GASTROINTESTINAL PARASITES IN MUSAHAR COMMUNITY IN BALAN BIHUL, SAPTARI, NEPAL

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

Submitted to
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September, 2023

DECLARATION

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

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RECOMMENDATION

This is to recommend that the thesis entitled "**Gastrointestinal parasites in Musahar Community in Balan Bihul, Saptari, Nepal**" has been carried out by Mr. Bhagwati Parsad Yadav for partial fulfillment of the requirement for Master's Degree in Zoology with the special paper of Parasitology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this work has not been submitted for any other degree in any institutions.

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On the recommendation of supervisor "**Assistant Professor Janak Raj Subedi**" this dissertation submitted by **Mr. Bhagwati Parsad Yadav** entitled **"Gastrointestinal Parasites in Musahar community of Balan Bihul, Saptari, Nepal"** is approved for the examination and submitted to Tribhuvan University in partial fulfillment of the requirement for the Master's Degree of Science in Zoology with special paper Parasitology.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Mr. Bhagwati Parsad Yadav" **Gastrointestinal parasites among Musahar Community in Balan Bihul, Saptari, Nepal** " has been accepted as a partial fulfillment for the requirement of Master's Degree of Science in Zoology with special paper Parasitology.

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
asl	Above sea level
CI	Confident Interval
CDZ	Central Department of Zoology
CDC	Centers for Disease Control and Prevention
GI	Gastro Intestinal
gm	Gram
$K_2Cr_2O_7$	Potassium dichormate
Kms	Kilometers
mins	Minute
ml	Milliliter
mm	Millimeter
NaCl	Sodium chloride
NS	Normal Saline
RT	Room temperature
rpm	Round per minute
spp.	Species
STH	Soil Transmitted helminths
Sq mi	Square miles
WHO	World Health Organization
^oC	Degree Centigrade
µ	Micrometer

ABSTRACT

Gastrointestinal parasites inhabiting the intestinal tract of hosts, including humans, directly or indirectly affect the growth, well-being and the survival of hosts. However, parasitism in humans, especially among the highly marginalized ethnic tribes in Nepal have not been fully illustrated. Therefore, current research aimed to determine the prevalence and diversity of gastrointestinal parasites among Musahar Community people living in Saptari District in eastern Nepal. A total of 200 fresh fecal samples of Musahar people of varying age and sex were collected via purposive sampling. The samples were then immediately preserved in 2.5% potassium dichromate solution in 30 ml sterile vials and transported to Central Department of Zoology. The laboratory analysis was carried out by direct wet mount and concentration (sedimentation and flotation) methods and examined under a compound microscope. Results showed 81% (162/200) and a total of 9 different species of parasites. The presence reported were *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%). The prevalence of helminths (62%) was higher than the single-celled protozoa (42%), and mixed pattern of infection was higher than single infection. GI parasitism is comparatively higher in those population who are habituated in consuming rats, walking barefoot, defecating haphazardly on open lands, living in mud-built houses and with unknown or more than one year earlier history of medication. In conclusion, Mushar community people harbor a significantly higher prevalence of parasites in their GI tract. Thus, an awareness campaign and arrangement of effective deworming is highly recommended.

सार

जठरांत्रीय परजीवीहरूले मानिस लगायत होस्टहरूको आन्द्रा मार्गमा बस्ने, प्रत्यक्ष वा अप्रत्यक्ष रूपमा होस्टहरूको वृद्धि, कल्याण र अस्तित्वलाई असर गर्छ। यद्यपि, मानवमा परजीवी, विशेष गरी नेपालका उच्च सीमान्तकृत जातीय जनजातिहरूमा पूर्ण रूपमा चित्रण गरिएको छैन। तसर्थ, हालको अनुसन्धानले पूर्वी नेपालको सप्तरी जिल्लामा बसोबास गर्ने मुसहर समुदायका मानिसहरूमा ग्यास्ट्रोइंटेस्टाइनल परजीवीहरूको व्यापकता र विविधता निर्धारण गर्ने लक्ष्य राखेको छ। बिभिन्न उमेर र लिङ्गका मुसहर व्यक्तिको कुल २ सय वटा ताजा मलको नमुना पर्पोसिभ नमूना संकलन गरिएको थियो । त्यसपछि नमूनाहरू तुरुन्तै २.५% पोटासियम डाइक्रोमेट घोल ३० मिलीलीटर बाँझ शीशीमा सुरक्षित गरियो र केन्द्रीय प्राणी विज्ञान विभागमा पठाइयो। प्रयोगशाला विश्लेषण प्रत्यक्ष भिजेको माउन्ट र एकाग्रता (सेडिमेन्टेशन र फ्लोटेशन) विधिहरूद्वारा गरिएको थियो र कम्पाउन्ड माइक्रोस्कोप अन्तर्गत जाँच गरियो। नतिजाहरूले ८१% (१६२/२००) र कुल ९ विभिन्न प्रजातिका परजीवीहरू देखाए। उपस्थिति रिपोर्ट गरिएको थियो *Ancylostoma sp.* (४१.५%), *Ascaris lumbricoides* (२९%), *Entamoeba sp.* (३१.५%)%), *Entamoeba coli* (२१.५%), *Trichuris trichuria* (१६%) *Strongyloides stercoralis* (८.५%), *Giardia sp.* (७%), *Hymenolepis nana* (१४%) र *Balantidium coli* (२%)। हेल्मिन्थको प्रचलन (६२%) एकल-कोशिका प्रोटोजोआ (४२%) भन्दा बढी थियो, र संक्रमणको मिश्रित ढाँचा एकल संक्रमण भन्दा बढी थियो। मुसा खाने, खाली खुट्टा हिड्ने, खुला जमिनमा जथाभावी दिसा गर्ने, माटोले बनेको घरमा बस्ने र औषधिको एक वर्षभन्दा अघिको इतिहास नभएको वा अज्ञात भएका मानिसहरूमा GI परजीवी तुलनात्मक रूपमा बढी हुन्छ। अन्तमा, मुसहर समुदायका मानिसहरूले आफ्नो GI पथमा परजीवीहरूको उल्लेखनीय रूपमा उच्च प्रकोप राख्छन्। तसर्थ, एक सचेतना अभियान र प्रभावकारी जुकाको व्यवस्था गर्न अत्यधिक सिफारिस गरिएको छ।

1. INTRODUCTION

1.1 Background

Center for Disease Control and Prevention (CDC) defines parasite as "an organism that lives on or in a host organism and gets its food from or at the expense of its hosts". Gastrointestinal (GI) parasites reside in the GI tract of the hosts including humans as well as domestic and wild animals. GI or gut infection is mainly caused by various species of protozoa and helminth parasites (Suntaravitun and Dokmaikaw, 2018) and human infection with these parasites is a common phenomenon. It is highly prevalent throughout the world and with greater dominance in the developing and underdeveloped countries (Hussain *et al.,* 1997; Adhikari *et al.,* 2021). Thus, in developing countries, GI parasitism is considered as one of the challenging threats to healthier life (Kia *et al.,* 2008), which significantly contributes to mortality and morbidity (Haque, 2007; Nugi *et al.,* 2011). Human acquisition of GI parasites is estimated to occur with greater prevalence ranging up to 50% in developed and upto 95% in developing and underdeveloped countries throughout the world (Baral *et al.,* 2017). Even recent WHO reports state that more than 1.5 billion people globally are infected with various species of STH and more than 880 million children need immediate treatment (WHO, 2020). In this circumstance, various types of risk factors contribute to the acquisition success of intestinal parasites and its burden in humans (Tigabu *et al.,* 2019; Adhikari *et al.,* 2021). These factors include socio-economic status, education, awareness, level of consciousness, housing, lifestyle, deworming practices, personal hygiene and the surrounding environment. Some medically significant

Species of human parasite includes protozoa (*Entamoeba, Giardia, Cryptosporidium, Cyclospora,* and *Balantidium*) and helmints (*Ascaris, Ancylostoma, Trichuris, Enterobius, Strongyloides, Fasciola, Taenia, Schistosoma*, and *Paragonimus*).

1.2 Musahar and human parasites

In Nepal, Musahar or Mushahar are classified as a broader social group of Madheshi Dalit, one of the most marginalised groups (Path & Kathmandu, 2014). Morphologically, these people have short sature, a long narrow headspape, and broad nose. They reside in the lowlands of Terai in Madhesh Province (3%), Koshi Province (1.4%) and Lumbini Province (0.1%). They represent 0.9% of the total population of Nepal with 234,490 individuals (CBS, 2011). They mostly inhabit the rural areas within the country and represent the most illiterate ethnic communities with literacy rate 21.82% and with the lowest ageing index of 7.22% (Path & Kathmandu, 2014). They are mostly landless (almost 99%) and primarily bonded agricultural labourers but sometimes they can be jobless even as much as eight months in a year (Rana, 2017; Bhatta and Shrestha, 2023; Poudel and Kattel, 2019). Interestingly, they possess rat hunting and consumption practices especially from agricultural fields in order to cope with hunger. It has been discussed that eating rats, porks and drinking alcohol are part of their major food culture (Sahay, 2019).

In Nepal a very few studies have been carried out considering the gastrointestinal parasitism in ethnic tribes including the Musahar tribes. In the year 2009 and 2021, parasitological surveys carried out among Musahar people living in Parsa (n=54) and Nawalparasi (n=103) districts respectively in Nepal revealed a lower prevalence rates ranging 28%-33.3% (Parajuli *et al.,* 2009; Khadka *et al.,* 2021). However, parasitism in similar ethnic tribes living in the tropical lowland of Terai in eastern Nepal is yet to be evaluated and discussed.

Feeding habits, socio-economic conditions and environmental factors are among the major causal factors to impact the prevalence of parasitic infections in the human population (Tigabu *et al.,* 2019; Adhikari *et al.,* 2021). In this scenario, the simple lifestyle adopted by the Musahar comprising poverty, illiteracy, ignorance, traditional feeding practices, lack of access to clean drinking water sources, simple housing, open defecation practices might favor a greater susceptibility to gastrointestinal parasitism. Thus, these people are hypothesized to be at greater risk of parasitic infection. This is why, the current study aimed to enlist prevalence and diversity of parasites including the assessment of few potential risk factors contributing parasitism among the Musahar Community people inhabiting the tropical lowlands of Terai in Balan Bihul, Saptari, in eastern Nepal.

1.3 Objectives of the study

1.3.1 General objective

i) To determine the prevalence of gastrointestinal parasites in Musahar Community in Balan Bihul, Saptari, Nepal.

1.3.2 Specific objectives

ii) To determine the diversity of protozoa and helminths parasites in Musahar Community.
iii) To determine age and sex-wise variation in the prevalence of gastrointestinal parasites among Musahar Community.

1.4 Rational of the study

The prevalence of intestinal parasites among indigenous groups of people is generally high, owing to their low socio-economic condition, illiteracy, ignorance and poor housing and lifestyle. Thus, the current study will be a milestone to know the scenario of GI parasitic infection among Musahar communities in eastern Nepal, which is yet to be evaluated and discussed. The findings of this work may provide a enlightment regarding the knowledge on parasitic relationship as well as pathogenic interrelationship among the different species of parasites and the indigenous people of varying age, sex and other socio-economic discrepancy. The real picture of parasitism will be important for treating the infected individuals and seeking appropriate measures to control parasitic infection and its burden in ethnic tribes within the country.

2. LITERATURE REVIEW

Survey on human infection with GI parasites has been an interesting area of research in worldwide context. It is evident with several surveys and researches being conducted and published globally in the national and international peer-reviewed journals. For instance, Kang and his team reported a higher (97.4%) prevalence of intestinal parasites among remote villagers in India (Kang *et al.,* 1998). They reported protozoa, like *Giardia lamblia, Entamoeba histolytica, Cryptosporidium* spp., and helminths, like hookworm, *Ascaris lumbricoides, Hymenolepis nana, Strongyloides stercoralis, Trichuris trichiura,* and *Enterobius vermicularis*. Following it, Siddiqui and his colleague reported (47.5%) prevalence rate of parasitic infection and interestingly, it was the first report to record such a higher prevalence of *Giardia lamblia* (50%) and *Entamoeba histolytica* (48.86%) from Karachi, Pakistan (Siddiqui *et al.,* 2002). In the same way, Papazahariou and team reported an overall 18.02% prevalence rate of intestinal parasites among the Greek population (11.36%) and refugees/foreigners (27.23%) from Greece (Papazahariadou *et al.,* 2004). They reported *Entamoeba coli, Cryptosporidium* spp., *Blastocystis hominis, Giardia lamblia, Ascaris lumbricoides,* hookworm, *Strongyloides stercoralis, Enterobius vermicularis, Trichuris trichiura* and *Schistosoma mansoni.* Interestingly, the prevalence of *Blastocystis* sp. (10.1%) was the highest and that of *Schistosoma* sp. (0.22%) was the least (Papazahariadou *et al.,* 2004).

Overall, 73.5% of Terena Indians population in Brazil were found to be infected with 11 diverse species of intestinal protozoa including *Blastocystis hominis, Entamoeba coli, Entamoeba histolytica/Entamoeba dispar, Endolimax nana, Giardia lamblia,* and *Iodamoeba buetschlii* and helminths, like *Hymenolepis nana,* hookworm, *Strongyloides stercoralis, Ascaris lumbricoides,* and *Enterobius vermicularis* (Aguiar *et al.,* 2007). In the same study, the prevalence of protozoa (66.50%) was significantly higher than helminths (18.50%) (Aguiar *et al.,* 2007). Similarly, Mapuera community people in Brazil were also reported with higher prevalence of 12 species of intestinal protozoa, like *Entamoeba coli, Blastocystis hominis, Endolimax nana, Entamoeba histolytica/ dispar, Iodamoeba buetschlii, Chilomastix mesnili, Giardia lamblia, Cyclospora cayetanensis,* and *Cryptosporidium* including three helminthic faunae namely *Hymenolepis nana, Ascaris lumbricoides,* and *Enterobius vermicularis* (Borges *et al.,* 2009). Six years later, Pestehchian and his team reported a higher prevalence of parasitic infection among inhabitants (67.7%) and tribes (43%) in Iran (Pestehchian *et al.,* 2015). The recorded parasites were *Giardia intestinalis, Blastocystis hominis, Endolimax nana, Entamoeba coli, Iodamoeba beutschlli, Entamoeba histolytica/E. dispar, Chilomastix mesnili* and *Hymenolepis nana* (Pestehchian *et al.,* 2015).

Notably, a low prevalence (10.2%) of intestinal parasites were reported among the food handlers in Iran (Arbat *et al.,* 2018). In the same year, a parasitological assessment conducted among Fortin Mbororé and Yriapú Jungle villagers in Argentina revealed 87.8% prevalence rate of intestinal parasites with the presence of 13 different species of intestinal parasites (Rivero *et al.,* 2018). They also highlighted the occurrence of multiparasitism (70%) being significantly higher than mono parasitism among the children of age $\leq$ 6 years

(Rivero *et al.,* 2018). Two years later, in the same geography, a survey on intestinal parasites, undernutrition and socio-environmental determinants in schoolchildren revealed 78.1% of the children were infected with 12 species of intestinal parasites with 70.8% multiparasitism (Zonta *et al.,* 2020). Furthermore, 17.5% of the children were also infected with STH and the authors concluded children who lived in houses with adequate solid waste disposal, whose parents were jobless or work on temporary basis pose a greater risk of parasitism (Zonta *et al.,* 2020). In the same way, a parasitological survey conducted among Kogui indigenous tribe in Colombia revealed the presence of protozoa, like *Blastocystis hominis, Giardia duodenalis* and *Cryptosporidium* spp., and helminths, like *Ascaris* spp., *Trichuris trichiura*, *Necator americanus*, *Taenia* spp., and *Strongyloides stercoralis* (Kann *et al.,* 2022).

Several parasitological surveys have been carried out in Nepal concerning the human and their gastrointestinal parasites. Though only two research works had been published in international peer-reviewed journals concerning the ethnic Musahar communities and their intestinal parasitism. In 2009, Parajuli and team examined the fecal sample of 47 Musahar individual of age (20-60) years in Parsa district for the presence of geo-helminths and reported the prevalence of geo-helminths, like *Ascaris lumbricoides*, Hookworm and *Trichuris trichiura* in about 28%, 13% and 11% of these population. They were of the idea that unhygienic behaviors and chronic malnutrition were closely associated with geo helminthic infection (Parajuli *et al.,* 2009). Similarly, Khadka and his colleagues conducted a cross-sectional combined study regarding intestinal parasitism among the Musahar and Chepang community people in Nawalparasi and Makwanpur district respectively in 2021. They reported 33% Mushar individuals out of 103 were positive with at least one species of intestinal parasites (Khadka *et al.,* 2021). However, in the absence of individual data regarding the presence of parasites in particular ethnic communities, the scenario of individual parasitic presence in Musahar people in this study is still uncleared (Khadka *et al.,* 2021).

Considering parasitological survey among other ethnic communities in Nepal, Parajuli and team reported 68.8% prevalence of geo-helminths, like *Ascaris lumbricoides*, Hookworm and *Trichuris trichiura* in about 25%, 6% and 2% out of 54 Tharu ethnic people from Parsa district. Following it, a survey was conducted among Kumal ethnic group in Chitwan district in 2013, which revealed almost 50% parasitic prevalence and the presence of parasites, like Hookworm (30.87%), *Ascaris* (16.10%), *Hymenolepis* (6.04%). *Trichuris* (3.55%), Strongyloide (2.68%) and *Taenia* (2.01%) (Gyawali, 2012).

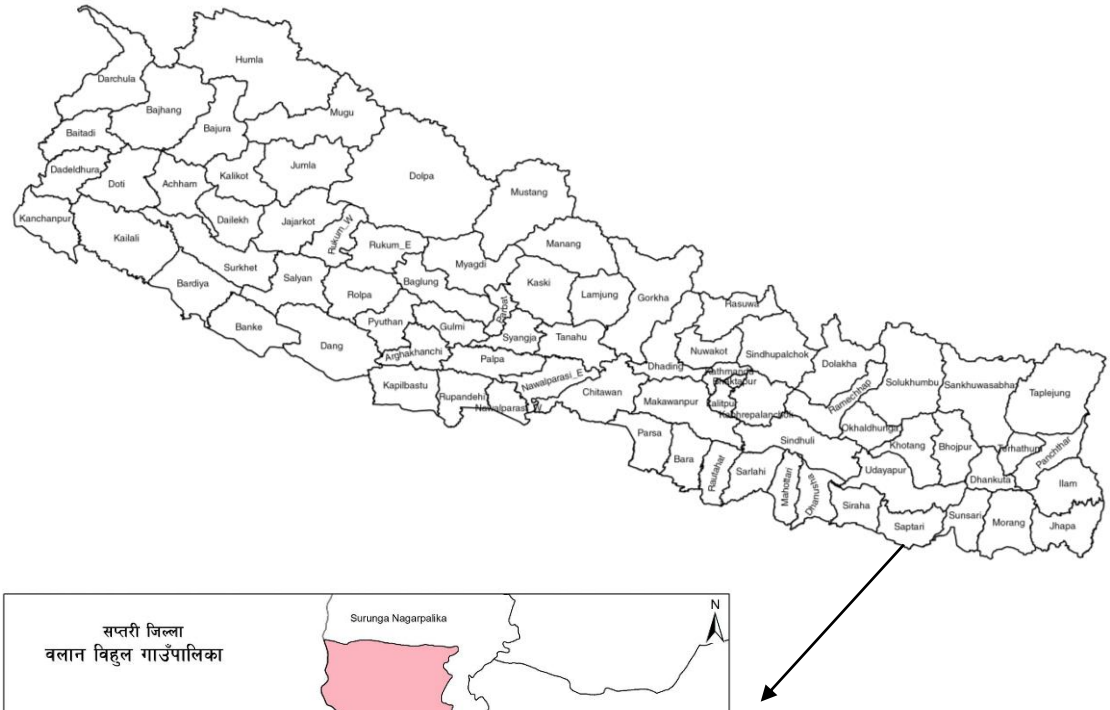
Following it, 63.35% of the Muslim community people out of 161 in Janakpurdham, were infected with at least one species of GI parasites (Yadav and Prakash, 2017). The authors reported a higher prevalence of parasitic infection in male population and the highly prevalent parasites were *Giardia lamblia* (28%) and Hookworm (10%). They were of the idea that parasitic prevalence was higher in those population who didn't wash their hands before and after meal, defecates in open area, didn't wash their hands and legs after defecation, didn't trim their nails and those who live in larger joint family with low income (Yadav and Prakash, 2017). Furthermore, intestinal helminth parasites among Satar and Chaudhary communities of Birtamod Municipality, in Jhapa was revealed in the

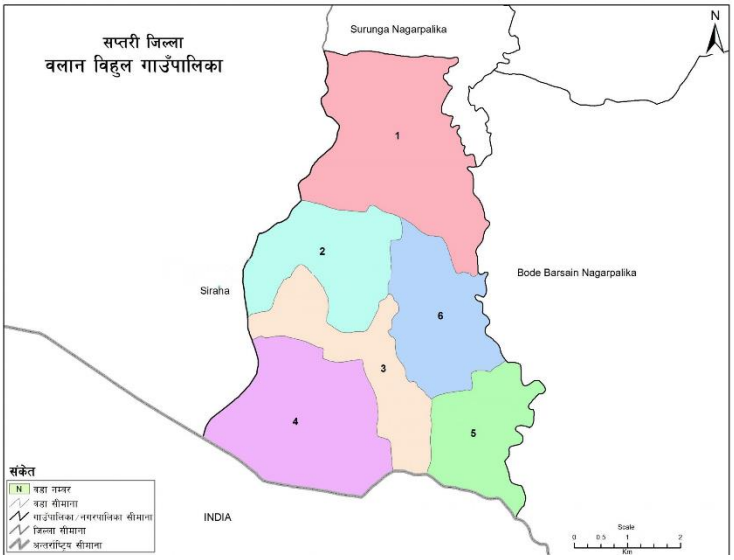
year 2020 (Chaudhary and Subedi, 2020). They reported an overall prevalence of 28.63% of GI parasites with the presence of parasites like, *Ascaris lumbricoides* (15.45%), *Trichuris trichiura* (8.18%), Hookworm (8.18%), *Strongyloides stercoralis* (2.73%), *Taenia* sp. (1.82%) and *Hymenolepis nana* (2.73%) in Satar communities and *Ascaris lumbricoides* (13.18%), *Trichuris trichiura* (7.27%), Hookworm (6.36%), *Strongyloides stercoralis* (5.27%) and *Taenia* sp. (1.82%) among Chaudhary (Tharu) Communities. They also concluded that handwashing habits, use of handwashing agents, defecation site preference and barefoot walking habits were associated with greater parasitic prevalence in these indigenous peoples (Chaudhary and Subedi, 2020). Following it, parasitism among Sarki ethnic groups was conducted in the year 2021 in Pala Rural Municipality, Baglung. The overall prevalence of parasitic infection was 31.32% and the reported parasites were *Trichuris trichiura* (53.20%) *Ascaris lumbricoides* (33.97%), *Entamoeba coli* (4.49%), *Taenia* sp. (3.21%), *Strongyloides stercoralis* (2.56%) and *Hymenolepis nana* (2.56%) (Thapa *et al.,* 2021). In the same way, Adhikari and team conducted a parasitological survey among indigenous Chepang of Shaktikhor Chitwan in central Nepal (Adhikari *et al.*, 2021). They reported 14 different genus of intestinal parasites, like *Balantidium coli, Blastocystis hominis, Cryptosporidium* sp., *Cyclospora cayetanensis, Entamoeba coli, Entamoeba histolytica, Giardia lamblia, Iodamoeba buetschlii, Ascaris lumbricoides,* hookworm, *Hymenolepis nana, Strongyloides stercoralis, Trichostrongylus,* and *Trichuris trichiura* and concluded that bat-consuming habit might contribute to acquisition of some zoonotic parasites to human (Adhikari *et al.*, 2021)

3. MATERIALS AND METHODS

3.1 Study area

The study has been conducted in Balan Bihul-5, Ramnagar village located in Balan Bihul Rural Municipality (26.59ºN and 86.51ºE) in Saptari district in Province No. 2 (Madhesh Province) of south-eastern Nepal. Its total area is 118.19 Km^2 (45.63 sq mi). It is named as Balan Bihul because it lies in between the Balan and Bihul river. According to Nepal Census, 2017, it had a population of 26,068 people and 6,560 individual households. Out of this population the total population of Musahar is 600. The population density is 220/Km^2 (570/sq mi). Balan Bihul Rural Municipality has six Village Development Committees (wards). It is attached with the open India border towards south. It is mixed with Indian culture and traditions due to the open border facility. Geographically the study area lies at the altitude of 61 to 610 ft. a.s.l. Ethnicity in this rural municipality includes, Yadav Sah (Teli), Mandals (Dhanuk), Muslims, Das (Baniya), Mehtas, Mushars, Maithil and Brahmins. The main religion followed is Hinduism and Islam.The main language spoken is Maithili and Nepali.





Balan-Bihul

Figure.1: Map of study site

3.2 Materials Required

3.2.1 Equipments / Apparatus

a) Cotton buds
b) Sterile vials
c) Forcep
d) Wooden applicator
e) Cool box
f) Glass slides
g) Cover slides
h) Glass rod
i) Tooth pick
j) Gloves
k) Masks
l) Compound Microscope
m) Centrifuge tube
n) Centrifuge machine
o) Plastic dropper
p) Weighing machine
q) Test-tube stand
r) Tea strainer
s) Measuring cylinder

3.2.2 Chemicals

t) Normal saline (0.9%)
u) 2.5% Potassium dichromate
v) Iodine solution (Gram's iodine)
w) Dettol hand wash

3.3 Methods

3.3.1 Preparation of 2.5% (w/v) Potassium dichromate

In order to prepare 2.5% potassium dichromate ($K_2Cr_2O_7$), 25 gm of potassium dichromate powder was weighed and dissolved in 1000 ml of distilled water. It was used for the preservation of parasitic bodies (eggs/larvae/cyst/trophozoites) present in the fecal matters (Zajac and Conboy, 2012). It also helps to maintain the integrity of parasitic bodies available in the fecal samples.

3.3.2 Preparation of (0.9%) Normal Saline

Normal saline (NS) was prepared by dissolving 9 gm of Sodium chloride in 1000 ml of distilled water. It was used in unstained preparation (Zajac and Conboy, 2012).

3.4 Collection, preservation and transportation of fecal samples

The human populations in the study area were into 3 groups based on their age; young and non-working group (10-20) years, adult/working group (20-59) years, and elderly or resting group (60 and above) years. Via purposive sampling, a total of 200 fresh fecal/stool samples were collected from various location within the study site. A day prior to sample collection, all the villagers were provided with 30 ml sterile vials and a wooden applicator. They were clearly instructed with the collection techniques. Finally, collection of sample was carried out early in the morning along with a structured questionnaire. The stool samples thus collected were immediately preserved in 2.5% $K_2Cr_2O_7$ solution and were carried to Zoology Laboratory at Central Department of Zoology for microscopic examination and further investigation.

3.5 Laboratory processing and identification

3.5.1 Macroscopic and microscopic examination of stool samples

Earlier to microscopic examination, the stool samples were macroscopically examined for the presence of mucus, segments of cestodes, and dead adult nematodes. Microscopically examination was carried out using direct wet mount and concentration techniques based on the procedure previously explained (Adhikari *et al.,* 2020; Adhikar *et al.,* 2021; Aryal *et al.,* 2021; Adhikari *et al.,* 2022; Adhikari *et al.,* 2023).

3.5.2 Preparation of unstained and stained smear

The stool sample preserved at 2.5% $K_2Cr_2O_7$ was stirred carefully with the help of a glass rod. A single drop of sample was taken with the help of plastic dropper and placced on a clean glass slide to make a smear. It was then covered with a clean coverslip. The excess of fluid was removed with the help of cotton and tissue paper. Similarly stained smear was prepared using 1-2 drops of Gram's iodine before smear preparation. The stained smear preparation will be helpful for the identification purpose and to study the nuclear matter of the protozoan cysts, and trophozoites.

3.5.3 Concentration technique

There are two types of concentration procedures; sedimentation and flotation. Both these techniques were used to concentrate helminthic eggs and larvae, protozoan cysts, (Garcia *et al.,* 2018).

In saturated salt flotation technique, the high specific gravity of a concentrated solution is used to float the lighter ova, cyst, eggs of nematode, and cestodes which are present in the stool sample. Its efficacy was further improved by centrifugation. It involved the following procedure.

a) 45 gm of NaCl was weighted in a weighing machine inorder to prepare the concentrated solution by dissolving it in 100 ml of distilled water. The solution was vigorously striated using the help of a glass rod.
b) About 2 gm of stool sample was taken and kept over the tea strainer fitted above a 15ml centrifuge tube fitted in a test tube stand tightly.
c) The stool sample was stirred carefully with the help of glass rod and filtered using 0.9% normal saline.
d) The 0.9% NS was further poured to centrifuge tube to make it full and centrifugation was performed at 1200 rpm for 5 minute at RT.
e) After that the supernatant was discarded immediately and recentrifugation was carried out after adding about 14 ml of saturated salt solution.
f) After recentrifugation, without removing the supernatant, the tube was filled completely with concentrated salt solution to develop a convex surface at top.
g) Then a clean cover slip was placed over the top of the tube, avoiding any bubbles being trapped and was left undisturbed for about 10 minutes.
h) The coverslip was gently removed and placed in a clean glass slide and observation was done under the compound microscope at 10x and 40x magnification of objective lens. The photographs of reported eggs, cysts, and oocysts were taken. using a mobile camera. The identification of parasitic eggs, cysts, and oocysts were taken and identification was done based on the morphological characters.

Sedimentation technique is primarily used to detect trematodes eggs in the stool samples; however, some eggs and larva of nematodes like unfertilized eggs of *Ascaris lumbricoides* and some cestodes eggs, like *Taenia* spp. are also better detected by this process because they do not float on concentration salt solution. It involves the following procedure.

i) About 2 gm of stool sample was taken and kept over the tea strainer fitted above a 15ml centrifuge tube fitted in a test tube stand tightly.
j) The stool sample was carefully stirred with the help of glass rod and filtered using 0.9% NS.
k) The 0.9% NS was further poured to centrifuge tube to completely filled it. It is then followed by centrifugation at 1200rpm for 5 minute at RT.
l) The supernatant was then discarded and the sediment was gently stirred and mixed with the help of glass rod.
m) A single drop of the sediment was then taken with the help of plastic dropper and using a drop of gram's iodine the stain smear was prepared.
n) Finally, microscopic examination was carried out under the compound microscope at 10x and 40x magnification of the objective lens and photographs of eggs, cysts and larva were taken.

3.5.4 Measurement and identification of cysts, oocysts, trophozoite, eggs, and larva

The measurement of reported protozoan cysts, oocysts, trophozoites, and helminthic eggs, and larva was done using oculo-micrometer and Imaje J software version ImajeJ 1.46r/Java 1.6.0_20 (64-bit). The identification of parasites were done comparing the obtained photographs and micrometry with the figures in published literatures, books, and CDC webpage (www.cdc.gov/parasites/).

3.6 Data analysis

In the current study, prevalence rate was measured by dividing the individual presence of parasites by the total number of samples and finally multiplied by 100. The collected data were encrypted and entered into Microsoft Excel spread sheet. Data were analyzed using Pearson's Chi-square test, using SPSS software. In all case, 95% confidence interval (CI) and $p<0.05$ was considered for statically significant difference.

3.7 Ethical consideration

To carry out the thesis work, written permission letter from concerned rural Municipality, Balan Bihul Rural Municipality (Permission No. 127/2080/081) for the collection of stool samples was taken.

4. RESULTS

4.1 Prevalence and diversity of GI Parasites in Musahar Community in Balan Bihul, Saptari, Nepal

The GI parasitic infection among Musahar Community in Balan Bihul, Saptari, Nepal revealed 81% (162/200) prevalence. These peoples were found to be infected with 9 different species of intestinal parasites. Among them four were protozoans like *Entameba* sp., *Entamoeba coli*, *Giardia* sp., and *Balantidium coli* and five were helminths, such as *Ascaris lumbricoides*, *Ancylostoma* sp., *Hymenolepis nana*, *Strongyloides stercoralis*, and *Trichuris trichiura* (Figure 5). The prevalence of *Ancylostoma* sp. (41.5%) was the highest followed by *Entamoeba* sp. (31.5%) and that of *B. coli* (2%) was the least. (Figure 2).

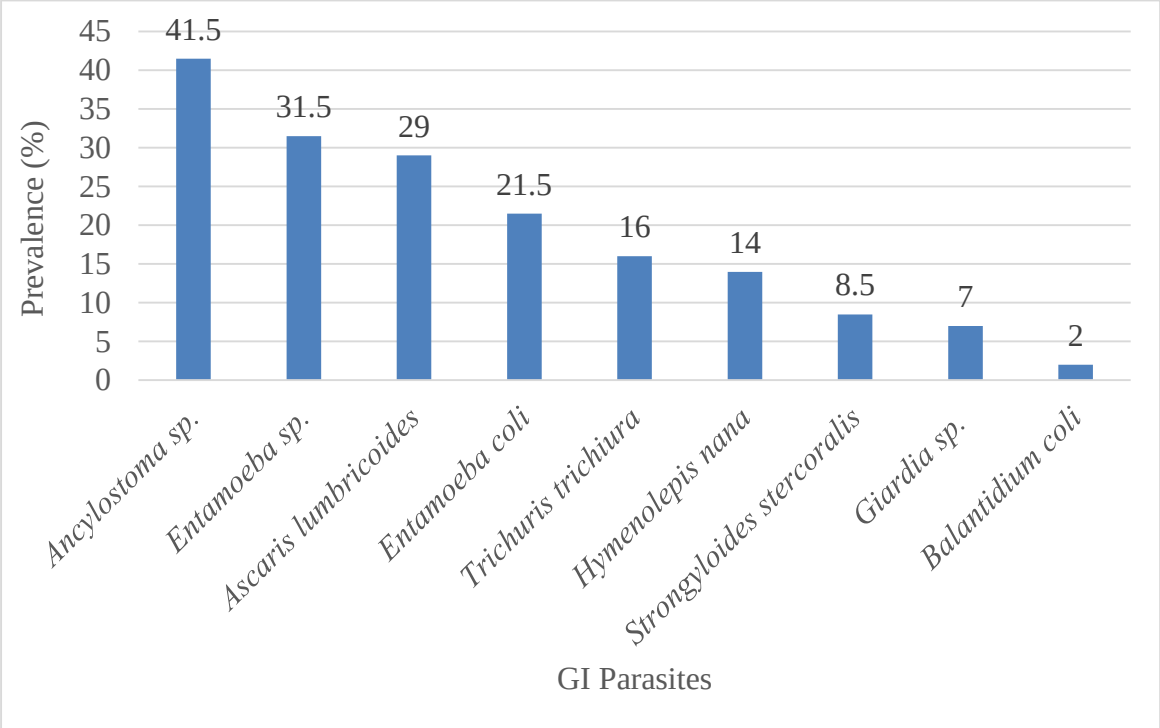


Figure 2: Overall GI parasitic prevalence in Musahar Community.

Furthermore, the reported parasites were classified as single cellular protozoa and multicellular helminths. The prevalence of helminth parasites (62%) out of 200 respondents was significantly higher than protozoa (38%) ($p<0.05$) (Figure 3). Considering the diversity two classes of helminths parasites were reported; cestoda (*Hymenolepis nana*) and nematoda (*Ascaris lumbricoides*, *Ancylostoma* sp., *Strongyloides stercoralis*, and *Trichuris trichiura).* Following it, protozoan type; Sarcodina (*Entamoeba* sp. and *E. coli*), flagellates (*Giardia* sp.) and ciliates (*B. coli*) were reported.

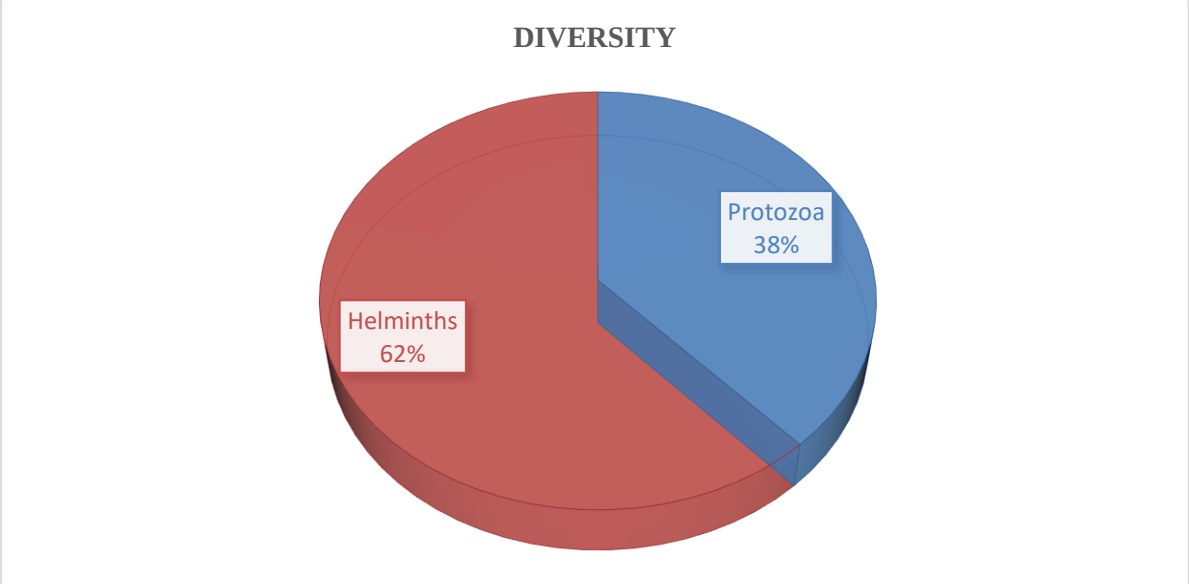


Figure 3: Prevalence of diverse protozoa and helminth parasites in Musahar Community.

4.2 Concurrency of GI parasitic infection

In the current study, the GI infection with multiple species of parasites is higher than infection with single species. Interestingly, the Musahar people were reported to be infected with a maximum of 4 species of parasites at a time. Double infection (43%) was the highest and Quadruplet infection (4%) was the least. The differences in the prevalence of single, double, triple and quadruple parasitic infection were found to be statically significant $p<0.05$ (Figure 4).

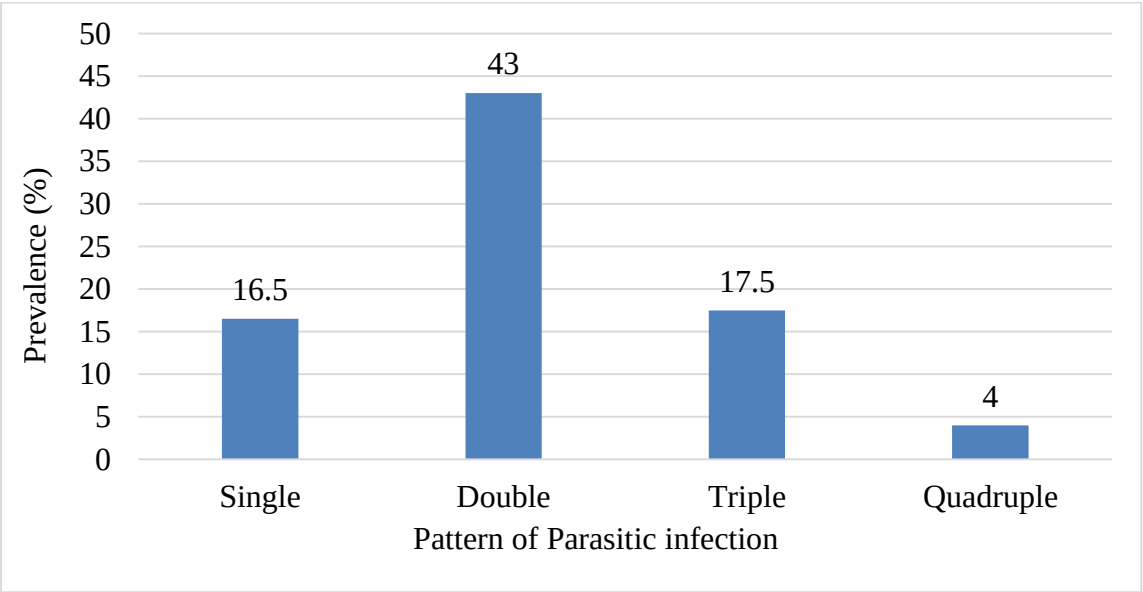


Figure 4: Concurrency of parasitic infection in Musahar Community.

4.3 Age and Sexwise parasitic prevalence of Parasites in Musahar Community

The current population of Musahar was categorized into three groups, such as young/non-working group (10-20) years, adult/working group (20-59) years and old/retired group (60 and above) years. Interestingly, parasitic prevalence was highest in the old /retired population (93.2%), followed by youngs/non-working population (85.7%) and that of adults/working population (73%) was the least (Table 1). Similarly, the female population (85%) has greater prevalence of GI parasites than male (75.6%) (Table 1).

4.4 Assessment of potential risk factors for parasitic infection

In the present study, five possible predictors of risk were assessed including feeding habit, house type, walking practices, defecation practices and history of medication /deworming. It was observed that the prevalence of GI parasites is higher in those populations who were rat-consumers, living in mud-built houses, always walking barefoot, defecating openly in open areas and having more than one year or unknown history of medication/deworming.
(Table.1).

Table 1: Assessment of Predictor of Risk Factors of Parasitism in Musahar

SN	Potential Risk	Categories	Total individual	Prevalence Rate	Statistics
1	Age	10-20 years 20-59 years 60 and above	56 100 44	48 (85.7%) 73 (73%) 41 (93.2%)	ns
2	Sex	Male Female	86 114	65 (75.6%) 97 (85.1%)	ns
3	Feeding habit	Rat-consumers Rat non-consumers	72 128	66 (91.7%) 96 (75%)	*p <0.05*
4.	House Type	Cemented Mud house/hut	34 166	22 (64%) 140 (84.3%)	*p <0.05*

5	Walking practice	Completely bare footers Occasionally wears shoes/sandal Always wear shoes/sandal	78 55 67	72 (92.3%) 48 (87.3%) 42 (62%)	*p <0.05*
6	Defecation practice	Open defecation Toilet users	154 46	136 (88.3%) 26 (56.5%)	*p <0.05*
7.	Medication/deworming	≤6 months ≤12 months >12 months/unknown history	52 106 42	38 (73.1%) 87 (82.1%). 37 (88.1%)	ns

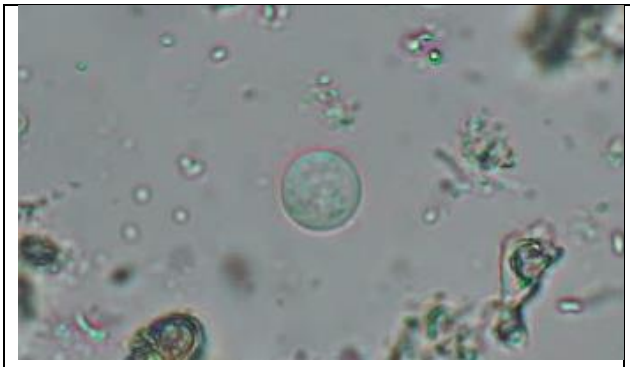
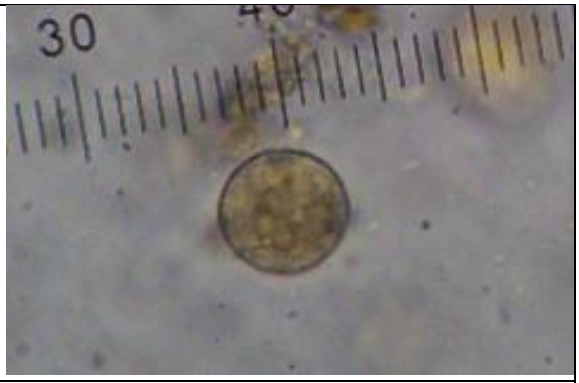
	
Cyst of *Entamoeba* sp. (12x11µm)	Cyst of *Entamoeba coli* (18x17 µm)
	
Egg of *Trichuris* sp. (54x23 µm)	Egg of *Hymenolepis nana (*43x36 µm)2
	
Egg of *Ascaris lumbricoides* (58x 40 µm)	Cyst of *Balantidium coli* (49x48 µm)
	
Egg of *Strongyloides stercoralis* (64x37 µm)	Egg of *Ancylostoma* sp. (66x38 µm)

Figure 5: Photographs and micrometry of human parasites (eggs, cysts, oocysts, and trophozoite

5. DISCUSSIONS

The current study indicates the prevalence of GI parasites in Musahar Community in eastern Nepal. The prevalence was recorded 81%. To the best of my knowledge, this study is the first of its kind in the country to report such a higher prevalence and greater diversity of intestinal parasites in the Musahar communities. Thus, the current study can work as a model for the study of parasitic diseases in indigenous communities in Nepal or else where.

In the present study, the overall prevalence of parasitic infection in a total of 200 stool samples of ethnic Musahar people was 81%. This prevalence rate is lower than the finding report from India (97.4%) (Kang *et al.,* 1998), Nepal (90-97%) (Adhikari *et al.,* 2021; Estevez *et al.,* 1983), but higher than the previous finding from Nepal (28%-31.32%) (Chaudhary and Subedi, 2020; Thapa *et al.,* 2021), India (75.8%) (Dhanabal *et al.,* 2007), Brazil (73.50%) (Aguiar *et al.,* 2007), Iran (56%) (Pestechaian *et al.,* 2015), and Pakistan (47.5%) (Siddiqui *et al.,* 2002). The dissimilarity in prevalence rate of GI parasites in these studies might be attributed to the discrepancy in sample size, sampling geography, employed laboratory based techniques, and the socio-economic as well as the existing environmental factors and the behavioural practice of ethnic peoples. We assumed higher prevalence in existing study might be due to sampling from the tropical lowland of Terai and from the socio-economically backward ethnic communities with existing poverty, ignorance and illiteracy. Interestingly, all three laboratory techniques were applied for parasitic assessment in each stool sample.

In the current research, the prevalence of helminth parasites (cestodes and nematodes) was higher over single-cellular protozoans. This finding of higher prevalence of helminths over protozoans is in accordance with the finding from Nepal (Rai *et al.,* 2008; Dhakal and Subedi, 2019) and India (Singh *et al.,* 1991). However, contrasting results were obtained from similar other studies from Nepal (Adhikari *et al.,* 2021; Baral *et al.,* 2017), Brazil (Aguiar *et al.,* 2007), and Iran (Pestehchian *et al.,* 2015). Even though, protozoan infections are usually associated with poor sanitary habits, lack of access to safe water, and improper hygiene (Amer *et al.,* 2015), the predominance of helminths particularly the soil transmitted helminths, like *Ancylostoma* spp., *Ascaris lumbricoides* and *Trichuris trichiura* are mainly attributed to the presence of optimal temperature that favoured the rapid embryonation of *Ascaris* and *Trichuris* eggs, egg hatching and conversion of rhabditiform larvae into infective filariform form larvae of hookworm and threadworms (Amadi *et al.,* 2010). Similarly, unhealthy feeding habits, i.e contaminated food and vegetables and drinking contaminated water contaminated with parasite's eggs favoured GI parasitism (Schuster and Ramiez-Avila, 2008; Adhikari *et al.,* 2021). Similarly, walking bare foot provides a favourable environment for filariform larvae of hookworm and threadworms to penetrate the host's unbroken skin and get entrance inside host body (Umbrello *et al.,* 2021). In the same way, occurrence of protozoa may be attributed to ignorance of people to personal hygiene, drinking of water from risk sources without any treatment, practice of open defecation and poor sanitary practices near the households. It

has been discussed that open defecation practices in the fields, near water sources, on roads increases the risk spread of mechanical vectors, like houseflies and cockroaches which transmit infective stages of parasites, such as cysts and trophozoites of protozoans like *E. histolytica*, *Giardia* spp., *Cryptosporidium* spp. and others (Adenusi and Adewoga, 2013; Issa, 2019). In addition, contaminated soil, air, water, foods and others contribute to spread of parasites (Fayer, 2004; Ghimire *et al.,* 2010). The ethnic people in current study were infected with nine different species of GI parasites. Among them *E. histolytica, E. coli, Ancylostoma* sp. *and Ascaris lumbricoides* were the most common parasites reported earlier from Nepal (Parajuli *et al.,* 2009; Adhikari *et al.,* 2021). Interestingly, the ciliated and larged-sized protozoa (*B. coli)* was also reported in the present study. This finding is supported by the fact that, ethnic people rear pigs and in close association to this animal which is the reservoir of this ciliates (Schuster & Ramirez-Avila, 2008). In addition, close contact with pigs also contributes to acquisition of *Ascaris lumbricoides* (Anderson, 1995).

It was observed that most of the respondents in the current study are habituated to walk bare foot or occasionally wear sandals/shoes. This is the main reason why they are infected with skin-penetrating parasites, like hookworms and threadworms, especially in muddy and sandy conditions. Remarkably, the only cestode parasite reported in the current study is *Hymenolepis nana*. 14% prevalence rate of this dwarf tape worm was higher than previous findings from Nepal (4%) (Ghimire and Mishra, 2005), (5.8%) (Agrawal *et al*., 2012) and slightly lower than from India (25.6%) (Kang *et al.,* 1998), and Brazil (20.5%) (Borges *et al.,* 2009). The presence of this tapeworm is attributed to poor hygienic and inadequate sanitary practices, overcrowding in the houses, which is a common problem in households in the current area. Person to person transmission is also a common phenomenon. However, we speculated that the habit of consuming rats, which is considered to be the reservoir of *H. nana*, might be a risk factor which is evident with the detection of this tapeworm only in the rat-consuming respondents. However, further epidemiological and molecular studies must be conducted to verify this concept.

In this study, the female population has higher parasitic prevalence than males (85.1% versus 75.6%). This result is in accordance with previous findings from Nepal (Dhakal and Subedi, 2019; Khadka *et al.,* 2021). However, another report from Nepal has contrasting findings (Adhikari *et al.,* 2021). Higher prevalence of GI parasites in females might have been possible due to their greater exposure to associated risk factors because females in study areas were mostly confined to household activities, child-caring, agriculture as well as animal husbandry, while male preferred outdoor works.

Similarly, old age/retired people of age 60 and above years were found to be highly infected with parasites (93.2%) than the young (10-20) years and adult/working population (20-59). It is speculated that immunity level decreases with age (DeVeale *et al.,* 2004). This might have resulted due to their unhealthy behavioural practices. They showed great ignorance in attaining healthy behavioural practices (Personal observation). These populations mainly feed on rat meat, prefer open defecation and always walk barefoot and show great negligence to follow personal hygienic habits, including wearing neat and clean dresses, nail cutting, and washing hands and legs after work and so on.

Furthermore, most of the respondents (64.5%) in the current study were reported with a concomitant pattern of infection. The maximum co-infection with four species of parasites was reported with double infection at the top. In general, concomitant infection is a rule in nature, but it has been discussed to have a synergistic effect to increase pathogenic virulence and infection severity in humans (Ezeamama *et al.,* 2008; Njunda *et al.,* 2015). Thus, it is obvious that the current Musahar community with high co-infection rate might have adverse effects on their GI health.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the current study, it is concluded that the Musahar Community people in Balan Bihul, Saptari, harbored a higher prevalence of GI parasites including nine diverse genus of intestinal parasites. They harboured higher prevalence and a wide variety of helminth parasites over protozoa. Parasitism varied with age sex and adopted behavioural practices. Parasitism is higher among female population, old age group peoples and among those who are habituated in consuming rats, walking barefoot (wearing no shoes/sandal), defecating haphazardly on open lands, live in mud-built houses and with unknown or more than one year earlier history of medication. In addition, the mixed pattern of parasitism was higher than the single infection.

6.2 Recommendations

Based on the outcomes of the study, following recommendations are made in order to reduce the prevalence of GI parasites ethnic Musahar community.

a) Local government bodies, parasitologists and public health workers should conduct a community-based antiprotozoal and anthelminthic chemotherapy campaign among the existing Musahar population.

b) The health awareness programs that basically focus on improvement of personal hygiene, ways of appropriate water management, like boiling and filtering, use of latrines, regular wearing of shoes/sandals, proper housing and environmental sanitation should be conducted on a regular basis which add to get rid of GI infections.

c) Molecular studies should be conducted for the identification of parasites.

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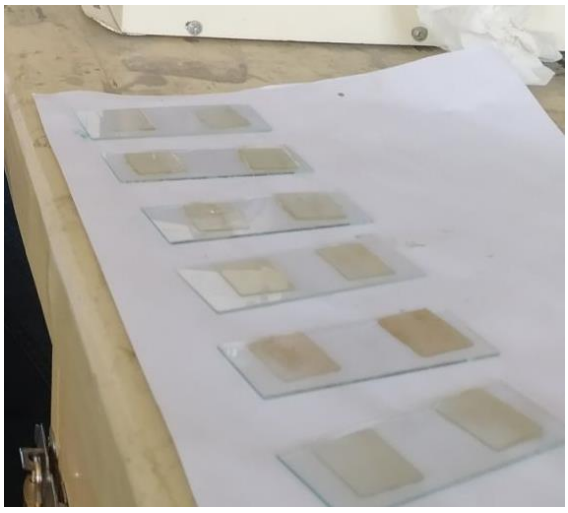
ANNEXES

Table.2: Morphometric features and micrometry of identified eggs, cysts and trophozoites of human parasites

Name of Parasites	Micrometry in average	Morphological characters	Reference (CDC), www.cdc.gov/parasites/)
E. histolytica (cyst)	(12x11) µm	Spherical or oval, may exist as uninucleated binucleated or quadrinucleated, stain reddish brown with iodine.	(10-20 µm). usual range (12-15 µm)
E. coli (cyst)	(18x17) µm	Spherical or oval, immature cyst contains two or more nuclei while mature cyst may contains eight nuclei, stain reddish brown with iodine.	(10-35 µm) usual (15-25 µm)
G. lamblia (cyst)	(12x8) µm	Oval or ellipsoidal, presence of four nuclei which may remain clustered at one end or lie in pairs at opposite ends but not visible in unstained preparation.	(8-19 µm) usual (11-12 µm)
B. coli (cyst)	(49x48) µm	Oval, surrounded by a thick and transparent double –layered wall. Nuclear dimorphism; consists of large kidney-shaped macro nuclus and small rounded micronucleus. Cilia is absent.	(40-60 µm) usual (45-54 µm)
A. lumbricoides (fertilized egg)	(58x40) µm	Round or ovoidal shape with thick shell, presence of mammillated albuminous coat or covering on outer shell, eggs may be in two, four or more cells or contain a fully developed larva when passed.	(45-70 µm) x (35-45µm)
Ancylostoma sp. (egg)	(66x37) µm	Oval or ellipsoidal, with a thin shell, embryo is divided into four to eight cell stages when passed in feces.	(57-76 µm) x (35-47 µm)
S. stercoralis (egg)	(56x32) µm	Oval, transparent and thin–shelled, contains a larva ready to hatch when passed.	(40-60 µm) x (20-40 µm)
H. nana (egg)	(43x36) µm	Oval shaped, egg shells are embryonated and contains two distinct membranes with six hooked oncosphere.	(40-60µm) x (30-50µm)
T. trichiura (egg)	(54x23) µm	Elongated, barrel-shaped with a polar plug at each end, egg shell is one celled or unsegmented when released.	(49-65 µm) x (20-29 µm)

Photo Plates



A. Preperation of fecal smears

B. Samples ready for centrifugation

C. Microscopic examination in lab

D. Centrifugation Process

QUESTIONNAIRE FOR SURVEY OF ……In

……. Nepal.

Name:

Age: ……. Sex…….

Locality……. Occupation: ……...… Caste……...

1.Where do you defecate?

i) Toilet ii) Open

2.Which source of water do you usually prefer?

i) Tube-well ii) Well iii) River iv) Pond

3.When do you wash your hand?

i) Before and after meal ii) After defecation iii) after work iv) All cases

4. Do you rear pigs at your home?

i) Yes ii) No iii) Sometimes only

5. Do you consume pork?

i) Yes usually ii) No iii) Sometime

6. Do you consume rat?

i) Yes ii) No

7. How often you wear shoes/ sandal?

i) Yes always ii) No never iii) Ocassionally

8. Do you have any GI problem right now?

i) Yes ii) No

9. Have you ever taken antiprotozoal/antihelminthic drugs?

i) 1 years ago ii) six month ago iii) once in a life (more than 2 years ago)

10. What will you do if you suffer from GI symptoms?

i) Do nothing ii) Consult witch doctor iii) Go to hospital

11. Do you know the cause of GI illness?

i) Yes ii) No

If yes, tell me what are they……

12. Do you know the method of prevention of GI infection?

i) Yes ii) No

If yes, tell me what are they……

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प.स २०८०/०८१
च.नं. १३६

मिति २०८०/०५/१२

विषय-अनुमति दिइएको बारे।

श्री जिव विज्ञान केन्द्रिय कार्यालय किर्तिपुर, काठमाण्डौं।

प्रस्तुत विषयमा जिल्ला सप्तरी बलान बिहुल गा.पा.वडा नं.- ५ रामनगर बस्ने भगवती प्रसाद यादवले यस कार्यालयमा पेश गरेको निवेदन माँग बमोजिम निज त्यस कार्यालयको चौंथो सेमेष्टरमा Parasitology विषय लिई अध्ययनगरिरहेकोमा निजले Prevelence of gastro Intestinal parasites among Musahar Community of Balan bihul RM Ward no-5, Saptari, Nepal शिर्षकमा शोध, अध्ययन, तथा अनुसन्धान गर्नको लागि अनुमति माँग गरेको हुँदा निजलाई सो कार्य गर्न गराउनको लागि यस कार्यालयबाट अनुमति प्रदान गरिएको व्यहोरा अनुरोध छ।

०८०/५/१२

विन्देश्वर यादव
वडा अध्यक्ष

विन्देश्वर यादव
वडा अध्यक्ष

"जातीय भेदभाव तथा छुवाछुत दण्डनीय अपराध हो ।"

S.N.	Name of the people	Age	Sex	Parasites	Remarks
1	Ram Kumari Sadey	55	Female		
2	Sarita Devi Sadey	58	F		
3	Ram Pyari Devi Sadey	72	F		
4	Arti Kumari Sadey	14	F		
5	Pragati Kumari Sadey	09	F		
6	Kriti Kumari Sadey	12	F		
7	Laxmi Kumari Sadey	65	F		
8	Nitish Kumar Sadey	12	M		
9	Gajendra Kumar Sadey	18	M		
10	Roopesh Kumar Sadey	22	M		
11	Shree Hari Devi Sadey	33	M		
12	Archana Kumari Sadey	23	F		
13	Usha Devi Sadey	35	F		
14	Sakuni Sadey	06	F		
15	Ajit Kumar Sadey	25	M		
16	Sapna Devi Sadey	35	F		
17	Sagar Sadey	65	M		
18	Amar Devi Sadey	70	F		
19	Dharmaniya Devi Sadey	30	F		
20	Indrajeet Sadey	23	M		
21	Meena Devi Sadey	22	F		
22	Akshay Kumar Sadey	07	M		
23	Rupesh Kumar Sadey	05	M		
24	[illegible] Sadey	[illegible]	F		
25	Parmeshwari Devi Sadey	65	F		
26	Rameet Sadey	25	M		
27	Radhika Devi Sadey	22	F		
28	Arjun Sadey	05	M		
29	Rachna Sadey	25	F		
30	Kanika Devi Sadey	25	F		
31	Mintu Sadey	46	M		
32	Indu Devi Sadey	40	F		
33	Raj Kumar Sadey	18	M		
34	Sapna [illegible] Sadey	13	F		
35	Shiv Kumar Sadey	08	M		
36	Mahendra Sadey	60	M		
37	Puja Devi Sadey	35	F		
38	Pooja Kumari Sadey	16	F		
39	Laxman Sadey	18	M		
40	Rohit Sadey	20	M		
41	Priyanka Kumari Sadey	22	F		
42	Sav Lal Sadey	63	M		
43	Gautam Devi Sadey	55	F		
44	Bittu Sadey	45	M		
45	Anupam Devi Sadey	32	F		
46	Sangita Devi Sadey	25	F		
47	Ankita Kumari Sadey	26	F		
48	Krishna Kumar Sadey	23	M		
49	Tarun Kumar Sadey	05	M		
50	Anjana Kumari Sadey	42	F		