:………………………….

APPENDIX IV



LOCATION OF VEGETABLE COLLECTION SITES