IDENTIFICATION OF GASTRO-INTESTINAL PARASITSES FROM THE FAECAL MATTERS OF RHESUS MONKEYS *Macaca mulatta* (Zimmerman, 1780) OF DHARAN

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DECLARATION

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

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RECOMMENDATION

This is to recommend that Jenisha Thakuri has completed this dissertation work entitled **"IDENTIFICATION OF GASTRO-INTESTINAL PARASITES FROM THE FAECAL MATTERS OF RHESUS MONKEYS *Macaca mulatta* (Zimmerman, 1780) OF DHARAN**" as a partial fulfillment of the requirements of B.Sc. degree in Biology (Zoology) under my supervision. To the best of my knowledge, this work has not been submitted for any other degree in any institutions.

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(Jenisha Thakuri)

ABSTRACT

The investigation was undertaken to study the prevalence of gastrointestinal parasites in Rhesus monkeys of Dharan. Altogether 130 samples of faecal matters were collected. The samples were collected in a sterile vial and were added with 10% formal saline. However, only 124 samples were selected for the further work. Due to the presence of contaminations in 6 samples with muds, dirt and maggots, etc. they were discarded. These samples were observed macroscopically at first and then by sedimentation and wet mount techniques in the laboratory.

Out of collected stool samples, 67 (54.03%) samples were found to be positive with at least one parasites. 31.76% samples were found to be positive with protozoans whereas 47.05% samples were found to be positive with helminthes (including both the single infection as well as multiple infections. 21.17% of them were infected with the unidentified parasites. The parasites included the four species of protozoa and six species of helminthes. The protozoans include *Entamoeba coli, E. histolytica, Balantidium coli* and *Eimera* sp. The helminthes include *Ascaris lumbricoides, Strongyloides stecolaris, Taenia* sp., *Enterobius vermicularis, Trichuris trichuria* and *Ancyclostoma duodenale*. The study shows that the *Ascaris lumbricoides* has the highest prevalence in the Rhesus monkeys of Dharan.

Similarly, 68% of the samples showed single parasitic infestation, 24% of the samples showed double parasitic infestation and 8% of the samples showed the multiple parasitic infestations. The present report showed that higher parasitic infestations in Dharan bazaar area in comparison to the Vijayapur forest area.

Key words: Rhesus monkeys, sedimentation and wet mount, *E.coli, B. coli, Ascaris lumbricoides,* Dharan

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LIST OF ABBREVIATIONS

Abbreviated form	**Details of abbreviations**
Min	minute
ml	milliliter
rpm	revolutions per minute
Prof.	Professor

1.INTRODUCTION

1.1 General introduction

Binomial name: *Macaca mulatta* (Zimmermann, 1780)

Systematic position:

Kingdom Animalia

Phylum Chordata

Class Mammalia

Order Primates

Suborder Haplorhini

Infraorder Simiiformes

Family Cercopithecidae

Genus *Macaca*

Species *mulatta*

Intestinal parasitic infection includes both the protozoans and helminthes which are the most common infections that occur worldwide. Protozoa are the parasites which consists of only one cell however helminthes consist of the worms and they consist of many cells (Haque 2007). Faeces are the most frequent specimen collected and examined for the presence of gastrointestinal parasites (Khanna et al. 2014).

The mammals that belong to the order primates include monkeys, apes, human, apes and other similar forms having dexterous hands and feet, binocular visions and well developed brains. They are commonly known as monkeys and exclude tree shrews; lemur- like forms, the apes and humans (Tattersall 1993). Of all the primates, monkeys have been successful next to humans to adapt best to widely diverse environmental conditions. They are found in tropical forests, dry savannas, mountains, villages, temples and even in large cities (Van Hoff 1990).

Macaques are the primates having very high adapting characteristics and are distributed more widely than any other non-primate genus. Macaques species occur in Asia ranging from Pakistan, India, Nepal, and Tibet of China in the west to the Wallace line tip of Japan and just south of the Wallace line in the Southeast (Thierry et al. 2004).

At present primates are found throughout the tropical zones of South America, Africa and Asia. Within those continental areas the types of habitat ranges from climax rain forest and moor land of high mountain ranges to open savanna and desert (Dunbar 1998). As a matter of fact, nowadays primates are confirmed between 40° N and 40° S of equator in the moderate habitat (Chalise 1999). The 2000 IUCN Red List threatened species had classified primate species and 224 species and sub-species as threatened (Rylands 2001). In Nepal, only three species of non-human primates (Hanuman Langur, Rhesus and Assamese monkeys) has been recorded till now (Chalise et al. 2005). The Rhesus monkeys (*Macaca mulatta*) are found freely living in wild as well as in the urban areas.

In order to survive, any species need a good diet to acquire all essential nutrients. All primates require similar general needs to obtain the energy but their individual requirements show variations and are obtained through various ways. No two species have an identical behavior and within the species as well there is a variation in diet within the social groups as well as within the populations (Oates 1987). Main differences in diet and feeding ecology of primates are usually determined by various factors such as body size, energetics, age or sex specific nutrient requirements, and anatomical specializations (Chivars and Hladik 1984). It reflects the evolutionary history of the family or the genus. The feeding habits of different species change seasonally as a response to short term fluctuations in the availability of preserved food items or resources (Torborg 1983). There are plenty of foods available in the evergreen and sub evergreen forests for the Rhesus monkeys. Trees are the main sources of foods for all the macaque. They depend upon various plant parts such as fruits, leaves, flowers, bark, etc. as well as vines and epiphytes. *Macaca mulatta* living in urban areas are to some extend dependent on human cooked foods such as chapatis, bread, roasted grains, ground nuts, splashed items, and even junk foods (Fooden 1980).

Parasites play a central part in the ecosystem and affect the ecology and species interactions (Esch and Fernandez 1993), host population and regulation (Hudson et al. 1998; Hochachka and Dhont 2000) and community diversity (Hudson et al. 2002). Parasites have always been an important part of the natural history of mammals. They form an important part of the ecosystem. Parasites are found to affect every group of organisms and monkeys are of no exception because of their feeding and dwelling habit. Rhesus macaque is an important part of the biodiversity and also forms a cognizable link between human and nature. They are found to be adapted well and co-existing with the human in both the urban and agricultural areas (Cawthon 2005).

Since the monkeys live near the human residents, they not only share human foods but also the parasites. Their close phylogenetic relationship with the human often results the high potential of pathogen exchange (Cheng 1999). Thus rhesus monkey population of Dharan may provide recent status of intestinal parasites, both of zoonotic and anthroponotic importance. Rhesus monkey and human are very close in terms of physiologic and genetic characters thus they share infectious agents like intestinal parasites along with the foods. There are many evidences showing that many emerging parasites in human have originated from the primates and in the same way there is also a great risk of transmission of human diseases to the primates (Jones-Engel et al. 2006).

Formalin (10%) is used for the preservation of the samples during the transportation. The faecal samples are examined both the microscopically as well as macroscopically for the presence of the parasites. Macroscopic methods can be used to detect the evidence of blood, mucus, parasitic segments, or whole parasites. Standard parasitological measures should be considered for the examination of the faecal samples which includes wet mount (saline mount and iodine preparation method) and by sedimentation method. Unstained saline wet mount preparation is done to detect the protozoan trophozoites and helminthic eggs and larvae. Iodine wet mount method helps to detect cysts. Similarly, a direct stained wet smear (saline mount) can be carried out where a drop of 1% Lugol's iodine is added at the edge of the cover slip and convert it into iodine mount (Mishra et al. 2013).

In a laboratory, two preparations of each specimen are usually made on each slide: one stained preparation while the other one is the unstained preparation. The saline

wet mount preparation is the unstained preparation made by the physiological saline. Its main advantage is that it helps to demonstrate the motility of the trophozoites. Iodine wet mount is the classical technique used for the microscopic examination which helps in the differentiation and identification of the parasites by the characteristics morphological features and the detail of the internal structures. The method is simple to perform as well as it is quick and inexpensive, facilitating direct visualization of the parasitic ova and cyst morphology. The disadvantage of this method is that the preparation dries within very few minutes, making it unreadable and unreliable to visualize live nematode larvae (Khanna et al. 2014).

This study mainly focuses to determine the current status of intestinal parasitic infections among the rhesus monkeys of Dharan. The study will give the information on identification of parasites in monkeys of Dharan and will support their ecological management in human proximity. This research also can be helpful for those who are interested in the similar fields.

1.2. Research objectives

a. General:

- To identify the gastrointestinal parasites from the faecal matter of the Rhesus monkeys of Dharan.

b. Specific:

- To examine the intestinal parasites in the stool of monkeys of Dharan.
- To compare the parasitic infection from different sampling stations.

1.3 Rationale of the study

Rhesus macaque is one of the species of old world monkey that reside near the human residents and often feed on plants, insects as well as human leftovers and refusals. Due to their unhygienic feeding habits it is presumed to have intestinal parasites which are deteriorating their health conditions. As it is a non-human primates living together with the humans, there is high chances of transferring of the zoonotic diseases. Human is always prone to be infected by most of the parasites of the rhesus thus they are of zoonotic importance. The main purpose of the study is to identify the

intestinal parasites from the faecal matter of the Rhesus monkeys of Dharan and its significance in the transfer of the various zoonotic diseases.

1.4 Limitations of the study

Due to the limited time and seasonal effects on the feeding behavior of the monkeys, the research has been carried out in only two distinct places of Dharan despite of abundance availability of Rhesus monkeys in different parts of Dharan.

2. LITERATURE REVIEW

2.1 Intestinal parasites

Parasites are the living organisms that that receive nourishment and shelter from the other organisms where they live. Intestinal parasites are those which must have an intestinal life cycle stage and live in either of the large or small intestine and receive nutrients, stool as well as blood from the intestinal wall and produces the traumatic damages to the intestinal villi. Some species of the intestinal parasites can cause haemorrhage into lumen of intestinal-mucosa due to the deposition of their eggs and some species penetrate and perforate the large intestines by secreting many lytic enzymes which digest intestinal tissues (Parija 2013). Transmission of the parasitic infection is mainly through oral route when they come in contact with the infected faeces (for example maybe through the contaminated food or contaminated water or soil). Intestinal parasites are broadly categorized into two categories which are protozoa and helminthes (Chatterjee 2009).

2.2 Distribution of intestinal parasitic infection

The survey conducted in Sri Lanka by Dewit et al. (1991) concluded the result gastro intestinal obtaine from toque macacaques(*macaca sinaca*) and langurs (*Presbytissenwx* and *P. entellus*) which were *Oesophagostomum aculeatum Streptopharagus pigmentata, Physaloptera* sp., *Enterobius vermicularis* and *Trichuris trichuria* and Hymenolepis.

According to the survey, the result showed the overall infection rate of 76.86% of all intestinal parasites where 53.72% was protozoans and 59.5% was helminthic parasites. (Chalise et al. 2011). The protozoans present were *E. histolytica, E. coli* and *B. coli*. The helminthes present were *strongyloides, Oesophagostomum, Trichostrongylus, Trichuris, Toxocora* and other *trichorids*.

Soulsby (1982) suggested the presence of *Toxocora, Entomoeba, Balantidium, Strongyloides, Oesophagostomum, Trichostrongylus* and *Trichuris* during the research.

Munene et al. (1998) reported the presence of the protozoan parasites, *E. histolytica, E.coli, B. coli,* and helminthic parasites as *Strongyloides, Trichuris, Oesophagostomum, and Trichostrongylus* in captive ad wild trapped non-human primates (Baboons, Vervets and Sykes) in Kenya.

The parasites present in the non-human primates as recorded by Munene et al. (1998) recorded *Strongyloids, Schistosoma mansoni, Streptopharagus* sp., *Entamoeba coli, Balantidium coli* and *E. histolytica* in non- human primates whereas Parmar (2012) recorded *Strongyloides* sp., *Trichuris* sp., *Entaemoeba histolytica* sp., *Ascaris* sp., *E.*coli, *Spirometra* sp. in non-human langur and rhesus monkeys in the Gujrat state, India.

In a survey conducted by Nath et al. (2012) found the prevalence of two parasitic specie viz. *Trichuris* sp and *Oesophagostomum* sp.

The result obtained by Goswami (1994) includes *Ascaris* sp., *Enterobius* sp., *Anchylostoma* sp., *Strongyloids* sp., *Entamoeba* sp., *Giardia and Balantidium* sp. in non human primates of Assam State zoo.

Hilser et al. (2011) recorded 62% langurs with positive result for helminthic infection while 82% for protozoan infection.

According to the survey carried out in the monkeys of Devghat, Chitwan were found to be infected with various protozoal and helminthic gastro-intestinal parasites viz. *B. coli, Eimeria* sp., *Entamoeba* sp., *Trichuris* sp., *Ascaris* sp., *Strongyloides* sp., *Oesophagostomum* sp., *Ancyclostoma* sp. *Trichostrongylus* sp. and Hymenolepis sp. (Adhikari et al. 2018).

The result revealed from the report Pokhrel et al. (2014) showed that a total of 10 different species of gastrointestinal parasites were found to be distributed among Assamese Macaque of SNNP. Distribution of helminth parasites (7 species) was found along with protozoan parasites (3 species) which were *B.coli, Entamoeba* sp., *Isospora* sp., *Ascaris*sp., *Trichuris* sp., *Strongyloides* sp., *Moniezia* sp., *Oesophagostomum* sp., *Hookworm and Physeloptera* sp.

The feral Bonnet Macaque in India (Varadharajan and Pythal 1999) reported the prevalence rate of 93% which is the highest prevalence of gastrointestinal parasites reported till date.

Various studies of the past done among the Rhesus Monkey from different area of Nepal has shown the positive result ranging from 60-85% (Malla 2007, Nepal 2010) though prevalence rates seem to vary according to locality.

3. MATERIALS AND METHODS

3.1 Materials

The list of materials, chemicals, equipment and reagents used during this study are listed in Appendix A.

3.2 Methods

Two methods were used for the study. They are macroscopic methods and microscopic methods. The microscopic methods included wet mount methods and sedimentation methods.

3.2.1 Study duration

The study was done from February to August 2019.

3.2.2 Laboratory setup

Laboratory setting was done in Biology laboratory some part was done in MSc. Microbiology laboratory, Central Campus of Technology, Hattisar, Dharan.

3.2.3 Study area

Dharan is a sub-metropolitan city located in province no. 1 of Nepal in the Sunsari district. It is situated at the foothills of Mahabharat range in the north while it joins the Terai at the tip of the south. It lies at an altitude of 1148ft (349m) from the sea level and has the coordinates of 26º 49' 0" N 87º 17' 0" E. The total population of Dharan is 137,705 (Census Bureau of Statistics 2012). The average temperature is 5 ºC to 35 ºC. Dharan is a religious place having different temples such as Budasubba, Dantakali, Panchakanya, etc. Monkeys tend to remain around the periphery of the temples thus Dharan consists of considerable number of monkeys.

Sample was collected from two different places of Dharan which are Dharan bazaar area and Vijayapur hill.

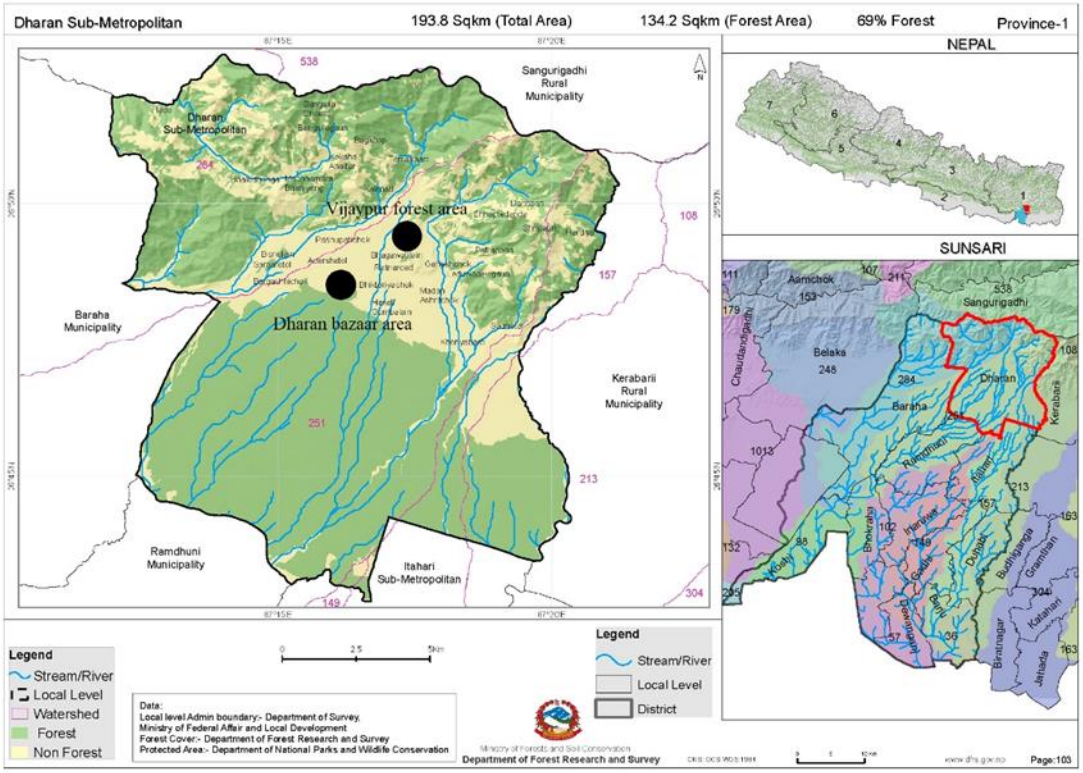

Figure 1. Map of Dharan showing sampling stations indicated by black dots.

3.2.4 Sample collection

About 5 gm of samples of the faecal matter of the Rhesus monkeys were taken in sterile vials or sterile plastic bags. This sampling was done twice a week in the morning from 6 am to 11 am along the Vijayapur hills and Dharan bazaar area.

3.2.5 Transportation of the sample

After collecting the stool sample from the Vijayapur hill and Dharan bazaar area, the samples were brought to the laboratory and fixed with 10% formal saline immediately.

3.2.6 Sample size calculation

Since the exact population of the monkeys of Dharan is unknown, the samples were taken by using random sampling method. For this research, 130 samples were collected of which 6 were found to be contaminated thus only 124 samples were taken for laboratory examinations.

3.2.7 Laboratory Processing of the sample (Parija, 2013)

Microscopic examination was done to detect and identify the cysts, oocytes and trophozoites of protozoan parasites and eggs and larvae of helminthic parasites. The process was carried out by saline wet mount method and sedimentation technique.

Saline Wet Mount

A drop of saline was taken in a clean, grease free slide and a small amount of stool sample was spread over it. The examination was first done under low power (10X) compound light microscope and then under high power (40X).

Formalin-ether Sedimentation technique

5 ml of 10% formal saline was taken and the preserved sample was taken and preserved sample was added to it and then shaken well. The suspension was sieved through the cotton guage in a funnel in a 15 ml centrifuge tube. After that 5 ml of ethyl acetate was added and shaken vigorously for 5 mins. Then the tube was centrifuged at 1000 rpm for 10 mins. The supernatant was decanted and 10 ml of 10% formalin was added to the sediment and was mixed thoroughly with the help of wooden applicator stick. Again the centrifuge was carried out at 1000 rpm for 10 mins.

After centrifugation four layers of suspensions were obtained.

a) A small amount of sediment was obtained at the bottom of the tube containing parasites.
b) On the upper layer of the sediment there was a layer of formalin.
c) On the top of the formalin layer, there was a plug of the faecal debris.
d) And there was a layer of diethyl ether on the topmost layer.

The plug of the debris was freed from the top of the tube by ringing the sides with the help of the applicator stick and also the top layer of the supernatant was decanted. The deposit after shaking was taken on the glass slide and the cover slip was placed over it and was examined by saline wet mount.

3.2.8 Recording of the Result

After the completion of the laboratory processing, the result so obtained was compared and identified based on Chatterji, D. (Protozoology and Helminthology). It was then recorded in thesis log book and later it was recorded and analyzed in the computer.

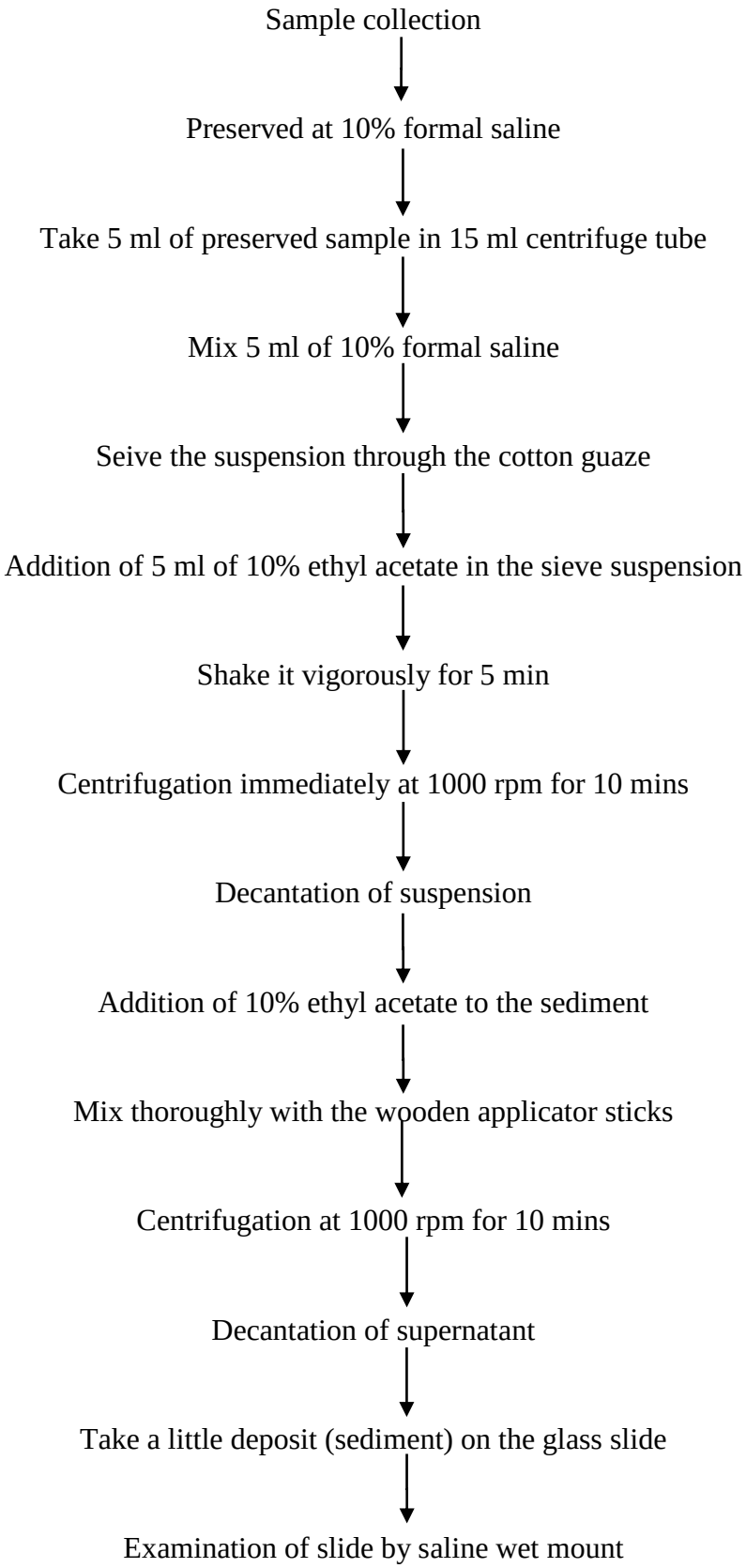

Figure 2. Flow chart of Formalin-ether sedimentation technique

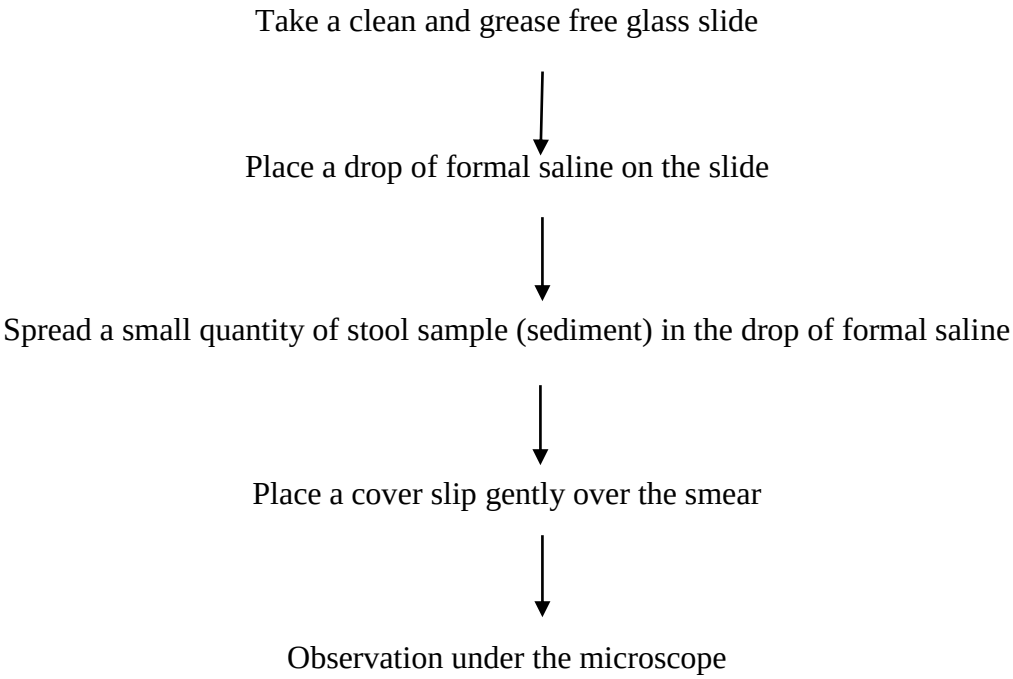

Figure 3. Flow chart of Saline wet mount technique

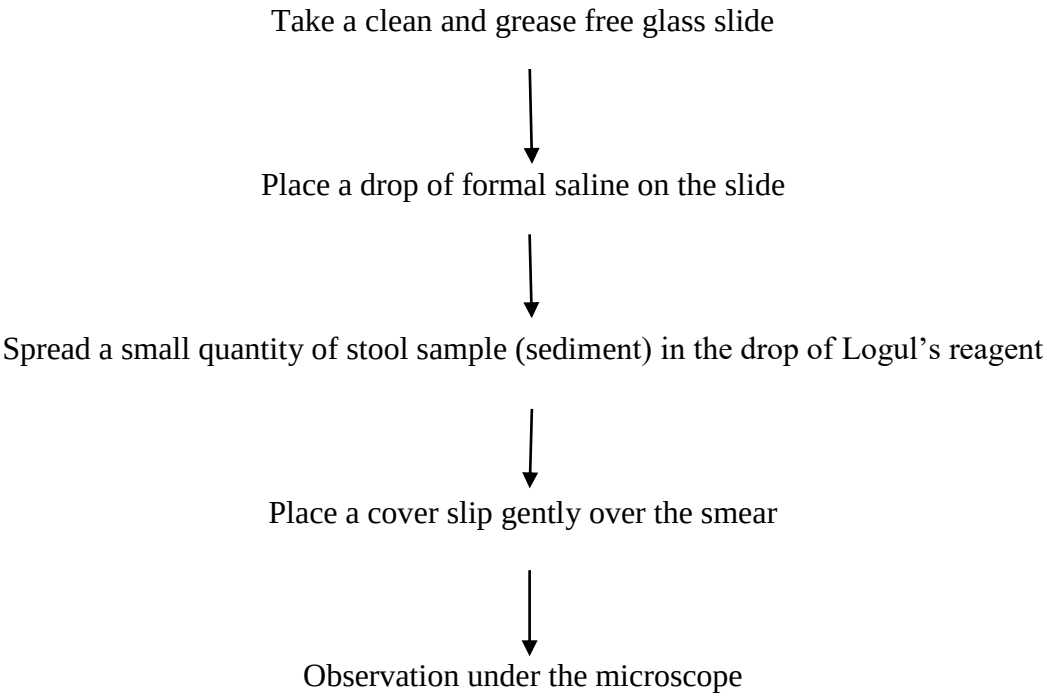

Figure 4. Flow chart of iodine wet mount technique

5. RESULTS

The laboratory examination of 124 stool samples of the rhesus monkeys was carried out in the laboratory of Biology Department at Central Campus of Technology, Dharan. Out of total collected samples, 6 (4.62%) samples were discarded due to their contaminations such as dirt, dust and maggots, etc. Altogether 67 (54.03%) samples were found to be positive for the presence of the at least one type of parasites. 39 (31.45%) samples were found to be negative for parasites. 18 (14.52%) samples contained possibly unidentified parasites (Figure 5).

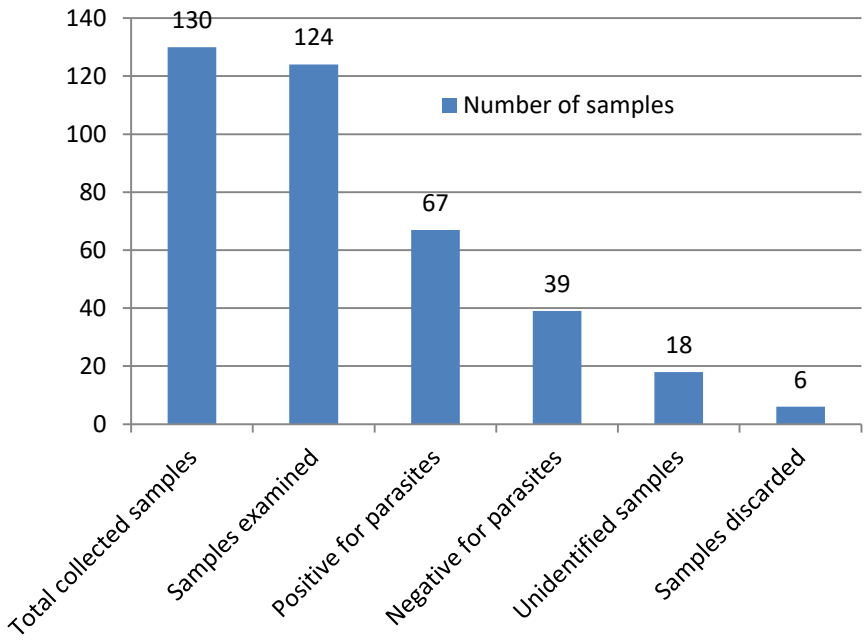

Figure 5. Result of examinations of collected samples.

4.1 Detection of parasites

Out of 85 positive stool samples, 27 (31.76%) samples were found to be protozoans and 40 (47.05%) samples were found to be helminthes. The present research result showed 18 (21.17%) samples unidentified species of parasites. The protozoans include the species as *E. histolytica* (7.46%), *E.coli* (25.37%), *Balantidium* (5.97%) and *Eimera* sp. (1.49%). The helminthes included *Ascaris lumbricoids* (34.32%), *Ancyclostoma duodenale* (17.91%), *Enterobius vermicularis (*4.47%), *Trichuris trichuria* (1.49%), *Strongyloids* sp. (1.49%), *Taenia* sp. (1.49%). The highest

prevalence was detected for *Ascaris lumbricoides* followed by *E.coli, Ancyclostoma duodenale, E. histolytica, Balantidium, Enterobius vermicularis, Eimerasps., Strongyloides stercolaris, Trichuris trichuria*and lastly *Taenia sp* (Figure 6). Statistical analysis of location vs parasite was found to be insignificant ($p>0.05$) (Appendix B)

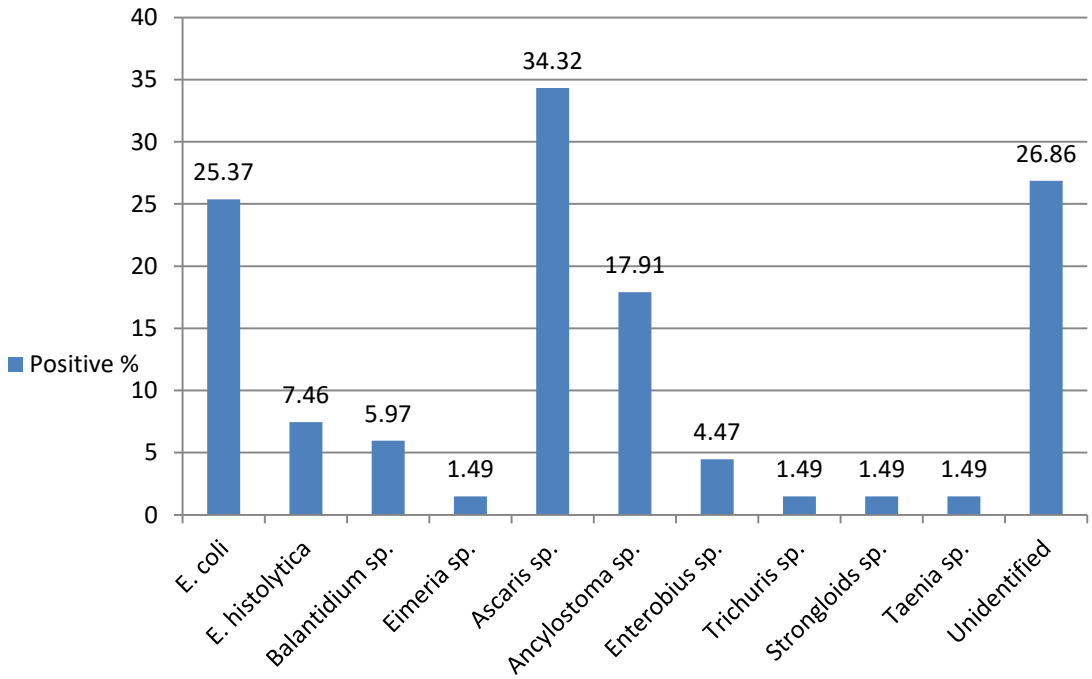

Figure 6. Prevalence of parasitic infestations.

Table 1. Prevalence of parasites in the collected samples

Intestinal Parasites	Number of samples		
	X	Y	Total
Protozoans			
E. coli	3 (1E, 2C)	14 (13C, 1T)	17
E. histolytica	0	5 (5C)	5
Balantidium	2 (2C)	2 (2E)	4
Eimeria sp.	0	1 (1E)	1
Total protozoans	**5**	**22**	**27**
Helminthes			
Ascaris lumbriciodes	8 (6O, 2A)	15 (12A, 3E)	23
Ancylostoma duodenale	3 (2A, 1E)	8 (7A, 1E)	11
Taenia sp.	0	1 (1A)	1
Enterobius vermicularis	0	3 (3A)	3
Trichuris trichuria	0	1 (1A)	1
Strongylois stecoralis	0	1 (1A)	1
Total helminthes	**11**	**29**	**40**
Unidentified	**4**	**14**	**18**
Grand total	**20**	**65**	**85**

A = Adult, C = Cyst, E = Egg, O = Ova, T = Trophozoite, X = Vijayapur Forest Area

Y= Dharan Bazaar Area

4.2 Prevalence of intestinal parasites on the basis of location

Out of 85 positive stool samples, 20 (23.53%) samples were obtained from Vijayapur forest area. The study result showed 5 (5.88%) samples were found to be protozoans and 11 (12.94%) samples were found to be helminthes. 65 (76.47%) samples obtained from Dharan bazaar area were found to be positive where 22 (25.88%) samples were found to be protozoans and 29 (34.12%) samples were found to be helminthes. 4 (4.71%) species of parasites were unidentified from Vijayapur forest area whereas 14 (16.47%) species of parasites were unidentified from Dharan bazaar area(Figure 6).

Out of 20 positive samples obtained from Vijayapur forest area, 5 (25%) stool samples were found to be protozoans, 11 (55%) samples were found to be helminthes whereas 4 (20%) samples contained unidentified parasites(Figure 7). Whereas out of 65 positive samples obtained from Dharan bazaar area, 22 (34%) samples were found to be positive with protozoans, 29 (45%) samples were positive with helminthic parasites and 14 (21%) samples contained unidentified species of parasites (Figure 7).

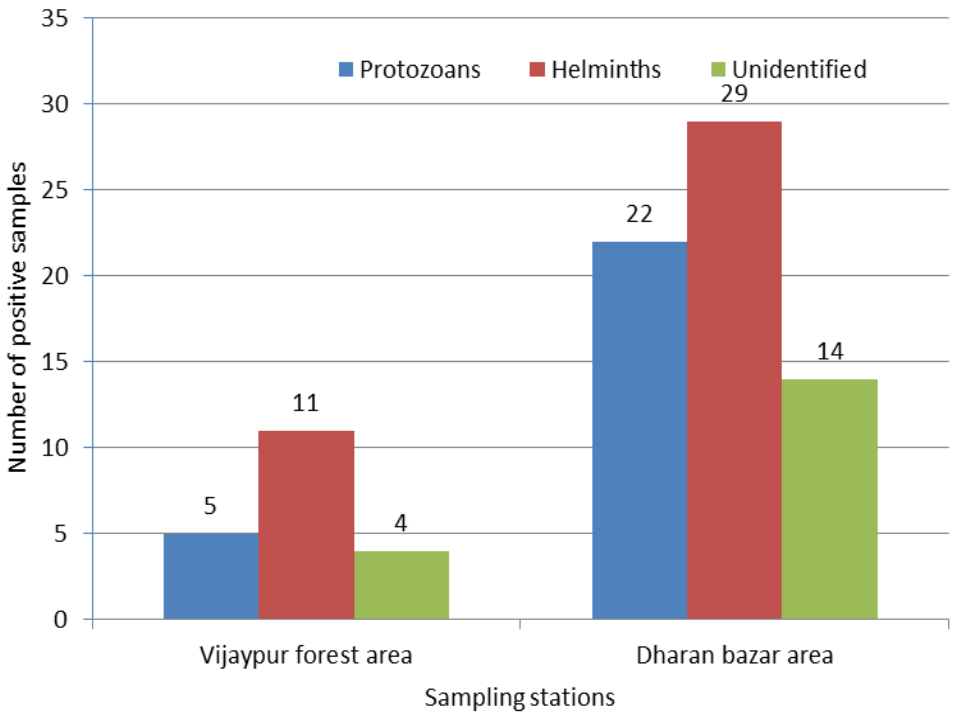

Figure 7. Prevalence of intestinal parasites on the basis of location

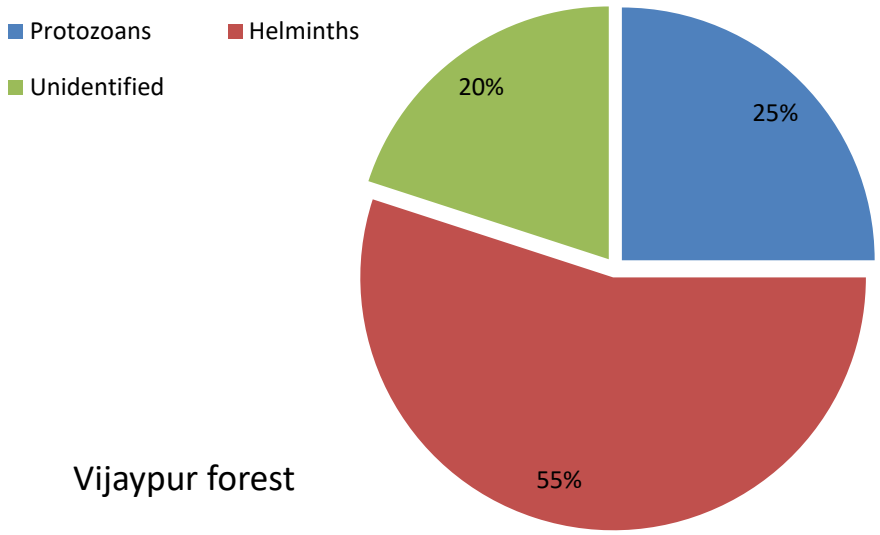

Figure 8. Prevalence of intestinal parasites in the rhesus monkeys of Vijayapur forest.

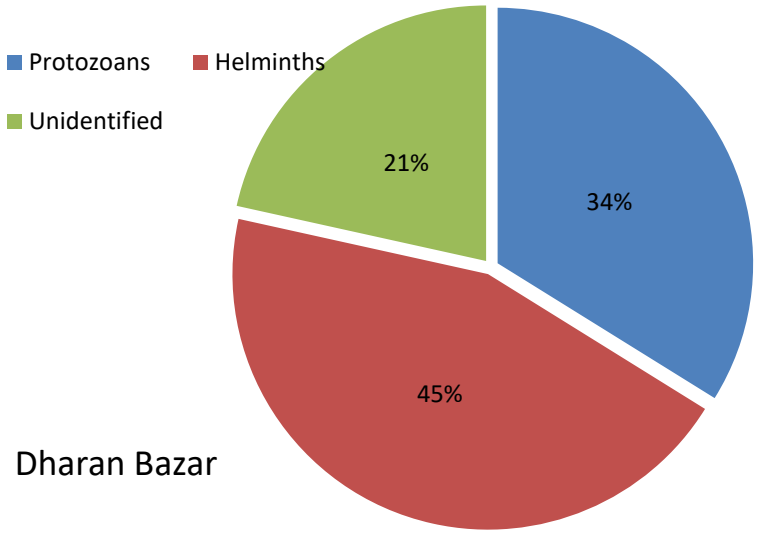

Figure 9. Prevalence of intestinal parasites in the monkeys of Dharan Bazaar.

4.3 Prevalence of intestinal parasites on the basis of type of infection

Out of 85 positive stool samples, 68% of the samples were found to have single parasitic infestation, 24% of them had double parasitic infestations whereas 8% of them had multiple parasitic infestation. In the recent study, 20 positive samples were obtained from Vijayapur forest area where 16.41% of them had single parasitic infestation, 2.9% of them had double parasitic infestations whereas none of them had multiple parasitic infestations. Similarly, out of 65 positive samples of Dharan bazaar area, 35.82% of them had single parasitic infestation, 14.92% of them had double

parasitic infestation and 29.85% of them had multiple parasitic infestation (Figure 10).

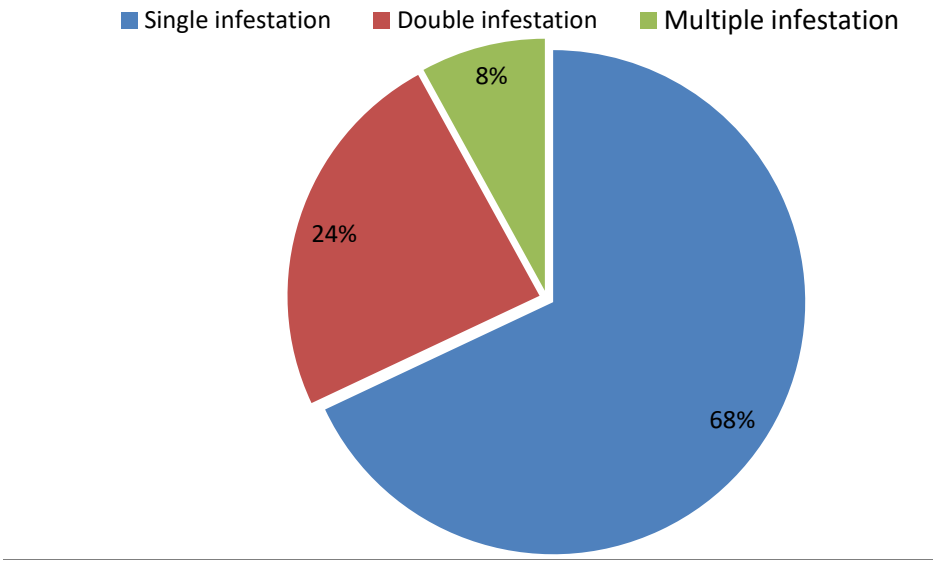

Figure 10. Prevalence of intestinal parasites on the basis of type of infection

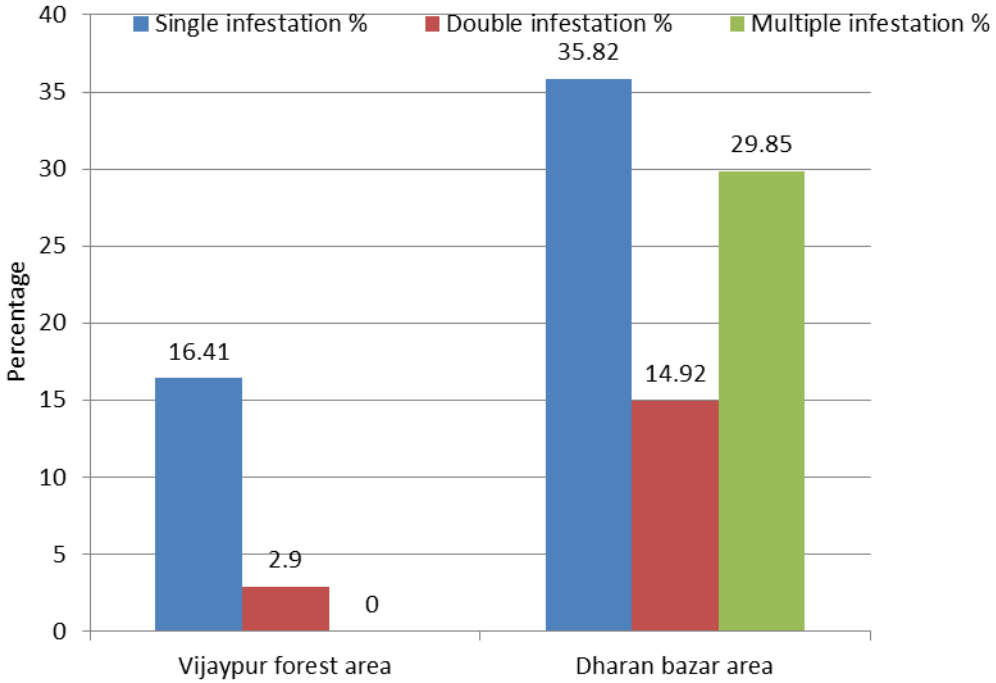

Figure 11. Types of parasitic infestations on the basis of location.

5. DISCUSSIONS

The laboratory examination of the total 124 samples of the faecal matters of the rhesus monkeys results that 67 (54.03%) samples were found to be positive for the presence of at least one type of parasites, 39 (31.45%) of the samples were found to be negative for the parasites whereas 18 (14.52%) of the samples contained possibly unidentified parasitic species.

The result of the present study showed the overall infection rate was found to be 68.55% including the identified as well as unidentified species of the parasites. The study showed the prevalence rate of 47.05% for the helminthes, 31.76% for the protozoans and 21.17% for the unidentified species of the parasites. The study also showed the absence of parasites. 31.45% of the samples were found to be negative for the presence of the parasites. The reason may be due to the less burden of parasitic infections or they are really parasites free. The reason is not clear because the experiment is solely based on the faecal matters of the Rhesus monkeys only.

The study showed the presence of four species of protozoa which includes *E. histolytica* (7.46%), *E. coli* (25.37%), *Balantidium* (5.97%)and *Eimera* sp. (1.49%).The helminthes includes six different species which are *Ascaris lumbricoids* (34.32%), *Ancyclostoma duodenale* (17.91%), *Enterobius vermicularis* (4.47%), *Trichuris trichuria* (1.49%), *Strongyloids* sp. (1.49%), *Taenia* sp. (1.49%). All the parasites in this study are in support with Soulsby (1982). He has listed *Entamoeba, Balantidium, Strongyloides, Oesophagostomum, Trichostrongylus* and *Trichuris* as infecting non- human primates. Thus these findings support the result of present study.

According to study carried out in Dharan, The prevalence of helminth infection (47.05%) was found higher than protozoal infection (21.17%).The result coincided with Jha *et. al.,* I (2011) who also reported similar result viz. 59.5% and 53.72% for helminth and protozoa infection respectively. The present study shows conflictions with the report of Hilser et al. (2011) who recorded that 62% langurs were positive for helminth infection and 82% were protozoans. These differences may be due to geographic condition, source of feeds and feeding behaviour of monkeys.

According to the research, the percentage of *Ascaris* sp. was found to be the highest prevalent species of helminthes which was 34.32%. The result was dissimilar to that of study carried out in Devghat, Chhitwan (Adhikari et al. 2018) who in the study found to be 11.82%. This was also confirmed from Red Langur (Hilser et al. 2011), Hanuman Langur and Rhesus Macaque (Parmar et al. 2012) and Assamese Macaque (Pokhrel & Maharjan 2014). But Arunachalam et al. (2015) reported it to be 5%. The overall infection of *Strongyloides* sp. was 149%. It was not supported by Adhikari et al. (2018) who reported it to be 10.75%. The result was also conflicted with the research done by Hilser et al. (2011) from Red Langur, and by Pokhrel and Maharjan (2014) from Assamese Macaque. Hookworm sp. was found to be 17.91% as found in the research. The result was found to be much higher than that of the report given by Adhikari et al. (2018) which was 3.22%. It is dissimilar to the reports of Pokhrel and Maharjan (2014) with 4.7% and contrary to the result of Hilser et al. (2011).

Regarding the intensity of infection, the study shows that 68% of the monkeys had single infection, 24% of them had double infections whereas 8% of them had multiple infections. The result shows conflictions with the result as given by Chalise et al. (2011) where 27.96% monkeys had single infection, 39.78% had double and 32.26% had multiple infections. Thus it makes it clear that 32% monkeys harboured more than one parasite. Thus the high rate of transmission might be possible either due to high population density or due to favourable environmental conditions for parasites. Thus, it would be rational to consider these monkeys populations as the reservoir hosts of several intestinal parasites of human.

The large density and diversity of the parasites can cause significant harm to the animals. It also represents large number of life cycles, transmission routes as well as pathogenicity. Multiple infections can be more harmful than the single infections. Multiple infections show impacts on growth pattern, reproduction, fecundity and establishment along with the death of the monkeys. Monkeys suffering from multiple infections are at higher risk than that of the monkeys with the single infections.

In this study, 78.82% samples were found to be positive for the presence of at least one type of parasites. The result coincides with the study done by Adhikari et al. (2018) where 74.20% of samples were found positive for single or multiple species of

parasites. Similarly, this result is similar to the investigation of Pokhrel and Maharjan (2014) and Jha et al. (2011) who revealed 72.94% and 76.86% positive cases from Assamese Macaque and Rhesus Macaque respectively. As for the parasitosis of the captive monkeys, the prevalence rate was lower (Nath et al. 2012). This could be due to regular screening of faecal samples and periodical anti-helmintic treatment in most of the zoos, as per the protocol of zoo authority.

6. CONCLUSION AND RECOMMENDATIONS

Thus it can be concluded that that the monkeys of Dharan are infected with various protozoa and helminthic gastro-intestinal parasites. Among the protozoal infections, *E. coli* was the highest prevalent protozoan as compared to other protozoa whereas among helminthes *Ascaris lumbricoides* showed the highest prevalence. Since 32% of the monkeys showed the multiple infections, it can be said that they are at higher risks to critical conditions of gastro-intestinal parasites.

Thus it is recommended that the local people be made aware about the transmission of various diseases through the contamination of faecal matters of the monkeys. The government should be made aware about the deteriorating health conditions of the monkeys and take effective actions against the control of such diseases. The monkeys should either be given vaccines or medicines to reduce the loads of the parasites in their bodies. The monkeys should be treated by the concerned authorities so as to prevent the transmission of the zoonotic diseases to the local people.

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APPENDICES

APPENDIX-A

LIST OF MATERIALS

Chemicals and reagents

Sodium chloride

Ethanol

Diethyl ether

Formaldehyde

Iodine crystals

Sulphuric acid

Methanol

Sucrose crystal

2.5% potassium dichromate

Ethyl acetate

Equipments

Microscope

Refrigerator

Centrifuge

Glass wares

Test tubes

Conical flask

Beaker

Measuring cylinder

Glass slide and cover slips

Droppers

Pipettes

Glass rods

Miscellaneous

Test tube stand

Wooden applicator

APPENDIX-B

STATISTICAL ANALYSIS

1. Statistical analysis between location versus prevalence of parasites

Location * Prevalance Crosstabulation

			Prevalance			Total
			protozoa	helminthes	unidentified	
Location	Vijayapur hill	Count	5_a	11_a	4_a	20
		Expected Count	6.4	9.4	4.2	20.0
	Dharan bazaar	Count	22_a	29_a	14_a	65
		Expected Count	20.6	30.6	13.8	65.0
	Total	Count	27	40	18	85
		Expected Count	27.0	40.0	18.0	85.0

Each subscript letter denotes a subset of Prevalence categories whose column proportions do not differ significantly from each other at the .05 level.

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.744[a]	2	.689
Likelihood Ratio	.753	2	.686
N of Valid Cases	85		

a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 4.24.

2. Statistical analysis between locations versus different stages of parasites

Crosstab

			P_stage					Total
			Egg	cyst	Ova	Adult	none	
Location	Vijayapur hill	Count	2_a	4_a	6_b	4_a	4_a	20
		Expected Count	2.1	5.2	1.4	7.1	4.2	20.0
	Dharan bazaar	Count	7_a	18_a	0_b	26_a	14_a	65
		Expected Count	6.9	16.8	4.6	22.9	13.8	65.0
	Total	Count	9	22	6	30	18	85
		Expected Count	9.0	22.0	6.0	30.0	18.0	85.0

Each subscript letter denotes a subset of P_stage categories whose column proportions do not differ significantly from each other at the .05 level.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	21.609[a]	4	.000
Likelihood Ratio	19.724	4	.001
N of Valid Cases	85		

a. 4 cells (40.0%) have expected count less than 5. The minimum expected count is 1.41.

LIST OF PHOTOGRAPHS

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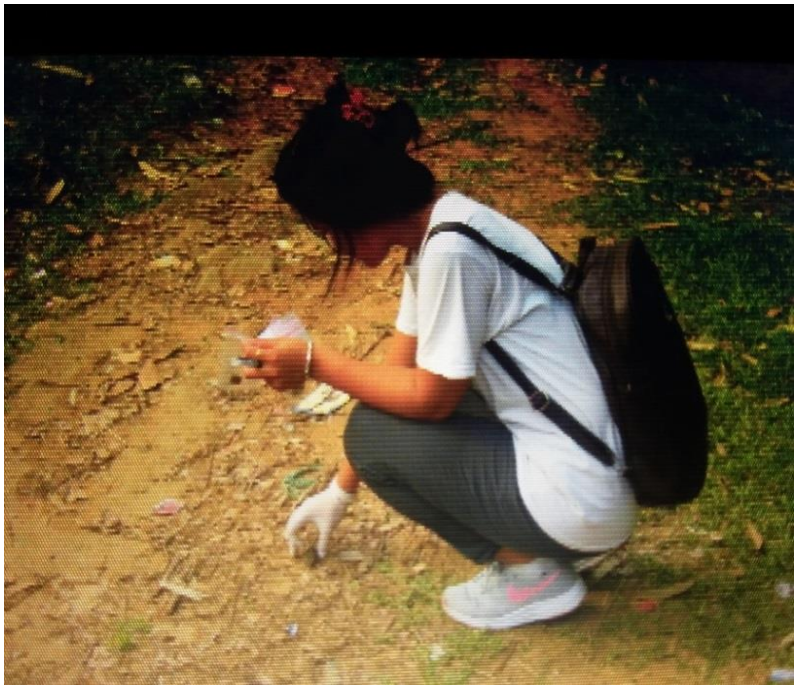
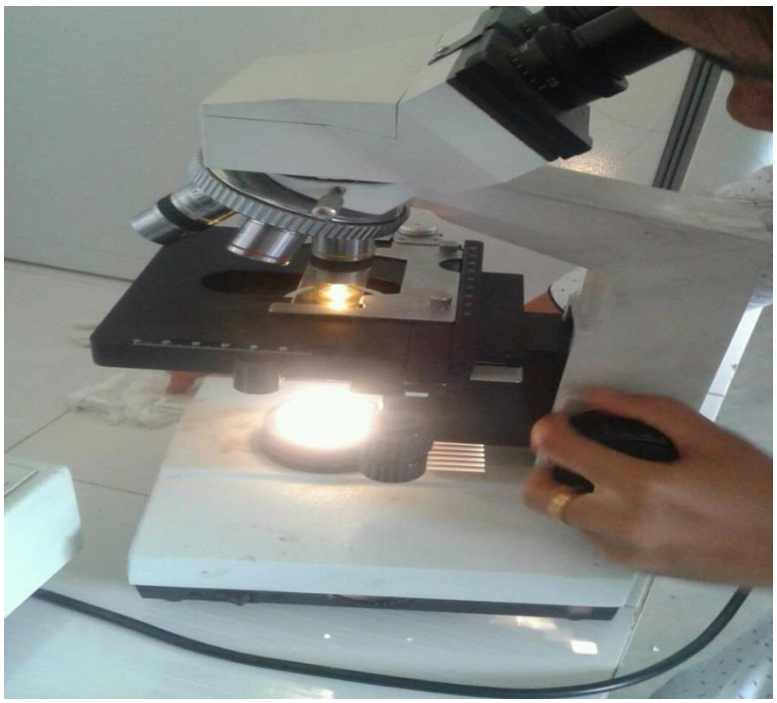
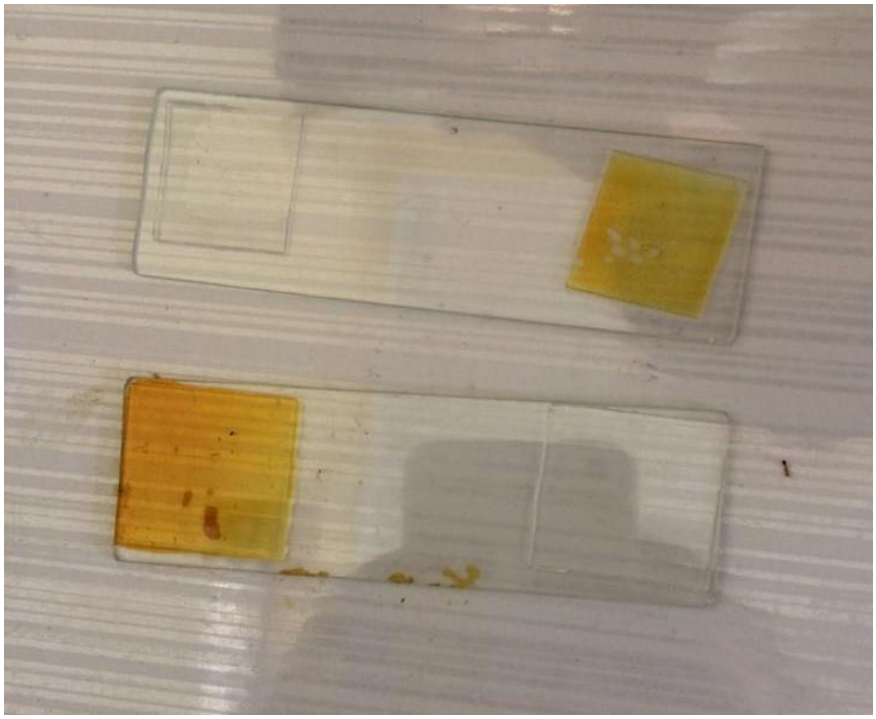


Photograph 1**.** Collecting samples



Photograph 2**.** Microscopic examination of stool sample

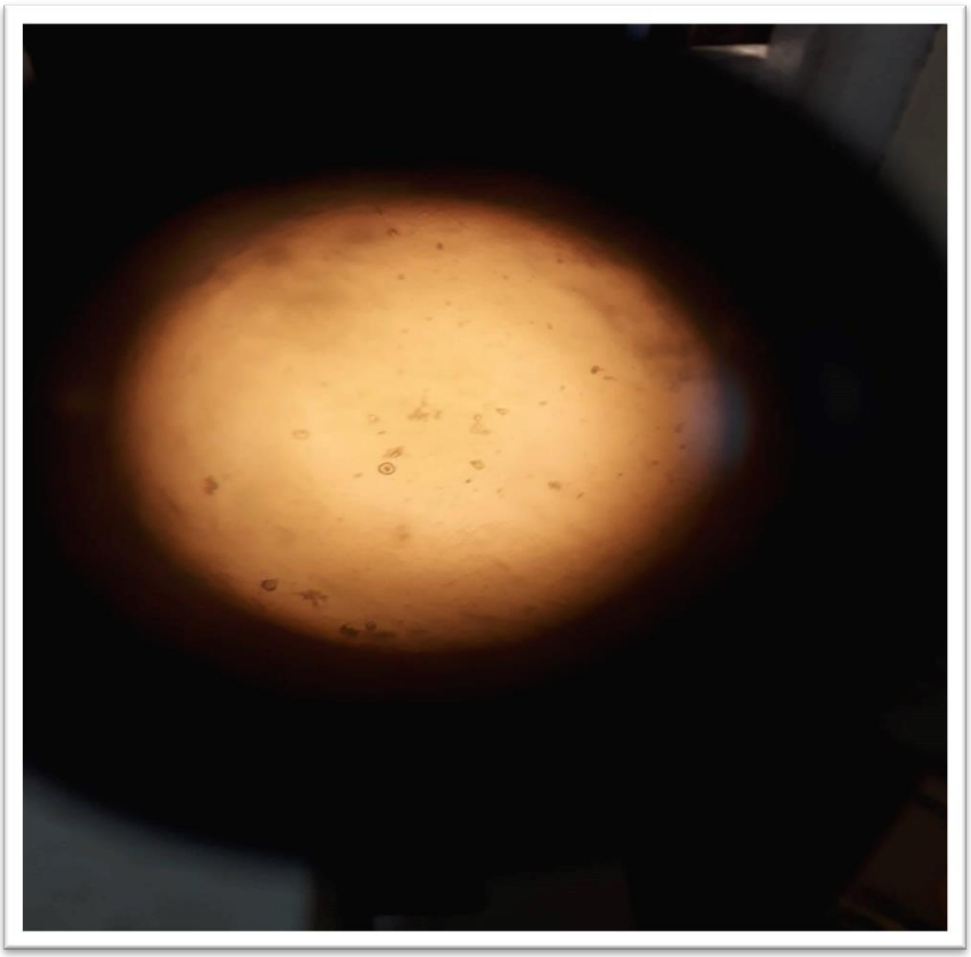


Photograph 3. Smear preparation of the stool samples

Photograph 4. Microscopic observation of *Taenia sp.*

Photograph 5. Microscopic observation of Hookworm (*Ancylostoma* sp)

Photograph 6. Microscopic observation of *Strongyloides stercolaris*



Photograph 7. Microscopic observation of *E. histolytica.*