A STUDY TO

DETERMINE THE PREVALENCE OF HELMINTHS PARASITES IN MULES FROM NEPALGUNJ, OF DISTRICT BANKE

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER'S DEGREE OF SCIENCE IN ZOOLOGY WITH SPECIAL PAPER PARASITOLOGY

BY

RADHA RANI

SUBMITTED TO

CENTRAL DEPARTMENT OF ZOOLOGY

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUWAN UNIVERSITY

KIRTIPUR, KATHMANDU

NEPAL

2010

SEASONAL COPROLOGICAL STUDY ON HELMINTH PARASITES OF MULES IN NEPALGUNJ, BANKE DISTRICT

A DISSERTATION

FOR THE PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE MASTER'S DEGREE OF SCIENCE

IN

PARASITOLOGY

BY

RADHA RANI

ROLL NO.365

BATCH 063-064

T.U. REGD.NO.5-3-28-6-2006

TO

CENTRAL DEPARTMENT OF ZOOLOGY

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY, KIRTIPUR

KATHMANDU, NEPAL

2010

TRIBHUVAN UNIVERSITY

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal

RECOMMENDATION

It is our pleasure to mention here that Miss Radha Rani has completed her dissertation work entitled "**SEASONAL COPROLOGICAL STUDY ON HELMINTH PARASITES OF MULES OF NEPALGUNJ, DISTRICT BANKE'' u**nder our supervison and guidance. It is her original work and brings out useful results and findings in the concerned field.

We strongly recommend this dissertation for approval for approval for the partial fulfillment of the requirements for the Master's Degree of science in Zoology with special paper Parasitolgy.

Supervisor	Co-Supervisor
Mr. Janak Raj Subedi	Dr. Kedar Bahadur Karki
Lecturer	Veterinary officer
CDZ, T.U. Kirtipur	Central Veterinary Laboratory
Kathmandu, Nepal	Tripureshwor, Kathmandu
Date: ………………………..	Date: ……………………

TRIBHUVAN UNIVERSITY

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal

LETTER OF APPROVAL

On the recommendation of supervisor Mr. Janak Raj Subedi and co-supervisor Dr. Kedar Bdr. Karki, this dissertation of Miss Radha Rani is approved for examination and is submitted to the Tribhuvan in partial fulfillment of the requirements for Master's Degree of science zoology (Parasitology).

Prof. Dr Ranjana Gupta

Head of the Department

CDZ, T.U., Kirtipur

Kathmandu, Nepal.

TRIBHUVAN UNIVERSITY

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal

APPROVAL

This dissertation presented by Miss Radha Rani entitled "**SEASONAL COPROLOGICAL STUDY ON HELMINTH PARASITES OF MULES OF NEPALGUNJ, DISTRICT BANKE"** has been approved for the partial fulfillment of the requirements for the Master's Degree in Zoology with Parasitology as specialization paper.

Evaluation Committee

______________________	______________________
Research Supervisor	Head of Department
Janak Raj Subedi	Prof. Ranjana Gupta, Ph.D.
Lecturer Zoology	Central Department of
Central Department of Zoology	Tribhuvan University
	Kirtipur, Kathmandu.
______________________	______________________
Co-Supervisor	External Examiner
Dr. Kedar Bdr. Karki	
Veterinary Officer	______________________
Central Veterinary Laboratory Tripureshwor,	Internal Examiner

TRIBHUVAN UNIVERSITY

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal

DECLARATION

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

Date: ___________________ ______________________

Radha Rani

ACKNOWLEDGEMENT

I owe a debt of gratitude to my Internal Supervisor **Mr. Janak Raj Subedi** Lecturer of Central Department of Zoology and my co-supervisor **Dr. Kedar Bahadur Karki**, Veterinary Officer of Central Veterinary Laboratory, Tripureshwor, for being so generous with their time and expertise. Their advices, suggestions and inspiration helped to attain the dissertation work successfully.

I would also like to extend my sincere gratitude to **Prof. Dr. Ranjana Gupta,** the Head of the Central Department of Zoology, for providing all the necessities for writing thesis work. I wish also express my appreaciation to all my teachers especially to **Prof. Dr. P.N. Mishra, Ashok Bam and Pitamber Dhakal** for their valuable support rendered to me to get my work done.

I also feel a deep sense of gratitude to Dr. Damodar Sedai, chief of central veterinary laboratory and all the laboratory staffs of Central Veterinary Laboratory, Tripureshwor for providing the laboratory fascility and for their kind support in the lab work.

My intimate friends Basanti Rizal, Radha Gaire, Y.P. Dakal and Sangita Nepal also could not be ignored for their constant and cordial support, encouragement and inspiration throughout the session.

Last but not the least I am much thankful to my dear bhanja **"Ajiv Thapa"** for his invaluable support who helped me throughout in arranging and formatting all the text to this final end.

Thank you all.

Radha Rani

Exam Roll No.365

Regd No.5-3-28-6-2006

Batch No.063-064

ABSTRACT

Internal and external parasitic diseases are common health problem in both domestic and wild animals. Parasitic infection decreases the performances; production and productivity in animals mainly in the reduction of body weight or even increases the mortality in acute cases. The current study was carried out in order to observe the seasonal prevalence of intestinal helmiths parasite in mules. The two different technique used during the detection of helmiths parasites were sedimentation and floatation technique. The samples were collected December\ January, May\ June, and August\September. The total number of samples had collected and examine for the study were 100,100 and 50 respectively for these study period. The overall prevalence of helminths parasites during December\Janurary were (53%), May\June (81%) and August\September (78%). A huge difference in the prevalence of helminth parasites in winter and summer, rainy season, but a very few difference between summer and rainy season were observed.During December\January (16.18%) of infections were caused by Cestodes, (50.86%) by terematodes and (69.81%) by nematodes. Like wise (19.73%), (56.79%) and (83.95%) of infection were caused by Cestodes, Trematodes and Nematodes respectively during May\June. Similarly in rainy season (28.51%), (53.84%) and (79.48%) cestodes, trematodes and nematodes infection in August\ September.*Taenia*, *Moniezia* and *Dipylidium* of Cestodes and *Schistosoma* and *Dicrocoelium* of trematodes genera were reported for the first time in mules from Nepal. Similarly, nematodes genera *Ancylostoma*,*Capillaria*,*Cooperia*,*Chabertia*, *Strongyloides*, *Trichuris* and *Toxocara* also reported for the first time in mules from Nepal.The prevalence percentage of identified geneara of cestodes were *Anoplocephala* (3.46%), *Dipylidium* (2.31%), *Moniezia* (2.31%) and *Taenia* (*8*.67%). Among trematodes, the genera identified with their prevalence percentage were found to be *Dicrocoelium* (9.24%), *Fasciola* (9.82%), *Gastrodiscus* (2.89%) and *Schistosoma* (32.36%). Similarly, the genera included in nematodes are *Ancylostoma* (1.15%), *Capillaria* (9.82%), *Chabertia* (4.04%), *Cooperia* (4.62%), *Dictyocaulus* (11.56%), *Oxyuris* (13.87%), *Parascaris* (10.40%), *Strongylus* (15.02%), *Strongyloides* (8.67%), *Trichostrongylus* (13.29%), *Trichuris* (9.24%), *Triodontophorus* (1.15%), *Toxocara*(5.78%).During winter, summer and rainy season 47.16%, 83.95% and 69.23% samples were found multiple infections respectively.

The difference in the prevalance of helminths parasites during winter, summer and rainy season were found statistically significant (χ^2=46.43, p<0.05, d.f. = 1).

Key word: Helminth, Trematodes, Cestodes, Nematodes, Parasite, prevalence, sedimentation, Floatation.

Introduction to reference.docx

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF MAPS

LIST OF PHOTOGRAPHS

ABBREVIATIONS

CBS – Central Bureau of Statistics

CDZ – Central Department of Zoology

CTVM – Centre of Tropical Veterinary Medicine

CVL – Central Veterinary Laboratory

TU – Tribhuvan University

CHAPTER - I

INTRODUCTION

1.1 Background

Despite the increase in mechanization throughout the world mules, donkeys and horses are still well deserving of the name 'beast of burden'. Still used as a means of transport for men and material and provide livelihood to a number of rural and semi-urban populace of Nepal. They have a prominent position in the agricultural systems of many developing countries. This is shown by the wide spread use of donkeys in rural and urban areas in Africa (Pearson *et al.*,1999) It is suggested that donkeys can play a great role in the frame works of food security and social equity of high food insecure countries. In areas away from roads, many Nepalese use mules or yaks to transport food and other supplies to villages. These mules are primarily used for domestic good transport in the track of high and mid mountain of Nepal where surface road transportation and air transport is not easily assessable. In Nepalgunj, Banke district, mules and donkeys are mostly used to transport the goods and passengers from Nepalgunj to Rupaidiya, the border of India. These animals not only provide the alternative entrepreneurship in these regions but also provide alternate employment opportunity. Most of the mules and donkeys being used in Nepal are being brought from neighboring part of India. Very few of them are natively breeded (Karki and Manandhar2006). Even though mules/donkeys have often been described as sturdy animals, they succumb to a variety of diseases and a number of other conditions (Svendsen1997). They do suffer from a number of diseases. Parasitic infestation is a major cause of illness. Documentation of parasitic infestation of equines in our country is lacking.

Horses have 64 chromosomes, while donkeys have 62. When horses and donkeys are mated, the mule offspring have 63 chromosomes. The gestation period in donkeys is 12 months on average, but it may vary from 11 to 14 months. Despite being considered sterile, mare mules and mare hinnies will have estrus cycles. These cycles can be regular, or erratic and variable. Female hinnies and mules can be used as embryo transfer recipients but care must be given to compatibility of donor and recipient. There have been documented cases of fertility in the female mule but not the female hinny. Donkeys and mules can survive on coarser pastures than a horse. Lush pastures suitable for horses may be too rich in protein and energy and, therefore, unsuitable for donkeys. Dry matter intake of feed as a percentage of body weight should be 1.75%-2.25% to meet the metabolic demands for maintenance for most donkeys and mules. Animals that are pregnant, nursing, growing, or used for heavy work, will have additional feed requirements (rolled oats, grain, hay or pasture) above their maintenance requirements (Sapkota 2009).

There are estimated to be 50 million donkeys (*Equus asinus*) and as many mules worldwide. They can be used for such applications as riding, driving, flock protection, companion, breeding, and training calves. Donkeys and mules are not small horses. They have anatomical and physiological differences compared to horses and their care requires special consideration. Structural differences compared to horses mean that they require specialized tack and harness for riding and driving.

There were 20,1000 horses, 60,000 mules and asses in Nepal(CBS, 2007). There has been a gradual decline in equine population in Nepal since 1980's due to road construction, decline in grassland and forest area and to some extent due to availability of air transport. Most of these horses and mules are used for transport in various remote districts of mountain region of Nepal such as Jumla, Humla, kalikot, Dolpa, Mugu, Mustang, Manang, Bajang, Bajura, Rasuwa, Taplejung etc. . Mules get a preference over hill ponies because of their greater strength, sure-footedness and sturdiness. The donkeys are also used for breeding to produce mules. The mules are produced by crossing donkeys (Jack) with mares.

1.2 Zoonosis

Mules spreading zoonotic diseases are rarely experienced. Lyme disease is an example of a disease that affects the mules and humans but Lyme disease is not transmitted to humans from mules. However in some cases mules serve as sentinels for human disease surveillance. For example, West Nile Fever and Eastern and Western Equine Encephalomyelitis are diseases that frequently appear in horses before cause are seen in humans. However, mules can also contract infectious disease that they can pass on or transmit to humans. Example of zoonotic diseases of mules includes Rabies, Arboviral encephalitis, Acute diarrhoea, Selmonellosis, Criptosporidosis, Leptospirosis, Anthrax, Dermatophytosis (ring worm disease), Brucellosis, Equine morbillivirus, Trauma etc. (www.ivis.org /proceeding /aaep/2002/910102000362.pdf)

1.3 Endoparasitism

Internal parasites are significant threat to mules. They are susceptible to more than 60 internal parasites and may harbor several species of worms at any time (Stelentenow and Purdy 2003).

Endoparasites are those organisms living within their hosts, in the gut, body cavity, liver, lungs, gall bladder and blood or 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., *Anoplocephala* sp. was typical endoparasites.

1.4 Cestoda

Cestodes, found in the gut are acquired by eating contaminated food or water found to be largely affecting the ruminants. In mules, *Anoplocephala* sp. mainly occurs in class cestoda. *Anoplocephala* sp. is an equine flat tape worm lives in intestine. It has mainly two hosts that is definitive host (mules) and intermediate host (oribatad mites).

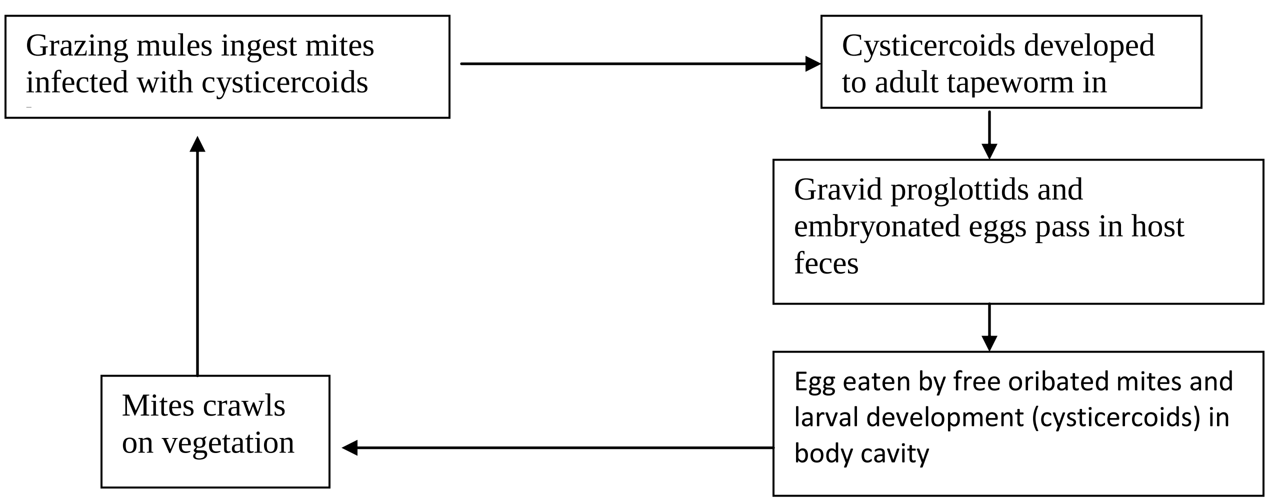


Fig 1: Life cycle of *Anoplocephala* sp.

Mules as they graze or eat other feed, accidently ingest oribatid mites infected with cysticercoids larva. Inside the mules, cysticercoids develop to adult tapeworm. Tapeworm eggs within gravid proglottid or free, passes in mules feces, are eaten by oribatid mites and infected mites eaten by mules and the tape worm cycle continuous.

It attaches to the ileo-ceacal valve. It may directly or indirectly reduces this opening. There may be ulceration, inflammation and formation of the diphthertic membrane, where the parasites attach. Other effects are perforation, intussusceptions (prolapse) of terminal ileum and ceacum and hypertrophy/hyperplasia (thickening) of the ileal walls. These symptoms seem to be more in weanling, yearling and adolescents.

1.5 Trematoda

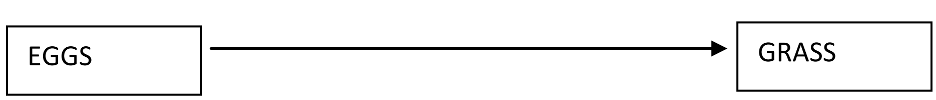
Trematode species play vital roles which are parasitic in livestock. In general, these trematodes commonly known as flukes, often live in the bile ducts or small intestine and may also affect the lungs. Some are ingested but some burrow into the skin for access. Their eggs are passed with the feces of the host. Trematodes specially include *Fasciaola* sp., *Dicrocoelium* sp*.*, *Schistosoma* sp*.*, *Gastrodiscus* sp*.*

Fascioliasis is a well known parasite of herbivorous animals. It has worldwide distribution on the animal reservoir host. A large variety of animals such as cattle, buffaloes and equines show infection rate that varies from 70% to 90% in some areas.

Infection of domestic ruminants with *Fasciola hepatica* and *Fasciola gigantica* causes significant loss estimated at over us$2000 per year to agriculture sector worldwide with over 600 million animals affected (Hensen, 1994).

Fasciola hepatica and *Fasciola gigantica* inhabit similarly in the bile duct of definitive host. The eggs produced by parasite are expelled with bile duct into the intestine. These in turn are shed in feces as:-

Eggs ⟶ free swimming⟶ miracidium ⟶ cercaria⟶ metacercaria⟶ adult.



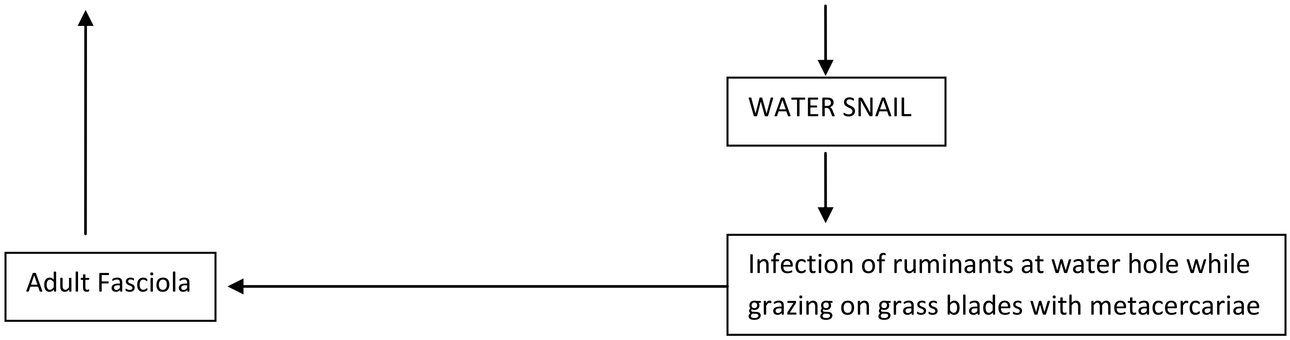


Fig. 2 Life-cycle of *Fasciola sp.*

The important species of snail involved in the transmission of fascioliasis vary in their geographical distribution in the world. Man and herbivorous animal acquires infections by ingestion of moist and raw aquatic plants, grass harbouring infective metacercariae. The metacercariae mature to become adult worms and lay eggs which are passed in faeces. On coming in contact with water they mature and invade the fresh water molluscan host snail. The mature cercaria emerge out of the snail and get encysted on aquatic grass, plants and dvelop into metcercariae which is the infective stage of the parasite.

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

Acute fascioliasis occurs seasonally and is manifest by anaemia and sudden death. Cases of chronic fasciaoliasis occur in all season and the clinical signs may include anaemia, reduced weight gain, unthriftiness, submandibular oedema and possibly death. The subsequent simultaneous migration of many immature flukes through the liver parenchyma causes severe destruction of liver tissue of the host. The penetration of the liver capsule by a large number of young flukes results in an inflammatory response of the capsule (peri-hepatitist). The blood sucking activities of the flukes irritate the lining of the ducts, resulting in an inflammatory respones and associated blood loss result in anaemia. Likewise the trematode *Dicrocoelium* sp., *Gastrodiscus* sp., the intestinal trematode and *Schistosoma* sp. the blood trematodes affects the host.

Schistosoma sp. is the only trematode living in the blood stream of warm blooded host. The blood stream is rich in glucose and amino-acids. So along with plasma and blood cell, it represents an environment which is suitable for egg producing trematode. *Schistosoma* sp., cause disease called Schistosomiasis or Bilharziasis and is the main helminthes disease. The infection are often manifest by acute intestinal signs, the mucosa of the intestine is severally damaged and the animal develop profuse bloody diarrhea, dehydration and loss of appetite. Not only goats, sheep, cattle or equines, over million people are infected in at least 75 countries with 500 million or more people exposed to infection (Acari 2000). Before, *Schistosoma* sp. were reported in buffaloes and goat but not reported in mules. But the first time in this study period *Schsistosoma* sp. reported in mules from Nepalgunj of district Banke.

1.6 Nematoda

The mostly prevalent nematodes in mules *Trichostrongylus* sp., *Strongylus* sp., *Strongyloid* sp., *oxyuris* sp., *parascaris* sp., *Dictyocaulus* sp., *Triodontophorus* sp., *Trichuris* sp. and *Habronema* sp. etc.

Fig. 3 Life cycle of gastrointestinal roundworms in general

Female round worms lay microscopic eggs that pass in the manure of mules. Within few days the larva hatches from the egg. The larva passes by 2nd and 3rd stage larva. They infect the pasture. Mules get infected when they graze on to contaminated pasture. The larva mature in the intestine, mate and begins laying eggs.

Trichostrongylus occurs in the stomach of mules. Most infections are chronic and mild. It induces typical lesions in mules. This condition has been described as ‘gastritis chronic hyperplastica et erovisa circumscripta’ for the main lesions is a pad-or-cushion-like thickening in the glandular part of the stomach. *Habronema* sp. also occurs in the stomach and transmitted by muscid dipterians and causes skin lesions, conjunctivitis, abscesses in lung tissues. When the adult worm imbed into the mucus lining of the stomach, passing of food completely blocked.

Strongylus sp. commonly called as blood worm is a common mule parasite. It generally lives in large intestine, causes significant loss of blood in intestine. Larvae migrates in mesenteric arteries, during migration, larvae damage blood vessel walls causing inflammation and increasing the risk of blood clots and aneurism (weak burging vessel that can burst), clots breaking away into the blood stream can results in blockage of artery. Adult worm are ‘plug feeder’ means that they feed by ingesting plug of mucosal tissues and capillaries.

Strongyloides sp. and *Trichuris* sp. commonly called ‘Threadworm’ and ‘Whipworm’ respectively, lives in intestine and causes acute diarrhea, inflammation of intestinal walls, haemorrhage, weakness and emaciation.

Oxyuris sp. cause ulceration in the intestine and nervousness, anorexia, rubbing and scratching of the peri-anal region causing irritation, dull hair coat and loss of hair, a condition known as ‘rat tail’. *Parascaris* sp. mostly affect the young mules, causes lethargy, loss of apettite, coughing, nasal discharge and decreased weight gain. In severe infections, the intestinal phase may cause impaction, rupture, peritonitis, intussusceptions and formation of abscesses and often death.

Dictyocaulus sp. resides in bronchial tree of the animals lung, so it is called ‘Lungworm’.It causes bronchitis, pneumonia,coughing, skin lesions, anaemia, gastric and enteric dysfunction, disturbances of ion-balance.*Triodontophorus* sp. lives in large intestine and cause deep ulcer. The damage thus caused results in formation of ‘crater like ulcer’. That might extend deep into the gut wall.

1.7 Gastro-intestinal helminth

Gastro-intestinal helminths include *Trichostrongylus* sp., *Habronema* sp., *Strongyloides* sp., *Strongylus* sp., *Gastrodiscus* sp., *Anoplocephala* sp. etc. Infections with gastro-intestinal helminths usually occur by the ingestion of eggs by human and animals, young animals are more sussceptible to the parasites. The effect of these parasites strongly depend on the number of parasites and the nutritional status of the animals, they are infecting. These helminths damage the stomach and intestinal mucous membrane, migrating larvae may cause damage to the liver, lungs and cause anaemia, diarrhoea. Mixed infection with gastro-intestitnal nematodes are very common.

Trichostrongyliasis is an infection of the gastro-intestinal tract of herbivorous animals and man is the accidental host caused by the members of the genus *Trichostrongylus.*

Strongyliasis is an intestinal infection of man and animals by the penetration of the skin by the filariform larvae of *Strongyloides stercoralis.*

1.8 Significance of study

Parasites and diseases can take a toll on both individual animal and on herd populations in general. An understanding of both the biology of the organisms involved and the methods of transmission helps to determine the significance of these types of infections in animals. Monitoring mule numbers and habitat condition as well as ascertaining parasite and diseases surveillance becomes extremely important in maintaining adequate numbers of healthy mules.

Nepal being a developing country, mostly goods are transported to backward or hilly area by mules. In Nepal there had been very less studies on endoparasites in mules which may be one of the factor in declining population of the equines. Parasitic infection decreases the production and productivity in the animals mainly in the reduction of body weight or failure to gain weight or even mortality in acute cases.

Very few studied have been conducted in Nepal to determine the potential losses in mule population. Thus, a need is felt to conduct a study regarding parasitic prevalence and its control which gives the suggestive guideline for vet-practitioner .In addition, the role and importance of mules for parasitic transmission and their significance in the continuity of some zoonotic diseases can be investigated. This study will provide objective of the study. The present study revealing the prevalence of helminth parasite seasonally that is during winter, summer and rainy season.

1.9 Limitations of the study

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

- The academic study has been carried out for the partial fulfillment of the requirements for the master's degree in zoology at Tribhuvan University, Kathmandu Nepal.
- The time for this study was also limited and carried out within three seasons only.
- The research has limited regarding finance and time constrains. Limited samples were taken for this study.
- This study also does not revel why some parasites were more predominant and other more not.
- Due to the lack of sophisticated instruments the identification of parasites was done up to genus level only.

CHAPTER - II

OBJECTIVES

The present study was done to fulfill the following objectives:

2.1 General Objective:

To determine the prevalence of gastro-intestinal helminths parasites in mules.

2.2 Specific Objective:

i. To identify the helminth parasites.

ii. To determine the seasonal prevalence of Cestodes, Trematodes and Nematodes in mules.

iii. To determine the rate of prevalence of gastrointestinal helminth parasites.

iv. To develop the recommendation for the planning regarding the control of helminth parasites in mules.

2.3 Hypothesis:

H0= There is no significant differences in prevalence of helminths parasites in winter, summer and rainy season.

H1= There is significant differences in prevalence of helminths parasites in winter, summer and rainy season.

CHAPTER - III

LITERATURE REVIEW

Parasitic zoonosis is distributed world wide and constitute an important group of disease affecting both human and animals. Many of the parasitic zoonosis produce significant mortality and morbidity in the human and are responsible for the health. Most of the papers have been presented and published largely after the outbreak of helminthic diseases among humans and animals.

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 *Trichina spiralis* were highly significant. His work with parasitism infections proved that *Taenia saginata* occurs only in cattle and that *Taenia solium* occurs only in pigs.

Parasites 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 host groups.

There are estimated to be 50 million donkeys and as many mules worldwide, who were suffering from internal parasites. They are susceptible to more than 60 internal parasites and may harbor several species of worms at any time. Literature reviews exist in helminth parasites as the disease continued to survive with new threats. Major research efforts that have been directed toward helminth parasites, the position of work and reports related to the epidemiology of helminth parasites have been mentioned here:

3.1 IN CONTEXT TO THE WORLD:

Matthee *et al*., (2000) studied on prevalence and biodiversity of helminth parasites in donkeys in South Africa. Quantitative samples were collected from the gastrointestinal tract of seven donkeys for recovery of helminth parasites. Fifteen genera and 29 species of helminths were identified comprising 27 species of nematodes in the Ascarididae, Atractidae, Habronematidae, Onchocercidae, Oxyuridae and Strongylidae. 1 species of cestode in the Anoplocephalidae and 1 species of trematode in the Paramphistomatidae, In addition, 2 species of oestrid fly larvae in the Gastrophiliidae were identified. The most abundant group in number of species was the cyathostomes (small strongyles) and of these, *Cyathostomum montgomeryi, Cylicocyclus* sp. and *Cylicostephanus minutus* were the most numerous. The most prevalent cyathostomes were *C. montgomeryi* and *Cylicocyclus* sp., *Strongylus vulgaris* was the most abundant and prevalent large strongyle species.

Eslami *et al***.,** (2000) worked on the parasitic infections in Iran.Over a period of 1 year from 1999 to 2000, fecal and blood samples and external surfaces of 290 racehorses on 20 private horse farms were examined. The alimentary canal was the only infected organ found. Fecal examination using a flotation method and saturated solution provided an indication of the *Parascaris equorum, Oxyuris equi*, and *Strongyle* eggs in 13.8%, 17%, and 28.3% of tested samples, respectively.

Holland *et al.,* (2001) studied on *Strongyle* infections in horses from North Vietnam .This study based on fecal egg counts and fecal cultures showed that *Strongyle* infections were a major health constraint in adult horses in North Vietnam. In packhorses from mountainous areas, the mean fecal egg count was 2053 eggs per gram (EPG), while in carriage-horses of the Red River delta it was 732. *Cyathostoma* sp. was found to be the most abundant in all fecal cultures, whereas *Strongylus* spp. prevalence was less than 7%. It appeared that 63% of all the animals were eligible for treatment since they had g counts higher than 500 EPG.

Hoste *et al*., (2002) studied on small *Strongyle* infections in France.Samples were collected from forty-two horses.Twenty species of Cyathostominae were identified. Ten most prevalent species were *Cyathostomum coronatum, Cylicocyclus nassatus, Cylicocyclus insigne, Cyathostomum catinatum, Cylicostephanus goldi, Poteriostomum imparidentatum, Cyathostomum labiatum, Cylicocyclus ultrajectinus, Cylicostephanus calicatus* and *Cylicostephanus minutus* which comprised 84% of the total adult population. Infections with singletons occurred in 12.5% of the positive horses while multiple infections were encountered in 87.5%.

Alayande *et al.,* (2003) studied on Prevalence of intestinal helminths of horse in sokoto.54 samples were collected randomly from horse stable within sokoto metropolis. The helminth ova identified were those of *Parascaris* sp, *Strongylus* sp, *Strongyloide* sp, *Panaplocephala* sp, *Dictyocaulus* sp, and *Gastrodiscus* sp. 84.4% of the samples examined were positive, out of which *Strongylus* sp. ova had the highest (75.5%).

Matthee *et al.,* (2004) studied on a comparison of the intestinal helminth communities of equidae in Southern Africa.The intestinal helminth communities of 8 horses, 12 donkeys, 21 Hartmann's mountain zebras, and 44 Burchell's zebras were compared using the original data from 6 studies in South Africa. Sixty helminth species (58 nematodes, 1 cestode, and 1 trematode species) were recorded. There were significant differences in the helminth community structures of the 4 *Equus* species. The helminth communities of the 2 closely related zebra subspecies were most similar, and they jointly shared 7 helminth species with donkeys and only 1 with horses. Geographic variation and host-mixing contributed to the helminth species composition. Multiple confamilial species infections were the norm in the donkeys and zebra subspecies, and no single-species infection was recorded for the Strongylidae. Congeneric species were commonly recorded in 3 genera (*Cyathostomum*, *Cylicocyclus*, and *Cylicostephanus*).

Yoseph *et al.,* (2005) worked on seasonal variation in parasite burden and body condition of working donkeys in east shewa and west shewa region of ethopia. A survey of donkeys arriving at markets in three localities in East and West Shewa regions of central

Ethiopia was carried out during 2002. Total faecal worm egg counts and body condition scores were measured for a total of 963 donkies over a 12-month period. Total faecal worm egg counts did not differ significantly between localities but there was significant (p<0.001) seasonal variation within localities. Levels of helminth infection closely followed rainfall patterns, being lowest (956 eggs per gram of faeces, e.p.g.) at the end of the long dry season (February) and highest (2022 e.p.g.) in the middle of the long wet season (August). Body condition score was associated closely with level of helminth infection (Goodman-Kruskal measure of association 0.60–0.80).

Veli *et al.*, (2005) studied the prevalence of *strongyle* infections and persistent efficacy of pyrantel embonate, ivermectin and moxidectin in Turkish horses.*Strongyle* infection was detected in 68% of 320 horses from 9 farms in western Turkey. Egg counts per gram of faeces (EPG) were more than 950 in half of the animals. The persistent efficacy of 3 different anthelmintics to suppress *Cyathostome* EPG was compared on a horse farm. One group of horses was treated with moxidectin on day 0, and the 2 other groups received pyrantel embonate or ivermectin on days 0 and 70. Pyrantel embonate reduced the EPG by >90% for 2 weeks; thereafter egg counts steadily increased. Ivermectin reduced the EPG by >98% for up to 6 weeks. In contrast, one treatment with moxidectin resulted in a persistent efficacy of >98% for at least 16 weeks.

Aydenizoz (2006) worked on to identify the helminth species present in horses from the Kirikkale province of Turkey as well as their prevalence. A total of 100 faecal samples were collected from horses in the Kirikkale province. Of the horses studied, 74% were infected with different helminth species. The helminth species found were Strongylidae (71%), *Parascaris equorum* (3%), *Anoplocephala perfoliata* (1%), and *Dicrocoelium dendriticum* (1%). Of these horses, 72% were infected with only one helminth species, and only 2% of the horses were infected with two species. *Strongylus vulgaris* (40.8%), *S. edentatus* (23.94%), *Trichonema* sp. (71.83%), *Triodontophorus* sp. (22.53%), *Gyalocephalus* sp. (2.81%) and *Poteriostomum* sp. (12.67%) were found in the faecal cultures of infected horses.

Pereira *et al.,* (2006) worked on gastrointestinal parasitic worms in equines in the Paraiba Valley, State of SaoPaulo, Brazil. A total of 20 individual equines (16 horses and

4 mules) were selected randomly. In the samples considered, the presence of parasites ranged from 155 to 1249 worms. Tapeworms (Cestoidea) were present in about 85% of the animals studied and roundworms (Nematoda) in 100% of the individuals. All the tapeworms collected were of one single species, *Anoplocephala perfoliata*. In the case of the roundworms, the prevalence of individual species was: 100% for Cyathostomineae, 90% for *Oxyuris equi*, 70% for *Strongylus vulgaris*, 45% for *S. edentatus*, 15% for *Strongylus equinus*, 60% for *Triodontophorus* sp., 50% for *Gyalocephalus capitatus*, 15% for *Oesophagodontus robustus* and *Craterostomum acuticaudatum*, and 5% each for *Parascaris equorum*, *Probstimayria vivipara*, *Habronema muscae*, and *Trichostrongylus axei*.

Ayele *et al*., (2006) worked on prevalence of gastrointestinal parasites of donkeys in durga bora district, Ethopia. A total of 339 faecal samples were collected randomly. The parasites encountered were *Strongyle* (100%), *Parascaris equorum* (50%), *Anoplocephala Spp*. (7.4%), *Gastrodiscus aegypticus* (6%), *Oxuris equi* (3%) and *Fasciola* (1.5%). Gross faecal examinations revealed *Gasterophilus intestinalis and Gasterophilus nasalis* (20.9%). 81.7% of donkeys sampled were severely infected, 8.3% heavily, 3.8% moderately and 6.2% mildly infected. Mixed infections were detected in 54.8% of the donkeys. Cultural identification of larvae (n=28) demonstrated *Strongylus vulgaris (*100%), *Cyathostomes (*100%), *Strongylus edentatus* (66.6%), *Trichostrongylus axei* (40%)*, Strongloides westeri* (33.3%*), Triodontophorus* (50%) and *Dictyocaulus arnfieldi* (20%).

Uslu and Guclu (2007) worked on prevalence of endoparasites in horses and donkeys in Turkey. Among the parasites determined in horses, the prevalence of *Strongylidae*, *Parascaris equorum*, *Strongyloides westeri*, *Fasciola sp*., *Anoplocephalidae*, *Oxyuris equi*, *Trichuris sp*., *Dicrocoelium dendriticum*, *Eimeria leucart*i, and *Eimeria sp*. was 100%, 10.81%, 7.2%, 3.6%, 2.7%, 1.8%, 0.9%, 0.9%, 4.5%, and 12.61%, respectively. In donkeys, the prevalence of *Strongylidae*, *S.westeri*, *P. equorum*, *Fasciola sp*., Anoplocephalidae, *Oxyuris equi*, *Dicrocoelium dendriticum*, *Eimeria leucarti*, and other *Eimeria sp*. was 100%, 12.34%, 9.8%, 6.17%, 6.17%, 1.23%, 1.23%, 3.7%, and 22.22%, respectively. According to faecal cultures, the prevalence of *Strongylus vulgaris*, *Strongylus edentatus*, *Trichonema sp*., *Triodontophorus sp*., and *Poteriostomum sp*. was 31.53%, 17.11%, 58.55%, 6.3%, and 5.40% in horses, respectively, and 23.45%, 14.81%, 74.07%, 4.93%, and 2.46% in donkeys, respectively.

Esmael (2007) studied on clinical and haematological study of the internal parasites in native donkeys in Mosul city. The study was included 70 native donkeys, corpological examinations revealed that the donkeys were infected with Nematodes such as Large strongyles *Strongylus spp*. 70%, *Triodontophorusspp*. 36.6%, Small *strongyles* (*caythostomines*) 33.3%, *Trichostrongylus axei* 33.3%, *Parascaris equorum* 20%, *Dictyocaulus arnfieldi* 13.3%, *Strongyloides westeri* 10%, *Habronema musca* 10%, *Oxyuris equi* 6.6% and Trematode worms *Gastrodiscus spp.*, *Dicroceolium spp*. 3.3%. The rate of a single infection was 20% and mixed infection was 80%.

Pandit *et al*., (2008) studied on the parasitic infestations of equines in Jammu and Kashmir by faecal samples examination. The overall infestation was found as high as 93.26%. *Trichonema sp.* (96.78%) dominated other types of parasites, *Strongylus sp*. (81.19%), *Triodontophorus sp*. (41.39%), *Dictyocaulus sp*. (14.10%), *Oxyuris sp.* (9.40%), *Paranoplocephala sp*. (8.14%), *Strongyloides sp.* (6.19%), *Parascaris sp*. (4.01%), *Amphistome sp.* (0.91%) and *Eimeria sp.* (0.34%) were also recorded.

Kuzmina and Kuzmin (2008) studied the species composition of the strongylid community of donkeys and explore the influence of anthelmintic treatments on the community structure.in Ukraine Seventeen species were found in donkeys studied: 16 species of Cyathostominae and 1 of Strongylinae. Between 2 and 7 species were found per donkey (average of 4.2 ± 2.8). *Cyathostomum tetracanthum, C. catinatum, Cylicocyclus nassatus, Cylicostephanus goldi and C. longibursatus* dominated in the community; they were found in 80-100% animals studied and comprised 91.7% of the total number of strongylids collected. Two species C. tetracanthum and Cylicocyclus auriculatus were found to be specific for donkeys. The results obtained showed a reduction of the species richness of the strongylid community in donkeys caused by lack of grazing and by regular anthelmintic treatments.

Getachew *et al.,* (2008) carried out a survey of seasonal pattrns in S*trongyle* faecal egg counts of working equids of the Central midlands and lowlands, Ethopia. A study was conducted for two consecutive years (1998–1999) to determine the seasonal patterns of *strongyle* infection in working donkeys of Ethiopia. For this purpose 2385 donkeys from midland and lowland areas were examined for the presence of parasitic ova. A hundred percent prevalence of S*trongyle* infection with similar seasonal pattern of *Strongyle* faecal worm egg output was obtained in all study areas. However, seasonal variations in the number of S*trongyle* faecal worm egg output were observed in all areas. The highest mean faecal worm egg outputs were recorded during the main rainy season (June to October) in both years in all areas. Although an increase in the mean S*trongyle* faecal egg output was obtained in the short rainy season (March–April) followed by a drop in the short dry season (May).

Mahfooz *et al*., (2008) worked on the prevalence and anthelmintic efficacy of Abamectin against gastrointestinal parasites under field conditions in Faisalabad (Punjab, Pakistan) was studied in 100 horses. The overall prevalence of gastrointestinal parasites was 75%, including *Strongylus spp*. (50%), *Oxyuris equi* (12%), *Parascaris equorum* (8%) and mixed infection (5%). Among these naturally infected animals, 15 were selected. These horses were assigned to three groups on the basis of prevalent species of gastrointestinal parasites. Each group had five animals, comprising four treatment horses and a control horse. Abamectin was evaluated against these gastrointestinal parasites with a single shot at the dose rate of 0.2 mg\kg body weight administered through subcutaneous route which resulted in 98% reduction in faecal egg count after day 14 post-treatment. Non-treated horses remained positive for gastrointestinal parasites. It was concluded that Abamectin is highly effective against gastrointestinal parasites in horses.

Shrikhande *et al*., *(*2009) studied on the incidence of helminth parasites in donkeys in Nagpur veterinary college.The faeces of 82 donkeys irrespective of sex and age were collected and examined for any parasitic ova. The faecal samples of 68 donkeys were infected with *Strongylus* sp. (54.87%), *Parascaris* sp. (29.26%), *Strongyloides* sp. (24.39%), *Trichonema* sp. (15.85%), *Oxyuris* sp. (8.53%), *Gastrodiscus* sp. (8.53%), *Entamoeba* sp. (8.53%), *Dictyocaulus* sp. (3.65%), and *Triodontophorus*sp. (2.43%)

Sinasu and Mustafa (2009) study was carried out to determine the prevalence of helminth species in horses, donkeys, and mules in the Central Black Sea region. For this purpose, 140 faecal samples were taken from horses (n = 83), donkeys (n = 31), and mules (n = 26). Infection rates were 91.57% (76 of 83) in horses, 96.77% (30 of 31) in donkeys, and 96.15% (25 of 26) in mules. The parasite species and their prevalence in examined animals were as follows: in horses *Strongylidae* spp. 77.10%, *Parascaris equorum*14.45%, *Fasciola* spp. 4.82%, *Oxyuris equi* 1.20%, *Anoplocephala* spp. 1.20%, *A. perfoliata* 1.20%, *A. magna* 1.20%, and *Dicrocoelium dentriticum* 1.20%; in donkeys *Strongylidae* spp. 96.77%, *P. equorum* 22.58%, *Strongyloides westeri* 22.58%, *Fasciola* spp. 16.13%, *Dictyocaulus arnfieldi* 9.67%, *O. equi* 6.45%, *Anoplocephala* spp. 6.45%, *D. dentriticum* 3.22%, and *Draschia\Habronema* spp. 3.22%; and in mules *Strongylidae* spp. 96.15%, *P. equorum* 15.38%, *Fasciola* spp. 11.53%, *Probstmayria vivipara* (adult) 3.84% and *A. perfoliata*3.84%. Faecal cultures from horses, donkeys, and mules showed infection rates of *Cyathostomum* spp. (33.88%,63.79%,69.07%), *Strongylus edentatus* (31.05%, 8.62%, 5.15%), *S. equines* (6.11%, 6.03%, 1.03%), *S. vulgaris* (3.52%, 3.01%, 8.76%), *Gyalocephalus* spp. (12.0%, 0.86%, 5.15%), *Poteriostomum* spp. (5.88%, 1.72%, 6.70%) and *Triodontophorus* spp.(1.41%,3.01%,4.12%), respectively. *Trichostrongylus axei* was not found in mules, and *S. westeri* was found (8.18%) only in donkeys.

Yanzhen *bu et al*., (2009) worked on *Strongyloid* nematodes in the caeca of 34 donkeys in Henan Province, China.Twenty-two species, including 18 *Cyathostominae* (small S*trongyles*) and 4 Strongylinae (large St*rongyles*), were identified. The five most prevalent *Cyathostominae* were *Cylicocyclus nassatus* (73.5%), *Coronocyclus labratus* (70.6%), *Coronocyclus labiatus* (67.6%), *Cyathostomum tetracanthum* (61.8%) and *Coronocyclus coronatus* (52.9%), accounting for 70.2% of all species identified; *C. labratus* (124.2 ± 256.4), *Cyathostomum tetracanthum* (96.4 ± 210.5) and *Cylicocyclus nassatus* (80.9 ± 117.1) had the greatest mean abundance, whereas *Strongylus vulgaris* was the most prevalent (88.2%) of the Strongylinae and had the highest mean abundance (34.9 ± 37.8), Only a small percentage (5.9%) of donkeys were infected by a single species, whereas the other donkeys had infections with multiple species.

Francisco and Arias *et al*., (2009) carried out a coprological survey to determine the influence of some intrinsic factors (breed, age, and sex) on the infection by helminth parasites in equine livestock under an oceanic climate area (NW Spain) was conducted. Faecal samples were individually collected and analyzed by the coprological techniques. The main *Strongylid* genera identified were *Trichonema* and *Cyalocephalus* spp (small *Strongyles*) and *Strongylus* and *Triodontophorus* (large S*trongyles*). The prevalence of gastrointestinal nematode was 89% and 1% cestoda. The percentage of horses with S*trongyloid* parasites was 89%, 11% for *Parascaris*, and 3% for *Oxyuris*. The highest prevalence for Ascariosis was observed in the youngest horses (<3 years), for Oxyurosis in the >10 years animals, and for Strongylosis in the 3–10 years ones. Females were significantly more parasitized than males.

Ggetachew *et al*., (2010) studied on gastrointestianal parasites of working donekey in Ethiopia. For the purpose 2935 working donkeys were coprologically examined for nematode and cestode, and 215 donkeys for trematode infections. Coprological examination revealed 99% *strongyle*, 80% *Fasciola*, 51% *parascaris*, 30% *Gastrodiscus*, 11% *strongyloides westeri*, 8% cestodes and 2% *Oxyuris equi* infection prevalence.

Khan *et a*l., (2010) studied on the prevalence of *Parascaris equorum* in paddock horses, donkeys, and mules (n = 150 for each) in Pakistan, was examined and the efficacy of two treatments, doramectin and garlic, measured on the basis of fecal egg counts. *Parascaris equorum* infection was found in 54 (36%) horses, 47 (31%) donkeys, and 42 (28%) mules. The final efficacy of treatment with doramectin was 92.5% in horses, 80.6% in donkeys, and 81.4% in mules, compared to 44.4%, 51.6%, and 37%, respectively, with garlic, making doramectin the more effective treatment.

Getachew *et a*l., (2010) studied on the epidemiology of Fasciolosis in working donkeys in Ethiopia. Faecal samples from 803 donkeys were collected and the number of liver flukes recovered from 112 donkeys at post-mortem. There was a high prevalence of Fasciolosis irrespective of the age of the donkeys. The overall prevalence of the infection was 44.4% in coprologically examined donkeys and the prevalence of donkeys examined by post-mortem was 41.9%. Both *Fasciola hepatica* and *Fasciola gigantica* were identified.

3.2 IN CONTEXT TO NEPAL

Most of the mules and horses being used in Nepal are being brought from neighboring part of India. Very few of them are natively breeded. Even though mules/donkeys have often been described as sturdy animals, they succumb to a variety of diseases and a number of other conditions. They do suffer from a number of diseases. Parasitic infestation is a major cause of illness. Documentation of parasitic infestation of mules in our Nepal is lacking.

In Nepal, the district livestock service office Udaypur reported the periodic death of mules, reason is that they were suffering from colic symptoms, which is find out by Karki and Manandhar. Poudel worked in horses with special references to *Strongylus* sp. in Chitwan. Ram sapkota also worked in helminths of mules in Lalitpur district. He worked sex wise and age wise. Sex wise, Female are found to have higher infection as they might have lower immunity due to gestation, lactation etc. Age wise, the highest prevalence is seen in animals of old age due to waning body condition and immunity.

Karki and Manandhar (2006) determined the prevalence rates and found associations between the acute sudden death of mules with colic symptom and parasites burden in udaypur. A total of 33 faecal samples were collected randomly for qualitative and quantitative faecal analysis. The parasites encountered were *Strongyle* (100%), *Parascaris equorum* (50%), *Anoplocephala* sp.(7.4%), gastrodiscus aegypticus(6%), *Oxuris equi* (3%) and *Fasciola* (1.5) 81.7% of mules sampled were severely infected, 8.3% heavily, 3.8% moderately and 6.2% mildly infected. Mixed infections were detected in 54.8% of the mules.

Paudel (2007) found the prevalence of gastrointestinal parasites as 80.48% (33\41) with *Strongylus* (48.78%), *Trichostrongylus* (31.70%), *Parascaris* (21.95%), *Trochonema* (17.07%), *Gastrodiscus* (7.31%), and *Habronema* (4.87%) in horses of Sainik stud farm Chitwan. The EPG count of Strongylus ranged between 200 and 800.

Sapkota (2009) studied on the prevalence of helminths parasites in mules in brick kilns of Lalitpur district. Out of 60 samples randomly taken, 27(45%) samples were positive. The samples were examined qualitatively by sedimentation and floatation method. Out of 27 infected samples the species of parasite found are *Gastrodiscus* (30%), *Strongylus* (22%), *Oxyuris* (30%), *Dictyocalus* (7%) and *Triodontoforus* (11%).

CHAPTER - IV

MATERIALS AND METHODS

4.1 Study Area

Nepal is well known for being a richest country in terms of biodiversity in the world having every possible habitat for living beings. It is located at 80° 4'to 88° 12' east longitude and 26° 22' to 30° 27' north latitude. It is one of the landlocked countries in Asia without any access to sea or ocean.Measuring 885 km and 193 km lengthwise and breadth wise respectively covering 147,181sq km.

Nepalgunj of Banke district, the specific study area is located at mid-western development region of Nepal. It is situated near the border of India. Nepalgunj is located at 81° 29' to 82° 80' east altitude and 27°51' to 28° 20' north latitude and elevation is 165 meters from sea level. It covers an area of 1,314 hecters.

This study was carried out for the seasonal prevalence rate of helminth infection in mules. The stool sample were collected from the study area and brought at central veterinary laboratory, Tripureshwor for laboratory diagnosis.

4.2 Study design:

Laboratory based diagnosis

4.3 Study period:

December/January 2008/2009, May/June (2009) and August/September 2009.

4.4 Sample size:

The total numbers of sample taken during winter, summer and rainy seasons were 100,100 and 50, respectively. Altogether 250 samples were collected for examination. These were collected from Nepalgunj of district Banke.

The image cannot be displayed. Your computer may not have enough memory to open the image, or the image may have been corrupted. Restart your computer, and then open the file again. If the red x still appears, you may have to delete the image and then insert it again.

4.5 Precautions and preservation:

To ensure the better condition during the sample collection, the following mentioned precautions were taken:

- Only fresh sample were collected.
- The sample was collected in air tight container to prevent desiccation.
- 3-4 drops of 10% formalin was used to fix the samples.
- Sampling was done randomly.

4.6.a Laboratory apparatus and materials:

a. Motor and pestle

b. Test tube

c. Cotton

d. Cover slip

e. Slides

f. Glass rod

g. Centrifugal machine

h. Gloves

i. Tea strainer

j. Microscope

k. Pasteur pipette

l. Refrigerator

m. Dropper

n. Rack

4.6.b Chemicals:

a. Formalin (10%)

b. Distilled water

c. Zinc sulphate solution (33%)

d. Methylene blue

4.7 Stool examination:

The examinations of stool sample were done by using differential floatation technique, sedimentation and Stoll's counting method.

4.7. a Flotation technique:

This technique is used widely for detecting eggs of nematodes and cestodes. As their eggs are lighter and small, they can float in the floatation liquid 42 ml of 33% zinc sulphate solution was added to the 3 gram of stool sample. The sample was grinded with the help of pistil, motor and filtered with a tea strainer. 15 ml plastic tube was used to pour the filtered solution. Then it was centrifuged at 1000 rpm for 5 minutes. To form convex surface at the top of the tube, some more zinc sulphate solution was added. Due to this cover slip touch the solution and after few minutes, the cover slip was taken off and placed on the slides for the examination at 10x.

4.7. b Sedimentation technique:

This technique is used for detecting trematodes eggs as they are heavier than the other eggs. The eggs get deposited at the bottom of the test tube after the centrifugation with zinc sulphate solution. With the help of pipette, a drop of deposited material was taken on the slid. On it, a drop methylene blue was added and was examined under a microscope at 4x and 10x.

4.7. c Stool's counting methods:

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

4.8. Key for trematodes, cestodes and nematods:

- Chaudhari, S.S, Gupta, S.K. Banarjee, D.P; Bhartnagar, P.K; and Ruprah, N.S,(2003). General helminthology. Manual of Veterinary Helminthology. 1:10 – 184. International Book Distributing company.Lucknow, India.
- Parija, S.C (1990). Helminthic infections: Trematode, Cestode and Nematode. Review of parasitic Zoonoses. 1:41-393. International Book Distributing Company. Lucknow, Indian.
- Soulsby, E.J.L (1982). Eggs of worm parasites. Helminthes, Arthopods and protozoa of Domesticated Animals; 7th edition. 1:24-345. The English Language Book Society and Bailliere Tindall. London.
- Gibbons, L.M; Jacobs, D.E.; fox, M.T. and Hansen, J. (2007). Faecal examination for Helminth parasites. The RVC/FAO guide to Veterinary Diagnostic parasitology, Electronic Media unit at the Royal Veterinary College.London.

CHAPTER - V

RESULT

Helminth parasitic infection is the common problem of mules and other domestic animals all over the world. This work has been done in order to identify the eggs of various types of helmith parasites. It also includes which parasite is found more in which season. Beside this the study also includes the multiple infections. This particularly deal with the rate of infection i.e. what number of parasites are able to cause infection or to what extent the particular helminth parasite is pathogenic. All this will serve as a medium for solving the problem of helminth parasites. Thus the study has been divided into the following major parts:

1. General prevalence of helminth parasites.
2. Seasonal prevalence of trematodes, cestodes and nematodes in Nepal
3. Class wise seasonal prevalence,
4. Identification of eggs of helminth
5. Multiple infection

1. General prevalence of helminth parasites:

Out of 100,100 and 50 samples from summer, winter and rainy season respectively, from 250 hosts, 173 hosts were found to be infected. Overall outcome to the study was 69.2% prevalence.This study showed 16.18% cestodes infection, 50.86% trematodes infection and 78.6% nematodes infection. The total number of genere observed during examination were 21 in number, out of which 4 genera of cestodes, 4 genera of trematodes and 13 genera of nematodes were observed. The general and overall prevalence percentage of each species is given in the following tables:

Table 1. Observed Genera of different classes with prevalence percentage:

S.N	Class	Genere of Helminth	Percentage
1	Cestoda	*Anoplocephala*	3.46
2		*Dipylidium*	2.31
3		*Moniezia*	2.31
4		*Taenia*	8.67
5	Trematoda	*Dicrocoelium*	9.24
6		*Fasciola*	9.82
7		*Gastrodiscus*	2.89
8		*Schistosoma*	32.86
9	Nematode	*Ancylostoma*	1.15
10		*Capillaria*	9.82
11		*Chabertia*	4.04
12		*Cooperia*	4.62
13		*Dictyocaulus*	11.56
14		*Oxyuris*	13.87
15		*Parascaris*	10.4
16		*Strongylus*	15.02
17		*Strongyloides*	8.67
18		*Trichostrongylus*	13.29
19		*Trichuris*	9.24
20		*Triodontophorus*	1.15
21		*Toxocara*	5.78

Highest prevalence shown by *Schistosoma* (32.36), lowest prevalence shown by *Ancylostoma* and *Triodontophorus* (1.15%).

2. Seasonal prevalence of helminth parasites in mules:

After collection of 250 samples from study area, out of which 100 samples collected in winter, 100 in summer and 50 in rainy season respectively. The examination of samples was carried out and examinations of samples were done by using the floatation and sedimentation technique. In winter 53 samples were positive (53%) out of 100 samples, in summer 81 samples were positive (81%) out of 100 samples and in rainy season 39 samples were positive (78%) out of 50 samples. The rate of prevalence of helminth were found more in summer than rainy and very less in winter season. The difference in the prevalence of different genus of helminthes parasites during three seasons altogether were found statistically significant (=46.43, $p<0.05$, d.f.=1).

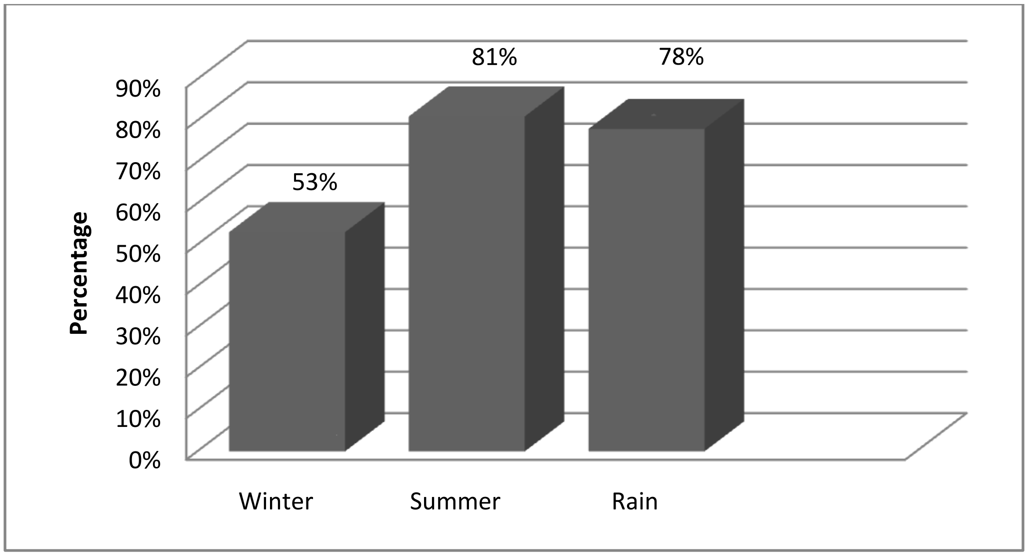


Fig: 4. General seasonal prevalence helminth parasites in mules

3. Class wise seasonal prevalence

During examination of samples overall 21 genera were observed. But on the seasonal basis 16 (76.19%) genera were found in winter, 20 (95.23%) genera were found in summer and 19 (90.47%) genera were found in rainy season.

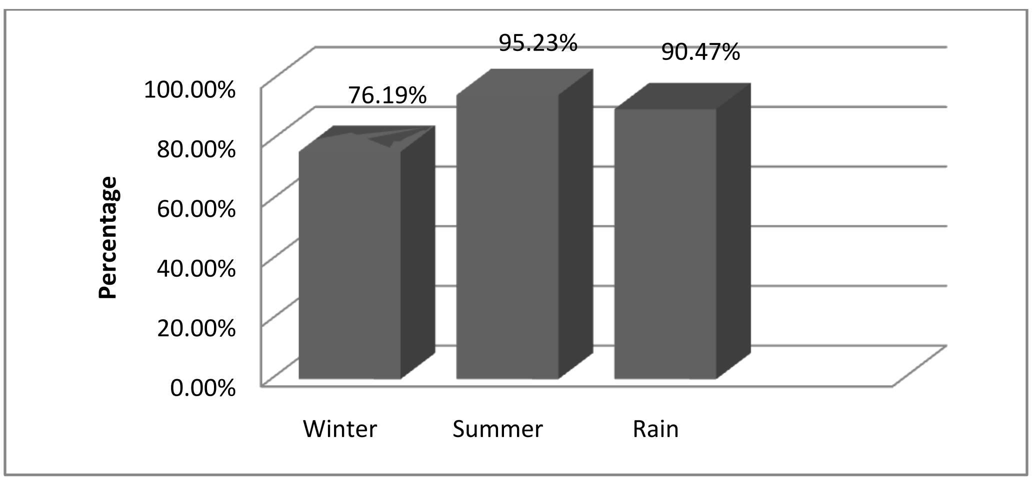


Fig: 5. Genera wise seasonal prevalence

3. a Seasonal prevalence of cestode genera in mules:

Altogether 28 (16.18%) samples were found positive for trematodes out of 173 positive samples. During winter 53(53%) samples positive out of 100 samples. From 53, 4(7.54%) samples were positive for cestodes. Out of 4 samples, 2(3.77) samples were positive for

Anoplocephala and 2(3.77%) samples were positive for *Taenia*. In summer 81 (81%) samples were positive out of 100 samples. From 81, 16 samples were positive for cestodes. Out of 16(19.73%), 4,2,2,9 samples were positive for *Anoplocephala* (4.93%), *Dipylidium* (2.46%), *Moniezia* (2.46%), *Taenia* (11.11%) respectively. In rainy season 39(78%) samples were positive out of 50 samples. From 39(78%) 8 samples were positive for cestodes. Out of 8 (28.5%) samples, 2,2,4 samples were positive for *Dipylidium*(5.12%), *Moniezia*(5.12%) and *Taenia* (10.25%) respecectively.The differences in the prevalence of different genera of cestodes during winter, summer and rainy season were found stastically in significant (= 4.71, $p<0.05$, d.f. =1)

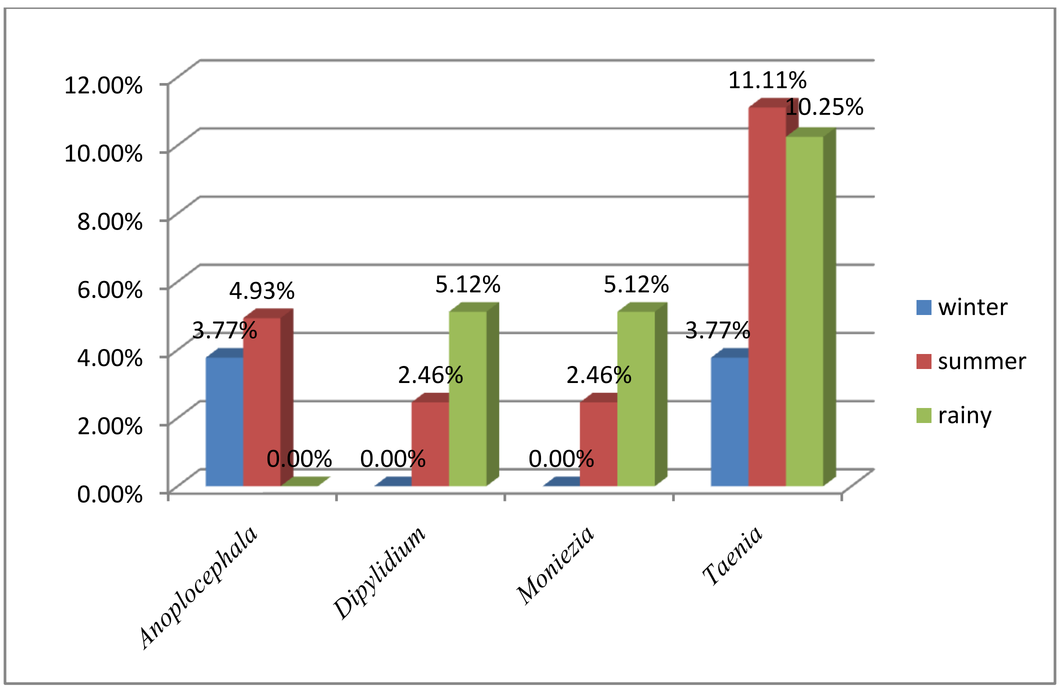


Fig: 6. Seasonal prevalence of cestodes genera

3.b Seasonal prevalence of Trematodes genera in mules:

Altogether 88(50.86%), samples were positive for trematodes out of 173 positive samples.During winter 53 (53%) samples were positive out 100 samples. From 53,21 (39.62%) samples were positive for trematodes out of 21 samples 3,2,17 samples were positive for *Dicrocoelium* (5.66%), *Fasciola* (3.77%), *Schistosoma*(32.07%) respectively.

In summer 81 (81%) samples were positive out of 100 samples. From 81, 46 (56.79%) samples were positive for trematodes, out of 46, 8, 12, 4, 26 samples were positive for *Dicrocoelium* (9.87%), Fasciola (14.81%), *Gastrodiscus* (4.93%) *Schistosoma* (32.09%) respectively. In rainy season 39(78%) samples were positive out of 50 samples. from 39 samples 21 (53.84%) samples were positive for trematodes. Out of 21-5, 3, 1, 13 samples were positive for *Dicrocoelium* (12.82%), *Fasciola* (7.69%), *Gastrodiscus* (2.56%) *Schistosoma* (33.33%) respectively.

The difference in the prevalence of different genra of trematodes during winter, summer and rainy seasons were found statistically insignifant (= 5.59, $p<0.05$, d.f. =1)

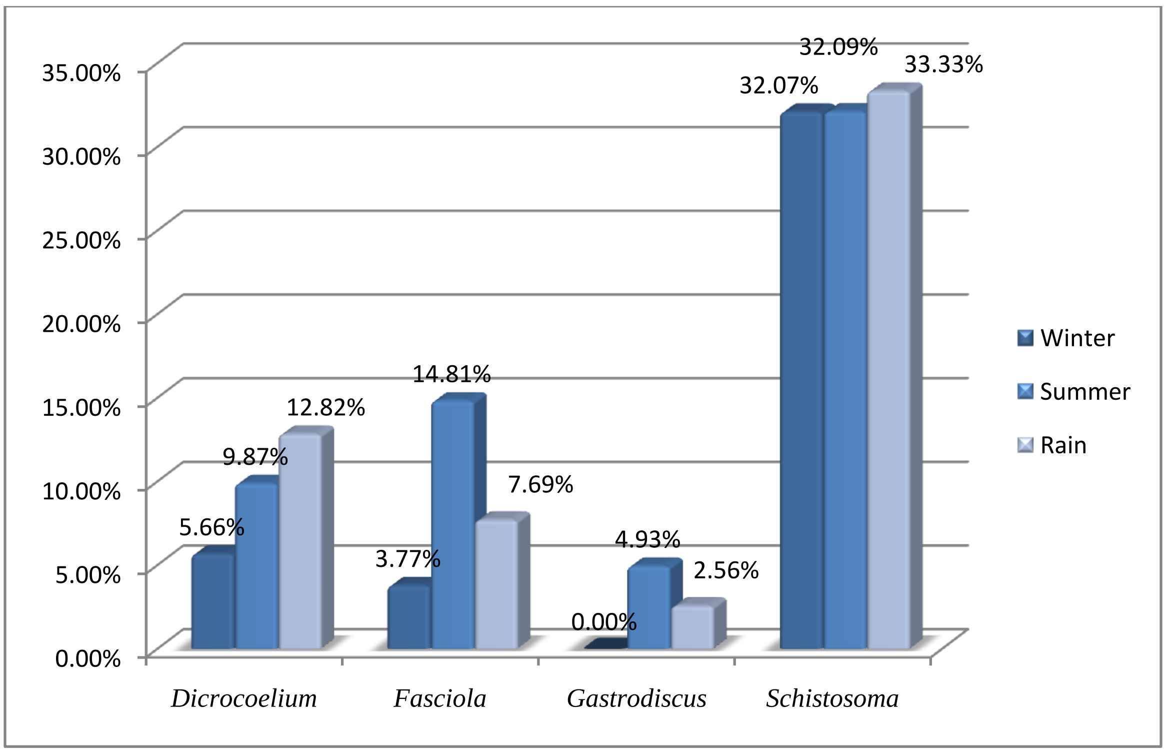


Fig: 7. Seasonal prevalence of trematodes genera

3.c Seasonal prevalence of Nematodes genera in mules:

Altogether 136 (78.61%) samples were positive for Nematodes out of 173 positive samples. From 136, 37 (69.81%) samples were positive in winter and 11 genera were observed.In summer 68 (83.95%) samples were positive for nematode and 12 genera were obsereved. In rainy season 31 (79.48%) samples were positive for nematodes and

12 genera were observed. The difference in the prevalence of different genera of nematodes during winter, summer and rainy season were found stastically in significant (= 19.60, $p<0.05$, d.f.=1)

Table 2. Prevalence of nematodes during winter:

S.no.	Nematode genera	during winter	prevalence
1	*Capillaria*	5	9.43%
2	*Cooperia*	1	1.88%
3	*Chabertia*	2	3.77%
4	*Dictyocaulus*	6	11.32%
5	*Oxyuris*	9	16.98%
6	*Parascaris*	5	9.43%
7	*Strongylus*	7	13.20%
8	*Strongyloides*	3	5.66%
9	*Trichostrongylus*	6	11.32%
10	*Trichuris*	5	9.43%
11	*Toxocara*	1	1.88%

Table 3. prevalence of nematode during summer:

S.no.	Nematode genera	Summer	prevalence
1	*Capillaria*	6	7.40%
2	*Cooperia*	5	6.17%
3	*Chabertia*	3	3.70%
4	*Dictyocaulus*	9	11.11%
5	*Oxyuris*	13	16.04%
6	*Parascaris*	9	11.11%
7	*Strongylus*	16	19.75%
8	*Strongyloides*	9	11.11%
9	*Trichostrongylus*	9	11.11%
10	*Trichuris*	7	8.64%
11	*Triodontophorus*	2	2.46%
12	*Toxocara*	7	8.64

Table 4. Prevalence of nematode in rainy season:

S.no.	Nematode genera	Rainy	Prevalence
1	Ancylostoma	2	5.12%
2	*Capillaria*	6	15.38%
3	*Cooperia*	2	5.12%
4	*Chabertia*	2	5.12%
5	*Dictyocaulus*	5	12.82%
6	*Oxyuris*	2	5.12%
7	*Parascaris*	4	10.25%
8	*Strongylus*	3	7.69%
9	*Strongyloides*	3	7.69%
10	*Trichostrongylus*	8	20.51%
11	*Trichuris*	4	10.25%
12	*Toxocara*	2	5.12%

4. Identification of eggs of Helminth:

During examination of winter samples 53% were found positive out of 100 samples, 81% samples of summer were found positive out of 100 samples and 78% samples of rainy season were found positive out of 50 samples. The total 21 eggs were identified and listed below in the table:

Table 5. Observed genera of different classes

S.no	Class	Genera of helminth
1	Cestoda	*Anoplocephala*
2		*Dipylidium*
3		*Moniezia*
4		*Taenia*
5	Trematoda	*Dicrocoelium*
6		*Fasciola*
7		*Gastrodiscus*
8		*Schistosoma*
9	Nematoda	*Ancylostoma*
10		*Capillaria*
11		*Chabertia*
12		*Cooperia*
13		*Dictyocaulus*
14		*Oxyuris*
15		*Parascaris*
16		*Strongylus*
17		*Strongyloides*
18		*Trichostrongylus*
19		*Trichuris*
20		*Triodontophorus*
21		*Toxocara*

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

CLASS-CESTODA

***Anoplocephala* sp.**

Family-Anoplocephalidae

Genus-*Anoplocephala* sp.

Description of egg: 50 to 80 µm in size and its distinguishing character is D- like shape

***Dipylidium* sp.**

Family-Dilepididae

Genus-*Dipylidium* sp.

Description of egg: Eggs are 12-15 µm in diameter, spherical, hyaline, thin shelled and with 3-pairs of hooklets.

***Moniezia* sp.**

Family – Anoplocephalidae

Genus – *Moniezia* sp.

Description of eggs: Eggs are 56-67 µm in diameter, triangle globular or quadrangular in shape, contain a well-developed pyriform apparatus

***Taenia* sp.**

Family – Tenidae

Genus – *Taenia* sp.

Description of egg: Eggs are 24-41 µm in diameter, spherical in shape, brown to dark-yellow in colur, thick shelled and contain an onchosphere.

CLASS-TREMATODA

***Dirocoelum* sp.**

Family – Dicrocoelidae

Genus – *Dicrocoelum* sp.

Description of eggs: Eggs are 36-45 by 23-30 μm in size, dark brown in colour, opercerlated, usually with a flattened side, contains miracidium when passed in to the faeces.

***Fasciola* sp**

Family – Fasciolidae

Genus – *Fasciola* sp

Description of eggs: Eggs are 130- 197 by 63-104 μm in size, oval shaped, and yellowish in colour, consist of embryonic

***Gastrodiscus* sp.**

Family-Gastrodiscidae

Genus-*Gastrodiscus*

Description of egg: 50-80 μ in size with three outer membrane with pyriform apparatus, pear shaped.

***Schistosoma* sp.**

Family – Schistosomitidae

Genus – *Schistosoma* sp.

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

CLASS-NEMATODA

***Ancylostoma* sp.**

Family – Ancylostomatidae

Genus – *Ancylostoma* sp.

Description of eggs: Eggs are 125-195 by 60-92 µm in size. They are eight celled when laid.

***Capillaria* sp.**

Family – Capillaridae

Genus – *Capillaria* sp.

Description of eggs: Eggs are 30-63 µm in size, barrel shaped, contain unsegmented embryo, colourless shell.

***Chabertia* sp.**

Family – Trichonematidae

Genus - *chabertia* sp.

Description of eggs: Eggs are 90-105 by 52-55 µm in size, oval shaped, laid in morula stage.

***Cooperia* sp**.

Family – Trichostrongylidae

Genus – *Cooperia* sp.

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

***Dictyocaulus* sp.**

Family – Dictyocaulidae

Genus – *Dictyocaulus*

Description of the eggs: Eggs are 71-84 by 46-52 µm in size, barrel- shaped, brownish yellow in colur and shell are pitted except at the poles.

***Oxyuris* sp.**

Family – Oxyuridae

Genus – *Oxyuris* sp.

Description of eggs: Eggs are 90 by 42 µm in size, elongate, slightly flattened on side, provided with a plug at one pole.

***Parascaris* sp.**

Family- Ascarididae

Genus- *Parascaris* sp.

Description of egg: 90 to 100 microns in size, spherical and have a brownish colour. The eggs contain a 1-celled zygote.

***Strongylus* sp.**

Family-Strongylidae

Genus- *Strongylus* sp.

Description of egg: 50 to 85 µm in size.It may be red or white in colour.

***Strongyloides* sp.**

Family – Strongylidae

Genus – *Strongyloides* sp

Description of eggs: Eggs are 40-64um by 20–40 µm in size, ellipsoidal, thin shelled embryonated when laid.

***Trichostrongylus* sp.**

Family – Trichostrongyloidae

Genus – *Trichostongylus* sp.

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

***Trichuris* sp.**

Family – Trichuridae

Genus – *Trichuris* SP.

Description of eggs: Eggs are 70-80 by 30-42 μm in size, brown in color, contain unsegmented embryo, barrel shaped with transparent plug at either pole.

***Triodontophorus* sp.**

Family- Strongylidae

Genus- *Triodontophorus* sp.

Description of egg: 60 - 130 μm in size.

***Toxocara* sp.**

Family – Ascaridae

Genus –*Toxocara* sp.

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

5.Multiple infection

0 to 1 = less than 2 ova per field. (Light infection)

2 to 4 = 2-4 ova per field. (Mild infection)

4 to 6 = 4-6 ova per field. (Moderate infection)

6 to 8 = 6 or more ova per field. (Heavy infection)

In the present study, rate of mixed infection was also observed. Altogether 173(69.2%) positive, 120 (69.36%) positive samples were examined mixed infection with 2-5 species in each samples.In multiple infections, the intensity of light infection shown by (0to1) that is less than two ova found per field, intensity of mild infection shown by (2to4) that is 2-4 ova found per field. Like wise, intensity of moderate infection shown by (4to6) that is 4-6 ova found per field and intensity of heavy infection shown by (6to8) that is 6-8 ova found per field.

Table no 6. Multiple infections in winter season

S.no.	Class	Name of the genera	0 to 1	2 to 4	4 to 6	6 to 8
1	Cestoda	*Anoplocephala*	1	1	-	-
2		*Dipylidium*	-	-	-	-
3		*Moniezia*	-	-	-	-
4		*Tenia*	1	1	-	-
5	Trematoda	*Dicrocoelium*	1	2	-	-
6		*Fasciola*	1	1	-	-
7		*Gastrodiscus*	-	-	-	-
8		*Schistosoma*	6	7	2	2
9	Nematoda	*Ancylostoma*	-	-	-	-
10		*Capillaria*	3	2	-	-
11		*Chabertia*	2	-	-	-
12		*Cooperia*	1	-	-	-
13		*Dictyocaulus*	3	2	1	-
14		*Oxyuris*	7	2	-	-
15		*Parascaris*	2	3	-	-
16		*Strongylus*	5	1	1	-
17		*Strongyloides*	2	1	-	-
18		*Trichostrongylus*	2	4	-	-
19		*Trichuris*	2	3	-	-
20		*Triodontophorus*	-	-	-	-
21		*Toxocara*	1	-	-	-

In winter 25(47.16%) samples were observed mixed infection out of 53 positive samples. Among winter helminthes, the intensity of light infection was noted due to *Oxyuris* wih 7(28%) (0-1) positive samples, mild infection shown by *Schistosoma* 7(28%) (2-4) positive samples. Moderate infection shown by *Schistosoma* with 2(8%) (4-6) positive samples and heavy infection shown by *Schistosoma* with 2 (8%) (6-8) positive samples.

Table 7. Multiple infections in summer season:

S.no.	Class	Name of the genera	0 to 1	2 to 4	4 to 6	6 to 8
1	Cestoda	*Anoplocephala*	2	1	1	-
2		*Dipylidium*	2	-	-	-
3		*Moniezia*	1	1	-	-
4		*Tenia*	2	5	2	-
5	Trematoda	*Dicrocoelium*	6	1	1	-
6		*Fasciola*	6	5	1	-
7		*Gastrodiscus*	1	2	1	-
8		*Schistosoma*	9	5	11	1
9	Nematoda	*Ancylostoma*	-	-	-	-
10		*Capillaria*	2	4	-	-
11		*Chabertia*	2	1	-	-
12		*Cooperia*	5	-	-	-
13		*Dictyocaulus*	6	2	1	-
14		*Oxyuris*	8	3	2	-
15		*Parascaris*	3	3	3	-
16		*Strongylus*	10	6	-	-
17		*Strongyloides*	4	4	1	-
18		*Trichostrongylus*	4	3	2	-
19		*Trichuris*	4	3	-	-
20		*Triodontophorus*	2	-	-	-
21		*Toxocara*	6	1	-	-

In summer 68(83.95%) samples were observed mixed infection out of 81 positive samples. In this time, light infection shown by *Strongylus* with 10(14.70%) (0-1) positive samples, mild infection shown by *Strongylus* with 6 (8.82%) (2-4) positive samples. Moderate infection shown by *Parascaris* with 3(4.4%) (4-6) positive samples and heavy infection shown by *Schistosoma* with 1(1.47%) (6-8) positive samples.

Table 8. Multiple infections in rainy season:

S.no.	Class	Name of the genera	0 to 1	2 to 4	4 to 6	6 to 8
1	Cestoda	*Anoplocephala*	-	-	-	-
2		*Dipylidium*	2	-	-	-
3		*Moniezia*	2	-	-	-
4		*Tenia*	1	2	1	-
5	Trematoda	*Dicrocoelium*	3	2	-	-
6		*Fasciola*	-	1	2	-
7		*Gastrodiscus*	-	1	-	-
8		*Schistosoma*	3	2	7	1
9	Nematoda	*Ancylostoma*	2	-	-	-
10		*Capillaria*	4	2	-	-
11		*Chabertia*	2	-	-	-
12		*Cooperia*	2	-	-	-
13		*Dictyocaulus*	4	1	-	-
14		*Oxyuris*	2	-	-	-
15		*Parascaris*	1	3	-	-
16		*Strongylus*	3	-	-	-
17		*Strongyloides*	2	1	-	-
18		*Trichostrongylus*	5	2	1	-
19		*Trichuris*	3	1	-	-
20		*Triodontophorus*	-	-	-	-
21		*Toxocara*	2	-	-	-

In rainy season 27(69.23%) samples were observed mixed infection out of 39 positive samples light infection shown by *Trichostrongylus* with 5(18.51%) (0-1) positive samples, mild infection shown by *Parascaris* with 3(11.11%) (2-4) positive samples. Moderate infection shown by *Schistosoma* with 7(25.92%) (4-6) positive samples and heavy infection shown by *Schistosoma* with 1(3.70%) (6-8) positive samples.

Plate 1

EGGS OF CESTODES OBSERVED

The image cannot be displayed. Your computer may not have enough memory to open the image, or the image may have been corrupted. Restart your computer, and then open the file again. If the red x still appears, you may have to delete the image and then insert it again.

Plate 2

EGGS OF TREMATODES OBSERVED

The image cannot be displayed. Your computer may not have enough memory to open the image, or the image may have been corrupted. Restart your computer, and then open the file again. If the red x still appears, you may have to delete the image and then insert it again.

Plate 3

EGGS OF NEMATODE OBSERVED

The image cannot be displayed. Your computer may not have enough memory to open the image, or the image may have been corrupted. Restart your computer, and then open the file again. If the red x still appears, you may have to delete the image and then insert it again.

Plate 4

EGGS OF NEMATODE OBSERVED

The image cannot be displayed. Your computer may not have enough memory to open the image, or the image may have been corrupted. Restart your computer, and then open the file again. If the red x still appears, you may have to delete the image and then insert it again.

CHAPTER - VI

DISCUSSION

The present study was carried out to determine the seasonal prevalence of intestinal helminths parasites of mules. It is thought that the prevalence of gastrointestinal parasites are considerably influenced by the climatic conditions and as far as possible the evidence for the distribution and prevalence of the diseases is presented by geographical area, roughly corresponding to climatic conditions. Generally the warm and humid conditions, which prevail in much of south-east Asia, provide good conditions for many gastrointestinal prevalence of flourish. Continuous high rainfall throughout the year in parts of the region means that there is no season during which the parasites are not problem (Tiyo *et al.,* 2008). The present investigation was carried out in the month of December\janauray, may\june, and August\september. The site of collection of samples was Nepalgunj of district Banke.

From the present study 53(53%) samples of winter found positive out of 100 samples, 81(81%) samples were found positive in summer out of 100 samples, and 39 (78%) found positive in rainy season out of 50 samples. The number of samples found positive during winter, summer and rainy seson for cestodes 4, 16 and 8, for trematodes 21, 46 and 21, for nematodes 37, 68, 31 respectively. (The number of samples exceeds total number of sample taken due to multiple infeciton).

In the present study two genera of cestoda, 3 genera of trematoda and 11 genera of nematode were observed during winter and during summer 4 genera of cestoda, 4 genera of trematoda and 12 genera of nematode were observed. Similarly 3 genera of cestoda and 4 genera of trematoda and 12 genera of neamatoda were identified. In the cestoda *Taenia* is very common in three seasons. *Anaplocephala* found in winter and summer seasons. *Moniezia* and *Dipylidium* found in summer and rainy season.

Among trematoda, *Schistosoma, Fasciola* and *Dicrocoelium* is very common found in winter, summer and rainy season. *Gastrodiscus* found in summer and rainy season. In nematoda, 11 genera were observed during winter namely-*Capillaria, Chabertia,* *Cooperia, Dictyocaulus, Oxyuris, Parascaris, Strongylus, Strongyloides, Trichostrongylus, Trichuris, Toxocara.* During summer 12 genera were observed namely - *Capillaria, Chabertia, Cooperia, Dictyocaulus, Oxyuris, Parascaris, Strongylus, Strongyloides*, *Trichostrongylus*, *Trichuris,Triodontophorus*, *Toxocara* during rainy season also 12 genera were observed namely- *Ancylosotoma,Capillaria, Chabertia, Cooperia*, *Dictyocaulus*, *Oxyuris*, *Parascaris*, *Strongylus*, *Strongyloides, Trichostrongylus, Trichuris, Toxocara.*

Tania, moniezia and *Dipylidium* of cestodes, *Schistosoma* and *Dicrocoelium* of trematodes genera is reported for the first time in mules from Nepal in the present study. Similarly, nematode genera *Ancylostoma*, *capillaria, Cooperia*, *Charbertia*, *Strongyloides, Trichuris* and *Toxocara* also reported for the first time in Nepal in the present study.

The seasonal prevalence of cestodes genera found in mules were *Anoplocephala*(3.77%\4.93%\0.0%), *Dipylidium* (0.0%\2.46%\5.12%), *Moniezia* (0.0%\2.46%\5.12%), *Taenia* (3.77%\11.11%\10.25%) during winter, summer and rainy season respectively. Similarly, the prevalence rate of trematodes were *Dicrocoelium (5.66*%\9.87%\12.82%), *Fasciola* (3.77%\14.81%\7.69%), *Gastrodiscus* (0.0%\4.93%\2.56%), *Schistosoma* (32.07%\32.09%\33.33%) during winter, summer, rainy season respectively.

similarly nematodes prevalence during winter, summer and rainy season recorded as follow:
Ancylostoma(0.0%\0.0%\5.12%),Capillaria(9.43%\7.40%\15.38%),Chabertia(3.77%\3.70%\5.12%),*Cooperia*(1.88%\6.17%\5.12%),*Dictyocaulus*(11.32%\11.11%\12.82%),*Oxyuris*(16.98%\16.04%\5.12%),*Parascaris*(9.43%\11.11%\10.25%),*Strongylus*(13.20%\19.75%\7.69%),*Strongyloides*(5.66%\11.11%\7.69%),*Trichostrongylus*(11.32%\11.11%\20.5%),*Trichuris*(9.43%\8.64%\10.25%),*Triodontophorus*(0.0%\2.46%\0.0%),(0.0%\2.46%\0.0%),*Toxocara*(1.88%\8.64%\5.12%).

Out of 250 selected samples 173 samples were positive four parasites of different genus showing a prevalence of 69.2%. The finding is much lower than that made by karki and Manandhar (2006) in mules of Udaypur who found the parasitic burden about 81.7% in mules and also lower than that observed by poudel (2007) who found the parasitic burden much higher about 80.48%. Ram sapkota (2009) also work done in mules, his finding show only 45% parasitic burden in mules which is lower than this present study.

During the study, out of 100 samples taken, during winter 53% were found positive and out of 53 positive samples, 25 (47.16%) had multiple infection. likely, during summer 81% samples were positive out of 100 samples. Out of 81 samples, 68 (83.95%) samples were found mixed infection. Similarly in rainy season 39(78%) samples were found positive out of 50 samples. Out of 39 samples, 27(69.23%) were found mixed infection. The study showed that the rate of multiple infection 69.36%was far greater than single infection 30.63%. The abundance of multiple infections mainly during summer and rainy season might be due to availability of suitable temperature and moisture. It may due to exposure of equines to highly infected pasture land, contaminated water or infected fodder. Mixed infection (54.8%) detected by karki and manandhar (2006). Esmael (2007) detected 80%.

Among cestodes Aydenizoz(2006) reported *Anoplocephala*(1%), again Ayele *et al*.,(2006) reported (7.4%) and in Nepal karki and manandhar (2006) found *Anoplocephala* (7.4%), which is harmony with the work of Ayele *et al*., present study shows the prevalence rate of *Anoplocephala* (3.77%) in winter, (4.93%) in summer and not found in rainy season. Over all prevalence was (3.46%), which is lower than the work done by Ayele *et al*., karki and manandhar. It might be due to the awareness about parasites and disease caused by them, using of effective medicines.

Similary in trematodes, the prevalence of *Dicrocoelium* was reported to be (1%) by Aydenizoz (2006), by Esmael (2007) found *Dicrocoelium* (3.3%). But the present study its prevalence were reported (5.66%), (9.87%), (12.82%) during winter, summer and rainy season respectively. Overall its prevalence was (9.24%) which is more than reported by Aydenizoz and Esmael. Presence of suitable temperature and moisture serve best for the breeding and development of these helminth parasites. So this could be the reason behind excessive prevalence of these helminth parasites.

The present study exhibited 3.77%, 14.81% and 7.69% prevalence rate of *Fasciola* during winter, summer and rainy season respectively. The increase in their prevalence during summer may be due to increase in humidity and availability of favourable temperature. Prevalence of *Fasciola* (1.5%) was reported from udayapur district, In Nepal (karki and manandhar, 2006) , 1.5% from Durga Bora district, Ethopia (Ayele *et al*.,2006), 11.53% from central black sea region (Sinasu and Mustafa,2009).

The present study shows the *Gastrodiscus* prevalence in summer 4.93% and in rainy season 2.56% and not found in winter. Their prevalence in summer and rainy season only might be due to favourable temperature and moisture during summer and rainy season. High prevalence of *Gastrodiscus* (30%) was reported by sapkota (2009), followed by Shrikhande *et al.*, (2009) 8.53%. Then 7.31% infectin reported by Poudel (2007). Karki and manandhar (2006) reported 6% infection, 3.3% infection reported by Esmael (2007).

Esmael (2007) in Mosul city reported the highest prevalence rate of *Strongylus* (70%), *Triodontophorus* (36.6%), *Trichostrongylus* (33.3%), *Parascaris* (20%), *Dictyocaulus* (13.3%), *Strongyloides* (10%), *Oxyuris* (6.6%). The present study shows the prevalence rate of *Strongylus* (15.02%), *Oxyuris* (13.87%), *Trichostrongylus* (13.29%), *Dictyocaulus* (11.56%), *Parascaris* (10.40%), *Strongyloides* (8.67%), *Triodontophorus* (1.15%). Prevalence were varies in parasites due to seasonal variations.

Uslu and Guclu (2007) reported *Trichuris* (0.9%) from Turkey. Present study found 9.43%, 8.64%, 10.25% during winter, summer and rainy season respectively. Over all its prevalence was 9.24% which is more greator than reported by Uslu and Guclu. It might be due to availability of favourable temperature and humidity in Nepal as compared to Turkey.

A reserch work by Pereira *et al.,* (2006) in equines in Brazil, showed highest infection rates of nematodes (100%) followed by Cestodes (85%) and trematodes were absent. But present study showed extremely higher overall prevalence rate of nematodes (78.6%), trematodes (50.86%) and cestodes (16.18%). The difference in the result could be due to the variation in weather condition and humidity in atomosphere.

According to Getochew *et al*., (2010) the most commonly occurring gastrointestinal parasites in donkey were *Strongyle, Fasciola, Parascaris, Gastrodiscus, Strongyloides and Oxyuris.* Similarly present study depicted *Stongylus, Oxyruris, Trichostrongylus, Parascaris, Fasciola, Trichuris and Strongyloides* as the most prevalent helminth parasites of mules.

CHAPTER - VII

CONCLUSION

The current study was carried out in order to observe the seasonal prevalence of intestinal helmiths parasite in mules. Samples were collected from Nepalgunj of district Banke.The two different technique used during the detection of helmiths parasites were sedimentation and floatation technique.The study period was December(08) to December(09).The samples were collected December\ January, May\ June, and August\September. The total number of samples had collected and examine for the study were 100,100 and 50 respectively for these study period. The overall prevalence of helminthes parasites during December\Janurary were (53%), May\June (81%) and August\September (78%). A huge difference in the prevalence of helminth parasites in winter and summer, rainy season, but a very few difference between summer and rainy season were observed.During, December\January (16.18%) of infection caused by Cestodes, (50.86%) by terematodes and (69.81%) by nematodes. Like wise (19.73%), (56.79%) and (83.95%) of infection were caused by Cestodes, Trematodes and Nematodes respectively during May\June.Similarly in rainy season (28.51%), (53.84%) and (79.48%) cestodes, Trematodes and nematodes infection in August\ September.*Teania*, *Moniezia* and *Dipylidium* of Cestodes *and Schistosoma* and *Dicrocoelium* of Trematodes genera were reported for the first time in mules from Nepal. Similarly, nematodes genera *Ancylostoma*,*Capillaria*,*Cooperia*,*Chabertia*, *Strongyloides*, *Trichuris* and *Toxocara* also reported for the first time in mules from Nepal.The prevalence percentage of identified geneara of cestodes were *Anoplocephala* (3.46%), *Dipylidium* (2.31%), *Moniezia* (2.31%) and *Taenia* (*8*.67%). Among Trematodes, the genera are identified with their prevalence percentage were found to be *Dicrocoelium* (9.24%), *Fasciola* (9.82%), *Gastrodiscus* (2.89%) and *Schistosoma* (32.36%). Similarly, the genera included in nematodes are *Ancylostoma* (1.15%), *Capillaria* (9.82%), *Chabertia* (4.04%), *Cooperia* (4.62%), *Dictyocaulus* (11.56%), *Oxyuris* (13.87%), *Parascaris* (10.40%), *Strongylus* (15.02%), *Strongyloides* (8.67%), *Trichostrongylus* (13.29%), *Trichuris* (9.24%), *Triodontophorus* (1.15%), *Toxocara* (5.78%).During winter, summer and rainy season 47.16%, 83.95% and 69.23% samples were found multiple infection respectively.

CHAPTER - VIII

RECOMMENDATIONS

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

- For the prevention of spread of gastro-intestinal parasites, the contamination of pastures should be prevented by treating the hosts with anthelmintics.
- The pastures can be made free of helminth parasites by braking their life cycle by eradication inter mediate host, snail through biological control method.
- Immigration of mules and other animals should be done after fulfilling the quarantine term and conditions.
- The sheds should not be kept wet, moist or humid. These all prove as heaven to helminth parasites for their growth and development.
- Treatment of infected hosts with anthelmintics and diagnosis could be done by taking help of nearby veterinary personnel.
- Awareness among the livestock farmers, and mule dealers regarding the ill effect of infection and zoonotic diseases by helminth parasites should be created.
- Impure water should not be supplied.
- Further research work should be carried out.

Reference

- Alayande, M.O., Onakpa, M.M. and Lawal, M.D. (2003). Prevalence of intestinal helminth of horses in SOKOTO. *The Nigerian Journal of Parasitology*, VOL.24: 191-193.
- Arias, M. and Cortinas, F.J. (2009). Intrinsic factors influencing the infection by helminth parasites in horses under an oceanic climate area NW Spain). *Journal of parasitology research*, VOL. 2009(5), Article ID 616173.
- Ayele, G. and Feseha, G. (2006). Prevalence of Gastrointestinal parasites of donkeys in Durga Bohara District, Ethiopia. (www.lrrd.org/lird 18/10/ayel18136 html).
- Aydenizoz, M. (2006). Prevalence of helmith in horses in Kirikkal, Turkey. *Indian veterinary Journal*.(www.cababstractplus.org/abstract/abstract.aspx,Acno=20043071 242)s
- Bu, Y., Niu, H. and Gasser, R.B. (2009). *Strongyloid* nematodes in the caeca of donkeys in Henan province, china. *Acta Parasitologica,* VOL. 54(3): 263-268.
- CBS Nepal 2007 Number of holiding with livestock and livestock number by livestock type national accounts of Nepal 2000-2007… www.cbs.gov.np/agriculture_monograph_contents.php.
- Hoste, H., Collobert- Laugier, C. and Dorchies, P. (2002). Prevalence, abundance and sit distribution of equine small *strongyles* in Normandy France. *Vet parastiol*, 110(1-2): 77-83.
- Eslami, A. and Bokai, S. (2000). Equines parasites in Iran. *Journal of equines veterinary science,* VOL. 25(4):143-144.
- Esmael, S.A. (2007). Clinical and haematological study of the internal parasites in native donkeys in Mosul city. (www.vetmedmosul.org/ijvs/media/10-2-11a.pdf)

- Fesecha, G., Getachew, M., Trawford, A. and Reid S.W.J. (2010). Gastrointestinal parasites of working donkey in Ethiopia. *Veterinary parasitology,* VOL. 42(6): 27-33.

- Getachew, M., Trawford, A.F. and love S. (2010). Epidemiological features of fascioliasis in working donkey in Ethiopia. *Veterinary parasitiology*, VOL.169:335-339.

- Getachew, M., Feseha, G., Trawford A. and Reid, S.W.J. (2008). A survey of seasonal patterns in *strongyle* faecal worm egg counts of working equides of the central midlands and lowlands, Ethopia. (www.biomedsearch.com/...seasonal-pattrns-in-strongyle/18975128.html-)

- Holland, W.G. and Dorny, P. (2001) *Strongyle* infections in horses from North Vietnam. *Revue Elev. Med. Vet. Pays trop.,* 54(1): 29-31.

- Karki and Manadhar (2006). Preliminary investigation of prevalence of gastrointestinal parasites of mule in Udaypur district.

- Khan, A., Khan, M.S. and mahmood, A.K. (2010). Prevalence & chemotherapy of *Parascaris equorum* in equines in Pakistan. VOL. 30(3):155-158.

- Kuzmina, T.A. and Kuzmin, YU.I. (2008). The species compositon of the *strongylid* community of donkeys and to explore the influence of anthelmintic treatment on the community structure. *Vstnik Zoologii* – 42(2).

- Lyons, E.T. and Tolliver, S.C. (1997).*Vet-32 tapeworm in horses.(*www.uky.edu/ag/animal science/pubs/vet 32.pdf)

- Mahfooz, A. and Masood, M.Z. (2008). Prevalence and anthelmintic efficacy of Abamectin against gastrointestinal parasites in horses. *Pakistan Vet. J.* 28(2): 76-78.

- Mathee, S., Krecek, R.C. and Miline, S.A. (2000). Prevalence and biodiversity of helminth parsites in donkeys from South Africa. *Journal of parasitiology*, 86(4): 756-762.

- Mathee, S., Krecek, R.C., Geoch, M.A. (2004). A comparision of intestinal helminth communites of equaidae in South Africa. *Journal of parasitiology,* 90(6): 1263-1273.

- Poudel, S. (2007). Prevalence of gastrointestinal parasites in horses with special reference to *strongylus* species of Sainik stud farm centre Bhartapur, Chitwan. *Blue-cross Annu. Bull. NVSA.*, VOL. 9:04-105.

- Pandit, B.A., Shahardar, R.A. and Jeyabal (2008). Prevalence of gastrointestinal parasitic infestation in equines of Kashmir valley, VOL. 3(1).

- Pearson, R.A., Nengomasha, E. and Krecek, R. C. (1999). The challenges in using donkeys for work in: Starkey PH, Kamubutho P (editors). Meeting the challenges of animals Traction. A resources book of the animal Traction network for Eastern and Southern Africa (ATNESA), pp. 190-198, Harare, Zimbabwe. Intermediate Technology publications, London.

- Pereria, J.R. and Vianna, S.S.S. (2006). Gastrointestinal parasitic worms in equines in the Paraiba Valley, state of Sao Paulo, Brazil. *Veterinary Parasitolgy,* Volume 140: 289-295.

- Syendsen, E. D. (1997) parasites abroad. The professional hand book of the donkey, 3rd edition. Pp.166-182, Whittet books limited, 18 Anley Road, London W14 OBY, pp.166-182.

- Shrikhande, G.B., Rewatkar, S.G., Deshmukh, S.S. and Raghorte, Y.M. (2009). The incident of helminth parasites in donkey in Nagpur. *Veterinary world*, VOL.2 (6):224.

- Sapkota, R. (2009). Prevalence of helminth parasites in mule sin brick kiln of Latlitpur district. Department of parasitology in Himalayan college of agriculture science and technology, Bhaktapur.

- Sinasi, and Mustafa, (2009). To determine the prevalence of helminth species in horses, donkeys and mules in the Central Black Sea region. *Turk J. Vet Anim. Sci*, VOL. 33: 373-378.

- Uslu and Guclu, F. (2007). Prevalence of endoparsites in horses and donkey in Turkey. *Vet inst pulway,* VOL.5:237-240.

- Veli, Y.Y and Ender, G. L. (2005). The prevalence of *strongyle* infection and persistent efficacy of pyrentel Embonate, Ivermectn and Moxidectin in Turkish horses. *Turk J. Vet Sci*, 29: 175-181.

- Weese, J.S. (2002). A review of equine Zoonotic disease: *Risk in Veterinary medicine AAEP proceeding*/ VOL. 48/2002. (www.ivis.org/proceeding/aaep/2002/910102000362 pdf.
- Yoseph, S., smith, D.G., Mengisty, A. and Betere, Y. (2005). Seasonal variation in the parasite burden and body condition of working donkeys in East Shewa and west shewa region of Ethiopia. *Journal of tropical animal health and production* VOL. 37.