Intestinal Parasitoses and Associated Risk Factors among Indigenous Community of Bhumikasthan Municipality, Arghakhanchi, Nepal

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

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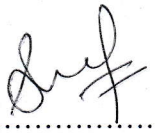
Declaration

I hereby declare that the work presented in this dissertation "Intestinal Parasitoses and associated risk factors among Indigenous community of Bhumikasthan Municipality, Arghakhanchi, Nepal" has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).



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Recommendation

This is to recommend that the dissertation entitled "Intestinal Parasitoses and associated risk factors among Indigenous community of Bhumikasthan Municipality, Arghakhanchi, Nepal" has been carried out by Sandhya Gautam for the partial fulfilment of master's degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this dissertation work has not been submitted for any other degree in any institutions.

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Letter of approval

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Certificate of acceptance

This dissertation work submitted by Sandhya Gautam entitled "Intestinal Parasitoses and associated risk factors among Indigenous community of Bhumikasthan Municipality, Arghakhanchi, Nepal" has been accepted as a partial fulfilment for the requirements of master's degree of Science in Zoology with special paper Parasitology.

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Abstract

Intestinal parasites affect individuals, especially different ethnic community across the globe and contributing health challenges. Present research aims to assess the prevalence of intestinal parasites conducted in Bhumikasthan Municipality, Arghakhanchi, Nepal, with nutritional, socioeconomic, sociodemographic, and behavioral factors. Total 200 fresh stool samples were collected along with structured questionnaires, utilizing a purposive sampling technique from individuals aged 18 and above. Stool samples were precisely preserved in a 2.5% potassium dichromate solution. The three different diagnostic techniques such as the direct wet mount, saturated salt floatation, and formal ether sedimentation techniques were employed. The study indicated that (n=44, 22%) of overall parasitic infection in the indigenous community was observed. The helminthic infection (n=32,16%) was larger in the studied community than protozoan infection with (n=12, 6%) ($p< 0.05$). As *Ascaris lumbricoides* showed infection in (n=29, 14.5%) being the predominance parasitic infection in community followed by *Giardia lamblia* (n=8, 4.5%), *Entamoeba histolytica* (n=4, 2%), and *Trichuris trichiura* (n=3, 1%). Female population were more exposed with (n=34, 23.8%) to the parasites due to their nature of work than males (n=10, 17.4%). Older aged people within age range of 60-80 years were observed with (n=18, 29.5%). The significant risk factors, includes lack of handwashing practices, consumption of unwashed fruits and vegetables, dietary habit and observation of worms in the stools made bad things worst. Therefore, it was clear that people were unaware of epidemiology of helminths and consequence of them that leads to intestinal parasitic infection in community.

शोध सारांश

यो अध्ययनमा भुमिकास्थान नगरपालिका, अर्घाखाँची नेपालमा परजीवी प्रसार र पोषणीय, अर्थसामाजिक, जानसंख्यिकिय, व्यवहारीक कारणको सम्बन्ध मुल्याङ्कन गर्ने प्रयास गरीएको छ। १८-८८ बर्षका व्यक्तिहरूबाट संरचनात्मक प्रश्नावली सहित कूल २०० दिसाका नमुना उचित प्रविधि प्रयोग गरेर संकलन गरीएको थियो । उक्त नमुनाहरुलाई २.५% पोटासियम डाइक्रोमेट घोलमा ध्यानपुर्वक सुरक्षित गरेर, विभिन्न निदानकारी प्रक्रियाहरू जस्तैः प्रत्यक्ष आर्द्र स्थापन पद्धति, सन्तृप्त लवण प्रवर्तन पद्धति र फर्मल ईथर अवसाधन पद्धति प्रयोग गरेर देखाइएको थियो । यस अनुसन्धानले उल्लेखनिय ४४ (२२%) आन्द्राको परजीवी संक्रमण देखाएको छ जसमा १२ (६%) प्रोटोजोवन र ३२ (१६%) हेल्मिथ देखिन्छन् जहाँ तथ्याङ्किय रुपमा हेल्मिथको संक्रमण प्रोटोजोवा भन्दा उच्च रहेको पाइएको छ । यस अध्ययनमा मुख्यतः अस्कारीस २९ (१४.५%) प्रमुख, दोस्रोमा गीयार्डिया ८ (४.५%), तेस्रोमा इन्टामोबिया ४ (२%) र चौथोमा ट्रिचुरीस ३ (१.५%) प्रजातीको बाहुल्यता देखिएको छ । तथापी लिङ्गले संक्रमणमा कुनै सम्बन्ध नदेखाइएको पाइयो । विभिन्न जनसाङ्खिकिय कारक र स्वास्थ्य अभ्यासहरू, जस्तै जनजातीय अभ्यास, माटोको घरमा आवास, धाराको पानी सिधै उपयोग गर्ने तथा स्वच्छता कायम नगर्ने, ले परजीवी संक्रमणमा महत्वपुर्ण प्रभाव पारेको देखिन्छन् । यसबाहेक, बहुविभिन्न विश्लेषणले विभिन्न व्यवहारका अभ्यास, जस्तै हात धुने, फलफूल र तरकारी धोएर मात्रै खाने, विभिन्न आहार बानीहरू र संक्रमणको गतिशीलतामा सम्बन्ध देखाएको छ । यस अध्ययनको निष्कर्षले परजिविक संक्रमणमा व्यवहारीक कारण र आहारबानीले अर्थसामाजिक, सामाजिक पक्षभन्दा धेरै प्रभावशाली भुमिका खेल्छन् र यसको नियन्त्रण विभिन्न निति नियमको आवश्यकतालाई जोड दिन्छ भन्ने देखाएको छ ।

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List of abbreviations

Abbreviated form	Details of abbreviations
SES	Socioeconomic status
IPIs	Intestinal parasitic infections
WHO	World Health Organization
IPs	Intestinal parasites
ELISA	Enzyme-linked immunoassay
IRC	Institutional review committee
IOST	Institute of Science and Technology
BMI	Body mass index
i.e.	That is
ºC	Degree Celsius
%	Percentage
Fig.	Figure
No.	Number
CDZ	Central Department of Zoology
TU	Tribhuvan University

1. Introduction

1.1 Background

Inhabitants of underdeveloped nations face a myriad of common diseases, with Intestinal parasitic infections standing out as a prevalent ailment caused by protozoans and helminths. Notably, developed nations tend to experience a higher prevalence of protozoan parasites compared to helminths, unlike endemic nations where intestinal parasites contribute significantly to diseases and mortality (Haque, 2007). The World Health Organization estimates that the prevalence of soil-transmitted helminthic infections is highest in tropical and subtropical regions, including sub-Saharan Africa, the Americas, China, and East Asia. Global estimates indicate that these infections impact about 24.0% of the world's population (WHO, 2020). Intestinal parasitic infection plays a pivotal role among infectious disorders impacting global health (Agrawal et al., 2012). Specifically, in underdeveloped nations, the overall frequency of parasitic infections is higher, while in industrialized nations, protozoans are more pronounced than intestinal helminths (Saud, 2017).

For many generations, indigenous groups in Asia have been self-sufficient and self-governing, with their own set of beliefs and customs that influence their way of life. (Magar, 2009). 35.81% of Nepal's population are indigenous people (CBS, 2021). The present study focuses on the indigenous communities of Magar, Mijar, Pariyar, and Bishwokarma, which account for 2013498 (6.9%) of Nepal's total population, 452229 (1.55%), 565932 (1.94%), and 1470010 (5.04%) respectively (CBS, 2021). The communities of Magar, Mijar, Pariyar, and Bishwokarma were known as Magar, Sarki, Damai, and Kami, respectively, prior to the census of 2078. (NSO, 2022). These individuals are susceptible to paratitosis because of their lifestyle, line of work, reliance on the natural world, and restricted access to medical care (Kunwar, 2013). In South Asia, exemplified by Nepal, intestinal parasitic infections are widespread among youngsters along with distinct ethnic community, impacting their health and development. These infections contribute significantly to nationwide public health issues, for instane, in the Chepang indigenous community of Nepal exhibited with a notable parasites including *Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis, Trichostrongylus, Entamoeba coli, Entamoeba histolytica, Giardia lamblia, Iodamoeba buetschlii, Cyclospora cayetanensis, Cryptosporidium species, Blastocystis hominis, and Balantidium coli* (Adhikari et al.,

2021). Factors such as inadequate drinking water, sanitation, hygiene practices, and gastrointestinal complaints, particularly in children, contribute directly to infections (Tiwari et al., 2019). Those with frequent symptoms often have parasitic infections, especially among Dalit communities, attributed to a lack of knowledge, non-use of anti-parasitic medication, lack of handwashing after toilet use, and contaminated drinking water (Yadav & Prakash, 2016). Moreover, socio-demographic factors such as gender, age, family size, and ethnicity, as well as practices like barefoot walking and inadequate hand hygiene, are identified as risk factors for parasitic infections in various community populations (Shrestha et al., 2012; Sah et al., 2013; Yadav & Prakash, 2016; Khadka et al., 2021). Intestinal parasitosis in adolescent's manifests as abdominal pain, anorexia, nausea, absenteeism, and weakened academic performance (Pandey et al., 2015). Additionally, low hemoglobin levels and body mass index (BMI) in adults are associated with IPs Infection (Wiria et al., 2013). These factors are found to be significantly associated risk factor with the intestinal parasitic infection in different studies within an indigenous group of people.

1.2 Statement of problem

The indigenous community in Bhumikasthan Municipality, ward number 08, Arghakhanchi, Nepal, through visiting a local health post, observed frequent patient with abdominal symptoms, which indicated potential linked to parasitic infections. Notably, factors such as poor sanitation, cultural practices, and existing poverty contribute to the prevalence of intestinal parasitic infection (IPIs) in this population. Despite numerous studies in different ethnic communities, the indigenous communities in this study site exhibit unique challenges, including walking barefoot in fields and cohabitating with free-ranging animals. This study aims to assess the prevalence and risk factors of dominant IPIs in this community, examining the association in between prevalence and with key risk factors such as dietary habits, drinking water quality, and handwashing practices.

1.3 Objectives

1.3.1 General objective

- To assess the prevalence and identify risk factors for Intestinal parasitic infections among the indigenous community of Bhumikasthan Municipality, Arghakhanchi, Nepal.

1.3.2 Specific objectives

- To determine the prevalence of Intestinal parasitic infections among indigenous communities in Bhumikasthan Municipality, ward number 08, Arghakhanchi, Nepal.
- To identify the risk factors associated with the prevalence of Intestinal parasitic infection.

1.4 Research question

What is the prevalence of intestinal parasitic infections (IPIs) among residents of Arghakhanchi, Nepal, and how are these infections associated with common risk indicators?

1.5 Significance of the study

Numerous studies have been conducted globally within indigenous communities. As, IPs infection has been notably linked to a range of enteric diseases and mortality in developing nations (Haque, 2007). This study evaluates parasite prevalence and risk factors in the indigenous community of Arghakhanchi district, ward 08. Notably, it is unique as no prior research has been conducted on ward 08 of Arghakhanchi district on the prevalence and risk factors of IPIs. This research gains further strength from examining the association between IPIs prevalence and key risk factors, such as dietary habits, drinking water quality, handwashing practices, and the observation of worms in stool. This straightforward approach investigate depth to the study. The findings are expected to aid in understanding population-specific parasites and their associated variables, enabling the development of strategies, plans, and policies to minimize risks.

1.6 Limitations of the study

The study's limitation stems from a small sample size (n=200) and its cross-sectional design, attributed to time constraints as a master’s degree student.

2. Literature review

2.1 Prevalence of Intestinal parasitic infection

Several investigations have been carried out in Southeast Asia, with a focus on parasitic infections within Indigenous Communities of different region of Malaysia. One particular study examined *Giardia* infection within these communities, involving 1330 participants across seven states. The findings revealed an infection rate of 11.6% with *Giardia* spp., notably higher among 12-year-olds. Diagnostic methods such as wet mount and formalin-ether sedimentation were utilized alongside a questionnaire to assess socio-economic status in rural Malaysia (Choy et al., 2014). Another study focusing on orang asli subgroups in Peninsular Malaysia found a 59.9% prevalence of *Trichuris trichiura*, with 95% of cases demonstrating co-infections with other parasites (Ngui et al., 2015).

In Selangor state, Malaysia, a study focused on the Temuan and Mah Meri indigenous groups, involving 186 participants, revealing the prevalence of various parasitic infections within these communities. Trichuriasis was the most prevalent among the Temuan community at 64.2%, followed by hookworm infection (34%), ascariasis (7.5%), amoebiasis (7.5%), and giardiasis (14.2%). And within the Mah Meri community, trichuriasis exhibited a higher prevalence at 77.5%, preceding ascariasis (21.3%), hookworm (15%), giardiasis (7.5%), and amoebiasis (3.8%). Co-infections were observed, with *Trichuris trichiura* and *Ascaris lumbricoides* being common among the Mah Meri community, while co-infection of *Trichuris trichiura* with hookworm prevailed in the Temuan community. (Chin et al., 2016). In Sarawak, Malaysia, among Indigenous Communities residing in longhouse communities, a study revealed an IPI prevalence of 57.5% among 341 individuals, highlighting the substantial burden of IPIs in these communities and emphasizing the necessity for ongoing monitoring and intervention efforts to address and manage these infections effectively (Rajoo et al., 2017). All four different studies in Malaysia emphasize the significance of comprehensively understanding parasitic prevalence and co-infection dynamics within indigenous communities, providing valuable insights into the parasitic landscape and highlighting age-specific vulnerabilities

In Hanoi, Vietnam, where wastewater is used for irrigation, revealing a significant prevalence of intestinal parasites (IPs) affecting 30% of individuals, with Hookworm at 25% and *Trichuris trichiura* at 5% being the predominant species. The study employed both the Kato-Katz technique and formalin-ether sedimentation for parasite detection,

highlighting the health risks associated with wastewater irrigation and emphasizing the need for comprehensive parasite detection methods, a study on a peri-urban population near the Red River (Fuhrimann et al., 2016). The other study conducted in the Kancheepuram district of Tamil Nadu, comparing urban and rural populations, finding distinct patterns with urban prevalence at 23.4% and rural at 50.8%, with *Entamoebia histolytica* and *Ascaris lumbricoides* being most prevalent. The study stressed the importance of efficient diagnostic methods for managing IPs infection (Mareeswaran et al., 2018). Also a study which focused on Blastocystis Subtype 1 in Eastern Communities of Thailand, revealing a 15.7% prevalence and 17 subtypes observed, employing various diagnostic methods like wet mount and formalin-ethyl acetate concentration, emphasizing the need for diverse diagnostic approaches for understanding intestinal parasitic infections comprehensively (Ruang-areerate et al., 2021).

In middle east asia, in marginalized rural communities spanning three villages in the Jordan Valley, Palestine, revealing a prevalence of parasitic infections at 48% (49/102). Predominantly, *Giardia lamblia* was identified as the leading species at 37% (37/102), followed by *Hymenolepis nana* at 9% (9/102). The research employed various diagnostic techniques such as wet fecal samples, ethyl acetate sedimentation, zinc sulfate flotation, conventional PCR, and real-time PCR (qPCR), emphasizing the importance of employing multiple methods for accurate parasite detection. This study not only underscored the significant burden of intestinal parasitic infections (IPIs) in marginalized rural communities but also highlighted the necessity for a comprehensive approach in understanding the parasitic landscape for effective intervention and management (Al-Jawabreh et al., 2019).

In the Africa, two different community reported where in Ebonyi State, Nigeria, conducted a study providing insights into the prevalence of ascariasis and other helminthic infections in a rural community, revealing a significant occurrence of ascariasis affecting 76.2% of the population examined (n=162). Hookworm infections were the most prevalent helminth observed, accounting for 25.5% (n=54), followed by *Trichuris trichiura* at 4.2% (n=9), and *Strongyloides stercoralis* at 3.3% (n=7), detected through the Kato-Katz method. The findings underscore the substantial burden of parasitic infections in the community, emphasizing the necessity for targeted interventions and public health initiatives to address the prevalence of helminthic diseases in rural areas of Ebonyi State, Nigeria (Caroline Okeke & Obiageli Ubachukwu, 2015). In South Ethiopia, focused on asymptomatic food handlers in meal serving facilities, revealing a significant prevalence of intestinal parasites

(IPs) affecting 41% of individuals, with *Ascaris lumbricoides* being the most predominant, followed by *Taenia* spp. and Hookworm. The study highlights the importance of regular screening and monitoring of food handlers for IPs to ensure food safety and public health in the region (Solomon et al., 2018).

In case of Nepal, rural hilly districts of Dhading, Sindhupalchok, and Ramechhap in Nepal, conducted an eye camp study among individuals undergoing cataract surgery, involving 221 participants, revealing a significant 66.9% of intestinal parasitic infection testing positive for ocular conditions. Ramechhap and Sindhupalchok showed higher positive rates (71.8% and 70.7%, respectively) compared to Dhading (60.0%). No statistically significant difference was observed. The Tibeto-Burman ethnic group exhibited the highest positive rate (70.1%) (Rai et al., 2008). In Chitwan district, a study among the Indigenous Kumal community was conducted, revealing a substantial burden of helminth infections, particularly hookworm, with a prevalence of 30.87%. The overall infection rate in the village was reported to be 54.0% (Gyawali, 2013). Also in Gaindakot VDC, Nepal, found a high prevalence of intestinal parasites (IPs) among Indigenous Community people aged 10-60 years, with approximately 54% showing helminth infections. They employed the Magnesium floatation method for diagnosis, highlighting its utility and underscoring the substantial burden of GI parasites within Indigenous Communities (Gyawali et al., 2013). Prevalence of intestinal parasites (IPs) was identified among children in the squatter community in Dharan Municipality, Nepal, with a prevalence rate of 41.4%. Males had a higher infection rate (45.8%) than females (37.5%), and children aged 4-8 years were most affected (48.1%). *Ascaris lumbricoides* (18.1%) and *Giardia lamblia* (74.02%) were the most common parasites, highlighting the urgent need for targeted interventions in this community, especially among young children (Chongbang et al., 2016).

In a recent study conducted in central Nepal, focusing on the Chepang Community, revealed a significant prevalence of intestinal parasites (IPs). The research, encompassing 100 individuals, found an alarming 97% affected by IPs, with protozoans and helminths comprising 8 and 6 different species, respectively. The investigation utilized diverse techniques for parasite detection, emphasizing the need for comprehensive diagnostic methods (Adhikari et al., 2021). In Makwanpur and Nawalparasi districts, examining Chepang and Musahar communities. With a sample size of 205 individuals, the overall prevalence of parasitic infections was 36.6%, slightly higher among Chepangs at 39.8% compared to Musahars at 33.3%. The research highlighted *Ascaris lumbricoides* as the

predominant helminth and *Entamoeba histolytica/dispar* among protozoans (Khadka et al., 2021). Lastly, investigated the Sarki ethnic group in Pala Rural Municipality, Baglung, examining 498 samples. They found an overall prevalence rate of 31.32%, with *Trichuris trichura* and *Ascaris lumbricoides* as the most prevalent helminths, and *Entamoeba coli* as the predominant protozoan. These studies underscore the importance of tailored public health interventions to address the prevalence and diversity of parasitic infections within indigenous communities in Nepal, highlighting the necessity of employing multiple diagnostic techniques for comprehensive understanding and effective intervention (Thapa et al., 2021).

2.2 Risks factors associated with Intestinal parasitic infection

Intestinal parasitic infection poses significant challenges in human life and are associated with various risk factors identified in several studies. Poverty has been identified as a risk factor for Intestinal parasitic infection in studies conducted by (Ngui et al., 2015; Chin et al., 2016). Personal sanitary behavior, including factors such as inadequate sanitation practices, has been implicated in the transmission of parasites in studies by (Choy et al., 2014; Caroline Okeke & Obiageli Ubachukwu, 2015; Ngui et al., 2015; Chongbang et al., 2016; Fuhrimann et al., 2016; Khadka et al., 2021; Ruang-areerate et al., 2021).

Sleeping in rooms with more than six persons has been identified as a risk factor in studies by (Caroline Okeke & Obiageli Ubachukwu, 2015; Chin et al., 2016; Ruang-areerate et al., 2021). Additionally, factors such as being a farmer and the age of children below 10 have been associated with increased risk in the study by (Caroline Okeke & Obiageli Ubachukwu, 2015). Consumption of specific non-vegetarian foods, such as bat meat, has been linked to parasite infections in the study by (Adhikari et al, 2021), while the source of drinking water has been identified as a risk factor in the study by (Khadka et al., 2021). Vegetarianism has been associated with lower risk in the study by (Rai et al., 2008). As non-vegetarian diet practice heightened risk, is attributed might be due to certain parasites thriving in animal tissues, and insufficient cooking or processing increases the likelihood of transmission to humans (Dardona et al., 2023).

Elder age, particularly above 40 years, has been identified as a risk factor in studies by (Gyawali, 2013; Al-Jawabreh et al., 2019). Specific occupational factors, such as being a laborer, having insufficient water sources, and living in mudded houses, were noted as risk factors in the study by (Gyawali et al., 2013). Female gender associated with children was

identified as a risk factor in the study by (Mareeswaran et al., 2018). Lack of proper sanitation facilities, association with animals, river bathing, and barefoot walking were associated with increased risk in studies by (Gyawali, 2013; Choy et al., 2014; Rajoo et al., 2017).

Factors such as not undergoing deworming in the past 12 months were identified as risk factors in the study by (Fuhrimann et al., 2016). Practices such as eating raw meat and inadequate handwashing after using the toilet or touching dirty materials were identified as risk factors in the study by (Solomon et al., 2018). Open pig farms were associated with increased risk in the study by (Ruang-areerate et al., 2021), while the lack of safe drinking water was noted as a risk factor in studies by (Choy et al., 2014; Chin et al., 2016). These findings collectively underscore the multifactorial nature of intestinal parasite infections and the importance of addressing various risk factors for effective prevention and control.

3. Materials and methods

3.1 Study area

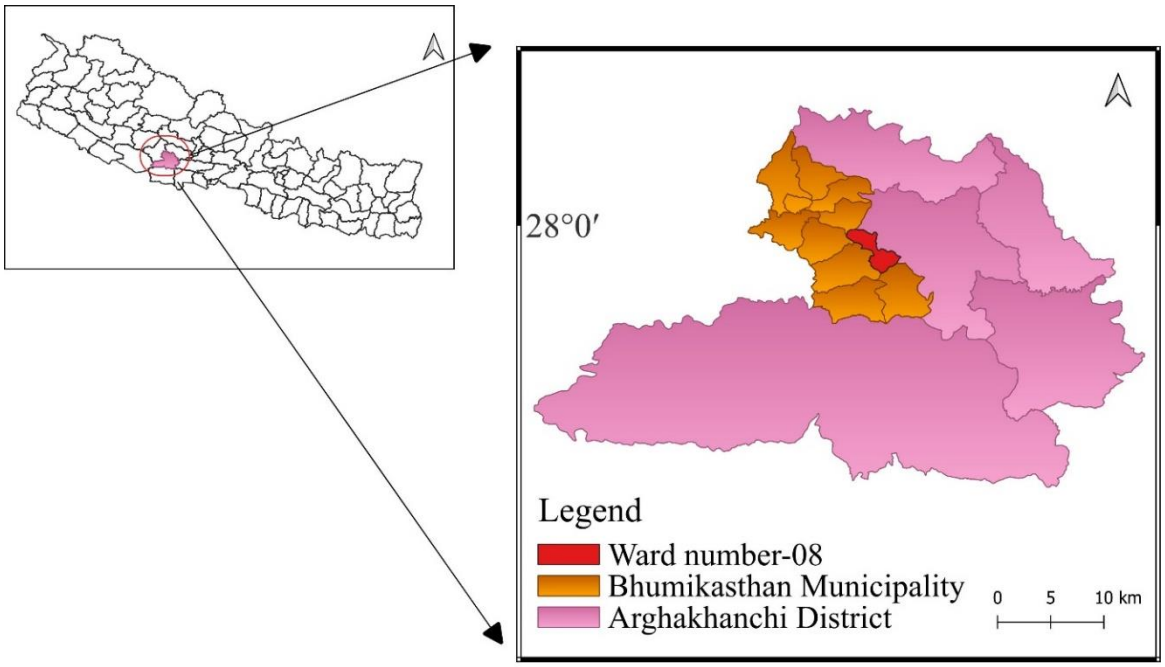

Figure 1: Geographic representation of the study area.

The research was conducted in ward number 08 of Bhumikasthan Municipality in Arghakhanchi, Nepal (Figure 1) situated at an elevation ranging from 305 to 2515 meters above sea level. The climate in this area varies from tropical to sub-tropical, with an average summer temperature of 29.3°C and a winter temperature of 9.4°C. According to the Central Bureau of Statistics Nepal in 2012, the reported annual average rainfall is 1750 mm. Arghakhanchi is geographically located between 27'45"N and 28'6"N latitude, and 80'45"E to 83'23"E longitude (Nepali et al., 2021). Out of a total population of 2123 individuals; 982 males, and 1141 females. The research conducted focused on individuals aged 18 and above, with a sample size of 200, representing approximately 9.42% of the total population (2123) of study site, from 86 households which was selected purposive sampling by lottery method from the study site. From bishwakarma community 92, Mijar 58, Pariyar 33 and magar 17 participants were selected through purposive sampling method. The diverse population in this region includes Hill Brahmin, Chhetri, Magar, Kami, Sarki, Kumal, Damai/Dholi, Newar, Gharti/Bhujel, Musalman, Thakuri, Sanyasi/Dasnami, Gurung, Terai Brahmin, Badi, Gaine, Tharu, and other ethnic groups.The diverse population in this region includes Hill Brahmin, Chhetri, Magar, Kami, Sarki, Kumal, Damai/Dholi, Newar,

Gharti/Bhujel, Musalman, Thakuri, Sanyasi/Dasnami, Gurung, Terai Brahmin, Badi, Gaine, Tharu, and other ethnic groups.

3.2 Study Procedure

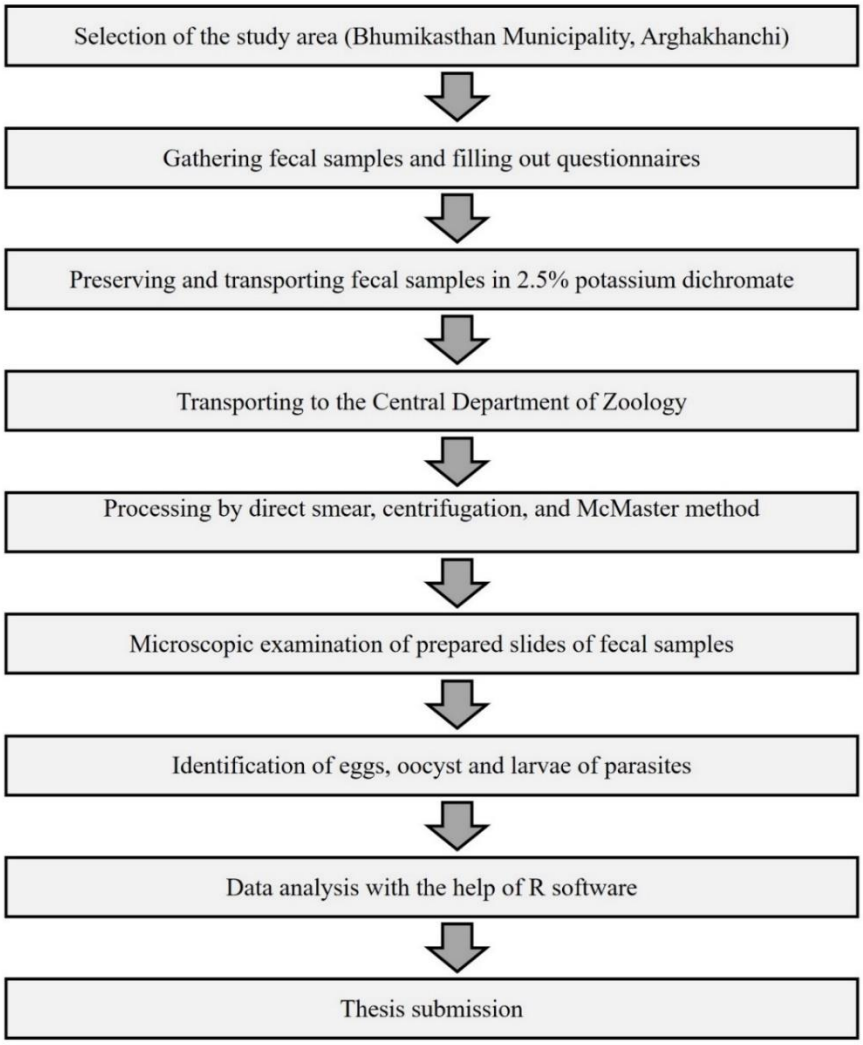

Figure 2: Flow chart of study procedure.

The flowchart in (Figure 2) outlines the process of the study conducted in Bhumikasthan Municipality ward 08, Arghakhanchi. It begins with the selection of the study area and progresses through the steps of gathering fecal samples and completing questionnaires. The collected samples are then preserved in 2.5% potassium dichromate and transported to the Central Department of Zoology (CDZ). Subsequent processing involves direct smear, and centrifugation. Microscopic examination of prepared slides follows, focusing on the identification of eggs, oocysts, and larvae of parasites. The final stages involve data analysis using R software, leading to the eventual submission of the thesis

3.3 Methods

3.3.1 Selection criteria

A convenient sampling method was employed for this study, involving participants aged 18 and older. Selection was conducted with the age range of 18 to 88, utilizing visits to local health centers where children were regularly administered anti-helminths parasitic medication.

3.3.2 Sample size

The findings from the 2021 national population and housing census for Ward Number 8 in Bhumikasthan municipality revealed that out of a total population of 2123 individuals, there were 982 males and 1141 females. The research conducted focused on individuals aged 18 to 88, with a sample size of 200, representing approximately 9.42% of the total population. To assess parasite prevalence and associated risk factors, a convenient sampling method was used to collect 200 fresh stool samples from the indigenous community.

3.3.3 Sampling and participants

The study was conducted during the first week of August 2023, involving the collection of 200 stool samples from the indigenous community, specifically focusing on individuals aged 18-88, including Magar, Mijar, Biswakarma, and Pariyar ethnic community. The sampling covered eight distinct villages within ward 08 of Bhumikasthan Municipality, Arghakhanchi District.

3.3.4 Sampling procedure

Initially, households within the selected communities were identified, with details such as the number of occupants and the household head's name documented. Each house in every community was assigned a code, and a purposive selection process was employed to choose households. A total of 83 households, comprising Magar, Mijar, Biswakarma, and Pariyar communities, were selected through convenient sampling from Lathebaj Kura, Bajakharka, Kimdadha, Nimdadha, Ghenchenbass, Seudeyni, Chiuramati, and Nuwakot, distinct villages.

3.3.5 Participant introduction and informed consent

Before proceeding with the study, all participants were briefed about its purpose, procedures, and the informed consent process. Participants were then requested to provide written informed consent for their involvement.

3.3.6 Data collection

Anthropometric assessments were conducted to gather data on height and weight. Structured questionnaires were administered to obtain information on socioeconomic status (SES), sociodemographic factors (age, gender, ethnicity, and family size), body mass index (BMI) calculated from height and weight, and behavioral practices. The latter included factors such as water consumption habits, outdoor activities (e.g., walking barefoot), personal hygiene practices (handwashing, nail trimming), among others, in line with insights from the literature review.

3.3.7 Stool sample collection and processing

Participants were given specially labeled plastic containers, free of disinfectants, to collect morning stool samples. The containers were marked for convenience, and participants were instructed to fill one-fourth of the provided container without contamination from different segments (first, middle, and last parts). Following this, the collected stool samples were mixed with a 2.5% potassium dichromate solution and transported to the CDZ laboratory, where they were preserved at 4°C for subsequent analysis.

3.3.8 Macroscopic examination

This process included the examination of stool attributes such as texture (solid, liquid, semi-solid, or other distinctive features), color variations (pale yellow, white, black, blood red, or any alternative color), and the identification of adult nematodes, trematodes, or cestodes.

3.3.9 Microscopic examination

Stool samples were subjected to microscopic examination for the detection and identification of intestinal protozoa, helminth eggs, and larvae. This process encompassed both direct wet mount and concentration methods. The microscopic analysis utilized three distinct techniques, namely the direct wet mount technique (Adhikari et al. 2021; Yadav &

Prakash 2016) formalin ether sedimentation technique (Bhattachan B et al. 2015), and the saturated salt floatation technique (Khadka et al. 2021).

3.3.9.1 Direct wet mount technique

Approximately 2 g of the fecal sample were precisely stirred and mixed. A small droplet from each sample was then placed onto a glass slide, with or without Gram's iodine stain. Subsequently, the sample was covered with a coverslip and examined under a microscope at magnifications of 10× and 40×, as described by (Adhikari et al. 2021).

3.3.9.2 Saturated salt floatation technique

Approximately one milliliter of the filtrate and 13 milliliters of sodium chloride (NaCl) was placed into a 15-milliliter centrifuge tube. This mixture was then undergoing centrifugation for a duration of 5 minutes, and the centrifuge tubes containing the mixtures was subsequently positioned within test tube stands. The concentrated NaCl solution was poured in its entirety to the brim, creating a convex surface at the top of the tube. The tube was sealed with a coverslip, ensuring that the solution meets the coverslip. After a waiting period of 15-20 minutes, the coverslip was removed and placed onto glass slides. These slides were then examined using a microscope, following the procedure outlined by (Zajac and Conboy 2012).

3.3.9.3 Formal ether Sedimentation technique

One milliliter of filtrate will be mixed with ten milliliters of 10% formal saline in a 15 ml centrifuge tube, and three milliliters of ether was added and thoroughly mixed. The mixture was then centrifuged for 5 minutes at 3000 rpm. Afterward, the supernatant liquid was discarded, and the settled solution was used for the experiment. Two drops of the solution were placed on a glass slide containing Lugol’s iodine, and parasitic stages will be examined under a microscope (Zajac and Conboy 2012).

3.3.10 Identification of intestinal parasites

During the microscopic examination, both unstained and stained stool smears were examined using 10x and 40x objectives. The identification process involved recognizing the cystic and trophozoites stages of protozoans, along with the egg and larva stages of helminths. This identification was carried out with reference to a medical laboratory manual and consultation with experts. Various characteristics, including size, shape, shell content,

color, exterior features, and hooks, were meticulously examined for accurate identification. To ensure precision, the observed features were compared with information from previously published literature, enhancing the reliability and thoroughness of the identification process.

3.3.11 Questionnaire survey

A concise set of questionnaires (refer to Appendix) was formulated to gather information on various aspects, including socioeconomic status (SES), sociodemographic factors (age, gender, ethnicity, and family size), body height and weight for BMI calculation, and behavioral practices. The latter encompassed associated risk factors such as the types of drinking water consumption, walking barefoot outdoors, hand-washing practices, nail trimming, and other relevant behaviors. The questionnaires were designed to comprehensively capture key data points essential for the study's objectives.

3.4 Ethical considerations

Approval for the ethical conduct of this study was secured from the Institutional Review Committee of the Institute of Science and Technology (IOST) at Tribhuvan University, with the assigned (Regd. No IRCIOST-23-0062). This authorization emphasizes the commitment to ensuring the research follows established ethical standards and safeguards the well-being and rights of the study participants.

3.5 Data analysis

The collected data was encrypted and entered a Microsoft Excel 2007 spreadsheet. Data were analyzed using Fisher's exact test (Two-sided) and Pearson's Chi-squared ($\chi 2$) test with R software. Significance was analyzed among different factors, also between total protozoan and total helminthic species. Association of GI parasitic infections with respect to demographic, socioeconomic, occupational, and behavioral characteristics among the studied populations were analyzed. In all cases, 95% confidence interval (CI) with $p<0.05$ were considered for the statistically significant difference.

4. Results

4.1 Analysis of the prevalence of Intestinal parasites

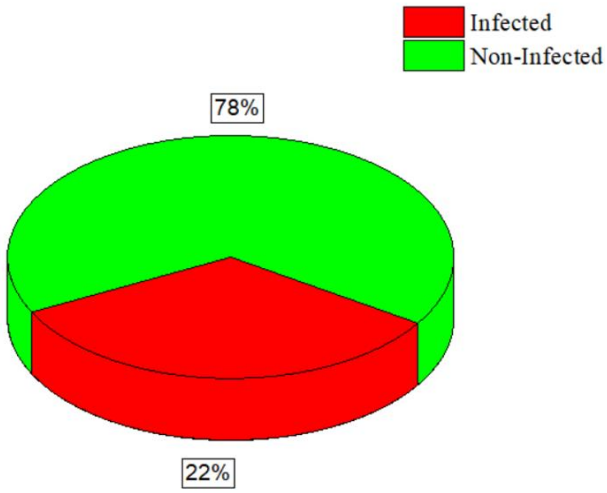

Figure 3: Pie chart showing the frequency of intestinal parasite infections within an indigenous community.

As illustrated in (Figure 3), from this study area, observing 200 freshly collected stool samples, which were subsequently examined for intestinal parasitic infections (IPIs). Among these, 44 samples from the indigenous community tested positive for infection. The findings revealed a 22% overall prevalence of intestinal parasites in the population under investigation.

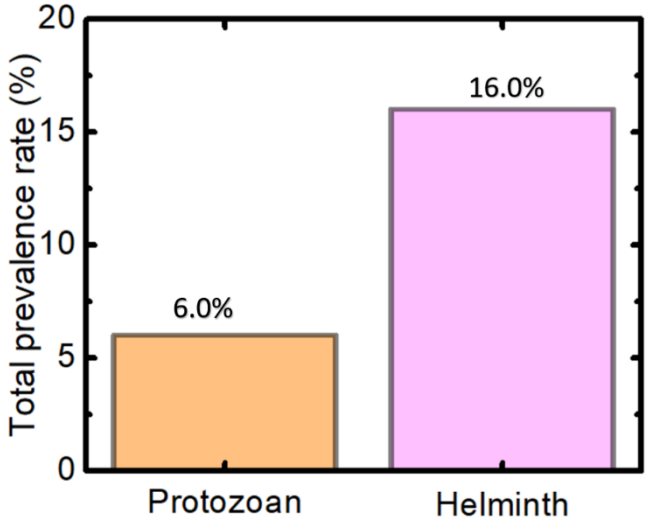

Figure 4: Bar chart displaying the incidence rates of protozoan and helminth infections within an indigenous community.

From the study site, 12 samples were found to be infected with protozoan parasites, representing a 6% overall prevalence of protozoan infections. Conversely, 32 samples were infected with helminth parasites, indicating a prevalence rate of 16%. Notably, helminth infections were more predominant compared to protozoan infections ($\chi 2 = 8.11$, $p < 0.05$), as illustrated in (Figure 4).

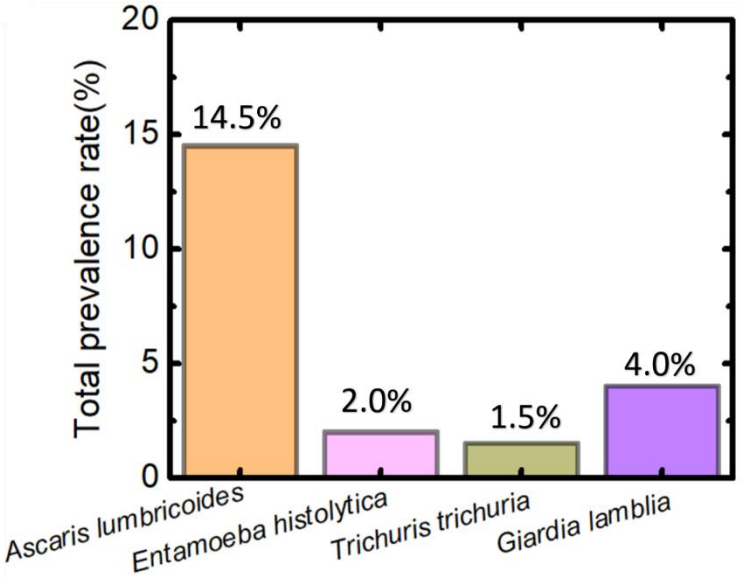

Figure 5: Bar chart illustrating the prevalence of infection by various species of intestinal parasites within an indigenous community.

Among the specific parasite species, *Ascaris lumbricoides* exhibited the highest prevalence of 14.5%(29), showing a statistically significant association ($\chi^2 = 41.51$, $p < 0.001$). However, *Entamoeba histolytica*, *Trichuris trichiura*, and *Giardia lamblia* had lower prevalence rates of 2%(4), 1.5%(3), and 4% (8), respectively. These findings underscore the substantial burden of IPs in the studied population, with *Ascaris lumbricoides* being a prominent contributor to the overall prevalence as shown in (Figure 5).

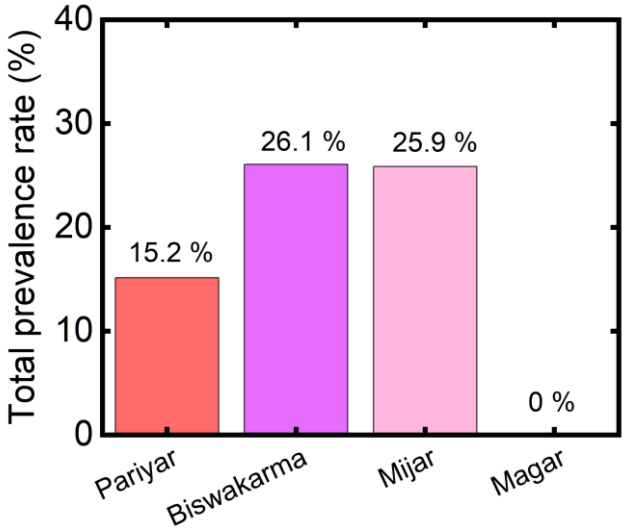

Figure 6: Bar chart illustrating the prevalence of intestinal parasite infections across different ethnic communities within the Bhumikasthan municipality.

Out of the total Pariyar community as shown in (Figure 6), only 5 individuals out of 33 were found to be infected, accounting for a prevalence rate of 15.2%. Similarly, among the Biswakarma community, 24 out of 92 participants were infected, representing a prevalence of 26.1%. In the Mijar community, 15 out of 58 participants were infected, resulting in a prevalence of 25.9%. Conversely, no infections were detected among the Magar community, with a prevalence rate of 0% among 17 participants. Significantly, the Biswakarma community exhibited the highest infection rate, with a prevalence of intestinal parasitic infection $p<0.045$, followed by the Mijar and Pariyar communities.

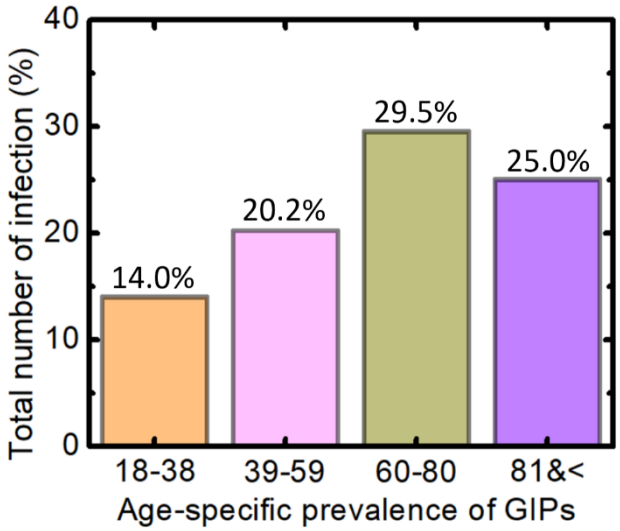

Figure 7: Bar chart depicting the prevalence of intestinal parasite infections across various age groups within the indigenous community.

The study covers an age range covering from 18 to 88 years old as shown in (Figure 7), revealing variations in parasitic infections across different age groups. Among participants aged 18-38 years (43 individuals), only 6 individuals were infected with parasites, representing a prevalence rate of 14.0%. Similarly, among participants aged 39-59 years (84 individuals), 17 individuals were infected, resulting in a prevalence rate of 20.2%. Furthermore, among participants aged 60-80 years (61 individuals), 18 were infected, accounting for a prevalence of 29.5%, while among those above 80 years (12 individuals), 3 were infected, yielding a parasitic infection rate of 25%. Although age is considered a risk factor associated with parasitic infection, this study did not reveal any statistical difference in intestinal parasitic infection across age groups.

(Table 1) showed, the gender-specific analysis revealed no statistically significant differences in the prevalence of IPs between males and females. For *Ascaris lumbricoides* 6 males tested positive out of 57, while 22 females tested positive out of 143, with a non-significant chi-square value of 0.45 ($p = 0.504$). Similarly, *Giardia lamblia* showed 1 positive case in males out of 57 and 8 positive cases in females out of 143, resulting in a non-significant chi-square value of 0.65 ($P = 0.45$). *Entamoeba histolytica* exhibited no significant gender-based difference, with 1 positive case in both males and females. *Trichuris trichiura* demonstrated 2 positive cases in males out of 57 and 1 positive case in females out of 143, yielding a non-significant chi-square value of 0.69 ($p = 0.196$).

Table 1: Parasite-specific prevalence with gender.

S.N.	Parasites	Male: N=57 Infected (%)	Female: N=143 Infected (%)	p-value
1	*Ascaris lumbricoides.*	6 (10.5)	22 (15.4)	0.504
2	*Trichuris trichiura.*	1 (1.7)	8 (5.6)	0.45
3	*Giardia lamblia.*	1 (1.7)	3 (2.1)	1
4	*Entamoeba histolytica.*	2 (3.5)	1 (0.7)	0.196
5	Total	10 (17.4)	34 (23.8)	0.337

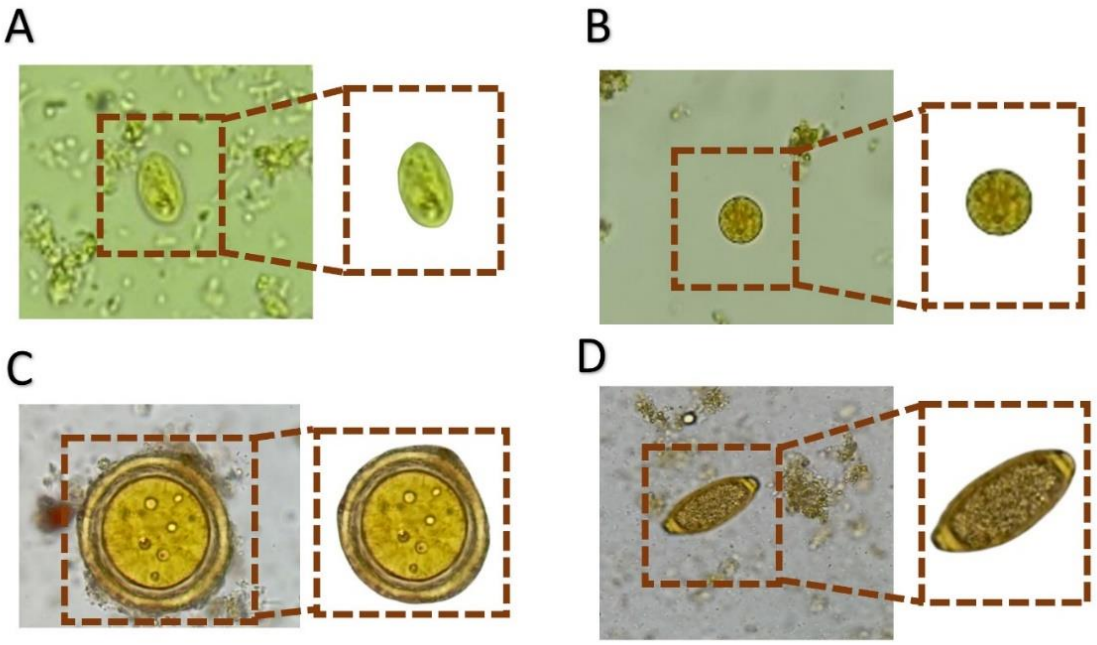

Figure 8: Microscopic photographic images of the different intestinal parasites. (A) *Giardia lamblia* (25.07 × 13.16 µm), (B) *Entamoeba histolytica* (10.45 × 8.71 µm), (C) *Ascaris lumbricoides* (89.66 × 53.12 µm), and (D) *Trichuris trichura* (53.41 × 21.63 µm).

(Figure 8) illustrates microscopic images of different IPs, labeled accordingly: (A) *Giardia lamblia* (25.07 × 13.16 µm), (B) *Entamoeba histolytica* (10.45 × 8.71 µm), (C) *Ascaris lumbricoides* (89.66 × 53.12 µm), and (D) *Trichuris trichiura* (53.41 × 21.63 µm). These images visually represent the morphological characteristics of each parasite, aiding in their identification and understanding.

4.2 Analysis of risk factors associated with prevalence of Intestinal parasites

(Table 2) reveals the results of the study, involving a total of 200 participants, revealed varying prevalence rates of IPs across different socio-demographic characteristics. Regarding family size, did not exhibit a significant association with infection prevalence. However, within muddy residences had a significantly higher prevalence compared to semi-concrete homes 26.61% and 12.06% respectively. Livestock sharing the same residence did not show a significant association with infection. Educational level, occupation, drinking water source, and raw meat consumption did not reveal significant associations with infection prevalence. However, individuals with tap water as a drinking source showed a significantly higher prevalence of 22.29%. The study did not find significant associations between infection prevalence and other factors such as knowledge on intestinal parasites, medication usage, and free-ranging poultry. These findings highlight the multifactorial nature of IPs prevalence, suggesting the importance of considering specific socio-demographic characteristics in public health interventions.

Table 2: Demographic characteristic features of participants analyzed using chi-square test to determine statistical significance.

Socio-demographic characteristics		Total persons N (200)	Infected (n)/ non-infected	Prevalence (n/N×100) %	p-values	Univariate OR (95% CI)
Age	18-38	43	6/37	14.00	0.278[¥]	Ref
	39-59	84	17/67	20.23		1.53(0.576-4.650)
	60-80	61	18/43	29.50		2.52(0.938-7.701)
	81≤	12	3/9	25.00		2.06(0.354-9.912)
Family member	1-3	31	9/22	29.03	0.523[¥]	Ref
	4-6	141	28/113	19.85		1.64(0.599-4.236)
	7-9	29	6/23	20.68		1.56(0.413-6.274)
	10≤	3	½	33.33		0.82(0.038-53.571)
Resident home	Muddy	139	37	26.61	0.017[@*]	Ref
	Semi-concrete	61	7	12.06		0.36(0.139-0.832)
Livestock with same resident	Yes	59	14/45	23.72	0.7026[@]	Ref
	No	141	30/111	21.27		1.15(0.545-2.359)
Education level	Can read	63	10/53	15.87	0.174[@]	Ref
	Cannot read	137	34/103	24.81		0.59(0.256-1.254)
Occupation	Farmer	146	35/111	23.97	0.171[@]	Ref
	Other	54	9/45	16.67		0.64(0.269-1.402)
Drinking water	Tap	118	33/85	22.29	0.014[@*]	Ref
	Boiled	82	11/71	21.15		2.47(1.193-5.489)
Raw meat	Yes	107	20/87	18.86	0.226[@]	Ref
	No	93	24/69	25.53		0.66(0.334-1.301)
Infection	Yes	50	11/39	22.00	1[@]	Ref
	No	150	33/117	22.00		1.01(0.446-2.143)
Knowledge on IPs	Yes	6	1/5	16.67	1[#]	Ref
	No	194	43/151	22.16		0.70(0.014-6.530)
Medication	Yes	15	2/13	13.33	0.529[¥]	Ref
	No	185	42/143	22.70		0.52(0.055-2.464)
Free ranging poultry	Yes	132	30/102	22.72	0.729[@]	Ref
	No	68	14/54	20.58		1.13(0.557-2.373)

Fisher's Exact Test: #, Pearson chi-square test: @, <0.05: *, and <0.001: **

(Table 3) presents the association between nutritional and hygiene practices and the prevalence of gastrointestinal parasitic infections (IPs) among the study participants. In terms of Body Mass Index (BMI), individuals categorized as overweight (BMI ≥25) showed a higher prevalence of 24.05%, while those categorized as underweight (BMI <18.5) had a prevalence of 25.00%, though neither were statistically significant. Covering food, frequency of nail trimming, nail biting, and walking barefoot were also examined. Covering food did not show a significant association with IPs prevalence. However, individuals who reported sometimes forgetting to cover their food had a higher prevalence of 29.72%. Nail trimming, both weekly and monthly, showed higher prevalence rates than when nails were long, but the differences were not statistically significant. Nail biting did not reveal a significant association with IPs prevalence. Walking barefoot, especially doing so sometimes, was significantly associated with a higher prevalence (25.92%) compared to those who did not walk barefoot as frequently (13.84%). These findings underscore the potential role of specific hygiene practices, such as covering food and regular nail trimming, in influencing the prevalence of IPs, emphasizing the importance of targeted interventions in these areas to mitigate the risk of infections.

Table 3: Nutritional status and hygiene practices features of participants analyzed using chi-square test (Fisher's Exact and Pearson chi-square) to determine statistical significance.

Nutritional and hygiene practices		Total N (200)				
Factors	**Subgroups**	**Total persons (N)**	**Infected/ non-infected**	**Prevalence (n/N×100) %**	**p-values**	**Univariate OR (95%CI)**
BMI	<18.5 (underweight)	12	3/9	25.00	0.819#	Ref
	18.5-24.99 (normal)	109	22/87	20.18		0.77(0.171-4.806)
	≥25 (overweight)	79	19/60	24.05		0.92(0.203-5.809)
Cover food	Yes	85	17/68	20.00	0.4523 @	Ref
	No	37	11/26	29.72		0.59(0.244 - 1.472)
	Sometime forget	78	16/62	20.51		0.97(0.446-2.106)
Nail trim	When long	74	18/57	24.32	0.141 @	Ref
	Weekly	39	4/34	10.25		2.72(0.912-10.313)
	Monthly	87	22/65	25.28		0.95(0.457-1.957)
Nail bite	Yes	42	10/32	23.8	0.707#	Ref
	No	158	34/124	21.5		1.32(0.520-3.151)
						Inf (0.061-Inf)
Walk barefoot	Sometimes	115	35/80	25.92	<0.001 @**	Ref
	No	85	9/76	13.84		3.63(1.690-8.571)

Fisher's Exact Test: #, Pearson chi-square test: @, <0.05: *, and <0.001: **

The multivariate logistic regression analysis provided valuable insights into the factors associated with IPIs. Gender, age, BMI, family size, socio-economic status, community, resident home construction, drinking water source, hygiene practices, education level, fruits and vegetable washing frequency, handwashing, walking barefoot, diet, and contact with soil showed no statistically significant association with the likelihood of infection. However, people following Vegetarian or non-vegetarian diet, noticing worms in the stool, washing fruits and vegetables before consumption, and handwashing practices, showcases the multivariate logistic regression with IPs prevalence as shown in (Table 4).

(Table 4) presents data on the association between dietary habits, awareness of parasites in stool, washing fruits and vegetables, handwashing practices before meals, and the incidence of intestinal parasitic infections (IPIs) among different groups. It reveals that adopting a vegetarian diet significantly reduces the likelihood of infection compared to a non-vegetarian diet, indicating a clear difference in infection rates between the two dietary groups. Similarly, individuals who noticed worms in their stool had a higher likelihood of infection compared to those who did not, emphasizing the role of awareness as a potential risk factor. Moreover, washing fruits and vegetables before consumption was associated with a lower infection rate, highlighting the importance of this practice in reducing the risk of IPIs. Additionally, maintaining good hand hygiene by washing hands before meals significantly decreased the odds of infection, underlining the crucial role of proper hygiene practices in preventing intestinal parasitic infections

Table 4: Significantly associated risk factors of participants analyzed using multivariate logistic regression.

Risk factors		Total persons N (200)	Infected (n)/ non-infected	Prevalence (n/N×100) %	p-values	Univariate OR (95% CI)	Multivariate AOR (95%CI)
Fruits & vegetables by wash	Always	86	16/70	18.60	0.028@*	Ref	Ref
	Sometime	114	28/86	24.56		0.70(0.346-1.399)	0.25(0.106-0.574)
Wash hand	Yes	96	6/90	8.10	0.001@**	Ref	Ref
	No	104	38/66	36.53		0.12(0.043-0.281)	16.19(6.812-38.471)
Diet	Veg	49	2/47	22.22	0.001#**	Ref	Ref
	Non-veg	151	42/109	21.98		0.12(0.0174--0.410)	9.89(4.009-24.407)
Worms in stool	Yes	38	14/24	36.84	0.025@*	Ref	Ref
	No	162	30/132	18.51		2.56(1.161-5.523)	0.33(0.076-1.472)

Fisher's Exact Test: #, Pearson chi-square test: @, <0.05: *, and <0.001: **

5. Discussion

5.1 Determining the prevalence of Intestinal parasites

This study examined the occurrence of intestinal parasitic infections in the indigenous community of Ward 08, Arghakhanchi district, Nepal. This study revealed a 22% prevalence of intestinal parasitic infections, aligning with similar rates reported in other studies. For instance, the prevalence of intestinal parasitic infections was 25.92% among indigenous Darai and Kumal communities of salyantar, Dhading, Nepal (Thapa, 2021) , 27.33% in Meche community (Dhakal & Subedi, 2019), 23.3% among Tibeto-Burman, Indo-Aryan, and Dalit school children in Chitwan district (Bhattachan et al., 2015), 31.32% among the Sarki ethnic group in Pala Rural Municipality, Baglung (Thapa et al., 2021), and 33.3% in the Musahar Community People of Makwanpur and Nawalsparasi districts (Khadka et al., 2021). In contrast to our study, a study conducted in the southern part of Tehran, Iran, reported a lower prevalence of IPIs at 10.7% (Arani et al., 2008). Conversely, other studies have indicated higher IPIs compared to our findings, with 81% reported among the Mushahar community in Balan Bihuli, Saptari, Nepal. Similarly, a study in central Nepal found a remarkably higher prevalence of 97% among the indigenous Chepang communities (Adhikari et al., 2021). Additionally, 54% within the Indigenous Community in Gaindakot VDC (Gyawali et al., 2013). The observed lower prevalence of GI parasites in our study may be attributed to ongoing routine deworming programs, variations in environmental factors at the study site, socio-economic conditions specific to the community, and prevalent hygiene practices among most individuals. However, it is crucial to acknowledge the limitation imposed by the small sample size, which hinders the drawing of definitive conclusions. Therefore, further studies are warranted to validate and reinforce these findings.

The analysis of 44 individuals in this study revealed a noteworthy difference in the distribution of intestinal parasitic infections, with *Ascaris lumbricoides* emerging as the significantly dominant species among the total infected with 14.5%. This prevalence supports with similar findings reported in a study among Chepang and Musahar community people of Makwanpur and Nawalparasi districts with 15.6%, in deula community as well, also in chepang community in saktikhor, chitwan district along with private and public school children and also in slum dwelling population in naya bazaar, kaski, Nepal. (Khadka et al., 2021; Subedi et al., 2021; Subedi et al., 2020; Adhikari et al., 2020; Tiwari et al.,

2018,). Moreover, upon comparing the prevalence rates of helminth and protozoal infections, the findings suggest a statistically significant predominance of helminth infections. This trend also mirrors a study conducted among the Chepang and Musahar communities residing in the Makwanpur and Nawalparasi districts of Nepal, in deula community as well, also in chepang community in saktikhor, chitwan district along with private and public school children and also in slum dwelling population in naya bazaar, kaski, Nepal (Khadka et al., 2021; Subedi et al., 2021; Subedi et al., 2020; Adhikari et al., 2020; Tiwari et al., 2018) This prevalence pattern may be influenced by various factors such as environmental conditions, sanitation practices, and geographical location (Ngui et al., 2015). Furthermore, study reveals higher infection rate in female with 23.8% and male with 17.4%. Intriguingly, gender did not show a significant association with the prevalence of intestinal parasitic infections in this study. However, similar trend of greater prevalence of infection was observed among females compared to males in other studies (Bhattachan et al., 2015; Saud, 2017; Tiwari, 2018). This trend could be attributed to traditional cultural customs assigning females the responsibility for household soil-related tasks and agricultural labor (Saud, 2017). Additionally, that male dominance in the workforce, particularly in least developed countries, may contribute to their lack of awareness regarding the transmission and epidemiology of helminth parasites (Rousham, 1994). Nevertheless, further research and larger sample sizes may be warranted to validate and generalize these findings. The study reveals parasitic infection in older age group people, with 29.5% of parasitic prevalence rate in 60-80 years of people followed by 25.0% in people category 80 years and above and 20.2% in 39-59 years of people. Where the least prevalence of infection was observed in 18-38 years of people with 14.0%. where similar result was seen in Chaudhary community of people in Nepal (Chaudhary & Subedi, 2020) were Elderly individuals were at a higher risk of contracting intestinal parasites due to factors such as retirement or lack of employment, often resulting in engagement in labor-intensive activities. Similar results were observed by other researcher (Khetan, 1980; Bangs et al., 1996).

5.2 Risk factors associated with prevalence of Intestinal parasites

In this study, several factors showed a notable correlation with the risk of parasitic infection. For instance, biswakarma community exhibited a 26.08% infection rate, followed by majir with 25.86% and pariyar community with 15.15%. Conversely, no parasitic infections were detected in the Magar community. A statistically significant correlation

was observed within the indigenous community, consistent with previous research, such as the study conducted on indigenous populations in rural Malaysia (Choy et al., 2014). As, IPs are widespread, they pose significant health risks, particularly to Indigenous communities who are highly susceptible to parasitic infections due to their low literacy rates, prevalent poverty leading to poor sanitation, and reliance on traditional remedies for treating infections (Thompson et al., 2001). Additionally, residing in muddy homes was significantly associated with parasitic infection, consistent findings was observed in one of the Indigenous Community of Nepal (Gyawali et al., 2013). Drinking untreated water, specifically tap water, was identified as another significant risk factor, supported by studies conducted in Giardia Infection among Indigenous Communities in Rural Malaysia (Choy et al., 2014) and study in intestinal parasitism among two indigenous sub- ethnic groups in Peninsular Malaysia (Chin et al., 2016). Furthermore, individuals who did not maintain proper hygiene practices were associated with an increased risk of intestinal parasitic infections, a trend agreed with several studies (Choy et al., 2014; Ngui et al., 2015; Caroline Okeke & Obiageli Ubachukwu, 2015; Chongbang et al., 2016; Fuhrimann et al., 2016; Khadka et al., 2021; Ruang-areerate et al., 2021). Walking barefoot was found to have a significant association with intestinal parasitic infections, where consistent finding was observed in different studies (Gyawali et al., 2013; Choy et al., 2014; Rajoo et al., 2017). Additionally, certain practices such as washing fruits and vegetables was also supported by similar result in the study conducted on evaluation of associated factors among fruits and vegetables collected from local markets of bule hora town, Southeast Ethiopia (Gemechu et al., 2023). A study focusing on asymptomatic food handlers in South Ethiopia also reported comparable findings regarding the significance of hand hygiene practices, particularly in relation to salmonellosis. (Solomon et al., 2018), and factor associated with infection, dietary habits, also illustrated similar finding with participant undergoing cataract surgery at the eye camps in rural hilly areas of Nepal (Rai et al., 2008). Moreover, Even, worms in stool were identified as significant factors contributing to the prevalence of IPs with similar to that of study conducted on children with gastrointestinal disorders (Kiani et al., 2016).

Assessed using multivariate logistic regression analysis. Proper washing of fruits and vegetables is essential to remove dirt, pesticides, and potential contaminants, including parasites (Osafo et al., 2022). However, this study reveals that individuals who do not wash their fruits and vegetables more regularly had a 0.25-fold (Ref 0.25(0.106-0.574)) greater

prevalence of IPIs compared to those who wash frequently. Similarly, the significance of maintaining good food hygiene is shown by the study's finding (Tadesse et al., 2019; Sitotaw et al., 2019). Suggesting, there might be due to consumption of contaminated fruits and vegetables due to lack of proper washing them consistently, which may lead to a risk of increased parasite infection (Sitotaw et al., 2019).

Data show that the presence of worms in the feces is indicative of a parasitic infection that is active. Ref 0.33(0.076-1.472), 0.33 times as many people affirmatively reported seeing worms in their feces as those who denied seeing any. Numerous things, such as inadequate sanitation, tainted food or water sources, and poor sanitation could be accountable for this (Sah et al., 2013; Narayan & Yadav, 2017; Khadka et al., 2021). The higher prevalence of IPs infection among participants reporting worms in their stool highlights the need for targeted interventions.

Regular handwashing with soap and water is a fundamental practice for preventing the transmission of various infections including those caused by IPs (Lee & Greig, 2010). The research finds, participants who were not consistently engaged in regular handwashing with soap and water hygiene practice before eating were more likely to introduce parasites into their bodies through contaminated hands P<0.001. Also, our study agreed with (Chongbang et al., 2016) which was on children of squatter community of dharan municipality. Similarly in other ethnic community, tharu community (Sah, 2012) and also in Satar and Chaudhary community (Chaudhary & Subedi, 2020). The reference value of 16.19 (95% CI: 6.812-38.471) suggests that individuals not adhering to regular handwashing with soap and water are 16.19 times more susceptible to an increased risk of infection. Meanwhile, the significant reduction in prevalence among participants who regularly practice handwashing with soap and water supports the essential role of personal hygiene in preventing gastrointestinal infections. The observed association between a non-vegetarian diet and a higher prevalence of IPs infection (Ref 9.89, 95% CI: 4.009-24.407, p<0.001) underscores the importance of dietary considerations in infection control. The 9.89 times increased susceptibility to infection among non-vegetarian participants emphasizes the potential risks associated with the consumption of contaminated food or water, for their non-vegetarian meal they depend on local hotels, prevalent in non-vegetarian diets. The study findings align with previous research, (Adhikari et al., 2021) focusing on indigenous Chepang communities in central Nepal.ss

6. Conclusions and recommendations

6.1. Conclusions

Overall, this study aimed to assess the prevalence of Intestinal parasites in the indigenous community of Bhumikasthan Municipality, Arghakhanchi district, Nepal. The analysis of fecal samples revealed an overall prevalence of 22% for GI parasites, with *Ascaris lumbricoides* exhibiting a higher prevalence rate compared to other intestinal parasites, while *Trichuris trichuira* had a lower prevalence rate. Gender-wise prevalence showed no significant association with infection, and helminth parasites were found to have a higher prevalence than protozoan parasites. Notably, only *Ascaris lumbricoides*, *Entamoeba histolytica*, and *Trichuris trichiura* were identified, indicating the absence of cestode and trematode parasites. Furthermore, this study examined handwashing habits, the washing of fruits and vegetables prior to consumption, identification of parasites in stool samples, and dietary behaviors as key factors associated with increased risk of parasitic infections. This research holds significance as it provides essential baseline data on the prevalence of IPs in an indigenous community that has not been previously studied in the Bhumikasthan Municipality of Arghakhanchi district, Western Nepal.

6.2. Recommendations

Effective control of Intestinal parasites necessitates a comprehensive strategy that addresses various aspects of prevention, monitoring, and treatment. Regular fecal examinations are a cornerstone in this approach, allowing for the early detection and identification of parasitic infestations. This diagnostic step is vital for initiating prompt and targeted treatment measures. Simultaneously, the implementation of health education and awareness programs within communities is essential. These initiatives empower individuals with knowledge about proper hygiene practices, sanitation, and preventive measures to reduce the risk of parasitic infections. By fostering a culture of awareness, communities can actively participate in their own health and well-being. Municipalities play a crucial role in public health infrastructure. This proactive measure ensures that interventions can be organized swiftly, reducing the spread of infections, and minimizing their impact on the community. Incorporating innovative approaches and medications aligned with contemporary healthcare standards is crucial for reducing the prevalence of gastrointestinal infections.

7. References

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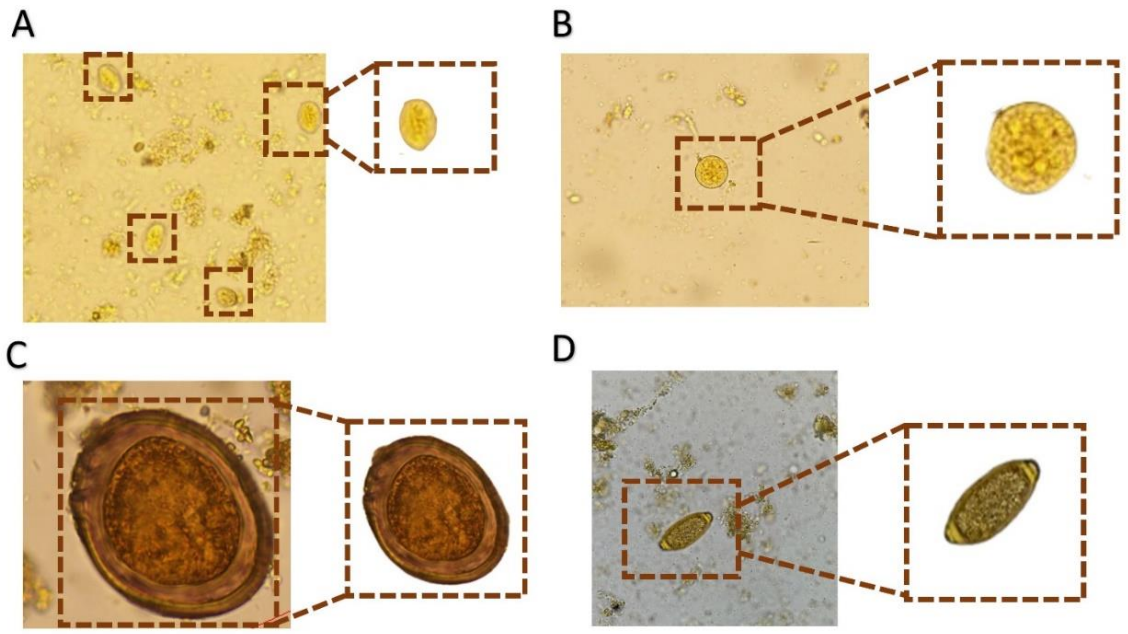
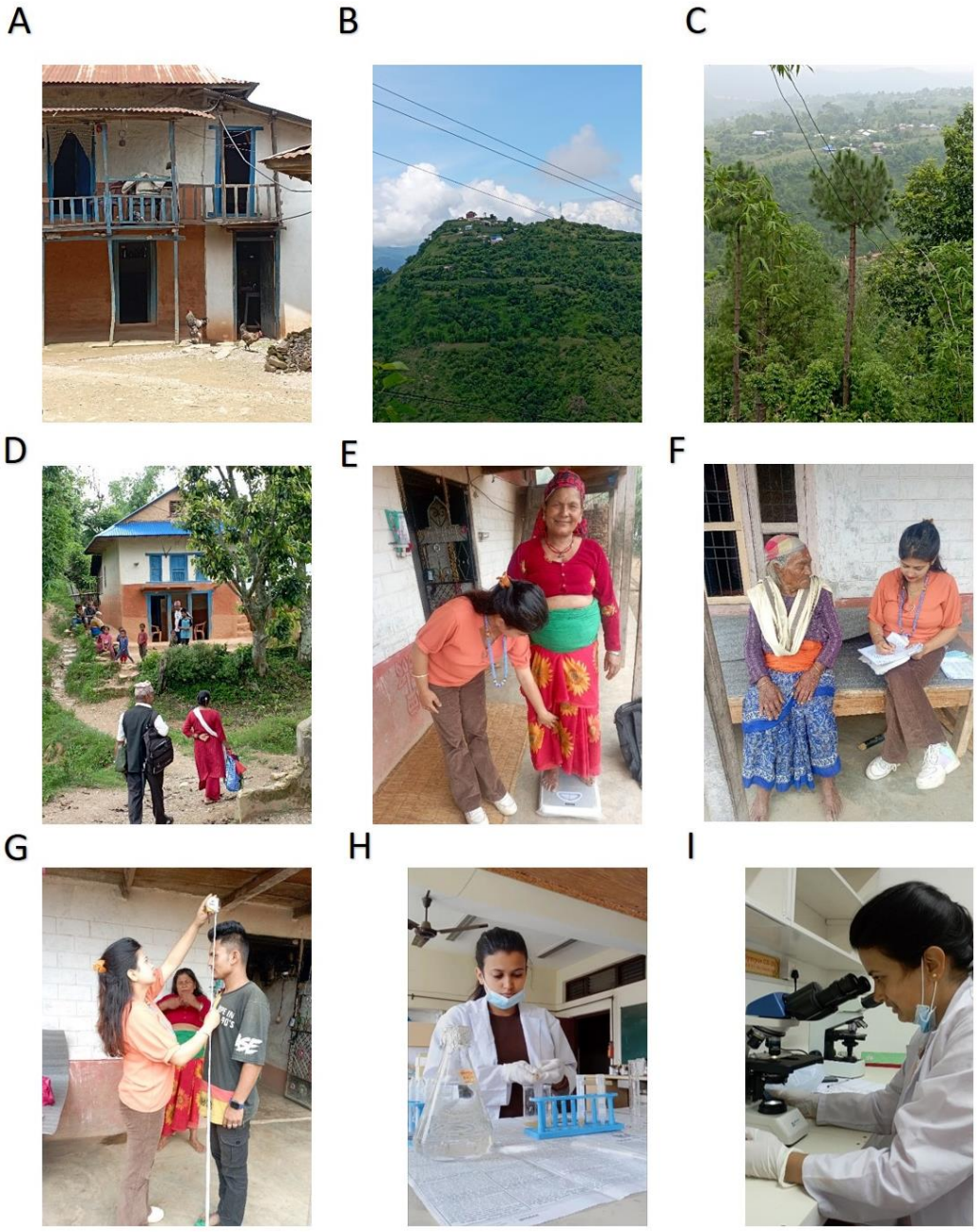
Appendices

Appendix 1.



Photograph 1: Microscopic images depicting diverse IPs, each accompanied by its corresponding size measurements. To elaborate (A) *Giardia lamblia* 15×13 µm, (B) *Entamoeba histolytica* 10×8 µm, (C) *Ascaris lumbricoides* 89×53 µm, and (D) *Trichuris trichiura* 53×21 µm, calibrated by B-view (Boec Germany)

Appendix 2.



Photograph 2: The process of collecting stool samples from the indigenous community. This includes various steps such as: (A) capturing a typical home of one of the study participants, (B) showcasing the study site in Nuwakot, (C) presenting the study site in Ghenchenbass, (D) heading towards the home of a participant, (E) measuring body mass, (F) filling out questionnaires, (G) measuring the body height of participants, (H) preparing microscopic slides for observing sample parasite under a microscope, and (I) conducting parasite observations through a microscope.

Appendix 3.

Questionnaires for participants

Household head name:

Participant code:

House number:

Phone number:

Gender: ☐ Male ☐ Female

Age:

1. The place you reside in

☐ Muddy ☐ Concrete ☐ Hut

2. Do your livestock occupy the same residence?

☐ Yes ☐ No

3. What type of drinking water do you prefer?

☐ Tap water ☐ River ☐ Boiled water ☐ Filtered water ☐ Ground water

4. What is your education level?

☐ Can read ☐ Cannot read and write

5. What is your occupation?

☐ Government employee ☐ Business ☐ Farmer ☐ Others

6. Do you use soap to wash your hands before eating?

☐ Yes ☐ No, but with water ☐ Sometimes ☐ Spoon

7. Do you cut and clean your nails once a week?

☐ Yes ☐ No ☐ Sometimes

8. Do you eat any fruits or green vegetables without washing?

☐ Always ☐ No ☐ Sometimes

9. Do you wear foot ware while outdoors?

☐ Yes ☐ No ☐ Sometimes not

10. How many family members are there in the house?

➢

11. Do you cover food from flies?

☐ Yes ☐ No ☐ Sometimes

12. Do you bite fingernails?

☐ Yes ☐ No ☐ Sometimes

13. Do you drink boiled water?

☐ Yes ☐ No ☐ Sometimes

14. Do you know at least a way to prevent intestinal helminthiasis?

➢

15. Did you consume any medication for intestinal helminths parasites in the last 6 months?

☐ Yes ☐ No

16. Do you have free-ranging poultry in the house?

☐ Yes ☐ No

17. Did you notice any worm in your stool?

☐ Yes ☐ No

18. How frequently have you experienced diarrhea or abdominal discomfort in a month?

-

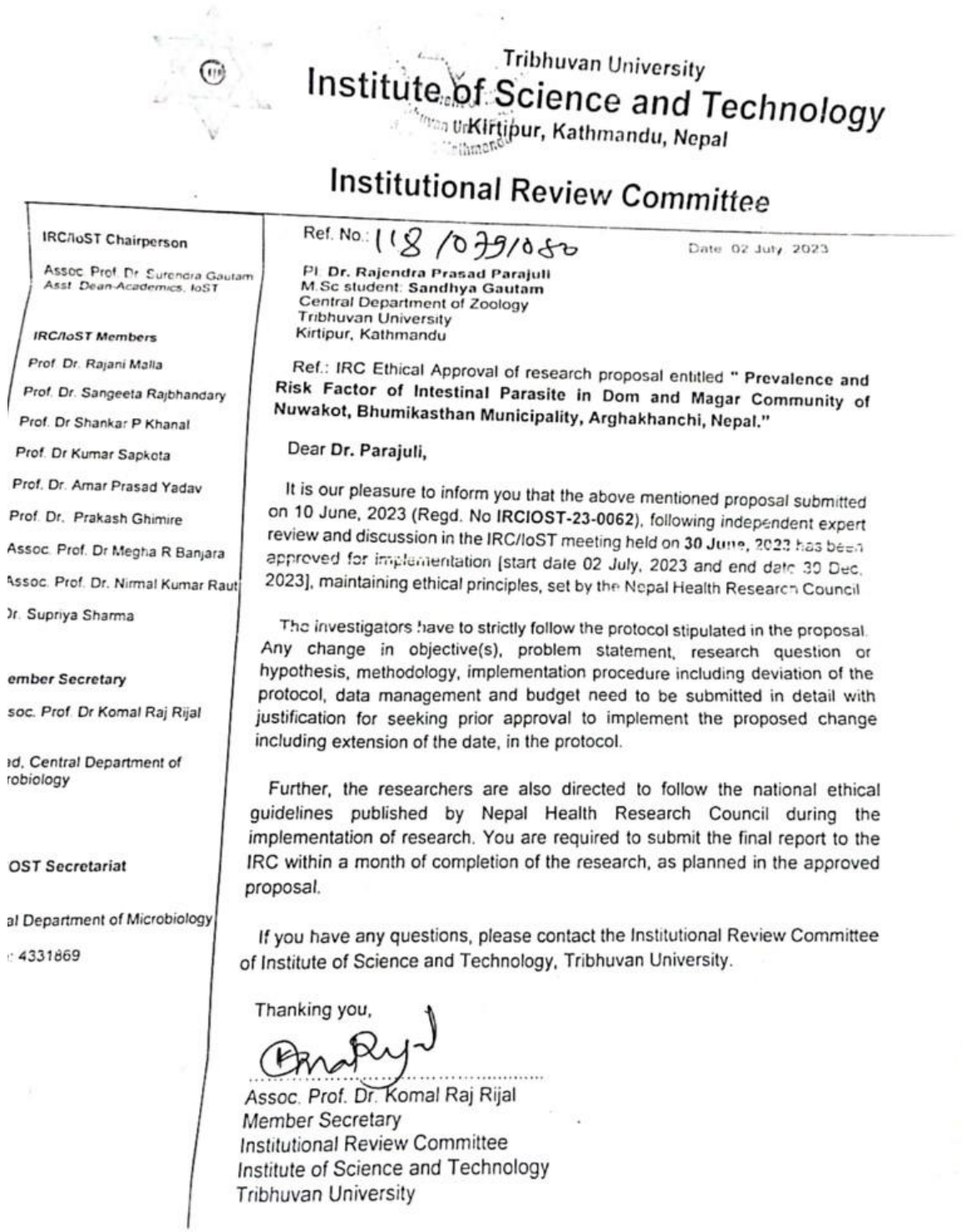
19. Do you follow a vegetarian or vegan diet?

☐ Vegetarian ☐ Non-vegetarian

20. Do you ever consumed raw meat?

☐ Yes ☐ No ☐ Maybe

Appendix 3.



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

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Asst. Dean-Academics, IoST

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OST Secretariat

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Ref. No.: 118 /079/080 Date: 02 July, 2023

PI **Dr. Rajendra Prasad Parajuli**
M.Sc student: **Sandhya Gautam**
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu

Ref.: IRC Ethical Approval of research proposal entitled **" Prevalence and Risk Factor of Intestinal Parasite in Dom and Magar Community of Nuwakot, Bhumikasthan Municipality, Arghakhanchi, Nepal."**

Dear **Dr. Parajuli,**

It is our pleasure to inform you that the above mentioned proposal submitted on 10 June, 2023 (Regd. No **IRCIOST-23-0062**), following independent expert review and discussion in the IRC/IoST meeting held on **30 June, 2023** has been approved for implementation [start date 02 July, 2023 and end date 30 Dec, 2023], maintaining ethical principles, set by the Nepal Health Research Council

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

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

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

Thanking you,

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

Photograph 3: Letter of Ethical Approval from IRC for research proposal