INTESTINAL PARASITOSIS AMONG HUMAN IMMUNODEFICIENCY VIRUS AND TUBERCULOSIS INFECTED PATIENTS OF DHARAN, NEPAL

A
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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 and is highly prevalent among the general population in Nepal. The presence study was done to find out the prevalence of intestinal parasitosis among HIV and Tuberculosis (TB) patients of Dharan total of 53 sample (<15 years) from HIV seropositive and 28 samples (<20years) from TB patients were collected in a clean, dry and capped fitted container and subjected to macroscopic and microscopic examination for ova, cyst, adult parasites and or segments of parasites. Samples were fixed in 10% formalin-ether solution. Sedimentation technique along with modified acid fast (Zeihl-Neelsen) staining method was performed for opportunistic intestinal parasites in both patients. Multiparasitic infection was noted in the study. The overall prevalence of intestinal parasites was found to be 54(66.67%) among 81 patients (53 HIV patients and 28 TB patients). The parasitosis in male was higher 25(30.86%) than female 15(81.51%). The study preval 18.51% (*G. lambia*), 14.81% (*E. histolytica*), 14.81% (*Cryptosporidium pavum*), 7.40% (*Isosporo beli*), 4.93% (*Microsporodium)*, 2.46% (Hookworm), 2.46% (*Taenia spp*.), 1.23% (*Blastocystctis homini*). In order to prevent this infection appropriate health education should be given to the patients concerning disease transmission, antiparasitic therapy, personal hygiene and safe drinking water.

Keywords: Parasitosis, HIV, TB, formal saline, formalin-ether, Zeihl-Neelsen,

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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*
HAART	:	Highly active antiretroviral therapy
HHs	:	Households
HIV	:	Human immuno deficiency virus
H. nana	:	*Hymenolepsis nana*
IPs	:	Intestinal Parasites
N. americanus	:	*Necator americanus*
NTD	:	Neglected Tropical Disease
NCC	:	Neurocysticercosis
STH	:	Soil Transmitted Helminthes
SPSS	:	Statistical Package for Social Science
S. stercorali	:	*Strongyloides stercoralis*
TB	:	*Tuberculosis*
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 are multicellular (Haque2007). Protozoa and helminthic parasites are the known parasites that affect the gastrointestinal cavity. Intestinal parasites such as *Ascaris lumbricoides, Trichuristrichiura* 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 young aged (Hailegenbriel 2017).In addition opportunistic parasites are also marked in immunocompetent patients. These include: *Cryptosporidium parvum, Isospora belli, Microsporidium* species, *, Cyclospora* species, *Blastocystis hominis* and *Dientamoeba fragilis* (Awole *et al*, 2003).

Taeniasis, amoebiasis, enterobiasis, ascariascis, giardiasis are the common intestinal parasitic infections found in human and transmission of these infections are mainly through oral route (Parija2013). Intestinal parasitic infection is one of the major health problems in developing countries. Human Immunodeficiency Virus (HIV) is a retrovirus, a member of the orthoretrovirinae subfamily, which has spherical virion measuring about 80-90 nm in diameter. It contains linear, positive sense, 9-10 kb, diploid, single stranded RNA as a genome (Awole *et al*, 2003).

Acquired Immunodeficiency Syndrome (AIDS) is a pattern of diseases which reflect the severe late manifestations of HIV. Patients with AIDS are considered to have CDC group IV and its diagnosis remains, in essence, a clinical definition. AIDS is one of the most important public health problems

worldwide at the start of 21st century. The national HIV/AIDS strategy (2002-2006) in Nepal has identified migrant populations, especially labour migrants to India, as one of the vulnerable groups for HIV infection (NCASC, 2003). As of June 30, 2006, 6990 HIV seropositive cases have been detected, with 1085 cases of AIDS; the estimated number is much higher than 60,000 (NCASC, 2006).

HIV infection leads to the depletion of CD_4+ T helper cells as it is the target cell for HIV infection. The depletion of T- helper lymphocytes results in the causation of opportunistic infections (OIs). OIs are those infections which are caused only when there is immunological and anatomical defects. When the immunological and anatomical defences are impaired or compromised as a result of congenital or acquired diseases or by use of immunosuppressive therapy or surgical techniques there is the manifestation of OIs. The OIs among HIV seropositive subjects and AIDS are caused by bacteria, fungi, parasites and other viruses as well. However, the frequency of OIS varies with the geographic area, the prevalence of microorganisms in an environment and the stage of HIV infection (Fleming, 1990).

Gastrointestinal involvement in HIV/AIDS is almost universal and significant disease occurs in 50-96% of patients. Diarrhoea can be a manifestation or a life threating complication of infection with HIV sometimes during the course of the disease. Infectious causes of diarrhoea have been found in 30-80% of patients depending on the extent of the study and patient characteristics. Such pathogens include opportunistic agents that consistently cause severe, chronic or frequent gastrointestinal disease and non opportunistic agents that usually cause acute, treatable diarrhoea illness (Fleming, 1990; Gilks and Ojoo 1991; Gurerrant and Boback 1990; Harries and Beeching 1991; Smith *et al*, 1988). The etiology for such diarrhoea could be either parasitic, bacterial, fungal, enteric virus or HIV itself may contribute to the diarrhoea. In addition to microbes, other factors such as medication, immune dysregulation, autonomic disorder and nutritional supplementation play substantial role in diarrhoea of HIV/AIDS patients (Essex *et al*, 1994; Harries 1991; Soave and Framm 1997).

Nematode like S*trongyloides stercoralis* can also cause diarrhoea and overwhelming infestation in patients with variety of immunosuppressive disorders including HIV/AIDS (Ambrioise, 2001; Pollock and Farthing 1997). Other nematodes such as hookworms, O*pisthorchis veverrini* and *Ascaris lumbricoides* can also be seen in stool of HIV patients (Wiwanitkit, 2001). Severe helminthic infection, expressing either as more egg/s in faeces or infestation simultaneously by several helminthes, correlated positively with the load of HIV particles in plasma (Fincham, 2003). This is because both progression of HIV infection to AIDS and helminth infections are associated with increased T helper cell 2 (Th_2) cytokine productions (Conlon *et al*, 1990). Helminths infection like ascariasis has also been shown to polarise the immune response in young adults to Th_2, which should increase the risk of sexual transmission of HIV. Ascariasis also suppresses interleukin-2, a Th_1 cytokine that can be used as a treatment for HIV/AIDS because it improves count of CD_4 T cells and restores immune function substantially (Fincham, 2003).

Several bacterial enteric infections occur with increased frequency in persons infected with HIV and some of them are more likely to be severe, recurrent, persistent and associated with extra-intestinal disease. Salmonellosis is likely to cause severe invasive disease in HIV infected persons. CDC surveillance definition has recently included recurrent non-typhoidal *Salmonella* septicaemia as an AIDS defining illness. *Shigella* bacteraemia, a rare complication of shigellosis in adults, may be more common in HIV infected patients. *Campylobacter* spp. are known to be one of the more prevalent organisms in HIV diarrhoea. *Campylobacter* enteritis and *Campylobacter* bacteraemia are more common in patients with AIDS than in the general public (Smith *et al*, 2002).

In Nepal many groups are considered as high-risk population to HIV infection. These include commercial sex workers, intravenous drug users, immigrants, highway truckers, blood and organ recipients, housewives, newborn and the clients of sex workers (NCASC, 2006). This study tried to reveal the prevalence of enteric parasitic infections among the high-risk group population.

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, Trichurisor* 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). Ziehl-Neelsen staining technique was used to stain coccidians. The smear is fixed with methanol ,1% carbol fuchsin as a primary stain, 1%v/v acid alcohol as a decoloriser and 0.25% malachite green as counter stain and were observed under 40X objective followed by 100X objective (Casemore DP, 1998).

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 nematodallarvae (Kanna et al 2014).

High prevalence of intestinal parasitic infection is reported among risk group. (Hailegebriel 2017). *Entamoeba histolytica* causes death more than 100,000 annually. Similarly, *Giardialamblia* affects approximately 200 million people oftheworld. 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.

The high prevalence of intestinal parasites indicated an increased morbidity in TB patients and emphasized the importance of continued stool analysis and treatment. Reducing the morbidity and mortality of TB and intestinal parasitosis co-infected persons requires an improved understanding of the prevalence of TB, intestinal parasites and their co-infection. The prevalence of smear positive tuberculosis and intestinal parasitosis co-infection predominantly. TB patients associated with intestinal parasitosis were found relatively higher which are the key facter for the morbidity (Alemayehu etal 20014). Prevalence of Smear Positive Tuberculosis, Intestinal Parasites and Their Co-Infection with TB are mostly a way to global health issues. Intestinal Parasitosis due to parasites such as *G. lamblia, A. lumbricoides* and *H. nana* in TB patients were significantly difference as compared with other suspects and shows multiple parasitic infection too (Alyinalem Alemu etal 2019). Based on the World Health Organization (WHO) estimates, 8.8 million new cases of TB and 1.1 million deaths occurred globally. The incidence of tuberculosis has been increasing dramatically throughout the world in the last decade.

Nepal is small improvised country where 70% of morbidity and mortality are associated with infectious diseases. Giardiasis, ascariosis, amoebiasis, ancyclostomiasis and taeniasis are common intestinal parasitic infections in Nepal. Among the whole population intestinal parasitic infections are highly prevalent in young aged, immunocompromised patients, socio-economically

suffered people, peoples who are out of reached of health facilities and environment sanitization knowledges.government has formulates the plan and policies to improve the health of public including improvement of water sanitation and hygiene measures but most of these measures have not been implemented in Nepal. This study aimed at implementing some of these measures as publichealth interventions to evaluate their effects on intestinal helminthic and protozoan infections among the TB and HIV/AIDS patients also to determine the prevalence of intestinal parasitic infection and associated risk factors among TB and HIV/AIDS patients. Hence this research was designed to study the intestinal parasitosis among HIV and TB patients from of Dharan, Nepal.

1.2 Objectives

1.2.1 General Objectives

- The general objective of this study is to find out the intestinal parasitosis among tuberculosis and human immunodeficiency virus infected patients of dharan.

1.2.2 Specific Objectives

- To study the intestinal parasitosis among the tuberculosis and HIV infected patients of Dharan.
- To study the socio-demographic distribution of intestinal parasitosis tuberculosis and human immunodeficiency virus infected patients 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. *Entamoebahistolytica*, *Giardialamblia*, *G. intestinalis* are the major protozoans which infect human intestine (Chatterjee2009). *Giardialamblia* is an important enteric protozoan which is worldwide in distribution and is common in warm moist climates. Giardiasis is the diarrheal diseasewhichis frequently caused by *Giardialamblia*. Intestinal protozoan is 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. *Entamoebahistolytica*, 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 Entamoebahistolytica*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 *Giardialamblia* (Sah et al 2013).

2.3.1 *Entamoebahistolytica*

Entamoebahistolytica 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. *Entamoebahistolytica*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 wall which surrounds the cyst. The mature cyst containing four nuclei of *Entamoeba histolytica* are mostly found in formed stool and can also be found in semi formed stool.

2.3.2 *Giardia lamblia*

Giardialamblia 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. *Giardialambila* 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. *Giardialamblia* 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 ≤ 5 years of age were must at risk of protozoan infections (Chatterjee 2009).

2.3.3 Intestinal Ciliates

Ciliates move by means of cilia and are also acquired by humans through fecal -oral transmission They have both cysts and trophozoite forms in their life cycle. Ciliates include *Balantidium coli* (Garcia 2009).

2.3.4 Intestinal Apicomplexa, Sporozoa (Coccidia)

These organisms are acquired by humans by ingestion of various meats or through fecal- oral transmission through contaminated food or water. Coccidia include -*Isospora belli, Cyclospora cayetanensis, Blastocystis hominis,*

Cryposporidium species such as *Crytosporidium parvum*, etc. Most of them complete their life cycle in single host. Among their different morphological stage oocysts are infective. The coccidia including *Microsporodium* particularly show high prevalence and cause opportunistic infection among immunocompromised people though it infects immunocompetent people as well, mainly children aged less than five years. In the case of immunocompromised patients, the intestinal opportunistic parasites play a major role in causing chronic diarrhea accompanied by weight loss (Gbakima 2007; Lee et al 2005; WHO 2010).

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 *Giardialamblia* was found to be 10.5% and 8% were *E. histolytica* (Sah et al 2013).

In Ethopia astudy 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 *Entamoebacoli* was found to be11.3% (Rangaiahagariet 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-Baidooet 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 was 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. histolytica*/*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).

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).

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 etal 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 (Kunwar2016). 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 walland 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. Ingiardiasis 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 faecal 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 (Parija2013).

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*, *Echinostomailo canum* 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.* s*olium*, *H. nana*, *T. trichiura* (Parija 2013).

2.12.1 *Ascaris lumbricoides*

Ascarislumbricoids 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 passes 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 be 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 anteriorend 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 chromictype (Chatterjee 2009).

2.12.3 *Trichuris trichiura*

*Tirchuristrichiura*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*

Hymenolepsisnana 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 parasitieshumans 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*

Strongyloidesstercoralis 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*

Taeniasolium is also known as pork tapeworm which causes intestinal taeniasis. Cysticercuscellulosae 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 faecescontaining 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

Sah et al 2016 carried out a survey among the school children of BiratnagarSubmetropolitan, 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.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).

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 infaecesand fertilize into infective stage within a few weeks in the soilwhere asunfertilize 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 produceeggs 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 cellophanetape 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 including severe health issues and fatal in some chronic diseases such HIV/AIDS and TB (Hall2008). 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 anemia in chronic diseases, women and children due to loss of blood which lead to increase mortality rate. (Arora 2014).

In addition, 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 *Taeniaspp*. 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 Soapand water.

2.20 Epidemiology of intestinal parasites

Intestinal Parasitic infections are globally endemic and constitute the greatest single worldwide cause of illness and diseases. Epidemiological researches in different countries have shown that the socioeconomic, sanitary and environmental conditions are important underlying causes in the endemicity of these parasitic infections (Baker *et al* 2009).

Intestinal Parasites are endemic in most tropical and subtropical countries, particularly in developing countries and are one of the major causes of diarrhoeal disease which may sometimes lead to death (WHO 2010). These intestinal infections pose a serious problem particularly in the developing countries with the people living in these countries more susceptible and

exposed to its predisposing factors. Due to diarrhoeal diseases, at least 5 million people deaths per year occur in developing countries (Shakya 2006). Outbreaks of intestinal infections such as cryptosporidiosis and giardiasis tend to be associated with municipal and recreational water systems and crowded human ecologies such as day care centers, orphanages or slums. Although IPIs especially helminths have been historically prevalent mainly in rural populations, the rapid urban migration in several low and middle-income countries, however, has resulted in the creation of urban squatter settlements with high rates of polyparasitism in these areas. Unmanaged urbanization in such nations with overcrowding and poor sanitation leads to the thriving of GI parasites and higher infection rates with the closer proximity of the infected mass to large vulnerable populations. Outbreaks usually occur during periods of heavy rain or monsoon when water management systems are overwhelmed such as the annual floods in Bangladesh or India (Harhay *et al* 2010).

According to WHO reports, in many countries malabsorption, diarrhoea, blood loss, impaired work capacity and reduced growth rate due to intestinal parasitic infections is taken as important health and social problems. Moreover, parasitic infections such as abdominal angiostrongyliasis, intestinal cyclosporiasis and strongyloidiasis are a common public health concern. The patterns of parasitic infection in the population may differ during a course of time due to the changes in the human behavior and lifestyles. So, periodic epidemiological study and transmission dynamics in parasitic infections will provide more accurate understanding (WHO 2014).

2.20.1 National Scenario

Dharan has lower prevalence rates of helminthic infections in comparison to protozoan infections as seen in the prior studies done in this region. A study conducted in Dharan in 2009 showed that *G. Lamblia* (11.5%) has been most frequently detected followed by *E. histolytica* (4.43 %) while intestinal helminths were detected at lower rates (0.04- 0.38%) (Gyawali *et al* 2009). The periodic administration of anti-helminthic drugs in the region could also

account for this difference in prevalence rates. The Nepalese government has initiated nationwide bi-annual deworming program, especially in schools to combat for the presence of intestinal helminthic infections. This program has been integrated with Vitamin A supplementation to minimize the helminthic infections associated with nutritional deficiencies. However, the issue has not

been brought under control as intestinal parasitosis is still one of the highly occurring infections occurring in the region, particularly among the school age children. This can be attributed to factors such as unsanitary disposal of garbage wastes in the area, lack of safe water supply as well as compromised sanitary habits among the children of this age group like failure to wash hands after defecation and other aspects of personal hygiene.

Among the different ethnic groups, the prevalence has been specially found intense among Dalits. This can be attributed to low socioeconomic status, literacy rate and health ignorance among the people of this ethnic group (Agrawal *et al* 2012; Ishiyama *et al* 2003). The prevalence rates are also generally high in the months of June to September i.e. summer with heavy rainfall. Rainfall may be a constraining factor that affects the parasite dynamics such as development, survival of parasite stages and movement of larval nematodes in the environment (Thapa *et al* 2012).

The prevalence is also reported to be more profound among people living within squatter communities (people unlawfully living in an unused land without any ownership). Most of the People of such communities are unable to access primary health care, basic education and safe drinking water due to poverty (IFAD 2010). They are socio-economically very backward living in extreme poverty and poor hygienic conditions, sharing their house with domesticated animals resulting in increased risk of infection. In a recent study conducted among the children of a Squatter community in Dharan, the overall prevalence of intestinal parasites was found to be (42.93%) with the highest prevalence of (48.8%) found in children of age group of 4-8 years (Chongbang *et al* 2016).

2.20.2 Global Scenario

Intestinal parasites are existent throughout the world. Despite intestinal parasitosis being preventable, treatable, and curable, it continues to be one of the leading causes of death from an infectious disease worldwide. IPIs are among the top ten most common infections in the world. According to World Health organization (WHO) estimate, globally there are 800-100 million cases of Ascariasis, 700- 900 million cases of hookworm infection, 500 million Trichuriasis, 500 million amoebiasis and 200 million Giardiasis (WHO 2014). Amoebic dysentery is reported to be the second most common cause of death from the parasitic disease worldwide after malaria, with its greatest impact on people of developing countries (Stanley 2003). The world health Organization (WHO) estimates that approximately 50 million people worldwide suffer from invasive amoebic infection each year resulting in 40- 100 thousand deaths annually. Cryptosporidiosis is becoming most prevalent in both developed and developing countries among patients with AIDS and among children aged less than five years. Several outbreaks of diarrhoeal caused by *C. cayetenensis* have been reported during the past few decades (Ortega and Sanchez 2010). They are most prevalent in tropical developing countries on the African, Asian and South American continents (WHO 2014).

In developed countries, IPIs are common in settings with poor sanitation standards such as the mental institutes and day care centers. In northern America and Europe these infections are reported to be most prevalent within immigrants or refugees While still found in North America and Europe, their prevalence is highest in areas of intense poverty and in low and middle-income countries in the tropical and subtropical regions of Sub-Saharan Africa, Asia and Latin America and the Caribbean (Harhay *et al* 2010). Hence, GI parasitic infections are known as infectious diseases of poverty. The poor people of the underdeveloped nations live in very congested settlements mostly of temporary in nature with compromise in sanitation. Therefore, these people are likely to have a higher rate of prevalence and experience a cycle where undernutrition and repeated infections result in excess morbidity that can continue from generation to generation (Mehraj *et al* 2008).

Soil-transmitted helminths (STH) are among the most prevalent organisms on the planet estimated to infect almost one -sixth of the global population (Hotez *et al* 2009). Reports from WHO estimates that more than 1.5 billion people or 24 % of the world's population are infected with soil-transmitted helminth infections worldwide. In addition, at least 120 countries across the tropics and subtropics are endemic and at least 1.3 billion people were estimated to be infected with at least on STH species. Infections are widely distributed with the greatest numbers occurring in Sub-Saharan Africa, China and East Asia. (WHO 2014). Epidemiological studies of STH infections have shown that the prevalence and intensity of these infections are highest among children of 4-15 years of age (Norhayati 2003).

A Significant association was reported between parasitic infections with parent's education, place of residence, washing hand habits (Hussein 2010). Likewise, an investigation conducted to determine the infection rate of intestinal parasites among 312 primary and post-primary school children in randomly selected schools in Ilesa West Local Government Area, Osun State, Nigeria, altogether 151 (48.40%) were infected. The most prevalent GI parasite found was *Ascaris lumbricoides* (39. 10%) followed by *E. histolytica* (9.29%) (Awolaju *et al* 2009).

2.21 Epidemiology of HIV/AIDS

Globally, only a small number of HIV infections are estimated to have occurred during the late 1970s and early 1980s. During the late 1990s, HIV prevalence increased remarkedly in Subsahran Africa. HIV has been well established in Asia for many years. The HIV/AIDS epidemic is spreading rapidly in all South Asian countries. India has the single largest proportion of HIV positive cases within its boarder, second globally to South Africa. Over 4 million estimated HIV infections are existing within the region and about 13,000 AIDS cases have been reported by the year 2000 (STC, 2003). The first case of AIDS in Nepal was reported in 1988. By mid 1990s, Nepal has entered the "Concentrated epidemic" stage with consistent HIV prevalence in female sex workers, injecting drug users and migrants. The HIV prevalence is

estimated around 0.5% in the general adult population (NCASC, 2003 and WHO/UNAIDS, 2003). There were around 60,018 people living with HIV/AIDS and 2,598 AIDS related deaths by 2002. Yearwise data indicates that the cases of HIV/AIDS have increased sharply since mid 1990s. In 1992, more than double members of new cases were reported than the formerly reported cumulative cases. Similarly, higher numbers of cases were reported in 2004 as compared to other years.

HIV continues to be a major global public health issue. In 2015, an estimated 36.7 million people were living with HIV (including 1.8 million children). A global HIV prevalence of 0.8%. the vast majority of this number live in low- and middle-income countries. In the year 1.1 million people died of AIDS related illness, since the start of the epidemic, an estimated 78 million people have become infected with HIV and 35 million people died of AIDS-related illness. An estimated 25.5 million people living with HIV live in sub-sharan Africa (WHO-2016). The vast majority of them (as estimated 19 million) live in eastern and southern Africa which saw 46% of new HIV infection globally in 2015. In 2015 there were roughly 2.1 million new cases, 150000 of which were among children. since 2010 the annual new infectious among adults (15+) has remained static at 1.9 million (USAIDS,2016).

2.21.1 National HIV/AIDS epidemiology

The case of aids in Nepal was reported in 1988(Gurubacharya et al, 1994). As of December 2007, National centre for AIDS and STD control (NCASC) has officially confirmed 10546 HIV positive cases and 1610 confirmed cases of AIDS among the total HIV in Nepal (NCASC,2007). USAID has estimated the adult (15-49yrs) HIV prevalence rate of 0.5% by the end the 2005 in general population where as the number of people living with HIV in the same time has been estimated to be 74000 of which 16000 are women. One third of HIV infection nationwide is among infecting drugs users. About 5100 AIDS death have been reported by the end of 2005(UNAIDS,2006).

Over the last few years HIV/AIDS epidemic in Nepal has gained ground and Nepal has progressed from a low prevalence country to a country with

concentrated epidemic. A situation analysis study of the young people, mobile populations, female sex workers, men who have sex with men, infecting drugs users, and children as the most vulnerable to HIV/AIDS in Nepal (NACSC, 2011). Around 10 years ago Nepal was described as a country having comparatively lower prevalence of HIV/AIDS compared to other countries in the southeast Asia. Seasonal migration to Indian cities for seeking jobs and sexual trafficking across a porous Indian border fuelled by the bloody maoist has raised Nepal's HIV prevalence second highest in the region after India (Singh et al, 2005). About 40% of an estimated 30,000 infecting drug users in Nepal have been reported to be infected with HIV (Karki,2000). The predominant mode of transmission of HIV has been reported that 38% of repatriated sex trafficked Nepalese women and girls have been tested positive for HIV (Silvermen et al, 2007). Similarly, 31% comprised of women aged 15-49 years and 6.5% children aged 0-4 years. Among Nepali migrants traveling to Indian cities, approximately 16% engage in high risk sexual behavior or visit sex workers (NCASC,2011).

2.21. Parasitic infections of the gastrointestinal tract associated with HIV/AIDS

Parasitic diarrhoeas are all important cause of morbidity in developing countries. Some of the parasites are well-established enteric pathogens eg. *E. histolytica*, *G. lamblia* and *Balantidium coli* and others are opportunistic pathogens i.e., *Cryptosporidium*, *Isospora*, *Microsporidium* and *Cyclospora* etc. Only a small percent of individuals harbouring the established enteric pathogenic parasites suffer from symptomatic disease in all immunocompetent hosts. However, with the advent of HIV/AIDS, the scenario has changed. Chronic, recurrent infections with all the enteric parasites have been reported from all over the world with varying frequencies. The rate of infection with a particular enteric parasite in HIV/AIDS patient will depend upon the endemicity of that particular parasite into community.

It has been estimated that the probability of developing a serious infection is 33% at 1 year and 58% at 2 years, if CD4 counts are below 200. Chronic parasitic gastro-intestinal infections mainly diarrhoea have been reported in

26% to 66% of patients with AIDS in North America and Europe and in 63% to 93% of patients in Haiti and Africa (developing countries). Protozoa are the most common cause particularly in developing countries. Most of the opportunistic enteric protozoa have gained importance during recent times on account of their association with HIV/AIDS.

Most common enteric opportunistic parasites which have been associated with HIV/AIDS include: *Cryptosporidium* spp, *Isospora belli, Microsporidium* spp, *Cyclospora* spp, *Strongyloides stercoralis, G. lamblia* and *E. histolytica.*

2.23. Gastrointestinal tract infection among HIV/AIDS patients

One of the major health problems among HIV infected patients is the intestinal parasite infestations. It can be seen that intestinal helminth infestation in HIV infected patients is common. However, the reported prevalence is usually similar to those of non HIV-infected patients in the same setting. The infestations are ordinary not opportunistic, hence, thus usually show no correlation to immune status of the patients. The suppression of immunity due to HIV infection shows no significant role in increasing the intestinal helminth infestations. On the other hand, having occult intestinal helminth infestations does also not worsen the outcome of HIV infection. Concerning the clinical manifestation, most of the helminth infestations are asymptomatic and the diagnosis is usually based on the stool examination. Treatments of the infestations as well as the outcomes are usually similar to immunocompetent host. Intestinal protozoal infections are also important problems for HIV-infected patients. Some infections are ordinary, while the others are opportunistic infections. The important opportunistic intestinal parasites including *C. parvum*, *I. belli*, *Cyclospora* and *Microsporidium* are found at high prevalence among the HIV-infected patients, especially in low immune cases with persistent diarrhoea. Concerning the clinical manifestation, most of the infections bring diarrhoea and the diagnosis is usually based on the stool examination with special stains. The treatment of the opportunistic infection can usually get control of the present illness but not prevent the re-infection. Luckily, with the present wide distribution of HAART, the prevalence of the

opportunistic intestinal protozoa infections is significantly decreased (Wiwanitkit, 2006).

A recent study on 22 HIV infected patients with diarrhoea in Thailand, *Microsporidium* was the most common pathogen (27%), followed by *Cryptosporidium* (9%) and *Isospora* (4.5%) (Punpoowong *et al*, 1998).

In situation of chronic helminthic infections, the Th2 subset dominates and thought to develop Th1 function facilitating HIV disease progression (Actor *et al*, 1993; Baum *et al*, 2003; Bentwich *et al*, 1996; Borkow *et al*, 2001). Th_2 response has been associated with wasting and more rapid HIV disease progression, confirming previous research that eosinophil percentages were directly and significantly correlated with viral load and inversely correlated with CD_4 cell count (Actor *et al*, 1993; Bentwich *et al*, 1996; Borkow *et al*, 2001; Kalinkowich *et al*, 1998). *C. parvum* was found infecting 12.5% of 80 AIDS patients in Tegucigalpa (Kaminsky, 1999) and 25.3% of 79 AIDS patients in San Pedro Sula but not 21 HIV positive nor 100 healthy commercial sex workers (Kaminsky 1999, Kaminsky et al, 2004, Gatechew et al, 2004).

In order to verify the occurrence of intestinal parasitic infections in HIV/AIDS patients, 100 HIV/AIDS patients (Group 1) and 85 clinically healthy individuals (Group 2) were submitted to coproparasitological examination. Intestinal parasites were detected in 27% of patients from Group 1 and in 17.6% from Group 2. In Group 1 the most frequent parasites were *S. stercoralis* 12%, with 2 cases of hyperinfection, *Isospora belli* 7%, *Cryptosporidium* spp. 4% with 1 asymptomatic case and hookworm 4%. Of the infected patients from Group 1 who reported to be chronic alcoholics, 64.3% had strongyloidiasis. Only 6 of the 27 infected patients from Group 1 were on HAART. In Group 2 the most frequent parasites were *S. stercoralis* 7.1%, hookworm 7.1% and *G. lamblia* 3.5% (Silva *et al*, 2005).

This became more evident when the appearance of a syndrome named "Slim Disease", characterized by an intense weight loss accompanied by chronic diarrhoea, prolonged fever and diffuse muscle weakness, was observed in Africa, especially in Uganda (Grunfeld and Schanbelan, 1994; Kanradt *et al*,

1985; Mhiri *et al*, 1992). Studies conducted in Zaire and Uganda have shown the presence of some pathogenic agents responsible for the "Slim Disease", such as *Isospora, Cryptosporidium, Salmonella, Shigella* and *Campylobacter* species, amounting to a prevalence of 60 to 80% (Bolinger and Quinn,1994). "Slim Disease" has been observed in advanced stages of HIV infection. The expression "Wasting Syndrome" was adopted in substitution by WHO in 1988 based on criteria laid down by the CDC (Mhiri *et al*, 1992).

In a study conducted in USA among one hundred individuals confirmed to be infected with HIV, the prevalence of enteric parasites was 55 (55%) for *G. lamblia*, 6 (6%) for *Cryptosporidium*, 10 (10%) for *I. belli* and 3 (3%) for *E. histolytica* (Esfandiari *et al*, 1995).

In France, 81 HIV/AIDS patients were studied for parasites, viruses, and bacteria. Pathogens were found in 70.6% of AIDS patients with diarrhoea or malabsorption. The respective prevalence of protozoa in AIDS patients with diarrhoea was *Cryptosporidium* spp. 37.3%, *B. hominis* 13.7%, *G. lamblia* 5.8%, *I. belli* 2% and *Enterocytozoon bieneusi* 2%. Microsporidia were noted in one patient with severe malabsorption but no diarrhoea. Other pathogens included cytomegalovirus in 27.4% and *Mycobacterium avium* in 5.8%. Patients with identified pathogens were more immunosuppressed and more severely malnourished than those with unexplained diarrhoea. Multiple pathogens were found in 13 of 81 patients (16%). Twenty-six of 66 identified pathogens (40%) were diagnosed only on biopsy specimens. Chronic diarrhoea in HIV patients could be explained in the vast majority by appropriate gastrointestinal investigations. Cryptosporidia played a major role, while microsporidia appeared to be less common (Cotte *et al*, 1993).

According to the study conducted in Brazil among AIDS patients, 40% were infected with at least one pathogenic species. The total prevalence of parasites was 16% for *G. lamblia*, 13% for *E. coli*, 7% for *C. parvum*, 3.5% for *H. nana*, 2.5% for *A. lumbricoides*, 2.5% for *S. stercoralis*, 2% for *I. belli*, and 0.5% for *B. hominis* (Cimerman *et al*, 1999).

A prospective observational study was conducted to determine the prevalence and the clinical impact of intestinal parasitic infections in diarrhoeal illness

among HIV-infected and HIV-uninfected children hospitalized with diarrhoea in Bangkok, Thailand, intestinal parasites were identified in the stool specimens of 27 of 82 (33%) HIV-infected and 12 of 80 (15%) HIV uninfected children. *Microsporidium* and *Cryptosporidium* were the most commonly found parasites. Eighty-two percent of HIV infected and 97% of HIV uninfected groups presented with acute diarrhoea and 76% of each group had watery diarrhoea (Chokephaibulkit et al, 2001).

According to the study conducted in North-Eastern Tanzania to determine the prevalence of pathogenic intestinal parasites among adult patients with enteropathic AIDS, a total of 352 patients were recruited of whom 158 (45%) had chronic diarrhoea. Of the 352 patients, 123 (35%) had intestinal parasites. Of the 123, 77 (62.6%) patients had chronic diarrhoea. The types of parasites detected were *Cryptosporidium*, *I. belli*, *S. stercoralis*, *Schistosoma mansoni*, *T. trichiura*, *A. lumbricoides*, hookworms and *E. histolytica*. The prevalence of intestinal parasites was significantly higher in patients with chronic diarrhoea than in those without diarrhoea (Tarimo *et al*, 1996).

A longitudinal study was carried in Bamako (Mali), concerning HIV positive patients suffering from diarrhoea. Opportunistic infections have been relatively frequent with *C. parvum* with 20%, *I. belli* with 8.5% and *Microsporidium* with 11.5% of cases. Other non-opportunistic microbes were found. The frequency and the danger of those opportunistic infections require their efficient diagnosis and care management (Konate *et al*, 2005).

Intestinal parasitosis is one of the major public health and socio-economic problems in Nepal though a hospital-based study had shown a declining trend during a period of ten years. Intestinal parasites even in low or moderate number affect on both nutritional and thereby on immune status of individuals leading to various morbidity and mortality and, therefore, considered to be "unremittingly corrosive". This is attributed to the reduction in appetite, digestion, absorption, acute phase status and increasing intestinal nutrient loses. The reported prevalence of intestinal parasitosis in Nepal varies considerably with over 90.0% in some areas. Overall, intestinal helminth infections alone rank fourth in "top ten" diseases in Nepal (Rai, 2005).

Sherchand *et al* (1997), reported the prevalence of intestinal parasites among children in southern Nepal as 60.1%. Hookworm infestation superseded all parasites by showing a positivity of 11.6% followed by *A. lumbricoides*. Among protozoan infestations, *G. lamblia* was at the top 9.9% followed by *E. histolytica*, 7.2%. The infestation of *Cryptosporidium*, *Cyclospora* and *Isospora* were 4.4%, 3.8% and 1.9% respectively. *H. nana* showed 3.3%, tapeworm 2.2%, *Opisthorchis* spp. 1.6% and *Schistosoma* 0.5% whereas 28.9% of infestation included two or more intestinal parasites.

Sherchand *et al* (1996), reported the prevalence of *Cryptosporidium* as 6.8% from the analysis of stool samples of the patients with diarrhoea. Sapkota *et al* (2004), reported the prevalence of enteric parasites as 32.0% among HIV/AIDS patients in Nepal. Among the parasite positive cases, 83.3% were diarrhoegenic and rest remained asymptomatic. The protozoan parasites detected were *C. parvum* (10.6%), *G. lamblia* (6.7%), *E. histolytica* (5.3%) and *C. cayetanensis* (2.6%). The helminthes detected were *S. stercoralis* and *T. trichiura* each with 2.6% prevalence, and a single case (1.3%) of hookworm.

Dhakal *et al* (2004), found that the prevalence rate for *C. parvum* infection was 10.4%; among which 56.3% specimens were found co-infected with other intestinal parasites such as *G. lamblia*, *E. histolytica*, *Cyclospora*, *E. coli*, *Ascaris*, hookworms, *T. trichiura* and *Trichomonas hominis*.

Opportunistic infections are the leading cause of mortality and morbidity among HIV/AIDS patients. The spectrum of opportunistic pathogens involved in such infections in Nepal is not well documented. A cross-sectional study was carried out at the AIDS clinic of Manipal Teaching Hospital, Pokhara, Nepal. A total of 45 opportunistic pathogenic isolates were recovered from the 74 patients. *Mycobacterium tuberculosis* was the commonest pathogen 60%, followed by *Cryptosporidium* spp. 13.3% and *Candida* spp. 11.1% (Das *et al*, 2005).

2.24 Epidemiology of tuberculosis

2.24.1Tuberculosis: global aspect

Tuberculosis is the world's second most common cause of death from infectious disease, after HIV/AIDS. Nearly one-third of the global population (2 billion persons) is infected with M. tuberculosis bacilli and is at risk of developing active clinical TB. Worldwide more than 16 million people are suffering from active TB disease (WHO, 2003). There were 8.8 million estimated new cases of TB (all types) in 2002, of which 3.9 million 16 were smear-positive (infectious type) (WHO, 2004a). Sub-Saharan Africa has the highest incidence rate (290 per 100000 populations), but the most populous countries of Asia have the largest numbers of cases: India, China, Indonesia, Bangladesh, and Pakistan together account for more than half the global burden. Eighty of new cases occur in 22 high-burden countries. Everyday more than 5000 people (approximately 2 million per year) are dying from the disease (WHO, 2003; WHO, 2004b).

2.23.2Gastrointestinal tract infection among TB patients

Tuberculosis, and a similar percentage with helminths, the majority of these infections are found in the developing countries. Mycobacterium tuberculosis usually enters the host through inhalation of droplets containing viable bacteria. The bacterium reaches alveolar spaces and is ingested by alveolar macrophages. This results in the induction of inflammatory responses with the consequent development of a glaucomatous lesion where different T-cell populations participate in protective immune response.The high prevalence of intestinal parasites indicated an increased morbidity in TB patients and emphasized the importance of continued stool analysis and treatment. Reducing the morbidity and mortality of TB and intestinal parasitosis co-infected persons requires an improved understanding of the prevalence of TB, intestinal parasites and their co-infection among tuberculosis suspects. Both TB and parasitic infections have been studied extensively, but information is scarce regarding the level of TB and parasitosis co-infection in co-endemic

regions. Therefore, this study aimed to determine the prevalence of smear positive tuberculosis, intestinal parasitosis and their co-infection among tuberculosis suspected patients to generate useful information of public health importance.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials:

The list of materials, chemicals, equipments reagents used in this study arelisted in Appendix A.

3.2 Methods:

3.2.1 Study Duration

The study was conducted from August 2019 to January 2020.

3.2.2 Laboratory Set up

Laboratory setting was done in Microbiology Laboratory, Central Camps of Technology, Dharan, Sunsari.

3.2.3 Area of Study

The stool samples were collected from HIV positive patients who are in touch of Dharan positive group and TB patients who were visiting public health post centre for DOTS.

3.2.4 Sample Collection

Each patient was 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 questionnaire with an inform consent was given to each patients accompanying the queries about their clinical history, hygienic practice and nutritional behavior was filled. A labeled dry, clean, disinfectant free wide mouth 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 the collected stool samples were brought to the laboratory and were fixed immediately with 10% formal saline mixed with equal volume of formal saline and stool.

3.2.6 HIV and TB Patients

HIV and Tuberculosis patients were obtained from different rehabilitation center and wards of Dharan Municipality.

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 gauge 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.

3.2.7.3 Modified acid fast (Ziehl-Neelsen) staining

It is required for accurate identification of the oocysts of *C. parvum*, *I. belli, C. cayetanensis* and the spore of *Microsporidium*. The oocysts are acid fast and stained red or pink against a blue background stained with methylene blue. Both hot and cold methods of staining can be used with equal sensitivity. This study followed the cold Kinyoun methods as follows.

1. A fecal smear was made on a glass slide and dried in air.
2. The smear was fixed in absolute methanol for 3 minutes.
3. The slide was flooded with carbol fuschin for 15-20 minutes and then washed with tap water.
4. The smear was then decolorized with 1% sulfuric acid for 15-20 seconds
5. The smear was washed with tap water and then counterstained with 0.5% methylene blue for 1 minute.
6. The slide was washed with tap water, air dried and examined under 40X followed by oil immersion.

3.2.8 Recording of the Result

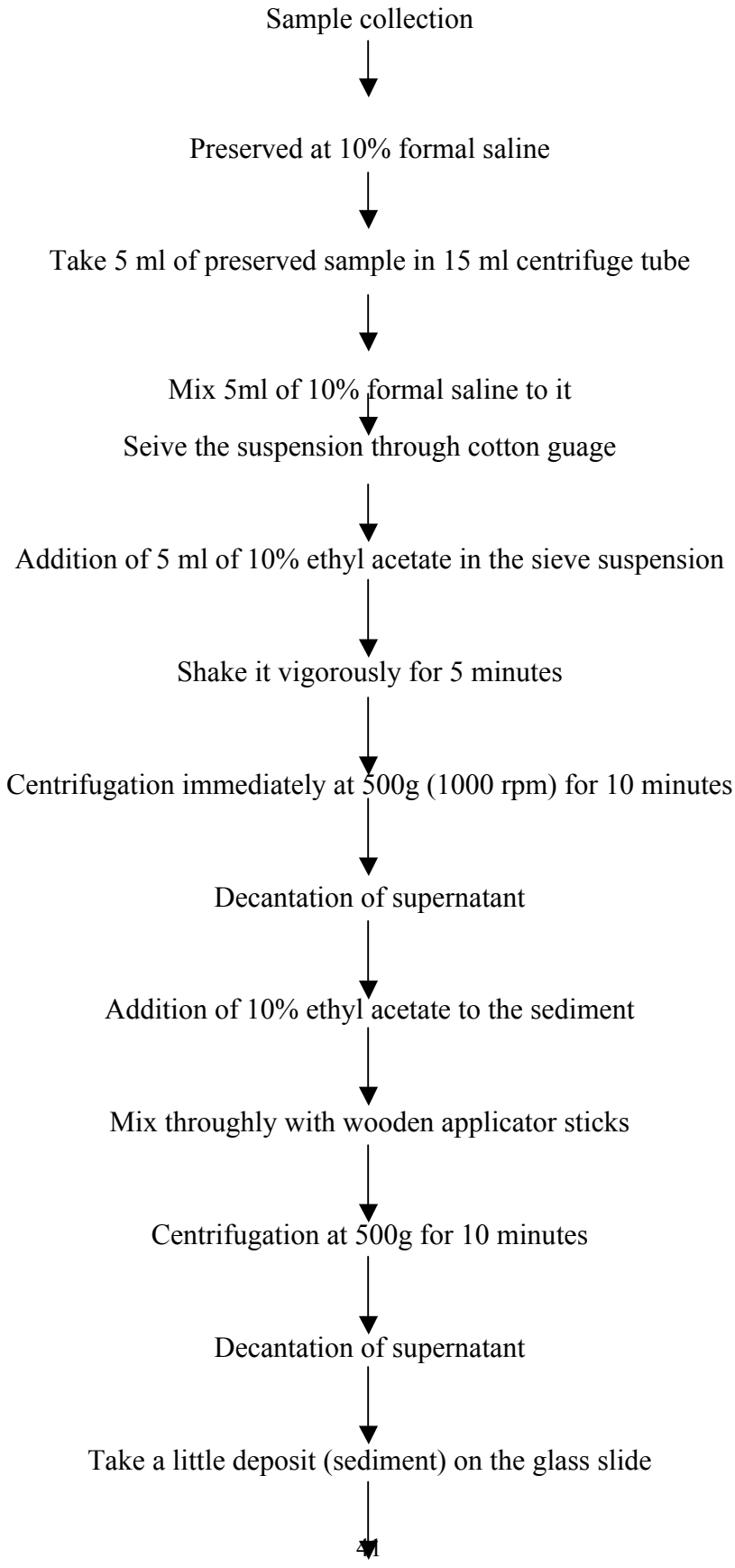
After laboratory processing of the stool sample, the result obtained was recorded in thesis log book. After then it was recorded in computer.

3.3 Report Distribution

After laboratory processing of the samples the result obtained was reported and the report was distributed to the patients. The patients 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.


Sample collection
Preserved at 10% formal saline
Take 5 ml of preserved sample in 15 ml centrifuge tube
Mix 5ml of 10% formal saline to it
Seive the suspension through cotton guage
Addition of 5 ml of 10% ethyl acetate in the sieve suspension
Shake it vigorously for 5 minutes
Centrifugation immediately at 500g (1000 rpm) for 10 minutes
Decantation of supernatant
Addition of 10% ethyl acetate to the sediment
Mix throughly with wooden applicator sticks
Centrifugation at 500g for 10 minutes
Decantation of supernatant
Take a little deposit (sediment) on the glass slide

Examination of slide by saline wet mount

Figure 3.1: Flow chart of Formalin- ether sedimentation technique

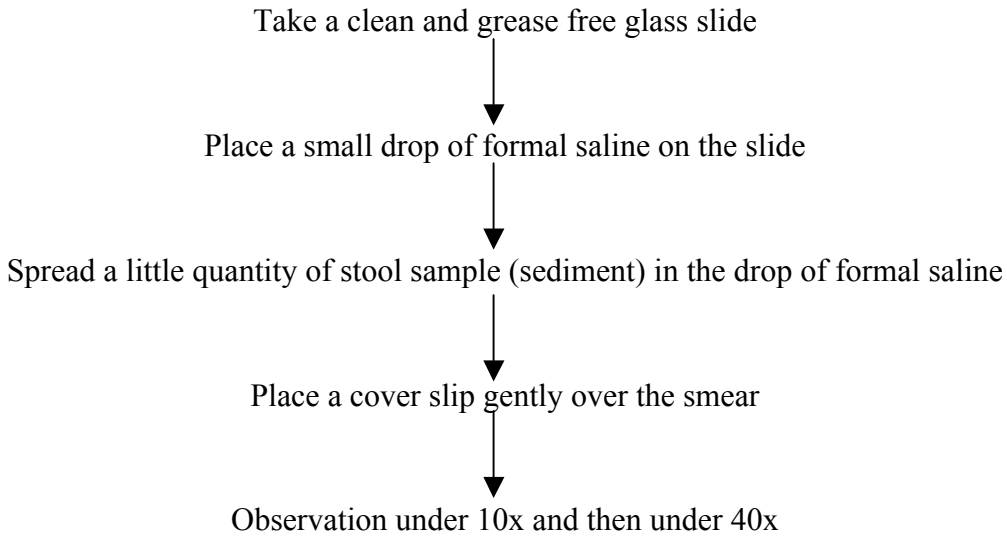


Figure 3.2: Flow chart of Saline wet mount technique

CHAPTER IV

RESULTS

Parasitosis among TB and HIV/AIDS was carried out by the examination of 81 stool samples. Altogether 54 samples were found to be positive for parasites.

4.1 Study Population

In this study, all together 81stool samples were examined. Among 81patients 50(61.72%)males were females and31(38.27%) were females. This result shows the participation of male patients was comparatively higher than female patients.

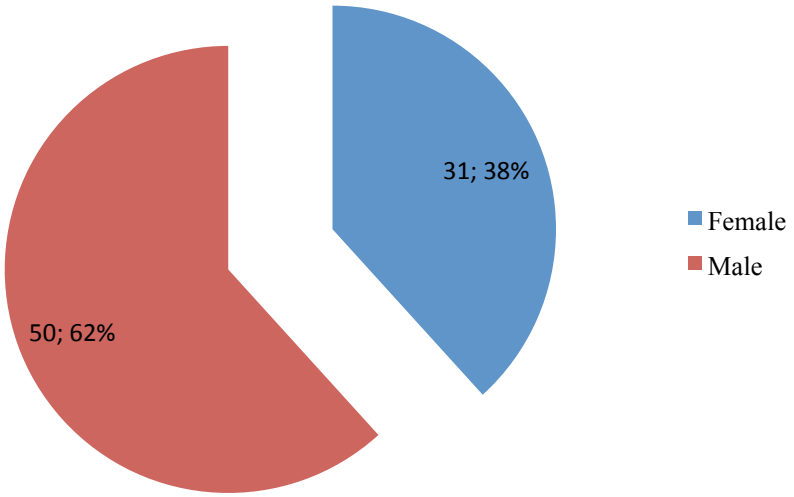


Figure: 4.1: Population under Study

4.2 Distribution of Students on the Basis of Age and Gender

On the basis of age, the highest number of patients who participated in this survey was of 30- 40years. In this age group, the total patients were 30 and among them 19 were males and 11 were females. The lowest number of patients who participated in this survey was below 10 years **(Figure 4.2)**

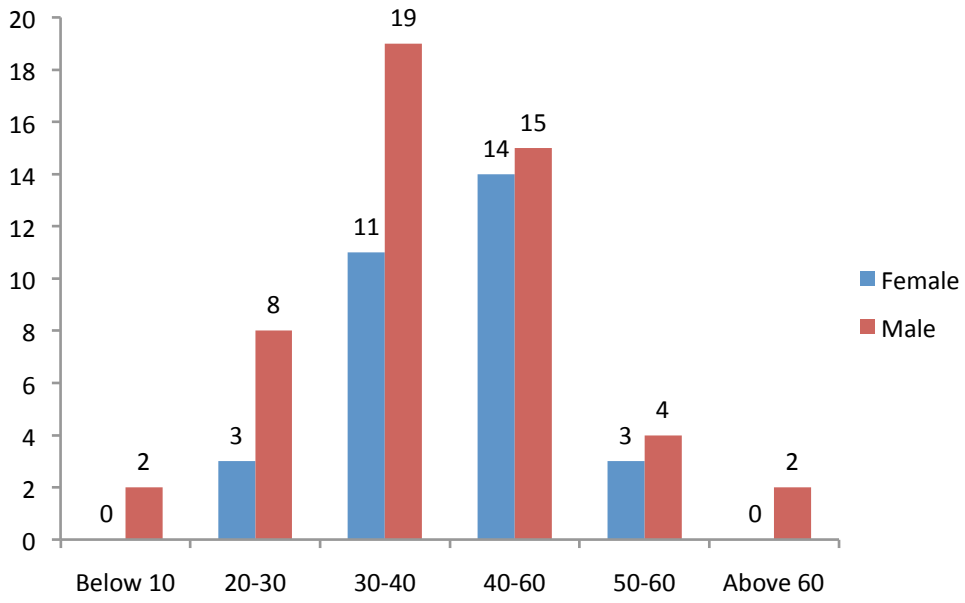


Fig. 4.2: Age and Gender wise distribution of population

4.3 Detection of Parasites

Out of 81 stool samples, 54 stool samples were found to be positive, 50 (92.59%) was protozoan parasites and 15 (27.78%) *G. lambia*, 12(22.22%) *E. histolytica*, 12(22.22%) *Cryptosporidium pavum*, 6(11.11%) *Isospora beli* 4(7.40%) *Microsprodium*, 1(1.85%) *Blastocytisis hominis*, 2(3.70%) *Tenia* spp, 2(3.70%) Hookworm among 54 positive stool samples was detected**.**

4.4 Giardiasis according to gender and type of patients

Prevelence of *G. lambia* was high in male (12.34%) patients then in female (7.40%) patients (**Table4.1a**). *G. lambia* was found 12(14.81%) in HIV and 4(4.93%) in TB patients (**Table4.1b**)

Table 4.1a: Giardiasis according to gender and type of patients

Patient	Parasitosis	Sex		Total	p- Value
		F	M		
HIV	Absent	15	26	41	0.369
	Present	5	7	12	
TB	Absent	10	14	24	
	Present	1	3	4	
Total		31	50	81	

Table 4.1b: Giardiasis according to type of patients

Parasitosis	Patient		Total	p- Value
	HIV	TB		
Absent	41	24	65	0.94
Present	12	4	16	
Total	53	28	81	

4.5 Cryptosporodiosis according to gender and type of patients

Prevelence of **Cryptosporodium** pavum was high in female8(9.8%%) patients then in male 4(4.9%) patients (**Table4.2a**). Cryptosporodium pavum was found 8(9.8%) in HIV and 4(4.93%) in TB patients (**Table 4.2b**)

Table 4.2a Cryptosporodiosis according to gender

Patient	Parasitosis	Sex		Total	p - Value
		F	M		
HIV	Absent	15	30	45	.031
	Present	5	3	8	
TB	Absent	8	16	24	
	Present	3	1	4	
Total		31	50	81	

Table 4.2b Cryptosporodiosis according to types of patients

Parasitosis	Patient		Total	p - Value
	HIV	TB		
Absent	45	24	69	.922
Present	8	4	12	
Total	53	28	81	

4.6 Prevalance of Isopora beli according to gender and type of patients

Prevalance of *Isopora beli* was high in male10(12.34%) patients then in female 2(2.46%)patients (**Table4.3a**). *Isopora beli* was found 6(7.40%) in HIV and was not found in TB patients (**Table4.3b**)

Table 4.3a Prevalance of Isopora beli according to gender

Patient	Parasitosis	Sex		Total	p - Value
		F	M		
HIV	Absent	19	28	47	0.922
	Present	1	5	6	
TB	Absent	8	16	24	
	Present	3	1	4	
Total	Absent	30	45	75	
	Present	1	5	6	
Total		31	50	81	

Table 4.3b Prevalance of Isopora beli according to types of patients

Parasitosis	Patient		Total	p-Value
	HIV	TB		
Absent	47	28	75	.064
Present	6	0	6	
Total	53	28	81	

4.7 Prevalance *Microsporidium* according to gender and type of patients

Prevalance of *Microsporidium* was high in male3(3.70%) patients then in male 1(1.23%) patients (**Table4.4a**). *Microsporidium* was found 3(3.70%) in HIV and 1(1.23%) in TB patients (**Table4.4b).**

Table 4.4a Prevalance *Microsporidium* according to gender

Patient	Parasitosis	Sex		Total	p - Value
		F	M		
HIV	Absent	19	31	50	
	Present	1	2	3	
TB	Absent	11	16	27	
	Present	-	1	1	
Total		31	50	81	

Table 4.4b Prevalance *Microsporidium* according to types of patients.

Parasitosis	Patient		Total	p-Value
	HIV	TB		
Absent	50	27	77	0.680
Present	3	1	4	
Total	53	28	81	

4.8 Teniasis according to gender and type of patients

Prevalance of *Teniasis* was in 2(2.46%) male patients where as was not found in female patients (**Table4.5a**). *Teniasis* was found 1(1.23%) in HIV and 1(1.23%) in TB patients (**Table4.5b**)

Table 4.5a Teniasis according to gender.

Patient	Parasitosis	Sex		Total	p - Value
		F	M		
HIV	absent	20	32	52	.260
	present	0	1	1	
TB	absent	11	16	27	
	present	0	1	1	
Total	absent	31	48	79	
	present	0	2	2	
Total		31	50	81	

Table 4.5b Teniasis according to types of patients

Parasitosis	Patient		Total	p-Value
	HIV	TB		
Absent	52	27	79	.642
present	1	1	2	
Total	53	28	81	

4.9 Prevalance of Hookworm according to gender and type of patients

Prevalance of **Hookworm** was 2(2.46%) in male patients and was not found in female patients (**Table4.6a**). **Hookworm** was found 2(2.46%) in HIV and was not fonnd in TB patients (**Table4.6b**)

Table 4.6a prevalance of Hookworm according to gender

Patient	Parasitosis	Sex		Total	
		F	M		
HIV	Absent	20	31	51	.260
	Present	0	2	2	
TB	Absent	11	17	28	
Total		31	50	81	

Table 4.6b prevalance of Hookworm according to types of patients

Parasitosis	Patient		Total	p- Value
	HIV	TB		
Absent	51	28	79	0.298
Present	2	0	2	
Total	53	28	81	

4.10 Prevalance *E. histolytica* of according to gender and type of patients

Prevalance of *E. histolytica* was high in male11(13.58%) patients then in female 1(1.23%)patients (**Table4.7a**). *E. histolytica* was found 8(9.87%) in HIV and 4(4.93%) in TB patients (**Table4.7b**)

Table 4.7a Prevalance *E. histolytica* of according to gender.

Patient	Parasitosis	Sex		Total	p- Value
		F	M		
HIV	Absent	20	25	45	.021
	Present	0	8	8	
TB	Absent	10	14	24	
	Present	1	3	4	
Total		31	50	81	

Table 4.7b Prevalance *E. histolytica* of according to patients

Parasitosis	Patient		Total	p - Value
	HIV	TB		
Absent	45	24	69	.922
Present	8	4	12	
Total	53	28	81	

4.11 Prevalance of *Blastocytis hominis* according to gender and type of patients

Blastocytis hominis was found 1(1.23%) in female patients and was not found in male (**Table4.8a**). ***Blastocytis hominis***was found 1(1.23%) in HIV and was not found in TB patients (**Table4.8b**)

Table 4.8a Prevalance of *Blastoc ytis hominis* according to gender.

Patient	Parasitosis	Sex		Total	
		F	M		
HIV	Absent	19	33	52	
	Present	1	0	1	.201
TB	Absent	11	17	28	
Total		31	50	81	

Table 4.8b: *Blastocytis hominis* infections according to Patients

Parasitosis	**Patient**		**Total**	**p- Value**
	HIV	**TB**		
Absent	52	28	80	.465
Present	1	0	1	
Total	53	28	81	

CHAPTER V

DISCUSSION

The overall prevalence of the parasitic infections was found 35.7% (26.7% among HIV seropositive subjects and 47.6% among high-risk group population). However, the prevalence of parasitic infections among HIV/AIDS subjects ranges from 18.4% to 76.2% in different parts of the world (Awole *et al*, 2003; Gomez Morales *et al*, 1995; Guk *et al*, 2005; Hunter *et al*, 1992; Modjarrad *et al*, 2005; Mohandas *et al*, 2002; Okodua *et al*, 2003; Tamiro *et al*, 1996; Zali *et al*, 2004). In Nepal, the prevalence of the parasitic infections in such population was found 30% (Sapkota *et al*, 2004). The finding of the present study was in agreement with the findings of Guk *et al* (2005), Modjarrad *et al* (2005), Mohandas *et al* (2002), Okodua *et al* (2002), Sapkota *et al* (2004) and Tamiro *et al* (1996) and lower than the findings of Awole *et al* (2002), Gomez Morales *et al* (1995) and Hunter *et al* (1992).

The finding was lower than in comparison to those studies. In comparison, the lower prevalence of intestinal parasitic infections among HIV/AIDS subjects could be correlated to the relatively high level of immune status among the study population, and the fact that diarrhoea and intestinal parasitic infections were strongly associated with lower CD_4 counts (Brink *et al*, 2002). Moreover, only one sample was taken from each patient, and it had been shown that second specimes yield more pathogen detections. In addition, incorporation of more sophisticated methods of diagnosis including jejunal fluid examination and biopsy increase the total yield of pathogens (Mukhopadhya *et al*, 1999). However, the immune status of the study population was not elucidated. The finding was higher in comparison to the finding of Zali *et al* (2004) in Iran. In addition to impaired defense mechanism in HIV/AIDS patients itself, poor hygiene and poverty might be important factors behind the burden of gastrointestinal infection in Nepalese HIV/AIDS patients. The increase in rainfall, temperature and relative humidity are contributing factors that help in rapid contamination and transmission of organisms in large dosages. Several other parameters including lack of health education, illiteracy, unsafe drinking

water, lack of sanitation and hygienic knowledge, undernutrition, overcrowding, superstition, widespread faecal contamination of the environment etc. are associated with risk of diarrhoea (WHO Report on Diarrhoeal Diseases, 1994).

The reported prevalence of intestinal parasitosis in Nepal varied considerably from one study to another (Ishiyama *et al*, 2001; Gianotti, 1990; Nepal and Palfy, 1980; Ono *et al*, 2001; Rai and Gurung, 1986; Rai *et al*, 1995, 2001, 2002; Reily, 1980; Sherchand *et al*, 1996; Takemasa *et al*, 2004) with nearly 100% in some rural areas (Estevez *et al*, 1983; Nepal and Palfy, 1980; Rai and Gurung, 1986; Reily, 1980). Most of the studies were focused on school children rather than the adult population eventhough, Sherchand *et al* (1996), reported higher prevalence of the parasitic infections among adults with abdominal discomfort. Developing countries face economical, sociological and medical burdens due to the increase of rural migrants to urban areas with deterioration of already poor general public health services. In this context overcrowding and insufficient health care promote the spread of intestinal parasites (Crompton and Savioli, 1993).

Present study revealed the prevalence of parasitic infections as 25.4% and 28.6% among male and female population respectively in case of HIV/AIDS subjects and 46.5% and 48.8% among male and female respectively in case of high-risk group population. Though the result showed the higher prevalence among female population, it was not significant statistically. The finding of the present study was in agreement with the findings of Hailemariam *et al* (2004), Okodua *et al* (2003) and Sapkota *et al* (2004). Similarly, the higher prevalence of the parasitic infections were seen in the age group of 20-39 years among male and above 40 years among female population in case of HIV/AIDS subjects respectively, though the result was not significant statistically. The result was in agreement with the findings of Awole *et al* (2003), Cruz *et al* (1996), Hailemariam *et al* (2004), Okodua *et al* (2003) and Sapkota *et al* (2004). In Nepal, around 80% of the total HIV positive are in the age group of 20-39 years (NCASC, 2006). The factors considered as major contributors for rapid spread of HIV and other infections in the country include poverty and the mobility of this economically productive age group

for the search of temporary jobs and low level of awareness on HIV/AIDS and the parasitic infections. This study included those HIV seropositive individuals who had the prior history of injecting drug use, commercial sex workers, clients of commercial sex workers and the housewives. Majority of the sample population were of this age group, so the prevalence was also found higher. The prevalence of parasitic infections among female was higher in the age group above 40 years. This unusual finding could be due to the low number of the sample from this age group.

In both the study population, the prevalence of parasitic infections were higher among the *Tibeto-Burmans* group, though the result was not significant statistically. The ethnicwise distribution of parasitic infections among the HIV/AIDS individuals in Nepal have not assessed yet so the findings of the present study was not able to compare with other findings. However, conflicting results had been reported by many investigators among HIV seronegative individuals in Nepal (Ishiyama *et al*, 2001; Rai *et al*, 2002; Rai *et al*, 2005; Sharma *et al*, 2004).

The prevalence of the multiple parasitic infections were found to be higher among the HIV/AIDS subjects in comparison to the high risk group population though the result was not significant statistically. Similar results had been reported by Awole *et al* (2003), Cotte *et al* (1993), Feitosa *et al* (2001) and Hailemariam *et al* (2004). The strong evidence had not been suggested but it indicated the facilitated establishment of parasites in immunocompromised patients (Hailemariam *et al*, 2004). Some reports from Nepal (Estevez *et al*, 1983; Rai *et al*, 2001) and elsewhere in the world (Kasuya *et al*, 1989; Rajeswari *et al*, 1994) had shown high levels of multiple parasitic infections. This was a clear indication of large numbers of various species of parasites in the local community (Sharma *et al*, 2004).

Present study found the prevalence of 20.5%, 4.4% and 1.8% helminthic, protozoal and mixed infections respectively among HIV/AIDS individuals. This study revealed that helminthic infections were more common than the protozoal infections. Similar results had been reported elsewhere in the world (Awole *et al*, 2003; Fontanet *et al*, 2000; Hailemariam *et al*, 2004; Kaminasky

et al, 2004; Modjarrad *et al*, 2005; Okodua *et al*, 2003; Wiwanitkit *et al*, 2001). The finding of the present study was not in agreement with the findings of most investigators who reported the highest prevalence of the protozoal infections among HIV/AIDS patients (Arinas-Pinto *et al*, 2003; Guk *et al*, 2003; Mohandas *et al*, 2002; Prasad *et al*, 2000; Sapkota *et al*, 2004; Zali *et al*, 2004). In Nepal, the annual rate of the soil transmitted helminthiasis ranged from 18% to 36.6%. The annual incidence decreased every successive calendar year in both adults and children, irrespective of sex (Rai *et al*, 1994). Due to the contamination of environment (soil/water), over 60% Nepalese are infected with some kinds of intestinal parasites (Rai, 2005). All helminths stimulate strong immune responses (Andreassen**,** 1997; Ishikawa *et al*, 1998; Loukas and Prociv, 2001; Malhotra *et al*, 1997; Soboslay *et al*, 1997; van Dam *et al*, 1996; White *et al*, 1997). Although these responses are useful for diagnosing infection, they frequently appear not to be protective. Moreover, damage also occurs indirectly as a result of the host defense mechanisms (Jusot *et al*, 1996; Stadecker *et al*, 1998). Immune-mediated inflammatory changes occur in the skin, lungs, liver, intestine, central nervous system, and eyes as worms migrate through these organs. Systemic changes such as eosinophilia, edema, and joint pain reflect local allergic responses to the parasites. The fact that many worms are extremely long-lived means that many inflammatory changes become irreversible, producing functional changes in tissues. All helminths release relatively large amounts of antigenic materials, and this voluminous production may divert immune responses or even locally exhaust the immune potential.

Present study revealed the lower prevalence of ascariasis in comparison to the other studies (Hailemariam *et al*, 2004; Kaminsky *et al*, 2004; Modjarrad *et al*, 2005; Okodua *et al*, 2003). The result was in agreement with the finding of Awole *et al* (2003) from Southwestern Ethiopia and that reported by Rai (2005), from Nepal. In Nepal, the prevalence of such soil transmitted helminthics is decreasing day by day with the increase in the level of awareness among the people who used to dwell in the urban areas. The present study had collected most of the sample from rehabilitation centre of the Kathmandu Valley. The HIV infected people living in such places had high

level of awareness as they were used to visit by the clinician once a week. They had better sanitary conditions in comparison to the other parts of the country. This study had collected single stool specimen for the investigation. This might be the reason of low prevalence. The geographical variation of the parasites might be another factor for the lower prevalence.

This study revealed the prevalence of 3.6% and 29.7% of hookworm infection among HIV seropositive subjects and high-risk group population respectively. The reported prevalence of hookworm infection among HIV/AIDS individuals in Nepal and elsewhere in the world varied from 0.8 to 39.2% (Awole *et al*, 2003; Hailemariam *et al*, 2004; Kaminsky *et al*, 2004; Modjarrad *et al*, 2005; Mohandas *et al*, 2002; Okodua *et al*, 2003; Sapkota *et al*, 2004). Hookworm is one of the commonest soil transmitted helminthes in Nepal. Rai (2005), reported the prevalence of 7% to over 80% in certain communities of Nepal. In Southern Nepal, the incidence ranges 11% to 29%. In Kathmandu Valley it ranged from 7% to 57.6%. Highest prevalence had been observed in hilly populations.

The finding of this study was in agreement with the finding of Hailemariam *et al* (2004) from Ethiopia and Sapkota *et al* (2004) from Nepal. Previous report did not show any interaction between HIV and helminthic infections (Awole *et al*, 2003). However the newly emerging relationship of helminthic infections to HIV and its potential impact on HIV progression, especially in countries where co-infection with HIV is a growing reality justify efforts for strengthening such programs and increasing measures to prevent and remedy conditions that foster transmission of parasitic infections (Kaminsky *et al*, 2004). The prevalence rate among the high risk group population was greater than the HIV infected population. This reflected the marked geographical variation, low level of awareness, provision of poor sanitary condition and high mobility (highway truckers and prostitutes) of the study population. As the sample had been collected from the rural area of Nepal, the health service provided by the government was not so satisfactory and this might be one factor for the higher prevalence.

This study revealed the prevalence of 2.7% and 1.2% of *E. histolytica* infection among HIV seropositive and high risk group population respectively. The reported prevalence of *E. histolytica* among HIV/AIDS individuals in Nepal and elsewhere in the world varied from 1.7 to 11.5% (Awole *et al*, 2003; Esfandiari *et al*, 1995; Hailemariam *et al*, 2004; Mohandas *et al*, 2002; Okodua *et al*, 2003; Prasad *et al*, 2000; Sapkota *et al*, 2004). The finding was in agreement with the finding of Mohandas *et al* (2002) from Northern India. It was somewhat lower in comparison with the previous study done by Sapkota *et al* (2004), in Nepal. The lower prevalence might be due to the study of single stool specimen and the use of traditional method of detecting cyst and trophozoite of the parasite. Incorporation of more sophisticated methods of diagnosis including jejunal fluid examination and biopsy increase the total yield of the pathogen (Mukhopadhya *et al*, 1999). Although *E. histolytica* was not classified under opportunistic parasites, it was significant compared to CD_4 counts (Sandraei *et al*, 2005). Similar result had been reported from Argentina and Brazil (Mendez *et al*, 1994; Moura *et al*, 1989). The protozoan infections such as *G. lamblia* and *E. histolytica* have not been found to be opportunistic in HIV infected patients because there is no evidence for an increased prevalence of these parasitic infections in HIV patients despite the fact that important immune defenses against them may be expected to be deranged by HIV infection. Hence, exposure to *G. lamblia* and *E. histolytica* are likely to occur independently of HIV infection, but heavier parasite loads may accumulate as well as experience delayed clearance of the parasite in individuals with concurrent HIV induced immunosuppression (Awole *et al*, 2003). However, in Nepal, Rai (2005), reported that *E histolytica* ranks second among the intestinal protozoan parasites. The reported incidence of intestinal amoebic infections ranged from less than 3 to 28.8%. In a hospital based study the year to year incidence ranged from 1.9% to 14.6%. Serological study revealed 24.6% of Nepalese have anti-amoebic antibody.

This study showed the prevalence of *Cryptosporidium* infection as 1.8% among HIV seropositive subjects whereas there was no infection among high risk group population. The reported prevalence of cryptosporidiosis among HIV/AIDS individuals in Nepal and elsewhere in the world varied from 1.2 to

11% (Awole *et al*, 2003; Ghimire *et al*, 2004; Guk *et al*, 2005; Mohandas *et al*, 2003; Okodua *et al*, 2003; Prasad *et al*, 2000; Ratnam *et al*, 1985; Sapkota *et al*, 2004; Uga *et al*, 1998; Zali *et al*, 2004). The incidence of *Cryptosporidium* in Nepal ranged from less than 1.0 to 10.4% in diarrhoeal stool samples (Dhakal *et al*, 2004; Rai, 2005; Sherchand *et al*, 1996). In a community in Southern Nepal, *Cryptosporidium* oocysts had been found in 4.4% (Rai, 2005).

The finding of the present study was in agreement with the finding of Zali *et al* (2004). The prevalence was lower in comparison with the previous study done by Dhakal *et al* (2004), Ghimire *et al* (2004), Sapkota *et al* (2004) and Sherchand *et al* (1996) from Nepal. The diarrhogenic cases in this study were very few and present study had examined the single stool specimen from the study population. This might be the reason of low prevalence. Moreover, oocyst excretion is usually low and variable hence multiple examinations and prior concentrations of stool specimens might be necessary (Awole *et al*, 2003). *C. parvum* infection alone had been associated with low CD_4 counts (Brink *et al*, 2002). This study had not collected the patients' CD_4 profile and include HIV positive rather than whether they developed the late sequlae or not. However, the number of AIDS patients with the complaint of diarrhoea were lower than in other studies done elsewhere in the world (Awole *et al*, 2003; Ghimire *et al*, 2004; Guk *et al*, 2005; Mohandas *et al*, 2003; Okodua *et al*, 2003; Prasad *et al*, 2000; Ratnam *et al*, 1985; Sapkota *et al*, 2004; Uga *et al*, 1998; Zali *et al*, 2004). The higher detection rate in other studies might be due to the use of highly sensitive methods of diagnosis.

Present study have found the prevalence of *G. lamblia* as 1.8% among HIV seropositive individuals and nil among high risk group population. The reported prevalence of giardiasis varied from 1.5 to 55% in Nepal and elsewhere in the world (Awole *et al*, 2003; Cotte *et al*, 1993; Guk *et al*, 2005; Hailemariam *et al*, 2004; Mohandas *et al*, 2002; Okodua *et al*, 2003; Sapkota *et al*, 2004; Zali *et al*, 2004). The finding was in agreement with the finding of Awole *et al* (2003) from Southwestern Ethiopia and Guk *et al* (2005) from South Korea but lower in comparison with studies in different other countries.

It did not occur in greater frequencies in HIV positive patients than in HIV negative individuals (Mohandas *et al*, 2002). Reports from other countries demonstrated that the prevalence of these pathogens was not influenced by moderate reduction of cell-mediated immunity (Brandonosio *et al*, 1999; Gomez Morales *et al*, 1995). However, Feitosa *et al* (2001), reported that giardiasis had been found in patients with immunological dysfunction other than HIV infection.

In Nepal, Rai (2005), reported G. lamblia as commonest intestinal protozoan parasite in Nepal. Symptomatic infections were mainly associated with diarrhoea and steatorrhoea. As shown by the literature published from Nepal during 1979 to 1995, *G. lamblia* constitutes most common intestinal protozoa infecting man. Similar finding was observed in a ten years study conducted in hospital population with a year-to-year incidence ranging from 5.2% to 26.4%. In terai area, incidence of *G. lamblia* was less than 14.0% whereas in hilly areas, it was less than 10.0%. In Kathmandu Valley, the incidence had been reported to be as high as 28.8%.

Present study found the prevalence of *B. hominis* as 0.9% among HIV seropositive individuals and nil among high-risk group population. The reported prevalence elsewhere in the world varies from 2.1 to 14.1% (Awole *et al*, 2003; Hailemariam *et al*, 2004; Mohandas *et al*, 2002; Prasad *et al*, 2000; Zali *et al*, 2004). Few reports from Nepal had reported *B. hominis*. The reported incidence ranged from less than 1.0% to 24.9%. Recently, in a remote hilly region in Far-western Region, it was found to be 1.3%. The prevalence of *B. hominis* in Kathmandu Valley in patients with diarrhoea and school attending children were 2.0% and 7.9% respectively. However, one report showed no association between *B. hominis* infection and diarrhoea in travelers (Rai, 2005).

Present study had found a lower prevalence of *B. hominis* in comparison with studies in other countries. In Nepal, no previous reports were found to assess the prevalence of *B. hominis* among HIV/AIDS individuals. However, the finding was in agreement with the overall prevalence as reported by Rai (2005) from Nepal. *Blastocystis* spp. was found to be significantly higher in

HIV/AIDS patients than in the control population (Hailemariam *et al*, 2004). Conflicting evidence exists as to whether *B. hominis* should also be considered as a significant cause of AIDS associated diarrhoea (Albrecht *et al*, 1995). Usually, the presence of more than 5 parasites/HPF of stool specimen was used as a criterion for positive reporting. Although the role of *B. hominis* as an emerging pathogen remains unresolved, the number of parasites excreted in stool might possibly be an indicator of its pathogenic role in HIV related diarrhoea (Prasad *et al*, 2000).

Present study had found the prevalence of *H. nana* as 0.9 % among HIV seropositive individuals and nil among high risk group population. Similar finding was reported by Awole *et al* (2003) from Southwestern Ethiopia. *H. nana* is a commonest tapeworm infecting man in Nepal. It was reported to be less than five percent; 3.3% in Southern Nepal and 4.9% in school children in Kathmandu Valley. Also a ten years study conducted in hospital populations had shown an incidence of less than five percent throughout the study period except in the very first year. Eggs of *H. nana* and *H. diminuta* had also been found in soil samples studied in Kathmandu. Very rarely, despite of therapeutic intervention, persistent hymenolepiasis for over three years had also been observed (Rai, 2005).

CHAPTER VI

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The intestinal parasites were detected from HIV and TB patients by microscopic examination and formalin-ether concentration method. The parasites identified were *G. lamblia*, *E. histolytica, Cryptosporidium* p*avum, hookworm, Tenia spp isosporo beli, Microsporidium and Blastocystis hominis*. Most of the parasites are opportunistic among immunocompramised patients...It may be concluded that in Nepalese HIV and TB infected patients, both intestinal helminthic and protozoal parasitic infections are still highly prevalent. Similarly, the higher prevalence of multiple parasitic infections among such population indicates the severe conditions. The high prevalence of helminthic parasites among such population indicates the adequate treatment and health education, provision of adequate toilet facilities and pipe borne water so that the continually contaminating the environment with ova and larvae of parasite would be greatly reduced. Finally, in the management of HIV and TB infected patients in Nepal with or without diarrhoeal symptoms, stool examination is still a useful investigation. There was no significant difference between age, sex, religious, ethnicity, bowels, syndrome, educational level of parents, family size and family type of patients and the prevalence of intestinal parasitic infection.

6.2 Recommendation

To improve the intestinal parasitic infections among HIV and TB, the following recommendation can be taken out.

1. It is recommended to include sensitive diagnostic tools for the diagnosis, as there may be prevalence of more opportunistic parasites as well.

2. It is to recommended the TB patients to take anti- tuberculosis drugs regularly and anti parasitic-drugs who had shown the prevalence of parasites.

3. Our data support the value of standard fecal examination in HIV as well as TB-infected patients even in the absence of diarrhoea so follows the regular health check-up and maintain the personal health hygiene.

4. The intestinal parasites in HIV and in TB infected persons lead to increase in morbidity and mortality of such individual. So, it is strongly recommended to follow the control measures such as appropriate education, deworming programme, incorporation of poverty alleviation techniques and effective sanitation and supply of clear water.

5. It is recommended for HIV infected subjects to go further diagnosis and take anti-parasitic drugs regularly as prescribed by physician as well as monitor their CD4 counts regularly.

6. *G. lamblia* was detected in high frequency among other parasites therefore, proper course of drug should be administered.

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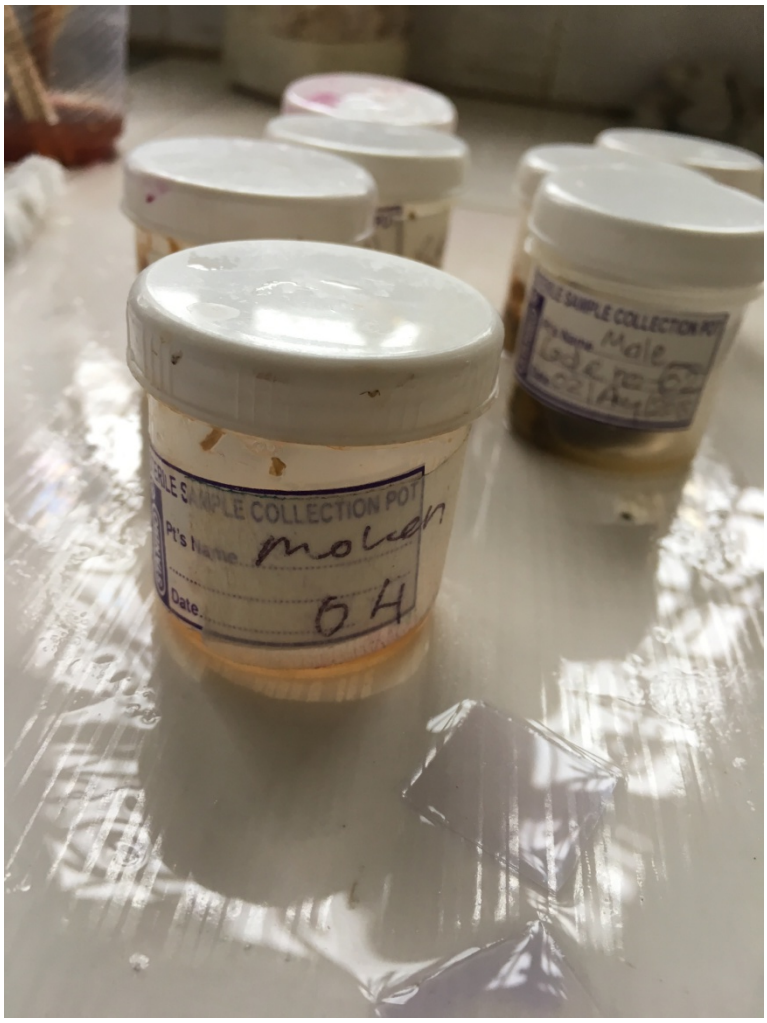
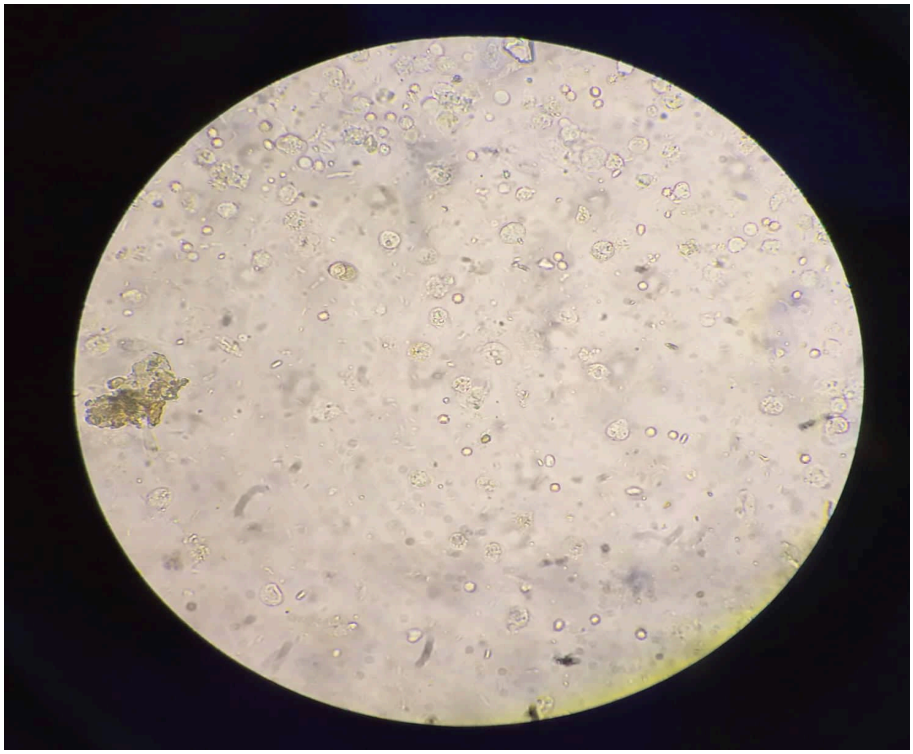
PHOTOGALLERY



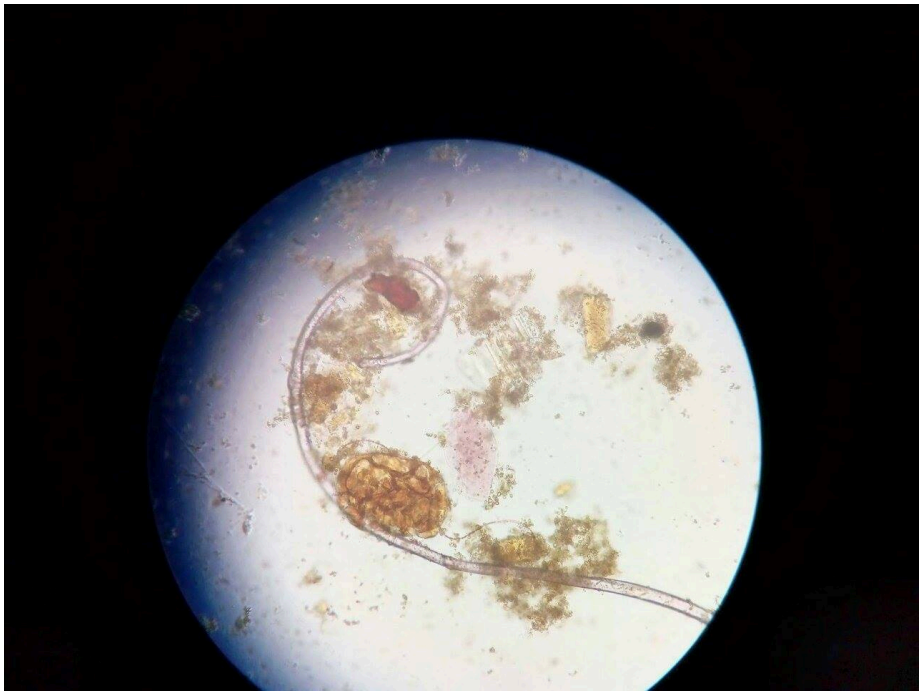
Photograph 1: Stool Samples Collected from patients

Photograph 2: Preparing Smear of Stool Samples

Photograph 3: Microscopic Examination of Stool Samples



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



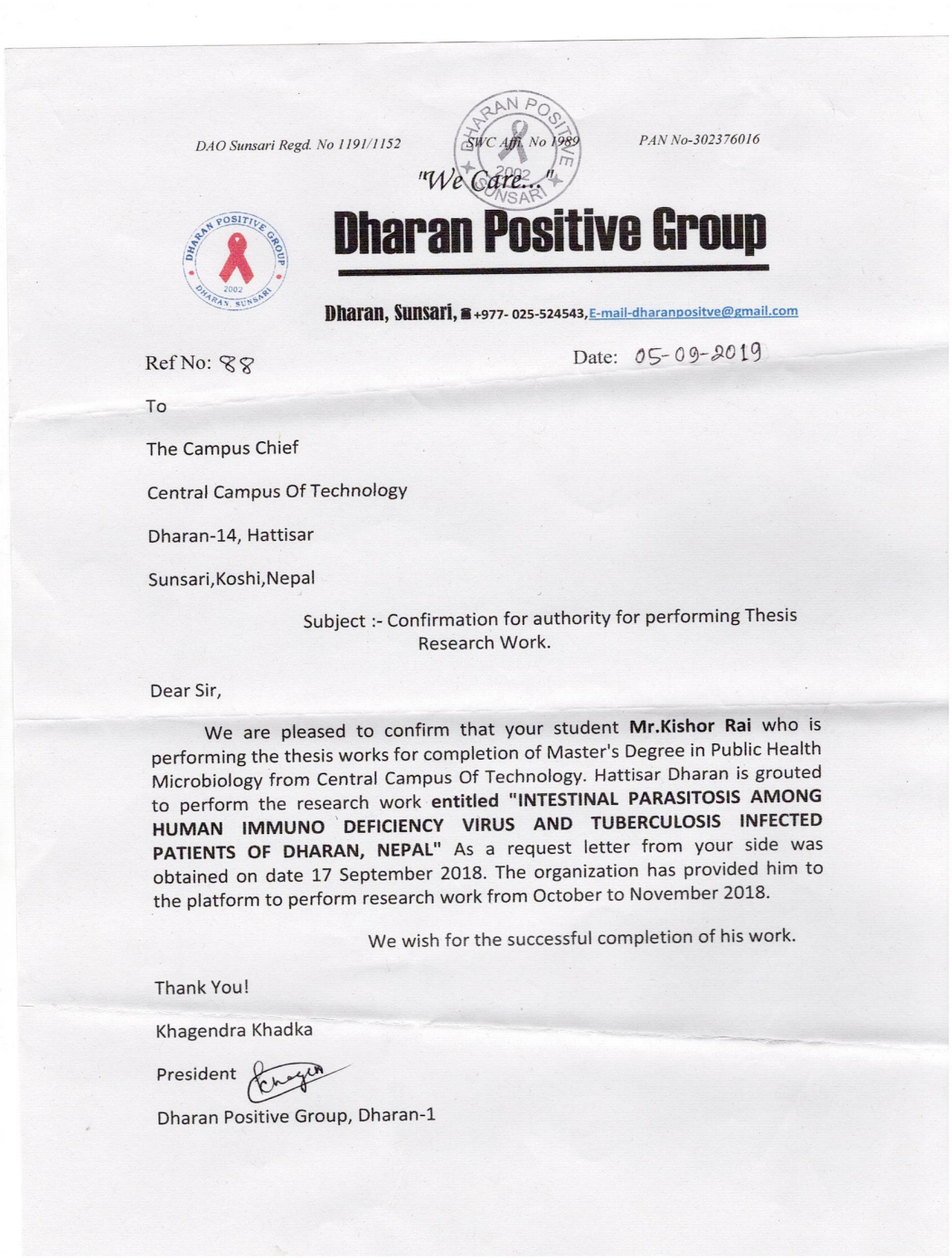
Photograph 5: An Ova of *Ascarislumbricoides* (Saline wet mount 40x)

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

APPENDICES

APPENDIX-A

(Conformation Letter from Dharan Positive Group, Dharan)



DAO Sunsari Regd. No 1191/1152 SWC Affi. No 1989 PAN No-302376016

"We Care..."

Dharan Positive Group

Dharan, Sunsari, ☎ +977- 025-524543, E-mail-dharanpositve@gmail.com

Ref No: ৪৪ Date: 05-09-2019

To

The Campus Chief

Central Campus Of Technology

Dharan-14, Hattisar

Sunsari,Koshi,Nepal

Subject :- Confirmation for authority for performing Thesis Research Work.

Dear Sir,

We are pleased to confirm that your student **Mr.Kishor Rai** who is performing the thesis works for completion of Master's Degree in Public Health Microbiology from Central Campus Of Technology. Hattisar Dharan is grouted to perform the research work **entitled "INTESTINAL PARASITOSIS AMONG HUMAN IMMUNO DEFICIENCY VIRUS AND TUBERCULOSIS INFECTED PATIENTS OF DHARAN, NEPAL"** As a request letter from your side was obtained on date 17 September 2018. The organization has provided him to the platform to perform research work from October to November 2018.

We wish for the successful completion of his work.

Thank You!

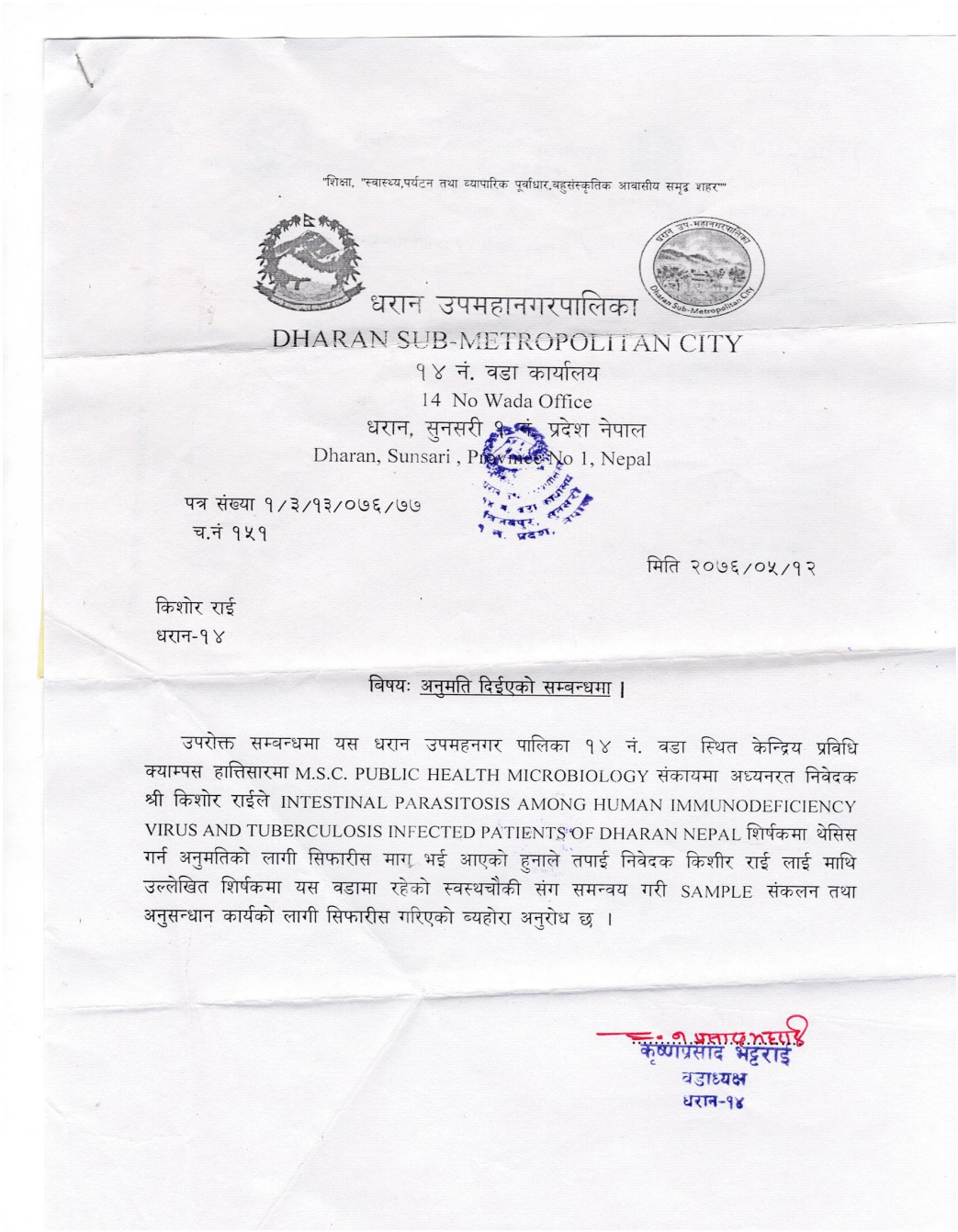
Khagendra Khadka

President

Dharan Positive Group, Dharan-1

APPENDIX -B

(Approval letter from Dharan sub-metropolitian ward- 14)



"शिक्षा, "स्वास्थ्य,पर्यटन तथा व्यापारिक पूर्वाधार,बहुसंस्कृतिक आवासीय समृद्ध शहर""

धरान उपमहानगरपालिका

DHARAN SUB-METROPOLITAN CITY

१४ नं. वडा कार्यालय

14 No Wada Office

धरान, सुनसरी २ नं. प्रदेश नेपाल

Dharan, Sunsari , Province No 1, Nepal

पत्र संख्या १/३/१३/०७६/७७

च.नं १५१

मिति २०७६/०५/१२

किशोर राई

धरान-१४

विषयः अनुमति दिईएको सम्बन्धमा ।

उपरोक्त सम्बन्धमा यस धरान उपमहनगर पालिका १४ नं. वडा स्थित केन्द्रिय प्रविधि क्याम्पस हात्तिसारमा M.S.C. PUBLIC HEALTH MICROBIOLOGY संकायमा अध्यनरत निवेदक श्री किशोर राईले INTESTINAL PARASITOSIS AMONG HUMAN IMMUNODEFICIENCY VIRUS AND TUBERCULOSIS INFECTED PATIENTS OF DHARAN NEPAL शिर्षकमा थेसिस गर्न अनुमतिको लागी सिफारीस माग भई आएको हुनाले तपाई निवेदक किशीर राई लाई माथि उल्लेखित शिर्षकमा यस वडामा रहेको स्वस्थचौकी संग समन्वय गरी SAMPLE संकलन तथा अनुसन्धान कार्यको लागी सिफारीस गरिएको व्यहोरा अनुरोध छ ।

कृष्णप्रसाद भट्टराई

वडाध्यक्ष

धरान-१४

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 Intermidate 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 Home made 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 hand regularly? Yes No
19. When do you wash your hand with soap and water?
    After work: Before eating: Both.

Health Status

1.Are you taking any antibiotics? Yes No

2. Do you have regular health check up? Yes No

3. Do you have any health complication? Yes No

APPENDIX E

SPSS Output

Frequency Table

Sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	F	31	38.3	38.3	38.3
	M	50	61.7	61.7	100.0
	Total	81	100.0	100.0	

G. lamblia

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	absent	65	80.2	80.2	80.2
	present	16	19.8	19.8	100.0
	Total	81	100.0	100.0	

E.histolytia

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	absent	69	85.2	85.2	85.2
	present	12	14.8	14.8	100.0
	Total	81	100.0	100.0	

cryptosporodium

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	absent	69	85.2	85.2	85.2
	present	12	14.8	14.8	100.0
	Total	81	100.0	100.0	

Isopora beli

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	absent	75	92.6	92.6	92.6
	present	6	7.4	7.4	100.0
	Total	81	100.0	100.0	

cryptosporodium * Sex * Patient

Patient			Sex		Total
			F	M	
HIV	cryptosporodium	absent	15	30	45
		present	5	3	8
	Total		20	33	53
TB	cryptosporodium	absent	8	16	24
		present	3	1	4
	Total		11	17	28
Total	cryptosporodium	absent	23	46	69
		present	8	4	12
	Total		31	50	81

Chi-Square Tests

Patient		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
HIV	Pearson Chi-Square	2.459[c]	1	.117		
	Continuity Correction[b]	1.375	1	.241		
	Likelihood Ratio	2.381	1	.123		
	Fisher's Exact Test				.137	.122
	N of Valid Cases	53				
TB	Pearson Chi-Square	2.496[d]	1	.114		
	Continuity Correction[b]	1.054	1	.305		
	Likelihood Ratio	2.469	1	.116		
	Fisher's Exact Test				.269	.153
	N of Valid Cases	28				
Total	Pearson Chi-Square	4.808[a]	1	.028		
	Continuity Correction[b]	3.500	1	.061		
	Likelihood Ratio	4.676	1	.031		
	Fisher's Exact Test				.050	.032
	N of Valid Cases	81				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.59.
b. Computed only for a 2x2 table
c. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 3.02.
d. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.57.

Isopora beli * Sex * Patient

Crosstab

Count

Patient			Sex		Total
			F	M	
HIV	Isopora beli	absent	19	28	47
		present	1	5	6
	Total		20	33	53
TB	Isopora beli	absent	11	17	28
	Total		11	17	28
Total	Isopora beli	absent	30	45	75
		present	1	5	6
	Total		31	50	81

Chi-Square Tests

Patient		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
HIV	Pearson Chi-Square	1.278[c]	1	.258		
	Continuity Correction[b]	.467	1	.494		
	Likelihood Ratio	1.424	1	.233		
	Fisher's Exact Test				.390	.255
	N of Valid Cases	53				
TB	Pearson Chi-Square	.[d]				
	N of Valid Cases	28				
Total	Pearson Chi-Square	1.280[a]	1	.258		
	Continuity Correction[b]	.483	1	.487		
	Likelihood Ratio	1.433	1	.231		
	Fisher's Exact Test				.399	.251
	N of Valid Cases	81				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 2.30.

b. Computed only for a 2x2 table

c. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 2.26.

d. No statistics are computed because Isopora beli is a constant.

Microsporidium * Sex * Patient

Crosstab

Count

Patient			Sex		Total
			F	M	
HIV	Microsporidium	absent	19	31	50
		present	1	2	3
	Total		20	33	53
TB	Microsporidium	absent	11	16	27
		present	0	1	1
	Total		11	17	28
Total	Microsporidium	absent	30	47	77
		present	1	3	4
	Total		31	50	81

Chi-Square Tests

Patient		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
HIV	Pearson Chi-Square	.026[c]	1	.871		
	Continuity Correction[b]	.000	1	1.000		
	Likelihood Ratio	.027	1	.870		
	Fisher's Exact Test				1.000	.684
	N of Valid Cases	53				
TB	Pearson Chi-Square	.671[d]	1	.413		
	Continuity Correction[b]	.000	1	1.000		

	Likelihood Ratio	1.022	1	.312		
	Fisher's Exact Test				1.000	.607
	N of Valid Cases	28				
	Pearson Chi-Square	.314[a]	1	.575		
	Continuity Correction[b]	.001	1	.974		
Total	Likelihood Ratio	.332	1	.564		
	Fisher's Exact Test				1.000	.504
	N of Valid Cases	81				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.53.

b. Computed only for a 2x2 table

c. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.13.

d. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .39.