PREVALENCE OF PINWORM (*Enterobius vermicularis*) AND OTHER INTESTINAL PARASITIC INFECTIONS AMONG THE CHILDREN OF BARBHANJYANG VDC, TANAHUN DISTRICT, NEPAL



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A thesis submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in Zoology with special paper Parasitology

> Submitted to Central Department of Zoology Institute of Science and Technology Tribhuvan University Kirtipur, Kathmandu Nepal December, 2014

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by references to the author(s) or institution(s).

Date: 29/12/2014

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RECOMMENDATION

This is to recommend that the thesis entitled "**Prevalence of Pinworm** (*Enterobius vermicularis*) and other intestinal parasitic infections among the children of **Barbhanjyang VDC**, Tanahun District, Nepal" has been carried out by Tara Dahal for the partial fulfilment of Master's Degree of Science in Zoology with special paper **Parasitology**. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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On the recommendation of supervisor "**Dr. Mahendra Maharjan**" this thesis submitted by **Tara Dahal** entitled "**Prevalence of Pinworm** (*Enterobius vermicularis*) and other intestinal parasitic infections among the Children of Barbhanjyang VDC, Tanahun District, Nepal" is approved for the examination and submitted to the Tribhuvan University in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper **Parasitology**.

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ACKNOWLEDGEMENTS

I am greatly indebted to my respected supervisor, **Dr. Mahendra Maharjan**, Associate Professor of Central Department of Zoology, T.U., Kirtipur for his considerable guidance, appropriate supervision, valuable suggestion and constant encouragement during research work. I am indebted to Prof. **Dr. Ranjana Gupta**, HOD of CDZ, T.U., for providing necessary materials for completing this research work and heartily co-operation and suggestions.

I am thankful all my friends and staffs of CDZ and all wisher who supported me in every step during the completion of this work.

I would like to express the special thanks to all the parents, especially to the mothers of the children from Barbhanjyang VDC for providing samples for this study without whom this task couldnot be completed.

I am grateful to my respected mother **Padam kumari Dahal** and all my family members for their heartily support and inspiration throughout my study period and academic career.

Tara Dahal

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
AIDS	Acquired Immune Deficiency Syndrome
BPKIHS	B.P. Koirala Institute of Health Sciences
CBS	Central Bureau of Statistics
CDC	Centers for Disease Control
CDZ	Central Department of Zoology
IPI	Intestinal Parasitic Infection
КАР	Knowledge, Attitude and Practices
P-Value	Probability Value
SPSS	Statistical Package for Social Science
STH	Soil Transmitted Helminthes
TU	Tribhuvan University
VDC	Village Development Committee
WHO	World Health Organization

ABSTRACT

Enterobius vermicularis infection is neglected but a major health problem in children of developing countries including Nepal. The present study was carried out to determine the prevalence of E. vermicularis along with other intestinal parasites in children of Barbhanjyang VDC, Tanahun, District, Nepal. A total of 110 Scotch tape (Cellophane tape) samples along with stool samples of children aged between 1-12 years were collected for detection of *E. vermicularis* and other intestinal parasites. Among them, 56 were male while 54 were female. Out of 110 samples examined, 14 (12.72%) were infected by E. vermicularis. Sexwise prevalence showed male (16.1%) were more infected than female (9.25%). Agewise prevalence showed among the age group 5-8 years old (5.45%). The infection rate was significantly associated with ethnic groups (Chi-square=11.824, df=2, P=0.003) since the prevalence rate was highest in Dalit children (64.28%) compared to others. Itching behaviour of children around the perianal region was directly associated with the prevalence rate of the E. vermicularis (P=0.0325). Nail biting habit of children also found to be statistically significant (P=0.024) with the prevalence of E. vermicularis. Other intestinal parasites by stool examination revealed seven different species of intestinal parasites. Among the protozoan parasites Entamoeba coli (29.62%) was most prevalent followed by Entamoeba histolytica (24.07%) and Giardia lamblia (11.11%) while in case of helminthes parasites, Ascaris lumbricoides (16.66%) was found to be most prevalent followed by Trichuris trichiura (11.11%), Hymenolepis nana (3.07%) and Hymenolepis diminuta (3.07%). The prevalence of intestinal parasites showed significant association with habit of use of toilet (P=0.037), methods of cleaning vegetables and fruits (P=0.05) and intake of anti-helminthic drugs (P=0.0091). The prevalence rate of intestinal parasites was increased due to lack of knowledge, poor sanitary condition and improper night soil disposal.

1. INTRODUCTION

1.1 Background

Enterobius vermicularis is commonly known as Pinworm, is a causative agent of enterobiasis, also known as *Oxyuris vermicularis* (Caldwell 1982) which is cosmopolitan in distribution and is more common in temperate and tropical regions. It is estimated that 200 million people are infected annually by this parasite particularly in crowded instituations such as day care centers, schools, hospitals and orphanages (Gulnaz and Nizami 2006).

It inhabits in the ceacum and right colon of the human and migrates out through the anus for the oviposition. It is a lumen dweller and hence does not cause medically serious infection although may cause troublesome on rare occasions when the worm invade the tissues (Hong et al. 2002). Infection occurs by ingestion of eggs via contaminated hands or food. Mature worms usually reside in the lumen of terminal ileum or ceacum (Heyman 2004) which after fertilization, the female worm migrates to the perianal region where air contact stimulates them to lay eggs (Murata et al. 2002, Zahariou et al. 2007). The presence of female *E. vermicularis* causes intense itching at the perianal regions. Scratching of the affected area will then transfer eggs to the finger and assist in the transmission of the eggs, both back to the original host which is termed as autoinfection (Horne 2002). Human acquire this parasite through direct contact with infected person or ingestion of contaminated food and water and rarely by inhalation of airborne eggs. Infection by this parasite is usually asymptomatic, however in prolonged infection they can cause anal itching and abdominal disturbances (Kim 2003).

The most commonly infected groups are children living in crowded environments such as summer camp and institutions, with hygiene and exposure being important factors (Cook 1994). Adults are the least common age group to experience the enterobiasis, with exception of mother whose children are infested (Devlin 1991). Occasionally, the adult stage migrates to unusual sites and produce granulomatous lesions (Chandrasoma and Mendis 1977), mostly involving female genital tract (Symmer 1950). The presence of pinworm inside the appendix at times may produce appendicitis (Yildrim et al. 2005) too.

The main clinical features experienced are pruritus and vulvitis and general symptoms such as insomnia and restlessness. A considerable proportion of children show loss of appetite and weight, irritability, emotional instability and enuresis (Manson-Bahr and Apted 1983). There are several methods for the detection of this parasites, such as direct examination of stool, concentration technique, etc but the most efficient one is the cellophane tape technique by which 99% of positive cases can be detected (Lee et al. 2000).

1.2 General Intestinal Parasites

Intestinal parasitic infections are one of the major health problems in several developing countries (Baragundi et al. 2011) which are more common in tropical and subtropical regions. Amoebiasis, ascariasis, trichuriasis and hookworm infections are the most common all over the world (Norhayati et al. 2003). Intestinal protozoans and helminthes parasites are widely prevalent and causing considerable medical and public health problems particularly to people belonging to illiterate and low socio- economic class (Bdir and Adwan 2010). Lack of awareness about importance of sanitation, personal and environmental hygiene with respect to health play the major factors in prevalence of intestinal parasites (Tariq and Zahid 2006). The intestinal parasitic infections are acquired by ingestion, inhalation or penetration of skin by infective forms. These infections are most common in school children and lead to nutritional deficiency, anemia, growth retardation and impaired learning ability (Baragundi et al. 2011).

World Health Organization (WHO) estimated that more than one billion people are suffering from chronically infections of intestinal parasites (WHO 1998). The common intestinal parasites includes: *Entamoeba coli, Entamoeba histolytica, Giradia lamblia, Ascaris lumbricoides, Trichuris trichiura, Hymenolepis nana, Hymenolepis diminuta, Ancyclostoma duodenale* and *Strogyloides stercoralis.*

1.3 Objectives of the Study

General objective

• To determine the prevalence of *Enterobius vermicularis* and other intestinal parasites in children of Barbhanjyang VDC, Tanahun District, Nepal

Specific objectives

- To determine the prevalence of *Enterobius vermicularis* among children of Barbhanjyang VDC, Tanahun District
- To determine the prevalence of intestinal parasites among the children of Barbhanjyang VDC, Tanahun District
- To correlate the socio-behaviour of children along with Knowledge, Attitude and Practices (KAP) in relation to *E. vermicularis* and other intestinal parasites.

1.4 Rationale of the Study

The hygiene and sanitary condition of the childrens of Barbhanjyang VDC seemed worse and some of them still defecate in open places and near water sources, which enhances the possible chances of occurring intestinal parasites. Similarly, the socio-economic condition of the children belonging to this VDC was not good. Most of the children's parents were illiterate, so they prefer traditional medical practices instead of consulting a doctor. Hence, observing this scenario during many visits, the present study was carried out in the Barbhanjyang VDC of Tanahun District to know the prevalence of *E. vermicularis* and other intestinal parasites in the children of this VDC.

2. LITERATURE REVIEW

2.1 Global Context

E. vermicularis is human nematode parasite belonging to family oxyuridae. The parasite is generally not recovered during the normal routine examination of stool samples. This parasite is most easily diagnosed by Scotch tape technique by which 99% of positive cases can be detected by applying adhesive cello tape around perianal regions early in the morning before going toilet or bathing since its eggs wash away by using water (Lee et al. 2000).

E. vermicularis is one of the common human intestinal parasites in the world (Burkhat and Burkhat 2005, Zahariou et al. 2007) particularly in temperate regions (Tandan et al. 2002). Several studies have been carried out regarding this parasite.

Enterobiasis cases have been reported from several countries of American continent such as Argentina, Peru, Venezuela etc. The overall prevalence of enterobiasis has been reported very high from among the children of aged between 5-14 years from Peru (Gilman et al. 1991) i.e. 42.00%, Argentina (Pezzani et al. 2004) i.e. 41.42%, Chile (Mercado et al. 1996) i.e. 35.2%. However, comparatively less prevalence of enterobiasis has been reported from United States (Schupf et al. 1995) i.e. 4.5% in mentally and developmentally retarded children, in Peru (Devera et al. 1998) which showed 1.1% in adult and children from different communities, in Argentina (Ilkow et al. 2000) i.e. 5% etc. Another study of Gamboa et al. (2011) in the same country showed that enterobiasis infection is correlated with nutritional status and socio-economic environmental conditions of children. It seems that enterobiasis is distributed throughout continent, one of the important public health indicators in America.

E. vermicularis infection has also been reported from various countries of the European continent like Turky, Italy. In Europe, the prevalence rates in some communities can reach 30-50 % and enterobiasis is more common in temperate than in tropical countries (Burkhat and Burkhat 2005). Children of age group 4-7 years old have been reported to be more susceptible enterobiasis and the cases have been found quite prevalent among the orphan children and nocturnal enuresis in Turkey (Culha and Duran 2006). The overall prevalence of enterobiasis has been reported high in children of aged between 1-15 years old in Denizli Turkey (Balci et al. 2010) i.e. 29.6%, which also showed the

correlation of the *E. vermicularis* to the socio-economic status and environmental factors. However, comparatively less prevalent has been reported in children aged between 7-15 years old in suphan of Turkey (Cengiz et al. 2010) i.e. 10.3% which also showed the prevalence rate high in male than in female and 7.3% in the same country (Alver et al. 2011). Another research of Gunduz et al. (2011) in same nation performed the biological investigation of stool in patients with acute diarrhea in different hospitals and found eight different species of microorganism including 1.6% of *E. vermicularis*. A suvey carried out among 514 immigrants living in South Italy during 2008 to 2009 showed 0.4% of *E. vermicularis* along with other intestinal parasites (Gualdieri et al. 2011).

Several studies have shown that E. vermicularis infection is highly prevalent in African continent. Since the most of the countries in Africa are below the poverty level. Large numbers of people suffer from various diseases including enterobiasis. Studies have been reported high prevalence of enterobiasis from Qwa-Qwa, South Africa (Mosala and Appleton 2003), Nigeria (Out-Basseg et al. 2011) which showed 6.8% and 35.6% for anal itching and enuresis respectively. Comparatively less prevalence rate (4.6%) has been reported from same country (Auta et al. 2013). Perianal itching was correlated with enterobiasis Out-Basseg et al. (2011) in Nigeria. No significant association between prevalence and hand washing after using toilet and washing of fruits and vegetables before eating reported by Auta et al. (2013) in Nigeria. Similarly, Alo et al. (2011) examined the eggs of E. vermicularis in the finger nails of primary school children and claimed that finger nails were the source of transmission of intestinal parasites in Ohaozara, Ebonyi state, Nigeria. On the other hand, Wumba et al. (2010) found the prevalence of the *E. vermicularis* in hospintalized AIDS patients along with other intestinal parasites in kinshana, Democratic Republic of Congo, Central Africa. Kotb et al. (2011) carried out an investigation in rural Assiut, Egypt, South Africa which showed that the E. vermicularis was more prevalent among the agricultural worked school children and concluded that the problem of child labor is serious, especially in rural area.

Several research reports have been published by different authors in Asia regarding the human intestinal nematode, *E. vermicularis*. Various studies have been showed that enterobiasis infection is highly prevalent when the most of the countries are below the poor socio-economic status. Large numbers of human population especially children have been infected from different intestinal infection including enterobiasis. The overall prevalence of enterobiasis has been reported high (33.8%) among the children in Iran

(Heidari and Rokni 2003), in the same country, 21.59% even in educated individuals and prevalence rate found high (73.69%) in female than in male (32.38%) children (Nourazian and Youssefi 2011), 25.5% and 49.1% respectively in elderly and mentally retarted person of Iraq (Rasti et al. 2012), 21.9% in children of aged between 5-10 years old in Thailand (Nithikathkul et al. 2001), 24.9% in children of aged between 1-12 years old in Iraq (Kadir and Amin 2011). Comparatively less prevalence of enterobiasis has been reported in several previous studies in Iraq (Raza and Sami 2009) i.e. 2.25% (Lee et al. 2011) showed the total prevalence of enterobiasis 10.5% in Korea, 12.67% among the children in India (Wani et al. 2009), 5.9% among the children of aged between 6-12 years old in South Jordan (Ammoura 2010).

Several previous studies have shown that male children more infected than female by enterobiasis in different countries of Asia. The egg positive rate of *E. vermicularis* has been found significantly higher in boys than that of girls in Korea (Park et al. 2005). Similarly, the prevalence of this parasite has been reported higher in boys than in girls in Korea (Lee et al. 2011) and in Taiwan (Chang et al. 2009) but statistically has not been explained clearly, in Iraq (Kadir and Amin 2011) which showed 26.57% in male and 22.83% in female children. The prevalence rate of *E. vermicularis* in male has been reported about 2.5 times more than in female in Iraq (Rasti et al. 2012). In contrast, however some other studies have been disagreemented with other studies and showed that girls were more susceptible than the boys for enterobiasis infection in Thailand (Tukaew et al. 2002) but statistically not explained. Similarly, the prevalence of enterobiasis has been reported high in female (73.69%) than in male (32.38%) in Iran (Nourazian and Youssefi 2011).

Regarding the age groups, the children of age group 6-10 years old has been reported more susceptible to enterobiasis followed by 11-15 years and 16-20 years old in Iraq (Raza and Sami 2009). The prevalence of enterobiasis has been reported higher in school children (29.5%) than in pre-school children (21.51%) in Iraq (Kadir and Amin 2011). Al- Taie (2008) showed that the most affected patients by enterobiasis were 11-20 years old in United Arab Emirates. Bustami and Khraisha (2010) showed that treatment of enterobiasis together with mebendazole, rectal enemas and health education achieved the higher cure rate than using mebendazole alone in Jordan. Significant association of anal itching as well as nail chewing behaviours of children have been shown in elderly and mentally retarded person of Iraq (Rasti et al. 2012). The research of Al-Mohammed et al.

(2011) showed the correlation between the prevalence of pinworm infection, sociodemographics and hygienic habits of male primary school children of aged between 7-12 years old in Saudi Arab (Al-Mohammed et al. 2011), water sources, defecation site and personal hygiene and extent of maternal education in India (Wani et al. 2010), the number of children per family, the frequency of washing linen and cleaning beds were the most important factors for the transmission of pinworm infection in Taiwan (Wang et al. 2010).

Studies have been reported the association between *E. vermicularis* and the appendix (Cook 1994, Da Siva et al. 2007, Ramezani and Dehghani 2007). Several researches have been reported association between *E. vermicularis* appendicitis from different parts of the world like in Iran (Mowlavi et al. 2004), in India (Madhukar et al. 2014), in Greek (Efraimidou et al. 2008) but extraintestinal localizations have also been reported such as female genital tract (Dundas et al. 1999, Hong et al. 2002, Smolyakov et al. 2003, Burkhat and Burkhat 2005) or other unusual sites (Mc Donald and Hourihane 1972, Gargano et al. 2003). Some studies have been reported of this parasite being located other sites rather than intestine like in the kidney (Estelle et al. 2010) in France, in male urinary tract (Zahariou et al. 2007) in Greece. Furthermore, ectopic infection seen most commonly in females may result in pelvic inflammatory (Tandan et al. 2002). *E. vermicularis* infection may associate with urinary tract infection (Ok et al. 1999).

Maraghi (1997) reported adult, larva and ova of *E. vermicularis* in cerebrospinal fluid of a 60 years old man who was admitted to the hospital with clinical manifestation of meningitis in Iran. On the other hand, Lakhnana et al. (2006) examined the perianal abscess of a 6 years old child due to the *E. vermicularis* in Pakistan. Another research of Shetty et al. (2012) in Mumbai, India examined the eggs containing larva of *E. vermicularis* in vaginal smear of a 35 years female patients presented with history of vulval pruritus and vaginal discharge. On the other hand, Gargano et al. (2003) examined the granulomatous sialoadenitis due to the presence of *E. vermicularis* in a 62 years old male presenting a mass in right madibular triangle and discussed that this was a very rare site and it would appear to the first report concerning enterobiasis in the salivary glands.

General Intestinal Parasites

Intestinal parasitic infection is one of the major problems in several developing countries (Baragundi et al. 2011) which are more common in tropical and subtropical regions. Amoebiasis, *ascariasis, trichuriasis* and hookworm infections are the most common all over the world (Norhayati et al. 2003).

Several studies have been carried out in American continent regarding the intestinal parasitic infection. A very high overall prevalence (61.6%) of IPI has been found in the children of rural villages Lucia (Kunup and Hunjan 2010) in which *A. lumbricoides* (15.7%) was found the most prevalent followed by hookworm (11.9%), *Strongyloides stercoralis* (9.9%), *Trichuris trichiura* (4.7%). Similarly, *A. lumbricoides* (91.00%) has also been reported as the most prevalent IPI followed by *Trichuris trichiura* (82.00%) in Guetemala (Watkins et al. 1996) while *Trichuris trichiura* (34.00%) as the most prevalent followed by *A. lumbricoides* (20.9%) and hookworm (11.2%) in America (De Rochars et al. 2004). It means helminthic parasitic infection is more prevalent among the population of America.

The prevalence of intestinal parasitic infection in children of aged 1-12 years have been found from a poor neighborhood and showed the correlation with their nutritional status and socio-environmental condition from a suburban neighborhood of La plata, Argentina (Gamboa et al. 2011).

Several studies have been carried out about intestinal parasitic infection in different countries of European continent. Even being the developed countries, many people have being suffering from different species of intestinal parasites. *Giardia lamblia* (15.4%) has been reported the most prevalent protozoan parasite in Turkey (Cengiz et al. 2010, Balci et al. 2010, Gunduz et al. 2011). But *Blastocystis hominis* has been reported as the most prevalent (Alver et al. 2011) followed by *E. vermicularis* (7.3%), *Hymenolepis nana* (6.2%), *Giardia intestinalis* (5.6%) *Entamoeba coli* (2.27%), *Entamoeba histolytica* (1.13%), *Entamoeba harmoni* (1.13%), *Taenia* spp (0.56%) among the people of the same country (Alver et al. 2011). However, another research of (Gualdieri et al. 2011) found hookworm was most prevalent among intestinal parasites in immigrants in the city of Naples, South Italy.

Cengiz et al. (2010) observed about socio-economic status had an important impact on the frequency of intestinal parasites among the primary school children aged between 7-15 years old in Suphan, Van, Turkey and the prevalence of intestinal parasites was slightly higher in boys (29.3%) than in girls (28.3%). Balci et al. (2010) showed the association of intestinal parasites with socio-economic status and environmental factors in children aged between 1-15 years old in Denizli, Turkey. It seems that protozoan parasites are still a major health problem among the population of the European countries.

There are several research work have been carried out in African continent regarding the intestinal parasites. The prevalence of intestinal parasites in the finger nails of the primary school children have been reported in Nigeria (Alo et al. 2013) which showed Ascaris lumbricoides (20.00%) was the most prevalent followed by E. vermicularis (17.8%), Trichuris trichiura (12.9%) and Ancyclostoma duodenale (6.5%). Similarly, Ascaris lumbricoides (30.7%) has been reported as a highly prevalent intestinal helminth parasites followed by Taenia spp (23.00%), Schistosoma mansoni (11.7%), hookworm (6.4%), Trichuris trichiura (4.9%), Enterobius vermicularis (4.6%), Strongyloides stercoralis (4.2%) and *Hymenolepis nana* (3.9%) among primary school children in Nigeria (Auta et al. 2013). Among the protozoan parasites, Entamoeba histolytica has been found as the most prevalent in Hospitalized AIDS patients in Congo (Wumba et al. 2010). On the other hand, E. histolytica and Cryptosporidium spp have been shown as the most common parasitic causes of diarrhea among the hospitalized attendees while Giardia lamblia among the primary school children in Vhembe District, South Africa (Samie et al. 2010). On the other hand another research in Egypt (Doaa and Said 2012) which examined the very high overall prevalence of intestinal parasites (31.7%) of contamination of the raw vegetables and the highest numbers of contaminated samples was detected in rocket (46.7%) while the least number in green onion (13.3%).

Finger sucking children were more susceptible to intestinal parasitic infection than in non finger sucking children and toilet facility of children also affected the infection in the finger sucking children who used pit latrines regarding higher prevalence of parasites in Nigeria (Idowu et al. 2011). On the other hand, agricultural worked school children aged between 6-17 years old has been found more infected by IPI than not worked school children and the problem of child labor was serious, especially in rural areas of Assiut, Egypt (Kotb et al. 2011). From the previous studies it is proved that helminthic as well as protozoan parasitic infection is the major health problems in the people of African countries.

Like in other continents, several researches have been carried out in Asian continents about intestinal parasites. Most of the developing countries remain in Asian continent. Majority of the people are illiterate. Poverty, illiteracy, ignorance etc. are common among the people. Many people, especially children are being the victim of different species of intestinal parasites. Several researches have been carried out regarding the intestinal parasites in different countries. Sanitary measurements should be conducted to decrease the parasitic infection (Heidari and Rokni 2003). The overall prevalence of the intestinal parasites has been found a very high (75.18%) in India (Wani et al. 2010), 64.1% in Malaysia (Lim et al. 2009). Comparatively less prevalence of intestinal parasitic infection has been showed 30.9% in Yemen (Alyousefi et al. 2011), 28.5% in Jordan (Ammoura 2010), in Saudi Arabia 27.2% (Al-Mohammed et al. 2011), 3.7% in Korea (Kim et al. 2009). Giardia lamblia (7.69%) has been reported as the highly prevalent protozoan parasite in Iraq (Raza and Sami 2009), in Jordan (Ammoura 2010) i.e.42.6%, in Yemen (Alyousefi et al. 2011) i.e. 17.7% while in some studies Entamoeba histolytica has been found as most prevalent protozoa like in United Arab Emirates (Al-Taie 2008), in India (Shrihari et al. 2010). Among the helminthes parasites, Ascaris lumbriciodes has been ranked as the most prevalent in India (Wani et al. 2009, Wani et al. 2010) i.e. 68.30% and 71.18% respectively. However, Trichiura trichiura has been found as the most prevalent helminthes parasite in Korea (Kyong-Rock et al. 2010), in Malaysia (Lim et al. 2009). On the other hand, Blastocystis hominis has been showed as the most prevalent among the primary school children in Saudi Arabia (Al-Mohammed et al. 2011). Regarding the sexwise distribution of IPI, the prevalence of infection has been reported higher in males than in females but statistically not explained in United Arab Emirates (Dash et al. 2010). The highest rate of the IPI has been recorded in 6-10 years old (34%) followed by 11-15 years old (20%) and 16-20 years old (17.5%) in Iraq (Raza and Sami 2009). The infection of intestinal parasites has been found more in rural areas (33.2%) than in urban areas (23.00%) in Jordan (Ammoura 2010).

Intestinal parasitic infections among the school children, coupled with the poor sanitary conditions in the schools in India (Sehagal et al. 2010). Similarly, IPI was directly correlated with drinking water sources in Jordan (Ammoura 2010). It seems that IPI is

distributed throughout the Asian countries and protozoan and helminthic infections still major health problem among the people especially in children.

2.2 National Context

Few studies were carried out in Nepal specially focusing E. vermicularis. Earlier studies had reported E. vermicularis infection in different parts of Nepal (Sharma et al. 1965, Cherchand 1997, Chaudhary 2003, Kunwar 2009, Shrestha and Maharjan 2013, Sah et al. 2013) along with other intestinal parasites. A high prevalence (16.19%) of enterobiasis has been reported from the children of Bhutanese Refugee camp of Jhapa (Sharma 2009). However, comparatively low prevalence of enterobiasis has been reported from many parts of Nepal, 5.52% in the school children of Chitawan (Pandit 2004), 2.8% in children of Bharatpur, Chitawa (Reddy et al. 1998), 0.3% among the school children of Bharatpur, Chitawan (Sah et al. 2013). Male children (5.20%) and female children (5.97%) have been equally infected and high rate of infection was found in the kids of labours (Pandit 2004). However, female children (17.54%) have been found highly infected with this parasite than male (14.58%) among the children of aged between 2-9 years old (Sharma 2009). In both of these studies, children from low socio-economic conditions have been more susceptible to the E. vermicularis infection. Rai et al. (1991) showed that the prevalence of E. vermicularis more among the children below 15 years old than in adult more than 15 years old in Kathmandu valley but statistically not explained. Similarly, the prevalence of enterobiasis has been found highest among the age group 5-14 years old (Singh et al. 2013). On the other hand, prevalence of *E. vermicularis* (1.62%) has been found among 624 patients in sursically removed appendices in the department of pathology BPKIHS, Dharan over the time period 2 years and 6 month and the parasite was mostly seen in histologically normal appendices and rarely associated with histological change of acute appendices (Sah and Bhadani 2006).

In 1965 Sharma worked on the intestinal parasitic infection from Bhaktapur over the period of five years. The intestinal helminthes parasites- *Ascaris lumbricoides, Trichuris trichiura*, hookworm and *Strongyloides stercorslis* are considered as the soil transmitted helminthes (STH).

Several studies have shown that a very high prevalence of intestinal parasites at different parts of Nepal. In Achham District, 76.4% IPI has been reported (Rai et al. 2001), 81.94% among the children of aged between 7-12 years old in Lalitpur District (Shrestha 2001), 60% among the school children in Dhading District (Rai et al. 2002), 67.6% among the people of Machhegaun VDC (Chaudhary 2003), 45.83% among the children of aged between 3-12 years old in Kirtipur area (Maharjan 2004), 45% among the kindergarden children of khusibbu, Kathmandu (Maharjan et al. 2013). However, comparatively less prevalence of IPI have been reported from some parts of countries, 36.5% among the people of Kathmandu Valley (Rai et al. 2000), 27.67% among the school children of Bhaktapur (Shrestha and Maharjan 2013), 11.3% among the school children of Dharan District (Sah et al. 2013), 20.5% among the children of aged between 5-15 years old in Gurukul High School, Jatuwa (Gupta 2009). Among the protozoan parasites, *Giardia lamblia* has been found as the most prevalent (Chandrashekhar 2005, Kunwar 2009 Maharjan et al. 2013, Tandukar et al. 2013), Enthelamoeba histolytica (Nayak 2008). Giardia lamblia (14.32%) has been reported as the most prevalent protozoan parasites followed Entamoeba histolytica (8.84%) in Kanti Children's Hospital, Maharajganj, Kathmandu (Kunwar 2009) however, Entamoeba histolytica as the most prevalent protozoan parasite among the children of aged between 5-14 years old in Nobel Medical College, Biratnagar (Singh et al. 2013). On the other hand, Entamoeba coli (21.00%) has been found as the most prevalent protozoan parasite followed by Giardia lamblia (13.7%), hookworm (13%) and Trichuris trichiura (3%) among the school children in the rural village of Chitawan (Tai-Soon et al. 2001).

Among the helminthes parasites, *Ascaris lumbricoides* has been ranked as the most prevalent (Reddy et al. 1998, Rai et al. 2001, Rai et al. 2002, Chaudhary 2003, Maharjan 2004, Shrestha and Maharjan 2013), hookworm (Sherchand et al. 1997, Khetan 1998, Young et al. 2000 and Sah et al. 2013). Apart from *A. lumbricoides* and hookworm, *Trichuris trichiura* has also been reported as the most prevalent helminth parasite (Pradhan 2001, Sharma et al. 2004, Rai et al. 2005, Khanal 2005, Sah 2009). *A. lumbricoides* (18.44%) has been reported as the most prevalent helminth parasite followed by *Ancyclostoma duodenale* (14.67%), *Trichuris trichiura* (10.2%), *Hymenolepis nana* (8.68%) and *Strongyloides stercoralis* (2.89%) among the people of Mithiawa VDC (Sah 2009) but *Taenia* spp (5.3%) as the most prevalent helminth

followed by hookworm (2%), *A. lumbricoides* (1.9%) among the school children Dharan District (Sah et al. 2013).

A significant association has been found in prevalence of IPI between urban and rural area in which rural area was more susceptible (Chandrashekhar et al. 2005). Single infection of IPI has been reported highest (69.23%) than double (27.27%) and triple infection (3.49%) in Kirtipur area (Maharjan 2004). Similarly, Sah (2009) also showed the high rate of single infection (65.2%) than double (22.66%) and multiple infections (12.3%).

Regarding the age groups, highest prevalence of IPI have been shown in the children of aged between 9-14 years old (Maharjan 2004) and among the children of age group 11-15 years old in Lalitpur District (Tandukar et al. 2013). In case of the sexwise distribution of IPI, prevalence rate of parasites has been found slightly higher in female than in male without statistically significant (Tai-Soon et al. 2001). Similarly, the prevalence of IPI has been found higher in female than in male with statistically significant in the people of Kirtipur, Kathmandu and Gunjnagar VDC, Chitawan District (Ghimire and Mishra 2005). However, the prevalence rate has been found high in male children (21.77%) than in female children (18.75%) in Biratnagar (Gupta 2009). Sah (2009) also showed that prevalence rate of IPI higher in male children (50%) compared to female children (21.62%) has been more infected by IPI than Yadav children (19.86%) among the school children Jatuwa, Biratnagar-18 (Gupta 2009) and higher prevalence rate of IPI in pode community (80.30%) than in non-Newar (36.84%) and Newar community (36.54%) in Kirtipur area (Maharjan 2004).

The parasitic infection was correlated with hygienic and food habit of children of aged between 0-14 years old in Kanti children's Hospital, Maharajgunj, Kathmandu (Kunwar 2009). Significant difference between the prevalence of IPI and hand washing practice as well as the source of drinking water and burden of parasitic infection was correlated with poor sanitary condition of school (Tandukar et al. 2013). From the previous studies, it shows that many people of different parts of Nepal are suffering from previous to still from different protozoan as well as helminthic parasitic infection due to illiteracy, poor hygienic, poor sanitary condition and low socio-economic condition.

3. MATERIALS AND METHODS

3.1 Study Area

Tanahun District lies in Gandaki Zone of Nepal, a landlocked country of South Asia. The District lies in the middle most of the country, covers an area of 1, 546km² and the population around 323,288 with absent population around 752 (CBS Nepal 2014). The headquarter of this District is Damauli, lies on Prithvi Highway. The study was done in children of Barbhanjyang VDC of this district, Eastern Region, Nepal with population around 5,074 and the population of children aged between 0-14 years old is about 1,596 (CBS Nepal 2014). Gurung, Magar, Newar, Chhetri, Thakuri, Sarki, Damai, Kami are the main ethnic groups people living in this VDC.



Figure 1: Map of Tanahun District



Figure 2: Map of Barbhanjyang VDC, Tanahun District

3.2 Materials Required

3.2.1 Equipments

- i. Compound Microscope
- ii. Sample Vials
- iii. Adhesive Cello tape (Scotch tape)
- iv. Filter paper
- v. Forceps
- vi. Cover slip
- vii. Glass slides
- viii. Gloves
 - ix. Tray
 - x. Mask

3.2.2 Chemicals

- i. Normal Saline
- ii. 2.5% Potassium dichromate
- iii. Dettol Soap
- iv. Iodine Solution (Lugol Solution)
- v. 70% alcohol.

3.2.3 Preparation of 2.5% Potassium Dichromate

2.5mg of potassium dichromate was weighted and dissolved in 100ml of distilled water. This solution was used for the preservation of parasites which contained in the feacal matters.

3.2.4 Preparation of Normal Saline

Normal Saline was prepared by dissolving 8.5gm of sodium chloride in 1000ml of distilled water which was used in unstained preparation.

3.2.5 Preparation of Iodine Solution

The iodine solution was prepared by dissolving 10gm of potassium iodide in 100ml of distilled water and 5 gm of iodine crystals (powered) are slowly added in it. The solution was solution was then filtered and kept in a stopper. It was diluted about 5 times distilled water. The solution was used in a stained preparation for the visualization of internalorganelle of the protozoan parasites as well as helminth eggs.

3.3 Samples Collection and Examination

3.3.1 Scotch tape Samples Collection and Observation

The present study was conducted among the children of aged between 1-12 years old in Barbhanjyang VDC, Tanahun District, Nepal during June to October 2013. The aim and objectives of the study was explained in detail to the parents of the children especially to mothers. After obtaining verbal consent, a glass slide and transparent adhesive (one side) tape with identification tags were given to the parents with detailed collection methods writing the numbers, name, age, sex and class of the children were recorded in the record file. They were requested to attach the adhesive cello tape around the perianal area of their child with sticky side of the Scotch tape that would be revealed by pulling back the tape kept on the glass slide. The procedure was clearly demonstrated to them with the use of their child. They were requested to use the procedure before the child went to the toilet or bathing early in the morning to prevent the eggs of *E. vermicularis* being wasted from the perianal area.

One hundred and twenty five materials were distributed and 116 were submitted for examination. Out of 116 were returned, six did not have the identification tags and were excluded from the analysis. The collected samples were placed in a slide box with identification tags. The samples were brought to the centre Department of Zoology, Tribhuvan, University, Kirtipur, Kathmandu, Nepal. The samples were examined microscopically without staining and identification was made based on shape and size of the eggs.

3.4 Stool Samples Collection and Examination

3.4.1 Samples Collection and Preservation

A total of 110 stool samples were collected from the children same children whose Scotch tape samples were collected. Sampling vials were distributed for each before a day and asked to collect about 5 gm of stool early in the morning with the help of clean stick provided with clean plastic vials. They were oriented to avoid contamination of stool sample with other matters. Immediately after collection, 2.5% potassium dichromate solution was put in the vials containing faecal matter for preservation of parasites present in the stool samples.

3.4.2 Macroscopic Examination

Stool samples were examined by naked eyes for adult parasites and parasitic segments as well as colour and nature of stool.

3.4.3 Microscopic Examination

All the necessary equipments were brought in the lab and unstained and stained smear were prepared.

Unstained Smear Preparation of Stool

A small portion of faecal matter was picked with the help of wooden applicator and emulsified with freshly prepared normal saline on a clean glass slide. A clean cover slip was placed it and excess of fluid was removed with the help of cotton the observed under the compound electric microscope.

Stained Smear Preparation of Stool

The prepared iodine solution (Lugol's iodine solution) was used for the stained smear preparation. The stained smear preparation was required for the identification and study of nuclear matter of the protozoan cyst and trophozoites. The stained smear was prepared in the similar manner as prepared to unstained smear.

Stained and unstained smear preparations were first observed under the low power (10X objective) of the compound microscope. Examination was started from one end of the slide to another that's why whole field was examined. Samples were examined under high

power (40X objective) of the microscope when required. Positive samples were recorded in the record file and photographs were taken by using digital camera. Identification was done with the help of medical parasitological book and supervisor.

3.5 Socio-behavioural Survey

The structured questionnaire was prepared related to co-relate socio-behavioural aspect along with Knowledge, Attitude and Practices regarding the *E. vermicularis* along with other intestinal parasites and interviewed to the parents of the study children. The obtained information was used for statistical analysis.



Photograph 1: Questionnaire survey

3.6 Statistical Analysis

The data obtained from the sdudy was analyzed by using SPSS version 16.00.

4. RESULTS

The present study was carried out among 110 children of age group 1-12 years old of Barbhanjyang VDC, Tanahun District over the period of six months, from June 2013 to October 2013. A total of 110 Scotch tape (Transparent adhesive cello tape) samples were collected and examined for finding the general prevalence of *Enterobius vermicularis* infection among the children along with stool sample examination. The parents of those children were interviewed using structural questionnaire to correlate behavioural practices of children related to *E. vermicularis* infection.

4.1 General Prevalence of Enterobius vermicularis

Out of 110 children examined, 12.72% were found to be positive for *E. vermicularis* infection.



Figure 3: General prevalence of *E. vermicularis* in children

Among them, 56 were of male children while 54 were of female children. It was found that among 56 male children, 16.1% were found to be positive for *E. vermicularis* infection. Similarly, among 54 female children, 9.25% were infected by *E. vermicularis*. The result indicated that male children were more infected by *E. vermicularis* than the

female children but statistically no significant difference was found in prevalence of *E*. *vermicularis* and sexes of children (Chi-squared=1.149, df=1, P=0.284) (Table1).

The agewise distribution of *E. vermicularis* showed high prevalence rate in 5-8 years old age group as compared to other age groups. But statistically, no significant difference were observed among different age group (Chi-square=0.284, df=2, P=0.868) (Table 1).

Age groups (years)	Male (N=56) %	Female (N=54) %	Total (N=110) %
1-4	2 (3.6%)	1 (1.85%)	3 (2.72%)
5-8	3 (5.3%)	3 (5.55%)	6 (5.45%)
9-12	4 (7.14%)	1 (1.85%)	5 (4.54%)
Total	9 (16.07%)	5 (9.25%)	14 (12.72%)

Table 1: Prevalence of *Enterobius vermicularis* in children of Barbhanjyang VDC,Tanahun District

Ethnically children were categorized into three groups i.e. Dalit, Janajati and Others. Sarki, Damai and Kami were kept in Dalit group. Magar, Gurung and Newar were kept in Janajati group where as Brahman, Chhetri and Thakuri were kept in Others. The highest prevalence of *E. vermicularis* was found among Dalit children (64.28%) as compared to Janajati (7.14%) and Others (28.57). Distribution of *E. vermicularis* to that of ethnic groups was statistically significant (Chi-square=11.820, df=2, P=0.003) (figure 4).



Figure 4: Prevalence of Enterobius vermicularis with ethnic groups

Intensity of the parasitic eggs directly co-relates with the degree of infection and the degree of infection has direct co-relation to that of the pathogenecity of the parasitic diseases. Hence numbers of eggs were counted among the positive cases and determined the intensity of the infection which was classified as light, mild and heavy infection. If there was less than 2 eggs, it was considered as light infection, 2-4 eggs mild and more than 4 eggs was considered as heavy infection.



Figure 5: Intensity of *Enterobius vermicularis* infection in children
Result revealed that among 110 children included in the present study, maximum had the mild infection (57.14%) followed by light (28.57%) and heavy infection (14.28%) (Figure 5).

4.1.1 Children's Behavioural Practices in Relation to E. vermicularis Infection

Questionnaire survey was conducted among the parents of 110 children of Barbhanjyang VDC, Tanahun District using the structural questionnaire related to the children's behavioral practices. The prevalence of *E. vermicularis* was found to be directly related to the itching behaviour of the children since *E. vermicularis* needs oxygen for laying eggs so come out around anal region for ovulation. The result indicated that the prevalence of *E. vermicularis* was found highest (85.71%) in those children who had itching habit around the perianal region. The result was found to be statistically significant (P=0.0325) (figure 6).



Figure 6: Enterobiasis in relation to children's bihavioural practices

Itching and chewing of thumb as well as nail biting behaviour of children directly influence the auto infection of *E. vermicularis*. The survey result indicated that children's having their nail biting habit was proportional to the prevalence of enterobiasis. The frequently nail biter children were more prone to parasitic infection than others (figure 7). The result was found statistically significant (P=0.024).



Figure 7: Nail biting habit and parasitic infection in children



Photograph 2: Observation of Scotch tape samples



Photograph 3: Eggs of *E. vermicularis* (10X x 10X)



Photograph 4: Eggs of *E. vermicularis* (10X x 40X)



Photograph 5: Single egg of *E. vermicularis* (10X x 40X)



Photograph 6: Larva of *E. vermicularis* (10X x 40X)

4.2 Prevalence of Intestinal Parasites in Children of Barbhanjyang VDC, Tanahun District

A total of 110 stool samples were collected from the same children from whom Scotch tape method was used for *Enterobius vermicularis*. The collected stool samples were brought in the parasitology laboratory of Central Department of Zoology, Kathmandu and examined by using electric (light) microscope by smear technique. Out of 110 children examined, 37 (33.63%) were found positive for intestinal parasitic infection in the children of Barbhanjyang VDC, Tanahun District (Figure 8).



Figure 8: General prevalence of intestinal parasites in children

The prevalence of intestinal parasites was higher in male 21 (37.5%) compared to females 16 (29.62%) (Table 2). Among the three age-groups categorized, the prevalence of intestinal parasites was found higher in 5-8 years old age group 14 (12.72%) followed by 9-12 years old age group 13 (11.81%), 1-4 age years old group 10 (9.09%) children (Table 2).

Age groups (years)	Male (N=56) %	Female (N=54) %	Total (N=110) %	
1-4	5 (8.92%)	5 (9.25%)	10 (9.09%)	
5-8	7 (12.5%)	7 (12.96%)	14 (12.72%)	
9-12	9 (16.07%)	4 (7.40%)	13 (11.81%)	
Total	21 (37.5%)	16 (29.62%)	37 (33.63%)	

Table 2: Prevalence of intestinal parasitic infection in children of Barbhanjyang VDC,

 Tanahun District

However, statistically not significant difference was observed between parasitic disease prevalence among age groups (Chi-square=0.037, df=2, P=0.982). Similarly, statistically no significant difference was observed between sexes and IPI (Chi-square=0.763, df=1, P=0.382). Among the three ethnic groups, the prevalence was found higher in Janajati group (48.64%) followed by Dalit (43.24%) and Others (8.10%) (Figure 9) which was statistically significant (Chi-square=5.812, df=2, P=0.05).



Figure 9: Prevalence of intestinal parasites with ethnic groups of children

Seven different species of intestinal parasites were found among the children of Barbhanjyang VDC, Tanahun District. Among them, three species were protozoan parasites which were *Entamoeba histolytica, Entamoeba coli* and *Giardia lamblia* where as four species were helminth parasites. Among the helminth parasites two species were cestodes which were *Hymenolepis nana* and *Hymenolepis diminuta* and two spesies were found nematodes which were *Ascaris lumbricoides* and *Trichuris trichiura*. Among the protozoan parasites, *Entamoeba coli* was highly prevalent 16 (29.62%) but it is not pathogenic. Then, *Entamoeba histilytica* was highest 13 (24.07%) followed by *Giardia lamblia* 6 (11.11%). The prevalence rate of cestoda was found same in *Hymenolepis nana* 2 (3.70%) and *Hymenolepis diminuta* 2 (3.70%). Btween two nematode, *Ascaris lumbricoides* was found highly prevalent 9 (16.66%) compared to *Trichuris trichiura* 6 (11.11%) (Table 3).

Parasites	Species	Prevalence
Protozoa	Entamoeba coli	16 (29.62%)
	Enmoeba histolytica	13 (24.075)
	Giardia lamblia	6 (11.11%)
Cestode	Hymenolepis nana	2 (3.70%)
	Hymenolepis diminuta	2 (3.70%)
Nematode	Ascaris lumbriciodes	9 (16.66%)
	Trichuris trichiura	6 (11.11%)

Table 3: Overall prevalence of intestinal parasites in children

Out of 110 children examined, the occurrence of single infection was highest 27 (60%) followed by double 14 (31.11%), triple 3 (6.66%) and quadruple 1 (2.22%) (Figure 10).



Figure 10: Concurrency of intestinal parasites

4.3 Assessment on intestinal parasites in relation to Knowledge, Attitude and Practices (KAP)

Most of the children and their parents /guardians did not have the knowledge about the intestinal parasites such as *Enterobius vermicularis, Entamoeba histolytica. Ascaris lumbricoides* etc. Most of them did not know how to prevent and control the parasitic infection.

4.3.1 Prevalence of Intestinal Parasites in Relation to Knowledge

The rate of infection was found highest in those children whose parents were not aware of intestinal parasitic infection.



Figure 11: Knowledge about intestinal parasites

The present study showed that the parasitic infections rate was highest 32 (86.48%) in those children whose parents did not have any knowledge about parasitic infection as compared to 5 (13.51%) who had the knowledge (Figure 11).

Majority of study population (parents) of the children of Barbhanjyang VDC, Tanahun District were illiterate. Most of them had not knowledge about the prevention and control of intestinal parasitic infection.

Our study had tried to explore the impact of parent's knowledge of transmission, infection, prevention and control of germ infection on parasitic prevalence.



Figure 12: Prevalence of parasitic infection in relation knowledge

Among the parents who were aware of transmission, prevention and control of parasitic infection had lower (21.62%) prevalence rate of intestinal parasites as compared to 78.37% who were not aware (Figure 12).

The personal sanitation and hygiene of the children of Barbhanjyang VDC, Tanahun District was not good. Still some children were found walking with their bare foot and the parasitic infection was higher in those children.



Figure 13: Prevalence of parasitic infection in relation to walking habit

The result showed that the rate of parasitic infection was found higher in those children who had the habit of walking with their bare foot, 86.48% (32/37) infection rate in comparison to 13.51% (5/37) of prevalence rate of intestinal parasitic infection who had the habit of walking with non bare foot (Figure 13).

4.3.2 Assessment Survey on the Prevalence of Parasitic Infections in Relation to Practices

Majority of the study children had the habit of using toilet at home i. e. 73.63% (81/110), while 26.36% (29/110) belong to that category who did not use toilet for defecation. Among the children, who did not use toilet, 11 (37.93%) had the habit of defecation in open place. Those children, who defecated in open place, had 54.54% (6/11) infection rate of intestinal parasites. Among those children, who did not use the toilet, 18 (62.06%) had the habit of defecation near the water sources. Those children, who defecated near water sources, had 38.88 % (7/29) prevalence rate of parasitic infection. The children who had habit of using toilet had lower infection rate 23.45% (19/81) in compared to 44.82% (13/29) of those who were not using toilet at home. The result revealed that lack of toilet at home was significantly associated with parasitic infection (P=0.037) (Table 4).

	Practices	Total	Infection	P
Defection place	Toilat	01	10 (22 45%)	value
Derecation place	Open place	01	19(23.4370)	0.037
	Neer water course	11	0(34.34%)	
	Inear water source	18	7 (38.88%)	
Hand washing	Water only	59	22 (37.28%)	0.623
Hanu washing	Soap and water	38	6 (15.78%)	0.023
	Ash and water	13	4 (30.76%)	
	Direct	66	26 (39.39%)	
Drinking water	Boiling	10	1 (10.00%)	
consumption	Filter	21	5 (23.80%)	0.371
	Sodish	2	0 (0%)	
	Adding Chemicals	11	0 (0%)	
	Rubbing clothes	23	9 (39.13%)	
Cleaning vegetables	Using tap/well water	73	17 (23.28%)	0.051
	Without cleaning	14	6 (42.85%)	
	Once a week	57	13 (22.80%)	
	Once in two weeks	31	11 (35.48%)	
Nail cutting habit	Sometimes	17	6 (35.29%)	
	When feels necessary	5	2 (40%)	0.189
	y			
	Before a week	15	0 (0%)	
Use of antihelminthic drugs	before two weeks	28	2 (7.14%)	0.0091
	Before a month	31	11 (35.48%)	0.0071
	Before six months	26	12 (46.15%)	
	Before one year and	10	7 (70.00%)	
	above	21	14 (45 100)	
Methods of treatment	Traditional methods	31	14 (45.16%)	
	Direct taking	16	5 (31.25%)	
	Consulting with	25	3 (12,00%)	0.198
	doctor	23	5 (12.0070)	
	Traditional methods	20	4 (20.00%)	
	and consulting with			
	Do not care	18	6 (33.33%)	1

Table 4: Assessment of intestinal parasitic infection in relation to practices

Nearly about half of the study children practiced soap water and ash water to wash hand after using toilet and before taking meal (51/110), acquired lower prevalence rate (19.60%) (10/51) as compared to 39.28% (22/59) of prevalence rate in those children who didn't practiced soap water and ash water for washing hand, however, not significantly association (P=0.623) (Table 4).

Out of 110 children, 66 children were using drinking water directly without any treatment, acquired higher infection rate i.e.39.39% of intestinal parasitic infection than the other conditions. Among the 44 children, parasitic infection rate was 10.00% while 23.80% parasitic prevalence among who use boiling water for drinking. Parasitic infection was not found among the children who use proper treatment of water for drinking which was not statistically significant (P=0.371) (Table 4).

There was significant association of prevalence of intestinal parasites in methods of cleaning vegetables and fruits (P=0.051). Out of 110 children, 23 children used the fruits and vegetables by rubbing on clothes only, had 39.13% (9/23) of prevalence rate of intestinal parasites. Among 110 children, 73 children used the fruits and vegetables by cleaning with tap/well water, had 23.28% (17/73) of parasitic infection where as highest infection rate i.e. 42.85% (6/14) of the children who used fruits and vegetables without cleaning were infected with parasitic infection (Table 4).

The present study was found that nail biting habit of the children was not significantly associated with IPI (P=0.189). Among the study children, 57 children was found to have nail cutting habit once a week had 22.80% of IPI, 31 children had the nail cutting habit once in two weeks, acquired 35.48% of IPI, 17 were found to have nail cutting habit sometimes had 35.29% of IPI where as 5 children were found to have the nail cutting habit when feel necessary had 40% of IPI (Table 4).

The present study showed that intake of anti-helminthic drugs before was significantly associated with the intestinal parasitic infection among the study children (P=0.0091). Out of 110 children, 15 children had taken deworming tablet before a week during the time of sample collection, had no any parasitic infection. Twenty eight (28) children had taken the anti-helminthes drugs before two weeks, had 7.14% IPI, Thirty one (31) children had taken the deworming tablet before one month during the time of sample

collection acquired 35.48 % of IPI. There was 46.15% (12/26) of prevalence rate of parasitic infection among the study children who had taken antihelminthic drugs before six month where as highest prevalent rate of infection was found among the children who had taken the deworming tablet before one year which was 70.00% (Table 4).

Method of treatment of intestinal parasitic infection was not significantly associated with the parasitic infection rate among the study children (P=0.198). Among the 110 children, thirty one (31) were found to follow the traditional methods for the treatment of intestinal parasitic infection, had 45.16% of parasitic infection rate where as sixteen (16) children of the total children were found direct taking medicine without consulting with doctors had 31.25 % of prevalent rate of IPI. In both above conditions the prevalence rate of parasitic infection were found higher in comparison to 12.00% and 20.00% among the children who consulted with doctor and both consulting doctors and following traditional respectively. On the other hand, 18 children did not care the parasitic infection acquired 33.33% of IPI among the study children (Table 4).



Photograph 7: Preservation and counting of stool samples



Photograph 8: Observation of stool samples



Photograph 9: Cyst of *Entamoeba coli* (10X x 40X)



Photograph 10: Cyst of *Entamoeba histolytica* (10X x 40X)



Photograph 11: Cyst of Giardia lamblia (10X x 40X)



Photograph 12: Egg of Ascaris lumbricoides (Decorticated) (10X x 40X)



Photograph 13: Egg of Ascaris lumbricoides (Corticated) (10X x 40X)



Photograph 14: Egg of Trichuris trichiura (10X x 40X)



Photograph 15: Egg of Hymenolepis nana (10X x 40X)



Photograph 16: Egg of Hymenolepis diminuta (10X x 10X)

5. DISCUSSION

Enterobius vermicularis is wordwide in distribution particuraly in temperate regions (Tandan et al. 2002). *E. vermicularis* pose serious threat in the physical well being of human, especially in children. Poverty, illiteracy and different socio-economic and socio-behavioural aspects of life may play leading role in increasing the rate of prevalence of this parasite. The Children compared to adult and old age group of population are more prone to enterobiasis and the rate of this parasite is higher in school children than preschool children (Raza and Sami 2009).

In order to access the enterobiasis among the children of Barbhanjyang VDC, Tanahun District, the present study was conducted. Out of 110 children, 12.72% were found to be infected by this parasite. This high prevalence rate which indicated the remarkable prevalence of this parasitic infection among the children may be associated with unsanitary living style, poor socio-economic condition, crowding, sharing of beds and consumption of raw vegetables and fruits, faecally contaminated water. However, enterobiasis in children is comparatively less than several other previous studies in Turkey (Culha and Duran 2006 and Balci et al. 2010) in Iraq (Kadir and Amin 2011), in Thailand (Nithikathkul et al. 2001), in Malaysia (Norhayati et al. 1994), in Egypt (Bahader et al. 1995), in Peru (Gilman et al. 1991), in chile (Mercado et al. 1996) and in Iran (Heidari and Rokni 2003) where as the overall infection rate among children was found comparatively more than in several earliar studies in Iraq (Raza and Sami 2009), in Taiwan (Chang et al. 2009), in Nepal (Kunwar 2009, Shrestha and Maharjan 2013, Sah et al. 2013), in Turkey (Alver et al. 2011) and in Srilanka (Gunawardena and Ismail 2013). Comparable prevalence of *E. vermicularis*, however, was reported in some other studies in Turkey (Cengiz et al. 2010), in Korea (Lee et al. 2011), in Nepal (Sharma 2009). This might be due to the difference in climatic condition, cultural behaviour and sanitary condition, density of population, economic status and geographical location. Pezzani et al. (2004) in Argentina showed 41.42% infection rate in children. Wani al. 2010 showed 13.92% of prevalence for E. vermicularis infection in India but Pandit (2004) found comparatively less prevalence i.e. 5.52% of infection in children of Chitawan District, Nepal.

Sexwise analysis of enterobiasis revealed higher prevalence (16.1%) in male children compared to female (9.25%), however there was not significant association in prevalence of *E. vermicularis* in between two sexes. Similar finding have also been reported previously in Nepal (Pandit 2004), in Turkey (Cengiz et al. 2010), in Nigeria (Auta et al. 2013), in Iraq (Kadir and Amin 2011), in Korea (Lee et al. 2011). The present result was in favour of the study done by Culha and Duran (2006) in Turkey which showed that the infection rate of *E. vermicularis* were 75.6% and 65.7% in male and female children respectively. Chang et al. (2009) also found statistically significant prevalence of *E. vermicularis* in boys and girls in Taiwan. Rasti et al. (2012) reported high prevalence of pinworm in male children about 2.5 times more than in female, however differential infection rate between sexes has not been explained. This may be due to the reason that children from both sexes share the similar type of environment and feeding habit and hence are equally susceptible for transmission of pinworm.

Regarding the age groups, though there was no significant association in prevalence rate in age groups, the high prevalence rate was found in 5-8 years old (5.45%) followed by 9-12 years old (4.54%) and 1-4 years old (2.72%) children. This might be due to the reseason that the children of this age group mostly spent their time outside the home playing in and out door games, crowding with large numbers of children, playing every day together and are in contact with contaminated soil as well as water which may facilitated transition and spreading of the infection. The minimum prevalence was observed in children of age group 1-4 years old (2.72%). The reason behind low prevalence rate in this age group could be that the children of this age group are fed breast milk and take care by their mothers properly. Similar result have also been reported by Raza and Sami (2009) in Iraq, Ammoura (2010) in south Jordan, Sing et al. (2013) in Nobel medical college, Biratnagar, Nepal.

Ethnicity play important role in dissemination of parasites and parasitic infection. Socioeconomic status, socio-cultural behaviour, personal hygiene and sanitation seem to be directly associated with the ethnicity of the community people. The present findings have been analyzed to find out the association between ethnicity and *E. vermicularis* infection. The infection rate *of E. vermicularis* was found to be significantly associated with ethnic groups. The highest prevalent rate was found among the Dalit children (64.28%) as compared to Others (28.57%) and Janajati (7.14%) children which was directly associated with poor personal hygiene, sanitary condition around the houses, illiteracy, poor socioeconomic condition etc. on the basis of direct field observation during the study period.

Enterobiasis infection among the family members as well as community and in most cases autoinfection of these parasitic diseases is influenced by the hygiene, sanitation and personal behaviour. In present study, the structural questionnaire survey was carried out to observe the linkage between personal practices and *E. vermicularis* infection. During the surveillance study, it was found that the prevalence of E. vermicularis with itching behaviour of children was statistically significant (P=0.0324) while highest prevalence (85.71%) among the children who had itching habit habit around the perianal regions compared to non-itching (14.28%). This may be due to crawling of adult female E. vermicularis around the perianal region for oviposition mainly during the early morning hours since it needs oxygen for laying the eggs. The present result was in agreement with the result of Out-Basseg et al. (2011) which showed the significant relation between perianal itching as well as nail chewing behaviour of children and enterobiasis. The present result also showed that the nail biting behaviour of children was statistically significant (P=0.024). The present finding coincides with result presented by (Alo et al. 2011) in Nigeria and Bustami and Khraish (2010) in Jordan where they found the children having their nail biting habit was proportional to the prevalence of enterobiasis.

Beside the enterobiasis, several other intestinal parasites influenced the socio-economic well being of the community. These infections have direct impact on health and mental development of the children. Human intestinal parasites are cosmopolitan in distribution, posing the serious health problems in developing countries as Nepal, where illiteracy, poverty and ignorance are interlocked. Among them, soil transmitted helminthes (STHS) and other helminthes parasites are serious threat in the physical well being of human. Poverty, illiteracy and different aspects of culture may play leading role in increasing rate of prevalence of intestinal parasites.

The present study indicated that the high prevalence of the intestinal parasites in children of Barbhanjyang VDC, Tanahun District. Out of 110 children, 37 (33.63%) were found to be infected by at least one species of parasites. Among the infected children, 64.86% belong to protozoan parasites where as 35.13% to helminthic parasites. This high rate of prevalence may be associated with unsanitary living style, poor socio-economic conditions, usual contact with soil and consumption of raw vegetables, fruits washed in

faecally contaminated untreated water. The total prevalence rate of IPI among the children showed comparatively less than several other studies in Nepal (Rai et al. 2001, Shrestha 2001, Rai et al. 2002, Chaudhary 2003, Maharjan 2004, Ghimire and Mishra 2005, Sah 2009, Maharjan et al. 2013), in India (Wani et al. 2010, Sehgal et al. 2010), in Nigeria (Idowu et al. 2011), in Italy (Gualdieri et al. 2011), in Iraq (Rasti et al. 2012, in Malaysia (Lim et al. 2009). However, similar prevalence of IPI was determined in some other studies in Nepal (Rai et al. 2000, Shrestha and Maharjan 2013), in Turkey (Cengiz et al. 2010), in south Jordan (Ammoura 2010), in Saudi Arabia (Al-Mohammed et al. 2011).

Though, the prevalence rate of IPI and sexes of children statistically not significant (chisquare=0.763, df=1, P=0.383), the prevalence of intestinal parasites was found higher in male children (37.5%) in comparison to the females (29.62%). This may be due to the reseason that both sexes share similar type of environment and feeding habit and hence equally susceptible for transmission and spreading of intestinal parasites. Similar finding have also been reported previously, AL-Taie (2008) in UAE, Rasti et al. (2012) in Iraq, Auta et al. (2013) in Nigeria, Cengiz et al. (2010) in Turkey, Ammoura (2010) in south Jordan, Gupta (2009) in Gurukul Madhyamic Vidhylaya, Jatuwa, Biratnagar, Nepal. The result also was in agreement with the result of Maharjan (2004) in which it was found that the prevalence of IPI 55.95% in male children where as 49.83% in female in children of Kirtipur area, Nepal. But in contrast, Ghimire and Mishra (2005) showed those females (46.8%) were more infected compared to the male (35.2%). But the parasitic prevalence associated with sexes has not been scientifically explained.

Ethinically children were categorized into three groups i.e. Dalit, Janajati and Others. The prevalence rate of IPI was found higher among the Janajati 18 (48.64%) followed by Dalit 16 (43.24%) and least prevalence rate was found among the Others 3 (8.10%). This may be related to the crowding index, educational levels of people, poor sanitary disposal of faecally contaminated water and poor hygiene around the area where infection rate were high. This result is similar with the result of Maharjan (2004) in which it was found that the children of Pode community were more infected (80.30%) than Newar (36.54%) and Non-Newar (36.84%).

In this study, seven different species of intestinal parasites were found to be distributed among the children of Barbhajyang VDC, Tanahun District. Three species of protozoan parasites includes *Entamoeba coli* (29.62%), *Entamoeba histolytica* (24.07%) and *Giardia lamblia* (11.11%). Four species of helminthes parasites were found. Among the helminth parasites, two species were cestodes which were *Hymenolepis nana* (3.70%) and *Hymenolepis diminuta* (3.70%) and two species nematodes which include *Ascaris lumbricoides* (16.66%) and *Trichuris trichiura* (11.11%). All these parasites had also been found in Kaski District (Chandrashekhar et al. 2005) as well as Nobel Medical College, Biratnagar (Sigh et al. 2013). The presence of protozoan parasites at high rates could be associated with quality of drinking water as well as unhygienic farming practices. During the field visit it was seen that community tap water is not treated. Most of the community people drink untreated water directly from sources. Farmers were using faecally contaminated water for irrigation etc.

Among the protozoan parasites, *Entamoeba coli* (29.62%) was the most prevalent intestinal protozoa parasite followed by *E. histolytica* (24.07%) and *Giardia lamblia* (11.11%). As by Tai-Soon et al. (2001) which showed *E. coli* was the most prevalent among the protozoan parasites. This might be due the drinking of untreated open sourced water. But other studies showed *E. histolytica* in Africa (Samie et al. 2010), in United Arab Emirates (Al-Taie 2008), in India (Shrihari et al. 2011), in Nepal (Nayak 2008, Singh et al. 2013) and *Giardia lamblia* in Turkey (Cengiz et al. 2010, Balci et al. 2010, Gunduz et al. 2011), in Iraq (Raza and Sami 2009), in Yemen (Alyousefi et al. 2011), in Jordan (Ammoura 2010), in Nepal (Tandukar et al. 2013) as the most prevalent protozoan parasites. *Giardia lamblia* and *Entamoeba histolytica* are the major causes of diarrhea and dysentery worldwide respectively.

In case of heminth parasites, *Ascaris lumbricoides* (16.66%) was the most prevalent intestinal parasite followed by *Trichuris trichiura* (11.11%), *Hymenolepis nana* (3.07%). This result is in favor with that result reported before in Turkey (Cengiz et al. 2010), in Nigeria (Auta et al. 2013), in India (Wani et al. 2009), including Nepal (Sharma 1965, Reddy et al. 1998, Pradhan 2001, Rai et al. 2001, Khanal 2005, Ghimire and Mishra 2005, Sah 2009, Maharjan 2004, Shrestha and Maharjan 2013) which also showed *A. lumbricoides* as the most prevalent helminth parasite. The previous studies highlighted that both protozoan and helminth parasites poses the serious public health threat globally, particularly developing countries like Nepal.

Out of 37 positive cases, highest prevalence was found with single parasitic infection (60%) followed by double, (31.11%), triple (6.66) infection and only one positive case was infected with four different species of intestinal parasites. This result resembles with Maharjan (2004) in which it was reported that single infection (69.23%), double (27.27%) and 3.49% triple infection (3.49%) in Kirtipur area Nepal. Bayoumy et al. (2011) also support this study where it was found that prevalence of single infection was higher (54.2%) as compared to mixed infection rate (12.9%).

The knowledge about the transmission, prevention and control about the intestinal parasites are directly related to the rate of the infection. The present result showed that the parasitic infection was higher 32 (86.48%) in those children whose parents did not have the knowledge as compared to 5 (13.51%) who had the knowledge of parasitic infection. Likewise, among the parents of children who were not aware of transmission, prevention and control of IPI, the prevalence rate was higher (78.37%) in comparison to 21.62% who were aware. This may be due to illiteracy, poverty, poor socio-economic conditions. The result was in agreement with earlier studies (Kuwar 2000, Maharjan 2004 and Sharma 2009).

The personal hygiene and sanitation of children of country seem pitifully. In rural villages, most children are found walking with bare foot, farmers most of time remained in bare foot working with faecally contaminated water. The present result showed that the rate of IPI was high in those children who had the habit of walking with bare foot (86.48%) in compared to 13.51% who had the habit of walking with non-bare foot. This might be due to poverty, illiteracy, negligence etc.

Intestinal parasitic infection is directly related to night soil disposal practices of the community people. In the present study, 73.63% children were found to be using the toilet for defecation while 26.36% were not. Among the toilet user, 23.45% had IPI where as 44.82% in non user. There was statistically significant association (P=0.037) in prevalence of IPI and defecation place. The presence of toilet at home may not ensure the prevention of intestinal parasitic infection. Improper use of toilet as well as open field defecation directly influences to increase rate of IPI. This result also was in agreement of result of Maharjan (2004) which showed that 95.83% had got the toilet while 3.53% were using open places for defecation. The present study was similar to other previous studies (Sherchand et al. 1999, Rai et al. 2001). The toilet facility of the children also affected

the infection in finger sucking children who used pit latrines regarding the higher prevalence of parasites (Idowu et al. 2011).

Regarding the hand washing behaviour after different activities particularly after toilet use it was found that 53.63% of children used water only to wash hand after using toilet and before meal while 46.36% used soap and ash water. It was found that prevalence rate of intestinal parasites was higher (37.28%) in those who were using water only compared to (19.60%) who were using ash and soap, though no significant association was found in prevalence rate (P=0.623). This result coincides with Idowu et al. (2011) in which it was found that 85% of the children did not wash hands after defecation. Maharjan (2004) also reported similar result in which it was found that only 28.52% of children used to wash their hands properly after defecation and Chaudhary (2014) in which it was reported that 27.2% of prevalence rate was found among the pregnant women who was used soap water to wash hand. Polluted hands might be the source of transmission of different intestinal diseases and promote to increase the infection rate.

Drinking water is the main source of intestinal parasitic diseases transmission. The prevalence of IPI depends on the quality of drinking water as other factors. The result revealed that 60% of children were using drinking water without any treatment while remaining by treating. Although, statistically not significant (P=0.371), there was higher prevalent rate (39.39%) in those who used drinking water without treatment as compared to 19.35% in those used with treatment. Maharjan (2004) and Chaudhary (2014) had also reported the similar result. The high rate of prevalence of intestinal parasites may also be related with consumption of vegetables, fruits water contaminated with infected faecal matter along with the consumption of faecally contaminated drinking water.

Nails play the important role in transmission of intestinal parasitic infection. Many people do not trim the nail regularly and may be the victim of different intestinal diseases. In this study, high infection rate (40%) was observed in children who neglected to trim the nail as compared 28.57% who trim, although no statistically significant (P=0.189). Long nail may accelerate the rate of transmission of IPI and may help the vectors to survive which also transmit the parasitic diseases. This result is similar to the result reported by Alo et al. (2013) in Nigeria which claimed that finger nails were one of the important sources of transmission of intestinal parasites.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The present study was carried out to determine the prevalence of *E. vermicularis* as well as other intestinal parasites among the children of Barbhanjyang VDC, Tanahun District, Nepal. Morning Scotch tape (cellophane tape) as well as stool samples were collected in a slide box and clean vials respectively from those children whose parent's orientation was done and materials were distributed a day before evening along with structured questionnaire survey to access knowledge, attitudes and practices in relation to the *E. vermicularis* and other general intestinal parasites.

Out of 110 Cello tape samples examined, the prevalence rate of *E. vermicularis* was found to be 12.72%. The male children were more infected by enterobiasis than the female without statistically significant (P=0.284). In case of sexwise distribution of enterobiasis, higher prevalence rate was found in 5-8 years old age group as compared to other without significant difference (P=0.868). Regarding the ethnical distribution, the highest prevalence of *E. vermicularis* was found among the Dalit children compared to Janajati and Others with statistically significant association (P=0.003). Mild infection of enterobiasis was found to be higher followed by light and heavy infection. In case of behavioural practices of children in relation to *E. vermicularis* infection, itching around the anal region and nail biting habit were found to be statistically significant.

Out of 110 stool samples examined, the prevalence of intestinal parasites apart from the *E. vermicularis* was found to be 33.63%. The prevalence of IPI was found higher in male children than the female but not significant difference was observed. However, no significant association was observed between IPI and age group, 5-8 years old age group was found to be more infected followed by 9-12 years and 1-4 years old age group. In case of ethnic groups, the highest prevalence of IPI was observed among the Janajati children followed by Dalit and Others with statistically significant difference. Regarding the intestinal parasites, the prevalence rate of *Entamoeba coli* was found to be highest followed by *Entamoeba histolytica*, *A. lumbricoides*, *Trichuris trichiura*, *Giardia lamblia*, *Hymenolepis nana* and *Hymenolepis diminuta*. The prevalence of the single infection of

intestinal parasites was higher followed by double, triple and quadruple infection in the children.

The prevalence of the IPI was observed highest in those children whose parents had less knowledge about the intestinal parasitic infection and their transmission, prevention and control. Similarly, the prevalence of IPI in children in relation to defecation place, methods of cleaning of vegetables and fruits and time of use of anti-helmithic drugs were statistically significant. The highest prevalence of IPI was seen those who didn't use soap for washing hand, drink water directly without treatment, not trim nail timely and followed the traditional methods only for the treatment of parasitic infection.

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6.2 RECOMMENDATIONS

For the effective prevention and control of the *E. vermicularis* as well as other intestinal parasitic infections among the children of Barbhanjyang VDC, Tanahun District, some recommendations are as follows

- i. People should be encouraged to improve sanitary environmental condition including personal hygiene and environmental sanitation
- ii. Trimming of the nails should be encouraged to the children to minimize the risk of infection
- iii. Nail biting habit should be discouraged
- iv. Parents must be educated that itching around the perianal region by the children are important symptoms of enterobiasis
- v. Basic health education programmes should be conducted time to time
- vi. Defecating habits of children in open field should be avoided and toilet construction should be promoted
- vii. Use of anti-helminthic drugs should be applied at least once within six months.

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ANNEX-I

QUESTIONNAIRE

Name:	Ag	ge:	Sex:				
Address:			Profession:				
Number of famil	ily members:	,	Гуре of school:				
Government/Bo	oarding/Montessor	i					
1. Do you have	toilet in your hom	e?					
a. Yes	b. No						
If yes, what type of toilet do you have?							
a. Dug well	b. Bore Hole	c. Water S	Seal				
2. Where does your child defecate?							
a. Toilet	a. Toilet b. Open place						
3. Where do you get drinking water from?							
a. Tap water	b. well						
4. Do you treat	drinking water?						
a. Yes	b. No						
If yes, how?							
a. Boiling	b. Filter c	. Piyush	d. Sodish				
5. Does your child have habit of hand washing after toilet and before meal?							
a. Yes.	b. No						
If yes, what does your child use for hand washing?							
a. Water only	b. Soap water	c. Ash w	ater				
6. How do you and your child clean vegetables/Fruits?							

a. Only rubbing on clothes	a. Only rubbing on clothes b. By using tap /well water							
c. Without cleaning	d. All above							
7. Does your child walk with bare foot?								
a. Yes. b. No								
8. Does your child cut nail regularly?								
a. Yes.	b. No							
If yes, when?								
a. Once a week	b. Once in two week							
c. Sometime	d. When he/she feels necessary							
9. Does your child have the habit of nail bite?								
a. Yes.	Yes. b. No							
If yes, when?								
a. Sometimes	b. frequently							
10. Does your child bath re	gularly?							
a. Yes.	b. No							
If yes, when?								
a. Once a week b	. Twice a week	c. Once in	two week					
11. Do you have pet in hom	e?							
a. Yes.	b. No							
If yes, what are they?								
a. Goats b. Dogs o	c. Cats d. Cows	e. Hens	f. All above					
12. Does your child play wi	th domestic animals?							
a. Yes. b. No								

If yes, what are they?

a. Goats	b. Dogs	c. Cats	d. Hens	e. O	thers (specify)			
13. What type of food habit does your child have?								
If non-vege	etarian, what typ	e of meat d	oes your ch	ild eat mostl	y?			
a. Boiled	b. Fried	c. Under-cooked d. Well-cooked						
14. Does your child itch around anus region?								
a. Yes.	Yes. b. No							
If yes, whe	n?							
a. Early in the morning. b. At day time c. At evening d. At night					d. At night			
15. Has your child take anti-helmintics before?								
a. Yes.		b. No						
If yes, how	long time befor	re?						
a. Before a week		b. Before two week c. Before one month						
c. Before six month		e. Before one year f. Others			f. Others			
KAP relate	ed questions:							
1. Do you know how dirrhoea and dysentery is called?								
a. Yes.	es. b. No							
If yes, indi	cate the causativ	ve agent?						
a. Entamoe	eba histolytica	b. <i>Gi</i>	ardia lambli	ia c. Ot	hers			
2. Do you know about intestinal parasites?								
a. Yes.	h. Yes. b. No							
If yes, wha	t are they?							
a. Roundw	orm b. Ta	peworm	c. Pinwor	n d. (Others			

3. Do you know what the causes of itching around the perianal region are?

a. Yes. b. No If yes, what are they? a. Scabies b. Pin worm c. Others..... 4. Do you know pin-worm? a. Yes. b. No If yes, what does it cause to human being? 5. How do you treat if your child get pin worm infestation? a. Direct taking medication b. Consultation doctor c. Traditional method Others 6. Do you know the method of prevention and control of germ infection? b. No a. Yes. If yes, what are they?

d.