Intestinal Parasites and Associated Risk Factors among the People of Squatter Community in Butwal, Rupandehi

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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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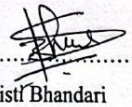
Declaration

I hereby declare that the work presented in this dissertation "Intestinal parasites and associated risk factors among the people of squatter community in Butwal, Rupandehi" 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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Certificate of Acceptance

This dissertation work submitted by Shristi Bhandari entitled "Intestinal parasites and associated risk factors among the people of squatter community in Butwal, Rupandehi" has been accepted as a partial fulfillment for the requirements of a Master's Degree of Science in Zoology with special paper Parasitology.

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Abstract

Intestinal parasitic infections are considered to be among the most prevalent illnesses infecting people causing major medical issues that affect billions of people worldwide, primarily in developing nations like Nepal. Increasing urbanization results in the formation of squatter communities along with health issues due to inferior quality of living. The objective of this study is to determine the prevalence and risk factors underlying parasitic intestinal infections in the squatter community of Butwal. A cross-sectional study with a total of 170 individuals were selected by convenience sampling strategy. During the month of October and November of 2023, a shortlists of questionnaires was prepared to evaluate demographic, socio-economic and behavioral factors to associate the parasitic infection. The collected stool samples were stored in a 2.5% potassium dichromate solution. Direct wet mount and formal ether sedimentation and flotation techniques were employed to determine the prevalence of intestinal parasites in the people of squatter community. Overall, the prevalence of intestinal parasitic infections was 28.8% (5.3% protozoa, 24.7% helminths). Altogether six species of intestinal parasites were detected. *Ascaris lumbricoides* (21.2%) was the most dominant helminth parasite followed by *Trichuris trichiura* (2.9 %), *Entamoeba histolytica* (2.4%), *Cryptosporidium* (1.8%), *Giardia lamblia* (1.8%) and Hookworm (1.8%). Multivariable regression analysis revealed that the participants who did not trim their nails regularly were more likely to get IPIs compared to the participants who trimmed their nails regularly. In addition, having open types of toilets might have more likelihood of getting IPIs but the association was borderline. Relatively high prevalence of intestinal parasites among squatter participants might contribute high risk of intestinal parasitic transmission as over one-quarter of participants neglected to trim their nails frequently, which is a significant risk factor of parasitic infections in this community. Simple health education regarding such crucial hygiene behavior might contribute significantly to reducing the parasitic burden in urban areas like Butwal where lots of people might be at risk of parasitic infections and transmission.

शोध सार

आन्द्राको परजीवी संक्रमण स्वास्थ्य समस्याहरू निम्त्याउने र मानिसहरूलाई असर गर्ने रोगहरू मध्ये एक प्रमुख रोग हो, जसले विश्वभरका अरबौं मानिसहरूलाई खास गरी विकासोन्मुख राष्ट्रहरूमा असर गर्छ । बढ्दो सहरीकरणका कारणले सुकुम्बासी समुदायको बन्छ जस्ले गुणस्तरहीन जीवनयापनका कारण स्वास्थ्य समस्याहरू निम्त्याउछ । यस अध्ययनको उद्देश्य बुटवलको सुकुम्बासी समुदायमा आन्द्राका परजीवीहरूको व्यापकता र तीव्रता पत्ता लगाउनु हो। एक सय सत्तरी व्यक्तिहरू convenience sampling विधि प्रयोग गरि छानिएको थियो । दिशाको नमूनाहरू २.५% पोटासियम डाइक्रोमेट घोलमा सुरक्षित गरि राखिएको थियो । Direct wet mount र formal ether sedimentation र floatation विधिहरूको प्रयोग गरि सुकुम्बासी जनसंख्यामा आन्द्राको परजीवी संक्रमणहरूको व्यापकता र तीव्रता पत्ता लगाइयको थियो । आन्द्राको परजीवी संक्रमणको समग्र प्रसार २८.८२ % (५.३% प्रोटोजोआ र २४.७% हेल्मिन्थ) थियो । आन्द्राका परजीवीहरूको कुल ६ प्रजातिहरू पत्ता लाग्यो। *Ascaris lumbricoides* (२१.२%) सबैभन्दा धेरै भेटिएको हेल्मिन्थ परजीवी थियो, त्यसपछि *Trichuris trichuria* (२.९%), *Entamoeba histolytica* (२.४%), *Cryptosporidium* (२.४%), *Giardia lamblia* (१.८%) र Hookworm (१.८%) थियो । मल्टिभेरिएबल रिग्रेसन विश्लेषणले नङ नकाँट्ने बानी र बन्द प्रकारको तुलनामा खुला प्रकारको शौचालयको प्रयोगमा रहेका सहभागीहरूमा आन्द्राको परजीवी संक्रमण व्यापकतासँग सम्बन्धित भएको देखायो । तुलनात्मक रूपमा उच्च प्रसार, सुकुम्बासी समुदायका सहभागीहरू बीच आन्द्राको परजीवी संक्रमणको उच्च जोखिममा योगदान गर्न सक्छ किनभने एक चौथाई भन्दा बढी सहभागीहरूले नियमित रूपमा आफ्नो नङ नकाटेको देखायो, जुन आन्द्राको परजीवी संक्रमणहरूको महत्त्वपूर्ण जोखिम कारक तत्व हो । स्वच्छता व्यवहार सम्बन्धी सरल स्वास्थ्य शिक्षाले परजीवीहरूको बोझ कम गर्न बुटवल जस्ता सहरी क्षेत्रहरू जहाँ धेरै मानिसहरू परजीवी संक्रमण र सर्ने जोखिममा छन्, एक महत्त्वपूर्ण योगदान पुर्याउन सक्छ ।

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List of abbreviations

Abbreviated form	Details of abbreviations
AD	Anno Domini
CBS	Central Bureau of Statistics
GIPs	Gastro-intestinal Parasitic Infection
IOST	Institute of Science and Technology
IPIs	Intestinal Parasitic Infections
mL	Milliliter
NaCl	Sodium Chloride
NHRC	Nepal Health Research Council
NS	Not Significant
rpm	Revolutions per minute
SES	Socioeconomic Status
SPSS	Statistical Package for Social Sciences
STHs	Soil-transmitted Helminths
WHO	World Health Organization
w/v	weight per volume

1. Introduction

1.1 Background

Squatters are people who unlawfully occupy uninhabited buildings or vacant land. These people reside on the property with no legal claim to the property. In other words, Squatter is also called Slums. They are a socio-economically underprivileged group of people. Squatters have lower-quality homes and working settings, associated with an overall unhealthy environment (Ooi & Phua 2007). They don't have a proper disposal system and proper sanitation.

Parasitic infections (Protozoan and Helminthic parasitic infections) are a global health problem. About 3.5 billion people have been infected with Intestinal (helminths and protozoan) parasites of which the largest majority of infections are among children (World Health Organization, 2023). Over a billion individuals live in slum areas, with the majority of them in developing countries, where slum dwellers make up more than 40% of the population living in urban areas (Gilbert, 2005). The quantity is rising and will keep on expanding unless local governments at all levels, co-operations, and the global community as a whole pay immediate and coordinated attention (Sultana et al., 2012). The most often mentioned risk factors for intestinal infection with parasites are age and gender-related lifestyle, dietary habits, financial status, limited access to clean drinking water and sanitation, and personal cleanliness (Hotez et al., 2014). Parasitic infection remains a significant global health concern, particularly in resource-limited settings (Esch & Petersen, 2013). Human intestinal helminthiasis is most commonly caused by soil-transmitted helminths (STHs), namely *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms (Zeleke et al., 2020).

According to World Health Organization (WHO) data from 2023, roughly 0.807-1.221 billion people were infected with nematode infection, 604-795 million with trichuriasis, and 576-740 million with hookworm infections worldwide. Annually, a minimum of 20,000 individuals die from such infection (WHO, 2023). The helminths *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms, *Hymenolepis nana* and the protozoan *Giardia duodenalis/Giardia intestinalis* are the common intestinal parasites parasitizing about 1 billion individuals globally (Bethony et al., 2006). Over a billion individuals can be infected with *Ascaris lumbricoides*, 795 million by *T. trichiura*, and 740 million by

hookworms (De Silva et al., 2003). *Giardia lamblia* affects about 280 million people worldwide (Esch & Petersen, 2013). Ascariasis is a widspread helminthic infection, particularly prevalent in impoverished areas and slums where sanitation conditions are not optimal. It's estimated that more than three billion individuals globally are infected, with children being majority because of their lifestyle habits as well as poorly developed immune mechanism (Vilwanathan et al., 2017). Poor sanitation and unmanaged ways of living cause a variety of health difficulties and problems in these settlements (Bhattachan et al., 2017).

Age and gender-related behavioral practices, dietary habits, socioeconomic status, limited access to clean water, improper sanitation in environment, and personal cleanliness are the most often stated cause of risk for infection with intestinal parasites. A range of factors identifies as risk factors for intestinal parasitic infections in slum settings. Poverty, illiteracy, poor hygiene, and lack of access to clean water are consistently associated with higher infection rates (El-Sherbini & Abosdera, 2013; Gyang et al., 2019). The high prevalence of these infections is closely related to poverty, illiteracy, poor hygiene, limited access to drinkable water and hot and humid tropical climate (Mehraj et al., 2008). In addition, specific behaviors, such as exposure to wastewater and feces have been associated to higher risk of parasitic infection in human (Fuhrimann et al., 2016). Poor personal hygiene, poor sanitation, lack of hand washing practices, and limited water supply in urban slums are risk factors related with intestinal parasites (Jamali et al., 2020).

These infections can cause a range of health problems, from mild discomfort to severe morbidity and mortality. Effective medication strategies are crucial for controlling and preventing parasitic infections. There is a proper need for targeted interventions to improve living conditions and reduce the burden of intestinal parasitic infections in slum communities. poor sanitation, inadequate environmental conditions, open air defecation, and the prevalence of protozoa and helminth infections (Shobha et al., 2013).

1.2 Statement of problem

In developing countries like Nepal, Urban Squatter is a major problem as it raises concerns about poor living conditions and serves as an ideal environment for many diseases in squatter settlements. It has been an ongoing issue in Kathmandu over the pervious two decades, including other major cities in developing countries. It is due to fast growth in population through migration and uncontrolled urbanization (Madai 2006). A slum community in a developing country is experiencing a high prevalence of parasitic intestinal

helminthic infection (Dhanabal et al., 2014). The presence, persistence, and spread of intestinal parasites serve as an indicator of both socioeconomic and environmental conditions (Horton, 2020). Insufficient environmental sanitation, drinking water, and personal hygiene activities are the most common risk factors for intestinal infection due to parasites in slum population (Hotez et al., 2014).

Despite the mass deworming and health education program implemented in the local level, intestinal parasitic infection (IPIs) is still the predominant cause of diarrheal illness in Nepal (Bhattachan et al., 2017). To address this issue of intestinal parasitic infection in the slum population, a comprehensive medication strategy should be implemented, aiming to treat existing infections and prevent reinfection.

1.3 Objectives

1.3.1 General objective

- To determine the prevalence of intestinal parasites and identify the associated risk factors among the people of the squatter community.

1.3.2 Specific objectives

- To find the intestinal protozoan and helminth parasites in people of slums.
- To access the associated risk factors with intestinal parasitic infection.
- To evaluate the prevalence of intestinal parasitic infection depending on socio-economic situations, socio-demographic and behavioral factors.

1.4 Research hypothesis

- People living in slum communities have a higher prevalence of intestinal parasitic infections compared to the general population due to various environmental and socio-economic risk factors.
- The absence of proper sanitation facilities (toilets, waste disposal) in slum communities increases the risk of exposure to fecal-borne parasites, leading to a higher prevalence of intestinal parasitic infections.
- Lower socioeconomic status (income, education level) among residents of slum communities is associated with an increased risk of intestinal parasitic infections due to limited access to preventative measures and healthcare.

1.5 Significance of the study

Despite existing research in major Nepali cities like Kathmandu and Dharan, this study will uniquely explore the squatter community of Butwal. It digs deeper than previous efforts by evaluating not just the presence (prevalence) of intestinal parasitic infections (IPIs) but also their associated factors. This approach will shed light on the parasitic burden within this specific community. Furthermore, the study goes beyond simple detection by pinpointing the socio-economic, socio-demographic, and behavioral factors that contribute to IPIs in this unique setting. By identifying these population-specific risk factors, the study shows the way for targeted interventions and policy development to minimize the risk of IPIs. Ultimately, this research not only addresses research gaps but also contributes to sustainable solutions for improving the quality of life in the squatter community of Butwal.

2. Literature review

2.1 Intestinal Parasitic Infection in Squatter People

In developing nations, parasitic infections that resulted from intestinal helminths and protozoan parasites are the most frequent infections. In squatter settelments, protozoan parasites causes higher gastrointestinal illness as compared to helminths. Environmental factors and personal hygiene practices contribute rise of intestinal helminths infections in developing countries. A study conducted on the prevalence of intestinal parasitic infections in Karachi found *Giardia lamblia* to be most prevalent (52.8%) followed by *Ascaris lumbricoides*, *Blastocystis hominis,* and *Hymenolepis nana* (Mehraj et al., 2008). Intestinal parasite prevalence is high in slum areas, with studies in Southern Delhi (Dudeja et al. 2012), Naya Bazar, Kaski, Nepal (Tiwari et al., 2018), and Bangladesh, Dhaka (Hosna et al., 2018) reporting high rates of intestinal parasites. The most common parasites identified were *E. histolytica*, *G. lamblia*, and *A. lumbricoides*. This is consistent with comparable findings in other South Asian nations, including Nepal and Bangladesh, where *E. histolytica* is also common intestinal protozoan parasite (Khanal et al., 2011; Khanum et al., 2016).

According to Chongbang et al. (2016), the ratio of infections below 8 years was almost equal to 48% in Squatter of Dharan, *Giardia lamblia* being the most prevalent parasite followed by *Ascaris lumbricoides*. A similar study done in Kaski reported a high rate of intestinal infection (24.1%) in slum-dwellers (26.9%) compared to males (Tiwari et al., 2018) and concluded that increased exposure to the contaminated water and gender disparity might have caused the variation. Due to the absence of moist soil in semi-urban areas, protozoan infection was more prevalent than helminth infections.

In Egypt slum, the most prevalent intestinal helminths were found to be *Ascaris lumbricoides* (46.5%) while the most prevalent protozoan parasite was found to be *Giardia lamblia* (22.6%). Polluted environment, contaminated domestic water supplies, and sewage problems were suggested as associated factors (Mahfouz et al., 1997). Several studies were conducted in urban slums of Brazil and reported a high prevalence rate of *E. Nana* (16%) followed by *E. coli* (10.9 %) and *G. lamblia* (4.0%) while in low frequencies was reported for *A. lumbricoides* (1.8%) and *Enterobius vermicularis* (1.7%). Children who played in the soil reported elevated infection compared to their counterparts (Korkes et al., 2009; Kuete et al., 2015) reported a low intestinal helminth prevalence rate compared to

protozoan infection in slum areas. The combination and integration of several social, behavioral, and environmental variables such as poverty, poor living lifestyle, and lack of personal cleanliness, both on personal and the communal levels were suggested as contributing factors.

Intestinal parasites constitute a major health problem in many developing countries, as prevalence values range from 19.3% to 70% in developing regions, such as Iran, northern Lebanon, Brazil, Nepal, Malaysia, and Saudi Arabia (Hossain et al., 2019). A range of studies has highlighted the prevalence of intestinal parasites in low-strata populations. For example, Dhanabal et al. (2014) found a high prevalence of parasites in low socioeconomic areas in South Chennai, with females and children particularly affected. Similarly, Wegayehu et al. (2013) reported a high prevalence of parasites in people of slum from highland and lowland areas in South Ethopia, with no significant difference between the two groups. Rudohradská et al. (2012) identified a high prevalence of parasites in children from a minority group with low hygienic standards in Slovakia, with *Ascaris lumbricoides* and *Trichuris trichiura* being the most common. These studies collectively underscore the need for improved hygiene and public health interventions in low-strata populations to reduce the burden of intestinal parasites.

A range of studies have found a high prevalence of intestinal parasites in squatter communities. In Malaysia, rural children had the highest infection rate, followed by urban squatters and children from flats (Sinniah et al., 2014). Different previous studies around the world especially in squatter communities reported a higher prevalence of gastrointestinal parasites (Ignacio et al., 2017). A much higher prevalence is being reported from squatter communities from developing countries compared to developed countries. Compared to the general population, the squatter community's participants reported inferior hygiene behaviors and inadequate access to sanitation, and clean water. However, few studies evaluated the association of such factors in IPIs burden (Gyang et al., 2019). Hence this study aimed to evaluate the burden of GIPs and their association with socioeconomic, sociodemographic, and behavioral factors prevalent in participants of the squatter community of Butwal, Rupandehi, Nepal.

2.2 Risk Factors of Intestinal Parasitic Infection

The most common associated risk factors of IPIs were family structure, socioeconomic status, and sanitization problems with improper sewage systems (Teixeira et al., 2007). Poverty was implicated as a major risk factor (Mehraj et al., 2008). Findings by Dudeja et al. (2012) and Tiwari et al. (2018) highlighted the urgent need for improved sanitation and access to clean water in squatter communities. Poor sanitation and inadequate personal hygiene were also suggested as associated factors (Shobha et al., 2013). El-Sherbini (2013) found that age factor mainly children, gender especially females, extreme malnutrition, poverty, lack of education, poor sanitation, lack of access to drinkable water, and hot and humid tropical climate, all contribute to higher infection rates. Among children, factors such as extreme malnutrition, poor hygiene, insufficient sanitation, behavioural habits and lack of clean drinking water are associated with a higher prevalence of these infections (El-Sherbini & Abosdera, 2013). The absence of a toilet and lack of hand washing after using the toilet has also been linked to a higher prevalence of intestinal parasitic infections (Abate et al., 2013).

Kotian et al. (2014) underscored the importance of safe drinking water, personal hygiene, and environmental sanitation in preventing and controlling intestinal parasitic infections. In the general population, low socioeconomic conditions, lack of access to clean water, poor personal hygiene, and environmental sanitation are key risk factors (Kotian et al., 2014). Several risk factors have been identified, including low education, occupational status, and poor sanitation (Mohammed et al., 2015). Poor hygiene and sanitary conditions, improper water supply, and uneducated families were reported as an associated risk factor by (Chongbang et al., 2016).

The IPIs are frequently related to low socioeconomic situations, such as insufficient sanitation and water availability (Ignacio et al., 2017). Infections due to intestinal parasites are considered as a significant public health concern, particularly in countries with slum areas particularly developing countries. A range of studies have identified various risk factors associated with intestinal parasite infections. Afidah Novitasari & Zainal Fatah (2021) further highlighted age, sex, residence, toilet facilities, hand washing with soap, shoe-wearing habits, nail trimming, consumption of undercooked food, personal hygiene, and source of drinking water as significant risk factors.

Factors such as frequent contact with soil, type of latrine used, lack of disinfection practices, presence of free-roaming animals, and open defecation were identified as associated risk factors for intestinal parasitic infections (Nath et al., 2022). These findings underscore the importance of addressing both individual and environmental factors in the prevention and control of intestinal parasite infections.

3. Materials and methods

3.1 Study area

Butwal Sub-Metropolitan City is one of the three primary cities of the rapidly expanding Butwal-Tilottama-Bhairahawa urbanized accumulation, which is specially connects Siddhartha Highway in Lumbini Province in West Nepal which have a total urban accumulated population of 421,018 as per the 2021 AD Nepal census (CBS, 2022). Butwal has a city population of 195,054 as per the 2021 AD Nepal census and considering the present situation, the population is rapidly increasing estimating near about 150,000. Also the highway connects Indian border at Sunauli and the hilly town areas of Tansen and Pokhara. Butwal, located in Rupandehi district of Nepal, is a city which is recognized for its quick expansion and developing educational sector.

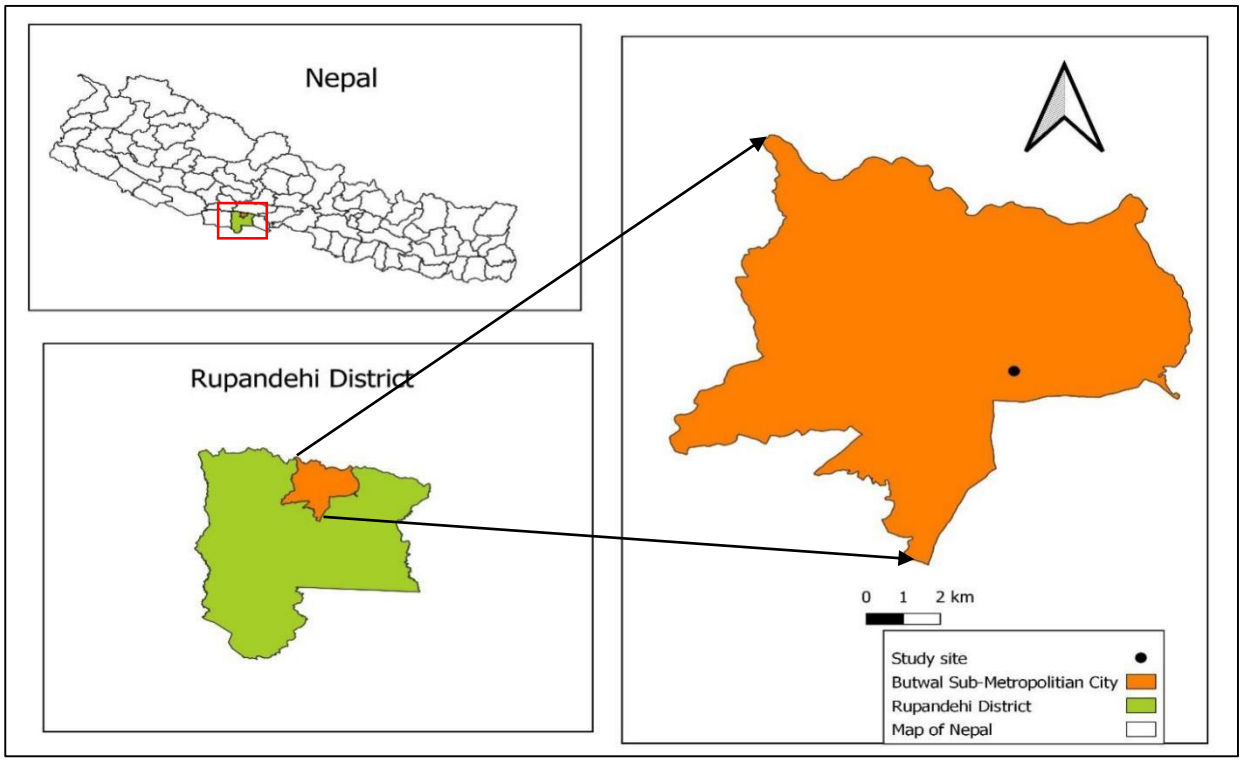

Figure 1. Map of study area of squatter community in Butwal-11, Rupandehi, Nepal.

Butwal city is located close to the bank of the Tinau River and on the northern border of the Terai plain beneath the Siwalik range. However, its administrative region stretches to the North at the slope above Siwalik. Butwal Sub-Metropolitan City is linked by Mahendra Highway (east to west) and Siddhartha Highway. The total coverage of area is 101.61 km² and is divided into a total of 19 wards with overall population of 138742 in 2011 (CBS,

2022). The Tinau River flows across the middle part of Butwal from north to south, separating it roughly into two equal portion. Many squatter places are present at the banks of the river. However, Butwal is comparatively recently urbanized city that has emerged and grown significantly since 1960 AD. The squatter community chosen for this study is Buddhanagar, which is one of the squatter territory situated in Butwal Sub-metropolitan city, ward-11 Devinagar that resides the Western portion of the Tinau River. The population distribution in ward 13 as per the census 2011 reported 7258 households with a population of 28193 of which the male population was 13834 and the female population was 14359 (CBS, 2022).

3.2. Sampling method

A convenient sampling method was used, with participants at the study location were chosen on a first come, first basis.

3.2.1 Selection criteria

As the routine national deworming campaign since 2004 is focused on younger populations, people below the age of 18 years were excluded. Participants above the age of 18 years were included in the study.

3.2.2 Sample size

Since we focused on a socio-economically underprivileged population, we selected a convenient sampling method given a sample size of 170 as determined by (Isreal 2003).

3.3 Methods

A cross-sectional study was conducted during the month of October and November of 2023. The participants were approached by convenience sampling method. Each participant was explained about the study objectives and process. Participants were requested to sign informed consent if they agreed to participate in the study. Once participants agreed and signed the written informed consent, the study participants were instructed to apply a clean sterile stick to collect a sample of feces approximately the size of a thumb from the first part, middle part, and final part of their faeces early in the morning and place it in the provided sterile vial, taking care not to contaminate it with urine or soil. The collected fecal samples were stored in 2.5% potassium dichromate solution to preserve the parasites before

transporting them to the Central Department of Zoology, Tribhuvan University for further investigation.

3.3.1 Macroscopic Examination

The process included examining the stool's consistency (solid, fluidy, semi-solid, or other texture), color (pale yellow, black, white, blood red, or any other colors), and also the presence of any juvenile nematodes, trematodes, or cestodes.

3.3.2 Microscopic Examination

Microscopic examination of feces was done to detect the presence of intestinal protozoan, helminth eggs, and larva. It involved direct wet mount method and concentration method.

3.3.2.1 Direct wet mount

About 2 grams of fecal samples were carefully stirred/mixed. A single drop of each sample, with or without Gram's iodine stain, was placed on the glass slide. The samples were then examined under a microscope by covering them with a coverslip.

3.3.2.2 Formalin-ether (FE) sedimentation

In a 15ml centrifuge tube, 2 grams of the fecal samples were thoroughly mixed with 12 ml of 0.9% w/v NaCl. After centrifuging the samples (1200 rpm for 5 minutes), the supernatant was discarded. The tube was then filled with 10 ml of 10% formalin and 3 ml of ether for centrifugation (1200 rpm for 5 minutes). After discarding the supernatant, the sediments were inspected under a microscope with 10X and 40X objectives.

3.3.2.3 Saturated salt floatation

After being filtered via a strainer into a 15 ml centrifuge tube, the fecal samples were thoroughly mixed in 12 ml of 0.9% w/v sodium chloride (NaCl). Then the filtrate was centrifuged (1200 rpm for 5 minutes). The tube was filled with 45% w/v NaCl after discarding the supernatant. The centrifuge tube was then centrifuged at 1200 rpm for 5 minutes. The tube was then completely filled with saturated NaCl and left undisturbed for 10 minutes by covering the rim of the tube with coverslip. Finally, the coverslip was removed and kept on a clean glass slide, and examined under the microscope with 10X and 40X objectives. (Adhikari et al., 2021).

3.3.3 Identification

Following the microscopic analysis of unstained and stained fecal slides with 10X and 40X objectives, the cystic and trophozoite phases of protozoans, along with the egg and larva stages of helminths, were identified by using a medical laboratory manual and specialists. Eggs and oocysts were identified based on their morphological traits, which included size, shape, shell content, color, external features and hooks (Soulsby, 1982).

3.3.4 Questionnaire survey

A short list of questionnaires was constructed addressing participant's behavioral, socioeconomic conditions, and socio-demographic information. (See Attached Appendix 1).

3.4 Ethical considerations

Ethical approval for this study was obtained from the Institutional Review Committee (IRC) of Institute of Science and Technology (IOST) of Tribhuvan University. (Approval number: IRCIOST-23-0061, see attached appendix 2).

3.5 Data analysis

All behavioral, demographic, and socioeconomic questions were coded, and the distributions of all variables were investigated. An Independent t-test was used for multiple group comparisons, analyzing continuous data and categorical data was analyzed using Chi-square/Fisher's Exact test. To investigate the association between the prevalence of overall IPIs and possible risk factors (i.e., hygiene behaviors, socio-demographic characteristics, and community), Multivariable logistic regression was used. The level of significance was set at $p<0.05$. All analyses were performed using Statistical analysis using SPSS Version 25.

4. Results

The study was carried out on the people living in the squatter community in Butwal. From October 2023 to November 2023, 170 stool samples of people above the age of 18 years and of both genders were collected and analyzed.

A total of 170 fecal samples were analyzed under the microscope with various methods as mentioned in method. Out of six identified species of parasites, 3 (i.e., *Entamoeba histolytica, Giardia lamblia, Cryptosporidium*) species belong to protozoa, 3 species to helminths (i.e., *Ascaris lumbricoides, Trichuris trichiura* and hookworms). Overall, *A. lumbricoides* has higher prevalence (21.2%) followed by *Trichuris trichiura* (2.9%), and hookworms (1.2%) as shown in (Figure 2).

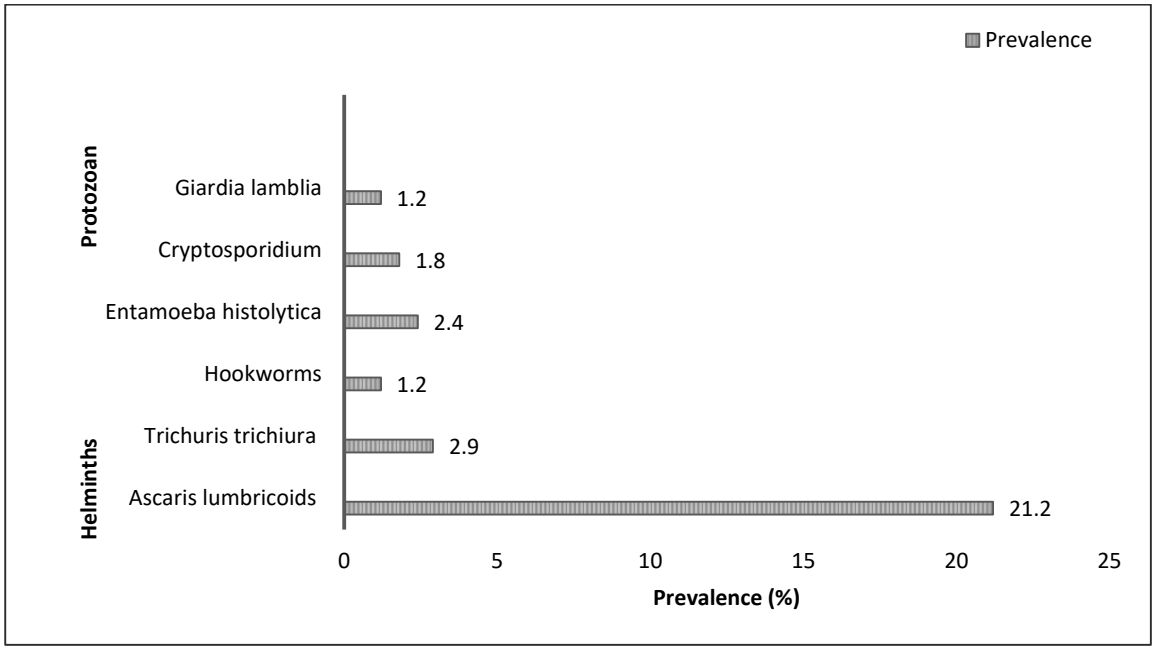

Figure 2. Bar graph showing the prevalence of the protozoan and helminth parasites found among the people of squatter community of Butwal.

More than a quarter samples (i.e., 49 (29%)) found infected with one or more species of IPIs (Table 1). Female participant indicated significantly high *T. trichiura* prevalence compared to male (Fisher exact test $p<0.030$) (Table 1). One out of 4 samples (i.e., 25%) indicated helminth parasites and while 5.3% samples indicated protozoan parasites. Three individuals (2%) indicated multiple infections where two (i.e., 2.4%) were male. Except *T. trichiura,* none of parasite or categories indicated any statistical difference between male and females.

Table 1. Prevalence of gastrointestinal parasites in participants (n = 170).

Parasite species	Male (%)	Female (%)	Chi-Square P value	Total n (%)
Protozoan Parasites				
Entamoeba histolytica	2 (2.4)	2 (2.3)	NS#	4 (2.4)
Giardia lamblia	1 (1.2)	1 (1.2)	NS#	2 (1.2)
Cryptosporidium	1 (1.2)	2 (2.3)	NS#	3 (1.8)
Helminth Parasites				
Ascaris lumbricoides	18 (21.4)	18 (20.9)	NS*	36 (21.2)
Trichuris trichiura	**0 (0)**	**5 (5.8)**	**0.03#**	**5 (2.9)**
Hookworms	2 (2.4)	0 (0)	NS#	2 (1.2)
Total infection	22 (26.2)	27 (31.4)	NS*	49 (28.8)
Total protozoan infection	4 (4.8)	5 (5.8)	NS#	9 (5.3)
Total helminth infection	19 (22.6)	23 (26.7)	NS*	42 (24.7)
Multiple Infection (2)	2 (2.4)	1 (1.2)	NS#	3 (1.8)

* Chi-square test, # Fisher Exact test while any cell has count less than 5.

The study participants were mostly of middle-age with a mean age of 41.87 years, having older male participants as compared to female participants in the study as illustrated in (Table 2). Women participants reported better self-rated health compared to male participants. Yet, all parameters including age, and self-rated were not significantly different between males and females. Similarly, socio-economic factors were also not significantly different between male and female participants. However, more illiteracy was observed in female participants as compared to male participants but poorer SES (Socio-economic status) was reported by male participants compared to female participants. Yet, 18% of participants reported that they did not have a closed type of toilet at home, and were using an open type of Toilets. Most of the participants reported neither pets nor livestock in their houses.

Table 2. Characteristic features of study participants (n = 170).

Characteristics	Male (n=84)	Female (n=86)	P-value	Total
Demographic Characteristics	Mean (SD)/n (%)	Mean (SD)/n (%)		Mean (SD)/n (%)
Age (in years)	43.52 (15.37)	40.26 (13.76)	NS$^{\$}$	41.87 (14.63)
Self-rated health (1 minimum-5 maximum)	3.73 (0.63)	3.81 (0.68)	NS$^{\$}$	3.77 (0.65)
Socioeconomic (SES) Characteristics				
Age Category				
Below 50	54 (64)	63 (73)	NS*	117 (69)
Above 50	30 (36)	23 (27)		
Can you read and Write?				
Yes	42 (50)	40 (46.5)	NS*	82 (48)
No	42 (50)	46 (53.5)		88 (52)
Self-reported SES				
Weak	45 (54)	48 (56)	NS	93 (55)
Very Weak	39 (46)	38 (44)		77 (45)
Do you Rear Pets in the House?				
No	69 (82)	71 (83)	NS*	140 (82)
Yes	15 (18)	15 (17)		30 (18)
Do you Rear Livestock as a Smallholder?				
No	61 (73)	65 (76)	NS*	126 (74)
Yes	23 (27)	21 (24)		44 (26)
Do you have your toilets in your home?				
Yes, I have a closed-type toilet	66 (79)	73 (85)	NS*	139 (82)
No, I have an open-type toilet	18 (21)	13 (15)		31 (18)

$: Independent T-test, *Chi-square test, $^{\#}$ Fisher Exact test while any cell has count less than 5

(Figure 3) shows the behavioral and lifestyle characteristics of study participants. There was no significant difference between male and female participants in any behavioral and lifestyle characteristics. Most of the participants reported a non-vegetarian diet, and most of them did not bite nails, use soap for hand washing, have knowledge about helminths, did not get frequent diarrhea, and covered food after cooking. However, about one out of three participants did not trimmed their nails on a regular basis and about half of the participants did not wash green vegetables and fruits before eating. About a quarter percentage of participants walked barefoot when outdoors, and consumed raw meat.

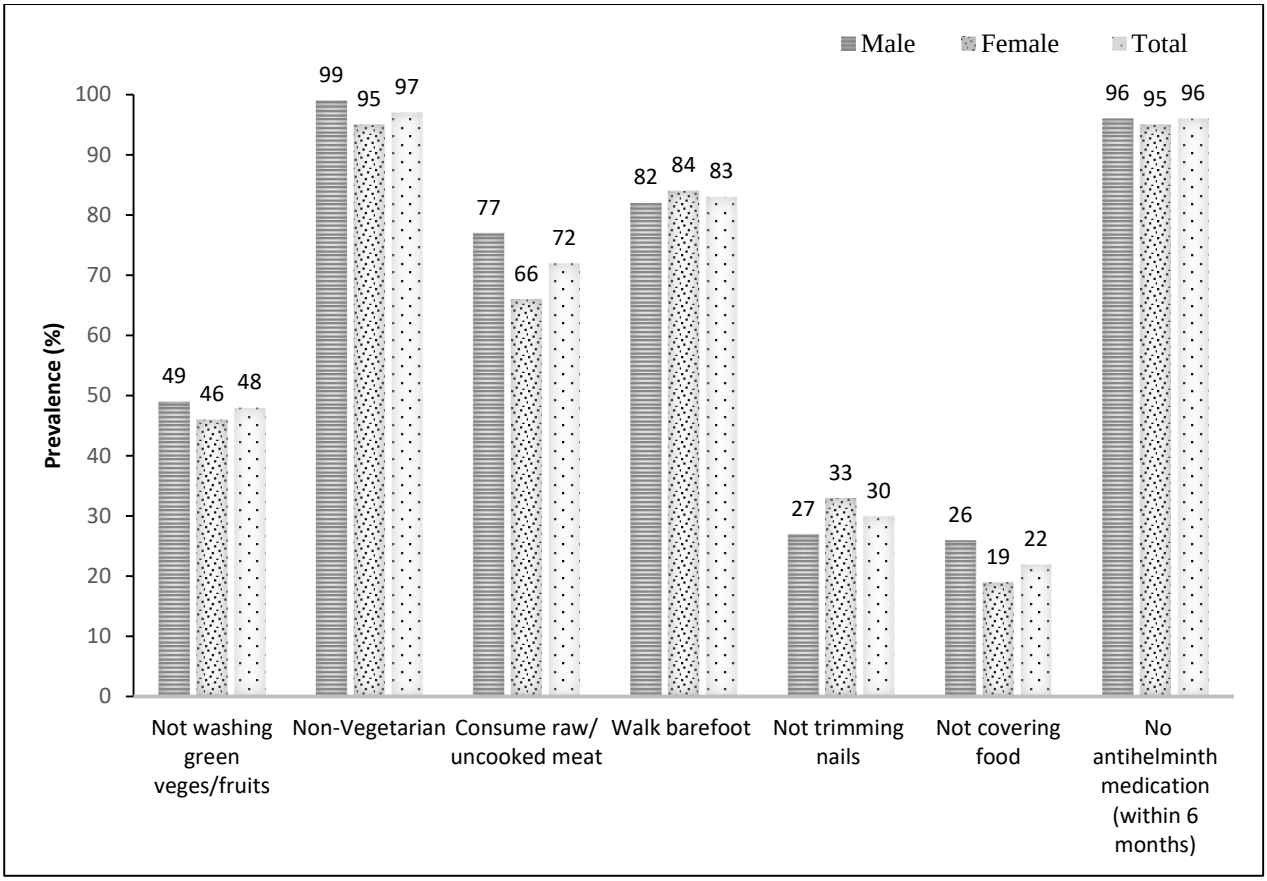

Figure 3. Bar graph representing behavioral, lifestyle and health status characteristics of the study participants.

The presence of any parasitic infection was higher among participants who did not trim nails once a week compared to participants who did rarely ($p < 0.001$) as shown in (Table 3). This study also shows that IPIs were significantly prevalent among people who own open-type toilets compared to closed type. Yet, both were borderline associations in univariate as well as adjusted multivariate models. However, prevalence of IPIs was not associated with any demographic, SES, lifestyle, or other behavioral characteristics evaluated.

Table 3. Prevalence and odds ratio of IPIs with respect to behavioral characteristics using logistic regression analysis (n=170).

	Any IPIs (*n* =170)		
		Univariate	Multivariate*
	%	OR (95%CI)	AOR (95%CI)
Socioeconomic (SES) Characteristics			
Age Category			
Above 50	30.2	ref	ref
Below 50	28.2	0.91 (0.45 to 1.85)	0.99 (0.42 to 2.30)
Gender?			
Male	26.2	ref	ref
Female	31.4	1.29 (0.66 to 2.51)	1.23 (0.59 to 2.57)
Can read and write			
Yes (Literate)	28.0	ref	ref
No (Illiterate)	29.5	1.08 (0.55 to 2.09)	1.25 (0.54 to 2.87)
Reported SES			
Weak	28.0	ref	ref
Very Weak	29.9	1.10 (0.56 to 2.14)	0.72 (0.31 to 1.66)
Do you wash Greens?			
Yes	25.8	ref	ref
No	32.1	1.35 (0.70 to 2.64)	0.99 (0.46 to 2.14)
Are you vegetarian?			
Yes	40.0	ref	ref
No	28.5	0.60 (0.10 to 3.70)	0.33 (0.04 to 2.49)
Do you eat Meat Raw?			
No	25.0	ref	ref
Yes	30.3	1.31 (0.61 to 2.79)	1.28 (0.53 to 3.13)
Use of soap for hand washing			
Yes	50.0	ref	
No	28.3	0.40 (0.05 to 2.89)	
Walk barefoot while outdoor			
No	21.4	ref	ref
Yes	30.3	1.59 (0.60 to 4.21)	1.38 (0.46 to 4.16)
Do you rear livestock?			
No	27.8	ref	ref
Yes	31.8	1.21 (0.58 to 2.56)	1.37 (0.61 to 3.06)
Do you get diarrhea every month?			
No	27.9	ref	ref
Yes	31.8	1.21 (0.46 to 3.17)	0.92 (0.31 to 2.73)
Did you trim your nails regularly?			
Frequently	**22.7**	**ref**	**ref**
Rarely	**43.1**	**2.59 (1.28 to 5.21)**	**2.78 (1.25 to 6.19)**
Took anthelminthic within 6 months?			
Yes	28.8	ref	ref
No	28.6	1.01 (0.19 to 5.40)	0.82 (0.13 to 5.22)
What type of Toilet do you ow			
Closed	**25.9**	**ref**	**ref**
Open	**41.9**	**2.07 (0.92 to 4.64)**	**2.29 (0.89 to 5.92)**

OR: Odds Ratio, AOR: Adjusted Odds Ratio, 95%CI: 95% confidence interval, prevalence percentage, ref: reference

*Model adjusted for all variables, Model Fit p <0.05, Significant, Negelkerke R square: 0.11

5. Discussion

This study evaluated the prevalence of IPIs on the participants of squatter community in Butwal. The overall prevalence of IPIs in our study (i.e., 28.8%) is closely comparable with the prevalence of IPIs as reported from other different studies as 25.88% prevalence was observed among the slum people of Southern Delhi (Dudeja et al., 2012), 25% among Brazilian urban slum (Ignacio et al., 2017), and 27.1% in slum area of Kathmandu (Bhattachan et al., 2017). As compared to our study, a lower prevalence of IPIs has also been reported among 428 participants (15.2%) in the urban setting of Cameroon (Kuete et al., 2015). Many earlier investigations, however have indicated high frequency of intestinal parasitic infections. For example, Chongbang et al. (2016), reported a 48% prevalence in Squatter of Dharan, *Giardia* being the most prevalent parasite followed by *Ascaris lumbricoides*. A similar study done by Tiwari et al. (2018) in Kaski reported a high rate of intestinal infection in slum-dwellers (24.1%) compared to the study done by Gil et al. (2013) (20.2%). This type of variation in the prevalence of IPIs might be attributed to different levels of awareness and varying climatic conditions (Adhikari et al., 2021).

Despite of ongoing routine deworming programs, the higher prevalence of IPIs among our study participants may be partially explained by poor sanitation in squatter communities observed by researchers during fieldwork and existing unhealthy hygiene behaviors. For example, one out of three participants did not trim their nails on a regular basis and about half of the participants did not wash green vegetables and fruits before eating. About a quarter percentage of participants walked barefoot when outdoors, and consumed raw meat. However, the majority of the participants reported healthier, behavioral and lifestyle characteristics too (i.e., handwashing before eating, etc.), knowledge of IPIs, and covering food. Hence, further study needs to be conducted to confirm this finding.

The overall prevalence of IPIs and other individual parasites was similar among males and females. Yet, females indicated higher *T. trichiura* infections compared to males. Several previous investigations revealed comparable results including higher *T. trichiura* prevalence in females as compared to males (Jamali et al., 2020). Yet, few studies reported a similar risk of *T. trichiura* between males and females in squatter communities (Mukutmoni & Khanum, 2018). In addition, a study by (Zeleke et al., 2020) reported a low prevalence of *T. trichiura* among females (Ignacio et al., 2017;Jamali et al., 2020). This may be due to geographical differences and the socio-economic and sanitary status of the study population.

In this study, although there was no statistical difference between males and females for any behavioral and lifestyle characteristics (i.e., handwashing before eating and wearing footwear while outdoors) but more women reported walking barefoot while outdoors and did not trim nail regularly, which may explain such discrepancy in *T. trichiura* infection by gender. Yet, the small sample size limits us to generalize the findings. Hence, further research is needed to validate this association.

This study also indicates a higher prevalence of IPIs among participants who do not own closed toilets. However, the association was borderline. Our findings agreed with previous studies by (Shobha et al., 2013) that reported a significant association of IPIs with toilet ownership. This is probably because the variables were proxy measures of poor socio-economic status, sanitation, and hygiene in general. Further, the odds ratios were always consistently greater for both univariate and multivariate models after adjustment. Since the participants are from squatter communities in Butwal, poor socio-economic status, sanitation and hygiene in general might be crucial among vulnerable populations.

6. Conclusion and recommendations

6.1 Conclusion

IPIs among one out of four participants from vulnerable populations like squatter communities in Butwal may pose a serious health problem. Multivariate logistic regression analysis showed that the IPIs were attributable to individual differences in behavior like "nail trimming habits". Simple health education regarding such crucial hygiene behaviors might contribute significantly to reducing the parasitic burden in urban areas like Butwal where lots of people might be at risk of parasitic infections and transmission.

6.2 Recommendations

- The status of the toilet should be maintained properly, i.e. closed type of toilet.
- Educate the public on enhanced personal and ecological hygiene, and improve sanitation control methods at both the home and community level.
- Routine medical examination, stool testing, and medication distribution must be done.
- Prioritize collaboration between public servants and health professionals to promote the environmental condition of the squatter community.

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Appendices

Appendix 1.

Questionnaires

Baseline Questionnaires related to demographic, socioeconomic and behavioral factors.

Participant Code/ Name:
Gender: ☐Male ☐Female Age:

1. In general, how would you rate your health on a scale of 1 to 5?
☐1 ☐2 ☐3 ☐4 ☐5

2. What type of drinking water do you prefer?
☐Tap water ☐Jar water ☐Boiled water ☐Filtered water

3. How frequently do you maintain your hygiene?
☐Always ☐Nearly always ☐Nearly never ☐Never

4. What is your Occupation?
☐Govt. Employee ☐Business ☐Farmer ☐other specify

5. Do you use soap to wash your hands before eating?
☐ Yes ☐No, but with water ☐sometimes ☐Spoon

6. Do you cut and clean your nails once a week?
☐ Yes ☐No ☐sometimes

7. Do you eat any fruits or green vegetables without washing?
☐Yes ☐No ☐sometimes

8. Do you wear foot ware while outdoors?
☐Yes ☐No ☐sometimes not
9. How many family members are in House?

10. Do you cover food from flies?
☐Yes ☐No ☐sometimes

11. Do you bite fingernails?
☐Yes ☐ No ☐sometimes

12. Do you play with Soils?
☐ Yes ☐No ☐sometimes

13. Do you eat food (any) dropped on the floor?
☐Yes ☐No ☐sometimes

14. Do you drink Boiled water?
☐Yes ☐No ☐sometimes
15. Do you know at least a way to prevent intestinal helminthiasis?

16. Did you consume any medication for intestinal helminths parasites in the last 6 months?
☐Yes ☐ No

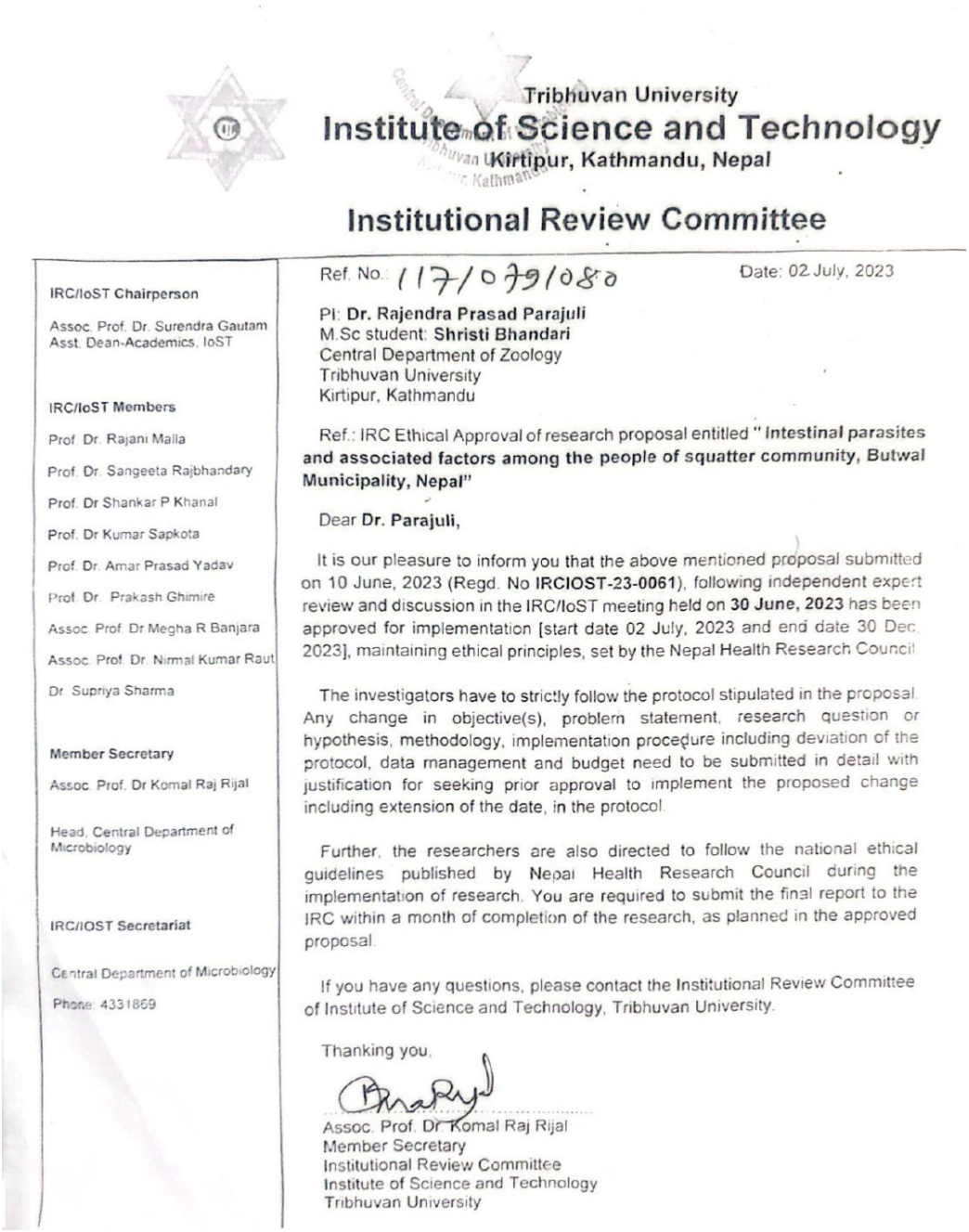
17. Do you have free-ranging pig or poultry in the house?
☐ Yes ☐ No

18. Did you notice any worm in your stool?
☐ Yes ☐ No
19. How frequently have you experienced diarrhea or abdominal discomfort in a month?

20. Do you ever consumed raw meat?
☐ Yes ☐No ☐Maybe

Appendix 2.

Ethical Approval of the study taken from IoST, Kirtipur, Nepal.



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

Institutional Review Committee

IRC/IoST Chairperson
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Ref. No.: 117/079/080 Date: 02 July, 2023

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

Ref.: IRC Ethical Approval of research proposal entitled **" Intestinal parasites and associated factors among the people of squatter community, Butwal Municipality, Nepal"**

Dear **Dr. Parajuli,**

It is our pleasure to inform you that the above mentioned proposal submitted on 10 June, 2023 (Regd. No **IRCIOST-23-0061**), 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.

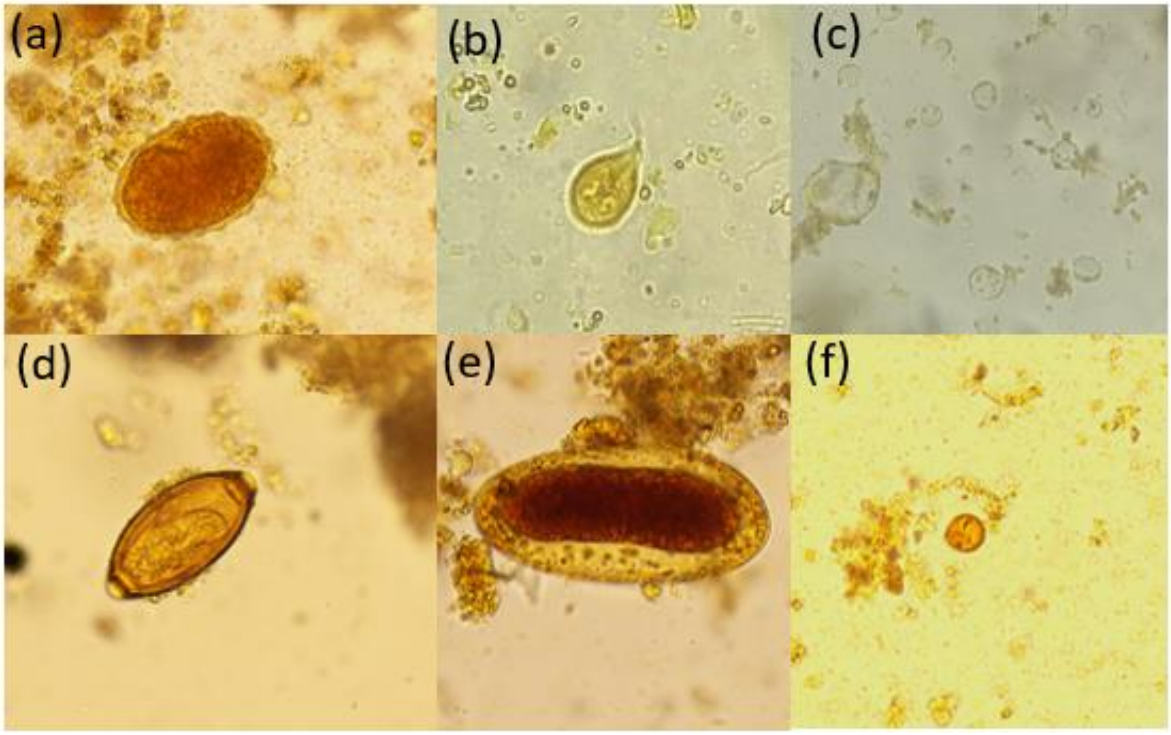
Further, the researchers are also directed to follow the national ethical guidelines published by Nepal Health Research Council during the implementation of research. You are required to submit the final report to the IRC within a month of completion of the research, as planned in the approved proposal.

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

8. Photographs



(a) Egg of *Ascaris lumbricoides* (b) Trophpzoite of *Giardia lamblia* (c) Cyst of *Entamoeba histolytica* (d) Egg of *Trichuris trichuria* (e) Egg of Hookworm (f) Oocyst of *Cryptosporidium*