CHAPTER 1

INTRODUCTION

1.1 Background:

Intestinal parasitic infection is the infection of gastrointestinal tract caused mainly by two groups of parasites namely protozoan and helminthic. It is one of the major public health problems in the world having cosmopolitan distribution as more than half of the population in the world are suffered from gastroenteritis and suffer great economic loss due to parasites. In addition, about one fourth of the world population is estimated to be infected by one or more species of parasites (Rai et al 1998; WHO 2000).

The host factors associated with predispotion of disease are age, level of immunity, co-existing disease which reduces immunity e.g. pregnancy, under nutrition etc. (Rai et al 2002). Some of the common clinical manifestations of the infection are diarrhea, dysentery, abdominal pain, anemia, vomiting, vitamin A deficiency, intestinal obstruction etc. (WHO 2010). Chronic infection impairs physical and mental development. Furthermore, it increases susceptibility to infections with other pathogens (Rai et al 2002). Thus the public health importance of intestinal parasitosis continues because of its high prevalence, virtually global distribution and effects on both nutritional and immune status of individuals (Rai et al 1995).

Over the last 100 years there has been a dramatic fall in the incidence and prevalence of parasitic diseases in the developed countries. However, in the developing countries like Nepal, parasitic diseases remain significant health problem (Kunwar et al 2004; Shakya et al 2012). The disease has caused a great economic loss and human health hazards in developing countries. In these less developed countries, poor environmental and personal hygiene, poor nutrition, overcrowding and climatic conditions that favor the development and survival of these parasites are some of the factors contributing to the high level of intestinal parasites transmission (Egwunyenga et al 2005; Obiukwu et al 2008; Vince et al 1991).

Nepal is a small impoverished country with infectious diseases including intestinal parasitosis, being highly prevalent (Rai et al 2001; 2002). Over 85.0% of the population are engaged in agriculture, live in village setting and have the low human development indexes (Rai et al 1998). Approximately 70.0% of the health problems in Nepal are infectious diseases (Rai et al 2001b). The reported prevalence varies considerably from one study to another (Ishiyama et al 2001; Rai et al 1995, 2001, 2002; Rai and Gurung 1986). On an average over 60.0% people are infected with some kinds of intestinal parasites.

Although, intestinal parasites target all age groups, children are more commonly affected than adults and suffer from malnutrition associated morbidity and mortality (Shrestha et al 2009). Children are among the most vulnerable to environmental threats as they are in a dynamic state of growth with their cells multiplying fast and their organ systems developing at a rapid rate (WHO 2000). It has been attributed to poor sanitation, poverty and lack of health education (Chaudhary et al 2004; Gyawali et al 2009; Heidari and Rokni 2003; Hussein 2011; Okyay et al 2004; Rai et al 2002) and water contamination (Adhikari et al 2007; Ono et al 2001; Shakya et al 2012). School children carry the heaviest burden of the associated morbidity due to their dirty habits of playing or handling of infested soils, eating with soiled hands, unhygienic toilet practices, drinking and eating of contaminated water and food (Malla et al 2004; Opara et al 2012; Östan et al 2007).

The World Health Organization estimates that over 270 million pre-school children and over 600 million of school children are living in areas where the parasites are intensively transmitted and are in need of treatment and preventive interventions (WHO 2010). At least 750 million episodes of diarrhea occur per year in developing countries that results in five million deaths (Shakya et al 2006). *Ascaris lumbricoides* and *Enterobius vermicularis* are two of the most prevalent intestinal helminths in school-age children (Bethony et al 2006). Worldwide, 320 million school-age children are infected with *A. lumbricoides* (WHO 2007). An analysis conducted in 2000 by Murray et al estimated that diarrhea accounts for 13.0% of all childhood deaths, amounting to 1.4 million deaths per year.

Nutritional status is a key indicator of health assessment (WHO 1994). Intestinal parasitosis even in low or moderate level, affect on host nutritional status by causing reduction in appetite, digestion, absorption and acute phase status and increasing intestinal nutrient losses which in turn leads to various morbidity and mortality (Rai et al 2004). Intestinal parasitosis contributes greatly to malnutrition in Nepal. A significant negative effect of intestinal parasitosis on some nutritional parameters in school children, pregnant women and general population in Nepal have been reported (Rai et al 2004). Malnutrition makes the children more vulnerable to intestinal parasites, which in turn leads to a poor nutritional status, creating a synergistic relation impairing growth (Gutiérrez-Cisneros et al 2010; Pedersen and Møller 2000).

There was strong association between giardiasis and malnutrition in many school children (Mahdy et al 2009). Malnutrition is more common among children aged less than five years and it is associated with child mortality (Rai et al 2002). In addition, emerging parasites have also been reported (Ono et al 2001; Sherchand et al 1996). Similarly, polyparasitism is common in some areas of Nepal (Rai et al 2001).

BACKGROUND INFORMATION OF THE STUDY POPULATION

Nepal is a land of cultural diversity and ethnic diversity. It is recognized as multi-caste, multi-lingual country. Different parts of Nepal are inhabited by people of different culture, ethnicity and caste. The racial, cultural, social and religious systems are diversified according to the diversity of the castes of living with respect to geography. The different multi-caste are assumed to be "*Tibeto-Burmans*" and "*Indo-Aryans*" including *Brahmin*, *Chhetri*, *Tharu*, *Teli*, *Dhobi*, *Muskar*, *Sharki*, *Damai*, *Badi* and *Kami*.

Being the capital city, Kathmandu is regarded as the city of opportunities and dreams. People from all over Nepal migrate to Kathmandu in search of job, higher education, health facilities etc. The unplanned and unsystematic urbanization has led to congested habitat, pollution, lack of potable water, improper sewerage system etc. which can contribute to cause various infectious diseases.

The establishment of private schools in Nepal had paralleled the advent of democracy more than half a century ago. In the years since, private schools have continued to proliferate all around Nepal and a trend of sending children to private schools has become popular which is more rampant in Kathmandu. Private schools are trusted for quality and standard education, proper management and discipline. So, every guardian whether economically sound or not aspire to send their children to private school hoping for their bright future. There are more than 1200 private schools against 300 public schools in Kathmandu Valley (DOE 2012). However, there is lack of proper and sufficient data about the condition of these schools and the health status of the students reading there.

Hence, this study is carried with a view to determine the prevalence of intestinal parasitic infection among the school children of private schools, possible risk factors and its impact on health status. These children are the future of the nation and the intestinal parasitic infections prevent their normal physical and mental development which are associated with a reduction in education and school performance, attendance and adverse effects on future earnings (Hotez et al 2009). This study will help the national regulatory bodies to know the health status of students of private schools and formulate plans and policies and implement them for the effective control of the infection. It will also help the researchers to carry out further studies in the related areas.

Objectives of the study

General objective

To determine the prevalence of intestinal parasitic infection among the private school children, related risk factors causing the infection and its possible impact on nutritional status of the host.

Specific objectives

1. To determine the prevalence of intestinal parasitic infection among private school children in Kathmandu.
2. To identify the major pre-disposing factors attributing to these parasitic infection.
3. To assess the possible impact of these parasitic infection on the nutritional status of the children.

CHAPTER 2

LITERATURE REVIEW

About 80.0% of all illness and disease in the world is caused by inadequate sanitation, unsafe water and unavailability of water, intestinal parasitosis being one of them (WHO 2000). Intestinal parasitic infection is one of the major public health problems in the world particularly in the developing countries approaching the prevalence of 100.0% in some areas. Both protozoa and helminthes are responsible for the intestinal infections leading to high mortality and morbidity, particularly in developing countries (Ishiyama et al 2001; Malla et al 2004; Rai et al 2004; Rai et al 2005; Rai and Gurung 1986; Sharma et al 2004; Shrestha 2001).

The prevention and control of the intestinal parasitic infection has been lot easier than before due to the production of efficacious drugs and improved diagnostic procedures. However, it has been a public health problem among developing and least developed countries as well as poor people and refugees among developed countries due to various risk factors and lack of necessary effort against the infection (Manganelli 2012).

2.1 Burden of Parasitic Infections

More than a decade ago, about 3.5 billion people in the world were estimated to be infected from intestinal parasitic infections and 450 million were ill as the result of these infections, the majority being children. The prevalence of hookworm, *A. lumbricoides* and *Trichuris* spp. infections were estimated to be 151 million, 250 million and 45.5 million, causing death of 65,000, 60,000 and 10,000 among them respectively. Similarly, *E. histolytica* was estimated to infect some 48 million people causing death of 70,000 and *G. lamblia* caused illness to about 500 thousand people (WHO 2000).

The burden of intestinal parasitic infection has decreased to some extent. Recent study carried out by Pullan et al (2014) reported that more than 1.5 billion people, or 24.0% of the world’s population are infected with soil-transmitted helminths. Infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, the Americas, China and East Asia. The study also showed that about 438.9 million people were infected with hookworm, about 819 million with *A. lumbricoides* and about 464.6 million with *T. trichiura* in 2010. Almost 70.0% of these infections are concentrated in Asia. Fletcher et al (2012) estimated that approximately 50 million people worldwide are infected annually with *E. histolytica* and the worldwide incidence of invasive disease is more likely to be 5 million cases annually, with global mortality rate at 100,000 per annum.

Expert reviews on epidemiology and control of human gastrointestinal parasites in children shows that the soil transmitted helminthes (*A. lumbricoides*, hookworm and *T. trichiura*) are the most prevalent, infecting an estimated one-sixth of global population. Infection rates are higher in children living in sub-Saharan Africa, followed by Asia and then Latin America and the Caribbean (Harhay et al 2010).

According to WHO (2015), about 266 million preschool-age children and over 609 million school-age children live in areas where these parasites are intensively transmitted, and are in need of treatment and preventive chemotherapy (PC), which is a periodic administration of antihelminthic chemotherapy as a public health intervention. In South-East Asian region only, more than 123 million school-age children are in need of PC were treated and Nepal maintained over 75.0% national coverage in this age group.

Many intestinal parasites, particularly the soil-transmitted helminthes have almost disappeared from industrialized countries. However, screening for parasitic infection remains a public health priority in US among serving refugees and immigrants, mostly immigrants from countries where intestinal parasitic diseases are endemic. The study reported 14.0% prevalence of intestinal parasites in stool samples of 533 refugees (Garg et al 2005). Similarly, among 247 immigrant children examined in Italy, 37 (15.0%) tested positive to the intestinal parasites (Manganelli et al 2012). Asymptomatic carriage of protozoan parasite is also common among developed countries. Júlio et al (2012) discovered the prevalence of *G. duodenalis* to be 1.9% among asymptomatic children when estimated by direct examination and increased to 6.8% (57/844) when ELISA results were added.

Intestinal parasitic infections are one of the major health problems in SAARC region. The infection causes a considerable morbidity and mortality in this region and the infection remains endemic in SAARC countries infecting mainly preschool and school level children (Rao et al 2003). In our neighboring country India, about 50.0% of the urban and 68.0% of rural populations were found to be affected with different species of intestinal parasites (Nagaraj et al 2004). Similarly, tapeworm infection was reported to be a major parasitic infection among children in Bhutan followed by other soil-transmitted helminthes. The high prevalence of tapeworm infection in Bhutan was attributed to beef eating habit of the people (Allen et al 2004).

An overall prevalence of 35.0% of intestinal pathogenic parasites were reported from Sukkur, Sindh, Pakistan (Sheikh et al 2009). Protozoan parasites were found to be dominating over helminthic parasites and was attributed to the contamination of drinking water with fecal matter.

Nepal is a small and impoverished land locked country located in South Asia. Like other developing countries, intestinal parasitic infection is a major public health problem in Nepal. The reported prevalence rates of enteric parasitosis in Nepal vary from considerably low (Rai et al 2002) to nearly one hundred percent (Estevez et al 1983) and have been associated with contamination of soil and water. Some emerging diarrheagenic enteric parasites have also been reported recently (Ono et al 2001). Many children die annually of easily preventable and treatable diseases including the intestinal parasitic infections (Rai et al 2002).

The prevalence of intestinal parasitic infection among the school children ranges from 13.9% to 43.3% (Gyawali et al 2009; Khanal et al 2011; Magar et al 2011; Mukhiya et al 2012; Regmi et al 2014; Shrestha et al 2012; Shrestha et al 2009; Tandukar et al 2013; Virtakoti et al 2010). The decreasing trend in prevalence rate has been attributed to the increased awareness regarding personal hygiene and environmental sanitation as well as the regular deworming program conducted during recent years especially in schools (Shakya et al 2012).

Intestinal protozoan parasitic infection is common health problem in HIV infected patients in Nepal. *E. histolytica* was the commonest protozoan infection in HIV seropositive subjects in Nepal (Adhikari et al 2007). Other parasites most commonly found in HIV patients were *G. lamblia* and *B. hominis.* The opportunistic protozoan parasite most commonly found was *C. parvum.* The prevalence of infection was found significantly lower among subjects dwelling at different rehabilitation centres of the Kathmandu Valley in comparison to those who did not. Infection rate was also found similar between males and females indicating an equal opportunity for acquiring parasitic infections.

Influx of parasites infected people in Kathmandu Valley from rural area coupled with unplanned urbanization was reported to be associated with a high prevalence of intestinal parasitic infection among school children in a sub-urban area of Kathmandu (Ishiyama et al 2001). The poor sewerage system and bad smelling street flood during and after rainy season provides additional conditions for heavy infection. Helminthic infections were more common than protozoan infection and male children had a greater burden of disease compared to female children.

Higher prevalence of infection was found in people of both sexes and from different occupations and age groups (Kia et al 2008; Niyatti et al 2009). Sharma et al (2004) had shown a higher prevalence of multi-parasitic infection than single parasitic infection. Higher prevalence of intestinal helminthic infection than that of protozoa has been reported by some studies (Ishiyama et al 2001; Rai et al 2005; Regmi et al 2014; Sharma et al 2004). However, protozoan infection has topped the list in other studies (Magar et al 2011; Malla et al 2004; Mukhiya et al 2012; Shakya et al 2012; Sharma et al 2004; Shrestha et al 2009; Tandukar et al 2013; Virtakoti et al 2010).

2.2 Pre-disposing Factors for Intestinal Parasitic Infections

2.2.1 Socio-demographic factors

Age

Intestinal parasitic infection was found to be higher in people of all age groups (Kia et al 2008, Niyatti et al 2009). However, according to Khanal et al (2011) and Shakya et al (2012), children below 10 years of age were found to be more infected with intestinal parasites. Similarly, those from grade 5 to grade 8 were less likely to be exposed to parasitic infection than those school children from grade 1 to 4. The possible reason might be the level of awareness about personal hygiene in children from grade 1 to 4 was lower than those from grade 5 to 8 (Workneh et al 2014). In contrast, children above 10 years of age were detected with intestinal parasitic infection in higher proportion according to studies conducted by Sharma et al (2004), Rai et al (2005) and Regmi et al (2014).

Gutierrez-Jimenez et al (2013) showed that preschool-age (2-5 years) children were more likely to be infected with intestinal parasites than breast feeding infants. Similarly, in a study in Brazil, children over 5 years of age had a higher risk of infection than younger individuals (Carneiro et al 2002). However, no such association regarding age, sex, were shown in most of the studies (Heidari and Rokni 2003; Ishiyama et al 2001; Magar et al 2011; Rai et al 2005; Shakya et al 2012; Sharma et al 2004; Shrestha et al 2012; Virtakoti et al 2010).

Gender

The association of boys to outdoor activities like playing different sports than girls make them more prone to intestinal parasitic infections (Tandukar et al 2013). The outdoor wandering nature of male children may also contribute to the infection (Ishiyama et al 2001). On the other hand, the more involvement of females in household works that make them frequently exposed with water is the major risk factor for parasitic infection in the case of females (Virtakoti et al 2010). Besides, they also have to take care of babies which increases the chance of transmission of the parasites (Malla et al 2004).

Financial status

The socio-economic factor like lower social economic status of father was significantly related with higher prevalence of intestinal parasitosis among children (Aksoy et al 2007; Östan et al 2007). Children from lower-income families were significantly more infected with intestinal parasites than those from normal or higher income families (Mengistu et al 2007; Quihui et al 2006). The risk of infection of *A. lumbricoides* was estimated to be 2.5 times more among children with low socio-economic index than those from middle category (Gutierrez-Jimenez et al 2013).

The children whose major family occupation was agriculture had significantly high positive rate of intestinal parasitosis compared to others (Tandukar et al 2013). Carneiro et al (2002) showed that children from families with a higher purchasing capacity had a lower risk of *A. lumbricoides* infection. Similarly, children with unemployed mothers also significantly showed a higher risk of intestinal parasitism (Östan et al 2007; Quihui et al 2006).

School children in Ethiopia who were not wearing shoes at the time of interview were significantly more infected with intestinal parasites than those who were wearing the shoes. The leading intestinal parasite in the study was hookworm and it was predicted that the students who do not wear shoe might be infected by soil transmitted helminthes through intact bare foot penetration (Workneh et al 2014).

Parent's literacy

Literacy means the ability to read and write. Parent's literacy plays a vital role in maintaining good health of children. A study conducted by Okyay et al (2004) revealed that health indicators for children whose mother’s education level is lower are always worse. Significant association between intestinal parasitic infection and lower education of mother was shown among school children (Mauro et al 2006; Okyay et al 2004; Östan et al 2007). Higher prevalence of intestinal parasitosis was found among children in rural area with less than primary school educated mother in Turkey (Okyay et al 2004). Similarly, father’s educational level and work status were also found to be the significant risk factors for the intestinal parasitic infections among school children (Al-zain 2009). A research in Pakistan also presented the literacy of mother as major risk factor for the increase in the prevalence of intestinal parasites in children as the children of educated mother had 3.5 times less chance of being infected with parasites (Chaudhry et al 2004).

In a study by Júlio et al (2012), parent's educational level was found to be highly associated with the prevalence of giardiasis among children. In addition to that, the illiteracy of parents had significant effect on the higher prevalence of intestinal parasitic infection among children (Fatemeh et al 2011; Heidari and Rokni 2003; Hussein 2011; Taheri et al 2011; Wani et al 2007). A significant association was observed in the decrease of worm infestation rate among Nepali children from Darjeeling, India with the increasing educational status of their mothers (Ram et al 2008). The children whose mother was educated has less burden of infection compared to the children whose mothers are uneducated or less educated.

According to a study carried out among school children in Nepal, a statistically significant relation was observed that indicated the poor socio-economic status and illiteracy of mothers to be the possible risk factors for the higher prevalence of intestinal parasitosis (Gyawali et al 2009). Children of illiterate parents and farmers were more infected than literate ones and non-farmers (Shakya et al 2012).

Availability of safe water

Children drinking untreated water or directly fetched from the water resources were significantly found to be a great risk factor for the intestinal parasitic infection than those drinking treated or filtered or boiled water (Quihui et al 2006; Tandukar et al 2013; Virtakoti et al 2010). The possible reason could be the contamination of water resources by the feces (Östan et al 2007). Unavailability of safe water supply among school children in Ethiopia was attributed for the very higher prevalence of the intestinal parasitic infection (Workneh et al 2014). Similarly, Wani et al (2007) detected children drinking water from rivers or streams and wells were found to harbor significantly greater prevalence of infection than those who had access to tap water.

Parasite egg contamination of water was reported to be a major cause of intestinal helminthic infection in a suburban area of Hanoi, Vietnam (Noda et al 2009). Water from pond and ditches used for drinking purposes in rainy season was found to be contaminated by helminthic eggs. Similarly, few filtered and non-filtered water samples were also found to be contaminated by parasites eggs which indicated that filtering mechanism was not working effectively and should be improved.

Very high prevalence of (71.2%) of intestinal parasitic infection was reported among school children in rural area of Kathmandu Valley (Rai et al 2005). Highest infection was observed in children drinking water from shallow well compared to natural spout, piped water and river water.

2.2.2 Environmental factors

Environmental sanitation

The poor sanitary conditions in the school was found to be the major risk factor for the higher prevalence of intestinal parasitic infections among the children of those schools (Rai et al 2002; Sehgal et al 2010). A study conducted by Quihui et al (2006) showed that defecation in open areas contributed to the increase in the intestinal parasitic infection. Prevalence of *T. trichiura* and *A. lumbricoides* was found to be significantly higher among students who had no latrine (Tsuyuoka et al 1999). In addition to that, studies in Nepal revealed that school children defecating in pit latrine and open field were more prone to intestinal parasitic infection than those using modern latrines (Gyawali et al 2009; Regmi et al 2014).

A high parasitic burden was observed during an unknown disease outbreak in rural hilly area of Western Nepal due to low environmental sanitation (Rai et al 2001). Helminthic parasites especially *A. lumbricoides* was found to be the major cause of outbreak of the infection and was attributed to the fact that half of the soil samples examined from household yard showed helminthic eggs. The environmental and sanitary condition was found extremely sleazy as over 80.0% of household had no latrine and practices open defecation.

Rural/Urban setting

Place of residence either rural or urban area has been a risk factor for intestinal parasitic infections (Fatemeh et al 2011). Okyay et al (2004) showed that the intestinal parasite prevalence among school children was significantly higher in rural areas of Turkey where there were no proper water network or sewerage system. The problem of the parasitic infection was significant among school children in rural parts of Iran (Sayyari et al 2005; Taheri et al 2011) and in the villages and refugee camps in Palestine (Hussein 2011). Slum dwellers in India have high rates of infestations due to poor sanitation, contaminated water supply and high population density (Nagaraj et al 2004).

Intestinal helminthic infection was reported to be highly endemic in Kathmandu due to poor sanitary conditions and unplanned urbanization. An overall prevalence of 31.0% of intestinal helminthic infection was reported among school children in Kathmandu Valley (Adhikari et al 2007).

Housing settings

In a study in Italy, the major risk factors leading the children to the infection of intestinal parasites were found to be housing, i.e. living in shacks, and cohabitation with other families. Children living in shacks were 2.7 times more prone to parasitic infection than others living in apartments (Manganelli et al 2012). A study in Turkey revealed that children living in crowded houses with insufficient indoor spaces were highly infected with intestinal parasites. In addition, increased number of family members than four was significantly related with higher incidence of intestinal parasites. The study also showed that the children having own bedroom and less than two siblings were significantly less infected with intestinal parasites (Östan et al 2007). Similarly, multiple parasitic infection has been reported to be most common in crowded families (Aksoy et al 2007).

Children living in houses with own drainage systems were significantly more protected from the *G. duodenalis* infection (Júlio et al 2012). Similarly, the risk of intestinal parasitic infection was lower in children with access to water in the washbasin than in those without it (Carneiro et al 2002).

2.2.3 Behavioral habits

Personal hygiene

Socio-cultural and behavioral activities are most commonly associated with the intensity of parasitic infection in a community. The high prevalence of parasitic infection was attributed to the unhygienic living condition in a study conducted by Bhatta et al (2007). Hygienic habit like washing hands with soap before eating and after toilet was significantly associated with the lower prevalence rate of intestinal parasitic infections among school children in Nepal (Gyawali et al 2009; Regmi et al 2014; Tandukar et al 2013). Similarly, in a study conducted in Palestine by Hussein (2011), habits like washing hands before eating and washing fruits and vegetables before eating were found to be significantly related with lower burden of intestinal parasitic infection among children.

A very high prevalence (91.0%) of *A. lumbricoides* infection among rural children of Northern Area, Pakistan was reported (Nishiura et al 2002). This was found to be associated with age, defecation practice, soil eating habit and habit hand washing after defecation. Similarly, a marginally high prevalence was observed in children washing their hands only before meal compared with those washing hands before eating anything Ishiyama et al (2001). Similarly improper toilets and failure to practice proper hand washing were perceived as contributors to the acquisition of intestinal parasitic infections in primary school-going children of the Dharan municipality (Amatya et al 2009).

Intestinal parasite prevalence was significantly higher in children who used hands for washing anal area after defecation than in children who use toilet paper sometimes or never. Similarly, unhygienic behavior like not washing hand with soap after defecation, before eating and preparing foods and cleaning anal area with the same piece of cloth were to be blamed for the higher *E. vermicularis* infection rate in the study in Turkey (Okyay et al 2004; Pinar et al 2004).

Lack of personal hygiene and sanitation behavior like trimming of nail among school children were found to have statistically significant association with the higher prevalence of intestinal parasitic infection than those with trimmed nails (Shrestha et al 2012). Similarly, Wani et al (2007) detected children with clean nails were significantly associated with the lower prevalence of intestinal parasitosis than the children with dirty nails.

Contact with pets

Prevalence of *G. duodenalis* was reported to be significantly higher among children with household pet contact, especially with dogs. The zoonotic transmission of *G. duodenalis* cysts from animals to humans has been raised and dogs are the most studied animals for giardiasis. The role of dogs as a definitive *G. duodenalis* host has been widely recognized as a public health problem (Júlio et al 2012).

Routine deworming

The children who recently administered anti-helminthic drug were less likely to suffer from intense infection of *A. lumbricoides* (Carneiro et al 2002). Similarly, school children in Nepal who have taken antihelminthic drug within past six months were less likely to have intestinal parasitic infection than those who have not taken the drug (Rai et al 2005). School children with gastro-intestinal symptoms were found to have relatively higher prevalence of intestinal parasitic infection in a study conducted by Gyawali et al (2009) and Tandukar et al (2013). Therefore, effective deworming program should be administered to minimize the infections among children in Nepal (Adhikari et al 2007).

2.3 Impact of intestinal parasitosis on nutritional status

A significant relationship was found between malnutrition (stunting) and parasitic infection in a study conducted by Taheri et al (2011). The prevalence of malnutrition, based on underweight, stunting and wasting was 6.5%, 6.5%, and 9.6%, respectively. The prevalence of malnutrition among the boys, based on wasting was higher than for girls. Similar statistically significant association between infection with intestinal parasites and malnutrition was observed when anthropometric analysis performed by Gutierrez-Jimenez et al (2013) among children in Mexico. The study revealed that more than 40.0% of children infected with intestinal parasites represented varying degrees of malnutrition and a marked delay in growth.

The intestinal parasitic infection in children is still quite common, even in a developed country like Italy and childrens' growth and parasitism may be related. Children classified in the lower height had a significantly greater prevalence of parasites than the others (Manganelli et al 2012).

Study by Yap et al (2012) in China detected a high prevalence of intestinal parasitic infection (86.0%), of which *T. trichiura, A. lumbricoides* and hookworm were 81.0%, 44.0% and 6.0% respectively. Children infected with *T. trichiura* had significantly lower body weight, height and BMI than their peers who were *T. trichiura* free. Stunting was observed in 59.0% of the participants, and 5.8% of them were wasted. No statistically significant associations between these two indicators and STH infection status were observed. Surprisingly, the mean hemoglobin level was 15.7 g/dl with no anemia observed regardless of children’s STH infection status.

Malnutrition resulting from various intestinal parasitic infections is common among women and children from rural area in Chad. The prevalence of malnutrition was 36.0% and 15.0% among total women and children examined, respectively. Similarly, 27.0% of children were found to be anemic. Malnourished women and children were found to be highly infected with intestinal parasites compared to those with normal nutritional status. Among malnourished subjects, anemic individuals were more frequently infected with intestinal parasites than those with normal hemoglobin status. The prevalence of intestinal parasitic infection was 63.0% among women and 60.0% among children. The predominant helminth species was *A. lumbricoides* while, *E. histolytica/dispar* was the most common protozoa. Co-existence of hookworm infection and malnutrition was found in 14.0% of women and in 18.0% of children. Out of the observed intestinal parasites, only *H. nana* was significantly associated with malnutrition (Bechir et al 2012).

Opara et al (2012) studied the impact of intestinal parasitic infections on the nutritional status of rural and urban area school children in Nigeria. The prevalence of parasitic infection was 67.4%, hookworm (41.7%) being the most common. The prevalence of intestinal parasites and under-nutrition was significantly higher in rural than in urban children. The prevalence of stunting, underweight and wasting for rural and urban children were 42.3% vs. 29.7%; 43.2% vs. 29.6% and 10.9% vs. 6.4%, respectively. With respect to nutritional indicators, the infected children had significantly higher z-scores than the uninfected children. Multivariate logistic regression analysis showed that only hookworm and *A. lumbricoides* were each significantly associated with stunting, wasting, and underweight.

Low blood hemoglobin and anaemia were the major consequences of the infection in children of Afghanistan (Gabrielli et al 2005). *A. lumbricoides* was reported to be the major helminthic parasite among the children and a considerable level of infection with *T. trichiura* and hookworm was also reported. The infection was most common in age group 8-15 years and anaemia was a major presenting symptom in them.

Multiple parasitic infection was found to be significantly associated with anemia among children in Gaza, Palestine. The prevalence of anemia among infected children (30.8%) was greater than among the non-infected (19.9%) in all age-groups. Infections with *A. lumbricoides* and *G. lamblia* were found to be significantly associated with low hemoglobin level in the participant children. Iron deficiency anemia was detected in 25.0% of the total sample. The mean hemoglobin was 11.3 g/dl with a range between 9.2 g/dl and 14.4 g/dl (Al-zain 2009). Similarly, in another study conducted by Le et al (2007), the chronic infection of *T. trichiura* was associated with double risk of anemia.

Demographic factors such as young age, wet season, female gender all correlated positively with increased rates of parasitic infection among young children in Guatemala. Malnutrition was associated with increased rates of infection for *G. lamblia* and *E. histolytica* (Cook et al 2009).

In Ethiopia, various nutritional indices like HAZ (stunting), BMIZ (wasting) and WAZ (underweight) of the school children were measured to find out the prevalence of malnutrition among them as well as to find out the significant association of malnutrition with respect to intestinal parasitic infection. The prevalence of stunting, wasting and underweight were measured to be 45.7%, 7.1% and 43.1% respectively (Asfaw and Goitom 2000).

Intestinal helminthic infestation bears a considerable burden among tribal population of Kottoor and Anchankovil areas of Kerala, India. Analysis of hemoglobin level of the 190 study population of showed that 66.3% were anemic (<11 gm/dl). Hemoglobin level in relation to helminthic infestation revealed that in the worm infested group, 81.1% (43/53) was anemic, as compared to only 60.0% (83/137) of non-worm infested group, showing significant difference (Farook et al 2002). Similarly, high prevalence of under nutrition in terms of underweight (61.7%), stunting (51.7%) and wasting (32.8%) was reported among tribal adolescents of Madhya Pradesh, India (Rao et al 2003).

Parasitic infestation was a common cause of morbidity and mortality in paediatric population of rural and urban areas of Pakistan. The common intestinal parasites causing abdominal discomfort among children of Quetta, Pakistan were *H. nana, G. lamblia, E. histolytica, A. lumbricoides* and *A. duodenale* (Wadood et al; 2005). Malabsorption and anemia were found to be the major consequences of intestinal parasitic infection among children of Quetta, Pakistan. Similarly, in Bhutan children bear the major burden of various parasitic infections. Malnutrition is a major consequence of the parasitic infections among the children in Bhutan. Tapeworm infection was reported to be a major parasitic infection among children in Bhutan followed by other soil-transmitted helminthes (Allen et al 2004).

The Nepal Demographic and Health Survey (2011) showed the prevalence of underweight, stunting and wasting among children was 29.0%, 41.0% and 11.0% respectively. Data were collected on the nutritional status of children by measuring the height and weight of all children under age 5 in the selected households and nutritional indices: weight-for-age, height-for-age, and weight-for-height were calculated (MoHP 2011). The survey also reported that 46.0% of children below 5 years of age were anemic in Nepal. Young people are more vulnerable to this disease due to their rapid growth need of high iron. Therefore, it is a critical health concern as it affects their growth and physical performance.

Anemia is common problem in the growing age group in developing countries like Nepal. In two different studies carried out in tertiary care hospital of Biratnagar and Rupandehi, the prevalence of anemia among the school-age children was found to be 24.4% and 46.0% respectively (Padmavathi et al 2014; Sinha and Majumdar 2012). Similarly, another study carried out among adolescent population in rural and urban area in Morang district showed higher prevalence of anemia (Baral and Onta 2009). In a cross-sectional study conducted among 618 school children from Morang, Udayapur, Bhojpur and Ilam districts, the overall prevalence of anemia was found to be 37.9%. The children of age group less than 10 years were found to be more anemic than those above the age of 10 years (Khatiwada et al 2015).

Rai et al (2000a) observed a significant association between Vitamin A deficiency and intestinal parasitic infection. There was significant difference in vitamin A levels between school children who tested positive and those who tested negative for intestinal parasitic eggs. Further, a significant increase in vitamin A levels was observed after deworming, proving the role of intestinal parasites in causing vitamin A deficiency.

There was no significant association between the prevalence of anemia and the prevalence of intestinal parasitic infection among school children in Brazil (Tsuyuoka et al 1999). The overall prevalence of anemia in the study population was 26.7% which was significantly associated with age of the children. Prevalence of height-for-age z-score (HAZ) (stunting), weight-for-height z-score (WHZ) (wasting), and weight-for-age z-score (WAZ) (underweight) was found to be 5.4%, 2.5% and 4.4% respectively. Children infected with intestinal parasites were observed with significantly lower nutritional indices (WAZ and HAZ scores) than those without intestinal parasites.

In a cross sectional study performed by Amare et al (2013), 405 children were examined. Of the total, 22.7% were found to be positive for intestinal parasites, *A. lumbricoides* being the most prevalent (7.6%). There was no statistically significant association between prevalence of malnutrition and the prevalence of parasitic infections. However, younger children with lower body weight and lower height were found to be more infected with intestinal parasites than children with higher anthropometric parameters.

No significant association with intestinal parasitic infection was shown when the nutritional parameters like height, weight, hemoglobin level, total protein, albumin, alb/globulin ratio, serum glutamate pyruvate transaminase, cholesterol and serum iron level were measured in school children in a research conducted by Rai et al (2004). Similarly, no statistically significant difference in hemoglobin level of school children in association with the intestinal parasitic prevalence was observed in a study conducted by Chandrasena et al (2004), Ghimire et al (2005) and Ishiyama et al (2001).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Design

It was a cross-sectional study of the prevalence of parasites in stool samples, the possible predisposing factors and their possible impact on the nutritional status of school children which was conducted from the month of July to November 2014.

3.2 Study Site

The samples were collected from four private schools of Kathmandu Valley- Rosebud School (Maharajganj), Oracle Academy (Samakhusi), Glenbird School (Samakhusi), Gyan-Mandir Secondary English School (Banasthali). The laboratory examination of the samples was carried in Shi-Gan International College of Science and Technology (SICOST), Maharajgunj, Kathmandu.

3.3 Study Population

Primary level school children (level 1-5) were included in this study.

3.4 Sample Size and Sampling Technique

The sample size was determined using Fisher's formula for sample size determination i.e.

$$n = Z_\alpha^2 pq/d^2$$

Where, n = required sample size

Z = z-score – set at 1.96 at 95% confidence level.

p = estimated proportion in the population having intestinal parasitic infection. Since, the prevalence of intestinal parasitic infection among school children in Kathmandu Valley ranges from 66.6% to 72.4% (average 69.5%); p will be estimated at 0.695.

q = 1-p

d= marginal error for the desired result i.e. ±6% (0.06)

The minimum required sample size was calculated to be 246. In this study, a total of 329 stool and blood samples were collected from the primary level school children. Blood samples were taken from only those students who had brought the stool samples.

3.5 Primary Data

Data collected and analyzed were as follows:

1. Parasites in stool samples
2. Hemoglobin level in blood samples
3. Age and height-weight measurement (Anthropometric Data)
4. Economic conditions, sanitation, personal hygiene, behavior and life style

3.6 Ethical Approval and Informed Consent Process

This study was approved by joint Institutional Review Board (IRB) of Shi-Gan Health Foundation and National Institute of Tropical Medicine and Public Health Research (NITMPHR) Kathmandu, Nepal. An official permission to conduct the study was obtained from SICOST. School Principal was informed about the study objectives and methods. A verbal permission was obtained to visit the classes and collect samples. The participants were also informed about the objectives and purpose of the study and verbal consent was obtained to conduct the study. Then a brief description about the intestinal parasitic infection and importance of the examination of stool to detect parasites was given to the participants. Confidentiality of information was assured. The result of the test was given to the participants promptly.

3.7 Sample Collection and Transportation

3.7.1 Stool Sample

A clean, dry, screw capped plastic container coded with roll number and class of the students and a wooden spoon was distributed to each of the student and were informed to collect stool containing mucus or blood, if present, up to the mark in the container. They were advised not to contaminate the stool with water and urine. The samples were collected next morning and were brought to the laboratory of SICOST. The stool samples were thoroughly mixed with 10% formal-saline in order to preserve the morphology and structure of eggs and cyst of the intestinal parasites

3.7.2 Blood Sample

Capillary blood was collected using micropipette after pricking the fingertip with sterile blood lancet using Clinical and Laboratory Standards Institute (CLSI) guideline 2013. For hemoglobin estimation, 20 µl blood was mixed with 5 ml of Drabkin's solution in a clean and dry test tube and labeled with students' roll number. This solution was brought to SICOST laboratory to measure the Optical Density (OD)/Hb Concentration colorimetrically as soon as possible.

3.8 Laboratory Processing of Sample

3.8.1 Stool Sample

Stool samples were processed for microscopy by Formal-Ether Concentration Technique. This is the most sensitive method for concentrating cysts, eggs, larva without distorting their morphology.

Procedures:

1. About one gram of stool sample was emulsified in about 3 ml of 10% formal saline solution, shaken well and was allowed to fixation.
2. Further 3-4 ml of 10% formal saline was added and shaken well.
3. The suspension was sieved through cotton gauge in a funnel into a test tube.
4. After filtration 3-4 ml of ether was added to the filtrate and shaken vigorously for 5 minutes.
5. The tube was immediately centrifuged at 2000 rpm for 5 minutes.
6. Four layers of suspension were obtained in the tube after centrifugation.
    a) A layer of diethyl ether at the top
    b) Underneath of it contains a plug of fecal debris

c) A layer of formalin below the debris

d) At last small amount of sediment at the bottom of the tube containing that may contain parasite eggs or cysts.

7. The plug of debris formed between diethyl ether and formalin was removed by rotating the tip of the applicator along the inner wall of the tube.

8. The supernatant layer of suspension were discarded and the sediment was examined by iodine wet mount.

Iodine wet mount

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

The cyst of the protozoan parasites were detected by observing the following characteristics:

i) Size, ii) Shape, iii) Nucleus number and visibility, iv) Peripheral chromatin, v) Karyosome, vi) Cytoplasam, chromatoidal bodies and vii) Glycogen

3.8.2 Blood Sample

Blood sample was processed to estimate hemoglobin concentration by cyanmethemoglobin method. In this method, when blood is mixed with Drabkin's solution, hemoglobin present in blood (Fe^{++}) is converted to methemoglobin with potassium ferricyanide. Methemoglobin is further converted to cyanmethemoglobin, after reaction with Potassium Cyanide. The OD of final pink colored complex is measured colorimetrically at 540 nm.

Procedures:

1. 5 ml of Drabkin's solution was pipetted in clean and dry test tubes.
2. 20 µl of capillary blood was collected and mixed with the Drabkin's solution.

3. The sample was allowed to stand at room temperature for about 10 minutes.
4. The OD of the solution is measured colorimetrically at 540 nm.
5. The value of hemoglobin concentration level was obtained from Hb. calibration graph.

3.9 Other Data Collection

3.9.1 Height and Weight (Anthropometric Data)

Height was measured by using measuring tape up to single decimal point in centimeter. Similarly, weight was measured by using a weighing machine up to single decimal point in kilogram. Body Mass Index (BMI), defined as the weight in kilogram of the individual divided by the square of the height in meter, will be used to determine the nutritional status of the school children as recommended by WHO.

3.9.2 Questionnaire

Questionnaire related to their name, age, sex, personal hygiene, toilet practices, housing condition, behavior habits, clinical history, etc. were asked.

3.9.3 Recording of the result

After laboratory processing of the samples, the result obtained was recorded in the log book and later recorded in the computer.

3.9.4 Report and medicine distribution

The report distribution was done as the result was obtained after laboratory processing of the samples. Every student with positive cases was given anti-parasitic drug along with the report. The complete dose of anti-parasitic drug distributed were Albendazole and Metronidazole according to the parasites detected.

3.10 Data Analysis

The data of parasites in stool, age, sex, Hb level in blood, height-weight measurements and possible risk factors were analyzed by using chi-square test and software IBM SPSS 16.0.

The data containing age, sex, height and weight was run in the macros developed for SPSS to calculate the indicators of the attained growth standards (length/height-for-age, weight-for-age, weight-for-length, weight-for-height, body mass index-for-age, head circumference-for-age, arm circumference-for-age, triceps skinfold-for-age and subscapular skinfold-for-age). The z-score values for height-for-age, weight-for-age and BMI-for-age relative to WHO 2007 reference were calculated using the SPSS software. Thinness/wasting (<-2SD of BMI-for-age), Underweight (<-2SD of Weight-for-age) and, stunting (<-2SD of Height –for-age) were defined according to WHO reference. Weight-for-age reference data cannot distinguish between height and body mass in an age period beyond 10 years where many children are experiencing the pubertal growth spurt and may appear as having excess weight (by weight-for-age) when in fact they are just tall. Therefore, BMI-for age is recommended by WHO to measure thinness/wasting in school children (WHO 2009). Similarly, mild, moderate and severe anemia was defined according to WHO reference. For children aged 5-11 years, the hemoglobin level for mild, moderate and severe anemia are 110-114g/l, 80-109g/l and <80g/l respectively whereas for children aged 12-14 years, the hemoglobin level for mild, moderate and severe anemia are 110-119g/l, 80-109g/l and <80g/l respectively (WHO 2011).

CHAPTER 4

RESULT

Within the study period from July 2014 to November 2014, a total of 329 stool samples, capillary blood samples and other data related to their age, sex, height, weight, socio-economic condition, personal hygiene and sanitation were collected. The prevalence of intestinal parasitic infection was calculated and its relation with various variables was assessed by using chi-square test to find out statistically significant association, if present.

Out of total 329 stool samples, 17.9% (n=59) were positive for intestinal parasitic infection. Of 59 positive samples, 69.5% were infected by single type of parasite either protozoa or helminthes and 30.5% by multiple parasites. Among single parasitic infection, 47.5% were protozoan and 22.0% were helminthic infection. Of the multiple parasitic infections, 8.5% were protozoan, 5.1% were helminthic and 16.9% were both protozoan and helminthic infections (Table 1).

Table 1: Pattern of intestinal parasitic infection among the study population

Types of infection	Frequency (n)	Percent
Single parasites	**41**	**69.5**
Protozoa	28	47.5
Helminthes	13	22.0
Multiple parasites	**18**	**30.5**
Protozoa	5	8.5
Helminthes	3	5.1
Protozoa + Helminthes	10	16.9
Total	**59 (17.9%)**	**100**

The prevalence of protozoan parasites was found to be higher (62.3%) than that of helminthes (37.7%). Among the protozoan parasites, *G. intestinalis* (29.9%) was the most common followed by *E. coli* (20.8%), *E. histolytica* (5.2%), *E. nana* (3.8%) and *B. hominis* (2.6%). Among helminthes, *T. trichiura* (24.7%) topped the list followed by *A. lumbricoides* (10.4%) and *H. nana* (2.6%) (Table 2).

Table 2: Prevalence of individual parasites found in the study population

Parasites	Total positive no. (n)	Percent
Helminthes	**29**	**37.7**
T. trichiura	19	24.7
A. lumbricoides	8	10.4
H. nana	2	2.6
Protozoa	**48**	**62.3**
G. intestinalis	23	29.9
E. coli	16	20.8
E. histolytica	4	5.2
E. nana	3	3.8
B. hominis	2	2.6
Total	**77**	**100**

Out of 59 positive cases, 34 (18.6%) cases were of boys and 25 (17.1%) of girls. The association of gender with prevalence of intestinal parasitic infection was not statistically significant ($p<0.05$) (Table 3).

Table 3: Gender wise distribution of intestinal parasitic infection

Gender	Total (N)	Positive (n)	Percent	p value
Female	146	25	17.1	**p = 0.732**
Male	183	34	18.6	
Total	**329**	**59**	**17.9**	

Of the 329 study population, 221 and 108 were of age group 6-10 years and 11-15 years respectively. A total of 54 (24.4%) children of age group 6-10 years were infected. Out of 108 children of age group 11-15 years, only 5 children (4.6%) were infected. The association between prevalence of intestinal parasitic infection and age was statistically significant ($p<0.05$) (Table 4).

Table 4: Age wise distribution of intestinal parasites

Age group	Total (N)	Positive (n)	Percent	p value
6-10 years	221	54	24.4	**p = 0.000**
11-15 years	108	5	4.6	
Total	**329**	**59**	**17.9**	

The prevalence of intestinal parasitic infection among children with literate parents was 40 (16.5%) and that of illiterate parents was 19 (21.8%). There was no significant association between the literacy of children's parents and the prevalence of intestinal parasitic infection ($p>0.05$) (Table 5).

Table 5: The prevalence of intestinal parasites in relation to parents' literacy

Parent's literacy	Total (N)	Positive (n)	Percent	p value
Illiterate	87	19	21.8	**p = 0.268**
Literate	242	40	16.5	
Total	**329**	**59**	**17.9**	

The prevalence of the intestinal parasitic infection was highest among those drinking tap water (19.0%) followed by underground water (18.2%) and jar water (12.5%) respectively. No significant association was found between the source of water and the prevalence of intestinal parasitic infection ($p>0.05$) (Table 6).

Table 6: The prevalence of intestinal parasites in relation to source of water

Source of water	Total (N)	Positive (n)	Percent	p value
Jar	40	5	12.5	**p = 0.624**
Tap	179	34	19.0	
Underground	110	20	18.2	
Total	**329**	**59**	**17.9**	

Of the total study population, 19.0% children drinking treated water and 14.9% drinking untreated water were detected with intestinal parasites. However, the treatment of drinking water prior to consumption was not significantly associated with the prevalence of intestinal parasitic infection ($p>0.05$) (Table 7).

Table 7: The prevalence of intestinal parasites in relation to treatment of water before drinking

Water treatment	Total (N)	Positive (n)	Percent	p value
Untreated	87	13	14.9	**p = 0.397**
Treated	242	46	19.0	
Total	**329**	**59**	**17.9**	

Prevalence of intestinal parasitic infection in the children with trimmed nail was 16.0% and in the children who had not trimmed nail was 20.4%. No statistically significant association was found between the habit of trimming nail and prevalence of intestinal parasitic infection ($p>0.05$) (Table 8).

Table 8: The prevalence of intestinal parasites in relation to trimming of nail

Trimming of nail	Total (N)	Positive (n)	Percent	P value
Untrimmed	142	29	20.4	**p = 0.305**
Trimmed	187	30	16.0	
Total	**329**	**59**	**17.9**	

A total of 274 children were found to have the habit of washing hands with soap before eating and after toilet and the prevalence of the intestinal parasitic infection among them was 16.8%. Parasite positive rate among those who did not have the habit of washing hands was 23.6%. There was no statistically significant relation between habit of washing hand with soap before eating and after toilet and the prevalence of intestinal parasitic infection (p>0.05) (Table 9).

Table 9: The prevalence of intestinal parasites in relation to habit of washing hand with soap before eating and after toilet

Hand washing habit before eating and after toilet	Total (N)	Positive (n)	Percent	p value
No	55	13	23.6	**p = 0.227**
Yes	274	46	16.8	
Total	**329**	**59**	**17.9**	

Among 223 children having the habit of eating foods from street vendors 15.2% were infected by intestinal parasites. In case of 106 children who had no such habits, 23.6% were found to be infected with intestinal parasites. The association between the street food eating habit and the prevalence of intestinal parasitic infection was not found to be statistically significant (p>0.05) (Table 10).

Table 10: The prevalence of intestinal parasites in relation to habit of eating street foods

Street food eating habit	Total	Positive	Percent	P value
No	106	25	23.6	**p = 0.065**
Yes	223	34	15.2	
Total	**329**	**59**	**17.9**	

The number of children having the habit playing on soil was 145 of which intestinal parasites were found among 15.2% (22/145) children. Of 184 children who had no such habit, 20.1% were found to be infected with intestinal parasites. There was no statistically significant association between habit of playing on soil and the prevalence of intestinal parasitic infection (p>0.05) (Table 11).

Table 11: The prevalence of intestinal parasites in relation to habit of playing on soil

Habit of playing in soil	Total (N)	Positive (n)	Percent	p value
Yes	184	37	20.1	**p = 0.247**
No	145	22	15.2	
Total	**329**	**59**	**17.9**	

The higher prevalence (26.7%) of parasitic infection was observed in students having family size more than five than in those having family size less than or equal to five (10.6%). A statistically significant relation was seen between the number of family members and the prevalence of intestinal parasitic infection (P<0.05) (Table 12).

Table 12: The prevalence of parasites according to family size

No. of family members	Total (N)	Positive (n)	Percent	p value
≤ 5	179	19	10.6	**p = 0.000**
> 5	150	40	26.7	
Total	**329**	**59**	**17.9**	

The prevalence of the parasitic infection was higher (19.5%) in children who hadn't taken antihelminthic drug within past 6 months. The prevalence was 17.0% among the one who had taken the drug. No significant relation was observed between the use of antihelminthic drugs within past 6 months and the prevalence of intestinal parasitic infection (p>0.05) (Table 13).

Table 13: The prevalence of intestinal parasites in relation to use of antihelminthic drugs within past 6 months

Use of drugs	Total (N)	Positive (n)	Percent	p value
No	123	24	19.5	**p = 0.564**
Yes	206	35	17.0	
Total	**329**	**59**	**17.9**	

Out of 329 school children, wasting was seen in 10.3% of which 9.6% were girls and 10.9% were boys. There was no significant difference in HAZ between boys and girls ($p>0.05$) (Table 14).

Table 14: BMI-for age z score (BMIZ) according to gender

Gender	Total (N)	Wasting (BMIZ< -2SD) (n)	Percent	p value
Female	146	14	9.6	**p = 0.692**
Male	183	20	10.9	
Total	**329**	**34**	**10.3**	

The rate of intestinal parasitic infection was 11.8% among the wasted children and 18.6% among the children with normal BMI-for-age z-scores. In case of height-for-age z-score, 16.9% of the total stunted children and 18.1% of the total children with normal height were infected with intestinal parasites. Of the total underweight children, 21.9% were infected with intestinal parasites and the rate of parasitic infection was 24.9% among normal weight children. None of the nutritional indices were found to have any statistically significant association ($p>0.05$) with intestinal parasitic infection (Table 15).

Table 15: Prevalence of intestinal parasitic infection in relation to different nutritional status

Nutritional indices		Total (N)	Positive (n)	Percent	P value
BMIZ	<-2 SD	34 (10.3%)	4	11.8	**p = 0.322**
	>-2 SD	295 (89.7%)	55	18.6	
HAZ	<-2 SD	59 (18.0%)	10	16.9	**p = 0.828**
	>-2 SD	270 (82.0%)	49	18.1	
WAZ	<-2 SD	32 (9.7%)	7	21.9	**p = 0.716**
	>-2 SD	189 (90.3%)	47	24.9	

The prevalence of anemia among the children was 20.5%. Among the total anemic children, 5.6% were moderately and 14.9% were mildly anemic. The mean hemoglobin level (± SD) was 12.4 ± 1.3. The average hemoglobin level among the children infected with intestinal parasites was slightly lower (12.2 ± 1.4) than that of non-infected children (12.5 ± 1.3). The prevalence of anemia was higher among children of age group 5-11 years (16.5%) than that of age group 12-14 years (4.0%) (Table 16).

Table 16: Prevalence of anemia in the study population

Age group	Moderate 8-10.9 g/dl	Mild*	Non**	Total
5-11	15 (4.6%)	39(11.9%)	167 (50.8%)	221 (67.3%)
12-14	3 (1.0%)	10 (3.0%)	95 (28.7%)	108 (32.7%)
Total	**18 (5.6%)**	**49 (14.9%)**	**262 (79.5%)**	**329 (100.0%)**

*11-11.4 g/dl in age group 5-11 and 11-12 g/dl in age group 12-14

** 11.5g/dl and above in age group 5-11 and 12 g/dl and above in age group 12-14

Among the anemic children (67/329), 12.0% were found to be infected with intestinal parasites. Of the 262 non-anemic children, 19.5% of them were found to be infected with intestinal parasites. There was no significant difference between the prevalence of intestinal parasitic infection and anemia in the children (p>0.05) (Table17).

Table 17: Prevalence of intestinal parasitic infection in relation to anemia in the children

Anemia	Total (N)	Positive (n)	Percent	P value
Anemic	67	8	12.0%	**p = 0.152**
Non-anemic	262	51	19.5%	
Total	**329**	**59**	**17.9**	

CHAPTER 5

DISCUSSION

Public health importance of intestinal parasitosis continues because of its high morbidity and mortality in developing countries (Rai et al 2005). Nepal is a small impoverished country where intestinal parasitosis is highly prevalent and varies considerably from one study to another (Rai et al 2001, Rai et al 2002). The present study was conducted to know the prevalence of intestinal parasitic infection among private school children which reflects the sanitary condition, socio-economic impact, environmental impact, consciousness about the factors causing the disease and health education among the school children.

The present study revealed overall prevalence of 17.9% (n=59) among the study population. The result was similar with the findings of the previous studies (Gyawali et al 2009; Khanal et al 2011; Mukhiya et al 2012; Shrestha et al 2012). However, Ishiyama et al (2001), Rai et al (2002), Rai et al (2004), Rai et al (2005), Sharma et al (2004), Shrestha et al (2002) have reported the higher prevalence of the parasite than the present finding. On the other hand, Garg et al (2005) and Júlio et al (2012) have reported a lower prevalence of parasitic infection among school children than present study. The analysis of recent reports have shown a decreasing rate of parasitic infection (Shrestha et al 2009; Bhatta et al 2007; Chandrashekhar et al 2005; Ghimire et al 2005; Shrestha et al 2002) compared to the past reports (Ishiyama et al 2001; Rai et al 2002b; Rai and Gurung, 1986; Sherchand et al 1996). The decrease in prevalence of parasitic infection in recent years could be due to increased health education of the people, increased standard of living of people, access to health services provided by government and private organizations, public awareness towards the prevention and control of disease (Khanal et al 2011; Shakya et al 2012).

The present study revealed the higher prevalence of protozoal infection compared to helminthic infection which was in agreement with the previous reports from Nepal (Chandrashekhar et al 2005; Magar et al 2011; Oda and

Sherchand 2002; Regmi et al 2014; Shakya et al 2012; Shrestha et al 2009; Shrestha et al 2012). Similar finding was also reported from other countries as well from India (Kaur et al 2002), Turkey (Aksoy et al 2007), Iran (Arani et al 2008), Thailand (Saksirisampant et al 2006). However, some studies from Nepal (Ishiyama et al 2001; Malla et al 2004; Rai et al 2004; Rai et al 2005; Sharma et al 2004; Uga et al 2004; Virtakoti et al 2010) have reported a higher prevalence of helminthic infection over protozoan infection. Prevalence of protozoan parasite was higher which could be due to the fecal contamination of drinking water, poor sanitary practices and poor health hygiene. This might also be due to the deworming program carried out frequently over past several years specially targeting the school age children (Khanal et al 2011; Rai et al 2001; Shakya et al 2012).

The climatic factor might be another reason for the high prevalence of protozoa compared to helminthes as the study was carried in the beginning of rainy season when the number of flies and insects increase and may be a possible vector of disease transmission and also due to active protozoal infection during rainy season (Rai et al 2001).

In this study, multi-parasitic infection was seen less than single parasitic infection which was in agreement with a lot of previous studies among school children in different parts of Nepal (Gyawali et al 2009; Ishiyama et al 2001; Khanal et al 2011; Regmi et al 2014; Sharma et al 2004; Shrestha et al 2012; Virtakoti et al 2010; Yong et al 2000). In similar studies carried in Cambodia (Lee et al 2002), Turkey (Aksoy et al 2007) and Mexico (Quihui et al 2006), high prevalence of monoparasitism has been reported. On the other hand, Rai et al (2001) and Sharma et al (2004) had shown a higher prevalence of multi-parasitic infection than single parasitic infection. Multiparasitic infection may be due to very poor health-hygiene and sanitary practices.

In this study, *Giardia* spp. (32.9%) was found to be the most prevalent intestinal parasite among the school children which was in agreement with various previous studies (Chandrashekhar et al 2005; Gyawali et al 2009; Magar et al 2011; Malla et al 2004; Shrestha et al 2009; Virtakoti et al 2010). It was also consistent with the report from India (Kaur et al 2002), Mexico

(Diaz et al 2003), Thailand (Saksirisampant et al 2006), Pakistan (Shaikh et al 2009), Iran (Sayyari et al 2005), Ethiopia (Mengistu et al 2007). *G. intestinalis* is considered as one of the important etiological agents of diarrhea in developing and developed countries (WHO 1994). This result can be attributed to poor quality and polluted drinking water supply system, poor drainage system and lack of hygiene and sanitation. Similarly, resistance of the cyst of *Giardia* spp. to the normal level of chlorination done in the drinking water from different sources with different pH and temperature can also contribute to the higher infection rate (Jarroll et al 1981) . Furthermore, flies and other insects sitting on faeces may be a vector in disease transmission. Also the cysts of *Giardia* spp. are more resistant to osmotic lysis could be a reason of its high prevalence (Rai et al 1994).

Similarly, among helminthes, higher prevalence of *T. trichiura* (16.4%) followed by *A. lumbricoides* (12.3%) was observed in this study which was in agreement with previous findings (Adhikari et al 2007; Ishiyama et al 2001; Jamaiah and Rohela 2005; Khanal et al 2011; Rai et al 2005; Sharma et al 2004; Sherchand et al 2007; Uga et al 2004). However, the result was in contrast with most of the previous findings by Rai et al (2004), Rai et al (2000a), Virtakoti et al (2010), Shrestha et al (2009), Shrestha (2001), Shakya et al (2012) and Gyawali et al (2009) in which *A. lumbricoides* was observed as the leading intestinal helminthes. This could be due to ineffective deworming with single dose of antihelminthic drug particularly in case of heavy infections (Rai et al 2000a). The high prevalence of *T. trichiura* is because of its special mode of attachment to cecal mucosa, longer lifespan of parasites as well as its refractory reaction to most antihelminthic and remains in intestine causing chronic infection (Magar et al 2011; Rai et al 2001). This can also be attributed to the possible contact of the school children to the soil containing the helminth eggs while playing and their ingestion by various unhealthy and unhygienic practices. The higher prevalence of *T. trichiura* in this study and other studies showed that *T. trichiura* is the most common intestinal helminth. Therefore, effective deworming of the parasites should be done.

The prevalence of intestinal parasitic infection was slightly higher among boys than girls. No statistically significant association was seen between gender and intestinal parasitic infection. Various previous studies in Nepal have also showed the higher rate of intestinal parasitic infection among boys than girls (Gyawali et al 2009; Ishiyama et al 2001; Magar et al 2011; Rai et al 2005; Rai et al 2004; Regmi et al 2014; Sharma et al 2004; Shrestha et al 2012; Shrestha et al 2009; Tandukar et al 2013). This higher prevalence of the infection among boys might be due to the boys being associated to outdoor activities like playing different sports than girls who are generally not allowed to do so. Similarly, the outdoor wandering nature of male children may also contribute to the finding (Ishiyama et al 2001). However, Malla et al (2004), Virtakoti et al (2010) and Shakya et al (2012), detected intestinal parasitic infections to be higher in girls than in boys. On the other hand, higher prevalence of infection has been reported in people of both sexes (Adhikari et al 2007; Kia et al 2008; Niyatti et al 2009).

The children of age group 6-10 years were observed with higher prevalence of intestinal parasitic infection than that of age group 11-15 years. This might be due to negligence of personal hygiene and sanitation, playing nature on soil among lower age group (6-10 years) and a bit more consciousness of personal hygiene and sanitation among the higher age group (11-15 years). The result was in agreement with previous reports by Chandrashekhar et al (2005), Khanal et al (2011) and Shakya et al (2012). Similarly, Rai et al (2002) has also reported a decrease in prevalence of infection significantly with the increase of age. However, various other findings were in contrast to this result (Rai et al 2005; Regmi et al 2014; Sharma et al 2004).

In this study, the prevalence of intestinal parasitic infection among the children of illiterate parents was higher than that of literate parents but no significant association was seen. Similar results have been seen in other studies from other parts of Nepal and various countries in the world (Al-zain 2009; Chaudhry et al 2004; Gyawali et al 2009; Heidari and Rokni 2003; Hussein 2011; Júlio et al 2012; Okyay et al 2004; Östan et al 2007; Quihui et al 2006; Wani et al 2007), and strong significant association was seen between the prevalence and the illiteracy of parents. This might be due to lack of awareness, health education and negligence of the parents toward the hygiene and sanitation.

This study showed the prevalence of the intestinal parasitic infection to be highest among the children drinking tap water followed by underground water and jar water respectively. However, there was no statistically significant association with the prevalence of the intestinal parasitic infection. The result was consistent with the one reported by Rai et al (2005) in which children drinking piped water was shown to be infected by intestinal parasitic infection in higher number but no significant association was observed. Fecal contamination of drinking water has been a major problem in Nepal, and conditions have not improved due to the rapid population growth, spread of slums, and poor sanitation. No proper treatment of water, no physical integrity of the distribution system, unplanned urbanization, poor sewerage system and poor hygienic and sanitary practice, blending of leaked sewerage with the water supply (Rai et al 2009; Rai et al 2012) might be the reason for the result in this study. In this study, children who consumed jar water also showed the prevalence of parasitic infection. That may be attributed to the haphazard production of jar water without maintaining any standards. These findings clearly indicated an urgent need of taking steps for improving quality drinking water (Rai et al 2012). Östan et al (2007) also reported similar finding and suggested to revise the tap water network in Turkey. However, the finding reported from India and Israel were inconsistent with the finding in this study (Al-zain 2009; Wani et al 2007).

Surprisingly, higher prevalence of intestinal parasitic infection was seen among children drinking treated water than those drinking untreated water but the result was not statistically significant. This result was in contrast with previous reports among school children in Nepal (Gyawali et al 2009; Ishiyama et al 2001; Tandukar et al 2013; Virtakoti et al 2010) and other countries (Östan et al 2007; Quihui et al 2006; Wani et al 2007). This higher proportion of infection among the children drinking treated water might be due to improper and insufficient treatment of water. The method of treatment of water and the efficacy of the chemicals used for the treatment of water plays a crucial role in the quality of water. Storing the treated water in dirty vessels and drinking water from glasses which are not washed properly might be another reason for higher infection rate among them.

In this study, the infection rate among the children with trimmed nails and having regular habit of trimming nails were found to be lower than those lacking such behavior but statistically significant association was not seen. This result was in agreement to the study done by Shrestha et al (2012) and Wani et al (2007). 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.

Children having the habit of washing hands regularly with soap and water before eating and after toilet were found to be infected with intestinal parasites in lower rate than those who had no such habit. However, there was no statistically significant association between the hand washing habit and prevalence of intestinal parasitic infection. This result was in agreement to several of the previous studies among school children in Nepal (Gyawali et al 2009; Östan et al 2007; Regmi et al 2014; Tandukar et al 2013).

Interestingly, in this study, the children with the habit of eating foods from street vendors were found to be less infected with intestinal parasites than those who had no such habit. But no statistically significant association was found between the street food eating habit and the prevalence of intestinal parasitic infection. However, there was a considerable number of cases of infection among children with the habit of eating foods from street vendors. These days street fast foods are being very popular among cities in Nepal most commonly among students. But there is lack of awareness among the street vendors in maintaining personal hygiene and sanitation as well as in maintaining good quality of the food they serve to local people. However, other risk factors contributing to the intestinal parasitic infection might be the reason for the surprising result in this study.

In this study, intestinal parasitic infection was highly prevalent among school children who have the habit of playing with soil than those not having such habits. The schools under study lacked well managed playground and less attention was given to environmental sanitation. The students had to play in a crowded playground during their break time. So, this result can also be attributed to the contact of children to the soil while playing which might contain different helminthes eggs.

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 one fourth 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 was consistent with the finding of others (Aksoy et al 2007; Östan et al 2007; Karrar and Rahim 1995). However, this result was not consistent to the finding of Rai et al (2005).

Although, no significant difference was observed, the prevalence of the parasitic infection was higher in children who had not taken antihelminthic drug within past 6 months than those who had taken the drug. The result can be attributed to the absence of any deworming program carried out recently from the time of interview of the children. There was not any routine deworming program in the school and whoever had taken the antihelminthic drugs within past 6 months had taken from local medical shops. The finding was consistent with the previous report by Rai et al (2005) whereas it was inconsistent with the finding by Adhikari et al (2007). Though, safe and efficacious broad spectrum anti-parasitic drug have been developed, their use in mass treatment programme and individual treatment has been limited by economic resources, existing manufacturing and distribution networks and national regulations.

Malnutrition makes the children more vulnerable to intestinal parasites, which in turn leads to a poor nutritional status, creating a synergistic relation impairing growth (Manganelli et al 2012). Intestinal parasitosis contributes to a great extent in the causation of malnutrition in Nepal (Rai et al 2004). A significant negative effect of intestinal parasitosis on nutritional parameters in school children in Nepal have been reported (Rai et al 2000) which adversely affects the education and school performance, attendance and cognitive development of school age children (Hotez et al 2009). In the present study, the overall prevalence of underweight, stunting and wasting was 9.7%, 18.0% and 10.3% respectively which was not in agreement with a nationwide survey conducted by MoHP (2011) among children below 5 years that showed higher prevalence of underweight, stunting and wasting i.e., 29.0%, 41.0% and 11.0% respectively. The findings were in agreement with a previous report from Ethiopia (Amare et al 2013). Higher prevalence of stunting and underweight (>30%) compared to this study has been recorded in previous studies from Vietnam and Nigeria by Le et al (2007) and Opara et al (2012) respectively. In this study, the prevalence of wasting were seen to be higher among boys than girls. Similarly, there was no significant association between the prevalence of intestinal parasitic infection and underweight, wasting as well as stunting.

Anemia is a major public health problem in Nepal, especially among young children and pregnant women (MoHP 2011) and is one of the major consequences of intestinal parasitic infection (Gabrielli et al 2005). The overall prevalence of anemia among the school children was 20.5% which was inconsistent with MoHP (2011). The result was inconsistent with previous studies carried out among school age children in different parts of Nepal which showed higher prevalence (Baral and Onta 2009; Khatiwada et al 2015; Padmavathi et al 2014; Sinha and Majumdar 2012). The difference in result might be due to the measurement of hemoglobin level in previous studies were done among school-age children visiting in hospital and use of some hemoglobin estimation methods having less specificity. Prevalence of anemia among the school children can be attributed to chronic parasitic infection that may obstruct proper absorption in intestine leading to nutritional deficiency. The lack of proper nutrients in the diet can also be the reason for the prevalence of anemia among the children. There was no significant association between the prevalence of intestinal parasitic infection and anemia.

Despite the government of Nepal and other private organizations are functioning for the promotion of health services through different programmes, no significant progress has been achieved in controlling the intestinal parasitic infection in Nepal. Many factors are involved in the failure of parasitic control programme such as human behavior, their religion and culture, natural phenomena (climate, rain, flooding), educational factors, political factors. Therefore more effort should be made and applicable plans and policies should be formulated and implemented to get the satisfactory achievement in control of parasitic infections. The present study will help the government regulatory bodies and other private and social organizations to formulate effective plans and policies regarding control of parasitic infection and it will also help to conduct further researches in related areas to other researchers.

Although present study reflects overall information on the prevalence of intestinal parasitic infection, various possible risk factors and nutritional status of private school children in Kathmandu, there are some limitation in this study:

1. Result would be more reliable if stool samples were collected from each individual on three consecutive days.
2. Information regarding type of toilet they use, occupation of the parents, number of family members to room ratio and number of rooms to toilet ratio was not accessed in this study which could have been a strong risk factor for the intestinal parasitic infection.
3. Detection of total protein level in serum could be done in the school children. That would have given more information on the relation of intestinal parasitic infection with nutritional status.
4. Limited age range of the study population as the target population was primary school children aged 3-15 years.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The parasitic infection among school children are closely related to their health hygiene, sanitary condition, socio-economic status and behavior as indicated by this study. The prevalence of intestinal parasitic infection among school children was 17.9%. *G. intestinalis* (29.9%) and *T. trichiura* (24.7%) were the commonest protozoan and helminthes found respectively. Monoparasitism was more common than polyparasitism among infected students and the prevalence of protozoan parasites was found to be greater than that of helminthes. The prevalence of infection was nearly equal in boys and girls. Children below the age of 10 years were highly infected than those above 10 years. Similarly, rate of the parasitic infection among the children of illiterate parents was higher than those of literate parents. Higher prevalence of the intestinal parasitic infection among the children drinking tap water indicates contamination of drinking water in reservoir or supplying pipes. Higher prevalence of intestinal parasitic infection was seen among children drinking treated water than those drinking untreated water which can be attributed to improper and insufficient treatment of water. Children who played outdoor games and those without the habit of trimming nails regularly were more infected with intestinal parasites. The infection rate was found lower among children who had taken anti-parasitic drugs within past 6 months compared to those who had not taken the drug within that period. In addition to these, a significant negative effect of intestinal parasitosis on nutritional parameters was seen. Based on these results, it can be concluded that the prevalence of parasitic infections is due to lack of proper environmental sanitation and hygienic practices as well as lack of periodic deworming. So, in order to minimize the rate of parasitic infections, proper planning and effective implementation should be done followed by routine surveillance and evaluation.

6.2 Recommendations

1. Higher prevalence of protozoan enteroparasite especially, *G. intestinalis* and higher rate of infection among the children drinking tap water indicates fecal contamination of drinking water in reservoir or supplying pipes. This emphasizes the need of appropriate surveillance and monitoring system of drinking water supply system.
2. Similarly, concerned authorities need to regularly monitor the jar water companies to check if they maintain the quality standard.
3. The higher prevalence of intestinal parasitic infection among children drinking treated water indicates improper and insufficient treatment of water. So, the parents as well as students should be made aware about the proper methods of water treatment. Similarly, efficacy of the chemicals available in the market for the treatment of water should be monitored regularly.
4. Periodic distribution of anti-parasitic drug and deworming programme should be conducted among school children as the study indicated very low prevalence of parasitic infection among children who have taken drug within past 6 months.
5. The parents and children must be educated about health hygiene and sanitary practices. Emphasis should be given to maintaining personal hygiene and environmental sanitation, drinking treated water, washing hands with soap after defecation and before meal and periodic stool examination.

REFERENCES

Adhikari N, Bomjan R, Khatri DB, Joshi DR, Dhakal P and Lekhak B (2007). Intestinal helminthic infections among school children in Kathmandu Valley. J Nepal Health Res Counc **5**: 17-21

Aksoy U, Akisu C, Bayram-ADelibas S, Ozkoc S, Sahin S and Usluca S (2007). Demographic status and prevalence of intestinal parasitic infections in school children in Izmir, Turkey. Turkish J Pediatr **49**: 278-282

Al-zain BF (2009). Impact of socioeconomic conditions and parasitic infection on hemoglobin level among children in Um-unnasser village, Gaza strip. Turk J Med Sci **39**: 53-58

Allen H, Sithey G, Padmasiri EA and Montresor A (2004). Epidemiology of soil-transmitted helminthes in the western region of Bhutan. Southeast Asian J Trop Med Public Health **35**: 777-779

Amare B, Ali J, Moges B, Yismaw G, Yeshambel G, Belyhun Y, Gebretsadik S, Woldeyohannes D, Tafess K, Abate E, Endris M, Tegabu D, Mulu A, Ota F, Fantahun B and Kassu A (2013). Nutritional status, intestinal parasite infection and allergy among school children in Northwest Ethiopia. BMC Pediatr **13**: 7

Arani AS, Alaghehbandan R, Akhlaghi L, Shahi M and Lari (2008). Prevalence of intestinal parasites in a population in south of Tehran, Iran. Rev Inst Med Trop S Paulo **50**: 145-149

Asfaw ST and Goitom L (2000). Malnutrition and enteric parasitoses among under-five children Aynalem Village, Tigray. Ethiop J Health Dev **14**: 67-75

Baral KP and Onta SR (2009). Prevalence of anemia amongst adolescents in Nepal: A community based study in rural and urban areas of Morang District. Nepal Med Coll J **11**: 179-182

Bechir M, Schelling E, Hamit MA, Tanner M and Zinsstag J (2012). Parasitic infections, anemia and malnutrition among rural settled and mobile pastoralist mothers and their children in Chad. Ecohealth **9**: 122-131

Bethony J, Brooker S and Albonico M et al (2006). Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet **367**: 1521–1532

Bhatta CP and KC B (2007). Study of intestinal parasitic infection in patients attending Kathmandu Medical College, Sinamangal, Kathmandu, Nepal. J Nepal Assoc Med Lab Sci **8**: 76-77

Chandrasena TGAN, Alwis ACDde, Silva LDRde, Morel RP and Silva NRde (2004). Intestinal parasitoses and the nutritional status of Veddah children in Sri Lanka. Southeast Asian J Trop Med Public Health **35**: 255-259

Chandrashekhar TS, Joshi HS, Gurung M, Subba SH, Rana MS and Shivananda PG (2005). Prevalence and distribution of intestinal parasitic infestations among school children in Kaski district, Western Nepal. J Med Biomed Res **4**: 78-82

Chaudhry ZH, Afzal M and Malik MA (2004). Epidemiological factors affecting prevalence of intestinal parasites in children of Muzaffarabad district. Pakistan J Zool **36**: 267-271

Cook DM, Swanson RC, Egget DL and Booth GM (2009). A retrospective analysis of prevalence of gastrointestinal parasites among school children in Palajunoj valley of Guatemala. J Health Popul Nutr **1**: 31-40

Diaz E, Mondragon J, Ramirez E and Bernal R (2003). Epidemiology and control of intestinal parasites with nitazoxanide in children in Mexico. J Trop Med Hyg **68**: 384-385

Egwunyenga OA and Ataikiru DP (2005). Soil-transmitted helminthiasis among school age children in Ethiope East Local Government Area, Delta State, Nigeria. Afr J Biotechnol **4**: 938-941

Estevez EG, Levine JA and Warren J (1983). Intestinal parasites in a remote village in Nepal. J Clin Microbiol **17**: 160-161

Farook MU, Sudharmini S, Ramadevi S and Vijayakumar K (2002). Intestinal helminthic infestations among tribal populations of Kottor and Achankovil areas in Kerala (India). J Commun Dis **34**: 171-178

Fletcher SM, Stark D, Harkness J and Ellisa J (2012). Enteric protozoa in the developed world: A public health perspective. Clinical Microbiology Reviews **25**: 420-445

Gabrielli AF, Ramsan N and Naumann C et al (2005). Soil-transmitted helminthes and haemoglobin status among Afghan children in World food programme assisted schools. J Helminthol **79**: 381-384

Garg PK, Perry S, Dorn M, Hardcastle L and Parsonnet J (2005). Risk of intestinal helminth and protozoan infection in a refugee population. Am J Trop Med Hyg **73**: 386-389

Ghimire TR and Mishra PN (2005). Intestinal Parasites and Haemoglobin Concentration in the People of two different areas of Nepal. J Nepal Health Res Council **3**: 1-7

Gutierrez-Jimenez J, Torres-Sanchez MGC, Fajardo-Martinez LP, Schlie-Guzman MA, Luna-Cazares LM, Gonzalez-Esquinca AR, Guerrero-Fuentes S and Vidal JE (2013). Malnutrition and the presence of intestinal parasites in children from the poorest municipalities of Mexico. J Infect DevCtries **7**: 741-747

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

Harhay MO, Horton J and Olliaro PL (2010). Epidemiology and control of human gastrointestinal parasites in children. Expert Rev. Anti Infect Ther **8**: 219-234

Heidari A and Rokni MB (2003). Prevalence of intestinal parasites among children in day-care center in Damghan - Iran. Iran J Public Health **32**: 31-34

Hotez PJ, Fenwick A, Savioli L and Molyneux DH (2009). Rescuing the bottom billion through control of neglected tropical diseases. Lancet **373**: 1570-1575

Hussein AS (2011). Prevalence of intestinal parasites among school children in northern districts of West Bank- Palestine. Trop Med Int Health **16**: 240-244

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

Jamaiah I and Rohela M (2005). Prevalence of intestinal parasites among members of the public in Kuala Lumpur, Malaysia. Southeast Asian J Trop Med Public health **36**: 68-71

Jarroll E, Bingham AK and Meyer EA (1981). Effect of Chlorine on *Giardia lamblia* Cyst Viability, App Env Microbiol **41**: 483-487

Júlio C, Vilares A, Oleastro M, Ferreira I, Gomes S, Monteiro L, Nunes B, Tenreiro R and Ângelo H (2012). Prevalence and risk factors for *Giardia duodenalis* infection among children: A case study in Portugal. Parasit Vectors **5**: 22

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

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

Khatiwada S, Gelal B, Gautam S, Tamang MK, Shakya PR, Lamsal M and Baral N (2015). Anemia among school children in eastern Nepal. J Trop Pediatr **61**: 231-233

Kia EB, Hosseini M, Nilforoushan MR, Meamar AR and Rezaeian M (2008). Study of intestinal protozoan parasites in rural inhabitants of Mazandaran Province, Northern Iran. Iranian J Parasitol **3**: 12-17

Kunwar CB, Shrestha M, Subba B, Jha B, Subedi J, Blangero J, Blangero SW and Towne B (2004). Intestinal parasitic infections among very young children in a rural community in Eastern Nepal. J Nepal Assoc Med Lab Sci **6**: 11-13

Lee KJ, Bae YT, Kim DH, Deung YK, Ryang YS, Kim HJ, Kyung IM and Yong TS (2002). Status of intestinal parasites infection among primary school children in Kampongcham, Cambodia. Korean J Parasitol **40**: 153-155

Le HT, Brouwer ID, Verhoef H, Nguyen KC and Kok FJ (2007). Anemia and intestinal parasite infection in school children in rural Vietnam. Asia Pac J Clin Nutr **16**: 716-723

Magar DT, Rai SK and Lekhak B (2011). Study of parasitic infection among children of Sukumbasi Basti in Kathmandu Valley. Nepal Med Coll J **13**: 7-10

Mahdy A, Surin J, Wan K, Mohd-Adnan A, Al-Mekhlafi M and Lim Y (2009). Giardia intestinalis genotypes: Risk factors and correlation with clinical symptoms. Acta Tropica **112**: 67-70

Malla B, Sherchand JB, Ghimire P, BC RK and Gauchan P (2004). Prevalence of intestinal parasitic infections and malnutrition among children in a rural community of Sarlahi, Nepal. J Nepal Health Res Counc **2**: 24-28

Manganelli L, Berrilli F, Cave DD, Ercoli L, Capelli G, Otranto D and Giangaspero A (2012). Intestinal parasite infections in immigrant children in the city of Rome, related risk factors and possible impact on nutritional status. Parasit Vectors **5**: 265

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

Mukhiya RK, Rai SK, Karki AB and Prajapati A (2012).Intestinal protozoan parasitic infection among school children. J Nepal Health Res Counc **10**: 113-117

Murray CJL, Lopez AD, Mathers CD and Stein C (2001). The global burden of disease 2000 project: aims, methods, and data sources. Geneva: World Health Organization

http://www.who.int/healthinfo/paper36.pdf

Nagaraj S, Raghavan R, Macaden R and Kurpad AV (2004). Intestinal parasitic infection and total serum IgE in asymptomatic adult males in an urban slum and efficacy of antiparasitic therapy. Indian J Med Microbiol **22**: 54-56

Nepal Demographic and Health Survey (2011). Population Division, MoHP, GoN, New ERA, ICF Intl, Kathandu, Nepal.

Nishiura H, Imai H, Nakao H, Tsukino H, Changazi MA, Hussain GA, Kuroda Y and Katoh T (2002). *Ascaris lumbricoides* among children in rural communities in the northern area, Pakistan: Prevalance, intensity and associated socio-cultural and behavioral risk factors. Acta Trop **83**: 223-231

Niyyati M, Rezaeian M, Zahabion F, Hajarzadeh R and Kia EB (2009). A survey on intestinal parasitic infections in patients referred to a hospital in Tehran. Pak J Med Sci **25**: 87-90

Noda S, Hoa NTV, Uga S, Thuan LK, Aoki Y and Fujimaki Y (2009). Parasite egg contamination of water and air in suburban area of Hanoi, Vietnam. Trop Med Health **37**: 55-61

Obiukwu MO, Umeanaeto PU, Eneanya CI and Nworgu GO (2008). Prevalence of gastro-intestinal helminth in school children in Mbaukwu, Anambra State, Nigeria. Ann Trop Med Parasitol **101**: 705-713

Oda Y and Sherchand JB (2002). Study on intestinal parasitic infection among students in Kathmandu related to their drinking water. J Nepal Assoc Med Lab Sci **4**: 36-41

Okyay P, Ertug S, Gultekin B, Onen O and Beser E (2004). Intestinal parasites prevalence and related factors in school children, a western city sample- Turkey. BMC Public Health **4**: 64

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

Opara KN, Udoidung NI, Opara DC, Okon OE, Edosomwan EU and Udoh AJ (2012). The impact of intestinal parasitic infections on the nutritional status of rural and urban school-aged children in Nigeria. Int J MCH and AIDS **1**: 73-82

Östan İ, Kilimcioğlu AA, Girginkardesler N, Özyurt BC, Limoncu ME and Ok ÜZ (2007). Health inequities: lower socio-economic conditions and higher incidences of intestinal parasites. BMC Public Health **7**: 342

Padmavathi P, Dubey RK, Jayan A, Neupane Y, Gautam N and Sinha AK (2014). Assessment of Anemia in School Aged Children Attending Tertiary Care Hospital. Int J Health Sci Res **4**: 80-84

Pedersen FK and Møller NE (2000). Diseases among refugee and immigrant children. Ugeskr Laeger **162**: 6207–6209

Pullan RL, Smith JL, Jasrasaria R and Brooker SJ (2014). Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasite & Vectors **7**: 46

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

Rai DR, Rai SK, Sharma BK, Ghimire P and Bhatta DR (2005). Factors associated with intestinal parasitic infection among school children in a rural area of Kathmandu Valley Nepal. Nepal Med Coll J **7**: 43-46

Rai SK, Bajracharya K and Budhathoki S et al (1995). Status of intestinal parasitosis at TU Teaching Hospital. J Inst Med (Nepal) **17**: 134-142

Rai SK and Gurung CK (1986). Intestinal parasitic infection in high school level students of Birgunj city. J Inst Med (Nepal) **8**: 33-37

Rai SK, Gurung R, Saiju R, Bajracharya L, Rai N, Gurung K, Shakya B, Pant J, Shrestha A, Sharma P, Shrestha A and Rai C (2008). Intestinal parasitosis among subjects undergoing cataract surgery at the eye camps in rural hilly areas of Nepal. Nepal Med Coll J **10**: 100-103

Rai SK, Hirai K, Abe A and Ohno Y (2002). Infectious diseases and malnutrition status in Nepal: an overview. Malays J Nutr **8**: 191-200

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

Rai SK, Hirai K, Abe A, Nakanish M, Rai G, Uga S and Shrestha HG (2004). Study on enteric parasitosis and nutritional status of school children in remote hilly areas in Nepal. Nepal Med Coll J **6**: 1-6

Rai SK, Matsumura T and Ono K et al (2001). Intestinal parasitosis in an "unknown disease outbreak" hit rural hilly area in Western Nepal. Nepal Med Coll J **3**: 69-73

Rai SK, Matsumura T, Ono K, Oda Y, Uga S, Rai N and Shrestha HG (2001b). Intestinal parasitoses in an "unknown disease outbreak" hit rural hilly area in western Nepal. Nepal Med Coll J **3**: 68-73

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

Rai SK, Ono K, Yanagida J-I, Ishiyama-Imura S, Kurokawa M and Rai C (2012). A large-scale study of bacterial contamination of drinking water and its public health impact in Nepal. Nepal Med Coll J **14:** 234-240

Rai SK, Ono K, Yanagida J-I, Kurokawa M and Rai C (2009). Status of drinking water contamination in Mountain Region, Nepal. Nepal Med Coll J **11**: 281-283

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

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

Ram R, Chakraborty M, Sarkar M, Bhattacharya SK, Bhattacharya KD and Roy M (2008). Parasitosis: A study among Nepali children in the district of Darjeeling. Indian J Med Sci **62**: 484-491

Rao VG, Aggrawal MC, Yadav R, Das SK, Sahare LK, Bondley MK and Minocha RK (2003). Intestinal parasitic infections, anaemia and undernutrition among tribal aldolescents of Madhya Pradesh. Indian J Community Med **18**: 26-29

Regmi P, Rai KR, Mukhiya RK, Tamang Y, Gurung P, Mandal PK and Rai SK (2014). Prevalence of intestinal parasites and associated risk factors among school children of Kalaiya in Bara district, Nepal. JSM Microbiol **2**: 1009

Saksirisampant W, Prowneborn, Kulkumthorn M, Yenthakam S, Janpla S and Nuchprayoon S (2006). The prevalence of intestinal parasitic infections among school children in the central region of Thailand. J Med Assoc Thai **89**: 1928-1933

Sayyari AA, Imanzadeh F, Bagheri Yazdi SA, Karami H and Yaghoobi M (2005). Prevalence of intestinal parasitic infections in the Islamic Republic of Iran. East Mediterr Health J **11**: 377-383

Sehgal R, Reddy GV, Verweij JJ and Rao AVS (2010). Prevalence of intestinal parasitic infections among school children and pregnant women in a low socio-economic area, Chandigarh, North India. Rev Infect **1**: 100-103

Shakya B, Rai SK, Rai G, Shrestha A and Singh A (2006). Study on intestinal infections by parasite and some bacteria among elderly people of Kathmandu valley. Nepal Med Coll J **8**: 243-247

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

Sharma BK, Rai SK, Rai DR and Choudhury DR (2004). Prevalence of intestinal parasitic infestation in school children in the Northeastern part of Kathmandu Valley, Nepal. Southeast Asian J Trop Med Public Health **35**: 501-506

Sheikh GS, Begum R, Hussain A and Shaikh R (2009). Prevalence of intestinal protozoan and helminth parasites in Sukkur, Sindh. Sindh Univ Res J **41**: 53-58

Sherchand JB, Larsson S and Shrestha MP (1996). Intestinal parasites in children and adults with and without abdominal discomfort from the Kathmandu area of Nepal. Trop Gastroenterol **17**: 15-22

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

Shrestha B (2001). Intestinal parasitic infestation in healthy school children of Lalitpur district. J Nepal Med Assoc **41**: 266-270

Shrestha A, KC N and Sharma R (2012). Prevalence of intestinal parasitosis among school children in Baglung district of Western Nepal. Kathmandu Univ Med J **37**: 3-6

Shrestha A, Rai SK, Basnyat SR, Rai CK and Shakya B (2007). Soil transmitted helminthiasis in Kathmandu, Nepal. Nepal Med Coll J **9**: 166-169

Shrestha SK, Rai SK, Virtakoti R and Pokharel P (2009). Parasitic infection in school children in Thimi area, Kathmandu Valley. J Nepal Asso Med Lab Sci **10**: 31-33

Sinha AK and Majumdar B (2012). Incidence of anemia based on haemoglobin levels in children 2-12 years of aged in Nobel Medical College Teaching Hospital, Biratnagar, Nepal. Journal of Nob Med Coll **1**: 36-38

Stanfield P, Brueton M, Chan M, Parkin M and Waterston T (1991). Diseases of Children in the Subtropics and Tropics. Vince J. Helminthiasis **17**: 633-648

Taheri F, Namakin K, Zarban A, Gholamreza and Sharifzadeh (2011). Intestinal parasitic infection among school children in South Khorasan Province, Iran. J Res Health Sci **11**: 45-50

Tandukar S, Ansari S, Adhikari N, Shrestha A, Gautam J, Sharma B, Rajbhandari D, Gautam S, Nepal HP and Sherchand JB (2013). Intestinal parasitosis in school children of Lalitpur district of Nepal. BMC Res Notes **6**: 449

Tsuyuoka R, Bailey JW, Guimarães AMdAN, Gurgel RQ and Cuevas LE (1999). Anemia and intestinal parasitic infections in primary school students in Aracaju, Sergipe, Brazil. Cad SaúdePública **15**: 413-421

Uga S, Rai SK, Kimura K et al (2004). Parasites detected from diarrheal stool samples collected in Nepal. Southeast Asian J Trop Med Public Health **35**: 19-23

Virtakoti R, Rai SK, Shrestha SK, Pokharel P, Ishiyama S, Kurokawa M, Yanagida JI and Ono K (2010). Intestinal parasitosis among school children in Pokhara Valley, Nepal. Souvenir of 15th Congress Nepal Pediatr Soc 2010 (March 25-7): 87-93

Wadood A, Bari A, Rhman A and Qasim FK (2005). Frequency of Intestinal Parasite Infestation in Children Hospital Quetta. Pakistan J Med Res **44**: 87-88

Wani SA, Ahmad F, Zargar SA, Ahmad Z, Ahmad P and Tak H (2007). Prevalence of intestinal parasites and associated risk factors among school children in Srinagar city, Kashmir, India. J Parasitol **96**: 1541-1543

Workneh T, Esmael A and Ayichiluhm M (2014). Prevalence of intestinal parasitic infections and associated factors among debreelias primary schools children, East Gojjam zone, Amhara region, North West Ethiopia. J Bacteriol Parasitol **5**: 1

WHO (1994). Readings on diarrhea: A student Manual, Geneva

WHO (2000). World health report Conquering suffering enriching humanity, Geneva, Switzerland

WHO (2007). Partners for parasite control: geographical distribution and useful facts and stats.

WHO (2009). WHO Anthroplus for personal computers Manual: Software for assessing growth of the world children and adolescents, Geneva, Switzerland

http://www.who.int/growthref/tools/en/

WHO (2010). Neglected Tropical Diseases - PCT Databank

WHO (2011). Hemoglobin concentrations for the diagnosis of anaemia and assessment of severity, Geneva, Switzerland

http://www.who.int/vmnis/.../haemoglobin.pdf 53

WHO (2015). Soil-transmitted helminthiases: number of children treated in 2013. Weekly epidemiological record **90**: 89-94

Yap P, Du ZW, Chen R, Zhang LP, Wu FW, Wang J, Wang XZ, Zhou H, Zhou XN, Utzinger J and Steinmann P (2012). Soil-transmitted helminth infections and physical fitness in school-aged Bulang children in southwest China: results from a cross-sectional survey. Parasit Vectors **5**: 50

Yong T-S, Sim S, Lee J, Ohrr H and Kim H (2000). A small- scale survey on the status of intestinal parasite infections in rural villages in Nepal. Korean J Parasitol **38**: 275-477

APPENDIX – A

Materials used

1. **Chemicals and reagent**

   Sodium chloride

   Diethyl ether

   Formaldehyde

   Drabkin's solution

2. **Materials**

   Test tube

   Conical flask

   Beaker

   Measuring cylinder

   Glass slides and cover slips

   Droppers

   Pipettes

   Test tube stand

   Screw capped plastic container

   Applicator

   Cotton gauze

3. **Equipment**

   Microscope

   Centrifuge

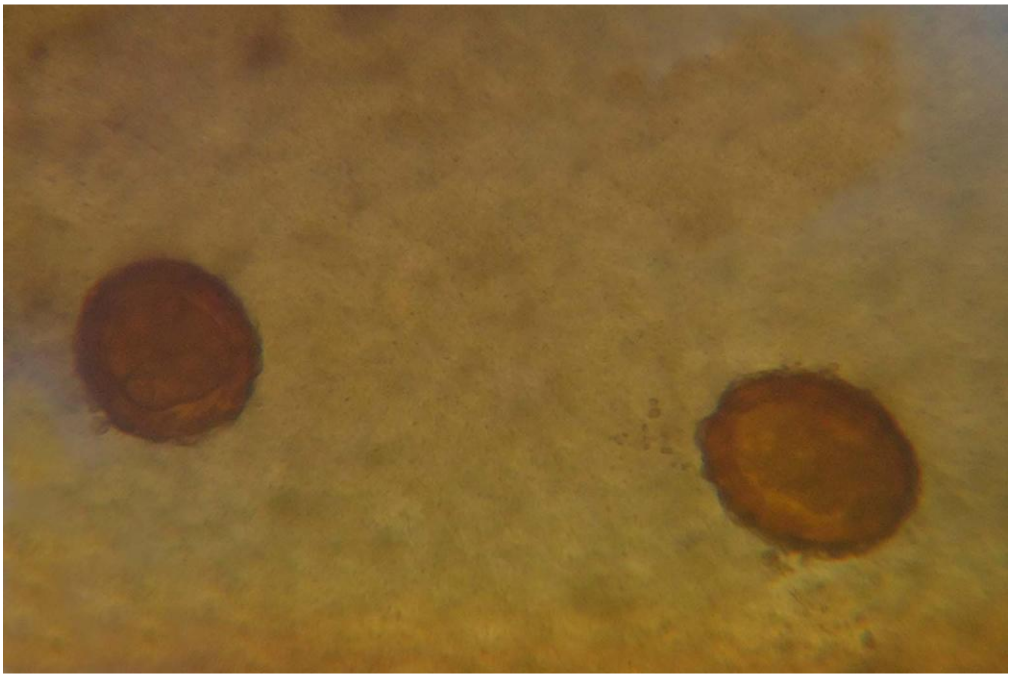
   Colorimeter

APPENDIX - B

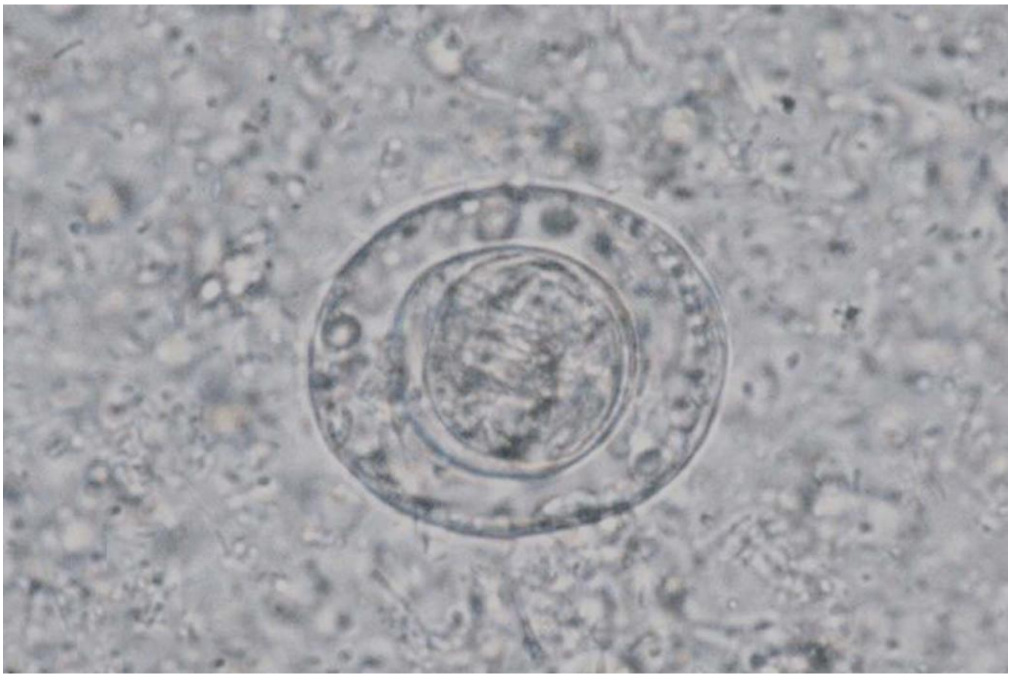
Questionnaire

Name:			
Address:			
Age:	Sex:		
Height:	Weight:		
Parents' literacy:	a) Literate	b) Illiterate	
Ownership of the house:	a) Self	b) Rental	
Source of drinking water:	a) Tap	b) Jar	c) Underground
Treatment of drinking water :	a) yes	b) No	
Hand washing with soap before eating and after toilet:	a) Yes	b) No	
Nails:	a) Trimmed	b) Untrimmed	
Habit of playing on soil	a) Yes	b) No	
Habit of eating foods from street vendors:	a) Yes	b) No	
Number of family members:	a) >5	b) ≤5	
Use of anti-helminthic drug	a) Yes	b) No	

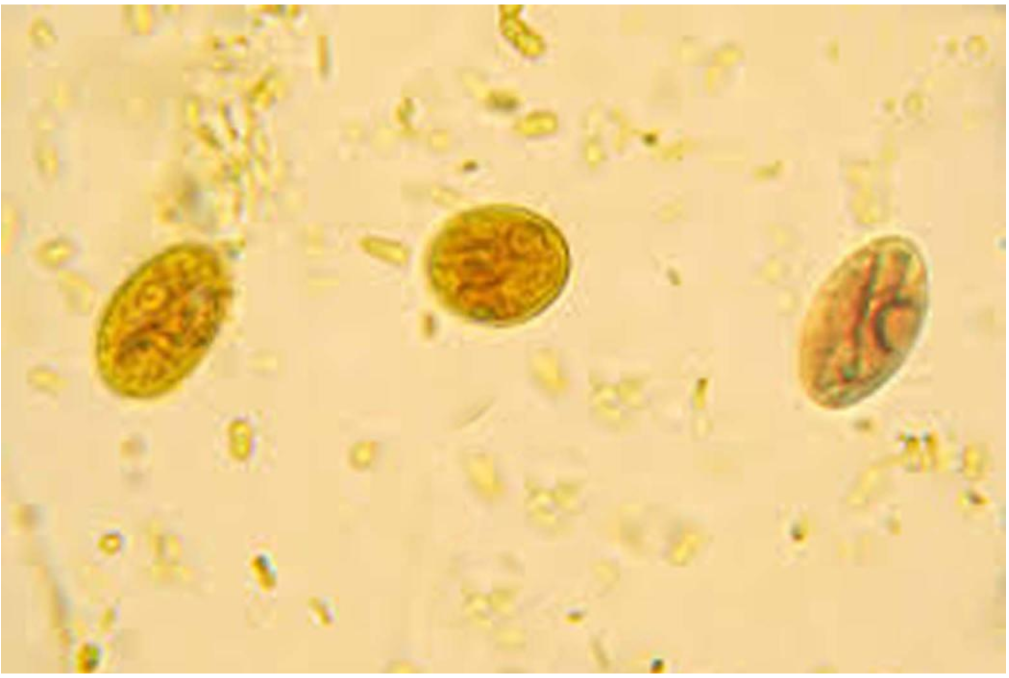
Photograph I: An egg of *T. trichiura* (Sample no. P12). It contains double shell, barrel shape, mucus plug at each pole and unsegmented ovum.



Photograph II: Fertilized eggs of *A. lumbricoides* (Sample no. T3). It contains bile stained brownish thick smooth translucent shell with albuminous coat thrown into mammilations and large unsegmented ovum.



Photograph III: An egg of *H. nana* (Sample no. F23). It contains outer thin and colorless membrane and inner membrane enclosing oncosphere with 3 pairs of hooklets.



Photograph IV: Cysts of *G.intestinalis*, (Sample no. A21). It is oval in shape with diagonally located axostyle.