Prevalence of Gastrointestinal Parasites in Rhesus Macaques (*Macaca mulatta*) and Human Community in Daunne, Nawalpur, Nepal

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Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University,

Kirtipur, Kathmandu, Nepal

April 2023

DECLARATION

I hereby declare that the work presented in this thesis "**Prevalence of gastrointestinal parasites in rhesus macaques (*Macaca mulatta*) and its comparison with human community in Daunne, Nawalpur, Nepal**" has been done by myself and has not been submitted elsewhere for the award of any degree. All sources of information have been acknowledged by reference to the authors or institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor "Associate Professor Laxman Khanal" this thesis submitted by Sangeeta Tandan entitled "**Prevalence of gastro-intestinal parasites in rhesus macaques (*Macaca mulatta*) and its comparison with human community in Daunne, Nawalpur, Nepal**" is approved for the examination in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Ecology and Environment.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Sangeeta Tandan entitled "**Prevalence of gastro-intestinal parasites in rhesus macaques (*Macaca mulatta*) and its comparison with human community in Daunne, Nawalpur, Nepal**" has been approved as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Ecology and Environment.

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Abbreviated form	Details of abbreviations
GI	gastrointestinal
RPM	revolutions per minute
C.F	calibration factor
µm	micrometer
S.D.	standard deviation

ABSTRACT

Rhesus macaques *(Macaca mulatta*, order: Primates, suborder: Haplorhini, family: Cercopithecidae) are distributed across Nepal which includes the whole Terai and Churia range as well as found within all of Nepal's protected areas up to 2,440 m elevation. Rhesus macaques and human have close relationship regarding their distribution, physiology and genetic characters which can cause transmission of infectious agents like gut parasites. This study aimed to provide information about prevalence of Gastro-intestinal (GI) parasites in rhesus macaques and its comparison with the parasitic prevalence of human community in Daunne, Nepal. A total of 120 rhesus macaque's fecal samples and 70 human fecal samples was collected from October 2021 to May 2022. Microscopical examination of fecal samples were done by direct wet mount and concentration method, floatation and sedimentation method. Parasitic prevalence rate of 39.2% was observed in rhesus macaques with highest prevalence of *Strongyloides* sp. (19.17%) followed by *Ascaris* sp. (13.33 %), Hookworm (10.83%) and *Trichuris* sp. (4.17%). Only single and double parasite infections were observed with rate of 30.83% and 8.33%, respectively, showing the mean richness of parasites 0.41 ± 0.49 (SD). Parasitic prevalence in the summer season (41.4%) was found to be higher than that in the winter season (36%) but no significant difference was observed (χ^2=0.361, df = 1, P=0.575). GI parasitic prevalence rates were 41.67% in adults, 30.77% in young and 27.27% in infants. Among 96 adult monkey samples, parasitic prevalence rate in adult females (52.46 %) was higher than in males (22.86 %) with significant difference (χ^2=8.018, P=0.005). Only one parasite species *Ascaris* sp. was detected in human samples with prevalence rate of 11.3% which is lower than in macaques of the study area. Findings of this study is useful for information on temple primates and adoption of effective management strategy.

1. INTRODUCTION

1.1 General background

Rhesus macaques (*Macaca mulatta*) are widely distributed across Nepal up to the elevation of 2,440 m. Its range includes the whole Terai and Churia range as well as area within all of Nepal's protected areas (Chalise 2013). The total population of rhesus macaques is estimated to approximately 100,000 individuals. Because of its large population, wide distribution, occurrence within several protected areas and lack of any major threats, this species is considered as least concern (Jnawali et al. 2011). Generally, rhesus macaques are found dwelling in different religious sites and cities in Nepal. Besides other nonhuman primates, they are also the natural and reservoir host of several gut parasites (Sapkota et al. 2020). Macaques are more likely to be infected with zoonotic and anthropogenic diseases and the probable cause of disease transmission is their frequent contact and habit of living in the religious and parkland of human proximity (Jha et al. 2011). Generally, primates live in closed group with frequent social interaction which make them more susceptible to the effects of parasitic transmission and infection (Stoner 1996). Around the globe, including Nepal, human and non-human primates are infected with parasitic diseases.

Gastrointestinal (GI) parasites like helminthes and protozoa are commonly found in human and non-human primates. Gastro-intestinal parasitic infections mainly include intestinal helminths and protozoan parasites which are the most prevailing infections world widely. One celled parasite which are capable of multiplying inside the body are protozoan parasites, however helminths are worms having many cells and are unable to multiply in the body. Intestinal helminthic parasites is also known as geohelminths and soil-transmitted helminths and includes species like Ascaris lumbricoides (roundworm), *Trichiuris trichiuria* (whipworm), *Ancylostoma duodenale*, and *Necator americanicus* (hookworms) (Haque 2007). GI parasites are found in the gastro-intestinal tract of the host and heavy parasitic infection can lead to anorexia, cachexia and severe ascites (Loukopoulos et al. 2007).Some of the parasites are non-pathogenic. However, large numbers of parasites have detrimental effect on the host which includes physiologic disturbances, nutritional loss and lesions. These effects depletes the host health and can be the cause of secondary infections (Toft & Eberhard 1998). GI parasites in non-human primates can be the major cause of gastro-enteritis, watery diarrhea, hemorrhage, dysentery and extra-intestinal infection such as liver abscess and even death (Akpan et al. 2010). In order to identify the gastrointestinal parasites, feces are the mostly collected and examined specimens (Khanna et al. 2014). Non-human primates (NHP) are the major reservoirs of parasites and this can transmit zoonotic diseases due to the close interaction between humans and NHP (Dawet et al. 2013). Parasitic interactions with host are believed to play a vital role in the diversification of natural populations. It was found that, particularly for virus, protozoa and specialist parasites, primate host sympatry is positively correlated with parasite species richness (Nunn et al. 2004). There is long term sympatry between human and non-human primates which results in a complex web of behavioral, ecological, and epidemiological relationships. The spatial overlap and interactions between humans and non-human primates creates a shared environment which can result in co-mingling of infectious agent (Fuentes 2006).

The GI parasites can be nonpathogenic or pathogenic. Pathogenic parasites may be the cause of disease in the host individuals but also, nonpathogenic parasites are important as their infection in the host shows the presence of fecal-oral transmission route in infected people (Sarkari et al. 2016). The transmission of GI parasites within the human community is through uncooked and unwashed food, contaminated water, poor hygiene, lack of cleanliness and fecal-oral route (Omalu et al. 2013). The transmission of gastrointestinal parasites is affected by the degree and nature of anthropogenic disturbance. Human activities like habitat fragmentation may increase sensitivity of primate populations to the risk of infection and in some cases can cause mortality (Chapman et al. 2005). Major elements responsible for parasitic transmission are increment in soil and water pollution by waste food and garbage, especially during the festive and picnic programs, and the occasional open defecation by visitors in the forest areas and nearby water sources. In this event, macaques are usually in contact with polluted soil and water, and consume garbage foods which may lead to the transmission success of gut parasites (Sapkota et al. 2020). Macaque and human share infectious agents like gut parasites, besides their food which is the result of their close relationship regarding their physiologic and genetic characters (Jha et al. 2011). The level of parasitic infection in rhesus macaques in mid-hill forests like Daunne is still unknown due to lack of scientific studies. So, this study will provide information about prevalence of gastrointestinal parasites in rhesus macaques and its similarity with parasitic prevalence among human community in Daunne.

1.2 Significance of study

Prevalence of GI parasites in rhesus macaques inhabiting the Daunne Forest area is unexplored so, the findings of this study can be a source of information regarding recent status of intestinal parasites in macaques. Study of parasitic prevalence of a species is vital to understand the health condition and adaptation of species in the environment. Many diseases were seen to be shared between human and non-human primate by exchange of parasites through fecal oral route, animal bite, nasal secretion and water-mediated route (Chapman et al. 2005). Human and macaque interaction can be seen in Daunne Devi Temple area as people and visitors around the Daunne Devi Temple feed the macaques with food. Macaques were observed to drink water which was also used by people for washing and in some case, drinking purpose. People were also observed to excrete and throw garbage in the forest around the temple which is also the habitat of macaques. This type of activities can be a route for parasitic transmission among human and macaques. So, knowledge of intestinal parasites in rhesus macaque and its comparison with human community can help to know the present status of possible transmission chance of parasites. It is also useful for examining the relationship between zoonotic disease in macaque and human community. Hence, this study is aimed to provide information about prevalence of GI parasites in rhesus macaques and its comparison with the parasitic infection in human community increasing the knowledge regarding health issues and status of macaques as well as chances of transmission of parasites and diseases. This study can play significant role for conservation and proper management of rhesus macaques of Daunne.

1.3 Research objectives

The general objective of this study was to determine the prevalence of gastro-intestinal (GI) parasites in rhesus macaques and humans of Daunne Devi Temple, Nawalpur.

The specific objectives are:

1. To determine prevalence, intensity, and richness of GI parasites in rhesus macaques and human community of Daunne Devi Temple area.

2. To identify seasonal and age-sex-based variation in GI parasites prevalence in rhesus macaques of Daunne Devi Temple.

2. LITERATURE REVIEW

2.1 Gastro-intestinal parasitic prevalence in rhesus macaques

Wild animal mainly non-human primates are the possible sources of bacterial, viral and parasitic zoonoses (Pokhrel & Maharjan 2014). In a study of intestinal parasitic prevalence of temple rhesus monkeys in Kathmandu, the infection of *Oesophagostomum* was the highest followed by *Balantidium coli*, *Strongyloides*, *E. histolytica*, *E. coli*, *Tichuris* and *Trichostrongylus* with the overall prevalence rate of 76.86% (Jha et al. 2011). In contrast to this, another study on Rhesus Macaque and Hanuman Langur of Devghat, Chitwan, highest infection was of *Balantidium coli* (27.95%) 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%) with the overall parasitic prevalence rate74.20% (Adhikari & Dhakal 2018). Similar study in Chitwan had reported highest infection of *Cryptosporidium* in Rhesus macaque with prevalence rate of gastrointestinal parasites 80% (Dhakal et al. 2018). However, in a study of rhesus macaques inhabiting Bajrabarahee Temple, 100% prevalence rate of intestinal parasites was observed and the highest infection was of *Entamoeba* spp. (66.7%) followed by *Balantidium coli* (59.5%), *Entamoeba coli* (57.1%), *Ascarid* spp. (21.4%), *Strongyloides* sp. (21.4%), hookworm (19%), *Trichuris* sp. (14.3%), *Cryptosporidium* sp. (11.9%), *Strongylid* spp. (9.5%), *Eimeria* sp. (7.1%), *Giardia* sp. (4.8%), and *Trichomonas* sp. (2.4%) (Sapkota et al. 2020). Study on Assamese Macaque of Shivapuri Nagarjun National Park (SNNP), parasitic infestation was observed in 28.24% of macaque and *B. coli* was found highly prevalent followed by other parasites which were *Entamoeba* sp., *Isospora* sp., *Ascaris* sp., *Trichuris* sp., *Strongyloides* sp., *Moniezia* sp., *Oesophagostomum* sp., Hookworm and *Physeloptera* sp. (Pokhrel & Maharjan 2014). A study of captive Rhesus macaque in Dhaka recorded seven species of parasites namely, *Hymenolepis* spp., *Taenia* spp., *Ascaris* spp., *Toxocara* spp., *Trichuris* spp., *Capillaria* spp. and Hookworm in (Tabasshum et al. 2018). Study of Rhesus macaque conducted in India found only 3 common parasites which were *Strongyle* spp., *Ascaris* spp. and *Eimeria* spp. and showed overall parasitic prevalence rate of 43% (Arunachalam et al. 2015). Another study in India reported four species of parasites from captive Pig-tailed Macaque namely, *Balantdium coli* (protozoa), and *Strongyle*, *Ascaris lumbricoides*, and *Trichiuris trichiura* (nematodes) with overall parasitic prevalence rate of 88.73% (Lalremruati & Solanki 2020). Among two different studies conducted on toque macaque, grey langur and purple-faced langur, one study (Ekanayake et al. 2006) reported infection of *Cryptosporidium*, *Enterobius*, *Strongyloides*, *Trichuris*, *E. histolytica*, *Iodamoeba* and *Balantidium* spp. while, another study (Huffman et al. 2013) reported *Entamoeba coli*, *Entamoeba histolytica / dispar*, *Trichuris* sp. and hookworm. The numbers of intestinal parasites were found higher in the summer and monsoon season as compared to winter season (Dhakal et al. 2018; Tabasshum et al. 2018; Kumar et al. 2019; Lalremruati & Solanki 2020). However, no significant difference is observed in the prevalence of parasites in Assamese macaques between summer and winter season (Pokhrel & Maharjan 2014).

2.2 Gastro-intestinal parasites of rhesus monkey interacting with humans

In developing countries, intestinal helminthes and protozoan parasites infections are found to be the most prevailing infections in humans. However, in developed countries, gastrointestinal infections are commonly caused by protozoan parasites compared to helminthes (Haque 2007). Macaque are considered as "sacred" and a part of religious structure by human community and are found to share water sources with human community in Bali. This type of contact between macaque and human community can substantially increase the spread of parasites, specifically gastro-intestinal parasites (Lane-DeGraaf et al. 2014). There is a long history and many cases regarding transmission and spreading of infectious disease from non-human primates to humans. Deadly pathogen is able to spread through human-to-human transmission or deadly pathogen can be transmitted from non-human primates to humans via a flying blood-sucking vector insect which is the most serious risk for public health (Devaux et al. 2019). It was reported that intestinal parasitic infections (IPIs) is one of the significant causes of illness among human communities and the major predictor of IPIs in the human community was close interaction with animals and absence of toilet which results in indiscriminate defecation (Rajoo et al. 2017). Intestinal parasitic infection is one of the serious health and socio-economic problem in developing countries (Shrestha et al. 2012). According to various literature reviewed, the most common parasites reported from different Asian countries were *Ascaris lumbricoides*, *Entamoeba coli*, *E. vermicularis*, *Hymonlepsis nana*, *Trichuris trichiura*, *Taenia* sp., *S. stercoralis* and hookworm (Steinmann et al. 2008; Taheri et al. 2011; Nasr et al. 2013; Kumar et al. 2014; Bora et al. 2016). According to several studies conducted in Nepal, it was concluded that low socio-economic status, poor housing and lifestyle, open defecation practices, close contact with domestic animals, not wearing shoes, habit of nail biting, consumption of unwashed fruits and vegetables, unhygienic skin and clothes, thumb sucking and poor personal hygiene increases the chance of infection with intestinal parasites (Shrestha et al. 2012; Sah et al. 2013; Sapkota et al. 2017; Adhikari et al. 2021). A research conducted in eastern region of Nepal among school going children, *Giardia intestinalis* (30.9%) was found as the most prevalent parasite followed by hookworm (18.6%), *A. lumbricoides* (15.5%), *H. nana* (6.2%) and *Entamoeba histolytica* (5.2%) (Ghimire et al. 2014). Another similar study conducted on patients attending to tertiary care hospital of eastern region of Nepal had reported *Giardia intestinalis* (3.34%) as the most prevalent parasite followed by *Entamoeba histolytica/E. dispar* (1.96%) and Hookworm (0.97%) (Baral et al. 2017). However, a study conducted in Bhaktapur district of Nepal, *Ascaris lumbricoides* (22.63%) showed the highest prevalence followed by *Trichuris trichiura* (6.06%), *Strongyloides stercoralis* (1.82%), Hookworm (1.62%), *Taenia* sp. (1.01%), *Hymenolepis nana* (0.81%) and *Enterobius vermicularis* (0.40%) (Shrestha & Maharjan 2013). In another study of Western Nepal, the most common parasite reported was *Entamoeba histolytica* (9.23%) followed by *Giardia lamblia* (5.76%), *Trichuris trichuria* (5%), *Ancylostoma duodenale* (2.65%) and *Ascaris lumbricoides* (2.3%). A study in indigenous community (Kumal) of Nepal reported high prevalence of Hookworm (30.87%) followed by *Ascaris*, *Hymenolepsis*, *Trichuris*, *Strongyloide* and *Taenia* (Gyawali 2012). *Giardia lamblia*, *Ascaris lumbricoides* and *Entamoeba histolytica* are the most common intestinal parasites found in different parts of Nepal (Das et al. 2006; Chongbang et al. 2016; Sapkota et al. 2017).

3. MATERIALS AND METHODS

3.1 Study area

This study was conducted in Daunne Forest of Nawalpur District. It lies in Gandaki Province at a geographic coordinates of 27°32′41″ to 27°33′27″ North latitudes and 83°50′10″ to 83°50′36″ East longitudes. The highest peak in Nepal’s Churia range, Mt. Devchuli (1,937 m) lies in this district. Daunne Devi temple area was selected as the study site. The temple lies in the hill pass at an elevation of 1033 meters above sea level in route from Bardaghat to Dumkibas. Daunne Devi Temple is a Hindu temple of Durga Goddess which also has Shivalaya. Since 1992, the user community has been taking care of the temple while, the present temple was built in 1998/99.

The Daunne Forest has subtropical to temperate climate. May and June are the hottest months, while December and February are the coldest months. In a year, average rainfall of about 1500 mm occurs. The temple is surrounded by huge forest with variety of flora and fauna. Vast diversity of butterfly, fish, amphibian, reptile, bird and mammal species such as Golden jackal (*Canis aureus*), Leopard (*Panthera pardus*), Rhesus macaque (*Macaca mulatta*), Common langur (*Semmenopithecus hector*), Jungle cat (*Felis chaus*), Common mongoose (*Herpestes edwardsii*) Common cobra (*Naja naja*), Indian python (*Python molurus*), Water snake (*Natrix piscator*), Golden monitor lizard (*Varanus bengalensis*) are found in the study area (Baral et al. 2003; Upadhyay 2008). The commonly found species of flora includes Sissoo (*Dalbergia sissoo*), Sal (*Shorea robusta*), Harro (*Terminalia chebula*), Barro (*Terminalia bellerica*), Saj (*Terminalia tomentosa*), Simal *(Bombax ceiba*), Jamun *(Syzigium cumini*), etc. (Subedi 2008).

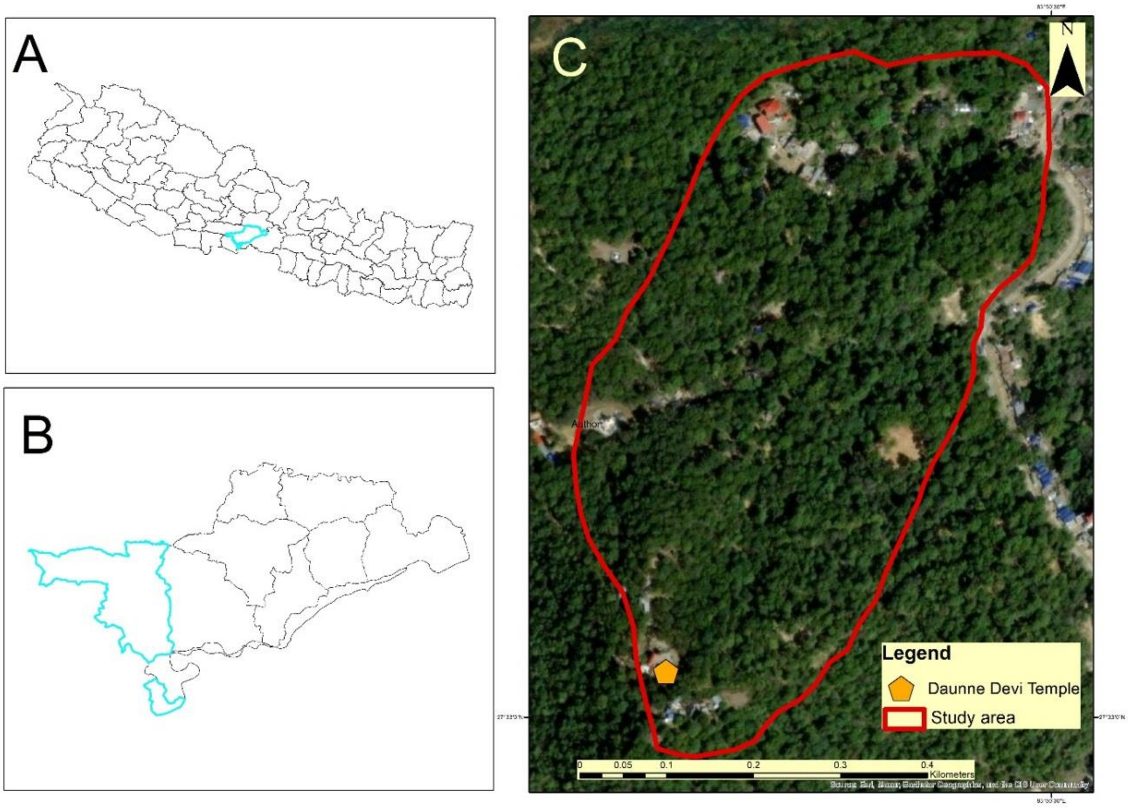

Figure 1. Map of study area; A. Map of Nepal showing the Nawalpur District. B. Map of Nawalpur District showing Binayee Tribeni Rural Municipality. C. Map showing the Daunne Devi Temple in the Daunne Forest.

3.2 Materials used

3.2.1 Apparatus used

- Vials
- Gloves and Mask
- Test-tube stand
- Centrifuge tube
- Tea strainer
- Beaker
- Coverslip
- Centrifuge machine
- Toothpick
- Weighing machine
- Conical flask

- Glass-rod
- Slide
- Dropper
- Microscope
- Ocular micrometer

3.2.2 Chemicals used

- 2.5% Potassium dichromate solution ($K_2Cr_2O_7$)
- Sodium chloride solution
- Lugol's Iodine solution
- Distilled water
- 10% Formalin
- Ethyl acetate
- Methylene blue

3.3 Methods

3.3.1 Preliminary field survey

Preliminary field survey was done during September 2021 to know about the study area, habitat and population status of rhesus macaques. Additional information related to rhesus macaque inhabiting the area was collected from the secondary sources and informal interviews with residents of the study area.

3.3.2 Sample collection and preservation

Fecal samples were collected from October 2021 to May 2022. Approximately 450 macaque individuals with 5 troops were counted randomly in the study area. Fecal samples of monkeys were collected from the Daunne Devi Temple area and the forest area around it by purposive method. Fresh fecal material was taken and placed in a vial containing 2.5% Potassium dichromate solution. Collected fecal samples were in semisolid or solid condition. We observed and followed the macaques to collect fresh fecal samples and identify their sex and age at the same time (Table 1).

Table 1: Age-sex categories used in the study

S.N.	Category	Description
1.	Adult male	Full grown with adult morphology (e.g., pink scrotum and prominent testes)
2.	Adult female	Full grown with adult morphology (e.g., elongated nipples, carrying baby)
3.	Young	Individuals lacking secondary sexual characteristics and have near adult size
4.	Infant	Small individuals nursed or are still in frequent proximity to a female

For the human fecal sample collection, sterile vials were provided to the targeted population. The targeted population includes the priest, caretakers of the temple, shop people near the temple and the local people living aside on the stairway of the temple. The targeted local people were mainly of temple, nearby shop and houses on the side of the temple stairway where, macaques were also observed to spend their time feeding, stealing food from shop and houses. The next morning the vials were collected and labelled with name, sex, and age. The samples were immediately preserved in 2.5% Potassium dichromate solution. Then, all the monkey and human fecal samples were transported to the lab of Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu.

3.3.3 Sample size

A total of 120 fecal samples (70 samples during summer and 50 samples during winter season) were collected by following the rhesus macaques in the study area. A total of 70 human fecal samples (40 samples during summer and 30 samples during winter season) was collected from purposively targeted human populations of the study area.

3.4 Laboratory processing and microscopic examination

For the identification of trophozoites, cysts, oocysts, eggs and larval stages of GI parasites, microscopical examination of fecal samples were done by direct wet mount and concentration method, floatation and sedimentation method. The slides were observed under microscope with low magnification of 10× first which is followed by high magnification of 40×.

3.4.1 Unstained smear preparation of stool/saline wet mount

A small amount of feces was taken and mixed with normal saline on the clean glass slide and then it was gently covered by cover slip. The excess fluid was removed using cotton filter paper and examined under the microscope with 10× and 40× magnification (Zajac & Conboy 2012).

3.4.2 Stained smear preparation of stool/Iodine wet mount

Two-three drops of stool sample was taken in the glass slide to which a drop of Lugol's iodine was added and mixed. Then a cover slip was placed, and excess fluid was removed using cotton filter paper. The smear was observed under the microscope with 10× and 40× magnifications. This technique helps in studying the internal structure of protozoans and identifying them (Zajac & Conboy 2012).

3.4.3 Sedimentation method

This method is used to detect eggs of flukes, tapeworms and nematodes whose eggs do not float promptly in common flotation solutions. Firstly, about two grams of the sample was filtered thoroughly and mixed with normal saline in a 15 ml centrifuge tube. At 1200 rpm, the sample was centrifuged for five minutes. The supernatant was discarded, and the sediment was mixed well. To this sediment, 10 ml of 10% buffered formalin and 3 ml of ethyl acetate was added in the tube and again centrifuged. Three layers of ethyl acetate containing upper layer of ethyl acetate and plug of debris, middle layer of 10% formalin and bottom layer of sediment were formed. An applicator stick was used to free the plug of debris and then all the supernatant fluid was decanted and discarded. At last, only the bottom sediment was remained in the tube. If sediments were too dry, one- two drops of 10% formalin were added and mixed well. A drop of sediment was kept on a clean slide, covered by a coverslip and observed under microscope both with and without Lugol's iodine with the microscope with 10× and 40× magnifications (Zajac & Conboy 2012).

3.4.4 Floatation method

This technique is used to float the less dense parasite on the fluid flotation medium with high density. Saturated salt solution is used as fluid floatation medium. Filtered two grams of the sample was mixed with normal saline in a 15 ml centrifuge tube and then centrifuged at 1200 rpm for five minutes. The supernatant was discarded and about 4-5 ml of floatation solution was remained in the tube which was mixed well. Further, concentrated NaCl solution was added and filled the tube up to 14 ml and centrifuged again at 1200 rpm for five minutes. After centrifugation, more concentrated NaCl was added drop by drop in the tube, until a convex surface was formed at the top. Over the top of the tube, a clean coverslip was placed avoiding any bubbles and was left undisturbed for at least 10 minutes. The coverslip was removed gently, avoiding the dropping of the sample from cover slip and then kept over a clean glass slide. The slide was examined under compound microscope 10× and 40× magnifications, both with and without Lugol's iodine (Zajac & Conboy 2012).

3.5 Identification of egg, cyst and larva

In order to detect and identify the possible parasitic stage, all the above techniques were used. The optical microscope was used to observe all the samples. The microscopic images were taken and eggs, cyst and larva were identified and confirmed by comparing their size, structure and color from different books, internet sources, published and unpublished articles (Soulsby 1968; Sapkota et al. 2020).

3.6 Calibration of egg, cyst and larva

Calibration of microscopic ocular and stage micrometer was done for the measurement of length, breadth and diameter of the parasite egg, cyst and larva. They were measure with the calibration factors (C.F).

C. F. = (No. of S.D/ No. of O.D) × 10 µm

C. F for 10× = 10 µm

C. F. for 40× = 2.6 µm

3.7 Data analysis

Prevalence of parasites was calculated by dividing the number of fecal samples infected with at least one parasite by the total number of fecal samples examined. The intensity of parasites was calculated as the total number of specific parasite species found in sample divided by the number of infected hosts with that parasite (Bush et al. 1997). Richness of parasites was measured as the number of parasite species detected in each sample (Turgeon et al. 2018). The collected data were coded and entered into Microsoft Excel spreadsheet. Using pie chart, table and bar diagram, interpretation of the data was done. Seasonal variation and age-sex based variation in GI parasite prevalence was tested for significance by Chi-squared test performed in "SPSS software". In all cases, 95% confidence interval (CI) and $p \leq 0.05$ was considered for statistically significant difference.

3.8 Ethical approval and consent to participant

The required permission for the collection of the fecal samples was obtained from the Department of Forest and Soil Conservation, Ministry of Forest and Environment, Government of Nepal. Human participation in the study was voluntary and the informed consent was obtained from each participant. Prior to the survey, the detailed purpose and procedures of the study were explained verbally to the participants in the Nepali language.

4. RESULTS

4.1 Prevalence of GI parasites in rhesus macaques

Among 120 fecal samples examined, 47 samples were positive for gastro-intestinal parasites showing prevalence rate of 39.2%. A total of four different species of only helminthes parasites were found to be distributed among rhesus macaques of Daunne Devi Temple which are *Strongyloides* sp., *Ascaris* sp., Hookworm and *Trichuris* sp.

4.1.1 GI parasites richness in rhesus macaques

Out of 47 samples positive to the GI infection, 37 samples of rhesus macaque were infected with one parasite species and 10 samples were infected with two different parasite species. So, only single parasite infection and double parasite infection was observed with rate of 30.83% and 8.33% respectively, showing the mean richness of parasites 0.41 ± 0.49 (SD).

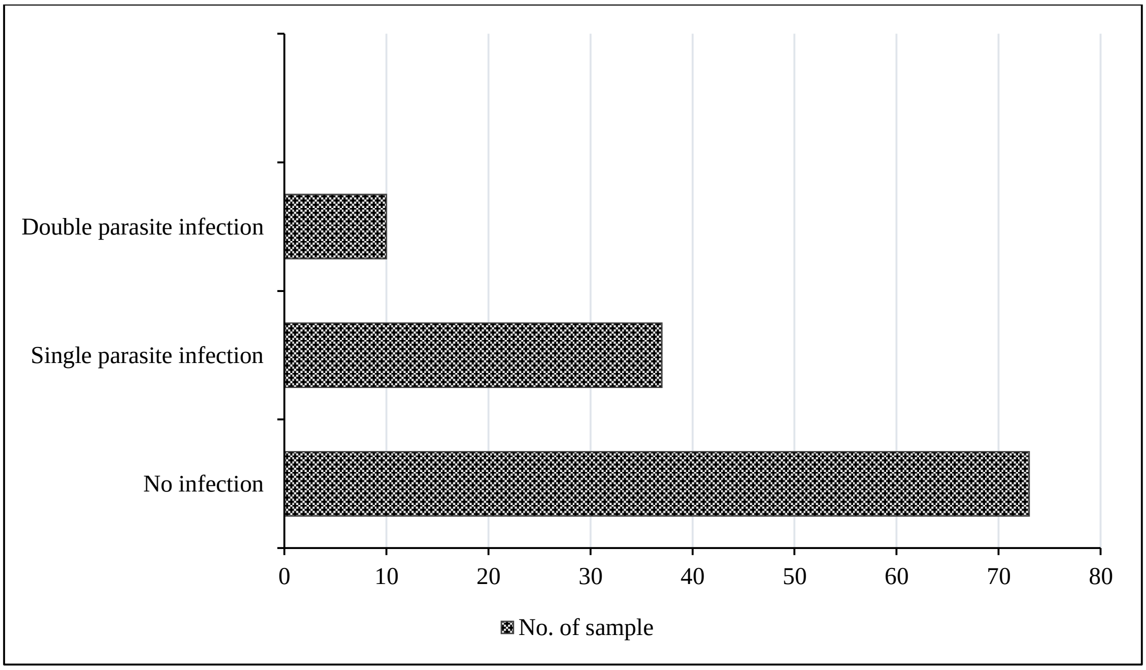

Figure 2. Infection status of GI parasites in rhesus macaque

4.1.2 GI parasite intensity in rhesus macaques

Among four helminthes, *Strongyloides* sp. has highest prevalence of 19.17% with intensity of 1.52. *Ascaris* sp. was found be second highly prevalent with 13.33 % and intensity of 1.38 followed by Hookworm with 10.83% prevalence rate and intensity of 1.46. *Trichuris* sp. was found in only in 5 samples with prevalence rate of 4.17%.

Table 2. Parasite prevalence and intensity of infection in rhesus macaque

Parasites	No. of positive samples	Prevalence (%)	Mean intensity
***Strongyloides* sp.**	23	19.17	1.52
***Ascaris* sp.**	16	13.33	1.38
Hookworm	13	10.83	1.46
***Trichuris* sp.**	5	4.17	1

4.1.3 Seasonal prevalence of GI parasites in rhesus macaques

During summer season, 29 samples (N= 70) of rhesus macaque were infected with at least one parasite showing prevalence of 41.4%. While during winter season, 18 samples (N= 50) were positive for parasitic infection with prevalence of 36%. The prevalence of parasites in summer season was higher than that in winter season but the difference was not statistically significant (χ^2=0.361, df = 1, P=0.575).

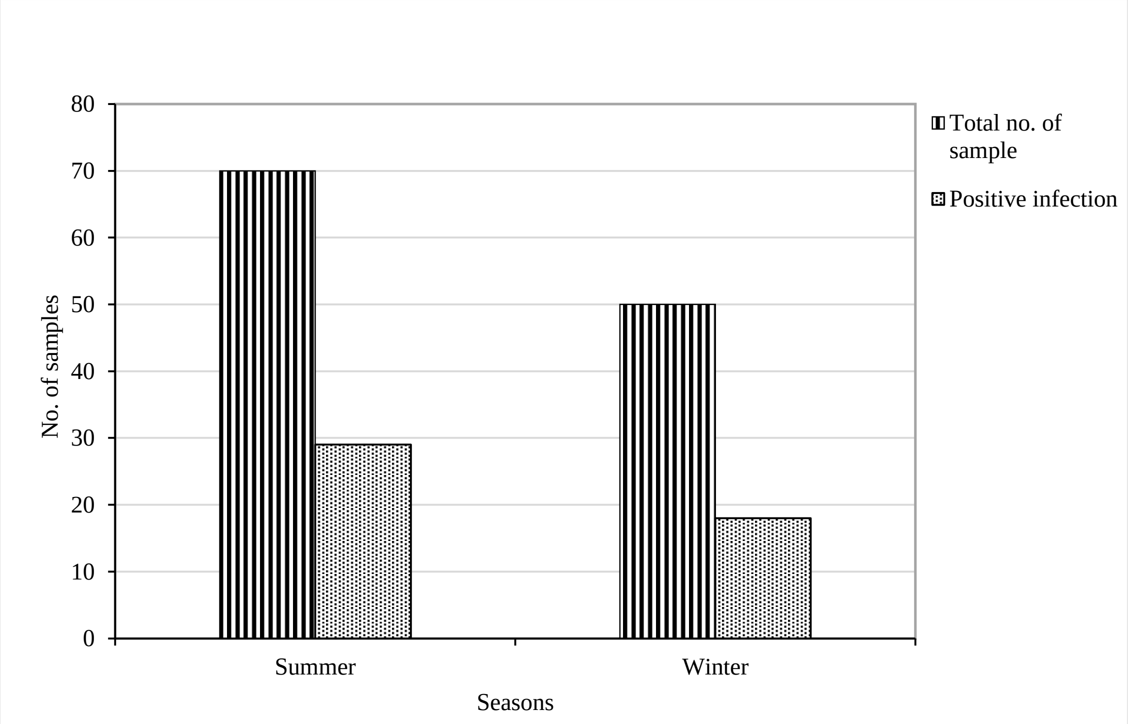

Figure 3. Seasonal prevalence of GI parasites in rhesus macaque

4.1.4 Age-group wise prevalence of GI parasites in rhesus macaques

Among 96 adult fecal samples, 40 samples were infected with 41.67% prevalence rate. For young macaques, among 13 sampled individuals, only 4 were infected with 30.77% prevalence rate. And among 11 sampled infants, only 3 were infected with prevalence rate of 27.27%. There is difference in the prevalence of different age groups, however no significant difference was observed statistically (χ^2=1.290, df = 1, P=0.581).

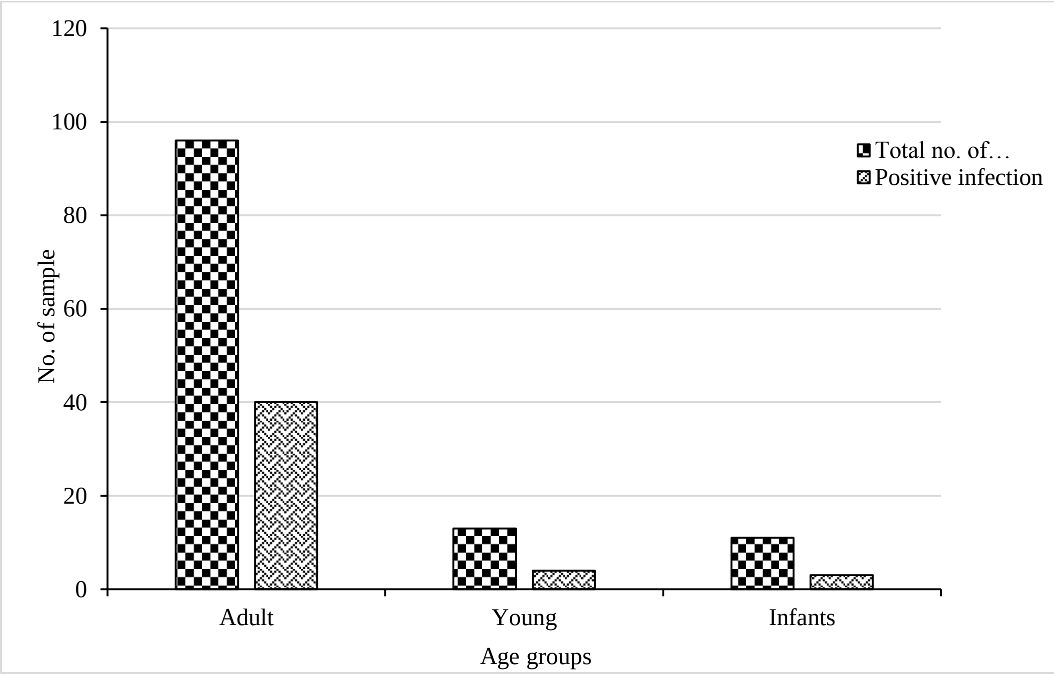

Figure 4. Age-group wise prevalence of GI parasites in rhesus macaque

4.1.5 Sex wise prevalence of GI parasites in rhesus macaques

Among 96 adult monkey samples, 35 were adult males and 61 were adult females. Parasite were present in 8 male samples and in 32 female samples. Parasitic prevalence rate in female was 52.46% which was higher than in male i.e. 22.86%. Significant difference was observed in the parasitic prevalence rate between the males and female macaques (χ^2=8.018, df = 1, P=0.005).

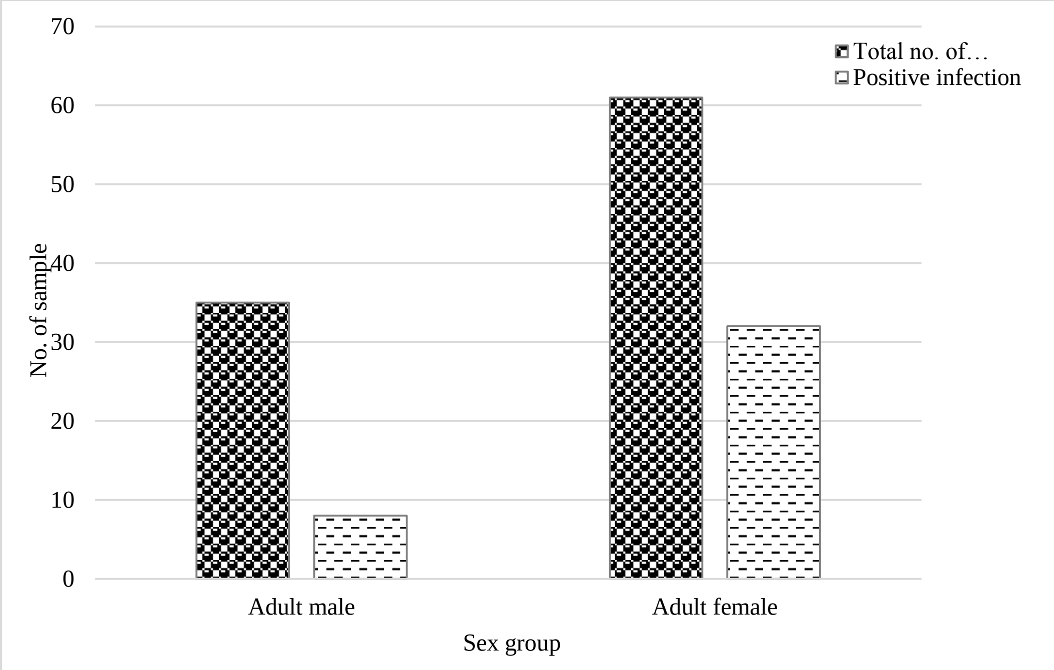

Figure 5. Sex wise prevalence of GI parasites in rhesus macaque

4.2 Parasitic prevalence in people of Daunne Devi Temple

Among 80 human samples, 8 samples were infected by parasites with prevalence of 11.3%. Only one parasite species *Ascaris* sp. were detected in human samples. Among infected 8 samples, one sample was of caretaker of temple area and remaining were of local people living around the temple stairway.

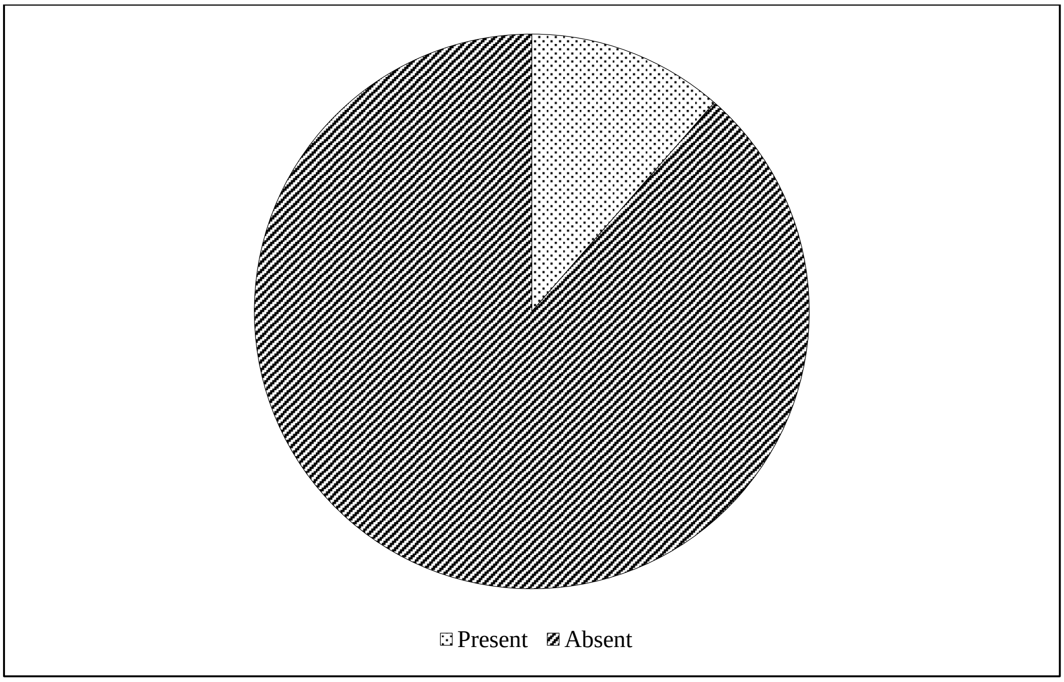

Figure 6. Parasitic prevalence in people living around Daunne Devi Temple

5. DISCUSSION

5.1 Prevalence, intensity and richness of GI parasites in rhesus macaques

This study showed 39.2% prevalence rate of GI parasites in rhesus macaque of Daunne Devi temple which was comparatively less than the prevalence rate reported in other studies on rhesus macaques of Nepal (Jha et al. 2011; Adhikari & Dhakal 2018; Dhakal et al. 2018; Sapkota et al. 2020) with parasitic prevalence rate of 76.86%, 74.20%, 80% and 100% respectively. Highest infection of *Strongyloides* sp. was reported in this study which was quite similar to the findings of (Arunachalam et al. 2015) who found 43% parasitic prevalence rate with highest infection of *Strongyloides* sp. in free living macaques of Namakkal district of Tamil Nadu. The possible reason for the low parasitic prevalence rate can be the presence of vast diversified forest as their habitat and consumption of variety of tree leaves that can have high nutrition and medicinal value. Four species of helminthes parasites were observed in rhesus macaque of Daunne Devi Temple namely, *Strongyloides* sp., *Ascaris* sp., Hookworm and *Trichuris* sp. which were also observed in other similar studies on parasites of rhesus macaques (Adhikari & Dhakal 2018; Dhakal et al. 2018; Sapkota et al. 2020). Among the gastro-intestinal parasites, *Strongyloides* sp. was found to be highly prevalent with rate of 19.17% which is similar to the reports of a study (Sapkota et al. 2020) in urban temple area with 21.4% and contrary to the results of other studies (Jha et al. 2011; Adhikari & Dhakal 2018) with 28.92% and 10.75% respectively. High prevalence of *Strongyloides* sp. in monkeys is also supported by studies (Mutani et al. 2003; Gillespie et al. 2005; Malla 2007). Second highest prevalent parasite was found to be *Ascaris* sp. with 13.33% which is similar to the results of a study (Adhikari & Dhakal 2018) with 11.82% whereas, lower than the results from other studies (Dhakal et al. 2018; Sapkota et al. 2020) with 22.22% and 21.4% respectively. In this study, the infection rate of Hookworm was found to be 10.83% which is higher rate than in other two similar studies (Adhikari & Dhakal 2018; Dhakal et al. 2018) with 3.22% and 6.67% respectively. Another study contradicts with this study showing higher infection rate of Hookworm i.e. 19% (Sapkota et al. 2020). Very low prevalence of *Trichuris* sp. was found with 4.17% which contradicts with other similar studies (Adhikari & Dhakal 2018; Dhakal et al. 2018; Sapkota et al. 2020) that had showed *Trichuris* sp. prevalence rate of 23.65%, 14.44% and 14.3% respectively. Out of 120 samples, single and double infection was found in 30.83% and 8.33% of samples respectively. The study conducted in temples of Kathmandu reported that 27.96% had single infection, 39.78% had double and 32.26% had multiple infection (Jha et al. 2011) which is higher than the infection status observed in this study. Similar study in urban temple macaques of Bajrabarahee found duplet infection of 26.2%, triplet infection of 57.1%, quadruplet infection of 11.9% and pentuplet infection of 4.8% (Sapkota et al. 2020). The parasitic prevalence, intensity and richness in this study was observed to be lower than in other studies which were compared above. It is common to report the presence of parasites in animals but the parasite infestation intensity and richness is manageable by behavioral strategies like grooming techniques, licking by the use of saliva and consumption of medicinal plant-based compounds (Hart & Hart 2018). Forest around Daunne Devi temple is community and protected forest having variety of plants and trees which was observed to be consumed by monkeys and also one of the major source of their food besides the human given food in temple. The infection rate of GI parasites is lower in natural habitat than in habitat close to resident area (Dhakal et al. 2018). Vast forest far from resident area and with low anthropogenic disturbances to monkeys around the study area was observed which eventually, provides them a thriving natural habitat. The rate of parasitic infestation was reported to be higher in populations of Howler monkey species living in fragmented habitats than in continuous and protected forest of Southern Mexico. As fragmented habitats with human settlements create disturbances and human interactions causing increase in contamination from human and domestic animals than in protected forest habitat (Trejo-Macías et al. 2007). So, protected diversified forest with variety of flora as their habitat can be a reason for the manageable parasitic load in monkeys of Daunne. It was reported that the monkeys of Daunne had no observable fitness issues as their body appeared healthy and in good condition. Also, the collected fecal samples were in solid to semi-solid form and no watery form, mucus or blood was observed in any fecal samples. This can be due to the presence of manageable intestinal parasite load which does not show impact in their fitness in a major way (Hart & Hart 2018).

In this study, the parasite infection rate was found to be more in summer season (41.4%) than in winter season (36%). This result is supported by a study of rhesus macaque in Chitwan-Annapurna landscape which also found higher infectious rate in summer season (protozoan 60%, helminthes 75.5%) than in winter season (protozoan 46.6%, helminthes 68.8%) (Dhakal et al. 2018). In hot days, it's easy for parasites to grow causing more contamination of food and water which can be a reason for increased chance of parasitic infection in rainy and summer seasons than in winter season (Gonzalez-Moreno et al. 2013). However, no significant differences was observed between parasitic prevalence rates of two seasons. This result of our study is supported by a similar study in Assamese monkeys of Shivapuri Nagarjun National Park in which high prevalence of parasites were recorded in summer season (72.50%) than in winter season (71.11%) but no significant difference was present in the prevalence of parasites between two seasons (Pokhrel & Maharjan 2014). In a similar study of lion-tailed macaque of India, overall parasitic load does not differ according to seasons and for parasite prevalence, no significant difference was found with monthly rainfall and mean maximum temperature (Kumar et al. 2019). The result of this study showed higher parasitic prevalence rate in adults with 41.67% followed by young with 30.77% and infant with 27.27%. This results contradicts with other similar studies on bonnet macaque and lion-tailed macaque of India which had reported higher parasitic load in immature macaques than in adult macaques (Kumar et al. 2018; Kumar et al. 2019). The difference in results can be due to the low number of collected fecal samples of young and infants than adults in this study. Parasitic prevalence in female was found to be 52.46% in our study, which is higher than the male with 22.86% which is supported by a study on bonnet macaque of India (Kumar et al. 2018), that showed higher parasitic load in female than male. This result contradicts with studies (Tabasshum et al. 2018; Kumar et al. 2019) on rhesus macaque and lion-tailed macaque respectively which shows higher prevalence of parasites in adult male than in adult female. The major reason this result of our study can be the less feeding on human given food and having an isolated dominant territory of male macaques than female macaques. Males are observed to have interactions with the group mainly during mating and fighting which can be the cause of less infection as compared to female (Kumar et al. 2018). Female macaques showed more close interaction with human beings around temple area during feeding which can be the cause of high chances of infection through contaminated food and water (Sapkota et al. 2020).

5.2 Comparison of GI parasites between rhesus macaques and human community

Total 190 fecal samples were collected and microscopically examined, among them, 120 were of rhesus macaque and 70 were of human beings. Intestinal parasitic prevalence rate in human beings was found to be 11.3% which is lower than in macaques (39.2%). The reason for this difference in parasitic infection rate in human beings and macaques can be the increased awareness, education in people and availability of modern health facilities for human while contaminated feeding food and water around temple area and lack of deworming program for macaques. Feeding of macaques by human increases interaction and direct contact with macaque body fluids and can transmit parasites (Jones-Engel et al. 2006) In present study, only *Ascaris* sp. was observed in humans which is common with rhesus macaques. Presence of *Ascaris* sp. as the commonest helminths was also observed in other similar kind of studies on parasitic prevalence of humans (Gyawali 2012; Shrestha & Maharjan 2013; Chongbang et al. 2016; Sapkota et al. 2017). Its presence can be due to the frequent fecal-oral spread of infection and contamination of soil by macaque feces (Sapkota et al. 2017). Presence of shared common parasites between human and monkey can be a matter of concern as it can transmit diseases and is detrimental for their health. Non-human primates act as the emerging source of infectious agents capable of infecting human because of similarity between human and non-human primate in genetic, physiologic and behavioral aspects (Jones-Engel et al. 2006). However, in this study, low parasitic load in human and presence of only one common parasites showed the less chance of diseases transmission between both species.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Parasitic prevalence in rhesus macaques of Daunne Devi Temple was found to be one third of the sampled number with highest prevalence of *Strongyloides* sp. followed by *Ascaris* sp., Hookworm and *Trichuris* sp. Single and double parasite infection was reported with the mean richness of parasites 0.41 ± 0.49 (SD). Parasite infection was higher in summer season than in winter season with no significant difference. Adult macaques showed more parasitic prevalence as compared to young and infants. Among adult macaques, adult female showed higher parasitic prevalence than adult males with significant difference. Parasite prevalence in human community of Daunne Devi Temple was less as compared to rhesus macaques showing presence of only one common parasite i.e. *Ascaris* sp.

6.2 Recommendation

A detailed study using different environmental variables and comparative study with other macaque groups would be appropriate to gain information about parasitic prevalence in rhesus macaques. Increase in population of rhesus macaque and their dependency on people for food at Daunne could cause the conflict between the macaques and the people. This type of interaction might increase the chance of contamination and disease transmission to both macaques and the locals of Daunne. Hence, the interaction between the macaques and the locals should be managed using effective management strategies for the wellbeing and benefit for both species.

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APPENDICES

Photo plates of parasites in rhesus macaques

Photo 1: Egg of *Strongyloides* sp. (60µm x 39µm, X400)

Photo 2: Egg of *Ascaris* sp. (52µm x 55µm, X400)

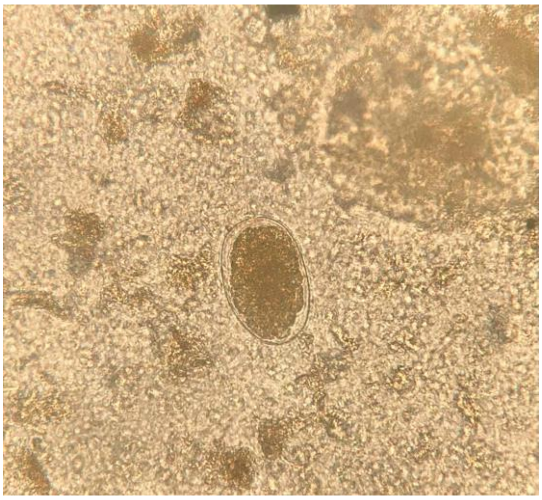

Photo 3: Egg of hookworm (75µm x 45µm, X400)

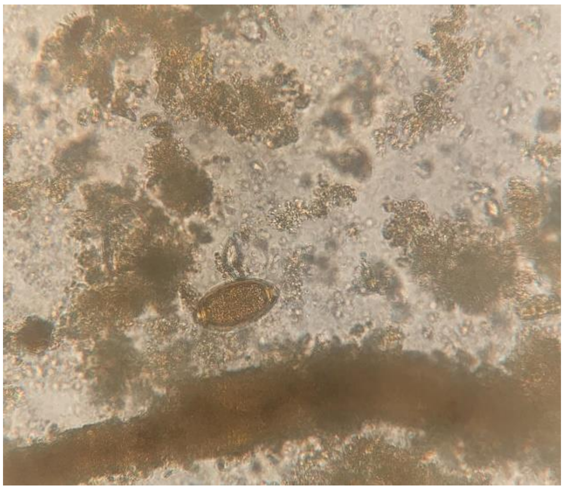

Photo 4: Egg of *Trichuris* sp. (55µm x 24µm, X400)

Photo plates of parasites in human

Photo 5. Egg of *Ascaris* sp. (52µm x 52µm, X400)

Photo plates of field visit and laboratory work

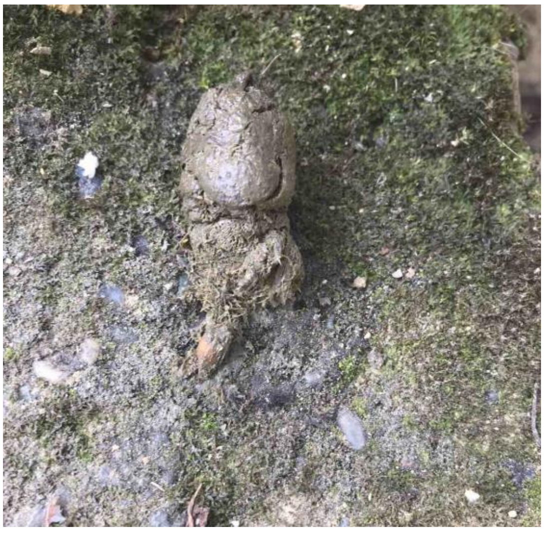

Photo 6. Fecal sample of rhesus macaque in semi-solid form

Photo 7. Fecal sample of rhesus macaque in solid form

Photo 8. Fecal sample collection

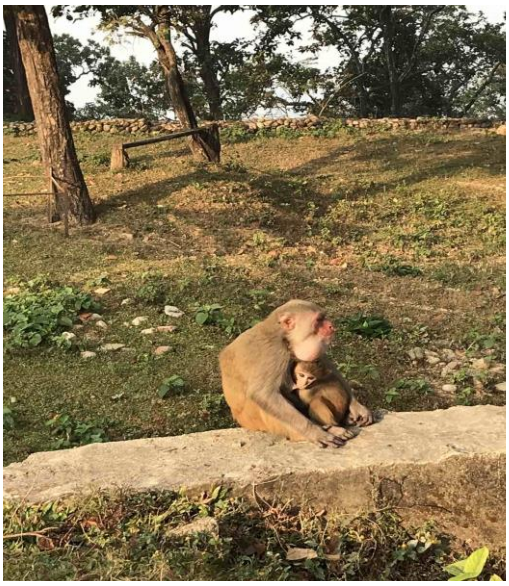

Photo 9. An adult female rhesus macaque breast feeding the baby

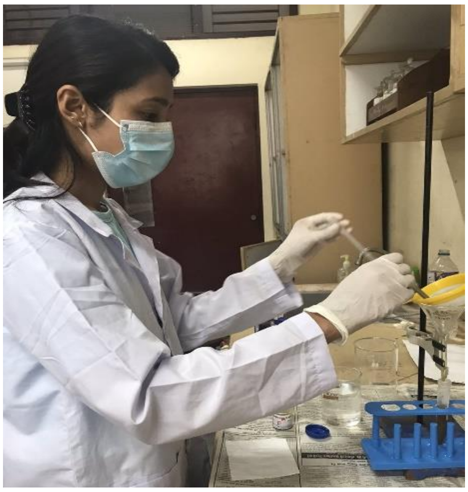

Photo 10. Laboratory work

Photo 11. Microscopic examination of fecal sample