PREVALENCE OF GASTRO-INTESTINAL PARASITES OF RHESUS MACAQUE (*Macaca mulatta* Zimmermann, 1780) AND HANUMAN LANGUR (*Semnopithecus entellus* Dufresne, 1797) IN DEVGHAT, CHITWAN, NEPAL

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Nepal

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DECLARATION

I hereby declare that the work presented in this thesis entitled **"Prevalence of gastro-intestinal parasites of Rhesus Macaque (*Macaca mulatta* Zimmermann, 1780) and Hanuman Langur (*Semnopithecus entellus* Dufresne, 1797) in Devghat, Chitwan, 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).

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RECOMMENDATION

This is to recommend that the thesis entitled **"Prevalence of gastro-intestinal parasites of Rhesus Macaque (*Macaca mulatta* Zimmermann, 1780) and Hanuman Langur (*Semnopithecus entellus* Dufresne, 1797) in Devghat, Chitwan, Nepal"** has been carried out by **Mr. Pujan Prasad Adhikari** 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.

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LETTER OF APPROVAL

On the recommendation of supervisor **Lecturer Mr. Pitambar Dhakal**, this thesis submitted by **Mr. Pujan Prasad Adhikari** entitled **"Prevalence of gastro-intestinal parasites of Rhesus Macaque (*Macaca mulatta* Zimmermann, 1780) and Hanuman Langur (*Semnopithecus entellus* Dufresne, 1797) in Devghat, Chitwan, 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.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by **Mr. Pujan Prasad Adhikari** entitled **"Prevalence of gastro-intestinal parasites of Rhesus Macaque (*Macaca mulatta* Zimmermann, 1780) and Hanuman Langur (*Semnopithecus entellus* Dufresne, 1797) in Devghat, Chitwan, Nepal"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

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Pujan Prasad Adhikari

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LIST OF ABBREVIATIONS

Abbreviated form	**Details of abbreviations**
µm	- Micrometre
AIDS	- Acquire Immune Deficiency Syndrome
ASP	- American Society of Primate
BPRCWR	- Barbados Primate Research Centre and Wildlife Research
BS	- Bikram Samvat
CDZ	- Central Department of Zoology
CI	- Confidence Interval
DADC	- Devgaht Area Development Committee
df	- Degree of freedom
DNPWC	- Department of National Park and Wildlife Conservation
et al.	- Et alia (and others)
GI	- Gastrointestinal
H_0	- Null hypothesis
H_1	- Alternative hypothesis
HIV	- Human Immuno-deficiency Virus
HMG	- His Majesty's Government
IUCN	- International Union for Conservation of Nature and Natural Resources
$K_2Cr_2O_7$	- Potassium dichromate
PLoS	- Public Library of Science
rpm	- Rotate per minute
SAZARC	- South Asian Zoo Association for Regional Co-operation.
SPG	- Specific Gravity
SSC	- Singapore Science Centre
SUFFREC	- Student Forum for Forestry Research and Environment Conservation
TU	- Tribhuwan University

ABSTRACT

Present investigation was undertaken to study the prevalence of gastrointestinal parasites in monkeys at Devghat, Chitwan. Altogether 93 fresh faecal samples were collected from 73 Rhesus Macaque belonging to five troops and 20 Hanuman Langur of two troops. About 10 gm of faecal material was collected in sterile vials with 2.5% potassium dichromate solution. These samples were examined microscopically by faecal concentration methods viz. floatation technique and sedimentation technique, in the laboratory of Central Department of Zoology, T.U. Kirtipur. Out of 93 samples, 69 (74.20%) were found positive for single or multiple species of parasites. Altogether, ten species of parasites including seven helminth (52.68%) and three protozoa (40.86%) were identified based on morphological characteristics of their eggs and cysts under light microscopy. The most commonly detected parasites were *Balantidium coli* (27.95%) followed gradually by *Eimeria* sp. (16.12%), *Entamoeba* sp. (13.97%), *Trichuris* sp. (23.65%), *Ascaris* sp. (11.82%), *Strongyloides* sp. (10.75%), *Oesophagostomum* sp. (5.37%), Hookworm sp. (3.22%), *Trichostrongylus* sp. (3.22%) and *Hymenolepis* sp. (1.07%). Single, double, triple and multiple species of parasites were found in 36.55%, 29.03%, 6.45% and 2.15% samples respectively. Comparatively, Rhesus Macaques were more infected (75.34%) than Hanuman Langur (70%). But this difference was not statistically significant P>0.05. Ten parasitic species were recorded from Rhesus Macaque but eight species were recorded from Hanuman Langur. In Hanuman Langur Hookworm sp. and *Hymenolepis* sp. were not detected. Six species of nematode (58.06%) parasites and one species of cestode (1.07%) parasite (*Hymenolepis* sp.) were found but trematode parasite was not found at all. *Hymenolepis* sp. has been reported for the first time in Nepal from Rhesus Macaque.

1. INTRODUCTION

1.1 Background

Primates are the highest order of mammals characterized by superior development of brains relative to other mammals, having dexterous hands and feet, binocular vision. It includes lemurs, monkeys, apes, humans and other similar forms (Tattersall, 1993). These features are more common and developed in monkeys and apes but noticeably less so in lemurs and lories. They evolved from arboreal animals and many species live most of their lives in trees. Most primate species live in tropical rain forests. The number of primate species within tropical areas has been shown to be positively correlated to the amount of rainfall and the amount of rain forest area (Reed and Fleagle, 1995). Till now, there are 633 identified species of primates and of those 54 % of them are threatened, endangered, or critically endangered (IUCN/SSC, 2012). Among them 25 primate species are considered to be the most endangered worldwide (Schwitzer *et al*., 2015).

The order Primates was systematically divided into two main groups: prosimians and anthropoids (simians). Prosimians have more like those of the earliest primate's features and include the lemurs of Madagascar, lorisoids and tarsiers. Simians include monkeys, apes and human. All the monkeys are excellent climbers and the most are primarily arboreal. Almost all of them live in tropical or sub-tropical climates. Their faces are usually flat and rather human in resemblance, their eyes point forward and they have greater degree of colour vision. Almost all monkeys have flat nails, hands and feet are highly developed for grasping, thumbs are opposable and habitually sit in an erect posture. They live in troops of up to several hundred individuals and travel about in search of food, having no permanent shelter. Female has a monthly reproductive cycle and mating may occur at any time but in some species mating is seasonal. Usually only one baby is born at a time and cared by the mother for a long period.

There are two large groups or superfamilies of monkeys: Old World monkeys (Cercopithecoidea) and New World monkeys (Ceboidea). Old World monkeys include the many species of macaque and others are langurs, baboons, drills, mandrills, guenons, cercocebus monkeys, colobus monkeys, proboscis monkeys. Rhesus Macaque is a member of the sub family Cercopithecinae and family Cercopithecidae and Hanuman Langur is a member of the sub family Colobinae and family Cercopithecidae of Primate order of class Mammalia. New Old monkeys include capuchins, squirrel monkeys, titi mokeys, spider monkeys, howlers.

Among the non-human primates, only three species of monkeys have been reported from Nepal (Chalise *et al*., 2005; Jnawali *et al*., 2011; Gewali, 2013); the Hanuman Langur (*Semnopithecus entellus* Dufresne, 1797), the Rhesus Macaque (*Macaca mulatta* Zimmermann, 1780) and the Assamese Macaque (*Macaca assamensis* McClelland, 1839).

1.2 Characteristics, distribution and habitat

1.2.1 Rhesus Macaque

The Rhesus Macaque (*Macaca mulatta*) is one of the well-known species of Old World monkeys. Rhesus Macaques are well known as Rato Bandar in Nepali, Rhesus Monkey in English, Lal Bandar in Hindi, Makkad in Marathi, Baojha in Hindko, Mankad in Oriya, Kathi in Telugu and Bandar in Urdu (Molur *et al*., 2003) and other local names are Hajariya, Aule bandar, Lalgandi (Gewali, 2013). They have pale face with pointed and protruding ears, not heavily pigmented, fur brown olive or brown and yellow brown, large area of naked skin in buttocks, no marked menstrual swelling but skin of buttocks becomes red during oestrus period (Chalise *et al.*, 2005; Gewali, 2013). Males have body length 48-64 cm and body wt. 6.5-12 kg, where as females have body length 45-55 cm and body wt. 5.5 kg (Chalise *et al.*, 2005).

Rhesus is the most common monkey species as least concern status. Rhesus Macaques are native to northern India, Bangladesh, Pakistan, Nepal, Burma, Thailand, Afghanistan, Vietnam, Southern China and some neighboring areas (Ciani, 1986). Rhesus Monkey exhibits appreciable adaptability within its wide range of distribution. They occur from low-lying flat lands to the foot of the Himalayas up to 4000m (Chalise *et al*., 2005). They exist in temperate coniferous, moist and dry deciduous forest, mangrove, scurb, rain forest, cropland, human habitation, temples, mixed and bamboo forest (Chalise *et al*., 2005; Gewali, 2013; Jnawali *et al*., 2011). They can tolerate much type of climatic and vegetative zones of South and East Asia from Afghanistan to Pakistan, India, Nepal, Bangladesh, Myanmar, Thailand, Laos and Vietnam (Roonwal and Mohnot, 1977). In Nepal, they occur in tropical rain forest of Tarai to the valleys across of higher elevation of Makalu-Barun, Langtang and coniferous, alpine forest of Rara area too (Southwick *et al*., 1982) and found in larger number in religious jungles and temples like Pashupati, Swayambhu, Sankhu-Bajrajogini etc of Kathmandu valley (Chalise, 1998; Chalise, 2001). In our country, Rhesus found in Kabhre-Balthai, Palpa-Matindanda, Chitwan- Ramnagar, Pokhara-Kalikhola, Sankhuwasabha-Lakuwa, Helambubeshi, Suklaphanta-Jhiljhile Tal, Kailali-Ghodaghodi Tal and Rasuwa-Ritthe Khola (Chalise *et al*., 2005). The total population of Rhesus Macaques is estimated to consist of approximately 100,000 individuals (Jnawali *et al*., 2011).

1.2.2 Hanuman Langur

The native name of langurs in Nepal is Dhedu, Langur, Kalomukhe bandar, Lampuchhre Bandar, Phetawal Bandar, Kaldhaure, Guna etc. The genus was formerly popular as *Presbytis* but now changed to *Semnopithecus* (Gewali, 2013). All the subspecies of *Semnopithecus* are langur in general terms. Three distinct species have been identified by Conservation Assessment and Management Plan workshop for Nepal (Sanjay *et al*., 2003).

Hanuman Langurs have black face, generally body hair are silver grey, dull and dim color in Tarai areas, while in upper highlands species have very bright white hair on head region, forehead and body parts are darker in color (Chalise *et al*., 2005; Gewali, 2013, Jnawali *et al*., 2011). In Hanuman Langur, limbs are slender and long, tail is longer than head and body length, hands and feet regions are black. The head-and-body length is from 51 to 79 cm, whiles their tails, at 69 cm to 102 cm are always longer than their bodies (Burnie and Wilson, 2005). The average weight of Hanuman Langur is 18 kg in the males and 11 kg in the females (Burnie and Wilson, 2005).

Hanuman Langurs are the most widely distributed in South Asia (Choudhury, 2007; Napier and Napier, 1967). They are dispersed throughout most of India and Sri Lanka (Ellerman and Morrison-Scott, 1966) and are also established in parts of Pakistan (Oates *et al*., 1994; Roonwal, 1984), Nepal (Chalise *et al.*, 2005; Gewali, 2013; Jnawali *et al*., 2011), Bhutan and Bangladesh (Choudhury, 2007). Langur monkeys are found in mountainous areas up to the Himalayan belt (Melamchi, Nepal) as well as in semi- desert areas (Rajasthan, India), Sub- tropical monsoon dry forests (Nepal Tarai) and tropical rain forests of Sri-Lanka (Chalise, 1995). Their habitats include a wide range of vegetation zone, semi-desert, dry open scrubs, open cultivated regions, open park woods, dry deciduous forests, moist deciduous evergreen dense forests and mountain forests up to the zones of rather homogeneous oak- coniferous forests, located from sea level up to the height of about 4000 m (Roonwal and Mohnot, 1977; Vogel, 1977; Kumar *et al*., 2008; Groves and Molur, 2008).

1.3 Ecology and behavior

1.3.1 Rhesus Macaque

Rhesus Macaques are diurnal animals exist in both arboreal and terrestrial condition. This species is highly adjustable to man-made environments and exists successfully in village, cities and towns area. It is omnivorous animal and its feeding habits were reported to feed on eggs, termites and moulds in addition to plants (Lindburg, 1971) while in human influenced areas it focus on cultivated crops, fruits, flowers, leaves, seeds, gums, buds, shoots, clover, roots, bark, pith and resin of angiosperms, gymnosperms and fungi (Jnawali *et al*., 2011) and also they supplement their food diet with grasshopper, ants, beetles and mushrooms (Lindburg, 1971; Fooden, 2000; Wolfe, 2002). In some areas, Rhesus Macaques depend directly as well as indirectly on parts of their diet from human activities (Richard *et al*., 1989; Southwick and Siddiqi, 1994).

1.3.2 Hanuman Langur

Hanuman Langurs are diurnal animal and live everywhere in Nepal except in permanent snowy area (Chalise *et al*., 2005; Gewali, 2013). They are shy, timid and less aggressive to human beings and mostly arboreal in comparison to Rhesus, moving tree to tree however, habitually come to the ground for easier movement if the condition is safe (Chalise *et al*., 2005; Gewali, 2013). They are leafivorous and insectivorous feed upon flower, leaf buds, ripe fruits, seeds, shoot, petiole, pulvinus, bark, gum, pith and evergreen mature leaves in winter; deciduous young leaves in spring and deciduous mature leaves in the monsoon and fall while in insects caterpillar, termite, ant, grasshopper (Sayers *et al*., 2008; Chalise *et al*., 2005; Gewali, 2013). They are found in dipterocarps forests of outer and inner Tarai, mixed deciduous and evergreen forest of *Schima-Castonopsis, Elaeocarpus-Macaranga* forests in mid-hills and mountains and Quercus-pine-rhododendron forest of high mountains and are adapted to the encroaching heat of Tarai, the harsh winter of mid-hills and chilly atmosphere by occasional snow at the lap of Himalaya (Chalise *et al.*, 2005; Gewali, 2013). They are not habituated in the human settlement in Nepal as in India and Shrilanka (Gewali, 2013).

1.4 Non-human primates health, parasitism and zoonotic importance

Parasites are excellently well-adapted organisms to their host. Due to intimate inter-relationship with their host, adaptation of parasites being complex, with which they co-evolved (Barnard and Behenke, 1990). Parasites richness and prevalence in wild animals is indicators of population status and ecosystem (Teichroeb *et al*., 2009). Wild primates can host maximum diversity of parasites. More than 50 different species of parasites were recorded in non-human primates (Nunn and Altizer, 2006).

The emergence of infectious disease in primates has become an intensive interest in recent years, in particular after several terrible events causing significant impact on wild primate population (Hilser *et al*., 2011). Among the best known examples are outbreaks of Ebola haemorrhagic fever in Gabon (Huijbregts *et al*., 2003; Walsh *et al*., 2003) and anthrax epidemics (Leendertz *et al*., 2004) in the Ivory Coast which resulted in the destroy the large proportion of African ape populations. The majority of primate parasites lead in chronic, sub-lethal infections (Goldberg *et al*., 2008). However, these infections may cause significant impairment to biological processes due to immunosuppression (Lafferty and Holt, 2003). Understanding, predicting and managing epidemics such as these are important issues for conservation and management for primates against parasitic infection.

Now, zoonotic disease emergence is a primary concern topic for public health (Jones *et al*., 2008; Wood *et al*., 2012). It has been recorded that 61% of pathogens that cause diseases in humans are transmitted from wildlife (Taylor *et al*., 2001). In addition, 25% of the parasites that infect in non-human primates also are found to infect humans (Pedersen *et al*., 2005).

Due to very close physiologic and genetic character between human and monkeys, monkeys become the intermediate host of many parasites results the high potential pathogen exchange (Ott-Joslin, 1993) and there are many diseases that are easily transmitted both human and non-human primates (Wolfe *et al*., 1998). Some known examples, Chimpanzees and Sooty mangabeys acts as reservoir host for HIV, AIDS (Gao *et al*., 1999). It may lead transmission of HIV/AIDS from non-human primates to human (Keele *et al*., 2006; Leroy *et al*., 2004). Similarly, parasitic infectious diseases including viral (e.g. respiratory viral infections), vector-borne diseases (e.g. malaria, yellow fever) or enteric parasitic diseases (e.g. giardiasis, amoebiasis and helminthiasis), epidemics of polio and scabies are an important threat to the health of human populations and to the conservation and survivorship of non-human primates (Kalema-Zikusoka *et al*., 2002 ; Chapman *et al*., 2005; Nunn and Altizer, 2006; Leendertz *et al*., 2006; Gillespie *et al*., 2008). Prevalence of gastrointestinal protozoa and helminths have been identified in baboons (Ghandour *et al*., 1995; Murray *et al*., 2000), Mountain Gorillas (Nizeyi *et al*., 1999; Nizeyi *et al*., 2002b), chimpanzees (Murray *et al*., 2000) and several species of monkeys (Soulsby, 1982). Several studies have confirmed that non-human primates may be carriers of human gastrointestinal parasites (Karere and Munene, 2002), ectoparasites (Kalema-Zikusoka *et al*., 2002) and bacteria (Nizeyi *et al*., 2001). Therefore people living in close proximity of such animals or individuals working in game parks, animal orphanages or research stations may be at risk of acquiring pathogens from infected animals. Hence parasitic impact on primate is higher concerning topic for research.

1.5 Risk factors connected to gastrointestinal parasites in monkeys.

The probability of parasite infection is affected by factors associated with host traits (dominance, sex, age) as well as external conditions such as seasonal changes in temperature, rainfall, resource availability, parasite life-cycles, distance to the nearest town, fragment size, fragment shape and total basal area of food (Wilson *et al*., 2002; Valdespino *et al*., 2010). For the primate species risk factors are parasite species richness and parasite prevalence associated with factors such as habitat condition, sex, age and seasonal variation (Martinez-Mota, 2015).

Anthropogenic habitat fragmentation may make primate populations more susceptible to risk of infection by parasites, and in some cases this may cause high mortality and morbidity (Chapman *et al*., 2005). Habitat reduction, a consequence of habitat fragmentation, occurs in restricted host distribution and in crowding host conditions at small area, enhancing the overlap among same species that make communication between pathogen (Gillespie and Chapman, 2007). The populations of the monkey species were more likely to be parasitized in fragmented habitat compared to continuous habitat (Trejo-Macias and Estrada, 2012).

Sex hormones also influence on parasite infections i.e., estrogen contributes with antibodies increase against specific antigens, a feature which could explain a more effective resistance in females against some parasite infections (Martinez-Mota, 2015).The prevalence and intensity of infection in females and juveniles are higher as compared to males and adults respectively (Stoner and Gonzalezdi-Pierro, 2006). The prevalence of the intestinal parasites were significantly higher in 1 - 2 year old juvenile macaques than in 3 - 4 year old juveniles and adults greater than 5 years (MacIntosh *et al*., 2010; Martinez-Mota, 2015).

Seasonal condition in climate plays an important role in the distribution, prevalence and transmission of parasites in animal populations (Altizer *et al*., 2006). For example in forests characterized by well-defined rainy and dry seasons, increased parasite prevalence, intensity and species richness have been associated with wetter periods of the year in (Wright *et al*., 2009; Milton, 1996; Cristobal-Azkarate *et al*., 2010; Huffman *et al*., 1997; Gonzalez-Moreno *et al*., 2013). Parasite species such as protozoa and helminth (nematode) only found or had a higher prevalence rate in the wet season (Trejo-Macias and Estrada, 2012). Due to increased humidity in wet season may favor the development rate and survival of intestinal parasites that are shed in feces such as nematodes (Trejo-Macias and Estrada, 2012; Huffman *et al*., 1997; O'Connor *et al*., 2006; Gillespie *et al*., 2010). However, higher parasite infection rates during drier periods of the year reported by Stoner and Gonzalezdi-Pierro (2006), Masi *et al*. (2012), Parr *et al*. (2013) form different species of primates. During dry periods animals experience nutritional stress, which is defined as an energy imbalance due to insufficient nutrient and energy intake associated with a restricted diet, resulting in reduced weight, fertility (Masi *et al*., 2012) and imbalance of health status (Lujan *et al*., 2005; Plowright *et al*., 2008; Jeanniarddu *et al*., 2009) and ultimately lead to a suppression of the immune system (Coe *et al*., 1992; Lloyd, 1995). Therefore may lead higher sensitive to parasitic infection.

Specific feeding and drinking behaviour of monkey also influence the parasitic infection (Pokhrel, 2014). Feeding habit of tree leaf, bark and fruits especially those of medicinal values, like neem and pomegranate leaves, which declined the parasitic infection (Parmar *et al*., 2012). Insectivorous consume arthropods (Beetle, Cockroach, Ants, Grasshopper etc.) which acts as intermediate host for various parasites that leads parasitic infection (Nunn and Altizer, 2006).

1.6 Objectives of the study

1.6.1 General objective

To study the prevalence of gastro-intestinal parasites of Rhesus Macaque (*Macaca mulatta* Zimmermann, 1780) and Hanuman Langur (*Semnopithecus entellus* Dufresne, 1797) in Devghat, Chitwan, Nepal.

1.6.2 Specific objectives

- To determine the overall prevalence of gastro-intestinal parasites of monkeys.
- To determine the species wise prevalence of gastro-intestinal parasites.
- To compare species wise prevalence rate of gastro-intestinal parasites between Rhesus Macaque and Hanuman Langur.
- To determined the structure, shape and size of eggs/cysts of gastro-intestinal parasites.

1.7 Significance of the study

In global context, some researches have been carried out regarding intestinal parasites of primates. In Nepal, few researches have been done on intestinal parasites in Rhesus Monkeys (*Macaca mulatta)* (Limbu and Pant, 2005; Malla, 2007; Dhoubhadel, 2007; Nepal, 2010 and Jha *et al*., 2011) and in Assamese Monkeys (*Macaca assamensis)* (Pokhrel, 2014). This study had been the first attempt on the prevalence of gastro-intestinal parasites of Rhesus Macaque (*Macaca mulatta* Zimmermann, 1780) and Hanuman Langur (*Semnopithecus entellus* Dufresne, 1797) in Devghat, Chitwan.

Parasites represent one of the most successful life forms and an important component of the biological diversity of tropical forests (Nunn and Altizer, 2006; Trejo-Macfas *et al*., 2007). Research on parasitic fauna can actually add a new direction to the understanding of ecological interactions, host distribution patterns and to the complex history of regions and habitats (Martinez-Mota, 2015). So Parasites and their impact on wild population should be highly considered. Therefore it is necessary to obtain accurate data on parasite diversity and abundance at local levels in order to understand the role of infectious agents in wildlife endangerment, declines and extinctions (Smith *et al*., 2009; Thompson *et al*., 2010). Furthermore the study of parasites in wild monkey populations provides knowledge for estimating the health and the infection risk in populations and establish general principles governing parasite occurrence which is critical for managing vulnerable wildlife populations and mitigating risks to human health (Chapman *et al*., 2006; Gillespie *et al*., 2005). This study has been carried out to understand the prevalence of gastro-intestinal parasites in monkeys and provides baseline data for further action plan.

1.8 Hypothesis

H_o= There was no significant difference in the prevalence of GI parasites between Rhesus Macaque and Hanuman Langur.
H_1= There was significant difference in the prevalence of GI parasites between Rhesus Macaque and Hanuman Langur.

1.9 Limitation of the study

- The study was design for the partial fulfillment of requirements for award of the degree of Master of Science in Zoology with special paper parasitology. Therefore, due to time and financial constraints, lack of resources intensive research could not possible.
- Faecal samples were collected only from a limited section of population within the study area.
- Identification of parasites was based on the morphometry of eggs/ cysts under light microscopy.

2. LITERATURE REVIEW

Parasite leads a major role in ecosystems, affecting the ecology of specific interaction (Esch and Fernandez, 1993), host population growth and regulation (Hochachka and Dhondt, 2000; Hudson *et al*., 1998), and community biodiversity (Hudson *et al*., 2002). Parasites can affect host survival and reproduction directly through pathological burden and indirectly by reducing host condition (Boyce, 1990; Chandra and Newberne, 1977; Coop and Holmes, 1996; Dobson and Hudson, 1992; Hudson *et al*., 1992). Severe parasitosis can lead to blood loss, tissue damage, spontaneous abortion, congenital malformations, and death (Chandra and Newberne, 1977; Despommier *et al*., 1995). However, less severe infections are more common and may impair nutrition, travel, feeding, predator escape and competition for resources or mates or increase energy expenditure (Coop and Holmes, 1996; Dobson and Hudson, 1992; Hudson *et al*., 1992; Packer *et al*., 2003).

Regarding these adverse conditions develop on the animals, Investigations of the animals's health status are important to improve conservation measures (Dobson, 1988; May *et al*., 1988; Thorne *et al*., 1988; Nizeyi *et al*., 1999; Nizeyi *et al*., 2002a, 2002b; Daszak *et al*., 2000; Graczyk *et al*., 2001). Parasitological studies have concentrated on large primates such as monkeys (Stuart and Strier, 1995; Gillespie *et al*., 2004; Gillespie *et al*., 2005; Boesch, 2008; Chapman and Huffman, 2009). This is probably due to Epidemiological interest because monkeys are genetically closer to humans and are known as a reservoir of certain pests and parasite fatal to humans (Wolfe *et al*., 1998). Due to this compliment many studies have been carried out regarding the gastro-intestinal parasites of wild or captive non-human primates i.e., monkeys in global context and national context. Some are as follows.

2.1 Global context

Altogether six helminth parasites viz. *Oesophagostomum aculeatum*, *Streptopharagus pigmentata*, *Physaloptera* sp., *Enterobius vermicularis*, *Trichuris trichiura* and *Hymenolepis* sp. were recovered from *Macaca sinica* and *presbytis* sp., whereas *Oesophagostomum* and *Strongyloides* were most abundant (Dewit *et al*., 1991). Gotoh (2008) studied gastrointestinal parasites in wild Japanese Macaques (*Macaca fuscata*) from 14 natural habitats, found highest prevalence rate of *Strongyloides fulleborns* followed by *Streptopharagus pigmentatus*, *Trichuris trichura*, *Oesophagostomum acculeatum*, *Gongylonema* sp. and *Berticella* sp. were detected. Mutani *et al*. (2003) observed parasites were *Strongyloides* (62.4%), *Physaloptera* (58.5%), *Trichuris* (52.8%), Hookworm (34.0%), *Oesophagostomum* (30.2%), *Trichostrongylus* (3.8%) and *Ascaris* (5.7%) with overall infection rate 88.7% from Green Monkeys of Barbados Primate Research Centre and Wildlife Reserve (BPRCWR). Out of the 108 fecal samples, 56 (51%) were positive with three parasite viz. the highest prevalence of *Oesophagostomum* 28% followed by Strongles sp. (14%) and *Entamoeba* sp. (14%) from the monkeys of Tamil Nadu. (Ponnudurai *et al*., 2003). The overall parasitization rate was 59.1 % found by Jones-Engel *et al*. (2004) from nine species of Pet Macaque in Sulawesi, Indonesia, Seven taxa of intestinal protozoa (*Blastocystis hominis* (43%), *Iodamoeba butschlii*

(22%), *Entamoeba coli* (14%), *Entamoeba hartmanni*, *Chilomastrix mesnili*, *Endolimax nana* and *Retortamonas intestinalis*) and three taxa of nematodes (Hookworm (6%), *Trichuris* spp. (3%) and *Ascaris* spp. (1%)) were detected. Kimberley *et al*. (2004) collected fecal samples from Red-howler Monkeys (*Alouatta seniculus*), Night Monkeys (*Aotus vociferans*), Spider Monkeys (*Ateles bezlebuth chamek*), brown Titi Monkeys (*Callicebus brunneus*) and Squirrel Monkeys (*Saimiri sciureus*) identified various protozoans *Ancyclostoma* sp., *Ascaris* sp., *Strongyloides stercoralis*, *Trichuris trichiura*, *Prosthenorchis elegans* and *Schistosoma mansoni*. Gillepsie *et al*. (2004) collected 293 faecal samples from four Guenon species (*Cercopithecus*) of Uganda, Six nematodes (*Strongyloides fsulleborni*, *Oesophagostomum* sp., strongyle sp., *Trichuris* sp., *Streptopharagus* sp., and *Enterobius* sp.), 1 cestode (*Bertiella* sp.), 1 trematode (Dicrocoeliidae) and 5 protozoans (*Entamoeba coli*, *Entamoeba histolytica*, *lodameoba butschlii*, *Giardia lamblia* and *Chilomastix mesnili*) were detected. Also another survey was carried out by Gillepsie *et al*. (2005) collected 2,103 faecal samples from three Colobus Monkey sepecies of Uganda, seven nematodes (*Strongyloides fsulleborni*, *S. stercoralis*, *Oesophagostomum* sp., strongyle sp., *Trichuris* sp., *Ascaris* sp. and *Colobenterobius* sp.), one cestode (*Bertiella* sp.), 1 trematode (Dicrocoeliidae) and three protozoans (*Entamoeba coli*, *Entamoeba histolytica* and *Giardia lamblia*) were detected.

A cross sectional survey launched (Ekanayake *et al*., 2006) in 125 monkeys (macaque and langurs) 27.00% (34 of 125) were positive for *Cryptosporidium* oocyst also co-infected with *E. coli*, *E. histolytica*, *E. hartmanni*, *Chilomastrix* sp. and *Balantidium* sp. and recorded helminthes were *Enterobius* spp., *Spiruroid* spp., *Strongyloides* spp., Strongyle spp. and *Trichuris* spp. Sing *et al*. (2009) collected 62 samples from Assamese Monkey (*Macaca assamensis*) found 48.38% (30) positive, 119 samples from Rhesus Monkey (*Macaca mulatta*) found 35.29% (42) positive, 12 samples from Hanuman Langur (*Semnopithecus*) found 25% (3) positive and 8 samples from Capped Langur (*Trachypithecus pileatus*) found 62.50% (5) positive for parasitic infection, recorded parasites were *Trichuris* spp., *Hymenolepis diminuata* and *strogyloides* spp. Wongsawad (2009) documented five species of protozoan (*Entamoeba coli*, *Entamoeba histolytica*, *Balantidium coli*, *Isospora* sp. and *Eimeria* sp.) and six species of helminthes (*Toxocaris* sp., *Toxocara* sp., *Oesophagostomum* sp., *Strongyloides* sp., *Trichuris* sp. and *Capillaria* sp.) from the Assamese Macaque (*Macaca assamensis*) in Thailand. Akpan *et al*. (2010) observed out of 300 samples, 120 (40%) samples from Drill monkeys were positive with high prevalence of *Entamoeba* sp. (40%) followed by *Strongyloides* sp. (22%), *Prosther* sp. (16%) *Necator* sp. (11%) and *Hymenolepis* sp. To determine the parasite richness and prevalence in Long tailed macaque (*Macaca fascicularis*) and Probosis Monkeys (*Nasalis larvatus*) by Salgado-Lynn *et al*. (2010) documented *Clonorchis* sp., *Fasciola* sp., *Taenia* sp., *Diphylidium* sp., *Strongyloides* sp., *Trichuris* sp., *Anatrichosoma* sp., *Ascaris* sp., *Strongylids* sp. and *Oxyrids* sp.

Perae- Rodriguez *et al*. (2010) collected 53 fecal sample of Owl Monkey (*Aotus azarai azarai*) in the Argentinean Chago, found (92%, n=49) contain parasites with prevalence rate was *Isospora* sp. (45%) followed by *Strongyloides* sp. (*30%)*, *Blastocystis* sp. (26%), *Trypanoxyrious* sp. (23%), *Entamoeba* sp. (23%), *Endolimax nana* (23%), *Uncinaria* sp. (17%), *Giarda* sp. (8%) and *Taenia* sp. (2%) and more than half of them (60%, n=32) had multiple infection. Hilser *et al*. (2011) collected faecal samples from five groups of Red Langurs (*Presbytis rubicunda*) inhabiting the Sabangau Peat- Swamp Forest, Central Kalimantan, recorded 97% parasitization including six nematode viz. *Trichuris trichuria* (28%), Hookworm sp. (28%), *Ascaris lumbricoides* (17%), *Strongyloides* sp. (13%), *Enterobius vermicularis* (8%) and *Trichostrongylus* sp. (5%), one trematode i.e., *Schistosoma mansoni* (10%) and four protozoa viz. *Entamoeba coli* (57%), *Entamoeba histolytica/ dispar* (52%), *Balantidium coli* (47%) and *Blastocystic hominis* (12%). Parmar *et al*. (2012) were found positive 34.14% in Hanuman Langur with presence of four species of helminthes viz. *Strongyloids* spp. (26.66%), *Trichuris* spp. (20%), *Ascaris* spp. (20%), and *Spirometra* spp. (13.33%) and two species of protozoan viz. *Entamoeba histolytica* (10%) and *Entamoeba coli* (10%), where 40.00% in Rhesus Macaque for the presence of same species of helminthes viz. viz. *Strongyloids* spp. (26.66%), *Trichuris* spp. (26.66%), *Ascaris* spp. (26.66%) and *Spirometra* spp. (20%). Nath *et al*. (2012) found 13.63% parasitic infection from Pig tailed Macaque (*Macaca nemestrina*), Stump-tailed Macaque (*Macaca arctoides*), Assamese Macaque (*Macaca assamensis*), Bonnet Macaque (*Macaca radiata*) and Golden Langur (*Trachypithecus geei*) in captive non-human primates of Assam state zoo, recorded only *Trichuris* sps. and *Oesophagostomum* sps. The occurrence of four parasitic species of zoonotic potential, *Entamoeba coli*, *Entamoeba histolytica* /*dispar*, *Trichuris* sp. and Hookworm sp. were investigated in the Toque Macaque, Grey Langur and the Purple-faced Langur at 32 sites across Sri Lanka (Huffman *et al*., 2013).

Khatun *et al*. (2014) showed *Balantidium coli* (83.3%) and *Trichuris* sp. (16.7%) from Rhesus Monkey at Rangpur recreational Garden and Zoo in Bangladesh. Maldonado-Lopez *et al*. (2014) identified three species of intestinal parasites (*Controrchis* sp., *Trypanoxyuris* sp. and *Strongyloides* sp.) in sympatric Howler Monkey (*Alouatta palliate*) and Spider Monkey (*Ateles geoffroyi*) populations in a tropical dry forest in Costa Rica. The most prevalent intestinal parasites were *Trichuris trichiura, Strongyle* sp., *Entamoeba* sp. and *Stronglyloides* sp. recorded by Adetunji (2014) from the Green Monkeys, Mandrill Monkeys, Mangabey Monkeys, Mona Monkeys and Putty-nose Monkeys of Zoological Garden in Ibandan, Nigeria. 60.00% samples of Rhesus Macaque were positive for the parasites with single infection of *Toxocara* spp (Thawait *et al*., 2014). But out of the 3142 samples of seven species of monkeys, all were infected by parasites and recorded : 9 protozoans (*Entamoeba coli*, *Entamoeba histolytica/dispar*, *Entamoeba hartmanni*, *Endolimax nana*, *Iodamoeba butschlii*, *Chilomastix mesnili*, *Giardia* sp., *Balantidium coli* and *Blastocystis* sp.) and 14 helminths (*Oesophagostomum* sp., *Ancylostoma* sp., *Anatrichosoma* sp., Capillariidae Gen. sp. 1, Capillariidae Gen. sp. 2, *Chitwoodspirura* sp., *Subulura* sp., *spirurids*, *Ternidens* sp., *Strongyloides* sp., *Trichostrongylus* sp., *Trichuris* sp. and *Dicrocoelium* sp.) (Kouassi *et al*., 2015). Arunachalam *et al*. (2015) collected 60 faecal samples from Rhesus Macaque, parasitic infection was 43% with prevalence of four parasitic species viz. high prevalence of *Strongyle* spp. (33%) followed by *Ascaris* spp. (5%), *Eimeria* spp. (3%) and *Coccidia* spp.(3%). Arjun *et al*. (2015) also investigated Ova of three gastrointestinal parasites were observed viz., *Strongyle* sp., *Strongyloides* sp. and *Enterobius vermicularis* from Bonnet Macaque (*Macaca radiata*).

Only one species of protozoa (*Coccidia* spp.) and three species of helminthes (*Trichuris* spp., stronglyle spp. and *Enterobius* spp.) investivagated by Bichi *et al*. (2016) from non-human primates by using floatation and sedimentation method.

2.2 National context

Non-human primates often share parasites with humans, understanding the ecology of infectious diseases in nonhuman primates is of paramount importance (Chapman *et al*., 2005). By considering this, a lot of research and investigation have been done in Non-human primates in the world. Some of the studies were performed in ecological basis like Characteristics, Distribution, Habit & Habitat, Ecology, Behaviors and Population status of monkeys in different ecological zones of Nepal (Ghimire, 2000; Bashyal, 2005; Wada, 2005; Nepal, 2005; Katiwada *et al*., 2007; Subedi, 2007; Chalise, 2008; Sayers *et al*., 2008; Regmi and Kandel, 2008; Chalise, 2010; Pandey, 2012; Gewali, 2013). But in Nepal, only little researches have been reported in monkeys in parasitic basis. In this section some research information related with the present work has been reviewed.

Strongyles and *paramphistome* were detected in Rhesus Monkey (*Macaca mulatta*) in Nilbarahi Community forest (Limbu and Pant, 2005). Malla (2007) was found 61.38% samples were positive for one or mixed infection of more than one helminthes viz., 16 nematodes; *Strongyloides fulleborni* was most prevalent with 51.61% followed by *Oxyuris* sp. (11.29%), *Ascaris lumbricoides* (10.48%), *Dictyocaulus* sp. (7.25%), *Chabertia* sp. (6.45%), *Toxacaris leonine* (6.45%), *Ostertagia* sp. (6.45%), *Trichuris ovis* (6.45%), *Trichuris trichura* (5.64%), *Trichostrongylus* sp. (4.83%), *Capillaria* sp. (4.03%), *Oesophagostomum* sp. (4.03%), *Ancylostoma duodenale* (2.41%), *Haemonchus contortus* (2.41%), *Cooperia* sp. (0.80%) and *Toxocara canis* (0.80%), 1 trematode; *Dicrocoelium* (4.83%) and 1 acanthacephala; *Prosthenorchis elegans* (5.64%) were detected from Rhesus Monkeys from Pashupati and Nilbarahi areas, of Kathmandu valley, and described the nematodes were more prevalence (89.51%) than trematodes (4.83%). Dhubhadel (2007) also found 62% samples were positive for parasites viz. highest prevalence of *Strongyloides fulleborni* (42.5%) followed by *Dictyocaulus* sp. (7.87%), *Taenia* sp. (7.08%), *Oesophagostomum* sp. (6.29%), *Trichuris ovis* (4.72%), *Capillaria* sp. (3.93%), *Ostertagia* sp. (3.93%), *Cooperia* sp. (3.93%), *Prosthenorchis elegan* (3.93%), *Dicrocoelium* sp.(3.14%), *Trichostrongylus* sp. (3.14%), *Oxyris* sp. (3.14%), *Toxocaries leonine* (3.14%), *Trichuris trichiura* (2.36%), *Ascaris lumbricoides* (1.57%), *Toxocara canis* (1.57%) and *Chabertia* sp. (1.57%) from Rhesus Monkeys of Swaymbhu and Nilbarahi area, of Kathmandu valley and demonstrated the presence of Nematode (85.82%), Tremtode (93.14%), Cestode (7.08) and Acanthocephala (3.93%). Malla (2007) and Dhubhadel (2007) were documented *Dictyocaulus* sp., *Taenia* sp., *Ostertagia* sp., *Cooperia* sp., *Prosthenorchis elegans, Dicrocoelium* sp., *Oxyuris* sp. and *Chabertia* sp for the first time in the Rhesus Monkeys from Nepal, whereas *Prosthenorchis elegans* was reported for the first time in Nepal .

Nepal (2010) recorded out of 300 samples 255 (85%) were found to be positive with intestinal parasites viz., 16 Nemotodes: *Strongyloides* sp. (27.06%), *Trichostrongylus* sp. (11.37%), *Dictyocaulus* sp. (7.45%), *Haemonchus* sp. (4.31%), *Ostertagia* sp. (5.88%), *Trichuris* sp. (9.80%), *Capillaria* sp. (6.27%), *Toxocara* sp. (12.94%), *Chabertia* sp. (8.63%), *Ascaris* sp. (7.45%), *Oesophagostomum* sp. (10.59%), *Ancylostoma* sp. (2.75%), *Bunostomum* sp. (1.96%), *Oxyuris* sp. (3.14%) and *Cooperia* sp. (4.31%), 3 Trematodes: *Dicrocoelium* sp. (9.80%), *Schistosoma* sp. (18.04%) and *Fasciola* sp. (9.80%), 2 Cestodes: *Taenia* sp. (9.80%) and *Dipylidium* sp. (9.80%) from the Rhesus Monkeys of Swaymbhu area, Kathmandu and the study revealed the prevalence of nematodes (75%), trematodes (15%) and cestodes (10%). In nematodes genera *Bunostomum* sp., cestodes genera *Dipylidium* sp., in trematodes genera *Schistosoma* sp., were reported for the first time in Nepal in Rhesus Monkey (Nepal, 2010). Jha *et al*. (2011) detected three species of protozoa: *Balantidium coli* (32.23%), *E. histolytica* (26.4%), *E. coli* (21.49%) and ten species of helminthes: *Oesophagostomum* was highest (35.54%) followed by *Strongyloides* (28.92%), *Trichuris* (14.05%), *Trichostrongylus* (11.57%), *Toxocara* (4.96%), Trichurid and other four species of unknown species from temple Rhesus Monkeys of Kathmandu valley and showed an overall infection rate of 76.86% for all intestinal Parasites with 53.72% for protozoan and 59.5% for helminthic parasites.

Pokhrel (2014) determined the distribution of intestinal parasites of 72.94% were positive for intestinal parasites viz. three protozoan parasites: *Balantidium coli* (28.24%) was the highly distributed parasites followed by *Entamoeba* sp. (20%) and *Isospora* sp. (3.53%), seven helminthes: *Ascaris* sp. (10.58%) was the most dominant helminth parasites followed by *Trichuris* sp. (9.41%), *Strongyloides* sp. (8.24%), *Moniezia* sp. (8.24%), *Oesophagostomun* sp. (4.7%), Hookworm sp. (4.7%) and *Physeloptera* sp. (1.17%) respectively and only one cestode parasite, *Moniezia* sp. was found but trematoda was not found in Assamese Macaque of Shivapuri Nagarjun National Park within two seasons.

3. MATERIALS AND METHODS

3.1 Study area

3.1.1 Background

Devghat Dham is the most sacred as well as an important religious pilgrimage site of Nepal. Located almost in the middle of Nepal, this shrine, which is a confluence of Kaligandaki and Trishuli Rivers, is regarded important not only in religious but also in natural, historical, cultural, archaeological and touristic point of view.

The government of Nepal has constituted a Devghat Area Development Committee in 2050 BS. For the development of the overall area of Devghat including the confluence of the Kaligandaki and Trishuli rivers 'Beni' at the meeting point of Tanahun, Chitwan and Nawalparasi districts of Nepal, and gave the responsibility of developing the area.

3.1.2 Geographical condition (Location):

Devghat lies in the Centre part of Nepal. It is located and covers the boarder area of Chitwan, Tanahun and Nawalparasi district as well as the junction of Gandaki, Limbini and Narayani Zones. Its geographical location is 85°22'30" to 84°30'00" east longitude and 27°42'30" to 27°47'30" north latitude (DADC. 2007). The geographical identity of Devghat area is the meeting point of Trishuli and Kaligandaki River and also meeting point of Tanahun, Chitwan and Nawalparasi district. This region covers inner Terai and hilly areas from 200m to 575m above sea level and occupies 54.34 km^2 (DADC. 2007). The study area lies in Bharatpur municipality of Chitwan -01.

For Rhesus Macaque, site 1 was selected and located 84°25'26.82" to 84°25'38.86" east longitude and 27°44'16.27" to 27°44'26.49" north latitude.

For Hanuman Langur, site 2 was selected and located 84°25'48.21" to 84°26'53.25" east longitude and 27°42'54.59" to 27°43'14.56" north latitude.

3.1.3 Climate:

As this area has mostly inner Terai areas, the climate here mostly resembles with that of Terai, but because of the large rivers flowing from this area, there is some variance in the climate.

According to the records on temperature and rain available from the Rampur campus, the maximum temperature is in the months of May and June at 36.6 degrees centigrade and the minimum temperature in the months of January and February at 6.2 degrees centigrade. Likewise, the maximum rain is 413.4 mm in June and July.

3.1.4. Monkey population in the study area:

In Devghat, Chitwan, Rhesus Macaque found in the human settlement and temples area whereas Hanuman Langur found in forest area. There was no research conduct till now so that there is no exact data about population of monkey in this area. According to the local people about more than 400 Rhesus Macaque are in human settlement area of Devghat, Chitwan. Subedi (2007) recorded only 43 Hanuman Langur belonging to four troops in Devghat Chitwan along the road side (Devghar marga).

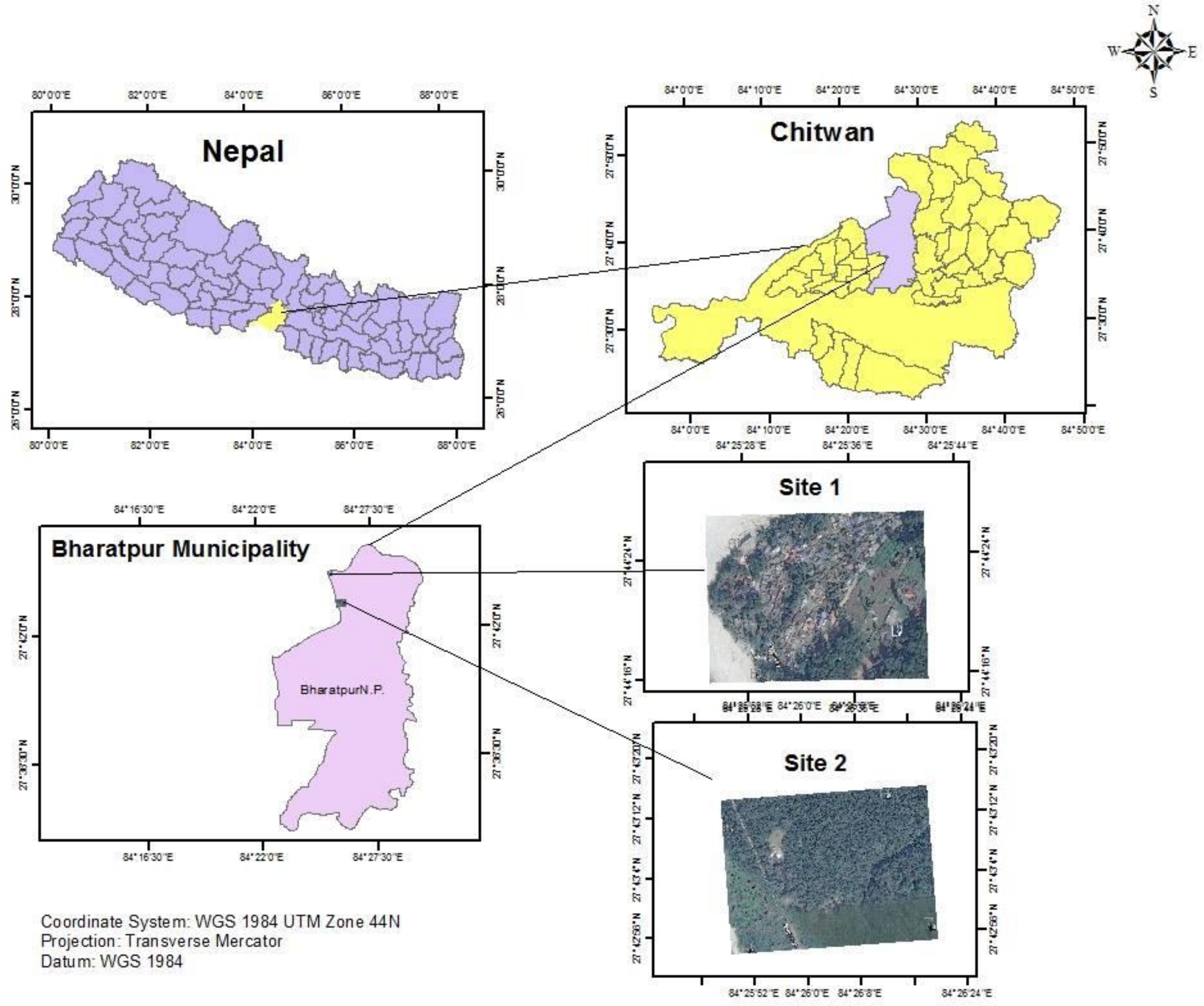


Figure 1: Showing the study area site 1 and site 2.

3.2 Materials required

3.2. Apparatus

- Vials
- Camera
- Gloves and masks
- Weighing machine
- Petri dish
- Conical flask
- Test-tube stand
- Glass- rod
- Test tube
- wooden box
- Tea strainer
- Forceps
- Beaker
- Needle
- Cover-slip
- Slide
- Centrifuge machine
- Centrifuge tube
- Toothpick
- Dropper
- Pipette
- Binocular- microscope

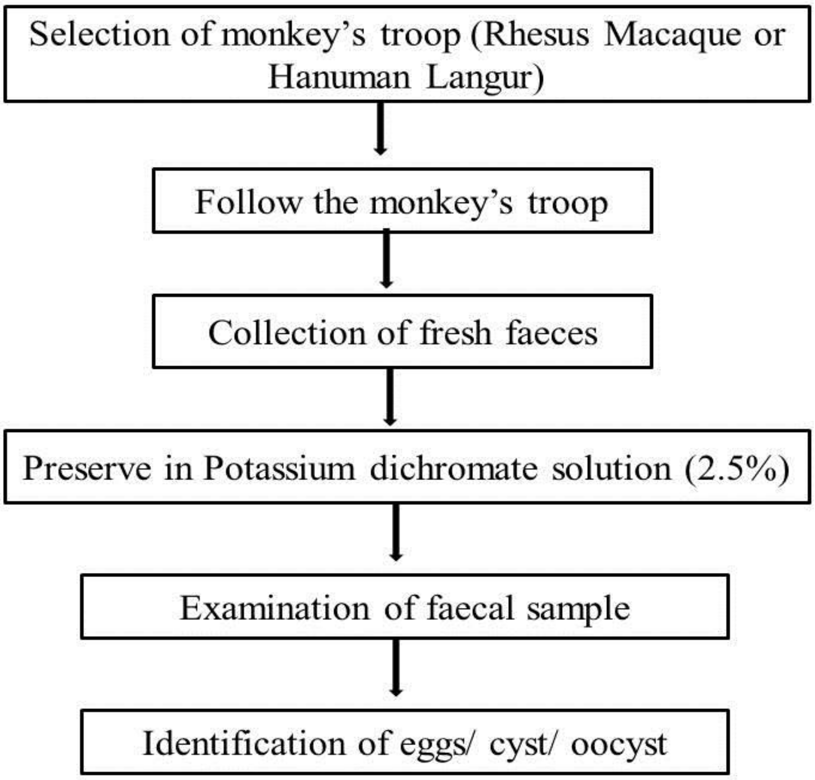
3.2.2 Chemicals

- Potassium dichromate solution ($K_2Cr_2O_7$) (2.5%)
- Sodium chloride solution (NaCl)
- Lugol's Iodine solution
- Methylene blue
- Distilled water

3.3 Field survey

Initially, on July 2015 preliminary survey was conducted to know the habitat, distribution and estimated troops (population) of Rhesus Macaque and Hanuman Langur in Devghat area. I consulted with the Executive Director and other members of Devghat Area Development Committee and other local people about monkey. Eventually, Chitwan side of Devghat area was selected for the fecal sample collection.

3.4 Research design



3.5 Fecal sample collection and preservation

Systematically troop followed method was applied for fresh faecal sample collection. Faecal samples were collected from March to April 2016 from monkeys during early morning (6:00am – 11:00am) and evening (3:00pm – 6:00pm).

73 fresh faecal samples were collected from 5 troops of Rhesus Macaque in village and temple area and 20 fresh faecal samples were collected from 2 troops of Hanuman Langur in forest area (along Devghat Marg forest) by followed each and every troops.

Before collection of the fresh faecal samples were carefully examined for colour, consistency, worm, cestode segments, blood and mucus. These were noted.

About 10 gram of faecal material was taken from the faecal mass with the help of wood spoon and placed in a 25ml vial containing Potassium dichromate solution (K2Cr2O7) (2.5%). Finally the sample was labeled according to species, serial number, location, date and time.

3.6 Microscopic examination of faecal sample

After collection and preservation, all faecal samples were examined at the laboratory of Central Department of Zoology (CDZ), T.U. Kirtipur, Kathmandu. The faecal samples were microscopically examined for trophozoites, cysts, oocysts, eggs and larvae of gastrointestinal parasites by concentration method viz. floatation technique and sedimentation technique (Soulsby, 1982; Zajac and Conboy, 2012).

3.6.1 Floatation technique

In the floatation technique, the fluid flotation medium i.e., saturated solution of sodium chloride (NaCl) (SPG 1.20) has higher specific gravity than parasitic forms. The higher the specific gravity (SPG) of the floatation solution, the greater the variety of parasite eggs that would float.

All the helminth eggs and protozoan cysts float in such a solution except the following eggs of *Ascaris lumbricoides*, eggs of *Taenia solium* and *Taenia saginata* and also the eggs of intestinal fluke. *Strongyloides* larvae do not float in salt solution.

Process

- About 3 gm of faecal sample was taken.
- The sample was kept on the beaker and grinded with about 20 ml of water.
- Filtrate the faecal solution by tea strainer and poured into centrifuge tube upto 12 ml and centrifuge at 1000 rpm for 5 minutes.
- The centrifuge tube was taken out and upper part of the water was removed with the help of pipette.
- The centrifuge tube was again filled with NaCl solution upto 12 ml and centrifuged at 1000 rpm for 5 minutes.
- The centrifuge tube was taken out and added more NaCl solution up to the tip of tube and a drop of methylene blue added upon it.
- A cover slip was placed over the top of the centrifuge tube so that the solution touched the cover slip and leaved for 5 minutes.
- Then, cover slip was taken gently and placed on a microscopic slide and examined under 10X and 40X. Finally, photographs were captured.

3.6.2 Sedimentation technique

A sedimentation procedure is used to isolate eggs of flukes, acanthocephalans, some other tapeworms and nematodes whose eggs are bit heavier than the others. For this technique, sediments of centrifuged contents were taken for eggs detection.

Process

- The centrifuge tube was taken out and upper part of the saturated NaCl solution was removed with the help of pipette, after examined the floatation.
- Remaining sediment content poured into the watch glass and stirred gently.
- A small drop of sediment mixture was taken with help of pipette and placed on the second slide, added one drop of iodine solution for staining.
- The specimen was stained by Iodine wet mount's solution and examined under 10X and 40X. Finally photographs were captured.

In this way, two slides were prepared from one sample (one from floatation and one from sedimentation) were examined microscopically at 10X and 40X to detect eggs of helminthes, protozoan's trophozoites or cysts of gastro-intestinal parasites.

3.7 Measurement of eggs, cysts and larva

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

3.8 Identification of the eggs, cysts and larva

The identification of the eggs, cysts and larva were confirmed by comparing the structure, color and size of eggs, cysts and trophozoites of published literature, journals and books (Soulsby, 1982; Gardiner *et al.*, 1988; Taylor *et al*., 2007; Hussam, 2015).

3.9 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 encrypted and entered into Microsoft Excel spread sheet. Data were statistically analyzed using Pearson's Chi-squared test with Yates' continuity correction, performed by "R", version 3.3.1 software packages. In all cases 95% confidence interval (CI) and $P<0.05$ was considered for statistically significant difference.

Photo no:1- Rhesus Macaques

Photo no:2- Faecal material of Rhesus Macaque

Photo no:3- Hanuman Langur

Photo no:4- Faecal material of Hanuman Langur

Photo no:5- collection of Faecal material

Photo no:6- Examination of Faecal sample

Photo no:7- Work at centrifuge machine

4. RESULTS

4.1 The overall prevalence of gastro-intestinal parasites in monkey

4.1.1 General prevalence rate of GI parasites

During this study, a total of 93 faecal samples were collected from Rhesus Macaque and Hanuman Langur of Devghat, Chitwan. Out of these 93 samples, 69 faecal samples were found to be positive for one or more than one GI parasites and remaining 24 samples were negative. Therefore, the overall gastrointestinal parasitic infection was 74.20% and negative was 25.80%. Hence, it revealed that there was a high prevalence rate of GI parasites in Rhesus Macaque and Hanuman Langur.

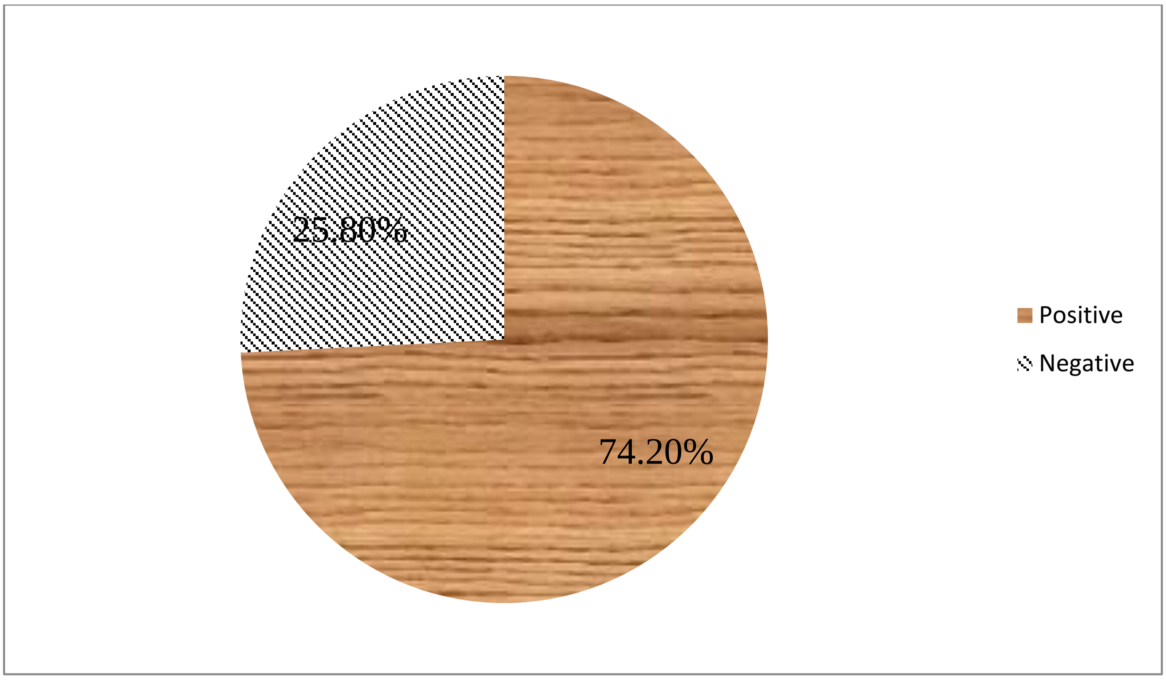


Figure 2: Overall general prevalence of GI parasite in monkeys

4.1.2 Phylum and class wise prevalence rate of GI parasites

The overall gastrointestinal parasitic infection was 74.20% (n=69) with 40.86% (n=38) protozoa and 52.68% (n=49) was helminths. Result indicated that helminth infection was more common as compared to protozoan infection. The prevalence rate of helminth was higher than protozoa (fig. 3) infection without statistically significance (χ^2 = 0.781, df= 1 and P> 0.05).

Monkeys were found to be infected with protozoan parasites belonging to three classes. Among them litostomata showed the highest prevalence (27.95%) followed by sporozoa (16.12%) and sarcodina (13.97%). Among the helminth parasites, nematode (58.06%) parasites were observed with highly prevalence rate (fig. 4). Only one species (*Hymenolepis* sp.) of cestode (1.07%) was found and trematode parasite was not observed from the monkeys.

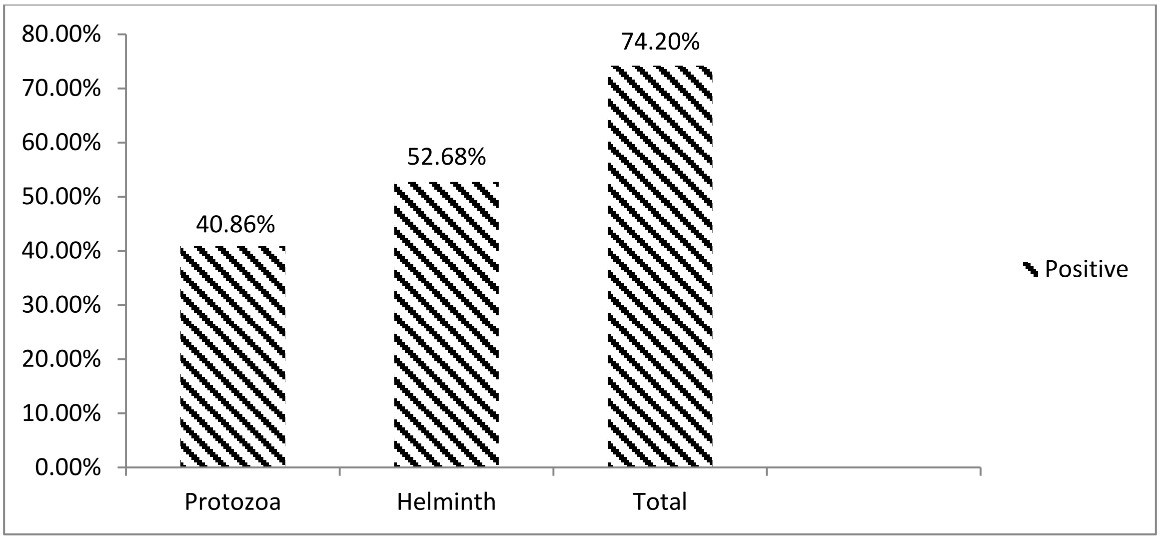


Figure 3: Overall phylum wise prevalence of GI parasites in monkeys.

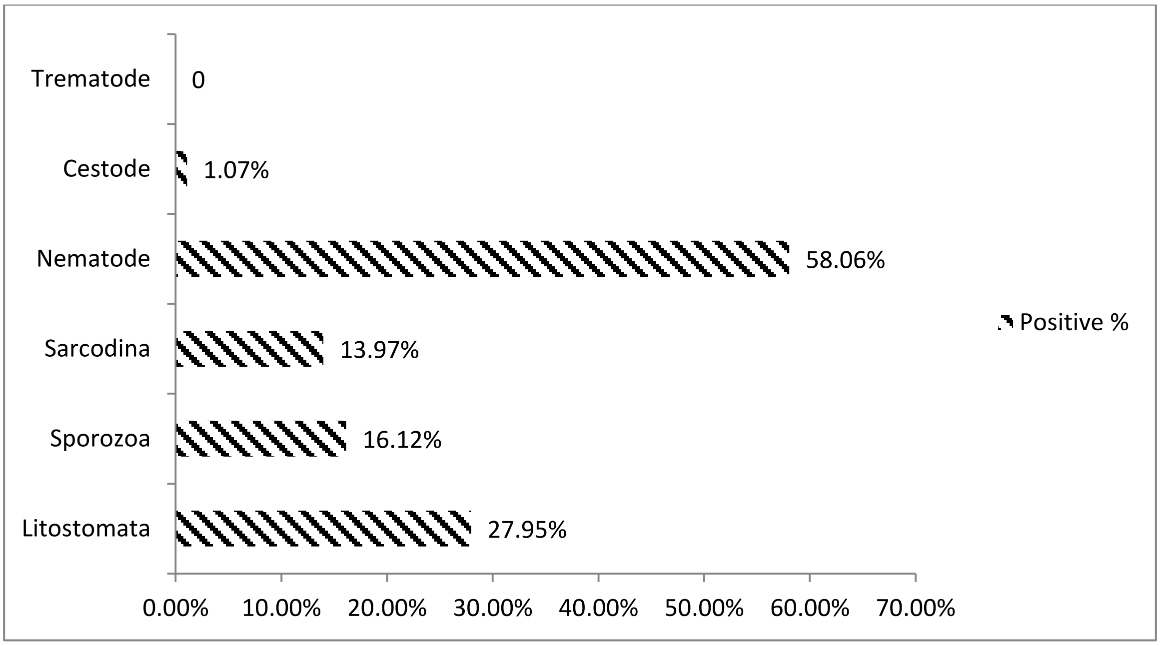


Figure 4: Class wise prevalence rate of GI parasites in monkeys

4.1.3 Overall prevalence rate of specific GI parasites of monkeys

Out of 93 collected samples, 69 were positive with 10 different GI parasites in the Rhesus Macaque and Hanuman Langur of Devghat, Chitwan. Identified parasites included three protozoan species (*Balantidium coli*, *Eimeria* sp. and *Entamoeba* sp.) and seven helminth species (*Trichuris* sp., *Ascaris* sp., *Strongyloides* sp., *Oesophagostomum* sp., Hookworm sp., *Trichostrongylus* sp. and *Hymenolepis* sp.).

Among the GI parasites a highest prevalence rate of 27.95% was detected for *Balantidium coli* followed gradually by *Trichuris* sp. (23.65%), *Eimeria* sp. (16.12%), *Entamoeba* sp. (13.97%), *Ascaris* sp. (11.82%), *Strongyloides* sp. (10.75%), *Oesophagostomum* sp. (5.37%), Hookworm sp. (3.22%), *Trichostrongylus* sp. (3.22%) and *Hymenolepis* sp. (1.07%). Unidentified nematode larvae also recorded at 6.45% of total samples (fig. 5). Overall prevalence of specific GI parasites were highly significantly different (χ^2= 57.987, df=10 and $P<0.05$).

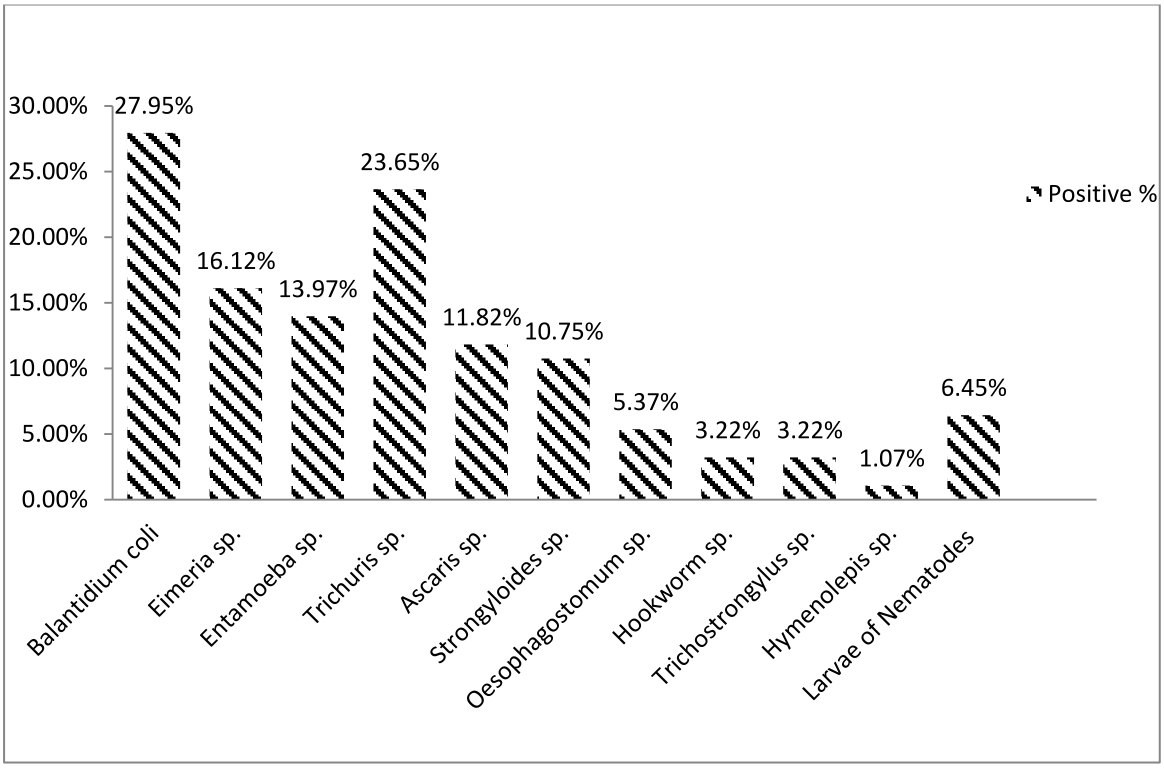


Figure 5: Overall prevalence of specific GI parasites in monkeys

4.1.4 Infection status of GI parasites

Out of 93 faecal samples, single, double, triple and more than triple species of parasites were observed in 34, 27, 6 and 2 samples respectively (fig. 6). The infection status of GI parasites were significantly difference (χ^2= 38.996, df=3 and P< 0.05).

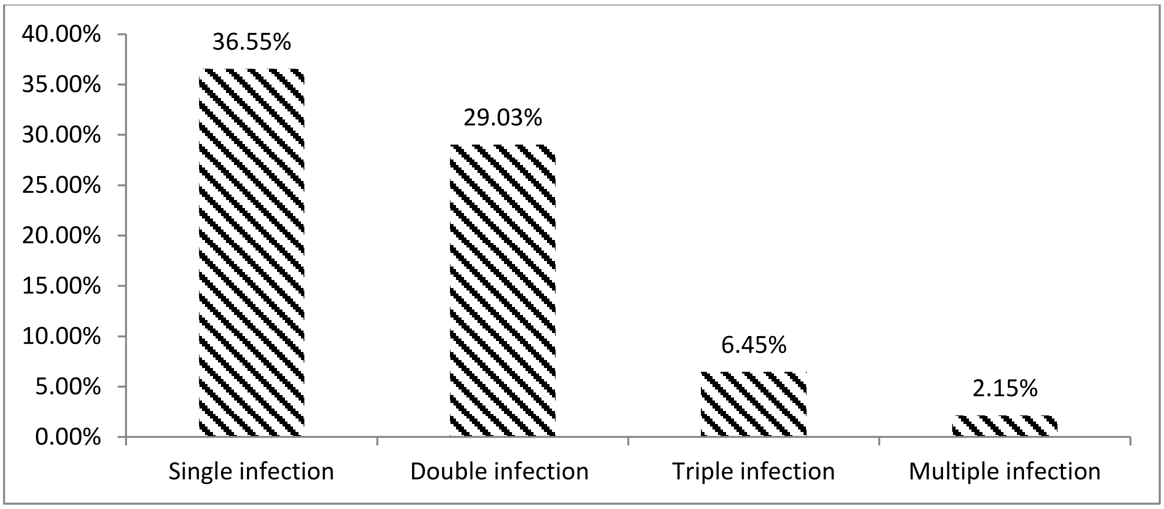


Figure 6: Infection status of GI parasites in monkeys

4.2. Prevalence of GI parasites in monkey species

4.2.1 General prevalence of GI parasites of Rhesus Macaque

Among the 73 faecal samples collected from Rhesus Macaque of Devghat, Chitwan, 55 samples were found to be positive 75.34% for at least one of the GI parasites and remaining 18 samples were negative (24.66%) (fig.7).

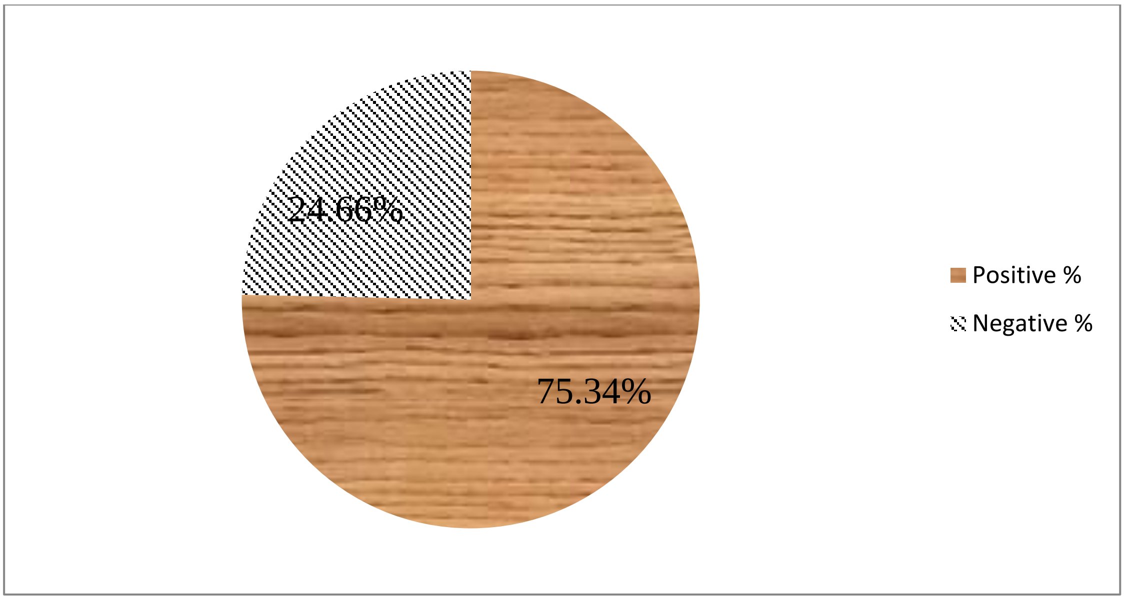


Figure 7: General prevalence rate of GI parasites in Rhesus Macaque

4.2.2 Phylum and class wise prevalence rate of GI parasites in Rhesus Macaque

The prevalence of GI parasitic infection was 75.34% (55 of 73) where helminths and protozoal infection were 52.05% (38 of 73) and 39.72% (29 of 73) respectively. The prevalence rate of helminth was higher than protozoa infection which was no significantly difference ($\chi^2 = 0.884$, df= 1 and P> 0.05) (fig. 8).

Rhesus Macaques were found to be infected with protozoan parasites belonging to three classes. Among them litostomata showed the highest prevalence (26.02%) followed by sporozoa (17.80%) and sarcodina (15.06%). In helminth infection, nematode parasite showed highest prevalence rate. Only one species of cestode parasite was observed and there was no observed trematode parasites (fig: 9)

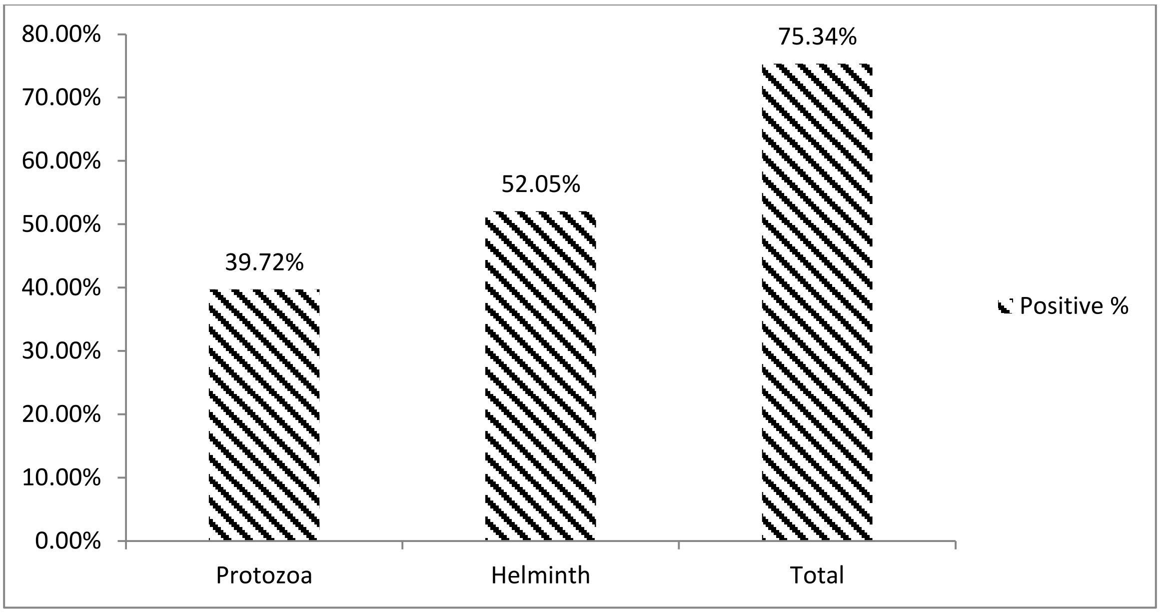


Figure 8: Phylum wise prevalence rate of GI parasites in Rhesus Macaque

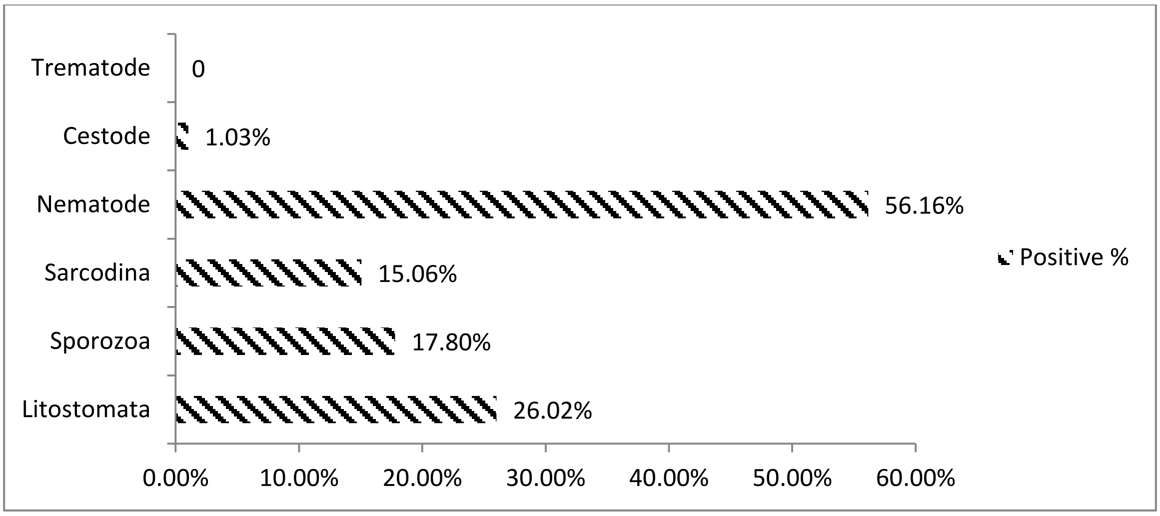


Figure 9: Class wise prevalence rate of GI parasites in Rhesus Macaque

4.2.3 Prevalence rate of specific GI parasites in Rhesus Macaque

Out of a 73 faecal samples collected from Rhesus Macaque of Devghat, Chitwan, 55 samples were positive for 10 different GI parasites.

Among the GI parasites *Balantidium coli* was found to be the most prevalent GI parasite with 26.02% followed gradually by *Trichuris* sp. (20.54%), *Eimeria* sp. (17.80%) and *Entamoeba* sp. (15.06%), *Ascaris* sp. (12.32%), *Strongyloides* sp. (10.95%), *Oesophagostomum* sp. (5.47%), Hookworm sp. (4.10%), *Trichostrongylus* sp. (2.73%) and *Hymenolepis* sp. (1.36%). Unidentified nematode larvae recorded at 8.21% of total samples (fig. 10). The prevalence of specific GI parasites of Rhesus Macaque found to be statistically significant (χ^2= 48.748, df= 10 and P<0.05)

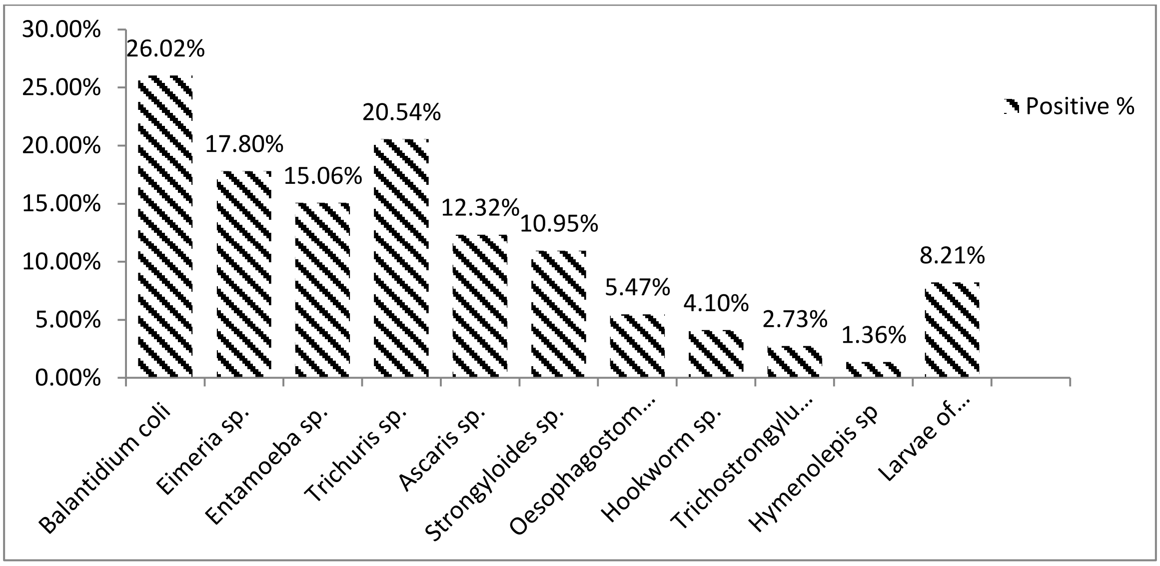


Figure 10: Prevalence of specific GI parasites in Rhesus Macaque

4.2.4. Infection status of GI parasites in Rhesus Macaque

Out of 73 faecal samples, single, double, triple and more than triple species of parasites were observed in 27, 22, 5 and 1 samples respectively (fig.11). The rate of infection of GI parasites were significantly difference (χ^2= 41.123, df= 3 and P< 0.05).

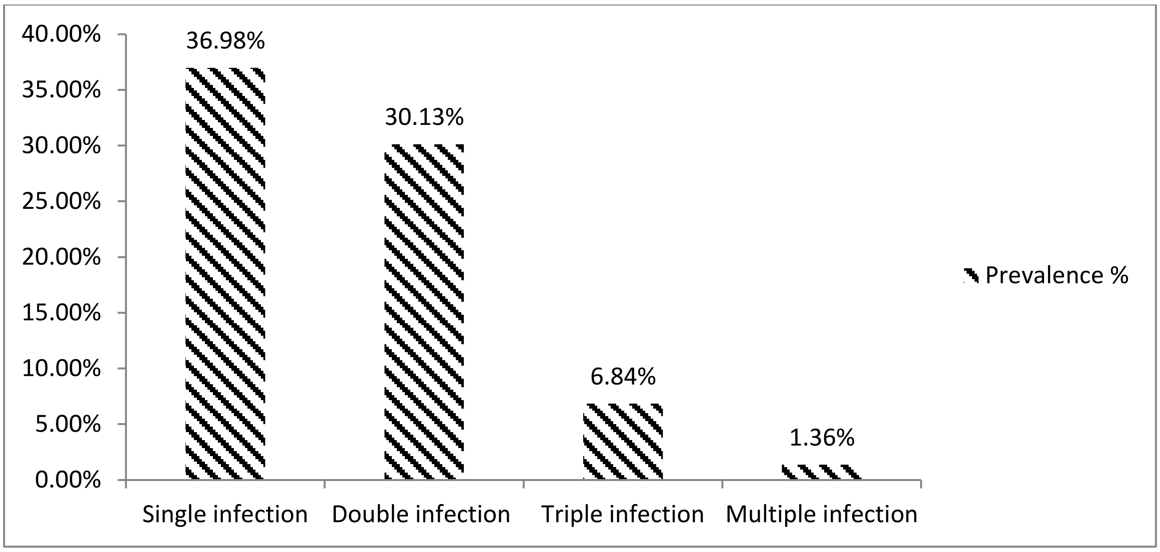


Figure 11: Infection status of GI parasites in Rhesus Macaque

4.2.5 General prevalence of GI parasites of Hanuman Langur

Among the 20 faecal samples were collected from Hanuman Langur of Devghat, Chitwan, 14 samples were found to be positive 70% for at least one of the GI parasites and remaining 6 samples were negative (30%) (fig. 12).

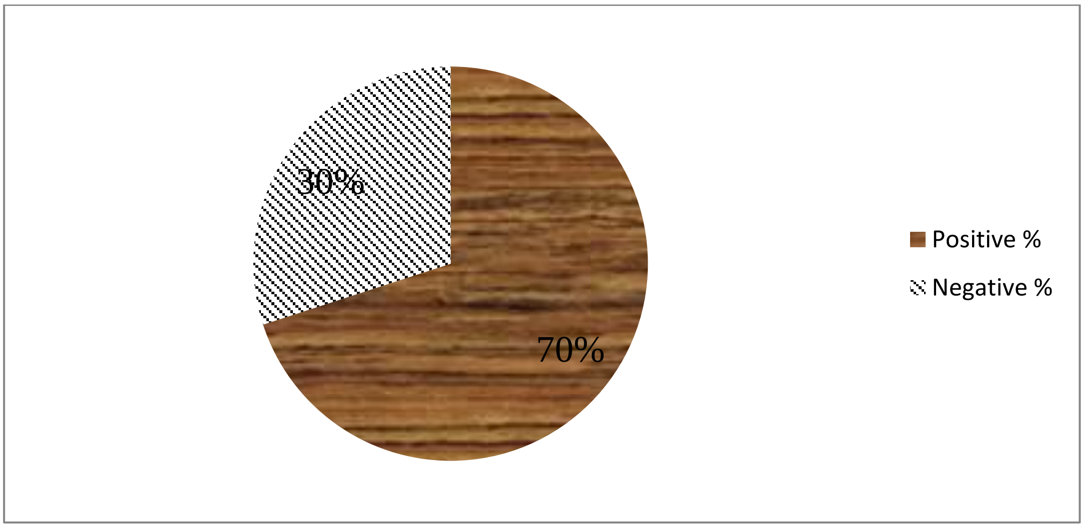


Figure 12: General prevalence rate of GI parasites in Hanuman Langur.

4.2.6 Phylum and class wise prevalence rate of GI parasites in Hanuman Langur

Out of 20 samples, prevalence of GI parasites in Hanuman Langur was 70%, where protozoal infection was 45% and Helminth infection was 55% (fig. 13). The prevalence rate of helminth was higher than protozoa infection which was no significantly difference ($\chi^2 = 0.4822$, df= 1 and P> 0.05).

Hanuman Langurs were found to be infected with protozoan parasites belonging to three classes. Among them litostomata showed the highest prevalence (35%) than sporozoa (10%) and sarcodina (10%). In regarding helminthes parasites nematode parasite were found with highly prevalence rate and other cestode and trematode parasites were not detect from Hanuman Langur (fig. 14)

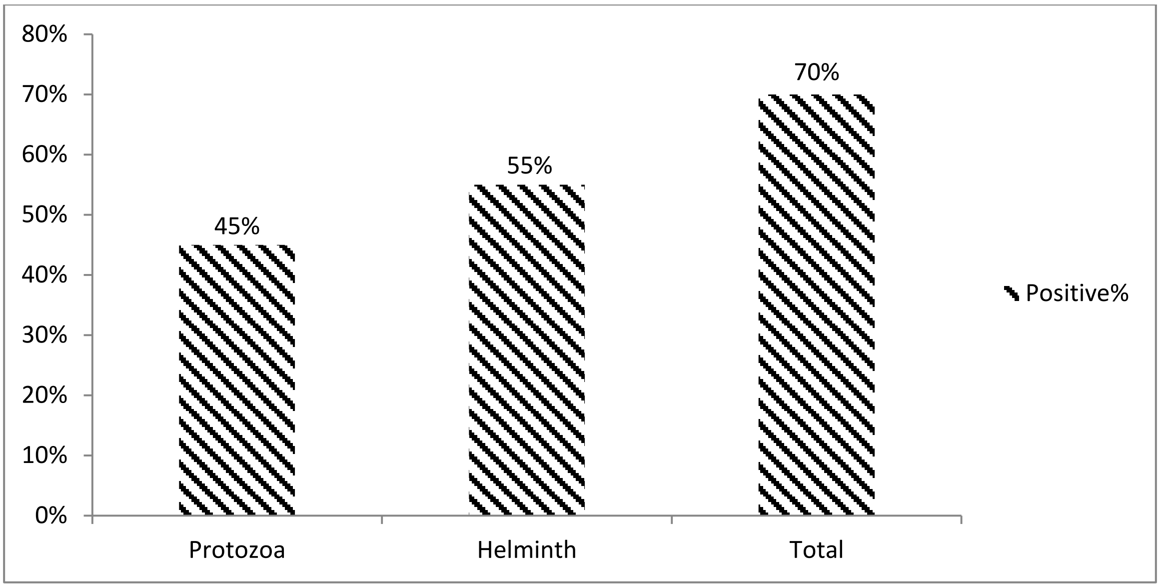


Figure 13: Phylum wise prevalence of GI parasites in Hanuman Langur

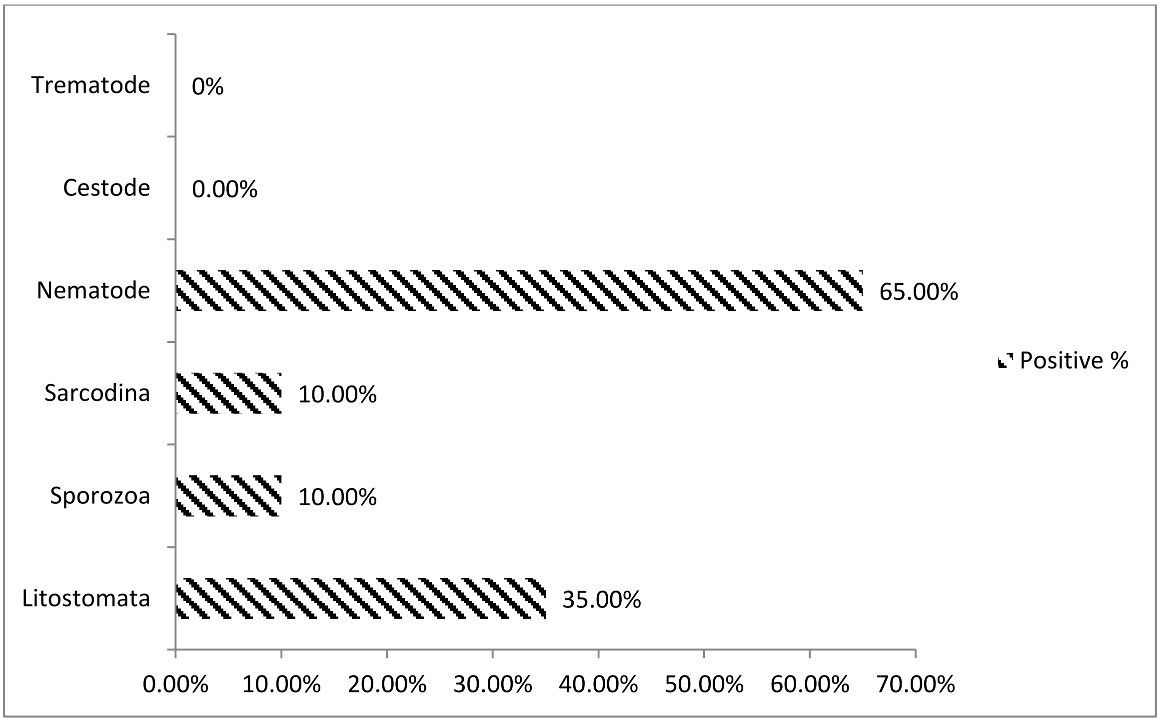


Figure 14: Class wise prevalence rate of GI parasites in Hanuman Langur

4.2.7 Prevalence rate of specific GI parasites in Hanuman Langur

A total of 20 faecal samples were collected from Hanuman Langur of Devghat, Chitwan. 14 samples were positive for 8 different GI parasites with three protozoans and five helminths. Among the prevalence of GI parasites *Balantidium coli* and *Trichuris* sp. were showed highest prevalent with 35% and followed gradually by *Eimeria* sp. (10%), *Entamoeba* sp. (10%), *Ascaris* sp. (10%), *Strongyloides* sp. (10%), *Oesophagostomum* sp. (5%) and *Trichostrongylus* sp. (5%) (fig. 15). The prevalence of specific GI parasites of Hanuman Langur found to be statistically significant difference (χ^2= 58.265, df= 7, and $P<0.05$).

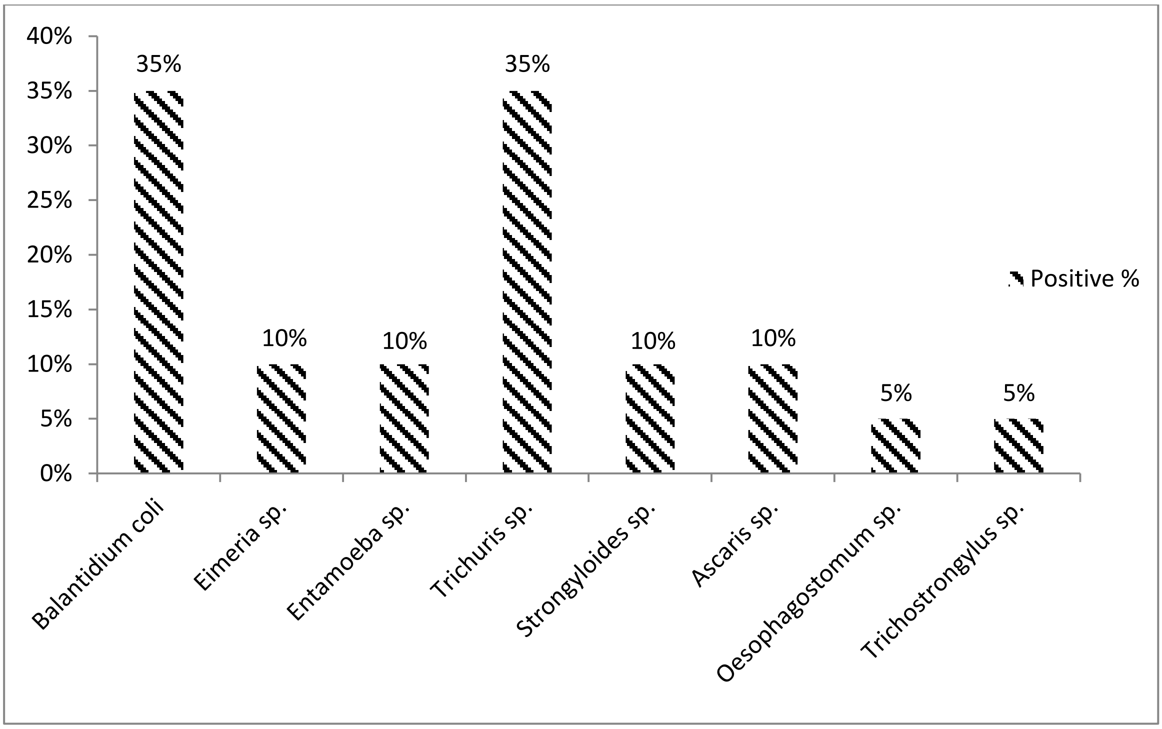


Figure 15: Prevalence rate of specific parasites in Hanuman Langur

4.2.8 Infection status of GI parasites in Hanuman Langur

Out of 20 faecal samples, single, double, triple and more than triple species of parasites were observed in 7, 5, 1 and 1 samples respectively. The infection status of GI parasites were significantly difference (χ^2= 32.541, df= 4 and $P< 0.05$).

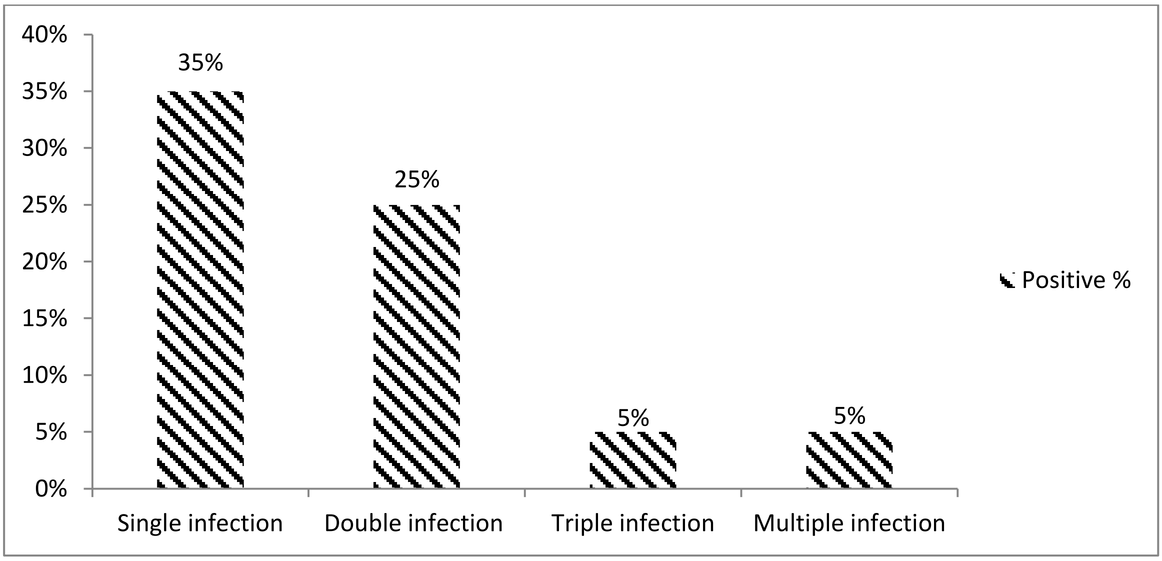


Figure 16: Infection status of GI parasites in Hanuman Langur

4.3 Comparative study of two species of monkey

The prevalence of parasite was slightly higher in Rhesus Macaque (75.34%) than in the Hanuman Langur (70%), but this difference was not statistically significant (χ^2=0, df=1 and $P> 0.05$).

The occurrence of protozoa and helminth in Rhesus Macaque were 39.72% and 52.05% respectively. For Hanuman Langur 45% of samples were positive with protozoa and 55% with helminth. There is no significantly difference of occurrence of protozoa and helminth in Rhesus Macaque and Hanuman Langur (χ^2= 0.237, df = 2 and P> 0.05).
Prevalence and association of specific GI parasites from Rhesus Macaque and Hanuman Langur were not significantly difference i.e., P>0.05 (Table 2).

Table 1: Overall prevalence of gastro-intestinal parasites among monkeys in Devghat, Chitwan.

Monkeys	Sample size	Protozoa positive (%)	Helminth positive (%)	Total positive (%)
Rhesus Macaque	73	29 (39.72%)	38 (52.05%)	55 (75.34%)
Hanuman Langur	20	9 (45%)	11 (55%)	14 (70%)
Total	93	38 (40.86%)	49 (52.68%)	69 (74.20%)

Table 2: Prevalence and association of specific GI parasites from Rhesus Macaque and Hanuman Langur in Devghat, Chitwan.

Parasites	Rhesus Macaque	Hanuman Langur	χ^2	Df	P value
Protozoa					
Balantidium coli	26.02%	35%	0.7405	1	0.3895
Eimeria sp.	17.80%	10%	1.4029	1	0.2362
Entamoeba sp.	15.06%	10%	0.5492	1	0.4586
Helminth					
Trichuris sp.	20.54%	35%	2.4566	1	0.117
Ascaris sp.	12.32%	10%	0.0588	1	0.8083
Strongyloides sp.	10.95%	10%	3.22×10^{-30}	1	1
Oesophagostomum sp.	5.47%	5%	7.52×10^{-31}	1	1
Hookworm sp.	4.10%	0%	-	-	-
Trichostrongylus sp.	2.73%	5%	-	-	-
Hymenolepis sp.	1.36%	0%	-	-	-
Larvae of Nematode	8.21%	0%	-	-	-
Total protozoa infection	39.72%	45%	0.1233	1	0.7254
Total helminth infection	52.05%	55%	0.0121	1	0.9121
Total parasitized	75.34%	70%	0.0520	1	0.8196
Single infection	36.98%	35%	0.0039	1	0.95
Double infection	30.13%	25%	0.2113	1	0.6457
Triple infection	6.84%	5%	0.0486	1	0.8255
Multiple infection	1.36%	5%	1.037	1	0.3085

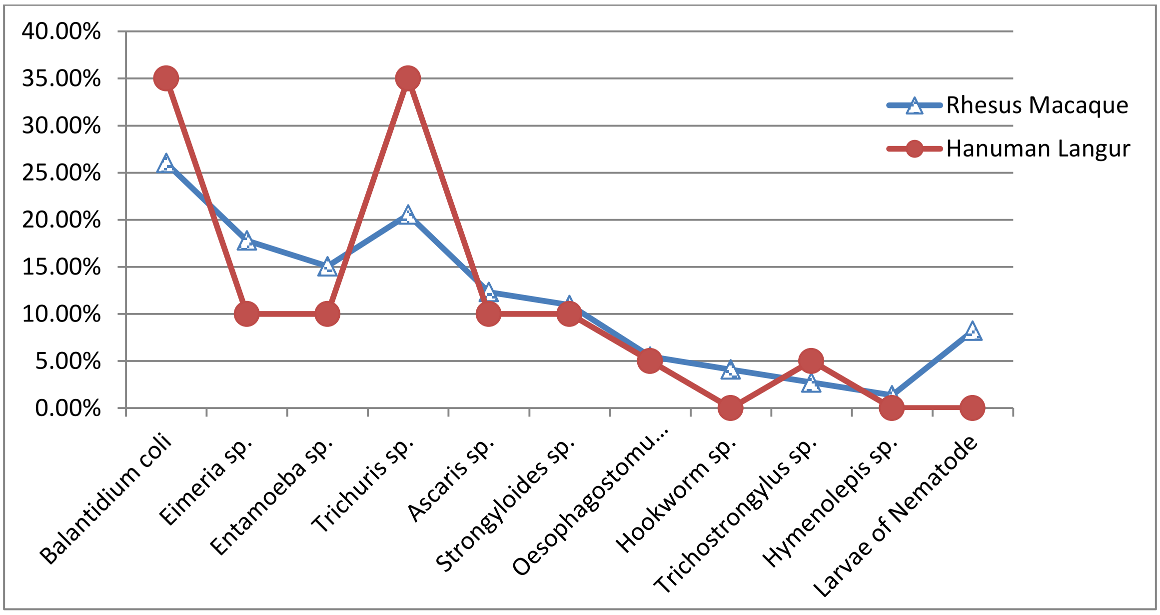


Figure 17: Comparison of specific GI parasites between Rhesus Macaque and Hanuman Langur.

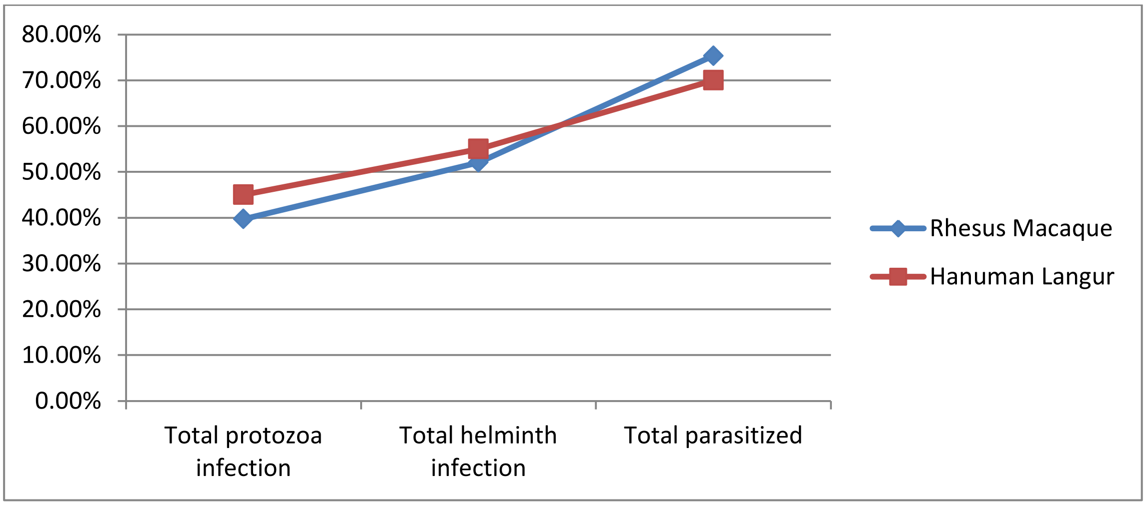


Figure 18: Phylum wise comparison of GI parasites between Rhesus Macaque and Hanuman Langur.

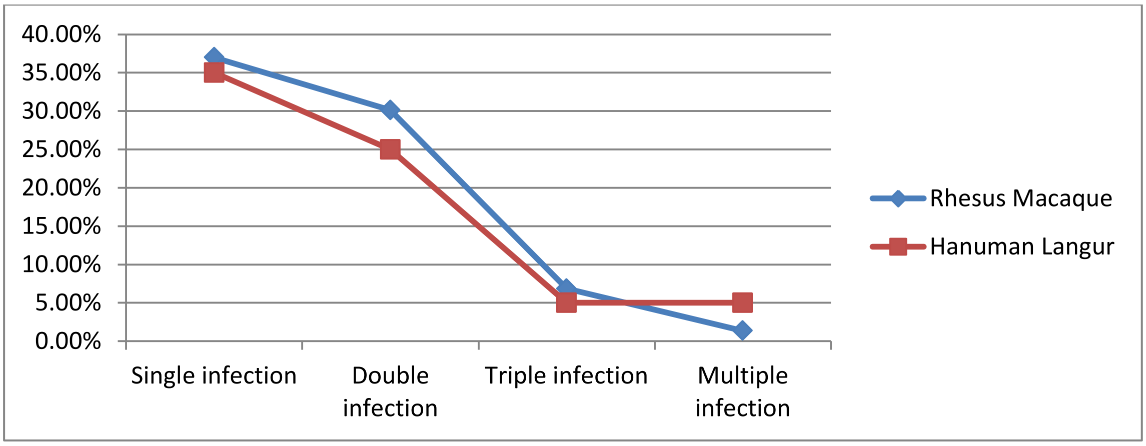


Figure 19: Comparisioan of infection status of GI parasites between Rhesus Macaque and Hanuman Langur.

4.4 Identified ova or eggs/cyst/oocysts of GI parasites from monkeys

Table 3:-The morphology of ova or egg/ cysts/ oocysts of different GI parasites of Rhesus Macaque and Hanuman Langur.

Name of parasite	Size (µm)		Morphological characters	Reference values (PV. 2012; Dewit *et al.*, 1991; Helenbrook *et al.*, 2015)
	Length	wide		
Balantidium coli ➢ Cyst ➢ Trophozoite	 40-45 25-40	 - 20-25	➢ It was spherical, yellow brownish colored. It contained one macro and micro nucleus. ➢ It was oval shaped covered in short cilia.	44.5 µm diameter
Eimeria sp.	23.29	-	Ovoid and spherical shaped, contained polar cap (micropyle) or without polar cap.	30×15 µm
Entamoeba sp.	15.52	-	Small spherical, reddish brown coloured contained one to four nucleus.	12 µm diameter
Trichuris sp.	50-55	20-25	Elongated barrel shaped, bile stained with bipolar plugs at each end. Plugs were colourless.	50-60 µm×21-25 µm
Ascaris sp. ➢ Fertile ➢ Infertile ➢ Decorticated	 (60-95.75) 80-95 30-45	 40-65 35-45 -	➢ Rounded or ovoidal shaped, Brown coloured with thick shelled. ➢ Elongated, kidney shaped. Internal material was a mass of irregular globules and granules. ➢ Both fertile and unfertilized eggs lacked their outer albuminous coats and were colourless.	50-75 µm×40-50 µm
Strongyloides sp.	48-54	25-30	Oval shaped, thin shell with smooth surface contained a short thick larva.	45-55 µm×26-35 µm
Oesophagostomum sp.	85-92	44-50	Ovoidal shaped, thin shelled with about 16-32 blastomeres were present.	88-105 µm ×44-65 µm

Hookworm sp.	50-65	33-40	Oval shaped, thin shelled, contained morula with 2-16 cells, without bile stained, Both pole rounded.	70.2×45.9 µm
Trichostrongylus sp.	85-95	40-45	Oval shaped, dissimilar and not very wide poles, one of which was more rounded than other. Thin shelled. There was usually a lot of clear space within an egg.	88×45 µm
Hymenolepis sp.	44	38.82	Oval shaped, shell consisted of two distinct membrane and embronated 6- hooked oncosphere inside shell.	40-60 µm×30-50 µm

4.5 Photo plates of GI parasites

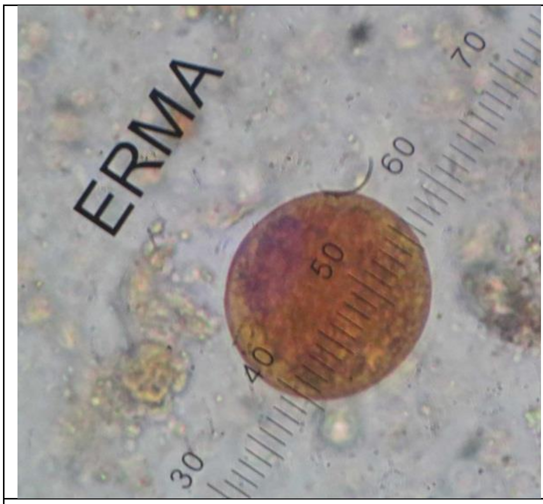


Photo no: 8- Cyst of *Balantidium coli* (10X×40X)

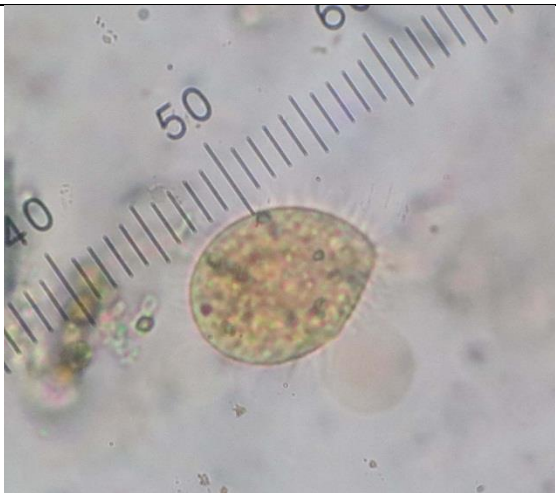


Photo no: 9- Trophozoite of *B. coli* (10X×40X)

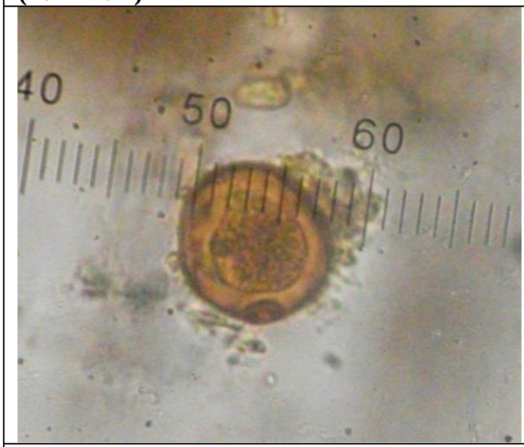


Photo no:10- Oocyst of *Eimeria* sp. (10X×40X)

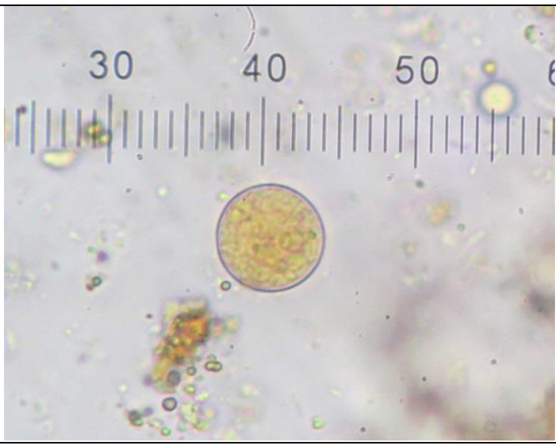


Photo no:11- Cyst of *Entamoeba* sp. (10X×40X)

Photo no:12- Egg of *Trichuris* sp. (10X×40X)

Photo no:13- Egg of *Ascaris* sp. (fertile) (10X×40X)

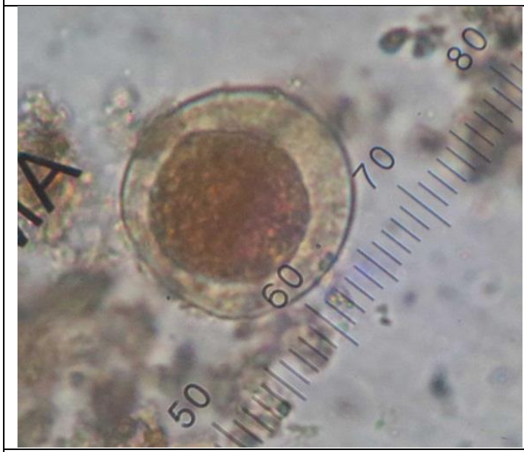


Photo no:14- Decorticated egg of *Ascaris* sp. (10X×40X)

Photo no:15- Unfertilized egg of *Ascaris* sp. (10X×40X)

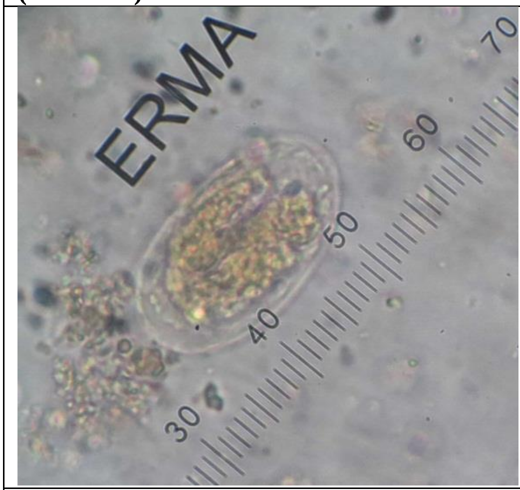


Photo no:16- Egg of *Strongyloides* sp. (10X×40X)

Photo no:17- Egg of *Oesophagostomum* sp. (10X×40X)

Photo no:18- Egg of Hookworm sp. (10X×40X)

Photo no:19- Egg of *Trichostrongylus* sp. (10X×40X)

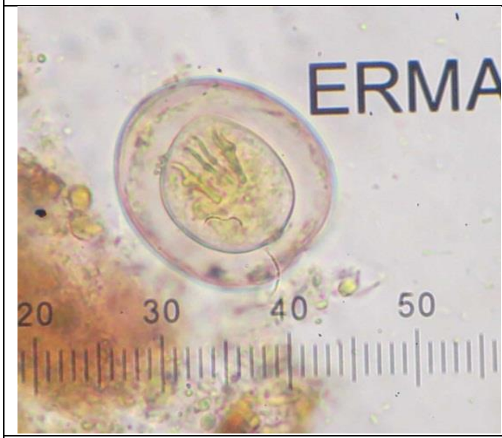


Photo no:20- Egg of *Hymenolepis* sp. (10X×40X)

Photo no:21- Larvae of unidentified nematode. (10X×40X)

5. DISCUSSION

The intension of the study was to investigate the prevalence of GI parasite Rhesus Macaque and Hanuman Langur in Devghat, Chitwan.

In the present study, total of 93 faecal samples were collected from the Rhesus Macaque and Hanuman Langur. After the laboratory examination, 69 (74.20%) were found to be positive for one or more than one GI parasites. Regarding high prevalence rate of monkey species, Kouassi *et al.* (2015) recorded 100% parasite infection from seven species of monkeys. About (60- 90)% parasitic infection recorded by Huffman *et al.* (2013) from Toque Macaque and langur, by Hilser *et al.* (2011) from Red Langur, by Sing *et al.* (2009) from Capped Langur, by Jones-Engel *et al.* (2004) from nine species of Pet Macaque, by Mutani *et al.* (2003) from Green Monkey, by Thawait *et al.* (2014) from Rhesus Macaque. In national context, the previous study recorded (60-85) % prevalence of parasite from Rhesus Macaque by Malla (2007), Dhoubhadel (2007), Nepal (2010) and from Assamese Macaque by Pokhrel (2014). But Nath *et al.* (2012) documented 13.63% of prevalence of parasitic infection in captive four *Macaca* species and one Golden Langur. In case of captive monkeys, the lower rate of prevalence could be due to regular screening of faecal samples and periodical anthelmintic treatment in most of the zoos, as per the protocol of zoo authority. Also Parmar *et al.* (2012) found 40% prevalence rate of parasitic infection in Rhesus Macaque and 34.14% in Hanuman Langur form forest area, Ekanayake *et al.* (2006) recorded 27% from Toque Macaque and langur in the Polonnaruwa Nature Sanctuary and Archaeological Reserve, from Assamese Macaque (48.38%), Rhesus Macaque (35.29%) and Hanuman Langur (25%) recorded by Sing *et al.* (2009) in Zoological park, from free living Rhesus Macaque recorded 43% of parasitic infection by Arunachalam *et al.* (2015). Low parasitic infection may be due to their natural feeding habit of tree leaf, bark and fruits especially those of medicinal values, like neem and pomegranate leaves, which declined the parasitic infection (Parmar *et al.*, 2012). The differences in the prevalence of GI parasites in wild and captive monkeys may be due to the different species of monkeys, age group, sex group, study area, climatic conditions, environmental condition and varied susceptibility of monkeys to the parasites.

The prevalence of helminth infection (52.68%) was found higher than protozoal infection (40.86%). Class wise protozoal infection recorded litostomata (27.95%), sporozoa (16.12%) and sarcodina (13.97%). This is more or less similar with the report of Adetunji (2014). Who revealed that 61.1% Non-human primate positive for helminth infection and 13.9% for protozoa in zoological Gardens in Ibadan, Nigeria. The present study conflict the report of Hilser *et al.* (2011) who recorded that 62% langurs were positive for helminth infection and 82% were protozoan infection. These differences may be due to geographic condition, source of feeds and feeding behaviour of monkeys. Among the helminth infection, the study showed higher prevalence of nematode species (58.06%) than cestode (1.07%) and was not found trematode species. The high occurrence of GI helminthes which included more of nematodes agrees with (Rossanigo and Gruner, 1995) who documented that nematodes are capable for most of the helminthes disease of veterinary importance. The higher occurrence of nematodes than cestodes and trematodes, agree with Pokhrel (2014), Nepal (2010), Malla (2007), Sing *et al.* (2009).

From the result of current study, ten different GI parasites were reported from Rhesus Macaque and Hanuman Langur. Among them three were protozoan and seven were helminthes. In protozoa, *Balantidium coli* was found in maximum positive samples i.e., 27.95%, which supports the findings of Pokhrel (2014), Jha *et al.* (2011) from Assamensis Monkeys and Rhesus Monkey respectively. It has a wide host range and possess a simple direct life cycle and it's occurrence in primates has been previously confirmed by Lim *et al.* (2008), Gomez *et al.* (2000) and Khatun *et al.* (2014). From the present study other protozoa viz. *Eimeria* sp. and *Entamoeba* sp. were found 16.12% and 13.97% respectively. That coincides the finding of Thawait *et al.* (2014), Wangsawad (2009). In some finding showed more than three protozoan parasites (Jones-Engel *et al.*, 2004; Ekanayak *et al.*, 2006; Sing *et al.*, 2009) and less protozoan parasites (Adetunji, 2014; Huffman *et al.*, 2013; Parmar *et al.*, 2012) from *Macaca* sp. and langur. The differences were might be due to the source of feeds, deworming environmental condition, climatic condition and also sample size is responsible for low and high prevalence of parasitic infection.

In helminthes infection *Trichuris* sp. showed the higher prevalence rate than other parasite i.e., 23.65%. This type of result also found by Pokhrel (2014) from Assamese Macaque, by Huffman *et al.* (2013) from *Macaca* sp. and langur monkeys, by Nath *et al.* (2012) from *Macaca* sp. and Golden Langur, by Hilser *et al.* (2011) from Red Langurs, by Parmar *et al.* (2012) also recorded 20% from Hanuman Langur, by Chapman *et al.* (2009) from Colobus Monkeys in more or less similar climatic condition. But Arunachalam *et al.* (2015) from Rhesus Macaque,Thawait *et al.* (2014) from Rhesus Monkey, Nepal (2010) from Rhesus Macaque, Ekanayake *et al.* (2006) from macaque and langur, Malla (2007) and Dhubhadel (2007) from Rhesus Monkey, Gotoh (2008) from *Macaca* sp. isolated another parasites for high prevalence. The differences between the results of high prevalence rate of *Trichuris* sp. might be due to climatic condition. Because *Trichuris* sp. well exist in a warm moist climate, low light, wet soil within temperate and tropical climates (Roberts and Janovy, 2000; Smyth, 1994). Chapman *et al.* (2009) recorded *Trichuris* sp. infection was higher abundance in the wet lowlands than highlands. The present study area (Devghat, Chitwan) was a lowlands and frequently wetter due to the Narayani River with Tropical forest. Due to this reason *Trichuris* sp. was possibly linked to higher prevalence for this area.

11.82% of *Ascaris* sp. was found in the total samples of the present study. Which is similar to the findings of Salgado-Lynn *et al.* (2010), Hilser *et al.* (2011), Parmar *et al.* (2012), Malla (2007), Pokhrel (2014) from Long tailed Macaque and Probosis Monkey, Red Langur, Hanuman Langur and Rhesus Macaque, Rhesus Macaque, Assamese Macaque respectively. But Arunachalam *et al.* (2015) documented 5%, Dhubhadel (2007) recorded only 1.57% from Rhesus Macaque. These types of variation occur due to sanitary condition of habitat and environment.

Present study revealed, the overall infection of *Strongyloides* sp. was 10.75%. Similarly Hilser *et al.* (2011), Pokhrel (2014) reported 13% and 8.24% from Red Langur and Assamese Macaque respectively, and less similar result of Akpan *et al.* (2010) recorded 22% from drill monkeys, Parmar *et al.* (2012) recorded 26.66% from Hanuman Langur. But this result is in contrast to the previous report of Mutani *et al.* (2003) showed that

62.4% of the Green Monkey, and other finding showed higher prevalence rate than present study (Nepal, 2010; Malla, 2007; Dhubhadel, 2007). This type of fluctuated result may be depends upon sanitary condition of environment, societies, health condition of other wild and domestic animal where they share habitat.

From the present study, *Oesophagostomum* sp. isolated 5.37% of total samples. It has been reported from *Macaca fuscata*, Green Monkey, Colobus Monkey, Assamese Monkey, Golden Langur, Rhesus Monkey, *Presbytis* sp. (Dewit *et al.*, 1991; Gotoh, 2008; Mutani *et al.*, 2003; Gillepsie *et al.*, 2005; Malla, 2007; Dhubhadel, 2007; Wongsawad, 2009; Nath *et al.*, 2012; Thawait *et al.*, 2014; Pokhrel, 2014). This previous result ranged was 4%-28% but Dewit *et al.* (1991) recorde 80% prevalence of *Oesophagostomum* sp. This difference might be due to feeding sources and sanitary condition of environment. It has been recorded that *Oesophagostomum* sp. found higher intensities during the wet season (Pettifer, 1984), similar that in this study, found low infection during dry seasons.

Hookworm sp. recovered 3.22% of total samples from present study. This finding is an agreement with the reports of Malla (2007), Nepal (2010), Pokhrel (2014) were recorded 2.14%, 2.75% and 4.7% respectively from Rhesus Macaque and Assmese Macaque. It was different with the previous report of Hilser *et al.* (2011) who found at 28% from Red Langur. This different depends on soil moisture, sanitary condition of environment and climatic condition was an important factor describing the differences of prevalence rates of parasite species among various geographical area (Nunn *et al*., 2005).

In this study, *Trichostrongylus* sp. was found 3.22% prevalence rate among the helminth parasites. This result was supported by Mutani *et al.* (2003) from Green Monkey recorded 3.8%, Hilser *et al.* (2011) from Red Langur recorded 5%, Malla (2007) and Dhubhadel (2007) recorded 4.83% and 3.14% respectively from Rhesus Monkey. But Nepal (2010) recorded slightly higher i.e., 11.75% from Rhesus Monkey. The prevalence of this parasites in monkey in interesting, because it is an important parasite of ruminant i.e., Grazzing mammals (Crockett and Dipeolu, 1984), prevalence of parasites because of contamination of their environment with ruminant waste (Munene *et al.*, 1998). So there was a low prevalence rate in other mammals due to different feeding behaviour.

Hymenolepis sp. has been reported from *Macca sinica* and *Presbytis* sp., Capped Langur, Drill Monkey (Dewit *et al.*, 1991; Sing *et al.*, 2009; Akpan *et al.*, 2010). As similar that, the present study revealed *Hymenolepis* sp. was least common parasite at 1.07% of total samples. It has been recorded for the first time in Nepal from monkeys. It is a common parasites of rodents. The beetle and fleas are act as intermediate host but not necessary i.e., also auto infecting parasite. Due to the insectivorous nature of monkey or accidently ingestion of fleas, they were become infecting. According to Beck *et al.* (2006), fleas were higher prevalence in warmer climate than other. Similar that, study area also provides the favorable condition for fleas.

The huge diversity and densities of pathogen species represent huge diversities of life cycle, transmission routes and pathogenicity that causes great harmful to animals and affecting wildlife can be threat to conservation (Woolhouse, 2002). So that single infection was not highly harmful as comparative to double, triple and multiple infections. Multiple infection may cause heavy loses i.e., in growth pattern, reproduction, fecundity, establishment and may cause death. During the study, out of the 93 samples, single, double, triple and multiple species of parasites were observed in 36.55%, 29.03%, 6.45% and 2.15% respectively. The ratio of infection status showed there was 2.15% samples were infected with more than three parasitic infection so they had higher risk to critical infection of GI parasites if untreatable condition. Similar that of study, Pokhrel (2014) recorded 43.53%, 17.65%, 4.7% and 1.17% for Single, Double, Triple and Quadruple infection from Assamese Macaque. Dhoubhadel (2007) observed 65.3%, 24.4%, and 7.08% for Single, Double and Multiple infections from Rhesus Macaque. But Nepal (2010) documented 39.61% had a single infection and 60.39% were multiple infections from Rhesus Macaque due to categorized into only two status i.e., Single and multiple infections.

During the study, 73 samples were taken from Rhesus Macaque and 20 from Hanuman Langur where parasitic prevalence rate were 75.34% and 70% respectively. The Hanuman Langurs are shy, mostly arboreal in comparison to Rhesus Macaque (Chalise *et al.*, 2005; Gewali, 2013). So that it is less contact to human, other animals and ground. Simply had low prevalence of parasitic infection (Dewit *et al.*, 1991; Parmar *et al.*, 2012). In the present study area also Rhesus Macaques were found in village and temple area whereas Hanuman Langurs are in forest area. Rhesus Macaque frequently contact with human and house wastage material but not Hanuman Langur. So the Rhesus Macaque had higher chance to parasitic infection as compare to Hanuman Langur. Gillepsie (2006) documented a result, that revealed sample size and prevalence rate were reciprocally related i.e., Minimum sample size showed maximum prevalence rate. According to that statement, the Hanuman Langurs are higher healthy than Rhesus Macaque. During the present study ten species of parasites were observed form Rhesus Macaque and eight species from Hanuman Langur. It also showed Rhesus Macaques were highly infected by parasitic species than Hanuman Langur.

Most noticeable was the prevalence of Hookworm sp. and *Hymenolepis* sp. where infections were not detected in the highly arboreal Hanuman Langur, in contrast to the less arboreal Rhesus Macaque.

The occurrence of GI parasites between two monkey species was not statistically significant difference i.e., $P>0.05$. But less similar reports (Hilser *et al.*, 2011) indicated that both significant and also not significant difference in occurrence of GI parasites between three species of primates viz. orangutan, gibbon and langur. In the present study due to same area, same feeding source, same climatic condition showed similar parasitic species in similar manner. Pearson's Chi-squared test with Yates' continuity correction revealed there was not significant difference of parasitic infection in Rhesus Macaque and Hanuman Langur of Devghat, Chitwan.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study, it is cleared that the GI parasites are highly prevalent (74.20%) in Rhesus Macaque and Hanuman Langur of Devghat, Chitwan. Among the GI parasites, helminths (52.68%) were more prevalent than protozoans (40.86%). Altogether seven helminthes and three protozoan parasites were identified. While concerning on protozoan parasites, *Balantidium coli* was the highest prevalent GI parasite, whereas *Trichuris* sp. was the highest prevalent helminthes parasites contributing 27.95% and 23.65% of total GI parasitic infection respectively. *Hymenolepis* sp. was the least prevalent GI parasite leading only 1.07% of total GI parasitic infection. The prevalence of other species of GI parasites were: *Eimeria* sp. (16.12%), *Entamoeba* sp. (13.97%), *Ascaris* sp. (11.82%), *Strongyloides* sp. (10.75%), *Oesophagostomum* sp. (5.37%), Hookworm sp. (3.22) and *Trichostrongylus* sp. (3.22%).

From the class wise analysis of parasitic infection litostomata, sporozoa, sarcodina, nematode and cestode were recorded 27.95%, 16.12%, 13.97%, 58.06% and 1.07% respectively where trematode was not detected. Monkeys of Devghat area were highly infected with single species and least infected with multiple species leading 36.55% and 2.15% of total parasitic infection. Double and triple species of parasites contributing 29.03% and 6.45% of total samples respectively.

Comparative finding of this study could be concluded that Rhesus Macaques were more infected by GI parasites than Hanuman Langur contributing 75.34% and 70% of infection respectively. But Pearson's Chi-squared test with Yates' continuity correction, revealed there was not significant differences of parasitic infection in Rhesus Macaque and Hanuman Langur ($P>0.05$). Totally ten specie of GI parasites were detected from the Rhesus Macaque whereas eight species of GI parasites were detected from Hanuman Langur. In Hanuman Langur Hookworm sp. and *Hymenolepis* sp. were not detected but found in Rhesus Macaque. *Hymenolepis* sp. recorded first time in Nepal from Rhesus Macaque.

6.2 Recommendations

Based on the finding of the present study following recommendations have been drawn:

- Further in depth studies must be conducted in large sample size.
- Research should be concentrated in molecular basis for identification of parasite species and understanding bacterial as well as viral disease of monkeys.

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