POPULATION STATUS AND THREATS TO SURVIVAL OF RHESUS MONKEY *Macaca mulatta* (Zimmermann, 1780) IN BAJRABARAHI AREA, LALITPUR, NEPAL

BIKRAM SAPKOTA
T.U. Registration No: 5-2-22-22-2010
Roll No: 409
Batch: 2073/74

A thesis submitted
in partial fulfillment of the requirements for the award of the Master degree
of Science in Zoology with special paper Ecology and Environment

Submitted to
Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
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DECLARATION

I hereby declare that the work presented in the thesis entitled "POPULATION STATUS AND THREATS TO SURVIVAL OF RHESUS MONKEYS *Macaca mulatta* (Zimmermann, 1780) IN BAJRABARAHI AREA, LALITPUR, 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)

.....

Bikram Sapkota

bik.saapkotaa@gmail.com

Date..12th Feb 2020.....

TRIBHUVAN UNIVERSITY
01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.

Ref.No.:

Central Department of Zoology T.U., Kirtipur

RECOMMENDATIONS

This is to recommend that the thesis entitled "POPULATION STATUS AND THREATS TO SURVIVAL OF RHESUS MONKEYS *Macaca mulatta* (Zimmermann, 1780) IN BAJRABARAHI AREA, LALITPUR, NEPAL" has been carried out by BIKRAM SAPKOTA Master's Degree of Science in Zoology with special paper Ecology and Environment. 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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Supervisor

Bishnu Prasad Bhattarai, PhD

Assistant Professor

Central Department of Zoology

Tribhuvan Tribhuvan University

Kirtipur, Kathmandu, Nepal

.....

Co-Supervisor

Tirth Raj Ghimire, PhD

Scientific officer

Nepal Academy of Science and Technology

Khumaltar, Lalitpur

TRIBHUVAN UNIVERSITY
01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.

Ref.No.:

Central Department of Zoology T.U., Kirtipur

LETTER OF APPROVAL

On the recommendation of supervisor Assistance Professor Dr. Bishnu Prasad Bhattarai, this thesis submitted as entitled ""POPULATION STATUS AND THREATS TO SURVIVAL OF RHESUS MONKEYS *Macaca mulatta* (Zimmermann, 1780) IN BAJRABARAHI AREA, LALITPUR, NEPAL" is approved for the examination ~~and submitted to the Tribhuvan University~~ in partial fulfillment of the requirement for master's degree of science in Zoology with special paper Ecology and Environment.

.....

Prof Tej Bahadur Thapa, PhD

Head of Department

Central Department of Zoology

Tribhuvan University Kirtipur

Kathmandu Nepal

Date: 12th Feb 2020

TRIBHUVAN UNIVERSITY
01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.

Ref.No.:

Central Department of Zoology T.U., Kirtipur

CERTIFICATE OF ACEEPTANCE

This thesis work submitted by Bikram Sapkota entitled ""POPULATION STATUS AND THREATS TO SURVIVAL OF RHESUS MONKEYS *Macaca mulatta* (Zimmermann, 1780) IN BAJRABARAHI AREA, LALITPUR, NEPAL" has been accepted as a partial fulfillment for the requirement of master's degree of science in zoology with special paper ecology and environment.

EVALUATION COMMITTEE

.....	.....
Supervisor	Head of Department
Bishnu Prasad Bhattarai, Ph.D.	Tej Bahadur Thapa, Ph.D.
Assistant Professor	Professor
Central Department of Zoology	Central Department of Zoology
.....	.....
External Examiner	Internal Examiner

Date: 2077/04/16

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ABSTRACT

The Rhesus macaques (*Macaca mulatta*) are well-known Old-World non-human primates which are commonly found to inhabit various religious sites and cities in Nepal. There are several types of threats found in them. The parasite and human monkey conflict are the major types of threats. This study was carried out in Bajrabarahi area Lalitpur to find the population status and possible threats of Rhesus monkey. Direct observation methods were used for population count, questionnaire survey was used for human monkey conflict and direct wet mount, sedimentation, floatation, and acid-fast staining techniques were used for the parasitic assessment. The collected data were analyzed with the use of Chi square test and MS EXCEL 2010 and data were presented in charts, Table, graphs and bar diagrams were used to present the data in a simplified and understandable form. The estimated population was 46 individuals. The human monkey conflict was increasing order. Total 80 respondents were participated in the survey the age from 15-60 years. Most of the respondents said that the human monkey conflict was due increasing the population and scarcity of the foods. Furthermore, cent percent prevalence with 12 varied species of the gut parasites were detected like Ascarid spp., *Balantidium coli*, *Cryptosporidium* sp., *Eimeria* sp., *Entamoeba coli*, *Entamoeba* spp., *Giardia* sp., hookworm, *Strongyloides* sp., Strongylid spp., *Trichomonas* sp., and *Trichuris* sp. suggesting parasitic infection as a major threat for the survival of these urban macaques.

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1. INTRODUCTION

1.1 Background of the study

The Rhesus macaques, *Macaca mulatta* (Zimmermann, 1780) are well-known Old-World non-human primates. They are physiologically and genetically similar to humans as both are believed to share a common ancestor that diverged about 25 million years ago and developed independently (Kumar and Hedges 1998, Gibbs et al. 2007) Compared with other macaques *Macaca mulatta* have a high level of adaptation and are well-adapted to co-exist with the human in urban settlements (Rathoure 2014; Kumar et al. 2013).

Monkeys have an important status in mythology and religion particularly, in Hinduism and Buddhism (Jokinen 2014; Fuentes 2017; Ale et al. 2020) and are commonly found in religious sites like temples, monasteries, and many urban areas (Ale et al. 2020). There is an extensive, unregulated, and close contact of macaques with humans because the religious sites are always full of local people, worshippers, and visitors (Fuentes 2005). Their feeding ecology and habitat are more or less similar to that of humans; thus, in the nearby areas, they often invade the houses, gardens, or agricultural fields for sharing the niches. They are also known to share disease-causing pathogens like the gut or gastrointestinal (GI) parasites, for example, from many years, these mammals have been linked for the outbreaks of emerging parasitic diseases in humans (Chapman et al. 2005, Jones-Engel et al. 2006, Ghimire et al. 2020). Gut parasitism has been evidenced that result in the high morbidity and mortality in nonhuman primates including many types of macaques (Fremming et al. 1955, Remfry 1978, Toft 1986, Chapman et al. 2005) as well as in human (Stauffer and Ravdin 2003, Haque 2007, WHO 2020) around the world. Thus, it is crucial to know the status of gut parasitism in the monkeys, especially in the anthropogenic ones, to reduce the possible health consequences in macaques as well as humans. The current study was conducted to assess the prevalence and diversity of gut parasites in the monkeys inhabiting Bajrabarahi, an urban temple area with increasing human–macaque interactions in the Lalitpur district, Nepal. They share infectious agents like intestinal parasites besides their food due to a very close evidences show that many emerging parasitic diseases in human are originated from primates on one side and on the other side, there is a great risk of human pathogen transmission to free ranging primates (Jones-Engel et al. 2006).

The lives and threats of the monkeys increase due to polluted water, unhealthy food, and poor health condition and habitat encroachment. The competition between human and money is the major problem in some areas because they share same food resources. The stealing food from the human settlement or garbage found around the forest and urban areas to supplement their natural diet so that the monkey shows the aggressive behavior to the human (Sharma et al. 2011). The monkeys which are found in the cities, village are very close to the human and have a competition between them and these monkeys are called urban monkeys (Rajpurohit et al. 2006). The massive cutting the fruit tree and plantation of exotic community that do not supply the food to the monkey and the monkeys enter to the field crops and human settlement area and that may cause the human monkey conflict (Ashan 2014).

1.1.1 Status of primates and their phylogeny

A total of 85 species of primates are recorded in the world. Primates that are found in the Madagascar include 28 species and about 50 species are found in each of the continents of the world accordingly, South America, Asia and Africa. All among them 44 species are recorded in south Asian countries (Sanjay et al. 2003). The primates are arranged into Strepsirhines and Platerhines-. Among these two the new world monkeys are belong to Strepsirhines and old-world monkey belong to Platerhines. All the apes follow the same root of old-world monkey. Among these primates the biggest apes' orangutans, gorilla, chimpanzees resemble with other nearest ancestors of human being in many capacities, social structure, and mental, emotional, reproductive and physiology development. The little-known pygmy chimpanzee that seems to be similar more than 98% with human beings, shows much more similar in locomotion, communications and sexual behaviors (Jolly 1985). Therefore, only three species (Rhesus, Assamese and Hanuman langur monkey) are recorded up to date in Nepal with their subspecies through density as a whole are not found (Chalise 2004).

1.1.2. Rhesus monkey

Rhesus monkeys are the best know species of Cercopithecoidea family. According to the IUCN the Rhesus macaques is one of the least concerned primates in the world (Timmins et al. 2008). They are distributed in Southeast Asia from northern Afghanistan in the east and south to the Godavari River in India, Thailand, Laos, Cambodia, Vietnam, Nepal, Bangladesh, Tibet and China in the west (Roonwal and

Mohnot 1997). It is most frequently kept in zoos even in smallest zoological gardens. Rhesus monkeys are considered pest species by their nuisance behavior. It is likely the most adaptable to a wide variety of habitats and elevations from high heat to snow fields to cities. It is partly migratory, sometimes ascending the Himalayas to an altitude of about 2500 meter during summer season.

Rhesus monkey are heavily built with compact robust limbs. The silky hair is yellowish brown, the necked skin is brown to yellowish brown, and the large posterior callosities are bright red no marked menstrual swelling occurs but skin of buttock becomes red during estrus period. The skin hangs in loose folds about the neck, breast and abdomen.

Rhesus monkey are characterized by a high degree of social flexibility four types of social groups can be described depending on the number of males in the groups. They are one male troop, multi-male troops, age-graded male troops and all male bands (Chalise 2004). Most social groups ranged from 8-10 individuals of both sexes, but there are generally 2-4 times as many females as males. Dominance hierarchy is more evident among small groups of male than those with more females who tend to live together more peacefully than the males. The gestation period of *Macaca mulatta* is 135-194 days and usually one baby is born in frequently a set of twins is produced babies are nursed for about one year, first clinging to their mother's bellies and later riding on her back. Sexual maturity in females is reached between the age of 2.5 and 4 years whiles males 2-3 years after that female reach menopause at the age of 25 (Southwick et al. 1992). Rhesus is ground feeder and is partly terrestrial and partly arboreal. Preferred food includes wild and cultivated fruits, berries, grains, leaves, buds, seeds, flowers and bark. They roost up peacefully in trees mid canopy to avoid their predators (Chalise 1998).

1.1.3 Gastrointestinal parasites in monkey

Gastro-intestinal parasites live in the intestinal tract of the host where they get nutrients and favorable conditions to complete their lifecycle. The gastro-intestinal parasite includes protozoa and helminthes. The gastro-intestinal parasitic infection is a major problem in the developing countries (Ngrenngarmlert et al. 2007). Wild primates can be the suitable host for the maximum number of parasites. They are particularly vulnerable to parasitic infection due to cohesive group characterized by frequent social infections that facilitate parasite transmission between individuals (Stoner 1996). More

than 50 different parasites were recorded in non- human primates (Nunn and Altiezer 2006).

Gut protozoan parasites such as *Entamoeba, Giardia,* and *Cryptosporidium,* similarly helminths like *Oesophagostum, Strongyloides, Trichostrongyle, Trichuris, Ascaris,* Hookworm, *Taenea* can cause the many infections to the non -human primates. As they cause the several complications like diarrhea, blood loss, enteritis, pulmonary lesions and other abdominal complications in non-human primates. The large number of the parasites can result in physiological disturbance, nutritional loss or may produce lesion that result in serious debilitation, and can create opportunistic for the secondary infection and that may lead to fetal however some of the parasites are non-pathogenic (Goldberg et al. 2008).

Parasites richness and prevalence is an indicator of the population status and ecosystem of wild animals (Teirchoeb et al. 2009). They harbor different gastro-intestinal parasites which affect their survival and reproductive activities by causing several diseases like Gastro intestinal hemorrhage, extra intestinal complications, spontaneous abortion and even that may lead to the death of an individual's (Colin et al. 2010, Akpan et al. 2010). Habitat fragmentation induced by human activities may make primate populations more sensitive to risk of infection by parasite.

1.2 Objectives

1.2.1 General objective

- To determine the population status and threats to survival of Rhesus monkeys (*Macaca mulata*) in Bajrabarahi area, Lalitpur, Nepal.

1.2.2 Specific objectives

- To determine the population status of Rhesus monkeys in Bajrabarahi area.
- To explore the status and situation of human-monkey conflict in Bajrabarahi area
- To investigate the prevalence and types of gastro-intestinal parasites in the macaques in Bajrabarahi area.

1.3. Rationale of the study

Many researches have been done about the population status of monkeys in different region of Nepal. Few researches have been done regarding the threats of monkeys. The scientific information regarding monkeys around Bajrabarahi temple was not available. Bajrabarahi area is a one of the religious places as well as famous picnic spot due to this people used to throw the wastage materials over there and the monkeys used to feed wastage materials and, they are suffering from parasitic infection. The study area is near to the human settlement area so that there is a conflict between human and monkey.

2. LITERATURE REVIEW

2.1. Population status of the Monkeys

In Nepal, Rhesus monkeys are found in tropical rain forest of Terai to the valleys across of the higher elevation of Makalu-Barun, Langtang and coniferous, alpine forest of the Rara area too (Southwick et al. 1982, Chalise 1998). They are in large number in religious jungle and temples like Pashupati, Swoyambhu, Shankkhu, Bajrayogani, etc. of Kathmandu valley (Chalise 1998). According to the classification of conservation assessment and management plan workshop 2002, status of available species has neen classified for Nepal (Sanjya et al. 2003). The conservation status of Rhesus monkey (*Macaca mulata*) as assessed as a list concern as it is widely distribution and abundant in its population. The Rhesus and Langurs are common. Assamese is strictly protected under the National Park and Wild life Conservation Act 1973, and has considered I endangered status (Chalise 1997 and 1998).

A stable population of Rhesus monkey around 450 individuals in religious places Pashupati and Swoyambhu area was estimated (Chalise 2004). He recommended that clean water supply and restoration of natural habitat are urgently needed to manage these populations, which research work on the title of a case of a population stability of semi provision free ranging temple Rhesus monkeys of Kathmandu valley, Nepal. The studied on the title of Habitat utilization of Rhesus monkeys and its conflicts with people in Shivapuri Nagarjuna National Park, Nepal found that the Rhesus monkeys were found to be distributed ranging from 1390 m to 2300 m in Sundarijal, Panimuhan and Rolche area of Shivapuri Nagarjuna National Park with total population of 125 species individuals during the study periods (Nepal 2005). Researchers also found that habitat type utilization was maximum towards tree shrubs area (44.82%) which was followed by rockey area (23.02%), smooth ground (14.60%), stream side (9.68%) and croup land (7.88%). (Singha et al. 2004) found a new species Arunachal macaque (*Macaca munzala*) from western Arunachal Pradesh north eastern India which share morphological characteristic independently with an Assam macaque or Assamese macaque (*Macaca* assamensis) and with a Tibetan macaque (*Macaca thibetana*).

2.2. Human monkey conflict

According to world conservation union, world park congress 2003, human-wildlife conflict occurs when wildlife's requirement overlaps with those of human population

creating cost to resident and wild animals. Direct contact with wildlife occurs in both rural and urban areas, it is mostly common inside and around protected area in the place where the density of population is higher and mostly in the cultivated and grazing area. One of the main challenges facing wildlife conservation in the twenty first century concerns the concerns the increasing interaction between people and wildlife and resulting conflict that emerge (Sillero and Switzer 2001). Conflict between wildlife and people is an important factor affecting the relationship between protected area and the people who live near those places (Stdusrod and Wegge 1995). Across the globe primates are more frequently found crop raiding animals, from Africa to the Arabian Peninsula to Southeast Asia to Japan, primates come into conflict with human due to the renowned crop raiding behavior of many species (Sillero and Switzer 2001). Conflict occurs when non–human primates raid crops (Forthman1986, Siex and Struhsakar 1999, Hill 2000). A large number of primates' raids crops, but appears that terrestrial species are more likely to damage crops than arboreal species and non-folivers are greater crop raider than folivores. Among the old-world monkey, the most common and better able to coexist with many species are from the genera *Macaca papio* and Cercopithecus in particular the several species of baboon (Papiospp), The Rhesus monkey (*Macaca mulatta*) and the Vervet monkey (Sillero and Switzer 2001). Rhesus monkey are the major crop pest in the hills and mountains of Nepal (Giri and shah 1992, Chalise 1997, 2001, 2003, Ghimire, 2000) increase in the population of Rhesus monkey (Malik 2001). Crop raiding by the Rhesus monkey is the serious problem in Bandhipokhara VDC Palpa as in other parts of Nepal (Chalise 1997). Human-monkey conflict in Jhor-mahakal and Gold dhunga area also facing conflict due to crop raiding (Air 2015). The Rhesus is the most common species than other primates in Nepal. It is also called the pest of farmers.

2.3. Gastro-Intestine Parasite in monkey

Gillespie and Chapman (2006) and (Parae-Rodgrigue et al. 2010) found that forest fragmentation and increase in the proportion of available forest and presence of human strongly influence the risk of parasitic infection. There would be a positive correlation between the human and non- human primate contacts with infections. (Whitter 2009, Lynn 2010). GI parasite in seven non- human primate of Tai National parkwhich reported 23 species of parasites among these nine Protozoans (*E. coli, E. histolytica, E.hartmani, E. nana, I. butschlii, Chilomastix mesenili, Giardia* sp, *Anatrichosoma* sp,

B. coli and Blastocystis sp.*),* 13 Nematodes (*Oesophagostomum* sp., *Ancylostoma* sp., Spirurids, *Ternidens* sp., *Strongyloides* sp., *Trichostrongylus* sp., and *Trichuris* sp., and 1 trematode (*Dicrocoelium* sp.) (Kauassi et al. 2015) in SriLanka. A study conducted among Rhesus macaque in India found most common *Strongyle,* (33%), followed by *Ascaris* sp. (5%) and *Eimeria* sp. (3%) (Arunachalam et al. 2015). Similarly, Parmar et al. (2012) found that Strongyloidessp., *Trichuris* sp., *E. histolytica, Ascaris* sp., *E. coli and Spirometra* sp. from Hanuman langur and Rhesus macaque of India. (Gillespie et al. 2004) reported that six nematodes (*S. fullberni, Oesophagostomum* sp., *unidentified* Strongyle*, Trichuris* sp., *Streptopharagouus* sp., *Enterobius* sp.*)* one cestode (*Bertella* sp.), one trematode *(Dicrocoeliidae),* and five protozoans (*E. coli, E. histolytica, Iodamobea butschii, G. lamblia* and *Cilomastix mesenili*).

Similar study conducted by Gillespie et al. (2005) reported that seven nematodes (*Strongyloides fulleborni, S. stercoralis, Oesophagostomum* sp., an unidentified Strongle, *Trichuris* sp., *Ascaris* sp., *and Colobenterobius* sp.) one cestoda (*Bertiella* sp.*),* one trematode *(Dicrocoeliidae),* and three protozoans (*E. coli, E. histolytica* and *G. lamblia*) from three different species of monkeys (the endangered red colobus, the eastern black and white colobus and Angolan white colobus) of Uganda

3. MATERIALS AND METHODS

3.1 Study Area

The study had been conducted in Bajrabarahi, a religious Hindu temple area located in Godawari Municipality, Lalitpur, Nepal. The area (2760610 N and 85.32930 E) is covered by a sacred forest and is a typical habitat of several species of birds, reptiles, and mammals, including the *Macaca mulatta*. By direct counting methods, we assessed 50 monkeys in the forest. The area is surrounded by agricultural land to the south and east, and human settlement to the north and west. Besides the religious importance, it is also a bird-watching and recreational spot, and thus many religious people, tourists, and local people usually visit the site. It has also been developed as a picnic spot, so macaque-human interaction is typical in the study

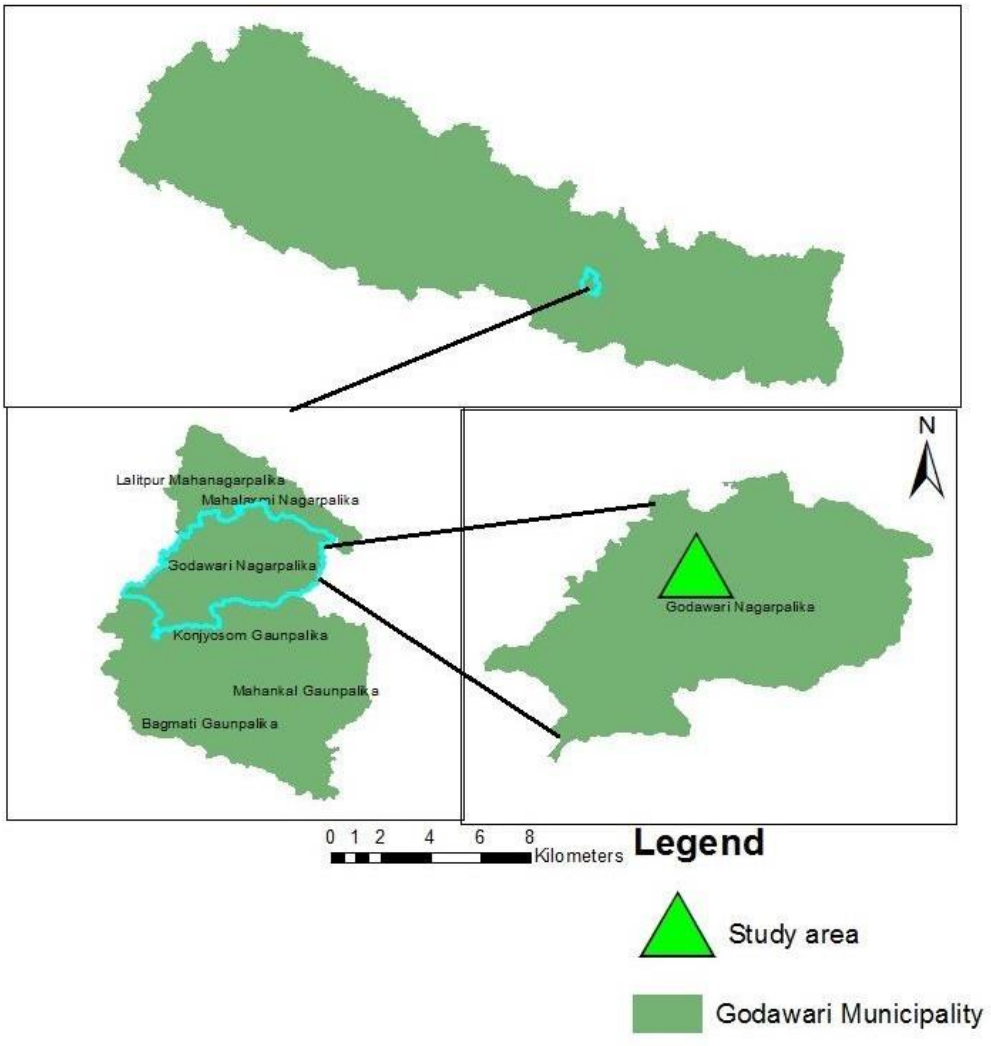


Figure.1 Map of study area

The major tree species includes *Myrsine capitellata, Schima wallichii, Neolitseaum brosa, Choerospondias axillaris, Castonopsis indica, Quescusseme carpifolia* etc. The shrubs include *Ageratum conyzoides, Berberisaristata and Jasminum humile.* Herbs species comprises of *Empatiens scabrida, Polygonatum verticillatum* and *Oxalis latifolia* etc.

3.2. Materials Required

- Electric microscope
- Sterile vials
- Cool box
- Centrifuge machine
- Gloves
- Forceps
- Wooden applicator
- Refrigerator
- Beaker
- Glass rod
- Tea strainer
- Cover slips
- Slides
- Data sheet
- Camera (Sony HD 12.0mp)
- Binocular (20x 100)
- GPS

Chemical required

- Lugol's Iodine Solution,
- 2.5% $K_2Cr_2O_7$
- Distilled water
- Saturated NaCl

3.3. Methods

3.3.1. For population status

The study was carried out from June 13th to 20th July 2019. The head count method of

monkey population was done with a help of binocular. First of all, regular observation was done early morning and early evening to locate the location of the monkeys in the study area. Five observers observed the monkeys from five different places simultaneously. The sex composition of the monkeys can be identifying by direct observation on the basis of shape of the head, testis and structure of the body.

3.3.2. For human monkey conflict

The semi questionnaire survey was done from 22th June to 23th July 2019. A survey was taken in different age group people from 15 years to 60 years. A total 80 questionnaire survey has been carried out in the study area to the local people. Special priority was given to the priest of the temple who had more knowledge about the human monkey interaction in the study area.

3.3.3. For the gastro intestinal parasite

Sample collection, preservation, and transportation,

From June to August 2019, a total of 42 fresh fecal samples of *M. mulatta* belonging to five different troops were collected non-invasively from various sites in the study area. The fecal samples were immediately preserved in 2.5% (w/v) potassium dichromate solution in 20 ml sterile vials and then, the samples were transported to the Animal Research Laboratory (ARL) of the Nepal Academy of Science and Technology (NAST) and stored in the refrigerator (4^{o} Celsius) for further analysis.

Microscopic examination of fecal sample

After the collection and preservation of the all-fecal samples, all the fecal samples were examined at the laboratory of Nepal Academy of Science and Technology (NAST). The fecal samples were microscopically examined for the trophozoites, cysts, oocysts, egg and larva of gastrointestinal parasites by the different methods such as direct wet mount and concentration method viz. floatation technique, and sedimentation technique and acid-fast staining techniques.

Direct wet mount

- This method involved stain and unstring smear preparation
- Stained smear preparation of stool/saline wet mount

- A portion of stool samples was taken on the glass slide and few drops of Lugol's iodine was added and mixed. Then a cover slip was placed over the mixture and excess of fluid was removed with a help of cotton. A smear was observed under microscope.
- Unstained smear preparation of stool/saline wet mount
- A small amount of the fecal sample was taken with a help tooth pick a and emulsified with a normal saline on a clean glass slide then cover slip was placed over it and excess of fluid was removed with cotton of filter paper. The smear was observed under a microscope for the demonstration of egg and larva of helminths.

Floatation technique

In the floatation technique, the fluid floatation medium i.e. saturated solution of sodium chloride (SPG1.20) has higher specific gravity then parasitic form higher the specific gravity (SPG) of the floatation solution greater the variety of parasite eggs that would float. All the helminths eggs and protozoan cysts floats in such a solution except the flowing eggs of *Ascaris lumbricoides* eggs of *Taenia solium*, *Tanea saginata* and also the eggs of intestinal fluke, strongyloid larva don't float on salt solution.

Process

- About 3 grams of fecal sample was taken
- The sample was kept on the beaker and grinded with about 20ml of normal saline.
- Filtrate the fecal solution by tea strainer and poured into centrifuge tube up to 12 ml and centrifuge at 1200 rpm for 5 minutes at room temperature.
- The centrifuge tube was taken out and upper part of the water was removed.
- The centrifuge tube was again filled with concentrated (45/100) NaCl solution up to 12 ml and centrifuged at 1200rpm for 5 minutes.
- The centrifuge was taken out and added more NaCl solution up to the tip of the tube.
- 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.
- Finally cover slip was taken gently and placed on microscope slide and examined under (10X and 40X) then photograph was captured.

Sedimentation technique

A sedimentation procedure is used to isolate eggs of Acanthocephalan, some other tapeworms and nematodes whose eggs bit heavier than others for these technique sediments of centrifuge contain were taken for eggs detection.

Process

- About 3 g of fecal sample was taken.
- The sample was kept on the beaker and grinded with about 20ml of normal saline.
- Filtrate the fecal solution by tea strainer and poured into centrifuge tube up to 12 ml and centrifuge at 1200 rpm for 5 minutes at room temperature.
- The centrifuge tube was taken out and upper part of the water was removed.
- The remaining sediment content was poured into a wash glass stirred gently.
- A small drop of sediment mixture was taken with a help of pipette and placed on the second slide, added one drop of iodine solution for staining.
- The specimen was stained by iodine wet mounts solution and examined under 10X and 40 X microscopes and 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 egg of helminths, protozoan, trophozoites or cyst of gastrointestinal parasite.

Acid fast staining

This technique of staining is quite effective for acid-fast organisms like *Mycobacterium, Cryptosporodium, Cyclospora, Isospora* and many more. The outer layer that means oocyst wall of these organisms contain large amounts of lipid substances called mycolic acids which resist the staining by ordinary stains like Gram stain (Morello et al. 2006). For this technique all the sample microscopically positive for *Cryptosporidium* and *Cyclospora* were selected and the laboratory processing was done on the basis of the procedure slightly modified by (Ghimire and Bhattarai 2019).

Initially 2-5 gram of stool sample was taken and filtered using tea strainer with 0.85% NaCl. Further, it was kept in a 15ml centrifuge tube along with 0.85% NaCl. Centrifugation was done at RT for 5 minutes at 1200 rpm, and the supernatant was

discarded.10% 10ml formalin and 4ml ethyl acetate was added in the centrifuge tube and centrifugation was again done at RT for 5 minutes at 1200 rpm. Supernatant was discarded. A thin smear with 1-2 drops of residual sample was prepared in the clean glass slide and was left to dry completely at RT (10-20mins). The dried specimens on slides were fixed with absolute methanol for 2 minutes. The sample was then stained with carbol fuchsin for about 10-15 minutes and then rinsed with distilled water. De-staining was done further with acid-alcohol (10ml H_2SO_4 + 90ml absolute ethanol) for 2 minutes and then rinsed with distilled water. Further staining with malachite green was done for about two minutes. Finally, the slides with specimen were rinsed with distilled water and left to dry over. After complete drying the slides was kept under microscope and observation was done at 100X magnification using immersion oil.

Measurement of eggs, cysts and larva

Eggs were measured by using image J software version image 1.46r/Java 1.6.0_20 (64 bit).

Identification of eggs for cysts and larva

The identification of egg cyst and larva were confirmed by comparing the structure color and size of egg, cysts and trophozoites of published literature journals and Books (Souls by 1982, Taylor et al.2007).

3.4. Data Analysis

Data were expressed as numbers of positive samples as well as prevalence rates in the table using Microsoft Word 2007. Prevalence rates were calculated by dividing the number of parasite positive samples (total or particular species) by the total number of samples observed (Ghimire and Bhattarai 2019)

Estimated population

$N=2n_k - n_{k-1}$

Where, N= estimated population.

n_k= highest Value of observed Population

n_{k-1}= second highest value of observed Population

4. RESULTS

4.1. Population Status of Rhesus Monkey

A total of 44 and 42 individuals of Rhesus macaques (*Macaca mulatta*) were counted in two different groups during the field. The Macaques were reported from the Bajrabarahi Temple area and near residential area. The estimated population of the rhesus was 46 (Group 1- 28 and 28 Group 2- 18).

Table 1. Total population of Rhesus Macaques in the study area.

Place	**GPS location**	**Altitude (m)**	**Troop name**	**1st height count (nk)**	**2nd highest count (nk-1)**	**Estimated population N=2nk - nk-1**
Bajrabarahi Temple area	27°41'26" N 85°18'60" E	1344	Group1	27	26	28
Residential area	27°41'28" N 85°18'59" E	1322	Group 2	17	16	18

Age-Sex Structure

Out of 44 individuals, the highest population was found in adult female (25%), followed by young female (20.45%), Juvenile (18.18%), adult male (13.63%), Infants (11.36%) and young male (11.36%) in first highest count (Table 2).

Table 2. Age-Sex structure of the macaques recorded within the study area on the basis of first highest count.

S. N	**Troop Name**	**Adult Male**	**Adult Female**	**Young Male**	**Young female**	**Juvenile**	**Infants**	**Total**
1	Group 1	2	5	2	3	3	2	17

2	Group 2	4	6	3	6	5	3	27
	Total	6	11	5	9	8	5	44
	Percentage (%)	13.6364	25	11.3636	20.455	18.182	11.364	100

Distribution of Rhesus Macaques in two divided groups.

Rhesus macaques were observed in two divided groups within the study area, areas around human settlement and Bajrabarahi temple area. One troop (Group 1) with 18 individuals were recorded around human settlement area where two adult males and six adult females with two young males, three young females, three juveniles and two infants were recorded.

Similarly, another troop (Group 2) of 28 individuals was observed around Bajrabarahi temple area, with five adult males, six adult females, three young males, six young females, five juveniles and two infants.

4.2. Human monkey conflict

Trends of monkey problem

Most of the respondents believed that the monkey problem was increasing order. Among 80 respondents 62 respondents said that human monkey conflict was in increasing order whereas seven respondents said that human monkey conflict was in decreasing order. Only the 11 respondents believed that the problem was constant from last few years (Figure 2).

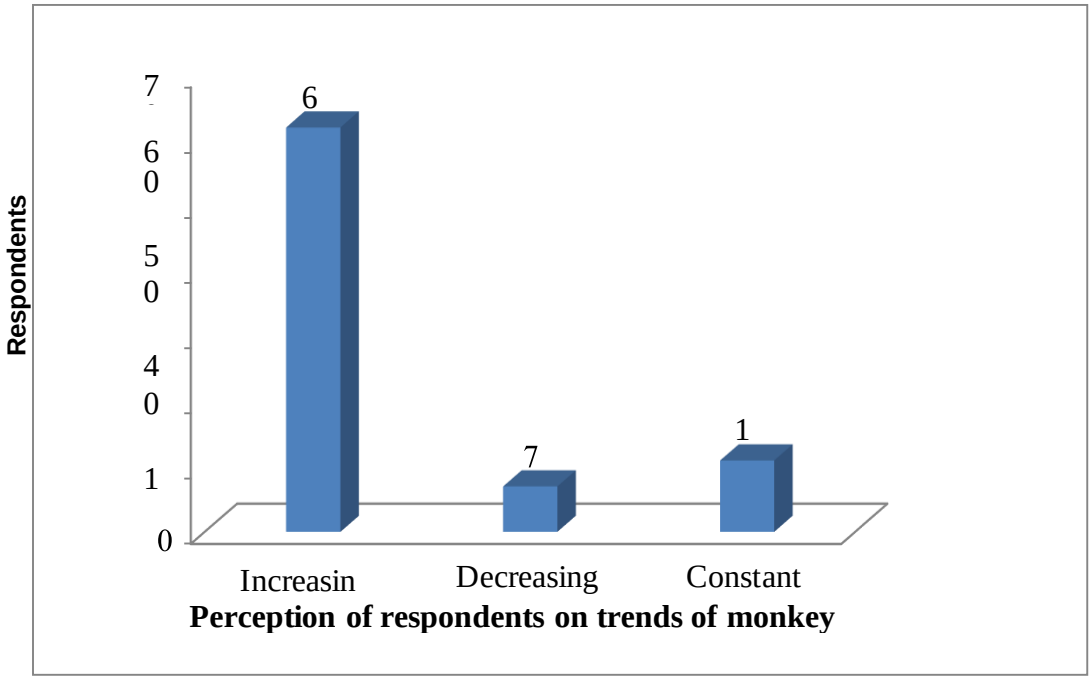


Figure 2. Trends of Monkey problem

Disturbance cause by monkey

The disturbance created by the monkey by grabbing (taking the foods, fruits and cloths) from a temple area by the monkey was found to be maximum and damage the electrical cable was found to be minimum (Figure 3).

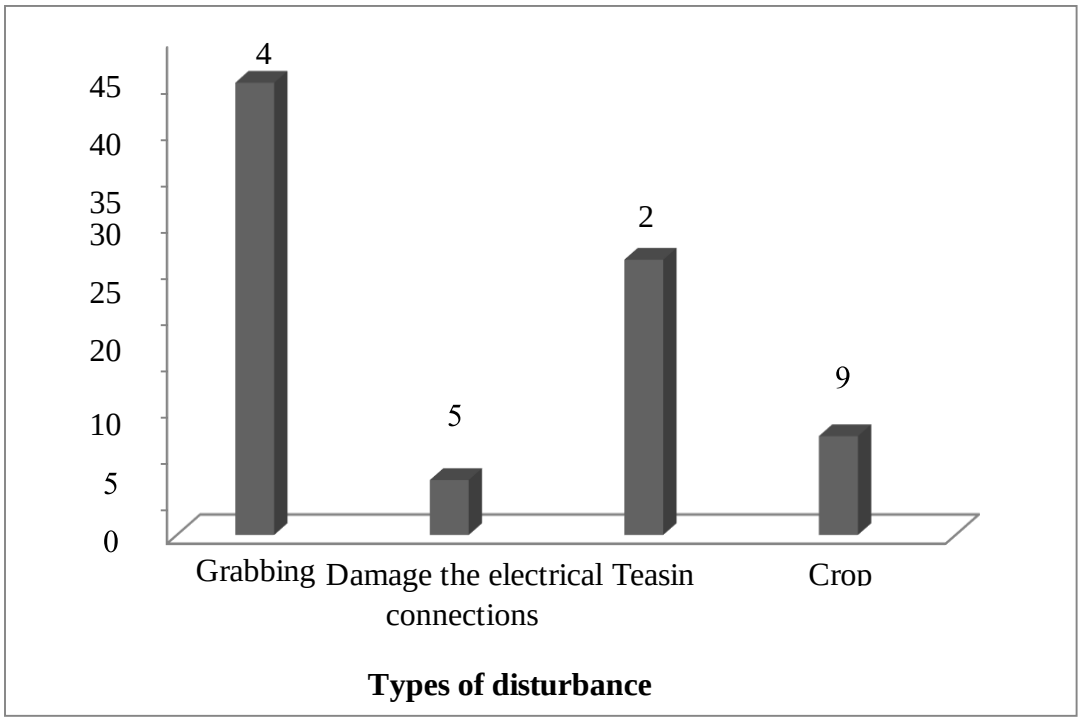


Figure 3: Different types of disturbances caused by monkey in study area

Human behavior towards monkey

Human perception towards monkey was also the important factors for the human monkey conflict. Among all the respondents, 38% respondents said that garbage throw was the major problem caused by the human to the monkey and others threats were noise (29%), chase (18%), and stone throw (15%).

Causes of increasing monkey problem

There are many factors that lead to the present increasing monkey problem. Among 80 respondents, highest number of respondents said that increasing monkey problem was caused by increasing population of monkey followed by food scarcity, lack of suitable habitat and internal migration (Figure 4).

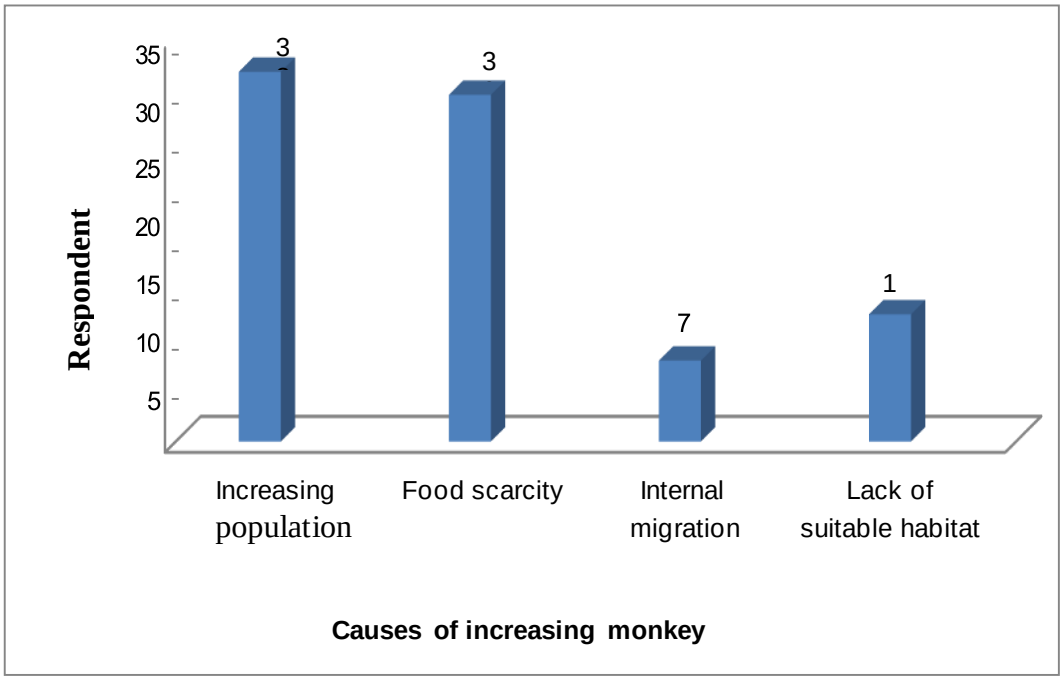


Figure.4: Causes of monkey problem in the study area

Monkey management

Among the total respondents, 40% of the respondents believed that fencing, 31% of the respondents believed that translocation, 20% of the respondents believed that visitor banned to the study area and 9% believed that killing was the major solution to manage the monkey problem (Figure 5).

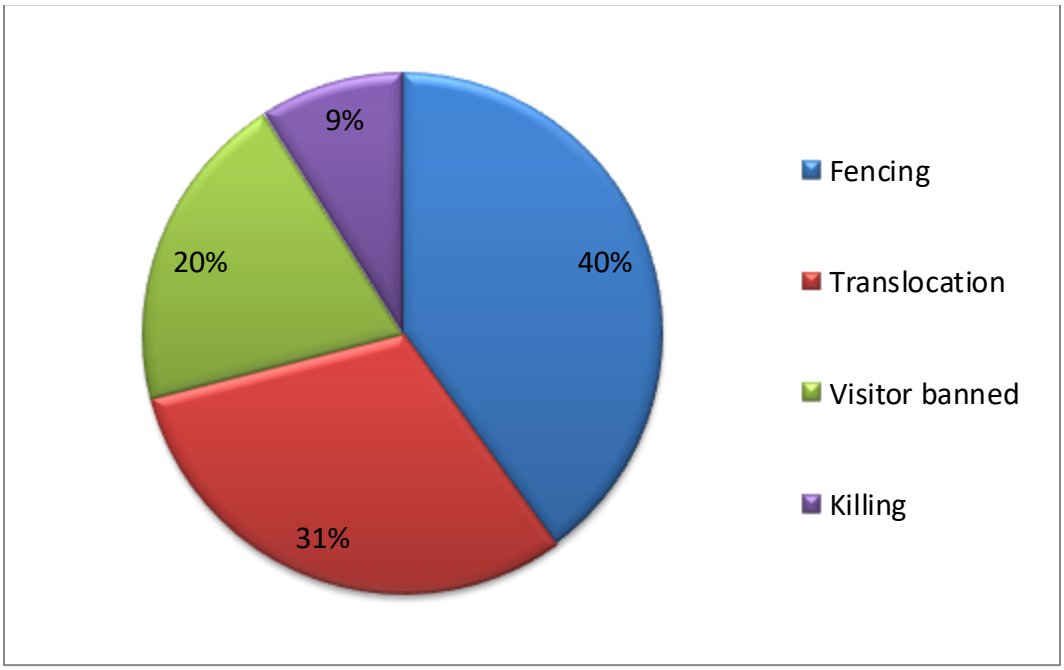


Figure. 5: Possible solution of monkey management in the study

4.3. Parasitic infection in monkey

In the current study, we reported a 100% prevalence rate of gut parasites. The prevalence of protozoa was higher (90.5%) compared to that of the helminths (47.6%). Furthermore, a total of 12 gut parasitic species were detected. They were protozoa - *Entamoeba* spp. (66.7%), *Balantidium coli* (59.5%), *Entamoeba coli* (57.1%), *Cryptosporidium* sp. (11.9%), *Eimeria* sp. (7.1%), *Giardia* sp. (4.8%), and *Trichomonas* sp. (2.4%) and helminths like Ascarid sp. (21.4%), *Strongyloides* sp. (21.4%), hookworm (19%), *Trichuris* sp. (14.3%), and Strongylid spp. (9.5%)

All samples were found to be mixed infections with two or more gut parasitic species. Triplet infection was the highest (57.1%) followed by the duplet (26.2%), and pentuplet (4.8%) infections were the least (Table 1). Further, two morphotypes of Ascarid eggs were detected. Some of these eggs were similar to human *Ascaris*, and others were similar to animal *Toxocara*. Similarly, based on the morphology and micrometry, three morphotypes of Strongylid eggs were detected in the current study.

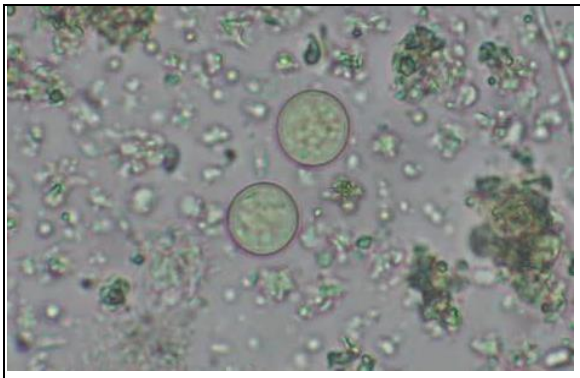
Gut parasitic species, their concurrency, and prevalence in *M. mulatta*. N represents total samples collected and n- represents number of positive samples.

Table 3: Infecting parasitic species with overall prevalence

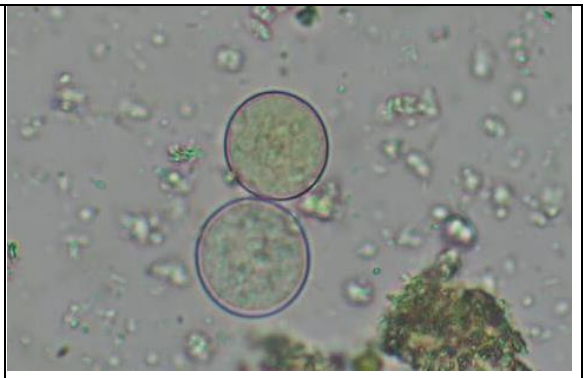
Infecting Parasitic species	**Overall Prevalence (nX100/N) (N=42)**
Protozoa	
Entamoeba spp.	28 (66.7%)
Entamoeba coli	24 (57.1%)
Eimeria sp.	3 (7.1%)
Cryptosporidium sp.	5 (11.9%)
Giardia sp.	2 (4.8%)
Balantidium coli	25 (59.5%)
Trichomonas sp.	1 (2.4%)
Helminths	

Ascarid spp.	24 (21.4%)
Strongylid spp.	4 (9.5%)
Hookworm	8 (19%)
Strongyloides sp.	9 (21.4%)
Trichuris sp.	6 (14.3%)
Total Protozoan infection	38 (90.5%)
Total Helminths infection	20 (47.6%)
Total Mixed infection	42 (100%)
Duplet Infection	11 (26.2 %)
Triplet Infection	24 (57.1 %)
Quadruplet Infection	5 (11.9%)
Pentuplet Infection	2 (4.8%)

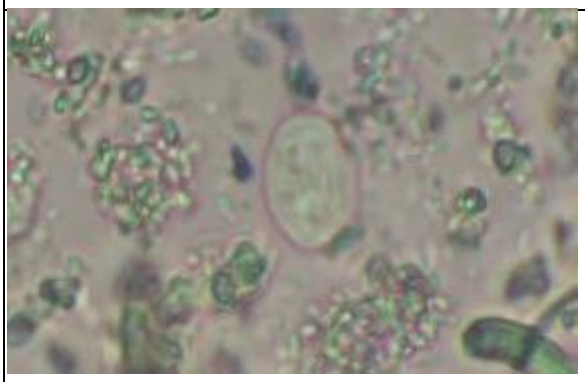
Photo plates of parasites



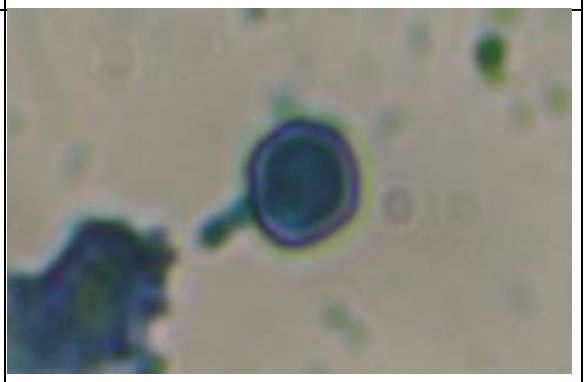
Entamoeba sp. (12x13 µm), iodine stain



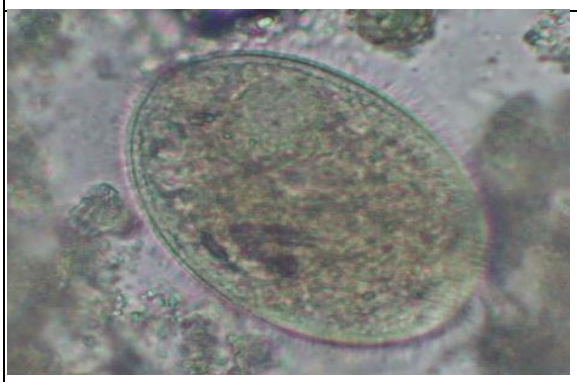
E. coli (22x21 µm), iodine stain



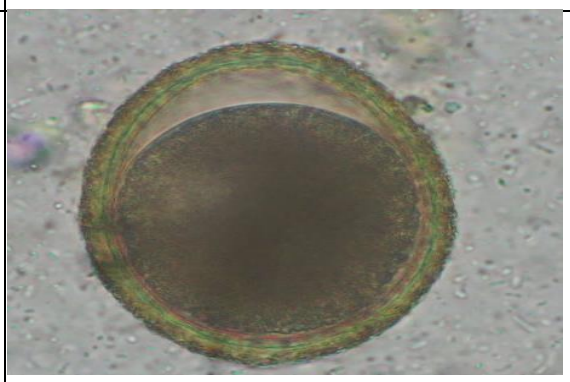
Giardia sp. (13x9 µm), direct wet mount



Cryptosporidium sp. (5x5 µm), acid fast stain



E. coli (68x40 µm), direct wet mount



Ascaris sp. (86x67 µm), flotation technique

	
Eimeria sp. (18x14 µm), flotation technique)	*Trichuris* sp. (51x23 µm), flotation technique)
	
Strongyle egg (82x46 µm) flotation technique	*Strongyloides* sp. (69x43 µm), direct wet mount)

5. DISCUSSION

During this study, Rhesus monkeys in Bajrabarahi area were found to be 46 individuals. They were divided into two groups one is near the residential area and another one is Bajrabarahi temple area. Similarly, the population of approximately 400 rhesus monkeys in 5- 9 social groups were recorded in the parkland of Swoyambhu Buddhist Stupa and a fluctuating population of 292 to 441 individuals with 5 to 9 groups were counted from Pasupathi Temple Area (Chalise 2008). As these monkeys are defensive in nature and opportunistic in crop raiding, to avoid the predator and to get food with less effort, they are likely to find in the periphery of human habitation (Van Hoof 1990). Bathyal (2005) also supports the situation of rhesus behaviors of this kind. He has recorded the Rhesus monkey in different sites of the Shivapuri Nagarjuna National Park mainly near the edge of cropland and human settlements areas. Kattel (1993) added that rhesus monkeys were mainly found in *Schima, Catatopsis* habitat on the lower periphery and Oak-laurel (*Quercus* sp.) in the middle elevation adjacent to human habitations. Soti (1995) had mentioned that Rhesus monkey were in Kakani area of SNP similar result that I found, Rhesus monkey of Bajrabarahi forest were near to the human resident.

The current study explores the status and diversity of the gut parasites in monkeys inhabiting an urban temple area situated in between human settlements in Nepal. In this study, the overall prevalence of the gut parasites was 100% which was similar to the result from National Zoo Dhaka (100%) (Tabasshum et al. 2018), higher than the findings from Nepal (61.9% – 86%) (Jha et al. 2011, Adhikari and Dhakal 2018, Bhattarai et al. 2019), and India (40%–66.5%) (Parmar et al. 2012, Jaiswal et al. 2014, Kumar et al., 2018). Besides, cent percent concomitant infections with maximum triplet co-infection rate suggested the parasitic richness in the gut of the macaques. The higher parasitic prevalence might be related to the applied study design, sampling techniques, laboratory-based techniques, sampling geography, including the climatic conditions and the lifestyle pattern of the existing macaque population. In the current study, we used a purposive sampling technique and collected the fresh fecal samples immediately after defecation by the macaques. The sampling period was warm and wet, the period favorable for the survival of larva, cysts, oocysts, and trophozoites of gut parasites (Zvinorova et al. 2016). Also, laboratory techniques like direct wet mount, concentrations (sedimentation and flotation), and acid-fast staining techniques were

used to enhance the detection of the parasites from each fecal sample. Macaques are the natural and reservoir hosts of many gut parasites. They live in a group and spend a semi-nomadic life that might massively increase the parasite dispersal (Macpherson 1994, Swedell 2012). The increasing soil and water pollution by waste foods, and garbage, especially during the festive and picnic programs, and the occasional open defecation by visitors/outsiders in the forest areas and Nearby water sources, are the risk factors of parasite transmission. In this scenario, macaques are usually contact with contaminated soil and water, and consumption of garbage foods may lead to the acquisition and transmission success of the gut parasites in them, which explains their species richness.

The current prevalence of gut protozoa was higher than that of the helminthes; however, this result contrasts with the previous findings (Adhikari and Dhakal 2018; Bhattarai et al. 2019) that recorded higher prevalence rates in helminths. Regarding protozoa, the prevalence of *Entamoeba* spp. in the current study was 66.7% which was lower than the findings from China (89.96%) (Zhang et al. 2019) and higher than those reported from Nepal (13.97% – 32%) (Jha et al. 2011, Pokhrel and Maharjan 2014, Adhikari and Dhakal 2018, Bhattarai et al. 2019) and from India (10% – 23.07%) (Parmar et al. 2012, Jaiswal et al. 2014). Several species of these pseudopodial amoebas like *Entamoeba histolytica, E nuttalli, E. dispar, E. moshkovskii, E. hartmanni, E. chattoni,* and *E. polecki* (Tachibana et al., 2007, Jiang et al., 2008, Tachibana et al., 2013, Guan et al., 2016, Zhang et al., 2019) have already been reported from macaques all over the world; however, majority of them are considered harmless and do not exhibit pathologic illness in the macaques. Pathologically, *E. histolytica and E. nuttali* are critical because they induce fatal intestinal and extraintestinal amebiasis (Fremming et al. 1955, Loomis et al. 1983, Haq et al. 1985, Beaver et al. 1988, Pang et al. 1993, Verweij et al. 2003, Tachibana et al. 2007, Jiang et al. 2008, Levecke et al. 2010). In our study, *Entamoeba coli* showed 57.1% prevalence rate, this finding was higher than the previous findings from Nepal (9.52% – 24.44%) (Jha et al. 2011, Bhattarai et al. 2019), India (10% – 26.92%) (Parmar et al. 2012, Jaiswal et al. 2014), and China (42%) (Guan et al. 2016). Although this species is typically asymptomatic in primates (Chapman et al. 2005), its presence should be taken as the indication of other pathogens inside the gut (Ghimire 2014). It was notable that, the rate of prevalence of *Cryptosporidium* sp. was 11.9%, which was lower than the findings from Nepal (41.1%) (Bhattarai et al. 2019) and India

(26.92%) (Jaiswal et al. 2014), and higher than the findings from China (10.94%) (Ye et al. 2012) and Thailand (1%) (Sricharern et al. 2016). This coccidian parasite causes highly fatal types of intestinal and extraintestinal pathologies (Kuhn et al., 1997, Kaup et al. 1998) and might be transmitted among humans and primates zoonotically (Ye et al. 2012, Zhao et al. 2019). Another coccidian parasite *Eimeria* sp. were reported in 7.1% of the fecal samples. This rate was lower than the findings from Nepal (16.12%) (Adhikari and Dhakal 2018) and higher than the results from India (3%) (Arunachalam et al. 2015). *Eimeria* sp. can cause severe pathologic consequences, especially in the young monkeys compared to the old ones (Burrows 1972).

Giardia and *Trichomonas* are the two flagellated parasites reported in the current study. The prevalence rate of *Giardia* sp. was 4.8% which was lower than the findings from India (31%) (Debenham et al. 2017), China (8.51%) (Ye et al. 2012), Nepal (6.67%) (Bhattarai et al. 2019), Thailand (7%) (Sricharern et al. 2016), and higher than the reports from India (1.2%) (Kumar et al. 2018). *Giardia* causes enteritis in macaques (Toft 1986, Chapman et al. 2005) and is a zoonotically critical parasite for the public and veterinary health (Ye et al. 2012, 2014). The prevalence rate of *Trichomonas* sp. was 2.2% when examined in the fecal samples. Reports of this species are found in *M. mulatta* following histopathologic studies in USA (Blanchard and Baskin 1988) and Germany (Blanchard and Baskin 1988, Kuhn et al. 1997, Kondova et al. 2005). This parasite is associated with mild to moderate gastritis (Blanchard and Baskin 1988, Blanchard 1993, Kaup et al. 1998), including many other severe GI pathologic consequences in immunocompromised macaques (Kondova et al. 2005). In the same way, *Balantidium coli*, a ciliate protozoan had a prevalence rate of 59.5% which was higher than the findings from Nepal (27.95% – 36%) (Jha et al., 2011, Pokhrel and Maharjan 2014, Adhikari and Dhakal 2018, Bhattarai et al. 2019) and India (8.7% – 19%) (Knezevich 1999, Kumar et al. 2018). This zoonotic parasite can also cause severe pathology in the intestinal tract of macaques, including diarrhea and rectal prolapse (Burrows 1972, Toft 1986, Kuhn et al. 1997).

Though significant number of people said that increasing of monkey's population was responsible for heighten the problem. The reintroduction and introduction of a species to an entirely new site must be carefully thought out so that the released species doesn't damage its new ecosystem or harm populations of any local endangered species (Olden

et al. 2011). Reintroduction of captives should not be considered a solution to the problem of surplus captive animals and a shortage of facilities. Releasing captive born animals without preliminary ground work and follow-up may turn out to be inhumane as well as seriously jeopardize the wild population (Kleiman 1989). However, this release is a part of conservation effort. Due to lack of proper habitat and their ability to integrate into a troop, these translocated monkeys seek provisional food, so they are constantly roaming around human residences and garbage raiding. Before these translocations occurred, there was no such problem with the native monkeys, either Rhesus or Assamese. Without properly assessing the carrying capacity of the habitat, translocations are wrong and almost certainly will increase the negative interactions between the monkeys and the local people (Imam et al. 2002). Here, adults and children simply have no idea about conservation of primates, or do they show any compassion towards animals. Lack of arms and no provision of killing the monkeys, increase of population of monkey, crop field very near to forest etc. were other major components responsible to heighten the problem of monkey as responded by the local farmers. In this study total 38 samples of Rhesus monkey were collected and examine on the basis of specific parasitic prevalence. After the laboratory examination the overall prevalence of intestinal parasites in the present study found 100%. Prevalence while Pokharel and Maharjan (2014) recorded 72.5% overall prevalence in *Assamese macaque* of SNNP, Kathmandu and Adhikari (2017) recorded 74.20% overall prevalence among Rhesus macaque and Hanuman Langurs of Devghat, Chitwan. In the same way, study conducted by Doubhadel (2007), Malla (2007) and Nepal (2010) found 60%-85% overall infection among the Rhesus macaque of Kathmandu valleys.

6. CONCLUSION AND RECOMMENDATION

From the current study, it was concluded that, the total number of estimated populations of the Rhesus Monkeys was 46. The increment in the number of monkeys in the study area was the major cause for human-macaque conflict. Cent percent prevalence rate and parasitic divergences (12 species) exist as a major health burden in these macaques. Translocation can serve as the best way of minimizing human–macaque conflict in the study area**.**

Recommendations

Some recommendations are put forwarded here, on the basis of study, for the effective management of least concerned Rhesus Monkeys in Bajrabarahi area.

- Research based on zoonotic transmission of GI parasites can be carried out further, since this host is implicated for transmission of GI parasites in human.
- Pollution should be controlled.
- Fencing should be done for the management of the monkey.
- Public awareness should be created to the visitors and local people
- Deworming the macaques must be done to reduce the burden of GI parasites and improving the health of macaques.

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Research Article

Diversity and prevalence of gut parasites in urban macaques

Bikram Sapkota[1,2], Roshan Babu Adhikari[3,4,6], Ganga Ram Regmi[4,5,7], Bishnu Prasad Bhattarai[1,8], and Tirth Raj Ghimire[3,9,*]

[1]Central Department of Zoology, Tribhuvan University, Kathmandu/NP.
[3]Animal Research Laboratory, Faculty of Science, Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal.
[4]Third Pole Conservancy, Bhaktapur-10, Nepal.
[5]Institute of Forestry, Tribhuvan University, Nepal.
[2]E-mail: bik.saapkotaa@gmail.com.
[6]E-mail: srkroshanbabu@gmail.com, ORCID ID: 0000-0002-5876-667X
[7]E-mail: gangarregmi@gmail.com, ORCID ID: 0000-0002-8392-5863
[8]E-mail: bpbhattarai@cdztu.edu.np, ORCID ID: 0000-0001-5741-6179
[9]E-mail: tirth.ghimire@nast.gov.np, ORCID ID: 0000-0001-9952-1786

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Abstract: Rhesus macaques (Macaca mulatta) are commonly found to inhabit various religious sites and cities in Nepal. Similar to other nonhuman primates, they are also the natural or reservoir host of several gut parasites. However, the status of gut parasitism, particularly in the urban dweller macaques, remains largely unexplored in the country. This study aimed to assess the prevalence and diversity of gut parasites in the monkeys inhabiting Bajrabarahee, an urban temple area in Lalitpur District, Nepal. A total of 42 fresh fecal samples of macaques belonging to five different troops, were collected and preserved in 2.5% (w/v) potassium dichromate solution. The fecal samples were processed by direct wet mount, concentration, and acid-fast techniques and examined under an optical microscope. All the fecal samples were positive with gut parasites. The parasites detected were Ascarid spp., Balantidium coli, Cryptosporidium sp., Eimeria sp., Entamoeba coli, Entamoeba spp., Giardia sp., hookworm, Strongyloides sp., Strongylid spp., Trichomonas sp., and Trichuris sp. Cent percent prevalence rate and high species richness with 12 parasites may indicate that they have impact on the gut health of these monkeys. This suggests the need of deworming the macaque population and enhancing public awareness for pro-active control of parasitic infection as well as of adopting the preventive measures to lessen the zoonotic transmission of the pathogenic parasites.

Keywords: Cryptosporidium, Entamoeba, gastro-intestinal (GI), Macaca mulatta, zoonosis.

सारांश : रातो बाँदरहरू प्राय: नेपालका विभिन्न धार्मिकस्थल र शहरहरूमा पाइन्छन् । अन्य गैर-मानव प्राइमेटहरू जस्तै तिनीहरू पनि पेटको धेरै परजीवीहरूको प्राकृतिक आश्रयस्थलहरु हुन्, यद्यपि, खासगरी नेपालको सहरवासी रातो बाँदरको पेटमा परजीवीको स्थिति भने अझै अन्वेषण गर्नुपर्ने रहेको छ । यसैले नेपालको ललितपुरस्थित शहरीक्षेत्रको मन्दिर बज्रबाराहीमा रहेको बाँदरको आन्द्रामा पाइने परजीवीहरूको व्यापकता र विविधताको आँकलन गर्नु यस अध्ययनको लक्ष्य हो । फरक-फरक पाँच प्रकारका समूहमा रहेका बाँदरहरुबाट कुल ४२ वटा ताजा दिसाका नमूनाहरू जम्मा गरि २.५% पोटाशियम डाइक्रोमेटमा संकलन गरी संरक्षित गरिएको थियो । उक्त नमूनाहरु डाइरेक्ट, कन्सनट्रेसन, र एसीड-फास्ट तरिकाहरुबाट एउटा अप्टीकल माइक्रोस्कोपद्वारा विश्लेषण गरिएको थियो, जसले आन्द्राहरुमा पाइने १२ वटा परजीवीहरु देखायो, जस्तै एस्कारिड, बालाण्टिडियम, क्रिप्टोस्पोरिडियम, आइमेरिया, एन्टअमिबा कोली, एन्टअमिबा इस्पेसिज, जिआर्डिया, अंकुशे जूका, स्ट्रङ्गाइलिड, स्ट्रङ्गाइलोइडिस, ट्राइकोमोनस, र ट्राइचुरिस थिए । धेरै परजीवीहरुको उच्च दर र व्याप्तताले गर्दा यी बाँदरहरुको आन्द्राको स्वास्थ्यमा नकरात्मक असर पर्न सक्छ र मानवमा परजीवीहरु प्रसारणको जोखिममा उल्लेखनीय वृद्धि गर्दछ । यसले परजीवीहरुको अग्रिम नियन्त्रणको लागि बाँदरहरुको जनसंख्यालाई औषधी खुवाउनु पर्ने र जनचेतना कार्यक्रमहरुको आवश्यकता र रोगजनक परजीवीहरूको जुनोटिक प्रसारण कम गर्नका लागि रोकथामको उपायहरू अनुकूलन गर्न सुझाव दिन्छ ।

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* Corresponding author, E-mail: tirth.ghimire@nast.gov.np; Tel.: +977-1-5547715

Appendix

Appendix 1: Questionnaire Format for respondents of Bajrabarahi area

Date…….

Name:…… Age:……..

Sex…….

Address:…….. Occupation:……..

1. Have you seen the monkey in Bajrabarahi area?

a) Yes b) No

2. What type of monkey have you seen?

a) Hanuman langur b) Ratobadar c) Paharabadar

3. Are they seen in all month?

a) Yes b) No

4. Do they create any disturbance in this area?

a) Yes b) No

5. If yes, what type of disturbance /damage do they create?

a) Grabbing/Taking the foods and fruits and cloths from a temple area

b) Damaging the network and electrical connections

c) Teasing the babies and girls

d) Damage the crop in the field

e) Other, specific

6. Which age groups monkeys are more destructive?

a) Adult b) Sub Adult c) Infant d) Juveniles e) All age category

7. What is the trend of problems caused by the monkey compared in three years?

a) Increasing b) Decreasing c) same

8. Where does the monkey more prefer to stay?

a) Deep in a forest b) In the periphery area of the forest

c)In the temple d) Near to the residential area

9. What are the main causes that make a monkey more destructive?

a) Increasing the population of the monkey b) Food scarcity in natural forest

c) Habitat destruction d) internal migration e) lack of suitable habitat

10. Problem caused by the human to the monkey

S. N	Caused by		Types			
	Local	Visitors	Chase	Stone/Catapult throw	Garbage throw	Unnecessary noise

11. What is the possible solution to manage the monkey in this area?

a) By killing

b) By translocation

c) By Fencing

d) Not allowed to the visitors to the temple area.

Appendix 2: Photoplates

Researcher working in the Laboratory

	
Photo1. Grooming	Photo 2. Researcher observing Monkeys

Photo 3. Viewing the visitor

Photo 4. Foraging

Photo 5. Monkey searching for food

Photo 6. Monkey searching for food