Copro-Microscopic Study of Gastrointestinal Helminth Parasites of Rodents (Family: Muridae) of Gokarneshwor Municipality, Kathmandu, Nepal

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DECLARATION

I hereby declare that the work presented in this dissertation "Copro-microscopic study of gastrointestinal helminth parasites of rodents (Family: Muridae) of Gokarneshwor municipality, Kathmandu, Nepal" 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 the dissertation entitled "Copro-microscopic study of gastrointestinal helminth parasites of rodents (Family: Muridae) of Gokarneshwor municipality, Kathmandu, Nepal" has been carried out by Rabina Gupta Kalwar for the partial fulfillment of Master's Degree of Science in Zoology with special paper parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this dissertation work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor "Mr. Janak Raj Subedi" this dissertation submitted by Rabina Gupta Kalwar entitled "Copro-microscopic study of gastrointestinal helminth parasites of rodents (Family: Muridae) of Gokarneshwor municipality, Kathmandu, Nepal" is approved for the examination in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper parasitology.

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This dissertation work submitted by Rabina Gupta Kalwar entitled "Copro-microscopic study of gastrointestinal helminth parasites of rodents (Family: Muridae) of Gokarneshwor municipality, Kathmandu, Nepal" has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper parasitology.

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ABSTRACT

Many parasites that are of medical importance have their definitive host as small mammals and are also zoonotic. Some of these rodent species are peri domestic meaning they are in close contact with humans and the intermediate host that help in the transmission of the disease. A cross sectional study was conducted between July to November 2022 to determine the gastrointestinal helminth parasites and their risks in rodents in Sundarijal, Kathmandu, Nepal. A total of 110 fecal samples were collected from rodents (73 rats, 46 *Rattus norvegicus* and 27 *Rattus rattus* and 37 house mice, *Mus musculus*), and examined using direct wet mount, floatation as well as formal-ether sedimentation techniques. The results showed that the overall prevalence of endoparasites was 29.09% with the following genera: Hymenolepis nana (17.27%), Hymenolepis diminuta (6.36%), Strongyloides stercoralis (4.54%) and Trichiuris spp. (0.9%). The result of the interview revealed that, walking bare foot and playing in open grassland has been identified as the major risk factors for the zoonotic diseases among the people living there. Thus, more studies should be conducted in the rural areas of Gokarneshwor Municipality with changing climate conditions to further explore the parasite diseases in wildlife and their impacts on human health and environment.

शोध सार

मानव स्वास्थ्यका लागि महत्वपूर्ण परजीवीहरू प्रायः साना स्तनधारी जनावरहरूमा पाइन्छन् र तिनीहरू जुनोटिक (जनावरबाट मानिसमा सर्ने) हुन्छन्। कतिपय कृन्तक (रोडेन्ट) प्रजातिहरू परिघरेलु (peri-domestic) प्रकृतिका हुन्छन्, जसले गर्दा तिनीहरू मानिसहरूसँग प्रत्यक्ष सम्पर्कमा रहन्छन् र रोग सार्ने मध्यस्थ (intermediate host) का रूपमा भूमिका खेल्छन्।

यो अध्ययन सन् २०२२ को जुलाईदेखि नोभेम्बर महिनासम्म काठमाडौँको गोकर्णेश्वर नगरपालिकाको सुन्दरीजल क्षेत्रमा गरिएको थियो। अध्ययन अन्तर्गत ११० वटा कृन्तकहरूबाट (७३ वटा मुसा, जसमा ४६ वटा *Rattus norvegicus* र २७ वटा *Rattus rattus* तथा ३७ वटा घरमा पाइने मुसा (*Mus musculus*) रहेका छन्) मल नमुना संकलन गरियो। संकलित नमुनाहरूलाई प्रत्यक्ष गीला माउन्ट (wet mount), फ्लोटेशन (floatation), तथा औपचारिक-ईथर अवसादन (formal-ether sedimentation) प्रविधिहरू प्रयोग गरी परीक्षण गरियो।

नतिजाबाट पत्ता लागेको थियो कि २९.०९% नमुनाहरू परजीवी संक्रमणयुक्त थिए। पहिचान गरिएका परजीवीहरूमा *Hymenolepis nana* (१७.२७%), *Hymenolepis diminuta* (६.३६%), *Strongyloides stercoralis* (४.५४%), तथा *Trichuris spp.* (०.९%) रहेका थिए।

यस अध्ययनमा गरिएको अन्तर्वार्ताका नतिजाहरू अनुसार नांगो खुट्टा हिँड्ने तथा खुला घाँसेमैदानमा खेल्ने बानी भएका व्यक्तिहरूमा जुनोटिक रोगहरू लाग्ने सम्भावना बढी देखिएको छ। तसर्थ, गोकर्णेश्वर नगरपालिकाका ग्रामीण क्षेत्रहरूमा थप अनुसन्धान आवश्यक छ, जसले वातावरणीय परिवर्तनसँगै वन्यजन्तुबाट सर्ने रोगहरूको विस्तृत अध्ययन गर्न र तिनको मानव स्वास्थ्य तथा वातावरणमा पार्ने असर पहिचान गर्न मद्दत पुर्याउनेछ।

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LIST OF ABBREVIATIONS

Abbreviated form	Details of Abbreviations
CDZ	Central Department of Zoology
Gm	Gram
Mg	Milligram
Ml	Milliliter
Mm	Millimeter
Rp	Rounds per minute
Spp.	Species
TU	Tribhuvan University
WHO	World Health Organization
VDC	Village Development Committee
GI	Gastro-intestinal
p-value	Probability Value
Ns	Non-significant

1. Introduction

1.1 Background

With almost 4,600 species, rodents (order Rodentia) are the biggest group of mammals. This vast animal order includes 27 distinct families, including porcupines, marmots, squirrels, and rats and mice (which belong to the Muridae family). Knowing the processes that occur in human and animal living tissues is greatly aided by studying rats (Casebolt et al., 1988, Dillehay et al., 1990). They play a crucial role in many ecosystems, either as carriers and reservoirs of disease or as prey to their predators (Okaye and Obiezue 2008). Additionally, they are employed in the safety evaluation of medications intended for human use (Clark et al., 1997). In many parts of the world, primarily in developing nations, rats are also used as an animal protein source (Clark et al., 1997). They are known to carry certain ecto and endo parasites, which puts human health at risk for those who reside near rodent populations (Zain et al., 2012).

Rodents are widely recognized as hosts and reservoirs of over 60 zoonotic diseases, posing a significant threat to human health (Meerburg et al., 2009; Kosoy et al., 2015). Various species of *Rattus*, including the Black Rat (*Rattus rattus*), Asian House Rat (*Rattus tanezumi*), Norway Rat (*Rattus norvegicus*), and Pacific Rat (*Rattus exulans*), have rapidly expanded their geographical range due to human activities (Aplin et al., 2011). These rodents have been implicated in the emergence and spread of several infectious diseases, such as plague (*Yersinia pestis*), murine typhus (*Rickettsia typhi*), scrub typhus (*Orientia tsutsugamushi*), leptospirosis (*Leptospira spp.*), hantavirus infections, and hemorrhagic fever (Mills, 2006; Han et al., 2015).

The ongoing global changes, particularly urbanization, have further facilitated the expansion of rodent populations beyond their natural habitats (Parsons et al., 2017). Due to their synanthropic nature, rodents thrive in human-inhabited environments, thereby increasing the risk of zoonotic disease transmission. Some of the most significant rodent-borne diseases affecting public health include salmonellosis (*Salmonella spp.*), plague, Lassa fever, leishmaniasis (*Leishmania spp.*), toxoplasmosis (*Toxoplasma gondii*), rat-bite fever (*Streptobacillus moniliformis*), and taeniasis (*Taenia spp.*) (Himsworth et al., 2013).

Additionally, rodents serve as reservoirs for complex bacterial pathogens such as *Mycobacterium tuberculosis*, *Mycobacterium microti*, *Escherichia coli*, *Coxiella burnetii* (Q fever), and the agents of tick-borne relapsing fever (Vladimirsky et al., 2014; Mori et al., 2017). Furthermore, ongoing research continues to isolate new bacterial species from rodents, whose pathogenic potential remains unknown, highlighting the need for further surveillance and study in this area (Hautvast et al., 2017).

Ecto-parasites which includes lice, ticks and mites can transmit a number of pathogens to man and animals (Soliman *et al.*, 2001). The parasitic eggs are exceeded out in rodent droppings on agricultural fields, saved grains and in numerous suitable for eating commodities in homes and hence accountable for spread of the disease (Khatoon *et al.*, 2004). Their ability to behave as a vector is significantly more suitable due to their physical similarities which they share with human (Kataranovski *et al.*, 2010). Hence increased rodent population in a place will be at once associated with elevated zoonotic sickness in human population (Stojcevic *et al.*, 2004).

According to Walsh et al. (1993) and Singleton et al. (2003), rats are known to spread illness and serve as reservoir hosts for a variety of zoonotic infections, including parasites that can be harmful to human health. Rodents' endoparasites are crucial to the zoonotic cycles of numerous illnesses, including as angiostrongyliosis and schismomiasis. There have been numerous researches conducted in Malaysia on the endoparasites of forest and commensal rats (Singh & Cheong, 1971; Leong et al., 1979; Yap et al., 1977; Krishnasamy et al., 1980; Ambu et al., 1996).

The main reason rats and mice are important to public health is that they are reservoirs or carriers of germs linked to illnesses and infections that humans can contract. Plague, Salmonellosis, Leptospirosis, Rickettsial pox, Murine typhus, Rat-bite fever, Hanta virus hemorrhagic pulmonary syndrome, and rabies are among these illnesses. Additionally, parasites like Trichinosis, eosinophilic meningitis, *Taeniasis, cryptosporidia*, and *Trypaonosoma lewisis* are also among them (Singleton et al., 2003).

Indirect contact is how such illnesses are spread to humans. Some spread by mosquito bites, others by fleas and lice, and still others by coming into touch with contaminated rodent urine or feces (Ruiz, 2004). Consumption of food or water tainted with embryonated eggs transferred from rodent droppings can infect humans. Contact with contaminated rodent urine or feces can spread some parasites, whereas contact with arthropods can spread others (Beg et al., 1983). The health of domestic animals and humans may be seriously jeopardized by *Rattus rattus*, *Rattus norvegicus*, and *Mus musculus*, which can act as vectors of zoonotic and numerous other diseases (Webster and Mac Donald, 1995).

In rats and other mammals, a consistent program of surveillance and control measures for gastrointestinal parasites, such as effective treatment and appropriate prevention based on accurate diagnosis, would undoubtedly improve the health status.

Keeping the significance in view, the existing paintings is therefore undertaken to recognize the generic species of helminth parasites in rodents in Gokarneshwor Municipality, Kathmandu, Nepal and thereby check their public health risks. This special issue calls for a more thorough investigation of rodents and rodents-borne diseases considering the ecology of rodents in their habitats. The need to identify the mechanism affecting pathogen diversity and infection within and between rodent species. Thus, in this study, prevalence of Gastrointestinal parasitic species in rodents of Sundarijal of Gokarneshwor Municipality, Kathmandu, Nepal will be investigated.

1.2 Statement of the Problem

Rodents serve as significant reservoirs for numerous zoonotic diseases, including gastrointestinal helminth infections; nevertheless, data on their prevalence in Nepal is scarce. The Gokarneshwor Municipality's Sundarijal area has a high risk of rodent-human contact, which raises the possibility of disease transmission. A knowledge vacuum is created by the paucity of thorough research on rodent-borne helminths in this field, which makes it challenging to evaluate the dangers to the public's health and put effective control measures in place.

By detecting and measuring gastrointestinal helminths in Sundarijal rats, this study seeks to close this gap. The study will shed important light on the epidemiology of rodent-borne parasites by examining their prevalence and species makeup. The results will raise awareness of public health issues and aid in the development of focused rodent control plans to reduce the dangers of the spread of zoonotic diseases.

1.3 Objectives

1.3.1 General objective

- To study gastro-intestinal helminth parasites of rodents of Sundarijal of Gokarneshwor Municipality, Kathmandu, Nepal.

1.3.2 Specific objectives

- To provide baseline data on fecal parasites in rats inhabiting Gokarneshwor Municipality, Kathmandu.
- To identify and quantify the prevalence of gastrointestinal parasites in mice residing Gokarneshwor Municipality, Kathmandu.

1.4 Significance of the study

- Limited Research in Nepal: Only one study has been conducted on gastrointestinal helminths in rodents in Nepal, highlighting a significant knowledge gap in this field.
- Zoonotic Disease Risk: Rodents are potential carriers of zoonotic parasites that can be transmitted to humans, posing a serious public health concern.

1.5 Limitation of the Study

There are clearly limitations to research investigations since they encounter numerous issues. Without a doubt, the current study has the following shortcomings:

- In order to partially complete the criteria for the Master of Zoology program at Tribhuvan University in Kathmandu, Nepal, an academic study was conducted.
- Additionally, this study had a limited time frame, being conducted over just two seasons.

2. Literature Review

Rodents are significant reservoirs of zoonotic diseases, hosting a wide range of parasites that pose serious risks to human and animal health (Walsh et al., 1993). Both ectoparasites and endoparasites found in rodents play a crucial role in zoonotic disease transmission, including plague, schistosomiasis, and hymenolepiasis (Bradshaw, 1999). Increased rodent populations have been directly associated with rising zoonotic disease cases in humans, often due to direct contact with contaminated feces, urine, or arthropod vectors (Beg et al., 1983). Among the common rodent species, *Rattus rattus*, *Rattus norvegicus*, and *Mus musculus* have been identified as potential vectors of zoonotic pathogens, leading to significant public health concerns (Webster & MacDonald, 1995). Understanding the lifecycle and transmission patterns of these parasites is essential for developing effective control measures in different ecological conditions.

Research across different geographical regions has highlighted the prevalence and diversity of gastrointestinal helminths in rodents. In Korea, *Capillaria hepatica*, *Hymenolepis diminuta*, and *Taenia taeniaformis* were reported in *Rattus norvegicus*, indicating high infection rates in urban rodent populations (Seong et al., 1995). Similarly, a study in South-West Iran detected four species of helminths and one protozoan in *Mus musculus*, *R. rattus*, and *R. norvegicus* (Kia et al., 2000).

A study in the Philippines examined *Rattus* spp. captured in wet markets, identifying two ectoparasites (*Echinolaelapus echidnius* and *Polyplax spinulosa*) and eight species of endoparasites. Despite a 100% infection rate with *T. taeniaeformes* and severe hepatic tissue damage due to *Capillaria hepatica*, the infected rodents displayed no visible signs of illness (Claveria et al., 2005).

In Sri Lanka, the common zoonotic parasites found in *R. rattus*, *M. musculus*, and *Bandicota* sp. included *Xenopsylla cheopis*, *H. diminuta*, *Monoliformes monoliformes*, *Cysticercus fasciolaris*, and *Raillietina* spp. (Sumangali et al., 2007). In Egypt, 10 trematodes, 4 cestodes, and 10 nematodes were identified in *R. norvegicus*, *R. r. frugivorous*, *R. r. alexandrines*, and *Mus musculus* collected from locations near garbage dumps and livestock farms (Kady et al., 2009).

A study in Taiwan found high infection rates of cestodes, nematodes, and protozoans in *R. norvegicus*, *R. rattus*, and *Suncus murinus* (Tung et al., 2009). Likewise, a comparative study in Pakistan demonstrated a correlation between helminth infections in rodents and humans, reinforcing the zoonotic significance of commensal rodents (Rafique et al., 2009).

A study in Thailand revealed that rodents were infected with 11 species of parasites, including two cestodes, eight nematodes, and one acanthocephalan. The prevalence of gastrointestinal helminths was recorded at 66.2%, with *Trichostrongylidae* (33.8%) being the most dominant species, followed by *Raillietina* spp. (20.6%), *Syphacia muris* (14.7%), and *Hymenolepis diminuta* (11.8%) (Chaisiri et al., 2010).

A survey in Nigeria examined 246 rodents, including *Rattus norvegicus*, *Thryonomys swinderianus*, and *Cricetomys gambianus*, and found that 54% were infected with gastrointestinal parasites. Notably, nematodes were detected in all rodents, while cestodes and protozoans were absent in some species. Female rodents exhibited a significantly higher prevalence of gastrointestinal parasites than males (Ayinmode et al., 2012).

Similarly, in Maiduguri, Nigeria, a study found that out of 85 trapped rats, 8.2% tested positive for gastrointestinal helminths, with *Hymenolepis diminuta* being the only helminth species identified (Paul et al., 2015). Another survey on the African giant rat (*Cricetomys gambianus*) found *Hymenolepis nana* to be the most dominant helminth species, followed by *Ancylostoma caninum*, *Strongyloides stercoralis*, and *Aspicularis tetraptera* (Mbaya et al., 2015).

Several studies have indicated a strong association between rodent-borne parasites and human infections. A 2009 study in Egypt reported a variety of zoonotic helminths in rodents from different environmental settings, reinforcing the role of rodents as reservoirs of medically significant parasites (Kady et al., 2009). Another study in Taiwan demonstrated significant infection rates of cestodes, nematodes, and protozoans in multiple rodent species, further establishing the need for effective rodent control strategies (Tung et al., 2009).

A study in Uttarakhand, India, investigated the biodiversity of helminths in *Rattus rattus* and *Mus musculus*, identifying *Hymenolepis nana*, *Hymenolepis diminuta*, *Syphacia muris*, *Capillaria hepatica*, and *Trichuris muris* as the predominant species (Deepesh et al., 2015). Additionally, research conducted in Gujarat, India, on zoo mammals found that 57.14% of fecal samples tested positive for parasitic infections, with nematodes being the most prevalent group (Parsani et al., 2015).

The most recent study on rodent-borne parasites in Nepal was conducted by Sharma et al. (2021), where they examined the prevalence of gastrointestinal helminths in commensal rodents from urban and rural settings. Their findings indicated a high prevalence of *Hymenolepis nana* and *Strongyloides stercoralis*, emphasizing the need for continuous surveillance and control programs in Nepalese communities.

The reviewed literature indicates that rodents are major reservoirs of zoonotic gastrointestinal helminths worldwide. The prevalence of parasites varies across geographical regions, host species, and environmental conditions. Several studies emphasize the strong correlation between rodent-borne helminths and human infections, highlighting the need for continuous monitoring and control measures. Given the ecological diversity and human-rodent interactions in Nepal, further research is essential to assess the public health risks posed by these parasites. The present study aims to fill this research gap by investigating the gastrointestinal helminths of rodents in Sundarijal, Kathmandu, and assessing their potential zoonotic implications.

3. Materials and Methods

3.1 Study Area

The study will be conducted in Gokarneshwor Municipality, Kathmandu, Nepal. It lies at coordinates 27°44′0″N 85°23′0″E with total area of 58.5 km^2 (22.6 sq mi). Gokarneshwar is a municipality in Kathmandu District in the Bagmati Province of Nepal that turned into set up on 2 December 2014 via merging the previous Village improvement committees Sundarijal, Nayapati, Baluwa, Jorpati and Gokarna. Municipality has a total population of 107,351 according to 2011 Nepal census with the density of 1,800/km^2 (4,800/sq mi). The office of the municipality is that of the former Jorpati village improvement committee. The river Bagmati has its foundation as the call Bagh Dwar located inside the center of the Shivapuri jungle on this municipality. In the village on the banks of the Bagmati River stands the Gokarna Mahadev temple, built in 1582.In overdue August or early September humans go to this temple to wash and make services in honor in their fathers, residing or useless, on a day referred to as Gokarna Aunsi.

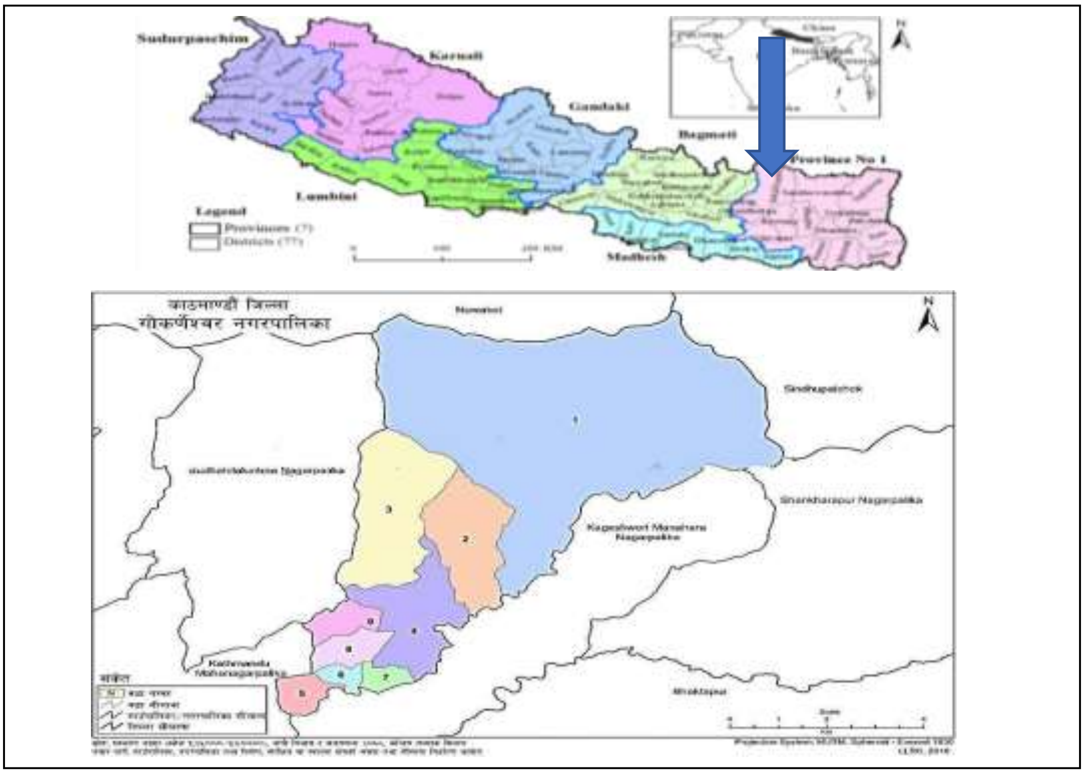


Figure 1: Map of Nepal showing Gokarneshwor Municipality

3.2 Study Design

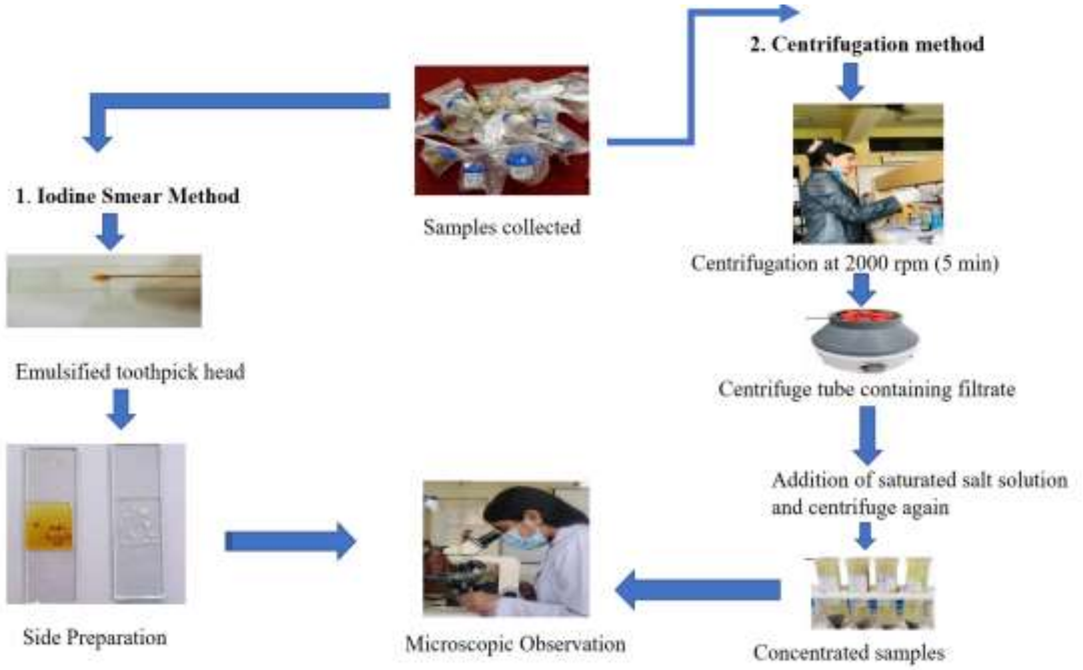


Figure 2: Flowchart showing the study design.

3.3 Sample Size

Samples were collected at seasonal variation both during winter and summer season. Sampling was done thrice a week and 24 samples were recorded as the repeated samples which were excluded in the data. A total of 110 fecal samples from rodents (rats and mice) were collected from Gokarneshwor Municipality, Kathmandu, Nepal.

3.4 Sample Collection, Preservation and Transportation

Feces of rodents; black rats (*Rattus rattus*), brown rats (*Rattus norvegicus)* and mice (*Mus musculus*) were collected from domestic places and agricultural fields of different parts of Gokarneshwor Municipality, Kathmandu, Nepal. Wire mesh traps were used to catch hold the rodents by the method of Asgari et al., (2007). The traps were set in the dark in the evening and when rodents get in traps were collected next morning. A thick bundle of papers was kept below each trap and were left for few hours at room temperature so as to collect fecal droppings. A mark was done in the ear/tail of the rodents with a nail polish to prevent repetition of samples.

The collected samples of rodents were immediately preserved at 2.5% potassium dichromate solution in 20 mL sterile vials. They were then transported to Laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur and further were stored at 4 degree (°) Celsius temperature.

3.5 Laboratory Processing and Examination

The collected samples of rodents were processed and examined by direct smear method for the detection of helminth's eggs (Battersby et al., 2002). Parasitic eggs were identified up to generic level using the key of Soulsby (1982), adult and segments passed were identified up to species level using standard keys.

3.5.1 Direct Wet Mount Technique

One to two drops of carefully stirred fecal samples were put in the slide with the help of a plastic dropper. The samples were observed directly at 2.5% (*w/v*) potassium dichromate, Gram's iodine stain and Giemsa's stain. (Zajac et al., 2021).

3.5.2 Saturated Salt Floatation Technique

This technique is used widely for detection of nematode and the cestode eggs. As, eggs of cestodes and nematodes are seemed to be very small in size and light so this technique ensure eggs to float in floatation liquid.

About two grams (gms) of the fecal samples were thoroughly mixed in a 13 milliliter (mL) normal saline (0.9% *w/v*) solution and were filtered with the help of a tea strainer. The solution was poured into a 15 mL conical centrifuge tube and proceeded to centrifuge (1200 revolutions per minute, rpm for 5 minutes). After discarding the supernatant, 12 mL of salt solution (45% *w/v*) was added in the tube to fill it, and a coverslip was placed on the mouth of the tube. After 10 minutes, the coverslip was carefully removed and put on the glass slide with or without Lugol's iodine for microscopic observation at 100x and 400x total magnifications. (Segura et al., 2023).

3.5.3 Sedimentation Technique

This technique is for detection of trematode eggs. It provides better results as trematode eggs are heavier than other eggs and they are deposited at the bottom of test tube after centrifugation with zinc sulphate solution.

About two gms of the fecal samples was thoroughly mixed in 13 mL normal saline (0.9% *w/v*), was filtered with the help of a tea strainer into a 15 mL centrifuge tube, and was proceeded to centrifuge (1200 rpm for 5 minutes). Then, the supernatant was discarded, and one to two drops of the sediment was put on a glass slide. Gram's iodine and Giemsa's stain (1/15 dilutions) was used differently in the deposits for the microscopic examinations at 100x and 400x total magnifications. (Zajac et al., 2021).

3.6 Statistical Analysis

Data on age, sex and those that were obtained during laboratory examinations of rodents were summarized in Microsoft Excel Spread sheet, 2010 version and presented in tables using descriptive statistics. Prevalence was estimated as P=d/n (%); where P=prevalence, d= number of individuals having disease at a particular point in time and n= number of individuals in the population at risk at that point in time.

3.7 Ethical Approval

All procedures for trapping and handling rodents followed the guidelines of the Department of Forest and Soil Conservation and followed standardized safety guidelines recommended by the Center of Disease Control and Prevention. Sampling procedures were authorized by the members of Shivapuri National Park, Nepal.

4. Results

The result of the study has been mentioned under the following sub headings:

4.1 Trapped Rodent Species

A total of 110 rodents comprising of 46 *Rattus norvegicus*, 27 *Rattus rattus*, and 37 *Mus musculus* were collected and examined.

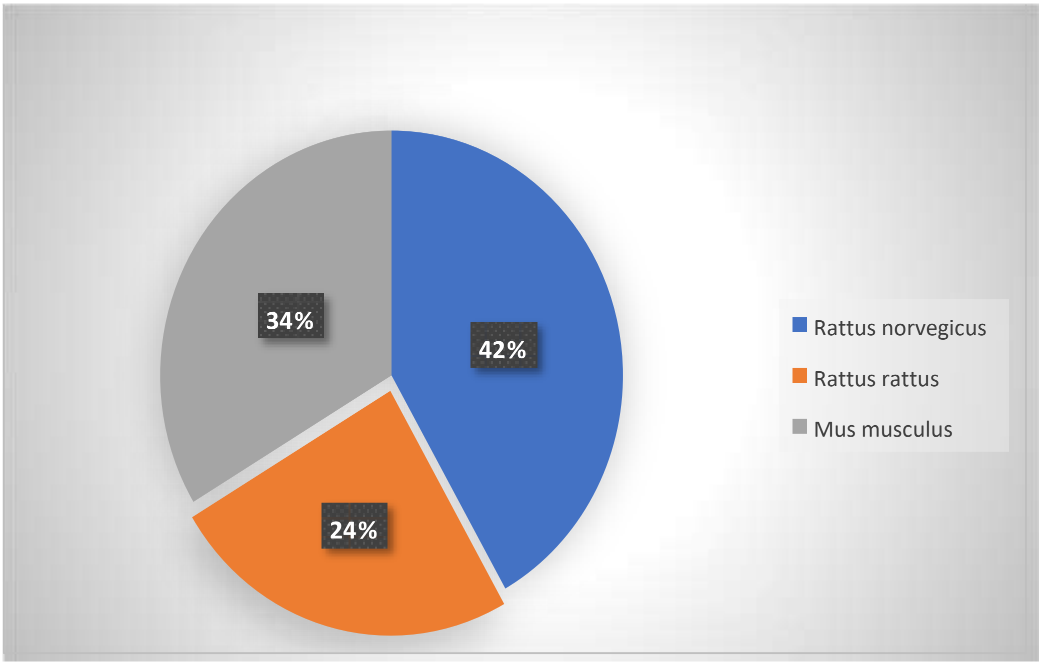


Figure 3: Species and number of trapped Rodent: Muridae

4.2 General Prevalence in Rodent Species

Out of 110 samples examined, only 32 samples (29.09%) were reported as infected samples. Among them, 27 from rats (16 *Rattus norvegicus* and 11 *Rattus rattus)* and 5 from house mice (*Mus musculus*) were infected.

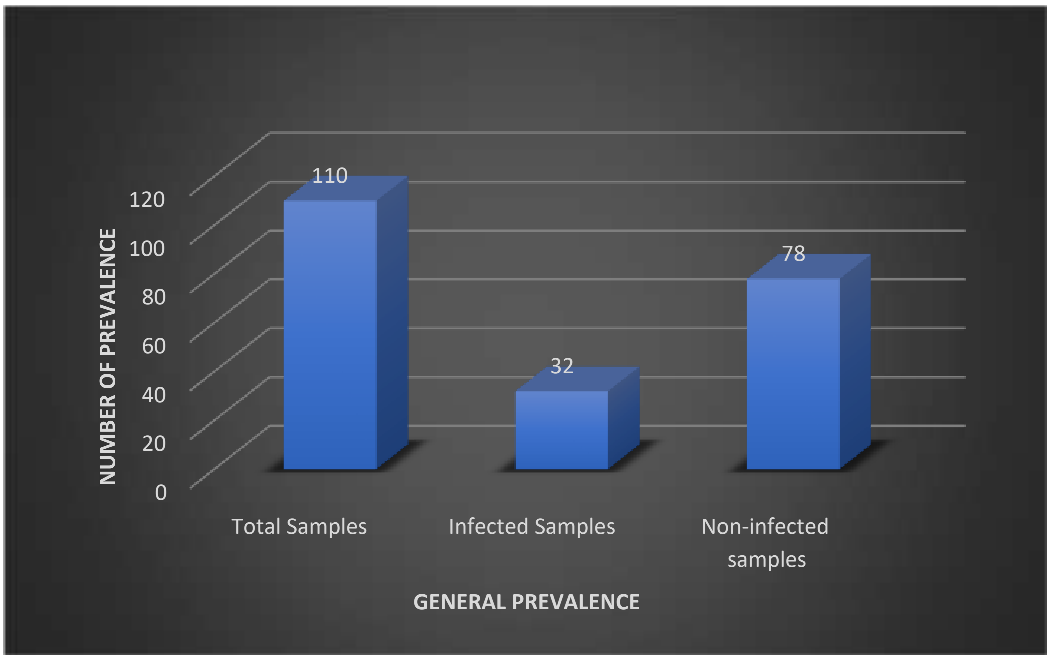


Figure 4: General Prevalence of Gastro-intestinal helminths parasites in rodents.

A total of 32 rodents (22 females and 10 males) infected with gastro-intestinal helminth parasites was found thus giving an overall infection rate of 29.09%. 4 species of helminths parasites were identified (70 cestodes and 13 nematodes). The identified cestodes were 48 *Hymenolepis nana* and 22 *Hymenolepis diminuta* followed by nematodes*;* 11 *Strogyloides spp* and 2 *Trichuris spp.*

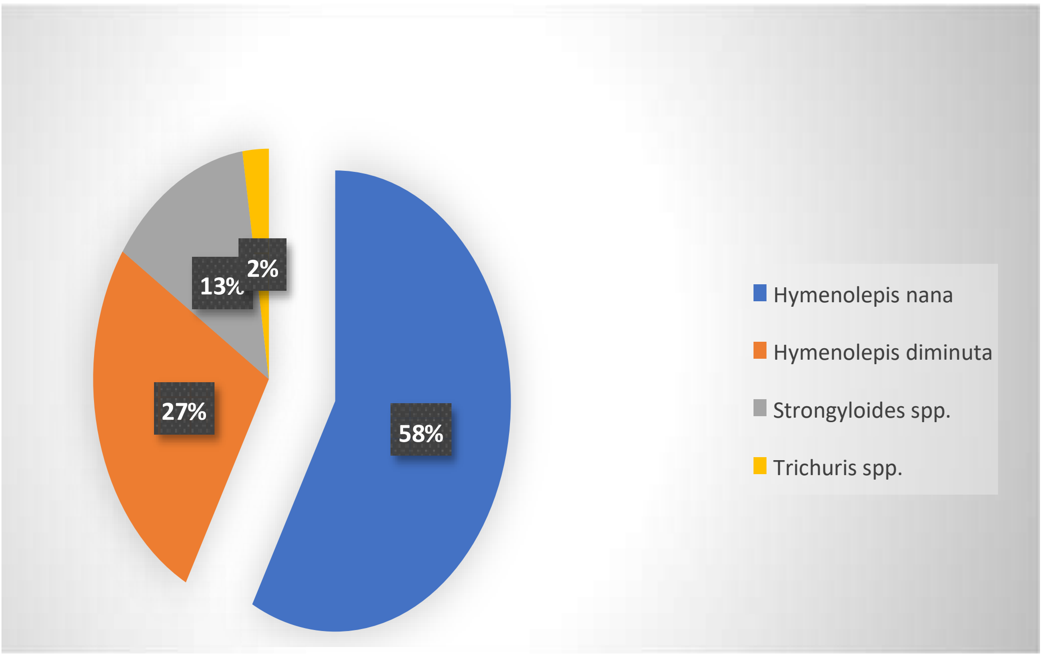


Figure 5: Number of GI parasites obtained from rodents.

4.3 Sex-Wise Prevalence

Out of 110 rodents captured, 60 were females (40 rats and 20 mice) and 50 were found to be males (33 rats and 17 mice). Among the infected 32 samples, 22 female rodents (20%) and 10 male rodents (9.09%) were infected with gastro-intestinal helminths parasites. Thus, making an overall infection of 29.09%. A significant difference in the prevalence of parasites was found between male and female rodents.

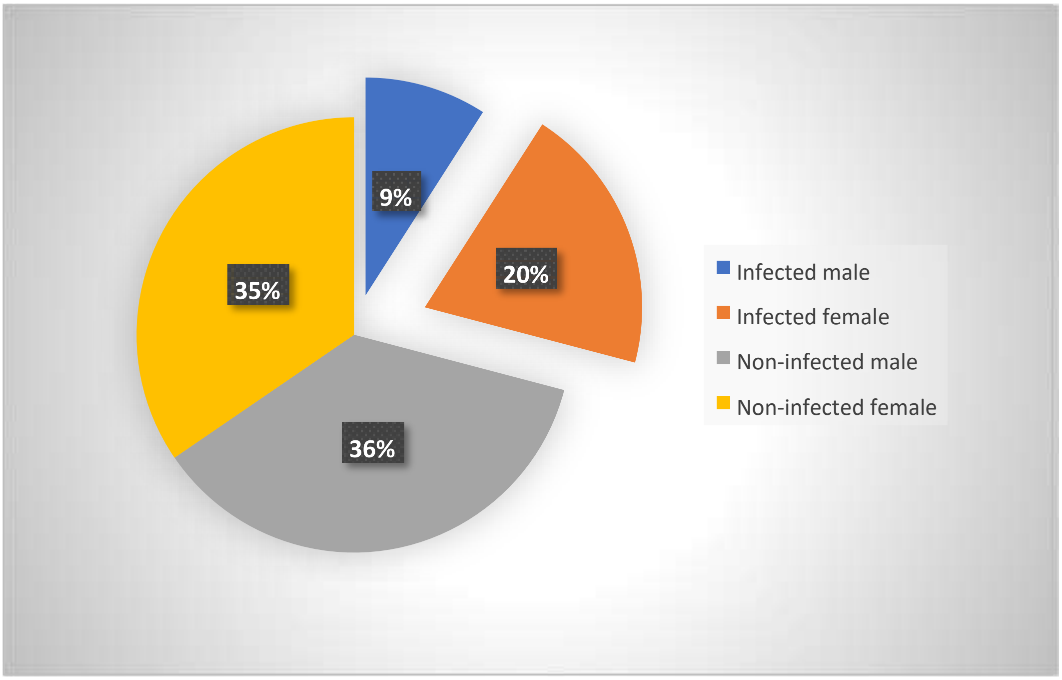


Figure 6: Sex-wise infected rodents

4.4 Concurrent GI helminths parasites infections in rodents

Among the 110 rodents captured, 70.90% was non-infected with parasites, 29.09% was infected, 29 with single infection (90.62%) followed by 3 with multiple infection (9.37%).

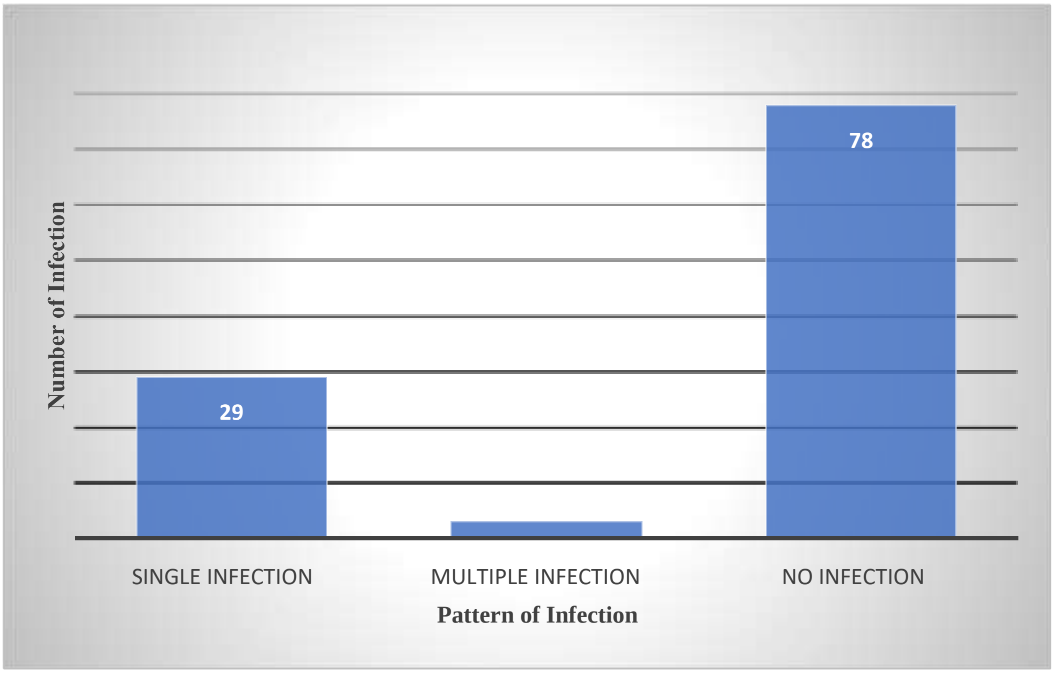


Figure 7: Pattern of infection

4.5 Class and genera wise infection

S.N	Categories	Genera	No. of infected samples	Prevalence % (infestation with in total)	Prevalence% (infestation within positive cases)
1	Nematodes	*Strongyloides stercoralis*	5	4.54%	18.51%
		Trichuris spp.	1	0.9%	3.70%
2	Cestodes	*Hymenolepis nana*	19	17.27%	59.37%
		Hymenolepis dimuntia	7	6.36%	21.8%

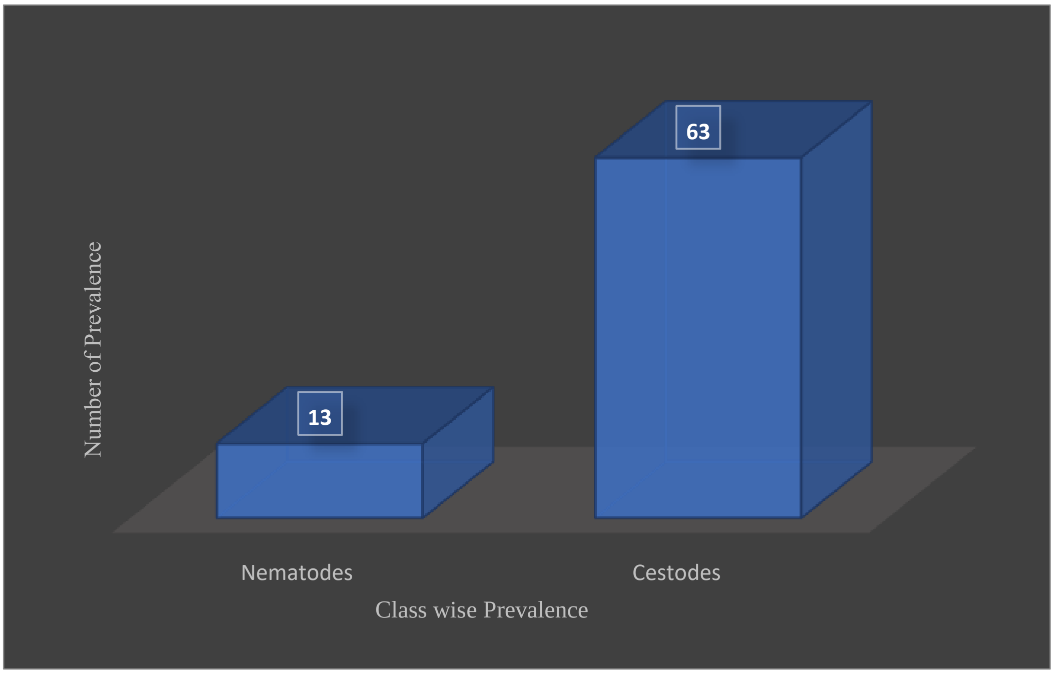


Figure 8: Class-wise Prevalence

4.5.1 Prevalence of nematodes in different rodent species

2 species of nematodes were found out of which prevalence of *Strongyloides* spp. (2 species) was found to be high in 18 species of rats followed by *Trichuris* spp. (1 species) in a rat.

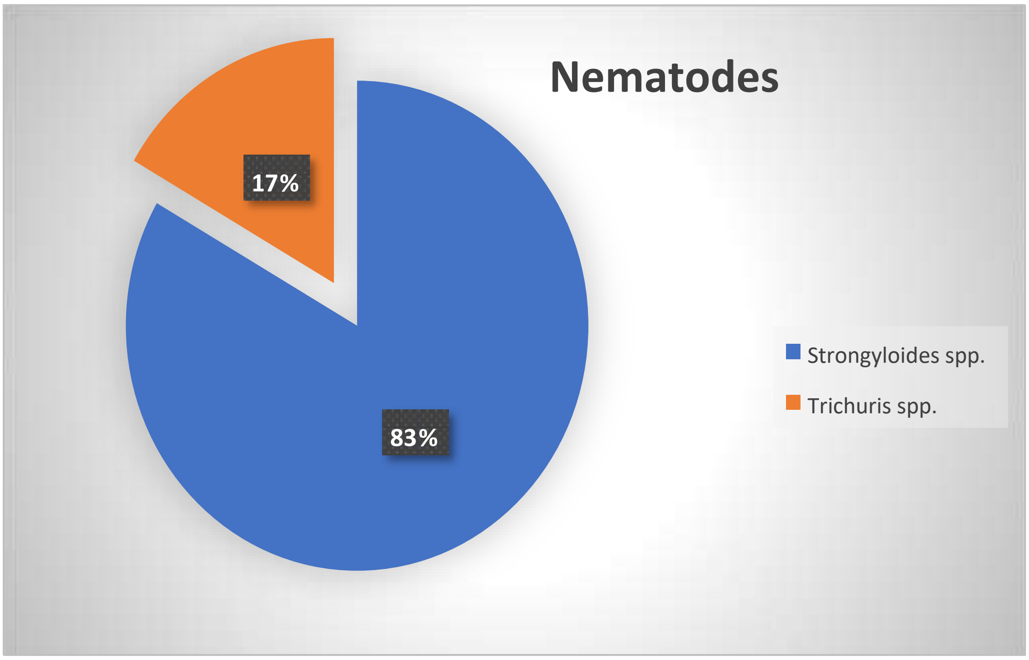


Figure 9: Prevalence of Nematodes in rodents.

4.5.2 Prevalence of Cestodes in different rodents

Likewise, 2 species of cestodes were reported out of which the prevalence of *Hymenolepis nana* (19) was found to be high in 18 species of rats and 1 in a house mouse followed by *Hymenolepis diminuta* (7) in 3 species in rats and 4 in house mice.

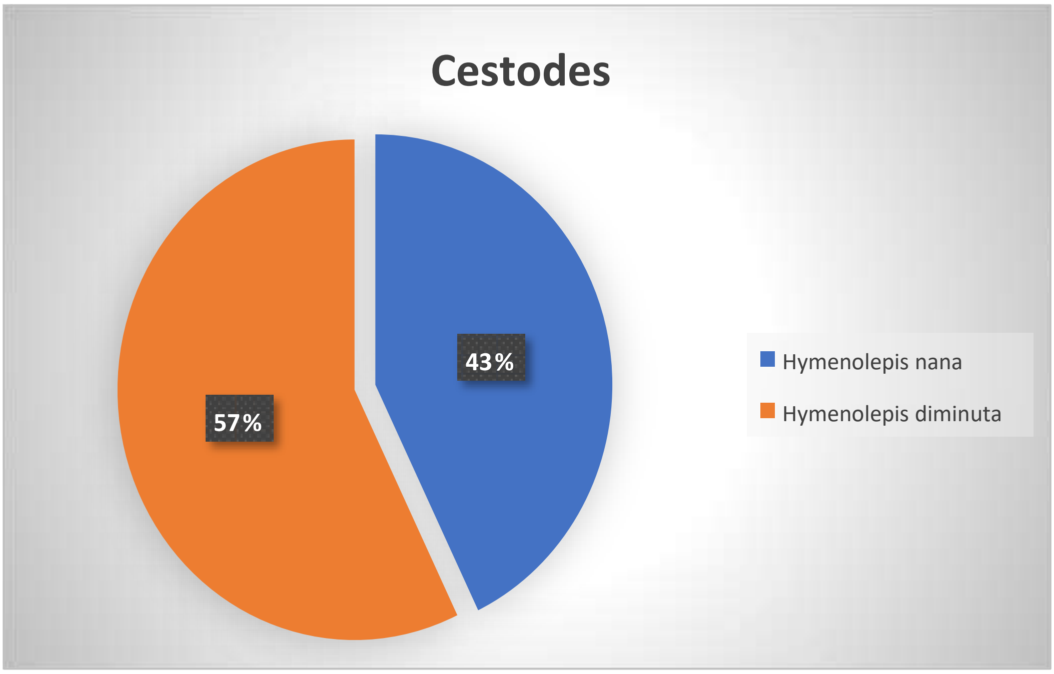


Figure 10: Prevalence of Cestodes in rodents.

4.6 Infection rates of GI helminths parasites in rodents when found alone and in concurrence to each other

Rodent species	GI parasites found	No. of host infected	% host infected	No. of parasites found	Mean Intensity	Mean abundance
Rattus rattus **(n=27)**	*H. nana*	5	45.45	23	4.6	0.85
	H. diminuta	3	27.27	7	2.33	0.26
	Strongyloides spp	2	18.18	4	2	0.15
	Concurrent Infection	1	9.09	2	2	0.07
	H. nana	7	43.75	13	1.86	0.28
	H. diminuta	3	18.75	8	2.67	0.17
Rattus norvegicus **(n=46)**	*Strongyloides spp*	3	18.75	7	2.33	0.15
	Trichuris spp.	1	6.25	2	2	0.04
	Concurrent Infection	2	12.5	5	2.5	0.11
Mus musculus **(n=37)**	*H. nana*	4	80	9	2.25	0.24
	H. diminuta	1	20	3	3	0.08
(n=110)	**Overall**	**32**	**29.09**	**83**	**2.5**	**0.21**

- ***H. nana*** is the dominant GI parasite across these rodent species, with a notable presence in both ***Mus musculus*** and ***Rattus rattus***. This suggests that ***H. nana*** may be of particular concern in environments where these rodents are common.

- The relatively low frequency of concurrent infections and moderate mean intensities suggest that while parasitic infections are present, they are not typically overwhelming in these populations.
- The results may point to species-specific susceptibility to different parasitic infections, highlighting the need for targeted control strategies based on the rodent species involved.

4.7 Prevalence in relation to different risk factors

Epidemiological Factors		Host Examined	Infected	% of host infected	Parasite number	Pvalue
Seasons	Summer	23	9	39.13	13	**0.488**
	Winter	52	14	26.92	51	
	Monsoon	35	9	25.71	19	
Location	Residence/Shops	47	9	19.14	28	**0.009**
	Poultry Farms	25	5	20	12	
	Agricultural Fields	38	18	47.36	43	
Species	*Rattus rattus*	27	11	40.74	36	**0.033**
	Rattus norvegicus	46	16	34.78	35	
	Mus musculus	37	5	13.51	12	
Sex	Male	50	10	20	27	**0.088**
	Female	60	22	36.66	56	
Age	Mature	73	23	31.5	68	**0.574**
	Young	37	9	24.32	15	

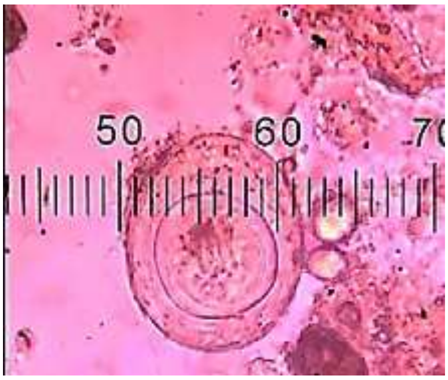
Significant associations ($p < 0.05$):

Location ($p = 0.009$): Infection rates significantly vary based on location.

Species (p = 0.033): Infection rates significantly differ between species.

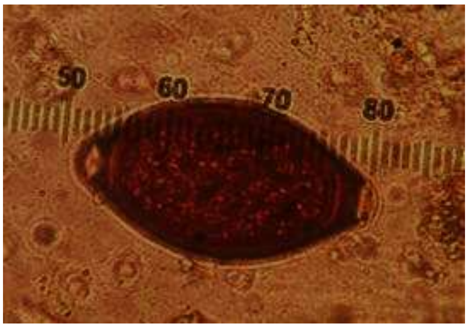
Non-significant associations (p > 0.05): Seasons, Sex, and Age do not show strong evidence of a significant effect on infection rates.

4.8 Photos of Egg/Oocyst/Larva of different parasites



Photograph 1: *Hymenolepis diminuta* (60-88 µm) at (10x X 40x).

Photograph 2: *Hymenolepis nana* (40-45 µm) at (10x X 40x).



Photograph 3: *Trichuris spp.* (30-42 µm) at (10x X 40x).

Photograph 4: *Strongyloides stercoralis* (23-79 µm) at (10x X 40x).

5. Discussion

In this investigation, parasite infections were detected in 29.09% of the fecal samples. With different diagnostic techniques employed in different research, our results show significant variety in prevalence rates among the nations. In Barbados, Mutani et al. (2003) reported 88.7% positive samples, indicating higher prevalence. On the other hand, Stuart et al. (1990) discovered that just 48% of the animals in Costa Rica had parasite infections, which is a far lower prevalence. These investigations, however, are not carried out in a captive environment but rather on animals that are allowed to roam freely. However, they show that even in animals that are allowed to roam freely, lower and higher prevalence of parasite infections can be identified.

According to this study, the overall prevalence of rat nematodes in Sundarijal, Gokarneshwor Municipality, was lower than that of cestodes and trematodes, which had been given greater attention in other comparable reports (Al-Aboody et al., 2020; Hotez, P.J. et al., 2012). Parasani et al. (2001) found that 68.8% of the animals in the Rajkot Municipal Corporation Zoo tested positive for helminthic diseases; our study is different from their findings. The disparity in results shows that, even in the case of an identical setting, the incidence of parasites may vary because of local conditions, management strategies, animal diets, and other factors.

Similar to previous workers, the current study found that the occurrence of cestodes is relatively higher in the summer and in homes, stores, etc. (Hilderbrand 2008, Kataranovski et al., 2011). However, Fichet et al. (2003) found that helminths were more common during the monsoon season than during other crop seasons. The summer and monsoon seasons' temperatures and relative humidity may be ideal for the parasites' maturation stages (Kreppel et al., 2016), increasing the likelihood of infection. According to Hancke and Saurej (2015), the level of urbanization may also have an impact on the number of arthropod intermediate hosts.

Similar findings have been found for R. rattus, R. norvegicus, and B. bengalensis in various environmental conditions, primarily in urban areas (Battersby et al., 2002; Abu-Madi et al., 2005, Easterbrook et al., 2014; Singla et al., 2016). A higher risk to public health is represented by the current study's findings of both single and concurrent *H.*

nana and *H. diminuta* infections (Stojcevic et al., 2004; Waugh et al., 2006; Easterbrook et al., 2007; Hancke et al., 2011).

The current investigation's 17.27% *H. nana* infection rate (including both single and concurrent infection) is very similar to that found in previous research by numerous researchers, such as 12.5% in South West Iranian rodents (Kia et al., 2001) and 11.0% in rats in the UK (Webster and MacDonald, 1995). Compared to our investigation, Gilioli et al. (2000) and Tanideh et al. (2010) showed a significantly higher prevalence in various laboratory rodents, 53.3% and 50.66%, respectively. The laboratory rodents' captivity may be the cause of this significant variation in rate.

The present investigation found a lower infection rate of 6.36% of *H. diminuta* (containing both single and concurrent infection), which is almost identical to the infection rates of 11.1% and 3.8% of *H. diminuta* reported by Kia et al. (2001) and Waugh et al. (2006), respectively. Compared to the current study, Kassen and Assefa (2000) from Addis Ababa, Ethiopia, Stojcevic et al. (2004) from Croatia, Abu-Madi et al. (2005) from Doha, Qatar, Kumarasinghe et al. (2006) from Sri Lanka, and Parasvaran et al. (2009) from Kuala Lampur documented higher infection rates of 30.7, 36.9, 35.8, 38.0, and 33.33% in various rodent species, respectively.

With a wide range of prevalence values, hymenolepiasis caused by both the cestodes species (*H. nana and H. diminuta*) is more prevalent in locations with poor structural and socioenvironmental conditions and where mice and people have close contact. Another source of infection is the environmental spread of *H. diminuta* eggs through beetle excrement (Pappas and Barley, 1999, Zhong et al., 2013, Makki et al., 2017). There have been reports of about 21 million cases of hymenolepiasis infection worldwide, primarily in tropical and subtropical areas. Because of auto-infection, the prevalence of *Hymenolepis* spp. in urban rodents is especially noteworthy. In *Hymenolepis* species, the ovum develops into an adult worm inside the host's gut without being expelled. The number of adult worms in the hosts' intestine rises as a result, increasing the likelihood that the environment would become contaminated with parasite eggs or ova in the stool (Tijani et al., 2020). Since gastrointestinal parasites are typically diagnosed using fecal microscopy, the affected population has been underreported (Stensvold et al., 2007). However, our study revealed that the number of parasite animals was the same when assessed using both the copro-parasitoscopical technique and gastrointestinal examination. Animals with fecal samples that tested positive for eggs were also discovered to have adult infections. Therefore, the copro-parasitoscopical approach offers good diagnostic sensitivity and specificity for cestodes, according to our study.

Compared to the current study, male rats had a marginally higher infection rate according to Sinnials et al. (1999) and Kia et al. (2010). This could be because males have longer exposure times to infections than females since they have larger territories (Brown et al., 1994) and overlapping home ranges (Ims, 1987).

A plan of action to control rodent helminths is necessary, since the evaluated articles in the current study identified some of the elements that can influence the population of rodent borne helminths in Kathmandu. Rodent-borne helminth abundance is dependent on the range and prevalence of the host organism (Antoniou et al., 2010; Eishhaziy et al., 2008). A rise in rodent populations may raise the possibility that humans will contract rodent parasites (Bordes, F., 2015). Animal zoonotic illnesses that use animals as hosts can be spread by rodents living in animal farms because they have easy access to animal feed (Alymeh, M., 2012).

The main strategy to prevent mouse zoonotic infections is to manage the rodent population (Eisen, R.J., 2013; Brown, L.M., 2015). Other contributing elements should also be considered when controlling rodent-related zoonoses, including rodent species, seasons, intermediate hosts, domestic rodent control management, and animal farms. Given that the majority of zoonotic helminths associated with rodents are associated with herbivores and carnivores (Centers for Disease Control and Prevention, 2020; Chaterjee, K.D. 2012; Sterba, J., 1976), controlling dogs, cats, and livestock animals is essential to halting the spread of helminth plague.

Accordingly, One Health practices are a useful strategy for reducing the frequency of rodent-borne helminths (Kaplan, B., et al., 2009). In order to achieve optimal health outcomes while acknowledging the interconnectedness of people, animals, plants, and their shared environment, "One Health is a collaborative, multisectoral, and transdisciplinary approach—working at the local, regional, national, and global levels" (Centers for Disease Control and Prevention, 2020). For the prevention and control of rodent helminths, One Health practices that connect the veterinary, medical, ecology, entomology, parasitology, zoology, and local populations are crucial.

6. Conclusion and Recommendation

6.1 Conclusion

Rodent species at Sundarijal of Gokarneshwor Municipality, were found to be infected with both nematodes and cestodes of helminth parasites. Overall prevalence of gastro-intestinal parasites was found to be 29.09%.

The current investigation verified that rodents are prone to contracting several gastrointestinal parasites. Although, Gokarneshwor Municipality has acceptable management methods, they still require improvement because GI parasite infections were discovered in the rodents.

This study provides baseline documentation for gastro-intestinal parasites. It will be important in developing control measures for GI parasites in rodents, as well as for other mammals over there.

6.2 Recommendation

The study's findings led to the formulation of the following recommendations:

- A complete picture of the rodents' natural zoonotic parasite infection could not be determined due to the study's tiny sample size. Therefore, it is important to maintain a suitable sample in relation to the study region.
- Additionally, rodent bacterial and protozoan parasites that are zoonotic to human health should be investigated.
- Given the dearth of scientific research on rats in Nepal and the possibility that they could serve as a reservoir for zoonotic diseases, this study is vital. There should be more research done in different cities to assess the possibility of human-to-zoonotic illness transfer.

Conflict of Interest

The study, the writing, and the publication of this work do not appear to have any potential conflicts of interest, according to the authors.

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Photograph Annex

Photograph 5: Materials used for the sampling.

Photograph 6: Samples collected, labelled and packed for the lab

Photograph 7, 8 and 9: Processing of fecal samples for centrifuging and examination under electronic microscope.

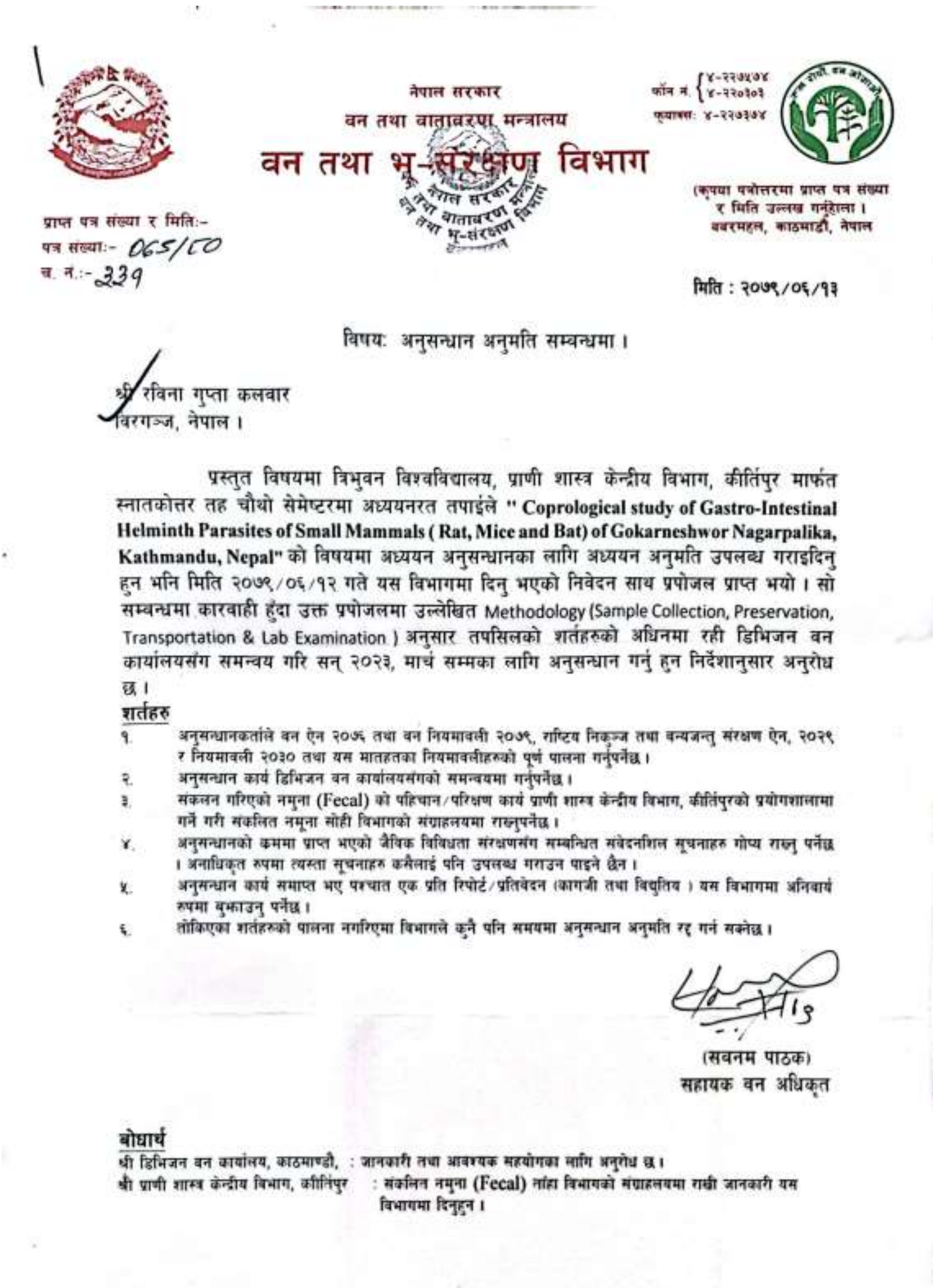
Photograph 10, 11: Field Visit at Sundarijal, Gokarneshwor Municipality,

Photograph12, 13: *Rattus norvegicus* Captured using Wire-mesh trap

Photograph 14: *Rattus rattus*

Photograph 15: *Mus musculus*

Photograph 16.17: Marking the ear/tail of rodents to prevent repetition of samples.



नेपाल सरकार
वन तथा वातावरण मन्त्रालय
वन तथा भू-संरक्षण विभाग

फोन नं. ४-२२७५७४, ४-२२०३०३
फ्याक्स: ४-२२७३७४

(कृपया पत्रोत्तरमा प्राप्त पत्र संख्या र मिति उल्लख गर्नुहोला । बबरमहल, काठमाडौं, नेपाल

प्राप्त पत्र संख्या र मिति:-
पत्र संख्या:- 065/80
च. नं.:- 339

मिति : २०७९/०६/१३

विषय: अनुसन्धान अनुमति सम्वन्धमा ।

श्री रविना गुप्ता कलवार
विरगञ्ज, नेपाल ।

प्रस्तुत विषयमा त्रिभुवन विश्वविद्यालय, प्राणी शास्त्र केन्द्रीय विभाग, कीर्तिपुर मार्फत स्नातकोत्तर तह चौथो सेमेष्टरमा अध्ययनरत तपाईंले " **Coprological study of Gastro-Intestinal Helminth Parasites of Small Mammals (Rat, Mice and Bat) of Gokarneshwor Nagarpalika, Kathmandu, Nepal"** को विषयमा अध्ययन अनुसन्धानका लागि अध्ययन अनुमति उपलब्ध गराइदिनु हुन भनि मिति २०७९/०६/१२ गते यस विभागमा दिनु भएको निवेदन साथ प्रपोजल प्राप्त भयो । सो सम्वन्धमा कारवाही हुँदा उक्त प्रपोजलमा उल्लेखित Methodology (Sample Collection, Preservation, Transportation & Lab Examination) अनुसार तपसिलको शर्तहरुको अधिनमा रही डिभिजन वन कार्यालयसँग समन्वय गरि सन् २०२३, मार्च सम्मका लागि अनुसन्धान गर्नु हुन निर्देशानुसार अनुरोध छ ।

शर्तहरु

१. अनुसन्धानकर्तांले वन ऐन २०७६ तथा वन नियमावली २०७९, राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण ऐन, २०२९ र नियमावली २०३० तथा यस मातहतका नियमावलीहरुको पूर्ण पालना गर्नुपर्नेछ ।
२. अनुसन्धान कार्य डिभिजन वन कार्यालयसँगको समन्वयमा गर्नुपर्नेछ ।
३. संकलन गरिएको नमुना (Fecal) को पहिचान/परिक्षण कार्य प्राणी शास्त्र केन्द्रीय विभाग, कीर्तिपुरको प्रयोगशालामा गर्ने गरी संकलित नमूना सोही विभागको संग्राहलयमा राख्नुपर्नेछ ।
४. अनुसन्धानको क्रममा प्राप्त भएको जैविक विविधता संरक्षणसँग सम्बन्धित संवेदनशिल सूचनाहरु गोप्य राख्नु पर्नेछ । अनाधिकृत रुपमा त्यस्ता सूचनाहरु कसैलाई पनि उपलब्ध गराउन पाइने छैन ।
५. अनुसन्धान कार्य समाप्त भए पश्चात एक प्रति रिपोर्ट/प्रतिवेदन (कागजी तथा विद्युतिय) यस विभागमा अनिवार्य रुपमा बुझाउनु पर्नेछ ।
६. तोकिएका शर्तहरुको पालना नगरिएमा विभागले कुनै पनि समयमा अनुसन्धान अनुमति रद्द गर्न सक्नेछ ।

(सवनम पाठक)
सहायक वन अधिकृत

बोधार्थ

श्री डिभिजन वन कार्यालय, काठमाण्डौ, : जानकारी तथा आवश्यक सहयोगका लागि अनुरोध छ ।
श्री प्राणी शास्त्र केन्द्रीय विभाग, कीर्तिपुर : संकलित नमुना (Fecal) त्यहा विभागको संग्राहलयमा राखी जानकारी यस विभागमा दिनुहुन ।

Photograph 18: Permission letter from Department of Forest and Soil Conservation