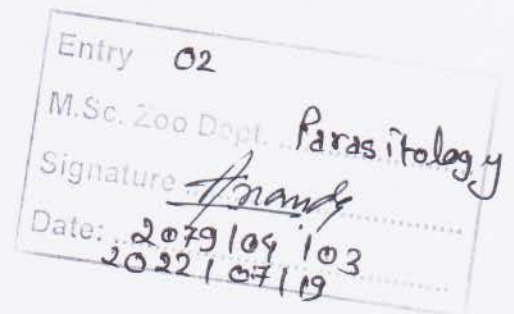


**GASTROINTESTINAL PARASITES IN HUMAN AND RHESUS
MONKEY OF NILBARAHI AREA, BHAKTAPUR, NEPAL**



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A thesis submitted

In partial fulfillment of the requirements for the award of the degree of Master of
Science in Zoology with special paper Parasitology

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kritipur, Kathmandu

Nepal

July, 2022

DECLARATION

I hereby declare that the work presented in this thesis entitled “**Gastrointestinal parasites in human and rhesus monkey of Nilbarahi area, Bhaktapur, 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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LISTS OF ABBREVIATIONS

Abbreviation form	Details of abbreviation
μm	Micrometer
CI	Confidence interval
df	Degree of freedom
E	Eastern
<i>et al.</i> ,	ET alia (and other)
GIT	Gastrointestinal Tract
GPS	Global Positioning System
HIV/AIDS	Human immunodeficiency virus infection/ acquired immunodeficiency syndrome
IPTs	Intestinal parasitic infections
$\text{K}_2\text{Cr}_2\text{O}_7$	Potassium dichromate
N	Northern
NaCl	Sodium Chloride
NAST	Nepal Academy of Science and Technology
NHP	Non-human primates
NHRC	Nepal Health Research Council
P- value	Probability value
PZs	Parasitic zoonoses
Rpm	Rotation per minute
Sp.	Species
SPG	Specific gravity

STH	Soil-transmitted helminth
TU	Tribhuvan University
VDC	Village Development Committee
WHO	World Health Organization
χ^2	Chi square

ABSTRACT

Gastrointestinal parasites are usual threats to primate populations. The study was conducted from March to April-2021 to determine the prevalence of gastrointestinal parasites in humans and monkeys in the Nilbarahi area, Bhaktapur, Nepal. Altogether 200 samples from humans (100) and monkeys (100) were collected and preserved in a 2.5% potassium dichromate solution. These samples were analyzed microscopic by direct wet mount method and concentration procedures, viz. flotation technique and sedimentation technique, in the laboratory of Nepal Academy of Science and Technology, Khumaltar, Lalitpur. Out of 100 samples of humans, 13(13%) samples were found positive, with the prevalence of protozoan parasites found higher compared to helminthic parasites. The most common protozoan parasites detected were *Cryptosporidium* sp. followed by *Ascaris lumbricoides* and *Taenia* sp. The infection rate was found higher in females than in males. The old age (above 60 yrs.) indicated the highest infection rate than other age groups. Only one parasite *Cryptosporidium* sp. showed heavy infection. The single parasitic infection was significantly high as compared to double infection. Similarly, in 100 fecal samples of monkeys examined, 81 (81%) were found positive prevalence with protozoan parasites indicated greater as compared to helminthic parasites. Among the protozoan infection, the most common parasites detected were *Entamoeba* spp. followed by *Entamoeba coli*, *Balantidium coli*, *Cryptosporidium* sp., *Cyclospora* sp., *Giardia* sp., *Eimeria* sp., and *Endolimax* sp. Among the helminthic infection, the infection of Ascarid spp. and Strongyle sp. were highest followed by *Taenia* sp., Hookworm whereas *Fasciola* sp., *Controrchis* sp. and *Capillaria* sp. infections were equal. All the identified parasites showed light infection. The single parasitic infection was high as compared to double, triple, and multiple infections. The common parasites were detected from two hosts were *Cryptosporidium* sp., *Taenia* sp., and Ascarid spp. due to poor sanitation habits, lack of safe drinking water, open place defecation, barefoot walking habits, demand for traditional practices, and sharing houses with animals increases the chance of infection with intestinal parasitic infection in people of the Nilbarahi area. There is a great need of generating awareness regarding periodic anti- parasitic treatment of such animals and other prevention and control measures.

Keywords: Human, Monkeys, Gastrointestinal parasites

1. INTRODUCTION

1.1 Background

Intestinal parasites can infect the host's gastrointestinal system, where they can obtain nutrition and suitable habitat in which to complete their life cycle. Gastrointestinal parasites include protozoa and helminths in human beings and other animals (Loukopoulos *et al.*, 2007). Intestinal parasitic infection is one of the significant health troubles in developing countries (Shrestha *et al.*, 2012). About 197 species of intestinal parasites have been reported in association with the human alimentary tract and their diseases are endemic all over the world (Ahmed and Siddiqu, 2013). It is estimated that greater than 24% of people worldwide get infected with intestinal parasitic infections (IPTs) (WHO, 2017). In Nepal, above half of the human beings are infected with one or more intestinal parasite species and in some rural areas, over 90 percent of locals are contaminated (Rai *et al.*, 1986).

In developed countries, protozoan parasites are more commonly gastrointestinal infections compared to helminths (Burgess *et al.*, 2017). *Giardia intestinalis*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, and *Cryptosporidium* spp. are the most prevalent intestinal protozoan parasites which cause diseases like giardiasis, amoebiasis, cyclosporiasis, and cryptosporidiosis respectively and they are associated with diarrhea (Davis *et al.*, 2002). Diarrheal diseases are a major food-borne public health problem throughout the world. (Li *et al.*, 2020). Each year, over 1.7 billion cases of childhood diarrhea are reported worldwide (WHO, 2017). Globally, *E. histolytica* remains one of the top three causes of parasitic mortality (Cui *et al.*, 2019). It affects roughly 50 million people each year, with 100,000 deaths annually (Bercu *et al.*, 2007) followed the second most prevalent cause of diarrhea in children is *Cryptosporidium* spp. (Bouzid *et al.*, 2018). Soil-transmitted helminth infections are among the most frequent infections in the world, affecting the poorest and most deprived communities. There are four species of intestinal helminthic parasites, also known as geohelminths and soil-transmitted helminths (STH): *Ascaris lumbricoides* (roundworm), *Trichiuris trichiuria* (whipworm), *Ancylostoma duodenale*, and *Necator americanus* (hookworm) (Haque, 2007). The World Health Organization estimates that more than 1.5 billion people become ill each year due to soil-transmitted helminths (WHO, 2020). According to global estimates of STH prevalence, Ascariasis (roundworm infection) is

the most prevalent (819 million), followed by Trichuriasis (whipworm infection) (464 million), and hookworm infection (438 million) (Pullan *et al.*, 2010). STH is a common disease in school-aged children, and it's often linked to stunted growth, decreased physical activity, and diminished cognitive function and learning ability (Stephenson *et al.*, 1998). Parasites can affect people of all ages, causing symptoms such as loss of appetite, vomiting, diarrhea, dysentery, fever, dehydration, and even mental issues (Bentley *et al.*, 2006). Intestinal parasites are spread via feces contamination in water, food, and soil, a lack of human cleanliness, and the intake of undercooked meat (Whittier *et al.*, 2000; Loukopoulos *et al.*, 2007). Severe parasitosis usually affects people due to social, geographical, economical, and inhabitant customs (Latham, 1997).

Nonhuman primates (NHP) are the closest living biological friends of humans, providing tremendous insights into human evolution, biology, and behavior as well as having integral roles in many nations' livelihoods, cultures, and religions (Tabassum *et al.*, 2018). Nepal has been reported to have five different monkey species: Assamese Macaque (*Macaca assamensis* Hodgson, 1840), Rhesus Macaque (*Macaca mulatta*, Zimmermann, 1780), Terai Grey Langur (*Semnopithecus hector* Pocock, 1928), Nepal Grey Langur (*Semnopithecus schistaceus* Hodgson, 1840), and Himalayan Grey Langur (*Semnopithecus ajax* Pocock, 1928) (Jnawali *et al.*, 2011). Rhesus macaques (*Macaca mulatta*) are synanthropic amongst NHPs, surviving in human-altered environments and becoming one of the world's greatest distributed and successful primates (Hasan *et al.*, 2014). It is the most adaptable and invasive of all primate taxa in terms of socioecological factors (Balasubramaniam *et al.*, 2020). The Rhesus macaque is a vital part of the biodiversity of the world, as well as a visible link between humans and nature. They have been discovered to be well adapted and coexist with people in both urban and agricultural settings (Cawthon, 2005).

In nonhuman primates, parasitic infections are among the most common diseases (Strait *et al.*, 2012). More than 50 different species of parasites were recorded in non-human primates (Nunn and Altiezer, 2005). They are infected with various parasites, which can be lethal depending on the type of parasite or the parasite load. Both protozoan and helminth parasites are very common (Munene *et al.*, 1998; Muriuki *et al.*, 1998). The most common intestinal protozoan parasites are *Giardia lamblia*, *Balantidium coli*, *Entamoeba histolytica*, *Cryptosporidium* spp, and helminth parasites including *Schistosoma mansoni*, *Oesophagostomum* spp, *Enterobius vermicularis*, *Strongyloides*

spp, *Trichostrongylus*, *Trichuris*, *Ascaris*, *Chaberti*, Hookworm, and *Taenia*. These parasitic infections can lead to diarrhea, blood loss, tissue damage, pulmonary lesions, abdominal complications, spontaneous abortion, congenital malformations, and death (Despommier *et al.*, 1995; Chandra and Newberne, 1977). On the other hand, massive anthropogenic changes to natural environments, such as increased host density, feeding platforms, rainy season, and climatic and temporal dynamics, may put primate populations in danger and modify parasite hazards on nonhuman primate reproductive and survival (Klaus *et al.*, 2018). This may result in a high proportion of mortality and morbidity (Chapman *et al.*, 2005).

A zoonosis is a disease or infection that is naturally transmitted from animals to human beings or from humans to animals. This consists of a wide range of bacteria, viruses, fungi, protozoa, parasites, and other pathogens (Rahman *et al.*, 2020). Parasitic zoonoses (PZs) are a serious but unnoticed public health issue, especially in growing countries (Devleeschauwer, 2014). Humans are hosts to almost 300 parasitic worm species and more than 70 protozoan species, some of which are transmitted from domesticated animals, and others are obtained when they come into contact with the source of infection (Ashford and Crewe, 1998). Approximately 61 percent of human pathogens are zoonotic (Taylor, 2001). Rhesus macaques are turning into a more vital source of zoonotic disease transmission (Wolfe *et al.*, 2007). They can transmit disease to human beings and serve as reservoirs for a range of emerging infectious diseases, such as malaria (Siregar *et al.*, 2015). The close evolutionary link between people and nonhuman primates (NHP), as nicely as human encroachment into herbal NHP habitats and proximity to them resulted in an established pathogen alternate (Ott- Joslon, 1993; Dawet *et al.*, 2013). Some studies have recognized rising human diseases, such as HIV/AIDS and Ebola that have non-human primate origins or are likely to be transmitted to people (Gao *et al.*, 1999; Leroy *et al.*, 2004). Likewise, proof from well-studied ape groups implies that polio, respiratory illnesses, and scabies originated from people (Hill *et al.*, 2001; Kalema-Zikusoka *et al.*, 2002).

1.2 Objectives

General objective

- To determine the prevalence of gastrointestinal parasites in human and rhesus monkeys of Nilbarahi, Bhaktapur.

Specific objectives

- To determine the prevalence of gastrointestinal parasitic infection with respect to age-wise and sex-wise in humans.
- To determine the intensity of gastrointestinal parasites among humans and monkeys.
- To compare the common gastrointestinal parasites of humans and monkeys.

1.3 Justification of the study

Intestinal parasites are widely prevalent in developing countries, probably due to inadequate sanitation and personal cleanliness. Approximately 3.5 billion people are infected by intestinal parasites and around 450 million children are ill due to these infections (WHO, 1998). Rhesus macaque is an important part of biodiversity and also forms a cognizable link between humans and nature. Many evidences demonstrate that many emerging parasites in humans are derived from monkeys and there is also a significant risk of human diseases being transmitted to primates (Jones-Engel *et al.*, 2006). Thus, there is a great need for studies about the prevalence of parasites among humans as well as animals. In Nepal, much research has been done on human and rhesus monkeys as well as their infectious diseases in different areas but the presence of zoonotically important GI parasites in human and monkeys are very less. To fulfill this gap current study have been conducted documentation to an understanding of the pathogenic interrelationship between humans and monkeys in the Nilbarahi of Bhaktapur which can be of great use for present and future generations.

2. LITERATURE REVIEW

Parasites have always been a significant part of the natural history of mammals. They form an important part of the ecosystem. Parasitism may rise when the density of intermediate host's increases, and it is also influenced by temperature, humidity, and rainfall (Lafferty, 1997; Sardar *et al.*, 2006). Parasites are spread by direct contact, alimentary (foodborne and waterborne), or aerogenic (airborne) routes and some agents are arthropod-borne and rodent-borne (Hubalek, 2003). It is estimated that as much as 60% of the world's population is infected with intestinal parasites (Ragunathan and Kalivaradhan, 2010).

Gastrointestinal (GIT) illnesses contribute significantly to the burden of illness from infectious diseases worldwide (Fletcher *et al.*, 2013). It causes significant morbidity and mortality in endemic countries. In developing countries, intestinal parasitic infections are one of the top ten major public health problems due to poor hygienic practices and unsanitary conditions (Ahmed and Siddiqu, 2013; Alwabr and Moayed, 2016). Dhanabal *et al.*, (2014) reported a higher GI prevalence of 75.7% from Chennai and recorded parasites like *Entamoeba coli*, *Cyclospora sp.*, *Entamoeba histolytica*, *Giardia intestinalis*, *Ascaris lumbricoides*, *Trichuris trichiura* and *Hymenolepis nana* by concentration techniques like sedimentation and floatation were performed. A similar method was used but a low parasitic prevalence (11.62%) was obtained by (Kotian *et al.*, 2014) in Uttarakhand hills. A similar study conducted by, Arshad *et al.*, 2019 reported (12.4%) parasitic prevalence and found nine different species of parasites like *Entamoeba histolytica*, *Giardia lamblia*, *Ascaris lumbricoides*, *Blastocystis hominis*, *Hymenolepis nana*, *Entamoeba coli*, *Iodamoeba butschlii*, *Ancylostoma duodenale* and *Taenia saginata* in Pakistan.

Subba and Singh, (2020) resulted in protozoan (29.3%) infection as dominant intestinal parasites than helminthic infection (4.6%) in East Sikkim. This result agrees with the data of a study done by (Sayyari *et al.*, 2005) reported in Iran. Whereas, (Nisha *et al.*, 2015; Irum *et al.*, 2021) recorded a higher prevalence of helminth infection compared to protozoan parasites among Malaysian and Pakistani people respectively. In Cambodia, (Liao *et al.*, 2017) found *Giardia intestinalis* was the most common intestinal parasite followed by *Entamoeba histolytica/dispar* but (Choubisa *et al.*, 2012) recorded *Entamoeba histolytica* was the commonest followed by *Entamoeba coli*,

Taenia solium, *Ascaris lumbricoides*, *Hymenolepis nana*, *Ancylostoma duodenale*, *Strongyloides stercoralis*, *Trichuris trichiura* and *Hymenolepis diminuta* in Rajasthan, India. Reports of various studies show that *A. lumbricoides* showed the greatest infection in different countries (Pandey and Mishra, 2000; Adefioye *et al.*, 2011; Da Silva *et al.*, 2016; Ganguly *et al.*, 2017). But, (Aimpun and Hshieh, 2004; Magaji and Magaji, 2021) found the most common infection was hookworm in the American and African continents respectively. A study conducted by (Oliveira *et al.*, 2016) in Africa found that protozoan parasites were more common among children and the frequency of helminths, was higher in adults which is compared between two indigenous ethnic groups in Amazonia. But, (Kirorei *et al.*, 2014) showed helminth infection was greater in primary school children from Western Kenya. This result was supported by (Hossain *et al.*, 2019) in Dhaka city which recorded eight species of parasites. i.e: *Entamoeba histolytica*, *Giardia lamblia*, *Diphyllobothrium latum*, *Paragonimus wastermani*, *Ascaris lumbricoides*, *Trichuris trichura*, *Ancylostoma duodenale* and *Enterobius vermicularis*.

Nepal is a small underdeveloped country in South Asia has also been affected by intestinal parasitic infections which are most prevalent in children (Chandrashekhar *et al.*, 2009). About, 70% of morbidity and mortality in Nepal are associated with infectious diseases (Agrawal *et al.*, 2012). In rural areas, open-air defecations are common and enhance parasites to invade individuals (Dhakal and Subedi, 2019). Giardiasis, Ascariasis, Amoebiasis, Ancylostomiasis, and Taeniasis are common intestinal parasitic infections in Nepal (Shrestha *et al.*, 2012). In the eastern region of Nepal, (Ghimire *et al.*, 2014) found a high prevalence of *Giardia intestinalis* (30.9%) followed by, Hookworm (18.6%), *Ascaris lumbricoides* (15.5%), *Hymenolepis nana* (6.2%) and *Entamoeba histolytica* (5.2%) among school-going children. Similarly, (Baral *et al.*, 2017) reported the same result among patients attending tertiary care hospitals. Rai *et al.*, (2017) described that the protozoan infection rate was higher than helminth infection in the rural area of school-going children of Lokhim VDC Nepal. But the result conflicted with the research done by (Yadav and Mahato, 2017) found a high prevalence of helminth infection in school children of Rangeli Municipality of Morang District. This result is supported by (Singh *et al.*, 2017; Dhakal and Subedi, 2019; Chaudhary and Subedi, 2020) showed *Ascaris lumbricoides* to be the most common helminth parasites from the different localities of eastern parts of Nepal.

Different research was done on indigenous people in the Central region of Nepal. The study conducted by (Gyawali, 2012; Thapa, 2018; Adhikari *et al.*, 2020; Khadka *et al.*, 2021) showed a high prevalence of helminthic infection than protozoan infection. Among different school children, the prevalence of parasites was found to be high in boys than in girls (Maharjan *et al.*, 2013; Pradhan *et al.*, 2014; Yadav and Prakash, 2016; Gurung *et al.*, 2019). This result was supported by (Adhikari *et al.*, 2020) which represented the highest infection rate in the male Chepangs group in Chitwan. Several studies conducted in western Nepal also conducted that poor sanitary conditions and education levels are the main cause of the parasitic infection. A study conducted by (Shrestha *et al.*, 2012) showed among school children in the Baglung district recorded a high prevalence showed *Entamoeba histolytica* (9.23%) followed by *Giardia lamblia* (5.76%), *Trichuris trichuria* (5%), *Ancylostoma duodenale* (2.65%) and *Ascaris lumbricoides* (2.3%). The study was also supported by (Oli, 2016; Jha, 2019). But (Chandrashekhar *et al.*, 2020) recorded the highest infection rate was *Giardia lamblia* (13.2%) followed by *Ascaris lumbricoides* (2.1%) and *Entamoeba histolytica* (1.7%) among school-going children in Western Nepal.

Parasites are found to affect every group of organisms and monkeys are no exception because of their feeding and dwelling habit. The third most important cause of the population decline of wildlife after hunting and habitat degradation is infectious diseases (Bengis *et al.*, 2004). Since the monkeys live near the human residents, they not only share human foods but also parasites. The close relationship between primates and social groups facilitates the transmission of the parasite (Stoner, 1996). In India, (Parmar *et al.*, 2012) and (Arunachalam *et al.*, 2015) conducted a study among free-range rhesus macaque and found a high prevalence of Strongyle spp. whereas, (Ye *et al.*, 2012) reported *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* of free-range rhesus monkeys in a popular public park in China. Li *et al.*, (2015) recorded a study in captive primates and the result showed *Trichuris* spp. was the most predominant in the primates, while *Entamoeba* spp. was the most prevalent in Old World monkeys of twenty-four zoological gardens in China. This result agrees with the data of a study done by (Nath *et al.*, 2012) that reported the prevalence of two parasitic species viz. *Trichuris* sp and *Oesophagostomum* sp. in captive monkeys of Assam State Zoo. Niranjana *et al.*, (2020) recorded protozoans infection were more than helminths in captive wildlife of Kanpur Zoological Park. But the result conflicted with

the research done by (Munene *et al.*, 1998) conducted a study between the two areas, in captive and wild trapped non-human primates (Baboons, Vervets and Sykes) detected helminths parasites higher than protozoa from Kenya. Two different studies conducted by (Ekanayake *et al.*, 2006) and (Huffman *et al.*, 2013) among toque macaque, grey langur, and the purple-faced langur reported co-infection of *Cryptosporidium* with *Enterobius* and *Strongyloides* in the former study whereas *Trichuris* sp. and *E. coli* were found out from later study in Sri Lanka.

Different research has been done on non-human primates in different parts of Nepal regarding intestinal parasites. Dhoubhadel (2007) found the highest prevalence of *Strongyloides fulleborni* (42.5%) whereas *Ascaris lumbricoides* (1.57%), *Toxocara canis* (1.57%), and *Chabertia* sp (1.57%) were found to be the least prevalent from Swoyambhu and Nilbarahi area. Another study conducted in the same year (Malla, 2007) also examined the same results which are compared between the two places of Pasupati and Nilbarahi area of Kathmandu valley. This kind of similar study conducted by (Nepal, 2010) found the maximum prevalence of *Strongyloides* sp. which is compared between the two seasons in the Swoyambhu area. A study conducted by (Pokhrel and Maharjan, 2014) and (Sapkota *et al.*, 2020) showed the highly prevalent protozoan parasites in primates. But the result conflicted with the research done by (Jha *et al.*, 2011; Adhikari and Dhakal, 2018; Thakuri, 2020) recorded helminths are higher than protozoan parasites in a different area of Nepal.

3. MATERIALS AND METHODS

3.1 Study area

The study area Nilbarahi temple is situated in the middle of the forest in Bode of Madhyapur Thimi. Madhyapur Thimi, also known as Thimi, is a municipality in Bhaktapur District in the Bagmati Zone of central Nepal. "Madhya" means center and "pur" means city; therefore, Madhyapur means city placed in the center. It is located in the center of the valley between Patan, Kathmandu, and Bhaktapur. It is one of the ancient cultural and historical places along the way from Bhaktapur to Kathmandu. The city is sited on elevated land and occupies an area of 11.47 sq. km, divided into nine administrative wards. Geographically, this municipality situates between 27° 40' 00" to 27° 40' 00" N Latitudes and 81° 22' 30" to 85° 25' 00" E Longitudes, at an average altitude of 1300 meters average mean sea level. Madhyapur Thimi is an ancient Newari town and is a center of Newar culture. Besides these other different ethnic/caste groups like Brahman, Chhetri, Damai, Sarki, and Kami inhabit.

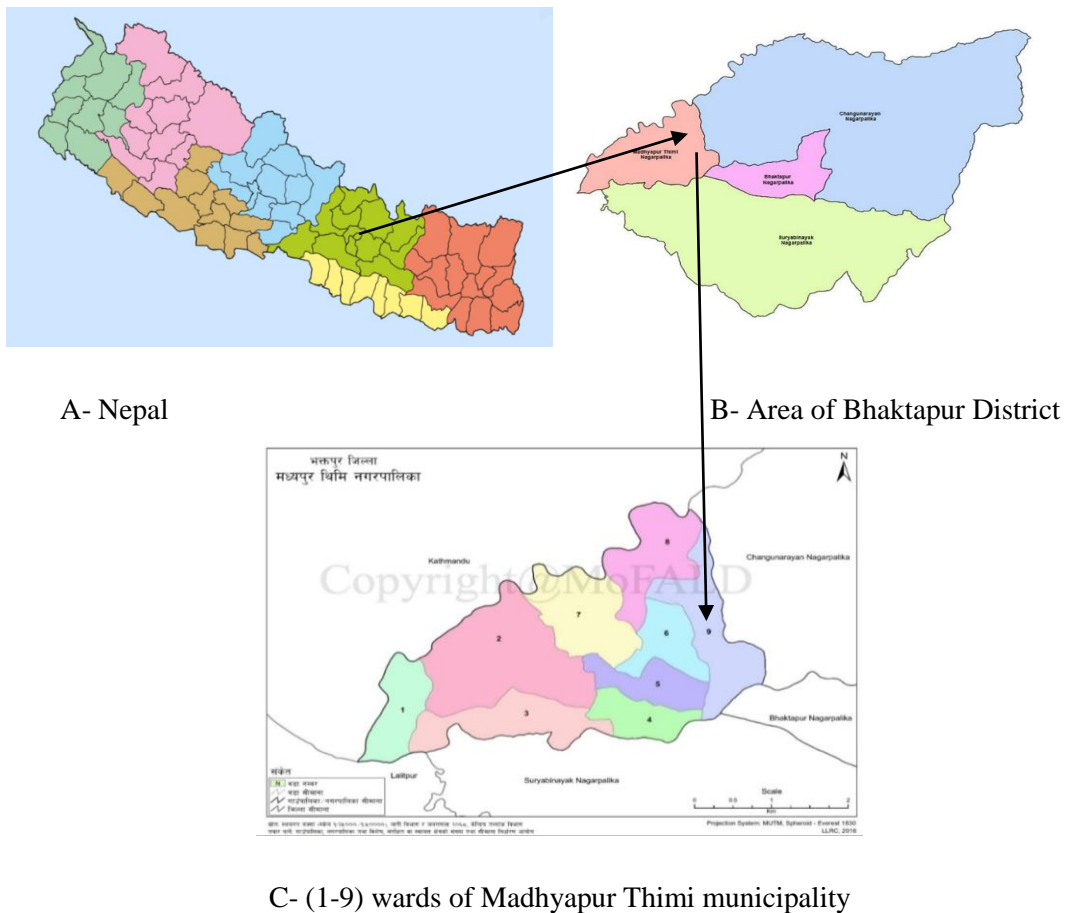


Fig 1: Map of the study area

3.2 Materials required

During the research, the materials used have been listed below:

3.2.1 Materials for field

- | | | |
|-------------------|-------------------------------------|--------------------|
| i. Measuring tape | ii. Global Positioning System (GPS) | iii. Sterile vials |
| iv. Glove | v. Camera | vi. Spoon |

3.2.2 Materials for Laboratory

- | | | |
|--------------------|--------------------------|--------------------------|
| i. Gloves | ii. Mask | iii. Electric microscope |
| iv. Spatula | v. Mortar and pestle | vi. Tea strainer |
| vii. Beaker | viii. Measuring cylinder | ix. Centrifuge tube |
| x. Test-tube stand | xi. Centrifuge machine | xii. Droppers |
| xiii. Glass slides | xiv. Toothpicks | xv. Coverslips |
| xvi. Cotton bud | xvii. Petri dish | xviii. Refrigerator |

3.2.3 Chemical required

- | | |
|--|---------------------|
| i. 2.5% Potassium dichromate ($K_2Cr_2O_7$) solution | ii. Distilled water |
| iii. 0.9% NaCl/ Saline solution | iv. Methylene blue |
| v. Lugol's Iodine solution | vi. Giemsa |
| vii. Concentrated NaCl (40%) | |

3.3 Methods

3.3.1 Field survey

The preliminary field visit was done in May-2020, to know about the study area, habitat, and population status of the host. The survey process was followed by field visits and interaction with the local people. Extensive field visits were made using the "Purposive Sampling Technique".

3.3.2 Study design

The study was designed to determine the pattern of parasitic infection in humans and monkeys in the Nilbarahi area from March to April 2021. The fieldwork involved two different designs to collect a sample of two different hosts.

Human host: The fieldwork involved house-to-house visits, information based on name, sex, and age was taken and samples were collected and finally preserved in 2.5% potassium dichromate solutions. The interview was taken among the owners by using pre-structured as well as open questionnaires.

Monkey host: Samples of monkeys were collected by purposive method from temples and forest areas. Fresh fecal samples were collected and preserved in 2.5% potassium dichromate solutions.

3.4 Ethical approval

To carry out the study, written permission from the Nepal Health Research Council and the Department of Forestry of Kathmandu was taken. As well as to carry out laboratory work permission letter from the Nepal Academy of Science and Technology was taken.

3.5 Sample size

Human:

First of all sterile vials and spatula were distributed to the targeted population. The next morning the vials were collected and coded with name, sex, and age. The samples were immediately preserved in a 2.5% potassium dichromate solution. A total no. of 100 fecal samples of humans was collected from purposively selected houses and targeted populations.

Monkeys:

A total of 100 fecal samples of monkeys were collected from the temple and forest area by the purposive method. Fresh fecal samples were collected from monkeys in the early morning (6:00 am - 10:00 am).

3.6 Sample collection and storage

Before collection of the fresh fecal samples, a carefully examined sample was done through the naked eye to know about color, consistency, worm, cestode segments, blood, and mucus as well as size was measured. It was noted about 10 grams of fecal material was taken from the fecal mass with the help of sterile forceps and gloved hand in sterile and air-tight vials. After collection of the sample in a 20ml vial, then preserved in Potassium dichromate solution ($K_2Cr_2O_7$) (2.5%) and brought to the Animal Research Laboratory of Nepal Academy of Science and Technology (NAST) for Laboratory diagnosis. The samples were kept in the refrigerator at 4°C.

3.7 Microscopic examination of fecal sample

Microscopic examination was done for the detection and identification of trophozoites, cysts, oocysts, eggs, and larval stages of the gastrointestinal parasites by direct wet mount and concentration method via, floatation and sedimentation technique. The slides were observed under low power first at 10X and followed to high power at 40X of the microscope.

3.7.1 Direct wet mount technique

This method involved stain and unstained smear preparation.

3.7.1.1 Unstained smear preparation of stool/ saline wet mount:

A small number of fecal samples were taken with the help of a toothpick and emulsified with normal saline on the clean glass slide. Then cover slip was placed over it and excess fluid was removed with cotton or filter paper. The smear was observed under the microscope to demonstrate helminthic eggs and larvae (Zajac and Conboy, 2012).

3.7.1.2 Stained smear preparation of stool/ Iodine wet mount:

A portion of stool sample was put on the glass slide to which a drop of Gram's iodine was added and mixed. Then cover slip was placed over the mixture and excess fluid was removed with cotton filter paper. The smear was observed under the microscope. Iodine wet mount was necessary for the identification and study of the nuclear character (Zajac and Conboy, 2012).

3.7.2 Flootation technique

In the floatation technique, the fluid floatation medium i.e., saturated solution of sodium chloride (NaCl) (SPG 1.20) has higher specific gravity than parasitic forms. The higher the specific gravity (SPG) of the floatation solution, the greater the variety of parasite eggs that would float. All the helminth eggs and protozoan cysts float in such a solution except the following eggs of *Ascaris lumbricoides*, eggs of *Taenia solium* and *Taenia saginata*, and also the eggs of an intestinal fluke. *Strongyloides* larvae do not float in salt solution (Zajac and Conboy, 2012).

Process

About 3 gm of the fecal sample was taken. The sample was kept in the beaker and grinded with about 20 ml of water. Filtrate the fecal solution in a tea strainer and poured into a centrifuge tube up to 12 ml and centrifuge at 1000 rpm for 5 minutes. The centrifuge tube was taken out and the upper part of the water was removed with the help of a pipette. The centrifuge tube was again filled with NaCl solution up to 12 ml and centrifuged at 1000 rpm for 5 minutes. The centrifuge tube was taken out and added more NaCl solution up to the tip of the tube and a drop of methylene blue was added to it. A cover slip was placed over the top of the centrifuge tube so that the solution touched the coverslip and left for 5 minutes. Then, the coverslip was taken gently and placed on a microscopic slide, and examined under 10X and 40X. Finally, photographs were captured.

3.7.3 Sedimentation technique

A sedimentation procedure is used to isolate eggs of flukes, acanthocephalans, some other *Taenia* sp. and nematodes whose eggs are a bit heavier than the others. For this technique, sediments of centrifuged contents were taken for egg detection (Zajac and Conboy, 2012).

Process

The centrifuge tube was taken out and the upper part of the saturated NaCl solution was removed with the help of a pipette, after examining the floatation. The remaining sediment content was poured into the watch glass and stirred gently. A small drop of sediment mixture was taken with help of a pipette and placed on the second slide,

adding one drop of iodine solution for staining. The specimen was stained by Iodine wet mount solution and examined under 10X and 40X. Finally, photographs were captured.

In this way, two slides were prepared from one sample (one from floatation and one from sedimentation) and were examined microscopically at 10X and 40X to detect eggs of helminths, protozoan trophozoites, or cysts of gastrointestinal parasites.

3.8 Measurement of eggs, cysts, and larva

Eggs were measured by using 'Image J' software version Image J 1.46r/ Java 1.6.0_20 (64-bit)

3.9 Identification of the eggs, cysts, and larva

The identification of the eggs, cysts, and larva was confirmed by comparing the structure, color, and size of eggs, cysts, and trophozoites in published literature, journals, and books (Soulsby, 1968; Taylor *et al.*, 2007).

3.10 Data analysis

For this study, the prevalence was measured as the percentage of host individuals infected with a particular parasite. The collected data were coded and entered into a Microsoft Excel spreadsheet. Thus analyzed data was interpreted by representing with a table, pie chart, and bar diagram. Data were statistically examined using Pearson's Chi-squared test, performed by "SPSS software". In all cases, a 95% confidence interval (CI) and $p < 0.05$ was considered for a statistically significant difference.

4. RESULTS

A total of 200 fecal samples were microscopically examined for parasitic infection including 100 samples from human beings and 100 from monkeys. Microscopic examination of fecal samples using the direct smear method, concentration, and flotation method revealed 13% prevalence rate in humans (Fig. 2) whereas 81% in monkeys (Fig. 3).

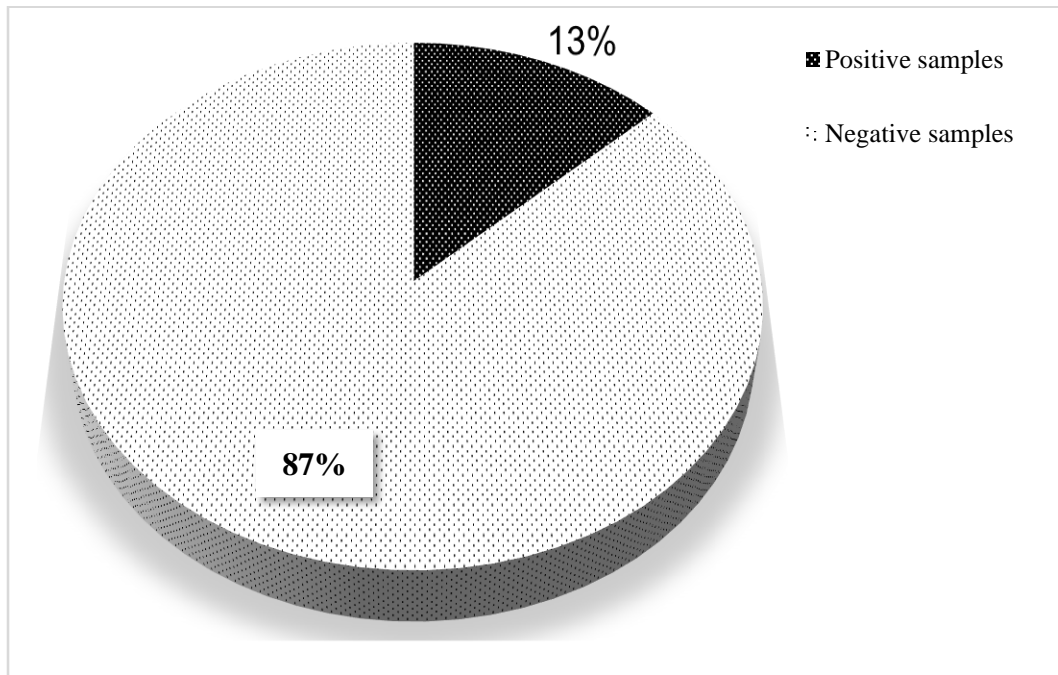


Fig 2: Prevalence of gastrointestinal parasites in human

GI parasites in monkeys were studied based on the prevalence and intensity of infections.

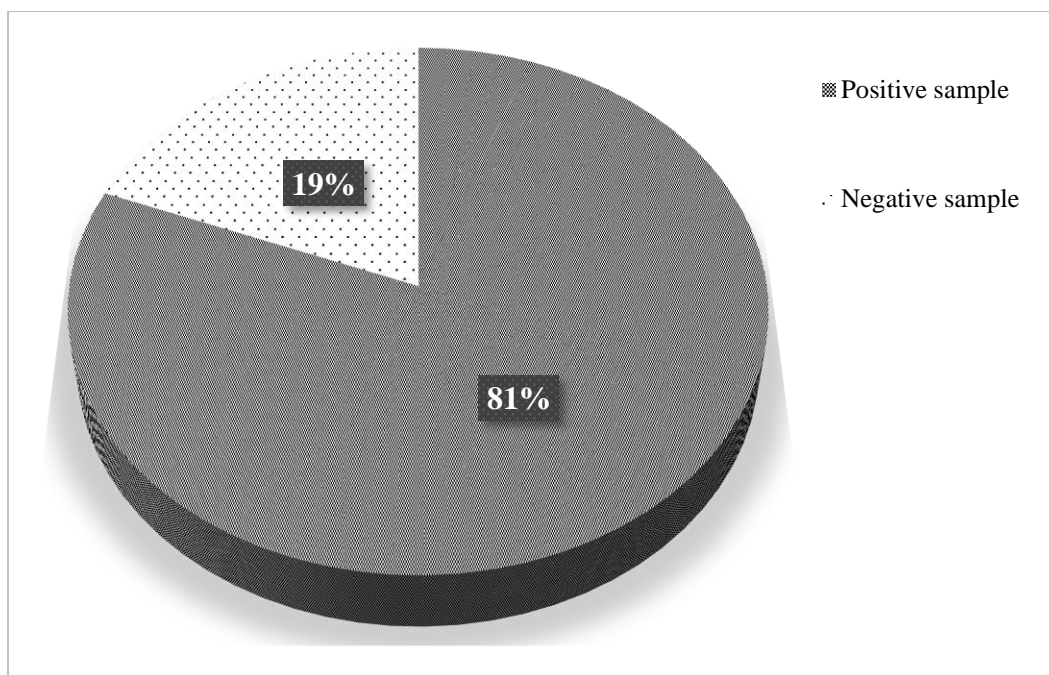


Fig 3: Prevalence of gastrointestinal parasites in monkey

4.1 Prevalence of gastrointestinal parasites in human

Out of 100 stool samples, the prevalence of protozoan was found higher compared to helminthic infection. The most common protozoan parasite detected was *Cryptosporidium* sp. Among the helminthic infection, the higher parasites detected were *Ascaris lumbricoides* followed by *Taenia* sp. (Table 1).

Table 1: Prevalence of gastrointestinal parasites in human

S.N	Class	Intestinal parasites	Total (n)	Overall Prevalence (nX100/N) (N=100)
1.	Protozoa		7	(7%)
		<i>Cryptosporidium</i> sp.	7	(7%)
2.	Helminth		6	(6%)
	1. Cestode	<i>Taenia</i> sp.	2	(2%)
	2. Nematode	<i>Ascaris lumbricoides</i>	5	(5%)

4.2 Sex-wise prevalence of gastrointestinal parasitic infection in human

Out of 100 stool samples examined, 46 were male and 54 were female participants. Out of 46 stool samples examined from males, 2 (4.35 %) were found to be positive. Likewise, among 54 stool samples examined from females, 11 (20.37 %) were found to be positive for intestinal parasites. Hence, the infection rate was found higher in females than in males. Statistically, there was no significant difference in the prevalence of gastrointestinal parasites between males and females ($\chi^2 = 3.433$, $df=1$, $p = 0.064$) (Table 2).

Table 2: Sex-wise prevalence of gastrointestinal parasitic infection in human

Gender	Total (n)	Positive (n)	Percentage	χ^2 value	P-value
Male	46	2	4.35%	3.433	0.064
Female	54	11	20.37%		
Total	100	13	13%		

4.3 Age-wise prevalence of gastrointestinal parasitic infection in human

The entire study population was categorized into three age groups which were 18-40 yrs., 41-60 yrs., and above 60 yrs. Out of 100 samples, 58 members were 18-40 yrs. age, 33 were 41-60 yrs. age and 9 were old age. Among 58 participant members in the 18-40 yrs. age group, five were shown to be a positive infection which was low as compared to other age groups. Out of 33 participating members in the 41-60 yrs. age group, six showed positive results and out of 9 participating members of the old age group, two showed positive infection which indicated the highest infection rate among them all. Statistically, there was no significant difference in the prevalence of intestinal parasites found according to the different age groups ($\chi^2 = 2.444$, $df= 2$, $p= 0.295$) (Table 3).

Table 3: Age-wise prevalence of gastrointestinal parasitic infection in human

Age groups	Total cases (N)	Positive case (n)	Percentage (%)	χ^2 value	P- value
18-40 yrs.	58	5	8.62%	2.444	0.295
41-60 yrs.	33	6	18.18%		
Above 60 yrs.	9	2	22.22%		
Total	100	13	13%		

4.4 Intensity of gastrointestinal parasites in human

Only one parasite *Cryptosporidium* sp. showed heavy infection (+++). None of the parasites showed mild infection (++). All identified parasites showed light infection (+) (Table 4).

Table 4: Intensity of gastrointestinal parasites in human

S.N	Class	Intestinal parasites	Light(+)	Mild (++)	Heavy (+++)
1.	Protozoa	<i>Cryptosporidium</i> sp.	6	0	1
2.	Helminth	<i>Taenia</i> sp.	2	0	0
		<i>Ascaris lumbricoides</i>	5	0	0
		Total	13	0	1

4.5 Mixed parasitic infection in human

Out of 13 positive fecal samples of humans, 12 samples were found to have a single infection while only 1 sample was found to have a double infection. The single parasitic infection was high as compared to double infection (Fig 4).

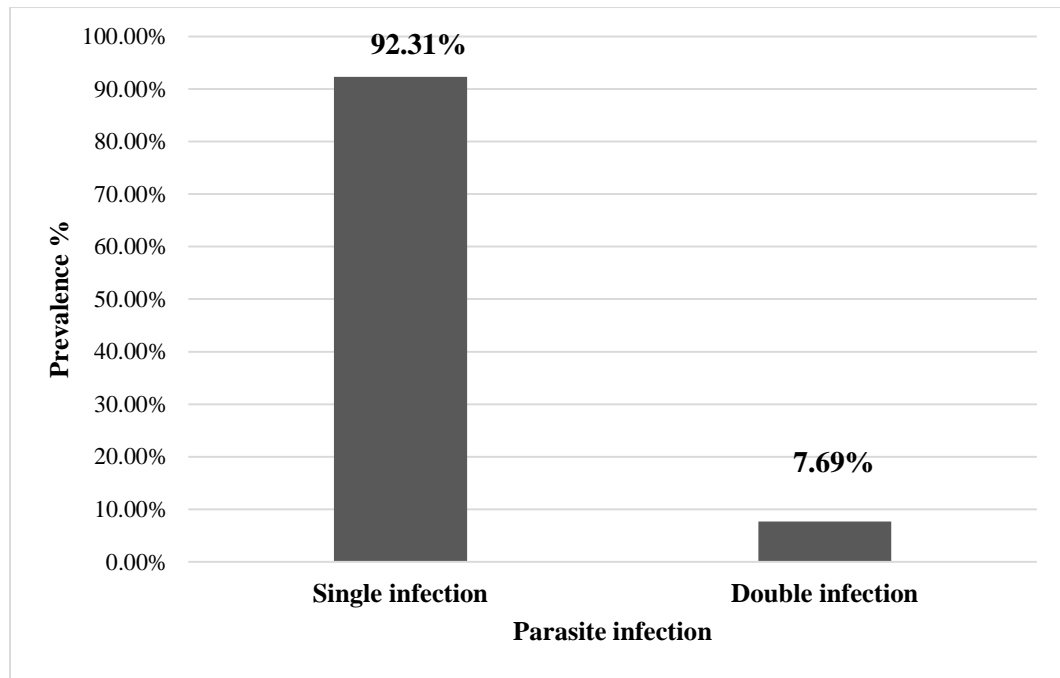


Fig 4: Mixed parasitic infection in human

4.6 Prevalence of gastrointestinal parasites in monkey

Out of 100 stool samples, the prevalence of protozoan parasites was found higher at 76% (76/100) as compared to helminthic parasites at 24% (24/100). Altogether, 15 species of intestinal parasites were detected among which 8 species were protozoa and 7 species were helminths. Among the protozoan infection, the most common parasites detected were *Entamoeba* spp. followed by *Entamoeba coli*, *Balantidium coli*, *Cryptosporidium* sp., *Cyclospora* sp., *Giardia* sp., *Eimeria* sp., and *Endolimax* sp. Among the helminthic infection, the infection of Ascarid spp. and Strongyle sp. were highest followed by *Taenia* sp., Hookworm where *Fasciola* sp., *Controrchis* sp. and *Capillaria* sp. infections were equal (Table 5).

Table: 5 Prevalence of gastrointestinal parasites in monkey

S.N	Class	Intestinal parasites	Total (n)	Overall Prevalence (nX100/N) (N=100)
1.	Protozoa		76	(76%)
		<i>Entamoeba</i> spp.	46	(46%)
		<i>Entamoeba coli</i>	37	(37%)
		<i>Balantidium coli</i>	31	(31%)
		<i>Cryptosporidium</i> sp.	18	(18%)
		<i>Cyclospora</i> sp.	5	(5%)
		<i>Giardia</i> sp.	4	(4%)
		<i>Eimeria</i> sp.	3	(3%)
		<i>Endolimax</i> sp.	1	(1%)
2.	Helminth		24	(24%)
	1. Trematode	<i>Fasciola</i> sp.	1	(1%)
		<i>Controrchis</i> sp.	1	(1%)
	2. Cestode	<i>Taenia</i> sp.	3	(3%)
	3. Nematode	Ascarid spp.	10	(10%)
		Strongyle sp.	10	(10%)
		Hookworm	2	(2%)
		<i>Capillaria</i> sp.	1	(1%)

4.7 Intensity of gastrointestinal parasites in monkey

All the identified parasites showed light infection (+). *Entamoeba* spp., *Balantidium coli*, and *Cryptosporidium* sp. showed all three types of parasitic infection (i.e. light, mild and heavy). *Entamoeba coli* showed light and mild infection (Table 6).

Table 6: Intensity of gastrointestinal parasites in monkey

S.N	Class	Intestinal parasites	Light(+)	Mild (++)	Heavy (++++)
1.	Protozoa				
		<i>Entamoeba</i> spp.	36	7	3
		<i>Entamoeba coli</i>	34	3	0
		<i>Balantidium coli</i>	29	1	1
		<i>Cryptosporidium</i> sp.	13	3	2
		<i>Cyclospora</i> sp.	5	0	0
		<i>Giardia</i> sp.	4	0	0
		<i>Eimeria</i> sp.	3	0	0
		<i>Endolimax</i> sp.	1	0	0
2.	Helminth				
	1. Trematode	<i>Fasciola</i> sp.	1	0	0
		<i>Controrchis</i> sp.	1	0	0
	2. Cestode	<i>Taenia</i> sp.	3	0	0
	3. Nematode	Ascarid spp.	10	0	0
		Strongyle sp.	10	0	0
		Hookworm	2	0	0
		<i>Capillaria</i> sp.	1	0	0
		Total	161	14	7

4.8 Mixed parasitic infection in monkeys

Out of 81 infected samples of monkeys, 29 were observed highly prevalent single infection followed by double infection 26, triple infection 15, and multiple infections 11 (Fig 5).

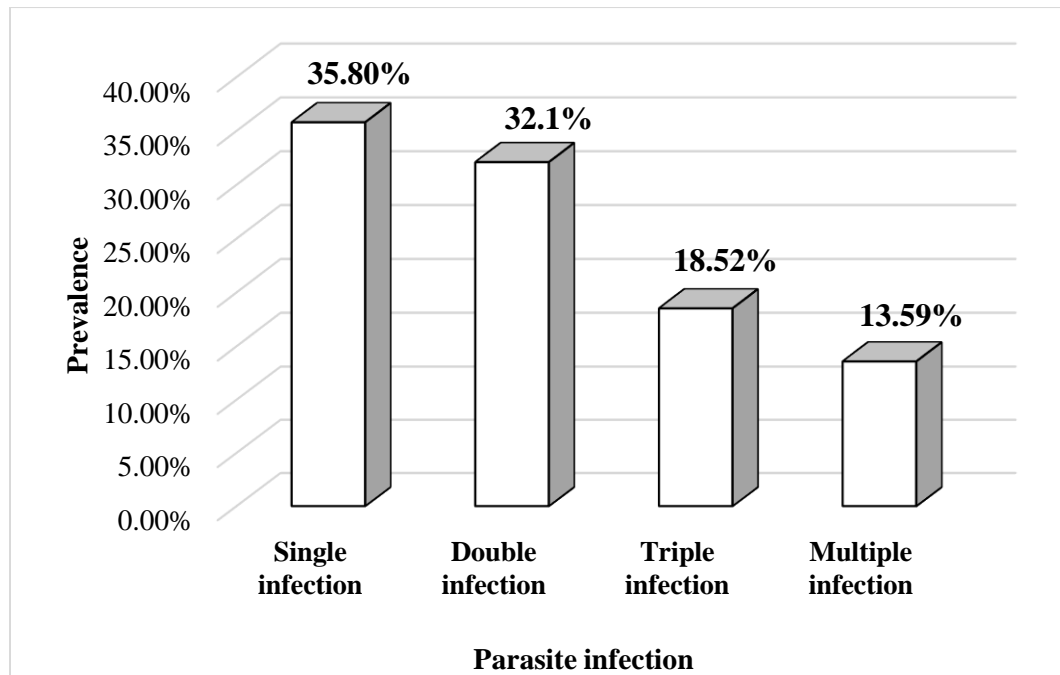


Fig 5: Mixed parasitic infection in monkeys

4.9 Comparison of gastrointestinal parasites between humans and monkeys

GI parasites of two hosts had been studied based on parasitic prevalence, comparison of parasites, and intensity of infections. In humans, only one genus of the protozoan parasite was detected and in monkeys, eight genera of protozoan parasites were found. The common protozoan parasite was *Cryptosporidium* sp. initiated in humans and monkeys which had a prevalence of (7%) and (18%) respectively. Two species of helminth were found in humans and seven species were recorded in monkeys. *Taenia* sp. and *Ascarid* spp. were noted as the common helminth parasites between humans and monkeys (Table 7).

Table 7: Comparison of gastrointestinal parasites between humans and monkeys

S.N	Class	Intestinal parasites	Human (N=100)	Monkeys (N=100)
1.	Protozoa		(7%)	(76%)
		<i>Entamoeba</i> spp.	0	(46%)
		<i>Entamoeba coli</i>	0	(37%)
		<i>Balantidium coli</i>	0	(31%)
		<i>Cryptosporidium</i> sp.	(7%)	(18%)
		<i>Cyclospora</i> sp.	0	(5%)
		<i>Giardia</i> sp.	0	(4%)
		<i>Eimeria</i> sp.	0	(3%)
		<i>Endolimax</i> sp.	0	(1%)
2.	Helminth		(6%)	(24%)
	1. Trematode	<i>Fasciola</i> sp.	0	(1%)
		<i>Controrchis</i> sp.	0	(1%)
	2. Cestode	<i>Taenia</i> sp.	(2%)	(3%)
	3. Nematode	Ascarid spp.	(5%)	(10%)
		Strongyle sp.	0	(10%)
		Hookworm	0	(2%)
		<i>Capillaria</i> sp.	0	(1%)

4.10 Measurements of gastrointestinal parasites between humans and monkeys

Oocyst of *Cryptosporidium* sp., the egg of *Taenia* sp., and the egg of *Ascarid* spp. were found common in both hosts. The parasites were examined microscopically at 10X and 40X after sedimentation at Gram's iodine.

Table 8: Measurements of gastrointestinal parasites between humans and monkeys

S.N	Class	Intestinal parasites	Measurements of human parasites	Measurements of monkey parasites
1.	Protozoa			
		<i>Cryptosporidium</i> sp.	2 μm x 2 μm, X400	1 μm x 1 μm, X400
2.	Helminth			
	1. Cestode	<i>Taenia</i> sp.	27 μm x 22 μm, X400	27 μm x 26 μm, X400
	2. Nematode	<i>Ascarid</i> spp.	20 μm x 18 μm, X400	32 μm x 27 μm, X400

4.11 Photo plates of GI parasites

4.11.1 Photo plates of human GI parasites

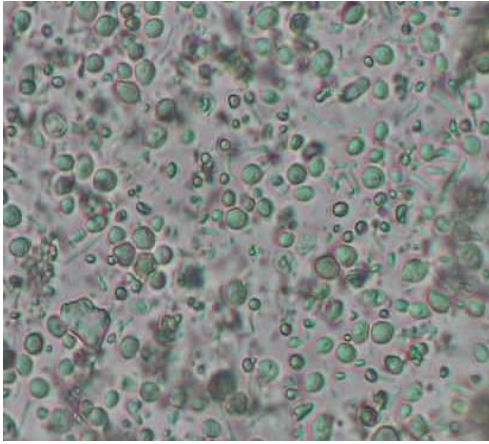


Photo No. 1: Oocyst of *Cryptosporidium* sp.
(2 μm x 2 μm , X400)

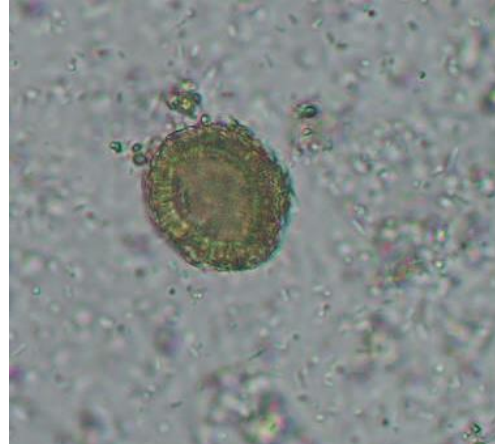


Photo No. 2: Egg of *Taenia* sp.,
(27 μm x 22 μm , X400)

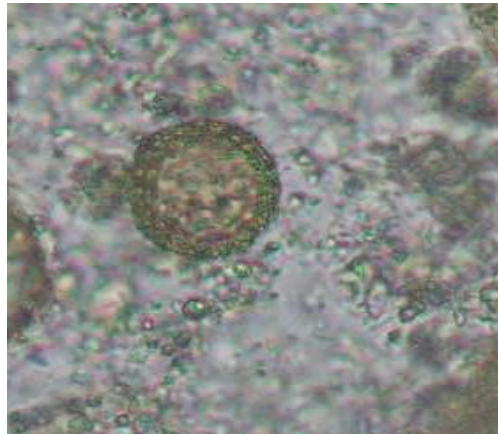


Photo No. 3: Egg of *Ascaris lumbricoides*,
(20 μm x 18 μm , X400)

4.11.2 Photo plates of monkey GI parasites

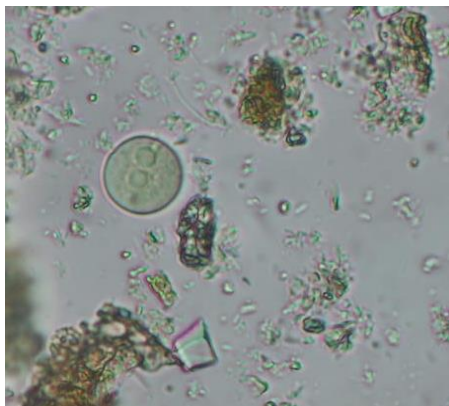


Photo No. 4: Cysts of *Entamoeba coli*
(17 μm x 17 μm , X400)

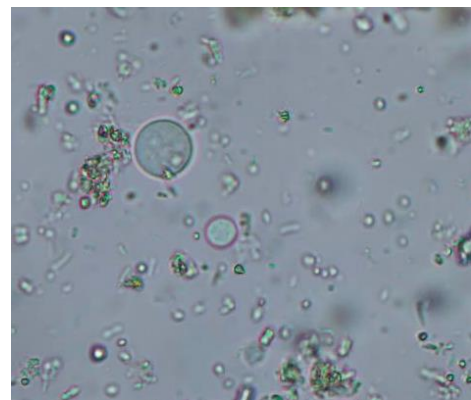


Photo No. 5: Cysts of *Entamoeba* spp.
(6 μm x 6 μm , X400)

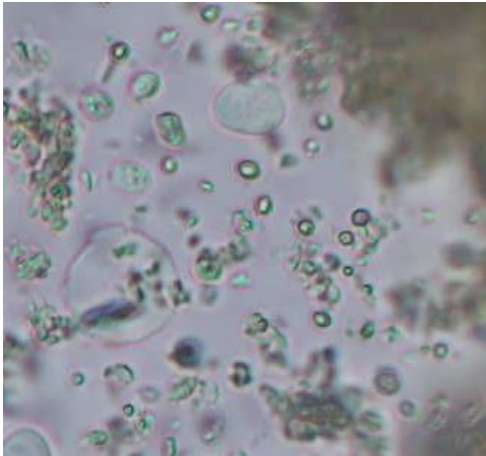


Photo No. 6: Oocyst of *Cryptosporidium* sp.
(1 μ m x 1 μ m, X400)



Photo No. 7: Oocyst of *Eimeria* sp. (18 μ m x 15 μ m, X400)

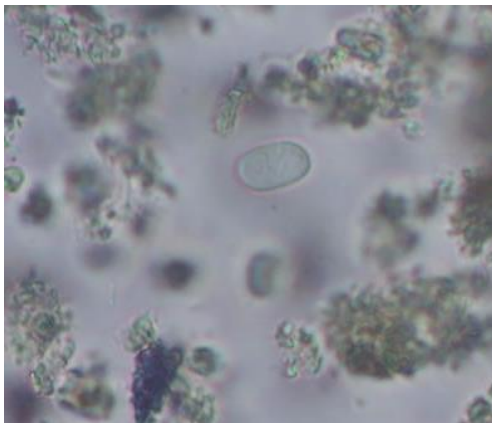


Photo No. 8: Oocyst of *Endolimax* sp.
(13 μ m x 7 μ m, X400)

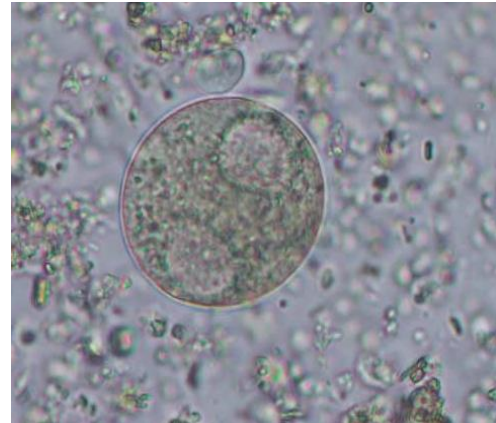


Photo No. 9: Cyst of *Balantidium coli*
(44 μ m x 42 μ m, X400)

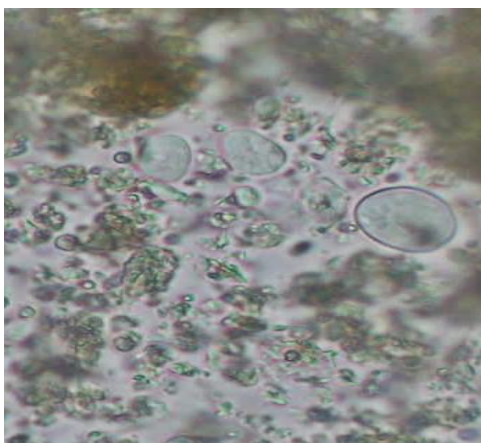


Photo No. 10: Cyst of *Giardia* sp.
(11 μ m x 8 μ m, X400)

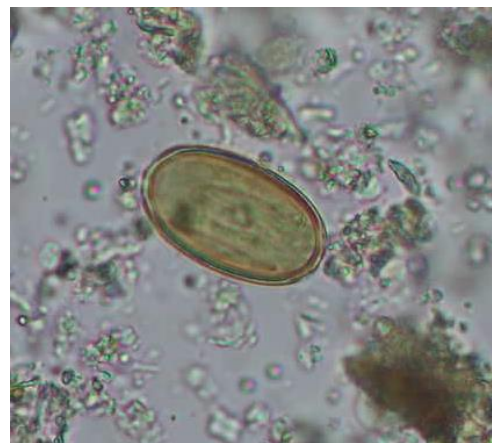


Photo No. 11: Egg of *Capillaria* sp.
(37 μ m x 21 μ m, X400)



Photo No. 12: Egg of *Controrchis* sp.
(37 μ m x 19 μ m, X400)



Photo No. 13: Egg of *Fasciola* sp.
(156 μ m x 75 μ m, X400)



Photo No. 14: Egg of *Strongyle* sp.
(69 μ m x 39 μ m, X400)

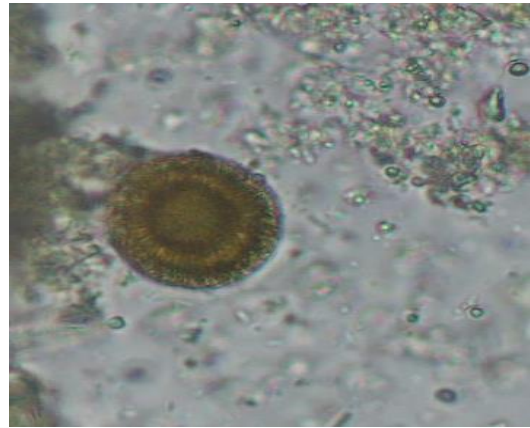


Photo No. 15: Egg of *Taenia* sp.
(27 μ m x 26 μ m, X400)

5. DISCUSSION

The current study indicates the prevalence of gastro-intestinal parasites concerning age and sex in humans, intensity, and mixed parasitic infection in humans and monkeys and compared the common gastro-intestinal parasites between two hosts in the Nilbarahi area. Several studies conducted worldwide concluded that poor sanitation habits, lack of safe drinking water, little or no access to primary health care, lack of proper education, open place defecation, barefoot walking habit, demand for traditional practice/treatment, and sharing houses with animals increases the chance of infection with intestinal parasitic infection (Whittier *et al.*, 2000; Loukopoulos *et al.*, 2007). In the present study, the prevalence of gastrointestinal parasites was recorded based on sex, age, intensity, and concurrency of mixed infections.

A total of 100 stool samples have been amassed in this study, of which 13% were positive cases with different groups of parasites. This result is determined to be less prevalent in parasitic infection than in research done by (Chandrashekhar *et al.*, (21.3%) in 2005; Malla *et al.*, 2008 (43.3%); Gyawali *et al.*, in 2009 (22.5%); Khanal *et al.*, (17.6%) on 2011). Adhikari *et al.*, (2021) recorded overall parasitic prevalence (97%) in their study among indigenous Chepangs which was significantly higher than the findings from other groups from various parts of Nepal. The difference in these results might be due to different sampling geographies and their climatic conditions, different socioeconomic conditions, behavioral practices by various indigenous groups, and the different laboratory techniques used in the fecal analysis.

The overall prevalence of intestinal parasites was 20.37% in female individuals and 4.35% in male individuals. Similar prevalence has been shown in the study done by (Malla *et al.*, 2004) with intestinal parasites in females (43.80%) showed high in comparison to males (39.42%) from Sarlahi and another study done by (Gyawali, 2012) found a greater prevalence of intestinal parasites in females (51.94 %) than in males (45.83%). A study conducted by (Ugbomeh *et al.*, 2018) also recorded parasitic infection rates high in females 57.2% more than males (52.4%) from Nigeria. Some studies conducted by (Karki, 2003; Tharu, 2006; Chin *et al.*, 2016; Muslim *et al.*, 2019) found similar records of parasitic infection in indigenous people of Nepal and other countries. The result once conflicted with the research was done by (Sah *et al.*, 2016) recorded a high prevalence of parasitic contamination among males (20.7%) in

comparison to females (19.1%) from the Eastern region of Nepal. A recent study showed that females were infected comparatively high than males this might be due to more females being more involved in agricultural labor in extra time as well as majority of females are illiterate and do not have any knowledge of parasites and parasitic infections. Based on the age of participant members in this study, parasitic infection was found to be highest among elder people age group above 60 years (22.22%) and found lowest among young age people of 18-40 years (8.62%). This result agrees with the data of a study done by (Gyawali, 2012; Oli, 2016; Thapa, 2018) showed the highest parasitic infection among elder people. High parasitic infection was found among elder people in the study might be due to their unhygienic behavior, lack of sanitation, and contaminated food and water. Reports of various studies show that the intensity of a single parasitic infection was higher than that of double and multiple infections in Bhaktapur (Shrestha *et al.*, 2013), Kathmandu valley (Thapa *et al.*, 2011; Ghimire *et al.*, 2014, and Pradhan *et al.*, 2014), Teaching hospital, Kathmandu, Nepal (Agrawal *et al.*, 2012), Dadeldhura ((Jha, 2018). The present study also resembles that single parasitic infection is higher than double infections which were 92.31% and 7.69% respectively. High single parasitic infection in the study area might be due to the use of specific anthelmintic drugs against specific parasitic infection, which might be due to auto-infection of specific parasites through the fecal-oral route and skin penetration. The present study recorded one species of protozoa and two species of helminths with a high prevalence of *Cryptosporidium* sp. (7%), *Ascaris* spp. (5%) and *Taenia* sp. (2%). *Cryptosporidium* sp. was recorded as the most prevalent parasite in this study which was in agreement with the previous study done in different countries (Alam *et al.*, 2013; Koffi *et al.*, 2014). *Cryptosporidium* sp. was found high in this study, this might be due to contaminated water, open defecation, unhygienic behavior, and lack of awareness are the possible risk factors for transmission of parasites and their eggs. In helminth infection, *Ascaris* spp. was recorded with high prevalence. This kind of similar study was conducted by other studies in Nepal (Rai *et al.*, 1986; Malla *et al.*, 2004; Shakya *et al.*, 2009; Sherchand *et al.*, 2010; Khanal *et al.*, 2011; Agrawal *et al.*, 2012; Shrestha *et al.*, 2012; Khadka *et al.*, 2013; Shrestha *et al.*, 2013; Tandukar *et al.*, 2015; Sah *et al.*, 2016). The high infection by *A. lumbricoides* found in the study area indicates water and soil contamination with helminths in the locality.

Similarly, another study was conducted on monkeys for the gastrointestinal prevalence of parasites. A total of 100 fecal samples of rhesus macaques were collected and examined based on prevalence, intensity, and mixed parasitic infection. The overall prevalence of intestinal parasites in the present study was found to be 81%, which was similar to the finding of (Bhattarai, 2019) who found an 80% overall prevalence in the Chitwan-Annapurna landscape, and (Nepal, 2010) who found an 85% overall prevalence in the Swoyambhu area of the Kathmandu valley. While studies in central Nepal (Shrestha, 2018; Pokhrel and Maharjan, 2014; Adhikari and Dhakal, 2018) found similar results, 72.5%, 72.94%, and 74.20%, Similar to the present study, almost all research conducted in Nepal among monkeys detected a high prevalence of GI parasites. Such research was conducted close to human-inhabited areas and the monkeys' fragmented natural habitats. With increasing soil and water pollution by waste food and garbage, especially during the festive and picnic programs, and open defecation by visitors in nearby water sources, forest and temple areas are the major risk factors for parasite transmission. The result was dissimilar to that of a study carried out by Nath *et al.*, (2012), which documented 13.63% of intestinal parasites from captive non-human primates of Assam State Zoo. Low parasitic prevalence in captive macaques might be due to regular husbandry practices, disease prophylaxis, and anthelmintic treatment administered in zoos.

Furthermore, protozoan parasitic infection was more common (76%) than helminthic infection (24%). The result coincides with the study done by (Lim *et al.*, 2008; Niranjana *et al.*, 2020; Sapkota, 2020) that found the prevalence of protozoa was higher compared to helminths. In contrast to these findings, a higher occurrence of helminths was reported than protozoans in non-human primates studied by (Munene *et al.*, 1998; Goossens *et al.*, 2005; and Bhattarai *et al.*, 2019) in different countries. The prevalence of protozoans was higher among all the helminths, which might be due to some of the protozoan parasites having a direct life cycle, i.e., without the involvement of intermediate hosts. They are transmitted by fecal-oral routes through contaminated food, soil, and water and can accumulate in that environment (Thawait *et al.*, 2014).

Altogether, 15 species of intestinal parasites were detected in monkeys. Among these, 8 species were protozoa and 7 species were helminths. The most common protozoan parasites detected were *Entamoeba* spp. 46 (46%), followed by *Entamoeba coli* 37 (37%), *Balantidium coli* 31 (31%), *Cryptosporidium* sp. 18 (18%), *Cyclospora* sp. 5

(5%), *Giardia* sp. 4 (4%), *Eimeria* sp. 3 (3%) and *Endolimax* sp. 1 (1%). *Entamoeba* spp. was found to be the highest in this research and a similar prevalence was recorded in Nepal (Sapkota, 2020; Pokhrel and Maharjan, 2014), China (Li *et al.*, 2015, Zanzani *et al.*, 2016, Dong *et al.*, 2017; Zhang *et al.*, 2019), the Philippines (Rivera *et al.*, 2010) and Bali (Lane *et al.*, 2011) in non-human primates. *Entamoeba* spp. is parasitic with a high potential for transmission to humans and animals because of their simple and direct life cycles (Pedersen *et al.*, 2005; Berrilli *et al.*, 2011; Morf and Sing, 2012). *Endolimax* sp. was found to be least in this research, whereas the result was dissimilar to that of the study carried out by (Zanzani *et al.*, 2016) which presented the most prevalent species. He also considered that *Endolimax* sp. was generally harmless for NHPs. Ascarid spp. and Strongyle spp. infections were the most common (10%), followed by *Taenia* sp. 3 (3%), Hookworm 2 (2%), *Fasciola* sp., *Controrchis* sp., and *Capillaria* sp. infections (1%). Ascarid spp. and Strongyle sp. were found to be the equal and most prevalent species of helminths. This result agrees with the data of a study done by (Sapkota, 2020) who found Ascarid spp. (21.4%), *Strongyloides* sp. (21.4%) showed equal and highest prevalence in his research. The maximum prevalence of *Strongyloides* sp. in non-human monkeys was supported by (Mutani *et al.*, 2003; Gillespie *et al.*, 2004; Gillespie *et al.*, 2005; Dhaubhadel, 2007; Malla, 2007; Nepal, 2010; Arunachalam *et al.*, 2015; Fernando *et al.*, 2022). *Strongyloides* are zoonotic and can cause serious illnesses, resulting in the deaths of macaques (Remfry, 1978; Toft, 1986; Chapman *et al.*, 2005).

The prevalence rate of Ascarid spp. was 10% in this research, which was similar to 10% (Nepal, 2010) and lower than the findings from Bangladesh (90.90%) (Tabasshum *et al.*, 2018), India (25.5%–26.66%) (Parmar *et al.*, 2012; Kumar *et al.*, 2018) and Nepal (10.48%–34.32%). (Malla, 2007; Bhattarai *et al.*, 2019 and Thakuri, 2019) and higher than the findings from India (5%) (Arunachalam *et al.*, 2015), Thailand (1%) (Schurer *et al.*, 2019) and Nepal (1.57%) (Dhaubhadel, 2007). Ascarid infection is one of the leading causes of high morbidity and mortality in macaques (Weiszer *et al.*, 1968; Richards *et al.*, 1983; Toft, 1986; Chapman *et al.*, 2005). The high prevalence of *Ascaris* spp. may have occurred due to the warm temperature of the study area, which is suitable for egg production. The current prevalence of *Fasciola* sp., *Controrchis* sp., and *Capillaria* sp. was equal, with the lowest 1% prevalence. The rate of *Capillaria* sp.

was lowest in this research, which was dissimilar to that of studies carried out in Nepal (Dhaubhadel, 2007; Malla, 2007; Nepal, 2010) and Sri Lanka (Thilakarathne, 2021).

Regarding intensity, the single parasitic infection was high in comparison to double, triple, and multiple infections. Many other studies, including this one, found a high prevalence of single parasitic infection (Pokhrel and Maharjan, 2014; Adhikari, 2017; Thakuri, 2019). This result shows conflict with the other studies (Chalise *et al.*, 2011; Nepal, 2010; Jha *et al.*, 2011) that found a high prevalence of multiple infections. Multiple infections can be more harmful than single infections. Monkeys carried multiple parasites, and this high rate of transmission could be attributed to either high population density or favorable environmental conditions for parasites. The infection shows effects on the growth pattern, reproduction, fertility, and establishment along with the death of the monkeys.

Altogether 200 samples were collected and examined microscopically, among them 100 were humans, and 100 were monkeys. The present study showed a high prevalence of GIPs in monkeys (81%) than in humans (13%) This present study showed high prevalence may be due to an increasing level of awareness, education in people, and increment of modern health facilities but lack of proper veterinary knowledge and deworming program in animals, increased host density and climatic condition may high proportion of parasitic infection in primates.

In the present study, *Cryptosporidium* sp., *Taenia* sp., and Ascarid spp. were commonly detected in humans and monkeys. The study conducted by (Rondon *et al.*, 2017; Shrestha, 2018) found Ascarid spp. was common in two hosts. Overlap of parasite species may occur both in captive and wild environments. In captivity, several parasites infect both non-human primates and humans (Flynn, 1973). *Entamoeba histolytica* was probably transmitted from infected colobus monkeys to a zookeeper (Loomis *et al.*, 1983). In the wild, the overlap of a helminth (*Strongyloides fuelleborni*) was exhibited in both mountain gorillas and humans recorded by (Ashford *et al.*, 1990). Moreover, *Balantidium coli* was probably transmitted from mangabeys to a researcher and indirectly to the person handling their fecal samples in Dzanga-Ndoki National Park, Africa (Lilly *et al.*, 2002).

This sharing of parasites in the two hosts that, and the exchange of disease is a matter of concern for wildlife due to contaminated soil, water, and consumption of garbage

foods. Human and non-human primate shares susceptibility to a wide range of fecal parasites (Jones- Engel *et al.*, 2006) as well as monkeys could act as a reservoir of some parasites that can infect man (Mutani *et al.*, 2003) such as protozoan parasites like *E. histolytica*, *G. lamblia* is consider to be easily transmissible from animals to human through contaminated waters, foods and hands (Smith and Meerovitch, 1985) as they caused dysentery and diarrhea in human.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study, it is concluded that the people of Nilbarahi were infected with different GI protozoan and helminth parasites. The common GI parasites were *Cryptosporidium* sp, Ascarid spp., and *Taenia* sp. between humans and monkeys. The overall prevalence of GI parasites in a human was 13%. Sex-wise prevalence of GI parasites indicated that females were more highly infected than the male with no significant difference. Similarly, above 60 yrs. age showed the highest infection rate than other age groups. The intensity of *Cryptosporidium* sp. showed heavy infection (+++). The study exposed single parasitic infection was significantly high as compared to double infection.

On the other hand, the prevalence of GI parasites in monkeys is highly prevalent at 81%. Altogether, 15 species of intestinal parasites were detected among which 8 species were protozoa and 7 species were helminths. Among the protozoan infection, the most common parasites detected were *Entamoeba* spp. followed by *Entamoeba coli*, *Balantidium coli*, *Cryptosporidium* sp., *Cyclospora* sp., *Giardia* sp., *Eimeria* sp., and *Endolimax* sp. Among the helminthic infection, the infection of Ascarid spp. and Strongyle sp. were highest followed by *Taenia* sp., Hookworm where *Fasciola* sp., *Controrchis* sp. and *Capillaria* sp. infections were equal. All the identified parasites showed light infection (+). Similar to humans, the double, triple, and multiple infections in monkeys were found to be low in comparison with the concurrency of single parasitic infection.

6.2 Recommendations

1. Among protozoans structurally similar *Cryptosporidium* sp., and among helminth parasites *Taenia* sp. and Ascarid spp. were found in humans and monkeys that need to be verified genetically similarity in future studies.
2. Further study based on molecular technique should be done for the identification of parasitic species as well as to know the potential source of zoonotic and anthroponotic risk.

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ANNEX 1

Intensity of parasites in human and monkeys

Note:

Protozoan cysts/ trophozoites (10 X 40)

Light infection (+) = 1-3 eggs/cysts/ larva per field

Mild infection (++) = 4-10 eggs/cysts/ larva per field

Heavy infection (+++) = ≥ 11 eggs/cysts/ larva per field

Helminths eggs/ B-coli cyst (10 X 10)

Light infection (+) = 1-3 eggs/cysts/ larva per field

Mild infection (++) = 4-10 eggs/cysts/ larva per field

Heavy infection (+++) = ≥ 11 eggs/cysts/ larva per field

ANNEX 2

PHOTOS



Photo No. 16: Interview with respondent



Photo No. 17: Distribution of vials to local people



Photo No. 18: Fecal materials of rhesus macaque



Photo No. 19: Monkey stool sample collection



Photo No. 20: Sedimentation technique



Photo No. 21: Centrifuge technique



Photo No. 22: Microscopic examination of stool sample



Photo No. 23: Identification of parasites