Gastrointestinal Parasitic Infection among Humans, Macaques, Dogs and Pigeons at Swayambhunath Mahachaitya, Kathmandu, Nepal

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A dissertation submitted
In partial fulfilment of the requirements for the award of the degree of Master of Science in Zoology with special paper Parasitology

May 2024

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Declaration

I hereby declare that the work presented in this dissertation "Gastrointestinal parasitic infection among humans, macaques, dogs and pigeons at Swayambhunath Mahachaitya, 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 "Gastrointestinal parasitic infection among humans, macaques, dogs and pigeons at Swayambhunath Mahachaitya, Kathmandu, Nepal" has been carried out by Anisha K.C. for the partial fulfilment 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 "Dr. Kishor Pandey" this dissertation submitted by Anisha K.C. entitled "Gastrointestinal parasitic infection among humans, macaques, dogs and pigeons at Swayambhunath Mahachaitya, 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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Abstract

Gastrointestinal (GI) parasites are the most common infectious agents in both humans and non-human animals. The interaction among humans and a wide variety of animals including wildlife, companion animals and avian species increases the likelihood of transmitting GI parasitic infections across different groups. They pose a significant but often neglected threat to public health, especially in developing countries. This study aimed to investigate the prevalence and diversity of GI parasites in humans, macaques, dogs and pigeons and to determine the risk of cross-species transmission. A cross-sectional study was performed from February 2023 to February 2024 at Swayambhunath Mahachaitya. A total of 200 fresh fecal samples were collected non-invasively comprising 50 from humans, 50 from macaques, 50 from dogs and 50 from pigeons. The samples were carefully labeled, preserved in 2.5% potassium dichromate and then transported to the laboratory at the CDZ. The samples were analyzed via iodine wet mount, floatation and sedimentation under the microscope at total magnifications of 100× and 400×. Data interpretation was carried out using venn diagram, tables and bar diagrams while statistical analysis was performed using R studio (version 4.3.2). The current study revealed an overall prevalence rate of 67% with specific rates of 16% in humans, 96% in macaques, 72% in dogs and 84% in pigeons respectively. Major GI parasites detected included *Ascaris lumbricoides* and *Entamoeba* spp. in humans. In macaques, *Balantidium coli*, Hookworm and *Strongyle* spp. were detected, while in dogs *Strongyloides* spp., Hookworm and *Strongyle* spp. were commonly found. Pigeons exhibited a higher prevalence of *Eimeria* spp., *Ascaridia* spp. and *Heterophyes* spp. Given the diverse range of parasites found across the study populations, this area presents a significant risk of cross-species transmission. Humans are susceptible to contracting parasitic infections from these animal populations, emphasizing the importance of implementing proper hygiene practices and parasite control measures. Further molecular studies will help characterization of parasite species and genotypes providing deeper insights into the potential for zoonotic cross transmission of parasites.

शोध सार

ग्यास्ट्रोइन्टेस्टाइनल परजीवीहरू मानव र गैर-मानव जनावरहरूमा सबैभन्दा सामान्य संक्रामक तत्व हुन् । तिनीहरूले विशेष गरी विकासोन्मुख देशहरूमा सार्वजनिक स्वास्थ्यका लागि महत्वपूर्ण तर प्रायः उपेक्षित खतरा खडा गर्छन् । यस अध्ययनको उद्देश्य मानिस, बाँदर, कुकुर र परेवाहरूमा जीआई परजीवीहरूको व्यापकता र विविधताको अनुसन्धान गर्नु र क्रस-प्रजाति प्रसारणको जोखिम निर्धारण गर्नु थियो । फागुन २०७९ देखि फागुन २०८० सम्म स्वयंभूनाथ महाचैत्यमा अन्तर-अनुभागीय अध्ययन गरिएको थियो । मानिसबाट पचास, बाँदरबाट पचास, कुकुरबाट पचास र परेवाबाट पचास सहित कुल दुई सय ताजा मल नमूनाहरू गैर-आक्रमणकारी रूपमा सङ्कलन गरिएको थियो । नमूनाहरू सावधानीपूर्वक लेबल गरियो, २.५% पोटेशियम डाइक्रोमेटमा संरक्षित गरियो र त्यसपछि केन्द्रीय प्राणी विज्ञान विभागको प्रयोगशालामा सारिएको थियो । १००× र ४००× को कुल आवर्धनमा माइक्रोस्कोप अन्तर्गत प्रत्यक्ष भिजेको माउन्ट, फ्लोटेशन र सेडिमेन्टेसनद्वारा नमूनाहरूको विश्लेषण गरिएको थियो । डेटा व्याख्या वेन रेखाचित्र, तालिकाहरू र बार रेखाचित्रहरू प्रयोग गरेर गरिएको थियो जबकि सांख्यिकीय विश्लेषण आर सफ्टवेयर प्रयोग गरेर गरिएको थियो । हालको अध्ययनले समग्र ६७% व्यापकता दर प्रकट गर्यो जसमा क्रमशः आठ (१६%) मानिस, अडचालीस (९६%) बाँदर, छत्तीस (७२%) कुकुर र बयालीस (८४%) परेवाहरू थिए । मानवमा पाइने प्रमुख जीआई परजीवीहरूमा एस्केरिस लुम्ब्रिकोइड्स र जियार्डिया ल्याम्ब्लिया समावेश थिए । बाँदरहरूमा, बालान्टिडियम कोलाई, हुकवर्म, र स्ट्रङ्गिलोइड्स एसपी पत्ता लगाइयो । कुकुरहरूमा स्ट्रङ्गिलोइड्स एसपी, हुकवर्म र स्ट्रङगाइल एसपी सामान्यतया पाइयो। परेवाहरूले इमेरिया एसपी, एस्केरिडिया एसपी, र हेटेरोफिस एसपीको व्यापकता प्रदर्शन गरे । अध्ययन गरिएको जनसङ्ख्यामा पाइने परजीवीहरूको ठूलो विविधताका कारण, यस क्षेत्रमा क्रस-प्रजाति सङ्क्रमणको जोखिम छ । उचित स्वच्छता अभ्यास र परजीवी नियन्त्रण उपायहरूको महत्त्वलाई जोड दिँदै मानिसहरूलाई यी जनावरहरूको जनसङ्ख्याबाट परजीवी सङ्क्रमण हुने खतरा हुन सक्छ । थप आणविक अध्ययनले परजीवी प्रजाति र जिनोटाइपहरूको चरित्र चित्रण गर्न मद्दत गर्नेछ र परजीवीहरूको जुनोटिक क्रस प्रसारणको सम्भाव्यतामा थप अन्तरदृष्टि दिनेछ ।

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List of abbreviations

Abbreviated form	Details of abbreviations
GI	Gastrointestinal
WHO	World Health Organization
UNESCO	United Nations Educational, Scientific and Cultural Organization
CDZ	Central Department of Zoology
NHPs	Non-Human Primates
TU	Tribhuvan University
IRC	Institutional Review Committee
IOST	Institute of Science and Technology
rpm	Rotation per minute
NaCl	Sodium Chloride
km	Kilometre
ml	Millilitre
m	metre

1. Introduction

1.1 Background

Gastrointestinal (GI) parasites are the most prevalent infectious agents of both humans and non-human animals including livestock, companion animals and wildlife particularly in rural Southeast Asian regions (Odermatt et al., 2010). Various GI parasites such as *Trichuris* spp., *Strongyloides* spp., *Ascaris* spp., *Schistosoma* spp. and hookworm often infect both humans and animals. Although the majority of people are asymptomatically colonized with parasites, the clinical presentation can range from mild abdominal discomfort or diarrhoea to serious complications such as perforation or bleeding (Hechenbleikner & McQuade, 2015). Domestic animals and wildlife play crucial roles as reservoirs that maintain zoonotic parasites under natural conditions from which humans might become infected (Youssef & Uga, 2014). These animal reservoirs frequently shed zoonotic parasites such as oocysts, eggs and larvae into the environment through their faeces (Ayinmode et al., 2016). In Nepal, diseases like taeniasis, leptospirosis, hydatidosis, brucellosis, toxoplasmosis and avian influenza have been recognized as priority zoonotic diseases due to their epidemic potential (Gautam et al., 2021). Parasitic zoonoses pose a significant yet often overlooked threat to public health, especially in developing countries (Devleesschauwer et al., 2014).

The term "Zoonoses" is derived from the Greek word "Zoon", which means animal and "nosos", which means illness. According to the World Health Organization (WHO), any disease or infection that is naturally transmissible from vertebrate animals to humans or from humans to animals is classified as a zoonosis (WHO, 2020). Zoonotic diseases may be bacterial, viral, parasitic, protozoan and fungal or they may involve unconventional agents that can cause a variety of illnesses in both humans and animals (Gautam et al., 2021). Infectious diseases have always been a part of human-animal interaction throughout history and almost half of all infectious diseases that affect humans have zoonotic roots (Rabinowitz et al., 2010). Around 60% of the emerging human infections are zoonotic in origin with over 70% of these pathogens originating from wildlife species (Esposito et al., 2023). Zoonoses pose a significant public health risk and directly threaten human wellbeing, potentially leading to death (Teichroeb et al., 2009).

Parasites are known to affect every group of organisms so, macaques, dogs and pigeons of Swayambhunath Mahachaitya are no exception because of their feeding and dwelling habits. Rhesus macaques, (*Macaca mulatta*, "Rato Bander" in Nepali) are distributed

widely across Nepal including the whole Terai region and Churia range. They are among the most widespread and evolutionary successful nonhuman primates (Xue et al., 2016). They often reside in close proximity to humans, forming probably the most intense relationship between human and non-human primates (Fuentes & Gamerl, 2005). Rhesus macaques often harbor a variety of parasites, some of which can be lethal depending on their type or the severity of infestation (Munene et al., 1998; Muriuki et al., 1998). Due to the close phylogenetic relationship between humans and macaques, there is considerable evidence of parasitic exchange between the two species (Chapman et al., 2005; Pedersen & Davies, 2009). Protozoan parasites such as *Giardia lamblia*, *Balantidium coli*, *Entamoeba histolytica* and *Cryptosporidium* spp. are frequently found in rhesus macaques along with helminth parasites like *Schistosoma mansoni*, *Oesophagostomum* spp., *Enterobius vermicularis*, *Strongyloides* spp., *Trichostrongylus*, *Trichuris*, *Ascaris*, *Chabertia*, hookworm and *Taenia*. These parasitic infections can lead to diarrhoea, hemorrhage, tissue damage, pulmonary abnormalities, abdominal issues, miscarriages, birth defects and mortality (Despommier et al., 2017) .

Dogs (*Canis lupus familiaris*) are the most widely distributed larger mammals in the world (Wynne, 2021). It is believed that dogs were among the first mammals to be domesticated, coexisting with humans across various eras and cultures since ancient times, including those of cave dwellers (Guedes et al., 2021). Therefore, they are known to regularly interact with humans, developing complex and multidirectional interspecific relationships (Bhattacharjee & Bhadra, 2020). GI parasites pose a significant concern for dogs, with a variety of species infecting them including *Giardia* spp., *Cystoisospora* spp., *Taenia* spp., *Echinococcus* spp., *Dipylidium* spp., *Toxocara* spp., *Ancylostoma* spp., *Capillaria* spp. and *Trichuris* spp. (Sukupayo & Tamang, 2023). These parasites are a source of several zoonotic diseases and the close proximity of dogs to humans can increase the risk of zoonotic disease transmission, especially when dogs are allowed to roam freely (Ortega-Pacheco et al., 2015).

Pigeons (*Columba livia)* are widely distributed avian species. Throughout history, humans have adopted pigeons as symbols of deities, peace, messengers, companionship, food and spiritual offerings. They are frequently seen in and around religious sites such as temples, gumbas, monasteries and urban areas all over the world (Adhikari et al., 2022). They are mucky birds responsible for disease and damage. They are a major source of infection and can transmit several diseases to humans and other animals (Patel et al., 2000). Among the

reviewed literature, Ascarid, *Capillaria* sp., *Raillietina* sp., *Echinostoma* sp., *Eimeria* sp., *Heterakis* sp., *Hymenolepis* sp., *Syngamus* sp. and *Tetrameres* sp. were commonly found GI parasites in pigeons (Gurung & Subedi, 2018). Pigeons have been positively identified as carriers of various diseases that can be transmitted to humans (Sukupayo, 2019).

The Swayambhunath area is characterized by frequent interactions between humans and animals including macaques, dogs and pigeons. These animals often inhabit religious sites and parklands close to human habitats, sharing community water sources which increases the likelihood of zoonotic disease transmission. The risk of such transmission may be particularly high in this area due to factors such as low levels of hygiene, overcrowded conditions, inadequate veterinary care and limited awareness of zoonotic diseases. These factors could contribute to the formation of new parasite-host relationships and new ecological niches in the disease transmission chain. Previous research has primarily focused on individual species and lacked comprehensive analysis of pathogen transmission risk between humans and animals that share the same environment. To address this gap, this study aims to explore parasitic infection among humans, macaques, dogs and pigeons at the popular UNESCO World Heritage Site, Swayambhunath Mahachaitya in Kathmandu, Nepal. The study seeks to establish baseline data on fecal parasites which will guide future research and help in the development of effective health strategies to prevent and control diseases.

1.2 Statement of the problem

Pathogens such as parasites can be transmitted between humans and animals, leading infectious diseases. Urban population growth and increased mobility of humans are two key factors that enhance the risk of parasite transfer by bringing humans and animals into close proximity. However, while urban wildlife and stray animals can serve as vectors of pathogens, the risks of transmission between wild-to-stray animal and animals-to-humans have often been overlooked. Previous research may have focused primarily on individual species and lacked a comprehensive analysis of potential cross-species transmission dynamics. Additionally, there may be limited data available on the prevalence of GI parasites present in the study area across different host species. Therefore, to date, there is limited understanding of the risk of pathogen transmission between humans, wildlife and stray animals that share the same environment. To address this gap, this study aims to explore the risk of parasitic infection among humans, macaques, dogs and pigeons at the

popular UNESCO World Heritage Site, Swayambhunath Mahachaitya in Kathmandu, Nepal.

1.3 Objectives

1.3.1 General objective

Gastrointestinal parasitic infection among humans, macaques, dogs and pigeons at Swayambhunath Mahachaitya, Kathmandu, Nepal.

1.3.2 Specific objectives

- To determine the overall prevalence of GI parasites among humans, macaques, dogs and pigeons.
- To identify the diverse GI parasites present within each study group.
- To identify the common GI parasites of zoonotic importance across different study groups.

1.4 Research questions

- What is the overall prevalence of GI parasites among humans, macaques, dogs and pigeons in the Swayambhunath area?
- What are the various types of GI parasites found in each species?
- Are there any common zoonotic GI parasites among the species identified in the study area?

1.5 Significance of the study

The study "Gastrointestinal parasitic infection among humans, macaques, dogs and pigeons at Swayambhunath Mahachaitya, Kathmandu, Nepal", holds significant implications for public health and wildlife conservation efforts. Firstly, understanding the prevalence and diversity of parasitic infections in multiple species residing in close proximity can provide valuable insights into the transmission pathways of these parasites, potentially shedding light on cross-species transmission and zoonotic risks. Such knowledge is crucial for developing targeted intervention strategies aimed at controlling parasite spread and minimizing the risk of human infections. The Swayambhunath area is culturally and religiously important and attracts many visitors from both within the country and internationally. Investigating GI parasite co-infections in this region is important for protecting public health. Furthermore, the study also holds relevance for wildlife conservation efforts as it can help identify parasitic threats to the local animal populations,

inform conservation strategies and contribute to the overall health and well-being of the ecosystem. Thus, this research has far-reaching implications for both human and animal health, as well as for the preservation of biodiversity in the Swayambhunath area and beyond.

1.6 Limitations of the study

- Though the study focuses on a specific area (Swayambhunath Mahachaitya), it would be interesting to see if the study and methodology could be replicated in other regions or specific eras.
- Fecal samples were collected from only a limited section of the population within the study area. This suggests the possibility of expanding sample collection to broaden the scope of further research.
- The study provides the groundwork for future research to employ molecular techniques, enabling species-level identification of eggs.

2. Literature review

Parasites are organisms that depend on other hosts for their survival. Certain parasites may not produce observable symptoms in their hosts whereas others can proliferate, reproduce or invade their host's organ system, causing illness and ultimately resulting in a parasitic infection (Kinman, 2018). GI or gut infection is mainly caused by various species of protozoa and helminth parasites (Suntaravitun & Dokmaikaw, 2018). GI parasitic infection widespread globally and constitute a major public health challenge. It is responsible for causing significant morbidity and mortality all over the world particularly in developing countries. Nepal is small improvised country in South-Asia, where 70% of morbidity and mortality are associated with infectious diseases. Among the various types of infectious diseases, GI parasitic infection alone constitutes one of the major causes of health problems (Agrawal et al., 2012). Human acquisition of GI parasites is estimated to occur with greater prevalence ranging up to 50% in developed and up to 95% in developing and underdeveloped countries throughout the world (Baral et al., 2017).

Approximately 3.5 billion peoples are infected with intestinal parasites and around 450 million children suffer from these infections (WHO, 1998). Intestinal parasites are widely prevalent in developing countries, likely due to poor sanitation and inadequate personal hygiene. It is estimated that up to 60% of the world's population is infected with intestinal parasites (Ragunathan et al., 2010). Adhikari et al. (2021) in his study, "Prevalence and risk factors of GI parasites in the Chepangs in Nepal" collected fecal samples from 100 indigenous Chepangs. They found that 97% humans harbored 14 different species of GI parasites including eight protozoan parasites (*Balantidium coli*, *Blastocystis hominis*, *Cryptosporidium* sp., *Cyclospora cayetanensis*, *Entamoeba coli*, *Entamoeba histolytica*, *Giardia lamblia* and *Iodamoeba buetschlii*) and six helminth parasites (*Ascaris lumbricoides*, hookworm, *Hymenolepis nana*, *Strongyloides stercoralis*, *Trichostrongylus* and *Trichuris trichiura*). Yadav (2023) examined GI parasites in the Musahar community in Balan Bihul, Saptari, Nepal. They observed a total prevalence of 81% with nine different parasite species including *Ancylostoma* sp. (41.5%), *Ascaris lumbricoides* (29%), *Entamoeba* sp. (31.5%), *Entamoeba coli* (21.5%), *Trichuris trichuria* (16%), *Strongyloides stercoralis* (8.5%), *Giardia* sp. (7%), *Hymenolepis nana* (14%) and *Balantidium coli* (2%). A study conducted to determine the GI parasitoses among the Chepang and Musahar community people of Makwanpur and Nawalparasi districts of Nepal revealed 36.6% with similar prevalence among Chepangs (39.8%) and Musahars (33.3%). The most

predominant helminth was *Ascaris lumbricoides* (15.6%), while the most prevalent protozoan was *Entamoeba histolytica*/*dispar* (5.4%). The study also found a significant association between parasite prevalence and socio-demographic factors, types of drinking water consumption and sanitation habits (Khadka et al., 2021). In Rupandehi district, Subedi et al. (2020) reported a 18.66% positive rate for the presence of at least one GI parasites. The prevalence rate was considerably higher in public school children (22.66%) compared to private school (14.66%). Four genera of parasites were identified, with *Ascaris lumbricoides* being the most common, followed by *Trichuris trichiura*, hookworm and *Taenia* sp.

In 2007, two separate studies were conducted on the prevalence of helminth infections among Rhesus macaques in the Kathmandu Valley. Dhoubhadel (2007), in the Swayambhu and Nilbarahi areas revealed a prevalence of 63.5%, with *Strongyloides fulleborni* being the most prevalent at 42.5%. Other identified parasites included *Dictyocaulus* sp. (7.87%), *Taenia* sp. (7.08%) and *Oesophagostomum* sp. (6.29%). Concurrently, Malla (2007) in the Pashupati and Nilbarahi areas reported a slightly lower prevalence of 61.38%. Here, *Strongyloides fulleborni* remained the dominant parasite at 51.61%, followed by *Oxyuris* sp. (11.29%) and *Ascaris lumbricoides* (10.48%). Interestingly, while some parasites appeared in both studies, their prevalence rates varied. Malla (2007) and Dhoubhadel (2007) documented *Dictyocaulus* sp., *Taenia* sp., *Ostertagia* sp., *Cooperia* sp., *Prosthenorchis elegans*, *Dicrocoelium* sp., *Oxyuris* sp. and *Chabertia* sp. for the first time in Rhesus macaques from Nepal whereas *Prosthenorchis elegans* was reported for the first time in Nepal. Furthermore, Nepal (2010) recorded overall prevalence rate of 85% among Rhesus macaques in the Swayambhu area of Kathmandu. The study identified 15 nematode species including *Strongyloides* sp. (27.06%), *Toxocara* sp. (12.94%), *Trichostrongylus* sp. (11.37%), *Oesophagostomum* sp. (10.59%), *Trichuris* sp. (9.80%), *Chabertia* sp. (8.63%), *Dictyocaulus* sp. (7.45%), *Ascaris* sp. (7.45%), *Capillaria* sp. (6.27%), *Ostertagia* sp. (5.88%), *Cooperia* sp. (4.31%), *Haemonchus* sp. (4.31%), *Oxyuris* sp. (3.14%), *Ancylostoma* sp. (2.75%) and *Bunostomum* sp. (1.96%). Additionally, three trematodes were identified including *Schistosoma* sp. (18.04%), *Dicrocoelium* sp. (9.80%) and *Fasciola* sp. (9.80%) along with two cestodes, *Dipylidium* sp. (21.57%) and *Taenia* sp. (9.80%). *Bunostomum* sp., *Dipylidium* sp. and *Schistosoma* sp., were reported for the first time in Nepal in Rhesus macaques. Additionally, Jha et al. (2011) detected three protozoan species: *Balantidium coli* (32.23%), *Entamoeba histolytica* (26.4%), *Entamoeba coli*

(21.49%) and 10 species of helminths: *Oesophagostomum* being highest (35.54%), followed by *Strongyloides* (28.92%), *Trichuris* (14.05%), *Trichostrongylus* (11.57%), *Toxocara* (4.96%), *Trichurid* and four unknown species in the temple of Kathmandu valley and showed an overall infection rate of 76.86% for all GI parasites with 53.72% for protozoan and 59.5% for helminthic parasites. Likewise, Sharma et al. (2013) recorded a prevalence rate of 59.32% in Rhesus macaques of Himachal Pradesh, India. A total of five different species of parasites were found to be distributed among Rhesus macaques including *Strongyle* spp. (55.42%), *Strongyloides* sp. (17.23%), *Trichuris* sp. (11.52%), Coccidia (8.78%) and *Ascaris* sp. (7.43%). Similarly, the study conducted in Namakkal, Tamil Nadu, India, revealed a 43% prevalence of endoparasitic infections among Rhesus macaques. It highlighted a high prevalence of *Strongyle* spp. (33%), followed by *Ascaris* spp. (5%) and *Eimeria* spp. (3%) in single infections. In cases of mixed infection, combinations of *Strongyle* sp. and *Ascarids* (1.7%), as well as *Strongyle* sp. and Coccidia (3%), were observed. Interestingly, none of the samples tested positive for multiple infections (Arunachalam et al., 2015). A similar study conducted on captive Rhesus macaques at Bangladesh National Zoo reported an overall parasitic prevalence of 100%. Six different types of helminth parasites were recorded with *Ascaris* spp. showing the highest infection rate in Rhesus macaques (Tabasshum et al., 2018). In the same year, another study on Rhesus macaques and Hanuman langur in Devghat, Chitwan, showed the highest infection of *Balantidium coli* (27.95%), followed by *Trichuris* sp. (23.65%), *Eimeria* sp. (16.12%), *Entamoeba* sp. (13.97%), *Ascaris* sp. (11.82%), *Strongyloides* sp. (10.75%), *Oesophagostomum* sp. (5.37%), hookorm sp. (3.22%), *Trichostrongylus* sp. (3.22%) and *Hymenolepis* sp. (1.07%) with an overall parasitic prevalence rate of 74.20% (Adhikari & Dhakal, 2018). A similar study conducted on Rhesus macaques in Chitwan-Annapurna landscape reported an overall parasitic prevalence of 80%. A total of 16 different types of parasites were recorded with the highest infection of *Cryptosporidium* in Rhesus macaques (Dhakal et al., 2018). However, in a study of Rhesus macaques inhabiting Bajrabarahee temple, 100% prevalence rate of parasites were observed with the highest infection of *Entamoeba* spp. (66.7%) followed by *Balantidium coli* (59.5%), *Entamoeba coli* (57.1%), *Ascarid* spp. (21.4%), *Strongyloides* sp. (21.4%), hookworm (19%), *Trichuris* sp. (14.3%), *Cryptosporidium* sp. (11.9%), Strongylid spp. (9.5%), *Eimeria* sp. (7.1%), *Giardia* sp. (4.8%) and *Trichomonas* sp. (2.4%). Most of them were zoonotically significant and can cause a serious harm to humans (Sapkota et al., 2020). In contrast, a study of Rhesus macaques at Dharan recorded a prevalence rate of 54.03%, with the most

commonly detected parasites being *Entamoeba coli* (25.37%), *Ascaris* sp. (34.32%) and *Ancylostoma* sp. (17.91%) (Thakuri et al., 2021). In a study of parasitic infestation in free ranging and captive Rhesus macaques in Bangladesh, Dhaka, Muznebin et al. (2022) identified parasites belonging to 30 species. Among these were six species of protozoa (*Entamoeba coli*, *Eimeria* sp., *Isospora* sp., *Toxoplasma gondii*, *Chilomastix mesnili* and *Gregarina* sp.), five species of cestodes (*Taenia* sp., *Moniezia* sp., *Reillietina* sp., *Bertiella* sp. and *Amoebataenia* sp.), 11 species of nematodes (*Ascaris lumbricoides*, *Toxocara* sp., *Trichuris trichiura*, *Strongyloides* sp., *Ancylostoma* sp., *Ascarops* sp., *Gongylonema* sp., *Gnathostoma* sp., *Subulura* sp., *Enterobius* sp. and *Capillaria* sp.) and seven species of trematodes (*Neoglyphe* sp., *Watsonius watsoni*, *Schistosoma mansoni*, *Paragonimus* sp., *Clonorchis sinensis*, *Brachylaemus* sp. and *Gastrothylax* sp.). Finally, one species of pentastomida, *Linguatula* sp., was identified. Chhetala (2022) recorded a prevalence rate of 13% in humans and 81% in Rhesus macaques in the Nilbarahi area, Bhaktapur, Nepal. The study in humans identified three species of parasites including *Cryptosporidium* sp. (7%), *Taenia* sp. (2%) and *Ascaris lumbricoides* (5%). In contrast, in Rhesus macaques, 15 species of GI parasites were detected among which eight species being protozoa and seven species being helminths. The most common protozoan infections were *Entamoeba* spp. followed by *Entamoeba coli*, *Balantidium coli* and *Cryptosporidium* sp., while the most common helminthic infections were *Ascarid* spp. and *Strongyle* sp. In contrast, a study conducted on free ranging Rhesus macaques in Bangladesh reported an overall parasitic prevalence of 54.4%. The study identified a total of six nematodes (*Toxocara* spp., *Trichuris* spp., *Strongyloides* spp., *Trichostrongylus* spp., *Dictyocaulus* spp. and *Ascaris* spp.), four cestodes (*Hymenolepis nana*, *Moniezia* spp., *Diphylobothrium* spp., *Taenia* spp.), one trematode (*Fasciolopsis buski*) and three protozoa (*Balantidium coli*, *Entamoeba* spp., Coccidia spp.) in the macaques (Islam et al., 2022). Adhikari et al. (2023) examined the prevalence and intensity of GI parasites in urban Rhesus macaques in the Kathmandu Valley. A total prevalence of 87.6% was observed with 14 different parasite species, comprising five protozoans, one coccidian and eight helminths. Notably, *Entamoeba coli* (54.71%) and *Balantidium coli* (44.33%) were the most prevalent protozoans while *Trichuris* spp. and *Strongyloides* spp. (both 31.13%) dominated among helminths. Additionally, *Iodamoeba buetschlii* was identified for the first time in Nepalese non-human primates (NHPs) with a prevalence of 10% across multiple sampled locations including Pashupatinath temple, Swayambhunath stupa and Tripureshwor Mahadev temple. Another similar study was conducted by Jain & Maharjan (2023) in Rhesus macaques of Shivapuri

Nagarjun National Park and temple areas of Kathmandu valley. A total five species of protozoan parasites and eight species of helminth parasites, indicating 100% prevalence rate where *Entamoeba coli* (67.14%) showed the highest prevalence followed by *Trichostrongylus* spp. (1.43%) with the lowest prevalence. *Nematodirus* spp. was identified for the first time from the samples of Rhesus macaques in Nepal. Tandan (2023) recorded a prevalence rate of 11.3% in humans and 39.2% in Rhesus macaques in the Daunne, Nawalpur, Nepal. Only one parasite species *Ascaris* sp. were detected in human samples. A total of four different species of helminths parasites were found to be distributed among Rhesus macaques including *Strongyloides* sp., *Ascaris* sp., hookworm and *Trichuris* sp. Sharma et al. (2023) conducted a study revealing 71.15% prevalence of parasitic infections among Rhesus macaques in Uttarakhand, India. The research identified seven genera of parasites including *Trichuris* spp. (23.07%), *Hymenolepis* spp. (5.76%), *Strongyloides* spp. (13.46%), *Ancylostoma* spp. (19.23%), *Entamoeba* cysts (26.92%), *Ascaris* spp. (7.69%), *Strongyle* eggs (13.46%) and unidentified cestode eggs (17.30%).

Traub et al. (2002), in his study, "The role of dogs in transmission of GI parasites in Northeast India" collected fecal sample from 101 dogs and 328 humans from the three tea estates over a three-month period. Nearly all, 99% dogs harbored one or more zoonotic species of GI parasites, with hookworm infection being most common (94%). Parasitic stages presumed to be host-specific for humans such as *Ascaris* spp. (31%), *Trichuris trichiura* (25%) and *Isospora belli* (2%) were also recovered from dog feces. Satyal et al. (2013) examined the prevalence of zoonotic GI helminths in dogs of Kathmandu, Nepal. A total prevalence of 46.7% was observed with five different parasite species including *Ancylostoma* spp. (52.0%), *Toxocara canis* (41.8%), *Taenia* spp. (15.3%), *Dipylidium caninum* (9.2%) and *Trichuris vulpis* (5.1%). The study conducted to investigate, "Zoonotic GI parasite burden of local dogs in Zaria, Northern Nigeria", revealed that zoonotic GI parasites of dogs are endemic in Zaria and the general public in the area are at high risk of being infected with these parasites. Out of 224 fecal samples analyzed, 76 (33.9%) were positive of at least one of the parasites. Of the 101 samples from streets and residential quarters of ABU, Zaria, *Isospora* spp. (11.9%) recorded the highest prevalence rate followed by *Taenia* spp. (5.9%), then *Toxocara canis*, *Ancylostoma caninum* and *Dipylidium caninum* were 5.0%, 4.0% and 1.0%, respectively (Ogbaje et al., 2015). In Rupandehi district, Yadav and Shrestha (2017) reported 58.75% positive samples for the presence of at least one of the zoonotic helminths. In that study, stray dogs had a

considerably greater prevalence of helminth parasites (78.5%) than pet dogs (39%) did. The common parasites observed in present study were *Ancylostoma* spp. (46.81%), *Toxocara canis* (37.87%), *Taenia* spp. (9.36%), *Dipylidium caninum* (22.98%), *Trichuris vulpis* (5.73%) and *Diphyllobothrium* spp. (2.98%). Bhattarai (2022) study revealed 27.5% prevalence of helminth infections, considerably higher prevalence in stray dogs (41.33%) than in pet dogs (13.33%). *Ancylostoma* sp. (39.39%) showed the highest prevalence among the five helminth parasites that were identified in this study, followed by *Taenia* sp. (24.24%), *Ascaris* sp. (15.15%), *Toxocara* sp. (12.12%) and *Trichostrongylus* sp. (9.09%). In a study conducted by Adhikari et al. (2023), among street dogs of Lalitpur had an overall prevalence of 95.7% with 23 diverse species of GI parasites (10 protozoa and 13 helminths). Among them, five protozoa (*Cryptosporidium* sp., *Entamoeba* sp., *Giardia* sp., *Sarcocystis* spp. and *Balantidium coli*) and 11 helminths (*Ancylostoma caninum*, *Ancylostoma braziliense*, Taeniid, *Toxocara canis*, *Trichuris vulpis*, *Strongyloides* sp., *Dipylidium caninum*, *Strongyle*, *Troglotrema salmincola*, *Capillaria plica* and *Capillaria aerophila*) possessed zoonotic potential and their overall prevalence was 92.5%. Since most of the reported parasites are zoonotic, dog density and parasitic richness indicate a greater spillover risk to humans and domestic animals. In contrast, Sukupayo and Tamang (2023) study revealed 59.50% prevalence of helminth infections, with a significantly higher prevalence in stray dogs (70%) than that in pet dogs (49%). *Ancylostoma* spp., *Toxocara* spp., *Trichuris* spp., *Capillaria* spp., *Dipylidium caninum* and *Taenia* spp. were six different zoonotic species found in the current study. The study showed the highest prevalence of *Ancylostoma* spp. (49.16%) and the least prevalence of *Capillaria* spp. (0.84%). The study highlighted the severe environmental contamination shed by dogs, causing a higher risk of zoonotic transmission. Bastakoti et al. (2023) investigated the prevalence of zoonotic helminths among street dogs in the Madi valley of Chitwan, Nepal. They found a high overall prevalence of 81.86%, identifying nine different parasite species including *Acylostoma* sp., *Toxocara* sp., *Taenia* spp., *Diphyllobothrium* sp., *Spirocera* sp., *Strongyloides* sp., *Spirometra* sp., *Dipylidium* sp. and *Echinococcus* sp. Also, 77.24% of these parasites, were identified as zoonotically significant, emphasizing the potential risk posed by these parasites to human health.

A study by Sukupayo (2018) investigated the prevalence of GI parasites among pigeons in Suryabinayak, Bhaktapur. A total prevalence of 53.24% was observed with various protozoan and helminth parasites. Protozoa such as *Eimeria* spp. (22.08%), along with

nematodes like *Ascaridia columbae* (27.27%) and *Capillaria* spp. (11.68%) and cestodes like *Raillietina* spp. (5.19%), were observed. In three temples of Pokhara valley, Gurung and Subedi (2018) reported 69.16% prevalence rate of parasitic infection in pigeons. Total of seven GI parasites that includes one subclass of protozoan: Coccidia (19.16%) and six genera of helminths: *Capillaria* sp. (31.67%), *Ascaridia* sp. (21.66%), *Echinostoma* sp. (7.50%), *Syangamus* sp. (5.83%), *Hymenolepis* sp. (3.33%) and *Hetarakis* sp. (2.50%) were identified and reported first time in Nepal. The higher prevalence of GI parasites was in Bhadrakali temple with (77.50%) followed by Tal Barahi temple (72.50%) and lowest in Bindhyabasini temple (57.50%). The study indicates that pigeons in three temples of Pokhara valley were highly susceptible to GI parasites. Adhikari et al. (2022) conducted a study showing a prevalence rate of 87.1% among pigeons found in Ratnanagar Municipality, Chitwan, including *Eimeria columbae* (46.5%), *Capillaria columbae* (29.7%), *Eimeria columbarum* (23.2%), *Ascaridia* sp. (22.6%), *Entamoeba* sp. (14.2%), *Eimeria labbeana* (10.3%), *Echinostoma* sp. (8.5%), *Heterophyes* sp. (7.7%), *Eimeria kapotei* (7.7%), *Isospora* sp. (7.1%), *Heterakis* sp. (7.1%), *Capillaria annulata* (5.8%), *Strongyle* (5.2%), *Caryospora* sp. (3.9%), *Cryptosporidium* sp. (3.9%) and *Hymenolepis* sp. (1.9%). They found that temple pigeons exhibited a higher prevalence rate and greater parasitic richness compared to household pigeons. According to the study conducted in Kerala by Thankachan et al. (2022), out of 91 pigeons tested, 79 were found positive for ova of GI parasites with a prevalence of 86.8%. *Ascaridia* spp. (59.3%) and *Capillaria* spp. (19.7%) were the most common nematodes observed. Oocysts of *Eimeria* spp. was observed in 39.5% of pigeons screened. This study depicts a very high occurrence of GI parasites among pigeons of Kerala. Jha et al. (2023) examined the prevalence of GI parasites of feral pigeon of Kathmandu, Nepal. A total prevalence of 90.83% was observed with six different parasite species including one genera of protozoa: *Eimeria* sp. (43.34%) and five genera of helminths: *Capillaria* sp. (51.67%) followed by *Ascaridia* sp. (27.50%), *Heterakis* sp. (19.17%), *Syngamus* sp. (4.17%) and *Tetrameres* sp. (1.70%). The prevalence rate of helminths (83.34%) was higher than prevalence rate of protozoan parasites (43.34%). The current study revealed heavy infection in feral pigeons at two temples of Kathmandu valley.

Various parasites that infect humans require animals in some stage of their life cycle (Devleesschauwer et al., 2014). Devleesschauwer et al. (2014) in a systematic review found that a large number of parasitic zoonoses are present in Nepal and are imposing an impact

higher than that of malaria and comparable to that of HIV/AIDS. In a study conducted by Zanetti et al. (2021) in different class of animal hosts, domestic and wild, from Brazil during 2019, an overall prevalence of 79.64% was recorded. *Blastocystis* sp. emerged as the predominant protozoan while *Ascaris* sp. was prevalent among helminths. Due to the great diversity of parasites found in the animal host, they can play an important role in the transmission and maintenance of the infection to other mammals, including humans. Similarly, Schär et al. (2014) in his study to determine the GI parasitic infections in humans and domestic animals including dogs in a rural Cambodian village, 87.2% humans and 81.9% dogs had at least one or more GI parasitic infections respectively. In total, 14 different parasite species were diagnosed, including eight helminthic and six protozoan parasites. Major GI parasitic infections found in humans included hookworms (63.3%), *Entamoeba* spp. (27.1%) and *Strongyloides stercoralis* (24.3%). In dogs, hookworm (80.8%), *Spirometra* spp. (21.3%) and *Strongyloides* spp. (14.9%) were most commonly detected. Eleven parasite species were detected in dogs (eight helminths and three protozoa), seven of which have zoonotic potential, including hookworm, *Strongyloides* spp., *Trichuris* spp., *Toxocara canis*, *Echinostoma* spp., *Giardia duodenalis* and *Entamoeba* spp. In a study to evaluate the risk of GI protozoan infection among laboratory macaques, animal facility workers and nearby villagers, Li et al. (2021) recorded an overall prevalence of 37.2% including 36.4% in macaques, 56% in facility workers and 28% in villagers respectively infected with one or more protozoa (*Enterocytozoan bieneusi*, *Cyclospora cayetanensis*, *Cryptosporidium* spp. and *Giardia intestinalis*). These results warrant the workers to limit contact with infected animals in order to minimize related health risks. Using an evolutionary framework, Pedersen and Davies (2009) identified Central Africa and Amazonia as hotspots for disease transmission among wild primates due to their high diversity of closely related species. Furthermore, regions in Central and Western Africa pose a higher risk of pathogen spillover to humans due to frequent interaction with wild primate populations, which is made worse by rapid human population growth, close proximity to apes and high-density population centres that promote increased contact rates among individuals. In between July 2017 to June 2018, the study was conducted in North-Eastern region of India to identify different GI parasites in various captive NHPs. Out of 145 NHPs examined, 32 (22.06%) were found positive with different parasites such as eggs of hookworms, *Trichuris* sp., *Ascarid*, cysts of *Balantidium coli* and *Giardia* sp. (Patra et al., 2018). In Bali, Indonesia, where agricultural-religious temples host long-tailed macaques near human communities, a study by Lane-deGraaf et al. (2014) examines

people's willingness to engage in behaviors risking exposure to GI parasites. Factors like age, education and occupation influence this willingness. The study highlights that certain activities like direct macaque contact and pet ownership may pose significant infection risks depending on local contexts. The study conducted to determine the prevalence of GI parasites of humans and dogs in Amazah District of Jos North, Nigeria recorded an overall prevalence of 41% and 52% respectively. Humans harbored hookworm, *Schistosoma* spp., *Giardia lamblia*, *Taenia* spp., *Strongyloides* spp., *Trichuris trichiura* and *Vampirolepis nana* whereas dogs harbored *Ancylostoma caninum*, *Isospora* spp., *Taenia* spp., *Dipylidium caninum* and *Toxocara* spp. (Golshang et al., 2023). A study was conducted to evaluate the prevalence of GI parasites in primates and their keepers from zoological gardens in Ibadan, by Adetunji (2014) which recorded the prevalence of 61.1% among NHPs. A total of four parasites were identified consisting of three nematodes species and one protozoa. The most prevalent GI helminths were *Trichuris trichiura* (47.2%), followed by *Strongyle* spp. (13.9%) and *Strongyloides* spp. (5.6%). *Entamoeba* spp is the only protozoa detected in 13.9% of the NHP's. Only one of the 19 zoo keepers screened was infested with *Ascaris lumbricoides* and two (15.4%) of the 13 members of control group were infested with *Ancylostoma duodenale*. There was no evidence of cross transmission of GI helminths between the NHP and the zoo keepers. Taylor et al. (2001) in his review identified 1415 species of infectious organism known to be pathogenic to humans including 66 protozoa and 287 helminths. Out of the identified species, 868 (61%) are zoonotic in nature. This study was conducted to determine the prevalence of potentially zoonotic GI parasites in domestic dogs and cats in Moscow (Russia). The total parasitic prevalence in dogs was; *Giardia* spp. (10.2%), *Cryptosporidium* spp. (2.7%), *Toxocara canis* (2%), *Strongyloides stercoralis* larvae (1.1%) (Kurnosova et al., 2023). For the detection of potentially zoonotic GI parasites in long-tailed macaques, dogs and cattle at Kosamphi forest park, Maha Sarakham, the study was conducted during November 2015 to April 2016. Parasites were found in 42 out of 67 long-tailed macaques (62.69%), 14 out of 32 dogs (43.69%) and 23 out of 35 cattles (65.71%). Three species of parasitic helminths including *Strongyloides* spp., *Trichuris* spp. and hookworm eggs which have been reported to be potentially zoonotic helminths were identified in all three animal species (Pumipuntu, 2018). In a study conducted by Schurer et al. (2019), fecal samples from 102 macaques and 115 people were analyzed to assess the presence of zoonotic parasites. The findings revealed that 44% of macaques and 12% of people were infected with GI helminths including *Strongyloides* spp., *Ascaris* spp. and *Trichuris* sp. These results highlight the potential for zoonotic

transmission of parasites between macaques and humans. According to the study conducted in Bangkok by Inpankaew et al. (2007), dogs in temple communities posed a potential zoonotic risk to humans and other animals for transmission of hookworms, *Giardia* (especially Assemblage A genotypes) and *Toxocara canis*. Out of 204 humans and 229 dog's fecal sample from 20 different temples, hookworms were the most common parasite in dogs (58.1%) followed by *Trichuris* (20.5%), *Isospora* (10%), *Giardia* (7.9%), *Toxocara* (7.4%), *Dipylidium caninum* (4.4%) and *Spirometra* (3.1%) whereas *Blastocystis hominis* (5.9%) was the most common parasite in humans followed by hookworms (3.4%), *Giardia* (2.5%), *Strongyloides* (2%) and *Cryptosporidium* (1.5%).

3. Materials and methods

3.1 Study area

Swayambhunath Mahachaitya is an ancient religious site situated atop a hillock in the Kathmandu Valley of Nepal. The temple sits at an elevation of 1524 m above sea level and 76.2 m above the Kathmandu Valley. The site is located in a region between 27.76-27.77 latitude North and 85.28-85.29 longitude East. It covers an area of 37 hectares (Figure 1), much of which is influenced by human settlement. Swayambhunath is surrounded by shops and houses while the parkland is bordered with timber-sized trees, small stupas and chaityas. Due to its religious significance, the temple attracts religious devotees, tourists and local residents, facilitating frequent interactions between humans and the diverse wildlife found in the area.

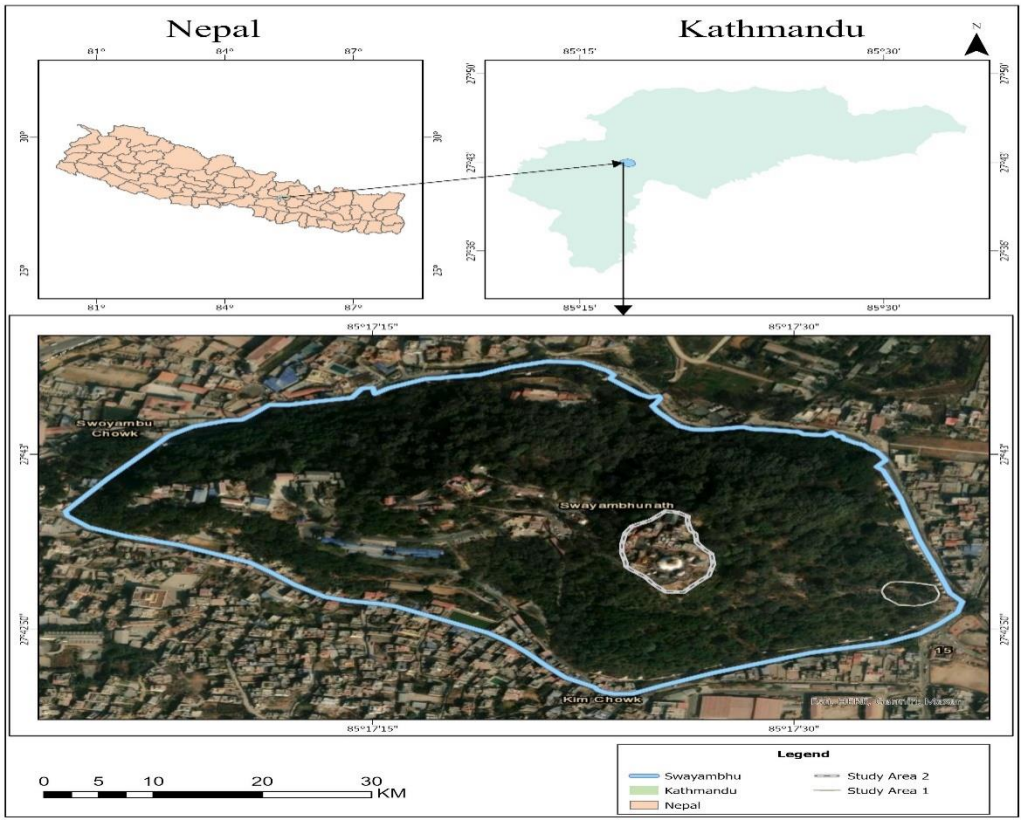


Figure 1. Map illustrating the geographic location of Swayambhunath Mahachaitya

3.2 Methods

3.2.1 Study design

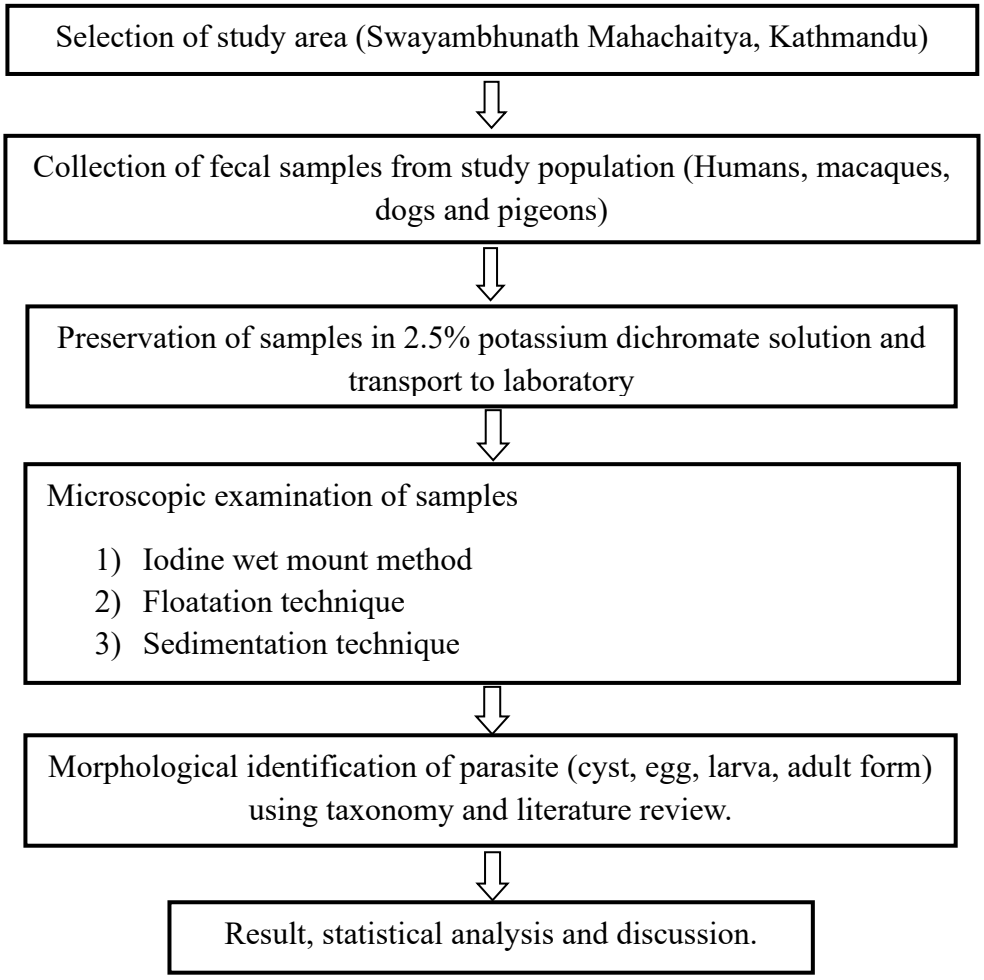


Figure 2. Research design of the study.

3.2.2 Ethical consideration

Ethical clearance for this study was obtained from the Institutional Review Committee (IRC) of the Institute of Science and Technology (IOST), Tribhuvan University (TU) with the approval number IRCIOST-23-0072. Additionally, an official permit (reference number 785/078-079) was acquired from the Department of Forest and Soil Conservation, Government of Nepal, ensuring compliance with ethical and legal regulations set by the government.

Participation of human subjects in the study was voluntary. Verbal and written informed consent were obtained from each participant. Prior to their participation, the detailed purpose and procedures of the study were explained. Those who expressed willingness to

participate in the research and completed written consent forms were enrolled. Moreover, to protect participant anonymity, no personal identifiers were associated with the samples.

3.2.3 Preliminary field survey

A preliminary field survey was conducted in February 2023 to understand the study area and it's population. Additional information regarding the identification of rhesus macaques and dogs inhabiting the Swayambhunath area was gathered during this survey.

3.2.4 Sample size and criteria for sample selection

A total of 200 fecal samples (50 from humans, 50 from macaques, 50 from dogs and 50 from pigeons) were collected non-invasively from June to October 2023. The decision to collect 50 samples from each species was made considering the considerably low population of dogs residing within one km of the Swayambhunath Mahachaitya area. This sample size was mirrored for humans, macaques and pigeons to ensure a balanced representation of the different populations in the study area.

3.2.4.1 Inclusion criteria

- Human participants must reside near Swayambhunath temple or visit at least three times a week.
- Only humans who were able to provide informed consent were included.
- To maintain the homogeneity of the sample, only macaques and dogs residing within Swayambhunath Mahachaitya temple were selected.
- Pigeons that visit the Swayambhunath temple were included in the research.

3.2.4.2 Exclusion criteria

- Human participants who were unable or unwilling to sign written consent were not included.
- Children (below 18 years of age) are excluded.

3.2.5 Sample collection, preservation and transportation

3.2.5.1 Humans

A total of 50 fresh fecal samples were collected from humans using a purposive sampling technique to ensure non-invasiveness. Prior to sample collection, participants were provided with brief instructions on how to collect fecal samples. A fecal collection

procedure sheet was prepared for human participants. They were given gloves, labeled collection vials, cotton, application sticks and zip lock bags for sample collection. Participants were instructed to collect approximately five grams of fecal sample. Fecal samples were collected the next morning and then placed in an icebox containing a 2.5% potassium dichromate solution and transported to the laboratory at the CDZ.

3.2.5.2 Macaques and dogs

Fecal samples from adult macaques and adult dogs were collected during both early morning and evening. Collection procedures ensured no harm or disturbance to the animals or their habitat. Using gloves and masks, approximately five grams of fecal material was carefully gathered and preserved in sterile vials containing 2.5% potassium dichromate, each labeled for proper identification. It was then transported to the laboratory at the CDZ.

3.2.5.3 Pigeons

A total of 50 fresh fecal samples were collected non-invasively from pigeons. The methodology involved a pooling approach, wherein temple pigeon samples were obtained by strategically placing clean plastics beneath roosting sites to efficiently capture fecal matter. Care was taken to avoid contamination of the fecal samples with droppings from other bird species in the temple. Using gloves and masks, approximately five grams of fecal material were collected, carefully preserved in sterile vials containing 2.5% potassium dichromate and labeled for proper identification. It was then transported to the laboratory at the CDZ.

3.2.6 Sample examination

All samples were tested in the CDZ laboratory at T.U, Kirtipur. The cysts, trophozoites, eggs and larvae of various parasites were identified using morphology and quantitative estimation employing the iodine wet mount method and the concentration technique (flotation and sedimentation).

3.2.7 Laboratory procedure and microscopic examination

The preserved fecal samples were initially examined macroscopically for the presence of mucus, segments of cestodes and dead adult nematodes. Then, microscopic examination was conducted using iodine wet mount and concentration techniques following the procedure outlined by (Zajac et al., 2012).

3.2.7.1 Iodine wet mount

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

3.2.7.2 Floatation method

This technique is used to float the less dense parasite on a fluid flotation medium with high density. A saturated salt solution serves as the fluid floatation medium. Two grams of the filtered sample were mixed with normal saline in a 15 ml centrifuge tube and then centrifuged at 1200 rpm for five minutes. The supernatant was discarded, leaving the sediment at the bottom of the tube, which was thoroughly mixed. Further, concentrated NaCl solution was added to fill the tube up to 13 ml and the mixture was centrifuged again at 1200 rpm for five minutes. Additional concentrated NaCl was added until a convex surface formed at the top of the tube. A clean coverslip was placed over the top of the tube, avoiding any bubbles and left undisturbed for at least 10 minutes. The coverslip was then gently removed, ensuring no sample was dropped and placed over a clean glass slide. The slide was examined under a microscope at 10× and 40× magnifications, with or without Lugol's iodine (Zajac et al., 2012).

3.2.7.3 Sedimentation method

This method is used to detect eggs of flukes, tapeworms and nematodes whose eggs do not float in common flotation solutions. Firstly, about two grams of the sample was filtered thoroughly and mixed with normal saline in a 15 ml centrifuge tube. At 1200 rpm, the sample was centrifuged for five minutes. The supernatant was discarded and the sediment was mixed well. To this sediment, 10 ml formalin and three ml of ethyl acetate were added in the tube and again centrifuged. Three layers of ethyl acetate containing the upper layer of ethyl acetate and plug of debris, the middle layer of 10% formalin and the bottom layer of sediment were formed. An applicator stick was used to free the plug of debris and then all the supernatant fluid was decanted and discarded. At last, only the bottom sediment was remained in the tube. If sediments were too dry, one to two drops of 10% formalin were added and mixed well. A drop of sediment was kept on a clean slide, covered by a coverslip

and observed under microscope at 10× and 40× magnification with Lugol's iodine (Zajac et al., 2012).

3.2.8 Identification of eggs/cysts/larvae

The identification of eggs, cysts and larva was confirmed by comparing their morphology and color with those published literature, journals and books (Zajac et al., 2012).

3.2.9 Data analysis

The prevalence of parasites was calculated by dividing the number of fecal samples infected with at least one parasite by the total number of fecal samples examined (Saville & Wittum, 2004). The collected data were coded and entered into Microsoft Excel (version 2013). Interpretation of the data was conducted using venn diagrams, tables and bar diagrams. Statistical analysis of the data was performed using R studio (version 4.3.2). Pearson's chi-square test was utilized for statistical analysis. In all cases, a 95% confidence interval and p-value ≤ 0.05 were considered indicative of a statistically significant difference.

4. Results

4.1 Overall prevalence of GI parasites

The examination of 200 fecal samples indicated an overall prevalence rate of 96% in macaques, 84% in pigeons, 72% in dogs and 16% in humans (Figure 3). A significant difference in the prevalence of GI parasites among humans, macaques, dogs and pigeons was observed (χ^2=84.93, p-value < 0.001).

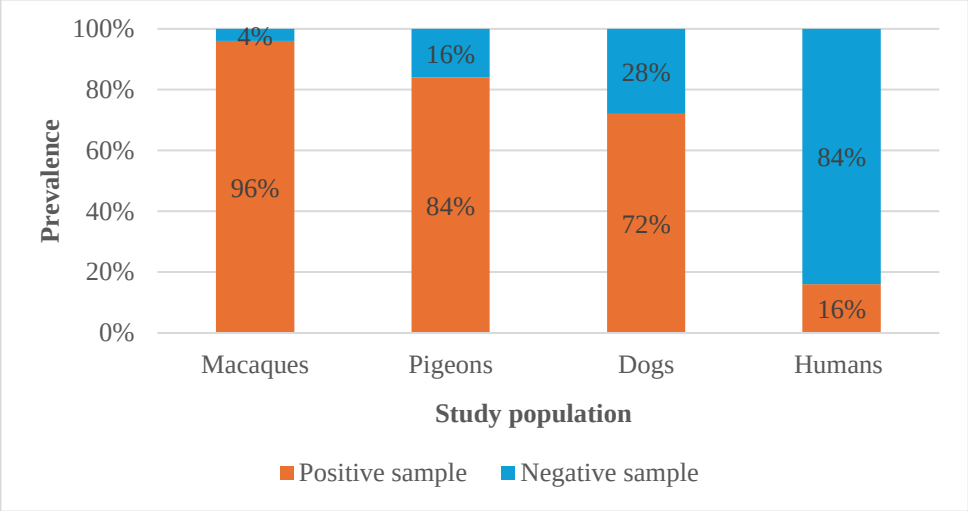


Figure 3. Overall prevalence of GI parasites among different study populations.

4.2 Diversity of GI parasites among the study population

4.2.1 Diversity of GI parasites in humans

Out of the eight positive samples, four different genera of parasites were observed. Among them, three (75%) genera tested positive for protozoa while one (25%) genus tested positive for nematodes (Table 1). The findings suggest that the highest prevalence of infection was observed within the phylum protozoa followed by nematodes.

Table 1. Diversity of parasites with prevalence rates in humans

Class	**S.N.**	**Name of the species**	**Positive samples**	
			No.	%
Protozoa	1	*Entamoeba* spp.	3	37.50
	2	*Giardia lamblia.*	2	25
	3	*Iodamoeba buetschlii*	1	12.50
Nematodes	1	*Ascaris lumbricoides*	4	50

4.2.2 Diversity of GI parasites in macaques

The analysis of fecal samples from macaques revealed a diverse range of parasite species. Out of the 48 positive samples, 16 different genera of parasites were observed. Among them, nine (56.25%) were found to be positive for protozoa, one (6.67%) for cestodes and six (37.5%) genera were found to be positive for nematodes (Table 2). The results of the study indicate that the maximum infection was found in the class protozoa followed by nematodes and cestodes.

Table 2. Diversity of parasites with prevalence rates in macaques

Class	**S.N.**	**Name of the species**	**Positive samples**	
			No.	%
Protozoa	1	*Balantidium coli*	32	66.67
	2	*Entamoeba* spp.	19	39.58
	3	*Entamoeba histolytica*	16	33.33
	4	*Entamoeba coli*	8	16.67
	5	*Iodamoeba buetschlii*	6	12.50
	6	*Chilomastix* spp.	3	6.25
	7	*Endolimax* spp.	2	4.17
	8	*Isospora* spp.	1	2.08
	9	*Giardia* spp.	1	2.08
Cestodes	1	*Bertiella* spp.	1	2.08
Nematodes	1	Hookworm	28	58.33
	2	*Strongyle* spp.	27	56. 25
	3	*Strongyloides* spp.	25	52.08
	4	*Trichuris* spp.	9	18.75
	5	*Physaloptera* spp.	4	8.33
	6	*Enterobius* spp.	2	4.17

4.2.3 Diversity of GI parasites in dogs

Out of 36 positive samples, nine different genera of parasites were observed. Among them, one (11.11%) tested positive for protozoa, one (11.11%) for cestodes and seven (77.78%) genera were positive for nematodes (Table 3). The results of the study indicate that maximum infection was found in class nematodes followed by protozoa and cestodes.

Table 3. Diversity of parasites with prevalence rates in dogs

Class	**S.N.**	**Name of the species**	**Positive samples**	
			No.	%
Protozoa	1	*Entamoeba* spp.	2	5.56
Cestodes	1	*Dipylidium caninum*	5	13.89
Nematodes	1	*Strongyloides* spp.	24	66.67
	2	Hookworm	19	52.78
	3	*Strongyle* spp.	17	47.22
	4	*Toxocara canis*	9	25
	5	*Trichuris* spp.	6	16.67
	6	*Capillaria* spp.	3	8.33
	7	*Ascaris* spp.	3	8.33

4.2.4 Diversity of GI parasites in pigeons

Out of 42 positive samples, six different genera of parasites were observed. Among them, two (33.33%) tested positive for protozoa, one (16.67%) for trematodes and three (50%) genera were positive for nematodes (Table 4). The findings suggest that the maximum infection was found in the class nematodes followed by protozoa and trematodes.

Table 4. Diversity of parasites with prevalence rates in pigeons

Class	**S.N.**	**Name of the species**	**Positive samples**	
			No.	%
Protozoa	1	*Eimeria* spp.	32	76.19
	2	Coccidian oocyst	11	26.19
Trematodes	1	*Heterophyes* spp.	16	38.10
Nematodes	1	*Ascaridia* spp.	18	42.86
	2	*Capillaria* spp.	13	30.95
	3	*Spirocerca* spp.	1	2.38

4.3 Common GI parasites found in different study groups

Common GI parasites found in humans, macaques, dogs and pigeons are illustrated in the venn diagram (Figure 4). *Entamoeba* spp. are common to humans, macaques and dogs. Hookworm, *Trichuris* spp., *Strongyloides* spp. and *Strongyle* spp. are common in both macaques and dogs, posing a zoonotic risk to humans. *Capillaria* spp. are shared by dogs and pigeons. *Ascaris* spp. are common to both humans and dogs.

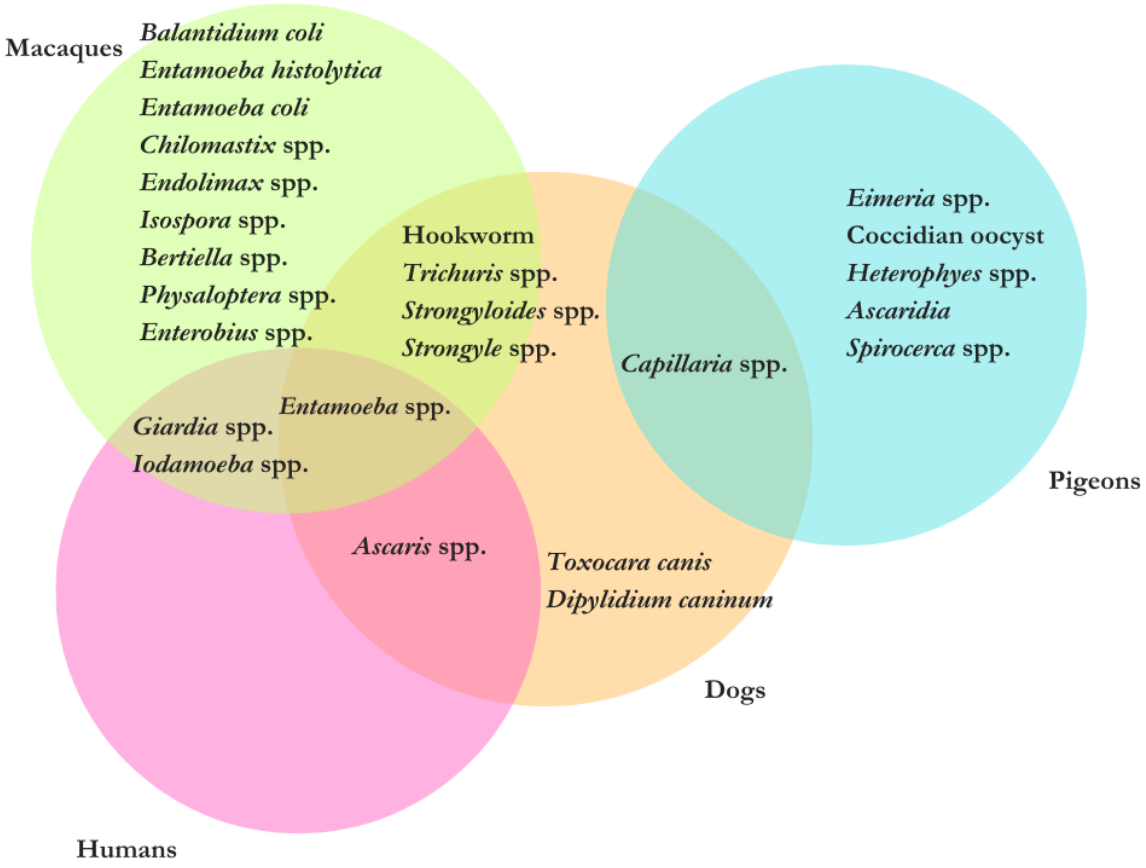


Figure 4. Venn-diagram showing common GI parasites in different study populations

5. Discussion

The current study revealed considerable prevalence rates of GI parasites among different species in the vicinity of Swayambhunath Mahachaitya, Kathmandu, Nepal; 16% in humans, 96% in macaques, 72% in dogs and 84% in pigeons. The prevalence rate observed in humans is comparable to the findings reported in school children from Rupandehi (18.66%; n=150) (Subedi et al., 2020), higher than those documented in humans from the Nilbarahi area (13%; n=100) (Chhetala, 2022), yet lower than rates recorded among the Chepangs of Central Nepal (97%; n= 100) (Adhikari et al., 2021), the Musahar community in Saptari (81%, n=200) (Yadav, 2023), the Chepang and Musahar communities of Makwanpur and Nawalparasi (36.6%; n=205) (Khadka et al., 2021), South (24.1%; n=166) (Wood, 1912), the people of the Deula community from Kirtipur (68%, n=150) (Subedi et al., 2021) and children of Nepal (50.5%; n=305) (Das et al., 2019). Differences in prevalence rates in humans could be attributed to variations in climate and topography or better hygiene practices and healthcare access. The lower prevalence in humans might be due to very low parasitic burdens resulting in low parasitic output or they could be truly parasitic-free; this remains unclear because the study was based only on fecal examination, not necroscopy. The prevalence of *Entamoeba* spp. was the highest among protozoa, similar to the findings of (Yadav, 2023) and (Khadka et al., 2021). Notably, the study reported *Iodamoeba buetschlii*, a large intestinal commensal, in 12.50% of the fecal samples, a rate lower than that found by (Adhikari et al., 2021) but higher than reported by (Nevin et al., 2024). Regarding flagellates, the prevalence rate of *Giardia lamblia* was 25% which is higher than the rates reported by (Adhikari et al., 2021) (Yadav, 2023) (Khadka et al., 2021) (Tharu, 2006) (Nevin et al., 2024) and (Sah et al., 2013). People will suffer from repeated severe diarrhoeal episodes that can be fatal. *Giardia* cysts are highly resistant to environmental conditions, being able to survive in cold mountain streams, stomach acid, chlorine and even in ultra violet-treated wastewater. The most predominant helminth was *Ascaris lumbricoides* (15.6%) which is similar to (Adhikari et al., 2021), (Khadka et al., 2021) (Tharu, 2006).

The overall parasite prevalence in Rhesus macaques (96%) is considerably higher than reported from other temple sites and cities in Nepal (54% - 88%) (Nepal, 2010; Adhikari et al., 2023; Malla, 2007; Jha et al., 2011; Dhoubhadel, 2007; Chhetala, 2022; Adhikari and Dhakal, 2018; Thakuri et al., 2021; Dhakal et al., 2018), yet lower than the rates recorded in rhesus macaques at Shivapuri Nagarjun National Park (100%; n=70) (Jain and Maharjan,

2023) and Bajrabarahee, Lalitpur (100%; n=42) (Sapkota et al., 2020). The present study reveals considerable diversity of GI parasites in rhesus macaques at Swayambhunath Mahachaitya, identifying 16 species in total. These include nine species of protozoa (*Balantidium coli*, *Entamoeba* spp., *Entamoeba histolytica*, *Entamoeba coli*, *Iodamoeba buetschlii*, *Chilomastix* spp., *Endolimax* spp., *Isospora* spp. and *Giardia* spp.), one species of cestode (*Bertiella* spp.) and six species of nematodes (Hookworm, *Strongyle* spp., *Strongyloides* spp., *Trichuris* spp., *Physaloptera* spp. and *Enterobius* spp.). The study shows a higher prevalence rate of protozoans compared to helminths, consistent with the findings of Sapkota et al. (2020) and Jain and Maharjan (2023). Among the protozoans, *Balantidium coli* (66.67%) showed the highest prevalence followed by *Entamoeba* spp. (39.59%) and *Entamoeba histolytica* (33.33%). *Balantidium coli*, the only ciliated protozoan commonly affecting humans and various animals, can cause balantidiasis, leading to dysentery and diarrhoea in both macaques and humans. The presence of *Iodamoeba buetschlii* aligns with previous studies from Nepal (Adhikari et al., 2023; Jain & Maharjan, 2023) and other regions (Levecke et al., 2007; Cordon et al., 2008; Kouassi et al., 2015). This parasite may be transmitted to rhesus macaques from infected humans or livestock, especially from swine farming around Kathmandu (Adhikari et al., 2023). Among the helminths, Hookworm (58.33%) showed the highest prevalence noted for it's zoonotic nature. The prevalence of *Trichuris* spp. (18.75%) is higher than reported by Nepal (2010), Jha et al. (2011), Sharma et al. (2013) and Sapkota et al. (2020) but lower than findings by Adhikari and Dhakal (2018), Sharma et al. (2023) and Adetunji (2014). The high prevalence of *Trichuris* spp. in this study may suggest changing climatic conditions in the Kathmandu Valley, as this parasite thrives in warm, moist environments with low light and wet soil. Notably, this study documents the presence of the cestode, *Bertiella* spp. in Nepal for the first time, though it has been reported in Bangladesh by Muznebin et al. (2022). In Swayambhunath, increasing soil and water pollution due to waste food and garbage, especially during festive and picnic programs, along with occasional open defecation by visitors and outsiders in the forest areas and nearby water sources has created a conductive environment for GI parasite transmission. Macaques, often in contact with contaminated soil and water and consuming garbage are at a high risk of acquiring GI parasites. These macaques depend on food brought by people, which can also be contaminated. Additionally, the favorable climatic conditions support the flourishing of these parasites. The high prevalence of GI parasites in macaques observed in this study is

likely due to these factors, with the densely populated environment and regular human-monkey interactions playing a crucial role in the natural and high rate of transmission.

Likewise, the prevalence rate (72%) observed in dogs in the current study was comparable with earlier studies conducted in South Africa (76%; n=63) (Minnaar et al., 2002), in stray dogs of Rupandehi, Nepal (78.5%; n=78) (Yadav & Shrestha, 2017) and Catalonia, Spain (71.6%; n=88) (Ortuño et al., 2014). However, the prevalence in the present study was lower than previous studies conducted in Lalitpur, Nepal (95.7%; n=332) (Adhikari et al., 2023), Chitwan, Nepal (81.86%; n=204) (Bastakoti et al., 2023) and Bangladesh (95%; n=60) (Das et al., 2012) whereas it was higher than in Mazandaran, Iran (59.50%; n =58) (Amouei et al., 2018), only in stray dogs of Kathmandu, Nepal (41.33%; n=60) (Bhattarai, 2022) only stray dogs of Kathmandu, Nepal (56.2%; n=105) (Satyal et al., 2013) and Bhaktapur, Nepal (59.5%, n=400) (Sukupayo & Tamang, 2023). The prevalence rate of GI parasites in dogs varies across different countries, influenced by geo-climatic factors, sample size, breed, sampling season, treatment strategies, and methodological differences. Noticeably, helminth parasites had a higher prevalence and a wider variety than protozoa. A higher prevalence of helminth parasite in stray dogs has already been reported by Adhikari et al. (2023). These regions exhibit subtropical to tropical climatic conditions which favor the egg hatching and larval development of helminths such as *Strongyloides* spp., the most prevalent parasite in this study, followed by hookworm. Hookworm induces threatening anaemia in canids and causes eosinophilic enteritis and larval migrans syndromes in humans. *Toxocara canis* found in the current study can easily cross the host barrier, infecting a wide range of hosts like cats, wild canids and humans. Similarly, *Ascaris* spp. found in this study can cause infections in humans and *Capillaria* spp. detected here have a history of human infection. Additionally, a few clinical cases have been attributed to *Trichuris* spp., indicating zoonotic transmission in endemic regions. The only cestode found in the study, *Dipylidium caninum* is also zoonotic. Regarding protozoa, only *Entamoeba* spp. was found, indicating zoonotic possibilities in nearby humans and animals. In Swayambhunath, the high prevalence of GI parasites in dogs can be attributed to their scavenging feeding habits and close association with humans, macaques and pigeons in the area. Dogs often consume animal carcasses, leftover foods, garbage, animal dung and human faeces and they drink water from contaminated sources, contributing to parasitosis. Despite the efforts of a few organizations working for the welfare of street dogs in Nepal, vaccination, deworming and castration are not optimal, leading to increased susceptibility

to parasitism. The availability of food in garbage, human waste and indiscriminate feeding, combined with the gathering of dogs near food sources, plays a significant role in parasitic infection.

The prevalence rate (84%) observed in pigeons in the current study was comparable to tumbler pigeons raised in Kırıkkale (82.9%; n=105) (Gökpınar et al., 2023) but lower than the findings from previous studies among feral pigeons from Bangladesh (100%; n=60) (Begum & Sehrin, 2013), household and temple pigeons in Central Nepal (87.1%; n=155) (Adhikari et al., 2022), Kerala, India (86.8%; n=91) (Thankachan et al., 2022) and two temples in Kathmandu valley (90.83%; n=109) (Jha et al., 2023). In contrast, the prevalence rate in this study was higher than in Suryabinayak, Bhaktapur (53.24%; n=77) (Sukupayo, 2019), Iraq (76%; n=50) (Issa et al., 2021) and three temples in Pokhara valley (69.16%; n=120) (Gurung & Subedi, 2018). The variation in these results can be attributed to differences in sampling techniques, sample sizes, examination methods and the types of parasites detected and the ecological characteristics of the sampling locations. For example, the study from Bangladesh, which reported a 100% parasite detection rate (Begum & Sehrin, 2013), used histopathologic findings, whereas, the current study utilized faecal sampling and potentially less sensitive microscopic techniques, although both iodine wet mount and concentrated methods were employed for each sample. The high rate of parasitic infection observed in the present study might be due to factors such as a constant source of infested droppings or infested intermediate hosts in the study area. Additionally, parasitic infection in pigeons can be influenced by food sources, geographic location, climatic conditions and the availability of intermediate hosts. In the present study, two genera of protozoa, one genus of trematodes and three genera of nematodes were observed. The two genera of protozoa found were *Eimeria* spp. and coccidian oocysts. Similarly, the one genus of trematodes found was *Heterophyes* spp. and the three genera of nematodes found were *Ascaridia* spp., *Capillaria* spp. and *Spirocerca* spp. This was the first record of *Spirocerca* spp. in pigeons in Nepal. The prevalence rate of *Eimera* spp. was found to be the highest in pigeons (76.19%) which is similar to reports by Adhikari et al. (2022) and Parsani et al. (2014) , while the least prevalence was shown by *Spirocerca* spp. (2.38%) out of the total 84% total prevalence of GI parasites. The high detection of *Eimeria* spp. in pigeons suggests water and food contamination in Swayambunath Mahachaitya. The prevalence of *Heterophyes* spp. (38.10%) is higher than reported by Adhikari et al. (2022). The prevalence of *Ascaridia* spp. (42.86%) is higher than reported by Adhikari et al. (2022) and

Jha et al. (2023) and comparable to that reported by Gökpınar et al. (2023), Gurung and Subedi (2018), Mohammed et al. (2019) and Mehmood et al. (2019). The prevalence of *Capillaria* spp. (30.95%) is comparable to that reported by Gökpınar et al. (2023), Marques et al. (2007) and Mehmood et al. (2019) but higher than that reported by Mohammed et al. (2019). The prevalence of helminths was higher than protozoan parasites in the present study, a finding consistent with previous reports by Patel et al. (2000), Jha et al. (2023), Gurung and Subedi (2018) and Parsani et al. (2014). The high prevalence of helminth infections recorded in this study could indicate a high incidence of the infective stages and intermediate hosts of the parasites in Swayambhunath Mahachaitya. The pigeon's scavenging behavior, traveling long distances and consuming whatever food is available, enhances their exposure to diverse environmental conditions including contact with other avian species, consequently increasing the acquisition of various parasites.

Due to the great diversity of parasites found in macaques, dogs and pigeons, these animals play an important role in the transmission and maintenance of infection to humans. For instance, *Giardia* spp. and *Iodamoeba* spp. are found in both humans and macaques suggesting a transmission flow between these species. Adhikari et al. (2023) reported that *Iodamoeba buetschlii* might be transmitted to rhesus macaques from infected humans or livestock, which can severely damage the macaque's GI tract, causing symptoms like diarrhoea and rectal prolapse. Li et al. (2021) confirmed that NHPs serve as reservoirs for *Giardi*a spp., highlighting the potential for interspecies transmission between humans and macaques. Similarly, *Balantidium coli* found only in macaques is zoonotic and can cause clinical side effects such as dysentery and diarrhoea in both macaques and humans (Jain & Maharjan, 2023). *Entamoeba histolytica*, though non-pathogenic to humans, is found in macaques, whereas *Entamoeba* spp. found in humans, macaques and dogs pose a zoonotic risk due to their simple and direct life cycles (Chhetala, 2022). Although *Entamoeba coli* in macaques is typically asymptomatic, other species like *Chilomastix* spp. and *Endolimax* spp. are zoonotic but non-pathogenic. *Isospora* spp. in macaques is asymptomatic and not zoonotic while *Physaloptera* spp. and *Enterobius* spp. are zoonotic but non-pathogenic. The lifecycle of *Physaloptera* spp. in humans remains undistinguished. Li et al. (2021) identified higher parasite prevalence among workers compared to villagers, attributing this to greater exposure to risk factors such as direct animal contact and environmental contamination. *Bertiella* spp., newly reported in rhesus macaques in Nepal, is zoonotic and can infect humans. Hookworm, *Trichuris* spp., *Strongyloides* spp. and *Strongyle* spp. found

in both macaques and dogs pose a zoonotic risk. Schär et al. (2014) identified seven GI parasites with zoonotic potential in rural Cambodian village dogs, including Hookworm, *Strongyloides* spp., *Trichuris* spp., *Toxocara canis*, *Echinostoma* spp., *Giardia duodenalis* and *Entamoeba* spp. *Ascaris* spp. common in both dogs and humans, is associated with intestinal pathology and respiratory symptoms in endemic regions (Jain & Maharjan, 2023). *Toxocara canis* and *Dipylidium caninum* found only in dogs are zoonotic in nature. *Toxocara canis* is non-infectious to humans while *Dipylidium caninum* is infectious to humans. Adhikari et al. (2023) reported *Toxocara* spp. being exchanged between macaques and stray dogs due to shared food and shelter. *Capillaria* spp. found in both dogs and pigeons may exchange between these animals and infect macaques, posing a zoonotic risk despite being non-infective. *Eimeria* spp. found only in pigeons can transmit to humans but is non-pathogenic. Parasites like, *Heterophyes* and *Spirocerca* spp. found in pigeons may also pose zoonotic risks. The interactions among humans, macaques, dogs and pigeons facilitate the transmission of parasites. Understanding these new relationships is crucial as approximately 90% of parasites described in humans have domestic and wild animals as definitive hosts in their life cycles.

6. Conclusions and recommendations

6.1 Conclusions

The prevalence rates of parasitic infections vary significantly among the different studied populations. Monkeys exhibit the highest prevalence rate (96%), followed by pigeons (84%), dogs (72%) and humans (16%). The prevalence rates suggest a host-specific pattern of parasitic infections. The relatively high prevalence rates observed in monkeys and dogs raise concerns about the zoonotic potential of certain parasites. Humans may be at risk of contracting parasitic infections from these animal populations, emphasizing the importance of proper hygiene practices and parasite control measures, particularly in areas where humans and animals coexist closely.

6.2 Recommendations

- Initiate comprehensive awareness campaigns targeted towards visitors and locals about the risks of GI parasitic transmission from close contact with animals such as macaques, dogs and pigeons.
- Provide regular veterinary care and health screenings for macaques living in the area to reduce the prevalence of GI parasites among them. This may include deworming treatments and vaccination campaigns.
- Establish a long-term monitoring program to assess the prevalence of GI parasites among humans, macaques, dogs and pigeons in the area.

7. References

Adetunji, V. (2014). Prevalence of gastro-intestinal parasites in primates and their keepers from two zoological gardens in Ibadan, Nigeria. *Sokoto Journal of Veterinary Sciences*, *12*(2), 25–30. https://doi.org/10.4314/sokjvs.v12i2.5

Adhikari, A., Koju, N. P., Maharjan, B., Khanal, L., Upreti, M., & Kyes, R. C. (2023). Gastro-intestinal parasites of urban rhesus macaques (*Macaca mulatta*) in the Kathmandu Valley, Nepal. *International Journal for Parasitology: Parasites and Wildlife*, *22*(October), 175–183. https://doi.org/10.1016/j.ijppaw.2023.10.007

Adhikari, P. P., & Dhakal, P. (2018). Prevalence of Gastro-Intestinal Parasites of Rhesus Macaque (*Macaca Mulatta* Zimmermann, 1780) and Hanuman Langur (*Semnopithecus Entellus Dufresne*, 1797) In Devghat, Chitwan, Nepal. *Journal of Institute of Science and Technology* *22*(2), 12-18. https://doi.org/10.3126/jist.v22i2.19590

Adhikari, R. B., Ale, P. B., Dhakal, M. A., & Ghimire, T. R. (2022). Prevalence and diversity of intestinal parasites in household and temple pigeons (*Columba livia*) in central Nepal. *Veterinary Medicine and Science*, *8*(4), 1528–1538. https://doi.org/10.1002/vms3.792

Adhikari, R. B., Dhakal, M. A., & Ghimire, T. R. (2023). Prevalence of intestinal parasites in street dogs (*Canis lupus familiaris*) with highlights on zoonosis in Lalitpur, Nepal. *Veterinary Medicine and Science*, *9*(6), 2513–2526. https://doi.org/10.1002/vms3.1258

Adhikari, R. B., Parajuli, R. P., Maharjan, M., & Ghimire, T. R. (2021). Prevalence and risk factors of gastrointestinal parasites in the Chepangs in Nepal. *Annals of Parasitology*, *67*(3), 387–405. https://doi.org/10.17420/ap6703.353

Agrawal, P. K., Rai, S. K., Khanal, L. K., Ghimire, G., Banjara, M. R., & Singh, A. (2012). Intestinal parasitic infections among patients attending Nepal Medical College Teaching Hospital, Kathmandu, Nepal. *Nepal Medical College Journal*, *14*(2), 80–83.

Amouei, A., Jahandar, H., Daryani, A., Sharif, M., Sarvi, S., Mizani, A., Hosseini, S. A., Sarafrazi, M., Siyadatpanah, A., Gohardieh, S., Bastani, R., & Gholami, S. (2018). Carnivores as important reservoirs of intestinal helminthic infections in Mazandaran

Province, Northern Iran. *Iranian Journal of Parasitology*, *13*(2), 251–257.

Arunachalam, K., Senthilvel, K., & Anbarasi, P. (2015). Endo parasitic infections in free living Rhesus Macaque (*Macaca mulatta*) of Namakkal, Tamil Nadu, India. *Zoos' Print*, *30*(6), 20–21.

Ayinmode, A. B., Obebe, O. O., & Olayemi, E. (2016). Prevalence of potentially zoonotic gastrointestinal parasites in canine faeces in Ibadan, Nigeria. *Ghana Medical Journal*, *50*(4), 201–206. https://doi.org/10.4314/gmj.v50i4.2

Baral, R., Jha, P., Amatya, R., & Khanal, B. (2017). Prevalence of intestinal parasitic infections among patients attending in a tertiary care hospital of eastern region of Nepal – A retrospective, laboratory based study. *Asian Journal of Medical Sciences*, *8*(3), 55–59. https://doi.org/10.3126/ajms.v8i3.16909

Begum, A., & Sehrin, S. (2013). Gastrointestinal helminths in pigeon *Columba livia* (Gmelin, 1789). *Journal of the Asiatic Society of Bangladesh, Science*, *38*(1), 93–98. https://doi.org/10.3329/jasbs.v38i1.15324

Bhattacharjee, D., & Bhadra, A. (2020). Humans Dominate the Social Interaction Networks of Urban Free-Ranging Dogs in India. *Frontiers in Psychology*, *11*, 1–11. https://doi.org/10.3389/fpsyg.2020.02153

Bastakoti, R., Pathak, C. R., Pandey, A., & Acharya, A. (2023). Prevalence of Zoonotic Parasites of Street Dogs and Its Threats in Madi Valley, Chitwan, Nepal. *Indian Journal of Veterinary Public Health*, *9*(3), 47–50. https://doi.org/10.62418/ijvph.9.3.2023.47-50

Bhattarai, G. P. (2022). *Gastro Intestinal Helminth Parasites in Stray and Pet Dogs of Kathmandu Valley, Nepal* (MSc dissertation). Central Department of Zoology, Tribhuvan University.

Chapman, C. A., Gillespie, T. R., & Goldberg, T. L. (2005). Primates and the ecology of their infectious diseases: How will anthropogenic change affect host-parasite interactions? *Evolutionary Anthropology*, *14*(4), 134–144. https://doi.org/10.1002/evan.20068

Chhetala, S. (2022). *Gastrointestinal parasites in human and rhesus monkey of Nilbarahi area, Bhaktapur, Nepal* (MSc dissertation). Central Department of Zoology, Tribhuvan University.

Cordon, G. P., Prados, A. H., Romero, D., Moreno, M. S., Pontes, A., Osuna, A., & Rosales, M. J. (2008). Intestinal parasitism in the animals of the zoological garden "Pena Escrita" (Almunecar, Spain). *Veterinary Parasitology*, *156*(3–4), 302–309. https://doi.org/10.1016/j. vetpar.2008.05.023

Das, R. K., Mahato, P., & Neupane, P. (2019). Contributing factors for intestinal parasitic infection in children: a study of Nepal. *International Journal Of Community Medicine And Public Health*, *6*(7), 2739. https://doi.org/10.18203/2394-6040.ijcmph20192803

Das, S., Alim, M. A., SIkder, S., Das Gupta, A., & Masuduzzaman, M. (2012). Prevalence and Worm Load of Enteric Helminthiasis in Stray Dogs of Chittagong Metropolitan, Bangladesh. *YYU Veteriner Fakultesi Dergisi*, *23*(3), 141–145.

Despommier, D. D., Griffin, D. O., Gwadz, R. W., Hotez, P. J., & Knirsch, C. A. (2017). Parasitc Diseases.

Devleesschauwer, B., Ale, A., Torgerson, P., Praet, N., Maertens de Noordhout, C., Pandey, B. D., Pun, S. B., Lake, R., Vercruysse, J., Joshi, D. D., Havelaar, A. H., Duchateau, L., Dorny, P., & Speybroeck, N. (2014). The Burden of Parasitic Zoonoses in Nepal: A Systematic Review. *PLoS Neglected Tropical Diseases*, *8*(1), 56. https://doi.org/10.1371/journal.pntd.0002634

Dhakal, D. N., Bhattarai, B. P., & Adhikari, J. N. (2018). Resource preference is the major determinant of gastrointestinal parasites prevalence in rhesus macaque (*Macaca mulatta*) in Chitwan-Annapurna landscape, Nepal. *Perspectives on Biodiversity of India, IV*, 154-158.

Dhoubhadel, M. (2007). P*revalence of intestinal helminth parasites in rhesus monkey (Macaca mulatta) of Swoyambhu and Nilbarahi area of Kathmandu valley* (MSc dissertation). Central Department of Zoology, Tribhuvan University.

Esposito, M. M., Turku, S., Lehrfield, L., & Shoman, A. (2023). The Impact of Human Activities on Zoonotic Infection Transmissions. *Animals*, *13*(10). https://doi.org/10.3390/ani13101646

Fuentes, A., & Gamerl, S. (2005). Disproportionate participation by age/sex classes in aggressive interactions between long-tailed macaques (*Macaca fascicularis*) and human tourists at Padangtegal monkey forest, Bali, Indonesia. *American Journal of Primatology*, *66*(2), 197–204. https://doi.org/10.1002/ajp.20138

Gautam, A., Upadhayay, P., Ghimre, D., Khanal, A., Gaire, A., & Kaphle, K. (2021). Prioritised Zoonotic Diseases of Nepal: A Review. *International Journal of Applied Sciences and Biotechnology*, *9*(1), 1–15. https://doi.org/10.3126/ijasbt.v9i1.34967

Gökpınar, S., Uysal, E., & Pusat, B. N. (2023). The prevalence of intestinal parasites in tumbler pigeons raised in Kırıkkale. *Van Vet J*, *34*(3), 219-223. https://doi.org/10.36483/vanvetj.1279215

Golshang, J. G., Patrobas, M. N., Oshadu, D. O., Dogo, G. A., Amuga, G. A., Lombin, L. H., & Nanvyat, N. (2023). Prevalence of Gastrointestinal Parasites of Humans and Dogs in Amazah District of Jos-North LGA, Plateau State, Nigeria. *Nigerian Journal of Parasitology*, *44*(2), 360–367. https://doi.org/10.4314/njpar.v44i2.8

Guedes, J. J. M., Assis, C. L., Feio, R. N., & Quintela, F. M. (2021). The impacts of domestic dogs (*Canis familiaris*) on wildlife in two brazilian hotspots and implications for conservation. *Animal Biodiversity and Conservation*, *44*(1), 45–58. https://doi.org/10.32800/abc.2021.44.0045

Gurung, A., & Subedi, J. R. (2018). Prevalence of gastrointestinal parasites of pigeons (*Columba* sp. Linnaeus, 1758) in three temples of Pokhara valley, Nepal. *Journal of Natural History Museum*, *30*(December 2018), 287–293. https://doi.org/10.3126/jnhm.v30i0.27604

Hechenbleikner, E. M., & McQuade, J. A. (2015). Parasitic colitis. *Clinics in Colon and Rectal Surgery*, *28*(2), 79–86. https://doi.org/10.1055/s-0035-1547335

Inpankaew, T., Traub, R., Thompson, R. C. A., & Sukthana, Y. (2007). Canine parasitic zoonoses in Bangkok temples. *Southeast Asian Journal of Tropical Medicine and Public Health*, *38*(2), 247–255.

Islam, S., Rahman, M. K., Uddin, M. H., Rahman, M. M., Chowdhury, M. N. U., Hassan, M. M., Magalhaes, R. S., & Islam, A. (2022). Prevalence and diversity of gastrointestinal parasites in free-ranging rhesus macaques (*Macaca mulatta*) in different land gradients of Bangladesh. *American Journal of Primatology*, *84*(1), 0–3. https://doi.org/10.1002/ajp.23345

Issa, A. R., Mero, W. M. S., Hasan, D. L., & Hameed, M. A. (2021). The prevalence of parasites in the domestic pigeons (*Columba livia domestica*) in Zakho City, Kurdistan-Iraq. *Baghdad Science Journal*, *18*(2), 210–216.

https://doi.org/10.21123/BSJ.2021.18.2.0210

Jain, P., & Maharjan, B. (2023). Gastro-Intestinal Parasites in Monkeys of Shivapuri Nagarjun National Park and Temple Areas of Kathmandu Valley. *Amrit Research Journal*, *4*(1), 79–90. https://doi.org/10.3126/arj.v4i1.61217

Jha, A., Chalise, M. K., Shrestha, R. M., & Karki, K. (2011). Intestinal Parasitic Investigation in Temple Rhesus Monkeys of Kathmandu. *The Initiation*, *4*(June), 1–7. https://doi.org/10.3126/init.v4i0.5530

Jha, B., Gupta, R., Subedi, J. R., & Chhetri, B. (2023). Gastrointestinal parasites of feral pigeon (*Columba livia* GMELIN, 1789) at two temples of Kathmandu valley. *Journal of Animal Science and Veterinary Medicine*, *8*(2), 23–28. https://doi.org/10.31248/jasvm2022.330

Khadka, S., Sapkota, S., Adhikari, S., Dubey, A. K., Thapa, A., Bashyal, R., & Bhusal, H. (2021). Intestinal Parasitoses among Chepang and Musahar Community People of Makwanpur and Nawalparasi Districts of Nepal. *Acta Parasitologica*, *66*(1), 146–154. https://doi.org/10.1007/s11686-020-00269-0

Kinman, T. (2018, September 17) Parasitic Infections. What is a parasitic infection? Healthline. https://www.healthline.com/health/parasitic-infections

Kouassi, R. Y. W., McGraw, S. W., Yao, P. K., Abou-Bacar, A., Brunet, J., Pesson, B., Bonfoh, B., N'goran, E. K., & Candolfi, E. (2015). Diversity and prevalence of gastrointestinal parasites in seven non-human primates of the Taï National Park, Cote d'Ivoire. *Parasite*, 22. https://doi.org/10.1051/parasite/2015001

Kurnosova, O. P., Panova, O. A., & Arisov, M. V. (2023). The prevalence of potentially zoonotic intestinal parasites in dogs and cats in Moscow, Russia. *Helminthologia (Poland)*, *60*(1), 44–51. https://doi.org/10.2478/helm-2023-0009

Lane-deGraaf, K. E., Putra, I. G. A. A., Wandia, I. N., Rompis, A., Hollocher, H., & Fuentes, A. (2014). Human behavior and opportunities for parasite transmission in communities surrounding long-tailed macaque populations in Bali, Indonesia. *American Journal of Primatology*, *76*(2), 159–167. https://doi.org/10.1002/ajp.22218

Levecke, B., Dorny, P., Geurden, T., Vercammen, F., & Vercruysse, J. (2007). Gastrointestinal protozoa in non-human primates of four zoological gardens in Belgium. Veterinary Parasitology, *148*(3–4), 236–246. https://doi.org/10.1016/j. vetpar.2007.06.020

Li, J., Ren, Y., Chen, H., Huang, W., Feng, X., & Hu, W. (2021). Risk Evaluation of Pathogenic Intestinal Protozoa Infection Among Laboratory Macaques, Animal Facility Workers and Nearby Villagers From One Health Perspective. *Frontiers in Veterinary Science*, *8*(September), 1–10. https://doi.org/10.3389/fvets.2021.696568

Malla, V. (2007). *Intestinal helminth parasites of (Macaca mulatta) (Zimmermann) from Pashupati (Kathmandu District) and Nilbarahi area (Bhaktapur District) of Nepal* (MSc dissertation). Central Department of Zoology, Tribhuvan University.

Marques, S. M. T., De Quadros, R. M., Da Silva, C. J., & Baldo, M. (2007). Parasites of pigeons (*Columba livia*) in urban areas of lages, Southern Brazil. *Parasitologia Latinoamericana*, *62*(3–4), 183–187. https://doi.org/10.4067/s0717-77122007000200014

Mehmood, S., Nashiruddullah, N., Ahmed, J. A., & Borkataki, S. (2019). Parasitic affections of domesticated pigeons (*Columba livia*) in Jammu, India. *Annals of Parasitology*, *65*(1), 53–64. https://doi.org/10.17420/ap6501.182

Minnaar, W. N., Krecek, R. C., & Fourie, L. J. (2002). Helminths in dogs from a peri-urban resource-limited community in Free State Province, South Africa. *Veterinary Parasitology*, *107*(4), 343–349. https://doi.org/10.1016/S0304-4017(02)00155-3

Munene, E., Otsyula, M., Mbaabu, D. A., Mutahi, W. T., Muriuki, S. M., & Muchemi, G. M. (1998). Helminth and protozoan gastrointestinal tract parasites in captive and wild-trapped African non-human primates. *Veterinary Parasitology*, *78*(3), 195–201. https://doi.org/10.1016/S0304-4017(98)00143-5

Muriuki, S. M., Murugu, R. K., Munene, E., Karere, G. M., & Chai, D. C. (1998). Some gastro-intestinal parasites of zoonotic (public health) importance commonly observed in old world non-human primates in Kenya. *Acta Tropica*, *71*(1), 73–82. https://doi.org/10.1016/S0001-706X(98)00040-0

Muznebin, F., Khanum, H., Jaman, M.F., & Shafiullah, Z.M. (2022). Endo-Parasitic Infestation in Captive and Free-Living Rhesus Macaques (*Macaca mulatta*) in Bangladesh. *Biomedical Journal of Scientific & Technical Research*, *40*(5). https://doi.org/10.26717/bjstr.2022.40.006528

Nepal, S. (2010). *Seasonal prevalence of intestinal helminth parasites in rhesus monkey (Macaca mulatta) of Swoyambhu area of Kathmandu valley* (MSc dissertation).

Central Department of Zoology, Tribhuvan University.

Odermatt, P., Lv, S., & Sayasone, S. (2010). Less Common Parasitic Infections in Southeast Asia that can Produce Outbreaks. *Advances in Parasitology*, *72*, 409–435. https://doi.org/10.1016/S0065-308X(10)72013-3

Ogbaje, C. I., Ofukwu, R. A., & Ajogi, I. A. (2015). Zoonotic gastrointestinal parasite burden of local dogs in Zaria, Northern Nigeria: Implications for human health. *International Journal of One Health*, *1*, 32–36. https://doi.org/10.14202/IJOH.2015.32-36

Ortega-Pacheco, A., Torres-Acosta, J. F. J., Alzina-López, A., Gutiérrez-Blanco, E., Bolio-González, M. E., Aguilar-Caballero, A. J., Rodríguez-Vivas, R. I., Gutiérrez-Ruiz, E., Acosta-Viana, K. Y., Guzmán-Marín, E., Rosado-Aguilar, A., & Jiménez-Coello, M. (2015). Parasitic zoonoses in humans and their dogs from a rural community of tropical Mexico. *Journal of Tropical Medicine*, *2015*, 1-6. https://doi.org/10.1155/2015/481086

Ortuño, A., Scorza, V., Castellà, J., & Lappin, M. (2014). Prevalence of intestinal parasites in shelter and hunting dogs in Catalonia, Northeastern Spain. *Veterinary Journal*, *199*(3), 465–467. https://doi.org/10.1016/j.tvjl.2013.11.022

Parsani, H. R., Momin, R. R., Lateef, A., & Shah, N. M. (2014). Gastro-intestinal helminths of pigeons (*Columba livia*) in Gujarat, India. *Egyptian Journal of Biology*, *16*, 63-71. https://doi.org/10.4314/ejb.v16i1.9

Patel, P. V., Patel, A. I., Sahu, R. K., & Vyas, R. (2000). Prevalence of gastro-intestinal parasites in captive birds of Gujarat zoos. *Zoos' Print Journal*, *15*(7), 295–296. https://doi.org/10.11609/jott.zpj.15.7.295-6

Patra, G., Lalremruati, P., Ghosh, S., Parida, A., Borthakur, S.K., & Behera, P. (2018). Prevalence of gastrointestinal parasites in captive non-human primates of zoological gardens in North-Eastern region of India. *Biological Rhythm Research*, *51*(5), 690–698. https://doi.org/10.1080/09291016.2018.1557854

Pedersen, A. B., & Davies, T. J. (2009). Cross-species pathogen transmission and disease emergence in primates. *EcoHealth*, *6*(4), 496–508. https://doi.org/10.1007/s10393-010-0284-3

Pumipuntu, N. (2018). Detection for potentially zoonotic gastrointestinal parasites in long-

tailed macaques, dogs and cattle at Kosamphi forest park, Maha Sarakham. *Veterinary Integrative Sciences*, *16*(2), 69–77. https://he02.tci-thaijo.org/index.php/vis/article/view/141526

Rabinowitz, P. M., & Conti, L. A. (2010). Zoonoses. *Human-Animal Medicine*, 105–298. https://doi.org/10.1016/B978-1-4160-6837-2.00009-9

Ragunathan, L., Kalivaradhan, S. K., Ramadass, S., Nagaraj, M., & Ramesh, K. (2010). Helminthic Infections in School Children in Puducherry, South India. *Journal of Microbiology, Immunology and Infection*, *43*(3), 228–232. https://doi.org/10.1016/S1684-1182(10)60036-9

Sah, R., Yadav, S., Baral, R., Bhattarai, S., Jha, N., & Pokharel, P. (2013). A study of prevalence of intestinal parasites and associated risk factors among the school children of Itahari, Eastern Region of Nepal. *Tropical Parasitology*, *3*(2), 140. https://doi.org/10.4103/2229-5070.122143

Sapkota, B., Adhikari, R. B., Regmi, G. R., Bhattarai, B. P., & Ghimire, T. R. (2020). Diversity and prevalence of gut parasites in urban macaques. *Applied Science and Technology Annals*, *1*(1), 34–41. https://doi.org/10.3126/asta.v1i1.30270

Satyal, R.C., Manandhar, S., Dhakal, S., Mahato, B.R., Chaulagain, S., Ghimire, L., & Pandeya, Y.R. (2013). Prevalence of gastrointestinal zoonotic helminths in dogs of Kathmandu, Nepal. *International Journal of Infection and Microbiology*, *2*(3), 91–94. https://doi.org/10.3126/ijim.v2i3.8211

Saville, W. J., & Wittum, T. E. (2004). Veterinary Epidemiology. *Equine Internal Medicine (Second Edition)*. https://doi.org/10.1016/B0-72-169777-1/50023-8

Schär, F., Inpankaew, T., Traub, R. J., Khieu, V., Dalsgaard, A., Chimnoi, W., Chhoun, C., Sok, D., Marti, H., Muth, S., & Odermatt, P. (2014). The prevalence and diversity of intestinal parasitic infections in humans and domestic animals in a rural Cambodian village. *Parasitology International*, *63*(4), 597–603. https://doi.org/10.1016/j.parint.2014.03.007

Schurer, J. M., Ramirez, V., Kyes, P., Tanee, T., Patarapadungkit, N., Thamsenanupap, P., Trufan, S., Grant, E. T., Garland-Lewis, G., Kelley, S., Nueaitong, H., Kyes, R. C., & Rabinowitz, P. (2019). Long-Tailed Macaques (*Macaca fascicularis*) in Urban Landscapes: Gastrointestinal parasitism and barriers for healthy coexistence in

Northeast Thailand. *American Journal of Tropical Medicine and Hygiene*, *100*(2), 357–364. https://doi.org/10.4269/ajtmh.18-0241

Sharma, D., Vatsya, S., Kumar, R. R., & Kumar, S. (2023). Gastro-intestinal parasites in free ranging Rhesus Macaque (*Macaca mulatta*) in Tarai region of Uttarakhand, India. *The Pharma Innovation Journal, 11*(8S), 1170-1173.

Sharma, P., Kumar, V., Pal, B., Mandial, R. K., Jithendran, K. P., & Rastogi, S. (2013). Prevalence of gastrointestinal parasitism in free ranging rhesus macaque (*Macaca mulatta*) of Himachal Pradesh. *Zoos' Print*, *28*(9), 26–27. http://www.zoosprint.org/ZooPrintMagazine/2013/September/26-27

Subedi, J. R., Gaire, K. P., & Devkota, R. P. (2020). Intestinal Helminthes Parasite among Public and Private School Children of Nepal. *Janaki Medical College Journal of Medical Science*, *8*(1), 5–13. https://doi.org/10.3126/jmcjms.v8i1.31551

Sukupayo, P. R. (2019). A Study on the Gastro Intestinal Parasites of Pigeons of Suryabinayak , Bhaktapur. *Anusheelan*, *10*, 86-91.

Sukupayo, P. R., & Tamang, S. (2023). Prevalence of Zoonotic Gastrointestinal Helminth Parasite among Dogs in Suryabinayak, Nepal. *Veterinary Medicine International*, *2023*. https://doi.org/10.1155/2023/3624593

Suntaravitun, P., & Dokmaikaw, A. (2018). Prevalence of intestinal parasites and associated risk factors for infection among rural communities of Chachoengsao province, Thailand. *Korean Journal of Parasitology*, *56*(1), 33–39. https://doi.org/10.3347/kjp.2018.56.1.33

Tabasshum, T., Mukutmoni, M., & Begum, A. (2018). Occurrence of gastrointestinal Helminths in captive rhesus macaques (*Macaca mulatta*). *Bangladesh Journal of Zoology*, *46*(2), 231–237. https://doi.org/10.3329/bjz.v46i2.39065

Tandan, S. (2023). *Prevalence of gastrointestinal parasites in Rhesus Macaques (Macaca mulatta) and Human community in Daunne, Nawalpur, Nepal* (MSc dissertation). Central Department of Zoology, Tribhuvan University.

Taylor, L. H., Latham, S. M., & Woolhouse, M. E. J. (2001). Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *356*(1411), 983–989. https://doi.org/10.1098/rstb.2001.0888

Teichroeb, J. A., Kutz, S. J., Parkar, U., Thompson, R. C. A., & Sicotte, P. (2009). Ecology

of the gastrointestinal parasites of *Colobus vellerosus* at Boabeng-Fiema, Ghana: Possible anthropozoonotic transmission. *American Journal of Physical Anthropology*, *140*(3), 498–507. https://doi.org/10.1002/ajpa.21098

Thakuri, J., Kharel, M., Tamang, G., Limbu, D.S., & Bantawa, K. (2021). Identification of gastro-intestinal parasitses from faecal matters of rhesus monkeys (*Macaca mulatta* Zimmerman, 1780) of Dharan, Nepal. *Integrating Biological Resources for Prosperity*, 424-431.

Thankachan, J., Tresamol, P. V., Vijayakumar, K., Vinodkumar, K., & Ajithkumar, S. (2022). Occurrence of gastrointestinal parasites among pigeons in Kerala. *Journal of Veterinary and Animal Sciences*, *53*(1), 39–43. https://doi.org/10.51966/jvas.2022.53.1.39-43

Traub, R. J., Robertson, I. D., Irwin, P., Mencke, N., & Thompson, R. C. A. (2002). The role of dogs in transmission of gastrointestinal parasites in a remote tea-growing community in Northeastern India. *American Journal of Tropical Medicine and Hygiene*, *67*(5), 539–545. https://doi.org/10.4269/ajtmh.2002.67.539

Wood, H. B. (1912). Intestinal parasites in the south. *Journal of the American Medical Association*, *LIX*(19), 1707–1708. https://doi.org/10.1001/jama.1912.04270110121017

World Health Organization. (1998). Control of Tropical Diseases. Geneva, Switzerland.

World Health Organization. (2020, July 29). *Zoonoses*. https://www.who.int/news-room/fact-sheets/detail/zoonoses

Wynne, C. D. L. (2021). Dogs' (*Canis lupus familiaris*) behavioral adaptations to a human-dominated niche: A review and novel hypothesis. *Advances in the Study of Behavior*, 53, 97-162. https://doi.org/10.1016/bs.asb.2021.03.004

Xue, C., Raveendran, M., Alan Harris, R., Fawcett, G. L., Liu, X., White, S., Dahdouli, M., Deiros, D. R., Below, J. E., Salerno, W., Cox, L., Fan, G., Ferguson, B., Horvath, J., Johnson, Z., Kanthaswamy, S., Michael Kubisch, H., Liu, D., Platt, M., … Rogers, J. (2016). The population genomics of Rhesus macaques (*Macaca mulatta*) based on whole-genome sequences. *Genome Research*, *26*(12), 1651–1662. https://doi.org/10.1101/gr.204255.116

Yadav, B. P. (2023). *Gastrointestinal parasites in Mushahar community in Balan Bihuli,*

Saptari, Nepal (MSc dissertation). Central Department of Zoology, Tribhuvan University.

Yadav, K.K., & Shrestha, B. (2017). Prevalence of Zoonotic Gastrointestional Helminth Parasites in Pet and Stray Dogs of Rupandehi District, Nepal. *Microbiology & Infectious Diseases*, *1*(2), 1-7. https://doi.org/10.33425/2639-9458.1014

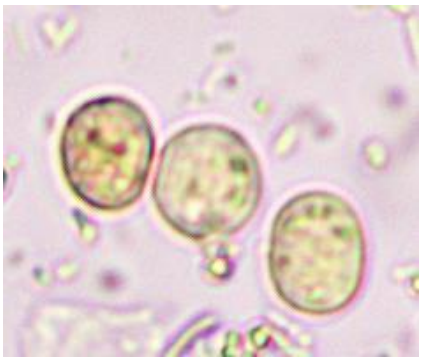
Youssef, A. I., & Uga, S. (2014). Review of parasitic zoonoses in Egypt. *Tropical Medicine and Health*, *42*(1), 3–14. https://doi.org/10.2149/tmh.2013-23

Zajac, A. M., Conboy, G. A., Greiner, E. C., Smith, S. A., & Snowden, K. F. (2012). Fecal Examination for the Diagnosis of Parasitism. In *Veterinary Clinical Parasitology*.

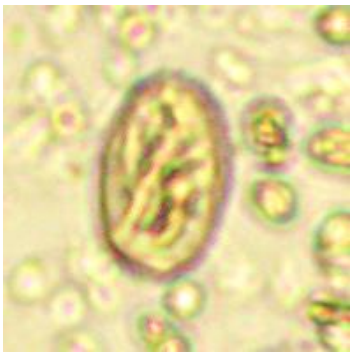
Zanetti, A. S., de Barros, L. F., de Araújo, M. S. M., Garcia, H. A., Aguiar, D. M., Espinosa, O. A., & Malheiros, A. F. (2021). Diversity and prevalence of intestinal parasites of zoonotic potential in animal hosts from different biomes in the central region of Brazil. *Annals of Parasitology*, *67*(1), 95–105. https://doi.org/10.17420/ap6701.316

Appendix 1. Photographs

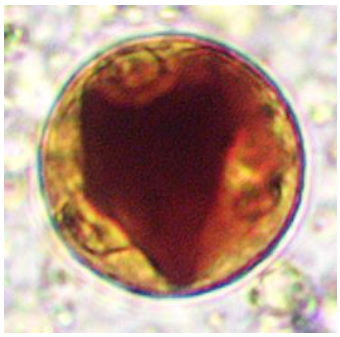
1. Photo plates of GI parasites in humans



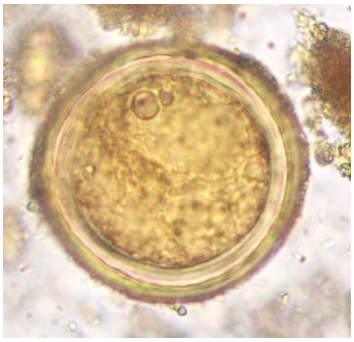
1. Cyst of *Entamoeba* spp.



2. Cyst of *Giardia lamblia*

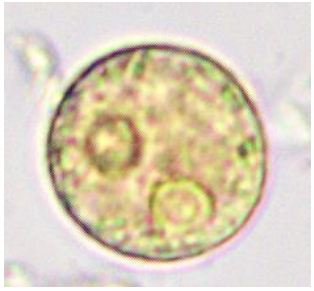


3. Cyst of *Iodamoeba buetschlii*

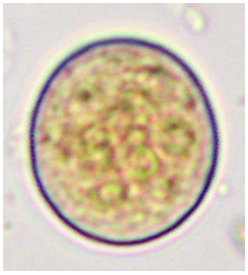


4. Egg of *Ascaris lumbricoides*

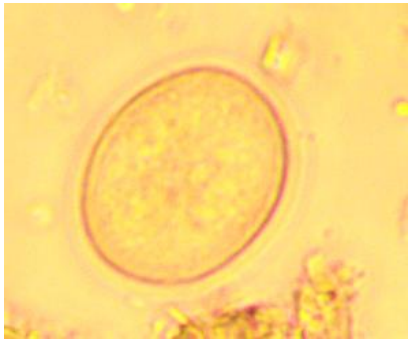
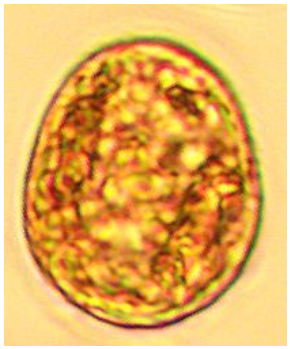
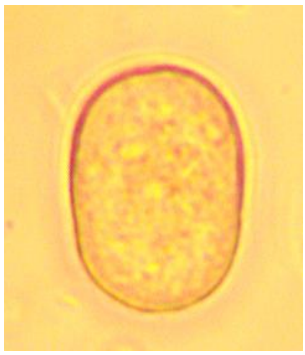
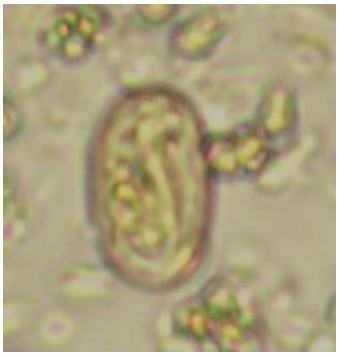
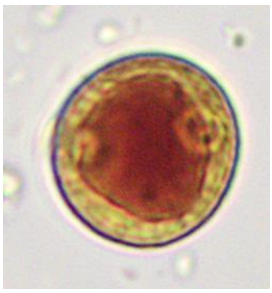
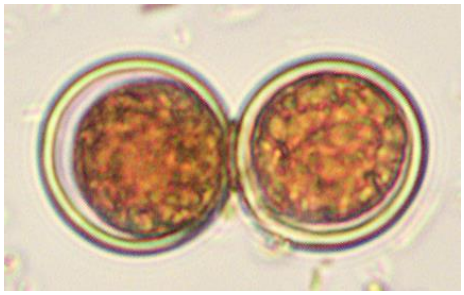
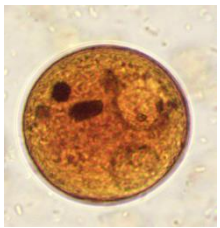
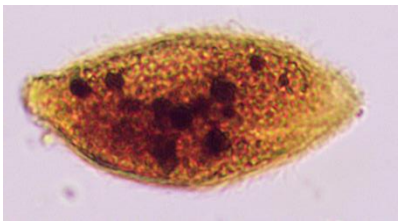
2. Photo plates of GI parasites in rhesus macaques

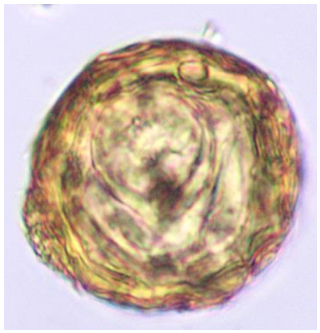


5. Cyst of *Entamoeba histolytica*

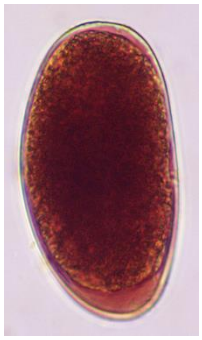


6. Cyst of *Entamoeba coli*

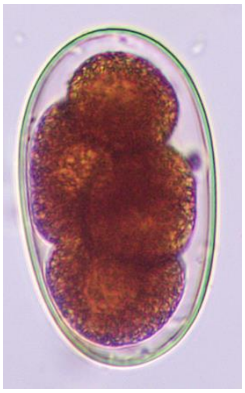
 7. Cyst of *Entamoeba* spp.	 8. Cyst of *Chilomastix* spp.
 9. Oocyst of *Endolimax* spp.	 10. Cyst of *Giardia* spp.
 11. Cyst of *Iodamoeba buetschlii*	 12. Cyst of *Isospora* spp.
 13. Cyst of *Balantidium coli*	 14. Trophozoite of *Balantidium coli*



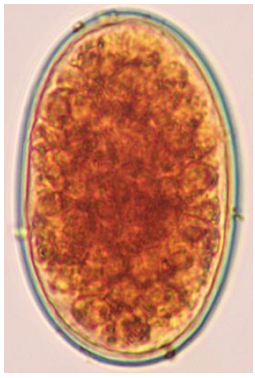
15. Egg of *Bertiella* spp.



16. Egg of *Enterobius* spp.

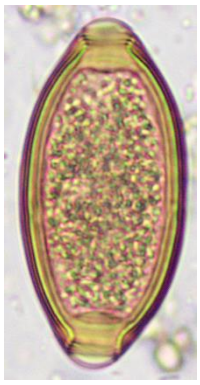


17. Egg of Hookworm



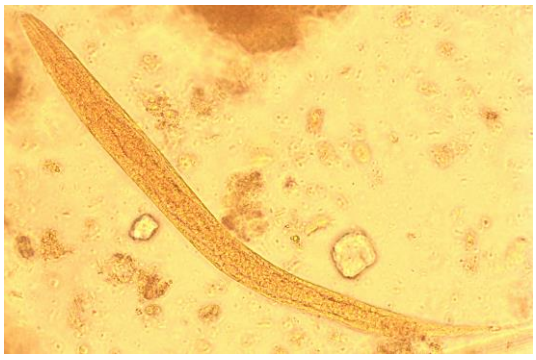
18. Egg of *Strongyle* spp.

19. Egg of *Physaloptera* spp.



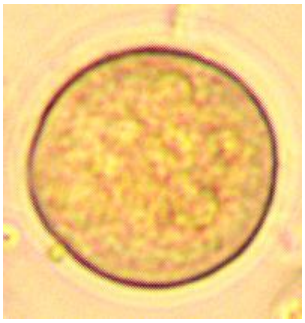
20. Egg of *Trichuris* spp.

21. Egg of *Strongyloides* spp.

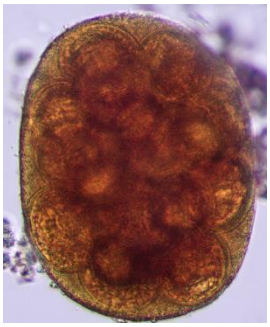


22. Larva of *Strongyloides* spp.

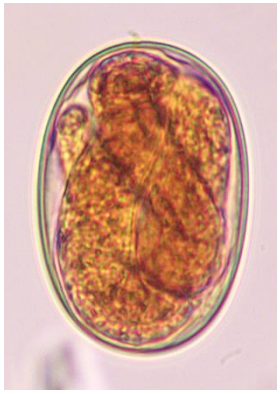
3. Photo plates of GI parasites in dogs



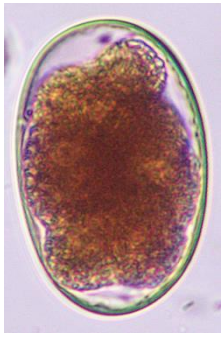
23. Cyst of *Entamoeba* spp.



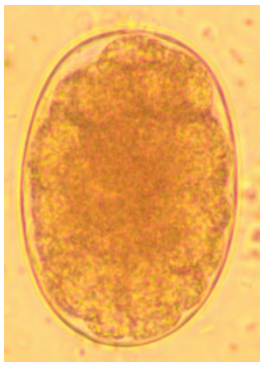
24. Egg of *Dipylidium caninum*



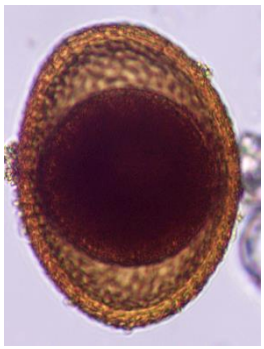
25. Egg of *Strongyloides* spp.



26. Egg of Hookworm

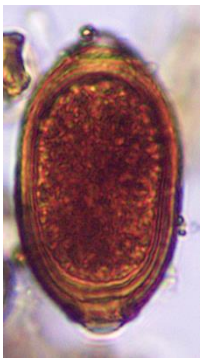


27. Egg of *Strongyle* spp.

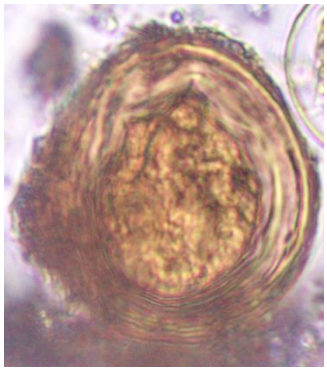
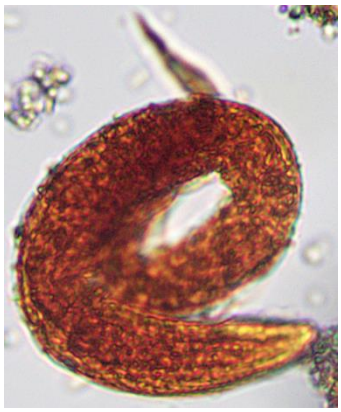


28. Egg of *Toxocara canis*

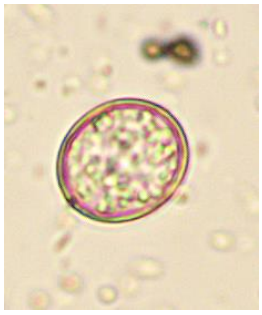
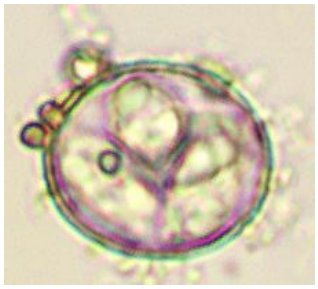
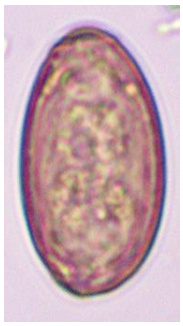
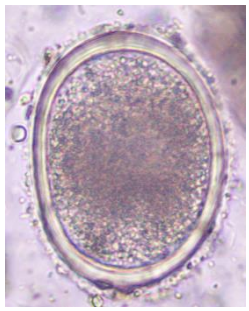
29. Egg of *Trichuris* spp.



30. Egg of *Capillaria* spp.

 31. Egg of *Ascaris* spp.	 32. Larva of *Strongyloides* spp.

4. Photo plates of GI parasites in pigeons

 33. Oocyst of *Eimeria* spp.	 34. Oocyst of coccidian
 35. Egg of *Heterophyes* spp.	 36. Egg of *Ascaridia* spp.
 37. Egg of *Capillaria* spp.	 38. Egg of *Spirocerca* spp.

5. Photos

39. Macaques consuming food provided by humans

40. Dogs consuming food provided by humans

41. Pigeons consuming food provided by humans

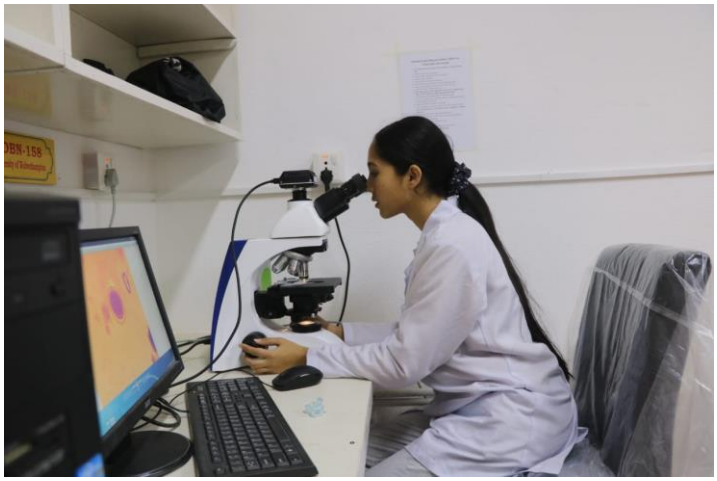
42. Macaque contaminating the water

43. Macaque and pigeons drinking water from the same water source

44. Humans, macaques, dogs and pigeons in a close proximity

45. Interaction between humans and macaques

46. Fecal sample collecting in vials



47. Microscopic examination of fecal samples

Appendix 2. Materials required

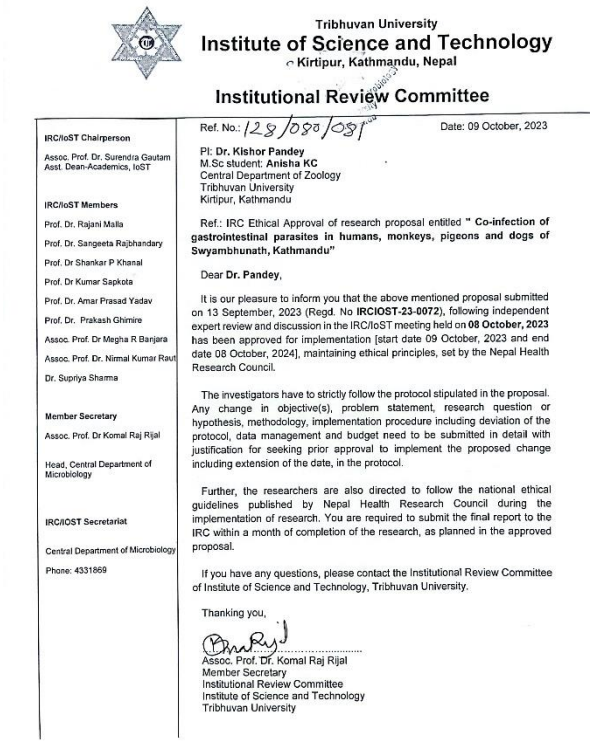
Apparatus required

- Gloves and masks
- Vials
- Tongue depressor
- Biohazard zip-lock bag
- Beaker
- Petri dish
- Conical flask
- Test tube stand
- Glass rod
- Tea strainer
- Spatula
- Glass slides
- Dropper
- Coverslips
- Graduated cylinder
- Cotton
- Fine mesh sieve tube
- Centrifuge tube
- Labeling tape
- Marker
- Forceps
- Centrifuge machine
- Weighing machine
- Toothpick
- Phase contrast microscope
- Ice box

Chemicals required

- 2.5% Potassium dichromate solution ($K_2Cr_2O_7$)
- Sodium chloride solution (Nacl)
- Lugol's iodine solution
- Distilled water
- 0.85% Normal saline
- 10% formalin
- Ethyl acetate

Appendix 3. Ethical approval Letter



Tribhuvan University
Institute of Science and Technology
Kirtipur, Kathmandu, Nepal

Institutional Review Committee

IRC/IoST Chairperson
Assoc. Prof. Dr. Surendra Gautam
Asst. Dean-Academics, IoST

IRC/IoST Members
Prof. Dr. Rajani Malla
Prof. Dr. Sangeeta Rajbhandary
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Prof. Dr Kumar Sapkota
Prof. Dr. Amar Prasad Yadav
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Assoc. Prof. Dr Megha R Banjara
Assoc. Prof. Dr. Nirmal Kumar Raut
Dr. Supriya Sharma

Member Secretary
Assoc. Prof. Dr Komal Raj Rijal
Head, Central Department of Microbiology

IRC/IOST Secretariat
Central Department of Microbiology
Phone: 4331869

Ref. No.: 128/080/081 Date: 09 October, 2023

PI: **Dr. Kishor Pandey**
M.Sc student: **Anisha KC**
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu

Ref.: IRC Ethical Approval of research proposal entitled **" Co-infection of gastrointestinal parasites in humans, monkeys, pigeons and dogs of Swyambhunath, Kathmandu"**

Dear **Dr. Pandey**,

It is our pleasure to inform you that the above mentioned proposal submitted on 13 September, 2023 (Regd. No **IRCIOST-23-0072**), following independent expert review and discussion in the IRC/IoST meeting held on **08 October, 2023** has been approved for implementation [start date 09 October, 2023 and end date 08 October, 2024], maintaining ethical principles, set by the Nepal Health Research Council.

The investigators have to strictly follow the protocol stipulated in the proposal. Any change in objective(s), problem statement, research question or hypothesis, methodology, implementation procedure including deviation of the protocol, data management and budget need to be submitted in detail with justification for seeking prior approval to implement the proposed change including extension of the date, in the protocol.

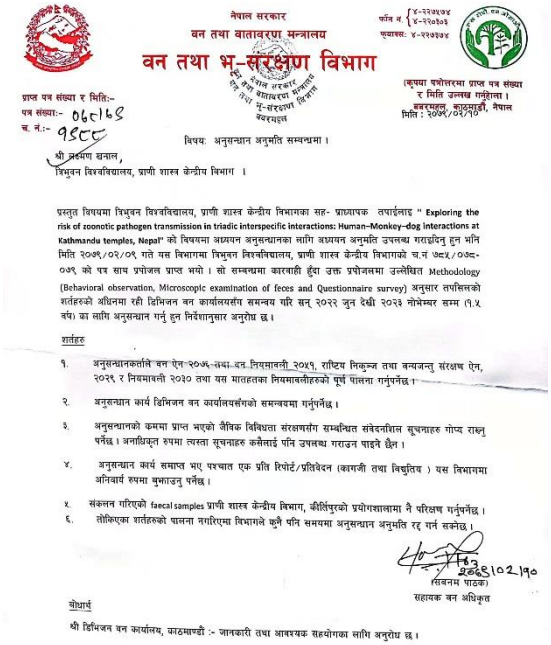
Further, the researchers are also directed to follow the national ethical guidelines published by Nepal Health Research Council during the implementation of research. You are required to submit the final report to the IRC within a month of completion of the research, as planned in the approved proposal.

If you have any questions, please contact the Institutional Review Committee of Institute of Science and Technology, Tribhuvan University.

Thanking you,

Assoc. Prof. Dr. Komal Raj Rijal
Member Secretary
Institutional Review Committee
Institute of Science and Technology
Tribhuvan University

1. Approval from IRC of IoST



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

फोन नं. ४-२२०५७४, ४-२२०८७३
फ्याक्स: ४-२२३३४४

(कृपया पत्रोत्तरमा प्राप्त पत्र संख्या र मिति उल्लेख गरिदिनुहोला ।)
बबरमहल, काठमाडौं, नेपाल
मिति : २०७९/०२/१०

प्राप्त पत्र संख्या र मिति:-
पत्र संख्या:- ०६८।६८
च. नं.:- ९५८८

विषय: अनुसन्धान अनुमति सम्बन्धमा ।

श्री अक्षण खनाल,
त्रिभुवन विश्वविद्यालय, प्राणी शास्त्र केन्द्रीय विभाग ।

प्रस्तुत विषयमा त्रिभुवन विश्वविद्यालय, प्राणी शास्त्र केन्द्रीय विभागका सह- प्राध्यापक तपाईलाई " **Exploring the risk of zoonotic pathogen transmission in triadic interspecific interactions: Human–Monkey–dog interactions at Kathmandu temples, Nepal**" को विषयमा अध्ययन अनुसन्धानका लागि अध्ययन अनुमति उपलब्ध गराइदिनु हुन मिति २०७९/०२/०९ गते यस विभागमा त्रिभुवन विश्वविद्यालय, प्राणी शास्त्र केन्द्रीय विभागको च.नं ७८१/०७८-०७९ को पत्र साथ प्रपोजल प्राप्त भयो । सो सम्बन्धमा कारवाही हुँदा उक्त प्रपोजलमा उल्लेखित Methodology (Behavioral observation, Microscopic examination of feces and Questionnaire survey) अनुसार तपसिलको शर्तहरुको अधिनमा रही त्रिभिजन वन कार्यालयसँग समन्वय गरि सन् २०२२ जुन देखी २०२३ नोभेम्बर सम्म (१.५ वर्ष) का लागि अनुसन्धान गर्नु हुन निर्देशानुसार अनुरोध छ ।

शर्तहरु

१. अनुसन्धानकर्ताले 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 त्रिभिजन वन कार्यालयसँगको समन्वयमा गर्नुपर्नेछ ।
३. अनुसन्धानको क्रममा प्राप्त भएको जैविक विविधता संरक्षणसँग सम्बन्धित संवेदनशील सूचनाहरु गोप्य राख्नु पर्नेछ । अनाधिकृत रुपमा त्यस्ता सूचनाहरु कसैलाई पनि उपलब्ध गराउन पाइने छैन ।
४. अनुसन्धान कार्य समाप्त भए पश्चात एक प्रति रिपोर्ट/प्रतिवेदन (कागजी तथा विद्युतिय) यस विभागमा अनिवार्य रुपमा बुझाउनु पर्नेछ ।
५. संकलन गरिएको faecal samples प्राणी शास्त्र केन्द्रीय विभाग, जीतपुरको प्रयोगशालामा नै परिक्षण गर्नुपर्नेछ ।
६. तोकिएका शर्तहरुको पालना नगरिएमा विभागले कुनै पनि समयमा अनुसन्धान अनुमति रद्द गर्न सक्नेछ ।

२०७९।०२।१०
(सबनम पाठक)
सहायक वन अधिकृत

बोधार्थ

श्री त्रिभिजन वन कार्यालय, काठमाण्डौं :- जानकारी तथा आवश्यक सहयोगका लागि अनुरोध छ ।

2. Approval from Department of Forest and Soil Conservation

Appendix 4. Sample collection procedure

Procedure to collect stool sample

Step 1
- If needed, urinate before starting to avoid contamination with feces
- Flush the toilet
- Wash your hands
- Put on the gloves provided

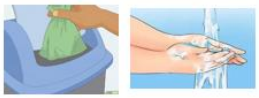
Step 2
- Place clean newspaper over the toilet seat opening under the lid. Also, place the toilet paper over it
- If the stool has been watery, put the paper bowl over the toilet paper

Step 3
- Defecate siting on the toilet seat onto the toilet paper or onto the paper bowl
- Make sure there is no urine or water in the toilet paper with the stool

Step 4
- Using the applicator, collect small scoop of stool (feces)
- Make sure no urine, water or other material gets in the container

Step 5
- Place the specimen container in the plastic bag provided
- Flush the toilet paper with stool down the toilet

Step 6
- Wrap the paper bowl or newspaper and gloves in clean newspaper and dispose it
- Wash hands with soap and warm water for 20 seconds



Step 7
Please do not forget to send/give your sample to the researcher as soon as possible (within a day)

दिसाको नमुना सङ्कलन गर्ने प्रक्रिया

प्रक्रिया १
- सर्वप्रथम शौचालय सफा गर्नुहोस्।
- साबुन पानीले हात धुनु होस्।
- उपलब्ध गराइएको पन्जा (Gloves) लगाउनुहोस्।

प्रक्रिया २
- शौचालयको सिट खोल्ने ठाउँमा सफा समाचार पत्र राख्नुहोस्।
- साथै, यसको माथि उपलब्ध गराइएको टाइलेट पेपर (toilet paper) राख्नुहोस्।
- यदि झाडापखाला भएको छ भने, कागजको कचौरा (Paper bowl) शौचालय पेपरमाथि राख्नुहोस्।

प्रक्रिया ३
- शौचालयको सिटमा बसेर टाइलेट पेपर वा कागजको भाँडोमा शौच गर्नुहोस्।
- टाइलेट पेपरमा पिसाब वा पानी छैन भनी सुनिश्चित गर्नुहोस्।

प्रक्रिया ४
- एप्लिकेटर प्रयोग गरेर, दिसाको थोरै मात्रा कन्टेनरमा सङ्कलन गर्नुहोस् ।
- कन्टेनरमा पिसाब, पानी वा अन्य सामग्री नपरोस् भनी सुनिश्चित गर्नुहोस्।

प्रक्रिया ५
- उपलब्ध गराइएको प्लास्टिकको झोलामा कन्टेनर राख्नुहोस्।
- शौचालयमा, दिसा सहित बाँकी रहेको टाइलेट पेपरलाई फ्लस (Flush) गर्नुहोस्।

प्रक्रिया ६
- कागजको कचौरा वा अखबार र पन्जालाई सफा अखबारमा बेरेर फाल्नुहोस्।
- साबुन र तातो पानीले २० सेकेन्डसम्म हात धुनुहोस्।

प्रक्रिया ७
- कृपया, जतिसक्दो चाँडो अनुसन्धानकर्तालाई आफ्नो नमूना पठाउन/दिन नबिर्सनुहोस् (एक दिन भित्र)।

Appendix 5. Identification sheet of rhesus macaques

Name of the monkey: Smiley

Code: Sm

Sex: Male

Age: Adult

- Smiley faced

- Black mole is present close to the right eye

- Three white spot is present above right mouth

Name of the monkey: Camel-humped

Code: Ch

Sex: Female

Age: Adult

- Camel hump shaped monkey

- Left mammary gland is larger
- Ring like shaped in left mammary gland

- Matted inner tail

Appendix 6. Identification sheet of dogs

Name of the dog: Butterfly

Code: Bf

Sex: Female

Age: Adult

- Forelimb = white coloured
- Hind limb = anterior black
- Belly region = all white

- Body colour = black and white

- Posterior part of the body is slightly brown
- Tail = 10% black, 90% white

Name of the dog: Kali

Code: Ka

Sex: Female

Age: Adult

- Forelimb = Black, left paw white coloured
- Hind limb = Black, paw has little white fur

- Black coloured with 5% burgundy
- Mouth has little white fur posteriorly

- Tail = fluffy