

I

INTRODUCTION

Primates are the highest order of mammals including lemurs, monkeys, anthropoid apes and man. This classification probably gives a pride of place for man in the animal kingdom. We can call it superior development of the brain and associated higher intelligence.

The major distinctive character in primates is the structure of their hands and feet. They are designed with the purpose of grasping objects. This is an adaptation to the particular habits and mode of life of these creatures. About 200 species of primates are recognized all over the world and they are grouped into 50 genera and almost 100 extinct genera (Tattersal, 1993), Up to date three species of macaques have been reported from Nepal, the Rhesus monkey (*Macaca mulatta*), Assamese monkey (*Macaca assamensis*) and Langur monkey (*Presbytis entellus*) (Chalise, 2004).

Macaca mulatta is one of the best known of the subfamily Cercopithecinae, family Cercopithecidae of primate order of mammalian (Chalise 1997, 1998). Rhesus monkeys are medium size animals with robust limbs of equal length. The head and body of Rhesus measure 45 cm and tail 21 cm. It weights about 8 kg. Its coat is generally of various shades of brown or black. The fore part of the body is grey; hind part rufous and under part pale. The face is bare and flesh colored and becomes reddish during the gesture period. The head of Rhesus monkey bears a crown of hair, which is directed backwards. The tail is well haired and pendulous measuring about half the head and body length. In the adult and adolescent females swelling and reddening of skin occurs which may invalue especially the region of thigh, buttocks and hips. (Shrestha, 1997)

The Rhesus monkeys *Macaca mulatta* are one of the most common of all Rhesus monkey in the world. They are distributed in South and East Asia i.e Afghanistan, Bangladesh, Burma, Hong Kong, Nepal, Srilanka, Thailand, and India etc.

In Nepal they are usually called Rato-bander and are found in jungles of religious spots like Pashupati , Swoyambhu, Daksinkali and Ram Mandir of Tirpureshwor, Thapathali, and Sankhu (Panthi,1997), (Chalise, 1998) and recently found in Phulchowki.

Rhesus monkeys are very important in view of the fact that they are always used as an experimental animal by medico- biological institute because monkeys belong to primate order and many of the organ system of monkeys are similar to human beings. Since we all know that every organisms whether invertebrates or vertebrates starting from protozoa to mammals have parasitic representative and these parasites are depended on other animals partially or fully for their survival.

Like other animals, Rhesus monkey also harbour many kinds of parasites of which, helminth parasites are one of them. They are worm like parasites that attack alimentary tract of monkeys and are the causative agent of terrible and there are list of debilitating, deforming and killing diseases. So studies in these regard is very important from epidemiological point of view. Further more, some helminth infection particularly due to intestinal parasites are one of the major causes of gross health problem in monkeys.

Recently, some Rhesus monkeys are taken under the captivity to study their breeding behavior at Lele VDC, Lalitpur, Kathmandu under the supervision of Doctor Swoyam Prakash Shrestha. The research is still under the process.

SIGNIFICANCE OF THE STUDY

Since monkey is a part of the ecosystem so we can't ignore its importance. It belongs to wild life populations, we all are aware that wild life population can serve as host reservoirs or vectors for some diseases that affect humans. In other cases, humans can inadvertently become involved in the lifecycle of the parasite and as a result, a disease situation may be created in the human.

Various researches have been done all over the world including Nepal in the field of human intestinal parasites, that's why we are well educated about the consequences caused by them. But very little is known about the intestinal parasite in animals like monkeys which have socio-religious value in our country. The Rhesus monkey (*Macaca mulatta*) is widely worshipped by Hindus as a devotee of lord Ram. Either mythological or biologically, the monkeys have a close relationship with human being and scientifically they are under the same primate order and they are probable to the disease or parasites similar to the human beings and they are the most important because they are used as experimental animals by medico-biology institute in different fields, the name Rhesus is coined after the discovery of Rhesus factor in them, that's why they should be protected. More over they came in frequent contact with human transmitting such parasites. The threat caused by the intestinal parasites to monkey and transmitting such parasite to human are least studied so still many more such studies have to be conducted. So from above reasons, it was found that it is necessary to target this species i.e. *Macaca mulatta* for research work.

II

OBJECTIVES

GENERAL OBJECTIVE

Prevalence of intestinal helminth parasites of Rhesus monkey (*Macaca mulatta*) from Swoyambhu and Nilbarahi areas.

SPECIFIC OBJECTIVES

1. Identification of the eggs/ ova.
2. To determine general prevalence rate.
3. To determine classwise prevalence rate.
4. To determine areawise prevalence rate.
5. To determine multiple infection.
6. To determine prevalence rate of specific helminth.
7. To determine zoonotically infective helminthes of monkey.

III

LITERATURE REVIEW

GLOBAL CONTEXT

Gruijter *et al.*, (2006) compared adult *Oesophagostomum bifurcum* from human and non-human primates from Ghana and found significant differences in morphological characters between *Oesophagostomum bifurcum* worm from humans, the Mona, Patas or green monkey. These findings suggest that *Oesophagostomum bifurcum* from different species of primate host represent distinct population variants.

Van Leishout *et al.*, (2005) examined 349 faecal samples of different primate from Ghana and found high prevalence of *Oesophagostomum bifurcum* (75.99%).

Philips *et al.*, (2004) collected faecal samples from 86 individuals of non-human primates from Tambopata Research Center, Tambopata National Reserve, Peru and analysed a concentration test, the result of which indicate the presence of various protozoans, *Ancylostoma* sp, *Ascaris* sp, *Strongyloides stercoralis*, *Trichuris trichura*, *Prosthenorchis elegans* and *Schistosoma mansoni*.

Ponnudurai *et al.*, (2003) examined 108 faecal samples of monkey in Tamil Nadu, out of which 56 (51%) samples were positive for parasitic infection. Among the parasitic infections, *Entamoeba* sp. accounted for 14%, *Oesophagostomum* sp. 28% and *Strongylus* sp. 14%. The incidence of all the 3 parasites was seen as mixed infections as 7%.

Michaud *et al.*, (2003) found that helminth parasites shared by non-human primates and man in Peru were *Ancylostoma brazilliensis*, *Asceris lumbricoides*, *Necator americanus*, *Hymenolepis diminuta* and *Trichuris* sp.

Hobbs *et al.*, (2003) found abdominal cysticercosis in rhesus macaque in Oregon National Primate Research Center, USA.

Gillespie *et al.*, (2003) collected 2,103 faecal samples from free-ranging individuals of the 3 colobus monkey of Uganda to determine the prevalence of gastrointestinal parasites. Seven nematodes (*Strongyloides fulleborni*, *Strongyloides stercoralis* and *Oesophagostomum* sp),1 Cestodes (*Berticella* sp.),1 Trematode (Dicrocoeliidae) and 3 protozoans (*Entamoeba coli*, *Entamoeba histolytica*, *Giardia lamblia*) were detected.

Plesker *et al.*, (2001) diagnosed 19 pig tailed macaques (*Macaca nemestrina*) at the Paul Ehrlich Institute, Germany and found three hydatid cysts in the liver which was confirmed as *Echinococcus granulosus*.

Eberhard *et al.*, (2001) suggested that *Oesophagostomum bifurcum* found in the human and monkeys in the same geographical region of northern Ghana and Togo are not likely to represent zoonotic infection acquired from monkeys.

Joseph *et al.*, (1999) informed on the parasitic load of two endangered primates, Lion-tailed Macaque and Nilgiri Langur, inhabiting Silent Valley National Park were collected during the period 1994 – 1995. Parasitic load within these primate species in captivity were also ascertained and compared with that of wild. The major intestinal parasites identified from the wild samples were *Trichuris* sp. and *Oesophagostomum* sp.

Horii et al., (1981) the incidence of eggs in individual faeces and the EPG of gastrointestinal nematodes for individually discriminated Japanese monkey of the Koshima troop were investigated monthly from October 1974 to June 1997. Eggs of 4 nematode species (*Oesophagostomum aculeatum*, *Trichuris trichura*, *Streptopharagus pigmentatus* and *Strongyloides fulleborni*) were frequently found and a few cestode segments of *Berticella* sp. were occasionally found. *Strongyloides fulleborni* had a high incidence (100%) in young monkeys, suggesting that its incidence might vary with the monkey's age.

NEPAL CONTEXT

Limbu et al., (2006) reported Strongylus and Paramphistome groups in Rhesus monkey (*Macaca mulatta*) for the first time in Nilbarahi in the community forest. No other work regarding intestinal parasite of Rhesus monkey was found except of Limbu (2006).

Work of Malla, (2007). is the joint work with the present work.

IV MATERIALS AND METHODS

STUDY SITE

Swoyambhu

Swoyambhu lies in Kathmandu district of Bagmati zone. It is an ancient religious place lying at the distance of 5 km west of Kathmandu city. Physically it is situated between 85°17'30" to 85°18'30" and 27°42'30" to 27°43'30" east longitude and north latitude respectively. The temple complex sits over top of hill rising 1,500 m above the sea level. The temple is about 2000 years old. Swoyambhu area occupies 37 hectares of land and most of the area is under the influence of human settlement. The forest in this area is sub tropical type. The monastery is bordered by shops and houses whereas the park land is bordered with timber size trees and small stupas and chaityas (Shrestha, 1991)

Nilbarahi

The name of the place is called after the name of goddess "Nilbarahi" devi. Physically it is situated between 85°23'30" to 85°24'30" and 27°40'30" to 27°42'30" east longitude and north latitude respectively. It lies 1335 meters above sea level. The Nilbarahi temple is located at the middle of the forest lying at the distance 12 km east of Kathmandu city. It is a Licchivikalin temple. There is a small village (Tigni) where majority of the people are Newars with few exceptions of Chhetries. According to the local people this area occupies 252 ropanies. There are about 500 monkeys in 4-5 groups. The forest in this area is sub tropical type. (Source: information from local people).

MATERIALS

-) Glass slides
-) Cover slips
-) Wooden sticks
-) Gloves
-) Disposable vials
-) Tube
-) Plastic centrifuge tube
-) Tape
-) Petridish
-) Beaker
-) Tray
-) Cotton
-) Porcelain basin
-) Filter

EQUIPMENTS

-) Refrigerator
-) Needle
-) Microscope
-) Centrifuge machine
-) Weighing machine
-) Camera
-) Forceps

CHEMICAL REAGENTS USED

-) 5% formalin solution used for preservatives.
-) 2.5% potassium dichromate solution used for the preservation of the parasites found in faecal.
-) Normal saline mixture and zinc sulphate mixture(sp gravity 1.2 and 1.3 respectively)
-) Iodine solution used for stain the preparation
-) Methylene blue solution used for stain the preparation

METHODS

The whole study was divided into four parts:

- ❖ Field surveillance
- ❖ Faecal sample collection
- ❖ Examination of Faecal samples
- ❖ Identification

❖ Field Surveillance

Initially a mini survey (pilot survey) was conducted to gather information about the study site, time of monkey's defecation and in which area maximum samples could be collected and also little information from local people after that final round of field survey was conducted.

❖ Faecal Sample Collection

The colour of the faeces were greenish black. The samples were collected from Swoyambhu area of Kathmandu district by several visits. Twenty fresh samples were collected from different sites to prevent the repetition of the faecal. Again the same process was repeated for Nilbarahi area.

Overall hundred samples were collected from each area and 2.5% potassium dichromate solution used for preservation and then refrigerated in Central Veterinary Laboratory (Tripureshwor).

❖ Examination of Faecal Samples

Examinations of collected faecal samples were done macroscopically as well as microscopically to identify the ova of helminth parasites.

Macroscopical Examination

Examination of faecal samples were done by naked eyes for the proper detection of whole or a part of the helminth parasites (adult worm of *Ascaris lumbricoides*, hookworm, *Trichuris trichura*, *Enterobius vermicularis* and other various intestinal flukes).

Microscopical Examination

Microscopical examination of collected faecal samples was carried out for the demonstration of eggs. Following two concentration techniques were followed i.e. flotation and sedimentation techniques. The faecal samples were examined under microscope at the laboratory of Veterinary in Tripureshwor by making two types of smear preparation i.e., unstained and stained.

Concentration Method

Following two techniques were followed:

(a) Flotation Techniques

In floatation technique, the suspending fluid (sodium chloride or zinc sulphate) has higher specific gravity than the parasitic forms which therefore, rise to the surface. All the helminthic eggs floats in such a solution except the following:- unfertilized eggs of *A. lumbricoides*, eggs of *T. solium*, and *T. saginata*, eggs of intestinal fluke. The strongyloid as larvae do not float in salt solution.

Saturated Salt Flotation Technique

- About 3 gm of faecal sample was taken.
- That faecal was kept on tea strainer and kept on porcelain basin and grinded. About 42 ml of water was then added and again grinded and filter.
- Filtrate of the faecal solution was mixed and about 15 ml of it was kept on plastic tube.
- The tube was centrifuged at 1000 rpm till 5 minutes.
- Then without disturbing the sediment, the above solution was removed slowly.
- The tube was kept on the stand and Sodium chloride solution was added till the upper level of it, so that the upper meniscus of water can be seen. Now cello tape was kept on it and was centrifuged at 1000 rpm till 5 minutes.
- Cello tape was taken out and kept on the slide and was observed under microscope.
- From this process eggs of Nematode, Cestode and Coccidia can be detected. For detection of lungworm half saturated salt solution is used.

Concentration Zinc Sulphate Solution Technique

This technique is used to detect tape worms.

- Sodium chloride solution was removed from the tube, which has been prepared from the above procedure.
- Faecal which has been sedimented at the bottom of the tube was stirred by rod and zinc sulphate solution was added on it

- The opening of the tube was covered by transparent cellotape so that the tape was in contact with the solution.
- It was then centrifuged at 1000 rpm for 5 mins
- Cellotape was taken out and kept on the slide and was observed under microscope.
- By this process eggs of *Fasciola* and *Paramphistome* can be observed.

(b) Sedimentation Technique

-) Suspension of faecal was prepared as flotation method. The suspension was filter through tea strainer in the beaker. The beaker was filled with water.
-) The solution was sedimented for 10-20 mins. Above water was thrown away. Again the beaker was filled with water.
-) Again allow the solution to sediment for 10-20 mins. Then the above water was removed and sediment was kept on a petridish. One drop of methylene blue was added on it and was observed under microscope.

❖ Identification

Ova were identified by the key of George, (1969), Soulsby, (1982) and Investigatio coprologica animalium domesticorum / Magnoum / Bos et ovis, Ianssen Pharmaceutica Ex Scientia Progressus at Central Veterinary Laboratory, Tripureshwor.

V

RESULTS

The study was conducted on the faecal samples of Rhesus monkey of Swoyambhu and Nilbarahi areas. From each study sites one hundred faecal samples of Rhesus monkey (*Macaca mulatta*) were examined at Central Veterinary Laboratory, Tripureshwor. The results of the study are presented under following headings.

A. Identification and Description of the ova and

B. Determination of the Prevalence rate of helminth ova at different levels

- ❖ General prevalence rate
- ❖ Classwise prevalence rate
- ❖ Areawise prevalence rate
- ❖ Prevalence rate of multiple infection of helminth
- ❖ Prevalence rate of specific helminth
- ❖ Prevalence rate of zoonotically infective helminth of monkey

A. IDENTIFICATION AND DESCRIPTION OF THE OVA RECOVERED

1. *Prosthenorchis elegans*

Description of the eggs

The eggs measure 65-81 μ m by 42-53 μ m containing the thick outer and thin inner shells enclosing the embryo (acanthor). Eggs are oval in shape.

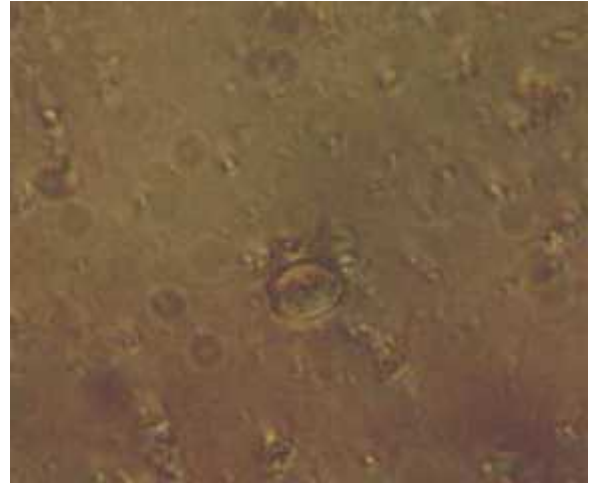


Plate No. 1 x400
An ovum of *Prosthenorchis elegans* 42~ x 65~

Discussion

Prosthenorchis elegans (Diesing, 1851) and *Prosthenorchis specula* (Olfers and Rudolphi, 1819) are very important parasites in the small intestine of Central and South American monkeys, *Prosthenorchis elegans* is very common and *Prosthenorchis specula* is less common. These parasites are now found through the world where primates are kept in captivity and where they have introduced the parasites.

This species has not been reported as yet from Nepal in any host.

Classification

Phylum : Acanthocephala	Rudolphi, 1808
Order : Archiacanthocephala	Meyer, 1931
Family : Oligacanthorhynchidae	Meyer, 1931
Genus : <i>Prosthenorchis</i>	Travassos, 1851
Species : <i>elegans</i>	Diesing, 1851

3. *Taenia* sp

Description of the eggs

The eggs are spherical, brown and are provided with a double layered shell. The external shell is thin and transparent. The inner shell containing a thick embryophore is brown, radially striated and measures 31µm -43µm in diameter.

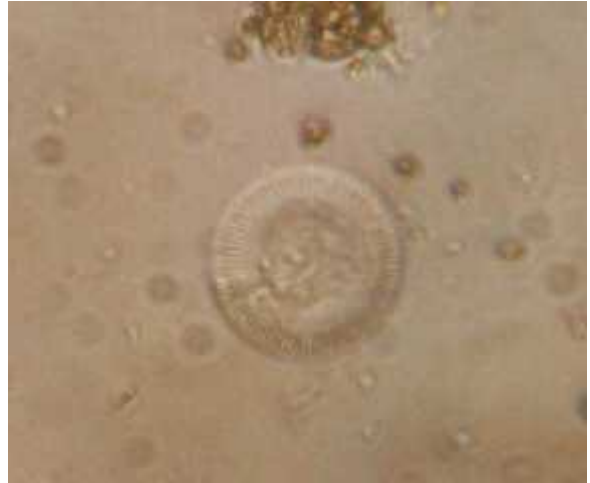


Plate No. 3
An ovum of *Taenia* sp

x400
35~ x 50~

Discussion

Taenia infection is world wide and endemic in the human population eating raw or inadequately cooked meat. It is hyper endemic in eastern Mediterranean countries, certain African countries and part of Russia. It is also common in India Japan Philippines and Latin America, Mexico.

From Nepal

In 1982, ADPCD reported *Taenia* species from dog, cat and human of Kathmandu. In the present study, *Taenia* sp. reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	: Eucestoda	Southwell, 1930
Order	: Taeniidea	Wardle, Meleod and Radinovsky, 1974
Family	: Taenidae	Ludwig, 1886
Genus	: <i>Taenia</i> sp	Linnaeus, 1758

5. *Toxascaris leonine*

Description of the eggs

The eggs are more or less round with smooth sides, brown or pale black in colour. They have a thick shell and contain a single cell when deposited in faeces but they may have undergone the first cell division. It measures 75-85µm in length and 60-75 µm in breadth.

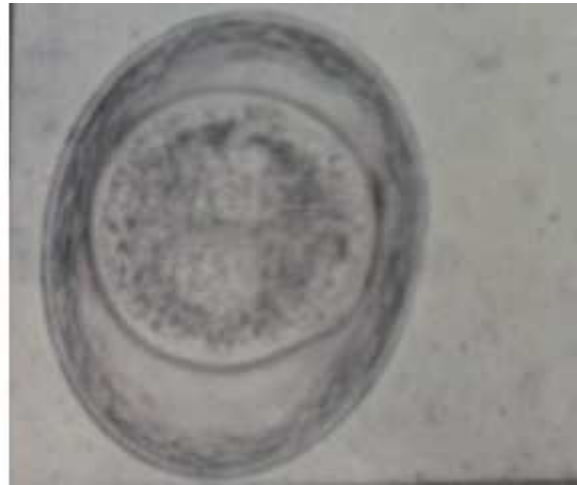


Plate No. 5
An ovum of *Toxascaris leonine* x400
60~ x 75~

Discussion

Toxascaris leonina occurs in the small intestine of the dog, cat, wild felidae and canidae in most parts of the world.

From Nepal

In 1970, Singh reported *Toxascaris leonine* in leopard from Kathmandu.

In 1999, Gautam et al., reported *Toxascaris leonine* 6.7% in pet dogs from Kathmandu.

In 2003, Khaniya and Sah reported *Toxascaris leonine* in dogs from Kathmandu.

In 2004, Khanal reported *Toxascaris leonine* in cat from Nawalparasi and Chitwan districts.

In the present study, *Toxascaris leonine* from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi 1808
Order	:	Ascaridida	Skrjabin and Schulz, 1940
Family	:	Ascaridae	Baird, 1853
Genus	:	<i>Toxascaris</i>	Leiper, 1907
Species	:	<i>leonine</i>	(V Linstaw, 1902) Leiper, 1907
Synonym	:	<i>Toxascaris limbata</i>	

6. *Ascaris lumbricoides*

Description of the eggs

Both fertilized and unfertilized eggs are present in the faeces.

Fertilized eggs

These eggs are oval to subspherical in shape and measure $45\mu\text{m}$ - $70\mu\text{m}$ in length and $35\mu\text{m}$ - $50\mu\text{m}$ in breadth.

They are bile stained and golden brown in colour.

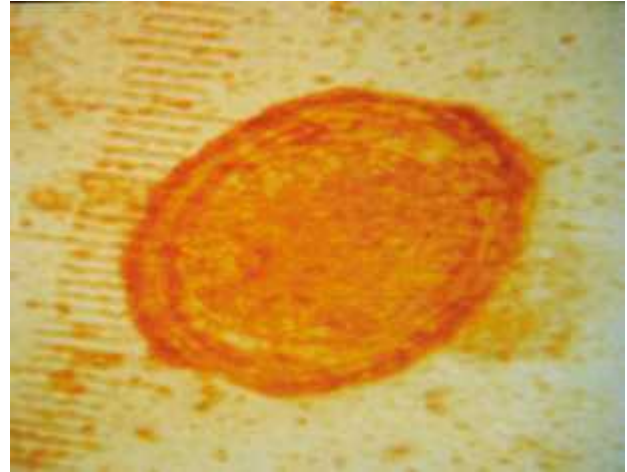


Plate No. 6

An ovum of *Ascaris lumbricoides*

x400

35~ x 45~

Unfertilized eggs

These eggs are brown thin shelled ellipsoidal and measure $78\mu\text{m}$ - $105\mu\text{m}$ in length and $38\mu\text{m}$ - $55\mu\text{m}$ in breadth. These are heaviest of all the helminthic eggs, hence do not float in saturated salt solution.

Discussion

Ascaris lumbricoides is distributed world wide approximately 25% of the world population are supposed to be suffering from ascariasis. The high incidence of infection is reported from China, Philippines, India, South East Asia, Middle East Africa and Central and South America. The infection rate is usually high in rural population of India and middle eastern parts of Africa (Nanda, 1990).

From Nepal

In 1965, Sharma et al., reported *Ascaris* in human from Kathmandu.

In 1965, Sharma reported *Ascaris lumbricoides* in human from Bhaktapur.

In 1975, Saulsa reported *Ascaris lumbricoides* in human from Pokhara.

In 1977, Shakya reported *Ascaris lumbricoides* in human from Surkhet.

In 1980, Khetan reported *Ascaris lumbricoides* in human from Narayani.

In 1981, Bol reported *Ascaris lumbricoides* in human from Narayani.

In 1982, IFP and PCP reported *Ascaris lumbricoides* in human from Panchkhal.

In 1982, ADPCD reported *Ascaris suis* in pigs from Kathmandu.

In 1982, ADPCD reported *Neoascaris vitulorum* in buffalo from Kathmandu.

In 1988, Gupta reported *Ascaris lumbricoides* in human from Kirtipur.

In 1997-1998, Sharma reported Ascariosis 43.69% in animals from Panchthar district.

In the present study, *Ascaris lumbricoides* reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi, 1808
Order	:	Ascaridida	Skrjabin and Schulz, 1940
Family	:	Ascaridae	Baird, 1853
Genus	:	<i>Ascaris</i>	Linnaeus, 1758
Species	:	<i>lumbricoides</i>	

7. *Oxyuris* sp

Description of the eggs

Eggs are asymmetrical, 80µm to 90µm in length and 40µm to 45µm in breadth, flattened on one side with operculum which is often plugged with mucus at one pole and passed in the faeces in an advanced morula stage.



Plate No. 7
An ovum of *Oxyuris* sp

x400
40~ x 80~

Discussion

It is almost reported from all parts of the world. Infection with the horse pinworm, *oxyuris equi* is extremely common and although of limited pathogenic significance in the intestine, the female parasites may cause an intense and pruritis during the process of egg laying.

From Nepal

In 2003, Rabwin, Joshi and Chhetri reported *Oxyuris equi* in horse from Kyanjin Gompa, Langtang.

In 1982, ADPCD reported *Oxyuris equi* in horse from Kathmandu.

In present study, *Oxyuris* sp. reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi, 1808
Order	:	Ascaridida	Skrjabin and Schulz, 1940
Family	:	Oxyuridae	Cobbold, 1864
Genus	:	<i>Oxyuris</i>	Rudolphi, 1803

8. *Ostertagia* sp

Description of the eggs

The eggs measures 74µm to 90µm in length and 40µm to 45µm in size and are elliptical in shape.



Plate No. 8
An ovum of *Ostertagia* sp

x400
40~ x 74~

Discussion

Distribution is world wide, *Ostertagia* is especially important in temperate climates and in sub tropical regions with winter rainfall. This genus is the major cause of parasitic gastritis in ruminants in temperate areas of the world.

From Nepal

In 1982 ADPCD reported *Ostertagia* sp in pig cattle and buffalo from Kathmandu.

In 1997, *Ostertagia nianquingtangluaensis* in goat and sheep from western hills of Nepal.

In 1997, Joshi reported *Ostertagia leptospicularis* in sheep from western hills of Nepal.

In 1999, Acharya reported *Ostertagia* sp. in sheep and goat from of IAAS live stock, central lab, Tripureshwor.

In 1999, Joshi reported *Ostertagia* sp in sheep and goat from Kaski district, Pokhara.

In the present study, *Ostertagia* sp. reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi, 1808
Order	:	Strongylida	(Diesing, 1851) Molin, 1861
Family	:	Trichostrongylidae	Leiper, 1912
Genus	:	<i>Ostertagia</i>	Ransom, 1907

9. *Cooperia* sp

Description of the eggs

Eggs are 70-80µm to 35-41µm in size, their sides are parallel and have less than 16 pale yellow blastomeres when laid.



Plate No. 9
An ovum of *Cooperia* sp

x640
36~ x 77~

Discussion

World wide in distribution in temperate areas, members of the genus *Cooperia* usually play a secondary role in the pathogenesis of parasitic gastroenteritis of ruminants. Although they may be the most numerous *Trichostrongyle* present. However, in some tropical and sub tropical areas some species are responsible for severe enteritis in calves.

From Nepal

In 1982, ADPCD reported *Cooperia* sp. in goat, sheep and buffalo from Kathmandu

In 1997, Joshi reported *Cooperia curticei* in sheep from western hills of Nepal.

In 1997, Joshi reported *Cooperia punctata* in sheep from western hills of Nepal.

In 1999, Acharya reported *Cooperia* sp. in sheep and goat of IAAS livestock, farm of central lab, Tripureshwor.

In the present study, *Cooperia* sp. reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi, 1808
Order	:	Strongylida	(Diesing, 1851) Molin, 1861
Family	:	Trichostrongylidae	Leiper, 1912
Genus	:	<i>Cooperia</i>	Ransom, 1907

10. *Dictyocaulus* sp

Description of the eggs

Eggs are 112-138 μ m to 69-90 μ m in size, ellipsoidal and contain fully developed larva when laid or first stage larva may pass in faeces.



Plate No. 10 **x400**
An ovum of *Dictyocaulus* sp **69~ x 112~**

Discussion

World wide in distribution, but especially important in temperate climates. This genus living in the bronchi of cattle, sheep, horses and donkey are the major cause of parasitic bronchitis in these hosts.

From Nepal

In 1982, ADPCD reported *Dictyocaulus* sp. in goat and sheep from Kathmandu.

In the present study, *Dictyocaulus* sp. reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi, 1808
Order	:	Strongylida	(Diesing, 1851) Molin, 1861
Family	:	Dictyocaulidae	Skrjabin, 1941
Genus	:	<i>Dictyocaulus</i>	Railliet and Henry, 1907

11. *Chabertia* sp.

Description of the eggs

Eggs are laid in morula stage and it measures 90-105 μm in length and 50-55 μm in breadth. Oval in shape.



Plate No. 11
An ovum of *Chabertia* sp.

x640
50~ x 90~

Discussion

World wide in distribution. Commonly known as large mouthed bowel worm. *Chabertia* sp is present, usually in low numbers, in the majority of sheep and goats.

From Nepal

In 1973, Singh reported *Chabertia* sp. in goat, cattle and sheep from Kathmandu.

In 1997, Joshi reported *Chabertia ovina* in goat and sheep from western hills of Nepal.

In 1999, Acharya reported *Chabertia ovina* in sheep and goat of IAAS livestock, farm of central lab, Tripureshwor.

In the present study, *Chabertia* sp. reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi, 1808
Order	:	Strongylida	(Diesing, 1851) Molin, 1861
Family	:	Trichonematidae	Witenberg, 1925
Genus	:	<i>Chabertia</i>	Railliet and Henry, 1909

12. *Oesophagostomum* sp

Description of the eggs

The eggs are usually indistinguishable from those of *Ancylostoma* and *Necator*. They measure 60-63 μm by 30-40 μm . the eggs are oval, thin-shelled and non-bile stained. A clear space is always present between the segmented ovum and the eggs shell.



Plate No. 12 x400
An ovum of *Oesophagostomum* sp 30~ x 60~

Discussion

Oesophagostomum sp are the natural intestinal parasites of apes and monkeys in Africa, Asia and South America. Human *Oesophagostomiasis* has been reported from East and West Africa and South America.

From Nepal

In 1982, ADPCD reported *Oesophagostomum* sp. in pig, cattle and buffalo from Kathmandu.

In 1997, Joshi reported *Oesophagostomum venulosm* in goat from western hills of Nepal.

In 1997, Joshi reported *Oesophagostomum asperum* in goat and sheep from western hills of Nepal.

In 1999, Acharya reported *Oesophagostomum* sp in sheep and goat of IAAS livestock, farm of central lab, Tripureshwor.

In 2003, Thakur reported *Oesophagostomum* sp. in pigs from Eastern hills of Nepal.

In the present study, *Oesophagostomum* sp. reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi, 1808
Order	:	Strongylida	(Diesing, 1851) Molin, 1861
Family	:	Trichonematidae	Witenberg, 1925
Genus	:	<i>Oesophagostomum</i>	Molin, 1861

13. *Trichostrongylus* sp.

Description of the eggs

Eggs are oval and bilaterally symmetrical. These are relatively larger (63-115 μm by 40 μm) than those of hook wormova. The shell has a thin transparent outer chitinous layer and a thin inner layer. The embryonic mass is multisegmented and varies from 16 to 32 in number. The space between the egg shell and embryonic mass is relatively conspicuous.



Plate No. 13 **x640**
An ovum of *Trichostrongylus* sp. 41~ x 87~

Discussion

Trichostrongylus infection is world wide in distribution. A wide variety of animals particularly the herbivorous animals, horses and rodents are infected by the worm. The case of human *Trichostrongyliasis* have been reported from Australia, China, Central Africa, Egypt, Indonesia, Iran, Iraq, Japan, Korea, Turkey, Russia and also India. (Chakarbarti 1990)

From Nepal

In 1967-92, Mainali reported *Trichostrongylus* sp. from Lulu cattle.

In 1973, Singh reported *Trichostrongylus* in cattle and buffalo from Kathmandu.

In 1998, Joshi reported *Trichostrongylus* sp in goats from Jamunapari of Nuwakot district.

In 1997, Joshi reported *Trichostrongylus orientalis* in goat and cattle from western hills of Nepal.

In 1997, Joshi reported *Trichostrongylus axei* in goat and cattle from western hills of Nepal.

In 1997, Joshi reported *Trichostrongylus colubiformis* in goat and cattle from western hills of Nepal.

In 1999, Joshi reported *Trichostrongylus* sp in goat and cattle from Kaski district , Palpa.

In 1999, Acharya reported *Trichostrongylus* sp in sheep and goat of IAAS livestock, farm of central lab, Tripureshwor.

In 2003, Thakur reported. *Trichostrongylus axei* in pigs from Eastern hills of Nepal.

In 2003, Rabwin, Joshi and Chhetri reported. *Trichostrongylus* sp in western hills of Nepal.

In the present study, *Trichostrongylus* sp. reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi, 1808
Order	:	Strongylida	(Diesing, 1851) Molin, 1861
Family	:	Trichostrongyloidae	Leiper, 1912
Genus	:	<i>Trichostrongylus</i>	Looss, 1905

14. *Strongyloides fulleborni*

Description of the eggs

The eggs are characteristically smaller, shorter and measure 35µm by 50µm. They are oval, transparent and very thin shelled, blunt ends, contain fully develop embryos when passed in the faeces.

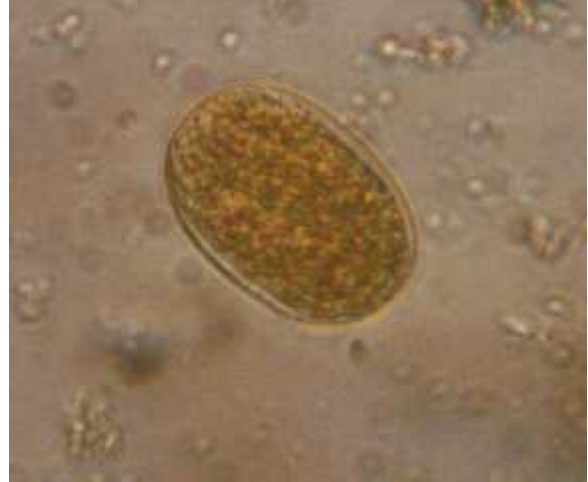


Plate No. 14 **x400**
An ovum of *Strongyloides fulleborni* 35~ x 50~

Discussion

Strongyloides fuelleborn, a natural parasite of monkeys and apes has been reported to cause zoonotic infection in man in Africa and parts Asia. *Strongyloides fuelleborni* infection is prevalent in tropical Africa and parts of Asia while fuelleborni like species infection is present in the parts of Papua New Guinea. (Jayaram 1990)

From Nepal

In 1973, Singh et al., reported *Strongyloides* sp in goat and sheep from Kathmandu

In 1977, Shakya reported *Strongyloides stercoralis* in human from Lalitpur.

In 1997, Joshi reported *Strongyloides papillosus* in goat and sheep from western hills of Nepal.

In 1999, Acharya reported *Strongyloides papillosus* in sheep and goat of IAAS livestock, farm of central lab, Tripureshwor

In 2003, Khakural and Khakural reported Strongyles in farm ruminant from Maldi VDC, Dhading.

In 2003, Rabwin, Joshi and Chhetri reported. Strongyles in horse from Kyanjingompa, Langtang.

In the present study, *Strongyloides. fulleborni* reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi, 1808
Order	:	Rhabditida	Chitwood, 1933
Family	:	Strongyloididae	Chitwood and Mc. Intosh, 1934
Genus	:	<i>Strongyloides</i>	Grassi, 1879
Species	:	<i>fulleborni</i>	Von Linstow, 1905
Synonyms	:	<i>Anguillula stercoralis</i>	

15. *Capillaria* sp

Description of the eggs

The eggs are more or less similar to that of *Trichuris* sp. but in comparison to *Trichuris* egg, the shell is almost colourless, the egg is barrel shaped, with the sides nearly parallel. The size of egg is smaller than that of *Trichuris trichura* i.e 44µm -50µm by 24µm to 33µm. The colour is pale brown but not bile stained. They had fine striations in the egg shell.



Plate No. 15 **x400**
An ovum of *Capillaria* sp **24~ x 44~**

Discussion

Capillaria sp are world wide in distribution. *Capillaria* sp infection is restricted to Northern Luzone area of Philippines and Thailand. Capillariasis is a newly recognized zoonotic helminthic infection of man caused by a few species of genus capillaria. (Bhatnagar 1990)

From Nepal

In 1967-92, Mainali reported *Capillaria* sp. from Lulu Cattle.

In 1982, ADPCD reported *Capillaria* sp. in poultry from Kathmandu.

In 2004, Khanal reported *Capillaria aerophila* in cat from Nawalparasi and Chitwan districts.

In the present study, *Capillaria* sp reported from Rhesus monkey is for the first time from Nepal.

Classification

Class	:	Nematoda	Rudolphi, 1808
Order	:	Enoplida	Schurmans, Stekhoven and Deconinck, 1933
Family	:	Capillariidae	Neveu-Lemaire, 1936
Genus	:	<i>Calillaria</i>	Zedar, 1800

B. DETERMINATION OF THE PREVALENCE RATE OF HELMINTH OVA AT DIFFERENT LEVELS

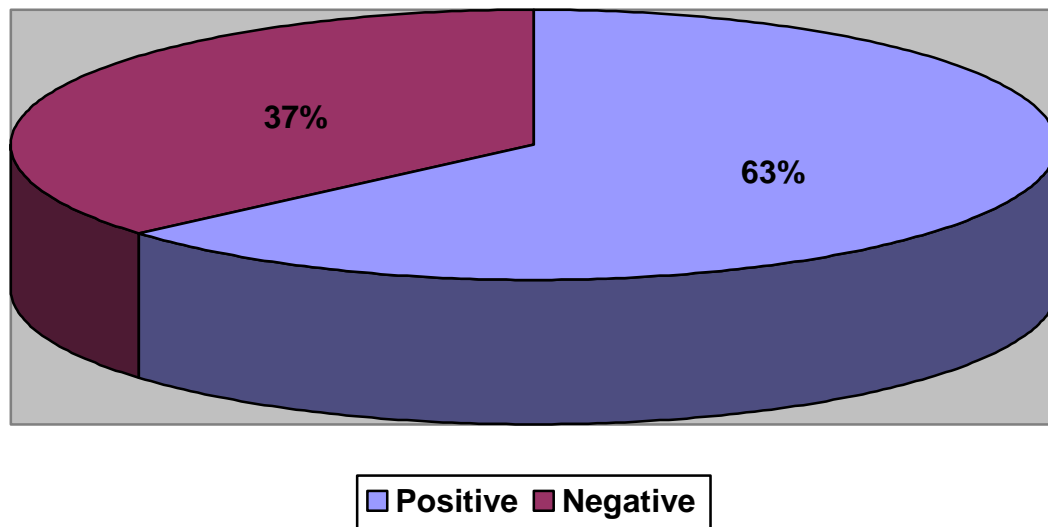
❖ General prevalence rate

Out of 200 samples examined 127 samples were found to be positive with one or more than one helminth parasites. Hence, positive percentage was 63.5% and negative percentage was 36.5%

Table 1: General prevalence of gastrointestinal helminth parasites of Rhesus monkey (*Macaca mulatta*)

Total samples	Positive samples		Negative samples	
	No	%	No	%
200	127	63.5	73	36.5

Fig 1: Diagrammatic representation of general prevalence of intestinal helminth parasites of Rhesus monkey (*Macaca mulatta*)



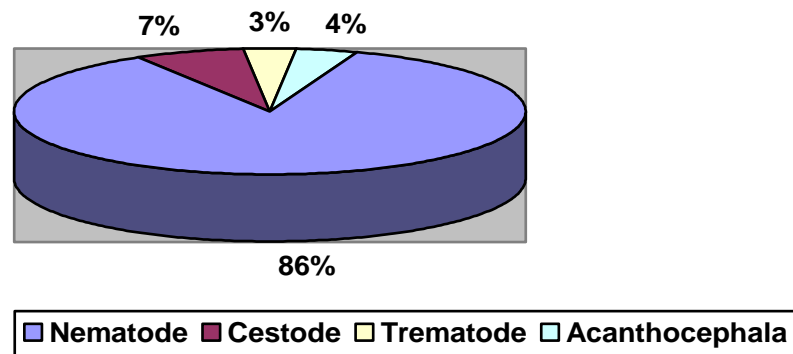
❖ Classwise prevalence rate

Out of 127 positive samples, 109 samples (85.82%) were positive for Nematodes, 9 samples (7.08%) were positive for Cestodes, 4 samples (3.14%) were positive for Trematodes and 5 samples (3.93%) were positive for Acanthocephalan. Statistically, significant difference was found in classwise parasitic infection ($X^2 = 495.7, p < 0.05$)

Table 2: Classwise prevalence of intestinal helminth parasites of Rhesus monkey (*Macaca mulata*)

S.n	Class	Positive samples	
		No	%
1	Nematodes	109	85.82
2	Cestodes	9	7.08
3	Trematodes	4	3.14
4	Acanthocephala	5	3.93

Fig 2: Classwise prevalence of intestinal helminth parasites of Rhesus monkey (*Macaca mulatta*)



❖ **Areawise prevalence rate**

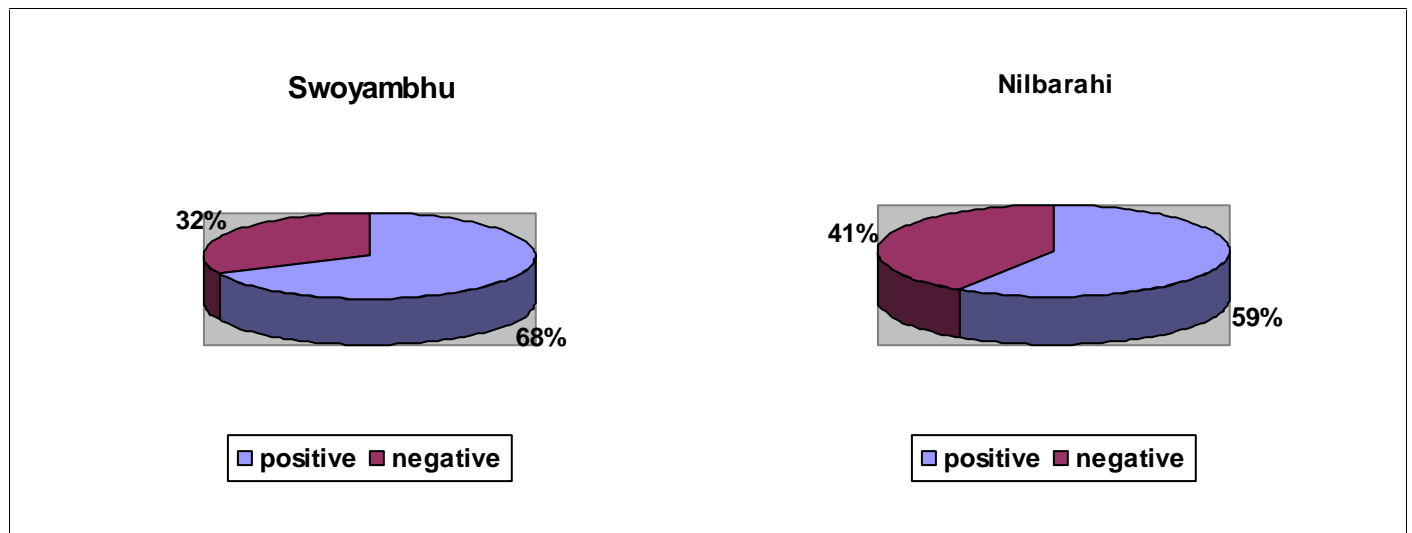
Out of 200 samples examined 100 samples belong to Swoyambhu area and 100 samples belong to Nilbarahi area. Prevalence rate of helminth parasites of monkey in Swoyambhu was found to be 68% and of Nilbarahi was 59%. Parasitization rate in Swoyambhu was found to be higher than that of Nilbarahi. Statistically, no significance difference was found in area wise parasitic infection ($X^2 = 1.74, p>0.05$).

Table 3: Areawise prevalence of gastrointestinal parasites of Rhesus monkey

(Macaca mulatta)

S.n	Area	Total samples	Positive samples		Negative samples	
			No	%	No	%
1	Swoyambhu	100	68	68%	32	32%
2	Nilbarahi	100	59	59%	41	41%

Fig 3: Diagrammatic representation of areawise prevalence of gastrointestinal parasites of Rhesus monkey (*Macaca mulatta*)



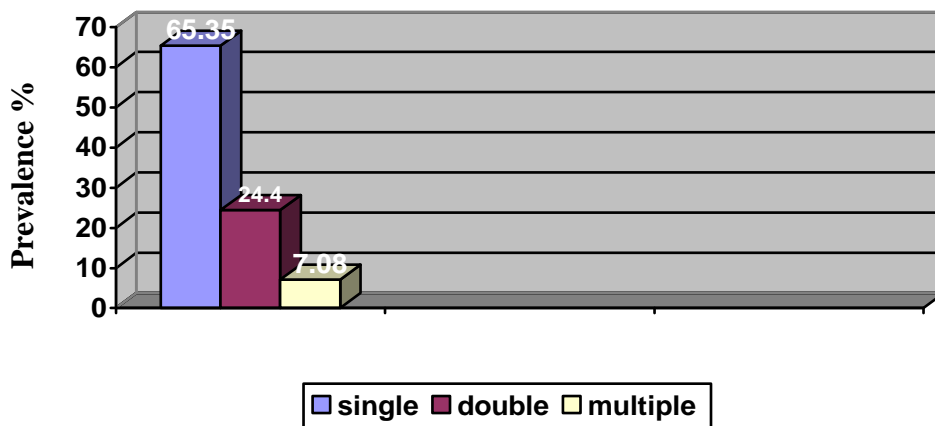
❖ **Prevalence rate of multiple infection of helminth**

Out of 127 positive samples, single infection of helminth parasite were found to be 65.35%, double infection was found to be 24.4% and multiple infection was found to be 7.08%. Single infections were of *Strongyloides fulliborni*, *Dictyocaulus* sp, *Oxyuris* sp, *Chabertia* sp, *Dicrocoelium* sp, *Trichostrongylus* sp, *Toxascaries leonina*, *Ostertagia* sp, *Oesophagostomum* sp, *Toxacara canis*, *Taenia* sp, *Prosthenorchis elegans*. Whereas *Oesophagostomum* sp and *Strongyloides fulliborni* were common in double infection when compared to other species.

Table 4: Concurrent infection of intestinal helminths

Single infection		Double infection		Multiple infection	
No	%	No	%	No	%
83	65.35	31	24.40	9	7.08

Fig 4: Diagrammatic representation of prevalence rate of single, double and multiple infections of intestinal helminth parasites of Rhesus monkey (*Macaca mulatta*)



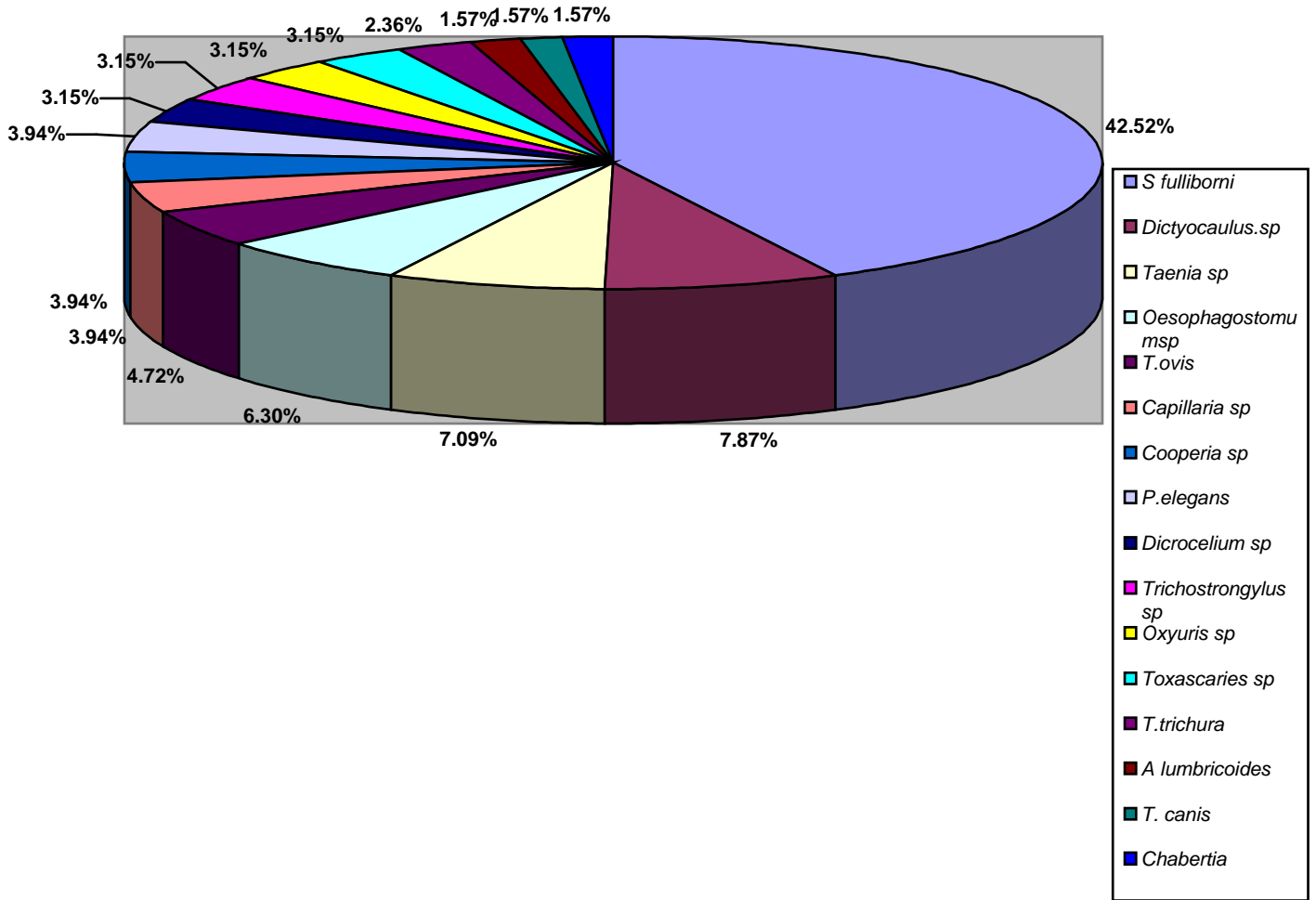
❖ **Prevalence rate of specific helminthes**

Out of 63.5% positive samples for helminth parasitic infections *Strongyloides fulleborni* accounted for 42.5%, *Dictyocaulus* sp (7.87%), *Taenia* sp (7.08%), *Oesophagostomum* sp (6.29%), *Trichuris ovis* (4.72%), *Capillaria* sp (3.93%), *Ostertagia* sp (3.93%), *Cooperia* sp (3.93%), *Prosthenorchis elegans* (3.93%), *Dicrocoelium* sp(3.14%), *Trichostrongylus* sp (3.14%), *Oxyuris* sp (3.14%), *Toxascaris leonina* (3.14%), *Trichuris trichiura* (2.36%), *Ascaris lumbricoides* (1.57%), *Toxocara canis* (1.57%) and *Chabertia* sp (1.57%).

Table no 5: Prevalence rate of specific helminthes

Sn	Class	Species	Positive samples	
			No	%
1	Nematoda	<i>Strongyloides fulleborni</i>	54	42.5%
		<i>Dictyocaulus</i> sp	10	7.87%
		<i>Oesophagostomum</i> sp	8	6.29%
		<i>Trichuris ovis</i>	6	4.72%
		<i>Capillaria</i> sp	5	3.93%
		<i>Ostertagia</i> sp	5	3.93%
		<i>Cooperia</i> sp	5	3.93%
		<i>Trichostrongylus</i> sp	4	3.14%
		<i>Oxyuris</i> sp	4	3.14%
		<i>Toxascaris leonina</i>	4	3.14%
		<i>Tichuis trichiura</i>	3	2.36%
		<i>Ascaris lumbricoides</i>	2	1.57%
		<i>Toxocara canis</i>	2	1.57%
		<i>Chabertia</i> sp	2	1.57%
		2	Cestoda	<i>Taenia</i> sp
3	Trematoda	<i>Dicrocoelium</i> sp	4	3.14%
4	A canthocephala	<i>Prosthenorchis elegans</i>	5	3.93%

Fig 5: Diagrammatic representation of prevalence of specific helminthes of gastrointestinal parasite of Rhesus monkey (*Macaca mulatta*)



❖ **Prevalence rate of zoonotically infective helminthes of monkey**

Out of total 127 positive samples, 106 (83.46%) were positive to zoonotically infective helminthes and 21 (16.53%) samples were negative to zoonotically non- infective helminthes. The list of zoonotically non-infective helminth was followed by Parija, 1980.

Table 6: Prevalence rate of zoonotically infective intestinal helminth parasites of Rhesus monkey (*Macaca mulatta*)

Total no of +ve faecal samples	Positive for			
	Zoonotically infective helminths		Zoonotically non-infective helminths	
	No	%	No	%
127	106	83.46	21	16.53

Fig 6: Diagrammatic representation of prevalence rate of zoonotically infective intestinal helminthes of Rhesus monkey (*Macaca mulata*)

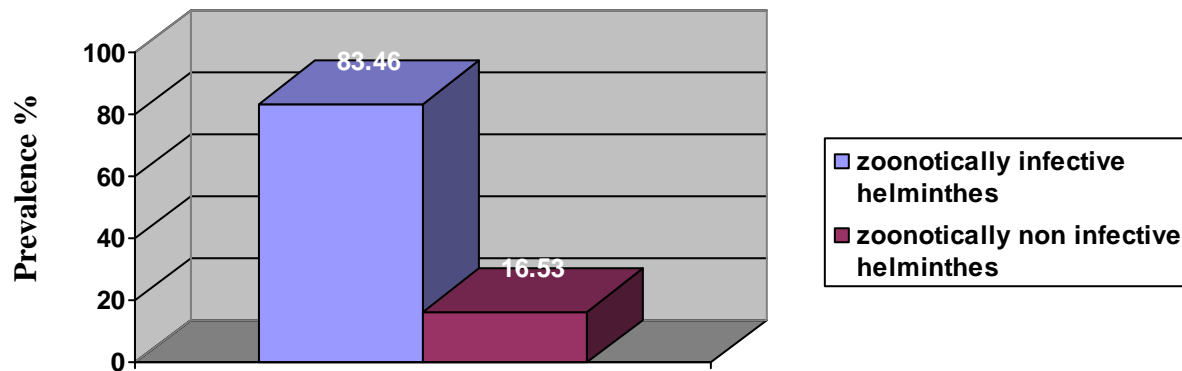




Plate No. 18
Monkeys of Swoyambhu Area



Plate No. 19
Monkeys of Nilbarahi Area



Plate No. 20
Collection of faecal samples at Swoyambhu



Plate No. 21
Collection of faecal samples at Nilbarahi Area



Plate No. 22
Lab work at CVL., Tripureshwor



Plate No. 23
Lab work at CVL., Tripureshwor

VI

CONCLUSION AND DISCUSSION

Parasites are the part of ecosystem without which no balance should be imagined. One at a time each and every animals were attached by different parasites. The topic is so vast that not all of the known human and animals intestinal helminthes parasitic infections have been surveyed properly throughout the world. From Nepal, there is no such studied have been found in case of Rhesus monkey (macaques). Hence, the findings of the prevalence figure of the present work are compared with the workdone in the Rhesus monkey (macaques) of different countries.

In the present study analysis of prevalence data showed that out of 200 samples collected from two different sites Swoyambhu and Nilbarahi area. 127 (63.5%) were found to be positive with one or more than one helminth parasites. This remarkable prevalence was found in these areas because of highly dense population around there and in other hand there was favourable climatic condition to flourish their habitat which favours the transmission of parasites. The macaques in the Swoyambhu and Nilbarahi area lives mostly in the outskirts of temple during daytime so they are mostly fed by the visitors this also helps to promote the parasitic infection in them.

In class-wise prevalence rate Nematodes with 85.82% was the highest followed by Cestodes (7.08%), Trematodes (3.14%) and Acanthocephala (3.93%). Present finding indicate that there were significant difference in class-wise prevalence rate of parasites. ($\chi^2 = 495.7$, $p < 0.05$). Such differences is due to the maximum use of natural water sources, which are often untreated and remain open with high chances being contaminated. Prevalence rate of Nematode was very high. We already know that Nematodes is transmitted through contamination of food and water and some of them can transmit themselves by penetration through skin also. Such differences may be supported due to geography and climatic condition.

The present result is more contradictory to those of earlier from different countries, but some of them are quite supportive. Among them Horii *et al.*, (1981) had frequently found four species of gastro-intestinal nematodes and a few cestode were occasionally found in Japanese monkey of Koshima troop.

Swoyambhu and Nilbarahi area are carried out to study the prevalence of intestinal parasites of Rhesus macaques in which the prevalence rate was found to be 68% and 59% respectively. Present findings indicate that there were not significant difference in area wise parasitic infection. The very much equal prevalence and distribution of parasites is due to same geography and climatic condition out there.

Philips *et al.*, (2004) collected faecal samples from 86 individuals of macaques from Tambopata Research Centre, Tambopata National Reserve, Peru and analysed, the result of which indicate the presence of various protozoans, *Ancylostoma* sp, *Ascaris* sp, *Strongyloides stercoralis*, *Trichuris trichura*, *Prosthenorchis elegans* and *Schistosoma mansoni*.

Out of 127 positive samples, there were 65.35% having single infection, 24.4% double infection and 7.08% multiple infection. The most common double infection was cooperated by *Strongyloides fulleborni* and *Oesophagostomum* sp with the other species and multiple infection were among the *Strongyloides fulleborni*, *Oesophagostomum* sp, *Taenia* sp, *Chabertia* sp, *Dictyocaulus* sp. This also shows that the *Strongyloides fulleborni* and *Oesophagostomum* sp were highly influenced among these concurrent groups.

The chance of infection cooperated by the *Strongyloides fulleborni*, *Oesophagostomum* sp were having because the Rhesus macaques are the specific host of these parasites.

The other non specific host parasites like *Toxocara Canis*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Toxascaris leonina* and so on were also found incooperated with or without the *strongyloides fulliborni* and *Oesophagostomum* Sp. This is because of contamination of faecal matter with soil.

Parasitic infection percentage goes high with the contaminated food and water. Water sources like rivers, streams, ponds were contaminated by the waste disposal, sewages, garbages deposited in these areas, that's why the more than one infection were having.

Out of 127 (63.5%) positive samples for helminth parasitic infections, *Strongyloides fulleborni* accounted for 42.5%, *Dictyocaulus* sp (7.87%), *Taenia* sp (7.08%), *Oesophagostomum* sp (6.29%), *Trichuris ovis* (4.72%), *Capillaria* sp (3.93%), *Ostertegia* sp (3.93%), *Cooperia* sp (3.93%), *Prosthenorchis elegans* (3.93%), *Dicrocoelium* sp (3.14%), *Trichostrongylus* sp (3.14%), *Oxyuris* sp (3.14%), *Toxascaris leonina* (3.14%), *Trichuris trichiura* (2.36%), *Ascaris lumbricoides* (1.57%), *Toxocara Canis* (1.57%), *Chabertia* sp (1.57%) respectively.

In the present study *Strongyloides fulleborni* with 42.5% highest prevalence obtained among other helminth parasite, it is due to Natural specific host of this parasite. We know that the Strangyloides group are th soil transmitted helminth parasites and as well as it can also transmit through contaminated food and water, so either they can easily get penetrated through the skin or easily established themselves through enoculation, but must of the other helminth species need contaminated food and water and some of them also need secondary or tertary host for their completion of life that's why *strongyloides fulleborni* have accounted in great number.

Horii et al., (1981) had found the high incidence (100%) *Strongyloides fulleborni* in young monkeys of Koshima troop of Japan but the other species were also frequently found.

Limbu et al., (2006) had also reported *Strongylus* and *Paramphistome* groups in Rhesus monkey for the first time in Nilbarahi in the community forest.

Out of total 127 positive samples 106 (83.46%) were positive to zoonotically infective helminthes and 21 (16.53%) samples were negative to zoonotically non-infective helminthes.

The prevalence rate of zoonotically infective helminthes was high because monkeys of the study area come into frequent contact with humans and domestic animals.

During faecal samples examination for intestinal helminth parasites of *Macaca mulatta* from both the areas some identified and unidentified protozoans were also detected. The prevalence rate and photographs of those intestinal protozoan parasites are shown in the annex.

All the genus and species of intestinal parasites are reported here for the first time from Rhesus monkey (*Macaca mulatta*) from Nepal whereas *Prosthenorchis elegans* is reported for the first time.

VII

RECOMMENDATIONS

1. Deworming program should be carried out regularly to improve monkey's health.
2. Deworming program should be conducted to reduce the transmission of zoonotically infectious disease.
3. Local people should be alert through awareness program about various zoonotic diseases, which may be transmitted through monkeys.
4. Researchers should also focus on wild life population because many researches have been done on humans and domestic animals but wild animals are still least studied.

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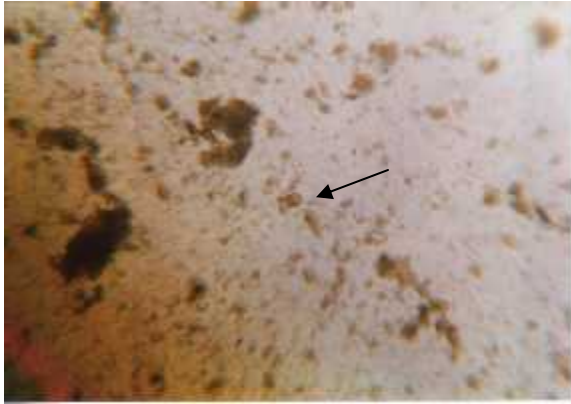


Plate No. 24
Cyst of *Entamoeba histolytica*



Plate No. 25
Trophozoite of *Balantidium coli*

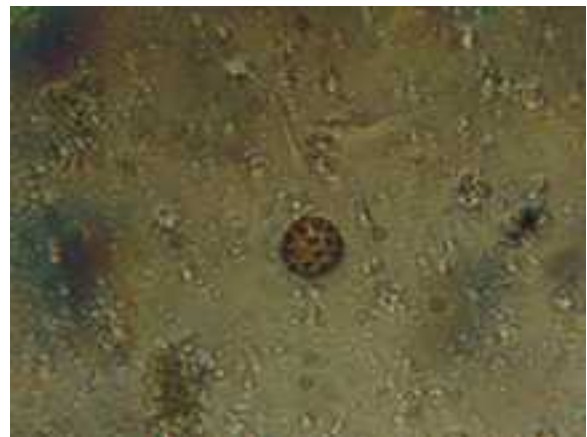


Plate No. 26
An unidentified protozoan egg

ANNEX

SOME PROTOZOAN PARASITES DETECTED

During faecal sample examination of *Macaca mulatta* from Swoyambhu and Nilbarahi area for the identification of intestinal helminth parasites, some identified and unidentified protozoan parasites were also detected.

Table no 7: Prevalence rate of identified and unidentified protozoan parasites

S.n	Total no of faecal samples examined	Total no of faecal samples positive to protozoan parasites	Protozoan parasites	No	%
1	200	92	<i>E. histolytica</i>	70	76.08
2			<i>B. coli</i>	12	13.04
3			Unidentified	10	10.86

Fig7: Diagrammatic representation of analysis of *E. histolytica*, *B. coli* and an unidentified protozoan parasites

