INTESTINAL PARASITIC INFECTIONS AMONG SCHOOL GOING CHILDREN IN HETAUDA, NEPAL

Rameshwor Parajuli

T.U. Registration No: 5-2-242-825-2015

T.U. Roll No: 839/076

Batch: 2076

2023/5/14

A thesis submitted in partial fulfillment of the requirements for the award of the degreeof Master of Science in Zoology with special paper Parasitology.

Submitted To:

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

May, 2023

DECLARATION

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

Date: 03/05/2023

Signature

Rameshwor Parajuli

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.

☎ ०१-४३३१८९६
01-4331896
Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology
T.U., Kirtipur

Entry
M.Sc. Zoo Dept. .....
Signature .....
Date: .....

RECOMMENDATIONS

This is to recommend that the thesis entitled **"INTESTINAL PARASITIC INFECTIONS AMONG SCHOOL GOING CHILDREN IN HETAUDA, NEPAL"** has been carried out by Rameshwor Parajuli for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

Date: 03/05/2023

Signature

Rajendra Prasad Parajuli, PhD

Associate Professor

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.

☎ ०१-४३३१८९६
01-4331896
Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology
T.U., Kirtipur

LETTER OF APPROVAL

On the recommendation of supervisor, Dr. Rajendra Prasad Parajuli, this thesis submitted by Rameshwor Parajuli entitled **"INTESTINAL PARASITIC INFECTIONS AMONG SCHOOL GOING CHILDREN IN HETAUDA, NEPAL"** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

Date: 03/05/2023

Signature

Prof. Kumar Sapkota, PhD

Head of Department

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.

☎ ०१-४३३१८९६
01-4331896
Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology T.U., Kirtipur

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Rameshwor Parajuli entitled **"INTESTINAL PARASITIC INFECTIONS AMONG SCHOOL GOING CHILDREN IN HETAUDA, NEPAL"** has been accepted as a partial fulfilment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

EVALUATION COMMITTEE

Supervisor

Rajendra Prasad Parajuli, PhD

Associate Professor

Head of Department

Prof. Kumar Sapkota, PhD

External Examiner

Tirtha Raj Ghimire, PhD

Assistant Professor

Internal Examiner

Kishor Pandey, PhD

Associate Profesor

Date of Examination: 07/07/2023

ACKNOWLEDGEMENTS

First of all, I am grateful to the Central Department of Zoology (CDZ), for all the help provided during the course of my research work. I am thankful to current and former head of department (Prof. Dr. Kumar Sapkota and Prof. Dr. Tej Thapa) for the continuous support, and encouragement during my field visit. I am thankful to my supervisor Dr. Rajendra Prasad Parajuli, Associate Professor of CDZ for supporting me in design, planning, execution of this research work until final interpretation and thesis writes up. I am thankful to the Institutional Review Committee of institute of science and technology (IRC of IOST) for providing ethical approval on time. I am grateful to my colleagues for their continuous encouragement during the thesis writing.

I am thankful to the Laboratory of Global hospital, Gwarko, Kathmandu for the assistance during the laboratory examination of samples. I am thankful to Mr. Sandeep Thapa, the lab incharge, for his help in the laboratory examination of samples.

Last but not the list, I am very thankful to the students, teachers, management committee, and respective principals of Hetauda Academy, Mahendra Kiran, Shiksha Bikash, Sunshine Boarding School and Makwanpur Multiple Campus for their participation in this study, support, and cooperation for the samples collection, and transportation.

Contents

LIST OF TABLES

List of Figures

LIST OF PHOTOGRAPHS

LIST OF APPENDICES

LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviation
TU	Tribhuvan University
CDZ	Central Department of Zoology
IOST	Institute of Science and Technology
IPIs	Intestinal Parasitic Infections
OWOB	Overweight and Obese
DBM	Double Burden of Malnutrition
HOD	Head of Department
IRC	Institutional Review Committee
WHO	World Health Organization
LMICs	Low and Middle Income Countries
BMI	Body Mass Index
DHS	Demographic Health Survery
STHs	Soil Transmitted Helminths
rpm	Revolution per minute
CDC	Center for Disease Control and Prevention

Abstract

The double burden of malnutrition (DBM) characterised by the co-existence of undernutrition along with overweight and obesity within populations is being a major problem in the developing world. Almost all countries are affected by obesity. With existing undernutrition in developing world, the global prevalence of obesity is increasing rapidly. Many earlier studies reported high prevalence of intestinal helminths in different groups of population. Some studies reported an association of intestinal parasitic infection (IPIs) with undernutrition while few recent studies reported inverse association of IPIs with overweight and obesity. This study evaluated the comparative risk and associated factors of IPIs among under [body mass index (BMI) <18.5], normal (BMI 18.5-24.9) and overweight (BMI>24.9) school aged adolescents. Anthropometric measurements were done for nutritional indicators and stool samples collected and examined for IPIs. Structured questionnaire survey was used to evaluate socio-economic, lifestyle, physical activity, and demographic information. One hundred five (n=105) school aged adolescents were selected matching their age, gender, and BMI categories. The concentration methods were used to assess the prevalence and intensity of IPIs in this population. Overall prevalence of IPIs was 5.71% with 3 parazoan (i.e., 2.86% *Giardia lamblia,* 1.90% *Entamoeba histolytica* and 0.95% *Endolimax nana*). Univariate and multivariable regression analysis indicated none of Social Economic Status (SES), demographic, lifestyle behavioral characteristics were significantly associated with the prevalence of overall IPIs. Yet, significant numbers of male reported undernutrition and elevated risk of IPIs in this study population.

1. Introduction

A living entity is referred to be a "parasite" if it occupies another organism (the "host") and feeds off of it. Ectoparasites, endoparasites, facultative parasites, obligatory parasites, and many more types of parasites can cause harm to humans. The prevalence of intestinal parasites is widespread. One of the main causes of public health issues worldwide, particularly in impoverished countries like Nepal, is intestinal parasitosis. Worldwide, 450 million individuals are suffering from intestinal parasitosis, the majority of them are children, and approximately 3.5 billion people are affected (Hajare et al., 2021). Parasites can mechanically injure their hosts by excavating into them or sifting through adjacent tissue, in addition to inducing negative inflammatory or immunological reactions. According to Taliaferro (2009), numerous of these conditions are brought on by most parasites. The most common IPIs detected in Nepal include *Ascaris lumbricoides, Hymenolepis nana,* Hookworm*, Trichuris trichiura, Giardia lamblia, and Entamoeba histolytica* (Dahal et al., 2022). According to Chandrashekhar et al. (2009), general poverty, poor environmental hygiene, and insufficient access to healthcare are all strongly linked to the high prevalence of these IPIs.

In the context of a shifting global sustenance landscape, predisposed by economic and income growth, urbanization, demographic change and globalization, nutritional epidemiology has seen a significant swing in recent decades. Malnutrition, in all its forms, comprises undernutrition as well as overweight and obesity (OWOB), affects all countries of the world (Fanzo et al., 2018). Many countries face a double burden of malnutrition (DBM), where both undernutrition and OWOB exist in the same population, even in same household (Popkin et al., 2020). Globally by 2020, 1.9 billion adults are OWOB, while 462 million are underweight (Dukhi, 2020). These mostly occur in low- and middle-income countries (LMICs). At the same time, in these same countries, rates of childhood overweight and obesity are rising. Double burden of malnutrition (i.e., both OWOB and undernutrition) is the burning problem globally. Specially in the context of our country which is still underdeveloped, this DBM can be devastating. If we could even understand one aspect of this problem it would be

truly contributing, which this study aims at. This study aims at finding the association between helminthiases and nutritional status. This study is likea dot on a paper which is merely one step towards the solution of the burning problem DBM but still holds a huge significance.

Studies commenced in Nepal have shown that although the prevalence of undernutrition in the population has mostly diminished over the last decades but the prevalence of OWOB hasincreased considerably (Balarajan and Villamor, 2009; Wei et al., 2019). Earlier 3 National surveys of (i.e., Demographic Health Survey (DHS) Nepal 2016, Micronutrient Survey 2016 and the STEPS survey 2019)), calculated that overall, 14.5–17% of the Nepalese adult women were underweight, while 22–25% were OWOB (Hasan et al., 2018; Mehata et al., 2021). Among men,17% were underweight and 17–23.4% were OWOB (Hasan et al., 2018; Mehata et al., 2021). In Nepal DHS 2016, there was no difference in the prevalence of OWOB between ecological zones or between rural vs. urban populations (Rawal et al., 2018). A nationwide survey of Nepal in 2016 showed that 31.16% population (women 38.87% and men 28.77%) were obese. In this survey study, 13,542 adults aged 18 years or above had their height and weight measured.

Overweight and obesity are associated with several other adverse health outcomes, including diabetes mellitus type 2, cardiovascular disease, certain types of cancer, musculoskeletal and mental disorders as well as pregnancy complications (Black et al., 2013; Collaborators, 2017; Schwinger et al., 2020). Amongst others, undernutrition is associated with an increased risk of infection (Harpsøe et al., 2016). The double burden of malnutrition (DBM) is characterized by the coexistence of undernutrition along with overweight and obesity, or diet-related non communicable diseases, within individuals, households and populations, and across the life course. In 2020, more than 1.9 billion adults worldwide, 18 years and older, were overweight while 462 million were underweight. More than 600 million were obese (Hashan et al., 2020). In the same year, 42 million children under the age of five were overweight or obese but 156 million were affected by stunting (low height-for-age). While 50 million children

were affected by wasting (low weight-for-height). Poor nutrition continues to cause nearly half of deaths in children under five, while low- and middle-income countries now witness a simultaneous rise in childhood overweight and obesity – increasing at a rate 30% faster than in richer nations (Hossain et al., 2020). Both types of population, OWOB and underweight, are at equal risk of parasitic infection. Not only BMI but hygiene habit and co-morbidities underlying disease also play role on parasitic infection risk (Dobner & Kaser, 2018). One study shows that GI parasites infection is high among underweight children of below 13 years age and severe stunting is high among girls than among boys (Amare et al., 2013).

Many studies on IPIs among school-aged children in Nepal have found prevalence rates ranging from 15% to 60%, with significant fluctuation over time and across the country (Kumar et al., 2021). Considering high prevalence of IPIs in Nepal with high DBM, this study aims to evaluate the comparative risk and associated factors of IPIs among under [body mass index (BMI) <18.5] and overweight (BMI>24.9) school aged adolescents. This study aims at finding the risk factors associated with helminthiases in overweight and underweight school aged adolescents. Further, studies have suggested an association of intestinal parasites with poor performance at school (Guan and Han, 2019; Akubuilo et al., 2020). Hence, this study will also see if the effect of IPIs in school performance altered among under or overweight participants.

Objectives

General objective

- To examine risk and associated factors of IPIs among Overweight and Obese (OWOB) participants compared to undernourished participants (i.e., school aged adolescent).

Specific objectives

- To investigate occurence and risk factors of IPIs in OWOB participants compared

to undernourished participants (i.e., school aged adolescent).

- To evaluate association between risk and associated factors of IPIs among OWOB participants compared to undernourished participants [i.e., school aged (10-19) adolescent] adjusting other suggested demographic variables.

2. LITERATURE REVIEW

Few studies reported association of certain viral and bacterial infections among participants with obesity (Atkinson, 2008; Dhurandhar, 2011; Yang et al., 2013). Only study evaluated associationbetween IPIs with obesity in Mexican children and adolescents and reportedhigher probability of IPIs among participants with higher BMI in the same year, 6, and 12 years later in life (Zavala et al., 2019). This study clearly shows a strong relationship between the parasitic presence and nutritional status. Not only this, but the study has also been done looking for later phase in life which adds more value to the literature itself. Similar interpretation (early child"insults" including infections to be associated to later overweight and obesity) was reported (Anguita et al., 1993; Picó et al., 2012; Guerrant et al., 2013). Early childhood caught infections from parasites eventually contributed to the OWOB later in life. This study is not only aware about the early childhood prevention of infection but also the possible risk and consequenceslater in the life.

Recent animal study reported helminth-dependent protection against obesity (Su et al., 2018). The study outcome contradicts with all the above study. Though the study is done on animals, the result obtained cannot be denied completely. Further study is necessary to meet a concluding statement regarding helminthes provokes or protects humans from obesity. Many studies on intestinal parasite infection among school-aged children in Nepal have found prevalence rates ranging from 15% to 60%, with significant fluctuation over time andacross the country. Nepal is an underdeveloped country with lack of knowledge about sanitation, hygiene, parasites and its effect on health. Such a high prevalence opens a whole new area of research and study.

Studies have suggested an association of IPIs with poor performance at school (Guan and Han, 2019; Akubuilo et al., 2020). School performance of adolescents does not only correlate with the grade or academic performance but also to the agility, presence of mind in classes, attendance, peer interaction, problem solving andstudent teacher interaction. A study done in Venezuela shows that 26.8%

households have DBM and also DBM households significantly linked with *G. lamblia* and Geohelminths (Campos Ponce et al., 2013). Thus, triple burden of disease seen, a very perturbing problem. A study in Mexico rural area shows that *Entamoeba coli* infection may contribute to fat deposition (Zavala et al., 2016). The same study shows that *Ascaris lumbricoides* as the most prevalent soil transmitted helminth (16%) followed by hookworm. *Entamoeba coli* was the predominant protozoa (20%) followed by *Endolimax nana, Balantidium coli, Entamoeba histolytica/dispar*, *Iodamoeba bütschlii* and *Giardia lamblia*. Most concerned protozoans are *Cryptosporidium*, *Giardia* and *Entamoeba* leading to stunting in children according to a recent metanalysis done (Hajissa et al., 2022). Protozoan parasites are widely spread and consist of a single cell-like unit that is morphologically and functionally complete (Chaterjee, 2009).

Giardia lamblia, Entamoeba histolytica, Entamoeba coli, Cryptosporidium, Cyclospora, Isospora, and Balantidium coli, are some common intestinal protozoan parasites. Interestingly, a very recent cross sectional study done among obese population shows that parasites do not correlate with nutritional deficiencies and body composition, but correlate to metabolic syndrome (Caudet et al., 2022). A research study in school children in Ethiopia concludes that roundworm, hookworm, and whipworm has no association with BMI (i.e., nutritional status) (Desta, 2014). A recent study done in Nepal to see the prevalence of STHs (Soil Transmitted Helminths) in 5 major community reveals that STH infection was associated with individual hygiene behavior, but not with nutritional status or socio-demographic characteristics (Parajuli et al., 2014). The studies previously done about the association of nutritional status with GI parasites have no linear results. They contradict with each other, which is obviously common in research. Yet, as IPIs and DBM actually exist together in many scenarios, further research is warranted in this research topic.

Numerous factors, including intestinal parasite infections, can cause serious problems in life. Low socioeconomic position is regarded to be the primary cause of IPIs, as are inadequate hygiene (Khanal et al., 2016), a lack of efficient sanitary disposal, a lack of clean drinking water (Rayapu et al., 2012), and a lack of health

education (Rashid et al., 2011). According to Karunaithas et al. (2011) and Sah et al. (2016), the act of washing one's hands with only water is also regarded to increase one's risk of contracting an intestinal parasite. Infection with an intestinal parasite connected to farming (Tandulkar et al., 2013). People who are unaware are more likely to contract parasites, according to some research (Pandey et al., 2015). Infections brought on by intestinal helminths and protozoan parasites are among the most prevalent in underdeveloped nations. More protozoan parasites than helminths are responsible for gastrointestinal illnesses in modern nations. In regions where they are endemic, IPIs significantly increase morbidity and mortality (Haque, 2007). Intestinal worm illnesses like roundworm, hookworm, and whipworm impact about one-fourth of the world's population (Else et al., 2020).

3. MATERIALS AND METHODS

3.1 Study area

Two schools (randomly selected 1 public – Mahendra Kiran Secondary School and 1 private – Hetauda Academy Secondary Residential School) were selected from Hetauda after consultation and approval with school authorities. Due to insufficiency of required number of sample students for our research, two more private boarding schools – Shiksha Bikas Shishu Sadan English Boarding School and Sunshine English Boarding School were added. All four schools are located in the periphery of Hetauda Sub-metropolitan city. Shiksha Bikas, Sunshine, and Mahendra Kiran are situated in the same ward, which is Hetauda-03. Hetauda Academy is located in Hetauda-11. All four schools run their classes from nursery to grade 10. Mahendra Kiran also has grade 11 and 12 as their regular classes.

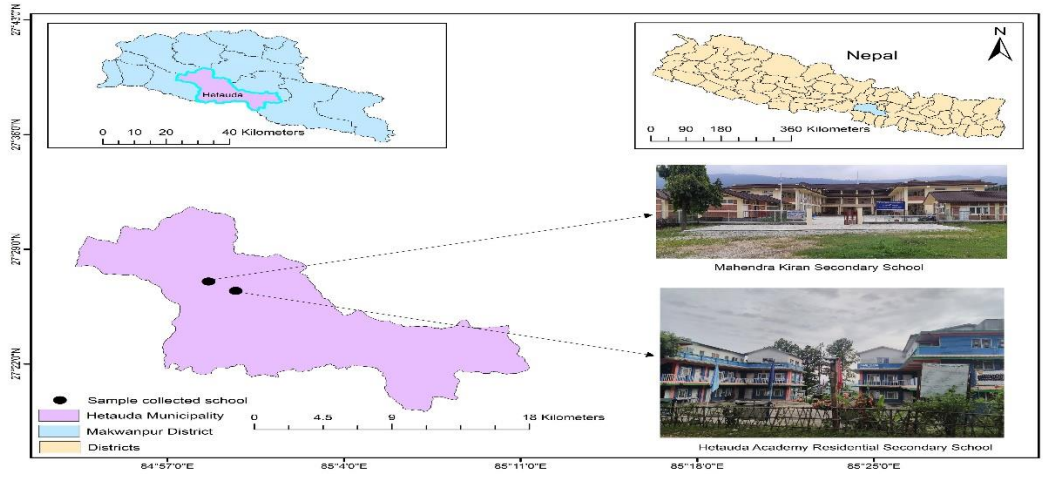


Fig 1: Map of Nepal showing study site

3.2 Study Method

First of all, all the selected schools were visited frequently to set up study camp in each school with minimum hamper in their study schedules. Each participants were explained about study protocol and asked if they and/or their parents (i.e., in case of non-adult participants) were agreed to participate in our study. They were

then asked to take the informed consent form with them for their guardians at home. Once signed informed consents were collected the day after distribution, questionnaires were then presented to the participants and parents separately in a different isolated place within the school premise. Participants were included in the study if they were of 6-19 years old and live in Hetauda for the last 3 years. While if potential participants reported recent (i.e., 1 month or less) intake of antihelminthics, or diagnosis of chronic disease like diabetes, they were excluded from study.

Assessment of nutritional status (height, weight, and BMI) was done by taking anthropometric measurements. BMI was evaluated with measured height and weight by using formula BMI = Weight (kg)/Height (m^2). Out of 319 eligible and agreed participants, 105 study participants were selected based on measured BMI following the screening criteria [i.e., 35 students were with body mass index (BMI) <18.5, 35 students were with normal BMI 18.5-24.9 and 35 overweight BMI>24.9] matching their grade, age, and gender equally from both public and private schools.

After the questionnairewas completed, sample students were asked to collect stool samples in the given vial along with wooden spatula and zipper bag. All vials were given unique code to avoid confusion and students names along with code were already noted before the vial distribution. Each collected samples were preserved in 2.5% potassium dichromate solution for the preservation of the parasites and brought to the laboratory of Central Department of Zoology, Tribhuvan University for microscopic examination.

3.3 Lab protocol and techniques

A strict lab protocol was followed during the lab processing. Direct wet mount, Saturated salt floatation technique, formal-ether sedimentation technique and acid fast staining technique were used for the laboratory examination of stool samples.

3.3.1 Direct wet mount

Direct wet mount was done first. Two grams (gms) of the fecal sample stirred carefully, a single drop of each sample then dropped on the glass slide with or without Gram's iodine. Then, by covering the sample with a coverslip, the sample was observed under a microscope at a total magnification of 100X and 400X (Adhikari et al., 2021).

3.3.2 Saturated salt floatation technique

In saturated salt floatation technique about two grams of the fecal sample was completely mixed in a 12 mL of 0.9% w/v sodium chloride (NaCl) following filtration through a strainer into a 15 mL centrifuge tube. The filtrate then proceeded to centrifuge at 1200 revolutions per minute (rpm) for 5 minutes. Then, the supernatant was discarded, and the tube filled with 45% w/v NaCl. The sample again centrifuged at 1200 rpm for more 5 minutes. Then, the centrifuge tube was completely and slowly filled with saturated NaCl and left undisturbed for 10 minutes covering its mouth with a coverslip. Finally, the coverslip was carefully removed and kept on a glass slide for microscopic examination at a total magnification of 100X and 400X with or without Gram's iodine. Thissaturated salt floatation technique is used to detect small eggs of parasites like nematodes and cestodes (Adhikari et al., 2021).

3.3.3 Formal-ether sedimentation technique

This is used for detecting large eggs of trematodes. Two gms of fecal sample were thoroughly mixed in 12 mL of 0.9%w/v NaCl in a 15 mL centrifuge tube. The sample then centrifuged (1200 rpm x 5 minutes) and the supernatant discarded. Then, 10 mL of 10% formalin and three mL of ethyl acetate were added in the tube for subsequent centrifugation at 1200 rpm for 5 minutes. Finally, the supernatant discarded, and the sediments examined under a microscope at a total magnification of 100X and 400X with or without Gram’s iodine (Adhikari et al., 2021).

3.3.4 Acid fast staining technique

Acid fast staining is used for detection of small protozoans eggs which are normally not detected even after the concentration technique done above. In acid fast staining, the sediments obtained following formal-ether method was used to prepare thin smears. The smears then dried at room temperature, fixed in absolute methanol for two minutes and then stained with carbol fucshin for 15 minutes. The smears then serially and gently washed with distilled water, acid alcohol, and distilled water for two minutes. The smears were restrained with malachite green for a minute and rinsed with distilled water later on. Finally, using immersion oil, the dry smears were observed under a microscope at a total magnification 1000X (Adhikari et al., 2021).

3.3.5 Parasites identification

The microscopic images compared with the images provided by Center for Disease Control and Prevention in its website (*CDC - DPDx - Parasites A-Z Index*, 2019).

3.4 Ethical considerations

The ethical approval for this study was obtained from the Ethical Committee of the institute of science and technology (IOST) of Tribhuvan University (approval no. 22-0024) and the selected schools supported our study.

3.5 Data Analysis

All questions were coded, and the distributions of all variables were examined. For multiple group comparisons (Table 1 and 2), independent t test was used for continuous data while a Chi-square test was used for analyzing categorical data. Multivariable logistic regression was used to investigate the association between prevalence of overall IPIs and possible risk factors, (i.e., hygiene behaviors, nutritional status, socio-demographic characteristics, and community). The level of significance was set at $p < 0.05$. All analyses were performed using the Statistical analyses were performed using IBM SPSS Statistic v25.

4. RESULTS

Table 1 illustrates that study participants mostly were teenagers male with the mean age of 14.24 years in the study. Female participants rated their health better than male participants though BMI, BMI category or other parameters were comparable with the male participants. Yet, height of male was higher than that of female, though age was similar between male and female participants. Similarly, none of the socio-economic factors were differed between male and female participants. GPA and perception about GPA were also similar between male and female participants and were from both private and public school, equally. Most of the participants reported households with less than 5 members in households, both parents can read and write with normal SES and frequent consumption of meat and fruits. Distribution of participants was not differed by schools and BMI category.

Table 1. Characteristic features of study participants (n = 105).

Characteristics	Male (n=63)	Female (n=42)	P-value	Total
	Mean (SD)/n (%)	Mean (SD)/n (%)		Mean (SD)/n (%)
Demographic Characteristics				
Age (in year)	14.27 (1.59)	14.19 (1.04)	NS$	14.24 (1.39)
Weight (Kg)	54.27 (18.16)	49.54 (12.60)	NS$	52.38 (16.26)
Height (Meter)	1.58 (0.11)	1.53 (0.06)	**0.010$**	1.56 (0.09)
Body Mass Index (BMI)(Kg/m2)	21.60 (6.24)	21.01 (4.76)	NS$	21.36 (5.67)
Self-rated health (Poor 1 to Excellent 5)	3.38 (1.01)	3.71 (0.71)	**0.049$**	3.51 (0.91)
School Parameter				
Grade (years)	8.08 (1.64)	8.38 (0.91)	NS$	8.19 (1.24)
GPA	2.49 (1.13)	2.21 (1.03)	NS$	2.37 (1.09)
Schools Participated				
Hetauda Academy (Private)	38 (36.19)	17 (16.19)		55 (52.38)
Mahendra Kiran Sec School (Pub)	17 (16.19)	19 (18.09)	NS*	36 (34.28)
Shiksha Bikas Shishu Sadan(Private)	2 (1.9)	2 (1.90)		4 (3.80)
Sunshine Boarding School (Private)	6 (5.71)	4 (3.80)		10 (9.52)
What do you think about your GPA?				
Ok	56 (53.33)	35 (33.33)	NS*	91 (86.67)
Not Good	7 (6.66)	7 (6.67)		14 (13.33)
Socioeconomic (SES) Characteristics				
Household (HH) Crowding				
Yes (≥5 member in HH)	12 (11.42)	10 (9.52)	NS*	22 (20.95)
No (<5 member in HH)	51 (48.57)	32 (30.47)		83 (79.05)
Reported SES				
Normal	59 (39.33)	38 (36.19)	NS*	97 (92.39)
Low	4 (2.67)	4 (2.67)		8 (7.61)
Do you consume Meat?				
Rarely	8 (7.61)	4 (2.67)	NS*	12 (11.43)
Frequently	55 (52.38)	38 (36.19)		93 (88.57)
Do you consume Fruits?				
Rarely	14 (13.33)	9 (8.57)	NS*	23 (21.91)
Frequently	49 (46.67)	33 (31.42)		82 (78.09)
Type of School now studying?				
Private	25 (23.80)	25 (23.80)	NS*	50 (47.62)
Public	38 (36.19)	27 (25.71)		55 (52.38)
BMI Category				
BMI <18.5 (Underweight)	22 (20.95)	13 (12.38)	NS*	35 (33.33)
BMI 18.5 to 24.99 (Normal)	18 (17.14)	17 (16.19)		35 (33.33)
BMI >25 (Overweight)	23 (21.91)	12 (11.43)		35 (33.34)

$: Independent T-test, * Chi square test

Table 2 showed behavioral and lifestyle characteristics of study participants, with risky behavioral and lifestyle characteristics (i.e., more than half participants did not use soap for handwashing before eating and walk barefoot while outdoor and did not trim nail regularly) in the study. Yet, most of participants reported recent consumption of anthelminthic drug, mostly covered food, did not consume dropped food, wash fruits and vegetables before eating. There was no significant different between male and female participants in different hygiene and lifestyle characteristics except consumption of pork and mothers occupation. Male

participants reported more pork consumption than female (p=0.05). More mother of female participants reported the farming as their main occupation than that of male participants (p<0.05).

Table 2. Behavioral, and lifestyle characteristics of study participants (n = 105).

Characteristics	Male (n=63)	Female (n=42)	Chi-Square P-value	Total
	n (%)	n (%)		n (%)
Behavioral/lifestyle characteristics				
Use of soap for handwashing				
Yes	24 (22.86)	16 (15.23)	NS*	40 (38.10)
No	39 (37.14)	26 (24.76)		65 (61.90)
Walk barefoot while outdoor				
No	26 (24.76)	23 (21.90)	NS*	49 (46.67)
Yes	37 (35.23)	19 (18.10)		56 (53.33)
Mothers Occupation				
Other	**48 (45.71)**	**24 (22.86)**	**0.039***	**72 (68.57)**
Farmer	**15 (14.29)**	**18 (17.14)**		**33 (31.43)**
Fathers Occupation				
Other	53 (50.47)	29 (27.62)	NS*	82 (78.10)
Farmer	10 (9.52)	13 (12.38)		23 (21.90)
Did you consume raw meat?				
No	57 (54.28)	41 (39.05)	NS*	98 (93.33)
Yes	6 (5.71)	1 (0.95)		7 (6.67)
Did you take anthelminthic within 6 months?				
Yes	54 (51.43)	35 (33.33)	NS*	89 (84.77)
No	9 (8.57)	7 (6.67)		16 (15.23)
Did you trim nails regularly?				
Yes	26 (24.76)	18 (17.14)	NS*	44 (41.90)
No	37 (35.23)	24 (22.86)		61 (58.09)
Did you notice worm in stool?				
No	29 (27.62)	23 (21.90)	NS*	52 (49.52)
Yes	34 (32.38)	19 (18.10)		53 (50.47)
Do you cover food regularly?				
Yes	55 (52.38)	37 (35.23)	NS*	92 (87.62)
No	8 (7.62)	5 (4.76)		13 (12.38)
Do you know about intestinal parasite?				
Yes	21 (20.00)	15 (14.29)	NS*	36 (34.28)
No	42 (40.00)	27 (25.71)		69 (65.71)
Do your pet entered to Kitchen?				
No	40 (38.09)	32 (30.47)	NS*	72 (68.57)
Yes	23 (21.90)	10 (9.5)		33 (31.42)
Do you consume dropped food?				
No	45 (42.86)	35 (33.33)	NS*	80 (76.19)
Yes	18 (17.14)	7 (6.67)		25 (23.81)
What type of water do you drink?				
Boiled or filtered	30 (28.57)	15 (14.28)	NS*	45 (42.86)
Jar	33 (31.42)	27 (25.71)		60 (57.14)
Do you wash fruits or green to eat raw?				
Yes	52 (49.52)	39 (37.14)	NS*	91 (86.67)
No	11 (10.48)	3 (2.86)		14 (13.33)
Do you consume pork?				
No	**38 (36.19)**	**33 (31.42)**	**0.05***	**71 (67.62)**
Yes	**25 (23.80)**	**9 (8.57)**		**34 (32.38)**

$: Independent T-test, * Chi square test

Table 3 shows prevalence of IPIs among study participants. A total of 105 faecal samples were evaluated under the microscope with different methods as described in method. Only 6 samples (5.71%) were found shedding 3 species of protozoa (i.e., *Giardia lamblia, Entamoeba histolytica and Endolimax nana*) (Table 3). Overall, *G. lamblia* has a higher prevalence (2.86%) followed by *E. histolytica* (1.9%), and *E. nana* (0.95%). Neither overall prevalence nor egg density differed by gender. Because of the small number of prevalence, statistical analysis was not run for individual parasites.

Table 3. Prevalence of gastrointestinal parasites in school aged participants (n = 105)

Parasite species	**Male** Mean (SD)/n (%)	**Female** Mean (SD)/n (%)	**Fisher's exact test P value**	**Total** Mean (SD)/n (%)
Giardia Lamblia	1 (0.95)	2 (1.90)	NS*	3 (2.86)
Entamoeba Histolytica	2 (1.90)	0 (0)	NA	2 (1.90)
Endolimax nana	1 (0.95)	0 (0)	NA	1 (0.95)
Infection density (0= not seen, 1= 1cyst per high power field(hpf), 2=2cyst per hpf	1 (0.50)	2.00 (0.00)	0.541$^{\$}$	1.83 (0.41)
Total infection	4 (3.81)	2 (1.90)	NS*	6 (5.71)

$: Independent T-test, * Fisher's exact test

Table 4 shows the association between parasitic infections and the associated factors. Presence of any parasitic infection was higher among participants from crowded household, who consumed pork, going to public school, who consumed raw meat, who do not use soap for hand washing before eating, underweight participants and male participants compared to their counterparts. Yet, none of the evaluated factors achieve statistical significance in univariate or multivariate model before or after adjustment to gender or BMI categories. This study also indicates that the prevalence of IPIs was significantly high among the undernutrition category compared to normal or overweight category. None of the evaluated demographic, SES, lifestyle, behavioral characteristics indicated any association with prevalence of IPIs in either uni/multi variate model.

Table 4. Prevalence and odds ratio of IPIS with respect to behavioral and individual characteristics using logistic regression analysis (n=105).

Socioeconomic (SES) Characteristics		Any IPIs (*n* =105) Univariate	Multivariate*
	%	OR (95%CI)	AOR (95%CI)
Household (HH) Crowding			
No (<5 member in HH)	4.82	ref	ref
Yes (>5 member in HH)	9.09	1.98 (0.34 to 11.56)	1.79 (0.29 to 10.90)
Can mother read and write?			
Yes (Literate)	6.49	ref	
No (Illiterate)	3.57	0.53 (0.06 to 4.78)	
Mothers Occupation			
Others	6.94	ref	
Farmer	3.03	0.42 (0.05 to 3.73)	
Fathers Occupation			
Others	6.09	ref	
Farmer	4.35	0.70 (0.08 to 6.31)	
Do you consume pork?			
No	5.63	ref	
Yes	5.88	1.05 (0.18 to 6.02)	
Types of School?			
Private	4.0	ref	ref
Public	7.27	1.88 (0.33 to 10.75)	3.08 (0.46 to 20.67)
Do you exercise regularly?			
Yes	5.55	ref	
No	5.88	1.06 (0.20 to 5.52)	
Do pet enter in Kitchen?			
No	5.55	ref	
Yes	6.06	1.10 (0.19 to 6.31)	
Do you consume raw Meat?			
No	5.10	ref	ref
Yes	14.28	3.10 (0.31 to 30.93)	3.37 (0.29 to 39.13)
Do you consume Fruits?			
Frequently	6.09	ref	
Rarely	4.34	0.70 (0.08 to 6.31)	
Use of soap for handwashing			
Yes	2.5	ref	ref
No	7.69	3.25 (0.37 to 28.88)	4.16 (0.43 to 39.82)
Walk barefoot while outdoor			
Yes	6.12	ref	
No	5.35	0.87 (0.17 to 4.51)	
BMI category			
Underweight	8.57	3.19 (0.32 to 32.24)	
Normal	2.86	ref	
Overweight	5.71	2.06 (0.18 to 23.83)	
Did you trim nails regularly?			
Frequently	6.82	ref	
Rarely	4.92	0.71 (0.14 to 3.68)	
Gender			
Male	6.34	ref	
Female	4.76	0.74 (0.13 to 4.22)	
Do you know about intestinal parasite?			
Yes	8.33	ref	
No	4.35	0.50 (0.10 to 2.61)	
What type of water do you drink?			
Boiled or filtered	6.67	ref	
Jar	5.00	0.74 (0.14 to 3.83)	

5. DISCUSSION

This study investigated the prevalence of IPIs among school aged adolescent from both private and public schools of Hetauda, Nepal. Prevalence of IPIs in our study (i.e., 5.71%) is comparablewith prevalence of IPI reported form different study. For example, Kunwar et al. (2016) reported a 4.12% prevalence of *E. histolytica* and 9.40% prevalence of *G. lamblia*. Similarly, Tandukar et al. (2013) reported 7.4% prevalence of *G. lamblia* followed by 3.4% prevalence of *E. histolytica* among more than 1300 stool samples evaluated from school children of Lalitpur district of Nepal. Comparable results were reported by Shrestha et al. (2012) among school children in Baglung districts of Western Nepal (i.e., *E. histolytica* (9.23%), *G. lamblia* (5.76%). In contrast, quite high prevalence of IPIs has also been reported by many previous studies. For example, a recent study (Shrestha et al., 2018) among schoolchildren in Dolakha and Ramechhap districts, Nepal reported quite high prevalence of IPIs. Such a discrepancy in the prevalence of IPIs might be due to the difference in the climatic conditions and different levels of awareness (Adhikari et al. 2021). The lower prevalence of IPIs in our study participants may be partially explained by ongoing routine deworming programs. In addition, most of participants reported healthier behavioral and lifestyle characteristics (i.e., frequent consumption of anthelminthic drug, mostly covering food, did not consume dropped food, wash fruits and vegetables before eating). However, small sample size limits us for any conclusion. Hence, further study needs to be conducted to confirm this finding.

Prevalence of overall IPIs was higher among males compared to females. Yet females indicated higher egg density compared to male, but both could not achieve statistical significance. A few earlier studies reported similar finding with elevated IPI prevalence among male compared to female. For example, in a study done among HIV patients it was found out that gender difference i.e., being male and diarrhea were strongly associated with an increased prevalence of intestinal parasites (Akinbo et al., 2010). Also, another study done in Islamic Republic of Iran found out that male gender was more infected with GI parasites in comparative to the female gender. Mainly *G. lamblia* was seen higher in male

gender and younger age group had more prevalence of infection than the older ones (Sayyari et al., 2005). Yet, few studiesreported similar risk of IPIs between male and female (Limbu et al., 2021). In this study, majorityof female reported healthier behavioral and lifestyle characteristics (i.e., less consumption of pork) compared to male participants ($p<0.05$), which may explain such discrepancy in IPIs infection by gender. Another study done in rural Nepal showed contrasting results to our study where younger girls were more infected (55.2%) than boys (44.8%). This study also found out that rural areas population were more infected by intestinal parasitic infections (52.3%) than urban areas (32.4%) (Bertoncello et al., 2021). Further, male indicated higher prevalence undernutrition and reported lower self-rated health compared to female, which may indicate weak nutritional as well as immunity for elevated IPIs infections. Yet, further study needs to be conducted to confirm this association.

This study indicated elevated risk of any parasitic infections (IPIs) among participants from crowded household, who consumed pork, going to public school, who consumed raw meat, who do not use soap for hand washing before eating, underweight participants and male participants compared to their counterparts. But none of the associations achieved statistical significance. Small sample size (n=105) might be behind such lack of statistical significance but consistent pattern in direction of association urges further study to confirm this association. Among the variables evaluated, only variables with highest OR (i.e., higher range of 10) were entered forcibly for mutual adjustment. Yet, association remains insignificant with consistent direction of association and AOR. Hence, the variables like “do not use soap to wash hand before eating” with highest OR/AOR in both univariate as well as adjusted multivariate model may represent as proxy measures of poor hygiene in general. Nutritional status (i.e., BMI categories) did not explain any variation in risk of IPIs. But study indicated elevated risk of IPIs among undernutrition category (BMI<18.5) compared to normal BMI (i.e., BMI 18.5 to 24.99) or overweight BMI categories (BMI>24.99). Further study with larger sample size is needed to confirm this association.

This study has lots of limitations like small sample size and cross sectional study design. Yet, design of the study from both public and private school with matched, age, gender and BMI categories (i.e., 35 participants in each BMI categories) enabled us to compare relative prevalence and associated risk factors. Further, low prevalence of parasites limits us for statistical evaluation causing lack of association and risk of individual parasites. On going deworming program with enhance hygiene behaviors might have been contributing to such decreased prevalence of IPIs in human population. Though we assumed IPIs might have some association in reported GPA, such association was not evident in our analysis in both univariate and multivariate models (data not shown). Again, low prevalence of parasites might have caused lack of association.

6. CONCLUSION AND RECOMMENDATIONS

CONCLUSION

IPIs among school aged populations may pose a serious health problem. Though school aged adolescent indicated low prevalence of IPIs but risk of IPI is attributable to individual differences in behavior like “not using soap for handwashing”. Relatively elevated malnutrition (reported under nutrition) with risky hygiene behaviors indicated male as risky cluster of school age population. However, small sample size limits us for generalization.

RECOMMENDATIONS

An in-depth health education and higher emphasis on IPIs should be given to school aged adolescents to minimize risk of IPIs.

7. REFERENCES

Adhikari, R. B., Parajuli, R. P., Maharjan, M., & Ghimire, T. R. (2021). Prevalence and risk factors of gastrointestinal parasites in the Chepangs in Nepal. Annals of Parasitology, 67(3), 387–405.

Akinbo, F. O., Okaka, ChristopherE., & Omoregie, R. (2010). Prevalence of intestinal parasitic infections among HIV patients in Benin City, Nigeria. Libyan Journal of Medicine, 5(1), 5506.

Akubuilo,U.C.,et al., 2020. Academic performance and intelligence quotient of primary school children in Enugu. African medical journal. 36, 129-129

Amare, B., Ali, J., Moges, B., Yismaw, G., Belyhun, Y., Gebretsadik, S., Woldeyohannes, D., Tafess, K., Abate, E., Endris, M., Tegabu, D., Mulu, A., Ota, F., Fantahun, B., & Kassu, A. (2013). Nutritional status, intestinal parasite infection and allergy among school children in Northwest Ethiopia. BMC Pediatrics, 13(1), 7.

Anguita RM, Sigulem DM, Sawaya AL. Intrauterine food restriction is associated with obesity in young rats. Journal of Nutrition 1993; 123: 1421-8.

Atkinson RL. Could viruses contribute to the worldwide epidemic of obesity? International Journal of Pediatric Obesity 2008; 3 Supplement 1: 37-43.

Balarajan Y, Villamor E. Nationally representative surveys show recent increases in the prevalence of overweight and obesity among women of reproductive age in Bangladesh, Nepal, and India. Journal of Nutrition 2009; 139: 2139-44.

Bertoncello, C., Amoruso, I., Moscardino, U., Fonzo, M., Maharjan, M., Buja, A., Baldo, V., Cocchio, S., & Baldovin, T. (2021). Sex-Biased Prevalence of Intestinal Parasitic Infections and Gender Inequality in Rural Nepal. International Journal of Infectious Diseases, 109, 148–154.

Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 2013; 382: 427-451.

Campos Ponce, M., Incani, R. N., Pinelli, E., Ten Kulve, N., Ramak, R., Polman, K., & Doak, C. M. (2013). Are intestinal parasites fuelling the rise in dual burden

households in Venezuela? Transactions of the Royal Society of Tropical Medicine and Hygiene, 107(2), 119–123.

Caudet, J., Trelis, M., Cifre, S., Soriano, J. M., & Merino-Torres, J. F. (2022). Presence and significance of intestinal unicellular parasites in a morbidly obese population. International Journal of Obesity, 46(1), Article 1.

Center for Disease Control and Prevention - Parasites A-Z Index. (2019, November 20). https://www.cdc.gov/dpdx/az.html

Chandrashekhar, T., Joshi, H., Gurung, M., Subba, S., Rana, M., & Shivananda, P. (2009). Prevalence and distribution of intestinal parasitic infestations among school children in Kaski District, Western Nepal. Journal of Medicine and Biomedical Research, 4(1), 78–82.

Chatterjee, K. D. 2009. Parasitology: Protozoology and Helminthology. 13th ed. CBS publishers. pp. 260-266.

Collaborators GO. Health effects of overweight and obesity in 195 countries over 25 years. New England Journal of Medicine 2017; 377: 13-27.

Dahal, A., Roka, D. B., Prasad, S. M., & Shrestha, S. (2022). Prevalence of intestinal parasitic infection among school children in Kirtipur Municipality, Kathmandu. Nepal Medical College Journal, 24(2), 121–126.

Desta, M. (2014). Prevalence of intestinal parasitic infections and their associations with anthropometric measurements of school children in selected primary schools, Wukro town, Eastern Tigray, Ethiopia.

Dhurandhar NV. A framework for identification of infections that contribute to human obesity. Lancet Infectious Disease 2011; 11: 963-9.

Dobner, J., & Kaser, S. (2018). Body mass index and the risk of infection from underweight to obesity. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases, 24(1), 24–28.

Dukhi, N. (2020). Global Prevalence of Malnutrition: Evidence from Literature. In Malnutrition. IntechOpen.

Else, K. J., Keiser, J., Holland, C. V., Grencis, R. K., Sattelle, D. B., Fujiwara, R. T., Bueno, L. L., Asaolu, S. O., Sowemimo, O. A., & Cooper, P. J. (2020).

Whipworm and roundworm infections. Nature Reviews Disease Primers, 6(1), Article 1.

Fanzo J, Hawkes C, Udomkesmalee E, Afshin A, Allemandi L, Assery O, et al. 2018 Global Nutrition Report: Shining a light to spur action on nutrition. 2018.

Gao, L., Bhurtyal, A., Wei, J., Akhtar, P., Wang, L., & Wang, Y. (2020). Double Burden of Malnutrition and Nutrition Transition in Asia: A Case Study of 4 Selected Countries with Different Socioeconomic Development. Advances in Nutrition (Bethesda, Md.), 11(6), 1663– 1670.

Guan, M., Han, B., 2019. Association between intestinal worm infection and malnutrition among rural children aged 9-11 years old in Guizhou Province, China. BMC public health. 19, 1204- 1204.

Guerrant RL, DeBoer MD, Moore SR, Scharf RJ, Lima AAM. The impoverished gut--a triple burden of diarrhoea, stunting and chronic disease. Nature reviews. Gastroenterology & hepatology 2013; 10: 220-229.

Gupta, R., Rayamajhee, B., Sherchan, S. P., Rai, G., Mukhiya, R. K., Khanal, B., & Rai, S. K. (2020). Prevalence of intestinal parasitosis and associated risk factors among school children of Saptari district, Nepal: A cross-sectional study. Tropical Medicine and Health, 48(1), 73.

Hajare, S. T., Gobena, R. K., Chauhan, N. M., & Eriso, F. (2021). Prevalence of Intestinal Parasite Infections and Their Associated Factors among Food Handlers Working in Selected Catering Establishments from Bule Hora, Ethiopia. BioMed Research International, 2021, 6669742.

Hajissa, K., Islam, M. A., Sanyang, A. M., & Mohamed, Z. (2022). Prevalence of intestinal protozoan parasites among school children in africa: A systematic review and meta-analysis. PLoS Neglected Tropical Diseases, 16(2), e0009971.

Haque, R. (2007). Human Intestinal Parasites. Journal of Health, Population, and Nutrition, 25(4), 387–391.

Harpsøe MC, Nielsen NM, Friis-Møller N, Andersson M, Wohlfahrt J, Linneberg A, et al. Body Mass Index and Risk of Infections Among Women in the Danish National Birth Cohort. Journal of Epidemiology 2016; 183: 1008-17.

Hasan M, Sutradhar I, Akter T, Das Gupta R, Joshi H, Haider MR, et al. Prevalence and determinants of hypertension among adult population in Nepal: Data from Nepal Demographic and Health Survey 2016. PloS one 2018; 13: e0198028.

Hashan, M. R., Das Gupta, R., Day, B., & Al Kibria, G. M. (2020). Differences in prevalence and associated factors of underweight and overweight/obesity according to rural–urban residence strata among women of reproductive age in Bangladesh: Evidence from a cross-sectional national survey. BMJ Open, 10(2), e034321.

Hossain, Md. T., Luies, S. K., & Biswas, T. (2020). Prevalence and Factors Associated with Overweight and Obesity among Primary School Children (9–14 Years) in a Selected Area of Dhaka, Bangladesh: A Cross-Sectional Study. Indian Journal of Community Medicine : Official Publication of Indian Association of Preventive & Social Medicine, 45(4), 429–434.

Karunaithas, R., Murugananthan, A. and Kannathasan, S. 2011. Prevalence and Aassociated factors of Soil transmitted Helminthes Infestation among Pre-school children of Vadamaradchi Educational Zone. Ving. Journal of Science, 10(1): 25-34.

Khanal, R., Upadhaya, S., Lamichhane, P., Karn, S. L., Pokhrel, S., & Pahwa, V. K. (2016). Enteric Parasitic Infections among School Children at Rupandehi, Nepal. Journal of Universal College of Medical Sciences, 4(2), Article 2.

Kunwar, R., Acharya, L., & Karki, S. (2016). Trends in prevalence of soil-transmitted helminth and major intestinal protozoan infections among school-aged children in Nepal. Tropical Medicine & International Health, 21(6), 703–719.

Limbu, D. S., Shrestha, S., Bantawa, K., Majhi, R., & Kharel, M. (2021). Intestinal Parasitic Infections and Associated Risk Factors Among School-going Children of Age 1-5 years in Dharan, Eastern Nepal. Himalayan Journal of Science and Technology, 5(01), Article 01.

Mehata S, Parajuli KR, Pant ND, Rayamajhee B, Yadav UN, Mehta RK, et al. Prevalence and correlates of Helicobacter pylori infection among under-five children, adolescent and non- pregnant women in Nepal: Further analysis of Nepal national micronutrient status survey 2016. PLOS Neglected Tropical Diseases 2021; 15: e0009510.

Pandey, S., Lo, A. L., & Shrestha, R. B. (2015). Intestinal parasitic infections among school children of Northern Kathmandu, Nepal. Asian Pacific Journal of Tropical Disease, 5, S89–S92.

Parajuli, R. P., Fujiwara, T., Umezaki, M., Konishi, S., Takane, E., Maharjan, M., Tachibana, K., Jiang, H. W., Pahari, K., & Watanabe, C. (2014). Prevalence and risk factors of soil-transmitted helminth infection in Nepal. Transactions of The Royal Society of Tropical Medicine and Hygiene, 108(4), 228–236.

Picó C, Palou M, Priego T, Sánchez J, Palou A. Metabolic programming of obesity by energy restriction during the perinatal period: different outcomes depending on gender and period, type and severity of restriction. Frontiers in physiology 2012; 3: 436-436.

Popkin BM, Corvalan C, Grummer-Strawn LM. Dynamics of the double burden of malnutrition and the changing nutrition reality. Lancet 2020; 395: 65-74.

Rashid, M.K., Joshi, M., Joshi, H. and Fatemi, K. 2011. Prevalence of Intestinal Parasites among School going Children in Bareilly district 2(1) Jan-March.

Rawal LB, Kanda K, Mahumud RA, Joshi D, Mehata S, Shrestha N, et al. Prevalence of underweight, overweight and obesity and their associated risk factors in Nepalese adults: Data from a Nationwide Survey, 2016. PLoS One 2018; 13: e0205912.

Rayapu, V., Dhandapany, S.P., Sheker, I.A. and Kasukurthy, S. 2012. Prevalence of Intestinal Helminthic Parasites in School going Children in Rural Area of Kuppam, Andhra Pradesh. International Journal of Basic Medical Sciences and Pharmacy (IJBMSP), 2(2): 76-79.

Sah, D. R. B., Baral, D. R., Shah, M. U., & Jha, P. N. (2016). A Study of Prevalence of Intestinal Helminthic Infections and Associated Risk Factors among the School Children of Biratnagar Submetropolitan, Eastern Region of Nepal. https://www.semanticscholar.org/paper/A-Study-of-Prevalence-of-Intestinal-Helminth.

Sayyari, A. A., Imanzadeh, F., Bagheri Yazdi, S. A., Karami, H., & Yaghoobi, M. (2005). Prevalence of intestinal parasitic infections in the Islamic Republic of Iran. EMHJ - Eastern MediterraneanHealthJournal,11(3),377-383, 2005. https://apps.who.int/iris/handle/10665/116956

Schwinger C, Chandyo RK, Ulak M, Hysing M, Shrestha M, Ranjitkar S, et al. Prevalence of Underweight, Overweight, and Obesity in Adults in Bhaktapur, Nepal in 2015-2017. Frontiers in nutrition 2020; 7: 567164-567164.

Shrestha, A., Narayan, K. C., & Sharma, R. (2012). Prevalence of Intestinal Parasitosis Among School Children in Baglung District of Western Nepal. Kathmandu University Medical Journal, 10(1), Article 1.

Shrestha, A., Schindler, C., Odermatt, P., Gerold, J., Erismann, S., Sharma, S., Koju, R., Utzinger, J., & Cissé, G. (2018). Intestinal parasite infections and associated risk factors among schoolchildren in Dolakha and Ramechhap districts, Nepal: A cross-sectional study. Parasites & Vectors, 11(1), 532.

Su CW, Chen CY, Li Y, Long SR, Massey W, Kumar DV, et al. Helminth infection protects against high fat diet-induced obesity via induction of alternatively activated macrophages. 2018; 8: 4607.

Taliaferro, W. 2009. Basic principles and concept I: Parasite systematic, Ecology and Evolution in foundation of parasitology, Eighth ed. II Dubuque IA New York, Sanfrancisco, 210 pp.

Tandukar, S., Ansari, S., Adhikari, N., Shrestha, A., Gautam, J., Sharma, B., Rajbhandari, D., Gautam, S., Nepal, H. P., & Sherchand, J. B. (2013). Intestinal parasitosis in school children of Lalitpur district of Nepal. BMC Research Notes, 6(1), 449.

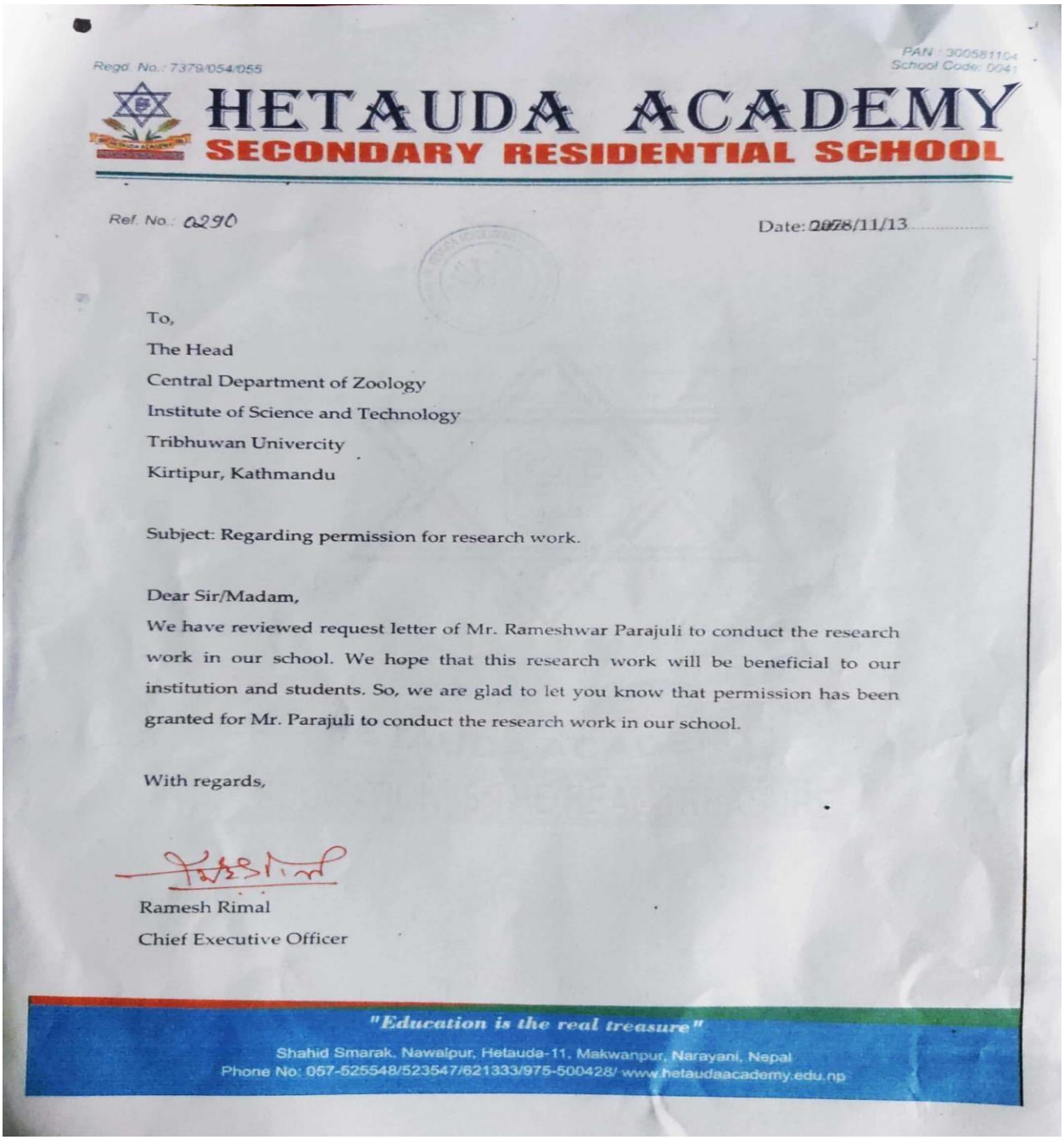
Wei J, Bhurtyal A, Dhungana RR, Bhattarai B, Zheng J, Wang L, et al. Changes in patterns of the double burden of undernutrition and overnutrition in Nepal over time. Obesity Review 2019; 20: 1321-1334.

Yang Z, Grinchuk V, Smith A, Qin B, Bohl JA, Sun R, et al. Parasitic nematode-induced modulation of body weight and associated metabolic dysfunction in mouse models of obesity. Infectious Immunology 2013; 81: 1905-14.

Zavala GA, Doak CM, Portrait F, Seidell JC, García OP, Rosado JL, et al. Are intestinal parasites associated with obesity in Mexican children and adolescents? Parasitology International 2019; 71: 126-131.

APPENDICES

1. Approval letter from Hetauda Academy Secondary Residential School



Regd. No.: 7379/054/055

PAN : 300581104
School Code: 0041

HETAUDA ACADEMY
SECONDARY RESIDENTIAL SCHOOL

Ref. No.: 0290

Date: 2078/11/13

To,
The Head
Central Department of Zoology
Institute of Science and Technology
Tribhuwan Univercity
Kirtipur, Kathmandu

Subject: Regarding permission for research work.

Dear Sir/Madam,

We have reviewed request letter of Mr. Rameshwar Parajuli to conduct the research work in our school. We hope that this research work will be beneficial to our institution and students. So, we are glad to let you know that permission has been granted for Mr. Parajuli to conduct the research work in our school.

With regards,

Ramesh Rimal
Chief Executive Officer

"Education is the real treasure"
Shahid Smarak, Nawalpur, Hetauda-11, Makwanpur, Narayani, Nepal
Phone No: 057-525548/523547/621333/975-500428/ www.hetaudaacademy.edu.np

2. Approval letter from Shiksha Bikas Shishu Sadan English Boarding School

PAN 3 0 0 8 8 6 1 0 8

Tel: 057-411066

SHIKSHA BIKAS SHISHU SADAN
ENGLISH BOARDING SCHOOL

Hetauda-3, Basamadi, Makawanpur
Estd.:- 2052

To,
Head of Department
Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur Kathmandu

Subject: Permission for Research work

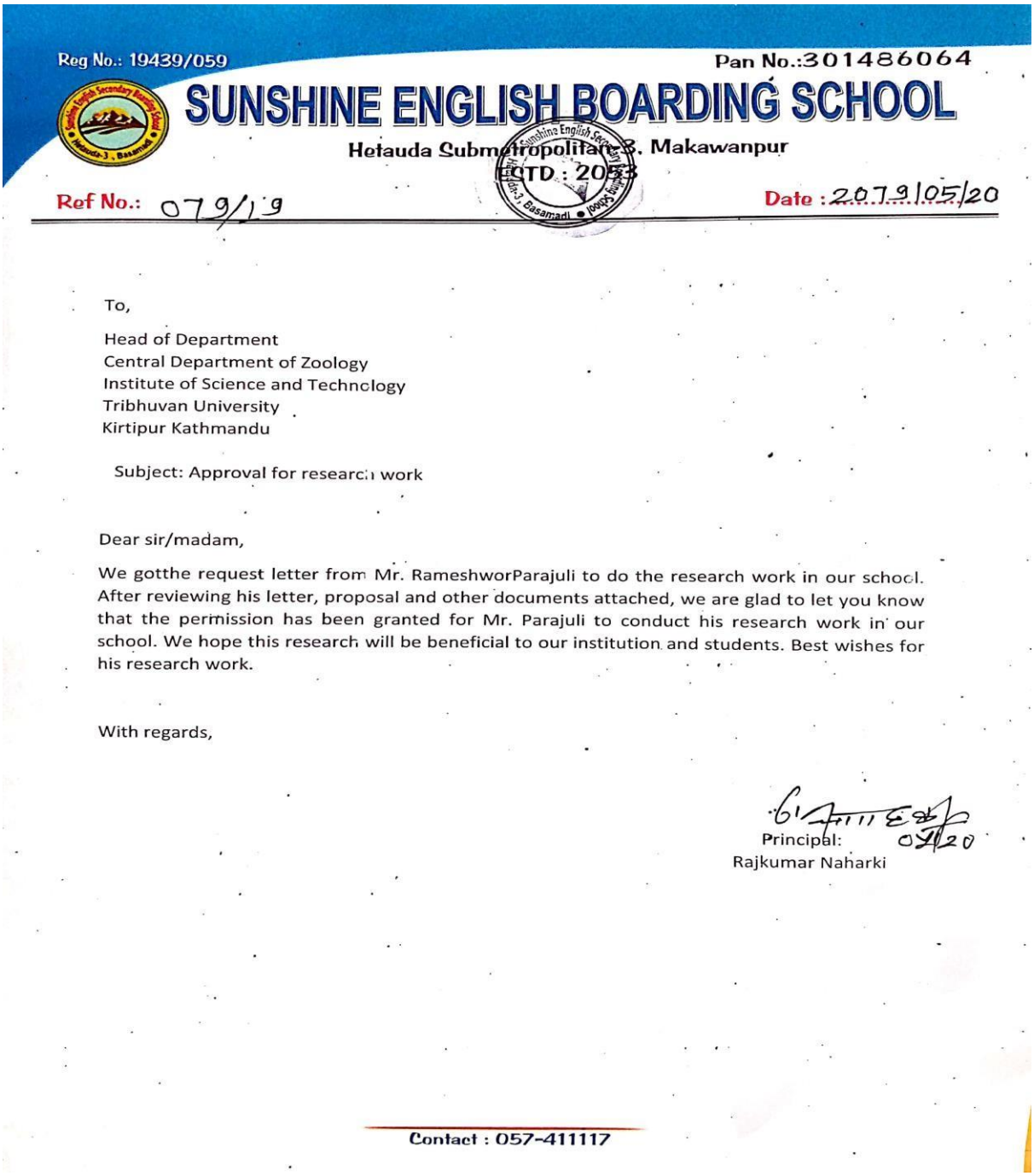
Respected Sir/Madam,

We reviewed the request letter of Mr. Rameshwor Parajuli to do the research work in our school. We are glad to let you know that the permission has been granted for Mr. Parajuli to conduct his research work in our school. We hope this research will be beneficial to our institution and students. Mr. Parajuli is our former student and we are happy to let him do his research work in our institution for his Post graduate degree.

With regards,

Grace Shrestha
Principal

3. Approval letter from Sunshine English Boarding School



Reg No.: 19439/059 Pan No.:301486064

SUNSHINE ENGLISH BOARDING SCHOOL

Hetauda Submetropolitan-3, Makawanpur

EIIN : 20533

Ref No.: 079/19 Date : 2079/05/20

To,

Head of Department
Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur Kathmandu

Subject: Approval for research work

Dear sir/madam,

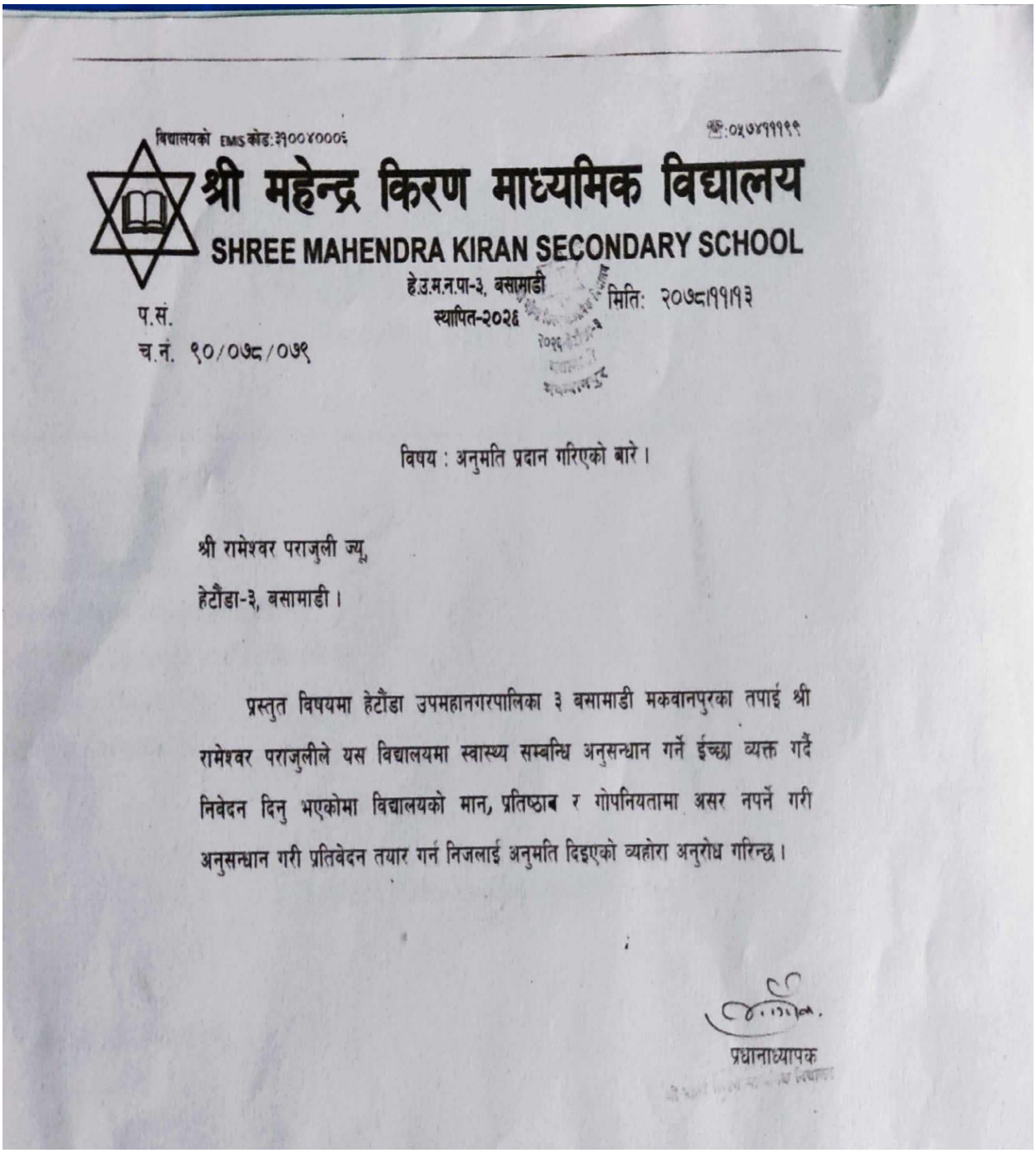
We gotthe request letter from Mr. RameshworParajuli to do the research work in our school. After reviewing his letter, proposal and other documents attached, we are glad to let you know that the permission has been granted for Mr. Parajuli to conduct his research work in our school. We hope this research will be beneficial to our institution and students. Best wishes for his research work.

With regards,

Principal: 05/20
Rajkumar Naharki

Contact : 057-411117

4. Approval letter from Shree Mahendra Kiran Secondary School



विद्यालयको EMS कोड:३१००४०००६ फोन:०५७४१११९९

श्री महेन्द्र किरण माध्यमिक विद्यालय

SHREE MAHENDRA KIRAN SECONDARY SCHOOL

हे.उ.म.न.पा-३, बसामाडी
स्थापित-२०२६

प.सं.
च.नं. ९०/०७८/०७९

मिति: २०७८।११।१३

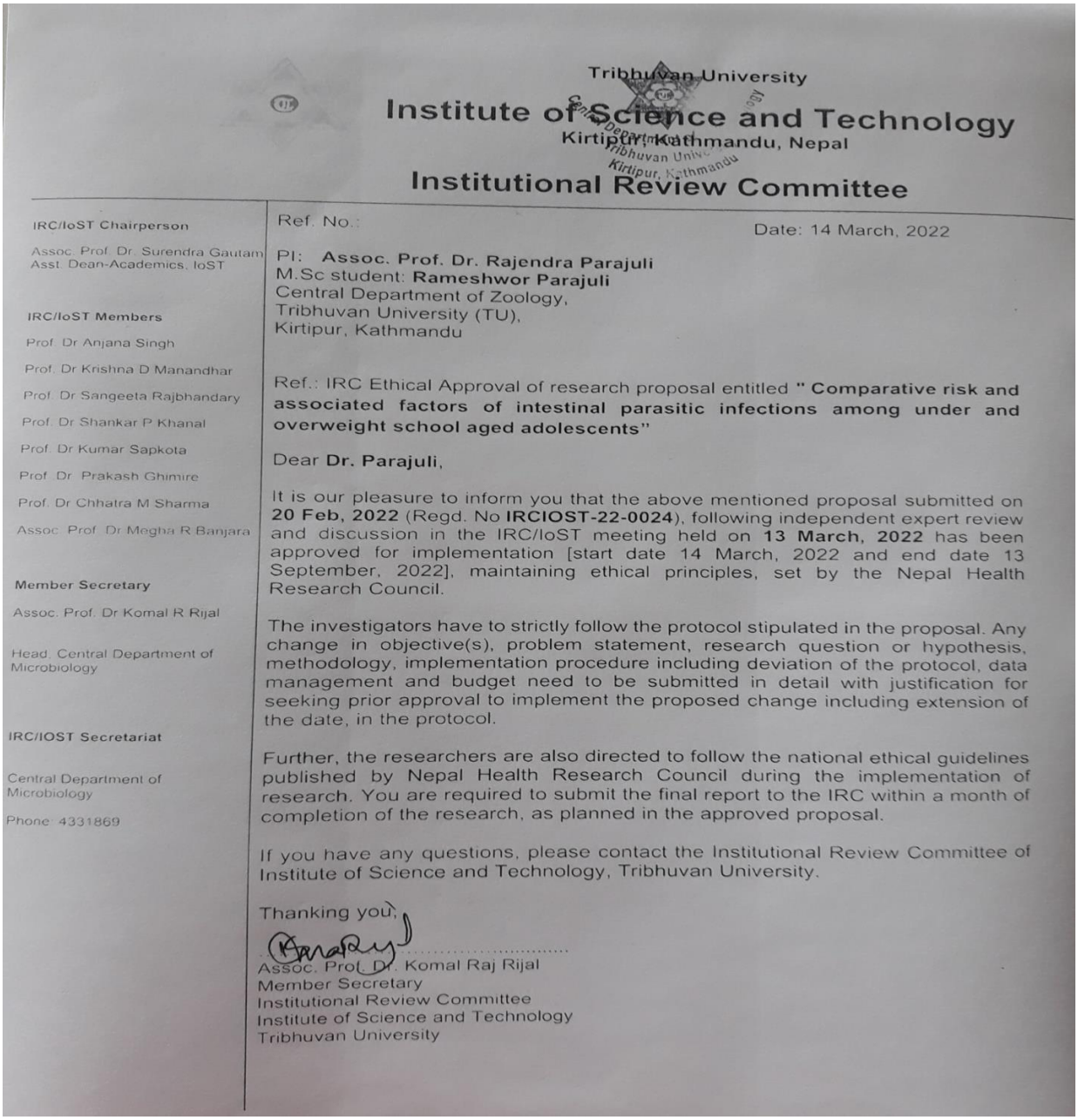
विषय : अनुमति प्रदान गरिएको बारे।

श्री रामेश्वर पराजुली ज्यू,
हेटौंडा-३, बसामाडी।

प्रस्तुत विषयमा हेटौंडा उपमहानगरपालिका ३ बसामाडी मकवानपुरका तपाई श्री रामेश्वर पराजुलीले यस विद्यालयमा स्वास्थ्य सम्बन्धि अनुसन्धान गर्ने ईच्छा व्यक्त गर्दै निवेदन दिनु भएकोमा विद्यालयको मान, प्रतिष्ठाब र गोपनियतामा असर नपर्ने गरी अनुसन्धान गरी प्रतिवेदन तयार गर्न निजलाई अनुमति दिइएको व्यहोरा अनुरोध गरिन्छ।

प्रधानाध्यापक

5. Ethical approval from IOST TU



Tribhuvan University

Institute of Science and Technology

Kirtipur, Kathmandu, Nepal

Institutional Review Committee

IRC/IoST Chairperson

Assoc. Prof. Dr. Surendra Gautam
Asst. Dean-Academics, IoST

IRC/IoST Members

Prof. Dr Anjana Singh

Prof. Dr Krishna D Manandhar

Prof. Dr Sangeeta Rajbhandary

Prof. Dr Shankar P Khanal

Prof. Dr Kumar Sapkota

Prof. Dr Prakash Ghimire

Prof. Dr Chhatra M Sharma

Assoc. Prof. Dr Megha R Banjara

Member Secretary

Assoc. Prof. Dr Komal R Rijal

Head, Central Department of Microbiology

IRC/IOST Secretariat

Central Department of Microbiology

Phone: 4331869

Ref. No.:

Date: 14 March, 2022

PI: **Assoc. Prof. Dr. Rajendra Parajuli**
M.Sc student: **Rameshwor Parajuli**
Central Department of Zoology,
Tribhuvan University (TU),
Kirtipur, Kathmandu

Ref.: IRC Ethical Approval of research proposal entitled **" Comparative risk and associated factors of intestinal parasitic infections among under and overweight school aged adolescents"**

Dear **Dr. Parajuli**,

It is our pleasure to inform you that the above mentioned proposal submitted on **20 Feb, 2022** (Regd. No **IRCIOST-22-0024**), following independent expert review and discussion in the IRC/IoST meeting held on **13 March, 2022** has been approved for implementation [start date 14 March, 2022 and end date 13 September, 2022], 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

6. Information letter to guardians/participants

अनुसूची १ सर्भेक्षणको सन्क्षिप्त विवरण

प्रिय माता-पिता/सहभागीहरू,

हामीहरु नेपाल स्थित त्रिभुवन बिस्वबिध्यालय ,प्राणीशास्त्र केन्द्रिय बिभागले किशोर-किशोरीहरूको स्कूलको स्वास्थ्य स्थितिबारे अध्ययन सञ्चालन गर्ने योजना बनाएको छ। तल बताएझैं यस सर्वेक्षणमा हामी तपाईं र तपाईंका छोराछोरीको सहभागिताको लागि हार्दिक अनुरोध गर्दछौं।

यो सर्भेक्षण का मुख्य मुख्य उदेश्य हरु तपसिल वमोजिम छन्।

१. न्यूनपोषित सहभागीहरूको तुलनामा ओभरवेट र मोटोपन का सहभागीहरुमा आन्द्रा परजीवीको जोखिम र त्यससँग सम्बन्धित कारकहरू को परीक्षण गर्ने ।
२. न्यूनपोषित सहभागीहरूको तुलनामा ओभरवेट र मोटोपन का सहभागीहरूमा आन्द्रा परजीवीको जोखिम र सम्बन्धित तत्त्वहरूबीचको सम्बन्धको मूल्यांकन गर्ने ।
३. न्यूनपोषित सहभागीहरूको तुलनामा ओभरवेट र मोटोपन का सहभागीहरूबीच आन्द्रा परजीवीको जोखिम र सम्बन्धित कारकहरूबीचको सम्बन्धको मूल्यांकन र अन्य सुझावित जनसांख्यिकीय चलहरू समायोजन गर्ने।

यो सर्भेक्षणमा देहाय बमोजिमको बिधि प्रक्रिया अपनाईने छ ।

१. रिपोर्ट गरिएको वा मापन गरिएको बिएमआइ (BMI) को आधारमा बिद्यार्थी चयन गर्ने ।
२. पोषण स्थितिको मूल्यांकन (उचाइ, वजन, BMI) मानविय नाप लिई गरिनेछ ।
३. छनौट भएका सबै विद्यार्थीहरुलाई दिइएको कप मा दिशा को नमुना संकलन गरिनेछ ।
४. प्रत्येक सङ्कलित नमूनालाई अध्ययन कोडद्वारा त्याग गरिनेछ र परजीवीहरूको संरक्षणका लागि २.५% पोटासियम डाइक्रोमेट रसायन मा संरक्षित गरिनेछ।
५. सहभागिहरुको सामाजिक, व्यवहारिक, जीवनशैली र जनसांख्यिक जानकारी संकलन गर्न संरचित प्रश्नहरु सोधिनेछ ।
६. सङ्कलित प्रत्येक नमूनालाई सूक्ष्म परीक्षणका लागि, प्राणीशास्त्र केन्द्रिय बिभाग त्रिभुवन विश्वविद्यालयको प्रयोगशालामा ल्याइनेछ ।

सङ्कलन गरिएका तथ्यांक तथा नमुनाहरू होसियारीसाथ ढुवानी, सञ्चय र उपचार गरिनेछ। हामीले प्रत्येक सहभागीलाई व्यक्तिगत परिणामहरू (feedback) सूचित गर्दा बाहेक व्यक्तिगत जानकारी कहिल्यै प्रकट गरिने छैन। प्राप्त तथ्यांक सहभागीहरुलाई जानकारी दिइनेछ । आन्द्रा परजीवीको व्यापकता, असामान्य मूल्य वा खतरनाक सामग्रीको उच्च जोखिमको संकेत भएमा, आ-आफ्नो व्यक्तिलाई त्यसै अनुसार सूचित गरिनेछ। सर्वेक्षणमा भाग लिनु पूर्णतया स्वेच्छिक छ; साथै, सहभागीलाई आफ्नो बाबु वा आमाले भाग लिन सहमत भएको बेलामा मात्र सर्वेक्षणमा समावेश गरिनेछ। सहभागिता अस्वीकार गरेको कारण कुनै पनि व्यक्तिलाई कुनै दण्ड, शुल्क वा हानि हुनेछैन।
हार्दिक धन्यबाद सहित

रामेसवोर पराजुली
MSc zoology, केन्द्रीय प्राणीशास्त्र विभाग,
त्रिभुवन विश्वविद्यालय, कीर्तिपुर, नेपाल
इमेल: rameshwor.parajuli1@gmail.com फोन; ९८४३७०२२२८

डा राजेन्द्र प्रसाद पराजुली
सह प्राध्यापक ,प्राणीशास्त्र केन्द्रिय बिभाग ,
त्रिभुवन बिस्वबिध्यालय ,काठमाडौँ ,नेपाल
इमेल: rajendra.parajuli@cdz.tu.edu.np फोन ;९८४५१७२४६३

7. Consent form

अनुसूची २ -सर्भेक्षणमा भागलिने सहमति पत्र

आदरणीय श्री.....

म यस सर्भेक्षण टोलि सदस्य) तपसिलका (ले देहाय बमोजिम ब्याख्या गरेको सर्भेक्षणमा भाग लिन मन्जुर छु।

तपसिल

१. यो सर्भेक्षणमा गरिने बिधि र लिइने नमुनाको बारे
२. यस सर्भेक्षणमा भाग नलिए बापत कुनै सजाय ,पूर्बाग्रह हुँदैन भन्ने बारे
३. यस सहमति पत्रमा हस्ताक्क्षर गरेता पनि सर्भेक्षण बिच मै छाडेपनि कुनै सजाय हुन्न भन्ने बारे
४. संकलित व्यक्तिगत सूचना र नमुनाको गोपानियताको बारे

सर्भेक्षण /अनुसन्धानको शिर्षक : **न्यूनपोषित र मोटोपन भएका किशोर-किशोरीहरू**को **आन्द्रामा हुने परजीवीहरूको सङ्क्रमण**को **तुलनात्मक रूपमा खतरा र त्यससित सम्बन्धित कारणहरू।**

सहभागीको नाम :

सहि ; मिति ;

सर्भेक्षण /अनुसन्धानको शिर्षक : **न्यूनपोषित र मोटोपन भएका किशोर-किशोरीहरू**को **आन्द्रामा हुने परजीवीहरूको सङ्क्रमण**को **तुलनात्मक रूपमा खतरा र त्यससित सम्बन्धित कारणहरू।**

मैले यस सर्वेक्षण को बारेमा अर्को पानामा उल्लेख बिस्तृत विवरण यस विवरणमा उल्लेख व्यक्तिलाई सुनाइ बुझाई सहमत भए पछि मात्र यो सहमति पत्रमा हस्तक्षर गरेको छु।

सर्भेक्षणको बारेमा बताउने व्यक्तिको नाम :

सहि ; मिति ;

8. Questionnaire

अनुसूची ३ आधारभूत स्वास्थ्य सर्वेक्षण प्रश्नावली

सहभागीको नाम/कोड : उमेर :लिंग : कक्षा :उचाई : तौल :

पछिल्लो तेश्रो बर्षको ग्रेड/प्रतिशत : पछिल्लो दोश्रो बर्षको ग्रेड/प्रतिशत : गत बर्षको ग्रेड/प्रतिशत

पछिल्लो तेश्रो बर्षको हाजिरी : पछिल्लो दोश्रो बर्षको हाजिरी : गत बर्षको हाजिरी

१. साधारणतया, १ देखि ५ को स्केलमा तपाईं आफ्नो स्वास्थ्यलाई कसरी रेट गर्नुहुन्छ?
☐१ ☐२ ☐३ ☐४ ☐५

२. प्राय **तपाईंले कस्तो खालको पिउने पानी** पिउनु हुन्छ ?
☐धराकोपानी ☐जारको पानी ☐उमालेको पानी ☐फिल्टर गरिएको पानी

३. तपाइले फलफुल तरकारी कतिपटक खानुहुन्छ|
☐दैनिक ☐हप्ताको एक पटक ☐ हप्ताको दुई पटक वा त्योभन्दा बढी ☐महिनाको एक पटक

४. फलफूल तरकारी पखालेर **खानुहुन्छ ?**
क) प्राय ख) कहिलेकाही

५. फलफूल तरकारी पकाएर **खानुहुन्छ ?**
क) प्राय ख) कहिलेकाही

६. हप्तामा कति पटक मासु खानुहुन्छ ?
☐ एक वा दुई पटक ☐तीन पटक ☐दैनिक ☐कुनै पनि होइन

७. बदेल पोर्क को मासु खानु भएको छ|
क) खाएको छु ख) छैन

८. हाल कुनै न कुनै प्रकारको व्यायाममा सहभागी हुनु भएको छ ?
☐छ ☐छैन

९. तपाईको तौलको सम्बन्धमा, तपाई के गर्न चाहनुहुन्छ ?
☐तौल घटाउन ☐ वजन बढाउन ☐वजन कायम राख्

१०. गएको १२ महिनामा विद्यालयको रिजल्टलाई **कसरी रेट गर्नुहुन्छ ?**
☐असन्तुष्ट ☐सन्तोषजनक ☐राम्रो/उत्कृष्ट

११ तपाईको परिवारको आर्थिक अवस्था कस्तो छ ?
☐गरिब ☐सामान्य ☐ राम्रो/उत्कृष्ट औशत बार्षिक पारिबारिक आम्दानी अनुमान गर्न सक्नुहुन्छ|

१२. नङ काट्ने बानी औसत/ प्राय
क) हप्तामा १ पटक ख) महिनामा १ पटक ग) बढेको थाहा पाए

9. Questionnaire continue

१३) बाहिर निस्कदा /बिस्तारा बाहेक चप्पल जुता लगाउनु हुन्छ|
क) बाहिर निस्कदा मात्र/घरमा लगाउदीन
ख) खेतमा/ बारीमा काम गर्दा लगाउदीन
ग) बिस्तारा मा बाहेक अन्यत्र सधै लगाउछु
घ)अन्य /ब्याख्या

१४) व्यक्तिगत सरसफाई
क) खाना खानु अघि सधै साबुन ले हात धुन्छु र चर्पी/ ट्वाइलेट पछी त अनिबार्य
ख) चर्पी/ ट्वाइलेट पछी त अनिबार्य धुन्छु तर खाना खानु अघि पछि पानी ले धुन्छ|
ग) साबुनले हात धोइदैन
घ) कहिलेकाही साबुनले हात धुन्छु/ कहिलेकाही बिर्सन्छु|
च) अन्य ब्याख्या

१५) खानेकुरा छोपिन्छ?
क) प्राय छोपिन्छ
ख) कहिलेकाही बिर्सिन्छ
ग) अन्य /विस्तृत

१६) घर मा कतिजना बसिन्छ:
१७) यहाँ लाई जुका परेको थाहा छ? छ? छैन? औषधी खाएको हो? छ? छैन?
हो भने कति महिना पहिले?
१८) घर भित्र कुखुरा/कुकुर/ हास सुँगुर? पसेको/पालेको? छ? छैन?

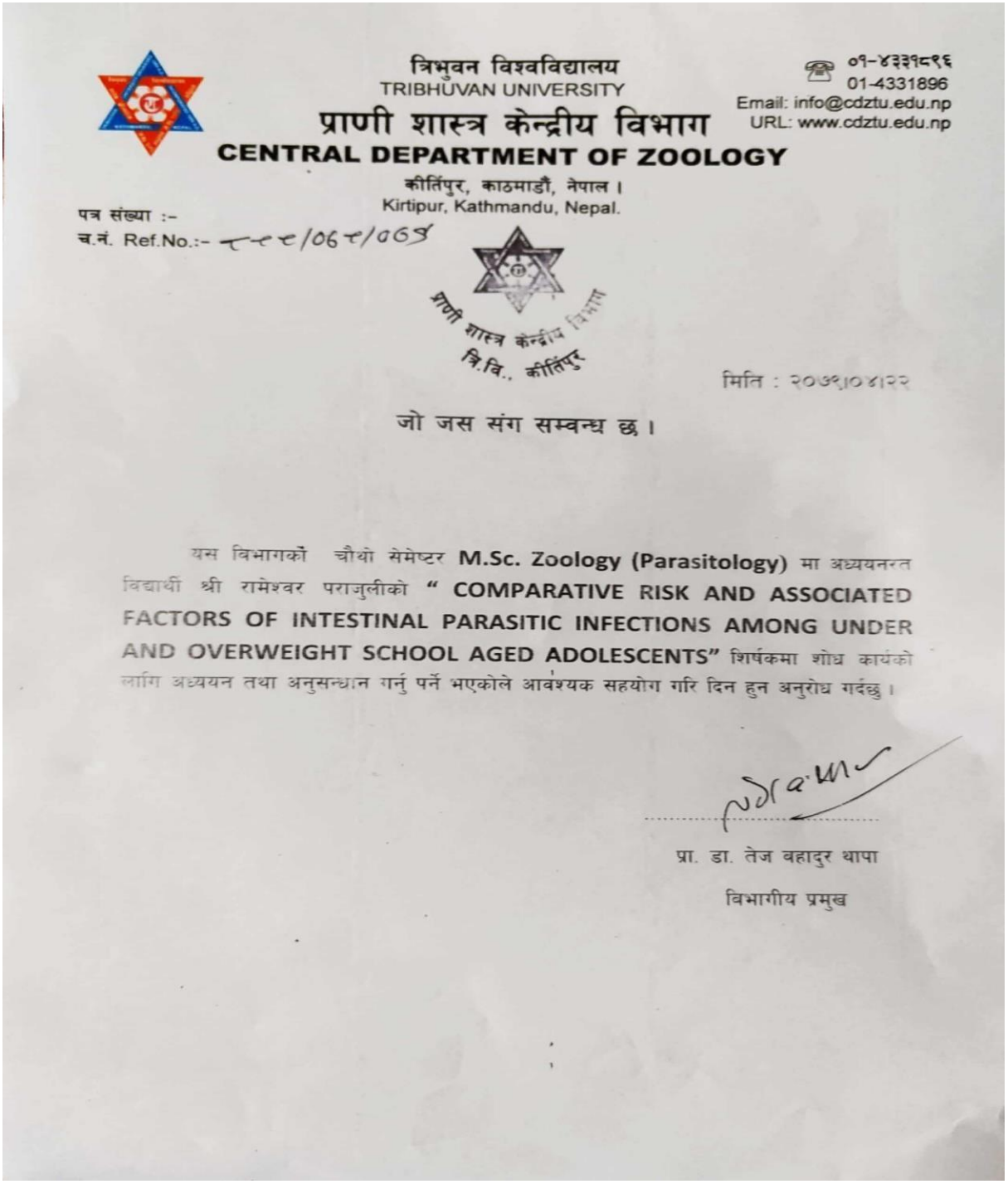
१९) काचो मासु खाएको याद छ? छ छैन?

२०. तपाईको बुवाको शिक्षा कस्तो छ ?
□ वास्तविक कक्षा/डिग्री..... □पढ्न र लेख्न सकिँदैन
२१. तपाईको आमाको शिक्षा कस्तो छ ?
□ वास्तविक कक्षा/डिग्री..... □पढ्न र लेख्न सकिँदैन
२२. तपाईंको बुवाको पेशा के हो ?
□सरकारी कर्मचारी □व्यवसायी □किसान □अन्य निर्दिष्ट
२३. तपाईको आमाको पेशा के हो ?
□सरकारी कर्मचारी □व्यवसायी □किसान □अन्य निर्दिष्ट
२४. के तपाई भुइँमा खसेको खाना (कुनै) खानुहुन्छ ?
□हो □कहिलेकाहीँ □होइन

२५. आन्द्राको हेलमिन्थियासिस (जुगा)रोक्ने कम्तीमा एक उपाय तपाईलाई थाहा छ ? छ? छैन?

२६. नङ टोक्ने बानि छ| छ? छैन?

10. Recommendation letter from Central Department of Zoology, TU



त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल।
Kirtipur, Kathmandu, Nepal.

०१-४३३१८९६
01-4331896
Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:- ८८८/०६८/०६९

प्राणी शास्त्र केन्द्रीय विभाग
त्रि.वि., कीर्तिपुर

मिति : २०७९।०४।२२

जो जस संग सम्बन्ध छ।

यस विभागको चौथो सेमेष्टर **M.Sc. Zoology (Parasitology)** मा अध्ययनरत विद्यार्थी श्री रामेश्वर पराजुलीको **" COMPARATIVE RISK AND ASSOCIATED FACTORS OF INTESTINAL PARASITIC INFECTIONS AMONG UNDER AND OVERWEIGHT SCHOOL AGED ADOLESCENTS"** शिर्षकमा शोध कार्यको लागि अध्ययन तथा अनुसन्धान गर्नु पर्ने भएकोले आवश्यक सहयोग गरि दिन हुन अनुरोध गर्दछु।

प्रा. डा. तेज बहादुर थापा
विभागीय प्रमुख

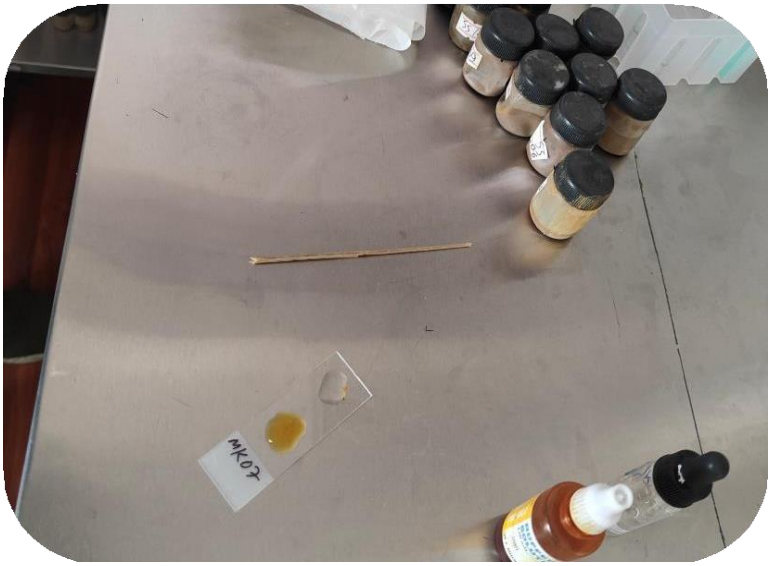
1. Hetauda Academy Secondary Residential School

2. Mahendra Kiran Secondary School

3. Shiksha Bikas Shishu Sadan Secondary English Boarding School

4. Students of Sunshine English Boarding School in assembly

5. Preparation of stool smear



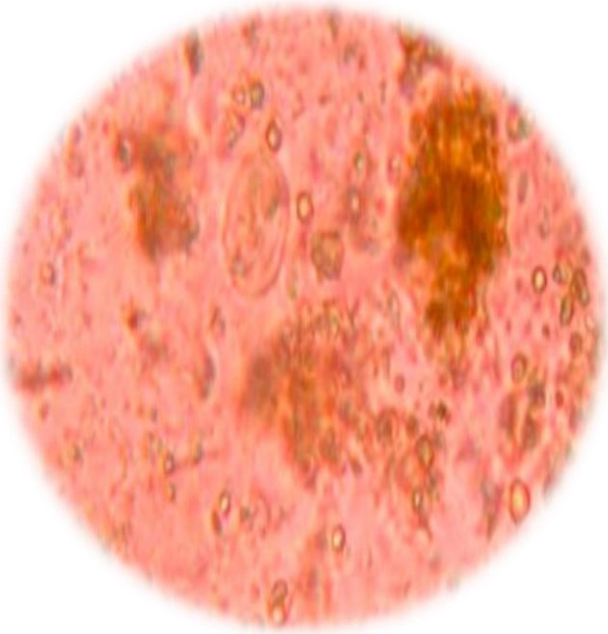
6. Stool smear ready to view under microscope

7. Height measurement of a student

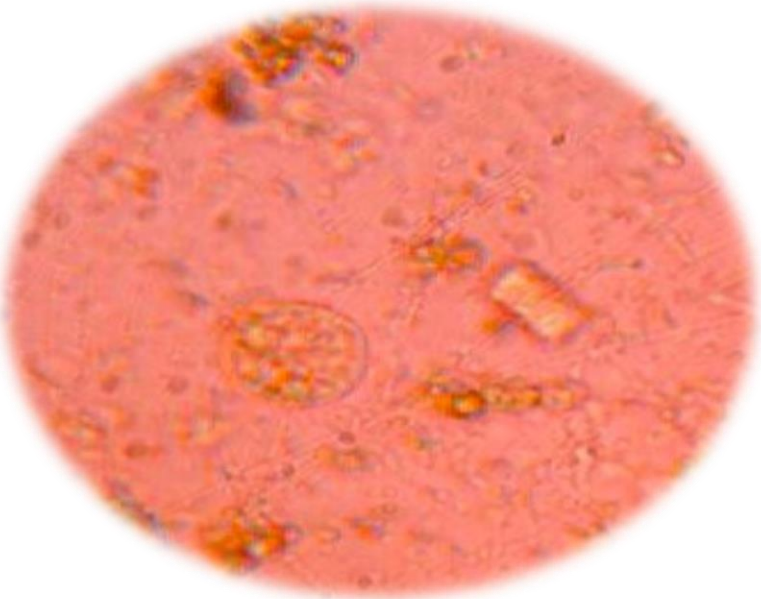
8. Weight measurement of a student

9. Lab work in central department of Zoology

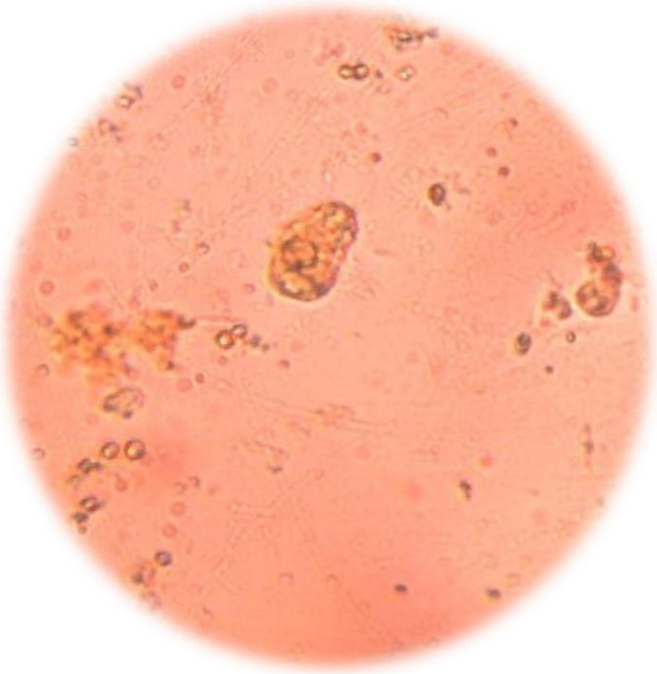
10. Microscopic examination of Stool sample



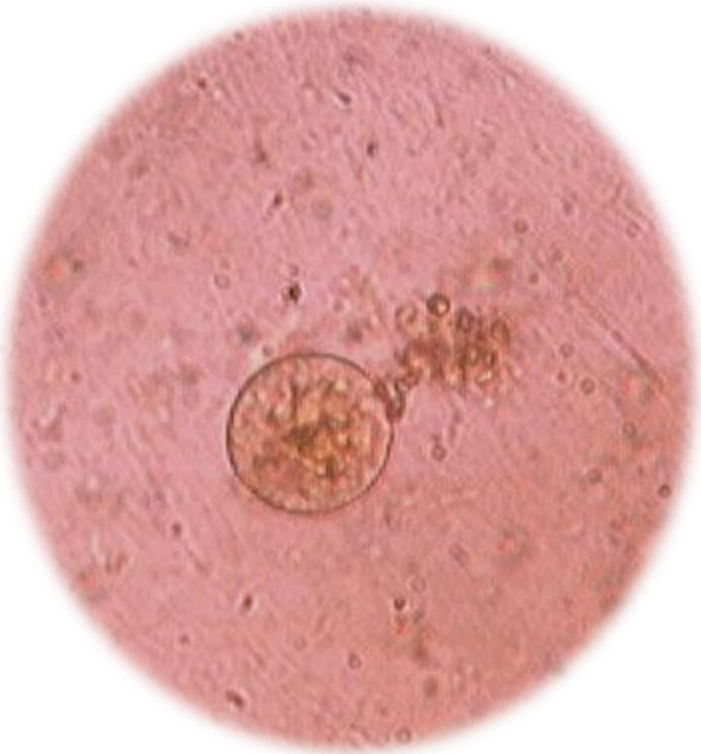
11. Cyst of *Giardia lamblia* seen in sample HA19



12.Cyst of *Entamoeba histolytica* seen in sample HA57



13. Cyst of *Endolimax nana* seen in sample HA32



14. Cyst of *Entamoeba histolytica* seen in sample HA38

15. Cyst of *Giardia lamblia* seen in sample MK29

16. Cyst of *Giardia lamblia* seen in sample SS06