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PREVALENCE OF GASTRO-INTESTINAL PARASITES OF RHESUS MACAQUE (*MACACA MULATTA* ZIMMERMANN, 1780) AND HANUMAN LANGUR (*SEMNOPIITHECUS ENTELLUS* DUFRESNE, 1797) IN DEVGHAT, CHITWAN, NEPAL

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ABSTRACT

The present investigation was undertaken to study the prevalence of gastrointestinal parasites in Rhesus Macaque and Hanuman Langur at Devghat, Chitwan. Altogether 93 fresh faecal samples were collected from Rhesus Macaque belonging to five troops and 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. Out of 93 samples, 69 (74.20%) were found positive for at least one parasite. Prevalence of helminth and protozoan parasites was 52.68% and 40.86% respectively. Altogether, 10 species of parasites including seven helminth and three protozoa 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 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%). Unidentified larvae of nematode which account for 6.45% of total samples were also recorded. Single, double, triple and multiple species of parasites were found in 36.55%, 29.03%, 6.45% and 2.15% samples respectively.

Keywords: Floatation and sedimentation technique, Gastro-intestinal parasite, Hanuman Langur, Macaque, Protozoan parasites.

INTRODUCTION

Rhesus Macaque and Hanuman Langur are diurnal animals which exist in both arboreal and terrestrial conditions. Hanuman Langurs are timid and less aggressive to human beings and hence are mostly arboreal in comparison to Rhesus Macaque (Chalise *et al.*, 2005; Gewali, 2013). They are leafivorous and insectivorous. Rhesus Macaques are adjustable to environment of human beings and successfully exist in village, cities and towns but Hanuman Langurs are found in mixed deciduous and evergreen forest (Chalise *et al.*, 2005; Gewali, 2013).

There are 633 identified species of primates in the world and 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). In Nepal, the estimated current

population of Rhesus Macaque is approximately 100,000 and that of Hanuman Langur is not well known (Jnawali *et al.*, 2011).

Parasites are one of the biotic factors which may influence their hosts in different ways (Borgsteede, 1996). 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 are the factors responsible for parasite infection in wild animals (Valdespino *et al.*, 2010). Parasites affect directly in host survival and reproduction through pathological effects and indirectly by reducing host's physical condition (Kalousova *et al.*, 2014). Severe parasitosis can lead to blood loss, tissue damage, spontaneous abortion, congenital malformations, and death (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 (Packer *et al.*, 2003).

Monkeys are particularly susceptible to parasitic infections because they live in cohesive groups characterized by frequent social interactions (Stoner, 1996) and specific feeding and drinking behavior (Pokhrel & Maharjan, 2014) which facilitate parasite transmission between individuals. This study was carried out to understand the prevalence of gastro-intestinal parasites in Rhesus Macaque and Hanuman Langur in Devghat, Chitwan and provide baseline data for further action plan.

MATERIALS AND METHODS

Study site

Devghat lies in the Central part of Nepal. Its geographical location is $85^{\circ}22'30''$ to $84^{\circ}30'00''$ East longitude and $27^{\circ}42'30''$ to $27^{\circ}47'30''$ North latitude (DADC, 2007). Devghat area is the meeting point of Trishuli and Kaligandaki River as well as three districts, namely Tanahun, Chitwan and Nawalparasi. The study area lies in Bharatpur municipality of Chitwan -01. Site 1 for Rhesus Macaque is located between $84^{\circ}25'26.82''$ to $84^{\circ}25'38.86''$ East longitude and $27^{\circ}44'16.27''$ to $27^{\circ}44'26.49''$ North latitude and site 2 for Hanuman Langur is located between $84^{\circ}25'48.21''$ to $84^{\circ}26'53.25''$ East longitude and $27^{\circ}42'54.59''$ to $27^{\circ}43'14.56''$ North latitude (Figure 1).

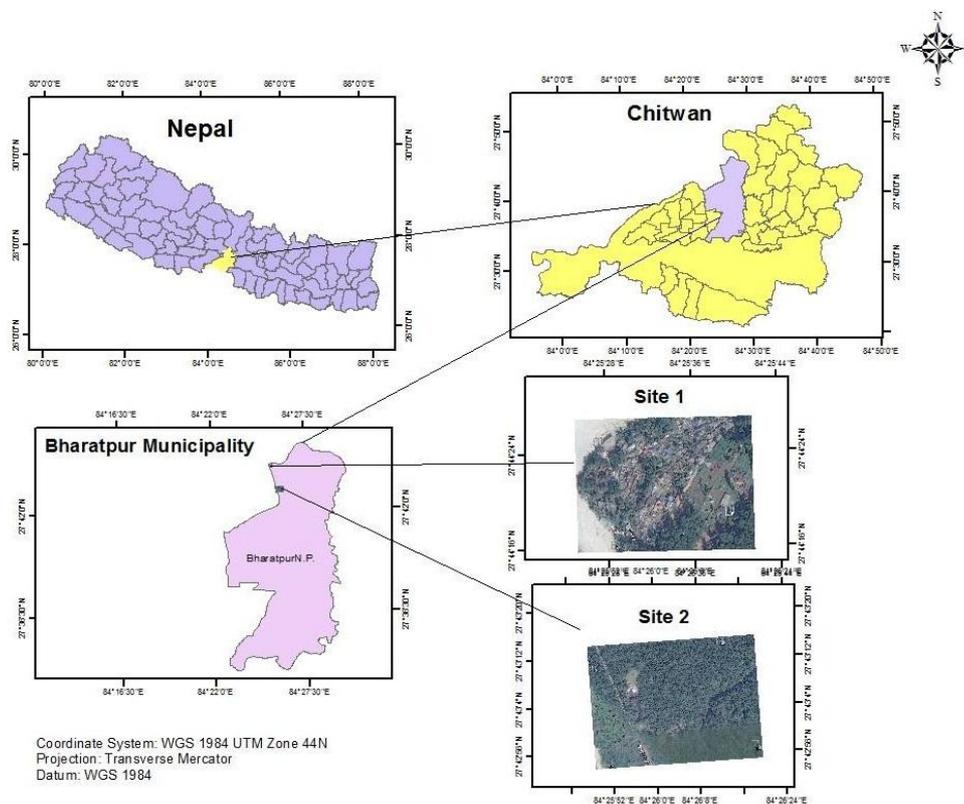


Fig. 1. Study area.

Faecal sample collection and examination

A total of 93 faecal samples were collected systematically by following the troops of Rhesus Macaque (n=5) and Hanuman Langur (n=2). 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 2.5% Potassium dichromate solution. The faecal samples were microscopically examined for trophozoites, cysts,

oocysts, eggs and larvae of gastrointestinal parasites by concentration methods; floatation technique and sedimentation technique (Soulsby, 1982; Zajac & Conboy, 2012). Identification of parasites was based on the morphometry of eggs/cysts under light microscopy (Soulsby, 1982; PV, 2012). By using ocular and stage micrometer, the length, breadth and diameter of parasites (eggs/cysts) were measured with calibration factor. Data were statistically analyzed using Pearson's

Chi-squared test with Yates' continuity correction, performed by "R", version 3.3.1 software packages.

RESULTS

Out of 93 fresh fecal samples, 69 (74.20%) samples were found to be positive for at least one

of gastro-intestinal parasites. Prevalence of protozoal infection was 40.86% and helminth infection was 52.68% (Figure 2). Prevalence of helminth was higher than protozoa but not significantly different ($\chi^2 = 0.884$, $df = 1$ and $P > 0.05$).

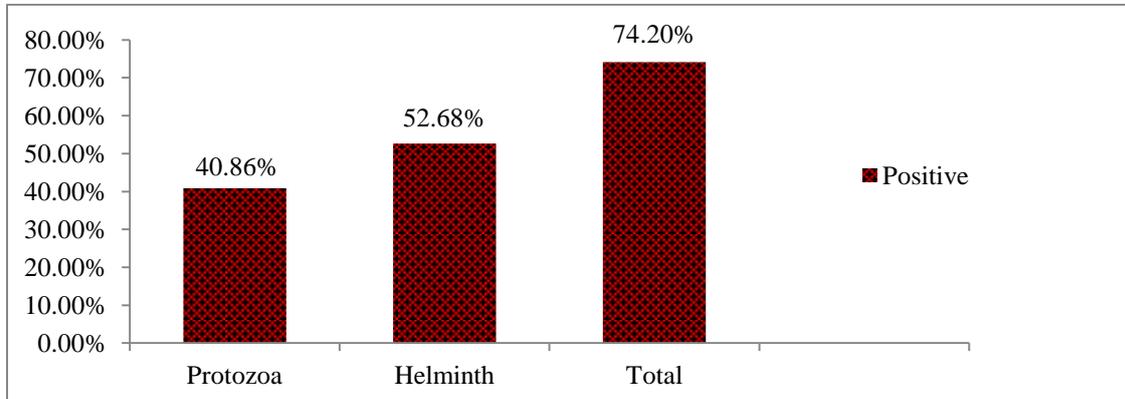


Fig. 2. Overall prevalence of parasitic infection.

Prevalence of specific gastro-intestinal parasites

A total of 10 different gastro-intestinal parasites were identified from 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 gastro-intestinal parasites a highest prevalence rate of 27.95% was detected for *Balantidium coli* followed 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 recorded were 6.45% of total samples (Figure 3). Overall prevalence of specific GI parasites were highly significance ($\chi^2 = 57.987$, $df = 10$ and $P < 0.05$).

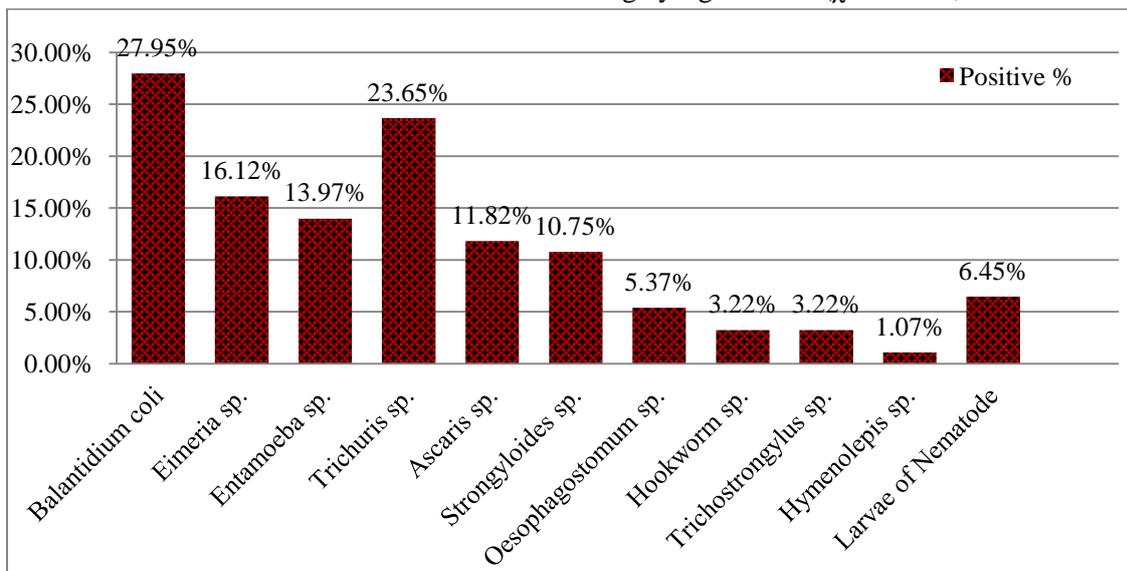


Fig. 3. Prevalence of specific gastro-intestinal parasites.

Infection status of gastro-intestinal parasites

The numbers of samples with single, double, triple and more than triple species of parasites was 34, 27,

6 and 2 respectively (Figure 4). The infection status of gastro-intestinal parasites were significantly different ($\chi^2= 38.996$, $df=3$ and $P< 0.05$).

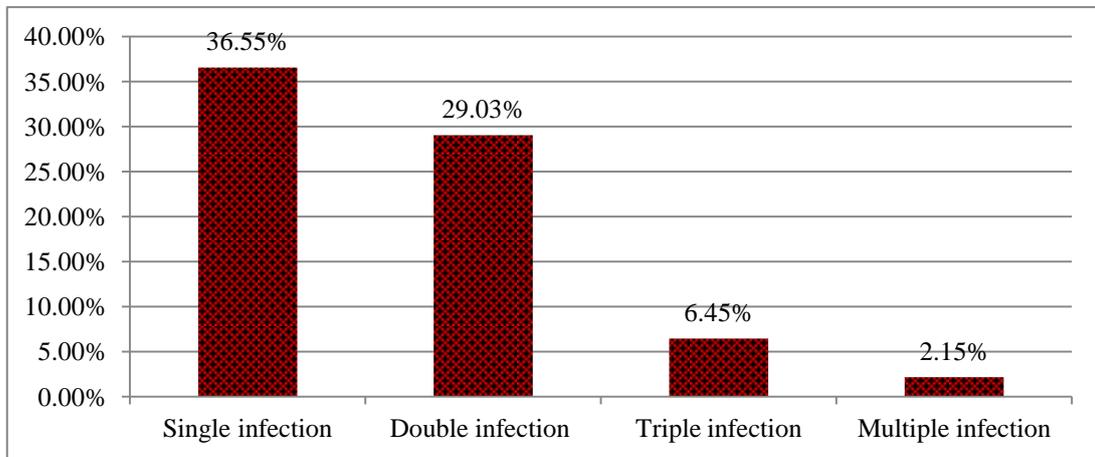


Fig. 4. Infection status of gastro-intestinal parasites.

Prevalence of gastro-intestinal parasites in Rhesus Macaque vs. Hanuman Langur

Among 93 fresh faecal samples, 73 samples were collected from Rhesus Macaque and 20 samples from Hanuman Langur. The prevalence of gastro-

intestinal parasite was slightly higher in Rhesus Macaque than in Hanuman Langur (Figure 5), but this difference was not statistically significant ($\chi^2=0$, $df=1$ and $P>0.05$)

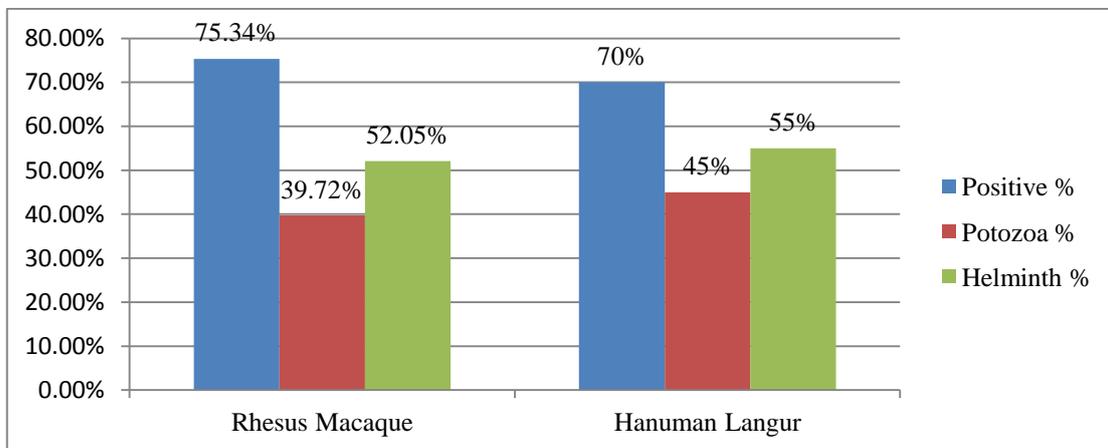


Fig. 5. Prevalence of gastro-intestinal parasites in Rhesus Macaque vs. Hanuman Langur.

DISCUSSION

In this study, 74.20% samples were found positive for single or multiple species of parasites. This result is similar to the investigation of Pokhrel and Maharjan (2014) and Jha *et al.* (2011) who revealed 72.94% and 76.86% positive cases from Assamese Macaque and Rhesus Macaque respectively. In case of captive monkeys, the lower rate of prevalence

(Nath *et al.*, 2012) could be due to regular screening of faecal samples and periodical antihelminthic treatment in most of the zoos, as per the protocol of zoo authority.

The prevalence of helminth infection (52.68%) was found higher than protozoal infection (40.86%). Jha *et al.* (2011) also reported similar result viz. 59.5% and 53.72% for helminth and protozoa

infection respectively. The present study conflict with 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.

From the result of current study, three protozoa and seven helminth gastro-intestinal parasites were reported from Rhesus Macaque and Hanuman Langur. In protozoa, *Balantidium coli* was found in maximum positive samples ie. 27.95%, this supports the findings of Pokhrel and Maharjan (2014) and 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) and Khatun *et al.* (2014). From the present study, other protozoa such as *Eimeria* sp. and *Entamoeba* sp. were found to be 16.12% and 13.97% respectively. In helminth, *Trichuris* sp. showed the higher prevalence rate than other parasite ie., 23.65%. This type of result also supported by Pokhrel and Maharjan (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 Langursand, by Parmar *et al.* (2012) from Hanuman Langur. The 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 & Janovy, 2000). The present study area (Devghat, Chitwan) is a lowland and frequently wet due to the Narayani River with Tropical forest. The percentage of *Ascaris* sp. in the present study was found to be 11.82%. This is also confirmed from Red Langur (Hilser *et al.*, 2011), Hanuman Langur and Rhesus Macaque (Parmar *et al.*, 2012) and Assamese Macaque (Pokhrel & Maharjan 2014). But Arunachalam *et al.* (2015) documented it to be 5%. The overall infection of *Strongyloides* sp. was 10.75%. It is supported by Hilser *et al.* (2011) from Red Langur, and by Pokhrel and Maharjan (2014) from Assamese Macaque. But, Parmar *et al.* (2012) showed higher prevalence (26.66%) from Hanuman Langur. *Oesophagostomum* sp. infection account for 5.37% of total samples. It has been reported from Assamese Monkey, Golden Langur, Rhesus Monkey, *Presbytis* sp. (Dewit *et al.*, 1991; Nath *et al.*, 2012; Thawait *et al.*, 2014; Pokhrel and Maharjan, 2014). Previous results ranged from 4%-

28% but Dewit *et al.* (1991) recorded 80% prevalence of *Oesophagostomum* sp. Hookworm sp. was found in 3.22% in present study. It is similar to the reports of Pokhrel and Maharjan (2014) with 4.7% and contrary to the result of Hilser *et al.* (2011) and Mutani *et al.* (2003) with 8% and 34% respectively. Soil moisture, sanitary condition of environment and climatic condition are important factors describing the differences of prevalence rates of parasite species among various geographical areas (Nunn *et al.*, 2005). Prevalence of *Trichostrongylus* sp. was found to be 3.22% among the helminth parasites. This result was supported by Hilser *et al.* (2011) from Red Langur. The prevalence of these parasites in monkey is interesting because it is an important parasite of ruminants (grazing mammals) (Crockett & Dipeolu, 1984). Therefore, prevalence of parasites can be due to contamination of their environment with ruminant waste. The present study revealed *Hymenolepis* sp. as the least common parasite with 1.07% prevalence. It has been reported from *Macaca sinica* and *Presbytis* sp. (Dewit *et al.*, 1991), Capped Langur (Sing *et al.*, 2009) and Drill Monkey (Akpan *et al.*, 2010). It is a common parasite of rodents with beetle and fleas as intermediate host. Due to the insectivorous nature of monkey or accidental ingestion of fleas, they are likely to be infected.

The huge diversity and densities of parasites represent enormous diversities of life cycle, transmission routes and pathogenicity that cause significant harm to animals. Heavy parasite infestation affects wild life severely and can be a threat to conservation (Woolhouse, 2002). Multiple infections are more harmful than single infection. Multiple infections may cause heavy losses through impact on growth pattern, reproduction, fecundity and establishment along with being the cause of death. Monkeys with multiple parasitic infections are at higher risk if untreated.

CONCLUSION

It is concluded that, the monkeys of Devghat, Chitwan are infected with various protozoa and helminth gastro-intestinal parasites. Among the protozoal infection *Balantidium coli* was highly prevalent compared to other parasites such as *Eimeria* sp. and *Entamoeba* sp. Among the helminth infection *Trichuris* sp. was found to be highly prevalent than other parasites such as *Ascaris* sp., *Strongyloides* sp., *Oesophagostomum* sp., Hookworm sp. *Trichostrongylus* sp. and

Hymenolepis sp. Among the parasitic infection 2.15% monkeys are at high risk to critical infection of gastro-intestinal parasites because they were found to have multiple infections.

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