PREVALENCE OF GASTRO-INTESTINAL PARASITES OF HORSES (*Equus caballus* Linnaeus, 1758) IN SEVEN VDCS OF RUKUM DISTRICT, NEPAL

NARESH OLI
T.U. Registration No: 5-2-54-233-2009
T.U. Examination Roll No: Zoo. 28
Batch: 2070/71

A thesis submitted
In partial fulfillments of the requirements for the award of the degree of Master of Science in Zoology with special paper Parasitology

Submitted To
Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur, Kathmandu
Nepal

December, 2016

DECLARATION

I hereby declare that the work presented in this thesis entitled **"Prevalence of Gastro-intestinal Parasites of Horses (*Equus caballus* Linnaeus, 1758) in Seven VDCs of Rukum District, Nepal"** has been done by myself, and has not been submitted elsewhere for the award of any degree. All the sources of the information have been specifically acknowledged by reference to the author(s) or institution(s).

Date: ....

Naresh Oli
Solitudenaresh2047@gmail.com

TRIBHUVAN UNIVERSITY
01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.
Institute of Science and Technology
Ref.No.:

RECOMMENDATION

This is to recommend that the thesis entitled **"Prevalence of Gastro-intestinal Parasites of Horses (*Equus caballus* Linnaeus, 1758) in Seven VDCs of Rukum District, Nepal"** has been carried out by **Mr. Naresh Oli** for the partial fulfillment of Master's 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.

Date: .....

.....

Supervisor
Mr. Janak Raj Subedi
Lecturer
Central Department of Zoology
Tribhuvan University
Kathmandu, Nepal

TRIBHUVAN UNIVERSITY
01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.
Institute of Science and Technology
Ref.No.:

LETTER OF APPROVAL

On the recommendation of supervisor **Lecturer Mr. Janak Raj Subedi**, this thesis submitted by **Mr. Naresh Oli** entitled **"Prevalence of Gastro-intestinal Parasites of Horses (*Equus caballus* Linnaeus, 1758) in Seven VDCs of Rukum District, Nepal"** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

Date: ....

Prof. Dr. Ranjana Gupta
Head of Department
Central Department of Zoology
Tribhuvan University
Kathmandu, Nepal

TRIBHUVAN UNIVERSITY
01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.
Institute of Science and Technology
Ref.No.:

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by **Mr. Naresh Oli** entitled **"Prevalence of Gastro-intestinal Parasites of Horses (*Equus caballus* Linnaeus, 1758) in Seven VDCs of Rukum District, Nepal"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

EVALUATION COMMITTEE

.....

Supervisor
Mr. Janak Raj Subedi
Lecturer
CDZ, TU

.....

Head of Department
Prof. Dr. Ranjana Gupta
CDZ, TU

.....

External Examiner

.....

Internal Examiner

Date of Examination: .....

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Naresh Oli
T.U. Registration No: 5-2-54-233-2009
T.U. Examination Roll No: Zoo.28
Batch: 2070/71

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LIST OF ABBREVIATIONS

Abbreviated form	Details of Abbreviations
H	Hukam
K	Kankri
Ko	Kol
M	Mahat
Mo	Morawang
OCHA	Office for the Coordination of Humanitarian Affairs
Rm	Ranmamaikot
rpm	Revolution per minute
SIONA	Statistical Information of Nepalese Agriculture
T	Taksera

ABSTRACT

Horse has been a loyal friend and trusted partner of human being. Horses are prone to infestation with both internal and external parasites. Present study was conducted in eastern seven VDCs of Rukum district to determine the prevalence of gastro-intestinal parasites in horses. The study was carried out from March 2016 to November 2016. A total of 105 faecal samples of horses (79 males and 26 females) were collected by using opportunistic random method during the month of April 2016. The collected faecal samples were preserved in 2.5% potassium dichromate and microscopically examined using concentration techniques. The overall prevalence of gastro-intestinal parasites was 84.76% (89/105). The total numbers of genera observed during faecal examination were 12 in numbers. Among them, the *Strongylus* sp. showed the highest prevalence (51.42%) followed by *Eimeria* sp. (20%), *Trichostrongylus* sp. (14.28%), *Trichonema* sp. (13.33%), *Parascaris equorum* (10.47%), *Balantidium* sp. (9.52%), *Dictyocaulus* sp. (8.57%), *Triodontophorus* sp. (7.61%), *Gastrodiscus sp.* (6.66%), *Oxyuris equi* (4.76%), *Entamoeba* sp. (3.80%), *Shistosoma* sp. (1.90%) and unidentified nematode larvae (7.61%). Three genera of parasites: *Shistosoma* sp., *Triodontophorus* sp. and *Dictyocaulus* sp. have been reported first time for Nepal in horses. Present study showed higher infection rate in females (92.30%) than in male horses (82.27%). No significant associations were observed between the prevalence of parasite rate with VDC-wise ($\chi 2 = 5.7161$; $p>0.05$) and prevalence rate with sex-wise ($\chi 2 = 0.3346$; $p>0.05$). But a significant association was observed between the infection status and study area ($\chi 2 = 84.277$; $p<0.05$).

1. INTRODUCTION

1.1 Background:

Ruminants possess unique types of digestive system compared to other higher mammals. Instead of one compartment of the stomach they have four compartments. Among four compartments, rumen is the largest part in the rumen partially chewed grass is stored and broken down into balls of cud. Many different species of ruminants are found around the world. Some of them are domesticated by human beings such as buffalo, cattle, goat, sheep, equines and most of them are in wild state like thar, deer, giraffes, ghoral, elephants, monkeys etc.

Horses, donkeys, mules, zebras and camels belong to the equine group. They are found mainly in temperate, semi-arid or highland areas (Alemayehu, 2004; Shiret and Samuel, 2015). Horses and donkeys are herd animals and will happily live in groups with donkeys or animals of a different species such as, sheep and goats. Donkeys and horse are very friendly animals and enjoy the company of humans. They are easily trained and are suitable for handling by children especially donkey. Before the development of firearms, the horse was crucial to warfare and before the invention of the steam engine, it was the fastest and most reliable form of land transport. Today its importance has scarcely diminished in parts of South America, Asia, Africa and Eastern Europe (Fitsum and Ahmed, 2015), and even elsewhere it is of great economic importance to sport and leisure industries.

Equines play an important role as working animals in many parts of the world, employed for packing, riding, carting and ploughing. Equine power is vital for both rural and urban transport system which is cheap and provides the best alternatives in places where the road network is insufficiently developed, the terrain is rugged and mountainous and in the cities where narrow streets mountainous and in the cities where narrow streets (Samuel *et al.*, 2015). Equids play crucial role in both urban and rural areas, providing agricultural energy and transport, and in many cases, the sole means of income generating for their resource limited owners (Getachew *et al*., 2014). Ever since it's domestication (Suteu, 1994; Khan *et al.*, 2015), the horse has been a loyal friend and trusted partner of man in day to day life, playing a vital role in many aspects of human life and evolution. Beauty, boldness and benefits of horse have been mentioned in the Bible and Quran. In the developed world, horses have great economic importance to sport and leisure industries.

The world population of equines (Abayneh *et al.*, 2002; Haimanot *et al.*, 2015) is 122.4 million (43.3 million horses, 40 million donkeys, 15 million mules, 24.1 million zebras and camel). Over 95% of all donkeys and mules and 60% of all horses are found in developing countries, which are playing a vital role in economies of developing countries through provision of 75% of traction energy (USCOTA, 1998; Goraya *et al*., 2013). Equines (horses, donkeys and mules) are raised primarily for draught and game purposes. As draught animals, equines play extremely important role in the daily livelihood of poor people. These animals are used for transportation of people, carriage and agricultural purposes.

Horse is a large land mammal notable for its speed, strength and endurance. Horses are members of the Equidae family, which also includes Zebras and Asses. Like all equids, the horse is extremely well adapted to travelling long distances with great efficiency and to surviving on a diet of nutrient-poor, high-fibre grasses. Horses were domesticated in Eurasia around 6,000 years ago (Biu *et al.,* 2014), they have provided humans with mobility and have served in agriculture, warfare, and sport. Horses were also used for drawing water from deep wells and in every kind of agricultural implement. They were also used for sports such as polo, hunting, racing, traction, teaching demonstration and scientific research, recreation purposes and as well as slaughtered for meat production and consume in some countries. The mounted troop police used them to help in crime control and land security.

Most equines are found in zone of high human population (highland) when agricultural system in which livestock involves and where these animals are used as source of draft powe (Alemayehu, 2004; Shiret and Samuel, 2015). In contrast, only 20% of all equines are known to live on low land areas (Alemayehu, 2004; Shiret and Samuel, 2015). Equine provides of power source for transportation, field operation and post harvest activities.

Nepal has a total of 52,577 horses/asses population that is reported from 51 districts of Nepal. Among these horses/asses population, Dolpa district has the highest numbers and Pyuthan district has the least number of horses/asses populations. In my study area, Rukum district has a total of 1,732 horses/asses population (SIONA, 2013/14). In our country Nepal, horses/asses are mainly kept for agriculture work and transportation work. In Nepal, the low level of development of the road transport network and the rough road of the country make the donkeys and the horses the most valuable, appropriate and affordable pack animals under the small holder farming or house wise rearing system. In my study area, VDCs are completely away from roads in rainy season but some VDCs always away from roads throughout the year. Therefore, many people use horses and mules to transport food and other supplies to villages. Long working hours and difficult conditions are experienced by horses and mules. These animals are often engaged in work for long hours and when get free they are left to browse and feed on grassland. These have the potential to affect negatively on their welfare of life and health. Despite their huge numbers and significant contribution to communities and the national economy, the attention given to the health aspects of working horses (equids) in Nepal is very minimal.

Rudolf Leuckart considered as "father of parasitology". The word 'parasites' derived from Greek which means situated beside. All over the world, horses are exposed to gastrointestinal parasites from many orders and genera resulting in significant morbidity and mortality (Hodgkinson, 2006; Goraya *et al.*, 2013; Tilahun *et al.*, 2014). Parasitism is the single most important impediment in successful horse rearing all over the world (Urquhart *et al*., 1996; Saeed *et al.*, 2010) and many species of parasites are found to infect horses (studies on prevalence of horse helminthes) in different parts of world have indicated varied prevalence under different management and parasite control systems (Chaudhry *et al*., 1991, Montinario *et al*., 2002, Champman *et al*., 2002, Boxell *et al*., 2004; Capewell *et al*., 2005; Saeed *et al*., 2010). Horses are prone to infestation by a mixture of internal and external parasites. An animal can harbor a great number of parasites without exhibiting any clinical signs (Claire and Masterson., 1987; Krecek *et al*.,

1987; Tolliver *et al*., 1987; Gasser *et al*., 2004; Hubert *et al*., 2004; Martin *et al*., 2007; Toscan *et al*., 2012; Khan *et al.*, 2015). Health issues affecting welfare of animals include acute diseases and disorders that cause immediate suffering and long-term progressive conditions leading to chronic pain. Equine parasitism is one of the important menaces affecting their working capacity and may result in mortality, For example, ticks, mites, lice, flies etc. cause irritation, weakness, emaciation, anemia, rough hair coat and disease transmission resulting in poor efficiency, stunted growth and even death of the animals. Mortality in equines (Hodgkinson, 2006; Goraya *et al*., 2013; Tilahun *et al*., 2014) has been frequently reported due to strongyles, tapeworms, ascarids, trypanosomes and *Babesia* sp.

1.2 Endoparasites:

Endoparasites are those organisms living within hosts i.e in the gut, body cavity, livers, lungs, gall bladder, blood or within the internal cavities, tissues or cells of their owns host. They totally depend up on the host for growth, development and other physiological processes and cause infection to them. Horses at times, suffer from various diseases such as parasitic infection. Endoparasites are significant threat to horses. They are susceptible to more than 60 internal parasites and harbor several species of worms at any time (Hodgkingson *et al.*, 2001; Lichtenfels *et al.*, 2008; Bodecek *et al.*, 2010; McWilliam *et al*., 2010; Lamb *et al.*, 2012; Guzel *et al.*, 2014). The common gastrointestinal parasites of equine are *Cryptosporidium parvum*, *Giardia* sp. (Butty, 2010), *Trypanosoma* sp., *Entamoeba* sp., *Balantidium* sp. (Wannas *et al.*, 2012), *Anoplocephala* sp., *Paranoplocephala* sp., *Cyathostomum* sp., *Trichonema* Sp., *Habronema* sp., *Draschia* sp., *Triodontophorus* sp., *Craterostomum* sp., *Oesophagodontus* sp., *Cylicocycus* sp., *Strongyles* species, *Oxyuris* sp., *Strongyloides*, *Trichostrongylus* sp., *Fasciola hepatica*, *Dicrocoelium* sp. etc (Taylor *et al.*, 2007).

Gastrointestinal parasites are one of the greatest limiting factors to successful horse rising throughout the world (Herd, 1986; Shiret and Samuel, 2015). They are worldwide problem for both small and large scale farmer with a greatest impact due to the availability of a wide range of agro-ecological factors suitable for diversified hosts and parasitic species. The majority of nematodes and other notable internal parasites such as cestodes, trematodes and coccidian are the major gastrointestinal parasites of horses (Taylor *et al.*, 2007; Alemayehu, 2004; Butty, 2010; Wannas *et al.*, 2012). The trematoda of important veterinary medicine may be found as adult in the intestine, bile duct, blood vessel or other organ of their final host. Adult cestodes are parasite of the intestine of vertebrate (Alemayehu, 2004; Shiret and Samuel, 2015).

The development and survival of helminthes and protozoans egg of larvae with faeces and on pasture are depending on temperature and moisture thus forming suitable environment for development of larvae of nematode and trematode to infected stage. Inadequate quality of water stored in the dam from which livestock area using directly for drink may also form suitable way for transmission of cestoda and coccidian. Many factors are known to influence the transmission and prevalence of gastrointestinal infection in grazing animals. Broadly the three influencing factors (Alemayehu, 2004; Shiret and Samuel, 2015) that can determine the occurrence of gastrointestinal tract infection could

be mentioned as environmental host interaction, environmental parasitic interaction and host parasitic interaction. Larval stages are responsible for the damage done to the host animal (Claire and Masterson., 1987; Krecek *et al*., 1987; Tolliver *et al*., 1987; Gasser *et al*., 2004; Hubert *et al*., 2004; Martin *et al*., 2007; Toscan *et al*., 2012; Khan *et al.*, 2015).
The effects of gastrointestinal parasites are more evident in young and under nourished horses. Small numbers causes minimal damage, but large number pose a risk for colic and other symptoms. As a rule, older horses appear to develop immunity against the common gastrointestinal parasites and tend not be affected by parasite related problems as commonly as younger horses (Alemayehu, 2004; Shiret and Samuel, 2015).
Strongyles are considered to be the most harmful, affecting horses of all ages and causing weight loss, weakness, anemia, diarrhea and even death. Larval stages are responsible for the damage done to the host animal (Nielsen *et al.,* 2006; Khan *et al.*, 2015). *Strongylu*s vulgaris has long been considered as one of the most common and pathogenic parasites of the horse (Claire and Masterson., 1987; Krecek *et al*., 1987; Tolliver *et al*., 1987; Gasser *et al*., 2004; Hubert *et al*., 2004; Martin *et al*., 2007; Toscan *et al*., 2012; Khan *et al.*, 2015). It is estimated that 45 to 90 percent of horses harbor *S. vulgaris* (McCraw and Slocombe, 1976; Jubb *et al*., 1985; Khan *et al.*, 2015). Ascarid worms have a high prevalence in foals, but can also affect young adult horses (Mitrea, 2011).

1.3 Intestinal protozoan parasites:
Protozoans are single celled organisms that can affect the body at a cellular level, causing problems especially in the circulatory, endocrine and gastrointestinal system (Goraya 2013). Protozoan parasites that commonly found in intestinal tract of equines are *Cryptosporidium* sp., *Isospora* sp. *Giardia* sp. (Butty, 2010), *Eimeria* sp., *Balantidium* sp., and *Entamoeba* sp. (Wannas *et al.*, 2012).

1.4 Intestinal helminth parasites:
Helminth are most important endoparaites mostly causing the infection in intestinal, and also in liver, lungs, lymphatic system, circulatory system, blood tissues, and skin. Helminths are parasitic worms, are large multi-cellular organisms. Helminths are generally endoparasites and parasitic forms except annelid worms (Morariu *et al.*, 2012).

1.5 Trematode parasites:
Trematode species play a vital role in the health of domesticate livestock and for wild animals. In general, trematodes commonly known as a flukes that live in the bile duct and intestine and can affect lungs also. Some of the infective stages of the trematode are ingested and some others penetrate the skins of host for entrance. The eggs are paased out thorough faeces of hosts. The major trematode parasites (Taylor *et al.*, 2007) of equines are: *Fasciola* sp, *Dicrocoelium* sp., *Gastrodiscus* sp. etc.

1.6 Cestode parasites:
These parasites are mainly found in the gut of hosts and hosts get infection by ingestion of contaminated food or water. The major cestode parasites (Taylor *et al.*, 2007) of

equines are *Anoplocephala* sp., *Echinococcus granulosus*, *Paranoplocephala* sp., *Taenia* sp., *Moniezia* sp. etc.

1.7 Nematode parasites:

The most common internal nematode parasites of the equines are strongyles (*Trichostrongyylus* sp., *Trichonema* sp., *Triodontophorus* sp. and *Strongylus* sp.), ascarids (*Parascaris equorum*), pinworms (*Oxyuris equi*), *Dictyocaulus* sp., *Probstmayria vivipara, Micronema deletrix, Draschia magastoma, Habronema* sp., *Onchocera* sp., *Craterostomum* sp., *Cylicocylus* sp., *Cylicodontophorus* sp., *Gyalocephalus* sp., *Cylindropharynx* sp., *Oesophagodontus* sp., *Poteriostomum* sp. and bots (*Gasterophilus* sp.) have the highest prevalence (Taylor *et al.*, 2007).

1.8 Objectives:

1.8.1 General objective:

- To find-out overall prevalence of gastro-intestinal parasites in horses of seven VDCs of Rukum district.

1.8.2 Specific objectives:

- To identify the gastro-intestinal parasites of horse (*Equus caballus* Linnaeus, 1758*)* by morphometry of eggs and larvae.
- To determine the rate of prevalence of gastro-intestinal parasites of horse in seven VDCs of Rukum.
- To compare the VDC-wise, sex-wise and infection-wise distributions of gastro-intestinal parasites of horse.
- To know the husbandry practice of horse and mules (equines).

1.9 Justification of the study:

Parasites and diseases can take a toll on both the individuals as well as herd population of animals. Nepal being a developing country, mostly goods are transport to backward of hilly and mountain regions by horses/asses. Horses are suffering from different parasitic diseases due to which their population had been decreasing in order. There had no study about gastrointestinal parasites of horses in relation to husbandry practice in national context of Nepal which may be one of the factors in declining population of the horses. Horses/asses population had been declining in order in my research studied area. In Rukum district, there had no research or study till now on horses/asses for gastrointestinal parasites of horses in relation to husbandry practice. For continued declining of horses/asses population in my studied area would make no population to Rukum district for the new research in the future time. The number of population had been declined due to different reason that is transportation facilities, poor husbandry practices, lack of manpower for care, mountain shifting, machine-able agro-facilities, nutritional factors and health related factors. Eastern part of Rukum district is major habitat of equines or horses. This study could be new research from Rukum district regarding the topic, which gives the suggestive guideline for the further research, locally horse reared people and also for veterinary practitioner. So it was realized that the research study should be

launched for health related diseases prevalence of gastrointestinal parasites in horses in relation to husbandry practices according to faecal examination in eastern seven VDCs (Mahat, Morawang, Kankri, Kol, Taksera, Hukam and Maikot) of Rukum district, Nepal.

1.10 Limitations of the study:

This study was designed to find-out prevalence of gastrointestinal parasites in horses in relation to husbandry practice with VDCs wise and sex wise. The identification of parasites was based on morphology and size of parasitic eggs and larva. The study doesn't reveal why some parasites were more predominant and others were not. This study was only limited to certain parameters related to the topic due to cost and time constraints. This study had been carried out for the partial fulfillment of the requirements for the Master's Degree in Zoology at Tribhuvan University, Kathmandu, Nepal.

1.11 Hypothesis:

1.11.1 Null hypothesis (H_0):

H_0: There were no significant associations of gastro-intestinal parasites and risk factors.

1.11.2 Research hypothesis (H_1):

H_1: There were significant associations of gastro-intestinal parasites and risk factors.

2. LITERATURE REVIEW

Domestic ruminants may get parasitic infections each other by sharing the food pastures or by grazing in the same habitats. Most of the researches have been carried out regarding to the other domesticated animals however few researches have been done to parasites of horse world widely. But in case of our country, very few studies have been done to parasites of horses. In case of Horses (*Equus caballus*), no more studies have been carried out regarding to the parasitic infection.

A large study looking at the association between poverty and animal disease (Perry *et al.*, 2002; Ali and Yagoob 2015) identified gastrointestinal (GI) parasitism as one of the most important problems for equids in developing countries. Mortality in equines has been frequently reported due to strongyles, tapeworms, ascarids, trypanosomiasis and babesiosis (Hodgkinson, 2006; Goraya, 2013; Tilahun *et al*., 2014). The natural host of the parasite (*Dictyocaulus arnfieldi*) is donkey, and comparably, large numbers of parasites can accumulate in the lungs of this host without clinical signs. Donkey can act as a reservoir for horses (Sharifi *et al*., 2010). Strongylosis has been reported from all parts of world and almost affects more than 90 % of horse population and *S. vulgaris* has long been considered as one of the most common and pathogenic parasites of the horse (Nielsen *et al.*, 2006; Toscan *et al*., 2012). Infestation with strongyles is complex and produces an inflammatory enteropathy which results in impaired intestinal motility and microcirculation (Bechera *et al*., 2010; Neils *et al*., 2011; Pilo *et al.*, 2012). After ingestion, larvae travel through the digestive system to the large intestine, *S. vulgaris* migrate to the anterior mesenteric artery, *S. endentatus* to the liver / flank area and S. equinus migrate to the liver and pancreas (Kuzmina *et al.*, 2012). Higher egg excretion has been recorded in spring and summer and higher EPG were recorded in young horse (≤ 3 year) as compared with older horses, no difference in the prevalence of strongyle infections as influenced by sex and excretion of eggs was also not affected by the sex of the animals (Saeed *et al*., 2010). Season has no impact on the prevalence of strongyle infections but shedding intensity of strongyle eggs is affected by season and significantly higher egg excretion was recorded in spring and summer (Nielsen, 2012). *Parascaris equorum* are highly prolific parasites, producing millions of extremely resistant eggs daily (Mitrea, 2011). Several studies have shown a marked decrease of S. vulgaris infection worldwide (Pilo *et al*., 2012). Determining the number of strongyle eggs per gram of faeces (EPG) has been the most widely used method for diagnosing infection with adult strongyles (Mahmood and Ashraf, 2010). Small strongyles involve 80% of the total parasite population in a horse and the highest incidence of infection in yearlings (nearly 90%) and the lowest (46.6%) in foals has been recorded (Kaplan and Nielsen, 2010; Andersen *et al*., 2013). Strongylidae family affecting more than 80% equids in the world and egg production varies seasonally and it has been demonstrated that least egg production occurs in winter, rising during the spring with maximal production during August / September (Claire and Masterson., 1987; Krecek *et al*., 1987; Tolliver *et al*., 1987; Peter and Waller, 1997; Gasser *et al*., 2004; Hubert *et al*., 2004; Martin *et al*., 2007; Toscan *et al*., 2012; Khan *et al.*, 2015). However, besides being species identification of

eggs is not possible, the identification of larvae is difficult and time-consuming using morphological parameters (Bodecek *et al.*, 2010; Lamb *et al*., 2012; Guzel *et al.*, 2014). It is estimated that 45 to 90 percent of horses harbor *S. vulgaris* (McCraw and Slocombe, 1976; Jubb *et al*., 1985; Khan *et al.*, 2015). Definitive diagnosis of strongylosis can be made by prove the presence of adult or larvae in the gastrointestinal tract during postmortem examinations (Hodgkingson *et al.*, 2001; Lichtenfels *et al.*, 2008; Guzel *et al.*, 2014). Infections with Nematodes of strongyles parasites are complex and more than 60 species have been documented (Hodgkingson *et al.*, 2001; Lichtenfels *et al.*, 2008; Bodecek *et al.*, 2010; McWilliam *et al*., 2010; Lamb *et al.*, 2012; Guzel *et al*., 2014). All over the world, horses are exposed to helminth nonparasites from many orders and genera resulting in significant morbidity and mortality (Hodgkinson, 2006; Tilahun *et al.*, 2014).

2.1 In the global context:

Overall positive prevalence 84.4% had been observed by using Direct and flotation techniques from Sokoto metropolis where *Strongylus* , *Ascaris* sp, *Strongyloide* sp, *Panaplocephala* sp, *Dictyocaulus* sp, and *Gastrodiscus* had been observed in where *Strongylus* prevalence 75.5% had the highest among identified helminth paraites but *Oxyuris* had not been observed (Alayande *et al.*, 2003). Overall 100% positive prevalence had been observed in horses and donkeys from Konya region of Turkey where prevalence of Strongylidae, *Parascaris equorum*, *Strongyloides westeri*, *Fasciola* , *Anoplocephalidae*, *Oxyuris equi*, *Trichuris* , *Dicrocoelium dendriticum*, *Eimeria leucart*i, and *Eimeria* had 100%, 10.81%, 7.2%, 3.6%, 2.7%, 1.8%, 0.9%, 0.9%, 4.5%, and 12.61%, respectively (Uslu and Guclu, 2007). Overall 93.26% positive prevalence had been observed in equines from Kashmir valley of Jammu and Kashmir state and *Strongylus* having the highest prevalence 81.19% followed by *Triodontophorus* (41.39%), *Dictyocaulus* (14.10%), *Oxyuris* (9.40%), *Paranoplocephala* (8.14%), *Strongyloides* (6.19%), *Parascaris* (4.01%), *Amphistome* (0.91%) and *Eimeria* (0.34%) had been reported (Pandit *et al.*, 2008). From slaughtered house of Poland, highest infection intensity had been observed in foals and yearlings of horses followed by 10.6% of *Gasterophilus intestinalis*, 7.9% of *Parascaris equorum* and 0.8% of mixed infection of *Oxyuris equi* and *Anoplocephala perfoliata* (Kornas *et al.*, 2010). The strongyles (small cyathostomes) had been considered as parasites with minimal pathogenic influence on the equine organisms and Cyathostomosis infection (Bodecek *et al.*, 2010) during the late winter and early spring months had been observed highest in young horses ($\leq$ 6 years). 50 species of subfamily Strongylinae and cyathostominae (family Strongylidae) parasites of nematode had been reported in which *Strongylus vulgaris* was the most pathogenic member of the large strongyles (Hassan *et al.*, 2010). In Kurfa chale of Ethiopia, 65.51% of positive infections of helminthes had been observed in horses from 200 faecal samples examination in which the highest prevalence infection of strongyle (58.5%) and diifferent species of helminth like *Parascaris equorum*, *Dictyocalus arnifieldi*, *Anoplocephla* species, *Gastrodiscus aegypticus*, *Strongyloides westeri*, *Trichstrongylus axei*, *Strongylus vulgaris* , *Strongylus edentatus*, *Strongylus equinus* and *Trichonema* sp. parasites had been observed from a year long research (Saeed *et al.*, 2010). *Giardia doudenalis* with 19.63% and *Cryptosporidium* sp. with 27.10% parasitic

infections had been observed (Butty, 2011) in equines from Nineveh, Iraq by using different methods (wet mount, flotation, lugol's iodine modified Ziehl Nelsecn (hot) and Giemsa stain). In Al Diwaniyah Governorate, 100% positive prevalence had been observed in horses and donkeys (Age between 2-6 years) where the prevalence of *Strongylidae* species had a highest (50%) followed by *Parascaris equorum* (40.90%), *Strongyloides westri* (22.72%), *Trichostrongylus axei* (25%), *Oxyuris equi* (11.36%), *Cryptosporidium* sp. (20.45%), *Balantidium coli* (15.90%) and *Eimeria* sp. (6.81%) in horses and the prevalence of *Strongylidae* species had a highest (57.14%) followed by *Parascaris equorum* (32.14%), *Strongyloides westri* (28.57%), *Trichostrongylus axei* (17.85%), *Oxyuris equi* (17.85%), *Dictyocaulus arnfieldi* (17.85%), *Cryptosporidium* sp. (19.64), *Balantidium coli* (17.85%), *Eimeria* sp. (10.71%) and *Entamoeba coli* (3.57%) in donkeys (Wannas *et al.*, 2012). Strongyles (87.97%), *Parascaris equorum* (13.9%), *Strongyloides westeri* (5.06%) and *Eimeria leuckarti* (1.09%), single parasite infection (79.85%), two multiple infection (19.42%) and three multiple infection (0.72%) had been observed (Ionita *et al*., 2013) in horses from from Romanian. 48 faecal samples had been collected from horses in Nigeria and examined by using floatation and sedimentation techniques and the highest prevalence rate of ciliates (81.3%) followed by *Strongylus* sp. (68.8%), *Oxyuris equi* (27.1%), *Strongyloides* sp. (25%), *Dictyocaulus* sp. (10.4%) and *Parascaris equorum* (6.3%) of different species had been observed where the higher infection had in young horses (5-16 years) than old horses (17-20 years) and in male horses than that of female horses (Umar *et al.*, 2013).

Out of 100 samples (53 horses, 41 donkeys and 6 mules) examination, 88.6% of horses, 85.3% of donkeys and 83.3% of mules had been observed from Aydin region of Torkey where the immature *Habronema* species had the highest prevalence (71.6%) followed by *Habronema muscae* (54%), *Trichostrongylus axei* (28.3%) and *Habronema majus* (50.9%) and the infection had not been affected by age factor but affected by sex factor (Aypak and Bergu, 2013). In North Darfun, 24.6% positive prevalence had been observed in equines from 1,400 animal examinations where the mild infection had the highest incidence (69.7%) and 84% followed by moderate infection (15.6%) and (8%), severe infection (14.7%) and (8%) for donkeys and horses respectively and *Strongylus* sp., *Trichostrongylus* sp., *Cyathostomes* sp. and *Strongyloides westeri* were the dominant genera of nematodes (Adam *et al.*, 2013). Out of 385 faecal samples (154 donkeys, 130 horses and 101 mule), overall prevalence (70.04%) followed by species specific prevalence 77.3%, 72.3% and 60.68% for donkeys, mules and horses respectively had been reported from south wolla, Ethiopia where Strongyle species had the highest prevalence 70.9%, 58.5% and 67.3% followed by 10.4%, 5.4% and 3.0% of *Parascaris quorum*, 4.5%, 3.8% and 4.0% of *Oxyuris equi* and 5.2%, 9.2% and 5.9%, of *Fasciola* sp. for donkeys, horses and mules respectively (Regassa and Yimer, 2013). Out of 384 samples of equines, 92.71% overall positive prevalence followed by species specific prevalence 80.95% of horses and 97.13% of donkeys had been observed from Gondar town, Ethiopia where prevalence of strongyle had the highest 66.67% followed by *Parascaris equorum* (43.8%), *Oxyuris equi* (0.95%), *Gastrodiscus aegyptiacus* (2.86%) and *Gastrophilus intestinalis* (0.95%) in horses and there was no statistical significant difference in prevalence of gastrointestinal parasites between age, sex and among body

condition (Mezgebu *et al.*, 2013). In Jabalpur, overall gastrointestinal nematode prevalence (59.25%) in horses where the highest prevalence in unorganized sector (96.45%) than organized sector (32%) and the mixed prevalence (47.50%) followed by Strongyle (25%), *Parascaris equorum* (18.75%). The maximum infection in age group of 1-6 years (69.09%) followed by 6-12 years (62.96%) and 12-18 years (34.78%) and the infection in females (60.97%) higher than in males (58.51%) had been observed (Yadav *et al.*, 2014). Out of 52 faecal samples (32 females and 20 males), 100% found to be positive (from 5 genera of nematode) in horses from Baquba city where the prevalence of *Strongylus* sp. (44.2%) was the highest and *Parascaris* sp. (3.9 %) the lowest followed by highest monthly prevalence (29.9%) in March and lowest monthly prevalence (14.3%) in December and highest infection (61.5%) in females and lowest infection (38.5%) in males (Hasson, 2014). From Formiga western city of Brazil, the prevalence of Cyathostominae was the highest (94.85%) in horses and identified species were *Trichostrongylus axei*, *Oxyuris equi*, *Triodontophorus serratus*, *Strongyloides westeri*, *Strongylus edentatus*, *Habronema muscae*, *Parascaris equorum*, *Probstmayria vivipara*, *Strongylus vulgaris*, *Gasterophilus nasalis*, *Anoplocephala magna* and *Anoplocephala perfoliata* (Weslen *et al.*, 2014). In schimoga region of Karnataka state, 84% of gastrointestinal helminthes from horses had been observed where the *Strongylus* sp. had the highest eggs prevalence (52.38%) followed by *Parascaris equorum (*10.71%), *Gastrodiscus* sp. (7.14%), *Oxyuris equi* (4.76%) and mixed infection (25%) of *strongylus*, *Strongyloides* and *gastrodiscus* of different species (Adeppa *et al.*, 2014).

In Hawassa town, overall prevalence of gastrointestinal parasites of horse and donkey (72.7%) and species specific prevalence of horses (63.7%) and donkeys (78.5%) had been observed by using floatation and McMaster techniques where the highest prevalence of strongyle had (76%) and (64.9%) and *Parascaris equorum* (26.2%) and (4.6%) in donkeys and horses respectively and the infection had been influenced by species and age factors but not by sex and body condition of the equines (Tesfu *et a*l., 2014). In Taiwan, 161 horse samples were positive from faecal examination of a total 436 samples by using acid-fast staining methods for *Cryptosporidium* (Guo *et al.*, 2014). Out of 159 faecal samples examination, 76.1% positive prevalence had been observed where *Strongylus* had the highest prevalence (55.3%) followed by *Oxyuris* (30.2%) and *Strongyloides* (10.7%) and the higher infection (80.8%) in females than male infection (58.8%) with mixed infection (17.6%) had been observed (Wosu and Udobi, 2014). In Kurfa chale of Ethiopia, 307 samples (75.62%) were positive (Sultan *et al.*, 2014) from a total of 406 samples where the species specific prevalence had (14.43%) in horses and (94.82%) in donkey and the strongyle had the highest prevalence (72.66%) followed by *Parascaris equorum* (15.51%), *Gastrophilus* (6.89%), *Anoplocephala* (2.2%) and *Oxiuris equi* (1.47%). Out of 190 faecal samples examination, 97.9% positive (Tilahun *et al.*, 2014) had been observed from Hawasa Town, Southern Ethiopia where the infection with one parasite (14.17%), two parasites (29.5%), three parasites (35.8%) and four parasites (17.9%) had and the *Parascaris equorum* (55.8%) had the highest infection followed by *Strongylus vulgaris* (44.7%), *Strongyle edentates* (36.8%), *Strongyle equines* (16.3%), *Oxyrus equi* (34.2%), *Strongyloides westeri* (28.4%), *Triodonthophorus tencollis* (33.2%) *and Dictyocaulus arnfieldi* (3.7%). In around Mekelle, 59.3% positive prevalence of

gastrointestinal parasites had been observed from horses by using direct, floatation and sedimentation techniques where strongyles sp. (27%), *Oxyuris equi* (8.8%), Anoplocephala sp. (2%), *Parascaris equirem* (1.8%), Gstrophilus (1.3%), *Strongyloid wester*i (0.8%), *Fasciola* sp. (0.5%), Eimeria species (0.5%) and mixed infections (16.8%) had been observed (Shiret and Samuel, 2015). From Kombolcha town, out of 384 samples (253 donkeys and 131 horses), 292 samples were positive after faecal examination by using floatation technique where the species specific prevalence had for donkeys (86.5%) and horses for (55.7%) and the Strongyle species was the highest prevalence (47.4%) followed by *Parascaris equorum (*4.7%), *Oxyuris equi* (2.8%), mixed infection (12.5%) of *Strongyle* and *Parascaris equarum*, (6%) of *Parascaris equi* and *Oxyuris equi* and (5.5%) of *strongyle* and *Oxyuris equi* (Samuel *et al*., 2015). Strongylosis had been reported from all parts of the world and almost affects more than 90% of horse population (Shite *et al.*, 2015).

Out of 390 equines (204 donkeys and 174 horses and 12 mules) from North-eastern Ethiopia, 64.61% (62.3%, 69%, and 41.7% in donkeys, horses and mules) of equines were positive and Strongyle infection was widely distributed (Molla *et al.*, 2015). In Israel, out of 485 samples, 144 samples were positives (Tiroshlevy *et al.*, 2015) and identified species were strongyle eggs (24%), Ascarids eggs (5%) and *Anoplocephala* (0.0042%). Out of 372 samples, 169 (51.7%) samples were positive for eggs of cyathostominae, *Strongylus* sp., *Parascaris equorum*, *Strongyloides westeri* and *Anoplocephala* from Warmia and Mazury (Sokol *et al.*, 2015) and the higher prevalence was in individual farms than in agrotouristic farms. In Danglia town, overall prevalence (5.73%) of strongyle was found to be positive in both species of horses and donkeys species and species specific prevalence (4.92%) and (5.83%) for horse and donkey respectively by using flotation technique (Haimanot *et al.*, 2015). Overall 20.40% positive prevalence of Equines from a total of 1,304 faecal samples examination in Mumbai and Pune region where *Strongyloides westeri* had the highest prevalence 13.19% followed by Strongyles (10.81%), *Parascaris equorum* (0.23%), *Dictyocaulus arnfieldi* (0.23%), Amhistomes (1.38%), *Schistosoma indicum* (0.31%) and *Anoplocephala* (0.07%) respectively had been observed (Matto *et al.*, 2015).

2.2 In context of Nepal:

Parasitic infestation is a major cause of illness but report of parasitic infestation of horses is lacking in our country Nepal.

Out of 33 samples examinations, 100% samples found to be positive (Karki and Manandhar, 2006) in mules from Udaypur district and the Strongyle species was encountered 100% infection followed by *Parascaris equorum* (50%), *Anoplocephala* sp. (7.4%), *Gastrodiscus aegypticus* (6%), *Oxyuris equi* (3%) and *Fasciola* (1.5%). In sainik stud farm of Bharatpur Chitwan, the gastrointestinal parasites of *Trichostrongylus* species having the highest prevalence 80.48% followed by *Parascaris* 21.95%, *Trochonema* 17.07%, *Gastrodiscus* 7.31%, *Habronema* 4.87% and *Strongylus* 48.78% had been observed (Paudel, 2007). Out of 60 faecal samples examination, 45% positive prevalence had been observed from Brick Kiln of Lalitpur district by using concentration technique

and found highest prevalence *Gastrodiscus* (30%) followed by *Strongylus* (22%), *Oxyuris* (30%), *Dictyocalus* (7%) and *Triodontophorus* (11%) had been observed (Sapkota, 2009). In Banke district, the highest infection (81%), moderate infection (78%) and lowest infection (53%) had been observed in May/June, August/September and December/January respectively from mules where has trematode of *Dicrocoelium* (9.24%), *Fasciola* (9.82%), *Gastrodiscus* (2.89%) and *Schistosoma* (32.36%), Cestode parasites of *Anoplocephala* (3.36%), *Diphylidium* (2.37%), *Moniezia* (2.31%) and *Taenia* (8.67%) and nematode parasites of *Ancyclostoma* (1.15%), *Capillaria* (9.82%), *Chabertia* (4.04%), *Cooperia* (4.62%), *Dictyocaulus* (11.56%0, *Oxyuris* (13.37%), *Parascaris* (10.40%), *Strongylus* (15.02%), *Strongyloides* (8.67%), *Trichostrongylus* (13.29%), *Trichuris* (9.24%), *Triodontophorus* (1.15%), *Toxocara* (5.78%) and 83.95%, 69.23% and 47.16% of multiple infections had been observed in summer, rainy and winter season respectively (Rani, 2010). The gastro-intestinal parasites had been researched (Shrestha, 2015) in horses from Illam community forest from where he had taken a total of 5 samples and found *Eimeria* (100%) having the highest followed by *Oxyuris* (80%), *Strongyloide* (80%), Hookworn (20%), *Ascaris* (80%), *Entamoeba* (40%) and *Balantidium* (20%).

3. MATERIALS AND METHODS

3.1 Study area:

Rukum district is a hilly mountain district which is situated in the Mid-Western Development Region of Nepal partially belonging to province number five and partially to province number six. It is extended over $28^{o}29'$N to $29^{o}0'$N latitude and $82^{o}12'$E to $82^{o}53'$E longitude. The altitude of this district ranges between 762 m to 6,072 m. It covers an area of 2,877 km^2 with population of 207,290. The temperature ranges between 0^{o}c to 34.4^{o}c and annual rainfall ranges between 1,600 mm to 2,400 mm. Rukum district is located at 571.86 km far distance from capital city Kathmandu (Source: https://en.m.wikipedia.org/wiki/Rukum-District, 2016).

Present study area is located in province number five that is eastern part of Rukum district. The present study area (Mahat, Morawang, Kankri, Kol, Taksera, Hukam and Ranmamaikot) is situated in the eastern part of Rukum district, which is located at 58 km to 125 km far distance from headquarter (Musikot Khalanga) of Rukum. The altitude of study area varies from 1,000 m to 4,000 m from sea level. Altitudinal range of Morawang, Mahat and Kankri VDCs ranges from 1000 m to 3000 m with subtropical to temperate climate but altitudinal range of Kol, Taksera, Hukam and Ranmamaikot VDCs ranges from 2,500 m to 4,000 m with temperate to subalpine climate. Majority of people live in these VDCs are Magar, Chhettri, Gurung and Dalit (Source: https://en.m.wikipedia.org/wiki/Rukum-District, 2016).

The study areas selected have contrasting features relating to geographic location, climatic condition, veterinary clinic, husbandry practice, fodder feeding and grazing land availability. Climatic condition of study area varies from subtropical climate to subalpine climate. Among all seven VDCs, veterinary clinic is only situated in Morawang VDC but all other six VDCs have no any clinic for veterinarian purpose. Most of people are not awareness towards veterinary treatments because veterinary clinics are available in very far away from local people of VDCs and available of only limited veterinary medicines and facilities. There is no horse farming supported by any other institution but only farmers own their private horses reared with very simple rearing management like cow and buffalo. Most of horses used for transportation purpose and feed on grains, agricultural byproducts, grass fodder and feed on open grass field during at evening and when they are in rest period. There has been observed no any veterinary related health program performed by both government sector as well as private sector in the study area till from many years during the study period. Horses get parasitic infection when they are grazing in contaminated open grass field and nearby rivers. The district has different domesticated animal's diseases like haemorrhagic septicemia, anthrax, pneumonia and worm's diseases according to office of Rukum district for animal's service (Source: https://en.m.wikipedia.org/wiki/Rukum-District, 2016).

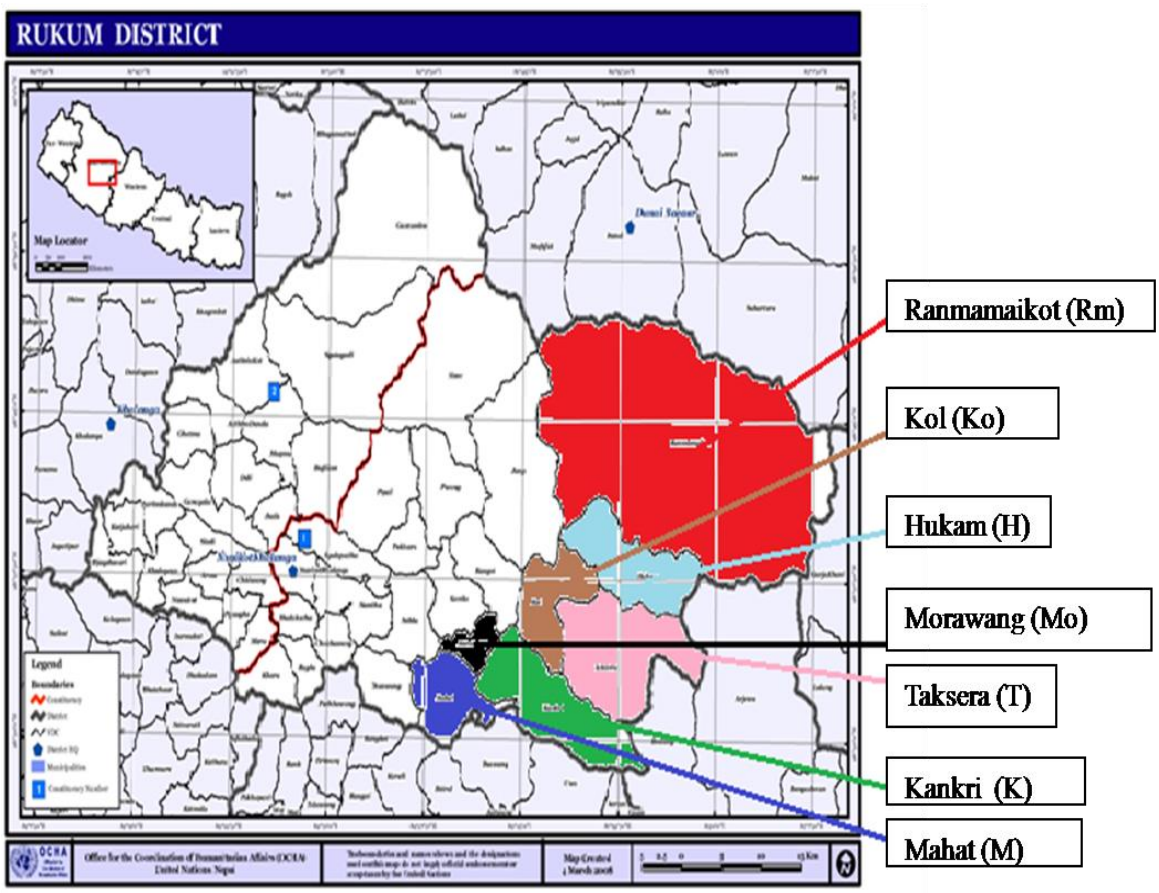

Figure 1: Map of Rukum district showing the study area (Source: OCHA 2008)

3.2 Materials used:

3.2.1 Materials for field:

I. Plastic vials
II. Medicate handplast

3.2.2 Materials for laboratory:

I. Electric microscope
II. Occular micrometer
III. Stage micrometer
IV. Volumetric flask
V. Centrifuge machine
VI. Centrifuge tubes
VII. Gloves
VIII. Beakers
IX. Cover slips
X. Slides
XI. Cotton
XII. Tea strainer
XIII. Glass rod
XIV. Cavity slide
XV. Watch glass
XVI. Rack
XVII. Dropper
XVIII. Tooth picks

3.2.3 Chemicals:

I. Potassium dichromate (2.5%)
II. Iodine solution
III. Nacl solution
IV. Methylene blue
V. Distilled water

3.3 Research design:

The present study was designed to assess the gastrointestinal parasitic infection in horse and mules in seven VDCs of Rukum district. The research comprises:

a) Selection of more horse habitat VDCs by direct observation.

b) Collection of 10 gram fresh faecal samples in sterile plastic vials (25 ml) in preservative by opportunistic random sampling.

c) Preservation of faecal samples in 2.5% of Potassium dichromate solution.

d) Examination of faecal samples by using concentration techniques that is flotation and sedimentation techniques.

e) Identification and measurement of eggs, cysts and larva of parasites.

f) Questionnaire survey (interview) for equines owners to know husbandry practice.

3.3.1 Study period:

The study was carried out from March 2016 to November 2016.

3.3.2 Sample and data collection methods:

The eastern parts of seven VDCs (Mahat, Morawang, Kankri, Kol, Taksera, Hukam and Ranmamaikot) of Rukum district were considered as the major habitats of horses/mules.

During the collection of faecal samples in seven VDCs, three day was given to each of them. Faecal samples were collected with two relatives and supported by other people in studied area. Fresh faecal samples were taken from behind just below of individual horse at early of the morning during time period between 4 am to 6 am. About 10 gram faecal sample from each horse was taken with help of disposable gloves and transferred inside the clean plastic vial having 25 ml. The same collection process was repeated for all collected faecal samples. Necessary informations were noted clearly, such as faecal samples collection date, collection VDC and sex of horse.

Both primary and secondary data sources were used in the study. Primary data was collected by using semi-structured questionnaire and direct field observation. Secondary data was collected from district. A formal type of questionnaire was used to collect data. The questionnaire was prepared and pretested before the actual beginning of the survey. A total of 48 respondent equines owners in the study area (VDCs) selected were interviewed. Data collected using questionnaire includes the major feed resources, management practice and healthcare. Field observation was made to enrich the data for husbandry practice.

3.3.3 Preservation of faecal samples:

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

3.3.4 Sample size:

A total of 105 faecal samples of horses (79 from males and 26 from females) were collected from seven VDCs (Mahat, Morawang, Kankri, Kol, Taksera, Hukam and Ranmamaikot) of Rukum district during the month of April 2016. The whole population

of seven VDCs was about 185. The sample size 105 which was occupied more than 56.75% as of whole population of seven VDCs. Among them, four VDCs (Mahat, Morawang, Kankri and Kol) were comprised 100% of total population, two VDCs (Taksera and Hukam) were comprised more than 90% of total population and one VDC (Ranmamaikot) was comprised more than 25% of total population (Table 1).

Table 1: Proportion of faecal samples of horses collected from seven VDCs of Rukum

VDC name	Collected sample number	Sample proportion (%)	Estimated population of horses in VDC
Morawang	9	8.57	9
Mahat	17	16.19	17
Kankri	5	4.76	5
Taksera	21	20	23
Kol	10	9.52	10
Hukam	15	14.28	16
Ranmamaikot	28	26.66	105

3.4 Laboratory examination:

The faecal samples were collected in plastic vials (25 ml), transported by bus from Rukum to Kathmandu and then brought to laboratory of Central Department of Zoology, Kirtipur, Kathmandu. The faecal samples were subjected to coprological examination by concentration technique (flotation and sedimentation).

3.4.1 Concentration techniques:

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

3.4.1.1 Differentiation Flotation Technique:

Nematode and cestode eggs present in horses faeces were detected through this technique. This technique ensures the eggs float in the floatation liquid, which helps to identify the eggs.

Approximately 3 gram of faecal sample was placed in a beaker and added 42 ml of water then the samples was grinded lightly with the help of rod and filter the solution by tea strainer. The filtrate solution was poured in to a centrifuge tube of 15 ml and centrifuged at 1,000 rpm for 5 minutes. The tube's water was replaced with more saturated Nacl solution and again centrifuged.

After centrifuged, super saturated Nacl solution was added to develop convex meniscus at the top of the tube and one drop of Methylene Blue (to stained) was added where a cover-slip can placed for a 5 minutes and then cover-slip was removed from tube and placed on slide and examined at 10Xx10X and 10Xx40X objectives. The photographs of eggs, cysts and larva of parasites were taken and identified based on colour, shape and size (morphometry).

3.4.1.2 Sedimentation Technique:

This technique is used for detection of trematode eggs. It provides a better result as the eggs of trematode are bit heavier than the other, sediments of centrifuged contents was taken for eggs detection.

Saturated Nacl solution was removed gently from the centrifuge tube after examined the flotation portion and pour the sediment content into the watch glass and stirred the content gently to mix it. One drop from the mixture was taken to prepare a second slide. The specimen was stained with Iodine wet mount's solution and examined at 10Xx10X and 10Xx40X objectives.

In this way, two slides were prepared from one sample (one from flotation and one from sedimentation) were examined at 10Xx10X and 10Xx40X objectives of microscope to detect eggs of protozoan, helminthes, protozoan's trophozoites or cysts of gastro-intestinal parasites.

3.5 Eggs, cysts and larva size measurement:

By using ocular and stage micrometer, the length and breadth (eggs, cysts and larvae) of parasites measured with calibration.

3.6 Eggs, cysts and larva size identification:

On the basis of shape and size of published literature journals and books (Gardiner *et al.*, 1988; Bevilaqua *et al.*, 1993; Lichtenfels *et al.*, 2002; Taylor *et al.*, 2007; Wannas *et al.*, 2012; Matto *et al.*, 2015; Ahmed *et al.*, 2011; Rahman *et al.*, 2014; Soulsby, 1982; Urquhart *et al.*, 1996; Hendrix and Robinson, 2006).

3.7 Data analysis:

For this study, prevalence was measured as the percentage of host individuals infected with a particular parasite (Margolis *et al.*, 1982; Bush *et al.*, 1997). The collected data were coded and entered into Microsoft Excel spread sheet. Data were statistically analyzed using Pearson's Chi-square test with Yates' continuity correction, performed by "R", version 3.3.1 software packages. Percentage was used to calculate prevalence. Data were statistically analyzed using Chi-square. In all cases 95% confidence interval (CI) and $p<0.05$ was considered for statistically significant difference.

4. RESULTS

4.1 Overall prevalence of gastro-intestinal parasites in horses:

Out of 105 samples, 89 (84.76%) samples were found to be positive (Figure 2) for parasitic egg, cyst and larvae.

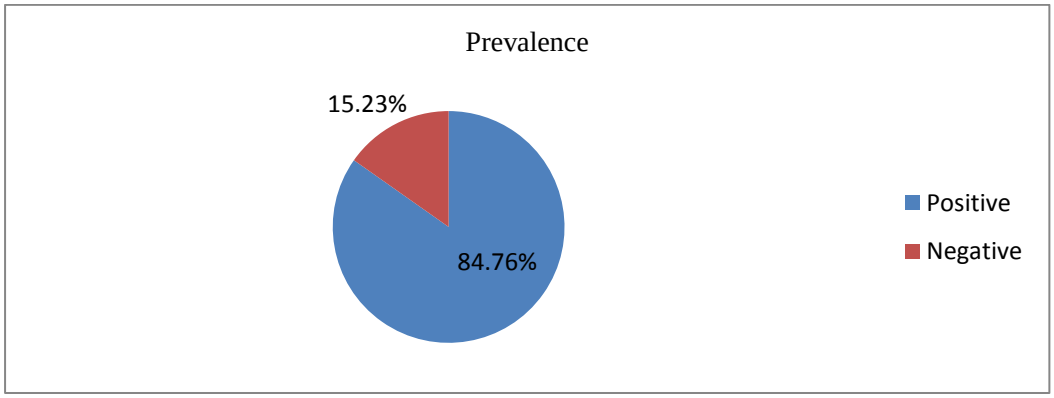

Figure 2: Overall prevalence

4.2. Prevalence of GI parasites in horses:

4.2.1 Protozoan parasites:

Overall, horses were found to be infected with protozoan parasites belonging to the three classes. Among them, *Eimeria* sp. showed the highest prevalence (20%) and *Entamoeba* sp. showed the lowest prevalence (3.80%) in Table 2.

Table 2: Overall protozoan parasites in horses

S.N.	Class	Parasite Name	Prevalence (%)
1.	Sporozoa	*Eimeria* sp.	21 (20%)
2.	Litostomatea	*Balantidium* sp.	10 (9.52%)
3.	Sarcodina	*Entamoeba* sp.	4 (3.80%)

4.2.2 Helminth parasites:

Table 3: Overall helminth parasites in horses

S.N.	Class	Parasite Name	Prevalence (%)
1.	Nematoda	*Strongylus* sp.	54 (51.42%)
2.		*Trichostrongylus* sp.	15 (14.28%)
3.		*Trichonema* sp.	14 (13.33%)
4.		*Parascaris equorum*	11 (10.47%)
5.		*Dictyocaulus* sp.	9 (8.57%)
6.		*Triodontophorus* sp.	8 (7.61%)
7.		*Oxyuris equi*	5 (4.76%)
8.		Unidentified nematode larva	8 (7.61%)
9.	Trematoda	*Gastrodiscus* sp.	7 (6.66%)
10.		*Schistosoma* sp.	2 (1.90%)

Overall, horses were found to be infected with helminth parasites belonging to two classes. Cestode parasites were not found during faecal examination (Figure 2). Trematode parasite revealed to be least infected to horses with two genera *Gastrodiscus* sp. (6.66%) and *Schistosoma* sp. (1.90%) but, horses were found to be highly infected with nematode parasites belonging to seven different genera (Table 3).

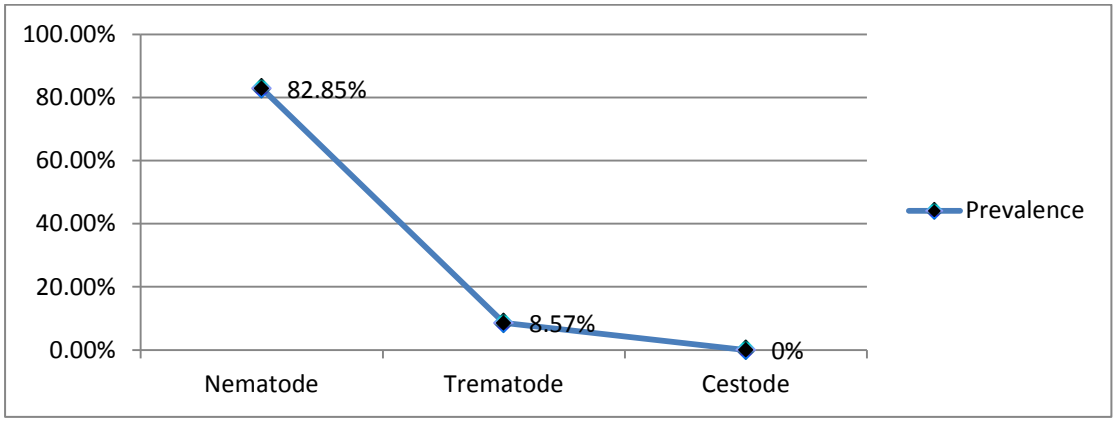

Figure 3: Overall class-wise helminth parasites in horses

Among nematode parasites, *Strongylus* sp. showed the highest prevalence (51.42%) followed by *Trichostrongylus* sp. (14.28%), *Trichonema* sp. (13.33%), *Triodontophorus* sp. (7.61%), *Parascaris equorum* (10.47%), *Oxyuris equi* (4.76%), *Dictyocaulus* sp. (8.57%) and nematode typed larva (7.61%) (Table 3).

4.2.3 VDC-wise prevalence of GI parasites in horses:

The highest prevalence (100%) was revealed in Kankri VDC followed by Ranmamaikot (96.42%), Kol (90%), Taksera (85.71%), Hukam (80%), Morawang (77.77%) and Mahat (64.70%) respectively (Table 4).

Table 4: VDC-wise prevalence of GI parasites in horses

VDC name	No. of examined	No. of positive	Prevalence (%)	$\chi 2$	P-value
Kankri	5	5	100%	5.7161	0.5733
Ranmamaikot	28	27	96.42%		
Kol	10	9	90%		
Taksera	21	18	85.71%		
Hukam	15	12	80%		
Morawang	9	7	77.77%		
Mahat	17	11	64.70%		

The study showed effects of VDCs (study areas) on the prevalence of gastro-intestinal parasite but, there was no statistical significant difference of the prevalence of intestinal parasite infection among VDCs ($\chi 2 = 5.7161$; $p>0.05$) (Table 4).

4.2.4 VDC-wise comparative prevalence of parasite species:

VDC wise, the highest prevalence of class sarcodina revealed in Kol (10%), sporozoa in Ranmamaikot (32.14%) and litostomatea in Kankri VDC (20%) but all classes of protozoan parasites were not found from Morawang VDC (Table 5). The highest prevalence of *Entamoeba* sp. revealed in Kol (10%), *Eimeria* sp. in Ranmamaikot (32.14%) and *Balantidium* sp. in Kankri VDC (20%) but protozoan parasites were not found from Morawang VDC. The highest infection of trematode of *Gastrodiscus* sp. revealed in Hukam (13.33%) and *Schistosoma* sp. in Ranmamaikot (7.14%) (Table 5).

Table 5: VDC-wise comparative prevalence of parasite species

Class	Parasite Name	K (n=5)	Rm (n=28)	Ko (n=10)	T (n=21)	H (n=15)	M (n=9)	M (n=17)
Sporozoa	*Eimeria* sp.	1 (20%)	9 (32.14%)	1 (10%)	2 (9.52%)	4 (26.66%)	-	4 (23.52%)
Litostomatea	*Balantidium* sp.	1 (20%)	4 (14.28%)	1 (10%)	2 (9.52%)	1 (6.66%)	-	1 (5.88%)
Sarcodina	*Entamoeba* sp.	-	1 (3.57%)	1 (10%)	1 (4.76%)	-	-	1 (5.88%)
Nematoda	*Strongylus* sp.	3 (60%)	19 (67.85%)	5 (50%)	11 (52.38%)	4 (26.66%)	6 (66.66%)	6 (35.29%)
	Trichostrongylus sp.	-	3 (10.71%)	3 (30%)	2 (9.52%)	2 (13.33%)	3 (33.33%)	2 (11.76%)
	Trichonema sp.	1 (20%)	3 (10.71%)	1 (10%)	2 (9.52%)	4 (26.66%)	1 (11.11%)	2 (11.76%)
	Parascaris equorum	-	3 (10.71%)	1 (10%)	3 (14.28%)	3 (13.33%)	-	1 (5.88%)
	Dictyocaulus sp.	-	4 (14.28%)	-	1 (4.76%)	2 (13.33%)	-	2 (11.76%)
	Triodontophorus sp.	1 (20%)	2 (7.14%)	1 (10%)	1 (4.76%)	2 (13.33%)	-	1 (5.88%)
	Oxyuris equi	-	2 (7.14%)	-	1 (4.76%)	2 (13.33%)	-	-
	Unidentified nematode larva	1 (20%)	2 (7.14%)	-	2 (9.52%)	1 (6.66%)	-	2 (11.76%)
Trematoda	*Gastrodiscus* sp.	-	3 (10.71%)	-	2 (9.52%)	2 (13.33%)	-	-
	Schistosoma sp.	-	2 (7.14%)	-	-	-	-	-

VDC-wise, the highest prevalence of class nematode revealed in Kankri VDC (100%) followed by Ranmamaikot (92.85%), Kol (90%), Taksera (80.95%), Hukam (80%), Morawang (77.77%) and Mahat (64.70%) (Table 5). The highest prevalence of *Strongylus* sp. (67.85%) revealed in Ranmamaikot followed by lowest in Hukam VDC (26.66%) and highest prevalence of *Trichonema* sp. (26.66%) in Hukam followed by lowest in Kol VDC (10%). Similarly, the highest prevalence of *Trichostrongylus* sp. revealed in Morawang VDC (33.33%) followed by equal prevalence rate of *Triodontophorus* sp. and nematode typed larva in Kankri (20%), *Parascaris equorum* in Taksera (14.28%), *Oxyuris equi* in Hukam (13.33%), *Dictyocaulus* sp. in Ranmamaikot (14.28%) and Nematode typed larva in Kankri VDC (20%) respectively (Table 5).

4.3 Overall sex-wise prevalence of GI parasites in horses:

Out of 105 horses samples collected, 79 were males and 26 were females. Sex-wise 65 males (82.27%) and 24 females (92.30%) were found to be positive for one or more parasites. The prevalence of intestinal parasitic infections in males and females is summarized in figure 3.

The study showed effect of sex on the prevalence of gastro-intestinal parasite infection. While absolute figure indicates a higher prevalence in females horses (92.30%), there was no statistical significant difference of the prevalence of intestinal parasite infection between males and females ($\chi 2 = 0.3346$; $p>0.05$) (Figure 4).

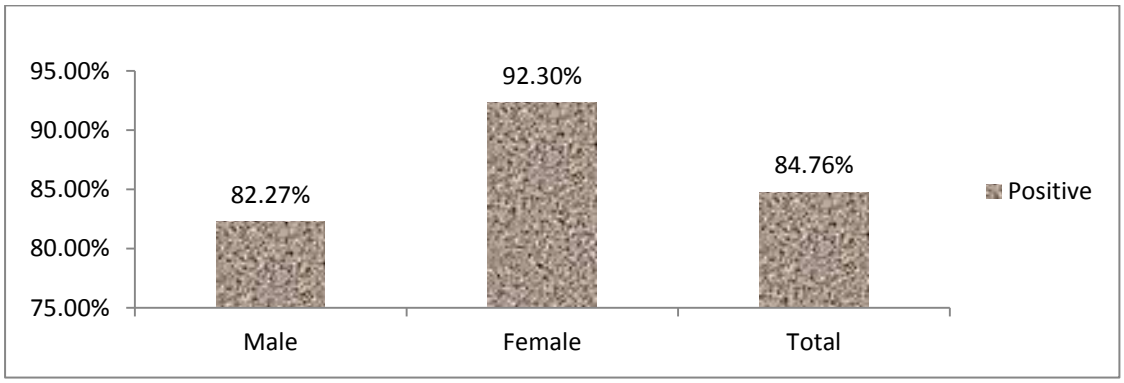

Figure 4: Overall sex-wise prevalence of GI parasites in horse

4.3.1 Sex-wise prevalence of GI parasites in VDCs:

Table 6: Sex-wise prevalence of GI parasites in horses in VDCs

VDC name	Sex	No. of examined	No. of positives	Prevalence (%)
Morawang	Male	8	6	75%
	Female	1	1	100%
	Total	9	7	77.77%
Mahat	Male	15	9	60%
	Female	2	2	100%
	Total	17	11	64.70%
Kankri	Male	4	4	100%
	Female	1	1	100%
	Total	5	5	100%
Taksera	Male	15	12	80%
	Female	6	6	100%
	Total	21	12	85.71%
Kol	Male	8	7	87.5%
	Female	2	2	100%
	Total	10	9	90%
Hukam	Male	11	10	90.90%
	Female	4	2	50%
	Total	15	12	80%
Ranmamaikot	Male	18	17	94.44%
	Female	10	10	100%
	Total	28	27	96.42%

Male sex-wise, the highest prevalence (100%) revealed in Kankri VDC and least prevalence (60%) in Mahat VDC and female sex-wise, the highest prevalence (100%) was revealed in six VDCs (MoMKTKoRm) and least prevalence (50%) revealed in Hukam VDC (Table 6).

4.4. Overall infection-wise prevalence of GI parasites in horses:

Overall, horses were found to be infected with single, double, triple and multiple gastro-intestinal parasites infections (Figure 4). The highest prevalence rate (42.85%) was noted for double infection followed by single (27.61%), triple (10.47%) and multiple (3.80%).
The study showed effects of study area on infections status of parasites and there was statistical significant difference among infection status in study area ($\chi 2$ = 84.277; $p<0.05$) (Figure 5).

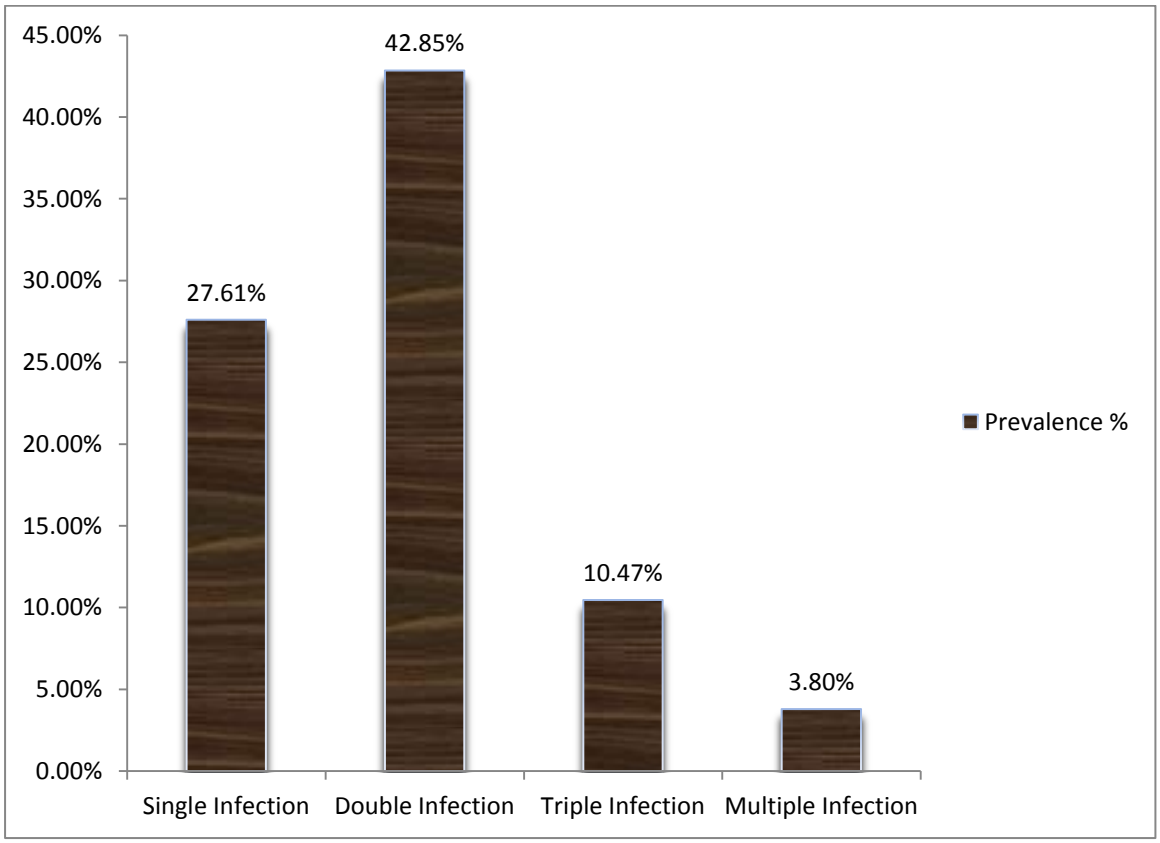

Figure 5: Overall infection status of GI parasites in horses

4.4.1 VDC infection-wise prevalence of GI parasites in horses:

Horses were found to be infected with single, double, triple and multiple GI parasites (Table 7). The highest prevalence of single infection revealed in Kankri VDC (60%) followed by no prevalence in Hukam VDC, highest double infection in Hukam VDC (60%) followed by lowest in Kankri VDC (20%), highest triple infection in Ranmamaikot VDC (21.42%) followed by no prevalence in Morawang VDC and highest multiple infection in Ranmamaikot VDC (7.14%) followed by no prevalence from four VDCs (MoMKKo).

Table 7: VDC-wise infection status of GI parasites in horses

VDC name	infection	No. of examined	No. of positives	Prevalence (%)
Morawang	Single	9	4	44.44%
	Double	9	3	33.33%
	Total	9	7	77.77%
Mahat	Single	17	1	5.88%
	Double	17	9	52.94%
	Triple	17	1	5.88%
	Total	17	11	64.70%
Kankri	Single	5	3	60%
	Double	5	1	20%
	Triple	5	1	20%
	Total	5	5	100%
Kol	Single	10	5	50%
	Double	10	3	30%
	Triple	10	1	10%
	Total	10	9	90%
Taksera	Single	21	9	42.85%
	Double	21	7	33.33%
	Triple	21	1	4.76%
	Multiple	21	1	4.76%
	Total	21	18	85.71%
Hukam	Double	15	10	66.66%
	Triple	15	1	6.66%
	Multiple	15	1	6.66%
	Total	15	12	80%
Ranmamaikot	Single	28	7	25%
	Double	28	12	42.85%
	Triple	28	6	21.42%
	Multiple	28	2	7.14%
	Total	28	27	96.42%

4.5 Identified eggs/cysts/larvae of parasites found in horses:

The range values of eggs, cysts and larvae were to be calculated. Reference values were given based on published books as in Table 8 and Table 9.

Table 8: Identified eggs and cysts of parasites found in horses

Name of parasites	Range of diameter of eggs and cysts (in µm)		Morphology characters	Reference values (Urquhart *et al.*, 1996; PV, 2012; Taylor *et al.*, 2007)
	length	width		
Entamoeba sp.	25-30	-	Cysts were small, rounded or spherical with four nucleus.	20-35 µm
Eimeria sp.	20-45	-	Eggs were elongated oval shaped, having prominent cyst wall with micropyle and protoplasmic mass was spherical in centrally or sub-centrally.	15-55 µm
Balantidium sp.	25-55	10-25	Cysts were spherical, faintly yellowish green in coloured and macronucleus but trophozoite were kidney or bean shaped.	10-55 µm×10-35 µm
Gastrodiscus sp.	55-65	35-40	Operculum was in one pole, pale grey or greenish in coloured, contained five blastomeres surrounded by about 50 yolk cells.	50-70 µm×30-45 µm
Schistosoma sp.	140-150	45-50	Lateral spine arised from the side of the egg.	110-150 µm× 40-55 µm
Strongylus sp.	80-90	45-50	Ovoid similar, similar or nearly similar pole, strongly barrel-shaped side-walls, small axis was longer than half the large axis and thin shelled with smooth surface.	75-92 µm×41-54 µm
Trichonema sp.	105-115	44-52	Ovoid the small axis was shorter than half the large axis nearly similar poles parallel side-walls.	100-120 µm×40-55 µm
Triodontophorus sp.	135-140	55-60	The small axis was shorter than half the large axis similar, nearly similar poles barrel-shaped side-walls smooth wall.	130-140 µm× 55-65 µm
Parascaris equorum	90-115	45-55	Nearly spherical or elongated, golden yellowish brown, granular contained, un-segmented and thick albuminous shelled.	90-120 µm×40-60 µm
Oxyuris equi	85-90	42-45	Slightly asymmetrical, dissimilar side walls, one was somewhat flattened, Polar plug was visible at one end and thick shelled with smooth surface.	80-95 µm×40-45 µm
Dictyocaulus sp.	80-90	52-55	Symmetrical side walls, thin shell.	80-100 µm×50-60 µm
Trichostrongylus sp.	90-100	35-40	Irregular ellipse dissimilar, kidney-shaped not very wide poles, one of which was more rounded than the other, dissimilar side-walls.	70-108 µm×30-40 µm

Table 9: Unidentified larvae of parasites found in horses

Name of parasites	Range of diameter of larvae found (in µm)		Morphology characters	Reference values (Taylor *et al.*, 2007)
	length	width		
Rhabdiasoidae larvae	350-500	15-20	Cuticle and smooth body, mouth beared lips and were short tail than others larvae.	300-600 µm×15-25 µm
Strongyloidae larvae	530-700	15-18	Mouth was without lips with leaf crowns. Buccal capsule well developed.	300-800 µm×15-20 µm
Dictyocaulidae larvae	200-400	10-15	Tail was a punctiform and transparent projection, no intestinal cells and grandular contents for L_2-larvae.	200-400 µm×10-15 µm

4.6 Husbandry practices in the study area based on questionnaires survey:

Out of 48 respondents interviewed, 47.91% were found to be the highest proportion that can read and write followed by primary (27.08%), above primary (20.83%) and illiterate (4.16%) respectively (Figure 6). 72.91% of respondents were found to be freshwater users, 22.91% river water users and 4.16% tap or pond water users for equines respectively (Figure 7). 62.5% of respondents were found to be used equines for transport income and 37.5% used for fertilizer as in soil to increase the soil fertility (Figure 8). Mixed feeding source was found to be highest proportion (56.25%) followed by natural (20.83%), shrubs and forest (12.5%) and grain residue (10.41%) respectively (Figure 9). Out of 48 respondents, 50% were found to be kept equines together with family, 31.25% with simple shed and 18.75% with separate house respectively (Figure 10). In the study area, 95.83% respondents were found to be responded that there was no veterinary clinic and only 4.16% respondents responded with available of veterinary clinic (Figure 11). 66.66% respondents were found to treat equines at home, 29.16% after serious infection and 4.16% in VDC veterinary clinic (Figure 12). The highest cleaning rate (52.08%) was noted for 4-7 days followed by 1-3 days (37.5%) and 8-15 days (10.41%) respectively (Figure 13). From the questionnaire, it was known that most of equines owner feed their equines twice a day (70.83%) followed by thrice (20.83) and four times a day (8.33%) during working days (Figure 14). Out of 48 respondents, 52.08% feed their equines thrice a day, 27.08% feed four times a day and 20.83% feed twice a day during resting period (Figure 15).

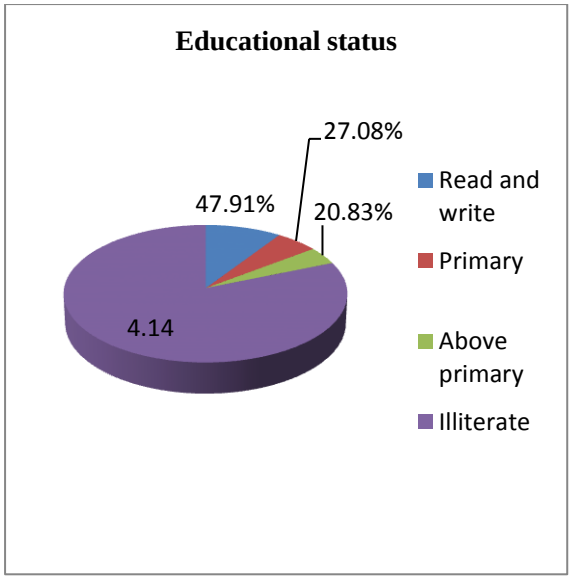

Figure 6: Educational status of respondents

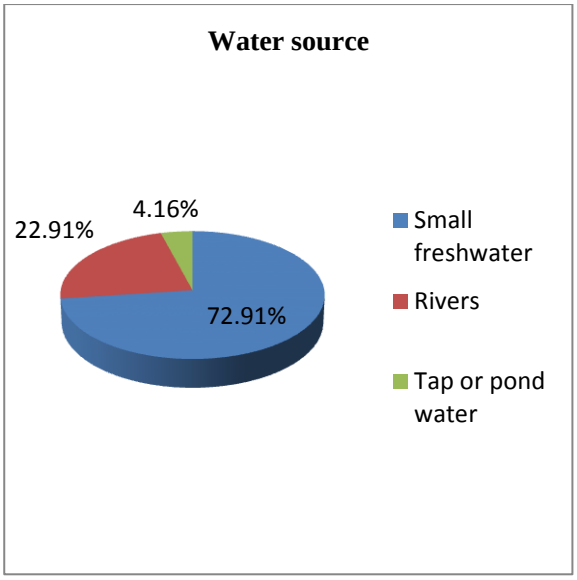

Figure 7: Feed of water sources for equines

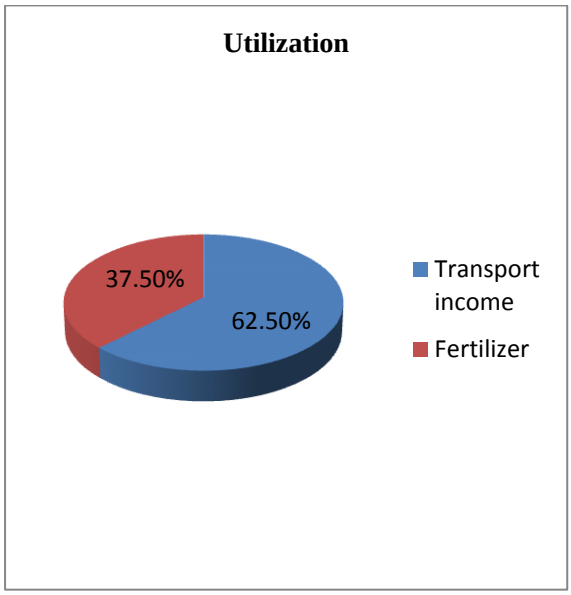

Figure 8: Utilization or purpose of equines

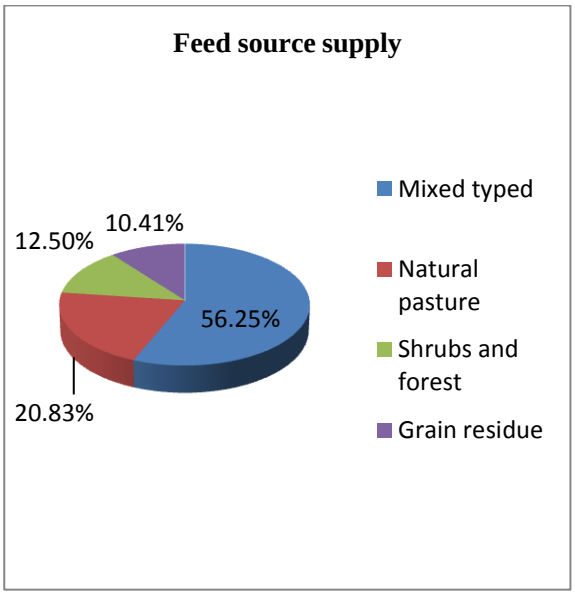

Figure 9: Major feed sources for equines

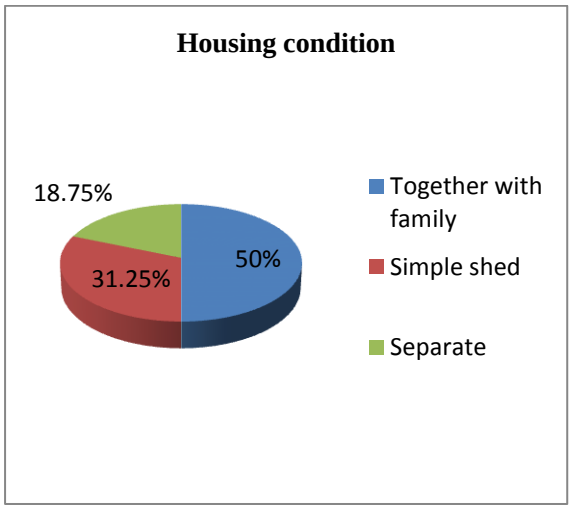

Figure 10: Housing conditions of equines

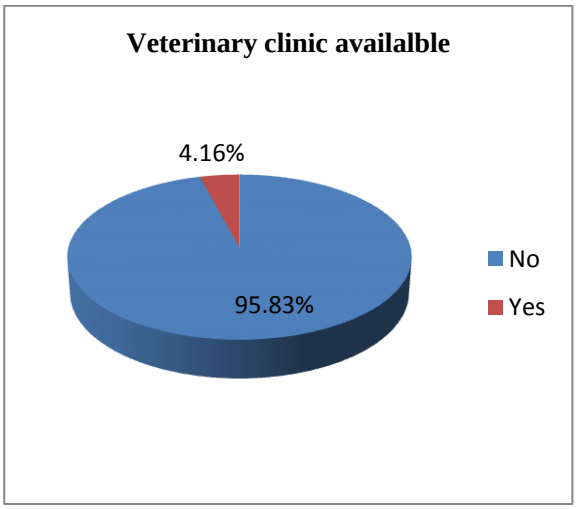

Figure 11: Veterinary clinic available

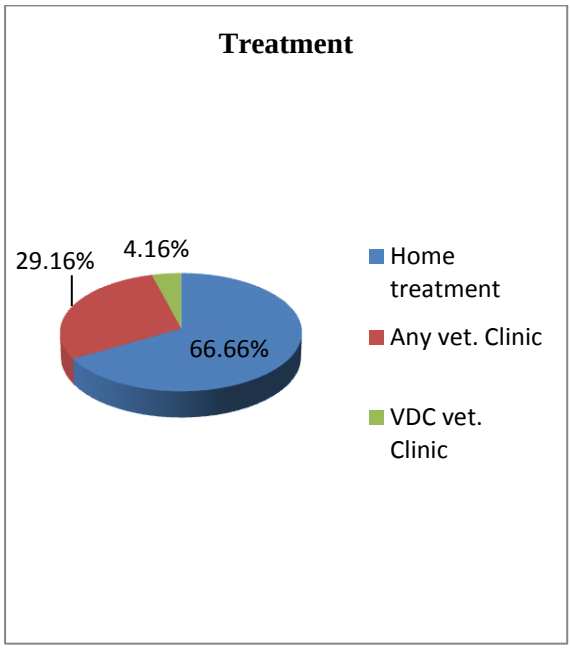

Figure 12: Treatment of equines

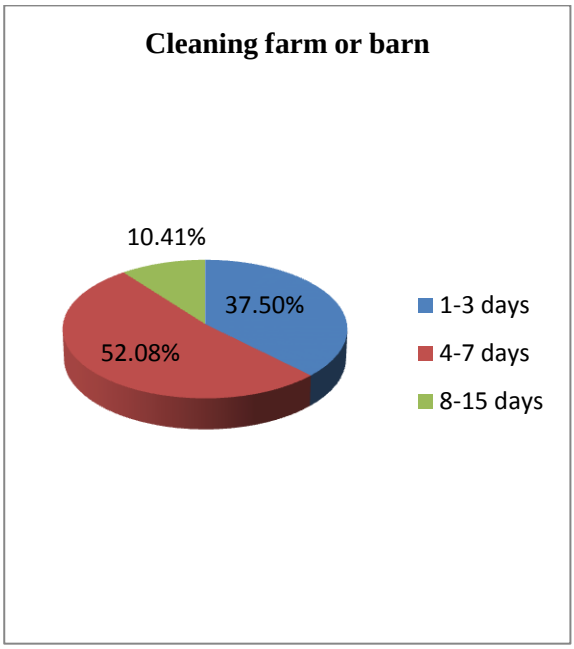

Figure 13: Cleaning frequent of equines farm

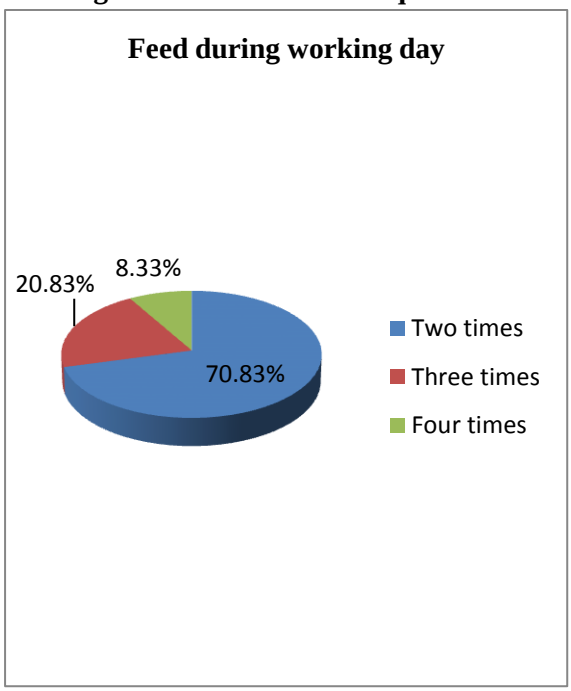

Figure 14: Feed times in a working day

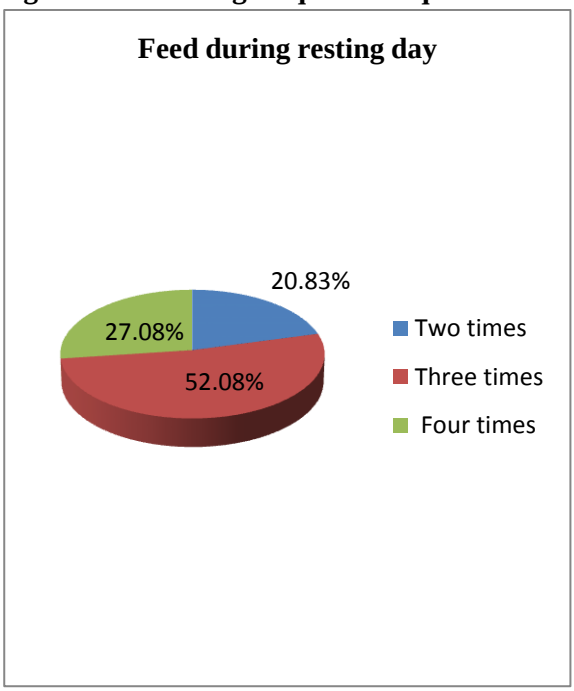

Figure 15: Feed times in a resting day

Photographs of identified eggs/cysts/larvae of parasites found in horses

Protozoan parasites (with 10Xx40X):

Photo 1: *Entamoeba* sp. (25 µm)

Photo 2: *Balantidium* sp. (35 µm) cyst

Photo 3: *Balantidium* sp. (45x22 µm) trophozoite

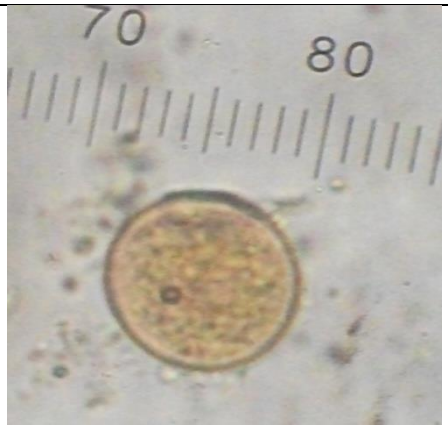

Photo 4: *Eimeria* sp. (22 µm)

Trematode parasites (with 10Xx 40X and 10Xx10X):

Photo 5: *Gastrodiscus* sp. (60x36 µm)

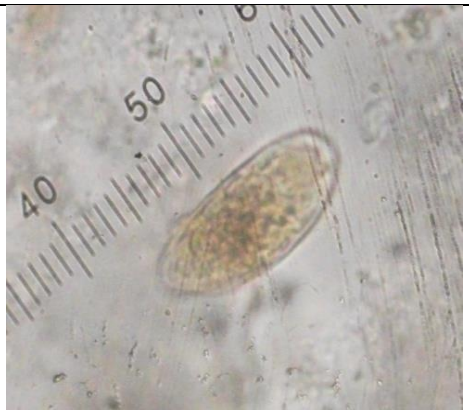

Photo 6: *Schistosoma* sp. (145x45 µm)

Nematode parasites (with 10Xx40X):

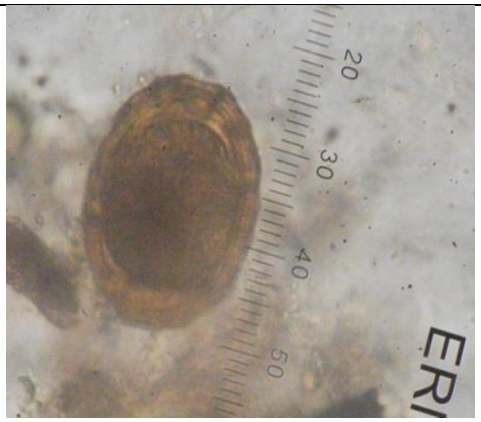

Photo 7: *Parascaris equorum* (90x49µm) corticated

Photo 8: *Parascaris equorum* (115x46µm) decorticated

Photo 9: *Strongylus* sp. (85x48 µm)

Photo 10: *Trichonema* sp. (113x51 µm)

Photo 11: *Triodontophorus* sp. (140x55 µm)

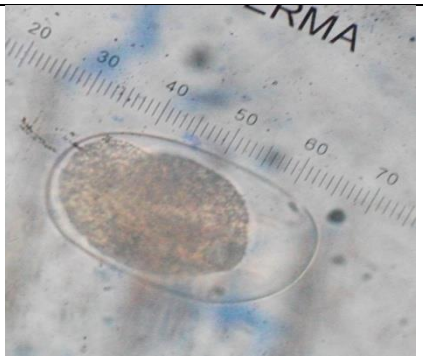

Photo 12: *Dictyocaulus* sp. (85x55µm)

Photo 13: *Oxyuris equi* (85x43 µm)

Photo 14: *Trichostrongylus* sp. (95x45 µm)

Nematode larvae (with 10Xx 40X and 10Xx10X):

Photo 15: Rhabdiasoidae larva (352x19µm)

Photo 16: Dictyocaulidae larva (204x10µm)

Photo 17: Strongyloidae larva (550x25µm)

Photographs of lab-work (CDZ) and faecal collection (study area)

Photo 18: Ready to centrifuge

Photo 19: Saturated solution addition

Photo 20: Microscopic faecal observation

Photo 21: Faecal collection in field

Photo 22: Click with horse owner

Photo 23: Horse riding enjoy in field

5. DISCUSSION

It is well known that parasites are cosmopolitan in distribution and all animals bear different kind of parasites. Many studies have been carried out regarding the gastro-intestinal parasites of domestic livestock. Veterinarians are always interested to research about domestic animals but very few researches have been carried out to equines population than other domestic animals. Decrease of grazing areas, transport facilities, lack of pony farmer, nutritional factors, machine-able agro-facilities, lack of manpower for care, lack of breeding and health related factors are identified as the major parameters for declining of horse population in context of our country. Horses are suffering from different type of parasites and they shed parasites mainly by defecation and urination and when other horses get infection from the contaminated area. Most of protozoa don't cause poor body condition but helminth parasites can cause death of horses when they are severely infected.

In the present study, the prevalence of gastro-intestinal parasites of horses (*Equus caballus*) has been carried out for the first time in Nepal as comprising large population samples and first time from Rukum district. The research so far has not been adequate enough, prevalence figures of the present research work is mostly compared with the research work done in other countries and only few with our national context regarding the topic. The present study explored parasites prevalence in relation to husbandry practice as in general.

The present study revealed that high prevalence of gastro-intestinal tract (GIT) parasites in horses (*Equus caballus*) from seven VDCs (Morawang, Mahat, Kankri, Taksera, Kol, Hukam and Ranmamaikot) of Rukum district. Overall positive prevalence rate was found to be 84.76%. The total numbers of genera observed during faecal examination were 12 in numbers. Among identified parasites, the *Strongylus* sp. showed the highest prevalence (51.42%) followed by *Eimeria* sp. (20%), *Trichostrongylus* sp. (14.28%), *Trichonema* sp. (13.33%), *Parascaris equorum* (10.47%), *Balantidium* sp. (9.52%), *Dictyocaulus* sp. (8.57%), *Triodontophorus* sp. (7.61%), *Gastrodiscus sp.* (6.66%), *Oxyuris equi* (4.76%), *Entamoeba* sp. (3.80%), *Shistosoma* sp. (1.90%) and unidentified nematode larvae (7.61%).

The prevalence of gastro-intestinal parasites in the horses used for the study was high (84.76%). The high prevalence of infection observed in the study agrees with works of Aypak and bergu (2013), Adeppa *et al.* (2014), Mezgebu *et al.* (2013), Sultan *et al.* (2014) and Wosu and Udobi (2014), who reported 88.6%, 84%, 92.71%, 75.62% and 76.1% in Aydin region of Torkey, Schimoga of Karnataka, Gondar town and Savannath zone of Nigeria respectively. The prevalence in horses (84.76%) observed in the present study is lower than Wannas *et al.* (2012), Tilahun *et al.* (2014) and Hassan (2014), who reported 100%, 97.9% and 100% in Aldiwaniyah Governate, Hawassa Town and Baquba city respectively and higher than Saeed *et al.* (2010), Adam *et al.* (2013), Regassa and Yimer (2013), Yadav *et al.* (2014), Tesfu *et al.* (2014), Shiret and Samuel (2015), Samuel *et al.* (2015), Molla *et al.* (2015) and Sokol *et al.* (2015), who reported 65.1%, 24.6%, 60.68%, 59.25%, 63.7%, 59.3%, 55.7%, 69% and 51.7% in Kurfa Chale of Ethiopia,

North Darfun, South Wolla Ethiopia, Jabalpur of North Indea, Hawassa Town, Mekelle Region, Kombolchu Town, Menzkeya Gerbil district and Warmia of Mazury respectively. The high prevalence rate of present study could be due to samples collection during spring because eggs excretion during spring and summer season was high (Saeed *et al.*, 2010; Nielsen, 2012). All most all horses used in the study were working horses that mainly fed poorly to grain and byproducts of grains and they are less exposed to pasture grazing in the rest time period. The high prevalence rate (84.76%) of present study characterized by also poor husbandry practice of the study area (Adebabay, 2009; Sisay *et al.*, 2007; Mezgebu *et al.*, 2013; Adeppa *et al.*, 2014). The difference among these findings from different regions might be due to variation in management system, geo-graphical climatic condition, sample size, sample collection period and sampling method differences (Wannas *et al.*, 2012; Tilahun *et al.*, 2014; Yadav *et al.*, 2014).

The prevalence rates of present study from seven VDCs were 100% (Kankri), 96.42% (Ranmamaikot), 90% (Kol), 85.71% (Taksera), 80% (Hukam), 77.77% (Morawang) and 64.70% (Mahat) respectively. Four VDCs (Taksera, Kol, Hukam and Ranmamaikot) were situated in the highland and three VDCs (Kankri, Morawang and Mahat) were situated in the low land of Rukum district. The prevalence rate variations in the present study from seven VDCs could be due to mainly altitudinal variations and habitat variations in their respective VDCs. From present study, highland of cooler region and habitat near to river or water source was more infected than lowland (Courtney, 1999; Holland *et al.*, 2001).

Detection of highest prevalence of Strongyle type was 72.38% in the present study agrees with works of Sultan *et al.* (2014), Mezgebu *et al.* (2013), Paudel (2007) and Tesfu *et al.* (2014), who reported 72.66%, 66.67%, 65.85% and 64.9% in Kurfa chale, Gondar Town, Bharatpur Chitwan and Hawassa respectively. The present result was also similar with the reports of different researchers (Relf *et al.*, 2013; Dopfer *et al.*, 2004; Gurler *et al.*, 2010; Hinney *et al.*, 2011; Kornas *et al.*, 2010; Papazahariadou *et al.*, 2009; Rehbein *et al.*, 2013), who reported the prevalence rate ranging from 61-100% of horses in most European countries. The present result is higher than works of Saeed *et al.* (2010), Wannas *et al.* (2012), Regassa and Yimer (2013) and Samuel *et al.* (2015), who reported 58.5%, 50%, 58.5% and 47.4% in Kurfa chale, Aldiwaniyah Governorate, South wollo and Kombolcha Town respectively but very low result with 25% (Yadav *et al.*, 2015) and 24% (Tiroshlevy *et al.*, 2015) reported from Jabalpur and Israel respectively. But this report is very less than the reports of Alemayehu (2004) and Getachew *et al.* (2010) from East Shewa and Adau and Bost of East Shewa that revealed 100% and 99% prevalence respectively. Over 94% of the horses passed strongyle eggs reported by Lan *et al.* (1996), Holland *et al.* (2001). The high prevalence of strongyle in the present study clearly demonstrates that strongyle infections are high in the study area. The high faecal eggs prevalence could be explained by the climate, geo-graphical area that are suitable to development, survival and transmission of pre-parasitic stages on pasture during most of the year and by the absence of anti-parasitic treatments (Nielsen *et al.*, 2006; Kaplan and Nielsen, 2010; Toscan *et al.*, 2012; Andersen *et al.*, 2013).

The highest prevalence of *Strongylus* sp. was 51.42% in the current study which is close agreement with works of Wosu and Udobi (2014) and Adeppa *et al.* (2014) and by Poudel (2007), who reported 55.3%, 52.38% and 48.78% in North Nigeria, Schimoga Kornataka

and Sainik stud farm of Bharatpur Chitwan respectively but was lower result than Nigeria (68.8%) by Umar *et al.* (2013) and was higher result than Baquba city (44%) by Hassan (2014). Although equine strongylosis was well documented in cooler climate region of North Vietnam but little information was available from tropical countries (Courtney, 1999; Holland *et al.*, 2001).

Trichonema sp. is another common nematode parasite and also called as small strongyle that prevalent in Horses. Small strongyles are considered to be the main parasitic pathogen of the Horses (Khan *et al.*, 2015). The parasite has been reported from Ethiopia (Saeed *et al.*, 2010), North Darfun (Adam *et al.*, 2013) and Chitwan (Paudel, 2007). The prevalence of *Trichonema* sp. was 13.33% which is similar with 17.07% recorded by Paudel (2007) from Bharatpur Chitwan. It was felt difficult do discuss present result with others because most of researchers has not specified the parasite and has included in strongyle type. The present similar result with Paudel (2007) indicates that *Trichonema* sp. was not affected by variation of climate, altitude and geographical areas.

Triodontophorus sp. is a large strongyle nematode prevalent in horses or equines. The *Triodontophorus* sp. reported first time for Nepal in horse. The prevalence of *Triodontophorus* sp. (7.61%) reported in the present study is close agreement with 11% (Sapkota, 2009) but was lower than with 33.2% (Tilahun *et al.*, 2014) and was higher than with 1.15% (Rani, 2010) prevalence reported in Lalitpur, Hawassa Town and Banke district respectively. Difference in prevalence rate could be due to climatic condition, altitudinal and geographical variances. The low prevalence rate of *Triodontophorus* sp. from present finding indicates that study area is less contaminated with parasite.

Gastrodiscus sp. had been reported from different authors (Karki and Manandhar, 2006; Paudel, 2007; Sapkota, 2009; Saeed *et al.*, 2010; Mezgebu *et al.*, 2013; Adeppa *et al.*, 2014) from different countries. The prevalence (6.66%) of *Gastrodiscus* sp. reported in present study is close agreement with 7.14% (Adeppa *et al.*, 2014), 7.31% (Paudel, 2007) 6% (Karki and Manandhar, 2006) from Schimoga of Kornataka, Bharatpur Chitwan and Udyapur district respectively. Present finding result was higher than findings of Mezgebu *et al.* (2013) and Rani (2010), who reported 2.86% and 2.89% from Gondar Ethiopia and Banke district respectively and was lower than with 30% (Sapkota, 2009). From these results, it was seen that cooler and temperate climate is suitable for development of *Gastrodiscus* sp. than warm and tropical climate.

The *Shistosoma* sp. was reported first time for Nepal in horse. The prevalence (1.90%) of *Shistosoma* sp. reported in present study close agrees with 0.31% (Matto *et al.*, 2015) from Mumbai and Pune state but was very less than with 32.36% (Rani, 2010) reported from Banke district. The result showed that present study area is also suitable for snail (intermediate host) development. Lower result of present study than Rani (2010) could be due to cooler and temperate climate is present in the present study area.

The prevalence of *Trichostrongylus* sp. in horses was found 14.28%. *Trichostrongylus* sp. reported in the present study was very less than finding of Paudel (2007) who reported 80.48% from Bharatpur Chitwan and was less than findings of Wannas *et al.* (2012) and Aypak and Bergu (2013), who reported 25% and 28.3% from Al Diwaniyah Governorate and Aydin Torkey respectively. The lower result of present study could be due to low temperature and cooler climatic geographic area than other regions as mentioned above.

The prevalence rate of *Parascaris equorum* is (10.47%) almost similar 10.71% (Adeppa *et al.*, 2014) 7.9% (Kornas *et al.*, 2010), 6.3% (Umar *et al.*, 2013), 13.9% (Ionita *et al.*, 2013) and 15.51% (Sultan *et al.*, 2014). But lower than 40.90% (Wannas *et al.*, 2012), 43.8% (Mezgebu *et al.*, 2013) 18.75% (Yadav *et al.*, 2014), 26.2% (Tesfu *et al.*, 2014), 55.8% (Tilahun *et al.*, 2014) and was higher than 4.5% (Regassa and Yimer, 2013), 3.9% (Hasson, 2014), 1.8% (Shiret and Samuel, 2015), 4.7% (Samuel *et al.*, 2015) and 5% (Tiroshlevy *et al.*, 2015). The lower prevalence of *Parascaris equorum* in present study could be due to collection of faecal samples mostly from adult working horses and few only from young horses. *P. equorum* mainly occurs in young horses less than 3 years old and infection with this parasite is restricted to animals less than 5 years old (Mfitilodze and Hutchinson, 1989; Bucknell *et al.*, 1995). The difference in prevalence of *Parascaris equorum* from different reports in developing countries is somewhat conflicting and this could be due to compromised immune responses relating to concurrent diseases, but is worthy of further investigation (Melissa *et al.*, 2010).

The prevalence of *Oxyuris equi* was 4.76% in horses. This result is similar with Regassa and Yimer (2013), Adeppa *et al.* (2014), Mezgebu *et al.* (2013), Sultan *et al.* (2014), Samuel *et al.*, (2015) and Fikru *et al.*, (2005), Shiret and Samuel (2015) who reported 4%, 4.7%, 0.95%, 1.47%, 2.8% , 2.1% and 8.8% respectively. But, lower than results of Wannas *et al.* (2012), Umar *et al.* (2013), Wosu and Udobi (2014) and Tilahun *et al.* (2014) who reported 11.36%, 27.1%, 30.2% and 34.2% respectively. The lower result (4.76%) of *Oxyuris equi* in present study might be due to low temperature and collection methods because all samples were collected from captivated horses from behind of individual horse (not from the direct rectum) of each of them. This parasite differs from most other equine nematodes since the worm does not shed any eggs into the faeces, instead the female deposit her eggs at the skin around the anus. Eggs stick to the surroundings when the horse rubs its tail. Eventually the skin dry and fall of eggs in the surrounding when the horse rubs it's tail (Reinemeyer and Nielsen, 2013; Taylor *et al.*, 2007).

The *Dictyocaulus* sp. was reported first time for Nepal in horse. In several studies, 50-80% of donkeys have been found infected with *Dictyocaulus* sp. because donkeys act as reservoir host for the parasite (Clayton and Duncan, 1981). *Dictyocaulus* sp. was reported from different authors from different countries (Saeed *et al.*, 2010; Umar *et al.*, 2013; Tolossa and Ashenafi, 2013; Tilahun *et al.*, 2014). The prevalence of 8.57% of *Dictyocaulus* sp. was recorded in the present study is similar with report of Umar *et al.*, (2013), who reported 10.4% in Nigeria but, was higher than with 3.7% (Tilahun *et al.*, 2014), 2.5% (Saeed *et al.*, 2010) and 0.5% (Tolossa and Ashenafi, 2013). The findings are relatively lower than the present finding, this could be due to good husbandry practice, climatic and environmental differences, between countries and differences in access to drugs may partly explain the variation (Mezgebu *et al.*, 2013; Adeppa *et al.*, 2014)..

Only few studies have to be done regarding protozoan parasites to horses all over the world. The prevalence of 20% of *Eimeria* sp. was recorded in the present study is very less than the report of Shrestha (2015), who reported 100% in Illam community forest. But higher than reports of Wannas *et al.* (2012), Alharis (2001) and Alaloust *et al.* (1994),

who reported 6.81%, 10.96% and 4% and was very high than reports of Epe *et al.* (1993) and Tavassoli *et al.* (2010), who reported 0.6% and 0.5% in Dtsch Tierazll and Poland respectively.

The *Balantidium* sp. was reported by Wannas *et al.* (2012) and Shrestha (2015). The prevalence rate of *Balantidium* sp. (9.52%) is nearly similar with 15.90% (Wannas *et al.*, 2012) in Al Diwaniyah Governorate and was lower than report of Shrestha (2015), who reported 20% in Illam community forest.

It was felt difficult to discuss about the present study of *Entamoeba* sp. (3.80%) with others due to lack of species finding regarding the parasite. But, Wannas *et al.* (2012) reported 3.57% in donkeys in Governorate which is almost similar with present result. From Nepal, Shrestha (2015) reported *Entamoeba* sp. (40%) from a total of 5 samples examination of horses which was very high than the present finding result.

The lack of cestodes in current study provides evidence to back up the scarcity of infection by this class of parasites which was also reported by Meana *et al.* (1998). Meana *et al.* (1998) pointed to the coprological methods have a lower likelihood of diagnosing cestode infection when horses have less than 100 tapeworms.

There was no significant association ($\chi 2 = 5.7161$; $p>0.05$) of VDCs (study areas) and parasitic prevalence, which was 7 (77.77%), 11 (64.70%), 5 (100%), 9 (90%), 18 (85.71%), 12 (80%) and 27 (96.42%). This type of result has also been reported by Shiret and Samuel (2015).

The infection rate in females (92.30%) higher than in male horses (82.27%) reported in present study is similar with 60.97% and 58.51% (Yadav *et al.*, 2014), 61.5% and 38.5% (Hasson, 2014) and 80.8% and 58.8% (Wosu and Udobi, 2014) for females and males respectively. Similar finding have been reported by Singh *et al.* (2012), who have found higher prevalence in female equines (75.73%) as compared to male (72.30%). The present result is in agreement with those reported by Love and Duncan (1992) and Smith (2002). But, Umar *et al.* (2013) reported male infection is higher than female infection. The prevalence of female horses than male horses could be due to hormonal activities as mentioned by Dietz *et al.* (1984). Since pregnant and lactating female are more susceptible to parasitic infections this may be the cause of higher prevalence in females than males. There was no significant association ($\chi 2 = 0.3346$; $p>0.05$) between male and female parasitic prevalence, which was 65 (82.27%) and 24 (92.30%) respectively. The finding of this type of result was reported by Saeed *et al.* (2010), Mezgebu *et al.* (2013), Aypak and Bergu (2013), Tesfu *et al.* (2014) and Haimainot *et al.* (2015) but, opposite result i.e. significant different result reported by Umar *et al.* (2013), Aypak and Bergu (2013) and Hasson (2014). The study showed effects of study area on infections status of parasites and there was statistical significant difference among infection status in study area ($\chi 2 = 84.277$; $p<0.05$).

In current study, mixed infection were detected in 57.14% of horses which is almost similar with the finding of Tolossa and Ashenafi (2013) in horses of Arsi-Bale highlands of Oromiya region and Uslu and Guclu (2007) in Torkey, who reported 59.1% and 50% respectively. Tilahun *et al.* (2014) reported 83.2% of mixed infection from Hawassa Town, which is higher than present study but, present result was higher than with 25% (Adeppa *et al.*, 2014), 20.14% (Ionita *et al.*, 2014), 16.8% (Shiret and Samuel, 2015) and

12.5% (Samuel *et al.*, 2015) in Schimoga, Romania, Mekelle and Kombolcha respectively. These lower result than present study could be due to use of anti-parasitic drugs and de-worming programs in their studied area (Wannas *et al.*, 2012; Regassa and Yimer, 2013). But present higher mixed infection (57.14%) could be due to lack of horse de-worming, very less use of anti-parasitic drugs, dietary deficiency, poor management system, lack of knowledge for sanitation and climatic and geographical variation of the present study area (Saeed *et al.*, 2010; Sisay *et al.*, 2007; Mezgebu *et al.*, 2013).

In the present study area, most of respondents were not found educationally qualified for management of equines. Most of respondents were found used open river water source for equines which made them easy to get different parasitic infections. The main purpose of keeping horses in their house was to get income from transportations of human needs where small road to be developed. Mainly mixed type of food particles was found to feed their horses especially residue of grains, grasses and shrubs that made also possibility of high parasitic infection to them. Most of respondents found to be kept their horses with together family that made easy to exchange of human parasites. All most all respondents responded that there was no available of veterinary clinic for treatments. In the study area, horses found to be treated at home with turmeric powder, garlic and plant medicines. Most of respondents found to be cleaned their barns within a week. It means that there was very poor management of horses. The carrier horse mainly found to feed (food grain particles) twice during working day but resting horse found to feed (mainly natural food particles) thrice a day. Here feeding frequent found more in resting horse than working horse because working horse gets more food particles at a time to transport different human needs. From these husbandry practices, there was possibility of high parasitic infection. The high parasitic prevalence rate of present study could be also related with poor management or poor husbandry practices of study area. The result noted in the current study related to husbandry practice was generally in agreement with the reports of Solomon (2004) from Sinana and Dinsho districts of Bale highland, Asamenew (2007) from Bahir and Belete (2006) from highland and rift valley of Ethiopia.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion:

Overall, present study demonstrates the prevalence of GI parasitic infection in horses in seven VDCs of Rukum district was 84.76% using floatation and sedimentation technique. The total numbers of genera observed during faecal examination were 12 in numbers. Among them, the *Strongylus* sp. showed the highest prevalence (51.42%) followed by *Eimeria* sp. (20%), *Trichostrongylus* sp. (14.28%), *Trichonema* sp. (13.33%), *Parascaris equorum* (10.47%), *Balantidium* sp. (9.52%), *Dictyocaulus* sp. (8.57%), *Triodontophorus* sp. (7.61%), *Gastrodiscus sp.* (6.66%), *Oxyuris equi* (4.76%), *Entamoeba* sp. (3.80%), *Shistosoma* sp. (1.90%) and unidentified nematode larvae (7.61%). Three genera of parasites: *Shistosoma* sp., *Triodontophorus* sp. and *Dictyocaulus* sp. have been reported first time for Nepal in horses.

The present study revealed infection rate higher in females (92.30%) than in male horses (82.27%). The present study shows horses were more infected with mixed infection than single infection. The prevalence is higher in the study area as a normal and has been characterized by poor husbandry practices due to lack of veterinarian information and lack of veterinary hospital. This study revealed that the predominant parasites were *Strongylus* sp. and *Eimeria* sp. The study confirmed that horses were found to be most susceptible and infested by various GI parasites. Management practices and different locations can be considered as the important factors which influence the prevalence of GI parasites. In present study, sex and location had statistically no influences on prevalence of parasites but infection status showed significant association with study area. The higher prevalence of present study shows an alarming situation in the study area. This finding might be considered while designing control strategies of gastro-intestinal parasites in horses and also be useful to other equines and ruminants as well.

6.2 Recommendations:

Based on the above conclusion, the following recommendations are forwarded:

- Further study or molecular level characterization of parasites should be done.
- Improved basic animal's husbandry management system should be practiced in the study area.
- Educate the horse owners regarding correct way to improve animal's management system, importance of parasites and it is preventive and controlling systems.
- Sufficient feed supply and minimizing contaminated open grazing of horse is important.
- Balancing of the work load and duration should be managed.
- By implementing veterinary health program, appropriate prevention and control strategy supported by both government as well as private sector to minimize the gastro-intestinal parasites of horses.

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8. APPENDIX: QUESTIONNAIRES

Questionnaires (interview typed) survey for husbandry practices in the study area (Eastern seven VDCs) of Rukum district, Nepal.

I. General information

1. Date…….. 2. VDC Name……

3. Owner's Name…….. 4. Sex…….

5. Educational status

A. Illiterate B. Read and write

C. Primary level D. Above primary level

6. For what purpose do you keep or rear your equines?

A. Transportation B. Fertilizer

7. What is the major feed resource for your equines?

A. Natural pasture B. Shrubs and forest

C. Grain residue D. Mixed type

8. What are the major water resources for your equines?

A. Small fresh water B. Rivers

C. Pond or tap water

9. What type of house do you have for your equines?

A. Together with family B. Simple shed

C. Separate house or cottage

10. How frequent do you clean your equine's barn or farm?

A. 1-3 days B. 4-7 days

C. 8-15 days

11. Where do you treat your equines after get diseased?

A. Home treatment B. Any veterinary clinic

C. VDC veterinary clinic

12. Is veterinary clinic available on your own village development committee?

A. No B. Yes

13. How many times do you feed your equines in a day during working period?

A. Two times B. Three times

C. Four times

14. How many times do you feed your equines in a day during resting period?

A. Two times B. Three times

C. Four times