

CHAPTER ONE

INTRODUCTION

1.1 Background

Nepal is an agricultural country with poor economy. Agriculture contributes 38% of the nation's Gross Domestic Product (GDP) and livestock contributing almost 11% to GDP (World Bank, 2002). Livestock population in Nepal for 2004/2005 was estimated to be 6.99 M cattle, 4.08 M buffaloes, 7.15 M goats and 0.86 M sheep. Roughly 70% of households keep some type of livestock, including cows, buffaloes, pigs and chickens (FAO, 2005). Mainly farmers in rural areas supplement income by the sale of plant and animal product.

Cattle generally referred to as cow (*Bos sp.*) are domesticated ungulates a member of sub family bovine of the family bovidae. They are raised as livestock for meat, dairy products, leather and draft animals.

The cow is the national animal of Nepal. It is domesticated since long years ago. Hindu people worship cow in the form of Laxmi, the goddess of wealth. It is also the symbol of strength, abundance, selfless giving, and a full earthy life. Many Hindu feel that at least it was economically wise to keep cattle for their nourishing milk rather than consume their meat for single use (Botany and Bartoli,1990). The cow is the best giver of plenty. Not only did she give milk but also made agriculture possible. She is the second mother to millions of mankind. Protection and care ness of cow means protection of whole silent creation of God.

1.2 Status of Cattle

Cattle today are they basis of multibillion dollar industry world wide. The international trade in beef for 2000 was over \$30 billion and represented only 23% of world beef production (Clay, 2004). The production of milk which is also made into cheese, butter, yogurt and other dairy products take an important part of food supply for many of the world's people. Cattle hides used for lather to make shoes and clothing. Cattle remain broadly used as draft animals (pulling carts, ploughing field) in many developing countries such as Nepal and India.

Cattle when kept on a large numbers together may act as contributing factor in the rise in green house gas emission. A 400 page United Nations report from the Food and Agricultural Organization (FAO) shows that cattle farming are responsible for 18% of green house gases.

For the first time Carolous Linnaeus identified 3 species of cattle. These were *Bos taurus*, the European cattle including similar type from Asia and Africa, *Bos indicus*, the zebu and the extinct *Bos primigemius* the auroaches. Nowadays these Three have been grouped as one species (They, 2003).

Locally in our community three species viz; local, Jersey and Holstein has been reared since long. Livestock farming is an integral part of Nepalese Agriculture in National economy. Among these cattle farming covers maximum percentage of livestock farming.

Diseases not only decrease the production but also may lead to the death of cattle. Helminthic diseases are must occurring diseases to cause economic loss in livestock farming. Manly trematodes, cestods and nematodes are mostly found helminth parasites in cattle

Zoonotic diseases are diseases that can be transmitted from animals to humans and from humans to animals. Zoonotic diseases may be acquired or spread in a variety of ways: through the air (aerosol), by direct contact, by contact with an inanimate object that harbors the disease (fomite transmission), by oral ingestion, and by insect transmission. There are fifteen cattle diseases with zoonotic potential in the United States, some of which are more common than others. They include anthrax, brucellosis, cryptosporidiosis, dermatophilosis, *Escherichia coli*, giardiasis, leptospirosis, listeriosis, pseudocowpox, Q fever, rabies, ringworm, salmonellosis, tuberculosis, and vesicular stomatitis.

1.3 Parasites and Parasitism

Parasitology is an essential branch of biology, ecology, economic zoology, and medical zoology, providing us about structure & lifecycle of parasites, etiology, epidemiology, origin of infection & transmission, risk factors, economic importance,

pathogenesis, clinical findings, and pathological diagnosis even treatments and controls that can be applied in our daily life.

Parasitism is a heterogenic association between organisms of which the parasite lives and feeds in or on the body of other comparatively larger partner called host. The host provides nourishment and shelter to the parasite. Parasitism can also be considered as an ecological between two populations of same or different species. The parasites are biologically, economically, ecologically, physiologically & metabolically closely connected with their hosts throughout their lifespan where the host resists by producing antibodies and become adopted or tolerated to the parasitic presence. The word parasite has been derived from the Greek words (Gr. Para - besides, situs – food) meaning eating in addition to food (Parija,1990).

Parasites are classified as endoparasites and ectoparasites on the basis where they live inside or on the body of host. Endoparasites are those organisms living within their host in gut, liver, body cavity, blood. Such forms nearly always exhibit a complete parasitic existence and they totally depend upon their host for food and shelter. Presence of endoparasites also referred to as infection. For example *Fasciola*, *Taenia*, are typical of endoparasites. Infection with gastrointestinal helminthes is referred as one of the important factor causing productivity loss (Shretha, 1994). These helminth parasites comprise trematodes, cestodes and nematodes.

The most important and widely prevalent nematodes are *Ostertagia* sp., *Trichostrongylus* sp., *Cooperia* sp., *Oesophagostomum* sp. etc. These nematodes in the small intestine may cause severe damage to the intestinal mucosal membrane.

Toxocara sp. and *Dictyocaulus* sp. have the world wide distribution and the prevalence is higher in cattle and buffaloes (Karki, 2005).

Trichostrongylias is an infection of the gastrointestinal tract of herbivorous animals and man is the accidental host caused by the species of *Trichostrongylus*. Infection is acquired by the ingestion of contaminated vegetables or drinks with the third stage larvae. Strongyloidiasis is an intestinal infection of man caused by the penetration of the skin by the filariform larvae of *Strongyloid stercoralis*.

Toxocariasis in human is widely distributed throughout the world in both temperate and tropical countries. Man acquires infection by the ingestion of larvae of this nematode present in the inadequately cooked food of paratenic host. (Williams, 1999)

Haemonchus is another important nematode parasite found in the abomasums of various ruminants. It causes severe blood and protein loss into abomasums and intestine due to damage caused by the parasite and often results in edema in the submandibular region.

Ostertagia occurs in the abomasums of goat, sheep, cattle etc. the infection with this parasite the functional gastric gland mass and large area of gastric mucosa may be affected. *Cooperia* is relatively small worm found in the small intestine, rarely in the abomasums of ruminants.

Regarding gastrointestinal round worms of cattle *Trichostrongylus* sp., hookworm *Ascaris* sp., *Strongyloide* sp., *Toxocara* sp., *Chabertia* sp. are the common occurrence. Female roundworm lays microscopic eggs that pass along with faeces of cattle. Within a few days larvae hatch from the eggs. The larvae pass via different stages for maturation. They infect the pasture and cattle become infected when grazes on contaminated pasture. In the intestine of cattle the nematode parasites become mature.

The pathogenic effect of gastrointestinal parasites may be sub clinical or clinical. The effects of these parasites mainly depend on the number of parasites and the nutritional status of the animal they are infecting. The clinical symptoms are weight loss, reduced food intake, diarrhea, loss of production etc and even death.

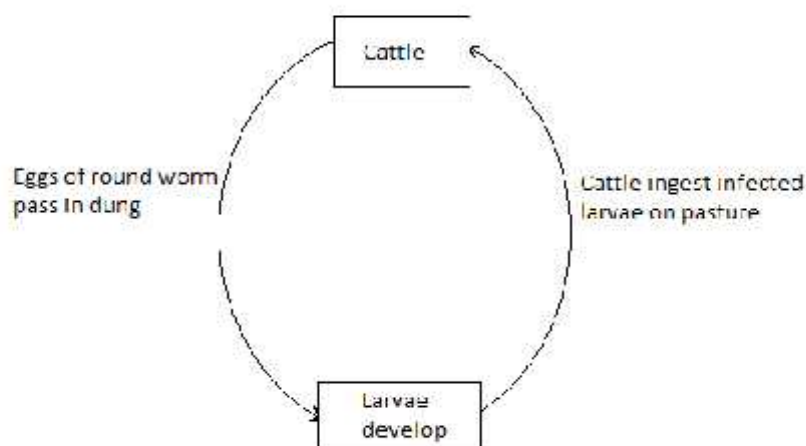


Figure 1: Lifecycle of gastrointestinal roundworm.

Cestodes found in gut are acquired by eating contaminated food or water found to be largely affecting the ruminants. This group comprises the genera *Moniezia* sp. which is cosmopolitan in distribution and *Taenia* sp. which is commonly found in the rumen of the domesticated and wild carnivores. They are reported from Asia and Africa (Karki, 2005).

Moniezia sp. infection in ruminants of cattle is caused by ingesting herbage containing mites carrying the infective stage of the parasite. Heavy infection cause poor growth and diarrhoea in lambs.

Taenia saginata usually called cow or buffalo tapeworm. It has two hosts viz; definitive host man and intermediate host cattle. It is also called beef tapeworm. The segments of worm pass out along with the faeces of the human being and when ingested by cattle, infect them on reaching alimentary canal of the host. The eggs hatch out and liberated, penetrate the gut wall and enter mesenteric lymphatics and finally reaches to the circulation. They invade muscular tissue and undergo further development.

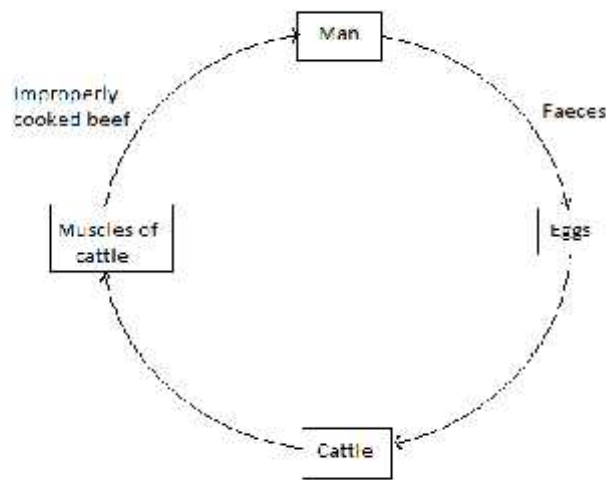


Figure 2: Lifecycle of *Taenia saginata*.

Trematodes commonly known as flukes often live in the bile duct or small intestine and may also affect the lungs. Their eggs are passed with the faeces of the host. Some are ingested but some burrow the skin after hatching for access. They especially

include *Fasciola* sp., *Schistosoma* sp. and *Paramphistomum* sp. (Shah and Agrawal, 1990)

Fasciola sp. is a well known parasite of herbivorous animal. It has worldwide distribution. A large variety of animals such as cattle, buffaloes show infection rate that varies from 70% - 90% in some areas. The different local names of this disease viz; namle, mate, lew etc. in different regions are proof of its continued existence for many years in animal population of the country.

Infection of domestic ruminants with *Fasciola hepatica* and *Fasciola gigantica* causes significant loss estimated at over US \$ 2000 million per year to agriculture sector worldwide with over 600 million animals affected (Hansen, 1994). In Nepal the economic loss due to fascialosis was estimated to be Rs 14.2 cororer (Lohani and Rasaili, 1995).

Fasciola hepatica and *Fasciola gigantica* inhabit similarly in the bile ducts of final host (cattle). *Fasciola* completes its lifecycle in two different hosts, snail (*Lymnea* sp., *Planorbis* sp.or *Bulinus* sp.) as an intermediate host and cattle as definitive host. The parasite eggs are expelled with the bile into the intestine and shed in the faeces. Eggs hatch into a free swimming ciliated miracidium larva which after active swimming penetrates the suitable intermediate host, snail by its boring apical organ. In snail larvae pass its sporocyst, radia and cercaria stages. Mature cercariae are shed out and are transformed into metacercariae a more resistant stage. Metecercaria is attached to the grass. Cattle acquire infection by ingestion of metacercaria stage of parasite through grazing. In the intestine young liver fluke are liberated and they reach to the liver via peritoneal cavity or through blood stream.

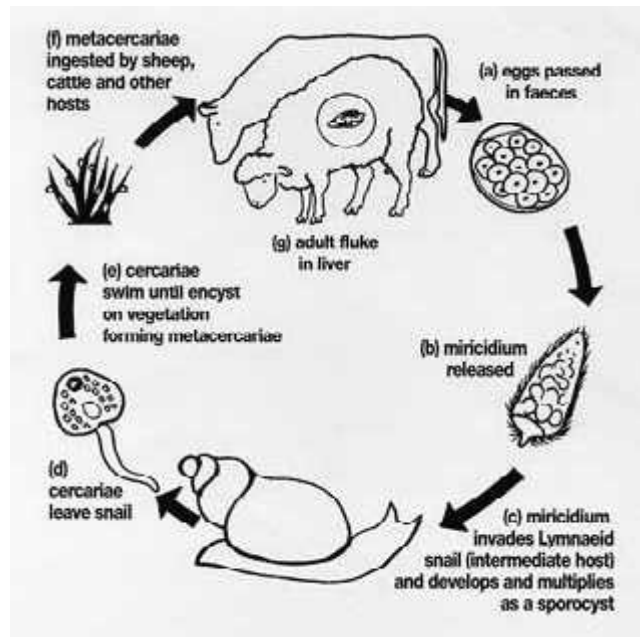


Figure 3: Lifecycle of *F. hepatica*.

The important species of snail involved in the transmission of fascioliasis vary in their geographical distribution in the world. Man and herbivorous animals (cattle) acquire infection by the ingestion of moist and raw aquatic plants harboring infective metacercariae.

Previous study at Lumle Agricultural Research Center (LARC) has identified the diseases prevalence mainly affected by the availability of khet and paddy cultivation and permanent water sources rather than altitude (Joshi, 1988) rice straw which is the major feed for livestock during winter months has been reported as the potential source of infection for fascioliasis (Joshi, 1987 and Mahato, 1993). Green grasses from near permanent water sources or water lodging area in monsoon are another potential source for *Fasciola* infection. Therefore the in Nepalese hills the major risk period of *Fasciola* infection is during post monsoon and winter months.

Dicrocoelium sp. was first reported by Mukhia in buffaloes in Nepal where prevalence rate was found 29.61%.

Schistosoma species are the only trematodes that live in the blood stream of warm blooded hosts. *Schistosoma* sp. causes schistosomiasis. The infections often manifest by acute intestinal signs, the mucosa of the intestine is severely damaged and the

animal develops profuse bloody diarrhoea, dehydration loss of appetite not only cattle over 200 million people are infected in at least 75 countries with 500 million or more people exposed to infection (Arcari, 2000). Most of the species like *Schistosoma spindalia*, *S. japonicum* and *S. bovis* has been reported among buffaloes from Surkhet district (Ghimire, 1987). In Satungal Kathmandu prevalence rate of *Schistosoma* sp. was found about 9% (Mukhia, 2007). Similarly in Anarmani Jhapa prevalence rate of *Schistosoma* sp. was found about 19.09% (Dhakal, 2009).

Table 1: Parasites and their hosts with location of infection.

S.N	Parasite	Definitive host	Location
a	<i>Trichostrongylus</i> sp.	Cattle, sheep, goat, man, pig & horse	Small intestine, abomasum & omasum
b	<i>Schistosoma</i> sp.	Cattle, buffalo & goat	Mesenteric veins
c	<i>Dipylidium</i> sp.	Man, cattle, dog & cat	Small intestine
d	<i>Toxocara vitulorum</i>	Buffalo, cattle, calves	Small intestine
e	<i>Fasciola</i> sp.	Sheep, goat, cattle, dog, cat & man	Liver, lungs, bile duct & kidney

Source : Chandler, ASA.C.

1.4 Significance of the Study

Cattle in our country is one of the way of having financial resource of people living therein especially in villages the infection of helminth parasites in cattle can cause significant economic loss leading to the poor health, reduced growth and fertility, reduction in production and death of animal. This study is an effort to identify and determine the seasonal prevalence of helminth parasite in cattle.

Nepal being a developing country depends on agriculture and animal husbandry for its bulk of economy. However the farming technique is rather un scientific. A cattle farming is also done in poor and unhygienic manner and hence is heavily infected

with different types of parasites including helminth parasites. In these circumstances this type of study plays a vital role.

This study will also help as a roadmap for future researchers & investigators and aware people about the helminthic diseases of cattle and their harmful aspects. The next steps to be considered are the most practical economic way by which goal can be achieved. Thus the study has got a great importance and significant in itself.

1.5 Limitations of the Study

This study was carried out to determine the prevalence of helminth parasites seasonally giving focus to the *Fasciola* sp. and also for the partial fulfillment of the requirement for Master's Degree in Zoology at Tribhuvan University. Due to the time and cost factors the minimum numbers of samples within two seasons only were taken for study so that future researchers can elaborate this study by approaching the untouched portion.

CHAPTER TWO

OBJECTIVES

Helminth infection in cattle directly or indirectly hampers livestock and its product. So, proposed study deals on the helminth parasites of cattle causing economic loss to the farmers.

2.1 General Objective

To determine the prevalence of intestinal helminth parasites of cattle.

2.2 Specific Objectives

- i. To identify the helminth parasites up to genus level.
- ii. To determine the prevalence of trematodes, cestodes and nematodes.
- iii. To determine the helminth parasites in two seasons viz; rainy and winter.
- iv. To develop the recommendation for further planning regarding to the control of helminth parasites in cattle emphasizing *Fasciola* sp.

2.3 Hypothesis

H₀: There is no significant difference in prevalence of two species of *Fasciola* in rainy and winter season.

H₁: There is significant difference in prevalence of two species of *Fasciola* in rainy and winter season

CHAPTER THREE

LITERATURE REVIEW

Before 17th century, knowledge of parasitology was limited to ectoparasites like lice & flies and few internal parasites like roundworms, pinworms and tapeworms

Linnaeus gave another view about these internal parasites that they originated from accidentally swallowed free living organisms. However this belief was erased in the later half of 17th century by Francisco Redei, the grandfather of parasitology. He demonstrated development of maggots from eggs of flies. He also proved that *Ascaris* had males and females and produced eggs. At the same time, Leeuwenhoek perfected microscope and discovered protozoan parasites.

Parasitic zoonoses are distributed worldwide and constitute an important group of diseases affecting both the human and animals. Many of the parasitic zoonoses produce significant mortality and morbidity in human and are responsible for the major economic loss by affecting the animal health. Most of the papers have been presented and published largely after the outbreak of helminthic diseases among human and animals (Pandey, 1998). Literature exists in helminth parasites as the disease continued to survive with new threats. Major research efforts that have been directed towards helminthic parasites, the portions of the works and reports related to the epidemiology of helminth parasites have been mentioned here.

3.1 Global Context.

In 1758, Linnaeus first reported the genus *Ascaris*.

In 1819, Rudolphi was first to report *Dicrocoelium*.

In 1851, Bilharz was the first to demonstrate the adult worm of *Schistosoma* in mesenteric veins of a man in Cairo.

In 1876, Newis and Mc. Connell were the first to describe trematode *Amphistom* from the caecum of an Indian patient.

In 1888, Claus reported about platyhelminthes.

In 1907, Sambom first pointed out that the egg belongs to a new separate species and named it as *Schistosoma mansoni*.

In 1917, Bhasker first reported *Taenia* sp. infection in man from India in the emigrant populations of Nagapathnam and Dindigul in Tamilnadu. Subsequently study was based on the routine microscopic examination of the faeces.

In 1926, Chandler initially recognized *schistosoma* sp. as a parasite of man and claimed the eggs of this parasite from human faeces.

In 1952, Beaner *et al.*, first recognized human *Toxocariasis* who found the larva of *Toxocara canis* the dog roundworm, in the liver and lung tissues of three children in New Orleans (USA).

Balmire *et al.*, (1970) conducted a study to assess the contamination rate for fascioliasis in adult cattle in England and Wales ranged between 22% and 35%. It then fell steadily from 20% in 1970, to 6% in 1978 (Balmire *et al.*, 1980). In 1978, the rate of fascioliasis in England and Wales was 5.6% (Maff, 1979) and in 1980 was 6.5% (Maff, 1980).

Lamenta and Manuel (1981) carried out a study on 330 cattle in Metro Manila abattoirs, Philippines where *Fasciola* sp. was found in 3.64%. *F. gigantica* was recovered in 3.03% of the 330 cattle. *F. hepatica* was found only in mixed infection with *F. gigantica* in 0.60% of cattle.

Baglapedia (1988) investigated the prevalence of *Schistosoma spindalis* and *S. indicum* among cattle all over Bangladesh. Mostly, adult cattle above 3 years of age were severely affected up to 25%. *S. nasalis* was widespread among cattle and buffaloes all over the country. Its occurrence was very high (60%) and was very common in the Southern districts of Bangladesh.

Anwar and Gill (1990) reported prevalence of schistosomes of veterinary importance in Pakistan and India. A total of 20,000 examined animals from different localities of Panjab province of Pakistan, 13% cattle and buffaloes infected with *S. indicum* and *S. spindalis*.

WHO (1993) reported *Schistosoma* sp. in about 25% of cow, water buffalo, dog and pig in Philippines.

WHO (1993) carried out a study in China where 40 species of wild and domestic animals have been found naturally infected with *S. japonicum*. The main animal reservoirs are cattle, buffalo, pig and dogs.

Iassan *et al.*, (2000) conducted a study on prevalence dynamics of fascioliasis versus other gastrointestinal helminthes in both buffaloes and cattle in Eliza Governorate. They collected 1042 buffaloes and cattle faecal samples. Their coprological examination revealed that 16.46% of the examined buffaloes and 10.35% cattle respectively were harbouring *Fasciola* sp. With the help of faecal test they found 2.07% of the examined animals have *Fasciola* sp. and 2.5% helminth eggs in their faeces. The helminthes included mainly other Gastro-intestinal parasites such as *Paramphistomum* sp. and *Moniezia* sp. Monthly and seasonal prevalence of parasites investigated the spring season was the most favorable one for infection with predominant one for infection.

Lezeriuc *et al.*, (2002) conducted a study in between 1995 and 2001. 28,878 cattle were slaughtered in their abattoir at Baean, Romania. During this period 2,220 cattle were diagnosed with fascioliasis and 5,120 cattle with dicrocoeliasis. The prevalence of parasitic infection in cattle was higher in 2001 compared to 1995, especially in the case of bovine fascioliasis which increased from 4.0-14% and bovine dicrocoeliasis which increase from 3.8-37.1%.

Mondal *et al.*, (2002) conducted a study of gastro-intestinal helminthes in livestock grazing in grassland of Bangladesh. They released two cow calves and two goats in a grassland used for communal grazing of livestock. After slaughtering of the tracer animals, their gastrointestinal examination revealed six species of trematode and one cestode. The nematode species were *Haemonchus controtus*, *Trichostrongylus axei*, *Oesophagostomum* sp., *Trichuris* sp. and *Bunostomum* sp. The cestode was one of the genuses *Moniezia*. With this study, grasslands are thought to be one of the main sources of gastrointestinal parasitic diseases of livestock in Bangladesh.

Yadav *et al.*, (2003) reported the prevalence rate of fascioliasis high in terai region of India followed by hills and plains respectively. Buffaloes were the most susceptible host followed by cattle and sheep.

Bory (2003) investigated the prevalence of liverfluke in buffaloes and beef cattle slaughtered at VISSAN. The infection in buffaloes and beef cattle were 14.83% and 22.92% respectively. *F. gigantica* infect both buffaloes and cattle where *F. hepatica* infect only buffaloes.

Basu *et al.*, (2003) reported *Fasciola hepatica* and *F. gigantica* to be the major parasites involved and causing economic losses to livestock in E. Africa. *F. hepatica* has shown to be the most important fluke species in Ethiopian livestock with distribution over 3 quarters of the nations except in the arid north-east and east of the country. The distribution of *F. gigantica* was mainly localized the western humid zone of the country. The prevalence of bovine fascioliasis has shown range from 11.05% to 81%. A rough estimate of the economic loss due to fascioliasis in bovine is about 3560 million billion per year.

Swai *et al.*, (2004) surveyed the prevalence of gastro-intestinal (GI) parasites in grazing cattle in pastoral farming area during the period of march 2004. Data were gathered from 17 herds/farms with a total of 90 cattle in five wards of Ngorongoro district. Trematode infections were found in 56.60% of the cattle. Most farms (94%) had trematode infection. Trematode infections were influenced by level of tick infections and the location of farm.

Yadav *et al.*, (2005) reported the highest incidence of gastro intestinal nematodiasis in goats followed by buffalo and cattle in India. *Haemonchus* sp., *Trichostrongylus* sp., *Bunostomum* sp., *Oesophagostomum* sp. and *Strongyloides* sp. were the main parasites recovered from the intestine of sheep, goats and buffaloes.

Muraleedharan (2005) observed the gastro intestinal parasites of livestock in a central dry zone of Karnataka, India. He reported the prevalence of gastrointestinal parasites in cattle (18.22%), buffaloes (20.85%), sheep (39.34%) and goats (46.12%) of southern taluks of central dry zone of Karnataka during drought period. *Strongyloides* were the most common nematodes *Fasciola*, *Amphistomes*, *Moniezia*, and *Entamoeba*

infections were low among livestock but *Fasciola* infection was not seen in sheep. Ova of *Gongylonema* were recorded from one cattle and *Strongyloides* were recorded only in sheep. Low incidence of *Trichuris* infection was noticed in cattle, sheep and goats. *Strongyloides* in cattle was found higher during south west monsoon.

Regessa *et al.*, (2006) conducted a study to determine the prevalence and risk factors associated with gastro intestinal parasitism in western Oroma, Ethiopia during 2004-2005. A total of 757 ruminants (257 cattle, 255 sheep and 245 goats) were included in the study using standard coprological parasitological procedure. The study showed that the overall prevalence of gastro intestinal parasites was 69.60% with 50.20%, 75.30%, and 84.10% in cattle sheep and goats respectively. *Strongyloides* and *Eimeria* were most common parasites encountered in the area. Season and age were shown to have associated with prevalence.

Nath (2007) pointed haemato-biochemical changes in cattle with paramphistomiasis. In the investigation the alternations in the haemato-biochemical parameters in spontaneous cases of paramphistomiasis in cattle were studied.

Fahrion *et al.*, (2008) reported patent *Toxocara canis* infections in previously exposed and in helminth free dogs after infection with low numbers of embryonated eggs and shows the prevalence rate of 18.18%.

Benavides *et al.*, (2008) conducted a study on *Anoplocephala perfoliata* in the Northwest of Colombia. 105 horses, 2 ass and 28 mules were sampled for the study. a total of 31.7% animals resulted positive. The coprological result showed significant difference ($P < 0.05$) between species with a high prevalence in equids of species *Equus caballus* (22.96%) followed by hybrid (*Equus caballus* × *Asinus asinus*) (8.15%) and none in *Asinus asinus*.

Mendes *et al.*, (2008) reported development of *Fasciola hepatica* in *Lymnaea collumella* infected with miracidia derived from cattle and marmoset infection. *Fasciola hepatica* released in cattle faeces were significantly bigger than those released in marmoset faeces.

Moses *et al.*, (2008) reported the presence of *Histophilus somni* in Nigerian dairy cattle. They showed infection in Holstein-friesian dairy cattle with prevalence of 40% and 50% respectively.

Thanh *et al.*, (2008) studied human fascioliasis and the presence of hybrid forms of *Fasciola hepatica* and *Fasciola gigantica* in Vietnam. The two species of common liver fluke *F. hepatica* and *F. gigantica* cause human fascioliasis. Hybrid between these species and introgressed forms of *Fasciola* are known from temperate and subtropical regions of eastern Asia.

Muhammad *et al.*, (2009) conducted a study on prevalence of *Fasciola* for a period of one year. Of the 4800 faecal samples analyzed, 25.46% were found positive for fascioliasis. The occurrence of *Fasciola gigantica* (22.40%) was higher than *F. hepatica* (3.06%). The highest month wise prevalence was found in winter (39.08%) followed in decreasing order by spring (29.50%), autumn (20.33%) and summer (12.92%) species wise prevalence of fascioliasis was found higher in buffaloes (30.50%) than in cattle (20.42%).

Hamed *et al.*, (2009) studied monthly sample of *Bulinus truncates* during a year from a cattle breeding farm located in the region of Sejname, North Tunisia. Of 763 bulinids 39% were infected with *F. hepatica*. Peak prevalence was found at June and October.

Yabe *et al.*, (2009) investigated the interactions among *F. gigantica*, *Schistosoma* sp. and *Amphistomum* species. Concurrent natural infection in zambian cattle based on egg worm counts in the abattoir 315 cattle were screened for worms a total of 32% harboured all three trematodes 66% had *F. gigantica* and *Amphistomum* sp. Interaction, 52% had *Schistosoma* sp. and *Amphistomum* sp. while 32% had *F. gigantica* and *Schistosoma* sp. A positive correlation ($p=0.014$) was found between *F. gigantica* and *Amphistomum* burden.

Kanyari *et al.*, (2010) studied prevalence of endoparasites in cattle with zoonotic potential within urban and peri-urban areas of Lake Victoria Basin, Kenya. The study was carried in six sites within Kisumu urban and peri-urban focused on the cattle parasites. Three different age groups were faecal sampled for determination of the

prevalence and infection levels of various helminth and protozoans especially those with zoonotic potential. Associations and correlations between different variables were determined. The prevalence of various parasite types was 51% *Strongyles*, 2% *Strongyloides papillosus*, 2% *Toxocara vitulorum*, 2% *Trichuris* species. Others were *Fasciola* 64.2%, *Paramphistomum* 31.3%, *Moniezia* species (16%) and *Entomoeba* 83%, *Coccidia* species 30%, *Giardia* species 14% and *Balantidium coli* 6.6%. The correlations in prevalence and intensity between age, breed, different parasite types and area of origin are discussed. *Strongyles* were the most common nematodes and especially among the under one year olds. Trematodes were significantly more frequent and intense in young stock and adults compared to the calves. Majority cattle had 2 to 3 parasite types while very few had none, single or multiple infections involving 4 parasite types.

Mekroud *et al.*, (2010) studied the prevalence and intensity of *Paramphistomum daubney* infection in cattle from North Eastern Algeria. 2,033 cattle were sampled over 14 month period from three Algerian slaughter houses. The prevalence of infection varied from different slaughter houses 1.2%, 7.5% and 12.1%. No significant correlation between prevalence, intensity of infection and age of cattle was noted.

Marc *et al.*, (2010) carried out a study on survey of gastro intestinal parasitic infections of beef cattle in regions under Mediteranean weather in Greece. A total of 262 samples were taken in 15 farms from three different regions of Greece within two years. Helminth eggs were detected in 42 (16%) samples. *Strongyle* type eggs were found in 28 (10.7%) samples, *Strongyloides* sp. and *Toxocara* sp. in 8 (3.1%) samples each and *Capillaria* sp. and *Moniezia* sp. eggs in 1 (0.4%) sample each.

Nasser and Khalifa (2010) investigated fascioliasis prevalence among animals and humans in Upper Egypt. 105 cows, 163 buffaloes and 29 sheep were sampled during study period. The overall prevalence was found 30.3% including 28.6% in cows, 33.7% in buffaloes and 17.2% in sheep.

Dornya *et al.*, (2011) conducted a study on Infections with gastrointestinal nematodes, *Fasciola* and *Paramphistomum* in cattle in Cambodia and their association with morbidity parameters and seasonal variations of helminth infections and their

association with morbidity parameters in traditionally reared Cambodian cattle. Four villages in two provinces of West Cambodia were visited on monthly intervals over a period of 11 months, during which 2391 animals were faecal and blood sampled for parasitological and haematological examinations. The overall proportion of samples that was positive for gastrointestinal nematodes was 52%, 44% and 37% in calves (from 1 to 6 months), young animals (6 to 24 months) and adults (over 24 months), respectively. The prevalences of *Fasciola* and *Paramphistomum* estimated by coprological examination, varied between 5-20% and 45-95% respectively.

3.2 Literature Review in Context of Nepal

Research work on parasitic diseases of farm livestock initiated during the decades of 1970 in Nepal. Surveys on common parasitic diseases were undertaken in Kathmandu valley and in few other districts representing hills, terai and high mountains (Singh *et al.*, 1973). This study determined the prevalence of parasitic diseases and carried out the identification of nematode parasites, snail species and some ectoparasites.

Inglis, and Ogden, (1935) worked on some *Strongyloides* (nematodes) from mammals in east Nepal with some other parasitic nematodes.

Malakar (1965) reported about some helminth parasites from domestic animals. These were *Fasciola* from cow and goat.

Ghimire (1987) conducted a study on incidence on of common diseases of cattle and buffaloes in Surkhet district. The endoparasitic infections recorded were fascioliasis, toxocariasis, paramphistomiasis, internal schistosomiasis and monieziasis.

Mahato (1993) reported *Fasciola* prevalence 57.9% in buffalo in the hills and 4.3% in terai.

Acharya (1996) conducted a study on efficacy of triclabendazole and oxcyclosanide against fascioliasis of lactating buffaloes and cattle. The study was conducted between January 1996 and March 1996. Of the 317 lactating cow and buffaloes examined 21.6% cows and 30% buffaloes were positive for *Fasciola* sp. infection. However, buffaloes did not response in either treatment groups as effectively as cows.

Regmi *et al.*, (1999) conducted a study to know the Fascioliasis prevalence in Thuladihi VDC of Syanja district. Coprological examination revealed that 67.66% buffalo and 62.10% cattle were affected with fascioliasis.

Sharma (1998-1999) conducted a study on parasitic infection in animals of Panchthar district, fascioliasis was found in 40.12%.

Nirmal (2000) conducted a study of major diseases of goats in far western region Nepal. In the study 71% cases were found having parasitic diseases among which 54.6% due to *Strongyloides* and 61% due to coccidians.

Pandey, Mahato and Gupta (2002) studied prevalence of *Fasciola* infection in *Lymnea* snails and buffaloes in Devbhumi Bhalua VDC of Kavre district. The infection rate in rice field was found 1.67% springs, 1.4% and in irrigation channels 0.99%.

CVL (2002/2003) conducted a study on the prevalence and diversity of *Fasciola* sp. in buffaloes in areas of Kathmandu valley. 92 faecal samples were analysed, where 56 (61%) were found positive for *Fasciola* sp.

Mishra (2003) studied blood parasites of domestic animals in Makawanpur district of Nepal. He reported 32.90% positive cases out of 240 total sample.

Maharjan (2004) reported the presence of *Ascaris* sp. (13.46%), *Trichuris* sp. (1.92%) and *Strongyloides* sp. (0.32%) in children of Kirtipur area with respect to their socio-cultural and socio-economic status.

Jaiswal (2006) carried out a study on fascioliasis in ruminants at Dhanusa district based on examination of faecal samples brought to DLSO, Janakpur from June 15 to November 15, 2005. A total of 2,655 faecal samples were examined out of which 70.70% positive for overall parasitic infestation. Among this prevalence of fascioliasis was found to be 43.43%, followed by paramphistomiasis 38.09% and round worms 13.43%. The prevalence of *Fasciola* infection found in goats was 31.25%, in cattle 49.36% and in buffalo was 56.02%.

Mukhia (2007) conducted a study and reported 90.90% samples positive for trematodes in buffaloes in Satungal VDC, Kathmandu. *Schistosoma* sp. was found in 46.94% followed by *Fasciola* sp. 32.60% and *Dicrocoelium* sp. 20.61%.

Parajuli (2007) studied the intestinal helminth parasite of goat and found 181 (81.53% positive samples among 222 total samples from Khasi bazaar Kalanki, Kathmandu.

Gurung (2007) conducted a study on the prevalence of eggs of three trematode genera *Fasciola* sp., *Dicrocoelium* sp. and *Schistosoma* sp. in buffaloes of Satungal Slaughter House in Satungal Kathmandu during the period of December 2006-January 2007. A total of 210 samples were collected during the study period and the overall prevalence was found 61.90% where infection by *Fasciola* sp. (38.57%) and *Schistosoma* sp. (28.10%) was noted.

Dhakal (2008) conducted a study on intestinal helminth parasites of cattle in Anarmani VDC of Jhapa. Altogether 200 dung samples were collected for the research work where *Dipylidium* (35.75%), *Schistosoma*, (2.7%), *Trichostrongylus* (26.57%), *Dicrocoelium* (12.32%), etc. were found in summer and *Trichostrongylus* (36.5%), *Fasciola* (10.00%), *Dicrocoelium* (8.00%) *Schistosoma* (7.1%) etc. were found as helminth parasites in winter. He found that 14% (21) samples with single infection and remaining 81% with mixed infection.

RVL Biratnagar ((2008-2009) conducted a study where 2446 faecal samples were brought to DLSO Biratnagar.among 2446 samples, 1883 samples (76.95) were positive. The result of faecal test revealed that fascioliasis (57%) is the most prevalent parasitic infestation followed by paramphistomiasis (22%) and nematodiasis (18%).

RVL Pokhara (2008-2009) studied helminth parasites of ruminants from Pokhara. Altogether 757 faecal samples were studied and 422 were found positive. The main helminth parasites found were *Fasciola*, *Paramphistomum*, *Strongyloides*, *Strongylus*, *Trichuris* and *Moniezia*.

Bashir (2009) conducted a study on goat in order to observe the seasonal prevalence of intestinal helminthes parasites in goat brought to Khashi-bazaar, Kalanki. The total numbers of samples collected and examined for the study were 100 and 124

respectively during study period. The overall prevalence of helminthes parasite during December and January were 46% and that in the month of May and June were 90.3%. Mixed infection was observed in 26% and 87.5% in the samples of winter and summer respectively.

Shrestha and Joshi (2010) conducted a study in Kirtipur Municipality. A total of 200 water buffaloes were slaughtered and examined of which 100 were observed during the winter time and 100 were observed during the summer time 2008. Out of them, 93 (46.50%) were male and 107 (53.50%) were female. 54 (27%) of them were calves, 51 (25.50%) were adults and 95 (47.50%) were olds. Female were found more infected with fascioliasis. 38.05% of the female had fascioliasis as compared to 16.09% of male. The difference in sex-wise prevalence of fascioliasis was found significant ($p = 0.0004$). Old animals (35.78%) were infected with *Fasciola* more often than calves (14.81%) and (29.41%) adults. Out of 35 positive cases of *Fasciola* infection observed during winter, 20 (57.14%) buffaloes had only *Fasciola hepatica* while 11 (31.42%) had only *F. gigantica* and 4 (11.42%) had both *F. hepatica* and *F. gigantica*. Similarly during summer, out of 22 animals that had fascioliasis, *F. hepatica* was found in 7 (31.81%) of them while *F. gigantica* was observed in 12 (54.54%) of them and 3 (13.63%) of them had both *F. hepatica* and *F. gigantica*. Infection with *Fasciola hepatica* (59.65%) was found slightly higher than *Fasciola gigantica* (52.63%).

CHAPTER FOUR

MATERIALS AND METHODS

4.1 Study Area

Nepal is rich in biodiversity due to its unique geographical position and latitudinal variation. Geographically it lies between 80⁰ 4” to 88⁰12” in East longitude and 26⁰22” to 30⁰27” in north latitude. It is land locked country occupying 1,47,181 sq. Km area and borders China to north and India to east west and south.

The research side Mukundapur VDC- 2 lies in Western development region under Nawalparasi district of Lumbini Zone. The district is surrounded by Bihar of India in the South, China in the east Tanahu and Arghakhanchi in the North and Rupandehi in the West.

Mukundapur VDC-02 is 17 Km western from Bharatpur (The head quarter of Chitwan) and 75 Km eastern from Parasi (The head quarter of Nawalparasi). The village is surrounded by VDC’s like Gaindakot, Amarapuri, Ratanpur.

The population of this village is mixed type. Ethnically Brahman, Chhettri, Tharu, Magar, Bote, Gurung, Damai, Kami, live in the VDC. Most of the houses rear cattle for their economic support and also for their daily needs. Cows are reared for milk and milk products while oxen for pulling carts and ploughing fields.

4.2 Study Design

Laboratory based microscopical examination of dung samples.

4.3 Study Period

September 2009 and January 2010.

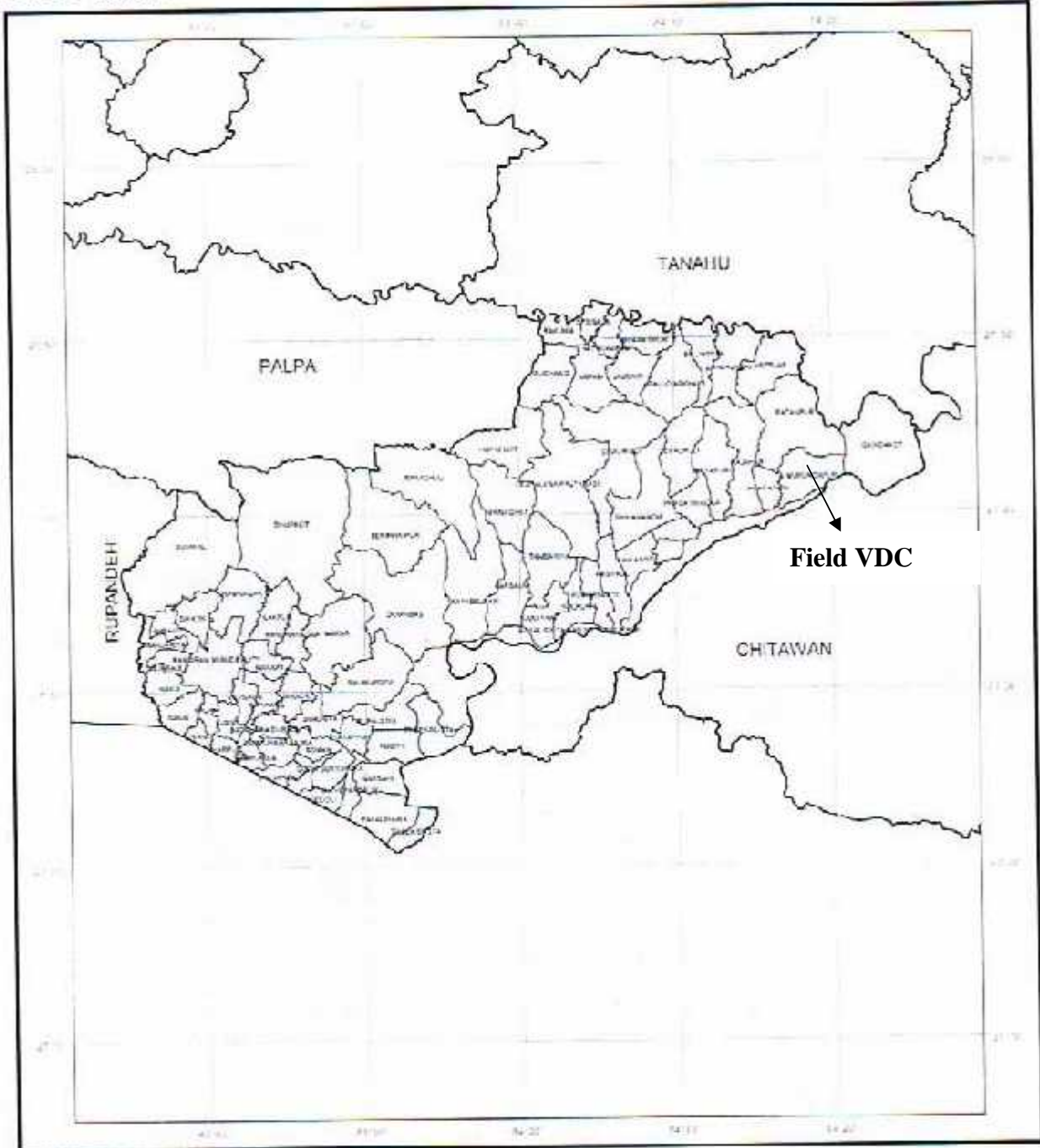
4.4 Sample Size

A total of 100 samples were taken randomly in rainy season and 100 samples were taken in winter season from ward no. 02 of Mukundapur VDC, Nawalparasi.

NAWALPARASI DISTRICT

ZONE : LUMBINI

District Code : 47



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SCALE 1 : 60000

LEGEND	
	District Boundary
	VDC Boundary
MORANG	District Name
	VDC Name

DISTRICT : NAWALPARASI
Area : 2162 Sq.Km.(Approx.)

12000 0 12000 24000 Meters

HORIZONTAL DATUM

Spheroid : Everest 1830
Projection : MUTM
Origin : Longitude 84° E, Latitude 0° N
False coordinates of origin : 500,000 m Easting, 0 m Northing
Scale Factor of Central Meridian : 0.99999

Map compiled from National Topographic Database at scales 1:25,000 and 1:50,000. Internal administrative boundaries are not demarcated on the ground. Map produced by the Survey Department, National Geographic Information Infrastructure Programme (NGIIP), Kathmandu.

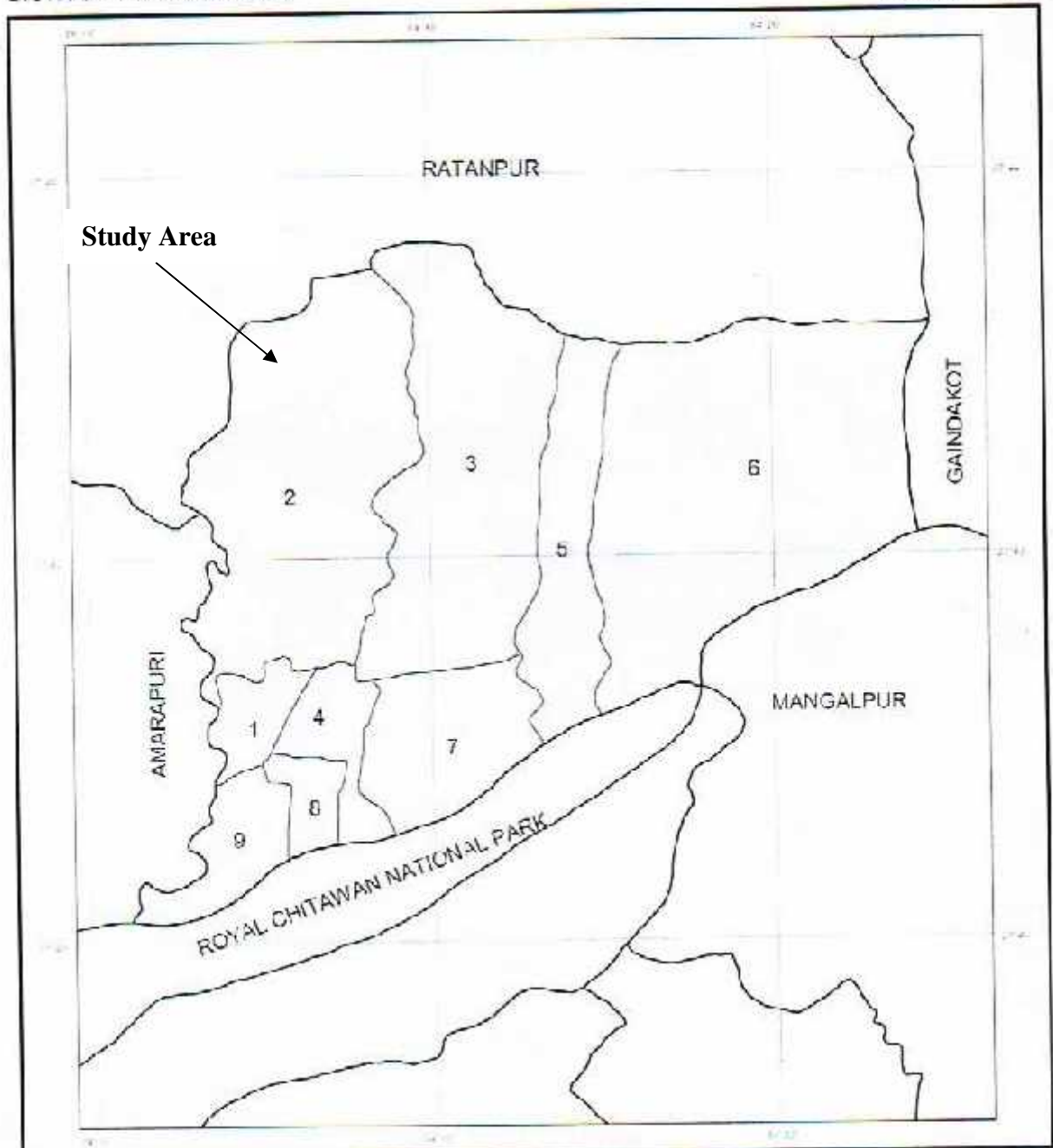
LOCATION MAP



MUKUNDAPUR VDC

DISTRICT : NAWALPARASI

VDC Code : 47041



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SCALE 1 : 55000

NAWALPARASI DISTRICT
VDC Location Map

LEGEND	
	VDC Boundary
	Ward Boundary
BUKHIL	VDC Name
5	Ward Number



HORIZONTAL DATUM
Spheroid: Everest 1830
Projection: MUTM
Origin: Longitude 84° E, Latitude 27° N
False coordinate of origin: 500 000 m. Easting, 0 m. Northing
Scale Factor at Central Meridian: 0.9999



MUKUNDAPUR VDC
Area: 27 Sq.Km. (Approx.)

Map compiled from National Topographic Database at scales 1:25 000 and 1:50 000, 2002. Internal administrative boundaries are not demarcated on the ground. Map produced by the Survey Department, National Geographic Information Infrastructure Programme (NGIIP), Kathmandu.

4.5 Precautions and Preservations

To ensure better condition following precautions were taken during sample collection and up to the time of lab examination.

1. Fresh dung samples were collected as far as possible.
2. Collected samples were thoroughly mixed with 2.5% potassium dichromate ($K_2Cr_2O_7$) for preservation.
3. The samples were kept in air tight container.
4. To check egg and larval development samples were preserved in refrigerator up to the time of laboratory examination.
5. Sampling was done randomly.

4.6 Laboratory Materials Required

4.6.1 Tools

- | | |
|--------------------|------------------|
| - Plastic bottles | - Cover sleeves |
| - Gloves | - Gloves |
| - Microscope | - Tea strainer |
| - Refrigerator | - Test tube |
| - Pipette | - Grinding basin |
| - Cotton | - Slides |
| - Volumetric flask | - Beaker |
| - Centrifuge tube | |

4.6.2 Chemicals

- 2.5% potassium dichromate solution
- Concentrated sodium chloride (NaCl) solution.

- 33% Zinc chloride solution

- Methylene blue

4.7 Dung Examination

The dung samples were collected preserved in potassium dichromate solution and refrigerated. These were examined by sedimentation and floatation technique.

4.7.1 Simple Floatation Technique

This technique is used for the detection of cestodes and nematodes eggs which are lighter than the floatation liquid and easily float in the floatation liquid.

4.7.1.1 Procedure

- a. Put approximately 3g of faeces (weigh or measure the faeces with a precalibrated teaspoon) into Container 1.
- b. Pour 50 ml of flotation fluid into Container 1.
- c. Mix (stir) the contents thoroughly with a stirring device (tongue blade, fork).
- d. Pour the resultant faecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2.
- e. Leave the container to stand for 10 minutes.
- f. Press a test tube to the bottom of the filtrate, lift it quickly and transfer a few drops adhering to the surface to a microslide.
- g. The test tube ought to touch the microslide for at least 2-4 seconds for the drops to run off.
- h. Mount the cover slip on the micro slide for microscopical examination.

4.7.2 Sedimentation Technique

This technique is used for the detection of trematode eggs. It provides good result for the trematode eggs such as *Fasciola* sp., *Schistosoma* sp., *Dicrocoelium* sp. which are heavier than the other eggs and deposit at the bottom.

4.7.2.1 Procedure

- (a) Weigh or measure approximately 3g of faeces into Container 1.
- (b) Pour 40-50 ml of tap water into Container 1.
- (c) Mix (stir) thoroughly with a stirring device (fork, tongue blade).
- (d) Filter the faecal suspension through a tea strainer or double-layer of cheesecloth into Container 2.
- (e) Pour the filtered material into a test tube.
- (f) Allow to sediment for 5 minutes.
- (g) Remove (pipette, decant) the supernatant very carefully.
- (h) Re-suspend the sediment in 5 ml of water.
- (i) Allow to sediment for 5 minutes.
- (j) Discard (pipette, decant) the supernatant very carefully.
- (k) Stain the sediment by adding one drop of methylene blue.
- (l) Transfer the sediment to a micro slide. Cover with a coverslip.

4.8 Key for Cestodes, Trematodes and Nematodes

-) Soulsby, E.J.L. (1982). Eggs of worm parasites. *Helminthes, Arthropods and Protozoa of domesticated animals*. 7th edition.1:24-345. The English Language Book society and Bailliera Tindall London.
-) Gibbons, L.M; Jacobs, D.E; Fox M.T; and Hansen, J. (2007). Faecal examination for helminth parasites. The RVC/FAO guide to Veterinary Diagnostic Parasitology . Electronic media unit at the Royal Veterinary College London.
-) Mahato, S.N. (2003). Veterinary Laboratory Techniques. 1:27-43. Department of Livestock services, Harihar Bhawan Kathmandu.

CHAPTER FIVE

RESULTS

Helminth parasitic infection is the common problem of cattle and other domesticated animals all over the world. This work has been done in order to identify the eggs of various types of helminth parasites found in the dung samples. It also includes which parasite is found more in which season. Beside this the study also includes the type of infection i.e. single and mixed caused by the parasites. All this will serve as a medium for solving the problems of helminth parasites of cattle. This study reveals the prevalence of intestinal helminth parasites of cattle as

1. Class-wise prevalence of helminthes in general.
2. Season-wise prevalence of helminthes.
3. General prevalence of helminthes.
4. Prevalence of *Fasciola* species.
5. Single and mixed infections.
6. Identification of eggs of helminthes in brief.

5.1 General Prevalence of Parasites.

Out of 200 total samples examined, 130 (65%) samples were found to be positive with intestinal helminth parasites. Among 130 positive samples 22 genera of helminthes were observed. Out of 22 genera of helminthes 5 (22.73%) were trematodes, 4 (18.18%) were cestodes and 13 (59.09%) were nematodes.

Graphical presentation of observed genera of different classes of helminthes.

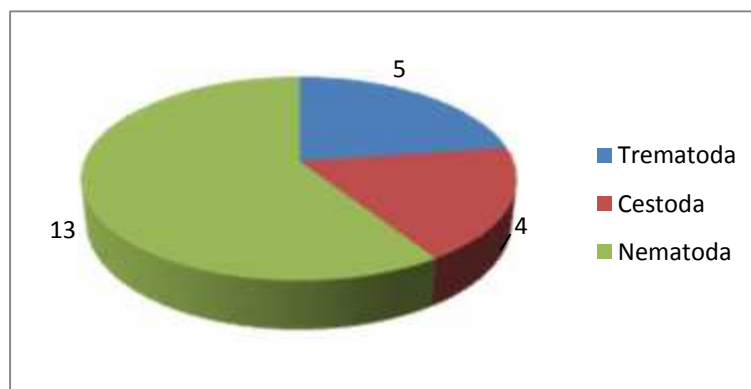


Figure 4: Observed genera of different classes of helminth

Table 2: General prevalence of helminth parasites

SN	classes	Helminth genera	Percnrtage
A	Trematodesg	1. <i>Fasciola</i> sp.	55.38%
		2. <i>Schistosoma</i> sp.	37.69%
		3. <i>Dicrocoelium</i> sp.	1.54%
		4. <i>Paramphistomum</i> sp.	3.85%
		5. <i>Ornithobilharzia</i> sp.	0.77%
B	Cestodes	1. <i>Dipylidium</i> sp.	7.69%
		2. <i>Taenia</i> sp.	4.62%
		3. <i>Moniezia</i> sp.	1.54%
		4. <i>Anoplocephala</i> sp.	0.77%
C	Nematodes	1. <i>Trichostrongylus</i> sp.	10.77%
		2. <i>Strongyloides</i> sp.	9.23%
		3. <i>Chabertia</i> sp.	5.38%
		4. <i>Toxocara</i> sp.	1.54%
		5. <i>Ostertagia</i> sp.	4.62%
		6. <i>Bunostomum</i> sp.	1.54%
		7. <i>Dictyocaulus</i> sp.	9.23%
		8. <i>Haemonchus</i> sp.	0.77%
		9. <i>Ascaris</i> sp.	4.62%
		10. <i>Capillaria</i> sp.	2.31%
		11. <i>Cooperia</i> sp.	3.85%
		12. <i>Oesophagostomum</i> sp.	0.77%
		13. <i>Trichuris</i> sp.	3.08%

The result indicates that maximum infection was caused by the genera of nematodes (59.09%) followed by trematodes (22.72%) and cestodes (18.18%). *Fasciola* sp. from trematodes, *Dipylidium* sp. from cestodes and *Trichostrongylus* sp. from nematodes have dominated their respective classes.

5.2 Season-wise Prevalence

5.2.1 Prevalence in Rainy Season

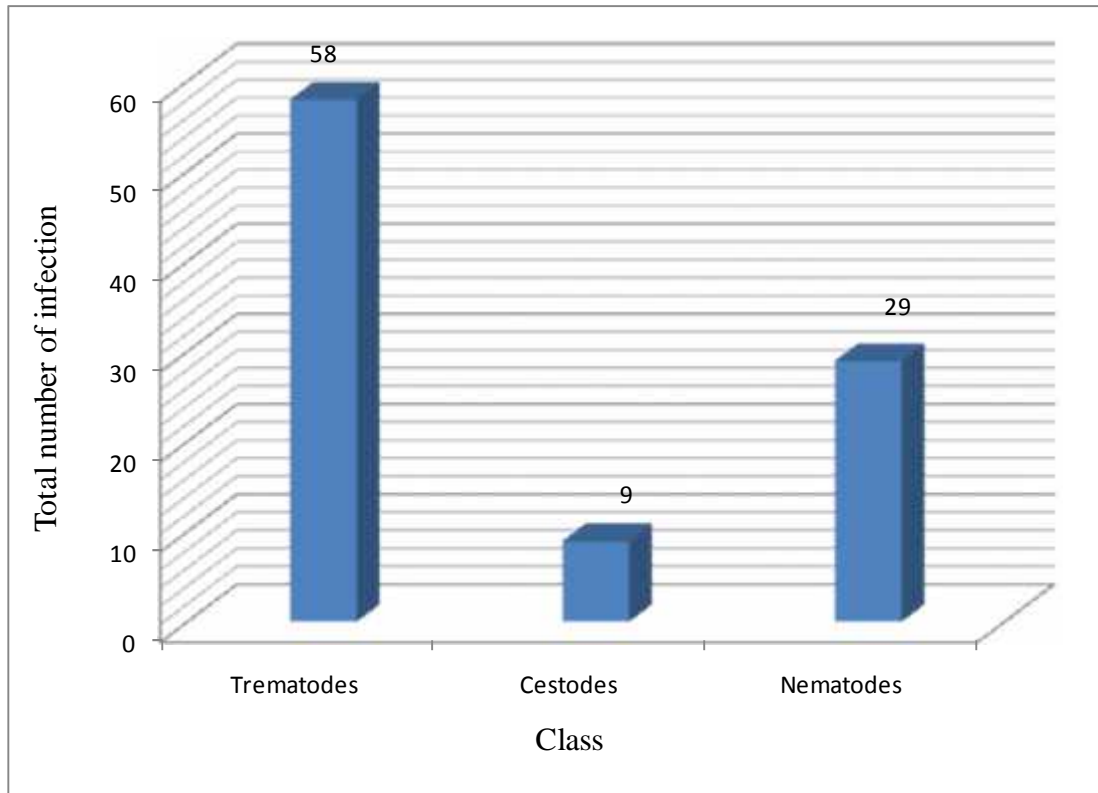


Figure 5: Number of infections by trematodes, cestodes and nematodes in rainy season.

In rainy season maximum no. of infections were due to the genera of class trematoda (63.20%) followed by nematoda (28%) and cestoda (8.80%).

Table 3: Prevalence of helminth parasites in rainy season.

S.N	Parasite	Percentage
1	<i>Fasciola</i> sp.	67.14%
2	<i>Schistosoma</i> sp.	37.14%
3	<i>Dicrocoelium</i> sp.	2.86%
4	<i>Paramphistomum</i> sp.	4.29%
5	<i>Ornithobilharzia</i> sp.	1.43%
6	<i>Dipylidium</i> sp.	7.14%
7	<i>Taenia</i> sp.	4.29%
8	<i>Moniezia</i> sp.	2.86%
9	<i>Anoplocephala</i> sp.	1.43%
10	<i>Trichostrongylus</i> sp.	7.14%
11	<i>Strongyloides</i> sp.	8.57%
12	<i>Chabertia</i> sp.	4.29%
13	<i>Ostertagia</i> sp.	4.29%
14	<i>Toxocara</i> sp.	1.43%
15	<i>Dictyocaulus</i> sp.	7.14%
16	<i>Haemonchus</i> sp.	1.43%
17	<i>Ascaris</i> sp.	7.14%
18	<i>Capillaria</i> sp.	4.29%
19	<i>Cooperia</i> sp.	2.86%
20	<i>Trichuris</i> sp.	1.43%

Out of 100 samples examined, 20 genera of helminth were reported. Among them *Fasciola* sp. (67.14%) was found in highest percentage followed by *Schistosoma* sp. Similarly *Ornithobilharzia* sp., *Moniezia* sp., *Anoplocephala* sp., *Haemonchus* sp. and *Capillaria* sp. were only recorded from rainy season. *Bunostomum* sp. and *Oesophagostomum* sp. were not reported in rainy season.

5.2.2 Prevalence in Winter Season.

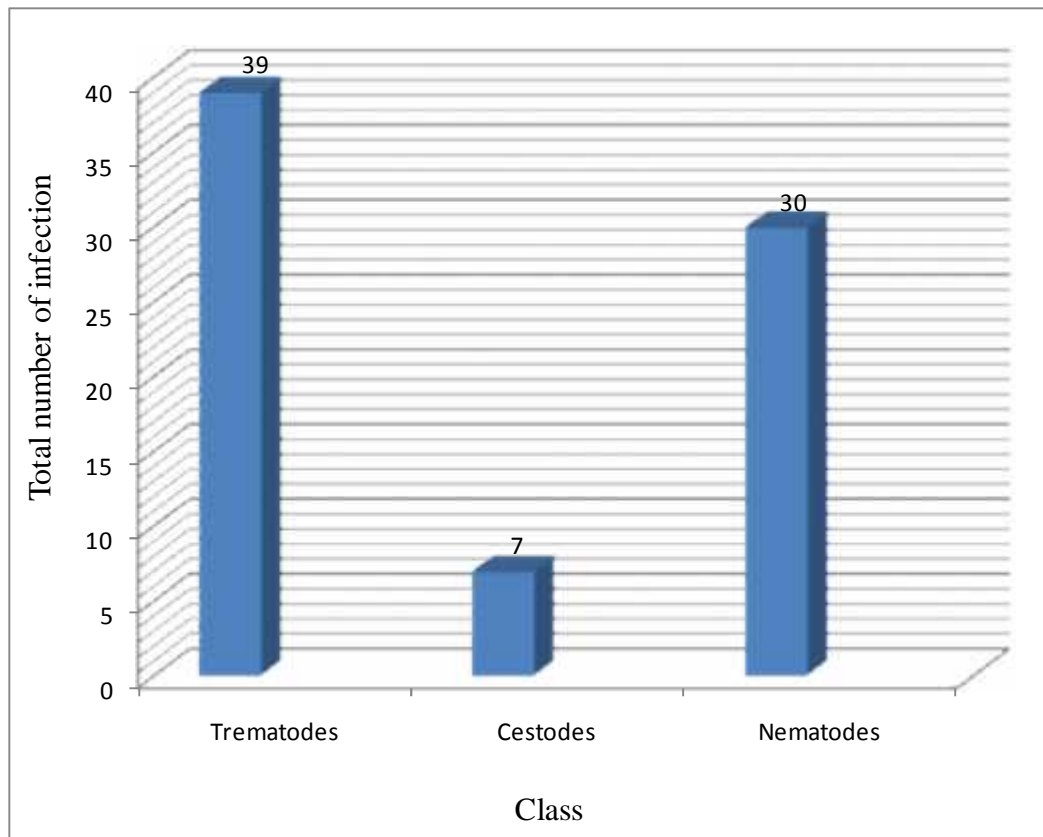


Figure 6: Number of infections by trematodes, cestodes and nematodes in winter season.

In winter season maximum numbers of infections were due to class trematoda (51.02%) followed by nematoda (40.82%) and cestoda (8.16%).

Table 4: Prevalence of helminth parasites in winter season.

S.N.	Parasite	Percentage
1	<i>Fasciola</i> sp.	25.51%
2	<i>Schistosoma</i> sp.	23.47%
3	<i>Paramphistomum</i> sp.	2.04%
4	<i>Dipylidium</i> sp.	5.01%
5	<i>Taenia</i> sp.	3.06%
6	<i>Trichostrongylus</i> sp.	9.19%
7	<i>Strongyloides</i> sp.	6.12%
8	<i>Chabertia</i> sp.	4.08%
9	<i>Ostertagia</i> sp.	3.06%
10	<i>Toxocara</i> sp.	1.02%
11	<i>Dictyocaulus</i> sp.	7.15%
12	<i>Bunostomum</i> sp.	2.04%
13	<i>Ascaris</i> sp.	1.02%
14	<i>Cooperia</i> sp.	3.06%
15	<i>Trichuris</i> sp.	3.06%
16	<i>Oesophagostomum</i> sp.	1.02%

Out of 100 samples, 16 genera of helminth parasites were recorded. Among them infection due to *Fasciola* sp. is highest followed by *Schistosoma* sp. *Bunostomum* sp. and *Oesophagostomum* sp. were only reported in winter season. Similarly *Ornithobilharzia* sp., *Moniezia* sp., *Anoplocephala* sp., *Haemonchus* sp. and *Capillaria* sp. were not recorded from winter season.

5.3 Class-wise Prevalence of Helminth Parasites.

A total of 200 samples were collected from Mukundapur VDC-02 within two seasons viz; rainy and winter. Samples were examined by sedimentation and flotation techniques in CVL Tripureshor Kathmandu. There were overall 130 positive and 70 negative samples found during lab examination.

Collectively 5 genera of trematodes, 4 genera of cestodes and 13 genera of nematodes were recognized.

5.3.1 Prevalence of Trematode Genera.

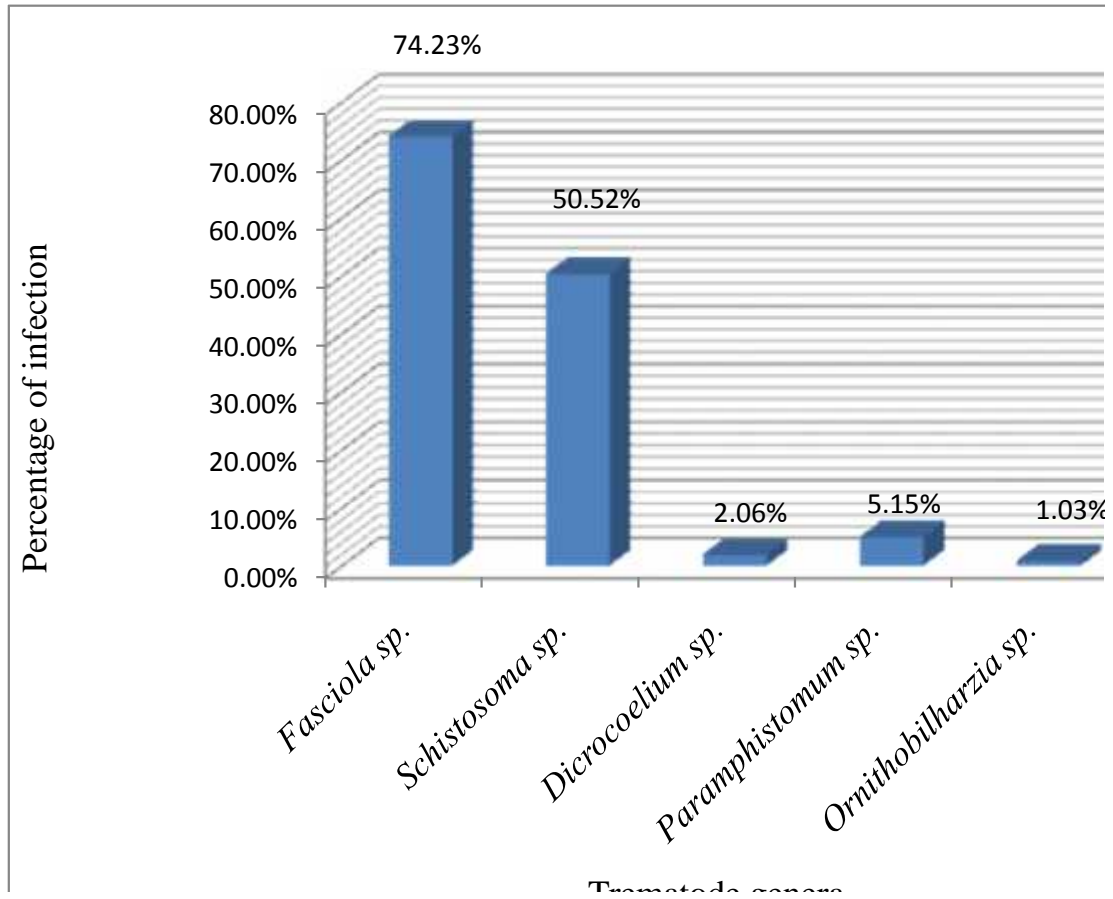


Figure 7: Prevalence of trematodes in cattle.

Out of 200 samples 97 samples were positive with trematodes. The infection by *Fasciola* is highest (74.32%) followed by *Schistosoma* (50.52%). Single infection by *Ornithobilharzia* sp. was noticed.

5.3.2 Prevalence of Cestode Genera.

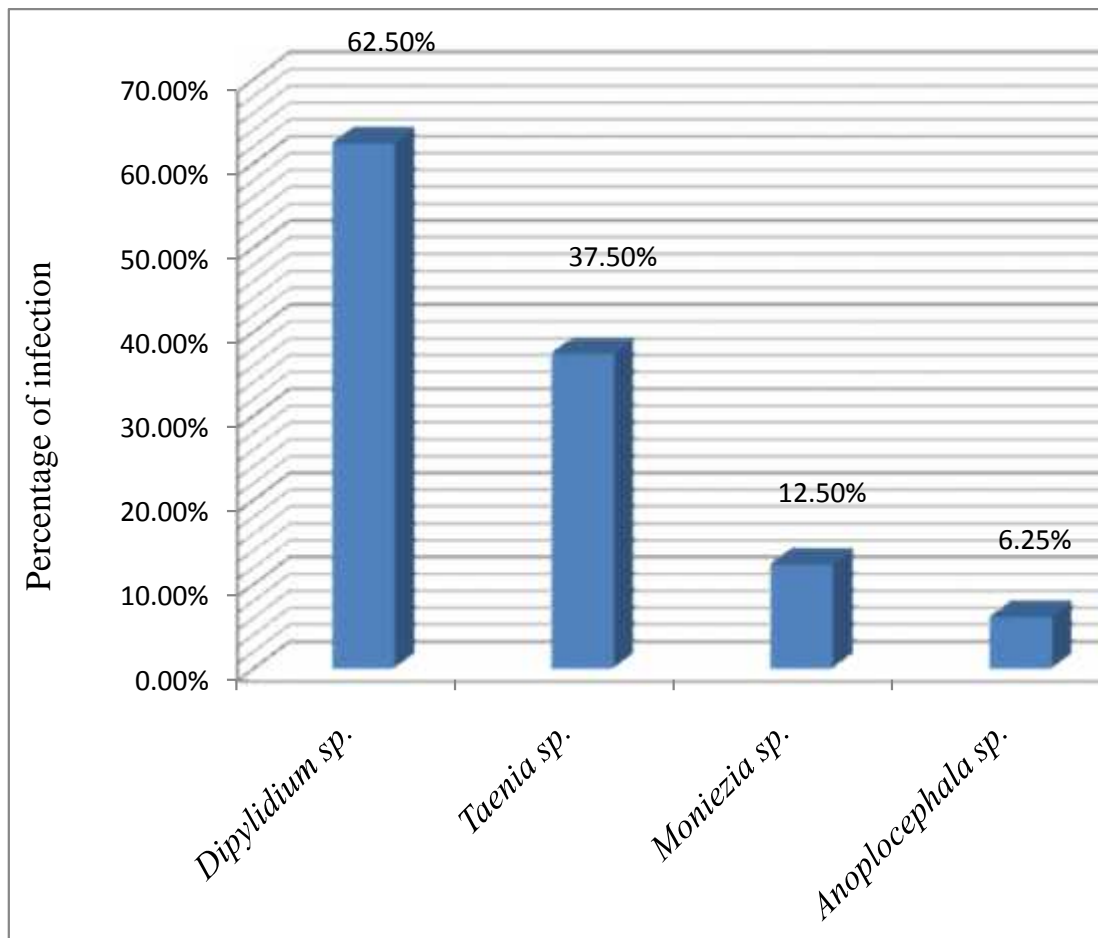


Figure 8: Prevalence of cestodes in cattle.

A total of 16 samples were found positive with cestodes. The highest infection was due to *Dipylidium sp.* (62.50%) followed by *Taenia sp.* (37.50%) and lowest infection was due to *Anoplocephala sp.* (6.25%) which was recovered from only one sample.

5.3.3 Prevalence of Nematode Genera.

Table 5: Prevalence of nematode genera.

S.N.	Parasite	Percentage
1.	<i>Trichostrongylus</i> sp.	23.72%
2.	<i>Strongyloides</i> sp.	20.34%
3.	<i>Chabertia</i> sp.	11.86%
4.	<i>Ostertagia</i> sp.	10.17%
5.	<i>Toxocara</i> sp.	3.39%
6.	<i>Dictyocaulus</i> sp.	20.34%
7.	<i>Haemonchus</i> sp.	1.69%
8.	<i>Ascaris</i> sp.	10.17%
9.	<i>Capillaria</i> sp.	5.08%
10.	<i>Cooperia</i> sp.	8.47%
11.	<i>Trichuris</i> sp.	6.78%
12.	<i>Bunostomum</i> sp.	3.39%
13.	<i>Oesophagostomum</i> sp.	1.69%

During the research period 200 samples were collected and 57 samples were positive with nematodes. The highest infection was due to *Trichostrongylus* sp. (23.72%) followed by *Strongyloides* sp. (20.34%). *Haemonchus* sp. (1.69%) and *Oesophagostomum* sp. (1.69%) each were recorded from only one sample.

The difference in the prevalence of helminth parasites of class trematodes, cestodes and nematodes were found statistically significant ($\mathcal{R}^2=57.29$, $P<0.05$, d.f =2).

5.4 Prevalence of *Fasciola* Species

Out of 130 positive samples 72 (55.38%) with *Fasciola* species infection were pragmated. The abundance of *Fasciola* species was different in different seasons. Two species of *Fasciola* were practical during lab examination. The table below shows its prevalence in rainy and winter.

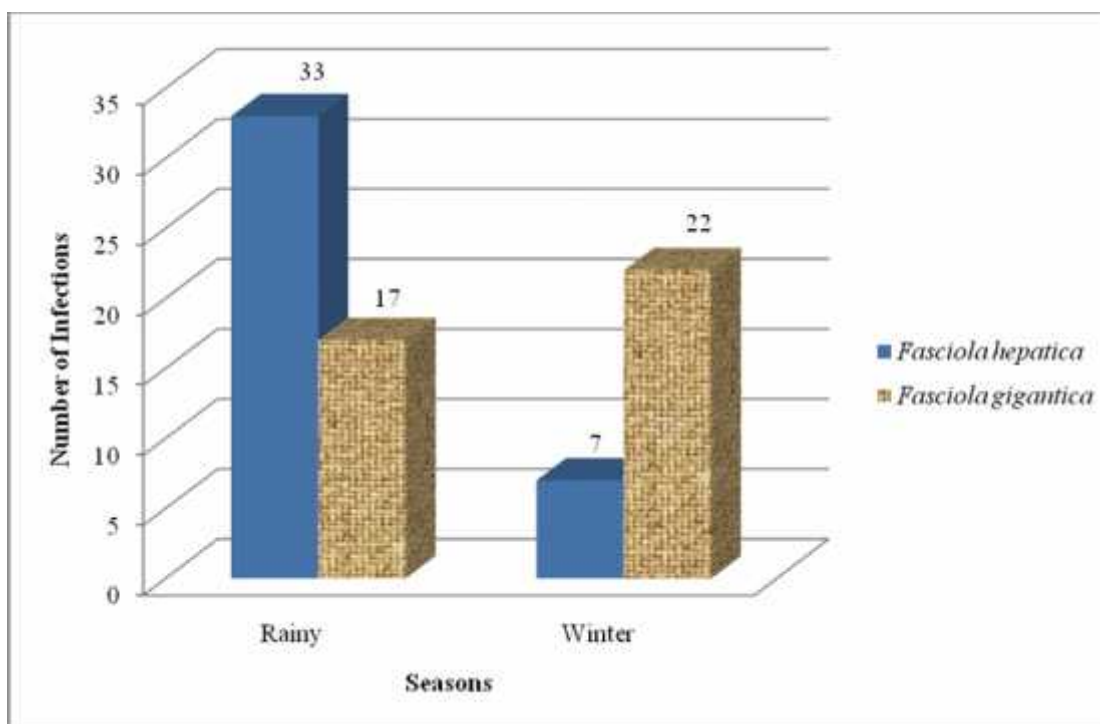


Figure 9: Prevalence of liver fluke in rainy and winter season.

In rainy season 47 (67.14%) samples were *Fasciola* positive out of total 70 positive samples. Of these, 33 (47.14%) samples were positive with *Fasciola hepatica* and 17 (24.29%) samples were positive with *F. gigantica*

In winter season 25 (41.67%) samples were *Fasciola* positive out of 60 positive. 22 (36.67%) positive samples were due to *Fasciola gigantica* and 7 (11.67%) samples were due to *F. hepatica*.

The prevalence of two species of *Fasciola* in summer and winter results statically as ($\chi^2 = 12.86, P < 0.05, d.f = 1$).

5.5 Single and Mixed Infections

In the present study 130 (65%) samples were positive and out of total 130 positive samples 66 (50.76%) samples were found to have single infection. Among positive samples with single infection the highest samples were credited to *Fasciola* sp. then *Schistosoma* sp. and *Trichostrongylus* sp. Rest 64 (49.23%) positive samples were found to have mixed infections. Most of the mixed infections were with *Fasciola* sp. (37%).

Approximately 3 gm of dung sample was taken for microscopical examination. Some samples were found having single parasite and some others were found with up to 20 parasites.

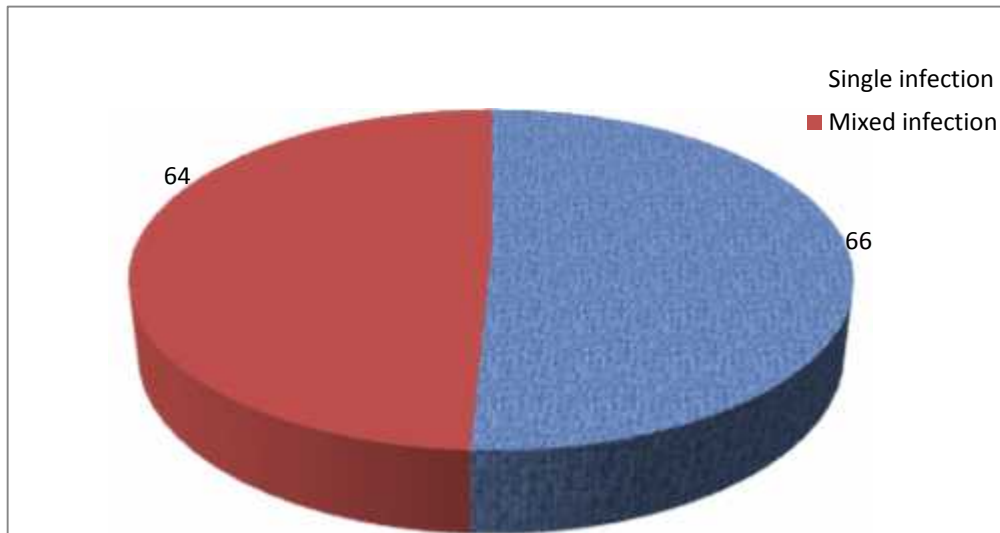


Figure 10: Total number of single and mixed infections.

5.6 Identification of Helminth Eggs in Brief

Identification of eggs of helminths was done on the basis of their morphology and characters. Identification of eggs of helminths in brief was done as follows:

5.6.1 Trematodes

***Dicrocoelium* sp. (Rudolphi, 1819).**

Family - Dicrocoelidae

Genus - *Dicrocoelium* sp.

Characters

Eggs are 36-45 μm by 23-40 μm in size, dark brown in colour, operculated, usually with a flattened side, contains miracidium when passed in the faeces.

***Fasciola* sp. (Linnaeus, 1758).**

Family - Fasciolidae

Genus - *Fasciola* sp.

Characters

Eggs are 1340 μm -197 μm by 63-104 on size oval shaped, yellowish (*Fasciola gigantica*) or reddish brown (*Fasciola hepatica*) in colour, consist of embryonic mass and shell, operculum usually indistinct.

***Schistosoma* sp. (Weinland, 1858).**

Family - Schistosomitidae

Genus - *Schistosoma* sp.

Characters

Eggs are 200 μm by 70-90 μm in size spindle shaped, flattened at one side, elongated or oval with a terminal spine.

***Paramphistomum* sp. (Fischoeder, 1901).**

Family - Paramphistomidae

Genus - *Paramphistomum* sp.

Characters

Eggs are 114 μm – 176 μm by 73 μm -100 μm in size, oval in shape, whitish to transparent in colour with distinct operculum, knob like thickening at the acetabular end of shell, embryonic cells distinct.

***Ornithobilharzia* sp. (Odhner, 1912).**

Family - Schistosomitidae

Genus - *Ornithobilharzia* sp.

Characters

Eggs are 72 μm - 77 μm by 18-26 in size, terminal spine, short appendage at the other end.

5.6.2 Cestodes

***Taenia* sp. (Linnaeus, 1758).**

Family - Taenidae

Genus - *Taenia* sp.

Characters

Eggs are 24 µm – 41 µm in diameter, spherical in shape, brown to dark yellow in colour, thick shelled and contain an onchosphere .

***Dipylidium* sp. (Leuckart, 1863).**

Family - Dipylidae

Genus - *Dypilidium* sp.

Characters

Eggs are 25 µm - 50 µm in diameter, 4-20 globular cells per capsule.

***Moniezia* sp. (R. Blanchard, 1891).**

Family - Anoplocephalidae

Genus - *Moniezia* sp.

Characters

Eggs are 56 µm - 767 µm in diameter, triangular, globular or quadrangular in shape, contains a well developed pyriform apparatus.

5.6.3 Nematodes

***Trichostrongylus* sp. (Looss, 1905).**

Family - Trichostrongylidae

Genus - *Trichostrongylus* sp.

Characters

Eggs are 79 µm - 92 µm by 32 µm -49 µm in size, oval and bilaterally symmetrical, shell has a thin and transparent outer chitinous layer and a thin inner lipoid layer, embryonic mass multi segmented and varies from 16- 32 in number.

***Strongyloides* sp. (Grassi, 1879).**

Family - Strongyloidae

Genus - *Strongyloides* sp.

Characters

Eggs are 40 µm -64 µm by 20-40 in size, ellipsoidal, thin shelled, embryonated when laid

***Chabertia* sp. (Railliet & Henry, 1909).**

Family - Trichostrongyloidae

Genus - *Chabertia* sp.

Characters

Eggs are 90 µm - 105 µm by 52 µm -55 µm in size, notched, and laid in morula stage .

***Toxocara* sp. (Stiles,1905).**

Family - Ascaridae

Genus - *Toxocara* sp.

Characters

Eggs are 75 µm -95 µm by 60 µm -75 µm in size , sub globular and have finely pitted albuminous layer, occasionally two celled.

***Ostertagia* sp. (Ransom,1907).**

Family - Trichostrongylidae

Genus - *Ostertagia* sp.

Characters

Eggs are 89 µm -100 µm by 40 µm – 50 µm in size, elliptical in shape, contains fully developed larva when laid.

***Bunostomum* sp. (Railliet, 1902).**

Family - Necatorinae

Genus - *Bunostomum* sp.

Characters

Eggs are 79 µm -106 µm by 47 µm - 50 µm in size, elliptical, have blunt ends and clearly pigmented embryonic cells.

***Dictyocaulus* sp. (Railliet & Henry, 1909).**

Family - Dictyocaulidae

Genus - *Dictyocaulus* sp.

Characters

Eggs are 82 µm -88 µm by 30-33 in size, ellipsoidal, contain fully developed or first stage larva when laid.

***Haemonchus* sp. (Cobb, 1898)**

Family - Trichostrongylidae

Genus - *Haemonchus* sp.

Characters

Eggs are 70 µm -85 µm by 42 µm -48 µm in size, embryo 16-32 celled when laid.

***Ascaris* sp. (Linnaeus, 1758).**

Family - Ascaridae

Genus - *Ascaris* sp.

Characters

Eggs are 40 µm -90 µm in diameter, sub globular, ornate laid in morula stage.

***Capillaria* sp. (Zeder, 1800).**

Family - Capillaridae

Genus - *Capillaria* sp.

Characters

Eggs are 30 µm -63 µm in length, barrel shaped, contains unsegmented embryo, colourless shelled.

***Cooperia* sp. (Ransom,1907).**

Family - Trichostrongylidae

Genus - *Cooperia* sp.

Characters

Eggs are 70 µm -82 µm by 35 µm - 41 µm in size. Their sides are parallel and have less than 16 pale yellow blastomeres when laid.

***Trichuris* sp. (Roederer, 1761)**

Family - Trichuridae

Genus - *Trichuris* sp.

Characters

Eggs are 70 µm -80 µm by 30 µm -42 µm in size brown in colour, contains unsegmented embryo, barrel shaped, contains mucous plug at either pole.

***Oesophagostomum* sp. (Molin, 1861).**

Family - Trichonematidae

Genus - *Oesophagostomum* sp.

Characters

Eggs are 70 µm -76 µm by 36 µm -40 µm in size, strongyle like.

CHAPTER SIX

DISCUSSION AND CONCLUSION

Present study was conducted to investigate the “Prevalence of intestinal helminth parasites in general and *Fasciola* in particular in cattle”. Altogether 200 dung samples were collected in two seasons summer and winter from Mukundapur-02 Nawalparasi. 100 samples were collected in each seasons. There were 130 (65%) positive and 70 (35%) negative samples practical. In general 22.73% trematodes, 18.18% cestodes and 59.09% nematode genera were identified in the lab study period.

Among 130 positive samples observed 97 samples were positive with trematodes, 16 samples were positive with cestodes and 57 samples were positive with nematodes.

In trematodes *Fasciola* sp., *Schistosoma* sp., *Dicrocoelium* sp., *Paramphistomum* sp. and *Ornithobilharzia* sp. were observed which were 5 in numbers.

In cestodes *Dipylidium* sp., *Taenia* sp., *Moniezia* sp. and *Anoplocephala* sp. were recorded which were 4 in number.

In nematodes *Trichostrongylus* sp., *Strongyloides* sp., *Chabertia* sp., *Toxocara* sp., *Ostertagia* sp., *Ascaris* sp., *Capillaria* sp., *Cooperia* sp., *Dictyocaulus* sp., *Bunostomum* sp., *Oesophagostomum* sp., *Trichuris* sp. and *Haemonchus* sp. were noticed which were 13 in number.

The research was done under different headings viz., season-wise, class-wise, general prevalence and prevalence of *Fasciola* in particular in cattle.

In season-wise prevalence - 25% trematode, 20% cestode and 55% nematode genera were concluded in rainy season. Among these the highest prevalence was found by *Fasciola* sp. (67.14%). In winter season 18.75% trematode, 12.50% cestode and 68.75% nematode genera were found. In the winter season the infection by *Fasciola* sp. was found to be the highest (41.67%).

Similarly there were many other parasites which showed their rare presence. *Ornithobilharzia*, and *Dicrocoelium* from trematoda, *Moniezia*, and *Anoplocephala*

from cestoda and *Haemonchus*, *Oesophagostomum*, *Bunostomum*, and *Toxocara* from nematoda have negligible infections. These parasites were observed from only one or two dung samples.

Due to the lack of advanced equipments during lab study identification of parasites other than *Fasciola* was possible up to genus level. No species and sub species have been identified except *Fasciola*.

Among 130 positive samples 50.77% with mixed infection and 49.23% with single infection were observed in the present study. The overall prevalence of helminthes parasite during December and January were 46% and that in the month of May and June were 90.3%. Mixed infection was observed in 26% and 87.5% in the samples of winter and summer respectively (Bashir, 2009). Dhakal (2008) reported altogether 14% single infection and 81% mixed infection in cattle from Anarmani, Jhapa.

Muraleedharan (2005) carried out a study in Karnatak India where 18.22% helminth parasitic infection was noted. 71% parasitic infection in goats was reported from far western region of Nepal (Nirmal, 2000). Regassa *et al.*, (2006) conducted a study to determine the prevalence and risk factors associated with GI parasitism in Western Oromia Ethiopia during 2003-2004. They showed the prevalence of helminth parasites in cattle 69.60%. Similarly 76.9% parasitic infections in ruminants from Biratnagar (RVL Biratnagar, 2008-2009) and 422 of 757 infections from Pokhara (2008-2009) were observed. Present study almost meets with this figure where overall prevalence of parasite was found 65%.

Similarly, *Trichostrongylus* sp., *Bunostomum* sp., *Oesophagostomum* sp. and *Strongyloides* sp. were major parasitic helminth in India (Yadav *et al.*, 2005). According to Muraleedharan (2005) the prevalence dynamics of GI parasites of livestock in Karnataka India during drought period and reported highest infection due to *Strongyloides* followed by *Fasciola* and *Amphistomum*. But present study shows difference with it. Here *Fasciola* is dominant parasitic species followed by *Schistosoma*. Presence of suitable temperature and moisture serve best for the breeding and development of helminth parasites and their intermediate hosts. So this could be the reason behind excessive prevalence of certain helminth parasites.

Swai *et al.*, (2004) reported 56.60% trematode infection in cattle from Ngorongoro district, Tanzania. Similarly fascioliasis, toxocariasis, paramphistomiasis, schistosomiasis and monieziasis was reported from Surkhet (Ghimire, 1987).

Among cestodes, *Dipylidium* (7.69%) shows highest prevalence in the present study. *Anoplocephala* was reported for the first time in cattle from Nepal. Dhakal (2008) also reported the highest prevalence of *Dipylidium* in summer in cattle from Anarmani, Jhapa.

The prevalence of intestinal helminth parasites in buffalo brought to Satungal for slaughter purpose was 83.96% the main helminthes were *Schistosoma* 46.94%, *Fasciola* 32.60%, *Dicrocoelium* 20.61% etc. It shows slight difference with the present study. The difference in the result could be the variation in weather conditions and humidity in atmosphere.

In the case of *Fasciola* two species were identified i.e. *Fasciola hepatica* and *F. gigantica*. Out of 70 positive samples in rainy season, 33 (47.14%) samples were found infected with *Fasciola hepatica* and 17 (24.29%) with *F. gigantica*. In winter season 7 (11.67%) samples were positive with *Fasciola hepatica* and 22 (36.66%) with *F. gigantica* infection. The occurrence of *Fasciola gigantica* (22.40%) was higher than *F. hepatica* (3.06%) in cattle (Muraleedharan, 2009).

The present study exhibited overall 55.38% fascioliasis prevalence. The prevalence of *Fasciola* sp. in rainy and winter season was found to be 67.14% and 41.67% respectively. High prevalence of *Fasciola* sp. was reported from terai region of Nepal than the hills (Mahato, 1993), followed by 21.6% fascioliasis prevalence in different parts of Nepal (Acharya, 1996), 62.10% prevalence from Syanja district (Regmi, 1999), 40.12% from Panchthar (Sharma,1998-1999), 43.43% from Dhanusa district (Jaiswal, 2006), and overall 25.46% fascioliasis prevalence in Zambia (Mohammad, 2009). In overall examination *Fasciola* sp. was dominant over other parasitic species in number of infection and density of infection. This might be due to exposure of cattle to highly infected pasture land, contaminated water or infected fodder which is favorable for *Fasciola* sp.

In the present study seasonal prevalence of two species of *Fasciola* significantly differs. Statically $t^2_{\text{cal}}=12.86$, d.f =1, $p < 0.05$ and $t^2_{\text{tab}}=3.841$. Here the calculated t^2 value is greater than the tabulated t^2 value. So the seasonal prevalence of two species of *Fasciola* is different. In the study the prevalence of two species of *Fasciola* i.e. *F. hepatica* and *F. gigantica* were opposite. In summer prevalence by *F. hepatica* was higher and in winter prevalence of *F. gigantica* was higher. This might be due to their same site location; liver and interspecific competition for survival. Meta-cercaria of *F. gigantica* live longer in drought than meta-cercaria of *F. hepatica* (Shiramizu and Abu, 1988). Thus the prevalence of *F. gigantica* in winter is attributed.

CHAPTER SEVEN

RECOMMENDATIONS

On the basis of the present study following measures are recommended:

- For the prevention and elimination of gastro-intestinal parasites, strategic anthelmintic treatment should be applied.
- The cattle grazing pasture should be made free of helminth parasites by breaking their lifecycle by eradicating intermediate host, snail.
- Parasite free fodder, water and grasses should be provided to the cattle.
- The shed should be kept clean and safe to check the growth and development of parasites.
- Awareness programme about meat borne and zoonotic diseases should be carried to the public and butcher who are directly connected with cattle.
- Further research work should be carried out.
- Stall feeding and biogas plant system is recommended.

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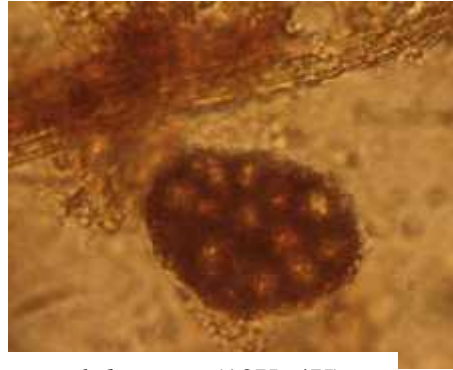
PHOTOGRAPHS

Plate 1

EGGS OF CESTODES OBSERVED

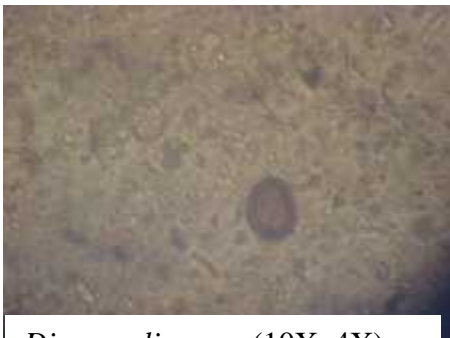


Taenia sp. (10Xx10X)



Dipylidium sp. (10Xx4X)

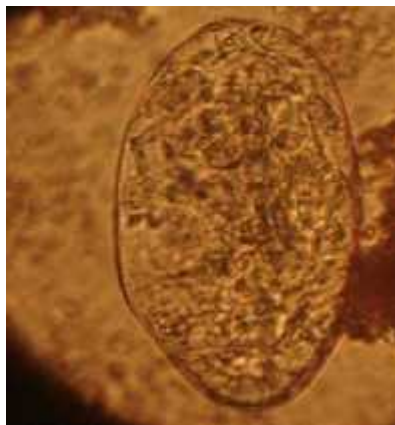
EGGS OF TREMATODES OBSERVED



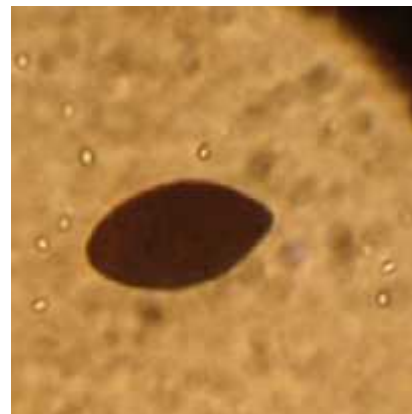
Dicrocoelium sp. (10Xx4X)



Schistosoma sp. (10Xx10X)



Fasciola .gigantica (10Xx4X)



Fasciola.hepatica(10Xx4X)

Plate 2

EGGS OF NEMATODES OBSERVED



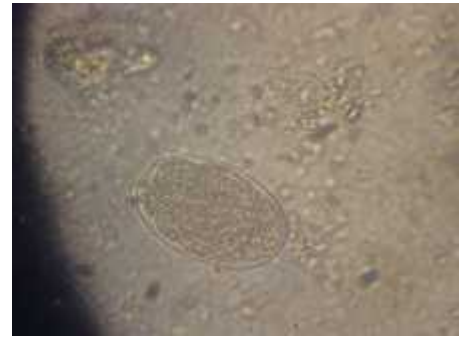
Strongyloides sp. (10Xx10X)



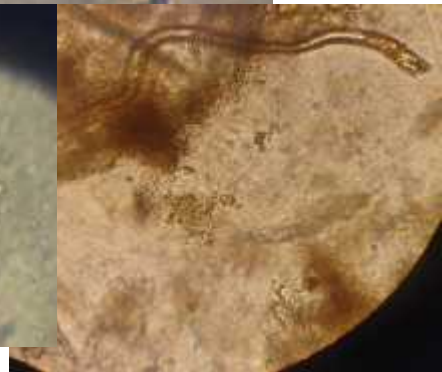
Trichostrongylus sp. (10Xx10X)



Haemonchus sp. (10Xx10X)



Dictyocaulus sp. (10Xx10X)



0Xx10X)

Dictyocaulus sp.larva (10Xx10X)

Plate 3



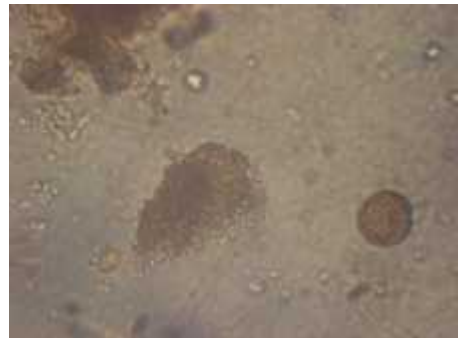
Trichuris ovis (10Xx10X)



Ascaris sp. (10Xx10X)



Trichuris sp. (10Xx10X)



Ascaris sp. (10Xx4X)



floatation and sedimentation



Taking aid of expert



collecting dung samples



Lab work at CVL, Tripureshwor, Kathmandu