1

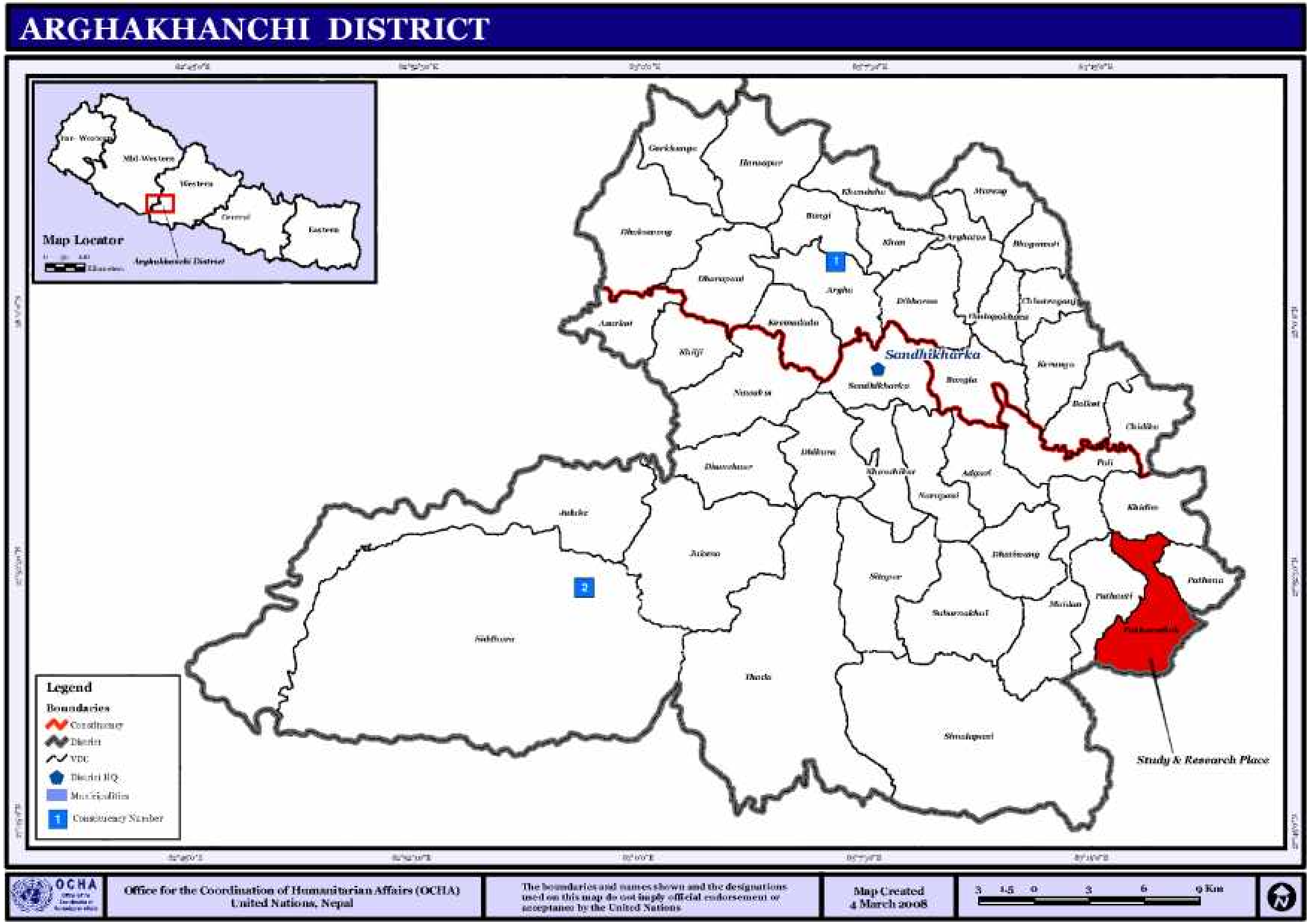
INTRODUCTION

1.1 Study Area

Nepal is a landlocked mountainous country surrounded by China to the north and India to the East, West and South. It is one of the richest countries in the world in terms of biodiversity due to its unique geographical position and latitudinal variation. Geographically, it is 80˚ 4” to 88˚ 12” East longitude and 26˚ 22” to 30˚ 27” North latitude. It is approximately 885 km in length and its mean width is 193 km with a total land area of 1, 47, 181 sq. km.

Arghakhanchi District, a part of Lumbini Zone, is one of the seventy-five districts of Nepal. The district with Sandhikharka as its district headquarters, covers an area of 1,193 km^2 and has population (2001) of 208,391. The district is bound by Palpa in the east, Gulmi in the North, Kapilbastu and Rupendehi in the south and Dang and Pyuthan in the west. About 68% of the total area lies in the Mahabharat Range, and the remaining lies in the Siwalik Region .It is situated between 305m-2575m above sea level, about 40% of its total area is covered by forest.

The study area Pokharathok VDC is located in Arghakhanchi and it is one of the small village of among 52 villages of Arghakhanchi district. It is situated at the mountainous region. In Pokharathok, people are mainly engaged in farming and domestication of buffaloes, cows and goats.


ARGHAKHANCHI DISTRICT
Map Locator
Far- Western
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Boundaries
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VDC
District HQ
Municipalities
Constituency Number
Office for the Coordination of Humanitarian Affairs (OCHA)
United Nations, Nepal
The boundaries and names shown and the designations used on this map do not imply official endorsement or acceptance by the United Nations
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1.2 <u>Background</u>

Nepal is an agricultural country with poor economy. Agriculture and livestock contributes almost 38% and 11%of the national GDP respectively (World Bank 2004). 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.

Buffalo (*Bubalus bubalis*) is one of the oldest and most important domestic livestock in Nepal. It belongs to the order Artiodactyla and family Bovidae. They are the traditional provider of milk, meat, manure and drought power. Many countries including Nepal is domesticating buffaloes since long time back, it's domestication was in evidence some 4,500 years ago in the Indus valley civilization (Cockrill 1970). Some of the Hindu religious book depicted it as a giant animal. Buffalo is known to thrive and adapt remarkably under severest climatic conditions where other domestic stock could barely exist.

Buffaloes are essential components of the mixed farming systems in the hills of Nepal, and are found in all parts of the country. They are distributed in more than 25 tropical countries stretching from southern Europe through India and China to the whole of south-east Asia .In Nepal majority of households raise at least one buffalo for milk and manure production and sell male calf or mature male for family income. Buffaloes are one of the most economically important livestock in Nepal as their products such as bones, skins, and goods made from their fetch are of great importance for the man. At present, about 4 millions buffaloes are found in Nepal, which is increasing every year in the last one and half decade (MOAC 2005).Epstein (1977) classified the buffaloes of Nepal into four regional types namely terai, hilly, midlands and Himalayan mountains on the basis of the size of their horns and the length and colour of their coats.

1.3 <u>Status of buffalo</u>

Livestock farming is an integral part of the farming system and buffalo contributes substantially in the livestock sector in Nepal. Buffalo, in Nepal has been acclimatized and adapted to wide range of environmental conditions. It is widely distributed from sub-humid regions of subtropics in terai to cool temperate regions in the hills and mountains (Shah and Shree G. 1981/82).

Buffaloes are the main producers of milk and preferred species as indicated by the increasing ratio of she-buffaloes to cows in many parts of the country. One million milking buffaloes are present in the country, where 70% of milk has been produced by buffalo only (MOAC 2005). The level of milk production also places Nepal among a few other countries, viz. India, Pakistan and Egypt where the buffalo milk has been evidently the main animal food (Moioli 1996). According to livestock production, Nepal has produced 8,94,591 metric tons buffalo milk in the year 2005 and the number of milking buffaloes was 10,50,977 in 2005 (VEC: national information 2005) and the annual production of milk was 300 kg per household in the year 2005(VEC : national information 2005).

Traditionally, meat and meat products originating from all domestic farm animals except cattle are consumed in Nepal. Animal slaughter is a common practice not only for consumption but also for religious sacrifices and other tradition ceremonies. According to livestock production, Nepal has produced 1,38,953 metric tons buffalo meat in the year 2005 and the annual production was 51 kg per household in the year 2005(VEC: national information 2005). The total population of animals that could contribute to national meat production is comprised of 3.4 millions buffaloes (CBS 2002). On analyzing the contribution of different animal species in national meat supply, it was evident that buffaloes contribute about 57.4% of total meat supply (Joshi et al. 2003). The demand for buffalo meat in Kathmandu is high. High demand of buffalo meat may be due to leanness, cheaper in price as compared to other meat and there is no religious taboo in buffalo meat (MOAC 2005).

1.4 <u>Endoparasitism</u>

Parasites are classified as endoparasites and ectoparasites on the basis where they live inside or on the body cavity. Buffaloes are susceptible to internal parasites and may harbor several species of worm at any time.

Endoparasites are those organisms living in their hosts, in the gut, body cavity, liver, lungs, gall bladder and blood and within the intestinal cavities, tissues or cell of the host. Such forms almost live a completely parasitic existence. They totally depend upon their host and causing infection to them. For example, *Tricostrongylus* sp., *Fasciola* sp., *Schistosoma* sp., are typical endoparasites.

Buffalo diseases have been identified as one of the major factor which have caused substantial economic loss to the poor subsistent farmers in the developing countries (Othman and Baker 1981).The parasitic diseases are usually very important factors which cause the death of many buffaloes yearly. Parasites usually include gastro-intestinal helminthoses, coccidiosis, fascioliosis and mange (Othman and Baker 1981). Cockrill (1974) stated that the buffalo is exposed to a higher risk of infection with snail born helminthes due to the animals propensity to seek rivers, pools or swamps for wallowing. The infection with fluke, tapeworms, and roundworms are responsible to lower the overall production both by way of morbidity and mortality.

1.5 Trematoda

Trematodes commonly known as flukes, often live in the bile ducts or small intestine and may also affect the lungs. Some are ingested but some burrow into the skin for access. Their eggs are passed with the faeces of the host. Trematode especially include *Fasciola* sp., *Dicrocoelium* sp., *Schistoma* sp. and *Paramphistomum* sp. (Shah and Agrawal 1990).

Fascioliasis is well known parasite of herbivorous animals. It has worldwide distribution on the animal reservoir host. A large variety of animals such as cattle, buffaloes show infection rate that varies from 70% to 90% in some areas. The different local names of this disease, such as namle, mate, lew etc. in different regions, are proof of its continued existence for many years in the 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 the agriculture sector worldwide with over 600 million animals affected (Hansen 1994).

The economic loss due to fascioliasis in Nepal was estimated to be Rs.14.2 crore (Lohani and Rasaili 1995). The prevalence of fascioliasis ranging between 50% to 90% has been reported in buffaloes (Mahato 1993). In addition fascioliasis is now recognized as an emerging human disease.

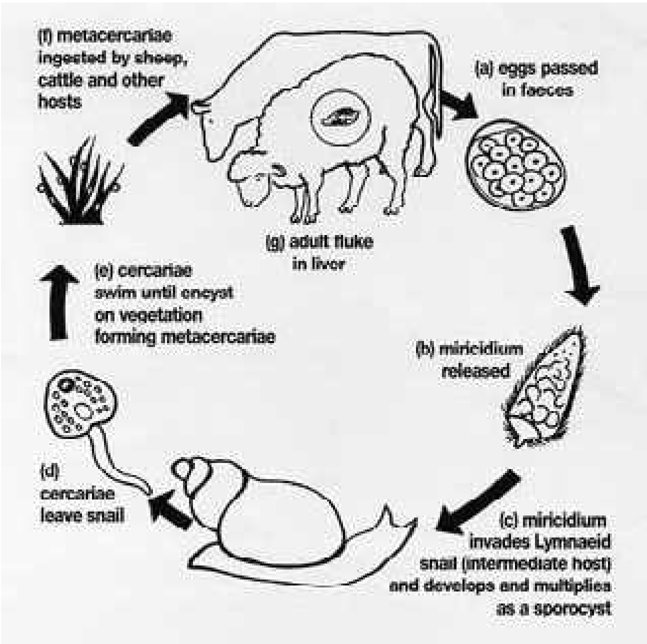


Fig. 1: Lifecycle of *Fasciola* sp.

The important *Lymnaeae* species of snail involved in the transmission of fascioliasis vary in their geographical distribution in the world. Man and herbivorous animals (sheep, cattle, buffalo, etc.) acquire infection by the ingestion of moist and raw aquatic plants such as water cress harbouring infective metacercariae. The metacercariae mature to become adult worms and lay eggs which are passed in the faeces. On coming in contact with water they mature and invade the molluscan host, the fresh water snail and undergo development. The mature cercariae emerge out of the snail and encyst on aquatic grasses, plants and develop into metacercariae which is the infective stage of the parasite. Similarly, dicrocoeliasis is caused by the liverfluke *Dicrocoelium dendriticum* a common parasite of the biliary passages of sheep and other herbivorous and omnivorous animals. Most of the human infections occurs by the ingestion of the liver of infected sheep.

Fascioliasis in ruminants ranges in severity from a devastating highly fatal disease in sheep to an asymptomatic infection in cattle. Acute fascioliasis occurs seasonally and is manifest by anemia and sudden death. Cases of chronic fascioliasis occur in all seasons and the clinical signs may include anaemia, reduced weight gain, decreased milk production, unthriftiness, submandibular odema and possibly death. The subsequent simultaneous migration of many immature flukes through the liver parenchyma causes severe destruction of liver tissue of the host. The penetration of the liver capsule by a large number of young flukes results in an inflammatory response of the capsule (peri-hepatitis). The blood sucking activities of the flukes irritate the lining of ducts, resulting in an inflammatory response and the associated blood loss results in anaemia. Likewise the trematode *Dicrocoelium*, gastrointestinal trematode *Paramphistomes* and *Schistosomes* the blood trematodes affects the host abundantly.

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 areas in monsoon are another potential source of *Fasciola* infection. Therefore in the Nepalese
hills, the major risk period of *Fasciola* infection is during post monsoon and winter months.

Schistosoma spp. are the only trematodes living in the blood stream of warm blooded hosts. The blood stream is rich in glucose and amino acids. So along with the plasma and blood cells, it represents an environment which is suitable for egg producing trematodes. *Schistsoma* spp. causes disease called Schistosomiasis or Bilharziasis and is the main helminth diseases. The infections are often manifest by acute intestinal signs, the mucosa of the intestine is severally damaged and the animal develops profuse bloody diarrhea, dehydration and loss of appetite. Most of the species like *Schistosoma spindalia, S. japonicum* and *S. bovis* has been reported among buffaloes from Surkhet district (Ghimire 1987).

1.6 Cestoda

Cestodes found in gut are acquired by eating contaminated food or water found to be largely affecting the ruminants. This group comprises of 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 have reported from Asia and Africa (Karki 2005).

Moniezia sp. in ruminants of the buffalo and cattle causes infections by ingesting herbage contaminated with the mites carrying the infective stage of the parasite. Heavy infections cause poor growth and diarrhoea in lambs.

Taenia saginata usually called cow or buffalo tapeworm has two hosts viz., definitive host man and intermediate host cow or cattle. It is also called beef tapeworm. The worms (segements) passes out along with the faeces of human being and when ingested by cattle, infects them on reaching alimentary canal of the host, the eggs hatch out and liberated, they penetrate the gut wall and enter mesenteric lymphatics and finally reaches circulation. Then they invade the muscular tissue and undergo further development.

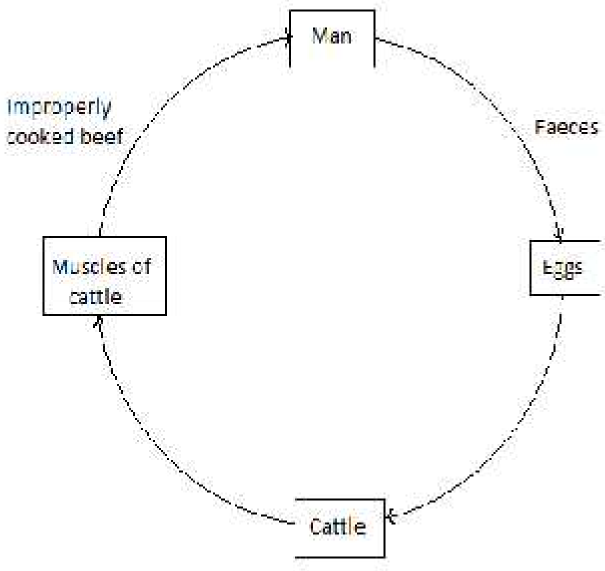


Fig. 2: Lifecycle of *Taenia* sp.

1.7 Nematoda

The most important and widely prevalent nematodes of buffalo are trichostrongyle group i.e. *Haemonchus* sp., *Ostertagia* sp., *Trichostrongylus* sp., *Cooperia* sp., *Oesophagostomum* sp.,etc. These nematodes in the small intestine may cause severe damage to the intestinal mucousal membrane with. *Toxocara* sp., and *Dictyocaulus* sp., has the worldwide distribution and the prevalence is higher in buffalo and cattle (Karki 2005).

Trichostrongyliasis is an infection of the gastrointestinal tract of herbivorous animals and man is the accidental host caused by the members of the genus *Trichostrongylus.* The infection is acquired by ingestion of contaminated vegetables or drinks with the filariform larvae. Strongyloidiasis is an intestinal infection of man caused by the penetration of the skin by the filariform larvae of *Strongyloides 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, buffalo 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.

Among roundworms of buffalo, the commonest are *Trichostrongylus* sp., *Ascaris* sp., *Strongyloides* sp. Female roundworms lay microscopic eggs that pass in the manure of buffaloes. Within few days the larva hatches from the egg. The larva passes via second and third stage. They infect the pasture. Buffaloes get infected when they graze on the contaminated pasture. The larva mature in the intestine, mate and begins laying eggs. Adult roundworms can cause anaemia, diarrhea, poor growth and even death.

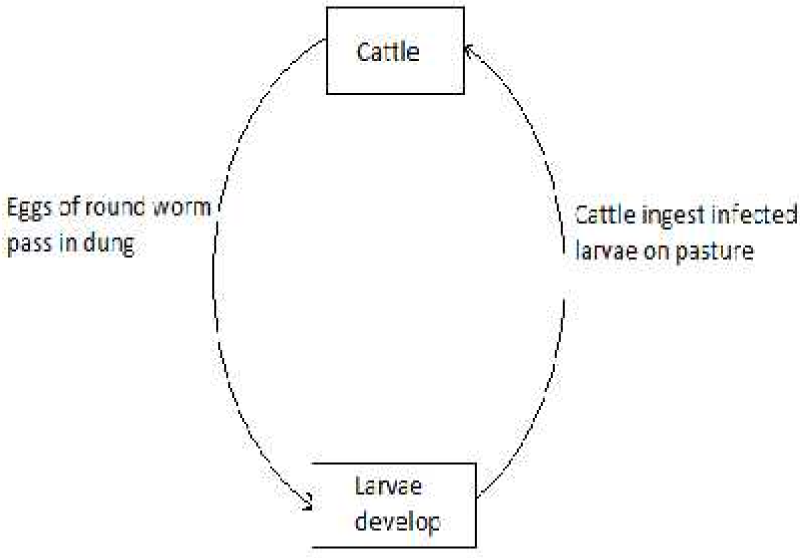


Fig. 3: Lifecycle of gastrointestinal roundworms in general

The pathogenic effect of gastrointestinal parasites may be sub-clinical or clinical. Young animals are most susceptible. The effect of these parasites is strongly dependent on the number of parasites and the natural status of the animals they are infecting. The clinical sign comprise of weight loss, reduced feed intake, diarrhea, mortality, reduced carcass quality and reduced wool production/quality. Severe blood and protein loss into the abomasums and intestine due to damage caused by the parasite often results in oedema in the sub-mandibular region. Some nematode species, especially *Haemonchus* is the most pathogenic of the blood suckers and infectious with large numbers of this parasite often result in severe anaemia in the host.

Cases of gastro-intestinal nematodes have been reported from several countries like Africa, Asia, Ethiopia, Sahel and parts of Somalia and Sudan.The widely prevalent nematodes *Haemonchus, Ostertagia, Trichostrongylus, Mecistocirrus, Cooperia, Nematodirus, Oesophagostomum* and *Bunostomum*. *Toxocara* and *Trichuris* are quite pathogenic to animals throughout the world. Infections with gastro-intestinal nematodes usually occurs by the ingestion of eggs by young calves.

Toxocara sp. cause damage to the liver and lungs. The presence of the adult parasites in the small intestine is often associated with diarrhea and reduced weight gain. In untreated cases and heavy infections, the mortality rate may be upto 35-40 percent of infected animals, and it is believed to be the most serious disease of buffalo calves in Southeast Asia.

But, gastrointestinal parasites are major constraint in livestock production in humid tropics of Southeast Asia. The parasitic infections are worldwide problem for both small and large scale farmers. They cause economic losses in a variety of ways: they cause losses through lowered fertility, reduced work capacity, involuntary culling, a reduction in food intake and lower weight gains, lower milk production, treatment costs and mortality in heavily parasitized animals .

1.8 Significance of the study

The infection of helminth parasites in buffaloes can cause significant economic loss leading to the poor health, reduced growth, decrease in milk production etc. This study is an effort to determine the different helminth parasites in buffaloes and the rate of infection in them.

This study has been focused on the intestinal helminth parasites of buffaloes in Pokharathok VDC .This study will be useful as it will give information regarding the prevalence of helminth parasites of buffaloes seasonally i.e. during summer and winter. Miss Gouri Mukhia has worked previously on the buffaloes intestinal parasites and it was found to be 83% positive cases. The present study is an illustration of the previous work in addition, revealing the prevalence of helminth parasite seasonally. This study will also form a base for the future researchers and investigators.

1.9 Limitations of the study

Research studies face many problems, so obviously have limitation to the study. The present study no doubt, bears the following limitations.

- This academic study has been carried out for the partial fulfillment of the requirements for the Master’s Degree in Zoology at Tribhuvan University, Kathmandu Nepal.

- The time for this study was also limited and carried out within two seasons only.
- The research has limited regarding finance and time constrains. The minimum percentage of the total cattle selected houses of the studied area is taken as key information for this study.
- Due to the lack of sophisticated instruments the identification of parasites was done upto genus level only.

1.10 Objectives

1.10.1 General Objective

To study the seasonal prevalence of intestinal helminth parasites in buffalo of Pokharathok VDC of Arghakhanchi district.

1.10.2 Specific Objectives

- To identify the helminth parasites.
- To determine the degree of seasonal prevalence of trematode, cestode and nematode.
- To determine the number of eggs present per gram of faeces.
- To develop the recommendations for the planning regarding the control of helminth parasites in buffalo.

1.10.3 Hypothesis

H_o = There is no significant difference in prevalence of helminthes parasites in winter and summer.

H_1 = There is significant difference in prevalence of helminthes parasites in winter and summer.

2
LITERATURE REVIEW

Before 17th century, knowledge of parasitology was limited to ectoparasites like lice and 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 Franscisco Redeii, 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, Leeuwonhoek perfected microscope and discovered protozoan parasites. Rudolf Leuckart considered as "father of parasitiology". The word 'Parasites' derived from Greek. It means situated beside. In the field of parasitiology, his studies of the liver fluke, *Taenia* and *Trichina spiralis* were highly significant. His work with parasitism infections proved that Taenia saginata occurs only in cattle and that *Taenia solium* occurs only in pigs.

Parasites are the organisms which depend on the host for their shelter, food and metabolic activities. The association between a parasite and a host is known as parasitism. Parasitism in actual sense can be defined as "an intimate and obligatory relationship between two heterospecific organisms during which the parasite, usually the smaller one of two partners, is metabolically dependent on the host".

Parasites originated from their free- living ancestors; they evolved along with their hosts. Consequently certain groups of parasites are limited to specific groups of hosts. This evolutionary relationship between parasites and their hosts may give valuable information about the relationship between different groups of hosts. For example, the moderately evolved monogentic trematodes parasitize only fish, while the highly evolved dignetic trematodes are found not only in fish but more commonly in higher vertebrates. Furthermore, the more advanced dignetic trematodes tend to occur in the highest hosts groups.

Parasitism is one of the major problems affecting buffaloes and cattle. The associated economic losses are inflicted in the form of low productivity, reduced product quality, high treatment cost and mortality (Gupta et al. 1978). Buffalo diseases have been identified as one of the major factor which have disrupted the development of the industry in Asia and have caused substantial economic loss to the poor subsistent farmers in the developing countries (Othman and Baker 1981).The parasitic diseases are not less important in buffaloes than other infectious diseases. Parasitic zoonoses are distributed worldwide and constitute an important group of diseases affecting both the humans and animals. Many of the parasitic zoonoses produce significant mortality and morbidity in the human and are responsible for the major economic losses by affecting the animal health. Most of the papers have been presented and published largely after the outbreak of the helminthic diseases among human and animals. Literatures exist in helminthic parasites as the diseases continued to survive with new threats. Major researchers efforts that have been directed towards helminth parasites, the portions of the work and reports related to the epidemiology of helminth parasite have been mentioned here.

2.1 Literature review in global context:

Srikitjakarn et al. (1987) conducted metaphylactic deworming program for buffalo calves in north-east Thailand. Hundred new born swamp buffalo calves from 3 villages in north-east Thailand were divided equally into treatment and control groups. Eggs excretion rates for the roundworms *Strongyloides papillosus* and *Toxocara vitulorum* were recorded as high as 85% and 58% respectively during the first 3 months of life.

Marques and Serofernerker (1999) conducted a study for *Fasciola hepatica* infection in the state of Rio Grande Do Sul, Brazil. They examined 105 slaughtered buffaloes at a meat packing plant between April 1999 and Nov 1999, in Viamao in Ria Grande Do Sul in Southern Brazil. The occurrence rate of Fasciola hepatica was 20% for buffaloes. The studied areas represent important endemic region of fascialosis.

Iassan et al. (2000) conducted a study on prevalence dynamics of fascioliasis versus other gastro-intestinal helminthes in both buffaloes and cattle in Giza 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 has *Fasciola* sp. and 2.5% helminth eggs in their faeces. The helminths included mainly other gastro-intestinal parasites such as *Paramphistomum* spp and *Moniezia* spp.

Monthly and seasonal prevalence of parasites investigated the spring season was the most favourable one for infection with predominant one for infection.

Oliveira et al. (2002) reported hepatic fascioliasis in buffaloes in Abattoir of Vale Do Ribeira, Sao Paulo-Brazil. Hepatic fascioliasis among 130 slaughtered buffaloes was studied at the Cajati Abbatoir from September 2002 to December 2002. The incidence of livers infected by *Fasciola hepatica* was 75%.

Maqbool et al. (2002) undertook an epidemiological studies at slaughter houses, livestock farms, veterinary hospitals and on household buffaloes under the different climatic conditions existing in Punjab province. Infection rate was 25.59% at slaughter houses, 20.16% at livestock farms, 13.7% at veterinary hospitals and 10.5% at household buffaloes. Overall highest 24% seasonal prevalence in all types of buffalo was recorded during autumn, spring and winter. It was found that a higher infection rate was recorded in older buffalo than in youngsters (below 2 years age). Buffalo of either sex were equally affected.

Bhutto et al. (2002) conducted a study on prevalence of gastrointestinal helminths in buffalo calves. A total of 200 faecal samples were randomly collected from either sexes of buffalo calves of different age groups. On microscopic examination of the samples, prevalence of helminths in buffalo calves was recorded as 47%. Out of these, only 18% buffalo calves excreted eggs in their faeces that ranged from 100-400 eggs/g (epg) while 7,13,5.5 and 3.5% calves excreted eggs in faeces that ranged from 401-800, 800-1200, 1201-1600,1601 and above eggs/g, respectively. Further it was observed that 43 and 4% buffalo calves were positive for nematodes and trematodes, respectively but mixed infections were observed in 1.5% calves. Cestodes were not detected in any samples examined. Four different species of helminths were identified, the species were: *Toxocara vitulorum*(33%), *Ostertagia ostertagi*(8%), *Trichuris ovis*(2%) and *Fasciola gigantica*(4%). The highest egg counts were recorded in 1-120 days old calves. A slightly higher prevalence(48.30%) of helminths was found in female than male(45.12%) calves.

Carabin et al. (2003) conducted a study to estimate the sensitivity and specificity of the Danish bilharziasis in buffaloes in Philippines. Faecal samples from animals were collected on five consecutive days in four villages between January to July 2003. The faecal samples were analysed with the filtration and sedimentation Danish bilharziasis laboratory technique. The prevalences and 95% credible intervals of *Schistosoma japonicum* adjusted for imperfect sensitivity and specificity in buffaloes were 6.3% respectively.

Akhtar and Mohammad (2003) conducted a study on prevalence of helminthiasis in buffaloes in colony, Hyderabad. The prevalence of helminthiasis was found to be 15.2% in buffaloes. Out of 500 samples examined, 9.2% were infected with nematodes and 5.4% were infected with trematodes respectively. Mixed infection was observed in 0.6%. The chief helminths identified were *Toxocara vitulorum* 3.6%, *Fasciola gigantica* 3.2%, *Oesophagostomum radiatum* 3%, *Strongyloides papillosus* 2.4%, *Fasciola hepatica* 2.2%, *Ostertagia ostertagi* 1%, *Paramphistomum cervi* 0.8% and *Trichuris* sp. 0.2%.

Magdoub AA-EI and Sayed IA-EI (2003) studied on relationship between one system of climatic conditions on the helminthic infection rate at middle delta, Egypt. 1178 buffaloes owned by farmers from 160 herds belong to 32 villages in Egypt were randomly chosen to study factors which influence the infection with gastro-intestinal parasites. Relationship between number of parasites, herd size resources of water and season of the year were investigated. The main results showed (i) *Fasciola gigantica* infection recorded the highest percentage 48.04% followed by Neoascaris *vitulorum* and *Eimcria* sp. (ii) percentage rates of parasitic infection (single, double or triple) in each animal were 62.80%, 29.43% and 7.77% respectively. (iii) Infection rate tended to increase with herd size in most cases. (iv) Resource of water had highly significant effect on infection rate.

Yadav et al. (2003) reported the prevalence rate of fascioliasis and amphistomiasis high in terai region of India followed by hills and plains, respectively. Buffaloes were the most susceptible hosts followed by cattle and sheep.The incidence of *Fasciola gigantica* infection was high in post monsoon season September to January with peak in January in the terai.

Yadav et al. (2004) reported the highest incidence of G.I nematodiasis in goats followed by buffaloes and cattle. *Haemonchus, Trichostrongylus, Bunostomum, Oesophagostomum* and *Strongyloides* species were the main parasites recovered from intestine of sheeps, buffaloes and goats.

Yadav et al. (2005) reported various species of Sarcocystis viz. *S.fusiformis* and *S. levinei* in buffaloes. The overall prevalence was 73.72% in buffaloes.

Borthakur and Das (2006) reported the incidence of monieziosis in cattle and buffalo calves of Guwahati. Faecal samples of calves of 240 cattle and 60 buffalo were examined for Moniezia spp. infection. The infection rate was 5%, 13.75% and 6% in indigenous, cross-bred cow calves and buffalo calves, respectively. Average eggs per gram of faeces was 56 in buffalo calves, 96.9 in crossbred calves and 19.6 in local cow calves, respectively in villages around Guwahati, Assam.

Singh et al. (2006) reported the prevalence of common helminthic infections in buffaloes was studied by examining the various organs and feacal samples (200) of buffaloes calves (below 3 months of age) collected from nearby villages of Mathura during different seasons.Maximum incidence of trematodes was detected during summer season (43.66%) followed by rainy (28.95%) and winter season (20.51%), while higher infection of cestodes was noticed during winter season (36.36%) than summer (26.67%) and rainy seasons (25%) while incidence of nematodes was found more during summer (71.59%) and rainy season (69.09%) as compared to winter season (21.05%).

Singh et al. (2007) conducted the efficacy of Dewormin, a broad spectrum anthelminthic was assessed in buffalo calves clinically infected with strongyles nematodes.Twelve buffalo calves were divided into two groups of 6 animals each. Eggs per gram (EPG) of faeces of all these calves were estimated, which ranged between 2400-9600 in group I and 2400-3500 in group II. Calves of group I were dosed with Dewormin @20g twice daily for two days and group II calves served as infected control. Efficacy of Dewormin was found cent-percent as EPG became zero from 7 to 28 days post-infection.

Kaur et al. (2008) reported the prevalence of gastrointestinal parasites in cow/buffalo (82.35%) has been reported from Patiala. The gastrointestinal parasites detected in cow/buffalo were *Toxocara vitulorum* (78.57%), *Haemonchus* sp. (57.14%) followed by *Cryptosporidium* sp. (50%),*Eimeria* sp. (50%), *Oesophagostomum* sp. (42.86%) and *Trichuris* sp. (14.29%).

Bilal (2009) reported the prevalence of gastrointestinal parasites on calves (80 of each buffalo and cow). The results indicated that 75% buffalo and 56.25% cow calves were positive for worm infestation. The highest prevalence of nematodes was recorded followed by mixed infection and cestodes, and no calf was found positive for trematodes. Buffalo and cow calves between 1 to 6 months of age exhibited highest prevalence (86.67, 69.05%) compared to the age group of 7 to 12 months (60, 42.10%). Calves on grazing were heavily infected 983.33% buffalo calves, 75% cow calves) than those of stall fed (70% buffalo calves, 46.16% cow calves). Buffalo male calves were more affected (88.38%) than female calves (59.46%) whereas, the same was for cow calves.

Sreedhar et al. (2009) worked on the influence of season on the prevalence of helminthic infection among cattle and buffaloes of Anantapur district of Andhra Pradesh indicated that the over all prevalence of parasitic infection was 336 (42.0%) out of 800 faecal samples. The highest incidence was observed by *Amphistomes* 180 (22.5%) followed by *Coccida* 65 (8.1%), *Strongyles* 61 (7.6%) and *Fasciola* 30 (3.8%). The incidence of parasitic infection was higher in monsoon season as compared to that of summer and winter.

Raza et al. (2010) reported the point prevalence of *Toxocara vitulorum* in buffalo and cattle slaughtered at Multan abattoir. Gastrointestinal tracts of 94 buffaloes and 48 cattle were examined for *Toxocara vitulorum*. Prevalence of *Toxocara vitulorum* was 63.83 and 37.50% in buffalo and cattle, respectively.

Wadhwa et al. (2011) conducted a study on prevalence of gastrointestinal helminthes in cattle and buffaloes in Bikaner, Rajasthan, India. In the year 2007, a total of 200 faecal samples, comprising of 100 samples each from cattle and buffaloes were analyzed to confirm the presence of gastrointestinal parasitic infection. 24 (12%) samples were found positive for *Strongyle* eggs. 11% cattle and 13% buffaloes were found to be positive for G.I helminthosis. The prevalence in cattle varied from 9.09 to 12.50 in different locations. Prevalence range was slightly higher in buffaloes which ranged between 10.52 to 14.81.

Athar et al. (2011) conducted a study to determine the point prevalence of various helminths of cattle and buffalo population of district Toba Tek Singh, Pakistan and economic benefits of deworming with oxyclozanide.Out of 540 faecal samples examined, 205 (37.96%)were found infected with helminths. Significantly higher prevalence of helminths was recorded in buffaloes (40%) 112/28) as compared to cattle (35.77%, 93/260). *Oesophagostomum, Cooperia, Trichostrongylus,Strongyloide, Ostertagia,* *Fasciola hepatica, Fasciola gigantica* and *Haemonchus contortus* were the helminth species identified in the study area. Oxyclozanide medicated buffaloes (E=96.66%) and cattle (E=95.64%) showed a significant decrease in faecal egg counts on day 14 post-treatment. An average daily increase of 0.89 and 0.71 liters of milk along with 0.42 and 0.37% more fat per buffalo and cattle, respectively was observed in oxyclozanide medication. It can be concluded that single dose of oxyclozanide is effective against all bovine helminths.

Samal et al. (2011) conducted a study on simultaneous infestation of a buffalo calf with *Ascaris* and *Strongyloides*: A case study. *Toxocara vitulorum* occurs in the small intestine of Indian buffalo, and is found in many places of the world. Small intestinal infestation with *Strongyloides papillosus* occurs in ruminants. However, we diagnosed a nondescript Indian buffalo calf infested simultaneously with *Toxocara vitulorum* and *Strongyloides papillosus* and treated it with standard regimen and found the subject cured.

2.2 <u>Literature review in context to Nepal</u>

Most of the Nepalese farmers are small land holder and they keep animals mainly cattles and buffaloes for converting low quality forage and crop-by-products to milk and meat product for home consumption, manure and power required for their livelihood. There are many factors responsible for limiting production of the ruminants. Parasitic infestation is one of the most important causes for this. The helminthiasis has been emerged as an important parasitic disease since from the past decades in the world, but in Nepal it had been reported upto certain extent.

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.In Nepal, Parajuli worked in buffaloes with special references to *Fasciola* sp. and *Paramphistomes* in Surkhet district. Lohani and Jaeckle also worked in *Fasciola* species in Palpa. Ghimire worked on incidence of common diseases of cattle and buffaloes in Surkhet district .Mahato reported that only the bottom portion of rice straw contains metacercariae of *Fasciola* and these species could remain viable until March. Therefore, the author recommended that feeding of the bottom portions of the rice straw should be avoided in order to control fascioliasis in stall fed animals.

Parajuli (1967-92) reported flukes in buffaloes from Surkhet district as *Fasciola* sp.56.75% and P*aramphistomes* sp. 35.13%.

Lohani & Jaeckle (1981-82) conducted a study to identify *Fasciola* species in Palpa. Liverfluke specimens were collected from five slaughtering places of Tansen in the last week of July and beginning of August, 1981. Identification was done by Hoerning Institute of Parasitology, University of Bern and results were mixed infections with predominance of *Fasciola gigantica*.

Ghimire (1987) conducted a study on incidence of common diseases of cattle and buffaloes in Surkhet district. The endoparasitic infections recorded were Fascioliasis, Toxocariasis, Paramphistomiasis, internal Schistosomiasis and Monieziasis.

Acharya (1996) conducted a study on efficancy of trichobendazole and oxyclozanide 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* infection. However, buffaloes did not response in either treatment groups as effectively as cows.

Mahato et al. (1997, 2000) reported on epidemiological basis of the control of fascioliasis in Nepal. Despite increased awareness of the diseases and massive increase in the use of anthelmintics, they found no impact on the prevalence of the disease in the last two decades. Failure to control the disease were mainly due to lack of information about its epidemiology in the country.

Shrestha and Joshi (1997) carried out a study to evaluate the effectiveness of a strategic drenching against fascioliasis in cattle in the western hills of Nepal. Faecal samples were collected at monthly intervals, and were examined by standard sedimentation method for the presence of *Fasciola* eggs. The strategic drenching reduced the overall infection in treated animals.

Sharma (1998-99) conducted a study on parasitic infection in animals of Panchthar district. Ascariasis (43.69%) was found to be the most common followed by Fascioliasis (40.12%) and *Paramphistomum* (16.20%).

Pandey; Mahato; and Gupta (2002) studied prevalence of *Fasciola* infection in *Lymnaea* snails and buffaloes in Devbhumi Baluwa VDC of Kavre district. The infection rate in rice field was found 1.67% in springs 1.40% and in irrigation channels 0.99%.

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

Adhikari et al. (2002/2003) conducted a study on the prevalence and diversity of *Fasciola* sp. in buffaloes and cattle in areas of Kathmandu valley from 23 April 2003 to 30 June 2003. The prevalence of *Fasciola* sp. was found to be 36% and 61% in cattle and buffaloes respectively. Other parasite were also found during the study which includes 48% *Paramphistomum* sp. in cattle. Similarly *Paramphistomum* sp. 11% and *Strongyloides* sp. 10% were isolated from buffaloes and cattle. The study concluded that the potential role of liver flukes in the livestock production and development is highlighted.

Jaiswal (2006) carried out a study on fascioliasis in ruminants at Dhanusa district based on examination for fecal sample brought to DLSO, Janakpur from June15 to November 15, 2005. A total of 2655 fecal samples were examined out of which 70.70% were positive for overall parasitic infestation. Among these prevalence of fascioliasis was found to be 43.43% followed by paramphistomiasis 38.09% and roundworms 13.43%. The prevalence of *Fasciola* infection found in buffalo was 56.02%, in cattle 49.36% and in goat was 31.25%.

Mukhia (2007) studied intestinal helminth parasite of buffalo and found 220 (83.96%) positive samples among 262 stool samples from Santungal, 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 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%).

3

MATERIALS AND METHODS

This study is carried out for determining the seasonal prevalence of helminthes parasites in buffaloes. The stool samples were collected from the study area and brought at Central Veterinary Laboratory, Tripureshwor for laboratory diagnosis. Samples were collected from buffaloes which were not treated with anthehelminthic.

3.1 Study design

The study design is based under laboratory examination.

3.2 Sample size

A total of 250 stool samples were collected from Pokharathok VDC. The number of samples taken during winter were 120 and during summer were 130.

3.3 Precautions and preservation

To ensure better condition during sample collection the following precautions were taken.

a) The fresh stool samples were taken.
b) The samples were collected in airtight container to prevent desiccation.
c) 3-4 drops of 10% formalin were used to fix stool samples.

3.4 Laboratory apparatus and materials

a. Motor and pestle
b. Test tube
c. Cotton
d. Cover slip
e. Slides
f. Glass rod
g. Centrifugal machine
h. Gloves
i. Tea strainer

j. Microscope
k. Pasteur pipette
l. Refrigerator
m Dropper
n. Rack

3.5 Chemicals

a. Formalin
b. Distilled water
c. Zinc sulphate solution (33%)
d. Methylene blue

3.6 Stool Examination

The stool samples were examined by simple floatation technique, sedimentation technique and stoll's counting method.

3.6.1 Simple Floatation technique

This technique is widely used for the detection of nematode and cestode eggs. As their eggs are lighter and small, they can float in the floatation liquid. 3 gm of stool sample was taken in a beaker and 42 ml of 33% zinc sulphate solution was added. The sample was grinded lightly with the help of pistil and motor and filtered with a tea strainer. The filtered sample was poured into plastic tube of 15 ml and centrifuged at 1000rpm for 5 minutes. To form convex surface at the top of the tube, some more zinc sulphate solution was added. A cover slip was placed over the top of the tube so that zinc sulphate solution touches the coverslip and then the coverslip was placed on a slide and examined at 10x.

3.6.2 Sedimentation technique

This technique is used for the detection of trematode eggs. It provides good results as the eggs of trematode is bit heavier than the other eggs and deposited at the bottom. 3gm of stool sample was taken in a beaker and 42 ml of 33% zinc sulphate solution was added. The sample was grinded lightly with the help of pistil and motor and filtered with a teastainer. The filtered sample was poured in a plastic tube of 15 ml and centrifuged at 1000rmp for 5 minutes. The eggs get deposited at the bottom of the test tube after the centrifugation. With the help of pipette, a drop of deposited material was taken on the slide and then added drop of methylene blue into it and examined under the microscope at 4x and 10x.

3.6.3 Stoll's counting method

It is the easiest quantitative method to count the number of eggs present in the field without the help of Mcmaster. The eggs of helminth parasites has been observed through the microscope present on the slide and were counted.The number of eggs of trematode, nematode and cestode was detected and counted. The total number of eggs determines the number of eggs present per gram of faeces.

3.7 Key for trematodes, cestodes and nematodes

- -Chaudhari, S.S; Gupta, S.K; Banerjee, D.P; Bhatnagar, P.K; and Ruprah, N.S. (2003). General helminthology. *Manual of veterinary helminthology; 1*: 10-184.

- -Investigatio coprologica animalium domesticum/magnorum/bos et ovis.*Ianssen Pharmaceutica Ex Scientia Progressus.*

- -Parijia, S.C. (1990). Helminthic infections: trematode, cestode and nematode. *Review of Parasitic Zoonoses*; 1: 41-393.

- -Soulsby, E.J.L. (1982). Eggs of worm parasites. *Helminths, Arthropods and Protozoa of Domesticated Animals;* 7th edition, 1: 24-338.

- -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.*

4

RESULTS

The result has been divided into five parts:

1. General prevalence of helminth parasites in buffaloes
2. Seasonal prevalence of helminth parasites
3. Class wise seasonal prevalence
4. Identification of the eggs of helminth parasites
5. Multiple and single infection.

4.1 General prevalence of helminth parasites

Out of 250 stool samples, 170 (68%) samples were found to be positive during both the seasons i.e. summer and winter. Therefore, the general prevalence rate of helminth parasites in buffaloes was found to be 64%. This study showed 74.11% trematode infection, 65.88% nematode infection and 15.88% cestode infection. The total numbers of genera observed during examination were 21 in number i.e. 8 genera of trematodes, 1 genera of cestode and 12 genera of nematodes were observed (Fig 4). The general and overall prevalence percentage of each species is given in table 1.

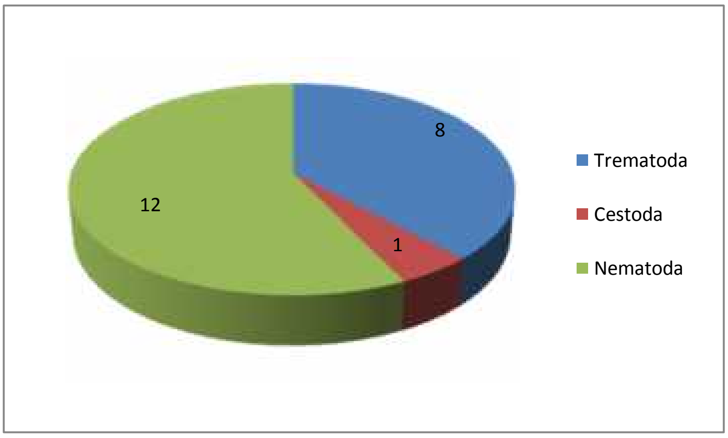


Fig. 4: Observed genera of different classes.

Table 1. General prevalence of helminth parasites

S.No.	Class	Genera of Helminth	Percentage
1.	Trematode	*Fasciola*	29.41
2.		*Paramphistomum*	11.76
3.		*Dicrocoelium*	12.94
4.		*Schistosoma*	38.23
5.		*Gastrothylax*	2.35
6.		*Fischoederius*	1.17
7.		*Ornithobilharzia*	0.58
8.		*Skrjabinema*	5.29
9.	Cestode	*Moniezia*	15.88
10.	Nematode	*Strongyloides*	13.52
11.		*Trichostrongylus*	5.88
12.		*Toxocara*	34.11
13.		*Ascaris*	18.23
14.		*Chabertia*	1.76
15.		*Trichuris*	5.88
16.		*Dictyocaulus*	0.58
17.		*Oesophagostomum*	1.17
18.		*Capillaria*	1.17
19.		*Haemonchus*	1.76
20.		*Ostertagia*	2.94
21.		*Cooperia*	0.58

The result indicates that maximum infection was caused by the genera of trematodes (74.11%) followed by nematodes (65.88%) and cestodes (15.88%). Highest prevalence was shown by *Schistosoma* (38.23%) and lowest prevalence was shown by *Ornithobilharzia* and *Dictyocaulus* (0.58%).

4.2 Seasonal prevalence of helminth parasites

Out of 120 samples of winter, 55 samples were positive (45.83%) and that of summer samples 115 samples were found positive (88.46%). The rate of prevalence of helminth was found more during summer i.e. 88.46% than in winter i.e. 45.83%. The difference in the prevalence of different genus of helminth parasites during both the seasons altogether were found statistically significant (χ^2 = 740.15, $P<0.05$, d.f. = 20).

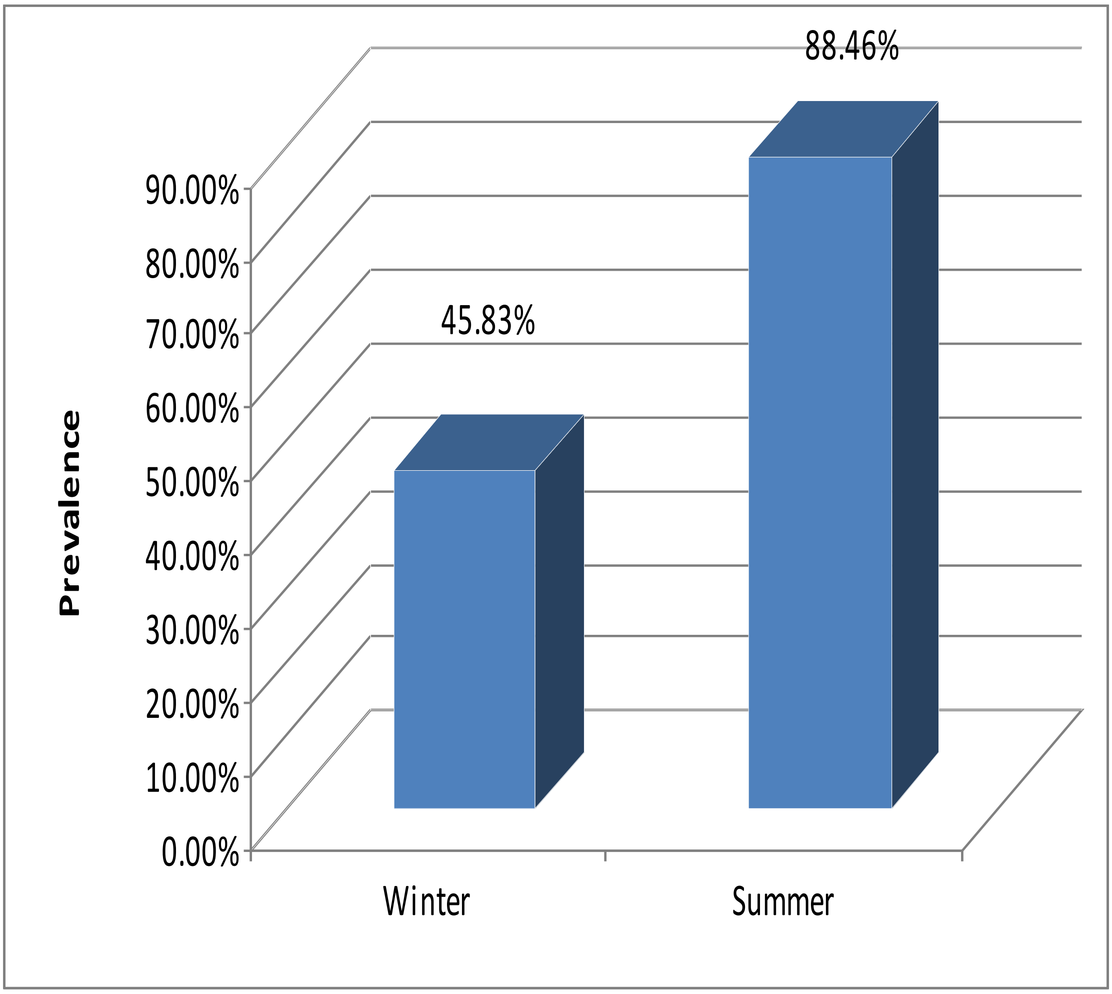


Fig: 5. General seasonal prevalence of helminth parasites in buffalo

4.3 Class wise seasonal prevalence

Altogether eggs of 21 genera were observed during examination of the samples. But on seasonal basis 17 (77.27%) genera were recorded during winter and 20 (90.90%) genera were observed during summer.

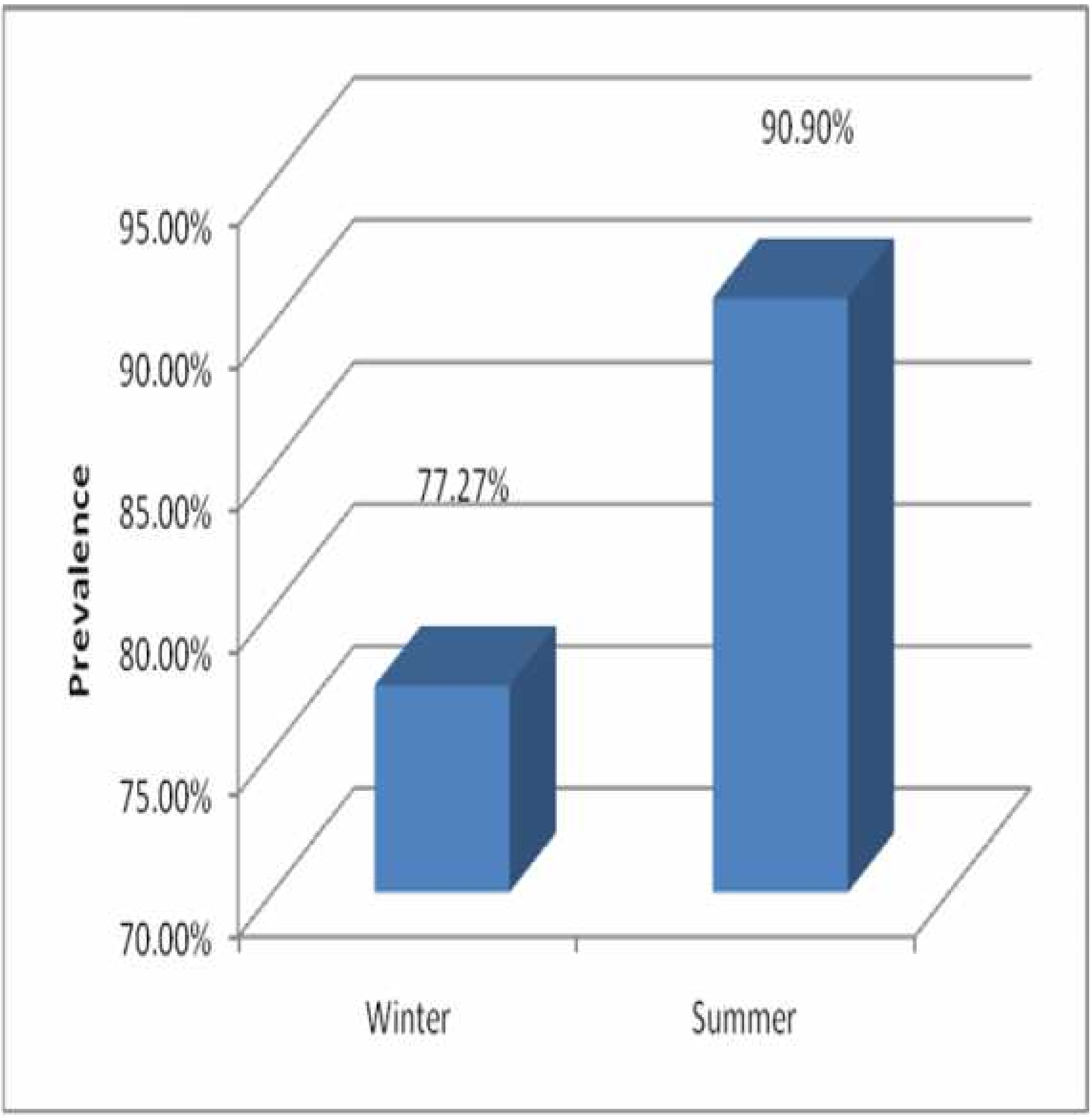


Fig: 6. Genera wise seasonal prevalence

4.3.1 <u>Seasonal prevalence of trematode</u>

Altogether, 126 (74.11%) samples were found positive for trematodes. Among winter 38 (31.66%) samples were found positive and 88 (67.69%) samples of summer were found positive. The difference in the prevalence of different genus of trematodes during summer and winter was found statistically insignificant. ($\chi^2 = 3.06$, $P<0.05$, d.f. = 7).

Table No. 2. Prevalence of trematodes

S. No.	**Trematode genera**	**During winter**		**During summer**	
		Nos.	**%**	**Nos.**	**%**
1.	*Fasciola*	15	12.5%	35	26.92%
2.	*Paramphistomum*	6	5%	14	10.76%
3.	*Dicrocoelium*	8	6.66%	14	10.76%
4.	*Schistosoma*	20	16.66%	45	34.61%
5.	*Gastrothylax*	1	0.83%	3	2.30%
6.	*Fischoederius*	1	-	2	1.53%
7.	*Ornithobilharzia*	-	-	1	0.76%
8.	*Skrjabinema*	-	0.83%	8	6.15%

4.3.2 ***Seasonal prevalence of cestode***

Out of 250 samples, 27 (15.88%) samples were found to be positive during both seasons. The prevalence rate during winter was 10% i.e. 12 samples were positive and that of summer was 11.53% i.e. 15 samples were found positive. The difference in the prevalence of different genus of cestodes during summer and winter result statistically insignificant . (χ^2 = 0.03, $P<0.05$) .

Table No. 4. Prevalence of cestodes

S.No.	**Cestode genera**	**During winter**		**During summer**	
		Nos.	**%**	**Nos.**	**%**
1.	*Moniezia*	12	10%	15	11.53%

4.3.3 Seasonal prevalence of nematodes

The total number of positive samples found during both the seasons were 112 (65.88%) out of 250 samples. The prevalence rate during winter was 32.5% i.e. 39 samples were positive and that of summer was 56.15% i.e. 73 samples were found positive. The difference in the prevalence of different genus of nematodes during summer and winter result statistically significant . (χ^2 = 4.75, P<0.05, d.f. =11).

Table No. 6. Prevalence of nematodes

S.No.	Nematode genera	During winter		During summer	
		Nos.	**%**	**Nos.**	**%**
1.	*Strongyloides*	9	7.5%	14	10.76%
2.	*Trichostrongylus*	5	4.16%	5	3.84%
3.	*Toxocara*	18	15%	40	30.76%
4.	*Ascaris*	11	9.16%	20	15.38%
5.	*Chabertia*	1	0.83%	2	1.53%
6.	*Trichuris*	4	3.33%	6	4.61%
7.	*Oesophagostomum*	1	0.83%	1	0.76%
8.	*Haemonchus*	1	0.83%	2	1.53%
9.	*Ostertagia*	2	1.66%	3	2.30%
10.	*Cooperia*	1	0.83%	-	-
11.	*Dictyocaulus*	-	-	1	0.76%
12.	*Capillaria*	-	-	1	0.76%

4.4 <u>Identification of eggs of Helminth</u>

Out of 120 stool samples, 55(45.83%) samples were found positive during winter and 115(88.46%) samples of summer were found positive among 130 samples. The total number of eggs identified were listed below in the table.

Table No. 8. Observed genera of different classes

S. No.	**Class**	**Identified helminthes**
1.	**Trematode**	***Fasciola***
2.		***Paramphistomum***
3.		***Schistosoma***
4.		***Dicrocoelium***
5.		***Gastrothylax***
6.		***Fischoederius***
7.		***Ornithobilharzia***
8.		***Skrjabinema***
9.	**Cestode**	***Moniezia***
10.	**Nematode**	***Strongyloides***
11.		***Trichostrongylus***
12.		***Toxocara***
13.		***Ascaris***
14.		***Chabertia***
15.		***Trichuris***
16.		***Dictyocaulus***
17.		***Oesophagostomum***
18.		***Capillaria***
19.		***Haemonchus***
20.		***Ostertagia***
21.		***Cooperia***

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

TREMATODES

Fasciola sp.

Classification:

Family: Fasciolidae

Genus : *Fasciola*

Description of the eggs:

Eggs are 30µm by 60-90µm in size, yellowish in colour, consist of embryonic mass and shell, operculum usually indistinct.

Discussion:

In 1758, Linnaeus, reported *Fasciola* from the bile ducts of the sheep and other ruminants.

In 1967-92, Parajuli reported *Fasciola* sp. 56.75% in buffalo from Surkhet district.

In 1987,Ghimire reported *Fasciola* sp.in cattle,buffaloes and goats from Surkhet district.

In 1999, Regmi, Dhakal and Sharma reported *Fasciola* infected 67.66% in buffalo and 62.10% in cattle from Thuladihi VDC, Syangja.

In 2002, Pandey, Mahato and Gupta reported *Fasciola* infection in Lymnaea snails and buffaloes from Devbhumi Baluwa VDC OF Kavre district.

In 2007, Mukhia reported *Fasciola* infection 32-06% among buffaloes brought to Satungal for slaughter purpose.

Paramphistomum sp.

Classification:

Family : Paramphistomatidae

Genus : *Paramphistomum* sp.

Description of the eggs:

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

Discussion:

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

In 1967-92, Parajuli reported *Paramphistomes* 35.13% in buffalo from Surkhet district.

In 1982, ADPCD reported *Paramphistomum* sp. in cattle and buffalo from Kathmandu.

In 2003, Khakural and Khakural reported *Paramphistomum* sp. in farm ruminants from Maidi VDC, Dhading.

In 2006, Jaisawal reported 38.09% *Paramphistomum* in ruminants from Janakpur district.

***Dicrocoelium* sp.**

Classification:

Family : Dicrocoelidae

Genus : *Dicrocelium* sp.

Description of the eggs:

Eggs are 36-45µm by 20-30µm in size, dark brown in colour, operculated and thick-shelled.

Discussion:

In 1899, Loss reported *D. lanceatum* from the bile ducts of the sheep, goat and cattle.

In 2007, Karki reported *Dicrocoelium* sp. in elephants of Nepal.

In 2007, Mukhia reported *Dicrocoelium* sp. in buffaloes of Satungal.

***Schistosoma* sp.**

Classification:

Family : Schistosomitidae

Genus : *Schistosoma* sp.

Description of the eggs:

Eggs are 200µm by 70-90µm in size, spindle shaped, flattened at one side, greatly elongated with straight slender terminal spine.

Discussion:

In 1851, Bilharz reported the adult worm of *Schistosoma* in mesenteric viens of a man in Cairo.

In 1993, WHO reported *Schistosoma* sp. in cow, water buffalo and dog and pigs in Phillipines.

In 2007, Karki reported *Schistosoma* sp. in buffaloes from Nepal.

Gastrothylax sp.

Classification:

Family : Gastrothylacidae

Genus : *Gastrothylax* sp.

Description of the eggs:

Eggs are 115-135µm by 66-70µm in size, distinct operculum, embryonic shells.

Discussion:

In 1847, Creplin reported *Gastrothylax* sp. from the rumen of sheep, cattle and buffalo.

In 1970, Singh reported *Gastrothylax* sp. in buffalo from Kathmandu.

In 1973, Singh et al. first reported *Gastrothylax* sp.

Fischoederius sp.

Classification:

Family : Gastrothylacidae

Genus : *Fischoederius* sp.

Description of the eggs:

Eggs are 125-152µm by 65-75µm in size, elliptical in shape, distinct acetabulum.

Discussion:

In 1883, Poirier reported *Fischoederius* sp. from the rumen of cattle.

In 1973, Singh et al. reported *Fischoederius* sp.from goat intestine.

Ornithobilharzia sp.

Classification:

Family : Schistosomitidae

Genus : *Ornithobilharzia* sp.

Description of the eggs:

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

Discussion:

In 1913, Skrjabin reported *Ornithobilharzia* sp. from the mesenteric viens of sheep and cattle.

In 2007, Mukhia reported *Ornithobilharzia* sp.in buffaloes from Kathmandu.

Skrjabinema sp.

Classification:

Family : Oxyuridae

Genus : *Skrjabinema* sp.

Description of the eggs:

Bean shaped in appearance, eggs are fully embryonated.

Discussion:

In 1915, Skrjabin reported *Skrjabinema ovis* from the sheep and goat.

In 1997, Joshi reported *Skrjabinema ovis* in goat from western hills of Nepal.

CESTODES

Moniezia sp.

Classification:

Family : Anoplocephalidae

Genus : *Moniezia* sp.

Description of the eggs:

Eggs are 56-67µm in diameter, triangular, globular or quadrangular in shape, contain a well developed pyriform apparatus.

Discussion:

In 1810, Rudolphi reported *M. expansa* from the small intestine of sheep, cattle and other ruminants.

In 1979, Moniez reported *Moniezia benedeni* from the cattle.

In 1981, ADPCD reported *Moniezia* sp. from calves and sheep.

In 1987, Ghimire reported *Moniezia* sp. in cattle, buffaloes and goats from Surkhet district.

In 1989, Gupta first reported *Moniezia expansa* from goat.

In 2001, Parajuli reported *Moniezia* sp. infection in goats of Khasibazar Kalanki brought for slaughter purpose.

In 2007, Mukhia reported *Moniezia* sp. infection 12.21% among buffaloes brought to Satungal for slaughter purpose.

NEMATODES

Strongyloides sp.

Classification:

Family : Strongylidae

Genus : *Strongyloides* sp.

Description of the eggs:

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

Discussion:

In 1856, Wedl reported *Strongyloides* sp. from the small intestine of sheep and cattle.

In 1973, Singh et al. reported *Strongyloides* sp. from goat and sheep of Kathmandu.

In 1997, Joshi reported *Strongyloides* sp. from goat and sheep of western hills of Nepal.

In 1999, Acharya reported *Strongyloides papillosus* in sheep and goat of IAAS livestock farm.

In 2002-03, Adhikari et al. reported 10% *Strongyloides* sp. among buffalo from areas of Kathmandu Valley.

In 2003, Khakural and Khakural reported strongyles in ruminants from Maidi VDC, Dhading.

Trichostrongylus sp.

Classification:

Family : Trichostrongyloidae

Genus : *Trichostrongylus* sp.

Description of the eggs:

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

Discussion:

In 1973, Singh reported *Trichostrongylus* from cattle and buffalo.

In 1997, Joshi reported *Trichostrongylus* sp. from cattle and goat from western hills of Nepal.

In 2003, Thakur reported *Trichostrongylus* sp. in pigs from eastern hills of Nepal.

In 2003, Rabin, Joshi and Chetri reported *Trichostrongylus* sp. in Yaks from Chandanbari, Langtang.

Toxocara sp.

Classification:

Family : Ascaridae

Genus : *Toxocara* sp.

Description of the eggs:

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

Discussion:

In 1782, Goeze reported *T. vitulorum* from the small intestine of cattle and buffalo.

In 1967-92, Joshi and Ghimire reported *Toxocara* sp. in buffaloes calves from Lumle, Pokhara.

In 1987, Ghimire reported *Toxocara* sp. in cattle, buffaloes and goats from Surkhet district.

In 2003, Khaniya and Sah reported *Toxocara* sp. in dogs.

Ascaris sp.

Classification:

Family : Ascaridae

Genus : *Ascaris* sp.

Description of the eggs:

Eggs are 50-90µm in size, laid in morula stage, subglobular.

Discussion:

In 1782, Goeze reported *Ascaris* sp. from the small intestine of cattle and buffalo.

In 1982, ADPCD reported *Ascaris* sp. in buffalo and chauri from Kathmandu.

In 2003, Karki reported *A. lumbricoides* in magar community, Palpa.

Chabertia sp.

Classification:

Family : Trichonematidae

Genus : *Chabertia* sp.

Description of the eggs:

Eggs are 90-105µm by 50-55µm in size, oval shaped, laid in morula stage.

Discussion:

In1790, Gmelin reported *Chabertia* sp. from the colon of sheep, cattle and other ruminants.

In 1997, Joshi reported *Chabertia* sp. in sheep and goat from western hills of Nepal.

In 1999, Acharya reported *Chabertia* sp. in sheep and goat of IAAS livestock farm.

In 2007,Mukhia reported *Chabertia* sp. infection 0.38% among buffaloes brought to Satungal, for slaughter purposes.

Oesophagostomum sp.

Classification:

Family : Strongyloidae

Genus : *Oesophagostomum* sp.

Description of the eggs:

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

Discussion:

In 1803, Rudolphi reported *O. radiatum* from the colon of cattle and water buffalo.

In 1982, ADPCD reported *Oesophagostomum* sp. in pig, cattle and buffalo from Kathmandu.

In 2006, Dhital reported *Oesophagostomum* sp. from goats of IAAS livestock farm and Manglaour VDC 2, Chitwan.

In 2007, Mukhia reported *Oesophagostomum* sp. from buffaloes brought to Satungal for slaughter purposes.

Haemonchus sp.

Classification:

Family : Trichostrongylidae

Genus : *Haemonchus* sp.

Description of the eggs:

Eggs are 70-85µm by 41-48µm in size, embryo 16-32 celled when laid.

Discussion:

In 1803, Rudolphi reported *H. contortus* from the abomasums of sheep, cattle and other ruminants.

In 1973, Singh et al. reported *Haemonchus* sp. in cattle, sheep and buffalo from Kathmandu.

In 1999, Joshi reported *Haemonchus* sp. in sheep and goat from Kaski district, Pokhara.

In 2007, Parajuli studied and reported the prevalence of *Haemonchus* sp. in the intestine of goats brought to Khasibazar for slaughter purposes.

Ostertagia sp.

Classification:

Family : Trichostrongylidae

Genus : *Ostertagia* sp.

Description of the eggs:

Eggs are 80-85µm by 40-45µm in size, elliptical in shape.

Discussion:

In 1907, Ransom reported *Ostertagia* sp. from the abomasums and small intestine of sheep, cattle and other ruminants.

In 1982, ADPCD reported *Ostertagia* sp. in pig, cattle and buffalo from Kathmandu.

In 1999, Acharya reported *Ostertagia* sp. in sheep and goat of IAAS livestock farm.

In 2006, Dhital reported *Ostertagia* sp. in goats of IAAS livestock farm and Manglapur VDC 2 Chitwan.

Cooperia sp.

Classification:

Family : Trichostrongylidae

Genus : *Cooperia* sp.

Description of the eggs:

Eggs are 68-82µm by 34-42µm in size, consist of a double layer.

Discussion:

In 1907, Ransom reported *Cooperia* sp. from the small intestine and abomasums of ruminants.

In 1982, ADPCD reported *Cooperia* sp. in goat, sheep and buffalo from Kathmandu.

In 2007, Mukhia reported *Cooperia* sp. in buffaloes brought to Satungal for slaughter purposes.

Dictyocaulus sp.

Classification:

Family : Dictyocaulidae

Genus : *Dictyocaulus* sp.

Description of the eggs:

Eggs are 82-88µm by 33-30µm in size, ellipsoidal, first stage larva may pass.

Discussion:

In 1809, Rudolphi reported *Dictyocaulus* sp. from the bronchi of sheep, goat and wild ruminants.

In 1982, ADPCD reported *Dictyocaulus* sp. in goat and sheep from Kathmandu.

In 2007, Mukhia reported *Dictyocaulus* sp. infection 0.76% in buffaloes brought to Satungal for slaughter purposes.

Trichuris sp.

Classification:

Family : Trichuridae

Genus : *Trichuris* sp.

Description of the eggs:

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

Discussion:

In 1795, Abildgaard reported *T. ovis* from the caecum of sheep, cattle and other ruminants.

In 1970, Singh reported *Trichuris globulosa* in goat from Kathmandu.

In 1982, ADPCD reported *Trichuris trichura* in cattle, sheep, goat and buffalo from Kathmandu.

In 2003, Thakur reported *Trichuris* sp. in pigs from eastern hills of Nepal.

Capillaria sp.

Classification:

Family : Capillaridae

Genus : *Capillaria* sp.

Description of the eggs:

Eggs are 30-63µm in size, unsegmented, barrel shaped, colourless shell.

Discussion:

In 1800, Zeder reported *Capillaria* sp. from the small intestine of dog and cattle.

In 1967-92, Mainali reported *Capillaria* sp. from Lulu cattle.

In 1982, ADPCD reported *Capillaria* sp. in poultry from Kathmandu.

In 2007, Mukhia reported *Capillaria* sp. infection 0.38% in buffaloes brought to Satungal for slaughter purposes.

4.5 Multiple and Single infection

In the present study, rate of mixed infection was also observed. Out of 170(68%) positive samples, 131(77.05%) positive samples were found mixed infections with 2-5 species in each samples. In multiple infections, the intensity of light infection shown by (0 to 2) that is 0-2 ova found per field, intensity of moderate infection shown by (2 to 6) that is 2-6 ova per field and intensity of heavy infection shown by (6 to 8) that is 6-8 ova per field.

Table No.9. Multiple infection during winter season

S.no.	Class	Name of the genera	Light Infection (0-1)	Moderate Infection (2-6)	Heavy Infection (6-8)
1.	Trematode	*Fasciola*	7	2	-
2.		*Paramphistomum*	4	2	-
3.		*Dicrocoelium*	5	2	1
4.		*Schistosoma*	15	2	3
5.		*Gastrothylax*	1	-	-
6.		*Fischoederius*	-	-	-
7.		*Ornithobilharzia*	-	-	-
8.		*Skrjabinema*	1	-	-
9.	Cestode	*Moniezia*	6	-	-
10.	Nematode	*Strongyloides*	6	-	-
11.		*Trichostrongylus*	3	-	-
12.		*Toxocara*	14	4	-
13.		*Ascaris*	11	-	-
14.		*Chabertia*	-	-	-
15.		*Trichuris*	4	-	-
16.		*Dictyocaulus*		-	-
17.		*Oesophagostomum*	1	-	-
18.		*Capillaria*	-	-	-
19.		*Haemonchus*	-	-	-
20.		*Ostertagia*	2	-	-
21.		*Cooperia*	-	-	-

Out of 55 positive samples of winter, mixed infection was observed in 33(60%) samples. Among winter helminths, the intensity of light infection was noted due to *Schistosoma* with 15(27.27%) (0 -1) positive samples. Moderate infection was shown by*Toxocara* with 4(7.27%) (2 - 6) positive samples and heavy infection was noted due to *Schistosoma* with 3(5.45%) positive samples.

Table No.10. Multiple infection during summer season

S.no.	Class	Name of the genera	Light Infection (0-1)	Moderate Infection (2-6)	Heavy Infection (6-8)
1.	Trematode	*Fasciola*	26	2	2
2.		*Paramphistomum*	12	2	-
3.		*Dicrocoelium*	10	2	2
4.		*Schistosoma*	40	5	-
5.		*Gastrothylax*	3	-	-
6.		*Fischoederius*	2	-	-
7.		*Ornithobilharzia*	1	-	-
8.		*Skrjabinema*	6	2	-
9.	Cestode	*Moniezia*	10	-	1
10.	Nematode	*Strongyloides*	14	-	-
11.		*Trichostrongylus*	3	-	-
12.		*Toxocara*	34	1	5
13.		*Ascaris*	18	2	-
14.		*Chabertia*	1	-	-
15.		*Trichuris*	6	-	-
16.		*Dictyocaulus*	1	-	-
17.		*Oesophagostomum*	1	-	-
18.		*Capillaria*	1	-	-
19.		*Haemonchus*	2	-	-
20.		*Ostertagia*	4	-	-
21.		*Cooperia*	-	-	-

Out of 115 positive samples of summer, mixed infection was observed in 98 (85.21%) samples. Among summer helminthes, the intensity of light infection was noted due to *Schistosoma* with 40(34.78%) (0 - 1) positive samples. Moderate infection were shown by *Schistosoma* with 5(4.34%) (2 - 6) positive samples and heavy infection was noted due to *Toxocara* with 5 (4.34%) positive samples.

Table No. 11. <u>Single Infection</u>

Genera	Total single infection		Positive samples	
	Winter	Summer	Winter	Summer
Fasciola	22	17	6	5
Moniezia	22	17	6	4
Strongyloides	22	17	5	5
Trichostrongylus	22	17	2	2
Haemonchus	22	17	1	-
Chabertia	22	17	1	1
Cooperia	22	17	1	-

Out of 120 samples taken during winter 55 were found positive and out of 55 positive samples, 22 were found to have single infection. The highest rate of single infection was due to *Fasciola* and *Moniezia* with 10.90%, *Strongyloides* 9.09%, *Trichostrongylus* with 3.63%,followed by *Haemonchus*, *Chabertia* and *Cooperia* with 1.81%. Similarly during summer, single infection was noted from 14.78% samples. Here *Strongyloides* and *Fasciola* with 4.34% showed higher rate of infection and *Moniezia* with 3.47% followed by *Trichostrongylus* 1.73% and *Chabertia* with 0.86%.

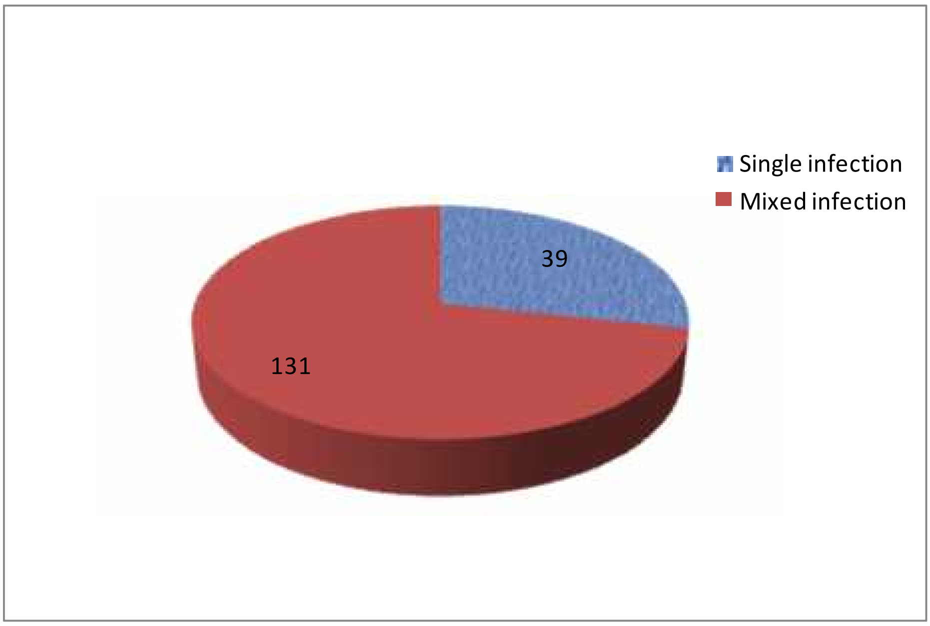


Fig:7. Total number of mixed and single infection.

PHOTOGRAPHS

EGGS OF TREMATODES OBSERVED

Dicrocoelium sp.(10Xx10X)

Schistosoma sp. (10Xx10X)

Fasciola sp. (10Xx10X)

EGGS OF NEMATODES OBSERVED

Trichostrongylus sp. (10Xx10X)

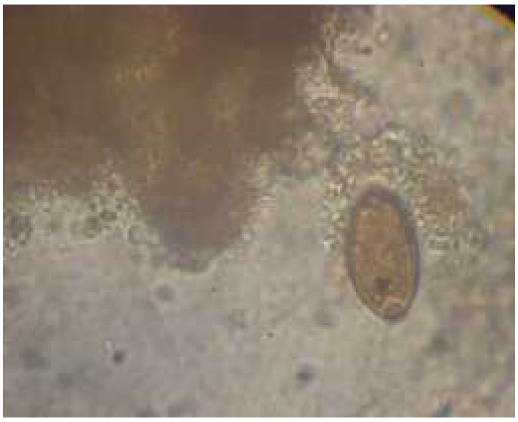
Strongyloides sp. (10Xx10X)

Haemonchus sp. (10Xx10X)

Dictyocaulus sp. (10Xx10X)

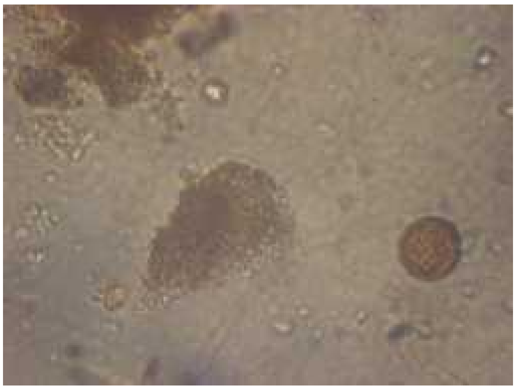
Toxocara sp. (10Xx10X)

Trichuris sp. (10Xx10X)



Capillaria sp. (10Xx10X)

Ostertagia sp. (10Xx10X)



Ascaris sp. (10Xx10X)

Samples in floatation and sedimentation techniques

Samples ready in slides to observe

Taking aid of an expert

Lab work at CVL, Tripureshwor, Kathmandu

DISCUSSION

The aim of the study was to investigate the seasonal prevalence of intestinal helminthes parasites in buffaloes. The present investigation was carried out in the month of December /January and May / June. The stool samples were collected from Pokharathok VDC. From the present study, 45.83% samples of winter were found positive out of 120 samples and 88.46% samples of summer were found positive out of 130 samples. The numbers of samples found positive during winter and summer for Trematoda were 38 and 88, for Cestoda 12 and 15 and for Nematoda were 39 and 73 respectively. (The number of samples exceeds the total number of samples taken due to multiple infections.)

The helminthiasis has been emerged as an important parasitic disease since from the past decades in the world, but in Nepal it had been reported upto certain extent. It can be said that the prevalence of any gastrointestinal parasite is influenced by the climatic condition and geographical factor. Like, the warm and humid condition of South East Asia harbour a suitable condition for most of the parasite to flourish well. The prevailing continous rainfall throughout the year in these regions, helps for the survival of such parasite.

In the present study 6 genera of Trematoda, 1 genera of Cestoda and 10 genera of Nematoda were examined during winter. Similarly from the summer samples, 8 genera of Trematoda, 1 genera of Cestoda and 11 genera of Nematoda were observed.

Among Trematodes, 6 genera were observed in winter samples, namely- *Fasciola, Paramphistomum, Dicrocoelium, Schistosoma, Gastrothylax* and *Skrjabinema*. Where as in summer samples, 8 genera were observed as *Fasciola, Paramphistomum, Dicrocoelium, Schistosoma, Gastrothylax, Fischoderius, Ornithobilharzia* and *Skrjabinema*. *Fischoderius* and *Ornithobilharzia* were not found in winter samples. In Cestodes, *Moniezia* was found in both the seasons.

In case of Nematodes, 10 genera were found in winter samples, namely- *Strongyloides, Trichostrongylus, Toxocara, Ascaris, Chabertia, Trichuris, Oesophagostomum, Haemonchus, Ostertagia,* and *Cooperia* . Where as in summer samples 11 genera were observed as- *Strongyloides, Trichonstrongylus, Toxocara, Ascaris, Chabertia, Trichuris,* *Dictyocaulus*, *Oesophagostomum*, *Capillaria*, *Haemonchus* and *Ostertagia*. *Cooperia* was not found in summer. Similarly *Dictyocaulus* and *Capillaria* were not observed during winter.

The seasonal prevalence of trematode genera found in buffaloes were *Fasciola* 12.5%\26.92%, *Paramphistomum* 5%\ 10.76%, *Dicrocoelium* 6.66%\ 10.76%, *Schistosoma* 16.66%\ 34.61%, *Gastrothylax* 0.83%\ 2.30%, *Fischoderius* 0.0%\ 1.53%, *Ornithobilharzia* 0.0%\ 0.76% and *Skrjabinema* 0.83%\ 6.15% during winter and summer respectively. Similarly the prevalence rate of cestodes during winter and summer were recorded as follows- *Moniezia* 10%\ 11.53% .

In nematode genera the seasonal prevalence rate during winter and summer were recorded as follows- *Strongyloides* 7.5%\ 10.76%, *Trichostrongylus* 4.16%\ 3.84%, *Toxocara* 15%\ 30.76%, *Ascaris* 9.16%\ 15.38%, *Chabertia* 0.83%\ 1.53%, *Trichuris* 3.33%\ 4.61%, *Dictyocaulus* 0.0%\ 0.76%, *Oesophagostomum* 0.83%\ 0.76%, Capillaria 0.0%\ 0.76%, *Haemonchus* 0.83%\1.53%, *Ostertagia* 1.66%\ 2.30%, and *Cooperia* 0.83%\ 0.0%. In overall *Toxocara* (34.11%) was the most encountered species followed by Ascaris (18.23%) and *Strongyloides* (13.52%) and among the least prevalence species was *Dictyocaulus* (0.58%).

Due to lack of sophisticated equipments in the laboratory the identification of parasites was possible up to genus level. No species and subspecies have been identified.

The present study exhibited 12.5% and 26.92% prevalence rate of Fascioliasis during winter and summer respectively. The increase in their prevalence during summer may be due to increase in humidity and availability of favorable temperature. High prevalence of *Fasciola* i.e. 67.66% has been reported from Thuladihi Syangja among buffaloes (Regmi et al. 1999), followed by 56.75% *Fasciola* infection from Surkhet district (Parajuli1967-92) and 40.12% infection in animals from Panchthar district (Sharma 1997-98) which were the highest infection among buffaloes comparing to the present study. Likewise, the prevalence of *Paramphistomum* i.e. 35.13% has been reported from Surkhet district (Parajuli 1967-92), 16.20% in animals from Panchthar district (Sharma 1997-98) and which were higher than the present prevalence i.e. 5% in winter and 10.76% in summer.

Similarly, *Schistosoma* has been reported among buffalo from Surkhet district (Ghimire 1987) which has been found in the present study and its prevalence has been reported i.e.21.66% in winter and 42.30% in summer. *Fischoederius* and *Gastrothylax* has been reported from Kathmandu among buffaloes by (Singh et al. 1973) and which is also found in the present study. As *Skrjabinema* has been reported from western hills of Nepal (Joshi 1997) which is also found in the present study and its prevalence i.e. 0.83% during winter and 6.15% during summer has been reported.

Maximum incidence of trematodes was detected during summer season (43.66%) followed by rainy (28.95%) and winter season (20.51%) from Mathura (Agrawal et al. 2006). In the present study the maximum incidence of trematodes was detected during summer (67.69%) followed by winter season (31.66%). Availability of favorable temperature and increase in humidity could be the reason for the high prevalence of trematodes during summer.The parasitic infection was higher in monsoon season as compared to that of summer and winter (Sreedhar et al. 2009).

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.

Among Cestodes, *Moniezia* has been reported by Malakar (1965), Ghimire (1987), Gupta (1989). In the present study 10% and 11.53% prevalence has been recorded during winter and summer respectively. Overall prevalence rate of cestodes in the current study has been found to be 15.88% and seasonally i.e. 10% in winter and 11.53% in summer has been reported. Presence of suitable temperature and moisture serve best for the breeding and development of the helminthes parasites. So this could be the reason behind excessive prevalence of certain helminthes parasites.

The prevalence of helminthiasis in buffaloes was found to be 15.2% in the colony of Hyderabad (Akhtar et al. 2003). The chief helminth were *Toxocara* 3.6%, *Oesophagostomum* 3.0%, *Strongyloides* 2.4%, *Ostertagia* 1.0% and *Trichuris* 0.2%. Comparing to the present study, the prevalence of *Toxocara*, *Oesophagostomum*, *Strongyloides, Ostertagia* and *Trichuris* were higher in both the seasons because these are the common nematodes of buffaloes and may occurred due to the ingestion of contaminated soil and herbage during grazing and propensity to seek rivers, pools or swamps for wallowing. *Oesophagostomum* 0.83% in winter and 0.76% in summer has been found lower comparing to the prevalence of colony of Hyderbad may be the infection of *Oesophagostomum* is less on the infected buffalo.

The gastro-intestinal nematodosis reported from terai, India (Yadav et al. 2004) were *Haemonchus, Trichonstrongylus, Oesophagostomum* and *Strongyloides* were the main parasites among buffaloes which were found similar to the present study because the strongyle groups are common in buffaloes.

Infection with *Strongyloides* 85% and *Toxocara* 58% among buffaloes has been reported from Thailand (Srikit Jakarn et al. 1987) similarly infection with *Toxocara* 63.83% has been reported from Multan abattoir which is greatly higher than the present study i.e. *Strongyloides* 13.52% and *Toxocara* 34.11%.

The prevalence of common helminthic infections in buffaloes was studied in Mathura during different seasons (Agrawal et al. 2006). The higher infections of nematodes was noticed during summer season (71.59%) and rainy season (69.09%) as compared to winter season (21.05%).In the present study the higher infections of nematodes was found during summer (56.15%) as compared to winter season (32.5%).

Ascariosis 43.69% reported Panchthar district (Sharma 1997-98) is found to be higher than the present study i.e. 9.16% in winter and 15.38% in summer may be the animals of Panchthar district were infected with more contaminated water and other *Ascaris* infected matters.

Similarly *Chabertia, Cooperia, Dictyocaulus* and *Capillaria* has been reported among buffaloes (ADPCD 1982) which is found to be bit similar to the present study.

The prevalence of gastro-intestinal parasites in buffaloes was found to be 82.35% from Patiala (Kaur et al. 2008). The parasites were *Toxocara* 78.57%, *Haemonchus* 57.14%, *Oesophagostomum* 42.86% and *Trichuris* 14.29% which were found to be greatly higher than the present study.

In the present study, the number of helminth parasites in two seasons (summer and winter) differs significantly. Statistically, ($\chi^2_{(cal)}$=740.15, $\chi^2_{(tab)}$=32.671, $P<0.05$, d.f.=21). The tabulated value is less than calculated value. So this result rejects the hypothesis of the study.

Mixed infections 0.6% with species of *Fasciola, Ostertagia, Paramphistomum, Trichuris, Oesophagostomum* and *Strongyloides* have been reported from the colony of Hyderabad among buffaloes (Akhtar and Mohammad 2003). Mixed infection was observed in 26% and 87.5% in the samples of winter and summer respectively (Bashir 2009). Comparing to it,the overall mixed infection 77.05% was noted higher in the present study.The abundance of multiple infections mainly during summer might be due to availability of suitable temperature and moisture. It also might be due to exposure of buffaloes to highly infected pasture land, contaminated water or infected fodder.

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The current study was carried out in order to observe the seasonal prevalence of intestinal helminths parasite in buffaloes. Samples were collected from Pokharathok-1of district Arghakhanchi. The two different technique used during the detection of helminths parasites were sedimentation and floatation technique. The samples were collected in the months of December/ January and May/ June. The total number of samples had collected and examine for the study were 120 and 130 respectively.The overall prevalence rate of helminthes parasites during December/ January was found to be 45.83% and that in the month of May/ June was studied to be 88.46%. A huge difference in the prevalence of helminth parasites in the both seasons were observed.The parasitic infection of trematode was studied to be 31.66%, cestode 10% and of nematode 32.5% during December/ January. Likewise 67.69%, 11.53% and 56.15% of infections were caused by trematodes, cestodes and nematodes respectively during May and June. The increase in the prevalence of helminthes parasites during summer i.e. May and June may be due to increase in the humidity and favorable temperature. The prevalence percentage of identified genera of trematodes are as follows; *Fasciola* 29.94%, *Paramphistomum* 11.76%, *Dicrocoelium* 1294%, *Schistosome* 47.64%, *Gastrothylax* 2.35%, *Fischoederius* 1.17%, *Ornithobilharzia* 0.58% and *Skrjabinema* 5.29% respectively.Among cestodes, the genera identified with their prevalence percentage were found to be *Moniezia* 12.94%.Similarly the genera included in nematodes are *Strongyloides* 13.52%, *Trichostrongylus* 5.88%, *Toxocara* 34.11%, *Ascaris* 18.23%, *Chabertia* 1.76%, *Trichuris* 5.88%, *Dictyocaulus* 0.58%, *Oesophagostomum* 1.17%, *Capillaria* 1.17%, *Haemonchus* 1.76%, *Ostertagia* 2.94%, and *Cooperia* 0.58%.Single infection was found in 40% samples during winter and during summer it was found in 14.78% samples. Mixed infections was observed in 60% samples in winter and during summer it was found in 85.21% samples.

6.2 Recommendations

- Anthehelmintics treatment should be applied to eliminate the parasite from the host.
- The sheds should not be kept wet, moist or humid. These all prove as heaven to helminth parasites for their growth and development.
- The pastures can be made free of helminth parasites by breaking their life cycle by eradicating intermediate host, snail through biological control method.
- Immigration of buffaloes and other animals should be done after fulfilling the quarantine terms and conditions.
- Impure water should not be supplied.
- Treatment of infected hosts with anthelminthics and diagnosis could be done by taking help of nearby veterinary personnel.
- The program for awareness of the ill effect of infection and zoonotic diseases by helminthes parasites to the livestock farmers, public butchers and buffaloes dealers should be developed.
- This study will be base for the future investigators and further research work should be carried out.

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