PREVALENCE OF INTESTINAL PARASITES IN THARU COMMUNITY OF PAWANNAGAR VDCs OF DANG DISTRICT IN RELATION TO THEIR SOCIO-ECONOMIC STATUS

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Submitted to:

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Kirtipur, Kathmandu

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DECLARATION

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

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CERTIFICATE OF ACCEPTANCE

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LIST OF ABBREVIATIONS

Abbreviated form	Details of Abbreviations
CDZ	Central Department of Zoology
VDC	Village Development Committee
WHO	World Health Organization
T.U.	Tribhuvan University
χ^2	Chi-square
d.f.	Degree of freedom
No.	Number
et al.	and his associates
Sqkm	Square kilometer
Km	Kilometer
ml	Milliliter
mm	Millimeter
gm	Gram
A.D.	Amino Domino
µm	Micrometer
E	East
N	North
IUCN	International Union for Conservation of Nature
BBC	British Broadcasting Corporation
IPI	Intestinal Parasitic Infection
IP	Intestinal Parasite

ABSTRACT

Intestinal parasitosis is becoming a common problem among people of least developing countries living under low socio-economic profile. This study was carried out to determine the prevalence of intestinal parasites in Tharu people of Pawannagar VDCs of Dang district in relation to their socio-economic status. The study was carried out with prepared questionnaire method to determine the knowledge, attitudes and practices between March to November, 2016. Altogether 200 stool samples were randomly collected from different age groups and sexes and were preserved in 2.5% potassium dichromate solution. These stool samples were examined by direct smear method in the Parasitology Laboratory of Central Department of Zoology, Kirtipur, Kathmandu. Out of 200 peoples, 59 (29.5%) were found to be infected with one or more intestinal parasites among them the prevalence rate of parasitic infection in males were 34 (31.19%), whereas females were 25 (27.47%) (χ^2=0.18017). Finding of the study showed that, highest prevalence rate was in the age group above 41 years was 33.33%, where lowest was 28.67% (χ^2=0.10876) in the age group 20-40 years. Altogether, five species of intestinal parasites were detected. *Entamoeba histolytica* was most predominant among protozoa whereas *Ascaris lumbricoides* was detected among helminth parasites. The distribution of helminthic infection 51 (86.44%) were higher than the protozoan infection 8 (13.55%). The prevalence of *A. lumbricoides* 27 (45.76%) was the highest whereas *Enteriobius vermicularis* 2 (3.38%) was the lowest parasites. But the study showed that single 52(88.13%), double 5 (8.47%) and multiple 2 (3.38%) parasitic infections in the Tharu communities. Tharu people were interviewed on the basis of parasitic infection, knowledge of the transmission, control and prevention of the parasites. Most 141 (70.5%) people were unaware and 59 (29.5%) people were aware about the intestinal parasitic worms. Female were more 31 (34.06%) aware than male 28 (25.68%).

Thus, the wide parasitic infection among Tharu people are attributed to poor hygienic condition, contaminated water, unhygienic food, poor sanitary disposal condition and low socio-economic status of these communities help to increase the high prevalence rate.

CHAPTER- 1

INTRODUCTION

1.1 Background of the study

Parasites are those living organisms that live in a host organisms and gets food from the expense of its host. There are different types of parasites such as ectoparasites, endoparasites, facultative parasites, obligatory parasites etc. that can cause disease in humans. They bring serious health problems in humans (CDC, 2014).

Intestinal parasitic infections are distributed virtually throughout the world. According to the World Health Organization (WHO) estimates, globally about 3.5 billon people are affected by intestinal parasitic infections and cause clinical morbidity in approximately 450 million, majorities of the cases occur among children (WHO, 1998). Intestinal parasitic infections such as Ascariasis, hookworm infection and Trichiuriasis are among the ten most common infections in the world and each constituting of 100 million, 900 million respectively, being responsible for considerable morbidity and mortality. Besides of causing morbidity and mortality infections with intestinal parasites have been associated with stunting physical weakness and low educational performance of the poor segments of the population and intimately linked with low economic level, poor personal and environmental tropical climate and low altitude (Desta *et al.,* 2014).

Intestinal parasitic diseases remain a serious public health problem in many developing countries especially due to fecal contamination of water and food (Engels and Savioli, 2006). More than 72 species of protozoa and helminth parasites can lodge in humans. Most are considered food and water borne zoonoses (Slifko *et al.,* 2000; Pozio, 2003 and Leelayoova *et al.,* 2004). Multiple socio-geographic and environmental factors determine the prevalence and intensity of a parasitic infection such as weather, that affects the time and intensity of outbreaks (Doligalska and Donskow, 2003; Siriski, 2003).

Intestinal parasites can infect the gastro-intestinal tract of humans and other animals. They can live throughout the body but most prefer the intestinal wall, means of exposure include; ingestion of undercooked meat, drinking infected water and skin absorption. The two main types of intestinal parasites are helminths and protozoans that reside in the intestines. An intestinal parasite can damage its host via an infection which is called helminthiasis in case of helminthes (Loukopoulos *et al.,* 2007).

The common intestinal parasites are *Ascaris lumbricoides, Strongyloids stercoralis, Giardia intestinalis, Ancylostoma duodenale, Entamoeba histolytica, Cyclosporidium parvum* etc. Among these certain types remain in the intestines and other travels outside the intestines to invade other organs. Some are so small they can only be seen under the microscope. Most tapeworms and roundworms develop in the human body and lay their eggs there. The eggs then pass out of the body through stool and can infest others (Birn and Armando, 1999).

Intestinal parasites cause significant morbidity and motility. Disease due to *Giardia intestinalis, Ancylostoma duodenale, Entamoeba histolytica* causes irritation, sleep disturbance, nausea, vomiting, malabsorption, diarrhea and weight loss. Treatment includes metronidazole, proper hand washing and consumption of bottled water can be preventive. An *Ancylostoma duodenale* causes blood loss and anemia. Treatment includes albendazole, mebendazole and blood transfusion (Kucik *et al.*, 2004).

In Nepal over 70% of morbidity and mortality are associated with infectious diseases and is also reflected in the "top ten diseases" of Nepal (Rai *et al.*, 2011). Diarrhea is produced by a variety of etiological agents of them, intestinal parasitic infection alone contributes a great extent in the cause of diarrhea and is one of the most common public health problems in Nepal (Rai *et al.*, 1998).The common intestinal helminthes reported from Nepalese children are *A. lumbricoides*, hookworm and *Trichuris trichuria* (Thapa *et al.*, 2011) with manifestations as varied as malnutrition, iron deficiency anemia, mal-absorption syndrome, intestinal absorption and mental and physical growth retardation. Protozoal infections, amoebiasis and giardiasis are most frequently reported. The agents spread faeco-orally through contaminated sources. Although people of all ages may be infected by these organisms. Children are more often infected due to compromise in sanitary habits (Saksirisampaint *et al.*, 2003).

Intestinal parasitosis appears as one of the major economic burden to the developing countries like Nepal. Dang district is the largest valley in south Asia. It contains highly developed to remote rural places. People in the city areas are more educated than the villages. People in Dang district are troubled by intestinal parasitic infection due to poor health habits, water pollution, unhygienic behaviour, low economic status (Khanal *et al.*, 2011).Mostly children are affected by the parasitic infections (Rai *et al.*, 1994).Water pollution is the main cause of parasitic infection. Diarrheal and gastro-intestinal tract diseases are the result of water pollution. Diarrhea (10%) is the disease caused by contaminated water in Nepal (DoHS, 1998 and SAEHN, 2002).

1.2 Introduction to Intestinal Parasites

The intestinal parasites are those parasites which inhibit the intestinal region of the host and get nourishment from there. The intestinal parasites are generally protozoan and helminthes.

1.2.1 Intestinal protozoan parasites:

Protozoa are unicellular, microscopic organisms which are widely distributed. There are about 65,000 species of protozoa of which 10,000 are parasitic. Each protozoan cell is able to perform all the vital functions of life. The mode of reproduction could be asexual or sexual.

Entamoeba and Amoebiasis

Entamoeba histolytica was described first by Losch in 1875 in the stool of Russian suffering from dysentery. The infection caused by *E. histolytica* is known as amoebiasis. It may manifest as diarrhea, dysenatry and hepatic or pulmonary amoebiasis.

The morphology of the parasites can be divided into trophozoite, precyst and cyst. Trophozoite is feeding stage, also known as growing stage, and measuring 15-30 µm in size. It has not fixed shape because of constantly changing position. Precyst is colourless, round or oval smaller than trophozoite but larger than cyst. It ranges between 10-20 µm in size. Cyst becomes rounded and is surrounding by a smooth wall. The cyst is initially unicellular and then develops into a binuclear after binary fission (Ichhpujani *et al*., 2002).

These amoebae are transmitted by oral-fecal route. Poverty, ignorance, impair personal hygiene are the major facilitate the spread of disease. Amoebas multiply rapidly in the tissue cells and use the cytolysed materials as their food.

Giardia and Giardiasis

Giardia intestinalis is also known as *G. lamblia, G. duodenalis and Lamblia intestinalis*. It was seen by Leeuwenhoeck in 1681 while examining his own stool. It is worldwide in distribution with highest prevalence in the tropics and subtropics area. It appears in all age groups but higher incidence in children. It is found in duodenum and the upper part of jejunum in man.

The morphology of *Giardia* exists in two forms: trophozoite and cyst. Trophozoite is feeding stage, measuring about 10-20µm in length, 6-15µm in width and 1-3µm in thickness. The parasite gives a typical monkey-face appearance on microscopic examination. Trophozoites multiply in the intestine by binary fission. Cyst convert from trophozoite when the environmental conditions are unfavorable. It is oval, measuring 8-14µm in length and 6-10µm in width. Cyst contains four nuclei. Cysts are the infective stages of man, which are transmitted through a water source (Ichhpujani *et al*., 2002).

The disease caused by *Giardia* is known as Giardiasis. This disease is asymptomatic to a malabsorption syndrome. Diarrhoea, weight loss, abdominal cramps, anorexia, headache, chills etc. are the symptoms produced in acute and chronic Giardiasis.

Cyclospora and Cyclosporiasis

Cyclospora cayetanensis is a coccidian parasite measuring 8-10µm in diameter. The potential sources of infections are faecally contaminated water and foods. Human excrete unsporulated cysts or oocysts into the environment. During sporulation the sporant divides into two sporocysts and each contain two sporozoites. Sporocysts exists in the gastro-intestinal tract and invade small intestinal epithelial cells. Cyclosporiasis have been recorded from around the world since 1990 outbreaks in poorly understand people. *Cyclospora* infect the small intestine and usually causes watery diarrhea and other symptoms are loss of appetite, loss of weight, low-grade fever, fatigue, and headache (Ichhpujani *et al*., 2002).

***Cryptosporidium* and Cryptosporidiosis**

Cryptosporidium is a coccidian parasite. *Cryptosporidium parvum* causes the parasitic diseases Cryptosporidiosis, which is high incidence in AIDS cases. *C. parvum* was discovered by Current and Upton in 1985.

Cryptosporidium exists in only one form: oocyst. The oocyst measures 4-5µm in diameter. Oocysts may contain 1-6 large dark granules and numerous small granules. In a mature post-sporulation oocyst, 2-4 sausage shaped sporozoites can be seen. Oocysts has two walls-thin and thick walled.

The infection is caused by ingesting oocyst which is passed in the faeces of infected humans or animals. It is transmitted through person to person.

Cryptosporidiosis is an opportunistic infection and causes infection in immune-competent persons. It appears cholera like diarrhoeal disease. It may manifest as respiratory illness. There is no effective treatment for Cryptosporidiosis in immune-compromised persons and seems to be self-limiting in immune-competent persons (Ichhpujani *et al*., 2002).

1.2.2 Intestinal helminthes parasites:

The term helminthes has been derived from a Greek word meaning worm. There are metrozoa and are classified into two phylums: Platyhelminthes and Nemathelminthes. Platyhelminthes is divided into two classes: Cestoidea and Trematodea while Nemathelminthes has only one class Nematodea.

***Ascaris* and Ascariasis:**

Ascaris lumbricoides commonly known round worm is the largest intestinal nematode. It was discovered by Linnaeus in 1758. It is cosmopolitan in distribution being especially prevalent in tropical countries like India and China. The adults are found in the intestine of man mainly in the jejunum and upper part of ileum. It is also found in gorilla, apes and pigs. These are large, stout, tapering at both ends. Females are slightly larger (200-400 × 3.6 mm) than males (150-300 × 2-4 mm).When freshly passed in the stool, the adult worm is light cream or pink in color. The mouth opens at the anterior end and possesses three finely toothed lips-one dorsal and two ventral. The body is filled with irritating fluid called as ascaron or ascarase. The posterior end of male worm is curved ventrally in the form of a hook having a conical tip. The posterior end of female worm is conical and straight. The anus is sub-terminal and opens directly on the ventral aspect in the form of a transverse slit (Ichhpujani *et al*., 2002).

Man acquires infection by the ingestion of eggs containing embryonated second stage infective larva in food, drinking water and vegetables. Great majority (about 85%) of the infections are symptomless. The symptoms can be produced by the migrating larva and adults worms. Fever, cough, dyspnoea, bloody sputum is seen due to larva and larva may settle in brain, heart, kidneys and spinal cord. Fevers, oedema of face, rashes, conjunctivitis, haemorrhagic pancreatitis, meningitis are seen due to adult worm.

***Ancyclostoma* and Ancyclostomiasis:**

Ancyclostoma duodenale was described by Dubini in 1843 in Italy. It is also known as Hook worm disease, prevalent throughout the tropics and sub-tropics. Adults are small, grayish, white or brown cylindrical worms. The anterior end is slightly bent. The hookworm species are differentiated by their buccal capsule and arrangement of rays in the bursa. The buccal capsule of *A. duodenale* is provided with 6 teeth, 4 hook like on the ventral surface and 2 knob like on the dorsal surface. There are 5 glands associated with the digestive system which secretes an anticoagulant substance. The male is smaller (8-11 mm × 0.45 mm) than female (10-13 mm × 0.66 mm). Posterior part of male is expanded in an umbrella like fashion whereas female is tapering and no expanded bursa.

The adult worm lives in the intestine of man particularly in the jejunum and less often in the duodenum. Each female can lays 15,000-20,000 eggs per day. Humans are exposed to hookworm infection when third stage filariform larvae penetrate the skin (most common mode) exposed to contaminated soil. Penetration may occur at any site on the skin. Infection also takes place by ingestion of contaminated food materials containing filariform larvae occasionally (Ichhpujani *et al*., 2002).

***Trichuris* and Trichuriasis:**

The disease caused by *Trichuris* is Trichuriasis or whipworm infection. It was first discovered by Linnaeus in 1771. It occurs worldwide but mostly prevalent where the sanitation is poor and warm moist climates. The adult worms live in the large intestine of man especially in the caecum and vermiform appendix. The male measures 30-40 mm with a tightly coiled posterior end and a single spicule. The female worm is slightly longer (40-50 mm) and both sexes have a narrow anterior portion. The anterior thin part remains buried in the intestinal mucosa. The intestine and reproductive organs are located in the posterior thick part of the body. The worm is oviparous. After fertilization the female begins to lays about 2000-10000 eggs per day which is barrel shaped eggs. Eggs when freshly passed are not infective to human beings. The eggs take about 3 weeks to develop in the moist soil and remain infective for about 2 weeks. When the embryonated eggs containing larvae are ingested by human hosts with food and water, the shell is digested by the digestive juices and larvae are liberated through one pole and migrate to caecum (Ichhpujani *et al*., 2002).

Patients with mild infections are usually asymptomatic. The clinical features are abdominal pain, weight loss, anaemia, malnutrition, bloody diarrhea, acute appendicitis.

***Hymenolepis* and Hymenolepiasis:**

The disease caused by *Hymenolepis nana* is Hymenolepiasis. The species nana meaning small is derived from the small size of the adult worm. The worm was first discovered by Bilharz in 1857. It is cosmopolitan and more common in warm climates. The adults are found in the lumen of the ileum with scolex embedded in the mucosa. It is short measures 20-40 mm in length with a diameter of 1 mm and hence is called a "dwarf tapeworm". It seems like a mucus thread when observation through naked eye. The scolex is 0.3 mm wide, bears 4 suckers and has a retractable rostellum with 20-30 "spanner shaped" hooks. The neck is long and slender. The strobila consists of about 200 proglottids. There are 3

round testes which lie in the posterior part of each proglottid. The eggs are spherical or oval measures 30-45 µm in diameter. Some eggs hatch out in the lumen of the small intestine and liberate embryos which directly invade the intestinal villi. Human strains can infect rats through rat fleas (Ichhpujani *et al*., 2002).

The infection is more common in children. The infection is symptomatic in malnourished and immuno-compromised children. Abdominal pain, diarrhea, weight loss and weakness are the major clinical features.

Strongyloides and Strongyloidiasis:

The disease caused by *Strongyloides* is Strongyloidiasis. The different stages of *S. stercoralis* are adult worms, eggs and larvae. Only female worms are seen in the intestine and are parthenogenetics i.e; they can produce offspring without being fertilized by the male. Females are boarder than male worms. These are not seen in human infections because they do not invade the intestinal wall and are eliminated from the intestine. Female adult worms are hardly visible to the naked eye, measures 2.4-2.7 × 30-40 µm. The mouth possesses 3 small lips. The cylindrical oesophagus is present in the anterior part of the body, while intestine is present in the posterior 2/3 rd of the body. The posterior end is pointed. The vulval opening is situated at the junction of the middle and posterior third of the body. The females are ovo-viviparous. There are two types of larvae-rhabditiform and filiariform larvae. Rhabditiform larvae are found in the lumen, measues 200-250 × 16 µm and are mortile. Filariform larvae are skin penetrating infective forms of the parasite which are longer and more slender then the rhabditiform larvae. They have short mouth and cylindrical oesophagus.

The infection is acquired when a man walks barefoot on the faecally contaminated soil. The filiariform larvae directly penetrate through the skin coming in contact with the soil. The filariform larvae invade the tissue, penetrate into the venous circulation and carried by the blood streams to the right heart and then to the lungs. They leave the pulmonary capillaries enter the lungs alveoli, migrate to the bronchi, trachea, larynx and epiglottis. The females burrow their way into the mucous membrane and begin to lay eggs in tissues. The males are avoided with the faeces and die.

It may cause mild to severe abdominal symptoms. It has opportunistic behavior in immune-compromised persons. Superficial ulceration in the intestine, mild eosinophilic infiltration, catarrhal is the major pathological features (Ichhpujani *et al*., 2002).

Taenia and Taeniasis:

Hippocrates gave the description of *Taenia saginata* and Goeze differentiated it from T. solium in 1782. It is worldwide in distribution.

The scolex of the adult tapeworm (*T. saginata*) is embedded in the mucosa of the wall of ileum. The adult worm is white, semi-transparent tape like worm. It measures about 5-12 meters but sometimes exceed 20 meters. The scolex of the adult worm is pear-shaped, 1-2 mm in diameter, with four prominent round suckers but with no rostellum or hooks. Proglottids are upto 2000. The length of a gravid segment is 3-4 times its breadth. The common genital pore is situated marginally near the posterior end of each segment. Eggs are liberated by rupture of the ripe proglottids. About 80000 eggs may be present in a

single proglottid. Eggs are spherical in shape measure 31-43 µm in diameter. The eggs are not infective to man. In the intestine, eggs rupture and onchospheres are liberated. These penetrate the gut wall with their hooks gain entrance into the portal vessels, finally reaching the systemic circulation. Humans are infected through eating the uncooked beef containing cysticerci called measly beef. The clinical features are usually asymptomatic but may be noticed abdominal discomfort, hunger pain, chronic indigestion, diarrhea, anorexia (Ichhpujani *et al*., 2002).

Linnaeus in 1758 discovered the parasite of *T. solium*. The adult worm lives in the small intestine of man measures 2-3 meters in length. Scolex is about "pin head" size. The head is provided with rostellum armed with a double row of alternating large and small hooklets. The neck is short, 5-10 mm in length. There are less than 1000 proglottids. Immature proglottids are broader than longer. The genital pore is present laterally at middle of each segment. The testes consist of 150-200 follicles. The ovary is situated in the posterior third of each proglottid. The larval stage known as cysticercus cellulose occurs in pig and also in man. It is small, ovoid, milky white bladder measuring 5 mm in length and 8-10 mm in breadth.it contains albumin and salts. Eggs are morphologically similar to that of *T. saginata*. The eggs are release through the ruptured wall. Man acquires infection either by eating the inadequately cooked pork containing cysticercus cellulosae or by ingesting the eggs of *T. solium* in contaminated food and water. Taeniasis is aymptomatic in cases of man. Occasionally mild diarrheas, abdominal pain, loss of appetite are the main symptoms due to cysticercus cellulosae.

1.3 Objectives

1.3.1 General objective

- To find out the prevalence of intestinal parasitic infections in Tharu communities of Pawannagar VDCs of Dang district.

1.3.2 Specific objectives

- To study the prevalence of different intestinal parasitic infection with respect to their socio-economic status as well as sex-wise and age-wise distribution.
- To study the knowledge and hygienic practices in Tharu communities.
- To know the awareness against the intestinal parasites infection and hygienic habits.
- To provide the recommendations for future planning to control the intestinal parasites.

1.4 Significance of the study

Nepal is a land locked country and most of the parts of the country are rural and village area due to conservative and unhygienic traditional habits plays a major role in increasing the parasitic infection.

Intestinal parasitic diseases remain serious public health problems in many developing countries especially due to fecal contamination of water and food (Engels and Savioli, 2006). Intestinal parasitic infection alone contributes a great extent in the cause of diarrhea and is one of the most common public health problems in Nepal (Rai *et al.*, 1998). People in Dang district are troubled by intestinal parasitic infection due to poor health habits, water pollution, unhygienic behavior, low economic status (Khanal *et al.*, 2011). Mostly children are affected by the parasitic infections that are the main cause of water pollution. Water pollution results the diarrheal and gastro-intestinal tract infection (Rai *et al.*, 1994).

Communicable diseases such as diarrhea, dysentery influence the most of the children and old persons are being affected on growth of the host, weakness the body. Contaminated water and consumption of uncooked vegetables and unhygienic habit play an important role in transmission and promote of parasitic infection. The present study may play a major role in awareness in the Tharu communities.

Poor economy, unhygienic habit, health education, sanitation behavior, defecating in open field have resulted high prevalence rate of parasitic infection in the country as well in the Tharu communities. Other causes might be low socio-economic status, lack of awareness etc. The feeding habit, personal sanitation, per capita income has observed poor. These determine the poor economic status. So, the present study is conducted to find out the prevalence of intestinal parasitic infection in relation to their socio-economic status of the Tharu people of Pawannagar VDCs of Nepal.

The study has been undertaken in different age groups and sex to find out the prevalence of intestinal parasites in relation to their socio-economic status. Awareness programme and its consequences are brought and this will help in minimizing parasitic diseases and personal as well as community sanitation habit.

Intestinal parasite is one of the world's major health problem factor as well as economic concern. Due to unhygienic food, contaminated water and poor sanitary disposal condition of Tharu communities were worse and most of the people defecate in open field near settlement area as well as river banks, which enhance the possible chances for the establishment of diseases. So, there is need to review the scope and extend of disease and find out the effective means of controlling the parasites. The study is fully specified for diagnosis of intestinal parasites in humans is the vital importance in relation to their socio-economic status. This type of survey is new in this area. So, it will help for making plan, prevention, control and treatment of parasites in Tharu communities. The findings of the study will be helpful for future action plan.

1.5 Limitation of the study

- This research has been carried out for the partial fulfillment of the requirements for the master's degree in Zoology. Therefore, due to time span, financial constraint and lack of resources, intensive research couldn't possible.
- Stool samples of all the individuals of the study area were not observed.
- The study was focused in different ages and sexes in limited population and focused on prevalence of intestinal parasites in relation to their socio-economic status, which may not be sufficient for description of possible sources of infection.

1.6 Hypothesis

The null and alternative hypothesis of this research work is:

H_0=There is no significant differences in prevalence rate of intestinal parasites among Tharu communities of Pawannagar VDCs of Dang district.

H_1= There is significant differences in prevalence rate of intestinal parasites among Tharu communities of Pawannagar VDCs of Dang district.

CHAPTER- 2

LITERATURE REVIEW

2.1 History of Parasitology

Up to the middle of 17th century knowledge of parasitology was limited to recognition of the existence of a few common external parasites such as lice, fleas and few internal parasites like tapeworms, *ascaris,* pinworms and guinea worms. They were present by the natural products of human bodies. Even Rudolphi and Bremser also supported this idea (Chandler and Read, 1961).

In Linnaeus time, people thought that internal parasite was originated from accidentally swallowed free living organisms (Chandler and Read, 1961).

During the later half of 17th century Franceso Redi, grandfather of parasitology started that maggots developed from eggs of flies. At the same time, Leeuwenhoek perfected microscopes and discovered *Giardia* in his own stool and other protozoan in rain water, saliva etc. (Chandler and Read, 1961).

Trichuris trichiuria was first discovered by Linnaeus in 1771. Grassi in 1887 and Fulleborn in 1923 carried out its complete life cycle.

Rudolphi (Linnaeus of Parasitology) classified all the parasites known up to his time. That was Nematoidea, Acanthocephala, Nematoda, Cestoda and Cystica. In 1800, Zeder recognized five classes of worms which were already named by Rudolphi. In 1773 Muller discovered cercaria larvae.

In 1782, Dubini discovered human hook worm. Similarly Leoss (1898) made the discovery of penetration of the skin by hook worm larvae.

In 1859, Lambl discovered the parasite *Entamoeba histolytica*.

Kuchenmeister in 1851, proved the nature of cysticerci by feeding of *Taenia* of rabbits to dogs and in 1853 he proved that bladder worm in pigs gave rise to tapeworms in man.

Kuchemeister in 1855 and Leuckart in 1856 investigated the life history of *T. solium.* Then later Leuckart workout the lifecycle of *Enterobius vermicularis* in 1865 and Losch proved its pathogenic nature in 1875.

In 1876, Normand first reported *Strongyloides stercoralis*.

In 1903, Schoudinn differentiated pathogenic and non-pathogenic types of amoebae.

In 1916, Stewart experimentally proved tissue migration of *Ascaris* whereas Ranson (1920), Stewart (1921) and Vokogawa (1923) conclusively demonstrated that only one host is required for *Ascaris.*

Human intestinal parasites have been studied by many workers. Some of the studies on human parasites and their infection are as follows:

2.2 Scenario of Intestinal Parasitic Infection.

Approximately 3.5 billion peoples are infected by intestinal parasites and around 450 million children are ill due to these infections (WHO, 1998). Intestinal parasites are widely prevalent in developing countries, probably due to poor sanitation and inadequate personal hygiene. It is estimated that as much as 60% of the world's population is infected with intestinal parasites (Ragunathan *et al.,* 2010). Intestinal parasitic infection especially helminthes occurs in a developing countries (Yasmeen *et al.,* 2015; Goli *et al.,* 2014).

A.lumbricoides, T. trichuria, Hookworm, *E. histolytica, H. nana* has been reported to be an important parasites among the children of developing countries (Opara *et al.,* 2007; Seghal *et al.,* 2010; Goli *et al.,* 2014; Wordemann *et al.,* 2006; Abahussain, 2005; Alamir *et al.,* 2013; Akingbade *et al.,* 2013). Not only in children *A. lumbricoides* and *E. histolytica* were the most predominant parasite in HIV positive patients (Akinbo *et al.,* 2010; Kipyengen *et al.,* 2012). Similarly, *A. lumbricoides* infection has also been reported in pregnant women (Sehgal *et al.,* 2010).

Globally, 1298 million people have hookworm infection (Crompton, 1999), most of the studies showed *A. lumbricoides* was the most prevalent intestinal parasite (Opara *et al.,* 2007; Goli *et al.,* 2014; Ullah *et al.,* 2009).

Karunaithas *et al.* (2011) done the investigation of soil transmitted helminthes infestation Jaffna district and showed that prevalence of transmitted was zero in this study.

Most of the literatures revealed that IPI is common in females (Akingbade *et al.,* 2013; Khanum *et al.,* 2013; Marothi *et al.,* 2011) has been shown to be infected with common intestinal parasites like *A. lumbricoides, T. trichuria*, Hookworm, *H. nana* and other author such as (Opara *et al.,* 2007; Sehgal *et al.,* 2010; Goli *et al.,* 2014) and Abahussain (2005) reported common intestinal parasites from population of Nigeria, North India, Bangalore and Saudi Arabia.

IPI in relation among HIV patients was carried out from Benin city, Nigeria and intestinal parasites has been reported from different states of India by different researcher such as (Frederick *et al.,* 2010; Marothi *et al.,* 2011) where Abahussain (2005) showed that prevalence of parasitic infection among expatriate workers was 31.4%, out of which 25.9% for *A. lumbricoides*, 26.7% for *T. trichuria*, 9.2% for *E. histolytica*. Similarly, Mohan *et al.,* (2016) showed rate of IPI among school children was 30.64% where 54.54% rural area was higher infection with *A. lumbricoides*. Alamir *et al.,* (2013) showed prevalence rate of IPI was 77.9% among school children in Dagi Primary School, Ethiopia were highly infected with *A. lumbricoides* 8.3%.

Significant association between male and females had been shown by different authors in different country. Al-Kanfaji *et al.* (2014) reported females are most infected with IPI

50.1% while Ojurongbe *et al.*, (2014) showed males are highly infected with IPI 28.4%. Similarly, another author (Mohammed *et al.,* 2014; Dash *et al.,* 2010; Yesmeen *et al.,* 2015; Wordemann *et al.,* 2006) has also been reported as a higher infected rate of parasitic infection in males.

Most of the research on the basis of IPI among school going children and other people in developed countries like Nigeria, India and Ethiopia and has been reported by different researcher such as (Alamir *et al.,* 2013; Abahussain, 2005; Opara *et al.,* 2007) revealed single IPI was higher than double and multiple infection. Similarly, these investigation resembles with another author Goli *et al.* (2014) showed that prevalence of single parasitic infection was 89.86%.

Nepal is a small improvised country located in South Asia, where 70% of morbidity and mortality are associated with infectious diseases (Rai *et al.,* 2005). Among the various types of infectious diseases, IPI is one of the major causes of health problems (Rai *et al.,* 2005). In Nepal, giardiasis, ascariasis, amoebiasis and taeniasis are common IPI (Acharya, 1997). Intestinal protozoan infection and helminthic infection rank third and fourth respectively in Nepal (DHS, 2004).

Most of the research on the basis of IPI was carried out from different parts of Nepal and has been reported by different researcher (Khanal *et al.,* 2011; Pokhrel *et al.,* 2004; Agrawal *et al.,* 2012; where Regmi *et al.,* 2004) showed that prevalence of parasitic infection among school going children of Kalaiya, Bara district was 31.7%, out of which 17.1% for *A. lumbricoides*, 22.8% for *H. nana*, 8.5% for *E. vermicularis*, 44.0% for *E. histolytica*.

Thapa *et al.* (2011); Pradhan *et al.* (2014); Regmi *et al.* (2014); Agrawal *et al.* (2012) showed that prevalence rate was highest in males than females. Some author revealed (Tiwari *et al.,* 2013; Sherchand *et al.,* 2010; Tandukar *et al.,* 2015) showed the highest prevalence of parasitic infection in females than in males. Similarly, Shakya *et al.* (2012) showed prevalence rate of parasitic infection in girls was 19.1% and decreased in boys 10.3% among school going children in a border town of Nepal.

Most of the research on the basis of IPI was carried out from different parts of the Nepal and intestinal parasitic infection has been reported by different researcher such as (Rai *et al.,* 1986; Shrestha *et al.,* 2013; Shrestha *et al.,* 2012; Malla *et al.,* 2004; Tandukar *et al.,* 2015; Pandey *et al.,* 2015) where *A. Lumbricoides* infection as most dominant parasite infection. Some author carried out study in children and adult showed *E. histolytica* as the major intestinal parasites (Sherchand *et al.,* 2012; Agrawal *et al.,* 2012; Shakya *et al.,* 2012; Shrestha *et al.,* 2012; Singh *et al.,* 2014).

Thapa *et al.* (2011) done the investigation of parasitic infection among young children in rural community of Nepal showed 45.4%. Over 60% of Nepalese were infected with intestinal parasites and the distributions of parasites in rural area were higher than Urban area, 90% people were infected with intestinal parasites in rural area (Estevez *et al.,* 1983). Similarly, another author (Shrestha *et al.,* 2001) reported prevalence of IPI was

73.45% and 71.66% from *A. lumbricoides* occurs in rural and urban children where Chandrashekhar *et al.,* (2005) reported higher in Urban than rural, 52.3% and 47.7% in Urban and Rural school going children.

Shrestha *et al.* (2014) has reported that soil transmitted helminth in Kathmandu, where contamination rate of soil with parasite eggs and larvae was found to be 28.5%. IPI was reported in Nepal by (Shakya *et al.,* 2012; Tandulkar *et al.,* 2013) among the parasites identified *A. lumbricoides* was dominant in soil outside the valley (Rai *et al.,* 2000).

Pokhrel *et al.* (2004); Agrawal *et al.* (2012); Uga *et al.* (2004) reported that protozoan infection rate was higher in school going children of Nepal than the helminth infection.

Most literatures were available regarding intestinal parasitic infection among single infection were more prevalent in people in developing country like Nepal (Agrawal *et al.,* 2012; Thapa *et al.,* 2011; Pradhan *et al.,* 2014; Shrestha *et al.,* 2013; Uga *et al.,* 2004).

Pokhrel *et al.* (2004) reported that 33.4% were infected with one or more kinds of IPI. Khanal *et al.* (2011) showed prevalence rate of children aged 6-16 years in a public school was 17.6%. Similarly, Agrawal *et al.* (2012) revealed 30.1 % infection rate was slightly similar 31.7% investigated by (Regmi *et al.,* 2014). Sherchand *et al.* (2010) showed prevalence rate of IPI among school children was higher 51.9% in which other author showed similar reports (Alamir *et al.,* 2013; Ullah *et al.,* 2009; Patel *et al.,* 2006).

A.Lumbricoides causes intestinal obstruction, liver abscess, irritation and damage of malabsorption with the infection (Rodriguez-Morales *et al.,* 2006). Most of the children were highly affected by parasites than other age groups and infections were caused by poor environmental and personal hygiene (Gebru *et al.,* 2015).

2.3. Risk Factor of Intestinal Parasitic Infection.

Intestinal parasitic infections cause the serious problems in life which have so many factors that stimulate. Low socio-economic status, poor hygienic condition (Khanal *et al.,* 2011; Ojurongbe *et al.,* 2014), lack of pure drinking water, lack of proper sanitary disposal (Rayapu *et al.,* 2012), lack of health education are supposed to be the root cause of parasitic infection (Rashid *et al.,* 2011). Studies have shown that soil transmitted helminth infections are widely distributed throughout the tropic and subtropics (Dada *et al.,* 2015). Other risk factors contributing the parasitic infection includes open ground defecation (Karunaithas *et al.,* 2011), poor quality housing, day-care attendance (Benicio *et al.,* 2004) and hand washing practices with only use of water (Sah *et al.,* 2016), types of drinking water (Thapa *et al.,* 2011; Shakya *et al.,* 2012).

Most of the author showed intestinal parasitic infection occurs in school going children (Sharma *et al.,* 2004; Ragunathan *et al.,* 2010; Shrestha *et al.,* 2012; Al-Kahfaji 2014; Ojurongbe *et al.,* 2014). IPI has been also reported from HIV positive patients (Kipyengen *et al.,* 2012; Akinbo *et al.,* 2010). Some studies showed malnutrition status

helps to promote the IPI in children and pregnant women (Malla *et al.*, 2004; Sehgal *et al.*, 2010).

Some author showed non-vegetarian was highly infected with parasitic infection (Pandey *et al.*, 2015). Well water, lack of pure drinking water or drinking untreated water consumption cause intestinal parasitic infection (Thapa *et al.*, 2011; Tandulkar *et al.*, 2013; Shakya *et al.*, 2012). Antihelminthic prophylaxis decreased the intestinal parasitic infection (Karunaithas *et al.*, 2011).

Hand washing behavior (only use of water) also considered as leading factors for intestinal parasitic infection (Karunaithas *et al.*, 2011; Sah *et al.*, 2016). Intestinal parasitic infection associated with the farming profession (Tandulkar *et al.*, 2013). Some studied showed unaware peoples are highly associated with parasitic infection (Pandey *et al.*, 2015). Karunaithas *et al.* (2011) showed intestinal parasitic infection was associated with absence of toilet.

Some researcher showed poor hygienic condition and low socio-economic status considered as leading factor of parasitic infection (Khanal *et al.*, 2011; Ojurongbe *et al.*, 2014). Improper health education and lack of proper sanitary disposal are also risk factors for causing the intestinal parasitic infection (Rashid *et al.*, 2011) as well as day-care attendance (Benicio *et al.*, 2004) was associated with parasitic infection.

Intestinal parasitic infection was highly found in school going children under the 15 years old (Opara *et al.*, 2007; Khanal *et al.*, 2011; Agrawal *et al.*, 2012; Thapa *et al.*, 2011; Singh *et al.*, 2014) as well as in pregnant women (Sehgal *et al.*, 2010) and HIV positive patients (Akinbo *et al.*, 2010; Kipyengen *et al.*, 2012).

CHAPTER- 3

MATERIALS AND METHODS

3.1 Study area

The study was conducted from March to November, 2016 in Pawannagar VDCs lies in the Dang district. Out of 34 village development committee and 4 municipalities in Dang district, Pawannagr VDCs lies in the western part of this district.

3.2 Introduction of Dang district.

Dang is a district of Rapti zone, administrative headquarters of this district Ghorahi, located in Mid-Western Development Region of Nepal. It is situated at the height of 610 meters to 1130 meters from sea level and its area is 2,955 sqkm^2. Total population of Dang district is 552,583 according to census of 2068 B.S. (CBS, 2012). Its agricultural land is 572,271 hector, there are 34 village development committies and 4 municipalities that are- Lamahi, Ghorahi, Tripur and Tulsipur.

Dang district is as site of study, which lies in inner terai valley of Rapti zone of Nepal, some 280 km west of the capital city, Kathmandu. Dang district lies between the coordinates 28°0′N 82°16 'E 28.000'N 82.267°E. (MCPL, 2014). To the south, the district boarder Uttar Pardesh, India and north are Pyuthan, Rolpa and Salyan. The summer temperature of the district is above 35℃ and winter temperature below 15℃. The climate zone of the district is lower tropical below 300 meters (18.1% area), upper tropical above 300-1000 meters (69.9% area) and subtropical 1000-2000 meters (12.0% area).

Throughout historical times and probably earlier the Dang and Deukhuri valleys were home to indigenous Tharu people. This region has majority of people of Tharu ethnicity. These steep, virtually uninhabited sourthern slopes of the Mahabharat ranges are another cultural buffer zone between traditional Tharu lands and the culturally distinct Middle Hills where Nepali is the dominant language, the homeland of Brahmins and Chhetris. The Kumal (potter) ethnic group is also semi-indigenous. Main resident of this district are Tharus, Brahmins, Chhetries, Magars, Kamis etc. Main professions of these residents in this district are agriculture, business and jobs (BBC, 2007).

3.3 Introduction of Pawannagar VDCs.

The study population comprised all Tharu people. The study area is Pawanagar VDCs of Nepal which is underdeveloped and rural area. It is situated 4 km. west of Tulsipur municipality. The total population of this VDCs is 8,673 among them 4,051 males and 4,622 are females (CBS, 2012). The main profession of the resident in this VDCs is agriculture. The main castes are Brahmin, Chettri, Magar, Tharu and others. The economic status of people of the Tharu people of the study area is poor. So, this area is backward in health, education and hygiene. Most of the Tharu people depend on agricultural work. They defecate in open fields due to their open defecate habit but almost of the houses had latrine.

DISTRICT : Dang [56]		V.D.C./MUNICIPALITY : Pawan Nagar [25]		
WARD	HOUSEHOLD	POPULATION		
		TOTAL	MALE	FEMALE
1	144	650	307	343
2	224	1,113	531	582
3	246	951	414	537
4	379	1,647	710	937
5	143	795	397	398
6	127	574	274	300
7	165	746	341	405
8	292	1,301	630	671
9	178	896	447	449
TOTAL	**1,898**	**8,673**	**4,051**	**4,622**

Source: CBS, 2012

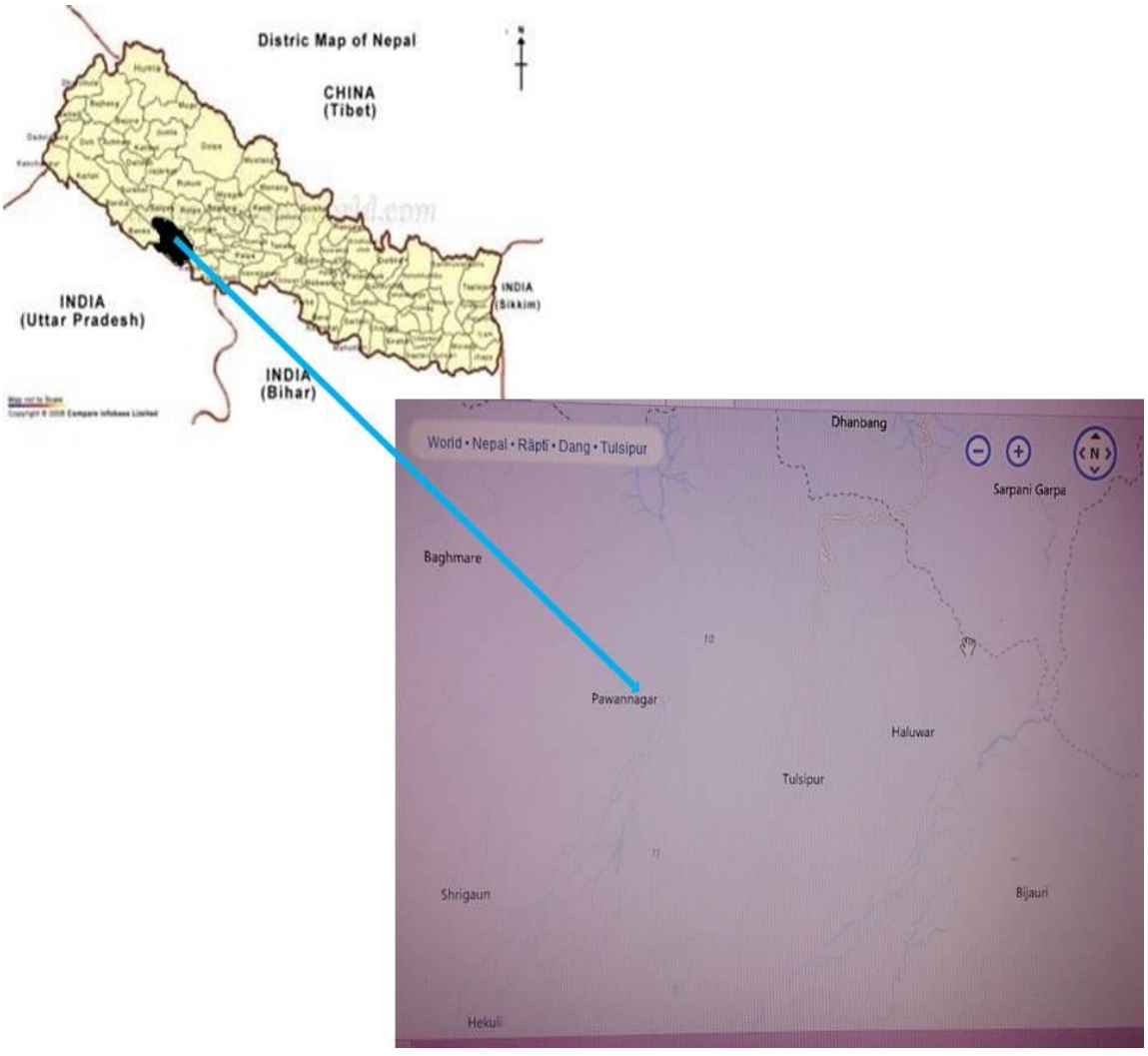

Fig.1: Pawannagar VDCs showing study area.

3.4 Study Duration

Random sampling survey for collecting information and stool was conducted in the study area with prepared questionnaires during March to November, 2016.

3.5 Study Design

The study design is based under the Laboratory examination.

3.6 Sample Size

Total sample size of the present study is 200 from different age and sex of Tharu peoples. The study of population were divided into 3 age groups i.e. 5 to 19 years, 20-40 years and above 41 years of age and stool samples were collected from the Tharu people of Pawannagar VDCs of Dang district. The data were collected by field worker.

3.7. Stool Sampling

Two hundred peoples were randomly selected for the study purpose. After proper instructions, vials were given to the children regarding collection of the stool sample, they were given sterile labeled vials and application sticks. From each Tharu person, about 2 gm. of fresh stool were collected. Each of the specimens was checked for its labeling and quantity. The collected stool samples were preserved in potassium dichromate (2.5%) and transported to the Parasitology Laboratory of Central Department of Zoology for further investigation of eggs, adult of intestinal parasites.

3.8. Materials

3.8.1 Equipments

- Compound microscope,
- Filter paper or Cotton,
- Tray, Coverslip,
- Forcep,
- Gloves,
- Needle and Sticks, Slides,
- Vials for sample collection.

3.8.2 Chemicals

- 2.5% Potassium dichromate,
- Dettol soap,
- 0.5% normal saline,
- 10% formaline,
- Iodine solution

3.8.3 Preparation of Potassium Dichromate:

2.5 gm of Potassium Dichromate was weighted accurately with the help of electric balance and dissolved in 100 ml of distilled water. This solution was used for the preservation of parasite found in the stool.

3.8.4 Preparation of Normal Saline:

Normal saline was used for observing the characteristics movement of parasites. This solution was prepared by dissolving 8.5 gm of Sodium chloride in 1000 ml of distilled water, which was used in unstained preparation (Zajac and Conboy, 2012).

3.8.5 Preparation of Iodine Solution:

Iodine solution was used for studying the internal characters of identification of the species of protozoan parasites as well as helminthes eggs, a stained preparation was required. Iodine solution was prepared by dissolving 10 gm of potassium iodine in 100 ml of distilled water and slowly adding 5 gm iodine crystals in it. The solution was filtered and then kept in bottle (Zajac and Conboy, 2012).

3.9 Laboratory Work

All the laboratory works were done in Parasitology Laboratory of Central Department of Zoology, TU, Kirtipur, Kathmandu under the supervision of supervisor.

3.10 Methods of Stool Examination

3.10.1 Macroscopic Examination

Firstly, the stool samples were examined by naked eyes for the eggs and adult helminth parasites.

3.10.2 Microscopic Examination

3.10.2.1 Unstained Preparation of Stool Smear:

A minute portion of stool was taken with the help of small stick and emulsified with normal saline (0.5) and a drop of it was taken on a clean glass slide. Then a cover slide was placed gently put over it so as to spread out the emulsion into a thin, fairly uniform and transparent layer and excess of fluid was removed with the help of filter paper(Zajac and Conboy, 2012).

3.10.2.2 Stained Preparation of Stool Smear:

Stained preparation was necessary for the identification and the study of the nuclear membrane and the iodine stained preparation was used for this purpose (Zajac and Conboy, 2012).

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3.11 Methods of Observation

Both stained and unstained preparation were first fixed in microscope and examined under the low power 10X objective. Observation was starting from end of the slide to another. When the parasites, eggs were seen then objects were centered and focused under the high power for detailed diagnosis.

3.12 Questionnaires

The questionnaires were done to know about the knowledge, attitude and practices of Tharu people which include population of different age groups and sex. Short questionnaires were designed which included **(a) Socio-demographic:** address, age, gender and socio-economic status. **(b) Behavioural data:** hand washing habits and types of drinking water**. (c) Participant's present medical history:** any complaints of abdominal pain/discomfort, nausea and vomiting. Children were interviewed in their mother tongue. The entire questionnaires were checked for accuracy and completeness. The questionnaires are shown in the annex.

3.13 Data analysis and Interpretation

All data as well as laboratory finding were analyzed according to their age, sex, feeding habit, and infection rate. Thus, analyzed data was interpreted by representing with table and pie-chart. Association of intestinal parasites with age–wise, sex-wise, occupation-wise, drinking water etc. prevalence were assessed by using "R", version 3.3.1 software packages whereas Pearson's Chi-square test were done. The total observed value is assumed as examined samples whereas the expected value is assumed as positive sample for the significance differences.

CHAPTER- 4

RESULTS

The study was conducted among the people of Pawannagar VDCs of Dang district. A total 200 stool samples of different age and sex groups were collected and examined from March to November, 2016. The result of the present study is divided into two categories.

4.1 Results of stool examination.
4.2 Results of questionnaires survey analysis.

4.1 Results of Stool Examination:

4.1.1 General Prevalence of Intestinal Parasites:

Out of 200 stool samples, the general prevalence of intestinal parasites of Pawannagar VDCs were found to be 59 (29.5%) positive.

Table 1: General Prevalence of Intestinal Parasites of Pawannagar VDCs.

Name of VDCs	Total no. of examined samples	Positive cases(200)	Positive %	Negative cases(200)	Negative %
Pawannagar	200	59	29.5	141	70.5

- **Distribution of Protozoan and Helminthic Infection:**
  Fig.2 reveals that, Out of 59 positive samples the distribution of helminthic infection 51 (86.44%) were higher than the protozoan infection 8 (13.55%) in the Tharu people of Pawannagar VDCs.

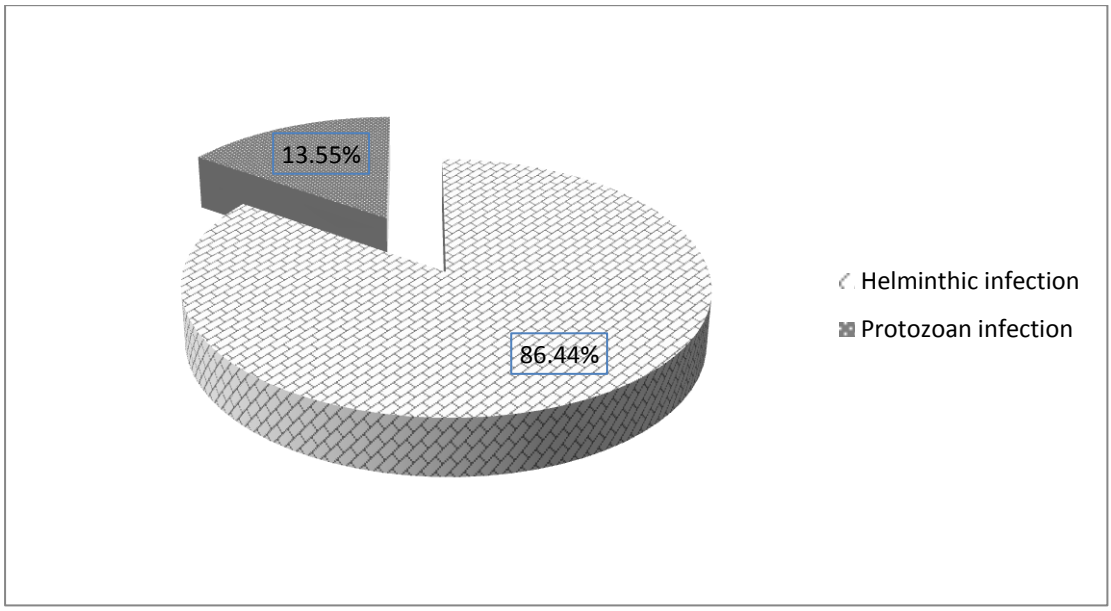

Fig.2: Distribution of Protozoan and Helminthic Infection.

4.1.2 Sex-wise Prevalence of Intestinal Parasites:

Out of 200 stool samples examined, 109 were of male persons and 91 were female person. Out of 109 stool samples examined from male, 34 (31.19 %) were found to be positive. Likewise, out of 91 stool samples examined from female, 25 (27.47 %) were found to be positive for intestinal parasites. Hence, infection rate was found higher in male people than female.

Statistically, there was no significant difference in the prevalence of intestinal parasites between male and female of Tharu people (χ^2 =0.18017, df=2, p=0.9139).

Table 2: Sex-wise Prevalence of Intestinal Parasites.

S.N.	Sex	Total examined samples	+ ve cases No.	+ ve cases %	_ ve cases No.	_ ve cases %
1.	Male	109	34	31.19	75	68.80
2.	Female	91	25	27.47	66	72.52
	Total	200	59	29.5	141	70.5

4.1.3 Age Group-wise Prevalence of Intestinal Parasites:

The entire study population was categorized into three age groups which were 5-19 years, 20-40 years and above 41 years old.

The distribution of intestinal parasites was maximum 7 (33.33%) in above 41 years of age group and minimum 39 (28.67%) in 20-40 years age group.

Statistically, there was no significant difference in the prevalence of intestinal parasites was found according to the different age-groups (χ^2 =0.10876, df=3, p=0.9908).

Table 3: Age Group-wise Prevalence of Intestinal Parasites.

S.N.	Age(Years)	Total No. of samples	+ ve No.	+ ve %
1.	5-19	43	13	30.23
2.	20-40	136	39	28.67
3.	Above 41	21	7	33.33
	Total	200	59	29.5

4.1.4 Prevalence of Specific Intestinal Parasites:

Out of 59 positive samples, 27 (45.76%) were infected with *Ascaris lumbricoides* followed by *H. nana* 17 (28.81%), *T. trichuria* 5 (8.47%), *Enteriobius vermicularis* 2 (3.38%) and *E. histolytica* 8 (13.55%).

Table 4: Infection rate of Specific Intestinal Parasites.

S.N.	Parasites	Numbers	Infected %
1.	*Ascaris lumbricoides*	27	45.76
2.	*Hymenolepis nana*	17	28.81
3.	*Trichuris trichuria*	5	8.47
4.	*Enteriobius vermicularis*	2	3.38
5.	*Entamoeba histolytica*	8	13.55
	Total	59	29.5

4.1.5 Intensity of Infection:

Out of 200 examined stool samples, the infection of single parasite was more common than double and multiple species infection. Among them 59 were positive cases, out of them there was 34 (31.19%) male and 25 (27.47%) female were infected with the intestinal parasitic infection. According to the positive samples, there was 52 (88.13%) single infection while 5 (8.47%) double infection and also 2 (3.38%) multiple infections in the Tharu people.

- **Intensity of Single Infection:**

Out of 56 single parasitic infection, the intensity of *Ascaris lumbricoides* was found to be maximum 25 (48.07%) cases was followed by *H. nana* 16 (30.76%), *E. histolytica* 6 (11.53%), *Enteriobius vermicularis* 2 (3.84%) and *T. trichuria* 3 (5.76%).

Table 5: Intensity of Single Infection.

S.N.	Parasites	No.	% of + ve cases (n=52)	No. of infected male	No. of infected female
1.	*Ascaris lumbricoides*	25	48.07	15	10
2.	*Hymenolepis nana*	16	30.76	9	7
3.	*Trichuris trichuria*	3	5.76	1	2
4.	*Enteriobius vermicularis*	2	3.84	1	1
5.	*Entamoeba histolytica*	6	11.53	4	2
	Total	52	88.13	30	22

❖ Intensity of Double infection:

Out of 3 double parasitic infection, the intensity of parasitic infection was found to be maximum in both *A. lumbricoides* + *T. trichuria* 2 (40.0%) and *A. lumbricoides* + *H. nana* 2 (40.0%) followed by *A. lumbricoides* + *E. histolytica* 1 (20.0%).

Table 6: Intensity of Double Infection.

S.N.	Parasites	No.	% of + ve cases (n=5)	No. of infected male	No. of infected female
1.	*A. lumbricoides* + *H. nana*	2	40.0	1	1
2.	*A.Lumbricoides* + *E. histolytica*	1	20.0	-	1
3.	*A. lumbricoides* + *T. trichuria*	2	40.0	1	1
	Total	5	8.47	2	3

❖ Intensity of Multiple Infection:

Out of 59 parasitic infection, the intensity of multiple infections was found to be 3.38%.

Table 7: Intensity of Multiple Infection.

S.N.	Parasites	No.	% of + ve cases (n=2)	No. of infected male	No. of infected female
1.	*A. lumbricoides* + *H. nana* + *E. histolytica*	1	50.0	1	-
2.	*A. lumbricoides* + *E. histolytica* + *T. trichuria*	1	50.0	1	-
	Total	2	3.38	2	-

4.2 Results of Questionnaire Survey Analysis:

Interview was carried out from the Tharu people whose stool was collected and examined for this a set of questionnaires were asked to them for the information of parasitic infection, behavior, economic status, occupation and other different habitual behaviors. The results from the questionnaires survey analysis are as follows:

4.2.1 Knowledge of Interviewed people:

Tharu people were interviewed on the basis of parasitic infection, knowledge of the transmission, control and prevention of the parasites. Most 141 (70.5%) people were unaware and 59 (29.5%) people were aware about the intestinal parasitic worms. Female were more 31 (34.06%) aware than male 28 (25.68%).

Statistically, there was no significant difference in parasitic infection rate between male and female awareness in Tharu people (χ^2 =0.90686, df=2, p=0.6354).

Table 8: Knowledge of Parasites among Tharu people and their Infection rate.

S. N.	Sex	No. of respondents	Aware				Unware			
			No.	%	Infected	%	No.	%	Infected	%
1.	Male	109	28	25.68	7	25	81	74.31	26	32.09
2.	Female	91	31	34.06	5	16	60	65.93	21	35.00
	Total	200	59	29.5	12	20.33	141	70.5	47	33.33

4.2.2 Hand Washing-wise Prevalence of Intestinal Parasites:

Out of 200 stool samples, the prevalence of parasitic infection was found to be maximum 43 (53.75%) in those people who used only water as cleaning agent to clean hands and minimum 3 (6.25%) in those people who used soap and water as cleaning agent.

Statistically, there was highly significant difference in the prevalence of intestinal parasitic infection among different agent for cleaning of hands (χ^2 =22.107, df=4, p=0.0001908).

Table 9: Hand Washing-wise Prevalence of Intestinal Parasites.

S.N.	Agent	Observation no.	+ ve cases	+ ve %	Infected male	%	Infected female	%
1.	Soap and water	48	3	6.25	1	33.33	2	66.66
2.	Ash and water	33	4	12.12	3	75.00	1	25.00
3.	Soil and water	39	9	23.07	4	44.44	5	55.55
4.	Only with water	80	43	53.75	26	60.46	17	16.27
	Total	200	59	29.5	34	57.62	25	42.37

4.2.3 Food-Habit wise Prevalence of Intestinal Parasites:

People who used pork meat for food were found to be highly infected 179 (90.86%)with intestinal parasites and less to be in people consume chicken 18 (9.13%). Similarly, non-vegetarian were slightly highly infected 57 (29.53%) than vegetarian 2 (28.57%) with intestinal parasitic infection.

Table 10: Food- Habit wise Prevalence of Intestinal Parasites.

S. N.	Sex	No. of interviewer	Vegetarian				Non-vegetarian			
			No.	%	Infected no.	%	No.	%	Infected no.	%
1.	Male	109	2	1.83	1	50.00	107	98.16	33	30.84
2.	Female	91	5	5.49	1	20.00	86	94.50	24	27.90
	Total	200	7	3.5	2	28.57	193	96.50	57	29.53

4.2.4 Occupation-wise Prevalence of Intestinal Parasites:

The prevalence of intestinal parasitic infection was found to be maximum 34 (35.05%) in farmer and minimum 9 (23.68%) was found in student whereas service holder had not found any intestinal parasites.

Statistically, there was no significant difference in the prevalence of intestinal parasites was found according to the occupation of the people (χ^2=1.81, df=7, p=0.9696)

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Table 11: Occupation-wise Prevalence of Intestinal Parasites.

S.N.	Occupation	No. of respondent	No. of + ve samples	%
1	Student	38	9	23.68
2	Farmer	97	34	35.05
3	House wife	45	11	24.44
4	Laborer	12	3	25.00
5	Driver	3	1	33.33
6	Businessman	4	1	25.00
7	Service holder	1	-	-
	Total	200	59	29.5

4.2.5 Defecation Place-wise Prevalence of Intestinal Parasites:

From the survey method, it was found that out of 200 people were interviewed, among them 160 (80.00%) persons were using toilet where 81 (74.31%) were males and 79 (86.81%) were females and 40 (20.00%) persons were using open field, near settlement area or river banks where 18 (64.28%) were males and 5 (41.66%) females. Among them male were more infected than female from parasitic infection.

Table 12: Defecation Place-wise Prevalence of Intestinal Parasites.

S. N.	Sex	No. of interviewer	Toilet user				Toilet non-user			
			No.	%	Infected no.	%	No.	%	Infected no.	%
1.	Male	109	81	74.31	16	19.75	28	26.60	18	64.28
2.	Female	91	79	86.81	13	16.45	12	12.08	5	41.66
	Total	200	160	80.00	36	22.5	40	20.00	23	57.5

4.2.6 Prevalence of Intestinal Parasites on the Basis of Drinking water:

Out of 200 stool samples, the distribution of intestinal parasitic infection was found to be maximum 9 (32.14%) in underground water drinking person whereas tap water drinking person were found to be less infected 50 (29.06%).Statistically, there was no significant difference in the prevalence of intestinal parasites was found among the peoples of using different drinking water sources (χ^2= 0.058531, df=2, p=0.9712).

Table 13: Prevalence of Intestinal Parasites on the Basis of Drinking water.

S.N.	Water sources	Observation no.	+ ve cases	+ ve %
1.	Tap water	172	50	29.06
2.	Underground water	28	9	32.14
	Total	200	59	29.5

4.2.7 Livestock and Domestic Animals Ownership-wise Prevalence of Intestinal Parasites:

Most of the interviewed people out of 200, 187 (93.5%) had livestock and domestic animals mainly pigs, goats, oxen, hens, cows and sheep while 13 (6.5%) had not livestock. The prevalence of parasitic infection was found 56 (29.94%) who had the livestock and domestic animals while 3 (23.07%) who didn't have livestock and domestic animals.

Statistically, there was no significant difference in the prevalence of intestinal parasites of the peoples on the basis of livestock and domestic animals ownership (χ^2 =0.15744, df=2, p=0.9243).

Table 14: Livestock and Domestic Animals Ownership-wise Prevalence of Intestinal Parasites.

S.N.	Animal husbandry	Observation no.	+ ve cases	+ ve %
1.	Having domestic animals	187	56	29.94
2.	Not having domestic animals	13	3	23.07
	Total	200	59	29.5

4.2.8 Treatment Method-wise Prevalence of Intestinal Parasites:

Out of 200 stool samples, it was found that most of the people 23 (31.5%) believed in traditional method (Dhami, Guruwa) treatments and 7 (26.92%) people believed in direct taking medicine. There was maximum intestinal parasitic infection 23 (31.5%) occurred in those people who believed in traditional methods for the treatment of intestinal parasites.

Statistically, there was no significant difference in prevalence of intestinal parasites of the people on the basis of treatment method (χ^2 =0.13838, df=3, p=0.9869).

Table 15: Treatment Method-wise Prevalence of Intestinal Parasites.

S.N.	Methods	No. of respondent	+ ve cases	%
1.	Direct taking medicine	26	7	26.92
2.	Traditional method	73	23	31.5
3.	Consulting doctor	101	29	28.71
	Total	200	59	29.5

CHAPTER-5

DISCUSSION

Intestinal parasites present throughout the world in various degree of prevalence are the major health problems in areas where there is overcrowding, poor environmental sanitation and personal hygiene practice especially in developing countries (WHO, 2010). The Global Burden of diarrheal disease was 72.7 million, among them 61.6 million of 0-4 years aged peoples and 3.5 million of 4-15 years aged peoples were suffering from diarrheal disease (WHO, 2004).

The overall prevalence of intestinal parasitic infection was found to be 29.5%. So, the finding is slightly similar to the reports of studies in different countries such as in Arba Minch town, Southern Ethiopia (Haffu *et al.,* 2014), in Pudunathan, South India (Rajunathan *et al.*, 2010), in Karaikal (Mohan *et al.*, 2016), in Al-Khobar, Saudi Arabia (Abahussain *et al.,* 2005). Likewise, the prevalence of intestinal parasites found in this study is much higher than that reported earlier in Deukhury valley, Dang, Nepal (Khanal *et al.,* 2011), among school going children of Bshaktapur district, Nepal (Shrestha *et al.,* 2013), in Kaski district (Chandrashekhar *et al.,* 2005), in school children of Lalitpur district of Nepal (Tandukar *et al.,* 2013), in Baglung district of Western Nepal (Shrestha *et al.,* 2012), Internal parasitosis (Shakya *et al.,* 2009), in a tertiary care hospital- a retrospective study (Singh *et al.,* 2014), school going children in Kathmandu Valley (Tandukar *et al.,* 2015), among school children in a rural village of Kathmandu Valley (Pradhan *et al.,* 2014), school children of Biratnagar Submetropolitan, Eastern Region of Nepal (Sah *et al.,* 2016), school going children in Pokhara, Nepal (Khadka *et al.,* 2013) and similar to few reports elsewhere in Nepal but still it seems high in comparison to internal (Chhetri, 1997; Rai *et al.,* 2000; Rai *et al.,* 1998) and external scenario such as in Breilley district (Rashid *et al.,* 2011), in Abeokuta, Ogun state, Nigeria (Akingbade *et al.,* 2013), at Ujjain, Mahya Pradesh, India (Marothi *et al.,* 2011), among primary school children in Bangalore (Golia *et al.,* 2014). These differences might be due to place and time differences of the study, health awareness, education and living standards of people.

The total prevalence of intestinal parasites in Tharu communities were *A. lumbricoides* (45.76%), *H. nana* (28.81%), *T. trichuria* (8.47%), *Enteriobius vermicularis* (3.38%) and *E. histolytica* (13.55%). These parasites were also reported by (Khanal *et al.,* 2011) among hospital visiting patients in Deukhury Valley, Dang, Nepal, Mengistu *et al.,* (2004) in a rural area close to the southeast of Lake Langano, Ethiopia, (lee *et al.,* 2000) in Chorwingun, Kangwon-do, Korea, (Zareef *et al.,* 2008) in stool samples, (Rashid *et al.,* 2011) in Breilley district, (Kipyengen *et al.,* 2012) among HIV patients in Baringo, Korea, (Desta *et al.,* 2014) in Arba Minch town, Southern Ethiopia, (Shrestha *et al.,* 2013) among school children of Bhaktapur district, Nepal, (Shakya *et al.,* 2009) internal parasitosis, (Pandey *et al.,* 2015) school children of Northern Kathmandu, Nepal, (Rayapu *et al.,* 2012) in rural areas of Kuppam, Andhra Pradesh, (Shakya *et al.* 2012) school children in border towns of Nepal, Singh *et al.,* (2014) in a tertiary care hospital. The finding of the study follows the other studies for the highest prevalence of *A.*

lumbricoides which shows that *A. lumbricoides* was the most common helminth in Nepal (Khanal *et al.,* 2011), and also reported from (Rai *et al.,* 1986), Bhaktapur (Shrestha *et al.,* 2013), Baglung (Shrestha *et al.,* 2012), Nepal (Shakya *et al.,* 2009), Sarlahi (Malla *et al.,* 2004), Khusibhu area (Maharjan *et al.,* 2013), Biratnagar (Singh *et al.,* 2014), Kathmandu Valley (Tandukar *et al.,* 2015), Biratnagar (Sah *et al.,* 2016), remote village of Nepal (Estevez *et al.,* 1983), Kathmandu (Sherchand *et al.,* 2010), Pokhara (Khadka *et al.,* 2013), Teaching hospital, Kathmandu (Agrawal *et al.,* 2012), Ethiopia (Addis *et al.,* 2015).

The high infection by *A. lumbricoides* found in the study area that indicates water contamination with helminthes in the locality. Similarly, higher protozoan infections particularly by *E. histolytica*. This study suggests contamination of soil and water with helminthes in Pawannagar VDCs. This reports seems similar to that of another study from Nigeria (Ojurongbe *et al.,* 2014), Sukumbasi Basti, Kathmandu (Thapa *et al.,* 2011) and this might be due to contaminated water, ineffective filtration, rapid, unplanned housing without proper defecation and water supply system in the rural area as well as open defecation, unhygienic behavior and lack of awareness in the rural area in the Tharu communities are the possible risk factors of transmission of parasites and their eggs. *Ascaris lumbricoides* infection was high detected among Tharu in this study might be related to low economy with poor farmers residing in the locality who usually work bare foot in the farm.

Gender-wise parasitic infection rate was found almost equal among males and females, through slightly higher in males (31.19%) which is similar to findings of other studies on general population in Nepal and other countries (Tandukar *et al.,* 2013; Khanal *et al.,* 2011; Batu *et al.,* 2004; Ojurongbe *et al.,* 2014; Pandey *et al.,* 2015; Mohammed *et al.,* 2014; Singh *et al.,* 2014; Dash *et al.,* 2010; Pradhan *et al.,* 2014; Makanga *et al.,* 2011; Wordemann *et al.,* 2006; Chandrashekhar *et al.,* 2005; Thapa *et al.,* 2011; Regmi *et al.,* 2014; Agrawal *et al.,* 2012). So, the present study shows agreement with suggestions of various studies regarding gender independence of parasitic infection

Reports of various studies shows that the intensity of single parasitic infection were higher than that of double and multiple infection in Puducherry, South India (Rajunthan *et al.,* 2010), Nepal (Uga *et al.,* 2004), Amalapuram (Padmaja *et al.,* 2014), Bhaktapur (Shrestha *et al.,* 2013), Kathmandu valley (Pradhan *et al.,* 2014), Al-Khobar, Soudi Arabia (Abahussain, 2005), Kathmandu, Nepal (Thapa *et al.,* 2011), Teaching hospital, Kathmandu, Nepal (Agrawal *et al.,* 2012), Imo state, Nigeria (Opara *et al.,* 2007). The present study also resembles that single parasitic infection is higher than other infection and their infection rate is in single, double and multiple parasitic infections were 89.83%, 5.08% and also 5.08% respectively in the Tharu communities. From the result it reveled that, *A. lumbricoides* parasitic infection was found to be highest than *H. nana*, *T. trichuria* and *E. histolytica* parasitic infection.

Based on the age of patients included in the study, parasitic infection was found to be highest among elder people age group above 41 years (33.33%) and it was lowest among

middle age people of 20-40 years (28.67%). Such infection indicates that lack of health knowledge regarding parasitic infection especially among Tharu communities, who mostly consume pork during festivals and other occasion and might be ingestion of improperly cooked pork. This finding is different to the reports of studies done in various places of Nepal (Ojurongbe *et al.,* 2014; Pandey *et al.,* 2015; Rayapu *et al.,* 2012; Addis *et al.,* 2015) was found highly in school going children under 15 years old. High parasitic infection was found among elder people in the study might be due to their unhygienic behavior, lack of sanitation and contaminated food and water.

In the present study, the prevalence of intestinal parasite was found to be higher 29.53% in the persons who are non-vegetarians and lower 28.57% in the persons who are vegetarians. Several of the studies showed that the highest prevalence of intestinal parasites infection was found to be non-vegetarians, which is similar with result shown by (Pandey *et al.,* 2015). This might be due to consuming infected raw meat, improperly cooked meat are the possible risk factors of transmission.

In the present study, (80.00%) people use toilet and only (20.00%) people use open field as defecation place. The prevalence of intestinal parasites was found to be higher (57.5%) in the persons who use open field as defecation place. It is an agreement with the report published by Karunaithas *et al.* (2011).

The prevalence of intestinal infection (53.57%) was found in those people who used only water as cleaning agent but it seems similar to that result by Sah *et al.* (2016). These might be due to personal behavior, lack of health awareness and health education.

In the present, the prevalence of intestinal parasites was greater (29.94%) in people having livestock and domestic animals ownership. This might be due to insufficient sanitary facilities, lack of personal hygiene and living nearby livestock and domestic animals.

Reports of various studies have shown that, the intestinal parasitic infection rate depends upon many factors including socio-economic status of people belonging to low socio-economic status (Khanal *et al.,* 2011; Ojurongbe *et al.,* 2014; Rayapu *et al.,* 2012). Almost Tharu people have low (31.95%) socio-economic status whereas (16.12%) have medium. The finding of the study also found similar report and which might be due to most of the people with low socio-economic status included in the study. This might be related to illiteracy, unhygienic practices, unawareness, open defecation and consumption of raw water, which is mostly contaminated in rural areas.

The present study revealed that the population had lack of awareness about the intestinal parasites among which (70.5%) were unaware about the parasitic infection whereas (33.33%) were infected. They do not have an idea of means and modes of internal parasitic transmission. This result resemble with the result by Pandey *et al.* (2015). The finding of the study shows that knowledge of parasitic infection is very poor in the Tharu communities due to lack of public health awareness and health education.

The finding of the study revealed that (50.5%) people believe in consulting with doctor among which (28.71%) were infected with intestinal parasites and (36.5%) believe in traditional methods such as Dhami, Guruwa among them (31.5%) were infected. So, maximum infection was observed in people who believe in traditional methods. It is due to lack of knowledge, attitudes and cultural and behavioral variations.

Although, the prevalence of intestinal parasites among Tharu people of Pawannagar VDCs reflects the need of public health awareness programs regarding protection of water source from fecal contamination, personal hygienic behaviors practice, use of latrine, proper environmental sanitation and continuous mass deworming programmes in the Tharu communities.

CHAPTER-6

CONCLUSION AND RECOMMENDATIONS

6.2 Conclusion

The study was carried out to observe the prevalence of intestinal parasites in Tharu communities of Pawannagar VDCs of Dang district in relation their socio-economic status. Out of 200 stool samples, 59 (29.5%) were positive and 141 (70.5%) were negative in which the infection rate was higher in male (31.19%) than that of female (27.47%). Age-wise intestinal parasites was found to be highest among elderly people age group above 41 years (33.33%) followed by teenage people age group 5-19 years (30.23%) and adult people age group 20-40 years (28.67%) respectively. Altogether five species of intestinal parasites were detected, the most common was *Ascaris lumbricoides*, and a helminthes parasite (45.76%) followed by protozoa *Entamoeba histolytica* (13.55%) and other helminthes namely *Hymenolepis nana* (28.81%), *Enteriobius vermicularis* (3.38%) and *Trichuris trichuria* (8.47%) respectively. But the study showed that single (88.13%), double (8.47%) and multiple (3.38%) parasitic infections were in the Tharu communities. The distribution of helminthic infection 51(86.44%) were higher than the protozoan infection 8 (13.55%) in Tharu people of Pawannagar VDCs.

In the present study, the prevalence of intestinal parasite was highest (35.05%) in those persons whose occupation was farmer. This is due to illitrate and do not have any knowledge of parasites and parasitic infections. The minimum prevalence of intestinal parasites was (23.68%) in those persons who were students. This is due to education and has knowledge of parasites and parasitic infections. They follow hygienic food, freshly cooked food, safe drinking water and healthy hygienic habits.

In the present study, (80.00%) people use toilet and (20.00%) people use open field for defecation. The highest prevalence (57.5%) of intestinal parasites was found in toilet non-user persons whereas less infected (22.5%) were found in toilet user persons. The toilet non-user person defecate in open field near settlement area, river banks, bushes where parasites are growing frequently that promote to risk of parasitic infection. This may be the reason of parasitic infection of toilet non-user persons.

In the present study, most of the people (70.5%) were unaware and (29.5%) were aware about the intestinal parasitic infection. Most of the unaware persons were highly infected (33.33%) than the aware persons (20.33%). The highest prevalence (32.14%) of intestinal parasites was found in underground water consuming people whereas tap water consuming people were found to be less infected (29.06%). The underground water sources are well, pond, stream that may contaminated with the parasites or by human activities like clothing, bathing near water sources. This may be the reason of underground water consuming people were more infected than tap water consuming people. The presence of parasites and their infection was minimized by using drink filtered water, boiled water or might be effective for other risk factors.

Intestinal parasitic infection is an important public health problem in Nepal. The present study reveals that intestinal parasites are abundant among Tharu people of Pawannagar VDCs. The situation strongly calls for the institution of control measures including treatment of infected individuals, improvement of health status of infected persons by health education, public health awareness and also develop the health care facilities, medical needs and aware the people for the utilization of health services. Poverty, lack of awareness, poor environmental sanitation, raw and uncooked food consuming habit and unsafe drinking water are some of the predisposing factors highlighted by this study as causes of parasitic infections. Thus, there is need for intensive and habitual health education for behavioral changes related to personal and mass treatment for the effective control of intestinal parasitic infections in the concerned area.

6.2 Recommendations

Followings are the recommendations for prevention and control of intestinal parasites.

- Public health education in the Tharu communities should be made compulsory for the awareness of people.
- Habit of defecating on open field, river banks etc. should be prohibited.
- Consumption of raw or undercooked meat, vegetables and contaminated water should be prevented.
- Well established sanitary toilet should be build up in each and every house.
- People should change their feeding behavior.
- Pure and safe drinking water facility should be made easily accessible for the tribal communities.
- Basic health programs should be launched time to time for raising awareness towards the parasitic infections, prevention and controls.
- The research work on the prevalence of intestinal parasites in the relation of socio-economic status and prevention should be encouragement.

CHAPTER-7

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ANNEX- 1

PHOTOGRAPHS

Photo No.1:

Photo No.2:

Photo No.3:

Photo No.4:

Photo No.5:

Photo No.1,2,3,4,5: Observation of stool sample under microscope in CDZs Laboratory.

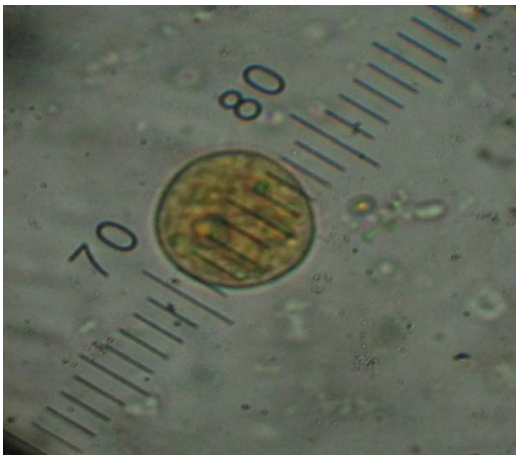

Photo No.6: Egg of *E. histolytica*

(10X×40X) 20.64µm

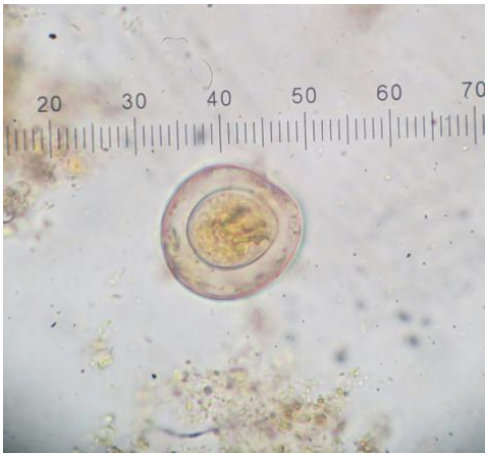

Photo No.7: Egg of *H. nana*

(10X×40X) 43.86µm

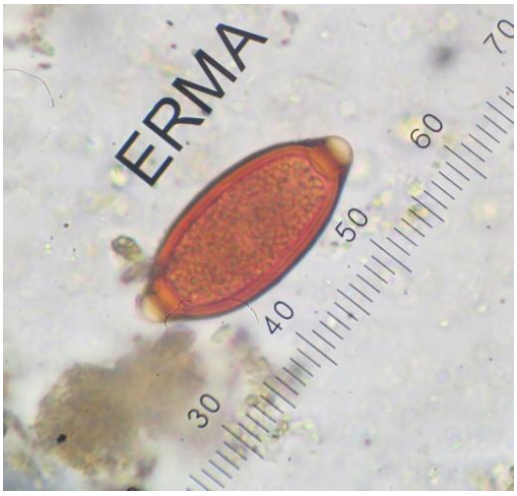

Photo No.8: Egg of *T. Trichuria*

(10X×40X) 59.34µm

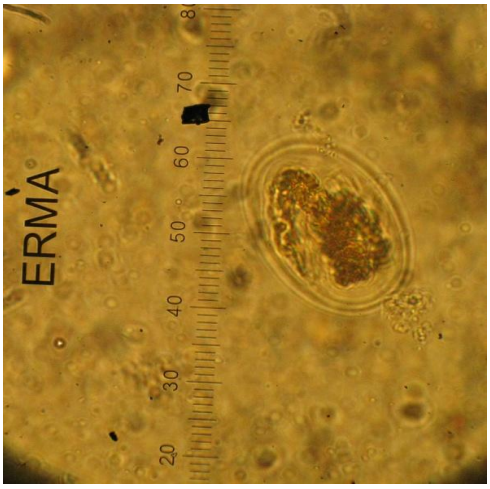

Photo No.9: *Enterobius vermicularis*

(10X×40) 64.5µm

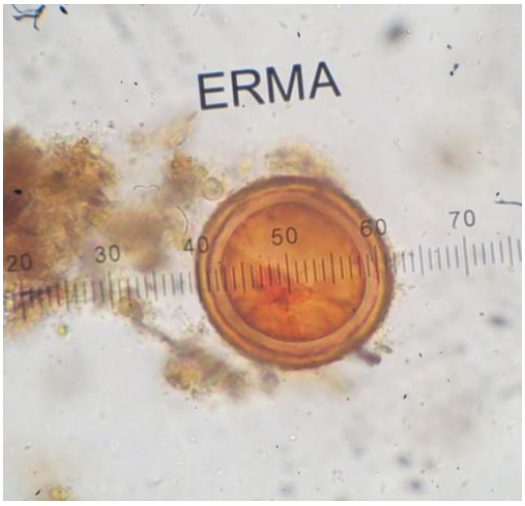

Photo No.10: Egg of *A. lumbricoids*

(10X×40X) 56.76µm

ANNEX- 2

QUESTIONNAIRES

1. S.No.

   Name: Age: Sex: M/F Education:

   Address: No. of family:

2. What is your main occupation?

   Teacher Farmer Other…….

   - What is your main source of income in your family?

   ……..

   - Are there any extra sources of income in your family?

   ……..

   - How many productive members in your family?

   …….

3. Drinking water

   Tap Tube well Well River Spring

4. How do you use water for family?

   Direct Boiling By adding water purifying chemicals

5. Where do you defecate?

   Toilet Open place River bank

6. When do you defecate?

   Early morning After breakfast After meal Afternoon

7. Do you wash your hand before meal?
   Yes/No

   If yes, what type?

   With soap with ash with soil Other……..

8. Do you have any domesticated animals?
   Yes/No

If yes, what type?.....

9. What type of food habit you have?

   Vegetarian Non-vegetarian

   - If non-vegeterian, which meat frequently you take?

   Pork Chicken Buffalo Mutton Fish Beef

   - How do you prepare your meat to eat?

   Boiled Well cooked Half cooked Sekuwa masu

10. How do you clean your vegetables and fruits?

    Rubbing on clothes Tap/well water Without washing

11. Have you suffered by diarrhoea /dysentery worm?
    If yes, when?

    1 week before 1month before 6 month before Now Don't know

12. How you taken deworming tablet?
    Yes/No

    If yes: 1 week before 2 week before 1 month before 6 month before

13. Do you know the cause of diarrhoe?
    Yes/No

    If yes, what are they?.....

14. Do you know the causes of worm infection?
    Yes/No

    If yes, what are they?.....

15. How do you treat in case of infection?

    Traditional methods By taking medicine By consult doctors

16. Do you know the methods of prevention of worm infection?
    Yes/No

    If yes, what are they?.....

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