PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG CANCER PATIENTS OF NEPAL CANCER HOSPITAL AND RESEARCH CENTER, LALITPUR

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July 17, 2023

DECLARATION

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

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RECOMMENDATION

This is to recommend that the thesis entitled **"PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG CANCER PATIENTS OF NEPAL CANCER HOSPITAL AND RESEARCH CENTER, LALITPUR"** has been carried out by Barsha Singh 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.

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LETTER OF APPROVAL

On the recommendation of supervisor "Dr. Rajendra Prasad Parajuli" this thesis submitted by Barsha Singh entitled **"PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG CANCER PATIENTS OF NEPAL CANCER HOSPITAL AND RESEARCH CENTER, LALITPUR"** 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.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Barsha Singh entitled **"PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG CANCER PATIENTS OF NEPAL CANCER HOSPITAL AND RESEARCH CENTER, LALITPUR"** has been approved as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviation
AIDs	Acquired Immunodeficiency Syndrome
BMI	Body Mass Index
CDC	Centre for Disease Control and Prevention
CDZ	Center Department of Zoology
DLC	Differential Leukocyte Count
ELISA	Enzyme-linked immunosorbent assay
g/dl	Gram per deciliter
gm	Gram
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
HM	Hematological malignancies
HoD	Head of Department
IoST	Institute of Science and Technology
IPIs	Intestinal Parasitic Infections
IRC	Institutional Review Committee
Kg	Kilogram
m	Meter
mm	Millimeter
ml	Milliliter
NCHRC	Nepal Cancer Hospital and Research Center
PCR	Polymerase chain reaction
rpm	Revolution per minute
SES	Socio-economic status
STH	Soil-transmitted helminth
TB	Tuberculosis
TLC	Total Leucocyte Count
WHO	World Health Organization
µm	Micrometer

ABSTRACT

Infection with intestinal parasites is widespread worldwide, especially in developing countries. Intestinal parasites are a major cause of diarrhea in both immunocompetent and immunocompromised people, but cancer patients are more prone to infection, which can be fatal. This study aimed to investigate the prevalence and examine the risk factors of intestinal parasitic infections (IPIs) among suspected cancer patients at Nepal Cancer Hospital & Research Center (NCHRC) in Nepal with suppressed immunity. One hundred individuals were selected with a convenience sampling strategy. The stool samples were preserved in a 2.5% potassium dichromate solution. Direct wet mount, sedimentation, flotation, and acid-fast methods were used to assess the prevalence and intensity of IPIs in this population. The overall prevalence of IPIs was 8% (4% Protozoan and 4% helminths). Altogether 3 species of intestinal parasites were detected. *Cryptosporidium* (4%) was predominant followed by *Trichuris trichiura* (2%) and *Ancylostoma duodenale* (2%). Multivariable regression analysis revealed that not using soap for hand-washing was significantly associated with the prevalence of overall IPIs. None of the other evaluated risk factors indicated an association with IPIs infection. In this study, the IPIs were attributable to individual hygiene behaviors, but not to nutritional status or socio-demographic characteristics. Yet, significant numbers of males reported anemia and undernutrition in this study population.

1. INTRODUCTION

1.1 Background

A "parasite" is a living entity that lives in another organism (the "host") and derives nutrients at the expense of the host. Numerous forms of parasites can harm people, including ectoparasites, endoparasites, facultative parasites, and obligatory parasites. They cause major health issues in people (CDC, 2014). Intestinal parasites are widely distributed. Intestinal parasitosis is one of the major causes of public health problems worldwide, particularly in developing nations such as Nepal. Intestinal parasitosis affects around 3.5 billion people worldwide, with 450 million being ill as a result of these infections,the vast majority of them are children (WHO, 2000). In addition to generating harmful inflammatory or immunological responses, parasites can also mechanically harm their hosts by excavating into them or digging into nearby tissue. The majority of parasites cause their host to experience a number of these conditions (Taliaferro, 2009).

Infection with intestinal parasites is common throughout the world, particularly in developing countries. Intestinal parasites are one of the leading causes of diarrhea in both immunocompetent and immunocompromised people, but cancer patients are especially vulnerable to contamination, which can be fatal (Salehi et al., 2020). In patients with a suppressed immune system, opportunistic parasitic infections are the most common cause of secondary infection (Uysal et al., 2017). Cancer patients receiving chemotherapy and other immunosuppressive medications are always vulnerable to infections, including opportunistic parasites (Salehi *et al*., 2018). If not diagnosed and treated early, intestinal parasite infections can cause major consequences in immunocompromised patients. Because of the high rate of enteroparasitosis in cancer patients, which is most likely due to their immunocompromised status, these people are more susceptible to becoming infected by a variety of parasitic species (Jeske et al., 2017).

Opportunistic parasites are usually associated with immunocompromised individuals due to immune system deficiencies. Lymphocytes (i.e., T and B cells) are impaired in their ability to fight opportunistic infections due to alterations in their cellular and humoral responses. As a result, immunocompromised persons are more vulnerable to microorganisms such as viruses, bacteria, fungi, and parasites (Hassanein & Fanaky, 2021). Immunocompromised individuals are more susceptible to opportunistic parasite infection when CD4+ T lymphocyte numbers drop below 200 cells/l. In underdeveloped countries, intestinal helminth infections are becoming more common due to poor environmental sanitation and personal cleanliness habits. In nations with limited resources and inadequate hygiene standards, there is a large overlap between chronic diseases including HIV, TB, cancer, and intestinal helminthic infections (Sitotaw et al., 2022).

In developing countries such as Nepal, intestinal parasite infections are a major cause of morbidity. *Ascaris lumbricoides*, *Hymenolepis nana*, hookworm, *Trichuris trichiura*, *Giardia lamblia*, and *Entamoeba histolytica* are the most prevalent intestinal parasites found in Nepal (Dahal et al., 2022). The high occurrence of these intestinal parasitic infestations is closely associated with overall poverty, poor environmental hygiene, and inadequate health care (Chandrashekhar et al., 2009). Earlier studies have suggested that many socio-economic and behavioral factors are associated with gastrointestinal parasites such as hygiene behaviors (Parajuli et al., 2009), eating raw or unwashed fruits and vegetables (Isazadeh et al., 2020; Azim et al., 2018; Mohamed et al., 2016), drinking water quality (Maharjan et al., 2013; KC et al., 2019), parent's occupation and education (Shrestha et al., 2019), family income (Quihui et al., 2006), children's hygiene and food habits (Maharjan et al., 2013) and malnutrition (Buzigi, 2015; Unachukwu & Nwakanma, 2018). Intestinal parasite infections (IPIs) prevalence is decreasing since the adoption of the national deworming program initiated in 2004. Some earlier studies among ethnic groups still reported a high prevalence (Parajuli et al., 2009; Adhikari etal., 2021). People with suspected cancer may have suppressedimmunity to opportunistic intestinal parasites. Hence, this study aims to evaluate such IPIs and associated factors. Further, our study will also allow seeing the prevalence and intensity of parasitic infection in association with suggested Socioeconomic status (SES), and socio-demographic and behavioral factors which may help to formulate relevant policies to support a vulnerable population of the society.

1.2 Objectives

1.2.1 General Objective

- To identify the prevalence of intestinal parasites in cancer patients

1.2.2 Specific Objectives

- To find the opportunistic parasitic infection in cancer patients.
- To assess associated risk factors with intestinal parasite infection.
- To evaluate the prevalence and intensity of parasitic infection in the association with suggested SES, sociodemographic and behavioral factors.

1.3 Significance of the study

Cancer is one of the most burning issues all across the world. Especially in developing countries, those immunocompromised people are more susceptible to parasitic infections and there are a lot of research gaps that need to be addressed. Detection of intestinal parasites in immunosuppressed people with cancer may help in treatment, care cost, and a further improvement in quality of life. This study may contribute to finding the prevalence of IPIs in the susceptive population and explore associated factors. It will help in sustainable solutions to the problems brought by this topic.

2. LITERATURE REVIEW

2.1 History of Parasitology

Prior to the middle of the 17th century, knowledge of parasitology was restricted to the recognition of a few common external parasites such as lice and fleas, as well as a few internal parasites such as *Ascaris*, tapeworms, pinworms, and guinea worms. They were present because of the natural products of human bodies. This concept was supported by Rudolphi and Bremser as well (Chandler and Read, 1961). People believed that internal parasites were caused by unintentionally swallowing free live organisms during the Linnaeus era (Chandler and Read, 1961).

Francesco Redi, the "Grandfather of Parasitology," discovered in the second half of the 17th century that maggots formed from flies' eggs and that Ascaris had male and female forms. He also searched for and discovered parasites not just in the human colon but also in other human organs, bird air sacs, and fish swim bladders (Chandler and Read, 1961). At the same time, Leewenhoek discovered the microscope, which helped him to describe many types of animalcules such as protozoa in rainwater, saliva, feces, and so on. Linnaeus identified *Trichuris trichiura* in 1771 (Arora and Arora, 2012), and Grassi and Fulleborn described its later life cycle. Zeder identified five different worm classes in 1800, and Rudolphi named them Nematoidea, Acanthocephala, Nematoda, Cestoda, and Cystica. Cercaria larvae were discovered by Muller in 1773 (Chandler and Read, 1961).

Dubini discovered the hookworm in 1782. In 1898, Leoss made the discovery that hookworm larvae could penetrate human skin. The first human amoeba, *Entamoeba gingivalis*, was discovered in 1849 by Gros. In 1859, Lambl discovered *Entamoeba histolytica*. In 1855 and 1856, respectively, Kuchemeister and Leukart investigated the life history of the *Taenia solium*. Leukart studied the life cycle of *Enterobius vermicularis* in 1865, and Losch later demonstrated its pathogenic character. In 1876, Normand discovered *Strongyloides stercoralis* (Arora and Arora, 2012). Schoudinn distinguished between pathogenic and non- pathogenic amoeba species in 1903 (Oli, 2016). In 1961, Stewart conducted an experiment to demonstrate *Ascaris* tissue movement.

2.2 Intestinal Parasitic Infection in Cancer Patients

In developing countries, parasitic infections caused by intestinal helminths and protozoan parasites are among the most common infections. In developed countries, protozoan parasitescause more gastrointestinal infections than helminths. In endemic countries, intestinal parasites cause significant morbidity and mortality (Haque, 2007). Around one-fourth of the world's population is affected by intestinal worm infections, such as roundworm, hookworm, and whipworm (Williams-Blangero et al., 1998). Cancer accounted for almost 13% of all deaths worldwide in 2008, with an estimated twelve million deaths by 2030 (Pestechian et al., 2020). Many environmental and physiological factors can cause cancer. Infections with viruses, bacteria, and parasites have been linked to human carcinogenesis for many years (Tong et al., 2017). When intestinal parasite infections in immunocompromised patients are not diagnosed and treated early, they can have catastrophic effects (Walton, 2021). Environmental factors and personal hygiene practices are significantly contributing to the rise of intestinal helminth infections in developing countries. There is a significant overlap between chronic illnesses like HIV, TB, and cancer and intestinal helminthic infections in countries with limited resources and inadequate hygiene standards. *Ascaris lumbricoides, Trichuris trichiura*, and hookworm are examples of intestinal helminths that impair nutrition and trigger a type-2 immune response that may worsen the severity and clinical results of cancer patients (Sitotaw et al., 2022).

In a study on the prevalence of enteric opportunistic parasitic infections in cancer patients ata teaching hospital affiliated with Iran University of Medical Sciences from July 2016 to December 2017, 23 patients were found to have intestinal parasites, with Blastocystis being the most common (14%) (Salehi et al., 2018).

In Turkey, a research on intestinal parasite infections in leukemic patients who had diarrhea revealed 9.9% parasitic infection out of 91 stool samples. The parasite that was found most commonly was *Cryptosporidium* (Uysal et al., 2017).

A case-control study on enteric protozoal infections in children receiving chemotherapy for cancer was conducted in Iraq which showed that 60 (60%) of the patients had enteric protozoal infections, compared to 5 (10%) of the control group. *Giardia lamblia* and *Cryptosporidium parvum* were found to be more prevalent in cancer children than in healthy children (Zainab & Al-mukhtar, 2015).

Fifty two stool samples from children aged 6 months to 16 years old were obtained as part of a study on intestinal parasite infection in children with cancer in Ahvaz, Southwest Iran2020. Among them, 38.38% were infected with intestinal parasites (Salehi et al., 2020).

The oocyst of *Cryptosporidium parvum* was analyzed by modified ziehl neelsen staining technique and corproantigen by ELISA in a study on *Cryptosporidium* Infection in Pediatric Patients with Lymphohematopoietic Malignancies. In children with cancer, 22% of 100 stool samples tested positive for *Cryptosporidium* infection (Fariba et al., 2007). A study on intestinal parasitism in pediatric oncology children receiving chemotherapy in Egypt found 6.6% unexpectedly low prevalence out of 137 stool samples (El-Badry et al., 2019).

A study on intestinal parasites in cancer patients in 15 municipalities in southern Brazil found that 61.65% of 73 samples were positive for parasites. *Ascaris lumbricoides* and *Giardia lamblia* were the most frequently associated parasites (Jeske et al., 2017). A study on the prevalence of intestinal helminths among cancer patients undergoing chemotherapy was conducted at the University of Gondar Comprehensive Specialized Hospital Oncology Clinic in Northwest Ethiopia. Intestinal parasites were found in 7/41 (17%) of the participants. The isolated parasites were hookworm 3/41 (7.3%), *Ascaris lumbricoides* 3/41 (7.3%), and *Hymenolepis nana* 1/41 (2.4%) (Sitotaw et al., 2022).

During a 5-year (2014-2019) investigation on the Prevalence of Parasitic Infections in Cancer Patients and Healthy Individuals in Isfahan, Iran, the prevalence of parasitic infection in cancer patients was 39%. In the control group, the infection rate was 28%. With prevalence rates of 18.7% and 13.2%, respectively, *Blastocystis hominis* was the most common parasite in both the cancer patient and control groups. Additional parasitic infections were *Entamoeba coli* (10.2%), *Endolimax nana* (6.4%), and *Giardia lamblia* (4.8%) (Pestechian et al., 2020).

A study conducted in Egypt on the prevalence of enteric opportunistic parasites in immunocompromised cancer patients with malignancy (150) and without malignancy (50) showed that opportunistic parasites were more prevalent in cancer patients (57%) than in the control group (43%) (Wassef et al., 2016).

From October 2010 to September 2011, a study on opportunistic Coccidian Parasites among Saudi Cancer patients Presenting with diarrhea: Prevalence and immune status was undertaken at King Khalid University Hospital in Saudi Arabia. The prevalence rates for *Cryptosporidium, Cyclospora*, and *Isospora belli* were 70.3%, 51.8%, and 25.9%, respectively, with an overall prevalence of 88.9% among cancer patients. Patients with lymphoma had a higher incidence of *Cryptosporidium* and *Cyclospora* (100 and 66.6%, respectively) (Sanad et al., 2014).

In Tehran, Iran, a study was undertaken on the prevalence of intestinal parasitic infection in cancer, organ transplant, and primary immunodeficiency patients. In general, parasites were found in 26/80 (32.5%) of patients with primary immunodeficiency, 22/85 (25.9%) of cancer patients, and 7/25 (28%) of organ transplant recipients. *Blastocystis hominis* was the most common intestinal parasite infection in cancer patients, followed by *Giardia lamblia* (2.3%) and *Dientamoeba fragilis* (1.1%) (Esteghamati et al., 2019).

In Poland (2017-2018), a study was undertaken on Protozoan intestinal parasitic infection in patients with hematological malignancies. Intestinal protozoans were found in 16% of patients with hematological malignancies (HM) and 6% of individuals in the control group. In stool samples from HM patients, cysts of *Giardia intestinalis* (2%), oocysts of *Cryptosporidium* spp. (10%), vacuolar forms of potentially pathogenic *Blastocystis* spp. (2%), and cysts of nonpathogenic *Entamoeba coli* (2%) were detected. Coproantigens of *Cryptosporidium* spp. and *Giardia intestinalis* were found in 5 (10%) and 1 (2%) patients with HM, respectively. Vacuolar forms of *Blastocystis* spp. were detected in three control group participants (Łanocha et al., 2022).

A cross-sectional study on An Association Between *Blastocystis* Subtypes and Colorectal Cancer Patients: A Significantly Different Profile from Non-cancer Individuals was conducted in Egypt from February 2019 to February 2021. *Blastocystis* was found in 52% of Colorectal Cancer patients and 42% of non-cancer persons, respectively(Ali et al., 2022).

A cross-sectional study on intestinal parasites in children with lymphohematopoietic malignancy was conducted in Mashhad, Iran. Three fresh stool samples were taken on three consecutive days. From October 2008 to October 2009, stool samples were obtained from 89 children aged 1 to 18 years with lymphohematopoietic malignancies receiving chemotherapy. The study found that 35.9% of patients had parasitic infections, with the following parasites identified: *G. lamblia* (the most common parasite in children) 16 (18%), and *Entamoeba coli* 6 (6.7%), *Iodamoeba butschlii* 2 (2.2%), *Blastocystis hominis* 5 (5.6%), *Chilomastics mesnili* (1.1%), *Hymenolepis nana* (1.1%), and *Enterobius vermicularis* (1.1%) (Zabolinejad et al., 2013).

In 2018, a study on intestinal protozoan infections in cancer patients undergoing chemotherapy was carried out in Shahrekord, Central Southwest Iran. Total 750 stool samples were collected from 250 chemotherapy patients (three fecal samples per patient). Intestinal parasites infected 12 (4.8%) patients, with 7 (2.8%) and 5 (2%) samples infected with *Blastocystis hominis* and *Giardia lamblia*, respectively (Banihashemi et al., 2020).

A descriptive, cross-sectional investigation was carried out on 52 stool specimens obtained from cancer patients hospitalized to the Oncology Ward of Shahid Baqaei 2 Hospital in Ahvaz, Iran, over six months. *Blastocystis* spp. was found in 21.1% of the cases, and 11.5% of them had gastrointestinal symptoms, indicating a significant relationship between *Blastocystis* infection and gastrointestinal symptoms (Salehi et al., 2021).

From February 2020 to October 2020, a study on the prevalence of intestinal parasites in cancer patients in the Al-Najaf Province was carried out in Iraq. 387 stool samples were collected, and 97 samples tested positive for intestinal parasites. According to the study, *Entamoeba histolytica* was the most prevalent intestinal parasite among cancer patients, accounting for 43.2% of cases (Alhuda & Neamah, 2021).

In Turkey, 94 patients with malignant solid tumors participated in a cross-sectional study on *Cryptosporidium* spp. during treatment. The prevalence was 2.1% (2/94), 5.3% (5/94), and 5.3% (5/94), as determined by Ziehl-Neelsen staining, real-time PCR, and ELISA, respectively. Using all of the findings from the three methods, the prevalence was 8.5% (8/94) (Karabey et al., 2021).

A study was conducted on the frequency of intestinal parasites in patients with malignancy in Ardabil Province, Northwest Iran. From February to September 2015, 100 fecal samples were collected from cancer patients during the cross-sectional research. The overall prevalence of intestinal parasite infections in cancer patients examined was 10%. The infection rates of identified intestinal parasites were 4% for *Cryptosporidium* spp. oocyst, 3% for *Blastocystis hominis*, 2% for *Giardia lamblia*, and 1% for *Taenia* spp. (Mohammadi-Ghalehbin et al., 2017).

2.3 Risk Factor of Intestinal Parasitic Infection

Intestinal parasite infections cause major issues in life, which are triggered by a variety of factors. Numerous conditions, including intestinal parasite infections, can lead to major health concerns. The most important risk variables for intestinal parasite infection were education level, raw vegetable usage, weight and upper arm circumference, animal keeping, water supply resources, and previously used medications (Khan et al. 2022). IPIs are thought to be caused mostly by low socioeconomic status, as well as poor hygiene (Khanal et al., 2016). Low socioeconomic status, poor hygiene (Khanal et al., 2011; Ojurongbe et al., 2014), a lack of effective sanitary disposal, a lack of pure drinking water (Rayapu et al., 2012), and a lack of health education (Rashid et al., 2011) are thought to be the underlying causes of parasite infection.

Hand-washing behavior (just using water) is also thought to be a risk factor for intestinal parasite infection (Karunaithas et al., 2011; Sah et al., 2016). Intestinal parasite infection has been also found associated with farming (Tandulkar et al., 2013). The IPI was linked to eating habits, hygienic conditions, and the quality of drinking water. Subjects receiving cataract surgery without bathrooms and vegetarians showed a marginally higher positive rate than those with toilets and non-vegetarians (Rai et al., 2008).

Some research has found that unaware people are more likely to be infected with parasites (Pandey et al., 2015). Soil transmitted helminth infections have been found to be widespread throughout the tropics and subtropics (Dada et al., 2015). Some authors found that non-vegetarians were highly infected with parasite infection (Pandey et al., 2015). Intestinal parasite infection is caused by consuming untreated water, well water, or a lack of pure drinking water (Thapa et al., 2011; Tandulkar et al., 2013; Shakya et al., 2012). Intestinal parasites are common in urban slums, and poverty has been linked to an increased risk of infection (Mehraj *et al.*, 2008).

In recent times, protozoan parasites cause more gastrointestinal disorders than helminths. IPIs considerably increase morbidity and death in areas where they are endemic (Haque, 2007). Intestinal worm diseases such as roundworm, hookworm, and whipworm affect almost one-fourth of the world's population (Else et al., 2020).

3. MATERIALS AND METHODS

3.1 Study area

Nepal Cancer Hospital and Research Center (NCHRC) is the first private-sector initiative to provide the best quality of innovative therapy and outstanding care to patients and their families from all over the world. The hospital has a dedicated interdisciplinary team of cancer specialists who provide cancer patients with inexpensive, safe, innovative, and compassionate care. In addition to innovative treatments and advanced equipment, the hospital goes beyond medicine by providing access to alternative medicine.

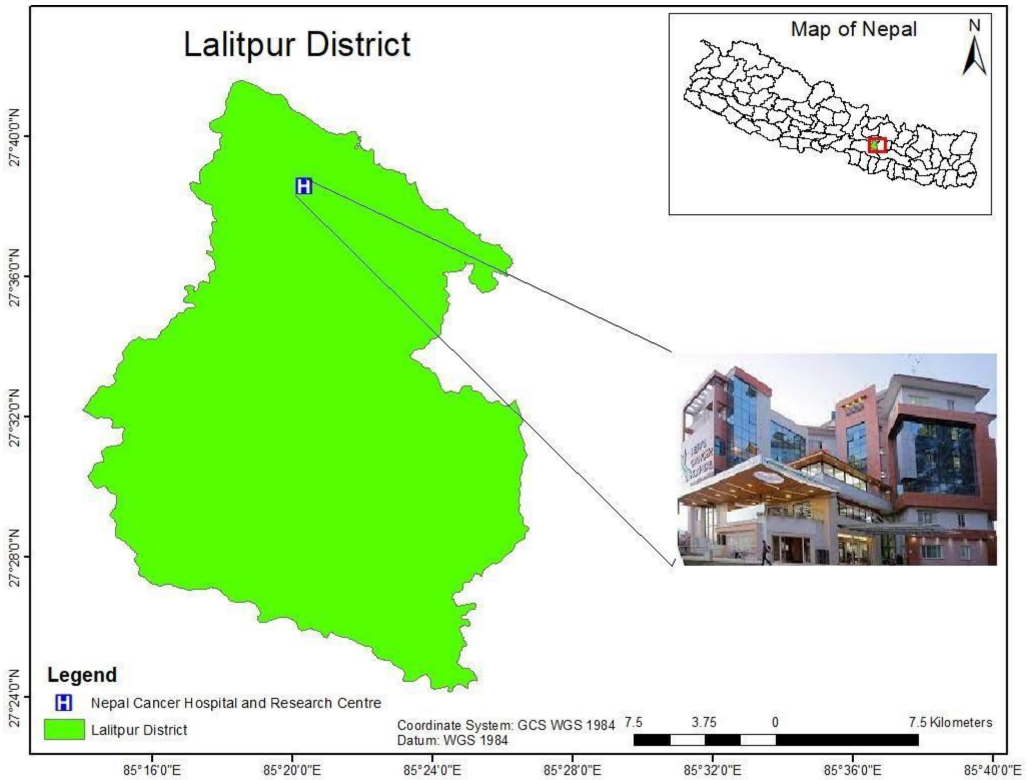

Figure 1- Map of study area

Nepal Cancer Hospital and Research Center is situated on 24 ropani land at Harisiddhi, Lalitpur, Nepal. The hospital's construction has been divided into two phases. The finished phase I hospital has 85 beds and covers an area of 50000 square feet. The Phase II hospital will be built within three years of the completion of the Phase I hospital, which will be 100,000 square feet and include 120 beds. In the future, the phase II hospital will be expanded to 300 beds.

NCHRC focuses on overall cancer care. Doctors have unparalleled abilities in detecting and treating all types of cancer, and the hospital employs modern equipment and the most innovative, advanced therapies to improve the chances of a cure. NCHRC has the most advanced radiation equipment, known as Linac True Beam-II with Rapid Arc. This has transformed Nepal's traditional radiotherapy treatment mode. Nurses and doctors collaborate as members of the healthcare team to provide the best course of treatment. They also have a group of social volunteers who are cancer survivors who work to guarantee that patients receive emotional support as well as information and experience about their cancer journey. They provide individual, family, and group counseling sessions both inpatient and outpatient. Patients who are interested in helping others can also join these groups.

3.2 Sampling Methods

Participants in the study site were recruited as first come first serve basis after taking informed consent. A convenient sampling technique was followed.

3.2.1 Selection Criteria

Patients who had taken an anthelmintic medication within the previous three months were excluded. Patients undergoing the treatment (diagnosed cases) were included.

3.3 Sample size

Since we focused on a special study population (i.e., Cancer patients undergoing Chemotherapy), we adopted a convenient sampling method with a sample size of 100.

3.4 Study design

i. Firstly, participants was recruited as first come first serve basis

ii. Informed consent was taken from the participants regarding the research

iii. A questionnaire survey was conducted with the participants regarding various socio-economic, demographic and behavioral factors

iv. Then, sterile vials was distributed for the collection of the stool sample and was labeled with the code name.

v. After the collection of the stool sample, it was preserved in 2.5% ofpotassium dichromate and stored at 4c

vi. Examined by direct wet mount, floatation, sedimentation, and acid-fast staining methods under laboratory conditions at central Department of Zoology, Tribhuvan University, kirtipur, Kathmandu and was observed under the microscope

vii. Identification and measurement of eggs, cyst and larva of parasites

viii. Data analysis

Figure 2- Study design

3.5 Methods

The participants were told to use a clean stick to scoop a sample of feces about the size of a thumb from the first, middle, and last parts of their stools early in the morning into the given sterile vial, being careful not to contaminate it with urine or soil. The collected sample was preserved in 2.5% of potassium dichromate solution to preserve the parasites and transported to the Central Department of Zoology of Tribhuvan University for further investigation. The blood parameter data was obtained from the complete blood count report of the patients.

3.5.1 Macroscopic Examination

This procedure involved looking at the stool's consistency (solid, liquid, semi-solid, or other characteristics), color (pale yellow, white, black, blood red, or any other color), and presence of any adult nematodes, trematodes, or cestodes.

3.5.2 Microscopic Examination

Microscopic examination of the stool can detect and identify intestinal protozoa as well as helminth eggs and larvae. It includes direct wet mount and concentration methods. Microscopic examination of the stool can detect and identify intestinal protozoa as well as helminth eggs and larvae. It includes direct wet mount and concentration methods.

3.5.2.1 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 sample was then examined under a microscope by covering it with a coverslip (Adhikari et al., 2021).

3.5.2.2 Formalin-ethyl acetate (FEA) sedimentation

In a 15 ml centrifuge tube, 2 grams of the fecal sample were thoroughly mixed with 12 mlof 0.9% w/v NaCl. After centrifuging the sample (1200 rpm for 5 minutes), the supernatant was discarded. In a 15 ml centrifuge tube, 2 grams of the fecal sample were well combined with 12 ml of 0.9% w/v NaCl. The material was centrifuged (1200 rpm for 5 minutes), and the supernatant was discarded. The tube was then filled with 10 ml of 10% formalin and 3 mlof ethyl acetate for centrifugation (1200 rpm for 5 minutes). After discarding the supernatant,the sediments were inspected under a microscope (Adhikari et al., 2021).

3.5.2.3 Saturated salt floatation

After being filtered via a strainer into a 15 ml centrifuge tube, the fecal sample wasthoroughly mixed in 12 ml of 0.9 % w/v sodium chloride (NaCl). The filtrate was then centrifuged (1200 rpm for 5 minutes) with the next step. After discarding the supernatant, the tube was filled with 45 % w/v NaCl. The sample was centrifuged for 5 minutes at 1200 rpm. The tube was then completely and gradually filled with saturated NaCl and left undisturbed for 10 minutes with its mouth covered with a coverslip. Finally, the coverslip was removed with care and placed on a glass slide, and was examined under the microscope (Adhikari et al., 2021).

3.5.2.4 Acid-fast staining

Thin smears were made from the sediments obtained using the formal-ether procedure. The smears were dried at room temperature, fixed in 100% methanol for 2 minutes, then stained with carbol fuchsin for 15 minutes. The smears were then rinsed serially and gently for 2 minutes with distilled water, acid alcohol, and distilled water. The smears were re-stained with malachite green for a minute and then washed with distilled water. Finally, the dried smears were examined under a microscope at a total magnification of 1000x using immersionoil (Adhikari et al., 2021).

3.5.3 Identification

During the microscopic examination of unstained and stained stool smears using 10x and 40x objectives, the cystic and trophozoite stages of protozoans, as well as the egg and larva stages of helminths, were identified using a medical laboratory manual and experts. Size, shape, shell content, color, exterior feature, and hooks were examined for identification and were compared with previously published different literature.

3.5.4 Questionnaire survey

A Shortlist of questionnaires (See Appendix) was prepared regarding participant's behavioral, socio-economic conditions, and socio-demographic factors.

3.6 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. 22-0058).

3.7 Data analysis

All behavioral, demographic, and socioeconomic questions were coded, and the distributions of all variables were examined. For multiple group comparisons (Tables 1 and 2), independent t test was used for continuous data while a Chi-square/ Fisher’s Exact test was used for analyzing categorical data. Multivariable logistic regression was used to investigate the association between the 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 Statistical analyses were performed using IBM SPSS Statistic v25.

4. RESULTS

The study was conducted on cancer patients at NCHRC in Kathmandu. From October 2022 to February 2023, 100 stool samples of all ages and genders were collected and analyzed. The overall number of positive cases was found to be 8%. There were 4% with protozoan infections and 4% with helminth infections. Seven of the positive individuals were men, and one was a woman. They all acknowledged not washing their hands with soap and water before eating and found themselves directly consuming jar water for drinking. In addition, 5 of the 8 positive cases did not have their nails cut regularly. Four participants were reported with opportunistic parasitic infection among which 3 were anemic.

Table 1 illustrates that study participants mostly were middle-aged with a mean age of 47.37 years, with older females compared to male participants ($p<0.001$) in the study. Women participants were heavier and with higher BMI compared to male participants. Yet, height and blood parameters including Hb, TLC, neutrophil, eosinophil, and monocytes were comparable between males and females. Similarly, none of the socio-economic factors differed between male and female participants except fruit consuming habits. More frequent fruit consumption was reported by female participants compared to male participants ($p\sim0.05$). Most of the participants reported households with more than 5 members in households, can read and write with normal SES and frequently consumption of meat. However, the majority of them do not exercise and do not have pets or poultry at home.

Table 1-Characteristics features of study participants (n = 100)

Characteristics	Male (n=50)	Female (n=50)	P-value	Total
	Mean(SD)/n (%)	Mean (SD)/n (%)		Mean(SD)/n (%)
Demographic Characteristics				
Age (in years)	40.40 (25.99)	54.34 (12.49)	0.001$	47.37 (21.47)
Weight (Kg)	50.32 (19.50)	59.18 (11.28)	0.007$	54.75 (16.46)
Height (Meter)	1.51 (0.25)	1.54 (0.06)	NS$	1.53 (0.18)
Body Mass Index (BMI)(Kg/m2)	20.93 (4.93)	24.86 (4.17)	0.000$	22.9 (4.95)
Blood Parameter				
Hemoglobin (Hb) gram per Deciliter (g/dl)	12.00 (1.64)	11.73 (1.65)	NS$	11.86 (1.64)
Total Leucocyte Count (TLC)	7762 (4821)	6820 (3592)	NS$	7290.61 (4256.21)
Differential Leucocyte Count (DLC)				
Neutrophils (%)	61.23 (11.05)	61.90 (10.74)	NS$	61.56 (10.7)
Lymphocytes	34.88 (10.74)	33.68 (11.60)	NS$	34.28 (11.14)
Monocytes	2.94 (2.56)	2.56 (2.04)	NS$	2.75 (2.31)
Eosinophils	1.57 (1.60)	1.70 (2.26)	NS$	1.63 (1.95)
Socioeconomic (SES) Characteristics				
Household (HH) Crowding				
Yes (>5 member in HH)	37 (74)	36 (72)	NS*	73 (73)
No (<5 member in HH)	13 (26)	14 (28)		27 (27)
Can read and write				
Yes (Literate)	44 (88)	45 (90)	NS*	89 (89)
No (Illiterate)	6 (12)	5 (10)		11 (11)
Reported SES				
Normal	34 (68)	35 (70)	NS*	69 (69)
Good	16 (32)	15 (30)		31 (31)
Rear Poultry or Pet in House				
Yes	4 (8)	3 (6)	NS*	7 (7)
No	46 (92)	47 (94)		93 (93)
Do you exercise regularly?				
Yes	8 (16)	12 (24)	NS*	20 (20)
No	42 (84)	38 (76)		80 (80)
Do you consume Meat?				
Rarely	21 (42)	14 (28)	NS*	35 (35)
Frequently	29 (58)	36 (72)		65 (65)
Do you consume Fruits?				
Rarely	9 (18)	2 (4)	0.051	11 (11)
Frequently	41 (84)	48 (96)		89 (89)

$: Independent T-test, * Chi square test

Table 2 showed behavioral and lifestyle characteristics of study participants, with healthier behavioral and lifestyle characteristics (i.e., handwashing before eating and wearing footwear while outdoor) among female compared to male participants ($p<0.05$) in the study. Yet, male reported frequent recent consumption of anthelminthic drug than female (p<0.05). There was no significant different between male and female participants in knowledge ofIPIs, nail trimming habits, covering food, and types of drinking water they consume. Most of the participants reported knowledge of IPIs, and covering food. However, more than one third of participants do not trim nails regularly. Two third of participants were anemic while male dominated the prevalence of anemia compared to female. Similarly, one third of participant were overweight and among them more than half were women. While male reported significantly high prevalence of underweight compared to women participants (p<0.001).

Table 2-Behavioral, lifestyle and health status characteristics of study participants (n = 100)

Characteristics	Male (n=50)	Female (n=50)	Chi-Square P-value	Total
	n (%)	n (%)		n (%)
Behavioral and lifestyle characteristics				
Use of soap for handwashing				
Yes	44 (88)	50 (100)	0.027	94 (94)
No	6 (12)	0 (0)		6 (6)
Walk barefoot while outdoor				
Yes	12 (24)	0 (0)	0.000	12 (12)
No	38 (76)	50 (100)		88 (88)
Did you trim nails regularly?				
Rarely	19 (38)	18 (36)	NS	37 (37)
Frequently	31 (62)	32 (64)		63 (63)
Did you consume anthelminthic within 6 months?				
Yes	15 (30)	1 (2)	0.000	16 (16)
No	35 (70)	49 (98)		94 (94)
Do you cover food regularly?				
Yes	48 (96)	49 (98)	NS	97 (97)
No	2 (4)	1 (2)		3 (3)
Do you know about intestinal parasite?				
Yes	45 (90)	50 (100)	NS	95 (95)
No	5 (10)	0 (0)		5 (5)
What type of water do you drink?				
Boiled or filtered	37 (74)	43 (86)	NS	80 (80)
Jar	13 (26)	7 (14)		20 (20)
Anemia				
Yes	38 (76)	26 (52)	0.012	64 (64)
No	12 (24)	24 (48)		36 (36)
BMI Category				
BMI <18.5 (Underweight)	19 (38)	3 (6)	0.000	22 (22)
BMI 18.5 to 24.99 (Normal)	24 (48)	21 (42)		45 (45)
BMI >25 (Overweight)	7 (14)	26 (52)		33 (33)

Table 3 shows the prevalence of IPIs among study participants. A total of 100 fecal samples were evaluated under the microscope with different techniques as described in the method. Only 8 samples (8%) were found shedding one or more species of IPIs (Table 3). Of the 1 species of parasites identified, *Cryptosporidium* species belong to protozoa, 2 species to nematodes. Overall, *Cryptosporidium* has a higher prevalence (4%) followed by *Trichuris trichiura* (2%), and hookworms (2%). The male participant indicated significantly high IPIs prevalence compared to females (Chi square p<0.030) (Table 3). Yet, egg density wasslightly higher among males compared to females (t test, p=0.06) but not statistically significant.

Table 3-Prevalence of gastrointestinal parasites in immunocompromised participants (n = 100)

Parasite species	**Male (%)**	**Female (%)**	**Fisher's Exact test P-value**	**Total**
Cryptosporidium	3 (6)	1 (2)	NA	4 (4)
Trichuris trichiura	2 (4)	0 (0)	NA	2 (2)
Hookworms	2 (4)	0 (0)	NA	2 (2)
Infection density	0.20 (0.54)	0.04 (0.28)	0.065$	
Total infection	7 (14)	1 (2)	0.03*	8 (8)
Protozoan	3 (6)	1 (2)	NA	4 (4)
Helminth	4 (8)	0 (0)	NA	4 (4)

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

Table 4 shows the association between parasitic infections associated factors. The presence of any parasitic infection was higher among participants doing frequent exercise compared toparticipants who exercise rarely. This study also indicates that the prevalence of IPIs was significantly high among participants who do not use soap to wash hand before eating (p <0.001) in both univariate as well as adjusted multivariate model. However, the prevalence of IPIs was not associated with any demographic, SES, lifestyle, or other behavioral characteristics evaluated.

Table 4-Prevalence and odds ratio of IPIs with respect to behavioral and individual characteristics using logistic regression analysis (n=100)

	Any IPIs (*n* =100)		
	%	Univariate OR (95%CI)	Multivariate* AOR (95%CI)
Socioeconomic (SES) Characteristics			
Household (HH) Crowding			
No (<5 member in HH)	11.11	ref	
Yes (>5 member in HH)	6.84	0.59 (0.13 to 2.65)	
Can read and write			
Yes (Literate)	7.87	ref	
No (Illiterate)	9.09	1.17 (0.13 to 10.52)	
Reported SES			
Middle High	3.2	ref	
Low	10.14	3.39 (0.40 to 28.79)	
Do you exercise regularly?			
Yes	20	ref	ref
No	5	0.21 (0.05 to 0.93)	0.08 (0.01 to 0.52)
Do you consume Meat?			
Rarely	14.28	ref	
Frequently	4.6	3.44 (0.77 to 15.38)	
Do you consume Fruits?			
Rarely	9.09	ref	
Frequently	7.9	1.17 (0.13 to 10.53)	
Use of soap for handwashing			
Yes	6.38	ref	ref
No	33.33	7.33 (1.11 to 48.45)	9.55 (1.03 to 88.46)
Walk barefoot while outdoor			
Yes	16.67	ref	
No	6.81	2.73 (0.49 to 15.41)	
BMI category			
Underweight	18.18	3.11 (0.63 to 15.34)	
Normal	6.67	ref	
Overweight	3.03	0.44 (0.04 to 4.41)	
Anemia			
No	2.77	ref	
Yes	10.94	4.30 (0.51 to 36.43)	
Did you trim nails regularly?			
Rarely	7.93	ref	
Frequently	8.10	1.02 (0.23 to 4.55)	
Did you consume anthelminthic within 6 months?			
Yes	18.75	ref	
No	5.95	0.27 (0.06 to 1.29)	
Do you know about intestinal parasite?			
Yes	7.37	ref	
No	20	3.14 (0.31 to 32.06)	
What type of water do you drink?			
Boiled or filtered	6.25	ref	
Jar	15	2.65 (0.58 to 12.47)	

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

*Model adjusted for gender

5. DISCUSSION

Intestinal parasites are a severe health problem in countries where there is overcrowding, inadequate environmental sanitation, and poor personal hygiene behavior, particularly in developing nations (WHO, 2010). Soil-transmitted helminth diseases causedby *Ascaris*, *Trichuris*, and hookworms are the most common, with more than two billion people believed to be infected (WHO, 2012). Coccidian protozoa and microsporidian fungi are opportunistic pathogens that are increasingly being implicated in infections in immunocompromised people. In most cases, these parasites invade the intestinal epithelium, causing secretory diarrhea and malabsorption. Immunocompromised individuals have a larger illness burden and a longer disease timeline (Einhorn et al., 2023).

This study investigated the prevalence of IPIs among suspected cancer patients at Nepal Cancer Hospital & Research Center (NCHRC) with suppressed immunity. The prevalence of IPIs in our study (i.e., 8%) is comparable with the prevalence of IPIs reported from different studies such as 10% among cancer patients in Ardabil Province, Northwest Iran (Mohammadi-Ghalehbin et al., 2017), 9.9% among leukemic patient in Turkey (Uysal etal., 2017) and 8.5% in Turkey (Karabey et al., 2021). A lower prevalence of IPIs compared to our study has also been reported among cancer patients like 2-5% in Shahrekord, Central Southwest Iran (Banihashemi et al., 2020). In contrast, quite a high prevalence of IPIs has also been reported by many previous studies. For example, in Ahvaz, Southwest Iran 2020, 38.38% were infected with intestinal parasites (Salehi et al., 2020). Similarly, in a study on intestinal parasites in cancer patients in 15 municipalities in southern Brazil 61.65% samples were positive for parasites (Jeske et al., 2017). Another study at King Khalid University Hospital in Saudi Arabia reported an overall prevalence of 88.9% among cancer patients. Patients with lymphoma had a higher incidence of *Cryptosporidium* and *Cyclospora* (100 and66.6%, respectively) (Sanad et al., 2014). Such a discrepancy in the prevalence of IPIs might be due to the difference in 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 the participants reported healthier behavioral and lifestyle characteristics (i.e., handwashing before eating and wearing footwear while outdoors), knowledge of IPIs, and covering food. However, the small sample size limits us from any conclusion. Hence, further study needs to be conducted to confirm this finding.

The prevalence of overall IPIs was higher among males compared to females. Yet males indicated higher egg density compared to females but could not achieve statistical significance. A few earlier studies reported similar findings with elevated IPIs prevalence among males compared to females (Derso et al., 2021; Sharma et al., 2021; Ulhaq et al., 2022; Ifeoma et al., 2022). Yet, few studies reported similar risks of IPIs between males and females among cancer patients (Al-Qobati et al., 2012). In this study, the majority of females reported healthier behavioral and lifestyle characteristics (i.e., handwashing before eating and wearing footwear while outdoors) compared to male participants ($p<0.05$), which may explain the discrepancy in IPIs infection by gender. Further, males indicated a higher prevalence of anemia and undernutrition compared to females, which may lead to weak nutritional as well as immunity for elevated IPIs infections. Yet, further study needs to be done to confirm this association.

The presence of parasitic infections (IPIs) was higher among participants doing frequent exercise compared to participants who exercise rarely. None of the previous studies evaluated such an association in our search. However, it can be assumed that regular exercise may enhance the chance of contamination of the hand with the surface and may also contribute to infestation by hookworm or Strongyloides if walked barefoot. About 24% of males reported barefoot walking while outdoors indicated higher IPIs compared to females. In addition, handwashing habits are consistently associated with IPIs prevalence in univariate and multivariate models after adjustment with gender and exercise. Yet, the small sample size limits us for generalization and further study needs to be conducted to confirm this association.

This study also indicates that the prevalence of IPIs was significantly high among participants who do not use soap to wash their hands before eating ($p <0.001$) in both univariate as well as adjusted multivariate models. However, the prevalence of IPIs was not associated with any demographic, SES, lifestyle, or other behavioral characteristics evaluated. Our findings agreed with previous studies (Olsen *et al*., 2001) that reported a significant association between “not using soap for handwashing” after defecation with roundworm infection among 2 to 12-year-old children in Egypt. A fecal-oral route of transmission is the established route of transmission of IPIs (Parajuli *et al*., 2009, 2015). This is probably because these variables were proxy measures of poor hygiene in general. However, the odds ratios were always consistently greater for both univariate and multivariate models after adjustment. Since the participants are cancer-diagnosed patients with compromised immunity, hand hygiene might be crucial among current vulnerable populations.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

IPIs among vulnerable populations pose a serious health problem. Multivariate logistic regression analysis showed that the IPIs were attributable to individual differences in behavior like “not using soap for handwashing”. High malnutrition (reported under, overnutrition, and high anemia) that stemmed from their dietary patterns, medication, and morbidity might worsen the situation of cancer patients together with IPIs. Yet, the small sample size and cross-sectional design limit us for generalization.

6.2 Recommendations

- It is important to promote research work on the prevalence of intestinal parasites andtheir prevention methods.
- Regular health examinations, stool testing, and medication distribution have to bedone without charge or at a substantially reduced cost.

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APPENDICES

Questionnaires

Baseline Questionnaires related to demographic, socioeconomic and behavioral factors

Participant Code/ Name:

Gender: ☐Male ☐Female Age: Height: Weight:

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 often do you consume fruits in your diet?

☐Every day ☐Once a week ☐Twice a week or more ☐Once a month

4. How often do you eat meat in a week?

☐ Once or twice ☐Thrice ☐Everyday ☐None

5. Have you currently participated in some form of exercise?

☐Yes ☐No

6. Concerning your weight, what would you like to achieve?

☐Lose weight ☐Gain weight ☐Maintain weight

7. What is your education?

☐ Actual Grade ☐Cannot read and write

8. What is the financial situation of your family?

☐Poor ☐Fair ☐ Good/Excellent☐

9. How frequently do you maintain your hygiene?

☐Always ☐Nearly always ☐Nearly never ☐Never

10. What is your father's education?

☐ Actual Grade ☐Cannot read and write

11. What is your mother's education?

☐ Actual Grade: ☐Cannot read and write

12. What is your occupation/ father's Occupation?

☐Govt. Employee ☐Business ☐Farmer ☐other specify

13. What is your occupation/ mother's Occupation

☐Govt Employee ☐ Business ☐Farmer ☐other specify

14. Do you use soap to wash your hand before eating?

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

15. Do you cut and clean your nail once a week?

☐ Yes ☐No ☐sometimes

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

☐Yes ☐No ☐sometimes

17. Do you wear foot ware while outdoor?

☐Yes ☐No ☐sometimes not

18. How many family members are in House?

19. Do you cover food from flies?

☐Yes ☐No ☐sometimes

20. Do you bite fingernails?

☐Yes ☐ No ☐sometimes

21. Do you play with Soils?

☐ Yes ☐No ☐sometimes

22. Do you eat food (any) dropped on the floor?

☐Yes ☐No ☐sometimes

23. Do you drink Boiled water?

☐Yes ☐No ☐sometimes

24. Do you have Reverse Osmosis Water Filter System in your House?

☐Yes ☐ No

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

26. Did you consume any medicine for intestinal helminths parasites in the last 6 months?

☐Yes ☐ No

27. Do you have free-ranging pig or poultry in the house?

☐ Yes ☐ No

28. Did you notice any worm in your stool?

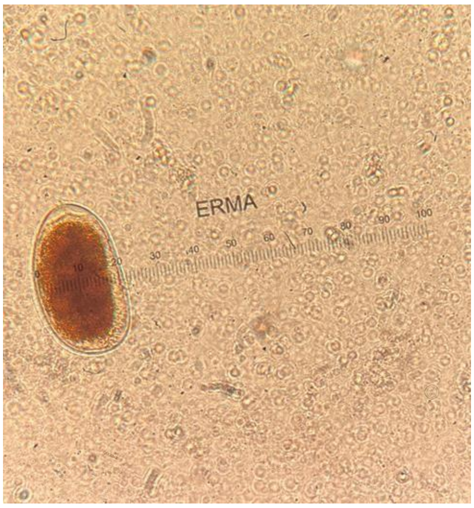
☐ Yes ☐ No

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

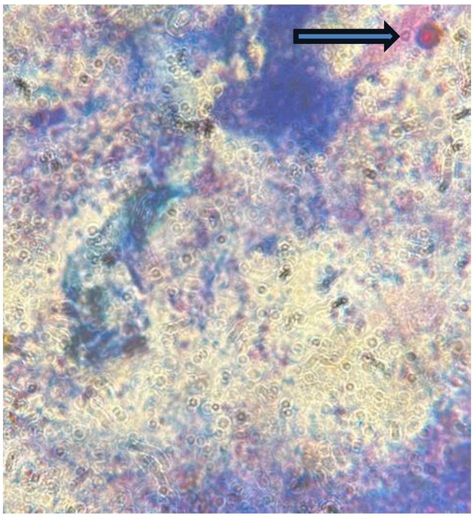
30. Do you ever consumed raw meat?

☐ Yes ☐No ☐Maybe

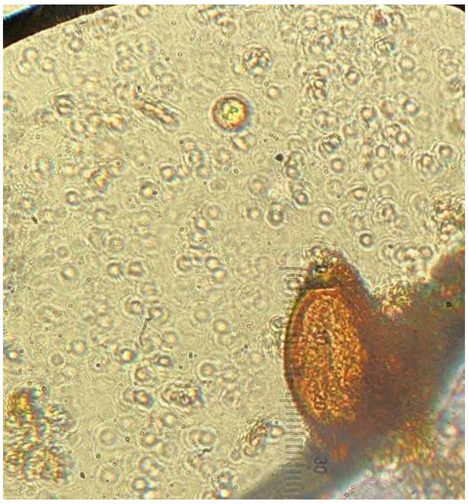
8. PHOTOGRAPHS



Photograph 1-Egg of hookworm
(10X *40X)



Photograph 2- Oocyst of *Cryptosporidium*
(10X *100X)



Photograph 3-Egg of *Trichuris trichiura*
(10X *40X)

Photograph 4- Microscopic examination