PREVALENCE OF INTESTINAL PARASITES IN WILD BUFFALO (*Bubalus arnee*, Kerr, 1792) OF KOSHI TAPPU WILDLIFE RESERVE, NEPAL.

Sushma Gupta
T.U. Registration No. : 5-2-54-668-2008
T.U. Examination Roll. No. : 55
Batch : 2070

A thesis submitted in partial fulfillment 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
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DECLARATION

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

Date: **……..** **……**

Sushma Gupta

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 Intestinal Parasites in Wild Buffalo (***Bubalus arnee,* **Kerr, 1792) of Koshi Tappu Wildlife Reserve, Nepal"** has been carried out by **Sushma Gupta** for the partial fulfillment of Master's Degree of Science in Zoology with special paper **Parasitology**. This is her 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: **……..**

LETTER OF APPROVAL

On the recommendation of supervisor **"Asso. Prof. Dr. Mahendra Maharjan"** this thesis submitted by **Sushma Gupta** entitled **"Prevalence of Intestinal Parasites in Wild Buffalo (*Bubalus arnee,* Kerr, 1792) of Koshi Tappu Wildlife Reserve, 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 UniversKirtipur,
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 **Sushma Gupta** entitled **"Prevalence of Intestinal Parasites in Wild Buffalo (*Bubalus arnee,* Kerr, 1792) of Koshi Tappu Wildlife Reserve, Nepal"** has been approved as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper **Parasitology**.

EVALUATION COMMITTEE

Date: ……

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Sushma Gupta
T. U Registration No: **5-2-54-668-2008**

T.U. Examination Roll No.: **55**
Batch: **2070/071**

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LIST OF ABBREVIATIONS

Abberviated form	Details of abberviations
µm	Micrometer
KTWR	Koshi Tappu Wildlife Reserve
DNPWC	Department of National Park and Wildlife Conservation
et al.	And his associates
GI	Gastrointestinal
gm	Gram
IUCN	International Union for Conservation of Nature
km	Kilometer
cm	Centimeter
m	Meter
mi	Mile
GON	Government of Nepal
ICZN	International Commission on Zoological Nomenclature
VDC	Village Development Committee
rpm	Rotation per minute
NaCl	Sodium chloride

ABSTRACT

Wild buffalo (*Bubalus arnee*) is endangered species which are conserved in Koshi Tappu Wildlife Reserve (KTWR), located in Sunsari, Saptari and Udayapur district of Eastern Terai Region of Nepal. In order to determine the prevalence of intestinal parasites of wild buffalo in KTWR, total of 160 fresh dung samples were collected by random faecal sampling method in March, 2016 and examined by floatation, sedimentation and Stool's counting technique using Lugol's Iodine mount following standard technique. Out of 160 dung samples examined, 76 (47.50%) dung samples were found positive for different parasitic infections. Nematode infection was found comparatively more (38.75%) than the protozoan (35%) infection. Seven different parasite species were revealed with one coccidian parasite: *Eimeria* 56 (35%) whereas six helminthes, including one species of trematode: *Paramphistomum* 37 (23%) and five species of nematode: *Trichostrongylus* 56 (35%), *Haemonchus* 47 (29.37%), *Toxocara* 39 (26.25%), *Strongyloides* 38 (23.75%) and *Oxyuris* 35 (21.88) were identified. The prevalence of various intestinal parasitic infection in wild buffaloes were found statistically insignificant (χ^2 = 0.15, P>0.05). Among, identified protozoan parasites, *Eimeria* without micropile and with micropile showed insignificant distribution (χ^2=0.83, P<0.05) whereas, the prevalence of parasites among protozoa (35%), trematode (11.87%) and nematode (38.75%) were significantly different (χ^2=12.48, P<0.05). Similarly, prevalence of mixed parasitic infections in wild buffaloes (χ^2 =11.806, P<0.05) also showed statistically significant different. However, no any activities on health care of wild buffalo regarding the intestinal parasites were found. Thus, this study indicated a higher prevalence of intestinal parasite in wild buffalo of KTWR.

1. INTRODUCTION

1.1 Background

Nepal is a small country but rich in biodiversity with variety of flora and fauna. The variety of biodiversity of Nepal is due to its unique land topography which ranges from lowlands with subtropical forest to arctic condition to the Himalayan highlands. For conservation and proper utilization of these wildlife and vegetation in 2037 B.S. Nepal Government established National Park and Wildlife Reserve. At present 10 National Park, three Wildlife Reserves, one Hunting reserve, and six Conservation Areas has been declared by Nepal government. Among three Wildlife Reserves, Koshi Tappu Wildlife Reserve (KTWR) is one which was established in 2032 B.S. (1976 A.D). It was established to conserve the Asiatic water buffalo (*Bubalus arnee*), locally known as Arna. Koshi Tappu wildlife reserve lies on the floodplain of Sapta Koshi River in including few area of Sunsari, Saptari and Udayapur district of Eastern Terai Region, Nepal. It is a smallest Wildlife Reserve with an area of 175 sq. km. and ranges in altitude from 75 to 81 m. Koshi Tappu is also known as the first Ramsar site of Nepal. Koshi Tappu was kept under the Ramsar Conservation in December 17, 1987 to achieve conservation and sustainable use of its wetlands. In 2004, about 173 km^2 of the reserve consisting 16 VDCs of Sunsari, Saptari and Udaypur district was declared as buffer zone. The vegetation of the reserve is mainly characterized by mixed deciduous riverine forest, grasslands, and marshy vegetation. The coverage of grasslands is 68%, compared to only about 6% of forest, which is predominated by Indian rosewood. Patches of catechu forest are more prevalent towards the northwestern part. The grasslands near the running water bodies are maintained by the annual flooding and grazing by wildlife (Karki, 2008). A total of 181 mammal species have been recorded to occur in Nepal (DNPWC 2012).Of them 23% are considered to be nationally threatened with extinction, 4% of species considered as critically endangered, 12% endangered and 7% vulnerable. A further 3% are considered near threatened, 35% of Nepal's mammals are considered least concern and 38% are considered data deficient (Jnawali *et al*., 2011). In recognition of the magnitude of biodiversity the Government of Nepal has established a network of 20 protected areas since 1973, consisting of ten national parks, three wildlife reserves, six conservation areas and one hunting reserve (GON, 2014). Additionally, nine Ramsar sites were declared between 1988 and 2008 (IUCN, 2016).

1.2 Wild buffalo (*Bubalus arnee* Kerr, 1792)

Carl Linnaeus applied the binomial *Bos bubalis* to the domestic water buffalo in his first description of 1758. In 1792, Robert Kerr applied the binomial *Bos arnee* to the wild species occurring in India from Bengal. Later authors subordinated the wild water buffalo under either *Bos*, *Bubalus* or *Buffelus* (Ellerman, *et* al., 1966). In 2003, the International Commission on Zoological Nomenclature placed *Bubalus arnee* on the Official List of Specific Names in Zoology, recognizing the validity of this name for a wild species (ICZN, 2003). Most authors have adopted the binomen *Bubalus arnee* for the wild water buffalo as valid for the taxon (Gentry, 2004). Wild water buffaloes (*Bubalus arnee*) also

called Wild Asian buffalo, Wild Water buffalo, and Asiatic buffalo. During the Pleistocene epoch the genus *Bubalus* was widely distributed throughout Europe and Southern Asia and contained forms nonspecific with *Bubalus arnee.* The wild water buffalo represents most likely the ancestor of the domestic water buffalo (Lau *et al.*, 1998). The wild buffaloes are categories in endangered on the IUCN Red list with remaining population.

1.3 Characteristic of wild buffalo

According to Nowak, 1999 the characteristic of wild water buffalo includes- the body weight 800-1200 kg, 240 to 300cm body length, massive, powerful animal with the widest horn span of any bovid (more than 2m) as the both sexes carry horns, 60 to 100 cm longs tail, a shoulder height with 150 to 190 cm, skin color is ash grey to black, contain moderately long coarse and sparse hair on their long and narrow head with small ears, the tip of the tail is bushy and the hooves are large and splayed.

1.4 Population status

The world population of wild buffalo is less the 4,000 animals occupying an area of less than 20,000km^2 in Nepal, India, Sir Lanka, Myanmar, Thailand and Bhutan (Heinen, 2002; Hedges *et al.,* 2008). They have been extirpated in Pakitan, Bangladesh, Laos and Vietnam (Hedges *et al.,* 2008; Choudhury, 2010).

1.4.1 Growth trend of wild water buffalo in Koshi Tappu.

In Nepal, the population of wild buffalo in Koshi Tappu was recorded since 1976. In 1976, there were 63 individuals and in 2009 it go increased with 219 individuals (Heinen, *et al.,* 1993; Aryal *et al.,* 2011). But now in 2016 its population has grown significantly and reached up to 432 individuals among them 120 males, 182 females and 130 calves (DNPWC, 2016).

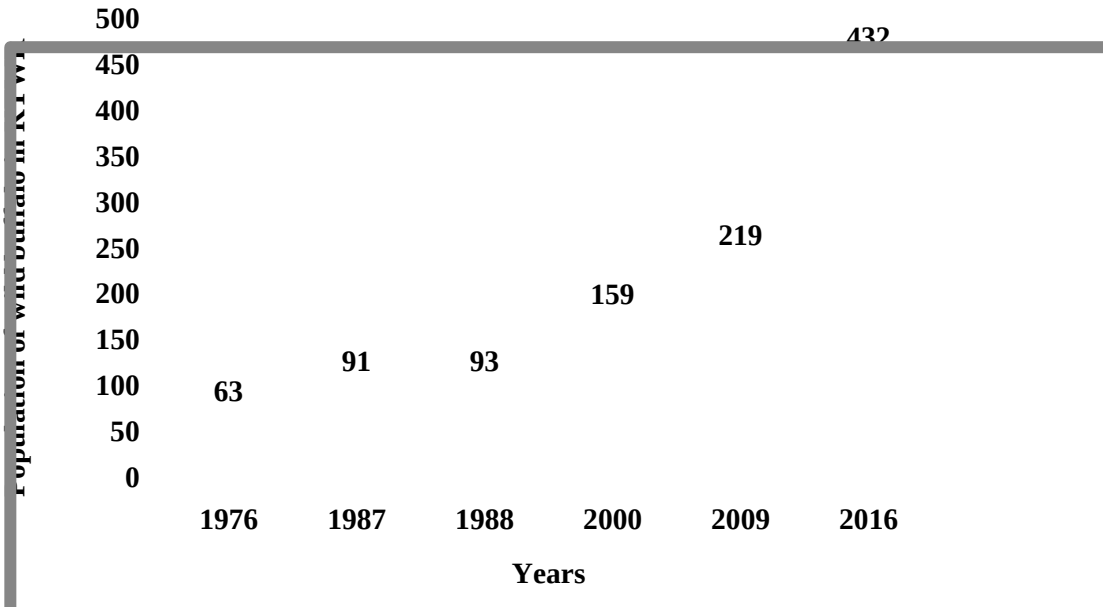

Figure 1: Population trend in wild buffalo of KTWR
(Source:-Aryal *et al*., 2011; DNPWC, 2016).

1.5 Ecology and behavior

Wild water buffaloes highly depend on the availability of water. Nowak, 1999 included some ecological behavior of wild water buffaloes as follows:- wild buffalo were both diurnal and nocturnal, they fed mainly in the morning, evening and night but in the mid day heat, they retreat to the shed or allows in water or muddy pools, (photo- 1) as the adult females and their young forms stable clans about 30 or many more individuals but while in resting area they gathered and formed a herd of 30 to 500 animals, (photo- 2) adult male formed (bachelor groups) 10 individuals but the older male often being solitary (photo- 3 and 4) and that stay apart from the female clans, the age at sexual maturity is 18 months for male and 3 years for females, Asian buffalo mainly breeds in October and November but some of them could bred year around, the gestation period was 10 to 11 months with an inter-birth interval of one year, they mainly gave birth to a single offspring but sometimes twins could be possible, the maximum life span of *Bubalus arnee* was 25 years. Wild water buffalo probably grazed by preference, feeding mainly on true grasses when available, but they also feed herbs, fruits and barks as well as brushy tree and shrubs (Daniel and Grubb 1966) listed *Cynandon dactylon, Themeda quadrivalvis* and *Coix* sp. as grasses known to be eaten by wild buffalo in India. A little information about the diet of wild buffalo of KTWR has been provided by some researcher as wild buffalo also feed on sedge *Cypersud corymbosus* (Dahmer, 1978; Shrestha, 1981; Kushwaha, 1986). Wild buffalo also feed on crops including rice, sugarcane, and jute, sometimes causing considerable damage (Lekagul and Mc. Neely, 1988; Kushwaha, 1986; Bauer, 1987).

1.6 Threats

As the wild water buffaloes were hunted for food, handcrafts products and sports. Cattle and domestic buffalo grazing inside the reserve is another serious problem, and local people keep domestic buffalo in a semi-wild or tended, free-ranging state to crossbreed with wild males because hybrid calves fetch higher prices (Heinen, 1993). The most important threats to wild Asian buffalo are interbreeding with feral and domestic buffalo, habitat loss/degradation, and hunting. Diseases and parasites transmitted by domestic livestock and competition for food and water between wild buffalo and domestic stock are also serious threats (Hedges, 2011).

1.7 Parasitic infections of wild buffalo

Parasite is living organisms that live in host organisms and gets food from the expense of its host. The water buffalo is susceptible to most diseases and parasites due to its natural habitat consisting of hot and humid regions that were very favorable to microorganism and parasite proliferation, some of the diseases affecting buffaloes have been subdivided as follows:-Viral diseases- Foot-and-mouth disease, Bovine viral diarrhea, Rabies; Neonatal diarrheal diseases- Cryptosporidiosis; Bacterial diseases- Bovine brucellosis, Tuberculosis; Parasitic diseases- Trypanosomiasis 249, Ascaridiosis, Fasciolosis, Babesiosis, Theileriosis, Strongilosis, Coccidiosis, Echinococcosis/hydatidosis, Mange; Fungal diseases- Deg Nala disease (Fangiolo *et al*, 2004). Coccidiosis causes severe economic losses due to reduction of feed efficiency, slower weight gain and increases

susceptibility to other diseases (Thomas, 1994). It occurs mainly in young animals because of lower immune competence. In buffalo calves, coccidiosis causes severe diarrhea, dysentery, dehydration, depression, anorexia, weakness (Ahmed and Soad, 2007). Coccidia replicate within the epithelial cells of the intestinal mucosa, often resulting in physical damage and activation of the mucosal immune system (Stewart and Penzhorn, 2004).

The earlier study has shown that roundworms such as *Toxocara vitulorum* and *Strongyloides papillosus* cause emaciation, reduced milk production, and immunity (Chittapanlapong *et al*., 1987). Trematode parasites caused the watery diarrhea, weakness, weight loss, decreased milk production, reduced product quality, mortality, and other secondary infections also (Soulsby, 1982). GI parasites can affect their hosts by directly consuming host resources or indirectly by damaging intestinal function (Stewart and Penzhorn, 2004), altering host behavior (Adelman and Martin, 2009), or disrupting the control of co-infecting parasites (Jolles *et al*., 2008). The host immune system plays an important role in the interactions between nematodes and coccidian because intracellular and extracellular parasites invoke opposite and cross-regulating immune responses (Morel and Oriss, 1998). Further, both coccidia and nematodes have a fecal-oral transmission route that results in seasonal variation in exposure (Horak *et al*., 2004; Stewart and Penzhorn, 2004).

1.8 Objective of the study

1.8.1 General objective

- To investigate the prevalence of intestinal parasites of wild buffalo (*Bubalus arnee*) in Koshi Tappu Wildlife Reserve, Nepal.

1.8.2 Specific objectives

- To identify the intestinal parasites of wild buffaloes by coprological examination.
- To determine the distribution and intensity of intestinal parasites.
- To determine the size of parasitic eggs and oocysts by micrometry.

1.9 Significance of the study

Wild buffalo is threatened species (IUCN 2008) which are valuable and important to maintain the ecological and biodiversity balance. Till date parasitic studies has not been carried out in KTWR. The most important threats to wild Asian buffalo are interbreeding with feral and domestic buffalo, habitat loss and hunting, diseases and parasites transmitted by domestic livestock and competition for food and water between wild buffalo and domestic stock are also serious threats (Hedges, 2008). As the parasites and infectious diseases of wildlife are major threats to conservation of threatened species (Lyles and Dobson 1993). Thus, there is a need for studying and documenting the prevalence of parasites among threatened species. From the conservation point of view, parasitological studies were important to understand mode of infection and potential transmission of parasites between species, both native and introduced (Begon *et al*., 1999). The present study provides some baseline information on the parasitic burden in wild buffalo and helped to formulate appropriate strategies to mitigate the endoparasitic problems of wild buffaloes in KTWR.

2. LITERATURE REVIEW

Parasites are the living organisms which depend on the host for their shelter, food and metabolic activities. Parasites are originated from their free living ancestors; they evolved along with their hosts. The association between a parasites and host is known as parasitism. Parasitism is one of the major problems affecting ruminants. Parasites can affect host survival and reproduction directly through pathological effects (blood loss tissue damage, spontaneous abortion, congenital malformations, and death) and also reduce the host's immunity by affecting on the physical condition (Thawait *et al*., 2014).

Among the other ruminants buffalo diseases have been identified as one of the major problem which have disrupted in the development of the industry in Asia and caused the substantial economic loss to the farmers in the developing countries (Othman and Baker, 1981). As all other domestic herbivores, wild ruminants are also suffered from different endoparasites since; they share land with other small and large ruminants. In case of wild buffalo, a very little research work has been carried out regarding parasitic infection. Wild ruminants can be infected by different parasites including protozoan, trematodes, cestodes and nematodes. Here, some of the important published work related with the present work has been reviewed.

2.1 Global context

2.1.1 Endoparasites of wild ruminants

Endoparasites are those organisms which inhabit in the gut, body cavity, liver, lungs, gall bladder, and blood or within the internal cavities and tissue or cell of their hosts causing parasitic infection. Parasites usually include gastro-intestinal helminthoses, coccidiosis, fascioliosis and mange (Othman and Baker, 1981). The suitable temperature and humidity play important role for the development of endoparasites. Some of the protozoan and helminthes parasites have been reported in wild ruminants. Among all the wild ruminants' wild buffalo were also susceptible to internal parasites because these animals seek rivers, pools or swamps for wallowing where the higher risk of infection with snail born helminthes.

a) Protozoan parasites

Wild as well as domestic ruminants have been reported to be highly infected with portozoan parasite. Among the protozoan parasites coccidiosis has been found in wild ruminants of different part of the world such as India (Varadharajan and Kandasamy, 2000), Pakistan (Azam *et al.,* 2002), Egypt (Wahed *et al.,* 2004), Spain (Vazquez *et al*., 2009). Coccidiosis causes severe economic losses due to reduction of feed efficiency, slower weight gain and increases susceptibility to other diseases (Thomas, 1994). In buffalo, coccidiosis cause severe diarrhea, dysentery, dehydration, depression, anorexia, as well as weakness (Ahmed and Soad, 2007). The major protozoan parasites that infect wild ruminants include *Eimeria* sp, *Blantidium* sp etc.

Eimeria sp. infects their hosts when water or foods contaminated with sporulated oocysts are ingested (Roberts and Janovy, 2005). Infection with the *Eimeria* sp. has been reported in several part of Indian subcontinents like Jabalpur (Marskole *et al.,* 2016), Tirupati (Sreedevi *et al.,* 2014), Haryana (Rana *et al.,* 2011) and in buffalo calves of Punjab (Jyoti *et al.,* 2014). Similarly, *Eimeria* infection has been reported from African countries like Tanzania (Swai *et al.,* 2013). Besides coccidian parasites, buffaloes have been found to be infected with *Balantidium* sp. in India (Sreedevi *et al.,* 2014) and Bangladesh (Biswas, 2012).

b) Helminths

i) Trematodes

Trematodes are commonly known as flukes and are found in the liver, bile duct or small intestine and sometimes present in lungs of buffaloes. They are the internal parasites of mollusca (snail) and vertebrates. Most of the trematode parasites have a complex life cycle as they complete their lifecycle within two or more than two hosts. The common trematode parasites represent *Paramphistomum, Fasciola, Schistosoma, Dicrocoelium* etc.

Fasciola are known as fluke that cause fascioliasis, commonly called as Namle, Mate, Lew, etc. *Fasciola* sp. has been identified in India (Swarnakar *et al.,* 2015; Mondel *et al.,* 2000), Pakistan (Bhutto, 2002), particularly *F. gigantica* has been reported from Europe, Italy (Cringoli *et al.,* 2009), Africa, Tanzania (Swai *et al.,* 2013), Egypt (Haridy *et al.,* 2006). *Fasciola gigantica* has been identified in India (Patel *et al.,* 2015; Gupta *et al.,* 2012), as well as Bangladesh (Saha *et al.,* 2013; Mamun *et al.,* 2011; Ahmedullah *et al.,* 2007).

Similarly, *Paramphistomum* is an intestinal fluke that inhabit mainly on a villi region of small intestine. *Paramphistomum* sp. has been reported from Asian countries like India (Marskole *et al.,* 2016; Sreedevi *et al.,* 2014), Bangladesh (Alam *et al.,* 2016; Fagiolini *et al.,* 2010), Europe, Italy (Cringoli *et al.,* 2009; Condoleol *et al.,* 2007), and some countries of the Africa, Tanzania (Swai *et al.,* 2013), Egypt (Haridy *et al.,* 2006). As the *P. cervi* was recorded in Pakistan (Kaza *et al.,* 2010), Bangladesh (Biswas, 2012; Mamun *et al.,* 2011), India (Patel *et al.,* 2012), Poland (Kobak *et al.,* 2012).

Schistosoma, also called as a blood fluke, is a genus of flatworms. This parasite lives in the mesenteric and hepatic veins of the host except *S. nasale,* which lives in the nasal veins. Three species of *Schistosoma* have been reported from buffaloes of Bangladesh, *S. spindale* and *S. indicum* has been reported by Mamun *et al.,* (2011) and *S. bovis* by Saha *et al.,*(2013). Similarly, *Dicrocoelium dentriticum* is a lancet liver fluke that tends to live in cattle and other grazing mammals. *Dicrocoelium* sp, need two intermediate host i. e snails and an ant to complete its life cycle (Junquera, 2015). *D. dendriticum* from buffalo of Italy (Condoleol *et al.,* 2007; Rinaldi *et al.,* 2009).

ii) Cestodes

Different type of tapeworm belongs to the class cestodes such as *Moniezia* sp. *Taenia* sp. etc. that inhabit in the small intestine of both the wild and domestic animals such as sheep, goat, cow, buffalo, and other ruminants. As the *Moniezia* is a large tapeworm and also known as sheep tapeworm or double pored tapeworm which causes poor growth, intestinal obstruction, weight loss, diarrhea during heavy infections. These cestodes are found in gut and are occurred by eating contaminated food and water. The earlier literatures have shown that cestode infections in buffaloes are not as common as nematode and trematode infections. Some of the researchers from Bangladesh indicated its presents in buffaloes (Biswas, 2012) while absence of cestode by others (Saha, 2013). Several studies have been shown on the *Moniezia* infection in buffaloes of India (Marskole *et al.*, 2016; Sreedevi *et al.*, 2014; Muraleedharan, 2005), Italy (Cringoli *et al.*, 2009), while some reports had shown absence of the cestode parasites (Mamun *et al.*, 2011), Pakistan (Bhutto *et al.*, 2002).

iii) Nematodes

There are numerous nematode parasites such as *Trichostrongylus* sp., *Strongyloides* sp., *Toxocara* sp., *Trichuris* sp., *Haemonchus* sp., *Oesophagostomum* sp., *Bunostomum* sp., and *Capillaria* sp. These nematodes are most important and widely prevalent in buffalo. These nematodes are found in the small intestine and may cause severe damage to the intestinal mucous membrane. These parasitic infections can be acquired by ingestion of egg through contaminated food, vegetable and water while some parasites are transmitted by penetration of filariform larvae.

Trichostrongylus, also called as hairworm**,** belonging to the family called *Strongylidae* and mainly affect the wild and domestic ruminants but in ruminants the worms are mostly found in mixed infections with other gastrointestinal roundworms such as *Cooperia, Haemonchus, Ostertagia* (Junquer, 2016). *Trichostrongylus* sp. has been reported in Buffaloes of Asian country like, Pakistan (Azam *et al.*, 2002 and Khan *et al.*, 2007), and African country like, Tanzania (Swai *et al.*, 2013). Two different species of *Trichostrongylus* have been reported from the buffaloes of India *T. axei* and *T. colubriformis* (Patel *et al.*, 2015).

Similarly, *Strongyloides* sp. is also known as the threadworm and also called as the common haematophagus parasitic nematode of cattle worldwide (Theodoropoulos *et al*., 2010). Its diagnosis is based on presenting clinical signs and conformation of eggs in fecal samples of infected ones (Keyyu *et al*., 2005). *Strongyloides* sp. has been reported in Buffalo of Asian countries like India (Marskole *et al.*, 2016; Jyoti *et al.*, 2014), Bangladesh (Alam *et al.*, 2016; Mamun *et al.*, 2011), Pakistan (Khan *et al.*, 2007, Azam *et al.*, 2002), European country like Italy (Condoleol *et al.*, 2007).

Toxocara virtulorum is a species of parasitic roundworms that infects cattle, buffaloes, bisons and other bovids and also known as *Neoascaris virtulorum* (Junquera, 2016) *Toxocara sp*. has been reported in India (Swarnaker *et al.*, 2015; Sreedevi *et al.*, 2014). As the *T. vitulorum* has been reported from India (Patel *et al.*, 2015, Jyoti *et al.*, 2014),

Pakistan (Azam *et al.*, 2002 and Bhutto *et al.*, 2002), Bangladesh (Biswas, 2012), Cambodia (Dorny *et al.*, 2015).

Trichuris sp. (whipworm) is a parasitic nematode of cattle worldwide (Matsubayashi *et al*., 2009). In heavy infections with this parasites caused diarrhea, anorexia and weight loss (Jiménez *et al* (2010). *Trichuris* sp. has been reported in India (Marskole *et al*., 2016; Sreedevi *et al*., 2014), Pakistan (Khan *et al.*, 2007, Azam *et al.*, 2002), Bangladesh (Biswas, 2012). *Haemonchus* sp. has been identified in Pakistan (Khan *et al*., 2007), Tanzania (Swai *et al.*, 2013). *Oesophagostomum* sp. has been identified in Tanzania (Swai *et al.*, 2013). *Bunostomum* sp. has been found in Tanzania (Swai *et al.*, 2013), Pakistan (Khan *et al*., 2007). *Capillaria* sp. has been found in Bangladesh (Biswas, 2012).

2.2 In National context

2.2.1 Endoparasites of buffalo

It includes both protozoan and helminthe parasites as follows

a) Protozoan parasites

In the context of Nepal, the studies were particularly focused on helminth parasites of buffaloes. None of the reports describing protozoan parasitic infection has been found except one report of Chalise, (2013) which described *Cryptosporidium* in wild buffaloes of KTWR.

b) Helminths

Helminthes parasites include trematode, cestode and nematode parasites that are found in the bile duct, liver and digestive system of both wild and domestic ruminants.

i) Trematodes

These are leaf like parasites which include *Fasciola* sp., *Paramphistomum* sp., *Schistosoma* sp., *Dicrocoelium* sp., *Gastrothylax* sp., *Fasciola* sp. is the most important species of pathogen that causes fascioliasis. *Fasciola* sp., *Paramphistomum* sp. and *Dicrocoelium* sp. were reported from buffaloes of Dhrampur, Dhanusa district, (Sah, 2015), Pokharathok, Arghakhanchi district (Devi, 2012), cattle of Anarmani V.D.C-2, Jhapa district (Dhakal, 2008) and buffaloes of Satungal, Kathmandu (Mukhiya, 2007). *Schistosoma* sp. has been identified in buffaloes of Pokharathok, Arghakhanchi district (Devi, 2012), in cattle of Anarmani V.D.C-2, Jhapa district (Dhakal, 2008) in buffaloes of Satungal, Kathmandu (Mukhiya, 2007).

ii) Cestodes

Cestodes are ribbon like tape worm such as *Moniezia*, *Taenia* etc. that are found in the gut of ruminants. These parasites require intermediate host for their transmission and some are transmitted through contaminated water and food. *Moniezia* sp. has been reported in buffaloes of Dhrampur, Dhanusa, Nepal (Sah, 2015), in Pokharathok, Arghakhanchi district (Devi, 2012), Satungal, Kathmandu (Mukhiya, 2007), and cattle of Anarmani V.D.C-2, Jhapa district (Dhakal, 2008).

iii) Nematodes

Nematodes are most important and widely prevalent of wild and domestic ruminants. These parasites have direct life cycle and do not involve any intermediate host and are transmitted by fecal contamination of food, water and soil. Some of these nematodes like *Trichostrongylus* sp., *Strongyloides* sp., *Toxocara* sp., *Trichuris* sp., *Haemonchus* sp., *Oesophagostomum* sp., *Ostestagia* sp., and *Capillaria* sp., have been reported from buffaloes of Dhrampur, Dhanusa district, (Sah, 2015), Pokharathok, Arghakhanchi district (Devi, 2012), Satungal, Kathmandu (Mukhiya, 2007), and in cattle of Anarmani V.D.C-2, Jhapa district (Dhakal, 2008).

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Koshi Tappu Wildlife Reserve located in the Saptari and Udaypur districts of Eastern Terai region Nepal (photo-5). KTWR is a protected area in the Terai of Eastern Nepal, covering 176 km^2 of wet lands in the Sunsari, Saptari and Udaypur district. Koshi-Tappu lies on the flood plains of Sapta Koshi River. It is the smallest wildlife reserve of Nepal. It ranges in the altitudes from 75-81 meters and lies between the coordinates 26'39'N., 87'0'E. The climate of this region is Tropical monsoon climate. The Koshi Tappu was declared as a first Ramsar site of Nepal, wetland of international importance under the Ramsar conservation in December 17, 1987 to achieve conservation and sustainable number of wetlands. The KTWR consists of 514 species of plants like *Dalbergia sissoo*, *Bombax ceiba*, *hydrilla* sp. (IUCN). Here about 502 species of birds out of which 12 species are globally threatened and 101 species are nationally threatened (photo-6). The animals like wild water buffalo (*Bubalus arnee*), elephant (*Elephus maximus*), gharial crocodile (*Gavialis gangeticus)* and Dolphin (*Platanista gangetica)* are the main protected animal of this reserve (DNPWC, 2016). As the KTWR was established to conserve the last remaining population of Asiatic water buffalo (*Bubalus arnee*), locally known as Arna (photo- 4).

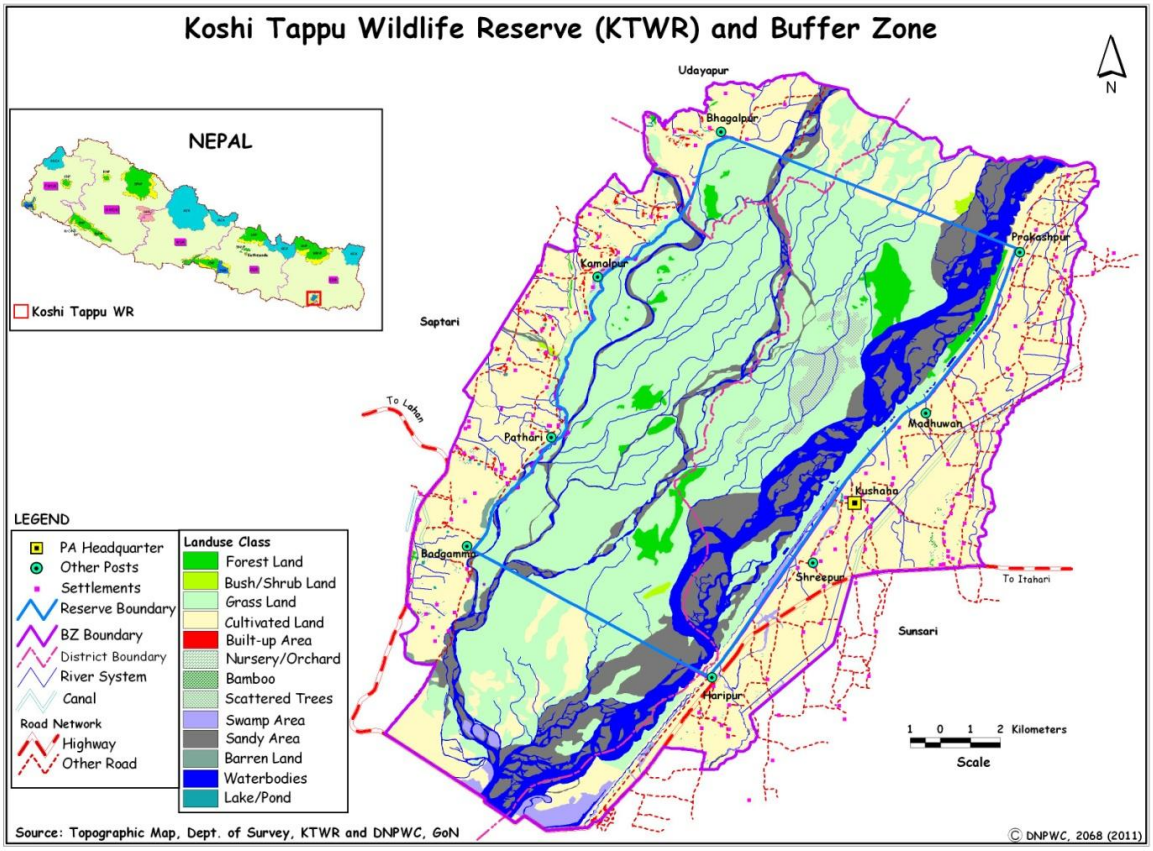

Figure 2: Map of Koshi Tappu Wildlife Reserve
(Source:-DNPWC).

3.2 Materials used

3.2.1 Equipments

I. Electric microscope
II. Ocular 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. Dropper
XVII. Tooth picks
XVIII. Masks
XIX. Glass vials
XXX. Camera
XXI. Gloves
XXII. Spatula

3.2.3 Chemicals

I. Potassium dichromate (2.5%)
II. Iodine solution
III. Saturated Sodium chloride (NaCl) solution
IV. Methylene blue
V. Distilled water

3.3 Study Design

3.3.1 Sample size

160 dung samples were collected from wild buffaloes of KTWR.

3.3.2 Identification of dung in the field:

Preliminary survey was carried out with the help of the staff of KTWR for the identification of major habitat of the wild buffalo, and dung identification. After the preliminary survey, some areas inside the reserve were selected, following the Chatara road - Pashchim Kushaha, Madhuwan and Parkashpur (photo- 7) and foot print of these animals (photo- 8 and 9) for the collection of fresh dung sample. As the wild buffalo drops large quantity of dung because of its volume of diet. The quantity of dung depends on the age and size of animals. The colour of dung depends on the fodder of animals. The colour of dung is dark green, dark brown and brown blakish (photo-10).

3.3.3 Collection and preservation of sample

In the next day early morning, before the collection of sample, dung was carefully examined for the adult parasites, blood mucus, as well as parasitic segments (photo- 11). Collected dung samples were preserved in 2.5% potassium dichromate that helps in maintaining the morphology of protozoan parasites and preventing of some helminth eggs and larvae, mucus as well as parasitic segments. With the help of spatula dung was carefully mixed and about 10 grams of each dung samples were collected in sterile vials.

The study was designed to determine the parasitic infection in wild water buffalo of KTWR as follows:-

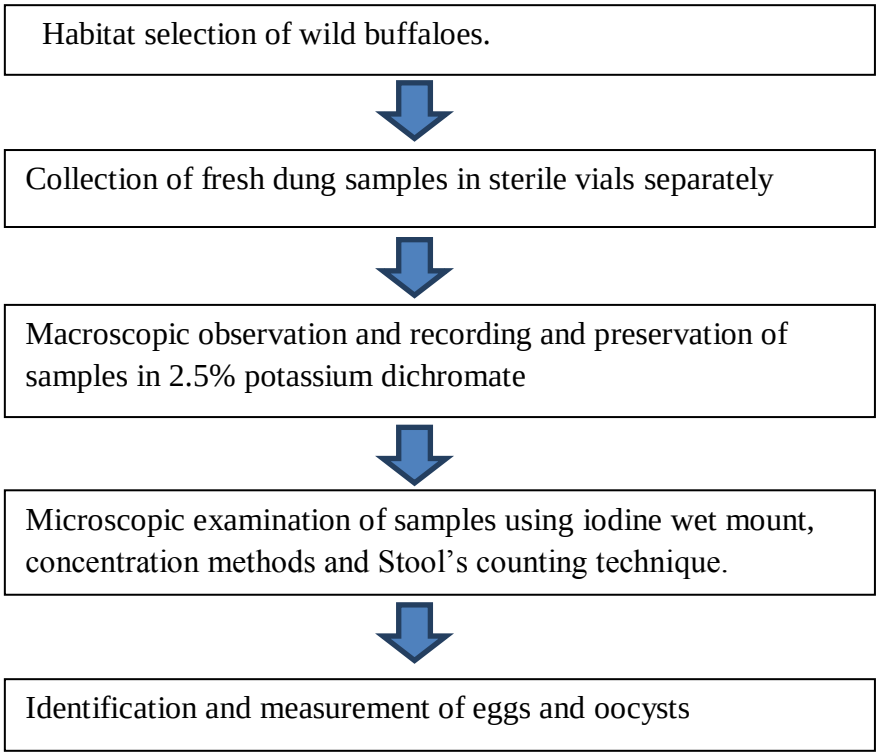

Figure 3: Flow chart showing research outline.

3.4 Microscopic examination

All the samples were examined in laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur, Nepal and Central Veterinary lab. Tripureshor, Kathmandu (photo- 12 and 13). The samples were processed for microscopic examination. The ova/cyst/oocyst and larva of different parasites will be identified according to shape, size and colour as the iodine wet mount, concentration methods (Floatation and sedimentation) and stool's counting technique were applied to determine EPG of dungs (Soulsby, 1982).

3.4.1 Iodine wet mount

About one tooth pick of samples were emulsified in a drop of Lugol's Iodine solution on a clean glass slide and then covered with a clean cover-slip. The smear was examined under electric microscope at 10X and 40X (Soulsby, 1982).

3.4.2 Concentration techniques

Eggs, cysts, and trophozoite were often in such low number in faeces, that they are difficult to be detected in direct smears or mounts. Therefore, these procedures were performed which includes floatation and sedimentation techniques (Soulsby, 1965).

3.4.2.1 Floatation technique

This technique ensures the eggs float in the floatation liquid, which helps to identify the nematode and cestode eggs present in *Bubalus arnee* dung. Approximately 2 gram of faecal samples were put in a beaker and 28 ml of water was added. The sample was grinded lightly with the help of rod or pistle and the solution was filtered by tea strainer. The filtrate solution was poured into a centrifuge tube of 15 ml and centrifuged at 1000 rpm for 5 minutes. The tube's water was replaced with super saturated NaCl solution and again centrifuged. After centrifuged, more saturated NaCl solution was added to develop convex meniscus at the top of the tube and one drop of Methylene blue (to stain) was also added. A cover-slip was placed for a 5 minutes. It was then removed from tube, placed on glass slide and examined microscopically at 10X and 40X. The photographs of eggs and cysts of parasites were taken and identified on the base of shape, shell and size (Soulsby 1982).

3.4.2.2 Sedimentation technique

Saturated Nacl solution was removed gently from the centrifuge tube after examination of the floatation portion and the sediment content was poured into the watch glass and the content was stirred 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 microscopically at 10X and 40X. This technique is used for detection of trematode eggs as the eggs of trematode are bit heavier than the other.

In this way, two slides were prepared from one sample (one from floatation and one from sedimentation), (photo- 14).

3.4.2.3 Modified Stoll's counting technique

The dung sample was first well mixed and then 3 gm. of dung were weighted with the help of a weighing balance and put in 100ml graduated beaker. The beaker was then filled with up to 45 ml marks. The dung was thoroughly mixed with water by a stirrer. The mixture was strained with a tea strainer. The strained mixture was again shaken and 0.15ml of suspension was taken onto a slide and covered with a cover slip. Then the slide was placed under microscope and intestinal parasitic eggs were identified and counted. Total number of eggs of parasites found in the slide was multiplied by 100 to get the eggs per grams of dung (Soulsby, 1982).

3.4.3 Eggs, cysts and larvae size measurement

Eggs and cysts size were measured by using micrometry. The calibration factor was found to be 10.37µm for 10X and 2.588µm for 40X.

3.4.4 Eggs, ocysts and larvae identification

The faecal samples were examined by direct microscopic method and concentration methods (sedimentation and floatation) to detect the presence of intestinal parasitic eggs protozoan and helminths parasites. (Soulsby, 1982; Urquhart *et al.*, 1987; Max *et al.*,

2006). The eggs, ocysts and larvae of parasites were identified from their morphological features such as shape, size and color (Soulsby, 1982).

3.5 Data analysis

Since the study was mainly focused on identification of different intestinal parasites, the Data were analyzed by using MS-Excel 2007 and statistical analysis was performed using "R", version 3.3.1 software packages. Chi-square test was used for statistical analysis of Data. In all cases 95% confidence interval (CI) and $P<0.05$ was considered for statistically Significant difference.

Photo 1: Herd of buffalo resting in water.

Photo 2: Herd of buffalo resting in sand.

Photo 3: Solitary male in water.

Photo 4: Solitary male in field.

Photo 5: Office of the KTWR Western Kushaha, Sunsari.

Photo 6: Species found in KTWR.

Photo 7: Fresh dung found on the Chatara road inside KTWR.

Photo 8: Pugmark of buffalo in moist soil.

Photo 9: Pugmark of buffalo in wet sand.

Photo 11: Collection of dung sample.

Photo 12: Sample examination at Parasitilogical lab. CDZ.

Photo 13: Sample examination at Central Veterinary Lab, Kathmandu.

Photo 14: Sample examination (centrifugation) at Parasitological lab, CDZ.

4. RESULTS

4.1 General prevalence of intestinal parasites.

Out of 160 faecal samples examined, 76 faecal samples were positive for one or more specific intestinal parasites, showing 47.5% prevalence of parasitic infection in wild buffaloes of KTWR.

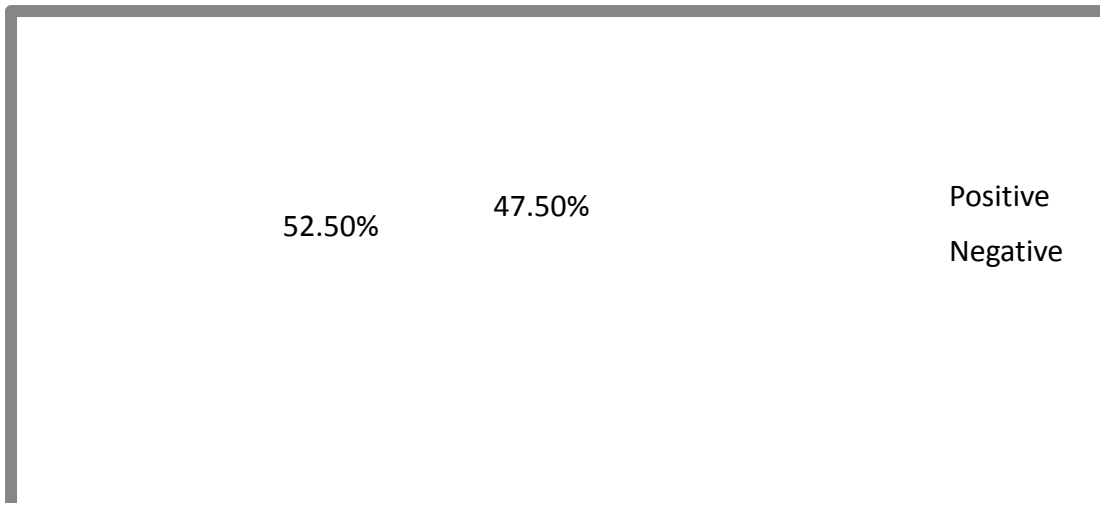

Figure 4: General prevalence of intestinal parasites.

4.2 Protozoan parasites

Among 160 samples 35% dung samples of wild buffaloes were found to be positives for protozoan parasites. Among, identified protozoan parasites, *Eimeria* without micropile 35% showed the comparatively high prevalence than *Eimeria* with micropile 25.62%. There is no significant difference between *Eimeria* without micropile and with micropile ($\chi^2 = 0.82691$, $P > 0.05$).

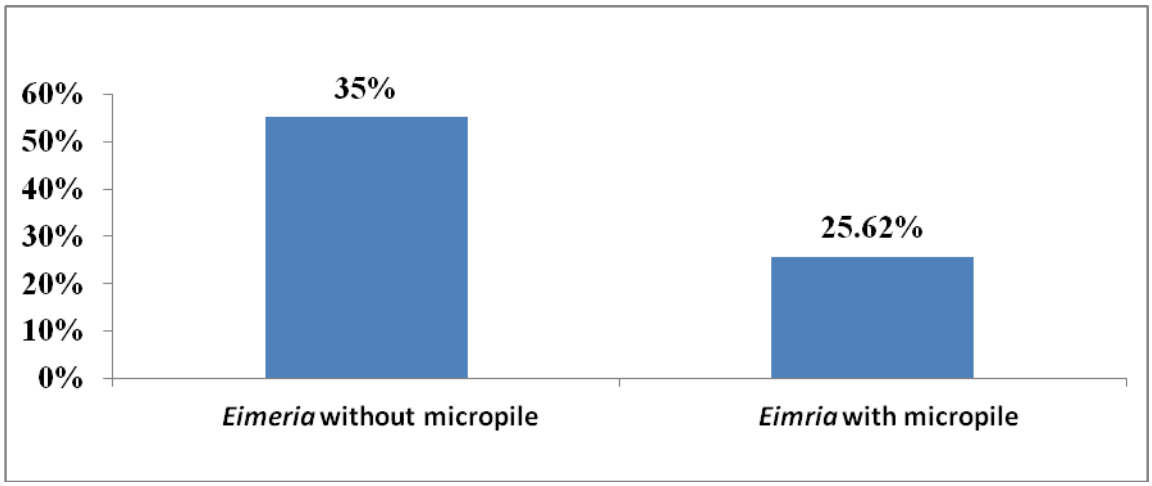

Figure 5: Protozoan parasites without micropile and with micropile.

4.3 Trematodes

Out of 160 samples, prevalence of trematodes observed in wild water buffalo of Koshi Tappu was 19 during the study only one Trematode parasitic infection was recorded in present study i.e. 37(23%) prevalence of *Paramphistomum*.

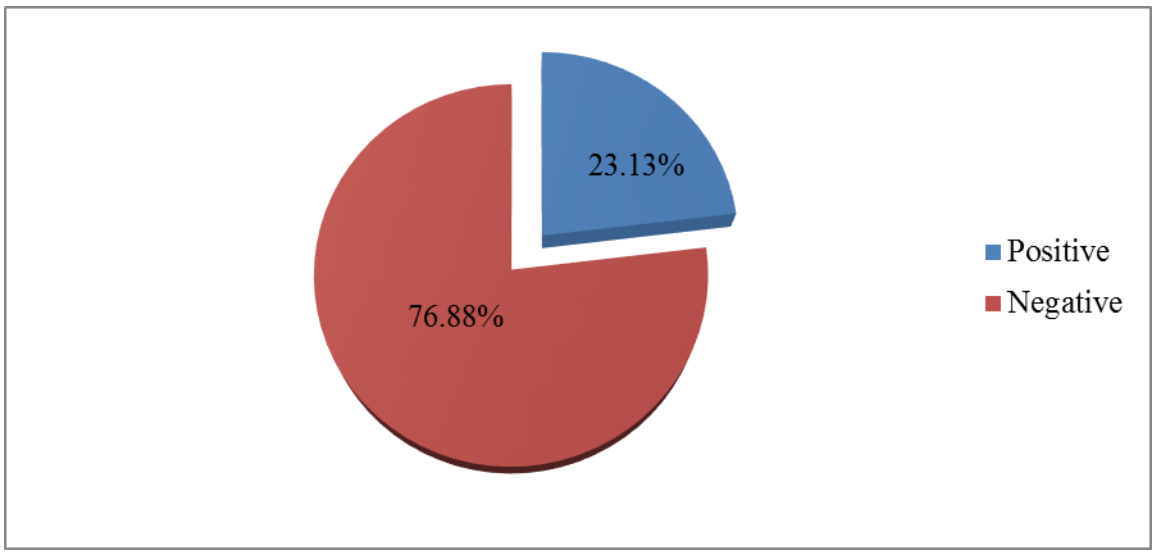

Figure 6: Trematode parasite in wild buffalo of KTWR.

4.4 Nematodes:

In 160 samples 62 samples were found to be positives for Nematodes. Among them *Trichostrongylus* 35% was highest prevalent in Wild water buffalo of Koshi Tappu Wild Life Reserve followed by *Haemonchus* 29.37, *Toxocara* 26.25% *Strongyloides* 23.75% *Oxyuris* like eggs 21.88%. Nematode parasitic infection in wild water buffaloes were not found to be statistically significant (χ^2= 3.0208, P> 0.05).

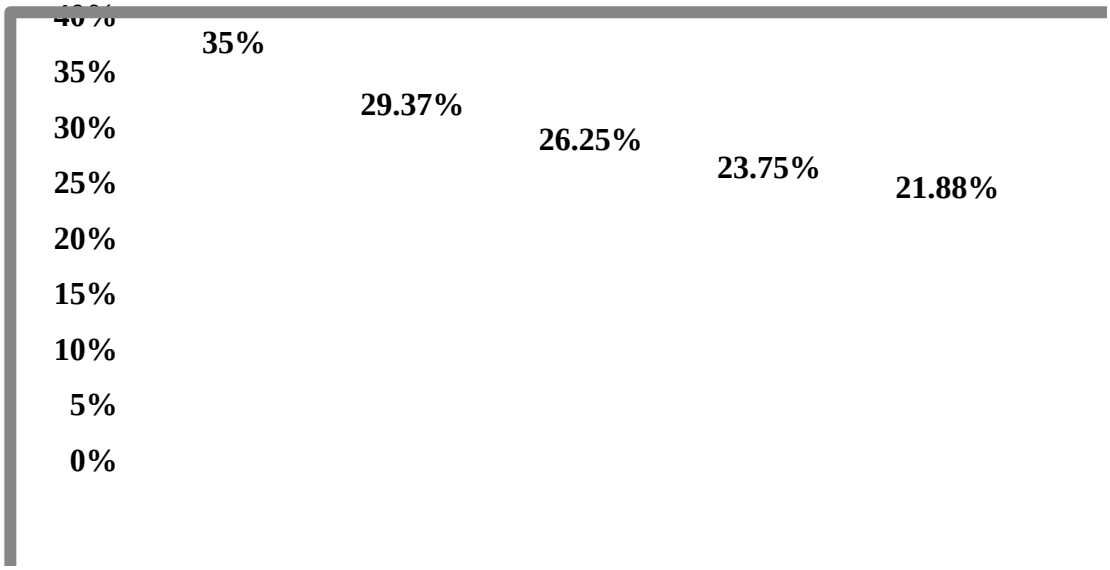

Figure 7: Prevalence of specific nematode parasites in wild buffalo of KTWR.

4.5 Mixed infection:

Out of 160 samples 76 samples were found to be infected with different intestinal parasites of wild buffalo of KTWR. Double infection showed the highest rate (38%) among other three infections. Multiple infection (28.94%), triple infection (22.36%) and single infection (11.84%) was encountered in this study. The result revealed the significant difference in mixed parasitic infection ($\chi^2 = 11.806$, $p<0.05$).

Table No: 1 Mixed parasitic infection in wild water buffaloes.

S.N	Types of infection	Total (%)
1	Single	9 (11.84%)
2	Double	29(38.15%)
3	Triple	17(22.36%)
4	Multiple	22(28.94%)

4.6 Intensity of Parasites:

In Koshi Tappu Wildlife Reserve, as the heavy parasitic infection was considered in those samples which have 6 or more ova or oocyst observed per field. Among the total positives sample (76), *Eimeria* without micropile (1.25%) and *Eimeria* with micropile (0.63%) belongs to Sporozoa; *Trichostrongylus* (1.88%), *Haemonchus* (0.63), *Toxocara* (0.63%), *Strongyloides* (0.63%) and *Oxyuris* like eggs (1.25%) belongs to Nematode revealed heavy infection. While maximum numbers of wild buffaloes were infected with light infection which was considered<2 ova or oocyst observed per field. Similarly, less number of wild buffaloes was found to be infected with mild i.e.2-4 ova or oocyst per field. Moderate number of infection was found to be less in compared to light and mild number of infection i.e. 4-6 ova or oocysts per field than mild infection.

Table No: 2. Intensity of parasitic infection

Class	Parasites	Light (+)	Mild (++)	Moderate (+++)	Heavy (++++)
Sporozoa	*Eimeria* without micropile.	24(15%)	19(11.88%)	11(6.88%)	2(1.25%)
	Eimeria with micropile.	18(11.25%)	16(10.1)	6(3.37%)	1(0.63%)
Trematode	*Paramphistomum* sp.	19(11.88%)	15(9.38%)	3(1.88)	
Nematode	*Trichostrongylus* sp.	29(18.13%)	17(10.63%)	7(4.38%)	3(1.88%)
	Haemonchus sp.	22(13.75%)	21(13.13%)	3(1.88%)	1(0.63%)
	Toxocara sp.	14(.75%)	22(13.75%)	2(1.25%)	1(0.63%)
	Strongyloides sp.	17(10.63%)	18(11.25%)	2(1.25%)	1(0.63%)
	Oxyuris like eggs	13(8.13%)	18(11.25%)	2(1.25%)	2(1.25%)

4.7 Distribution of intestinal parasites

In the present study, out of 160 faecal samples 62 (38.75%) faecal sample is positive for nematode which is followed by protozoa 56 (35%) and trematode 19 (11.87%). Statistically there is significance different between distribution of intestinal parasites (χ^2= 12.48, P>0.05).

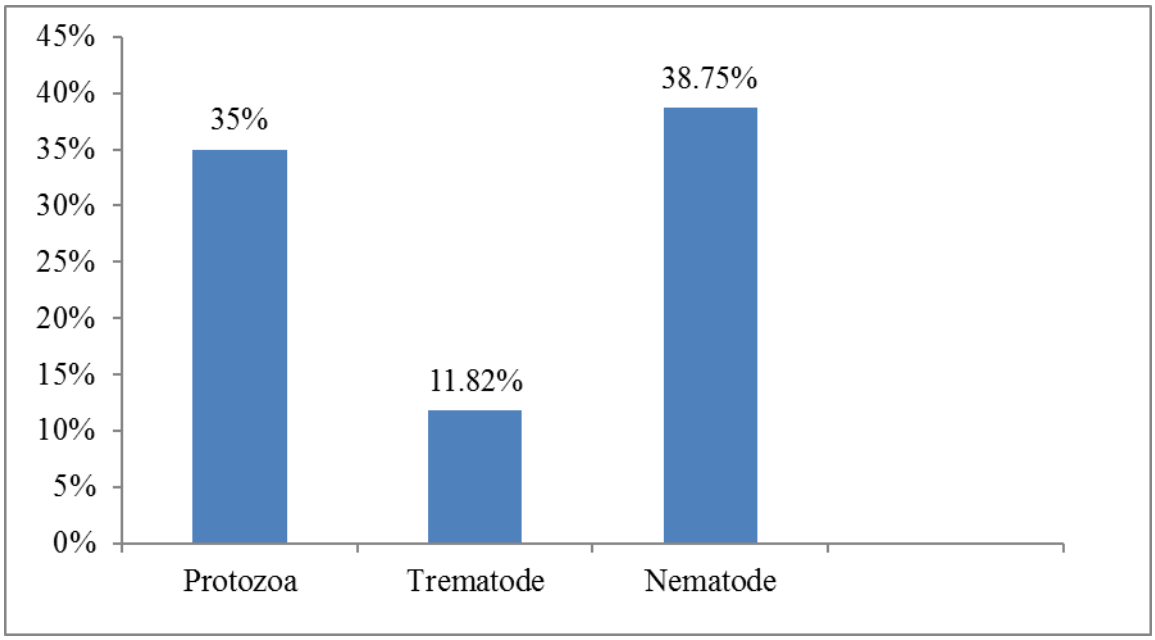

Figure 8: Distribution of parasites by group.

4.8 Diameter of Eggs/oocyst of different intestinal parasites of wild buffalo.

1. The diameter (length by width) of eggs/oocyst of different gastrointestinal parasites of wild buffalo which were measured during this study were given below-

2. *Eimeria* sp.
   The egg of oocyst of *Eimeria* sp was 27.16±12.388µm according to without micropile (photo- 15, 16 and 17), while 31.7±9.9 µm with micropile (photo- 18, 19 and 20). An egg of *Eimeria* are small in size and it contains morula which is located centrally or sub centrally or completely filled up, it is pink in color and the egg of *Eimeria* may contain micropile which is occurred one side of egg.

3. *Paramphistomum* sp.
   Eggs are 146.22±8.05 µm in size. The adult is conical in shape with operculum in one pole. The anterior end of egg is tapering and the posterior end is broad, eggs are pink in color due to its on hemoglobin (photo- 21).

4. *Trichostrongylus* sp.
   An egg are77±7.73 µm in size, which is oval or kidney bean shaped, with thin and transparent outer chitinous layer and wrinkled inner membrane, it is bilaterally symmetrical, colorless, with embryonic mass and multi segmented and varies from 16-32 in number. The space between the egg shell and embryonic mass is relatively conspicuous. Some of the eggs are rounded in one side than other (photo- 22).

5. *Haemonchus* sp.
   Eggs are thin shelled hyaline, oval, elongated, larger and rounder in shape which measure75.7±6.47µm in size. Eggs are with embryonic mass and multisegmented. It is resemble with those of *Trichostrongylus* when deposited (photo- 23).

6. *Strongyloides* sp.
   Eggs are small, measure 42.14±14.87µm in size oval with thin shelled, ellipsodal or round edges which contain fully developed larvae and that larva can be seen under low power on microscope (photo- 24,) along with the eggs of *Strongyloides*, few larvae were observed (photo- 25, 26, 27 and 28).

7. *Taxocara* sp.
   Eggs are 34.79±7.10 µm in size nearly spherical, yellowish brown, unsegmented and have finely pilled albuminous layer (photo- 29).

8. *Oxyuris* like eggs.
   Eggs are 46.58±2.14 µm in size, oval and bean shaped having segmented embryonic mass surrounded by sticky fluid (photo- 30).

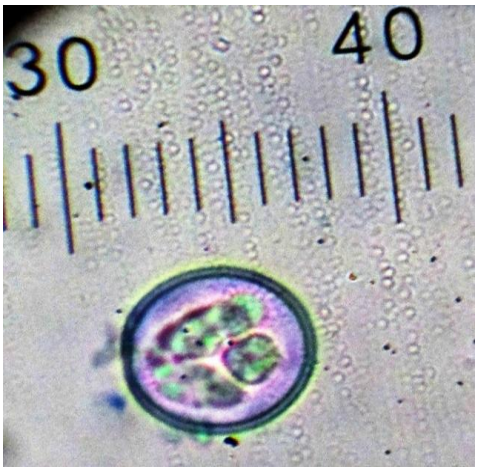

Photo 15: Oocysts of *Eimeria* sp. without micropile; (16µm).

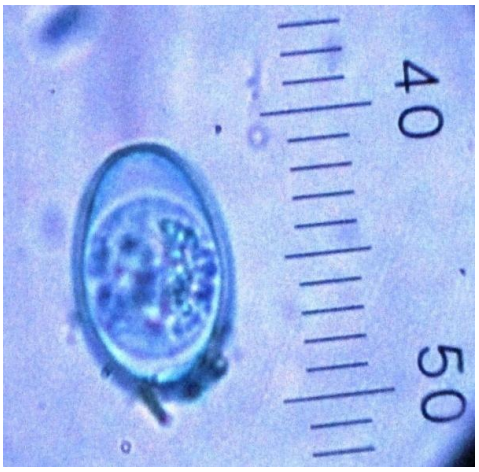

Photo 16: Oocysts of *Eimeria* sp. without micropile; (23µm).

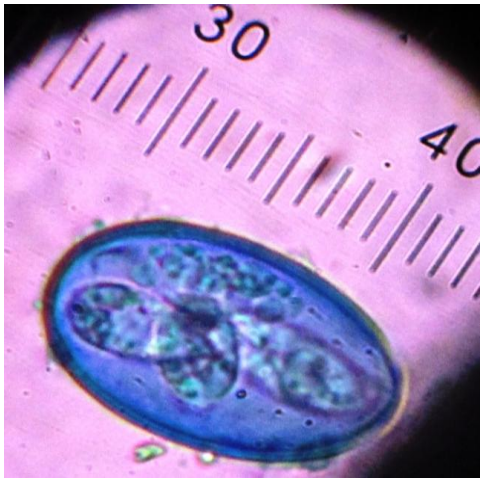

Photo 17: Oocysts of *Eimeria* sp. without micropile; (36µm).

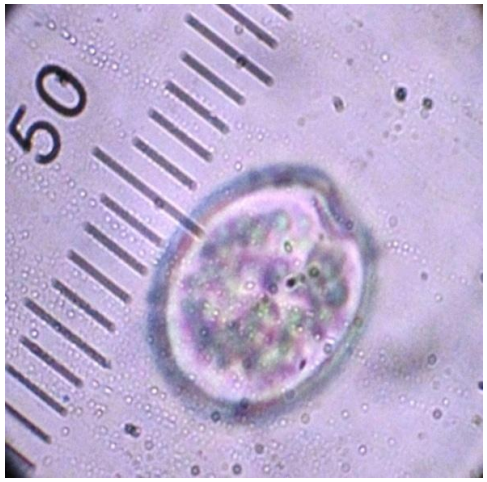

Photo 18: Oocysts of *Eimeria* sp. with micropile; (23µm).

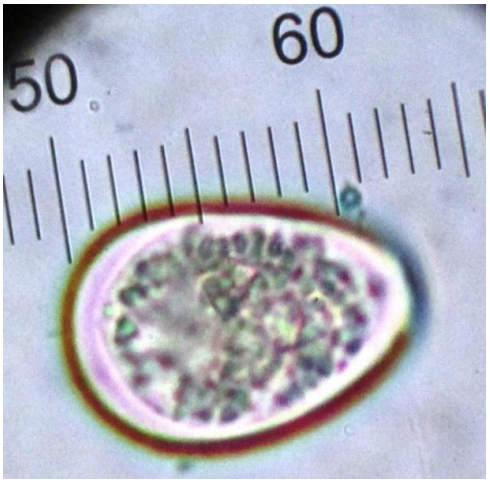

Photo 19: Oocysts of *Eimeria* sp. with micropile; (34µm).

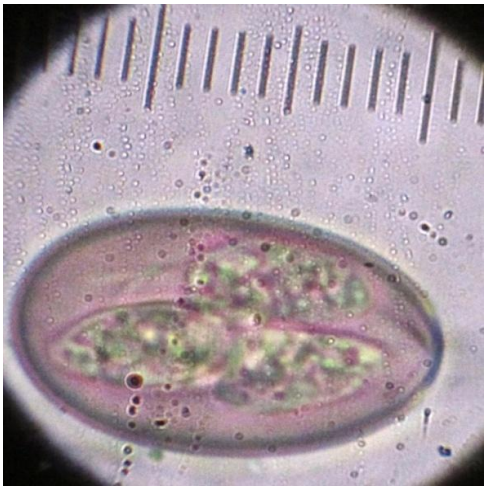

Photo 20: Oocysts of *Eimeria* sp. with micropile; (44µm).

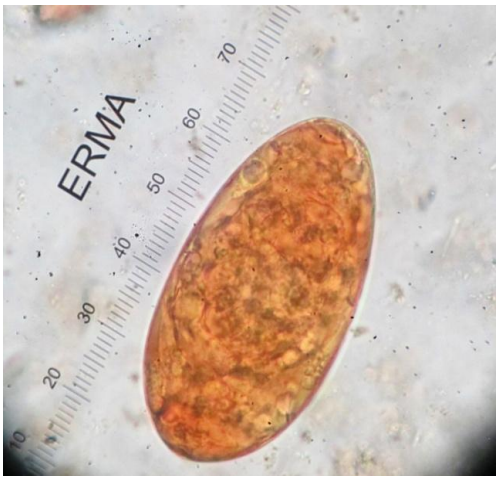

Photo 21: *Paramphistomum* sp. (134µm).

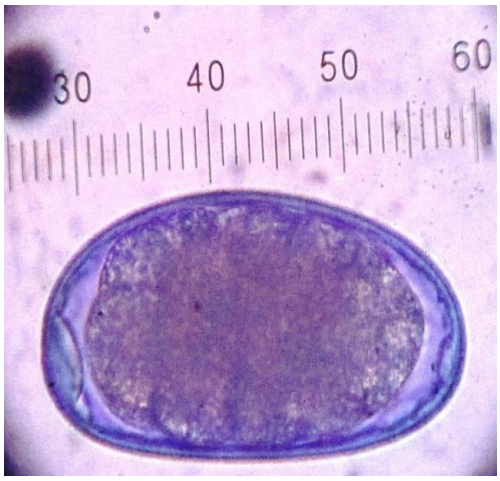

Photo 22: *Trichostrongylus* sp. (85µm).

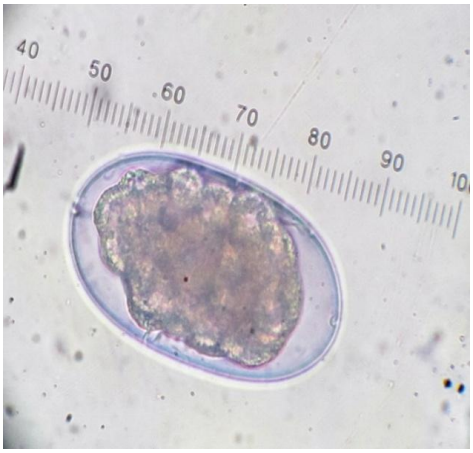

Photo 23: *Haemonchus* sp. (90µm).

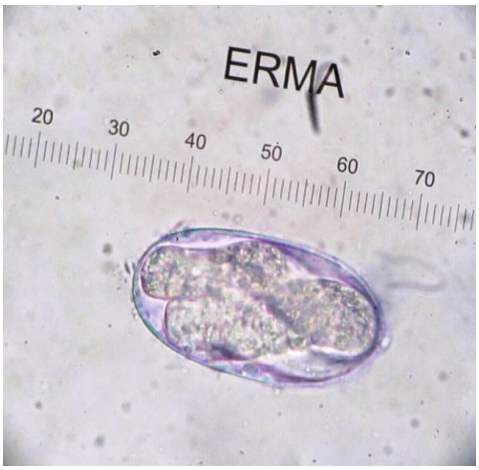

Photo 24: *Strongyloides* sp.

Photo 25: Larva of *Strongyloides* (Whole body).

Photo 26: Larva of *Strongyloides* (Mouth part).

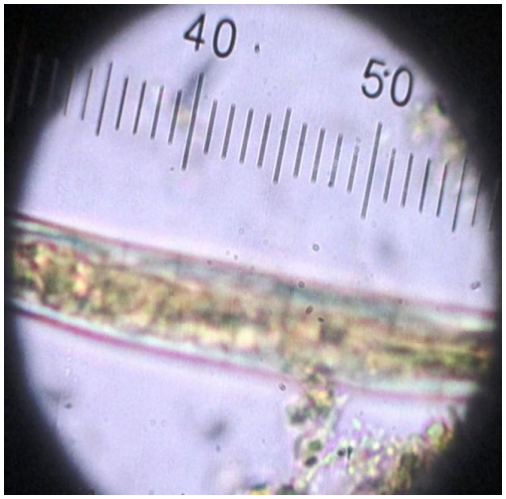

Photo 27: Larva of *Strongyloides* (Middle portion).

Photo 28: Larva of *Strongyloides* (Tail portion).

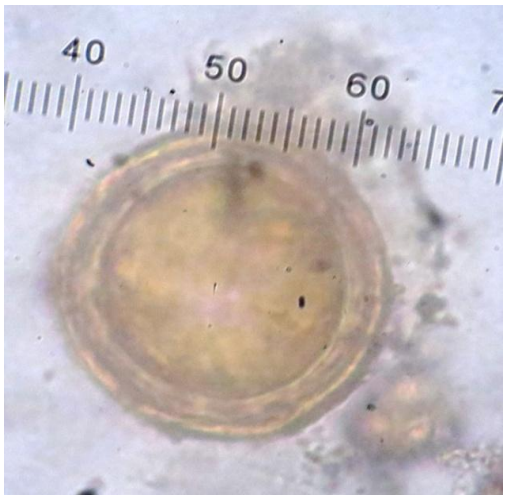

Photo 29: *Toxocara* sp. (85µm).

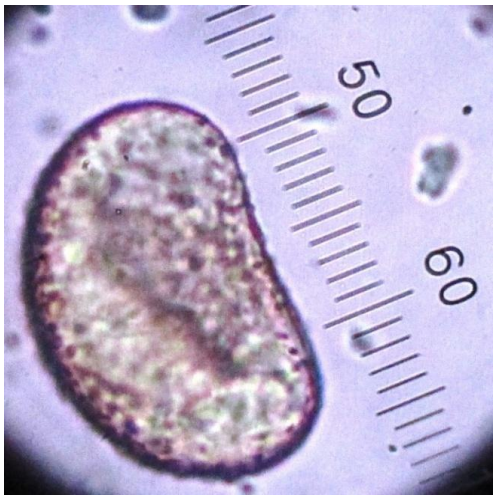

Photo 30: *Oxyuris* like egg. (90µm).

5. DISCUSSION

Wild life reserve is an area of land managed to conserve wildlife habitat. Wild water buffalo are associated with wet grasslands, swamps, and densely vegetated river valleys. Different kinds of disease and parasites are transmitted by domestic livestock. The animals suffer from a variety of infectious and non-infectious diseases, particularly that of parasitic origin (Iqbal *et al*., 2000; Akhter and Arshad, 2006). Change in environment and living conditions from freedom to captivity influences the animal's ecology and might increases the susceptibility to parasitic infections (Gossensa *et al.*, 2005; Jyoti *et al*., 2006).

The common parasitic disease of buffaloes includes gastro-intestinal helminthiasis, coccidiosis, fascioliosis and mange (Griffiths, 1974). Moreover, some helminthes of buffaloes are also transmissible (directly or indirectly) to humans where they can cause significant clinical diseases, such as schistosomiasis and fascioliasis in a number of countries (Wang *et al*., 2006; Tum *et a*l., 2007). In excess of helminthes, buffaloes are suffered from various intestinal protozoan infections also *(*Azam *et al*., 2002; Nalbantoglu *et al.*, 2008).

In the present, study the gastrointestinal parasites were found to be positive (47.5%) out of 160 dung samples. This prevalence rate was higher than 44%, 38.70%, 40.20%, 13%, 20.45% and 34.1% by Kobak *et al*., 2012 from Poland, Mir *et al*., 2013; Swai, 2013 from Tanzania, Sreedevi *et al*., 2014; Ashutosh, 2011; Muraleedharan, 2005 from India, but it was low as compared to 61.02%, 70.45%, 84.30% GI parasites by Mamun *et al*., 2011 from Bangladesh, Marskole *et al*., 20116 from India and Biswas, 2012 from Bangladesh. This variation in finding results might be due to the difference in the number of fecal sampled examined; samples had been taken from different host. In the present study, 35% fecal samples were found to be positive for protozoan parasites in wild buffalo of KTWR. The present prevalence was found to be less than 75% protozoan reported by Nalbantoglu *et al*., 2008 in Turkey while higher than the reports of Sreedevi *et al*., 2014 from India and Swai *et al*., 2013 from Tanzania who revealed 6.48% and 8.13% respectively. The variations might be due to the difference in sample size, selection of samples, techniques of sample collection, period and place of study, environmental factors breed of the buffaloes etc.

From the economic and sanitary points of view, coccidian parasites are the most prevalent among protozoa on the basis of morphological structure *Eimeria* can be differentiating in two types i.e. with micropile and without micropile but according to the previous reports, *Eimeria* had been differentiated into species level using culture technique. In the present study, *Eimeria* was found to be 35% in wild buffalo of KTWR, which showed the lowest prevalence rate as compared to 54.55% in buffalo calves by Jyoti *et al*., 2014 and 37.40% by Biswas, 2012 and higher than 29.55%, 1.15%, 8.1% and 16.10% recorded by Marskole *et al*., 2016; Sreedevi *et al*., 2014; Swai *et al*., 20113 and Rana *et al*., 2011 respectively. The diameter of oocyst of *Eimeria* sp. was 31.7± 9.9um according to

micropile while 27.16±12.39um without micropile observed in many faecal samples of wild buffaloes of KTWR.

Trematode parasites cause watery diarrhea, weakness, weight loss, decreased milk production, reduced product quality, mortality and other secondary infections (Soulby1982). The prevalence of trematodes observed in KTWR was (11.87%) which is similar to the report of Swai *et al*., 2013 who had revealed 12.2%. The present prevalence rate (11.87%) was found to be more than 2.11% and 7.1% trematode reported by Cringoli *et al*., (2009) and Condoleol *et al.*, (2007) but less as compared to previous prevalence rates 64.44%, 60.75% and 20% encountered by Sah, 2015; Saha, 2013 and Raza *et al*., 2010. In this study, only one species of treamatode, *Paramphistomum* was reported (11.87%) from the wild buffalo of KTWR. This prevalence rate was much similar to 11.76%, 11.06% and 11%observed by Devi, 2012; Swarnakar *et al*., 2015 and Kobak *et al*., 2012 but lowered than 26.98%, 17.66%, 60.75%,45%,25.40%,29.24% ,20% and 5.64% than encountered by Alam *et al*., 2016; Sah, 2015; Saha *et al.*, 2013; Gupta *et al.*, 2012; Biswas, 2012; Mamun, 2011; Raza *et al.*, 2010 and Mukhia, 2007 while higher than 5.27%,4.9%,10%and 7.1% observed by Marskole *et al*., 2016; Swai *et al.*, 2013, Condoleol *et al*., 2007 and Haridy *et al*., 2006. This difference could be due to the availability of the suitable intermediate host as well as management practices of different country. The size of egg was found to 146.22±8.05 µm.

No any cestodes was recorded in the present study in wild water buffalo of KTWR which supported the findings the result of Saha *et al.*, 2013; Mamun *et al.*, 2011; Liu *et al*., 2009; Azam *et al*., 2002 and Bachal *et al*., 2002. This is due to that buffalo are usually raised in animal houses and seldom accessible to intermediate hosts of cestodes.

Most of the studies on GI nematode ecology in cattle have concluded that climatic conditions play an important role in the survival and transmission of parasites, eggs and larvae (Rivera *et al .,1983*). The nematode species identified in the faecal examination of wild buffaloes of KTWR includes *Trichostrongylus* sp., *Haemonchus* sp., *Toxocara* sp., *Strongyloides* sp. and *Oxyuris* sp. The wild buffaloes of KTWR were found to be infected with 38,75% nematode parasites which was lower than 46.6% reported by Khan *et al.*, 2007 but higher than 28.57%,26%, 30%, 10% and 33.% nematodes observed by Sah, 2015; Patel, 2015; Swai *et al.*, 2013; Kobak *et al.*, 2011 and Cringoli *et al*., 2009 respectively.

Trichostrongylus sp. is common parasitic round worms of cattle and the various sp of these adult worms are mainly found in the small intestine, cecum and stomach. This parasite have a direct lifecycle and livestock become infected after ingesting the larva while grazing or with contaminated soil, as the larva can survive in the environment and can remain infective for up to six months (Junquera, 2016). *Trichostrongylus* sp. has been reported in Tanzania (Swai *et al*., 2013), Pakistan (Khan *et al*., 2007; Azam *et al*., 2002), Turkey (Guzel *et al*., 2013) and other various countries. *Trichostrongylus* eggs

(77±7.73µm) were isolated with prevalence rate 35% in wild water buffalo of KTWR in the present study. The prevalence rate 36.36% obtained by Guzel *et al*., 2013 was comparatively similar to the present study. The prevalence rate 21.19%, 20.3%, and 21.03% obtained by Azam *et al.,* 2002; Swai *et al*., 2013and Khan *et al*., 2007 were less than the present study. On the other hands, the much lower prevalence rates i.e. 2.7%, 10%, 9.33%, 5.88% and 1.90 recorded by Wahyudin *et al*., 2016; Sah, 2015; Devi, 2012, and Mukhia, 2007 respectively than present study.

Haemonchus sp. is one of the most important pathogens for water buffaloes producing a disease known as haemonchiasis which can cause anemia, growth loss, edema, emaciation, etc. and even death . It was recorded in Pakistan (Azam *et al*., 2002; Khan *et al*., 2007), Turkey (Guzel *et al.,* 2013), Bogor, Demark East Java (Wahyudin *et al.,* 2016), including from Nepal (Mukhia, 2007; Devi, 2012; Sah, 2015). I n the present study 29.37% of *Haemonchus* with average size (75.7±6.47µm in wild water buffalo of KTWR. The rate of (18.18%) and (16.30%) of *Haemonchus* recorded by Guzel *et al*., 2013 and Khan *et al*., 2007 was comparatively similar to 18% *Haemonchus* in wild water buffalo of KTWR but less than 24.1% and 26% recorded by Wahyudin *et al.,* 2016 and Aken *et al*., 2000 respectively. The rate 13.3%, 10.8%, 8.47%, 0.66%, 1.76%, and 1.14% as recorded by Wahyudin *et al.,* 2016; Azam *et al*., 2002; Sah, 2015; Devi, 2012, and Mukhia, 2007 were lowered than present study. Although the parasite in the common nematode parasites prevalence rate reported was much varied that could be due to the variation in sample size taken during study.

Strongyloide causes strongyloidiasis. *Strongyloides* worms may be present in a host as a parasitic and free living form in the soi,l these parasite can penetrate through skin by filariform larva. *Strongyloides* sp. has been reported from water buffaloes in India (Jyoti *et al.,* 2014; Patel *et al*., 2015) with the prevalence rate 28.45% and 46.39% respectively. These rates were comparatively higher than the present study. The present prevalence of *Strongyloides* was 23.75% in wild water buffaloes of KTWR that was less than the earlier reports of Mukhia, 2007; Khan *et al.,* 2007; Condoleol *et al.,* 2007; Bacha *et al.,* 2014, Swarnakar *et al.,* 2015; Sah, 2015; Alam *et al*., 2016 who recorded as 4.19%,7.72%,3.1%, 1.40%,13.52%,2.0%, 4.1%, 2.59%, 2.6%, o.49%, 2.66%,and 3.85% respectively. This could be due to the difference in the habitat and physiological condition of the buffaloes. This parasitic eggs size (42.14± 14.87µm) was reported in the present study.

Toxocara vitulorum is an important intestinal nematode that commonly infects ruminants such as cattle and buffalo in tropical and sub-tropical countries (Roberts 1990) Bovine toxocariosis is the most economically important diseases, affecting animals mainly at a young age due to maternal infection, and frequently causing death (Devi et al. 2000). *Toxocara sp*. has been reported from water buffalo from India (Swarnakar *et al.,* 2015; Patel *et al*., 2015; Sreedevi *et al*., 2014; Singh *et al*., 2014), Central Cambodia (Dorny *et al*., 2015), Bangladesh (Biswas, 2012, Khan *et al*., 2007, Mamun *et al*., 2011and Nepal (Sah, 2015; Devi, 2012; Mukhia, 2007), with prevalence rate i.e.0.099%,7.33%, 12.4%,13.14%, 2.54%, 5.66%, 34.11% and 22.90% respectively. The

prevalence rate of *Toxocara* was higher than 26.25% in KTWR as compared to all previous finding rates except 34.11% which is higher than 26.25%. This result showed that the water buffalo of conservation area were highly infected with *Toxocara* eggs. *Toxocara* egg with size (34.79±7.10µm) was found to be distributed in many faecal samples of the present study.

About 21.88% of *Oxyuris* like egg has been reported from wild water buffaloes of KTWR with size 46.58±2.14µm.The evidence of this parasitic egg was not reported before in any wild water buffalos during my study period.

In the present study, the mixed parasitic infections were found to be more common in wild water buffalo due to common pasture land and contaminated water bodies. Single infection was observed11.84%, double 38.15%, triple 22.36% and multiple 28.94% in wild buffalo of KTWR which is different with Swai *et al*., 2013 who found single infection 52%, and concurrent infection with two, three, four and five parasites were recorded 19%, 11.9%, 14.3% and 2.3% respectively. 3.17% and 22.73% mixed infection revealed by Sreedevi *et al*., 2014; Marskole *et al*., 2016 were less than the present study 28.94% in wild buffaloes of KTWR. The present study on single infection 11.84% was less than the 47.73% recorded by Marskole *et al*., 2016.

The intensity of different parasites in wild buffalo of KTWR was observed in this study. According to the results, maximum numbers of wild water buffalo were found to be infected with light infection which is asymptomatic condition and cannot cause the diseases in animals while less numbers of wild water buffalo were infected with heavy infection revealed by *Trichostrongylus, Eimeria, Haemonchus* and *Paramphistomum* in KTWR. The heavy infection is symptomatic condition and can cause serious disease in animals.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The present study showed that overall prevalence of intestinal parasites of wild buffaloes (*Bubalus arnee*) was found to 47.5% with higher prevalence rates of nematodes (38.75%) followed by protozoa (35%) and trematode (11.87%) but no cestode was recorded during the study period. Seven different parasitic species were revealed in wild buffalo of KTWR such as *Eimeria* among protozoa; *Paramphistomumm* among trematode; *Trichostrongylus, Haemonchus, Toxocara, Strongyloides* and *Oxyuris* like eggs. Out of all these identified intestinal parasites, *Trichostrongylus* and *Eimeria* showed the highest prevalence in wild buffalo of KTWR. As *Eimeria* (1.25%), *Trichostrongylus* (1.88%) *Haemonchus* (0.63%), *Toxocara* (0.63%), *Strongyloides* (0.63%) *and Oxyuris* like egg (1.25%) revealed heavy infection in wild buffalo of KTWR. The study indicated that wild buffalo of KTWR were highly infected to intestinal parasites. Therefore, sustainable ways is implement to control policy of the parasitic infection and further studies need to be designed and for the health and conservation of wild buffalo of KTWR.

6.2 Recommendations

Based on the outcome of the present study, the following recommendations have been made to reduce the risk of intestinal parasitic threat in the conservation of wild buffalo (*Bubalus arnee*).

- Proper pasture management programmed should be conducted on Koshi Tappu Wildlife Reserve for the parasites control.
- Further study for the identification and verification of *Oxyuris* like eggs needs to be carried out.
- Detailed study on seasonal, age and sex wise prevalence of intestinal parasites of wild water buffaloes should be carried out.

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