INTESTINAL PARASITOSIS AMONG SCHOOL GOING CHILDREN OF COMMUNITY SCHOOL OF DHARAN

A

Dissertation Submitted to the Department of Microbiology, Central Campus of Technology, Dharan, T.U, Nepal in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Microbiology

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ABSTRACT

Intestinal parasites are those which must have an intestinal life- cycle stage and usually attach in small and large intestine and produces traumatic damage in the intestinal villi. Parasitic infections caused by protozoa and helminths are the most common infections worldwide. The prevalence of parasitic infections varies with the level of sanitation andis highly prevalent among the general population in Nepal. The present study was done to find out the prevalence of intestinal parasitosis among school going children of community school of Dharan. Total of 301 stool samples (< 16 years children) were randomly collected (all the students from class I-VIII) and examined for intestinal parasitosis from April to September 2018. The samples were collected in clean, dry and capped fitted container and were subjected to macroscopic examination for ova, cysts, adult parasites and/or segment of parasites. Samples were fixed in 10% formal-saline and parasites were examined microscopically after concentration by formal- ether sedimentation technique. The overall parasite positive was found to be 26 (8.64%) among 301 students. Parasitosis in male was higher 16 (61.54%) than in female 10 (38.46%). Only monoparasitic infection was found in this study. Altogether 4 species of parasites were detected of them *Giardia lamblia* was the most common parasites followed by *Hymenolepsis nana*, *Ascaris lumbricoides* and hookworm. Younger children aged (≤9 years) had marginally higher positive rate (8.82%) than in children aged (>9 years) that is (8.61%).The intestinal parasitosis was higher in the students using tap water 25(8.9%) than those using mineral water. There was higher rate of intestinal parasites in Janjati 18(12.5%) than other ethnic group. The prevalence of intestinal parasitic infection was found to be lower in this study. In order to prevent this infection appropriate health education should be given to the students and their parents concerning disease transmission, personal hygiene and safe drinking water.

Key words: Parasitosis, school children, *G. lamblia*, *H. nana, A. lumbricoides*, hookworm, formal saline, formalin-ether.

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ABBREVIATION

A.lumbricoides	-	*Ascaris lumbricoides*
B. hominis	-	*Blastocystis hominis*
C. mesnili	-	*Chilomastic mesnili*
E.coli	-	*Entamoeba coli*
E. dispar	-	*Entamoeba dispar*
E. histolytica	-	*Entamoeba histolytica*
HHs	-	Households
H. nana	-	*Hymenolepsis nana*
IPs	-	Intestinal Parasites
N. americanus	-	*Necator americanus*
NTD	-	Neglected Tropical Disease
NCC	-	Neurocysti cercosis
PSC	-	Preschool Children
SAC	-	School Age Children
STH	-	Soil Transmitted Helminths
SPSS	-	Statistical Package for Social Science
S. stercoralis	-	*Strongyloides stercoralis*
T. trichiura	-	*Trichuris trichiura*
WHO	-	World Health Organization

CHAPTER I

INTRODUCTION

1.1Background

Intestinal parasitic infection includes both protozoa and helminthes which is the most common infection that occurs worldwide. Protozoa are parasites which consist only one cell where as helminths are worms which have many cells (Haque 2007). Protozoa and helminthic parasites are the known parasites that affect the gastrointestinal cavity. Intestinal parasites such as *Ascaris lumbricoides, Trichuris trichiura* and hookworm are the most prevalent and affect about one-sixth of the world population. *A. lumbricoides* is responsible for about 1.2 billion infections globally while *T. trichiura* and hookworm infection accounts about 795 million and 740 million, respectively. Among the protozoan parasite, *E. histolytica* and *Giardia lamblia* are the most dominant cause of intestinal morbidity in children (Hailegenbriel 2017).

Taeniasis, amoebiasis, enterobiasis, ascariascis, giardiasis are the major intestinal parasitic infections found in human and transmission of these infections are mainly through oral route (Parija 2013). Intestinal parasitosis is most common in school going children and higher prevalence is found in this age group (Khadka et al 2013). Intestinal parasitic infection is one of the major health problems in developing countries.

The WHO estimates that over 270 million pre-school children and over 600 million of school children are living in areas where the parasites are intensively transmitted and are in need of treatment and preventive interventions in the world (Shrestha et al 2012). Intestinal parasitosis, a major health problem in developing countries is aggravated by hot and humid climate, poverty, malnutrition, high population density and poor health (Ghimire et al 2014). It is estimated that more than 2 billion people world-wide (24% of the world's population) are infected with soil-transmitted helminths. Helminths are the main cause of disease burden in school-age children in developing countries as children bear the greater burden often

resulting in anaemia, lethargy, growth stunting, and malnutrition (WHO 2012).

Faeces are the most frequent specimens collected and examined for demonstration of parasites of gastrointestinal tract (Khanna et al 2014). The fecal oral route is significant in the transmission of parasitic infections to human to the poor personal hygiene and environmental conditions such as contaminated soil and water sources (Nxasana et al 2013). Intestinal parasitic infections are more prevalent among children as compared with the general population. About 12% of the global disease burdens caused by intestinal parasites are observed among children in the age ranges from 5 to 14 years in developing countries (Hailegenbriel 2017).

Lack of toilet at home and hand washing practice with soap after defecation were found to be significantly associated with parasitosis (Regmi et al 2014). Formalin (10%) is used for preservation during transportation. Fecal samples are examined for the presence of parasites both macroscopically and microscopically. The samples are examined by standard parasitological examination which include wet mount (saline mount and iodine preparation method) and by formalin-ether concentration method. Macroscopic examination is done to look for structures like proglottids, scolices, adult tapeworm, *Enterobius, Ascaris, Trichuris* or hookworm. Unstained saline wet mount preparation is done to detect protozoal tropozoites and helminthic eggs or larvae. Iodine wet mount is done to detect cysts. The Formalin- Ethyl acetate concentration technique is also performed for those cases which are not properly visible by saline mount. Initially, a macroscopic examination of the stool can be performed to find evidence of blood, mucus, parasitic segments or whole parasites. Next, a direct unstained wet smear (saline mount) examination is carried out and a drop of 1% Lugol's iodine is placed at the edge of cover slip to convert it into iodine mount (Mishra et al 2013).

In a parasitology laboratory, routinely two preparations of each specimen are usually made on each slide: one unstained preparation and another temporarily stained preparation. The saline wet mount is an unstained preparation made by using physiological saline. The advantage of saline preparation is that it helps

to demonstrate the motility of trophozoites. The classical technique described for the microscopic examination of parasites is the iodine mount method, which aids in the differentiation and identification of parasites by characteristic morphological features and details of internal structures. The method is simple to perform, quick and inexpensive, facilitating direct visualization of parasitic ova and cyst morphology. The disadvantage of this technique is that the preparation dries within a few minutes, rendering it unreadable and unreliable to visualize live nematodal larvae (Kanna et al 2014).

High prevalence of intestinal parasitic infection are reported among school children (Hailegebriel 2017). *Entamoeba histolytica* causes death more than 100,000 annually. Similarly, *Giardia lamblia* affects approximately 200 million people of the world. However, intestinal helminthes, *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* infect 1.4 billion, 1.3 billion and 1.0 billion people worldwide, respectively (Regmi et al 2014). An intestinal worm has been one of the major causes for the visit to health care facilities in the country. Intestinal parasitic infections are endemic worldwide and constitute a major public health problem and considered as cancers of developing countries.

Nepal is small improvised country where 70% of morbidity and mortality are associated with infectious diseases. Giardiasis, ascariasis, amoebiasis, ancyclostomiasis and taeniasis are common intestinal parasitic infections in Nepal. Among the whole population intestinal parasitic infections are highly prevalent in school going children. School health policy has detailed the public health measures including improvement of water sanitation and hygiene measures which should be implemented in schools to promote students' health but most of these measures have not been implemented in the schools in Nepal. This study aimed at implementing some of these measures as public health interventions to evaluate their effects on intestinal helminthic and protozoan infections among the children and also to determine the prevalence of intestinal parasitic infection and associated risk factors among school going children because children are the future pillar of the country. All the community schools of Dharan were not included due to cost problem. The

students suffering from fever were not included in this study and also due to time limitation formalin- ethyl acetate sedimentation technique could not be perform for all the samples. This study is done to find out the prevalence of the intestinal parasitosis and associated risk factors among school going children of Eastern Nepal.

1.2 Objectives

1.2.1 General Objectives

- The general objective of this study is to find out the prevalence of intestinal parasites among school going children of community school of Dharan.

1.2.2 Specific Objectives

- To study the prevalence of intestinal parasitosis among the school going children of Dharan.
- To study the sociodemographic distribution of intestinal parasitosis among school going children of Dharan.

CHAPTER II

LITERATURE REVIEW

2.1 Intestinal Parasites

A living organism which receives nourishment and shelter from another organism where it lives is called parasites. Intestinal parasites are those which must have an intestinal life- cycle stage and usually attach in small and large intestine to receive nutrients, stool and blood from intestinal wall and produces traumatic damage in the intestinal villi. Some sp. of intestinal parasites causes haemorrhage into lumen of intestinal mucosa due to deposition of their eggs and some sp. penetrate and perforate the large intestine by secreting many lytic enzymes which digest intestinal tissue (Parija 2013). Transmission of parasitic infection from one person to another is mainly through oral route when someone comes in contact with infected faeces (for example through contaminated soil, food and water). Parasites found in the intestine can be catagorized into two groups; they are protozoa and helminths (Chatterjee 2009).

2.2 Intestinal Protozoan infection

Protozoal parasite consists of single cell which is morphologically and functionally a complete cell. There exist two stages of protozoal parasites: tropozoites and cyst. Tropozoite is an active stage, only in this stage the potozoal parasites can multiply while cyst is an inactive or resistant stage. The protozoalparasites lose its power of growth and multiplication but is infective to human. *Entamoeba histolytica, Giardia lamblia, G. intestinalis* are the major protozoans which infect human intestine (Chatterjee 2009). *Giardia lamblia* is an important enteric protozoan which is worldwide in distribution and is common in warm moist climates. Giardiasis is the diarrheal disease which is frequently caused by *Giardia lamblia*. Intestinal protozoan are distributed worldwide. Intestinal protozoan infections are common in school-aged children in the developing world. They are responsible for clinically important infections such as acute and chronic diarrhea in both the developed

and the developing world. *Entamoeba histolytica*, which affects the colon, can spread to involve the liver if left untreated for a long period (Sah et al 2013). Amoebiasis is caused by *Entamoeba histolytica* which is the third leading parasitic cause of death in developing countries. *E. histolytica* is an important cause of diarrhoea in homosexual men those are suffering from AIDS (Parija 2013).

2.3 Some common Protozoa of Human Intestine

Intestinal parasites are the major problem of public health in tropical region although they seem to rise in less interest than do AIDS and tuberculosis. The third greatest parasitic disease responsible for death in the world is intestinal amoebiasis caused by *Entamoeba histolytica*. Also *Giardia lamblia* is worldwide in distribution and is common among important enteric protozoa. The frequent cause of diarrhea is also due to giardiasis caused by *Giardia lamblia* (Sah et al 2013).

2.3.1 *Entamoeba histolytica*

Entamoeba histolytica is the human intestinal protozoa, the trophozoits of which are present in the lumen, mucosa and the submucosa of the large intestine. The disease amoebiasis is caused by *Entamoeba histolytica* which is the third leading parasite cause of death in the developing country. *Entamoeba histolytica* mainly occurs in three stages: trophozoite, precyst and cyst. Trophozoites have a single nucleus that is spherical in shape and measures 3.5 µm in size Ithas a fine spherical chromatin and a central karyosome. Amoeba trophozoites are anaerobic, obligate fermenters and are actively motile with the help of their pseudopodia. The main characteristic features of *Entamoeba histolytica* is the presence of ingested erythrocytes which is not in *E. dispar*. The trophozoites cannot survive outside the body it quickly dies when excreted out of the body. Precyst is the stage between trophozoite and cyst and contains a single nucleus. The infective forms which excreted in the faeces are cyst the cyst is highly resistant to gastric acid, diverse environmental conditions and the chlorine concentration found in portable water due to smooth, refractile 0.5 µm thin chitinous walls which surround the cyst. The

mature cyst containing four nuclei of *Entamoeba histolytica* are mostly found in formed stool and can also be found in semiformed stool.

2.3.2 *Giardia intestinalis* (*Giardia lamblia*)

Giardia lamblia is an enteric parasite that causes giardiasis which is one of the frequent causes of diarrhoea in human, pets and livestock. *Giardia* is flagellate protozoa which have two nuclei each with four associated flagella and lack both mitochondria and golgi apparatus. *Giardia lambila* show two stages in their lifecycle: trophozoite and cyst. The trophozoites always have four pairs of flagella and the cysts typically have a thick cyst wall which is separated from the cytoplasm by a clear space. *Giardia lamblia* is worldwide in distribution and particularly common in the tropics and subtropics, in areas where water supply and the environment become faecally contaminated. Young children are more frequently infacted than adults in endemic areas, particularly those that are malnourished. The flagellate inhabits the small intestine of human and the trophozoites and cysts are present in the duodenum, jejunum and upper ileum. Cyst is oval or ellipsoidal in shape which is infective stage of parasite. A thick cyst wall surrounds the cyst and on staining with iodine the cyst becomes brown. Further, children $\leq$ 5 years of age were must at risk of protozoan infections (Chatterjee 2009).

2.4 Distribution of Intestinal Protozoa

Intestinal protozoa are worldwide in distribution. They are frequently associated with nutrition malabsorption syndromes and gastrointestinal morbidity. The prevalence of protozoan infections varies with the level of sanitation and is generally higher in the tropics and sub-tropics than in more temperate climates. In 2002, WHO estimated 3.5 billion of people are infected by digestive tract parasites and 450 million of people made ill by them. In Itahari out of 200 stool sample, 18.5% intetinal protozoan infection was found among them *Giardia lamblia* was found to be 10.5% and 8% were *E. histolytica* (Sah et al 2013).

In Ethopia a study done by Hailegebriel among school children out of 359 students 24.5% were infected by *E. histolytica* (Hailegebriel 2017). Also the

study done by Rangaiahagari in Andhra Pradesh, India out of 208 children the prevalence of *E. histolytica* was 30.8%, *G. lamblia* was 18.8% and *Entamoeba coli* was found to be11.3% (Rangaiahagari et al 2013).

A study done in Ghana a total of 485 patients including 365 diarrhoea and 120 non-diarrhoea children the prevalence of *G. lamblia* infection in diarrhoea non-diarrhoea children were 5.8% and 5% respectively (Anim-Baidoo et al 2016). Also the study done by Ghenghesh in 2016 in Libya, the prevalence rate of *E. histolytic* /*dispar* was 19.9% and for *G. lamblia 0*.9- 13% (mean 3.4%) was studies (Ghenghesh et al 2016).

Mukhiya et al 2016 surveyed 342 stool samples 68 (19.8%) were protozoan parasites. The study conducted by Popruk et al 2011 showed that prevalence of intestinal protozoan were assessed in Bangkok, Thailand by examination of stool from 432 individual using formalin other contraction technique. The population had *G. duodenalis* (11.67%) and 10.63%, 12% and 15% PK, TMK and MHK orphanage respectively (Mukhiya et al 2016).

In 2013, the study done in the urban slams of city in western India, among 880 participants 70.71% infection were comprised protozoan parasites. Among protozoan parasites *E. histolytic*/*dispar* and *G. lamblia* were identified. In 2.14% dual protozoan infections were observed with *E. histolytic*/ *dispar* and *G. lamblia* (Shobha et al 2013).

In a survey in Kalaya in Bara district of Nepal, 296 stool samples of school children were examined by using direct smear and formalin- ether concentration technique of protozoan parasites, *G. lamblia* was highly prevalence i.e 45.7% and *E. histolytica* was found to be 44% and 10.1% was *E. coli* (Regmi et al 2014).

The prevalence of intestinal parasite was determined for 2,515 stool samples in Tabriz Imam Reza Hospital, Iran. For stool sample inspection, direct smear microscopy and sedimentation technique were used.The highest rate of infection related to *E. histolytica* cyst was 0.79%. Many other protozoa including *E. histolytica* trophozoites was 0.07%, *G. lamblia* cyst was 0.59% ,

Giardia trophozoites 0.03%, *E. coli* cyst 0.31%, *Endolimax nana* cyst 0.43% respectively (Anvarian 2010).

The study was conducted by Sah et al 2013 among school children of Itahari, Eastern Region of Nepal in which 18.5% of the total population were found to be infected with intestinal protozoan infections among which 18.4% were male and 18.6% were female. Within the study group 10.5% were *G. lamblia* and 8% were found to be *E. histolytica* (Sah et al 2013).

2.5 Transmission of Intestinal Protozoan Infections

Feacal-oral contamination of water and food is generally the main mode of transmission of intestinal protozoan infections (Shrihari et al 2011). Faecally contaminated food or water is the most frequent route of transmission of *G. lamblia*, through drinking contaminated tap water or recreational exposures in lakes, rivers, or swimming pools (Kunwar 2016). Since *G. lamblia* also infects birds, cows, sheep, deer, dogs and cats, transmission of *G. lamblia* can also occur through contact with pets and domestic animals (Wiser 2015).In developing countries, socio-demography: improper sanitation, bad personal hygiene, eating of unwashed fruits and vegetables, and drinking of contaminated tap water are the common risk factors associated with *G. lamblia* infection (Anim-Baidoo et al 2016).

2.6 Life Cycle of intestinal protozoa

According to Shrihari (2011), feacal-oral contamination of water and food is generally the main mode of transmission of intestinal protozoan infections. Life cycle of the intestinal protozoa (amoeba, flagellates and ciliates) is simple type and is completed in a single host, the human. The protozoal parasites are infective at cyst stage and multiply at trophozoits stage. Mostly in trophozoits stage, the parasites replicate by asexual method of replication. After ingestion by an appropriate host, the cysts transform into trophozoites which show an active metabolism and are usually motile. The parasite undergoes asexual replication during the trophozoite stage after taking up nutrients from the host. The trophozoites are predominantly found attached to epithelial cells of the small intestine and are rarely found in stools, except in

the cases of severe diarrhea. Some of the trophozoites develop into cysts instead of undergoing replication. Cysts are characterized by a resistant wall and maturation involves two rounds of nuclear replication without cell division and is excreted with the faeces. In general, situations involving close human to human contact and unhygienic conditions are the main cause to promote transmission of protozoal infection. The protozoal parasites mostly reproduce a sexually by binary fission and rarely by multiple fission. Trophozoites occur freely in the human intestine and after encystation, the cysts excreted outside in the faeces are resistant to the environmental conditions. Cysts are immediately infective upon excretion with the feaces and remain viable for weeks-to-months depending on environmental conditions. A new cycle of infection is initiated by the cyst if ingested by a susceptible host (Parija 2013).

2.7 Symptoms of Intestinal Protozoan Infection

The spectrum of intestinal protozoan infections can range from asymptomatic to invasive disease to severe and/or chronic. Moreover, this protozoan eventually produces intermittent and chronic gastrointestinal symptoms leading to public health problems. Invasive forms of amoebiasis include intestinal amoebiasis or amoebic colitis, acute fulminant or necrotizing colitis, ameboma, and liver abscess. Symptoms of intestinal Amoebiasis can range from 1-3 weeks of diarrhea to grossly bloody dysenteric stools with abdominal pain and weight loss in some cases.

For giardiasis, asymptomatic carriers exhibit no symptoms at all. Symptomatic infections are noted more frequently in children than in adults. They include acute infectious diarrhea characterized by short-lasting acute diarrhea, nausea, abdominal distension, greasy stools, and anorexia. General symptoms include fever, anaemia and allergic manifestations. Chronic giardiasis is usually associated with intermittent, loose, foul-smelling stools that resemble those of acute enterocolitis and malabsorption states (Chatterjee 2009).

2.8 Diagnosis of Intestinal Protozoa

Diagnosis is confirmed by finding cysts or trophozoites in feaces or in duodenojejunal aspirates or biopsies microscopically. In giardiasis watery or loose stools may contain motile trophozoites which are detectable by the immediate examination of wet smears. The demonstration of *E. histolytica* cysts or trophozoites in feaces or tissues is required for the definitive dignosis of amoebiasis. Stool specimens should be preserved, stained and microscopically examined. Cysts are predominant in formed stools and trophozoites in diarrheic stools. For motile trophozoites fresh stools can also be immediately examined which exhibit a progressive motility (Arora 2014).

2.9 Treatment of Protozoan Infection

For the treatment of protozoal infection chemotherapy is the best method. In giardiasis nitroimidazole derivatives, acridine derivatives and nitrofurans are the three major classes of drugs used. Metronidazole is used for giardiasis whose recommended dosage is 400 mg three times per day for five days (or at least >3 days).For children, 15 mg/kg/d in three doses is recommended (Wiser, 2015). Tinidazole is also used in treatment of non-dysenteric colitis, dysentery, and extra-intestinal infections caused by *E. histolytica*. Initially the recommended dose is 2gm. For serious infections, Tinidazole is administered for three days, 2gm taken twice a day.

2.10 Prevention and Control of Intestinal Protozoa

The infection is acquired through the ingestion of cysts through contaminated food and water. The contamination of food or water with fecal material should be avoided to control intestinal protozoa. Health promotion and education should be given to improve personal hygiene, and emphasizing hand washing with soap and water, proper sanitation and food handling. They are effective control activities for the reduction of person-to-person transmission. There are no safe or effective chemoprophylaxis drugs for intestinal protozoa. Protecting water supplies can also lower endemicity and epidemics. *Giardia* and *Entamoeba* cysts are resistant to standard chlorine treatment, but are killed by

iodine or boiling. Sedimentation and filtration processes are quite effective at removing *Entamoeba* cysts (Parija 2013).

2.11 Intestinal Helminthes

The helminthes parasites are worm like, multi cellular, bilaterally symmetrical and elongated, flat or round bodies. They are classified into two types: Flat worms or platy helminthes and Round worms or nematodes. The important morphological forms of the helminthes are adult, larva and egg. The adult worms are macroscopic but can be often visible with our naked eye. All the helminthes produce eggs and are excreted out in fasces. Life-cycle of helminthes may be completed in one or more than one host. Generally, Cestodes and Trematodes complete their life- cycle in two different host but Nematode complete their lifecycle in one host (Parija 2013).

The intestinal helminthic infections are common in the world and are responsible for considerable morbidity and mortality. Approximately 2 billion people are infected with soil-transmitted helminthes worldwide. Over 880 million children need treatment for these helmenthic parasites. Nationwide roughly 50% of children and adolescent are estimated to be infected by intestinal parasitic infections (Yadav et al 2017).

The common nematodes include *A. lumbricoides*, *T. trichiura*, *E. vermicularis* (pinworm), *A. duodenale* and *N. mericanus* (Hookworms) and *S. stercoralis*. Cestodes include *T. solium*, *T. saginata*, *H. nana*, *H. diminuta* and others. Similarly, Trematodes include *Fasciolopsis buski*, *Echinostoma ilocanum* and *Heterophyes heterophyes* (Arora 2014).

2.12 Some Common Intestinal Helminthes

According to WHO (2012) estimates indicate that more than 2 billion people are infected are infected with soil transmitted parasites. The major intestinal helminthes are *A. lumbricoides, Hookworms, T. saginata, T. Solium, H. nana, T. trichiura* (Parija 2013).

2.12.1 *Ascaris lumbricoides*

Ascaris lumbricoids is cosmopolitan and having a world-wide in distribution. It is present in about 25% of human population and occurs in persons with unhygienic habits. The adult worm of *A.lumbricoides* lives in the lumen of the small intestine (jejunum) of human. *A. lumbricoides* is the largest intestinal nematode parasitizing and resembles an ordinary earthworm. In fresh from the intestine it is light brown and pink in colour and also it is round in shape tapers at both ends. The tail end of male is curved ventrally in the form of hook having a conical tip. The female is longer stouter than the male and the posterior extremity is neither curved nor pointed but is conical and straight. There is present vulvar waist in female. A fertilized female pass the eggs from the human host with the faces. The eggs are round or oval in shape always bile-stained is golden brown in colour. The egg is surrounded by a thick smooth translucent shell with an outer albuminous coast which floats in saturated solution of common salt but unfertilized egg doesnot float in salt solution because it is the heaviest of all helmenthic eggs. Both fertilized and unfertilized eggs may found in stool sample. The presence of unfertilized egg shows that the host is harboring female *Ascaris* i.e. mating between male and female has not occurred. Infection of *A. lumbricoides* in human is known as ascariasis (Chatterjee 2009).

2.12.2 Hookworm

Hookworm is worldwide in distribution in all tropical and subtropical countries especially in such places wherever humidity and temperature are favorable for the development of larvae in the soil. Particularly the jejunum of small intestine of human is the best habitat of adult worm and less often in the duodenum and rarely in the ileum. *Ancylostoma duodenale* and *Necator americanus* are the two genus of hookworm which are mostly similar in all aspects having little different in morphology.

Adult hookworm is small, grayish white, cylindrical worm. In freshly passed stool, the worm has reddish brown in colour due to the ingestion of blood in its intestinal tract.

The anterior end of the worm is bending slightly dorsally, hence the name hookworm. The life span of adult worm in human intestine is estimated to be 3 to 4 years. The eggs are passed out with the faeces which are oval or elliptical in shape, colourless (not bile-stained), surrounded by a transparent hyaline shell-membrane and contains segmented ovum usually with 4 blastomeres. When the eggs are passed out with the faeces, are not infective to human. A heavy infection with symptoms of anaemia may result from the failure of development of immunity.

No intermediate host is required for the life-cycle of hookworm and like other helminthes, multiplication of worms doesn't occur inside the human body. Ancylostome larvae may cause ancylostome dermatitis and creeping eruption as skin disease. Bornchitis and broncho-pneumonia may occur in the lungs. The adult worm may cause a severe progressive anaemia of microcytic hypo chromic type (Chatterjee 2009).

2.12.3 *Trichuris trichiura*

*Tirchuris trichiura*is whip-like in shape so its name is also whipworm. An intestinal infection caused by the invasion of the muscosa of the colon by the adult worm in human is trichinellosis. *T. trichiura* is relatively smaller in size and it lacks tissue migration phase in its life cycle. The adult worms are whip like and the anterior end is three-fifth being long, thin and hair like and the posterior one-two-fifth being short, thick and stout. Their entire anterior ends are deeply embedded into the mucosa.

The eggs are barrel-shaped with a colorless protruding mucus plug at each end. The eggs are yellowish- brown and double shelled. The freshly passed eggs are not infective to human and contain an unsegmented ovum.

The eggs in the faeces, deposited in damp warms, oil, develop to embryonated eggs in 10-14 days. The embrocated eggs contain rhabditiform larvae and are infective to humans. The infection occurs by the ingestion of these embryonated eggs. The adult worm invades the intestinal mucosa by its thin, thread-like anterior end and feeds on tissue secretions but it doesn't feed on blood (Parija 2013).

2.12.4. *Hymenolepsis nana*

Hymenolepsis nana is the common and smallest cost ode that infect human. The parasite is also known as dwarf tapeworm which is the only cost ode that parasities humans without requiring an intermediate host. The ileal portion of the small intensive of human is the major resident of adult worm. *H. nana* is a small and thread like which consists of a scolex, a long neck and nearly 200 proglottids.

The egg is colourless, oval and has two distinct membranes. The outer membrane is thin and colour less while inner membrane known as embryophore encloses an oncosphere with three pairs of hooklets. There is a clear space between the inner and the outer membranes which is filled with yolk granules. Two poles are present on the inner membrane from which 4-8 thread-like polar filaments emerge and fill the space between the two membranes. Life cycle of *H. nana* is completed in a single host. *Hymenolepsis* occurs commonly in 4-10 years children. *H. nana* is the most common helminthes which cause inflection in human. About 36 million people are infected with *H. nana* world-wide (Parija 2013).

2.12.5 *Strongyloides stercoralis*

Strongyloides stercoralis is also known as the dwarf thread worm which cause strongyloidiasis. This parasite has both parasitic and free-living generation and is the smallest pathogenic nematode known to cause infection in humans. The mucosa of the small intestine is the site where female parasite inhabits. Parasitic males are absent in infected humans. The parasitic females are very small 2.5 mm in length and 40-50 mm in breath and are translucent.

The eggs of *S. stercoralis* are just like those of hookworm but are smaller. The eggs are transparent, oval and very thin shelled and they contain larvae ready to hatch. Instead of eggs larvae are mostly found in the faeces because as soon as eggs are laid, the rhabditiform larvae hatch out of the eggs and migrate back to the lumen of the intestine from where they are excreted out with the faeces. The infective stage of the parasites is filariform cylindrical oesophagus and cause infection by penetration of the skin. *Strongyloides* shows heterogenic

and complex life cycle with its alternation between free-living cycle in the soil and parasitic cycle in the human body. Autoinfection is the unique features *S. stercoralis*. Both larvae and adults are pathogenic (Arora 2014).

2.12.6 *Taenia solium*

Taenia solium is also known as pork tapeworm which causes intestinal taeniasis. Cysticercus cellulosae is the larvae of the worm and also causes serious disease, in humans known as cysticercosis. Human is both the definitive host (harbouning the adult worm) and the intermediate host of the parasite *T. solium*. The adult worm inhibits the small intestine of humans. In adult *T. solium* there are scolex, neck and strobila consisting of segment. Scolex is round hooklets. Both the eggs and the larvae of *T. solium* are infective to humans. Humans acquire intestinal taeniasis by the ingestion of inadequately cooked pork that is infected with *C. cellulosae* and cysticercosis and also by food and water that are contaminated with human faeces containing the eggs of *T. solium*.

Intestinal taeniasis is a mild condition in humans as compared to cysticercosis, especially neurocysticercosis (NCC), is the most serious disease in humans.

2.13 Distribution of Intestinal Helminthic Infection

More than 2 billion people are infected with soil transmitted parasites (WHO 2012). Over 600 million school age children and over 270 million pre-school age children live in those areas where these parasites are intensively transmitted. The areas where sanitation is inadequate and water supplies are unsafe, the high prevalence of intestinal helminthes occurs. They are widely distributed in tropical and subtropical areas with greatest numbers occurring in sub- Saharan Africa, The Americas, China and East Asia.

A study of prevalence and intensity of soil-transmitted helminthes among urban slum children of Kenya age of preschool age children and school age children approximately 40% had STH infection of PSC and 1.1% of SAC. Also malnutrition among PSC and SAC was anemia (38.3% and 14.0%) respectively (Suchdev et al 2014).

Hossain et al 2018 conducted an investigation to determine the prevalence of *T. trichiura* infection and associated determinants in rural tea garden areas of sylhet, Bangladesh out of 300 participants 14% were found robustly infected with *T. trihiura* of the age group 11-20 years show highest prevalence i.e. 31.58% (Hossain et al 2018).

Fransis et al 2012 surveyed 432 primary school children, within age group 6-14 years living in Wakiso District, central Uganda. The study revealed the maximum (10.9%) prevalence rate of hookworm followed by *T. trichiura*, *Schistosoma mansoni* and *A. lumbricoides* by 3.1%, 1.9% and 0.2% respectively. The study revealed that 26.6%, 46% and 10.3% of incidences of stanting, underweight and MAM respectively were attributable to helminthic infection (Fransis et al 2012). Ensink et al 2008 carried out of survey among the sewage farming families in Hyderabad, India. The study found the increased risk of hookworm was 29.8% followed by *A. lumbricoides* and *T. trichiura* was 5.6% and 3.1% respectively (Ensink et al 2008).

Sah et al 2016 carried out a survey among the school children of Biratnagar Submetropolitan, Eastern Region of Nepal. The prevalence of intestinal helminthic infections was 15.5%. Hookworm was found high (6.5%), *A. lumbricoides* (5.5%), *T. trichuria* (2.5%) and *H. nana* was (1.0%) (Sah et al 2016). Similarly, another study done by Silver et al 2018 the Soil transmitted helminthes (STH) infections are among the most prevalent neglected tropical disease (NTD) worldwide. Overall prevalence of *Ascaris* was 18% followed by *Trichuris* 14% and hookworm was found to be 12% (Silver et al 2018).

The study done among 162 school children *A. lumbricoides* was found 29.0% (Nxasana et al 2013). In Rivers state, Nigeria in 2015, out of 3826 stool samples of school children prevalence of *A. lumbricoides* was 51.78%, hookworm was 25%, *T. trichura* was found to be 15.18%, *S. stercoralis* 7.14%, *Taenia spp* 0.89% and *E. vermicularis* was found to be 0.01% (Abah et al 2015). In 2012, the study done among 260 school children in Baglung district of western Nepal, the total prevalence of the intestinal parasitosis was found to be 21.05%. Among then 5% was *T. trichuria*, 2.65% was *A.duodenale* and 2.3% *A. lumbricoides* was found (Shrestha et al 2012).

The prevalence of intestinal parasite was determined for 165 stool samples in Birjung, Nepal. The prevalence rate of *A. lumbricoides* was 28.0% (Shakya et al 2012). Also the study done in Bhandarkhal Kathmandu, among 300 school children 29.1% were positive for one or more parasites. Out which 40.2% were helminthes in which hookworm was 18.6% which was the commonest helminthes (Ghimire et al 2014).

Golia et al 2014 conducted an investigation among primary school children in Bangalore, India. The cross- section study including 258 primary school children, there was 26.74% intestinal parasitic infection. Among them the highest prevalence was in the age group 6-8 years. And also among helminthes, the prevalence of *A. lumbricoides* was highest (27.63), followed by *T. trichiura*(18.42%), *H. nana* (15.8%) was found (Golia et al 2014).

The study conducted by Misra et al 2013 in the urban slums of a city in western India. Overall 1872 participants from 30 clusters and 409 house hold (HHS) the prevalence rate of helminths was delected in 25.71%. Among them *A. lumbricoides* was 12.85%, *H. nana* was 7.14% *Taenia spp* was 4.28%, *E. vermicularis* was 0.71% and *A. duodenale* was 0.71% (Misra et al 2013).

Ashok et al 2013 carried out a survey among 208 school going children in Amalpuram, Andhra Pradesh, India. The prevalence rate of *A. lumbricoides* was found to be 1.5%, 5.3% was hookworm, 0.8% was *E. vemicularis* and 0.8% *T. trichiura* was found in the stool sample (Ashok et al 2013).

A study of helminthic infection in Morang District of Eastern Nepal, the positive rate was found to be 833%. Among 3000 school children of Rangeli Municipality out of which 50.92% were *A. lumbricoides*, 44.56% were *A. duodenale*, 1.96% was *T. trichiura*, 1.44% was *E. vermicularis* and only 1.12% was *H. nana* (Yadav 2017).

2.14 Transmission of Intestinal Helminths

Intestinal helminthic infections are mostly spread by faecal-oral contact or contamination of water or food due to poor sanitation and hygiene practices.

The infections are acquired by ingestion or penetration of skin by infective forms (Suchadev et al 2014).

Most people become infected with *A. lumbricoides* and *T. trichiura* by ingesting the eggs of parasites. The gees which are attached to vegetables are ingested when the vegetables are not carefully cooked, washed or peeled. Eggs can also be ingested through contaminated water sources. The children who play in the soil may ingest eggs when they put their hands in their mouth without washing them (Parija 2013).

Person to person may also occur transmission of intestinal heminthic infection while handling of contaminated cloths or bed linens. *T.* solium and *T. saginata* are also transmitted to humans when they ingest uncooked infected pork and beef respectively.

2.15 Life Cycle of Intestinal Helminths

There are three main life cycle stages of intestinal helminthes: eggs, larvae and adults. In nematodes the infection starts with the invasion of larvae info the human body, either of through the skin penetration. The parasites produce eggs when becomes mature within the human body which are disposed with the faecal matter. The helminthes complete their life cycle in approximately three months depending upon the specific organism.

Cestode eggs released from gravid segments embryonate to produce embryos which are ingested by intermediate hosts, then later penetrate hast tissues where they excyst and forms adult tapeworms. Trematodes have more complex life-cycles where 'larval' stages undergo asexual amplification in snail intermediate hosts. Some species form encysted matacercariae on aquatic vegetation which is eaten by definitive hosts.

In *A. lumbricoides* when eggs are swallowed the larvae hatch from the eggs. They break into the alveoli and travel up the respiratory system to the throat to be swallowed again. The migration is needed for the larvae to develop into adult. A female produces about 200000 microscopic eggs per day that are

passed in faeces and fertilize into infective stage within a few weeks in the soil where as unfertilize eggs are not infective.

The life cycle of hookworm starts when the filariform larva penetrates the skin. It travels in the blood stream to the small pulmonary capillaries and breaks into the lung alveoli and migrates towards the trachea while coughing it is swallowed through the oesophagus to the stomach where it hooks into the intestinal mucosa in the small intestine and the intestinal mucosa in the small intestine and starts sucking blood. After that they become adult and produce eggs which are excreted out in the faeces where the larvae hatch in the faeces or in warm, moist, sandy soil within two days. They feed on organic matter and grow rapidly and within 10 days they become filariform larvae that are infective (Arora 2014).

2.16 Diagnosis of Intestinal Helminths

Intestinal helminthes can be diagnosis by following methods.

2.16.1 Microscopic Stool Examination

For most helminthes, direct observation of the parasite from stool is the confirmatory diagnostic method. It is also done by centrifugal sedimentation in a formalin ether system.

2.16.2 Collection of Eggs on Cellophane

Diagnosis of pinworms is made by identifying pinworms or their eggs. Eggs can be collected using a transparent cellophane tape by pressing the sticky side of the tape by pressing the sticky side of the tape to the anal skin. The eggs stick to the tape which can be place on a slide and examined under microscope.

2.16.3 Examination of Segments and Embryophores

Diagnosis of taeniasis can also be done by identification of segments and embryophares in the stool which are observed microscopically (Parija 2013).

2.17 Treatment of Intestinal Helminths

A single tablet of albendazole (400mg) and metronidazole (500mg) has been recommended WHO (2012) for the treatment of soil transmitted helminthes (WHO 2012). In case of hookworms, a confirmatory stool test is required after treatment to make sure all hookworms are dead. For taeniasis, albendazole can also be administered for three days, a tablet taken daily. Treatment of taeniasis can be done by administering nicolsamide carefully adhering to the dosage. This requires fasting in the morning and slowly chewing the tables with a spoonful of water (Parija 2013).

2.18 Public Health Importance of Intestinal Helminths

Helminthic infections with heavy intensity impair physical growth and cognitive development and are the cause of micronutrient deficiencies including iron deficiency leading to anaemia which results poor school performance and absenteeism in children reduce work productivity in adults and also effects pregnancy (Hall 2008). Parasitic infections can cause deficiencies in vitamins and minerals (iron, calcium and magnesium) block nutrient absorption, diminish immunity and also leading to serious disease. Especially, hookworms cause anaemia in women and children due to loss of blood (Arora 2014).

The helminthic infections lead to nutritional deficiency, anaemia, growth retardation and impaired learning ability among school age children (Baragundi et al 2011). If treatment is not given in time for heavy and long-term infection, it may result in death. Heavy infection with *T. solium* includes cysticercosis, which is the development of numerous vesicles that destroy different organs, eyes, nervous system and skin.

2.19 Control of Intestinal Helminths

The best method to control intestinal helminthes is the interruption of the transmission cycle of the parasites. They include chemotherapy, improvement in sanitation and health education. By deworrning and treating asymptomatic carriers, spread of intestinal helminthic infections can be controlled. Infections

with *Taenia spp*. can be present by thoroughly cooking beef and pork walking with bare foot should be reduces for preventing hookworm and *Strongyloids* infestation (WHO 2012).

Health education should discourage open defection and emphasize hygienic measures such as washing hands with Soap and water.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials:

The list of materials, chemicals, equipments reagents used in this study are listed in Appendix C.

3.2 Methods:

3.2.1 Study Duration

The study was conducted from April to September 2018.

3.2.2 Laboratory Set up

Laboratory setting was done in Sunsari laboratory, Bhanuchok, Dharan-9 and also some part of work was conducted in Microbiology Laboratory, Central Camps of Technology, Dharan.

3.2.3 Area of Study

The stool samples were collected from two community school children of Shree Panchayat Basic School, Dharan-16, Janpath and Shree Public High School, Dharan-12.

3.2.4 Sample Collection

Each school children were given the brief description about the importance of the examination of stool to detect the parasite. They were clearly advised not to contaminate with water and urine and the containers were with patient's name, code number, date and time of collection with their age. A qestionnaire with an inform consent was given to each child accompanying the queries about their clinical history, hygienic practice and nutritional behavior was filled. A labeled dry, clean, disinfectant free wide moth glass container was distributed and was asked them to bring about 20 grams (a marble size) stool sample next morning.

3.2.5 Transportation of the Sample

After collecting the stool sample from school children the collected stool samples were brought to the laboratory and was fixed immediately with 10% formal saline mixed with equal volume of formal saline and stool.

3.2.6 Sample Size Calculation

Sample size was obtained using the formula used by Golia et al (2014) as used in other health related studies.

According to Golia et al 2014, 26.74% school children were infected with intestinal parasitosis.

So, P value =0.2674, allowable error= 0.05,at 95% confidence interval Z= 1.96. Therefore, sample size was calculated as follows.

$$n= z^2pq/e^2$$

$$= (1.96)^2 0.2674(1-0.2674)/(0.05)^2$$

$$= 3.8416 \times 0.2674 \times 0.7326/0.0025$$

$$= 301.023 \quad \sim 301$$

3.2.7 Laboratory Processing of the Samples (Parija 2013)

Each stool sample was processed by microscopic examination. Microscopic examination was carried out for the detection and identification of cysts, oocysts and trophozoites of protozoan parasites and eggs and larvae of helminthic parasites.

3.2.7.1 Saline Wet mount

First of all a drop of saline was taken in a clean, grease free slide and a small quantity of stool sample was spread over it and then first examination was done under low power (10x) compound light microscope and the under high power (40x). The oil immersion objective (100x) was not recommended usually for the examination of wet mount of the stool.

3.2.7.2 Formalin- ether Sedimentation Technique

The technique was performed as follow:

1. 5 ml of 10% formal saline was taken and the preserved sample was added to it and then shaken well.
2. The suspension was sieved though cotton guage in a funnel into a 15 ml centrifuge tube.
3. After that 5ml of ethyl acetate was added and shaken vigorously for 5 minutes.
4. Then the tube was immediately centrifuge at 500g for 10 minutes or 1000 rpm for 10 minutes.
5. The supernatant was decanted and 10 ml of 10% formalin was added to the sediment and was mixed thoroughly with wooden applicator sticks.
6. Again centrifuge was carried out at 500g for 10 minutes.
7. After centrifugation four layers of suspension were obtained.
    a) A small amount of sediment was obtained at the bottom of the tube containing parasites.
    b) On the upper layer of sediment there was a layer of formalin.
    c) On the top of formalin layer, there was a plug of fecal debris.
    d) And there were a layer of diethyl ether on the topmost layer.
8. The plug of debris was freed from the top of the tube by ringing the sides with an applicator stick and also the top layer of supernatant was decanted.
9. The deposit after shaking was taken on the glass slide and the cover slip was placed over it and was examined by saline wet mount.

3.2.8 Recording of the Result

After laboratory processing of the stool sample, the result obtained was recorded in thesis log book. After then it was recorded in computer.

3.3 Report Distribution

After laboratory processing of the samples the result obtained was reported and the report was distributed to the children. The children with positive cases were given anti protocol drug along with the report. For protocol infection metronidazole was given and for helminthic parasite they were suggested to visit to the doctor for their better treatment with the report.

3.4 Statistical Analysis

Chi-square test was applied for statistical analysis of results using SPSS version 16. Association of intestinal infections with different variables was tested. Results were significant if p values were less than 0.05.

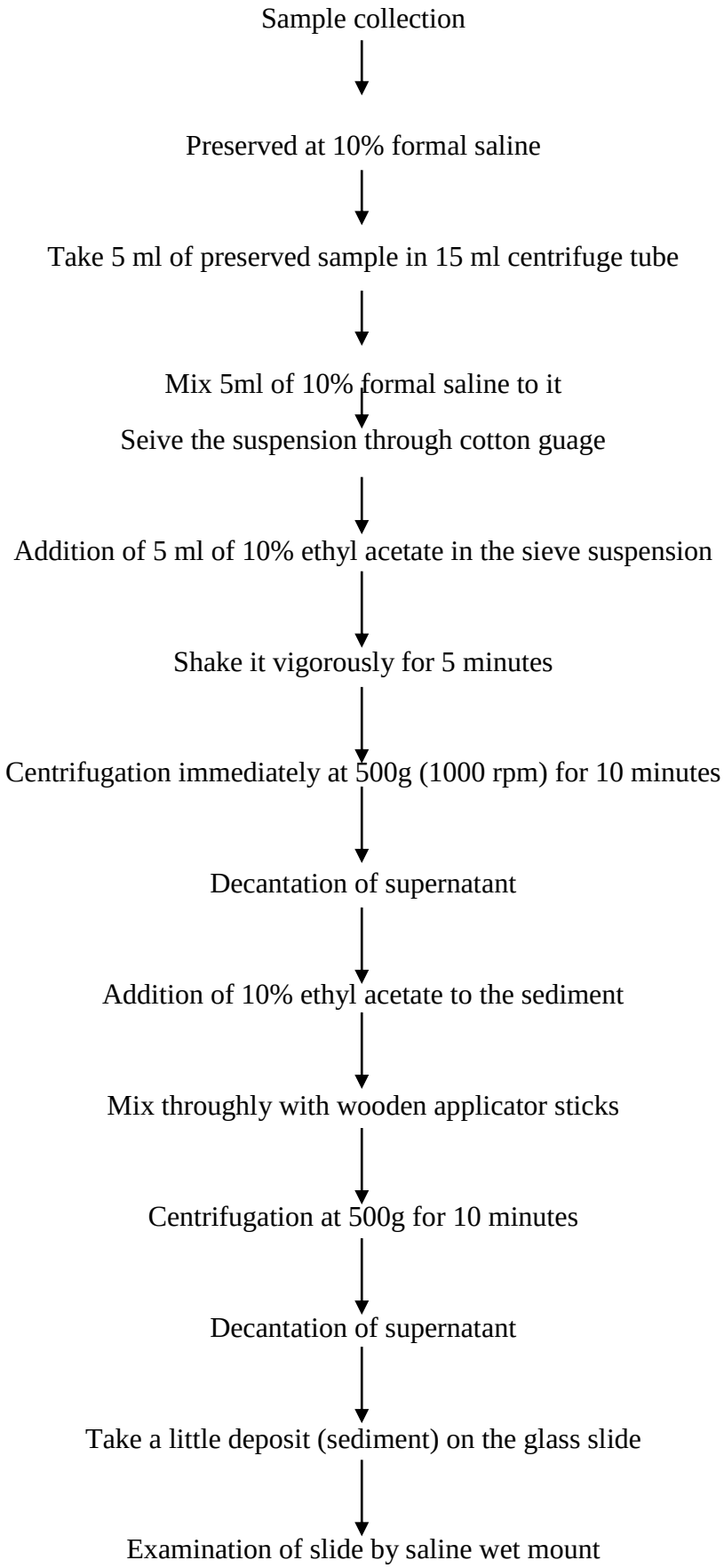


Figure 3.1: Flow chart of Formalin- ether sedimentation technique

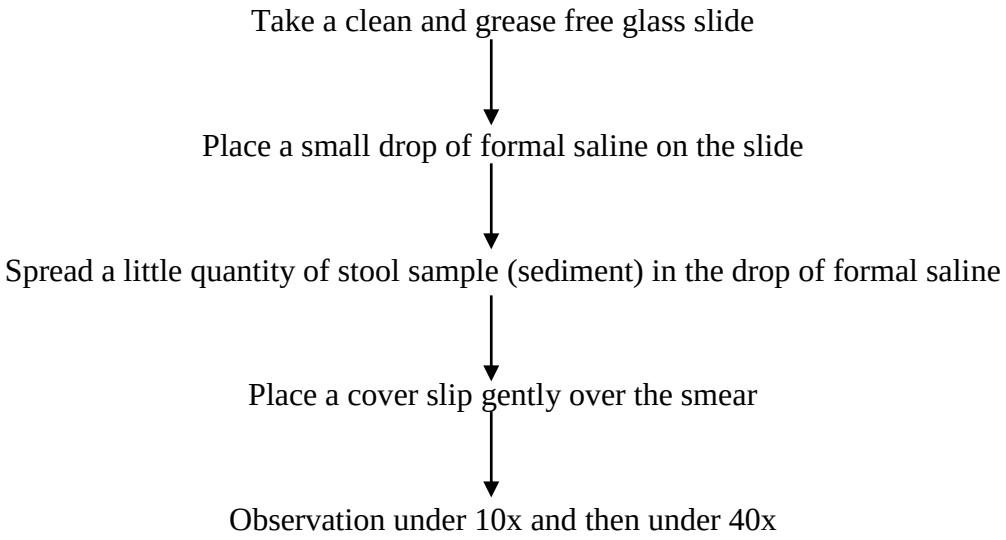


Figure 3.2: Flow chart of Saline wet mount technique

CHAPTER IV

RESULTS

Parasitosis among school going children was carried out by the examination of 301 stool samples. Altogether 26 samples were found to be positive for parasites.

4.1 Study Population

In this study, all together 301 students stool samples were examined. Among 301students 164 (54%) were females and 137(46%) were males. This result shows the participation of female students was comparetively higher than male students **(Figure 4.1)**.

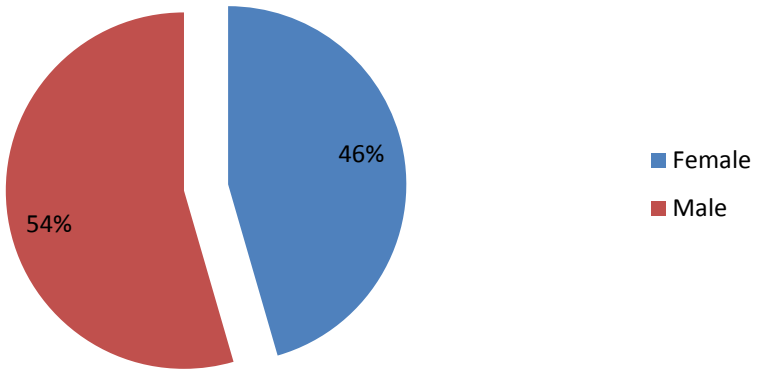


Figure: 4.1: Population under Study

4.2 Distribution of Students on the Basis of Age and Gender

On the basis of age, the highest number of students who participated in this survey was of 12- 13years. In this age group, the total students were 130 and among them 73 were males and 57 were females. The lowest number of students who participated in this survey was below 6-7 years **(Figure 4.2)**

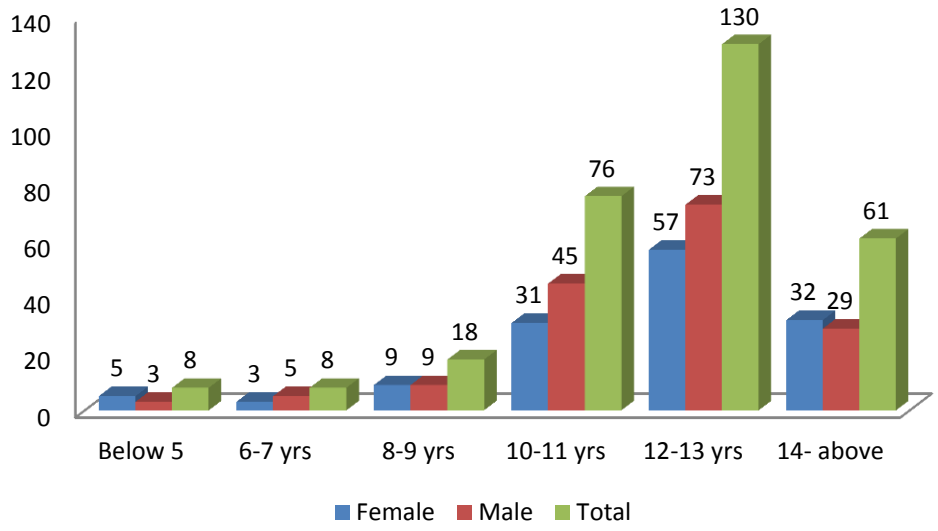


Figure 4.2: Age and Gender wise distribution of students

4.3 Detection of Parasites

Out of 301 stool samples, 26 stool samples were found to be positive, 19 (73.07%) was protozoan parasites and 7 (26.92%) was helminthes. Among 26 positive stool sample 5(19.23%) *H.nana*, 1(3.85%) *A. lumbricoides* and1 (3.85%) *hookworm* was detected **(Figure 4.3).**

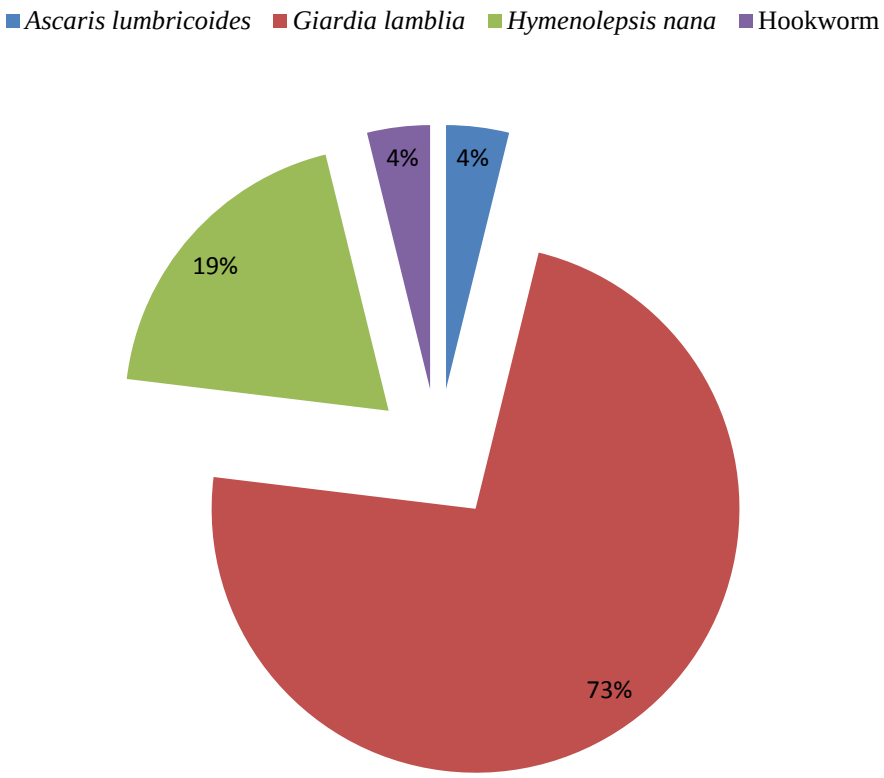


Figure 4.3: Prevalence of intestinal parasitosis

4.4 Prevalence of Intestinal Parasites according to the Gender

Out of 301 stool samples, the number of males and females were 164 (54.5%) and 137 (45.5%) respectively. Also among 26 positive cases the rate of intestinal parasitic infection was high in males as compared to the female. The intestinal parasitic infection was 16 (61.54%) in male students and 10 (38.46%) in female students **(Figure 4.4).**

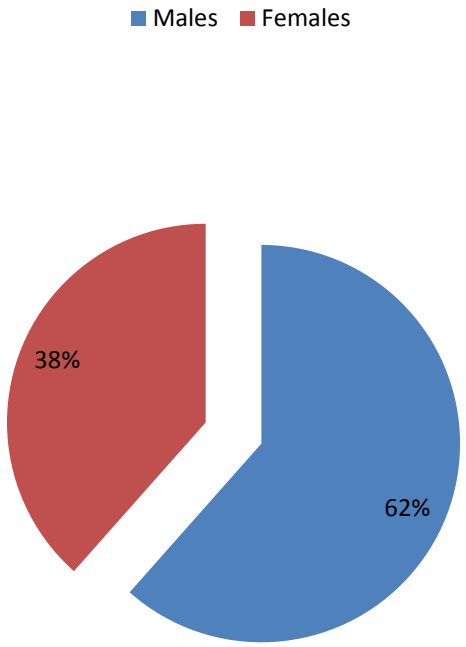


Figure 4.4: Gender wise prevalence of intestinal parasitosis

4.5 Prevalence of Intestinal Parasites according to Age Group

The rate of intestinal parasitosis was higher in the age group > 9 years than the age group ≤ 9 years old students. Intestinal parasitosis was found 3(8.82%) in students whose age was ≤ 9 and there was 23(8.61%) students positive > 9 years age **(Table 4.1)**.

Table 4.1: Prevalence of intestinal parasites according to age group

Age group	**Intestinal parasites**		**Total**	**Percent (%)**	**p- value**
	Absence	**Presence**			
≤ 9 Years	31	3	34	8.82	0.000
>9 Years	244	23	267	8.61	
Total	275	26	301	8.64	

4.6 Parasitosis according to the Ethnic Group

The rate of intestinal parasitic infection was 18 (12.5%) in Janjati, 4 (8.89%) was in Madeshi and 4 (5.48%) in Dalit. The prevalence rate of intestinal parasitosis was higher in Janjati **(Table 4.2)**.

Table 4.2: Parasitosis according to ethnic group

Ethnicity	Parasitosis overall		Total	Percentage	P- value
	Absence	Presence			
Bhramin	15	0	15	0	0.000
Chhetri	18	0	18	0	
Dalit	69	4	73	5.48	
Janjati	126	18	144	12.5	
Madeshi	41	4	45	8.89	
Others	6	0	6	0	
Total	275	26	301	8.64	

4.7 Parasitosis according to the Family Type

According to the family type, out of 232 students from nuclear family 23 (9.91%) were infected from intestinal parasites. Also among 69 students from joint family only 3 (4.35%) students were infected from intestinal parasites and the p-value was found to be 0.148 which shows that there is no significant difference between the family type and intestinal parasitosis **(Table 4.3)**.

Table 4.3: Parasitosis according to Family type

Family Type	Parasitosis		Total	Percent	p-value
	Absence	Presence			
Joint	66	3	69	4.35	0.148
Nuclear	209	23	232	9.91	
Total	275	26	301	8.64	

4.8 Parasitosis according to the Type of House

The overall 13 (10.74%) students out of 121 were infected with intestinal parasites having own house and 13 (7.22%) students out of 180 were found to be infected with intestinal parasites who lives in rented house and the p-value was found to be 0.00 **(Table 4.4)**.

Table 4.4: Parasitosis according to house type

House Type	Parasitosis		Total	Percent	p-value
	Absence	Presence			
Own	108	13	121	10.74	
Rented	167	13	180	7.22	0.00
Total	275	26	301	8.64	

4.9 Parasitosis according to Family Income

The overall parasitosis among low class family was found to be 13 (8.18%) out of 159 and among middle class family was found to be 13(9.15%) out of 142. Family whose yearly income was less than 1 lakh was considered as low class and family having yearly income between 1-3 lakh was considered as middle class. The result is as in **Table 4.5**.

Table 4.5: Parasitosis according to Family income

Family Income	Parasitosis		Total	Percent	p-value
	Absence	Presence			
Low class	146	13	159	8.18	
Middle class	129	13	142	9.15	0.746
Total	275	26	301	8.64	

4.10 Parasitosis according to Education Level of Parents

In this study, the occurrence of intestinal parasitosis was fond to be 6 (10.71%) among those students whose parents education level is high school, 1 (2.5%) among illiterate parents, 3 (30%) among intermediate parents, 4 (11.11%) among middle school parents and 12 (7.55%) among students whose education level is primary school **(Table 4.6)**.

Table 4.6: Parasitosis according to Education level of parents

Education Level	Parasitosis		Total	Percent	p-value
	Absence	Presence			
Illiterate	39	1	40	2.5	
Primary	147	12	159	7.55	
Middle school	32	4	36	11.11	0.074
High school	50	6	56	10.71	
Intermediate	7	3	10	30	
Total	275	26	301	8.64	

4.11 Parasitosis according to the Bowel Syndrome

Of total 301 students 177 (58.80%) students do not have any bowel syndrome and 124 (49.20%) students have got bowel syndrome. Overall high rate of infection was found in students having bowel syndrome 13 (10.48%) and 13 (7.34%) infection was found in those who were not suffering from bowel syndrome **(Table 4.7)**.

Table 4.7: Parasitosis according to bowel syndrome

Bowel Syndrome	Parasitosis		Total	Percent
	Absence	Presence		
No	164	13	177	7.34
Yes	111	13	124	10.48
Total	275	26	301	8.64

4.12 Parasitosis according to the source of Drinking Water

Amongst 301 stool samples 281 students use tap water and only 20 students use mineral water. The prevalence of intestinal parasitosis was high in the students using tap water 25 (8.9%) than the students using mineral water whose infection rate was only 1 (5%) **(Table 4.8)**.

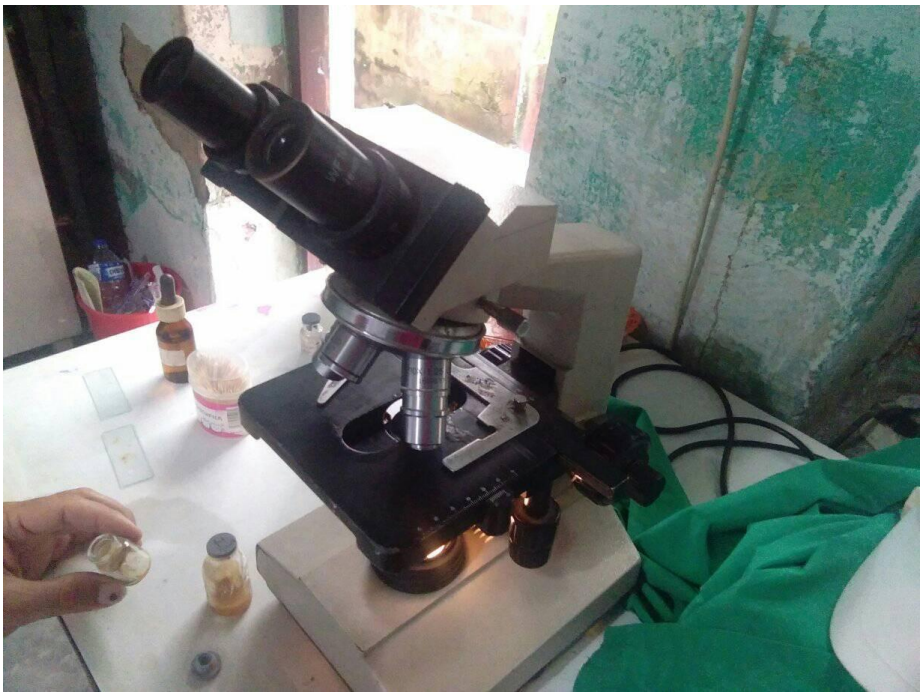
Table 4.8: Parasitosis according to the source of drinking water

Source	Parasitosis		Total	Percentage
	Absence	Presence		
Tap water	256	25	281	8.30
Mineral water	19	1	20	0.34
Total	275	26	301	8.64

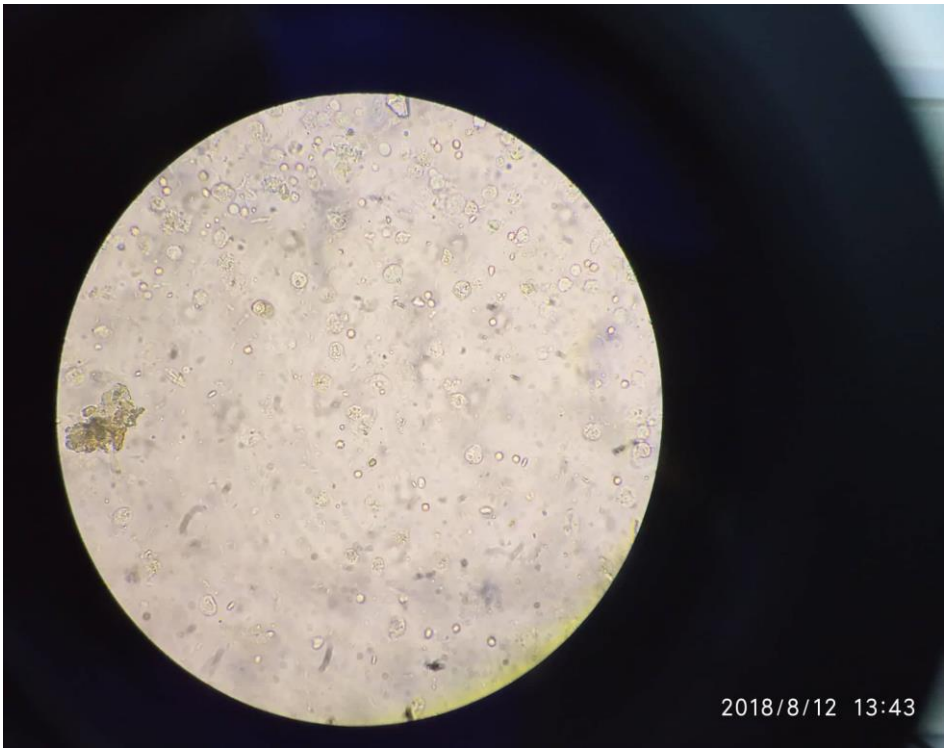
PHOTOGRAPHS

Photograph 1: Stool Samples Collected from School Children

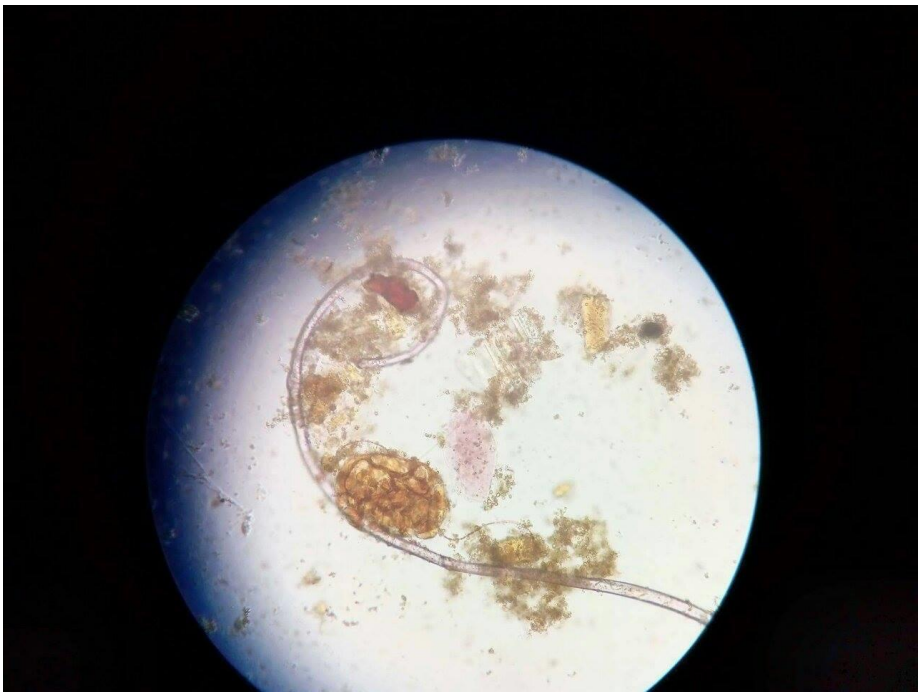
Photograph 2: Preparing Smear of Stool Samples



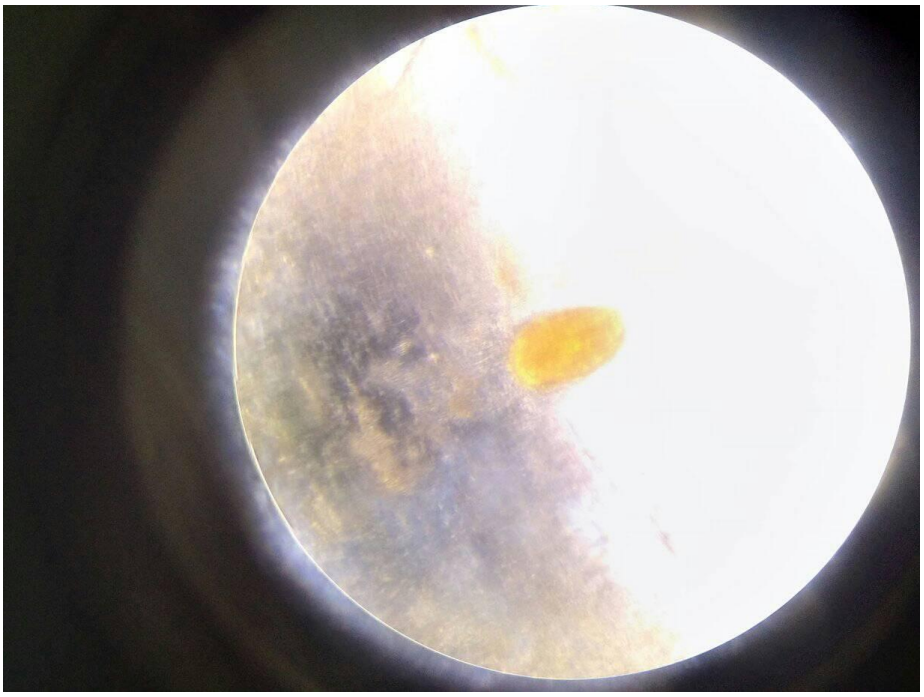
Photograph 3: Microscopic Examination of Stool Samples



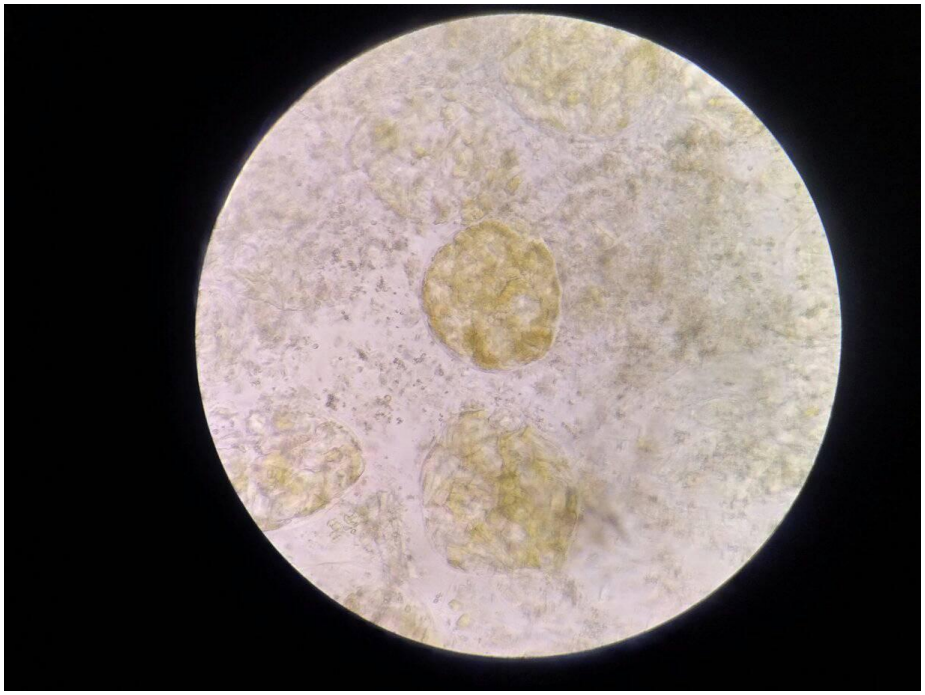
Photograph 4: Cysts of *Giardia lamblia* (Saline wet mount 10x)



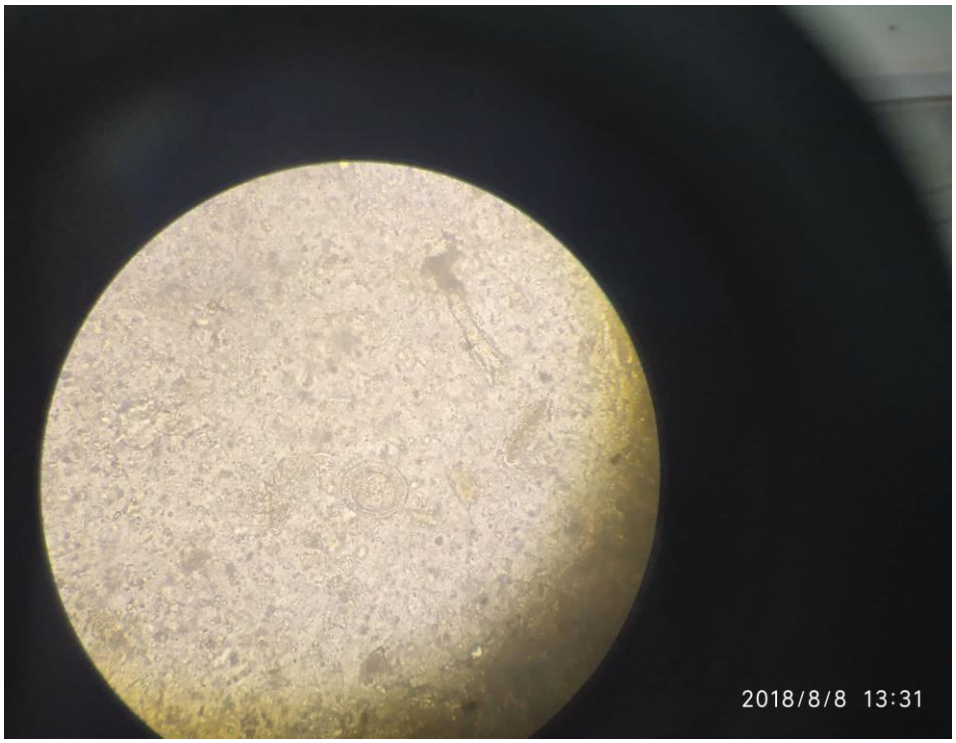
Photograph 5: An Ova of *Ascaris lumbricoides* (Saline wet mount 40x)



Photograph 6: An Ova of *Hookworm* (Saline wet mount 40x)



Photograph 7: Egg of *Hymenolepsis nana* (Saline wet mount 40x)



Photograph 8: Egg of *H. nana* (saline wet mount under 40x)

CHAPTER V

DISCUSSION

School children are the people who come to the school to gain knowledge from different places, community, family and ethnic groups. Moreover, they live in varying community they have different nutritive and hygienic and non-hygienic lifestyles. Therefore, it is like to have a higher rate of intestinal parasitic infection among the children. However, this study showed not very high (8.64%) positive rate of intestinal parasitosis. To the best of our knowledge, no such study on intestinal parasitic infection was available from Nepal. Hence, here are no such data for comparison.

The prevalence of protozoan is higher than helminths in this study. In this study, there was not the prevalence of multi-parasitism only mono-parasitism was detected. Among them *G. lamblia* was the most common 19 (73.07%) of total children under studied. The finding was in harmony with other previous result (Shobha et al, 2013; Sahetal, 2013; Khadka et al, 2013; Ashok et al, 2013).

In stool samples studies, 4 species of intestinal parasites were detected, 3 helminths and 1 protozoa. *G. lamblia* was detected the most among the parasites from Nepal (Sah et al, 2013; Khadka et al, 2013 and Rijal et al, 2013). It would be due to ineffective deworming with the single dose of antiprotozoal drug particularly in case of heavy infections. The high prevalences of giardiasis are because of without periodical diagnosis of the people may serve as the reservoir of *G. lamblia* and pass on the parasite to the susceptible host.

G. lamblia is the medically important gastrointestinal protozoa associated with diarrhoea, especially in communities without proper sanitation and protable water. The rate of protozoal infection is higher than helminthic in this study. The higher rate of protozoal infection might be due to the presence of land and water contaminated with faecal matter resulted due to the drainage of defecated faeces in open land and water, swimming in Sardu River, collecting mud from the river and use of contaminated drinking water.

The study done by Sah et al 2013 has also shown higher protozoal infection rate (18.5%) than the helminthes in school gong children. However, other studies in Nepal among school children have found higher prevalence of helminthic infection (Rijal et al, 2014; Kunwar et al, 2016 and Regmi et al, 2013). The prevalence rate of *E. histolytica* was not shown in this study.

The second highest rate of infection was *H. nana* in this study. It was found to be 5(19.23%) and this was inagreement with report given previously by other researcher (Regmi et al, 2014). It was reported to be 22.8% in Bara district, Nepal. The prevalence of *hookworm* and *A.lumbricoides* were low and almost similar 1(3.85%) for both which is different from the other result. These differences might be due to environmental, geographical and climatic condition of the study place and the technique is used for the identification. The majority of the studies on intestinal parasitosis in Nepal have been conducted by employing "direct smear" technique that might be the case discrepancy in the findings reported by various investigators in the past. The reason for the low prevalence of *hookworm* and *A. lumbricoides* might be due to use of shoes and slippers during most of their times which prevent the skin from the penetration of larvae. The transmission rarely occurred from the ingestion of food contaminated with fleas harbouring the cysticercoid larvae. The increasing use of toilet in the study area might also be the reason behind it. Ingestion of the filariform larvae present in the soil, breast milk from the mother to infants and transplacental might also is the reason to cause helminthic infection. The low prevalence of intestinal parasitic helminths would be due to deworming of antihelminthic drug and facilities of toilet in each and every house.

In this study, the distributions of males were slightly higher 164 (54.5%) than females137 (45.5%). A marginally higher positive rate among males 16 (61.54%) compared to females 10 (38.46%) was observed in this study. The result was in agreement with the report from Nepal by Ghimire et al 2014 in which 37.9%were males and 21.9% were females. However, in some studies from Nepal had shown higher positive rate among females (Khadka et al 2013; Sah et al 2016; Rijal et al 2013). It might be due more active, outdoor wondering nature of the males and usually working outsides the houses and in

dumping sides of Sardu River while female are limited to the household works. The study done by Yadav et al 2017 the prevalence rate was higher in males (54.72%) than in females (45.28%). The prevalence rate of boys was higher as compare to girls (Shrestha et al 2012; Abah et al 2015; Yadav et al 2016; Regmi et al 2016).

Similarly, higher positive rate was found in children less than or equal to 9 years than those greater than 9 years was in agreement with previous report from Nepal (Chongbang et al 2016; Shakya et al 2013). The parasitic infection rate was higher in children of this age which shows ≤ 9 years age was highly prevalent 3(8.82%) and lower rate was found in > 9 years 23(8.61%). Higher prevalence among younger children might be associated with their age activities and behaviour, children in this age group usually play and move around covering wider territory. Whereby possibilities of acquairing infections are increased. Also, this might be due to the activity of children like eating, swimming, playing on mud, playing on Sardu River and dumping sites, walking with bare foot that causes larvae to penetrate into the skin of the children.

Slightly higher positive rate of stool sample was found among children from smaller family. The school going children living in large family members that is joint family members had lower percentage of infection 3(4.35%) than those of living in small family member that is nuclear family 23(9.91%) (P=0.148). Statistically, there is no significant difference between family type and prevalence of intestinal parasitosis. This could be due to lack of proper care by the parents and inadequate hygiene and sanitation in small family. However, the result was insignificant with some of the reports which showed a marginally lower positive rate among small family and higher in large family (Shrestha et al 2012; Regmi et al 2014; Shakya et al 2012).

In this study, incidences rate of parasites were higher in Janajati 18 (12.5%) and then the second most prevent ratewas found in Madeshi 4 (8.89%).For Dalit the prevalence rate was 4 (5.48%). The prevalence rate of Bhramin was null ($p< 0.05$). This study was in agreement with other findings in Nepal (Yadav et al 2016; Khadka et al 2013). This might be due to lower socio-

economics, poor health and sanitation. This may also be due to uneducated lower cast parents who are not aware of using well managed latrine, unhygienic personal habits due to lack of knowledge awareness and also indirectly to their occupation. The helminthic infection among Janjati might also be due to intake of not properly cooked pork and beef meat mostly taken by Janjati.

The prevalence of intestinal parasitosis were found higher in the students who live in their own house 13(10.74%) and in rented houses that was 13(7.22%) out of 26 positive infected students ($p< 0.05$). This might be due to their cleaning activities of their houses and their hygienic activities.

The result presented in table 8 revealed the parasitosis according to the family income. The middle class family (income 1-3 lakh per year) showed 13(9.15%) students infected with intestinal parasitosis and 13(8.18%) infected students were from low class family (income less than 1 lakh)which is not in agreement with any other study of Nepal. The reason might be due to literacy of both family income types. Now a day, they were aware about their health. There was no significant difference between the family income and the occurrence of intestinal parasitic infections ($P=0.746$).

The prevalence of intestinal parasites was found to be higher in those students whose parent's education level is intermediate and lower in those whose parent's education is illiterate that is only one student was from illiterate parents. This study might not be in agreement with any other findings of Nepal. This might be due to their illiteracy and low level education for their health and hygienic practice. This study does not show any significant difference between education level of parents and the prevalence of intestinal parasites ($p=0.74$).

In this study, the prevalence rate was higher in those children who are suffering from bowel syndrome 10.48% (13/124) than those who do not have any bowel syndrome 7.34% (13/177) which is in agreement with Tiwari et al 2013. This might be due to the symptoms such as private part itching, nausea, abdominal discomfort, diarrhea, cough, dyspaenia and anemia are the primary indicator of parasitic infestation.

G. lamblia 13(9.03%) was the highly occurring protozoa in Janajati than other parasites. In Madeshi also the occurance of *G. lamblia* was high and secondly *H. nana* was found to be most prevalent.

Amongst 301 students most of them drink tap water and only few of them drink mineral water. The highest prevalence was found in the students who drink tap water 25(8.9%) than in mineral water 1(5%). This study was in agreement with previous report from Nepal (Sah et al 2016; Tiwari et al 2013; Shakya et al 2012; Sah et al 2013). The high prevalence might be due to contamination of municipal water supplies with human waste, poor quality of water, faulty of sewage line and insufficient level of chlorine.

The prevalence of the intestinal parasites both the protozoa and the helminthes found in this study was lower (8.64%) in comparision to other studies which may be due to the regular biannual deworming program of the government of Nepal along with vitamin A supplement.

CHAPTER VI

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The intestinal parasites were detected from school going children of community school by microscopic examination and formalin-ether concentration method. The parasites identified were *G. lamblia*, *H. nana*, *A. lumbricoides* and hookworm. *G. lamblia* was found to be major causative agent of intestinal parasitosis and among helminthes *H.nana* was common to cause parasitic infection. The parasites could infect both sex and of all age group in children. The parasitic infection was lower in children maintaining personal hygiene and having good-socio economic status.

The prevalence of intestinal parasitic infection in school going children is decreasing but it is still prevalent as major public health problems in school going children. Thus, effort from the municipality to improve the quality of drinking water supply should be done to decrease the parasitic infections. There was no significant difference between age, sex, religious, ethnicity, bowels, syndrome, educational level of parents, family size and family type of the students and the prevalence of intestinal parasitic infection.

6.2 Recommendation

To improve the intestinal parasitic infections among school going children, the following can be taken out.

1. *G. lamblia* was detected in high frequency among other parasites therefore; proper course of drug should be administered.
2. The children of either sex or age were infected by parasites. So, health promotion activities should be initiated in respective of their sex and age.
3. Defecation should be done in only toilet and should be cleaned regularly by using disinfectant.
4. Intake of raw pork meat should be avoided and the green vegetables should be cooked after proper washing.
5. All the parents should be provided awareness programme related with parasitic infection.
6. In order to prevent this intestinal parasitic infection appropriate health education should be given to the children and their parents concerning disease transmission, personal hygiene and safe drinking water.

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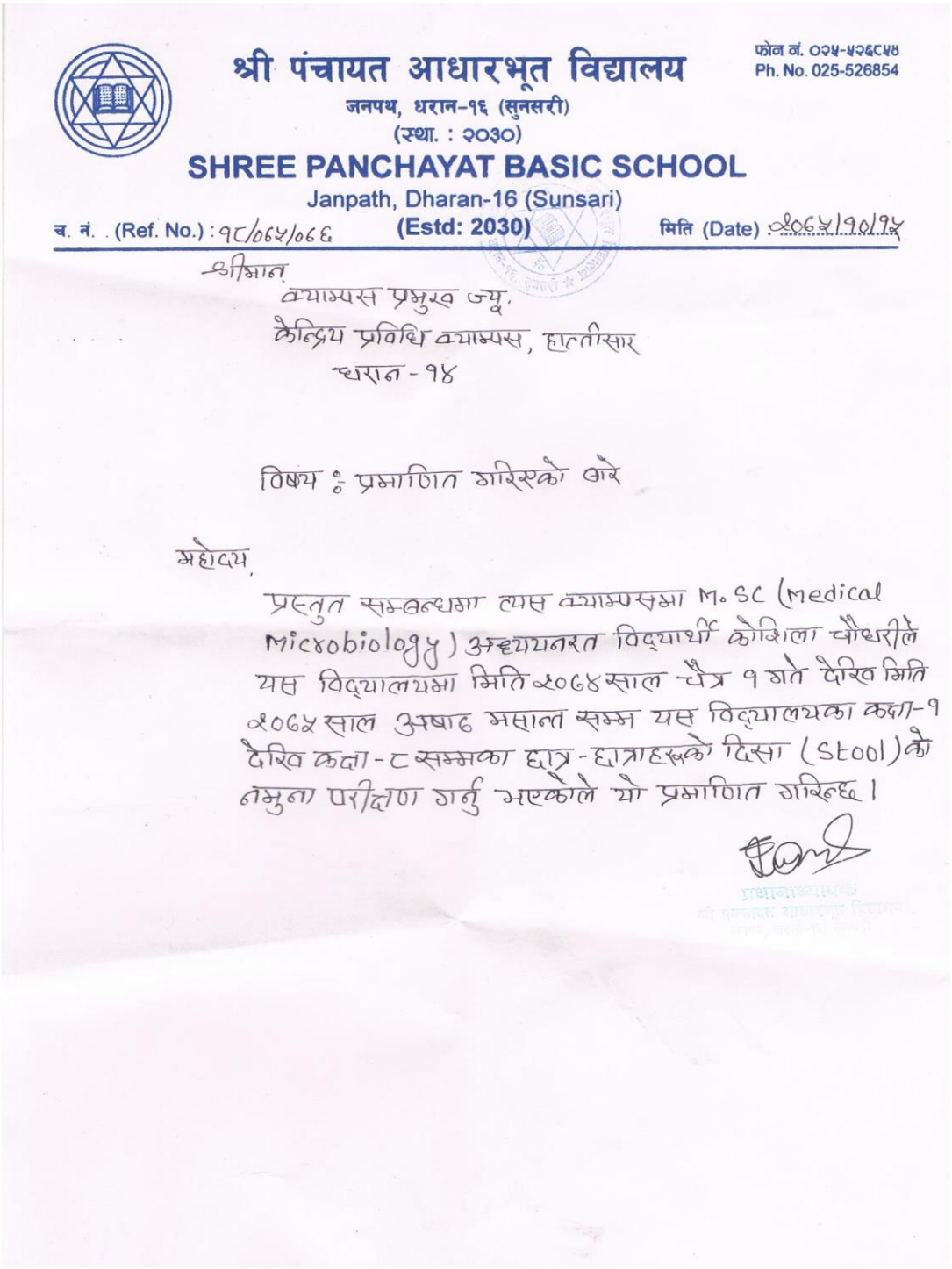
APPENDICES

APPENDIX-A (Conformation Letters from Schools)



श्री पंचायत आधारभूत विद्यालय
जनपथ, धरान-१६ (सुनसरी)
(स्था. : २०३०)
SHREE PANCHAYAT BASIC SCHOOL
Janpath, Dharan-16 (Sunsari)
(Estd: 2030)

फोन नं. ०२५-५२६८५४
Ph. No. 025-526854

च. नं. . (Ref. No.) : १८/०६५/०६६ मिति (Date) : २०६५/१०/१५

श्रीमान्
व्यायमस प्रमुख ज्यू,
केन्द्रिय प्रविधि व्यायमस, हात्तीसार
धरान - १४

विषय : प्रमाणित गरिएको बारे

महोदय,

प्रस्तुत सम्बन्धमा यस व्यायमसमा M. SC (Medical Microbiology) अध्ययनरत विद्यार्थी कोशिला चौधरीले यस विद्यालयमा मिति २०६४ साल चैत्र १ गते देखि मिति २०६५ साल असार मसान्त सम्म यस विद्यालयका कक्षा-१ देखि कक्षा-८ सम्मका छात्र-छात्राहरुको दिसा (Stool) को नमुना परीक्षण गर्नु भएकोले यो प्रमाणित गरिन्छ ।

School Code No. 060180009

श्री पब्लिक हाइ स्कुल
SHREE PUBLIC HIGH SCHOOL

चतरालाइन, धरान-१२, सुनसरी
Chataraline, Dharan-12, Sunsari

☎: 025-520267
Mob. : 9852025261

L.No. :- १९६
S.No. :- २०७५/०७६

मिति : २०७५/१०/१६

श्रीमान् क्याम्पस प्रमुख ज्यू
केन्द्रिय प्रविधि क्याम्पस,
हात्तिसार, धरान

विषय : प्रमाणित गरिएको बारे ।

महोदय,

उपर्युक्त सम्बन्धमा त्यस क्याम्पसमा M.sc.(Medical Microbiology) अध्ययनरत विद्यार्थी कोसिला चौधरीले यस विद्यालयमा मिति २०७५/०३/०५ देखि मिति २०७५/०५/२६ गते सम्म यस विद्यालयका कक्षा ४ देखि ८ सम्मका छात्र/छात्राहरुको दिसा (Stool) को नमूना परीक्षण गर्नु भएको व्यहोरा प्रमाणित गरिन्छ ।

२०७५-१०-१६

प्रधानाध्यापक

HEADMASTER
SHREE PUBLIC HIGH SCHOOL
Chataraline, Dharan-12,

APPENDIX-B

(NHRC Approval)

Government of Nepal

Nepal Health Research Council (NHRC)

Estd. 1991

Ref. No.: 1696

Date: 20 December 2018

Ms. Koshila Chaudhary
Principal Investigator
Central Campus of Technology
Dharan

Ref: **Approval of thesis proposal** entitled **Interstinal parasitosis among School going children of different community school of Dharan,Nepal**

Dear Ms. Chaudhary,

It is my pleasure to inform you that the above-mentioned proposal submitted on **22 August 2018** (**Reg. no. 537/2018**) has been approved by Nepal Health Research Council (NHRC) National Ethical Guidelines for Health Research in Nepal, Standard Operating Procedures Section 'C' point no. 6.3 through Expedited Review Procedures.

As per NHRC rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in objective(s), problem statement, research question or hypothesis, methodology, implementation procedure, data management and budget that may be necessary in course of the implementation of the research proposal can only be made so and implemented after prior approval from this council. Thus, it is compulsory to submit the detail of such changes intended or desired with justification prior to actual change in the protocol.

If the researcher requires transfer of the bio samples to other countries, the investigator should apply to the NHRC for the permission. The researchers will not be allowed to ship any raw/crude human biomaterial outside the country; only extracted and amplified samples can be taken to labs outside of Nepal for further study, as per the protocol submitted and approved by the NHRC. The remaining samples of the lab should be destroyed as per standard operating procedure, the process documented, and the NHRC informed.

Further, the researchers are directed to strictly abide by the National Ethical Guidelines published by NHRC during the implementation of their research proposal and **submit progress report in between and full or summary report upon completion.**

As per your thesis proposal, the total research budget is **NRs 16,000** and accordingly the processing fee amounts to **NRs 1,000.** It is acknowledged that the above-mentioned processing fee has been received at NHRC.

If you have any questions, please contact the Ethical Review M & E Section at NHRC.

Thanking you,

Prof. Dr. Anjani Kumar Jha
Executive Chairperson

Tel: +977 1 4254220, Fax: +977 1 4262469, Ramshah Path, PO Box: 7626, Kathmandu, Nepal
Website: http://www.nhrc.gov.np, E-mail: nhrc@nhrc.gov.np

APPENDIX-C
LIST OF MATERIALS

Chemical and Reagents

Sodium Chloride
Ethanol
Diethyl ether
Formaldehyde
Iodine crystals
Sulphuric acid
Methanol
Sucrose Crystal
2.5% potassium dichromate
Ethyl acetate

Equipments

Microscope
Refrigerator
Centrifuge

Glass wares

Test tubes
Conical flask
Beaker
Measuring Cylinder
Class slide and cover slips
Droppers
Pipettes
Glass rods

Miscellaneous

Test tube stand
Wooden applicator

APPENDIX-D

QUESTIONNAIRES

General characteristics

Name: Sex: Male / Female Age in years:

Religion	Hindu	Buddhist	Christian	Muslim	Others	
Ethnicity	Bhramin	Chhetri	Dalit	Janajati	Madeshi	Others
Measurements	Height (cm)	Weight (kg)	BMI	Waist/hip ratio		

Address:

Family size: ….No. of males: ….No of females: ….Family type: **N / J**

Income of family per year: less than1 lakh 1-3 lakhs more than 3lakhs

Is the family income sufficient for living: Yes No

How much do you spend on food?

Do you have problems in satisfying food for family? Yes No

Type of house: Own Rented

Marital status: Married Unmarried

Education level:

Primary	Intermediate	Middle school	high school	Illitrate

Any exercise: Yes No

How many hours do you sleep at night? …….hr

24 HOUR DIETAEY RECALL

Meals	Description	Amount
Breakfast		
Lunch		
Snacks		
Dinner		
Before bed		

FOOD CONSUMPTION PATTERN

1. Are you?

Vegan	Lacto vegan	Non vegan	Lacto-ovo vegan

2. How many meals do you have in a day? One Two Three
3. How many glasses of water do you drink? .....
4. Do you have breakfast? Yes No Sometimes
5. Do you eat junk foods?

   No: Daily: twice a day: Thrice a day: Frequently:
6. What kind of food do you prefer?

   Processed fast food Homemade cereals
7. Do you eat together with your family? Before Together After
8. Do you eat leftovers? Yes No Sometimes
9. Do you replace your food with other items? Yes No Sometimes
10. Do you have same eating time every day? Yes No
11. What is the source of your food?

    Kitchen garden: Purchasing:
12. Do you have any Bowel complications? Yes No

Medical complications	When	Any treatment	Medicines	Change in diet
Diarrhea				
Dysentery				
Stomach Ache				
Blood in stool				
Vomiting				

Hygiene and sanitation

13. What is the source of drinking water?

    Tap Water Mineral Water Others
14. Any purification method used?

    Filtration Boiling No
15. Do you have any uniform for work? Yes No
16. Toilet using Habit:

Use toilet always Never Sometimes

17. Soap After Toilet

Use always Never Sometimes

18. Do you wash and regularly? Yes No

19. When do you wash your hand with soap and water?

After work: Before eating: Both:

APPENDIX- E

STATISTICAL ANALYSIS

1. Statistical analysis of intestinal parasitosis

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	**Absence**	276	91.7	91.7	91.7
	Ascaris Lumbric	1	.3	.3	92.0
	Giardia lamblia	19	6.3	6.3	98.3
	H. nana	5	1.7	1.7	100.0
	Total	301	100.0	100.0	

2. Statistical analysis of ethnicity verses intestinal parasitosis

Ethnicity intestinal parasitosis crosstabulation

		Parasitosis_overall		Total
		Absence	Presence	
Ethnicity	**Bhramin**	15	0	15
	Chhetri	18	0	18
	Dalit	69	4	73
	Janajati	0	18	18
	Janjati	126	0	126
	Madeshi	41	4	45
	Others	6	0	6
Total		275	26	301

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	206.911[a]	6	.000
Likelihood Ratio	119.029	6	.000
N of Valid Cases	301		

a. 5 cells (35.7%) have expected count less than 5. The minimum expected count is .52.

3. Statistical analysis of family income verses intestinal parasitosis

Parasitosis overall Family income crosstabulation

		Family income		Total
		Low class	Middle class	
Parasitosis overall	Absence	146	129	275
	Presence	13	13	26
Total		159	142	301

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.091[a]	1	.763		
Continuity Correction[b]	.009	1	.923		
Likelihood Ratio	.091	1	.763		
Fisher's Exact Test				.838	.460
N of Valid Cases	301				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 12.27.

b. Computed only for a 2x2 table

4. Statistical analysis of family type verses intestinal parasitosis

Parasitosis * Family type crosstabulation

		Family type		Total
		Joint	Nuclear	
Parasitosis overall	Absence	66	209	275
	Presence	3	23	26
Total		69	232	301

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.088[a]	1	.148		
Continuity Correction[b]	1.442	1	.230		
Likelihood Ratio	2.397	1	.122		
Fisher's Exact Test				.221	.111
N of Valid Cases	301				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.96.

b. Computed only for a 2x2 table

5. Statistical analysis of education level verses intestinal parasitosis

Education_Level * intestinal parasitosis crosstabulation

		Parasitosis overall		Total
		Absence	Presence	
Education_Level	High school	50	6	56
	Illetrate	39	1	40
	Intermediate	7	3	10
	Middle school	32	4	36
	Primary	147	12	159
Total		275	26	301

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.517[a]	4	.074
Likelihood Ratio	7.127	4	.129
N of Valid Cases	301		

a. 4 cells (40.0%) have expected count less than 5. The minimum expected count is .86.

6. Statistical analysis of purification of water verses intestinal parasitosis

Purification of Water * Parasitosis overall Crosstabulation

Parasitosis_overall			Bowel_Syndrome		Total
			No	Yes	
Absence	Purification.of.Water	Boiling	1	1	2
		Filtration	99	63	162
		No	64	47	111
	Total		164	111	275
Presence	Purification.of.Water	Filtration	11	10	21
		No	2	3	5
	Total		13	13	26
Total	Purification.of.Water	Boiling	1	1	2
		Filtration	110	73	183
		No	66	50	116
	Total		177	124	301

Chi-Square Tests

Parasitosis_overall		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Absence	Pearson Chi-Square	.404[b]	2	.817		
	Likelihood Ratio	.402	2	.818		
	N of Valid Cases	275				
Presence	Pearson Chi-Square	.248[c]	1	.619		
	Continuity Correction[d]	.000	1	1.000		
	Likelihood Ratio	.249	1	.618		
	Fisher's Exact Test				1.000	.500
	N of Valid Cases	26				
Total	Pearson Chi-Square	.367[a]	2	.832		
	Likelihood Ratio	.366	2	.833		
	N of Valid Cases	301				

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is .82.

b. 2 cells (33.3%) have expected count less than 5. The minimum expected count is .81.