

# 1. INTRODUCTION

## 1.1 Background:

The biological subfamily bovidae includes a diverse group of 10 genera of medium to large sized ungulates including domestic cattle, bison, African buffalo, the water buffalo, the yak, and the four –horned and spiral – horned antelopes. The water buffalo or domestic asian water buffalo (*Bubalus bubalis*) is a large bovine animal, frequently used as livestock in southern Asia, and also widely in South America, southern Europe, northern Africa, and elsewhere. There are established feral populations of buffaloes in northern Australia, but the dwindling true wild populations are thought to survive in India, Bangladesh, Pakistan, Nepal, Bhutan, and Thailand. All the domestic varieties and breeds descend from one common ancestor, the wild water buffalo, which is now an endangered species. The domestic water buffalo, although derived from the wild water buffalo, is the product of thousands of years of selective breeding in either South Asia or Southeast Asia.

(*Bubalus bubalis*), also known as Asian animal, plays an important role in farmer economy. In addition to milk, meat, draft facilities, buffaloes provides organic wastes like dung and urine for fuel and fertilizer, dead animals and bone for feeding to poultry and hide, horns and fallow for industrial use. The buffaloes work slowly and without any argument. The working life of buffalo is longer than that of cattle, usually more than 17 years and up to 25 year of age. Because of this excellent draft and pulling capacity buffaloes are called as the “living factor of the East” (Cockrill, 1968).

Human societies have relied for centuries on animal agriculture to provide food, fiber, power, fertilizer and employment. This is more important in developing countries when human activities are interwoven with the raising of livestock. The world population of buffaloes (*Bubalus bubalis*) has been estimated at over 172 million head, more than 97% (167.6 million) of which are in Asia and Pacific region, mainly in India (97.7 million), Pakistan (25.5million) and Bangladesh (0.83 million) (FAO, 2004). In general the buffalo is regarded as more productive, healthier and more useful than the cow, especially for the poorest “backyard” farmers in Asia (Bhat, 1999). According to the 1997 FAO report, the buffalo are recognized as “Black Gold of Asia” (Khushik and Menon, 2004).

Buffalo (*Bubalus bubalis*) is one of the most important species of domestic livestock as a source of dairy, meat, manure and drought power and plays an important role in Nepal rural economy. Buffaloes are substantially distributed in more than 25 tropical countries stretching from Southern Europe through India and China to the full of South-East Asia.. Buffaloes in Nepal are widely distributed from sub humid regions of subtropics in Terai to cool temperate regions in the hills and mountain (Shah, 1981 and Shree,1982). Epstein (1977) has classified Nepalese buffaloes in four regional types; Terai, hilly, midland and Himalayan Mountain. These types are distinguished by size, shape, curvature and length of their horns and the colour of their coats.

Nepal is an agrarian country where 82% of people depend on agriculture activities. The Ministry of Agriculture and Cooperatives (MOAC,2008/2009) has estimated agricultural contribution to be 32.82% the national GDP. Buffalo farming is an integral part of Nepalese livestock industry. Buffaloes are multipurpose and economically most important livestock species raised under the farming system of a Nepal. Buffalo have strong body, broad hooves, flexible pastern and fetlock joints (Hibler, 2000). Karanth,(2002) has reported that buffalo's dung is extensively used as fuel for cooking in India, Pakistan, Bangladesh, and Nepal and in almost all countries. The first European pioneers on the great plains of the USA also used dried buffalo dung called buffalo chips for fuels because of the lack of wood.

Buffaloes have been raised under farming systems of Nepal throughout the known history. They are the traditional provider of milk, meat, hides, manure, draft power and also the reserve capital for the farm families. They are mainly raised by the smallholder farmers across all the physio-graphic agro-ecological (Agro-eco) zones of the country. Due to a key role played by buffalo in the farming systems of the country from the time immemorial to date, interest in this livestock species is ever growing as more systematic planning is adopted for growth of the country's agrarian economy.

Rasali ,(2000) observed the growth was not uniform in buffalo rearing countries in Asia. Although spectacular increases were observed in South Asia, countries in Eastern Asia exhibited a considerable decline in buffalo population, which is a matter of concern.

Parasitic infections are one of the major constraints for profitable dairy industry in tropical and subtropical countries including Nepal. Gastrointestinal (GI) parasites cause considerable global economic losses as a consequence of reduced weight gain, digestive disturbance, lowered production, impaired reproductive performance, condemnation of affected organs, and mortality in infected animals . In addition, the diverse

agroclimatic conditions, animal husbandry practice, and pasture management largely determine the incidence and severity of various parasitic diseases in certain area

### **1.2 Endoparasitism:**

The condition of parasite living inside a host is called endoparasitism. Endoparasites are those organisms which totally depends on the host for its growth, development and other physiological processes and can cause infection to them. These endoparasites lives inside host body like gut, body cavity, liver, Lungs, gallbladder, blood, or within the internal cavities, tissues or cells of their host.

The ten most reported disease in the year 2002-2009 in Nepal were liver fluke, fascioliasis, distomatosis, wound, diarrhoea, parasitic gastrornteritis, infertility, mastitis haemorrhagicsepticemia, ephemeral fever, foot and mouth disease, black quarter (www.moac.wfo.gov.np). Endoparasites are those organisms living within their hosts, in the gut, body cavity, liver, lungs, gall bladder and blood or within the internal cavities, tissues or cell of the host. Such forms nearly always live a completely parasitic existence. Since they totally depend upon their host, endoparasitism is also referred to as infection. For example- *Fasciola* spp., *Trichostrongylus* spp., *Schistosoma* spp. etc. are the typical endoparasites. A significant loss caused by *Fasciolagigantica* and *Fasciola hepatica* due to the infection in domestic ruminants was estimated over US\$ 200 million per annum to the agriculture sector worldwide with over 600 million animals affected (Boray, 1985 and Hiller and Apt, 1997). The economic loss due to fascioliasis in Nepal was estimated as Rs 14.2 crore (Lohani and Rasaili, 1995).

### **1.3 Intestinal helminthes parasite:**

Helminth are most important endoparaites mostly causing the infection in intestinal, and also in liver, lungs, lymphatic system, circulatory system, blood tissues, and skin. Helminths are parasitic worms, are large multi-cellular organisms. Mainly the helminthes parasites belong to three classes – Trematodes, Cestodes and Nematodes

### **1.4 Trematoda**

Trematodes species which are parasitic in livestock belongs to subclass Digenea. Trematodes are dorsoventrally flattened, some being leaf shaped and some long and narrow, the gastrointestinal fluke have thick fleshy bodies. The *Schistosoma*, which also belong to this group are elongated and almost roundworm like in appearance. The flukes that parasitize livestock are hermaphrodites (except *Schistosoma*) but they requires

intermediate host to complete their life cycles. The habitats of trematodes, also known as flukes, are the bile ducts, small intestine and also lungs. Some of them may be ingested and may borrow in the skin for access.

The potential source of *Fasciola* infection is green grasses from nearby permanent water sources or water loading areas in monsoon, so in Nepalese hills, post monsoon and winter months are considered as major *Fasciola* infection period. Acute fascioliasis occurs seasonally is manifest by anaemia and sudden death and chronic fascioliasis occur in all seasons having following clinical symptoms are anaemia, reduced weight gain, decreased in milk production, unthriftiness, submandibular oedema and possibly death. Severe destruction of liver tissue of the host is caused due to subsequent simultaneous migration of many immature flukes through the liver parenchyma as well as an inflammatory response of capsule (peri-hepatitis) is caused by penetration of liver capsule by large number of young flukes. The blood sucking activities of the flukes results in irritation of the lining of ducts followed by inflammation and blood loss, finally causing anaemia.

Similarly, dicrocoeliasis is a disease caused by liver fluke. *Dicrocoelium* spp., a common parasite in the biliary passage of sheep other herbivorous and omnivorous animals. Clinical signs and loss of production are rare in cattle; however older cattle may suffer reduced milk production and loss of weight. *Schistosoma* spp. are trematode living in the blood stream hence known as blood fluke. The disease caused by it is known as schistosomiasis. As the blood stream is rich in glucose and amino acid, plasma and blood cells, is very essential environment for producing egg by this trematode. Not only ruminant but also humans are also infected by it. The clinical symptoms are bloody diarrhoea as the mucosa of the intestine is severely damaged, dehydration and loss of appetite.

### **1.5 Cestodes**

Cestodes are commonly known as tapeworms found in the gut. The ruminant is largely affected by eating contaminated food and water. This genera includes the species of *Moniezia* and *Taenia*. *Moniezia* are commonly found in rumen of the domesticated and wild carnivores. The ingestion of herbage having mites carrying the infective stage of parasite is the main cause of infection in ruminants like buffaloes and cattles by *Moniezia*. Light to moderate infection are non-pathogenic but heavy infection causes poor growth and diarrhoea in lambs.

Similarly tapeworm's species *Taeniaovis*, *Taeniamulticeps*, *Echinococcusgranulosus* which are parasite in the small intestines of domesticated and wild carnivores, *Taeniasaginata* which is tapeworm of man, cysticercous, coenurus and hydatid cysts affects various parts of hosts. Taeniasis caused by infection due to *Taeniasaginata* and *Taeniasolium* is considered as zoonotic disease important, acquired by ingestion of unadequately cook beef and pork containing the infective cysticerci larvae. Echinococcosis is another important cestode infection of man and animals caused by ingestion of food and drinks with *Echinococcusgranulosus*. Here the egg passes in the faeces of dog.

### **1.6.Nematodes**

The important nematodes which are widely prevalent is Trichostrongyle group (*Haemonchus*, *Ostertegia*, *Trichostongylus*, *Cooperia* and *Nematodirus*,) *Oesophagostomum*, *Bunostomum*, *Toxocara*, *Dictyocaulus* species and Filarial nematodes greatly affecting the host. *Toxocaravitulorum*, *Dictyocaulus* species and Filarial nematodes has worldwide in distribution and the prevalence is higher in buffalo and cattle (Karki,2005). Some nematode species, especially *Haemonchus* is the most pathogenic bloodsucker, and infectious with large numbers of this parasite often result in severe anaemia in the host. Blood losses from *Oesophagostomum* and *Bunostomum* infections may add to the severity of the anaemia. Causes of gastro-intestinal nematode have been reported from several countries like Africa, Asia, Ethiopia, Sahel and parts of Somalia and Sudan (Mukhia,2007). Infections with gastro-intestinal nematodes usually occur by the ingestion of eggs by young calves. These nematodes damage the intestinal mucous membrane of the small intestine; migrating larvae may cause severe anaemia and diarrhoea to the host.

Trichostrongyliasis is an infection of the gastrointestinal tract of herbivorous animals and man is the accidental host caused by the members of the genus *Trichostongylus*. 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 *Strongyloidesstercoralis*. Toxocariasis (visceral larva migrans) in humans is widely distributed throughout the world, in both temperate and tropical countries. Man acquires infection accidentally by ingestion of the larvae ascarid of the dog present in the inadequately cooked food of paratenic host.

The pathogenic effect of gastro-intestinal parasites may be subclinical or clinical. Young animals are most susceptible. The effect of these parasites strongly dependent on the number of parasites and the nutritional status of animals they are infecting. The clinical signs comprise weight loss, reduced feed intake, diarrhoea, mortality, reduced carcass, quality and reduced wool production and quality. Severe blood and protein loss into the abomasum and intestine due to damage caused by the parasite often results in oedema in the sub-mandibular region.

## **1.7 OBJECTIVES**

The present study was done to fulfill the following objectives.

### **1.7.1 General Objective**

- To study the prevalence of gastrointestinal helminth parasites of buffaloes in Jhalari, Kanchanpur, Nepal.

### **1.7.2 Specific Objectives**

- To determine the overall prevalence of gastrointestinal helminth parasites in buffaloes.
- To determine season-wise prevalence of helminth parasites in buffaloes.
- To determine sex-wise prevalence of helminth parasites in buffaloes.
- To determine age-wise prevalence of helminth parasites in buffaloes.

## **1.8 JUSTIFICATION**

Parasites and diseases can take a toll on both the individuals as well as herd population of animals. Buffaloes are substantially distributed in more than 25 tropical countries stretching from Southern - Europe through India and china to the full of South -East Asia. Nepal being a developing country depends on agriculture and animals husbandry for its bulk of economy. However the farming technique is rather unscientific. The infection of helminthes parasites in buffalo can cause significant economic loss in the form of mortality, lowered general health condition, retarded growth, lower output of work and decrease in production of meat.

The buffalo farming is also done in poor and unhygienic manner and hence in heavily infected with different parasites. This study is an effort to determine the age, season and sex-wise prevalence of helminth parasite in buffalo which gives the suggestive guideline for the further researcher investigators. So it was realized that the research study should be

launched for health related diseases prevalence of gastrointestinal parasites in buffalo in relation to husbandry practices according to faecal examination.

### **1.9 Limitations of the study:**

This study was carried out to as “Coprological study of gastro-intestinal helminth parasites in seasonwise, agewise and sexwise prevalence of gastrointestinal helminthes parasites of buffaloes (*Bubalus bubalis*, Linnaeus 1758) in Jhalari VDC, ward no.1 and 2 in Mahendranagar, Kanchanpur, Nepal”. But the study doesn’t reveal why some parasites were more prominent and others were not. This study is limited to certain parameters and some of the part of the study were left untouched due to time and cost factors so the future researchers can elaborate this study by approaching the untouched portion. This study had been carried out for the partial fulfillment of the requirements for the Master’s Degree in Zoology at Tribhuvan University, Kathmandu, Nepal.

### **1.10 Hypothesis:**

**1.10.1 Null hypothesis (H0): H0:** There were no significant associations of gastrointestinal parasites and age, sex and season.

**1.10.2 Research hypothesis (H1): H1:** There were significant associations of gastrointestinal parasites and age, sex and season.

## 2. LITERATURE REVIEW

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”.

Ruminants are the primary consumer and the secondary producers. They play a vital role in the ecosystem by providing the food or being food for the prey. They get infected with different parasitic diseases and spread to their surrounding animals (Huchinson 2009, Boomker 2013).

### 2.1 Important findings in global context

Iassanet *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 examined buffaloes and 10.35% cattle respectively were harbouring *Fasciolasp.* With the help of faecal test they found 2.07% of the examined animals has *Fasciolasp.* and 2.5% helminthes eggs in their faeces .The helminthes includes 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.

Van *et al.* (2000) observed the overall prevalence of *Strongyles* infection was 53% for cattle and 28% for buffaloes in Mindanao, Philippines. The prevalence decreased with increasing age in both animal species. Faecalegg counts were significantly lower in buffaloes than in cattle. Adult animals had lower egg counts than calves. This age effect was most pronounced in buffaloes. Six genera of *Strongyles* nematodes were identified from the faecal cultures in both animal species, *Mecistocirrus*, *Haemonchus*, *Tristrongylus*, *Cooperia*, *Bunostomum* and *Oesophagostomum*.

Azamet *al.* (2002) investigated the prevalence ofecto-parasites of buffalo calves in 50 buffalo farms in Khadagzai area of Dir district in North West Frontier Province, Pakistan, in April and May [year not yet given]. Faecal examination of calves (n=118: age < less or > 1 year) revealed that 64.41% of the calves were positive for internal parasites. The worm load varied significantly (P<0.05) among the farms and was high (1600-3600 EPG)



in 2%, moderate (800-1600EPG) in 22%, low (200-800 EPG) in 42% of farms. Among the calves examined, 50.84% had worm burden of 200-800 EPG and 13.56% was observed only in 0.85% of the calves. Six species of nematodes and one species of trematode were identified. No cestodes infection was encountered during the study by *Trichuris* (9.32%), *Haemonchus* (8.47%), *Strongyloidespappillosus* (5.93%), *Ostertagia*(5.08%), *Toxocaravitulorum*(1.70%), *Fasciolasp.*(5.93%) and mixed infection (6.78%).

Azharet *al.* (2002)reported 25.59%, 26.16%, 13.7% and 10.5% infection rate of fascioliasis, respectively in slaughtered buffaloes, at livestock farms, veterinary hospital and in household buffaloes in Punjab province, Pakistan. Overall highest (24.0%) seasonal prevalence in all types of buffaloes was recorded during autumn, followed by spring (20.0%), winter (13.0%), while the lowest (9.0%) was recorded during summer. It was noticed that a higher infection rate was recorded in older buffaloes than in youngsters (below 2year of age) where as sex showed no significant difference. Buffaloes of either sex are equally affected.

Maqboolet *al.* (2002)under took an epidemiological studies at slaughter houses, livestock farms, veterinary hospital and on household buffaloes under the different climatic conditions existing in Punjab province. Infection rate was 25.5% 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 youngsters (below 2years of age). Buffalo of either sex were equally affected.

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

Bhutto *et al.* (2002) conducted a study on prevalence of gastrointestinal helminths in buffalo calves in Sindhi province of Pakistan.. 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: *Toxocaravitulorum*(33%), *Ostertagiaostertagi*(8%), *Trichurisovis*(2%) and *Fasciolagigantica*(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.

Magdoub and Sayed (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) *Fasciolagigantica* infection recorded the highest percentage 48.04% followed by *Neoscarisvitulorum* and *Eimcriasp* (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.

Akhtar and Mohammad (2005)conducted as study on prevalence of helminthiasis in buffaloes in colony, Hyderabad. The prevalence of helminthiasis was found to be 15.2% in buffalo. 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 helminthes identified were *Toxocaravitulorum*3.6%, *Fasciolagigantica*3.2%,*Oesophagostomumradiatum*3%,*Strongyloidespapillosus* 2.4%, *Fasciola hepatica* 2.2%,*Ostertagiaostertagia*1%,*Paramphistomumcervi*0.8% and *Trichurissp*0.2%.

Morsyet al. (2005) investigated on natural infection with *Fasciola* species in Tamyia Center, Egypt by stool examination and reported 20% infection 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 indigenou,

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.

Asif *et al.* (2007) recorded the overall prevalence of helminthiasis was 51% in cattle, 47% in buffaloes, 62% in sheep and 52% in goat, with nematodes being the most common helminthes in an irrigated area of lower Punjab, Pakistan. The prevalence of helminthes was higher in young animals compared with adults in cattle ( $P < 0.0001$ ), buffaloes ( $P < 0.0001$ ), sheep ( $P < 0.059$ ) and goats ( $P = 0.010$ ). The prevalence of different species of helminthes also varied in different age groups, with *Toxocaravitulum* being higher in calves than adult both in cattle ( $P = 0.017$ ) and buffaloes ( $P < 0.0001$ ). Sex-wise prevalence of helminthes was higher in males than females for buffaloes ( $P < 0.0001$ ) and sheep ( $P = 0.014$ ) in contrast to cattle and goats.

Condoleo *et al.* (2007) detected gastrointestinal *Strongyles* (33.1%), *Strongyloides spp.* (3.1%), *Fasciola hepatica* (7.1%), *Dicrocoelium dendriticum* (2.4%), *Paramphistomidae* (7.1%) and *Moniezia spp.* (2.4%) in buffaloes of central Italy.

Kaur and Kaur (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 *Toxocaravitulum* (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 (60.00%, 42.10%). Calves on grazing were heavily infected (88.33% 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.

Raza *et al.* (2010) reported the prevalence of *Toxocaravitulum* in buffalo and cattle slaughtered at Multan abattoir. Gastrointestinal tracts of 94 Buffaloes and 48 cattle were

examined for *Toxocaravitulorum*. Prevalence of *Toxocaravitulorum* 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. Twenty-four (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 and 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 oxclozanide. Out of 540 faecal samples examined, 205 (37.96%) were found infected with helminths. Significantly higher prevalence of helminths was recorded in buffaloes (40%, 28/112) as compared to cattle (35.77%, 93/260). *Oesophagostomum*, *Cooperia*, *Trichostrongylus*, *Strongyloide*, *Ostertagia*, *Fasciola hepatica*, *Fasciolagigantica* and *Haemonchus contortus* were the helminth species identified in the study area. Oxclozanide 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% increase fat for buffalo and cattle respectively was observed in oxclozanide medication. It can be concluded that single dose of oxclozanide is effective against all bovine helminths.

Sama *et al.* (2011) conducted a study on simultaneous infestation of a buffalo calf with *Ascaris* and *Strongyloides*. *Toxocaravitulorum* 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, they diagnosed a nondescript Indian buffalo calf infested simultaneously with *Toxocaravitulorum* and *Strongyloides papillosus* and treated it with standard regimen and found the subject cured.

## **2.2 Literature review in context to Nepal**

Helminthiasis has been reported as important parasitic disease in the world whereas in Nepal it has been reported upto certain extent. The farmers of Nepal are mostly small

proprietor thus mainly hold cattles like buffaloes for milk and meat by providing low grade fodder n crop by products in order to provide manure, for home consumption and to earn livelihood.

In Nepal, research works on parasitic diseases of livestock has been introduced during 1970, thus the surveys on common parasitic diseases were endeavored in Kathmandu valley and some other district representing hills, terai and high mountain(Singh *et al.* 1973) which accomplished the identification of nematode parasites, snail species and some ectoparasites.

Parajuli (1967-92) conducted a study in buffaloes from Surkhet district and reported *Fasciolasp* 56.75% and *Paramphistomessp* 35.13%.

Lohani&Jaeckle (1981-82) conducted a study to identify *Fasciola* species in Palpa. Liver fluke 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 *Fasciolagigantica*.

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.

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. Failures 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%).

Regmiet *al.* (1999) conducted a study to know the Fascioliasis prevalence in ThuladihiVDC of Syanja district. Coprological examination revealed that 67.66% Buffalo and 62.10% cattle were affected with Fascioliasis.

Pandeyet *al.* (2002) studied prevalence of *Fasciola* infection in *Lymnaea* snails and buffaloes in DevbhumiBaluwaVDC of Kavre district. The infection rate in rice field was found 1.67% in springs 1.40% and in irrigation channels 0.99%.

Adhikariet *al.* (2002, 2003) conducted a study on the prevalence and diversity of *Fasciolasp.* in buffaloes and cattle in areas of Kathmandu valley from 23 April 2003 to 30 June 2003. The prevalence of *Fasciolasp.* was found to be 36% and 61% in cattle and Buffaloes respectively. Other parasites were also found during the study which includes 48% *Paramphistomumsp.* in cattle. Similarly *Paramphistomumsp.* 11% and *Strongyloidessp.* 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 June 15 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. *Schistosomasp.* was found in 46.94% followed by *Fasciolasp.* 32.60% and *Dicrocoeliumsp.* 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 *Fasciolasp.*, *Dicrocoeliumsp.* and *Schistosomasp.* in buffaloes of Satungal Slaughter House in Satungal Kathmandu during the period of December 2006 to January 2007. A total of 210 samples were collected during the study period and the overall prevalence was found 61.90% where infection by *Fasciolasp.* (38.57%) and *Schistosomasp.* (28.10%) was noted.

Dhakal (2008) conducted a study on intestinal helminth parasites of cattle in AnarmaniVDC 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. The author 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 Khashibazaar, Kalanki, Kathmandu. 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%).

Pathak(2011)conducted a study of helminthes where he found 79.70% of overall prevalence with trematoda 5.94%, cestodes4.45% and nematodes 69.30% was found in the goat of bagbazar.

Dhakal(2011)reported cestode*Anoplocephala sp.* for the first time in Nepal from cattle.

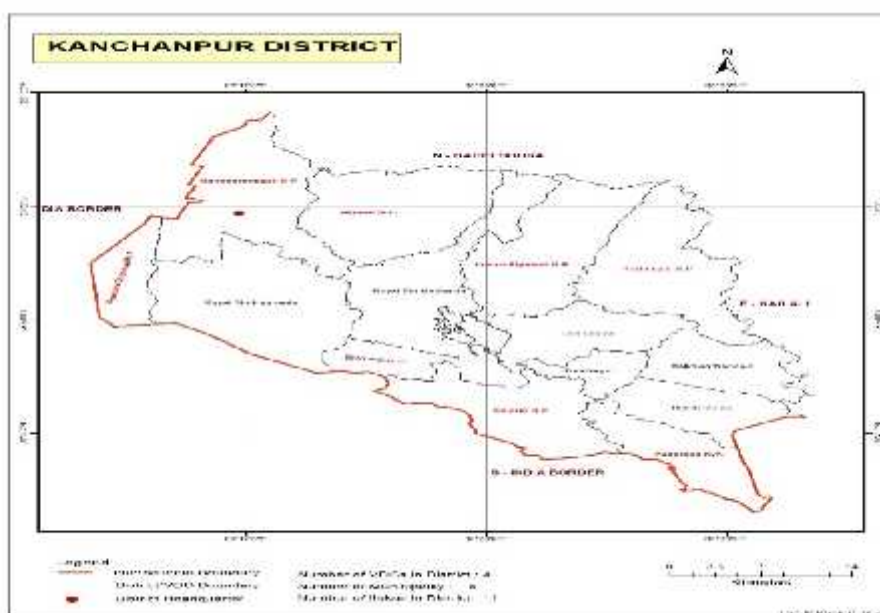


### 3. MATERIALS AND METHODS

#### 3.1 Study area

Kanchanpur district belongs to the far western region of Nepal which is the one of the district of Mahakali zone joined by the border of India. Although it is a small district it is comparatively progressive regarding communication, transportation and irrigation. It stands at the elevation of 159m to 228m from the sea level. Geographically it is 28<sup>0</sup>33"- 29<sup>0</sup>8" north latitude and 80<sup>0</sup>3"- 80<sup>0</sup>33" east longitude. The climate of the district is considered as hot, temperature maximum 43°C and minimum of 6.96°C with rainfall of 3000mm approximately. In Kanchanpur district there is one municipality in which there is 19 wards and 19 VDC are present. Among which Shreepur and JhalariVDC are developing. This district is also famous for having Suklaphata Wildlife reserve.

The present study was done in JhalariVDC of Mahendranagar municipality which is located 20.5km from the Mahendranagar town and is situated near Sayali River. Jhalari is being developed as the main market of kanchanpur district .JhalariVDC is divided in 9 wards as per rule.The study was done in ward no. 2 of JhalariVDC.At the time of the year 2011 Nepal census,JhalariVDC had a population of 24,347 people living there and 4,782 individual household present. In this VDC the buffalo are mainly used for milk, ploughing and social cultural rituals and not much for meat. ([https://en.wikipedia.org/wiki/Jhalari-Pipaladi\\_Municipality](https://en.wikipedia.org/wiki/Jhalari-Pipaladi_Municipality)).



[lgcdp.gov.np/GIS district](http://lgcdp.gov.np/GIS%20district)

### **3.2. Materials used**

#### **3.2.1. Materials used in laboratory:**

- |                        |                         |
|------------------------|-------------------------|
| a. Plastic bags        | i. Slides               |
| b. Forceps             | j. Cover slips          |
| c. Gloves              | k. Beaker               |
| d. Cotton              | l. Small flat top tubes |
| e. Dropper             | m. Mortar and pestle    |
| f. Electric Microscope | n. Strainer             |
| g. Refrigerator        | o. Glass rod            |
| h. Centrifuge machine  | p. Rack                 |

#### **3.2.2. Chemicals:**

- a. 2.5% Potassium dichromate
- b. Methylene blue
- c. ZnSO<sub>4</sub> 33%
- d. Distilled water
- e. NaCl solution

**3.3 Research design:** The present study was designed to assess the gastrointestinal parasitic infection in buffalos of JhalariVDCs 1 and 2 of kanchanpur district. The research comprises:

- a) Selection of buffaloes habitat in JhalariVDCs by direct observation.
- b) Collection of 10 gram fresh faecal samples in sterile plastic vials (25 ml) in preservative by opportunistic random sampling.
- c) Preservation of faecal samples in 2.5% of Potassium dichromate solution.
- d) Examination of faecal samples by using concentration techniques that is flotation and sedimentation techniques.
- e) Identification of eggs .

#### **3.3.1. Study Period:**

January/ February 2015 and June/July 2015

#### **3.3.2 Sample and data collection methods:**

During the collection of faecal samples two days were given in each season in JhalariVDC 1 and 2. Faecal samples were collected in study area. Fresh faecal samples were taken from behind just below of individual Buffalo at early of the morning during time period

between 4 am to 6 am. About 10 gram faecal sample from each buffalo was taken with help of disposable gloves and transferred inside the clean plastic vial having 25ml. The same collection process was repeated for all collected faecal samples. Necessary informations were noted clearly, such as faecal samples collection date, tentative age and sex .

### **3.3.3 Preservation of faecal samples:**

Collected faecal samples of buffalo were preserved in 2.5% Potassium dichromate that help in maintaining morphology and preventing further development of helminth eggs and larva

### **3.3.4 Sample size:**

The total samples taken during winter and summer were 104 and 120 respectively. Altogether 224 samples were taken for examination. The samples were collected from JhalariVDCward no. 1 and 2,Kanchanpur, Nepal.

**3.4Laboratory examination:** The faecal samples were collected in plastic vials (25 ml), transported by bus from Kanchanpur district to Kathmandu and then brought to Central laboratory of Veterinary of Tripureswor laboratory, Kathmandu. The faecal samples were subjected to coprological examination by concentration technique (flotation and sedimentation)..

**3.4.1Concentration techniques:** Eggs/cysts were often low number in faeces that they were difficult to be detected in direct smear. Therefore fecal samples were examined using flotation and sedimentation techniques .(Soulsby, 1982; Zajac and Conboy, 2012).

### **3.4.2. Differential floatation technique:**

Nematode and cestode eggs present in buffalofaeces were detected through this technique. This technique ensures the eggs float in the floatation liquid, which helps to identify the eggs. Approximately 3 gram of faecal sample was placed in a beaker and added 42 ml of water then the samples was grinded lightly with the help of rod and filter the solution by tea strainer. The filtrate solution was poured in to a centrifuge tube of 15 ml and centrifuged at 1,000 rpm for 5 minutes. The tube's water was replaced with more saturated NaCl solution and again centrifuged. After centrifuged, super saturated NaCl solution was added to develop convex meniscus at the top of the tube and one drop of Methylene Blue (to stained) was added where a coverslip can placed for a 5 minutes and

then cover-slip was removed from tube and placed on slide and examined at 10Xx10X and 10Xx40X objectives. The photographs of eggs, cysts and larva of parasites were taken and identified based on colour, shape and size (morphometry).(Soulsby, 1982; Zajac and Conboy, 2012).

#### **3.4.3. Sedimentation technique:**

The technique was used for the detection of trematode eggs. It provides good results as the eggs of trematode are a bit heavier than the other eggs and deposited at the bottom

Three grams of stool sample was taken in a beaker, 42 ml of water was added and grinded lightly with the help of mortar and pestle. The sample was filtered with a tea strainer and the filtered sample was poured in a plastic test tube, centrifuged at 1000rpm for 5minutes. The tube was taken out and the upper water was removed with the help of a pipette. Zinc Sulphate solution was filled in the tube and again centrifuged at 1000rpm for 5minutes. A drop of deposited materials was taken out from the test tube with the pipette and placed on the slide, a drop of methylene blue was added into it then the slidewas examined under the microscope at 10Xx10X and 10Xx40X objectives.(Soulsby, 1982; Zajac and Conboy, 2012).

#### **3.5.Data analysis:**

For this study, prevalence was measured as the percentage of host individuals infected with a particular parasite (Margolis *et al.*, 1982; Bush *et al.*, 1997). The collected data were coded and entered into Microsoft Excel spread sheet. Data were statistically analyzed. Percentage was used to calculate prevalence. Data were statistically analyzed using Chi-square. In all cases 95% confidence interval (CI) and  $p < 0.05$  was considered for statistically significant difference.

#### **Key for trematode,cestode and nematode.**

- ChaudhariS.S., Gupta S.K., Banerjee D.P., BhatnagarP.K., and RuprahN.S.(2003).*General helminthology.Manual of veterinary helminthology*; 1:10-184.
- SoulsbyE.J.L. (1982). *Eggs of worm parasites.Helminthes, Arthropods andProtozoan of Domesticated Animals*; 7<sup>th</sup> edition, 1:24-338.

## **4.RESULTS**

The study has been divided into four parts:

- 4.1. General prevalence of helminths parasite in buffaloes.
- 4.2. Seasonal prevalence of helminths parasites in buffaloes.
- 4.3. Age-wise prevalence of helminths parasite in buffaloes.
- 4.4. Sex-wise prevalence of helminths parasite in buffaloes.

### **4.1 General prevalence of helminths parasite in buffalo**

Out of 224 stool sample,186(83.03%) were found infected with one or more species of helminths parasites.

#### **Figure 1:General prevalence**

### **4.1.2 Overall class-wise prevalence of helminths parasite of buffalo**

A total of eleven parasites (ova/egg) were identified of which six genera of trematodes, one genus of cestode and four genera of nematode.

#### **Figure 2: Overall class-wise prevalence of helminthes parasite in buffaloes**

#### 4.1.3: Overall prevalence of different parasite in buffalo

Overall, buffaloes were found to be infected with helminths parasites belonging to three classes. Trematode parasites were found to be highly infected belonging to six genera followed by nematodes, i.e. four genera and least prevalence was seen in case of cestode with one genera.

**Table 1: Overall prevalence of different parasite in buffalo**

S.N	Class	Parasites Name	Prevalence	
			NO.	%
1.	Trematode	<i>Fasciolagigantica</i>	44	19.64
2.		<i>Fasciola hepatica</i>	28	12.5
3.		<i>Dicrocoelium lanceatum</i>	49	21.87
4.		<i>Ornithobilharzia pierce</i>	32	14.28
5.		<i>Schistosomamansoni</i>	9	4.02
6.		<i>Schistosomabovis</i>	16	7.14
7.	Cestode	<i>Anopocephalasp.</i>	1	0.45
8.	Nematodes	<i>Tristrongyluscolumbiformis</i>	3	1.34
9.		<i>Strongyloidessp.</i>	1	0.45
10.		<i>Ostertagiasp.</i>	1	0.45
11.		<i>Toxocaravitulorum</i>	2	0.89
	Total		186	83.02

Trematode parasite revealed to be highly infected to buffalo with six genera. The highest prevalence was showed by *Dicrocoelium lanceatum* (21.87%) followed by *Fasciolagigantica* (19.64%), *Ornithobilharzia pierce* (14.28%), *Fasciola*

*hepatica*(12.5%),*Schistosomamansoni*(4.02%)but, buffalo were found to be least infected with cestode with one genera *Anopocephalasp.*, (0.45%) and nematodes with four genera *Tristrongyluscolumbiformis*(1.34%), *Toxocaravitulorum*(0.89%), *Strongyloidessp.* and *Ostertagiasp.* (0.45%) .

#### **4.2. Seasonal prevalence of helminths parasites in buffaloes**

Out of 224 total samples examined in two season, 120 samples were observed in summer and 104 in winter respectively. Among them 107(89.10%) host were found positive in summer and 79(75.90%) host were found positive in winter season. In this study it was observed that the season of the year had profound effect on the prevalence of endo parasite in buffaloes. The seasonal prevalence of different helminth parasites result statistically significant in buffaloes ( $\chi^2=3.841$ ,  $P<0.05$  and  $d.f=1$ ).

#### **Figure no.2 Seasonal prevalence of helminth parasites in buffaloes**

##### **4.2.1 Class-wise seasonal prevalence of helminth parasite in buffaloes**

Altogether eggs of 11 genera were observed during examination of the samples. On seasonal basis ten species(90.90%) were recorded during summer and seven species(63.63%) were observed during winter.

### Figure no.3 Class--wise seasonal prevalence

#### 4.2.2 .Seasonal prevalence of trematodes during summer and winter

A total of 120 samples were examined in summer and 104 samples were examined during winter. Among the summer samples 100(83.33%) samples were found to be positive for trematodes in summer and 78( 75%) samples were found positive for trematodes in winter.

The difference in the prevalence of different genus of trematode during summer and winter was found not statistically significant. ( $\chi^2=1.78$ ,  $P<0.05$  and  $d.f=1$ ).

**Table no.2 Seasonal prevalence of trematodes**

Trematode	Summer	Percentage	Winter	Percentage
<i>Fasciolagigantica</i>	28	23.3%	16	15.38%
<i>Fasciola hepatica</i>	20	16.6%	8	7.69%
<i>Dicrocoelium lanceatum</i>	23	19.1%	26	25%
<i>Ornithobilharzia pierce</i>	22	18.33%	10	9.61%
<i>Schistosomamansoni</i>	2	1.66%	7	6.73%
<i>Schistosomabovis</i>	5	4.1%	11	10.57%
Total	100	83.3%	78	75%

The highest prevalence was shown by *Fasciolagigantica* 28(23.3%) and the lowest prevalence was shown by *Schistosomamansoni* 2(1.66%) in summer season where in



winter the highest prevalence was shown by *Dicrocoelium lanceatum* 26(25%) and the lowest prevalence was shown by *Schistosoma mansoni* 7(6.73%).

#### 4.2.3. Prevalence of cestode during summer and winter

Out of 120 samples, one sample (0.83%) was found to be positive for *Anoplocephala* sp. (cestode) in summer season and none of cestodes were recorded in winter.

#### 4.2.4 Prevalence of nematodes during summer and winter

The total number of positive samples found during both the seasons were 186(83.03%) out of 224 samples. The prevalence rate for nematodes during winter was 0.96% i.e. one sample was positive and that of summer was 4.86% i.e. six samples were found positive. The difference in the prevalence of different genus of nematodes during summer and winter result statistically insignificant. ( $\chi^2 = 3.57, P < 0.05, d.f. = 1$ )

**Table.4: Prevalence of nematodes during summer and winter.**

Nematode	Summer	Percent	Winter	Percent
<i>Trichostrongylus columbiformis</i>	2	1.6%	1	0.96%
<i>Strongyloides</i> sp.	1	0.83%	0	0%
<i>Ostertagia</i> sp.	1	0.83%	0	0%
<i>Toxocaravitulum</i>	2	1.6%	0	0%
Total	6	4.86%	1	0.96%

Among nematodes the highest prevalence was shown by *Trichostrongylus columbiformis* and *Toxocaravitulum* 2(1.6%) and the least prevalence is shown by *Strongyloides* sp. and *Ostertagia* sp. for summer i.e. 1(0.83%). and in winter season only one genera was found positive i.e. is *Trichostrongylus columbiformis* 1(0.96%).

#### 4.3. Age-wise prevalence of helminths parasite in buffaloes

In the present study there were total 224 samples out of them 70 were calves, 70 were young and 84 were older. Here buffaloes were distinguished in three categories, age from 0.5 to 2 years (calves), age > 2 to 5 years (young) and age > 5 years (older). The age relate

prevalence of different helminthes parasites results statistically significant in buffaloes ( $\chi^2=15.07$ ,  $P<0.05$ , d.f.=2).

Prevalence of endoparasites was higher (94.05%) in older animals followed by young animals (88.57%) and then calves (64.28%). Calves and young were infected by highest number (eight species) of parasites followed by older (seven species) but *Fasciolagigantica*, *Fasciola hepatica*, *Dicrocoelium lanceatum*, *Ornithobilharzia pierce*, *Schistosomamansoni* and *Schistosomabovis* were common among all age group.

**Figure No.3 :Age wise prevalence of helminths parasite**

**Table 5.Age-wise prevalence of helminthes parasite in buffaloes**

S.No.	Parasites recovered	Calves(0.5-2)years		Young(>2-5)years		Older(>5years)	
		N=70	%	N=70	%	N=84	%
1	<i>Fasciolagigantica</i>	11	15.71	13	18.57	20	23.81
2	<i>Fasciola hepatica</i>	9	12.85	6	8.57	13	15.48
3	<i>Dicrocoelium lanceatum</i>	7	10	15	21.43	27	32.14
4	<i>Ornithobilharzia pierce</i>	8	11.43	14	20	10	11.90
5	<i>Schistosomamansoni</i>	2	2.86	5	7.14	2	2.38

6	<i>Schistosomabovis</i>	4	5.71	7	10	5	5.95
7	<i>Anopocephala</i> sp.	0	0	1	1.43	0	0
8	<i>Trichostrongyluscolumbiformis</i>	3	4.29	0	0	0	0
9	<i>Strongyloides</i> sp.	0	0	1	1.43	0	0
10	<i>Ostertagi</i> asp.	1	1.43	0	0	0	0
11	<i>Toxocaravitulorum</i>	0	0	0	0	2	2.30
	Total	45	64.28	62	88.57	79	94.05

In calves(0.5 to 2 years), prevalence was relatively highest in case of *Fasciolagigantica*(15.71%),andlowestin*Ostertagi*asp.(1.43%).Similarly*Anopocephal*asp., *Strongyloides* sp. and *Toxocaravitulorum*were absent in calves.

In young animals (> 2 to 5 years) prevalence was relatively higher in case of *Dicrocoeliunlanceatum*(21.43%),*Anopocephala* sp. and *Strongyloides*sp.(1.43%). Interestingly*Trichostrongyluscolumbiformis*and*Toxocaravitulorum*were absent in young animals.

In older (> 5 years), prevalence was relatively higher in case of *Dicrocoeliunlanceatum*(32.48%) and lowest was*Schistosomamansoni* and*Toxocaravitulorum* (2.38%). Here*Anopocephal*asp.,*Strongyloides* sp., *Trichostrongyluscolumbiformis* and *Ostertagi*asp. were found absent in older animals.

#### **5.4Sex-wiseprevalence of helminths parasites in buffaloes**

Among 224samples examined 162(72.32%) samples belong to female buffaloes and 62(27.65%) samples belong to male buffaloes.Sex-wise136females(83.95%)and 50males(80.64%) were found to be positive for one or more heminths parasites.. In the present study the sex-wise prevalence of different helminthes parasites results significant in buffaloes (  $\chi^2=44.64$ ,  $P<0.05$ , d.f.=1).

**Figure.4: Sex-wise prevalence of helminths parasites in buffaloes**

**Table. 6:Sex-wise prevalence of helminths parasites in buffaloes.**

S.No.	Parasites recovered	Female		Male	
		N=162	%	N=62	%
1.	<i>Fasciolagigantica</i>	36	22.22	8	12.90
2.	<i>Fasciola hepatica</i>	22	13.58	6	9.68
3.	<i>Dicrocoelium lanceatum</i>	40	24.69	9	14.52
4.	<i>Ornithobilharzia pierce</i>	20	12.34	12	19.35
5.	<i>Schistosomamansoni</i>	4	2.46	5	8.06
6.	<i>Schistosomabovis</i>	10	6.17	6	9.68
7.	<i>Anoplocephalasp.</i>	1	0.61	0	0

8.	<i>Tristrongyluscolumbiformis</i>	1	0.61	2	3.23
9.	<i>Strongyloidessp.</i>	1	0.61	0	0
10.	<i>Ostertagiasp.</i>	1	0.61	0	0
11.	<i>Toxocaravitulorum</i>	0	0	2	3.23
	Total	136	83.95	50	80.64

In this study, it was detected that the prevalence of helminths parasites were higher in female (83.95%) buffaloes than in male (80.65%) buffaloes. It was interesting that, *Anoplocephala* sp., *Strongyloides* sp., *Ostertagia* sp. were observed only in female animals. In female animals, the prevalence was relatively higher in case of *Dicrocoelium lanceatum* (24.67%) followed by *Fasciolagigantica* (22.22%), *Fasciola hepatica* (13.6%), *Ornithobilharzia pierce* (12.34%), *Schistosomabovis* (6.17%), *Anoplocephalasps*, *Tristrongyluscolumbiformis*, *Strongyloidessp.* and *Ostertagiasp.* (0.61%). *Toxocaravitulorum* was found absent in female buffaloes.

In male animals, the prevalence of helminths parasites was relatively higher in case of *Ornithobilharzia pierce* (19.35%) followed by *Dicrocoelium lanceatum* (14.52%), *Fasciolagigantica* (12.90%), *Fasciola hepatica* (9.68%), *Schistosomabovis* (9.68%), *Schistosomamansoni* (8.06%) and *Toxocaravitulorum* (3.23%). The higher prevalence of trematodes was 81.48% followed by nematodes (1.85%) and cestode (0.61%) in female buffaloes. In case of males the highest prevalence was shown by trematodes (74.19%) followed by nematodes (6.45%) and cestodes were found absent in male buffaloes.

## IDENTIFICATIONS OF HELMINTH'S EGGS

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

Name of parasites	Morphological characters

<i>Fasciolagigantica</i> ,(Linnaeus, 1855)	yellowish in colour, consist of embryonic mass and shell, operculum usually indistinct.
<i>Fasciolahepatica</i> ,(Cobbold,1855)	Yellow to brown ,unembryonated,thinshell,operculated,oval
<i>Dicrocoeliumlanceatum</i> ,(Rudolphi, 1819)	dark brown in colour, operculated and thick- shelled
<i>Ornithobilharzia pierce</i> ,(Stiles andHassall,1998)	terminal spine, short appendage at the other end
<i>Schistosomamansoni</i> ,(Sambon, 1907)	spindle shaped, flattened at one side, greatly elongated with straight slender terminal spine
<i>Schistosomabovis</i> ,(Bilharz,1852)	Fusiform in shape and very much narrowed at the extremities and always carries pyriform shape.
<i>Anopocephalasp</i> ,(Blanchard1848)	Eggs pyriform apparatus with hyaline –liked walled and contain six hooklets (hexacanth embryo)
<i>Tristrongyluscolumbiformis</i> , (Giles, 1892)	oval and symmetrical, shell has a thin and transparent outer chitinous layer and a thin innerlipoidal layer, embryonic mass multisegmented and varies from 16-32 in number
<i>Strongyloidessp.</i> (Chitwood and McIntosh,1934)	ellipsoidal,thin shelled, embryonated when laid
<i>Ostertagiasp</i> (Stiles 1892)	elliptical in shape
<i>Toxocaravitulorum</i> ,(Goeze,1782 and Travassos,1927)	sub-globular and have finely pitted albuminous layer

Reference:SoulsbyE.J.L(1982).*Eggs of worm parasites. Helminthes,Arthropods and Protozoan of Domesticated Animals*;7<sup>th</sup> edition,1:24-338.

## **List of Photographs**

**Trematode parasites (10Xx10X):**





## 5. DISCUSSIONS

It is well known fact that parasites are cosmopolitan in distribution and all animals bear different kind of parasites. Many studies have been carried out regarding the gastrointestinal parasites of domestic livestock.

The aim of this study was to investigate the prevalence of helminthes parasite in buffalo. During the study period, stool samples were collected from Jhalari VDC of Kanchanpur district. Out of 224 samples examined 186 samples were positive. The research work indicates that the buffaloes are very much susceptible to endoparasites infection. About 83.03% buffaloes were found to be infected with endoparasite. Prevalence of trematodes namely *Fasciolagigantica* (19.64%), *Fasciolahepatica* (12.5%), *Dicrocoelium lanceatum* (21.87%), *Ornithobilharzi pierce* (14.28%), *Schistosomamansonii* (4.02%), *Schistosomabovis* (7.14%), Cestode namely *Anoplocephalasp.* (0.45%), and Nematodes namely *Trichostrongyluscolumbiformis* (1.34%), *Strongyloidessp.* (0.45%), *Ostertagiasp.* (0.45%), *Toxocaravitulorum* (0.89%) were recorded. Similar studies were conducted by some scientist in Nepal, India, Pakistan, Bangladesh and abroad.

Similar finding was reported by Mukhia (2007) who revealed that 83.96% of the buffaloes were positive for internal parasites in Kathmandu, Nepal. Gurung (2007) revealed 61.90% of overall prevalence of helminthes parasites in buffalo from Kathmandu, Nepal. Here Azam *et al.* (2002) and Bachal *et al.* (2002) recorded 64.41% and 47% buffaloes suffered from different types of helminthes in Pakistan, respectively, which were quite lesser than present study

The present finding is contrast with the earlier finding of Morsy *et al.* (2005) and Shazly *et al.* (2007) who recorded 20% and 9.73% infection of fasciolosis in Bangladesh and Egypt, respectively which were lesser than the present finding. From Nepal, the highest prevalence of *Fasciola hepatica* i.e., 67.66% has been reported from Thuladihi, Syangja among buffaloes (Regmi *et al.*, 1999). Similarly 40.12% infection in animals from Pachthar district (Sharma 1997-98), which were the highest infection among buffaloes comparing to the present study i.e. *Fasciolasp.* (32.14%). Similar type of prevalence of the *Fasciola sp.* has been reported by Mukhia (2007) in buffaloes which is 32.06% in Satungal, Kathmandu, Nepal.

For *Dicrocoelium lanceatum*, Mukhia (2007) reported 20.61% and Gurung(2007) reported 18.10% which is similar to the present study i.e.21.87%.

Most of the species like *Schistosoma mansoni*, *Schistosoma bovis*, *Schistosoma japonicum* has been reported from Surkhet district Ghimire (1987). Similarly Mukhia G. (2007) reported *Schistosoma mansoni* (4.19%), *Schistosoma bovis*, (46.56%), *Schistosoma japonicum* (0.38%) from Satungal, Kathmandu, Nepal. Gurung(2007) also reported *Schistosoma* spp. (28.10%), which were higher than the present study, here *Schistosoma mansoni* (4.02%) and *Schistosoma bovis* (7.14%). The difference among these findings from different regions might be due to geographical variation, sample size, variation in management system, sample collection period, and sampling method., differences (Wannas *et al.*, 2012; Tilahun *et al.*, 2014; Yadav *et al.*, 2014).

During present study Cestodes, *Anoplocehala* sp. (0.45%) was only reported. Previously, Uslu and Guclu (2007) found *Anoplocehala* sp. (2.7%) and (6.17%) in horses and in donkeys respectively in Konyu region. Dhakal (2011) had found *Anoplocehala* sp. (0.77%) in host cattle. It may be due to contamination while drinking water from the same water sources.

Among Nematodes, *Trichostrongylus columbiformis* (0.76%), *Strongyloides* sp. (4.19%), *Ostertagia* sp. (1.52%), *Toxocara vitulorum* (20.99%) were reported by Mukhia (2007) from Kathmandu, Nepal which were similarly to present finding i.e. *Trichostrongylus columbiformis* (1.33%), *Strongyloides* sp. (0.45%), *Ostertagia* sp. (0.45%), *Toxocara vitulorum* (0.89%). But the prevalence rate of *Strongyloides* sp. (4.19%) and *Toxocara vitulorum* (20.99%) which were quite higher in Mukhia (2007) than present study. The presence of these common nematodes might be due to the ingestion of contaminated soil and herbage during grazing as well as the propensity of buffaloes to seek rivers, pools or swamps for wallowing.

The variations among the findings might be due to the difference in the sample size, selection of samples, techniques of sample collection, period and place of study, environmental factors, breed of the animals etc

The present finding was done between two seasons winter and summer. It was found that seasonal fluctuation also affects the prevalence of endoparasitic infection in buffaloes. There was significant ( $P < 0.05$ ) difference in between season and parasitic infection. A relatively higher infection with endoparasites was observed in summer season (89.10%) than in winter season (75.90%). However the finding of Mamun (2008) observed a relatively higher infection with endoparasites were observed in rainy season (71.70%),

followed by summer(58.90%) and winter (52.27%) season in Bangladesh. Azhar *et al.*(2002) who reported the overall highest (24.0%) seasonal prevalence in all types buffaloes during autumn, followed by spring (20.0%), winter (13.0%) while the lowest (9.0%) was recorded in summer in Pakistan.

Here the present study has been done between two season, summer and winter, but the previous studies were done either in three seasons (summer, winter and rainy) or in four season seasons(summer, autumn, winter and spring). The contrast in between the present and earlier finding can be explained by the fact of variation in the geographical location of the experimental area and also the methods of study. However, the highest prevalence in summer season may be due to more temperature which favours dessication of egg and parasitic growth and multiplication.

During the study it was revealed that, there was significant difference between the age of the buffalo and the parasitic infection. It was found that older animals (94.05%) were more susceptible to infection than younger animals (88.57) and calves(64.28%) Azhar *et al.* (2002) who reported that the higher prevalence of helminthes infection in older animals compared to younger (below 2years of age) in buffaloes of Punjab province, Pakistan. The present finding is in agreement with the earlier report of Alim (1997) who also reported that highest rate of infection was found in the Buffaloes of above 10 year of age (65.63%). The finding of Shrestha and Joshi (2010) also support the present finding which report that older animals (35.78%) were infected with *Fasciola* infection than young buffaloes (29.41%) and calves (14.81%).

But the present finding is in contrast with the previous finding of Asif *et al.*(2007)and Mamun(2008) who noticed that higher prevalence of helminthes infection in youngeranimals compared to older buffaloes. The cause of this variation in the prevalence of infection in different age groups is difficult to explain but it might be due to an immunological phenomenon.

There was significant ( $P<0.05$ ) difference in the rate of infection in between the male and female buffaloes. It was observed that the prevalence of endoparasitie was slightly higher in female (83.95%) than in male buffaloes (80.64%) which supported the finding of Bachal *et al.* (2002) who reported a slightly higher prevalence (48.30%) of helminthes in females than in males (45.12%) in buffalo calves. Similarly Alim (1997) also reported that females (52.65%) were more susceptible to *Fasciola* infection than males (47.76%). The finding of Shrestha and Joshi (2010) also support the present finding which report that female (38.05%) was found more infected with fascioliasis than males (16.09%). But

present finding is contrast with the earlier finding of Mamun (2008) who reported that the prevalence of endoparasite was slightly higher in males (61.34%) than females (59.52%).

On the other hand, Azharet *al.* (2002) reported no sex variation. He noticed that buffaloes of either sex were equally affected. This disparity among the findings cannot be explained exactly but it might be assumed that hormonal influence may be associated with this. Methods of study, selections of samples and breed of buffaloes may also be associated with this.

## 6. CONCLUSION AND RECOMMENDATIONS

### 6.1. Conclusion

In the study, the age, sex and season related prevalence of helminths parasites was studied and identification of different helminths parasites was done. Among 224 samples 83.03% of buffaloes were found to be infected with different gastrointestinal helminth parasites, where 79.44% were trematodes, 0.45% were cestodes, 3.13% were nematodes. In season-wise prevalence, 120 samples were examined in summer and 104 samples were examined in winter. Among them 107 (89.10%) were found positive for summer and 79 (75.90%) host were found positive for winter. In class-wise seasonal prevalence of different helminth parasites, 100 (83.33%) and 78 (75%) samples were found positive for trematodes in summer and winter season respectively, for cestodes only one sample was found positive in summer season and for nematodes 6 (4.86%) and 1 (0.96%) samples were found positive for summer and winter respectively. In age-wise prevalence of different helminth endo-parasites older buffaloes were highly infected i.e., (94.05%) followed by younger (88.57%) and then calves (64.28%). In sex-wise prevalence, among 162 (72.32%) female 136 (83.95%) were found positive for different helminth parasites and among 62 (27.67%) males, 50 (80.64%) were found positive. In female buffaloes the prevalence was found higher than males.

The prevalence of endoparasites was studied by examination of faeces only, through ova detection which makes it difficult to study the species wise identification of the parasite (like *Strongyles*). The detection of pseudo parasites makes the identification even more difficult. Very less work has been done on pseudo parasites. Besides this study, the actual costs have to be determined. It would help to justify the necessity of control program against the parasitic disease. So, further study should be conducted to assess the economic losses due to parasitic diseases of buffaloes to find out effective control strategies against it. Even more work like detection of ecto-parasites and blood parasite can also be done.

### 6.2. Recommendations

Minimizing contaminated open grazing, appropriate feed supply to the buffaloes to prevent frequent infection and increase immunity.

Infected buffaloes with helminth parasites should be treated with anti-helminths under the proper supervision of veterinary personals

Water being the main source of contamination of various GI parasites, the animal should be supplied with treated water instead of letting them to drink from different water sources.

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