

COPROLOGICAL SURVEY OF GASTRO-INTESTINAL HELMINTH IN BUFFALO (*Bubalus bubalis*) OF DHRAMPUR, DHANUSHA, NEPAL



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Kirtipur, Kathmandu

Nepal

October, 2015

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author or institution.

Date:..... October 2015

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RECOMMENADATION

This is the recommended that the thesis entitled " **Coprological survey of Gastro-intetinal helminth in Buffalo (*Bubalus bubalis*) of Dhrampur Dhanusha, Nepal**" has been carried out by **Prabhaw kumar Sah** for the partial fulfilment of Masters Degree of science in Zoology with Special paper **Parasitology** . This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any Institutions.

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LETTER FOR APPROVAL

On the recommendation of supervisor "**Janak Raj Subedi**" this thesis submitted by **Prabhaw Kumar Sah** entitled "**Coprolological Survey of Gastro-Intestinal Helminth in Buffalo (*Bubalus bubalis*) of Dhrampur, Dhanusha, Nepal**" is approved for the examination and submitted to the Tribhuvan University in partial of the requirements for the Master's Degree of Science in Zoology with special paper **Parasitology**.

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LIST OF ABBREVIATION

ADPCD: Animal Disease Protection and Control Division

CBS: Central Bureau of Statistics

CVH: Central Veterinary Hospital

FAO: Food and Agriculture Organization

GDP: Gross domestic product

GI: Gastro Intestinal

IAAS: Institute of Agriculture and Animal Science

MOAC: Ministry of Agriculture and Cooperative

rpm: rotation per minute

VDC: Village Development Committee

VEC: Veterinary Epidemiological Center

ABSTRACT

Buffalo (*Bubalus bubalis*), an important species of domestic livestock, is generally affected by helminth parasites. Present study was carried out to find the prevalence of gastrointestinal helminth parasites of buffaloes. Out of a total of 300 fecal samples collected 150 each during summer and winter season, were tested for the presence of gastrointestinal helminths using sedimentation and flotation techniques and the parasites were identified morphologically under microscope. The test revealed 74% and 84.66% positive for the presence of eggs of gastrointestinal helminths for the winter and summer season respectively. The fecal samples showed the prevalence of trematodes (47.01%), cestodes (2.66%) and nematodes (10.66%) during winter season.

Likewise, the helminth eggs of trematodes, cestode and nematodes were 52.99%, 3.66% and 12 % of respectively during summer. Among the trematodes, *Fasciola* sp showed a higher (23.33%) prevalence followed by *Paramphistomum* (17.66%) and *Dicrocoelium* (9.66%). Among cestodes, the only eggs detected belonged to *Monezia* (6.33%) sp. Among nematodes *Trichostrongylus* sp showed a higher (9.33%) prevalence followed gradually by *Toxocara* (5.66%), *Strongyloides* (2.66%), *Trichuris* (1.66%), *Ostertagia* (1%), *Haemonchus* (0.66%), *Chabertia* (0.66%), *Cooperia* (0.33%), and *Capillaria* (0.33%) spp. The parasite eggs were identified morphologically under microscope.

The different in the prevalence of positive and negative samples were found statistically insignificant ($\chi^2 = 51.62$, $P < 0.05$, d.f. = 14). Single infection was observed in 216 (90.76%) samples, moderate infection in 14 (5.88%) samples and heavy infection 8 (3.66%) samples.

1. INTRODUCTION

1.1 Background

Bubalus bubalis (buffaloes) is one of the most important species of the domestic livestock as a source of dairy, manure, meat and drought power. It is the one of the oldest most important domestic livestock in Nepal. It belongs to the order Artiodactyla. The family Bovidae is known to feature in the folklore, literature and religion of many lands its domestication was in evidence some 4,500 year ago in the Indus valley civilization (Cockrill, 1970). Livestock farming is an integral part of the farming system and buffaloes contribute substantially in the livestock sector in Nepal. Buffalo in Nepal has been acclimatized and adapted to a wide range of environmental conditions. It is widely distributed from sub-humid regions of sub-tropics in terai to cool temperature regions in hill and mountains (Shah, 1981/82). Epstein (1977) has classified Nepalese buffaloes into four regional types: Terai, Hilly, Midland and Himalayan mountain. These type are distinguished by the size, shape curvature and length of their horns and the colour of their coats.

Bubalus bubalis (buffaloes) are essential components of the mixed farming system in Nepal and are found in all parts of the country. Buffaloes are substantially distributed in more than 25 tropical countries, stretching from southern-Europe through India and China to the whole of the south-east Asia. The world population of domestic buffalo in 1979 has been estimated at 126 million of which 122 million are in Asia including 60.7 million head of the buffalo in India alone (Kim and Ali, 1981). Therefore, nearly half of the world buffalo population is in Indian sub continent and yet scientific knowledge concerning this animal has not been commensurate with its increasing numbers and importance. Buffaloes are main producers of milk preferred species an indicated by the increasing ratio of she-buffaloes to cause in many part of country.

Nepal produced about 1,72,414 metric tons buffaloes meat and 11, 53,838 metric tons buffaloes milk in year of 2013. The number of milking buffaloes in the year 2013 was 51, 33,139. According to livestock production Nepal has been produced 1,75,232 metric tons buffalo meat and 11,88,433 metric tons buffalo milk in the year 2014 and the number of

milky buffalo 52,41,873 in 2014. The annual production of milk was 380 kg per household in the year 2014 (VEC, 2014).

1.2 Buffalo (*Bubalus bubalis*)

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 condition. It is widely distributed from sub-humid regions of subtropics in terai to cool temperature region in the hill and mountains (Shah 1981/82).

Buffalo are the main producers of milk and preferred species as indicate by the increasing ratio of sea buffaloes to cow in many part of the country. The number of buffaloes which are produced meat and milk per year give below:

Table no 1. Number of buffaloes which produce milk and meat

Year	No of buffaloes	Milk production in metric tons	Meat produced in metric tons
2008	44,96,507	9,87,780	1,11,209
2009	46,80,486	10,31,500	1,16,627
2010	48,36,984	10,66,867	1,62,213
2011	49,93,650	11,09,325	1,67,868
2012	51,33,139	11,53,838	1,72,416

Source: VEC, 2013.

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 produced 11,53,838 metric tons buffalo milk and 1,12,414 metric tons buffaloes meat (VEC 2013). The total production of buffaloes that contributes to the national meat production is composed of 16,68,820 buffaloes (CBS 2012) on the analyzing the contribution of different animal species in nation meat supply. It was evidenced that buffalo contributed about 57.4% of total meat supply (Joshi et. al., 2003).

1.3 Endoparasitism

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

The parasites which are living in the gut, body cavity level, lungs, gull bladder and the blood and within the internal cavity, tissues of the host which organism are endoparasites. They are totally dependent upon the host causing infection of them. They mainly depend upon the food and shelter eg. *Fasciola*, *Moniezia* and *Ascaris* etc.

Most of the buffaloes disease have been identified as one of the major factor which has caused substantial economic loss to the poor subsistent farmers in the developing countries (Othman and Beaker 1981). The parasite diseases are usually very important factor which cause the death of many buffalos yearly. Parasite usually includes gastro intestinal helminthes, coccidiosis, fascialosis and mange (Othman and Beaker 1981). Cockrill (1974) states the buffalo is exposed to a higher risk of infection with snail born helminth due to the animals propensity to seek river, pools or swamps for wallowing. The infection with fluke, tapeworms, and roundworms are responsible to the lower the overall production both by way of morbidity and mortality.

A parasite is an organism that lives in or on and takes its nourishment from another organism. Intestinal parasite includes helminth. Helminthes are worm such as tapeworm, pinworm and roundworm. (Modi at al. 1977).

1.4 Objective

1.4.1 General objective:

To study the coprological survey of gastro-intestinal helminths in buffaloes during winter and summer seasons in Dhanusha, Dhrampur.

1.4.1 Specific objectives

- To identify the eggs of gastrointestinal helminth parasites in buffaloes.
- To determine the seasonal prevalence of trematodes , cestodes and nematodes.
- To determine the number of egg present per gram of faeces.
- To develop the recommendation for planning and control of helminth parasite in buffaloes.

1.4.3 Hypothesis:

H_0 = The number of significant different in prevalence of helminth parasite in winter and summer.

H_1 = There is significant difference in prevalence of helminth parasite in winter and summer.

1.5 Limitation of the Study

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

- This academic study has been carried out for the partial fulfillment of requirement master's in degree in Zoology at Tribhuvan University Kathmandu Nepal.
- The time for the study was also limited and carried out within two seasons only.
- The identification of parasites was done up to genus level only based on microscopic examination of egg morphology.

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 Francisco Redei, the grandfather of parasitology. He demonstrated development of maggots from eggs of flies. He also proved that *Ascaris* had males and females and produced eggs. At the same time, Leeuwenhoek perfected microscope and discovered protozoan parasites. Rudolf Leuckart considered as “Father of parasitology”. The word ‘Parasites’ derived from Greek. It means situated beside. In the field of parasitology, his studies of the liver fluke, *Taenia* and *Trichinella 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 monogenetic trematodes parasitize only fish, while the highly evolved digenetic trematodes are found not only in fish but more commonly in higher vertebrates. Furthermore, the more advanced digenetic 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. 1989). 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 helminth diseases among human and animals. Literatures exist in helminth 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 parasites have been mentioned here.

2.1 In Global Context

2.1.1 Endoparasites of the buffaloes

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, *Trichostrongylus* 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 (1970) 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.

Helminths

i. Trematodes

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 includes *Fasciola* sp., *Dicrocoelium* sp. and *Paramphistomum* sp.

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).

Fasciola, are known as fluke that cause fascioliasis, commonly called as Namle, Mate, Lew, etc. *Fasciola* sp. has been identified 15.33% in buffaloes from India (Petal et al. 2015), 9.8% in buffaloes were observed in Tanzania (Mahanga et al. 2013), 22.46% of *Fasciola gigantica* has been identified in buffaloes from Bangladesh (Mumun et al. 2011), *Fasciola* has been identified in India (Mondal et al. 2000 and Sing et al. 2009), in South Nigeria (Opara et al. 2010 and Kingsely et al. 2013), in Pakistan (Farooq et al. 2013) and Bangladesh (Rahman Mustafizur 2012 and Hossain 2012) *Fasciola hepatica* has been reported.

Similarly, *Paramphistomum* sp. has been reported 29.24% in buffaloes from India (Petal et al. 2015), 4.9% were observed in buffaloes from Tanzania (Mahanga et al. 2013), 22.94% in water buffaloes from Bangladesh (Mumun et al. 2011), in India (Mandal et al. 2000, Singh et

al. 2009 and Broghare et al. 2009), in Bangladesh (Kehungo et al.2010, Rahaman and Mustafizer 2012), in Italy (Fagiolini et al. 1010) and it has been reported in cattle in Pakistan (Naeem et al.2011). Similarly, *Dicrocoelium* sp. was reported in cattle in Nigeria (Kingsely et al. 2013), *Dicrocoelium dendriticum* (2.6%) in buffaloes reported from Italy (Condoteo et al. 2010) followed by 2.4% in buffaloes from Italy (Rinaldi et al. 2009).

ii. Cestodes

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 *Moniezia* sp. in ruminants of the buffaloes 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 cows or buffaloes tapeworm has two hosts viz., definitive host man and in trematodes host cow or cattle. It is also called beef tapeworm. The worms (segments) passes out along with the stool of human being and when ingested by cattle, infects them on reaching alimentary canal of the host, the eggs hatch out and liberated and they penetrate the gut wall and enter mesenteric lymphatics and finally reaches circulation. Then they invade the muscular tissue and undergo further development.

Different type of tapeworm belongs to the class cestodes as *Moniezia* sp., *Taenia* sp. etc. They are habited in the small intestine of domestic as well as wild animals such as buffalo, goat, cattle, deer, sheep etc. *Moniezia* sp. is the largest tape worm also called as sheep tape worm that cause by poor growth, intestinal obstruction, weight loss diarrhoea during heavy infection. (2.66%) *Moniezia benedeni* has been reported in India (Petal et al. 2015), *M. expansa* and *M. benideni* repotted in Pakistan (Raza et al. 2013), 4.65% has been reported in cattle in Nigeria (Kingsely et al. 2013), 20.95% repotted in goats in Nigeria (Adoun et al. 2012) and Ukraine (Kuzmina et al. 2010).

iii. Nematodes

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 mucosal membrane with. *Toxocara* sp., and *Dictyocaulus* sp., has the worldwide distribution and the prevalence is higher in buffaloes and cattle. *Trichostrongylus* sp. is an infection of the gastrointestinal tract of herbivorous animals and man is the accidental host caused by the members of the genus *Trichostrongylus* sp. The infection is acquired by ingestion of contaminated vegetables or drinks with the filariform larvae. *Strongyloides* sp. is an intestinal infection of man caused by the penetration of the skin by the filariform larvae of *Strongyloides stercoralis*.

Toxocara sp. 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. *Haemonchus* sp. 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 sub-mandibular region (Singh et al. 2006).

Ostertagia sp. 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* sp. is relatively small worm found in the small intestine, rarely in the abomasums of ruminants.

Among roundworms of buffaloes, the commonest are *Trichostrongylus* sp., *Ascaris* sp., *Strongyloides* sp. etc. 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 anemia, diarrhea, poor growth and even death.

Strongyloides sp. (46.39%) has been reported in young buffaloes in India (Patel et al. 2015), 2.6% were reported in cattle in Ethiopia (Bacha et al. 2014), 4.1% were reported in buffaloes in Tanzania (Mahanga et al. 2013), 54.29% were reported in goats in Nigeria

(Adoun et al.2012) and 0.85% were reported in buffaloes in Bangladesh (Mamun et al. 2011), *Toxocara vitulorum* (2.54%) has been reported in Buffaloes in India (Petal et al. 2015), 2.4% were reported in buffaloes in Tanzania (Mahanga et al. 2013). Likewise *Toxocara vitulorum* has been observed in cattle in Pakistan (Raza et al. 2013) and *Toxocara vitulorum* were reported in buffaloes in Bangladesh (Mamun et al. 2011) and *Trichostrongylus axei* (5.33%) were reported in Buffaloes in India (Petal et al. 2015), in cattle from Ethiopia (Bacha et al. 2014), in buffaloes in Tanzania (Mahanga et al. 2013), *Trichostrongylus* sp. (4.75%) in South-South Nigeria (Kingsely et al. 2013) and *Trichostrongylu scolubri* form is (16.24%) were reported in cattle in Pakistan (Rafiullah et al. 2011), *Trichuris* sp. (3.1%) were reported in cattle in South-South Nigeria (Kingsely et al. 2013). 8.88% of *Trichuris ovis* reported in cattle in Nigeria (Endosomwan et al. 2012) and 6.24% of *Trichuris* has been reported in cattle in Pakistan (Rafiullah et al. 2011).

Similarly, *Osophagostomum* spp. (1.3%) were reported in cattle in Ethiopia (Bacha et al. 2014), *Osophagostomum* (7.10%) in Tanzania (Mahanga et al. 2013), *Osophagostomum* sp. were reported in cattle in Pakistan (Rafiullah et al. 2011). *Haemonchus* sp. (11.77%) were reported in cattle in Ethiopia (Bacha and Haftu 2014), (18.9%) reported in cattle in South-South Nigeria (Kingsely et al.2023) and in Pakistan (Rafiullah et al. 2011). *Ostertaga* spp. (1.8%) has been reported in cattle in Ethiopia (Bacha and Haftu 2014), *Ostertaga* sp. (4.10%) has been reported in buffaloes in Taliban (Mahanga et al. 2013), 10.3% in cattle in South-South Nigeria (Kingsely et al.2013), *Ostertagia ostertaga* (5.92%) has been reported in cattle in Nigeria (Edosomwan et al. 2012), 6.52% in Pakistan (Rafiullah, et al. 2011) and *Dictyocaulus* sp. (3.9%) has been reported in cattle in South-South Nigeria (Kingsely et al.2013), in Pakistan (Rafiullah et al. 2011). *Capillaria* sp. (3.7%) were reported in cattle in Nigeria (Edosomwan et al. 2012) and 11.1% in cattle in South-South Nigeria (Kingsely et al.2013).

2.2 In National Context

Helminths

It conations tematodes, cestodes and nematodes parasite which are found in the liver, bile duct as well as digestive system of both domestic and wild animals.

i. Tematodes

These are leaf like parasite including *Fasciola* sp., *Paramphistimum* sp., *Schistosoma* sp., *Dicrocoelium* sp. etc. *Fasciola* sp. is most important species of pathogen that causes fascioliasis. *Fasciola* sp. (29.41%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 6.13% in cattle in VDC Anarmani-2, Jhapa, district (Dhakal 2008), 5.40% in goats in Khasibazar, Kalanki, Kathmandu (Parajuli 2007), 32.6% in buffaloes in Satungal, Kathmandu (Mukhia 2007). Likewise different species had been conducted for *Paramphistimum* sp. (11.76%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 4.11% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008), 15.64% in buffaloes in Satungal, Kathmandu (Mukhia 2007), and 2.70% in goats in Khasibazar, Kalanki, Kathmandu (Parajuli 2007), *Schistosoma* sp. (38.23%) has been reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 5.38% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008) and 46.94% in buffaloes in Satungal, Kathmandu (Mukhia 2007) and *Dicrocoelium* sp. (12.94%) reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 5.38% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008) and 20.61% in buffaloes in Satungal, Kathmandu (Mukhia 2007).

Similarly, *Gastrothylax* sp. 2.35% reported were in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 1.27% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008) and 0.76% in buffaloes in Satungal, Kathmandu (Mukhia 2007) followed *Fishoederius* sp. (1.17%) were reported in buffaloes in VDC Pokharathok, Arghakhanchi district (Devi 2012), 0.07% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008) and 0.38% in buffaloes in Satungal, Kathmandu (Mukhia 2007), *Ornithobilharzia* sp. (0.58%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 1.15% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008) and 0.76% in buffaloes in Satungal, Kathmandu (Mukhia 2007). *Skrjabinema* sp. (5.29%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012) 2.46% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008) and 6.87% in buffaloes in Satungal, Kathmandu (Mukhia 2007).

ii. Cestodes

It is ribbon like tape worm such as *Moniezia*, *Taenia* etc. They are found in gut, are acquired by eating contaminated food or water found to be largely affecting the domestic as well as wild ruminants. *Moniezia* sp. were common cestodes in the buffaloes, cattle, goats, sheep etc. *Moniezia* sp. (31.66%) reported in buffaloes in Pokharatok VDC Arghakhanchi district (Devi 2012), 3.88% in cattle in VDC Anarani-2 Jhapa district (Dhakal 2008), 13.35% in buffaloes in Satungal, Kathmandu (Mukhia 2007) and 5.4% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007).

iii. Nematodes

The most important and widely prevalent nematodes of buffaloes 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 buffaloes and cattle (Karki 2005), *Strongyloides* sp. (13.52%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 7.77% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008), 9.45% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and 4.19% in buffaloes in Satungal, Kathmandu (Mukhia 2007) and *Trichostrongylus* sp. (5.88%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 23.63% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008), 17.56% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and 1.90% in buffaloes in Satungal, Kathmandu (Mukhia 2007).

Similarly, *Toxocara* sp. (34.11%) were reported in buffaloes (Devi 2012), 4.93% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008), 1.89% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and 22.90% in buffaloes in Satungal, Kathmandu (Mukhia 2007). *Ascaris* sp. (18.23%) were reported in buffaloes (Devi 2012), 0.89% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008), 3.15% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and 6.87% in buffaloes in Satungal, Kathmandu (Mukhia 2007), *Chabertia* sp. (1.76%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 14.86% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and 0.38% in buffaloes in Satungal, Kathmandu (Mukhia 2007) and *Trichuris* sp. (5.88%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 1.78% reported in cattle

in VDC Anarmani-2, Jhapa district (Dhakal 2008), 5.85% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and 2.29% in buffaloes in Satungal, Kathmandu (Mukhia 2007).

Dictyocaulus sp. (0.58%) were reported in buffaloes (Devi 2012), 1.34% in cattle (Dhakal 2008), 2.7% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and 0.76% in buffaloes in Satungal, Kathmandu (Mukhia 2007), *Oesophagostomum* sp. (1.17%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 0.37% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008), 8.11% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and 0.76% in buffaloes in Satungal, Kathmandu (Mukhia 2007), *Capillaria* sp. (1.17%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 0.29% in cattle (Dhakal 2008), 2.25% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and 0.38% in buffaloes in Satungal, Kathmandu (Mukhia 2007), *Haemonchus* sp. (1.76%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 3.36% in cattle in VDC Anarmani-2 Jhapa district (Dhakal 2008), 19.36% in goats (Parajuli 2007) and 1.14% in buffaloes in Satungal, Kathmandu (Mukhia 2007), *Ostestagia* sp. (2.94%) were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 4.41% in cattle in VDC Anarmani-2, Jhapa district (Dhakal2008) and 9.00% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and *Cooperia* sp. were reported in buffaloes in VDC Pokharatok, Arghakhanchi district (Devi 2012), 0.74% in cattle in VDC Anarmani-2, Jhapa district (Dhakal 2008) 4.05% in goats in Khasibazr, Kalanki, Kathmandu (Parajuli 2007) and 0.76% in buffaloes in Satungal, Kathmandu (Mukhia 2007).

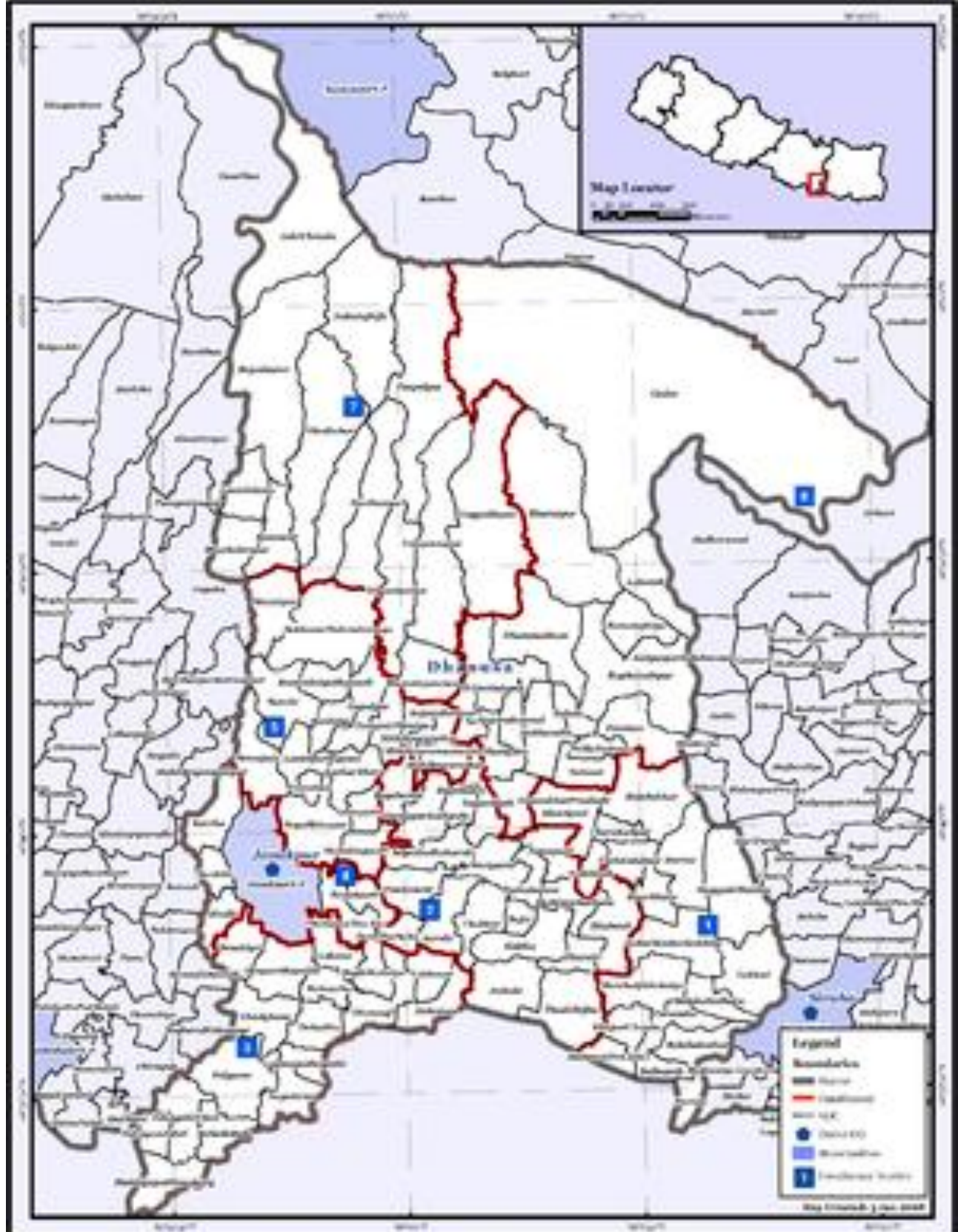
3. MATERIALS AND METHODS

3.1 Study area

Dhanusha district is the part of Nepal. Dhanusha district is one of the seventy five district of Nepal. The Janakpur is the headquarter of Dhanusha, covers an area of 1,180sq.km. and population of 7,54,777. The district is bounded by Siraha in the east side, Mahottari in the west side, Sindhuli in the north side and India (Bihar in the south side). About 25,021 sq.km. of the total area of Terai and situated 59-600m above the sea level.

The study area Dhanushadham municipality (Dhrampur) is located in Dhanusha district, it is 16 km east from the headquarter (Janakpur). The Dhanusha district contain six municipality. It is situated in main terai. The Dhanushadham (Dhrampur) people are mainly engaged in farming and domesticated of the buffaloes, cows and goat. The most animal are grazing in Dhanusha jungle and open fields.

DHANUSA DISTRICT



3.2 Materials

The materials used during research work have been listed below

3.2.1 Materials for Laboratory

Cotton, Refrigerator, Cover slip, Centrifuge machine, Slide, Beaker, Cello tape, Glass rod, Stick, Measuring cylinder, Needle, Volumetric flask, Gloves, Electronic weigh machine, Mask, Plastic centrifuge tube, Tea strainer, Microscope, Pasteur pipette, Rack, Motor and Pistle.

3.3 Chemicals

10% formalin, Distilled water, Sodium chloride solution, Zinc chloride solution, Methylene blue.

3.4 Study Design

The study design is based under laboratory examination

3.4.1 Sample Size

A total of 300 stool samples were collected from Dhanushadham municipality, (Dharmpur). The numbers of samples taken during winter were 150 and during summer were 150.

3.5 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) 2.5% potassium dichromate was used to fix stool samples.

3.6 Stool Examination

The stool samples were collected and brought to laboratory in preservatives and refrigerated. The stool samples were examined by differential floatation technique, sedimentation technique and Stoll's counting method.

3.6.1 Differential Floatation Technique

The differential floatation technique is widely used for the detection of nematode and cestode eggs. It provides good results among other floatation technique and is one of the easiest and short ways for identifying and counting the eggs.

Method

3 gm of stool sample was taken in a beaker and 42ml of water was added. With the help of motor and pistle, the sample was grinded lightly and filtered with a tea strainer. The filtered sample was poured into plastic tube of 15ml and centrifuged at 1000 rpm for 5 minutes. The tube was taken out and the upper part of the water was removed with the help of a pipette. The tube was noted filled with sodium chloride solution and centrifuged at 1000 rpm for five minutes. More NaCl solution was added upto the tip of the tube. A cover slip was placed over the top of the tube so that the NaCl touches the coverslip for a few minutes and then the coverslip was placed on a slide and examined at 10x.

3.6.2 Sedimentation Technique

The 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 (Source: Veterinary Lab. Techniques, 2003).

Method

3 gm of stool sample was taken in a beaker; 42 ml of water was added and grinded lightly with the help of motor and pistle. The sample was filtered with a tea strainer and the filtered sample was poured in a plastic test tube, centrifuged at 1000 rpm for 5 minutes. 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 1000 rpm for 5 minutes. A drop of deposited materials was taken out from the test tube with the pipette and placed on the slide, 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 species wise 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). Helminth 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.



Photo no: 1 Group of buffaloes grazing.



Photo no: 2 Collection of stool sample.



Photo no:3 Sample preservation.



Photo no:4 Processing of stool sample in lab.



Photo no: 5 Microscopic Examination.



Photo no:6 Samples ready in slides to observe.

4. RESULTS

Among 300 samples collected from Dhrampur, Dhanushadham, Dhanusa. 150 samples were collected in winter season and 150 were collected in summer season. Out of 300 samples were examined with the help of sedimentation and flotation techniques, 238 (79.33%) samples were found to be positive and 62 (20.77%) samples were found negative. The eggs of different genera of helminthes were identified according to their characters and morphology. Study had been done under different heading.

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

4.1 General Prevalence of Helminth Parasites

Among the 300 fecal samples collected from study area, Dhrampur, Dhanushadham, Dhanusha, 238 (79.33%) samples were found to be positive during both winter and summer seasons.

The difference in general prevalence of helminth parasites was found statistically insignificant ($\chi^2 = 51.62$, $P < 0.05$, d.f. = 1).

Therefore, the general prevalence rates of helminth parasites in buffaloes were found to be 79.33%.

4.2 Class-wise prevalence of helminth parasites

Table 2:- Class-wise prevalence of helminth parasites in buffaloes

S.N	Classes	Name of Genera	Total samples examined	Total No. of genera	Percentage (%)
1.	Tr em at od es	<i>Fasciola</i>	300	70	23.33

2.		<i>Paramphistomum</i>	300	52	17.66
3.		<i>Dicrocoelium</i>	300	31	9.66
4.	Cestodes	<i>Moniezia</i>	300	19	6.33
5.	Nematodes	<i>Strongyloides</i>	300	8	2.66
6.		<i>Toxocara</i>	300	17	5.66
7.		<i>Trichostrongylus</i>	300	28	9.33
8.		<i>Trichuris</i>	300	5	1.66
9.		<i>Chabertia</i>	300	2	0.66
10.		<i>Oesophagostomum</i>	300	2	0.66
11.		<i>Haemonchus</i>	300	2	0.66
12.		<i>Ostertagia</i>	300	3	1
13.		<i>Cooperia</i>	300	1	0.33
14.		<i>Dictyocaulus</i>	300	1	0.33
15.	<i>Capillaria</i>	300	1	0.33	

This study showed 63.44% trematodes infection, 28.57% nematodes infection and 7.89% cestodes infection. The total numbers of genera observed during examination were 74 i.e. 3 genera of trematodes, 1 genus of cestodes and 10 genera of nematodes.

The result shows that the maximum infection was caused by the class trematodes 63.44% followed by nematodes 28.57% and cestodes 7.89%. The highest prevalence was shown by *Fasciola* (23.33%), and lowest prevalence was shown by *Cooperia* (0.33%), *Capillaria* (0.33%) and others (0.33%).

4.3 Seasonal Prevalence

In this study, total 14 genera of eggs were observed on the seasonal basis in which 13 (86.66%) genera of eggs were recorded during winter and 14 (93.33%) genera eggs were observed during summer (Fig. 1).

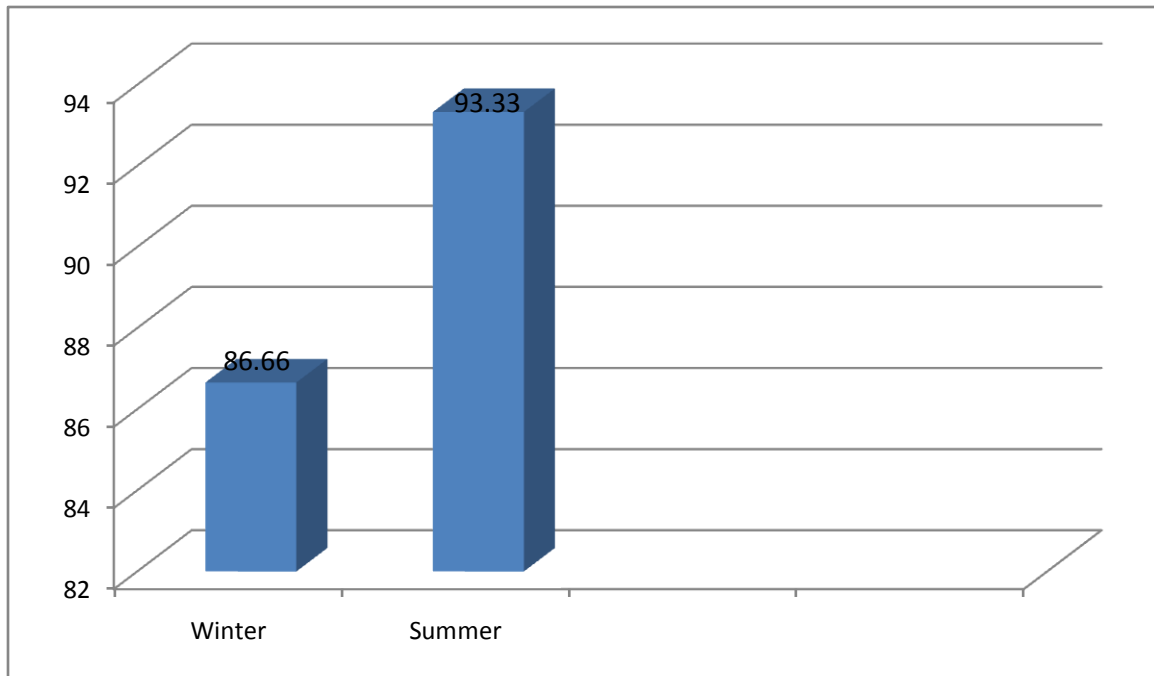


Fig.1 Seasonal Prevalence

4.3.1 Seasonal Prevalence of Trematodes

Among the 238 (79.5%) positive samples, 151 (50.33%) samples were found positive for trematodes. During winter 71 (47.01%) samples were found positive and 80 (53.33%) samples were found positive in summer (Table 3). The differences in the prevalence of different genera of trematodes during summer and winter was found statistically insignificant ($\chi^2 = 3.38$ $P < 0.05$, d.f.=2).

Table 3: Prevalence of Trematodes

S.N.	Name of genera	Total Sample s Examined in winter	Positive Samples During Winter		Total Samples Examined in Summer	Positive Samples During Summer	
			Nos	%		Nos	%
1.	<i>Fasciola</i>	150	32	21.33	150	38	25.33
2.	<i>Paramphistomum</i>	150	25	14.66	150	27	18
3.	<i>Dicrocoelium</i>	150	14	9.33	150	15	10

4.3.2 Seasonal Prevalence of Cestodes

Out of 300 samples 19 (6.33%) samples were found to be positive to cestodes during both seasons. The prevalence rate of *Moniezia* sp was recorded to be 8 (5.33%) and 11(7.33%) for winter and summer seasons respectively. The difference in the prevalence of different genus of cestodes during summer and winter result statistically insignificant ($\chi^2= 0.03$, P<0.05).

Table No 4: Prevalence of Cestodes

S.N.	Name of genera	Total Sample Examined Winter	Positive Sample During Winter		Total samples examined during summer	Positive Samples During Summer	
			NOS	%		NOS	%
1	<i>Moniezia</i>	150	8	5.33	150	11	7.33

4.3.3 Seasonal Prevalence of Nematodes

Out of 300 samples examined, altogether 238 (79.33%) samples showed the presence of nematode eggs where 68 (22.66%) samples were found positive for nematodes during both season. Among 32 (21.33%) samples were found positive during winter season. And 36 (24%) samples were found positive during summer season. The difference in the prevalence of different genera of nematodes during summer and winter results found statistically significant ($\chi^2=4.74$, $P<0.05$, d.f.=10).

Table No: 5. Prevalence of Nematodes

S.N.	Name of genera	Total Samples Examined in Winter	Positive Samples During Winter		Total Samples Examined in Summer	Positive Samples During Summer	
			Nos	%		Nos	%
1.	<i>Strongyloides</i>	150	3	2	150	5	3.33
2.	<i>Taxocara</i>	150	7	4.66	150	8	5.33
3.	<i>Trichostrongylus</i>	150	15	10	150	13	8.66
4.	<i>Trichuris</i>	150	2	1.33	150	3	2
5.	<i>Chabertia</i>	150	1	0.66	150	1	0.66
6.	<i>Oesophagostomum</i>	150	1	0.66	150	1	0.66
7.	<i>Haemonchus</i>	150	1	0.66	150	1	0.66
8.	<i>Ostertagia</i>	150	1	0.66	150	2	1.33
9.	<i>Cooperia</i>	150	1	0.66	150	-	-
10.	<i>Capillaria</i>	150	-	-	150	1	0.66
11.	Others	150	-	-	150	1	0.66

4.4 Seasonal prevalence of eggs of helminth parasite

Out of 150 samples of winter, 111 samples were observed positive i.e. 74% and out of 150 samples of summer, 127 samples were found positive i.e. 84.66%. The rate of prevalence of helminth parasites were found during summer 84.66% and winter 74%. The difference in the

prevalence of different genus of helminth parasite during both season altogether were found statistically signification ($\chi^2 = 738.58$, $P < 0.05$, d.f. = 15).

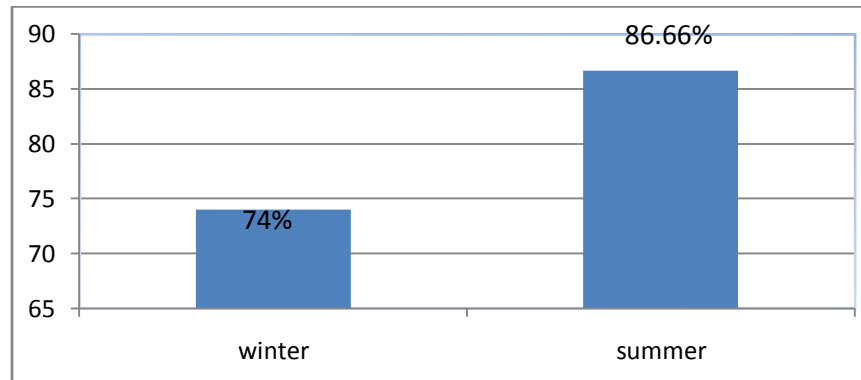


Figure: 2. Seasonal prevalence of eggs of helminth parasites

4.5 Identification of eggs of Helminth:

Among the 300 fecal samples examined, 268 (79.33%) samples were positive. 111 (46.63%) stool samples were observed positive during winter and 127 (53.37%) samples were found positive during summer. The total numbers of identified eggs were listed below (Table 6).

Table No: 6 Observed genera of different classes of helminth parasites

S.N.	Classes	Identified Helminth
1	Trematodes	<i>Fasciola</i>
2		<i>Paramphistomum</i>
3		<i>Schistosoma</i>
4		<i>Dicrocoelium</i>
5	Cestodes	<i>Moniezia</i>
6	Nematodes	<i>Strongyloides</i>
7		<i>Taxocara</i>
8		<i>Trichostrongylus</i>
9		<i>Trichuris</i>
10		<i>Chabertia</i>
11		<i>Oesophagostomum</i>

12		<i>Haemonchus</i>
13		<i>Ostertagia</i>
14		<i>Cooperia</i>
15		<i>Capillaria</i>

TREMATODES

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

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* sp. 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* sp. infected 67.66% in buffalo and 62.10% in cattle from Thuladihi VDC, Syangja.

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

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

In 2009, Bajracharya reported *Fasciola* sp. infected 35% among buffalo brought to Krirtipur (Kathmandu) for slaughter purpose.

In 2008, Dhakal reported *Fasciola* sp. infection 11.26% in cattle from Anarmani VDC-2 Jhapa.

In 2012, Devi reported *Fasciola* sp. infection 29.41% in buffaloes from Pokharatok VDC in Arghkhanchi.

***Paramphistomum* sp. (Zeder 1790)**

Classification:

Family: Paramphistomatidae

Genus: *Paramphistomum* sp.

Description of the eggs:

Eggs are 114-176 μ m by 73-100 μ m 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 *Paramphistomum* sp. 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* sp. in ruminants from Dhanusha district.

In 2007, Parajuli reported 20.70% *Paramphistomum* sp. in goats from kalanki (kathmandu).

In 2008, Dhakal reported 4.92%, *Paramphistomum* sp. cattle from Anarmani VDC-02, Jhapa.

In 2012, Devi reported 10.78% *Paramphistomum* sp. buffalo from Pokharatok VDC in Arghakanchi.

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

Classification:

Family: Dicrocoelidae

Genus: *Dicrocoelium* 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 buffalo of Satungal, Kathmandu.

In 2008, Dhakal reported *Dicrocoelium* sp. 5.38% in buffalo from Anarmani -02, Jhapa.

In 2012, Devi reported *Dicrocoelium* sp. 12.94% in buffalo from Pokharatok VDC in Argahakanchi.

CESTODES

***Moniezia* sp. (Rudolphi 1810)**

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.

In 2008, Dhakal reported *Moniezia* sp. 30.88% among cattle from Anarmani VDC-2, Jhapa.

In 2012, Devi reported *Moniezia* sp. infection 15.88% among buffalo from Pokharatok VDC Aghakhanchi.

NEMATODES

Strongyloides* sp. (Wedi 1856)*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 *Strongyloides* sp. in ruminants from Maldi VDC, Dhading.

In 2008, Dhakal reported 7.7% *Strongyloides* sp. among cattle from Anarmani VDC-2, Jhapa.

In 2012, Devi reported *Strongyloides* sp. among buffalo Pokharatok VDC Arghakhanchi.

In 2013, Arhali reported *Strongyloides* sp. among Himalayan Tahr and deer of Rara National Park.

***Toxocara* sp. (Werner 1782)**

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.

In 2007, Mukhiya reported *Toxocara* sp. in buffalo from satugal Kathmandu.

In 2007, Parajuli reported 1.80% *Toxocara* sp. in gaots from Khasibazar Kalanki (Kathmandu).

In 2008, Dhakal reported 4.93% *Toxocara* sp. in cattle from Anarmani VDC-2, Jhapa.

In 2012, Devi reported 34.11% *Toxocara* sp. in buffalo from Pokharatok VDC in Arghakhanchi.

***Trichostrongylus* sp. (Zeder 1800)**

Classification:

Family: Trichostrongyloidae

Genus: *Trichostrongylus* sp.

Description of the eggs:

Eggs are 79-92 μ m by 32-49 μ m in size, oval and bilaterally symmetrical, shell has a thin and transparent outer chitinous layer and a thin inner lipoidal layer, embryonic mass multi segmented 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.

In 2007, Mukhia reported 1.99% *Trichostrongylus* sp. in buffalo from Satungal, Kathmandu.

In 2007, Parajuli reported 17.56% *Trichostrongylus* sp. in goat from Khasibazar, Kalanki, Kathmandu.

In 2008, Dhakal reported 23.63% *Trichostrongylus* sp. in cattle from Anarmani VDC-2, Jhapa.

In 2012, Devi reported 13.52% of *Trichostrongylus* sp. in buffalo from Pokharatok VDC in Arghakhanchi.

In 2013, Arghali reported 11.76% *Trichostrongylus* sp. in deer and Himalyan Tahr from RARA national Park.

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

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.

In 2007, Mukhia reported 2.33% *Trichuris* sp. in buffalo from Satungal, Kathmandu.

In 2007, Parajuli reported 5.85% *Trichuris* sp. in goat from the Khasibazar, Kalanki, Kathmandu

In 2008, Dhakal reported 1.78% *Trichuris* sp. in cattle from Anarmani VDC, Jhapa.

In 2012, Devi reported 5.88% *Trichuris* sp. in buffalo from Pokharatok VDC Arghakhachi.

In 2013, Agrhali reported 8.82% *Trichuris* sp. in Himalayan Tahr and deer from RARA national parks.

***Chabertia* sp. (Gmelin1790)**

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:

In 1790, 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 buffalo brought to Satungal, for slaughter purposes.

In 2008, Dhakal reported *Chabertia* sp. infection 2.54% in cattle from Anarmani VDC-2, Jhapa.

In 2012, Devi reported *Chabertia* sp. infection 1.76% buffalo from Pokharatok VDC Arghakharchi.

***Oesophagostomum* sp. (Giles 1892)**

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

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

In 2008, Dhakal reported *Oesophagostomum* sp. in cattle from Anarmani VDC-2, Jhapa.

In 2012, Devi reported *Oesophagostomum* sp. in buffalo from Pokharathok VDC in Arghakhanchi.

***Haemonchus* sp. (Rudolphi 1803)**

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.

In 2008, Dhakal studied and reported the prevalence of *Haemonchus* sp. in coprological study from Anarmani VDC- 2 Jhapa.

In 2013, Devi reported *Haemonchus* sp. infection 1.76% among buffalo from Pokharathok VDC in Arghakhanchi.

Ostertagia* sp. (Stiles1892)*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.

In 2007, Mukhia reported *Ostertagia* sp. in buffalo from Satangal Kathmandu.

In 2008, Dhakal reported *Ostertagia* sp. in cattle Anarmani VDC-2 Jhapa.

In 2012, Devi reported *Ostertagia* sp. in buffalo from Pokharathok VDC in Arghakhanchi.

***Cooperia* sp. (Gordon 1932)**

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.

In 2008, Dhakal reported *Cooperia* sp. in cattle from Anarmani VDC-2 Jhapa.

In 2012, Devi reported *Cooperia* sp. in buffalo from Pokharathok VDC in Arghakhanchi.

***Dictyocaulus* sp. (Rudolphi 1809)**

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.

In 2008, Dhakal reported *Dictyocaulus* sp. infection 1.34% in cattle from Anarmani VDC-2, Jhapa.

In 2012, Devi reported *Dictyocaulus* sp. infection 0.58% in buffalo from Pokharathok VDC in Arghakhanchi.

***Capillaria* sp. (Rudolphi 1819)**

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.

In 2008, Dhakal reported *Capillaria* sp. infection 0.29% in cattle from Anarmani VDC-2 Jhapa.

In 2013, Devi reported 0.17% in buffalo from Pokharatok VDC Arghakhachi

4.5 Multiple and Single Infection

Table No 7. Multiple and Single Infection

S.N.	Classes	Name of genera	Light infection	Moderate Infection	Heavy Infection
1.	Trematodes	<i>Fasciola</i>	60	5	2
2.		<i>Paramphistomum</i>	45	4	4
3.		<i>Dicrocoelium</i>	26	2	1
4.	Cestodes	<i>Monirezia</i>	19		
5.	Nematodes	<i>Strongyloides</i>	8		
6.		<i>Taxocara</i>	5	2	1
7.		<i>Trichostrongylus</i>	27	1	
8.		<i>Trichuris</i>	5		
9.		<i>Chabertia</i>	2		
10.		<i>Oesophagostomum</i>	2		

11.		<i>Haemonchus</i>	2		
12.		<i>Ostertagia</i>	3		
13.		<i>Cooperia</i>	1		
14.		<i>Dictyocaulus</i>	1		
15.		<i>Capillaria</i>	1		

4.5.1 Single Infection

In the present study, out of 238 (79.33%) positive samples, 216 (90.76%) samples were found to be single infection. Among positive samples with single infection was recorded with *Fasciola* sp. (25.21%) followed gradually by *Paramphistomum* sp. (18.90%), *Trichostrongylus* sp.(11.34%), *Dicrocoelium* sp. (10.92%) and *Moniezia* sp. (7.98%) .

4.5.2 Multiple Infections

In the present study, the prevalence of mixed infections were also observed among 238 (79.33%) positive samples. 22 (9.24%) samples were found to be infected by multiple infections. Out of which moderate infections were observed 14 (5.88%) i.e. *Paramphistomum* sp. 4 (1.68%), *Fasciola* sp. 5 (2.10%), and the heavy infections were observed 8 (3.66%) i.e. *Paramphistomum* sp. 4 (1.68%), *Fasciola* sp. 2 (0.84 %) and *Toxocara* sp. 1 (0.42%).

PHOTOGRAPHS

EGGS OF TREMATODES AND CESTODES OBSERVED

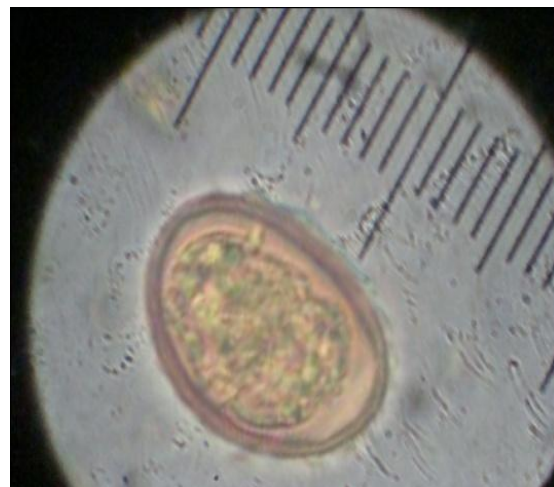
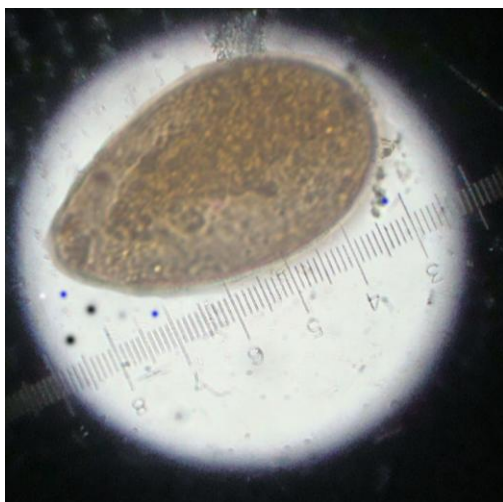


Photo no:7 *Fasciola* sp.(10X x10X)

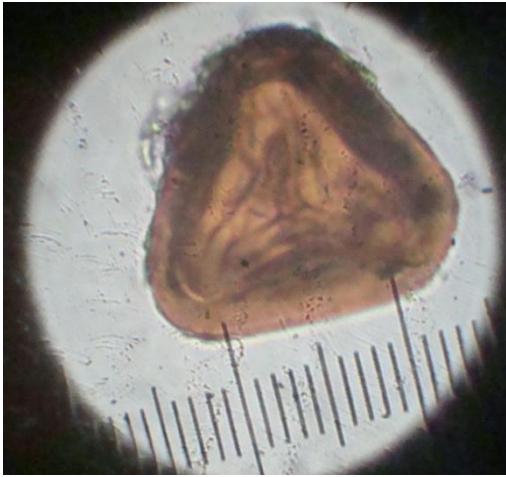
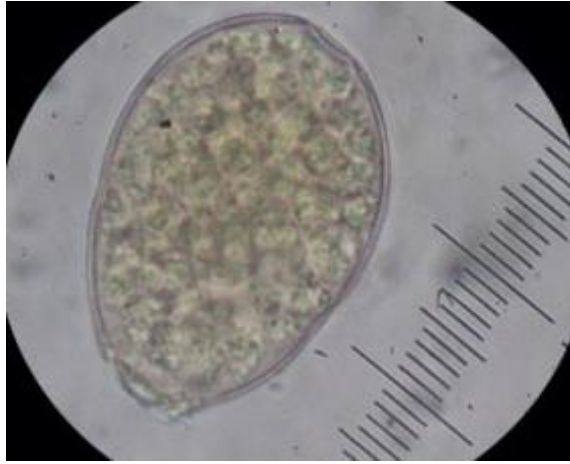


Photo no: 8 *Dicrocoelium* sp. (10X x10X)



Photo

no:9 *Moniezia* sp.(10X x10X)

Photo no:10 *Paramphistomum* sp.(10X x10X).

EGGS OF NEMATODES OBSERVED

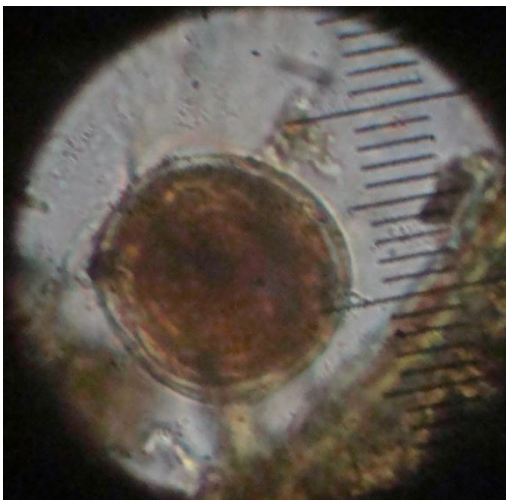


Photo no:11 *Toxocara* sp.(10X x10X)

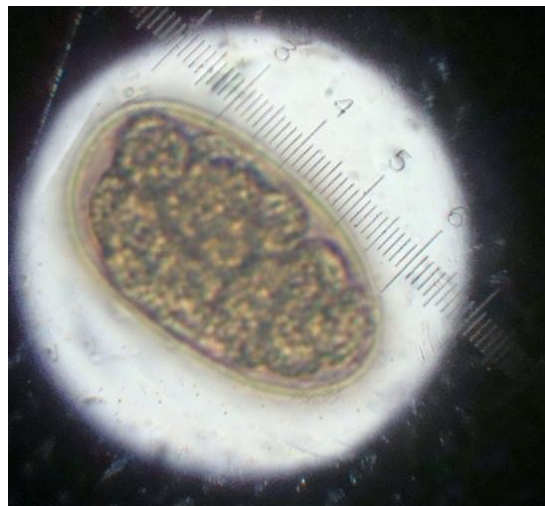


Photo no:12:*Trichostrongylus* sp.(10X x10X)



photo no.13: *Capillaria* sp.(10Xx10X)

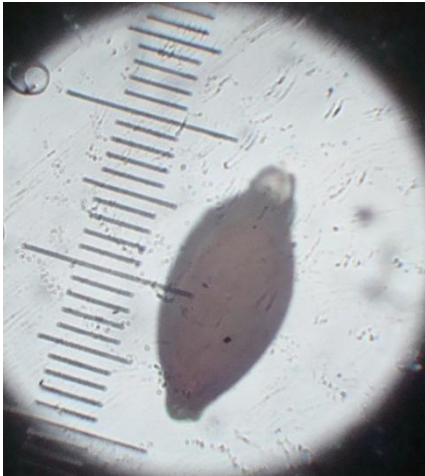


Photo no: 14 *Trichuris* .(10X x10X) Photo no:15*Strongyloides* sp. .(10X x10X).

5 DISCUSSION

The aim of study was to investigate the prevalence of helminth parasites in buffaloes in two seasons likewise summer and winter. The present investigation was carried out in the month of December to January and May to June. The stool samples were collected from Dhanushadham municipality (Dhrampur). In the present study, 79.33% samples of winter found positive out of 300 samples. 84.66% samples of summer were found positive out of 150 samples and 74% samples of winter were found positive out of 150. The numbers of samples were found positive during winter and summer for trematodes were 80 (53.33%) and

71 (74.01%), cestodes 11 (7.33%) and 8 (5.33%) for nematodes 36 (24%) and 32 (21.33%) respectively.

The helminths have been emerged as an important parasitic disease since from the past decades in the world, but in Nepal it had been reported up to certain extent. It can be said that the prevalence of any gastrointestinal parasite is influenced by the climate condition and geographical factors. Like the warm and humid condition of south East Asia harbour a suitable condition for most of the parasite to flourish well. The prevailing condition rainfall thought the year in these region helps for the survival of such parasite.

The study shows 3 genera of trematodes, 1 genus of cestodes and 10 genera of nematodes were examined during winter. Similarly from the summer sample 3 genera of trematodes, 1 genus of cestodes and 10 genera of nematodes were observed.

The study showed 3 genera of trematodes in winter, *Fasciola*, *Paramphistomum*, and *Dicrocoelium*, where as in summer 3 genera were indicates that *Fasciola*, *Paramphistomum*, and *Dicrocoelium*. In the case of cestodes only one genus *Moniezia* was found in the both season.

Similarlly, the preset study showed 9 genera of nematodes during winter samples as *Strongyloides*, *Toxocara*, *Trichostrongylus*, *Trichuris*, *Chabertia*, *Oesophagostomus*, *Haemonchus*, *Ostertagia*, and *Cooperia* where as in summer 11 genera were observed as *Strongyloides*, *Toxocara*, *Trichostrongylus*, *Trichuris*, *Chabertia*, *Oesophagostomus*, *Haemonchus*, *Ostertagia*, and *Capillaria*. In addition the *Capillaria* were not found in winter similarly, *Cooperia* was not observed during summer.

Coprological survey of gastro-Intestinal helminth in buffaloes revealed the presence of the eggs of *Fasciola* (21.33 % and 25.33 %), *Paramphistomum* (14.66% and 18%), *Dicrocoelium* (9.33% and 10%) and *Moniezia* (5.33% and 7.33%) spp. during winter and summer respectively.

Among the nematodes, *Strongyloids* 2% and 3.33%, *Toxocara* 4.66% and 5.33%, *Trichostongylus* 10% and 8.66%, *Trichuris* 1.33% and 2%, *Chabertia* 0.66% and 0.66%, *Oesophagostomum* 0.66% and 0.66%, *Haemonchus* 0.66% and 0.66% and *Ostertagia* 0.66%

and 1.33% were recorded in the fecal samples of buffaloes. In this study overall *Trichostrongylus* 9.33% were the most encountered species followed by *Strongylodes* and *Ascaris* was 2.66% and *Toxocara* was 2.33%.

The study exhibited 21.33% and 25.33% prevalence rate of fascioliasis during winter and summer respectively. A slight higher, their prevalence during summer may be due to increase in humidity and availability of favorable temperature and intermediate host. High prevalence of *Fasciola* (67.66%) has been reported from Thuladihi Syangia among buffaloes (Regmi et al. 1999), followed by *Fasciola* (56.75%) infection from Surkhet district (Parajuli 1967-92) and 40.12% infection in animal from Panchthar (40.12%) district (Sharma 1997) The higher prevalence of *Paramphistomum* (35.13%) has been reported from Surkhet district (Parajuli 1967-92) 16.2% and Panchthar district (Sharma 1998) which were higher than the present prevalence.

Maximum incidence of trematodes were detected during summer season (43.66%) followed by rainy (28.95%) and winter season (20.51%) from Mathura area (Agrawal et al. 2006). In this study maximum incidence of trematodes detected during summer (67.69%) followed by winter season 31.66%.

A higher (90.90%) prevalence of trematodes were reported earlier by Mukhia (2007). Devi (2012) reported 74.11% prevalence of trematodes. Availability of favorable temperature and increase in humidity could be the reason for the high prevalence of trematodes during summer.

The cestodes *Moniezia* has been reported by Ghimire (1987), Gupta (1989), Mukhia (2007) and Devi (2012). In this study 2.66% and 3.66% prevalence has been recorded during winter and summer respectively overall prevalence rate of cestodes in the current study has been found to be 6.33% and seasonally i.e. 6% during winter and 11% during summer has been reported. Presence of suitable temperature and moisture serve best for the breeding and development of the helminth parasites so this causes be the reason behind excessive preparation of certain helminth parasites.

In this study, the nematodes reported highest percentage was *Trichostrongylus* 9.33% followed by *Strongyloides* and *Toxocara*.

A higher prevalence (82.35%) of gastrointestinal helminth parasites in buffaloes was reported earlier (Kauret et al. 2008) who reported the prevalence of *Toxocara* (78.57%), *Haemonchus* (57.14%), *Oesophagostomum* (42.86%) and *Trichuris* (12.26%).

The mixed infection 0.6% with species of *Fasciola*, *Ostertagia*, *Paramphistomum*, *Trichuris*, *Oesophagostomum*, and *Strongyloides* have been reported from the colony of Hyderabad among buffalo (Akhtar and Mohommad 2003). Mixed infection was observed in 26% and 87.5% in the sample of winter and summer respectively (Bashir 2009). Compared 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 might be due to exposure of buffalo to highly infected pasture land, contaminated water or infected fodder.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion:

This study was carried out in order to observe the coprological survey of gastro-intestinal helminth in *Bubalus bubalis* (buffalo) samples were collected from Dhrampur, Dhanusha district. Sedimentation and flotation techniques were used to detect the eggs of helminths. The samples were collected during month of December to January and May to June.

The total number of samples collected and examined for the study were 150 during summer and 150 during winter. The infection of trematodes were observed to be 41.01%, cestodes 2.66% and nematodes 10% during winter season 52.99%, 3.66% and 12% of infection caused by trematodes, cestode and nematodes respectively during summer season.

The prevalence percentage of identified genera of tematodes was as follows: *Fasciola* 23.33%, *Paramphistomum* 17.33% and *Dicrocoelium* 9.66%.

Among cestodes, the genera identified with their prevalence percentage found to be *Moniezia* 6.33% only.

Similarly, the genera include in nematodes were *Strongyloids* 2.66%, *Toxocara* 5.66%, *Trichostongylus* 9.33%, *Trichuris* 1.66%, *Ostertagila* 1%, *Chabertia* 0.66%, *Oesophagostomum* 0.66%, *Haemonchus* 0.66% and *Cooperia* 0.33% and *Capillaria* 0.33%.. Single infection was found to be 90.75% and multiple infections were observed to be 9.24%.

6.2 Recommendations

- Antihelminth treatment should be applied to eliminate the parasite from the host.
- The programmes for awareness about meat borne disease and Zoonotic disease to public and butcher should be developed.
- Clear and safe grazing ground should be used for the buffaloes.
- The pasture can made free of helminth parasite by breaking their life cycle by eradication intermediate host, snail through biological control methods.
- Pure water should be supply for the drinking of buffalo.

- Treatment of infection host with anthelmintic and diagnosis could be done by taking help of hereby veterinary hospital.
- Parasites free fodder and grasses should be provided the buffaloes.
- This study best for the future investigators and further developed study can be done on the species.

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