

Research article

# Gastrointestinal parasites among Musahar Community in Balan-Bihul, Saptari, Nepal

Bhagwati Prasad Yadav<sup>1</sup> | Janak Raj Subedi<sup>1\*</sup>  | Roshan Babu Adhikari<sup>2,3,4</sup> 

<sup>1</sup> Central Department of Zoology, Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal

<sup>2</sup> Nepalese Army Institute of Health Sciences (NAIHS), Kathmandu, Nepal

<sup>3</sup> Department of Zoology, Alka Health Institute, Pvt. Ltd., Lalitpur, Nepal

<sup>4</sup> Third Pole Conservancy, Bhaktapur, Nepal

\* **Correspondence:** [janak.subedi@cdz.tu.edu.np](mailto:janak.subedi@cdz.tu.edu.np)

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## Abstract

Gastrointestinal parasites inhabiting the intestinal tract can directly or indirectly affect the growth, well-being, and survival of hosts, including humans. In this context, the Musahar, highly marginalized ethnic tribes with existing discrepancies, like illiteracy, low socio-economic conditions, and lack of awareness concerning healthy hygienic practices, may be at an increased risk of infectious parasitic diseases. Therefore, the current aim is to determine the diversity and frequency of gastrointestinal parasites among the Musahar residing in the Saptari district in eastern Nepal. Using purposive sampling and non-invasive techniques 200 fresh fecal samples and epidemiological data for microscopic examination and identification of risk factors were collected. The coproscopy was carried out using direct wet mount and concentration techniques. The results showed an 81% (162/200) prevalence and the presence of nine different species of parasites, including *Ancylostoma* sp. (41.5%), *Ascaris lumbricoides* (29%), *Entamoeba* sp. (31.5%), *Entamoeba coli* (21.5%), *Trichuris trichiura* (16%), *Strongyloides stercoralis* (8.5%), *Giardia* sp. (7%), *Hymenolepis nana* (14%), and *Blastocystis* sp. (2%). The prevalence of helminths (67%) was higher than that of single-celled protozoa (42%). Furthermore, parasitism was significantly higher among individuals who consumed game meat, defecated in open spaces, lived in mud homes, and had an unknown history of medication. In conclusion, the Musahar community exhibits a higher prevalence of gastrointestinal parasites. Therefore, implementing community-based health awareness programs concerning the cause, transmission route, effects as well as preventive measures for parasitism is highly recommended. In addition, the One Health study approach is essential to address the root causes of parasite transmission and ways to prevent them.

**Keywords:** *Ancylostoma*; *Blastocystis*; Game meat; *Hymenolepis*; Musahar

## 1 | Introduction

Gastrointestinal (GI) parasites inhabit the digestive tract of hosts, including humans and domestic and wild animals. These organisms have a global distribution; however, they are particularly common in the hosts of developing and underdeveloped countries with higher prevalence rates (Adhikari et al. 2021; Opara 2012). Therefore, owing to induced morbidity and deaths (Haque 2007; Ngui et al. 2011; Torgerson et al. 2015), they represent an important threat to a healthier life and survival (Lanker et al. 2023). According to recent reports from WHO, more than one and a half billion people, representing 24% of the global population, are parasitized with various species of soil-transmitted helminths, and almost 900 million children require immediate treatment (Jourdan et al. 2018; WHO 2023). However, several risk factors influence the likelihood of infection and the burden of these parasites in humans (Adhikari et al. 2021; Tigabu et al. 2019). 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 parasites include protozoa such as *Entamoeba*

*histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, and *Cyclospora cayetanensis*) and helminths (including *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Trichuris trichiura*, *Enterobius vermicularis*, *Strongyloides stercoralis*, *Fasciola hepatica*, *Taenia solium*, and *Schistosoma* spp.).

In Nepal, Musahar, or Mushahar, is a part of the Madheshi Dalit social group and is one of the most marginalized groups in the country (Path & Kathmandu 2014). They mostly reside in the lowlands of Terai in Madhesh Province (3%), Koshi Province (1.4%), and Lumbini Province (0.1%) and make up just 0.9% of Nepal's total population of 234,490 individuals (Gurung 2014). They mainly inhabit the rural areas within the country and represent the most illiterate ethnic communities, with a literacy rate of 21.82% and the lowest aging index of 7.22% (Path & Kathmandu 2014). They are mostly landless (almost 99%) and primarily bonded agricultural laborers, while some remain jobless for up to eight months a year (Poudel & Kattel 2019; Rana 2017; TTID et al. 2022). Interestingly, they practice hunting and consuming rats from agricultural fields to cope with hunger. Eating game meat (rat), also referred to as “Dhanchari” in certain regions, which are typically found in paddy fields, consuming pork, and drinking alcohol are part of their major food culture

and eating habits (Sahay 2019). These circumstances indicate that the Musahar community, in particular, leads a simple lifestyle characterized by poverty, illiteracy, ignorance, traditional feeding practices, a lack of access to clean drinking water sources, simple housing, and open defecation practices (TTID et al. 2022), which might be critical to enhance their susceptibility to GI parasitism resulting in chronic health problems such as malnutrition, anemia, and developmental issues in growing children.

In Nepal, limited studies have been available on GI parasitism among ethnic tribes, including the Musahar tribes. Previously, in the years 2009 and 2021, parasitological surveys were conducted among Musahar people living in the districts of central Terai, like Parsa (n = 54) and Nawalparasi (n = 103) (Khadka et al. 2021; Parajuli et al. 2009). These surveys revealed low prevalence rates of GI parasites, ranging from 28% to 33.3%, and suggested that behavioral practices, like walking barefoot, not using soap for handwashing, drinking untreated drinking water, and inadequate sanitation habits might have favored parasitism in them (Khadka et al. 2021; Parajuli et al. 2009). However, parasitism among the same ethnic tribes living in the tropical Terai plains of eastern Nepal is yet to be assessed and discussed. Feeding habits, socioeconomic conditions, and environmental factors are among the significant causal factors that impact the prevalence, transmission, and severity of parasitic infections in the human population (Adhikari et al. 2021; Tigabu et al. 2019). Understanding these factors only provides the foundation for developing effective, sustainable, and equitable solutions to control parasitic infections. Therefore, the current study aims to determine the prevalence and diversity of gastrointestinal parasites in the Musahar community, known for their unique practice of hunting and consuming rats. Additionally, the study seeks to identify the potential risk factors contributing to parasitism in this ethnic tribes residing in the tropical lowlands of Terai in eastern Nepal.

## 2 | Materials and methods

### 2.1 | Study area

The study was conducted in Balan-Bihul-5, Ramnagar village, located in Balan-Bihul Rural Municipality (26.59°N and 86.51°E) in Saptari district in Province No. 2 (Madhesh Province) of south-eastern Nepal. Its total area is 118.19 km<sup>2</sup>. It is named Balan-Bihul because it lies between the Balan and Bihul rivers. According to the National Population and Housing Census of 2021, a total of 46,581 individuals of the ethnic Musahar people live in the Saptari district, out of which about 600 individuals are the inhabitants of Balan-Bihul Rural Municipality (NPHC 2021). Geographically, the study area lies at 61 to 610 feet. above sea level and is attached to the open India border towards the south. This is why mixed Indian culture and traditions can be experienced. The major ethnicities include Yadav, Sah (Teli), Mandals (Dhanuk), Muslims, Das (Baniya), Mehtas, Musahar, Maithil, Tharu, and Brahmins. The main religions followed are Hinduism and Islam, and both Maithili and Nepali languages are spoken the most (Karki 2019).

### 2.2 | Collection, preservation, and transportation of fecal samples

It is a cross-sectional type of study. We conducted fecal sampling and collection of epidemiological data from ethnic Musahar people above 10 years of age. At the same time, we avoided sampling from children below 10, disabled individuals, and

menstruating females during the study period. For the collection of fecal samples, the current Musahar population in the study sites was classified into three groups based on their age: young and non-working group (10–19) years, adult/working group (20–59) years, and elderly or resting group (60 and above) years. A total of 200 fresh fecal samples, one from each individual, were collected via purposive sampling from nine different locations within the study site. A day before sample collection, all the interested villagers were provided 30 ml sterile vials and a wooden applicator. They were clearly instructed on the appropriate ways and amount of stool collection in the vials. Finally, the next morning, we visited door-to-door to collect the fecal samples and conducted a structured questionnaire survey among the participants to collect epidemiological data for risk assessment. The stool samples thus collected were immediately preserved in 2.5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution and carried to the Zoology Laboratory at the Central Department of Zoology for microscopic examination and parasitological investigation.

### 2.3 | Laboratory processing and identification

The laboratory processing in the current study involved direct wet mount, saturated salt flotation, and sedimentation methods based on the procedures previously explained (Adhikari et al. 2024; Aryal et al. 2022; Adhikari et al. 2020). The identification of parasites was based on their morphology, as explained in the CDC webpage ([www.cdc.gov/parasites/](http://www.cdc.gov/parasites/)).

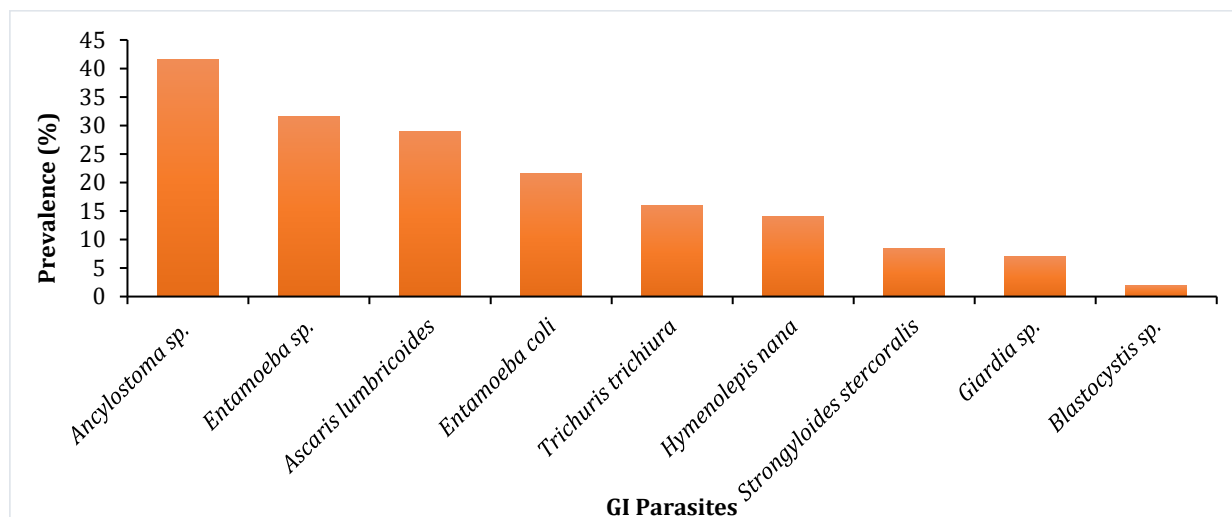
For the direct wet mount, the stool sample preserved at 2.5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> is stirred carefully with a glass rod. A single drop of the sample was sucked up using a plastic dropper and then placed on a clean glass slide with or without an iodine stain for microscopic observation (40×). In the saturated salt flotation technique, a high-specific gravity flotation medium (42% w/v NaCl) was used to float the lighter ova and cyst of GI parasites. Two grams of fecal samples were centrifuged (2000 rpm for 3 minutes) initially with normal saline and then with flotation media. After subsequent centrifugation, the flotation media was further added to fill the centrifuge tube, and a coverslip was placed at the mouth. After 10 minutes, the coverslip was gently removed and observed under the microscope. Similarly, for the sedimentation technique, the sediment obtained after initial centrifugation is smeared with iodine solution on a glass slide and observed under the microscope (40×).

### 2.4 | Data analysis

In the current study, all the parasitological data was encrypted and entered into a Microsoft Excel 2016 spreadsheet. Percentage prevalence was calculated by dividing the individual presence of parasites by the total number of the sampling population and finally multiplied by 100. SPSS software version 16 was used to analyze the data using Pearson's Chi-square ( $\chi^2$ ) test to test the association between the outcome and predictors. Statistically significant differences were considered at  $p < 0.05$ .

## 3 | Results

The copromicroscopic parasitological survey conducted among Musahar community members in Balan-Bihul, Saptari, Nepal, revealed 81% (162/200) prevalence rate of GI parasites. Additionally, a total of nine different species of parasites were reported. This includes protozoa such as *Entamoeba* sp., *Entamoeba coli*, *Giardia* sp., and *Blastocystis* sp. and helminths like *Ancylostoma* sp., *Ascaris lumbricoides*, *Hymenolepis nana*,



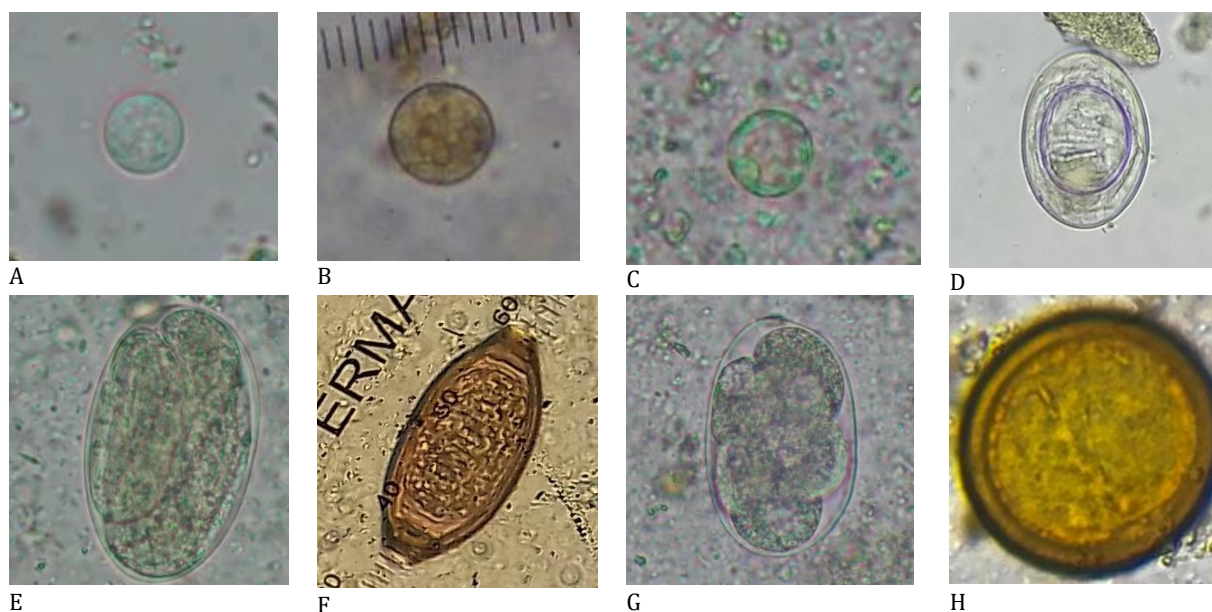
**Figure 1.** Overall GI parasitic prevalence in Musahar community people.

*Strongyloides stercoralis*, and *Trichuris trichiura* (Fig. 1). The prevalence of *Ancylostoma* sp. (41.5%) was the highest, followed by *Entamoeba* sp. (31.5%), and *Blastocystis* sp. (2%) was the least common parasite (Fig. 2). Similarly, the prevalence of single-celled protozoa (38%) was much lower than that of multi-cellular helminths (67%), showing that the difference is statistically significant ( $p < 0.05$ ).

Additionally, GI parasitism by multiple intestinal parasites has been reported to be more common than parasitism by a single species, suggesting a higher prevalence of polyparasitism. Notably, reports of co-infection with up to four distinct parasite species in a single individual have been made. In this case, co-infection with two species of parasites resulted in the highest proportion of infection (43%), followed by three parasitic species (17.5%) and one parasitic species (16.5%). However, co-infection with four parasitic species was the least common (4%). These results demonstrate that the pattern of parasite infection among the Musahar people differs statistically significantly ( $p < 0.05$ ).

Based on age, the current population of Musahar was categorized into three groups: the young/non-working group (10–19 years old), the adult/working group (20–59 years old), and the old/retired group (60 years and above). It is noteworthy that the old/retired population had the highest rate of parasitic prevalence (93.2%), followed by the young/non-working population (85.7%), and the working population (73%) had the lowest rate. These findings indicate a significant difference in the prevalence of GI parasites ( $p < 0.05$ ) (Table 1). Similarly, 85% of females had GI parasitism, which was substantially greater than 75.6% of males ( $p < 0.05$ ), suggesting older or younger participants compared to middle-aged and female participants compared to male participants were more likely to get GI infection.

Likewise, other possible risk predictors like feeding habits, house type, walking practices, defecation practices, and medication or deworming schedules have also been assessed. It revealed that participants who consumed game animals lived in mud-built houses, walked barefoot all the time, reported open defecation, and had an unclear history of deworming or more



**Figure 2.** Microscopic images of cysts and eggs of reported GI parasites.

than a year earlier history of deworming were more likely to get GI infection than their counterparts (Table 1).

#### 4 | Discussion

The current study indicates the prevalence of GI parasites in Musahar communities in Nepal. As far as we know, this is the first attempt to investigate the presence and divergence of GI parasites among Musahar communities in the eastern Terai region of Nepal. Therefore, it can serve as a model for future investigations on parasitic diseases affecting indigenous communities within the country and in other areas. The findings of the current study revealed an overall 81% prevalence rate of GI parasites. This rate is notably higher than those reported in previous studies among the same ethnic groups in the central Terai regions of Nepal, where the prevalence rate ranged from 28% to 33.3% in the Parsa (Parajuli et al. 2009) and Nawalparasi district (Khadka et al. 2021). When comparing the current findings to those from other ethnic communities globally, the 81% prevalence rate is lower than reports from India, which indicated a rate of 97.4% (Kang et al. 1998), and Nepal, with a rate of 97% (Adhikari et al. 2021). However, it is higher than earlier findings from Nepal (28%–31.32%) (Chaudhary & Subedi 2020; Thapa et al. 2021) and from other countries, such as India (75.8%) (Dhanabal et al. 2014), and Iran (56%) (Pestehchian 2015). The dissimilarity in the prevalence rate of GI parasites in these studies among different ethnic groups might be attributed to the inconsistency in sample size, variable sampling geographies, methodological contrasts, as well as the differences in socioeconomic, environmental, and behavioral factors. In the current case, sampling has been carried out from the tropical lowlands of Terai, and these people have deficient socio-economic status and discrepancies, like poverty, ignorance, and illiteracy. Notably, all three standard laboratory techniques for parasitic examination have been employed in each stool sample, which might have ensured higher positive cases and higher prevalence of the GI parasites in the current study.

Here, the prevalence of multicellular helminth parasites (cestodes and nematodes) was higher than that of single-cellular protozoans. Similar results have been observed in previous studies from Nepal (Dhakal & Subedi 2019; Rai et al. 2004). However, some similar studies from Nepal (Adhikari et al. 2021;

Baral et al. 2017), and Iran (Pestehchian 2015), have contrasting results. Despite the favorable factors for protozoan parasites, like poor sanitary habits, lack of access to safe water, and improper hygiene (Amer et al. 2016), exist in the study population (personal observation), the predominance of helminth parasites, particularly soil-transmitted helminths like *Ancylostoma* sp., *Ascaris lumbricoides*, and *Trichuris trichiura*, has been observed. In this case, the presence of sub-tropical to tropical climates in the study area may have ruled for a greater prevalence of STHs, in line with the assumptions made by other studies based on tropical locations (Adhikari et al. 2023; Steinbaum et al. 2016). This is because optimal temperatures favor the rapid embryonation of nematode eggs, such as *Ascaris* and *Trichuris*. Additionally, these conditions also facilitate the hatching of the eggs and the transformation of rhabditiform larvae into infectious filariform larvae, particularly in the case of *Ancylostoma* sp. and *Strongyloides* sp. (Amadi & Uttah 2010). However, STH infection and its greater incidence rates are also caused by several other variables. This encompasses behavioral, social, biological, and other environmental elements such as poverty, poor housing, and inadequate personal hygiene and sanitation at the individual and communal levels (Kattula et al. 2014). For instance, walking barefoot increases the risk of contracting *Ancylostoma* sp. (Chopra et al. 2023).

Here, the ethnic people were infected with nine different species of GI parasites. Previously, GI parasites like *E. histolytica*, *E. coli*, *Ancylostoma* sp., and *Ascaris lumbricoides* had been reported in them in Nepal (Khadka et al. 2021; Parajuli et al. 2009). However, the presence of protozoa, like *Blastocystis* sp., and helminths, like *Hymenolepis nana*, had been additionally reported in the present study. Considering *Blastocystis* sp., it is the only stramenopile well-known to cause infection in humans associated with irritable bowel syndrome and cutaneous lesions (Wawrzyniak et al. 2013). Meanwhile, the fecal-oral route is the primary way of transmission (Boutahar et al. 2023); poor hygienic practices, close contact with infected animals, and intake of contaminated food or drinks contributed to the possible risk factors (Li et al. 2007; Nagel et al. 2012). On the other hand, *Hymenolepis nana* mainly infects children belonging to rural communities, owing to the lack of sanitation and safe water, as well as crowded housing conditions and contact with rodents (Coello Peralta et al. 2023; Thompson 2015). The associated clinical manifestations of hymenolepiasis include headache, weight loss, anorexia, reduced growth, rashes, anal

**Table 1.** Assessment of potential risk factors of parasitism in Musahar

SN	Potential Risk	Categories	Total individual	Prevalence (%)	X <sup>2</sup> (p-value)
1	Age	10-19 years	56	48 (85.7)	26.078 (p=0.001)
		20-59 years	100	73 (73)	
		60 and above	44	41 (93.2)	
2	Sex	Male	86	65 (75.6)	5.84 (p=0.02)
		Female	114	97 (85.1)	
3	Feeding habit	Consumers (game meat)	72	66 (91.7)	15.68 (p=0.001)
		Non-consumers (game meat)	128	96 (75)	
4.	House Type	Cemented	34	22 (64)	87.12 (p=0.001)
		Mud house/hut	166	140 (84.3)	
5	Walking practice	Completely barefooters	78	72 (92.3)	3.96 (p =0.20)
		Occasionally wears shoes/sandals	55	48 (87.3)	
		Always wear shoes/sandals	67	42 (62)	
6	Defecation practice	Open defecation	154	136 (88.3)	58.32 (p=0.001)
		Toilet users	46	26 (56.5)	
7.	Medication/ deworming	≤6 months	52	38 (73.1)	35.64 (p=0.001)
		≤12 months	106	87 (82.1)	
		>12 months/unknown history	42	37 (88.1)	



and nasal itching, abdominal pain, nausea, flatulence, bloating, and diarrhea (Coello Peralta et al. 2023; Shahnazi et al. 2019).

Furthermore, the risk factor assessment revealed that GI parasitism among the Musahar community significantly varies with several factors, like age, sex, diet, house type, and deworming practices, including their habit of using slippers and latrines. It was observed that respondents preferring to consume game meat (rat), living in mud-built houses, waking barefoot, defecating in open spaces, and having an unknown or more than one year earlier history of deworming have significantly higher rates of parasitic prevalence than others. This indicates that the above-mentioned behavioral practices adopted by the Musahar community make them prone to GI parasitism. For instance, Musahar, preferring barefoot or occasionally wearing shoes and sandals, may quickly contact infective filariform larvae, particularly in areas with poor sanitation, open defecation, and inadequate waste disposal systems. Remarkably, Musahar people, who have a unique practice of eating game animals, reported a high prevalence of GI parasites. Notably, the parasite *Hymenolepis nana* is particularly common among them. While close contact with rodents and consumption of feed contaminated with their feces have been associated with hymenolepiasis (Coello Peralta et al. 2023), we speculated that their practice of consuming game meat (rats) could also contribute to the risk of meat-borne zoonosis. However, further epidemiological and molecular evidence is needed to confirm this hypothesis.

In this study, the female population has a higher parasitic prevalence than males (85.1% versus 75.6%). This result is on par with the previous findings from other ethnic communities in Nepal (Dhakal & Subedi 2019; Khadka et al. 2021). However, contrasting findings have also been reported among the Chepang population in central Nepal (Adhikari et al. 2021). A higher prevalence of GI parasites in females in the current study might have been possible because of the occupational exposure of females to potential risk factors. This includes household activities, child care, agriculture, and animal husbandry, while males prefer the rest of the outdoor work. Likewise, elderly and retired people aged 60 and older were found to be more highly infected with parasites (93.2%) than the young and adult/working population. Generally, it is speculated that immunity levels decrease with age (DeVeale et al. 2004), which is why the old Musahar becomes highly susceptible to GI infection. Moreover, in our observation and experience of working with the Musahar population during the study period, this age group showed greater negligence in attaining healthy behaviors and preferred unhealthy behaviors, like walking barefoot, defecating in open spaces, wearing untidy dresses, and seldom washing their hands and legs after work.

Additionally, Musahar living in mud-built houses (huts) have a significantly higher prevalence rate of parasites than those residing in cemented houses. A similar association was also obtained among the ethnic Chepang community living in central Nepal (Adhikari et al. 2021). Since mud houses retain greater moisture than cemented houses, a more favorable environment is maintained for GI parasites thriving in damp conditions. Moreover, mud houses provide niches for pests, such as rodents and vectors, like houseflies, that can carry parasitic ova and cystic stages (Issa 2019; Morand et al. 2015) and thus increase the risk of parasitic transmission. Moreover, living in mud-built houses in crowded conditions also favors the transmission of parasites that can quickly transfer person-to-person, such as *Hymenolepis nana*, *Entamoeba* spp., *Giardia* sp., and *Blastocystis* sp. In this circumstance, the predominance of such parasites in

the current Musahar population suggested a positive relationship between housing type and parasitism.

Similarly, consistent with the findings of earlier studies, among schoolchildren in the same district (Gupta et al. 2020), we reported a significantly higher prevalence of GI parasites among open-defecating Musahar than those using latrines. Open defecation generally favors completing the life cycle of GI parasites by contaminating soil and water sources with parasitic eggs, cysts, or larvae. However, latrines act as a barrier and cut off the life cycle of many parasites by reducing fecal contamination with soil and water. Moreover, several interconnected factors related to environmental contamination, hygiene, and exposure to parasitic life cycles, open defecators are more likely to have higher rates of parasitic infections compared to latrine users.

Interestingly, a noticeably higher number of individuals were positive for GI parasites compared to individuals with no known history of deworming or more than a year earlier history of deworming. This indicates that despite the deworming policies adopted by the government of Nepal, either the programs do not reach every marginalized person or these people show ignorance in taking the medicine due to a lack of health knowledge. Regular and frequent deworming can reduce the rate of parasitic infection by effectively killing adult worms and breaking their life cycle patterns; it also enhances immune function, allowing the body to resist future infections (Hamory et al. 2021; Welch et al. 2017). On the other hand, individuals with no deworming history remain at a higher risk of persistent infections and related health issues because parasites continue to reproduce inside the host and contribute to persistent infection and environmental contamination, perpetuating the reinfection cycle. Therefore, it is essential to prioritize integrating deworming programs into public health policies. These programs need to reach marginalized communities like the Musahar, and health awareness needs to be generated so that they take anti-helminthic drugs and maintain positive health.

Furthermore, we observed that most of the respondents (nearly 65%) in the current study showed a concomitant pattern of GI infection. This specifies the existence of dominant polyparasitism rather than monoparasitism in the current Musahar population. Despite concomitant infection being a natural phenomenon, it may contribute to the synergistic, antagonistic, as well as neutral interaction among the cohabitant parasites within a host (Hoarau et al. 2020). However, in most human and animal studies, positive interactions leading to greater virulence and infection severity caused by the co-infecting parasites have been observed (Adhikari & Ghimire 2021; Ezeamama et al. 2008; Njunda et al. 2015). Thus, it indicates that the current Musahar community with dominant polyparasitism may have impaired GI health. However, further histopathological studies must be conducted to understand the effect of polyparasitism by GI parasites on a human host.

This study is subjected to a few limitations. First are the methodological constraints of microscopy, which may have lower sensitivity than molecular detection and lead to an underestimation of the prevalence rate. Secondly, the possible effect is the selection of participants on a first-come-first basis, and there may be a chance that individuals in remote or underserved areas may likely be unassessed. Similarly, risk factors assessment, such as hygiene practices, housing, diet, and medicine intake, often depend on self-reported data, which can be prone to inaccuracies. However, we have strictly secured quality control during field surveys and laboratory techniques throughout the study period. We believe this copromicroscopic

examination addressed the unique GI parasites in ethnic Musahar people from eastern Nepal.

## 5 | Conclusions

The current study concluded that the Musahar community in Balan-Bihul, Saptari, harbored an alarmingly higher prevalence of GI parasites, which included nine diverse species, marking the first detection of *Blastocystis* sp. and *Hymenolepis nana* among these ethnic tribes in Nepal. Helminthic infection is more common than protozoa, and the prevalence of parasitism varies according to age, sex, and adopted behavioral practices, like walking barefoot, defecating in open areas, living in mud-built houses, and having an unknown history of medication and consuming game meat remains the significant risk for parasitism. In this circumstance, community-based health awareness and the One Health research concept must be conducted to develop a holistic understanding of socioeconomic, cultural, and ecological risk factors associated with parasitism, especially targeting the marginalized population.

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## Authors' contributions

B.P.Y., R.B.A., and J.R.S. designed the research. BPY collected data and did field and laboratory work. B.P.Y. and R.B.A. analyzed the data and wrote the manuscript. J.R.S. supervised the work. All authors read and gave final approval for publication.

## Ethical consideration

The Balan-Bihul Rural Municipality, Ward Number 05 (Permission Number 127/080/081), issued the required permission for field survey and fecal sample collection.

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## Consent

No people are identifiable with this publication.

## Conflicts of interest

The authors declare no conflict of interest.

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