PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AND ASSOCIATED RISK FACTORS AMONG PREGNANT WOMEN HETAUDA, NEPAL

Dipa Dhakal

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Submitted to

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Tribhuvan University

Kritipur, Kathmandu

Nepal

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DECLARATION

I here declare that the work presented in this thesis has been done by myself, and has not been submitted anywhere for the ward of any degree. All the source of information has been specifically acknowledged by reference to the authors or institutions.

Date: 2080/01/21

Dipa Dhakal

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T.U Regd. No: 5-2-242-804-2015

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त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.
☎ ०१-४३३१८९६
01-4331896
Email: info@cdz.tu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology
T.U., Kirtipur

RECOMMENDATION

This is to recommend that the thesis entitled **"PREVALENCE OF GASTROINTESTINAL PARASITES AND ASSOCIATED RISK FACTORS AMONG PREGNANT WOMEN HETAUDA, NEPAL"** has been carried out by Dipa Dhakal for partial fulfillment of Master`s Degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this work has not been submitted for any other degree in any institutions.

Date...2080/01/21.....

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Supervisor

Mr. Janak Raj Subedi

Lecturer

Central Department of Zoology

Tribhuvan University

Kritipur, Kathmandu, Nepal

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

फोन ०१-४३३१८९६
01-4331896
Email: info@cdz.tu.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 Mr. Janak Raj Subedi this thesis submitted by Dipa Dhakal entitled "**PREVALENCE OF GASTROINTESTINAL PARASITES AND ASSOCIATED RISK FACTORS AMONG PREGNANT WOMEN HETAUDA, NEPAL**", is approved for the examination and in partial fulfillment of requirements for Master`s Degree of Science in Zoology with special paper parasitology.

Date..2080|03|19

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Prof. Dr. Kumar Sapkota

Head of Department

Central Department of Zoology

Tribhuvan University

Kritipur, Kathmandu, Nepal

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

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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 Dipa Dhakal entitled **"PREVALENCE OF GASTROINTESTINAL PARASITES AND ASSOCIATED RISK FACTORS AMONG PREGNANT WOMEN HETAUDA, NEPAL."** has been accepted as a partial fulfillment for the requirement of Master's Degree of Science in Zoology with special paper Parasitology.

EVALUATION COMMITTEE

.....

(Supervisor)

Janak Raj Subedi

Lecturer

Central department of zoology

Kritipur, Kathmandu, Nepal

.....

External examiner

.....

(Head of Department)

Prof. Dr. Kumar Sapkota

Professor

Central Department of Zoology

Kritipur, Kathmandu, Nepal

.....

Internal Examiner

Date..2080|03|19

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Exam Roll N0. – 820/076

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LIST OF ABBREVATIONS

Abbreviated form	Details of Abbreviations
ANC	Antenatal Care
CNS	Central Nervous System
FHD	Family Health Division
GI	Gastrointestinal
GON	Government of Nepal
HIV	Human Immunodeficiency Virus
IPIs	Intestinal Parasitic Infections
IUGR	Intra-Uterine Growth Restriction
LBW	Low Birth Weight
ns	Non-significant
PNC	Postnatal Care
P-value	Probability value
qPCR	Quantitative Polymerase Chain Reaction
UNICEF	United Nations International Children`s Emergency Fund
WHO	World Health Organization

ABSTRACT

Intestinal parasitic infections affect pregnant women all over the world. The infection has been linked to the development of life-threatening conditions in both pregnant women and their developing fetus. Risk factors during pregnancy can be seen more in developing countries like Nepal. This study was conducted to determine the prevalence, intensity of infection and associated risk factor among pregnant women of five different wards of Hetauda sub-metropolitan city, Makawanpur, Bagmati province, Nepal. A cross-sectional study was carried out among (100) conveniently sampled pregnant women receiving antenatal care services at Rural Urban health care center of Two, four, five, ten and 11 numbers wards of Hetauda. Structured questionnaires were administered to study participants to assess socio-demographic and other possible factors. Stool samples were collected from each pregnant woman and examined for the presence of intestinal parasites by microscopy using direct wet mount, floatation as well as formal-ether sedimentation techniques. The study revealed that of the 100 samples examined, 19 samples (19%) were found to be positive for gastrointestinal parasites covering five genera. *Ascaris lumbricoides* (8%) was most predominant followed by *Entamoeba histolytica* (4%), *Strongyloides stercoralis* (3%), *Trichuris trichiura* (2%) and *Hymenolepis nana* (2%). There is no significant difference between age wise prevalence and parasites presence. Financial condition is identified as significant factor associated with intestinal parasite infection. Screening of the women for intestinal parasites and provision of health education during their Antenatal care (ANC) visit is essential to prevent the adverse effects caused by parasites on maternal and fetal health.

1. INTRODUCTION

1.1 Background

Pregnancy is the state of conception in which a fetus develops inside a woman's womb which causes a series of changes in a woman's body. It can be confirmed through a blood test, urine test, and ultrasound. Pregnancy usually lasts for 270-290 days beginning from the first day of a women's last menstrual period. Pregnancy is divided into three trimesters each lasting approximately three months that are; the first trimester (week 1 to week 12), the second trimester (week 13 to week 28), and the third trimester (week 29 to week 40). Every pregnancy carries its risk. Risk during pregnancy arises due to factors like age, weight, and overall health status of a pregnant lady. Advanced maternal age, lifestyle choices, maternal health problems, pregnancy complications, multiple pregnancies, pregnancy history, anemia, and malnutrition are the specific risk factors that contribute to high-risk pregnancy. Women's health plays a significant role in the advancement of society and the country. Women's health must be prioritized in every community because a healthy mother gives birth to a healthy child. Due to various factors, including differences in socioeconomic conditions, lifestyles, and health-seeking behaviors across cultures, women's communities are more susceptible to various diseases than men's communities. This has become a significant challenge in developing nations like Nepal. There are 380 new pregnancies worldwide every 60 seconds, 110 complicated pregnancies, 40 of which result in abortions, and a pregnant woman dies (Fathalla, 2020). The majority of these deaths are caused by preventable causes, with developed countries accounting for the remaining 1% of deaths. Of these, 99% occur in developing countries. In a similar vein, every year more than 14 million teenagers worldwide become mothers. Of course, there are such births in every society, but 12.8 million, or more than 90%, of adolescent mothers, live in developing nations. Pregnancy in women over 35 has gradually increased over the past ten years, with more women over 35 reporting pregnancy in their late 30s (Tozzo et al., 2019).

Risk factors during pregnancy can be seen more in developing countries like Nepal. In Nepal, various factors contribute to increased risk factors among pregnant women and including poverty, illiteracy, women’s low social status, limited access to basic maternal

health care, poorly developed transportation systems, cultural backgrounds, and a diverse population (Pokharel, 2020). The government of Nepal has a policy of providing four transportation incentives to pregnant women who visit government health facilities to decrease maternal death due to the above-mentioned risk factors and by lacking proper antenatal care (Sharma et al., 2021). Each municipality has its rural-urban health care center which provides the ANC services along with others. To encourage women to give birth with a trained attendant in a medical facility, antenatal care (ANC) is a crucial factor in determining a safe delivery. This will ultimately help to lower the rate of maternal mortality (Simkhada et al., 2010). Maternal health care practices of Nepalese women include four ANC visits as per national protocol in the fourth, sixth, eighth, and ninth months of pregnancy, institutional delivery, and PNC check-ups every seven days for pregnant women(Sharma et al., 2021). In order to remove financial constraints from women choosing institutional delivery, Nepal launched the Aama Program (Maternity Incentive Scheme) in 2005. Incentives range from Nepali Rupees (NRs) 3000, 2000, and 1000 in the Mountain, Hill, and Terai districts, respectively, to (NRs) 800 for women who complete four ANC visits following national protocol, according to the Family Health Division (FHD)(Sharma et al., 2021). Offering health information and services that can significantly improve women's and infants' health is one of ANC's key roles. Additionally, ANC use has a positive effect on the uptake of postnatal services. For its citizens, the Government of Nepal (GoN) offers a variety of health policies. One of the programs under which the GoN offers financial incentives to women who utilize maternal health services, such as ANC/PNC and the delivery facilities at the health institutions, is the Safe Motherhood Program. Women are reportedly going to a health facility to receive money and baby clothes despite giving birth at home (Sharma et al., 2021).

Intestinal parasites are the organisms that live in the gastrointestinal tract of animals including humans. Two types of intestinal parasite that is helminths and protozoans are present in human that causes infection. Helminths are large organisms with many cells that can be seen with the naked eye and are present in the human gut. Species of intestinal helminthic parasites are known as geo-helminths and soil-transmitted helminths; *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Ancylostoma duodenal*, and *Nector americanicus* (Hookworm). Protozoans are called microscopic organisms with

only one cell and they can multiply. The most common one includes *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium*, and *Cyclospora cayetanesis* (Haque, 2007).

Intestinal parasitic infections (IPIs) caused by protozoa and geo-helminths are common problems in the human population, more in developing countries. The top ten intestinal parasitic infections worldwide include trichuriasis, hookworm infection, amoebiasis, and ascariasis (Hailu et al., 2020). Inadequate sanitization in developing countries makes IPIs very common which can be spread via the fecal-oral route through contaminated water, food, or surface such as toilet handles. It has the potential to spread from person to person. IPIs are interconnected with socio-economic and environmental factors. Overcrowding, limited access to pure drinking water, and poor personal hygiene make IPIs prevalent (Siziya et al., 2013). Intestinal parasitosis could be associated with conditions for the development of anemia during pregnancy that can result in detrimental fetal and maternal outcomes (Brooker et al., 2008). Parasitic infection can happen at any time during the three trimesters, but the effects on the fetus and placenta are more severe when it happens in the first trimester. Additionally, among women who are expecting for the first time, the infection worsens (Muhangi et al., 2007).

During pregnancy, the Helminths parasite usually causes mild symptoms, and iron deficiency anemia, however, a protozoan infection can cause severe symptoms including watery diarrhea, abdominal pain, and nausea in pregnant women. Hookworm infection causes deficiencies of iron, protein, and zinc which leads to low pregnancy weight gain and low birth weight(LBW), (Zapardiel et al., 2010). Hookworm infection in humans is brought on by infection with the parasitic helminths Necator americanus and *Ancylostoma duodenale*, and it is spread through contact with contaminated soil. Although it has long been known that hookworm infection is one of the main causes of anemia in underprivileged areas, knowledge of the advantages of managing the infection during pregnancy has lagged behind that of the other primary causes of maternal anemia (Zapardiel et al., 2010). A lighter hookworm burden, however, has been demonstrated to be associated with anemia in other investigations. The level of iron deficiency anemia brought on by hookworms varies depending on the species since infection with A. duodenale results in more blood loss than infection with N.americanus (Hotez et al.,

2004). In contrast to hookworm infection, which is linked to delayed first pregnancies and longer inter-birth intervals, roundworm infection (*Ascaris lumbricoides*) is associated with earlier first births and shorter inter-birth intervals (Blackwell et al., 2015). Anemia in pregnancy is associated with higher levels of Trichuris and hookworm infection. Trichuris infections during pregnancy require renewed attention. Increased hookworm and Trichuris infection levels during the second trimester were linked to a higher risk of anemia during the third trimester (Gyorkos et al., 2011). Helminths may have a significant impact on fertility in humans, reflecting the physiological and immunological effects of infection. Nervous system disorders, anemia, and gastrointestinal disturbances are the hallmark signs of ancylostomiasis. The amebic disease seems to be more frequently linked to acute disease exacerbations and more pronounced symptoms.. Infected pregnant women may have tenderness and mild abdominal pain, as well as bloody, dysenteric stools. The diarrhea is marked, as secondary signs including fluid loss and electrolyte imbalance, whose effect is severe on the outcome of pregnancy. Strongyloides can cause "autoinfection," which has been shown to persist in humans for more than 30 years without treatment. However, compared to Ascaris, Trichuris, or hookworm infections, *Strongyloides stercoralis* is the fourth most significant intestinal nematode infection that causes Strongyloides (Stephenson et al., 2000). A mild case of anemia is frequently the result of acute Schistosoma infection. Infertility and ectopic pregnancies are linked to tubal granulomas, which affect tubal motility and patency. Infection by schistosomes of the placenta and fetus can result in intra-uterine growth restriction IUGR, LBW, preterm labor, and stillbirth (Friedman et al., 2007). Many mothers in developing nations experience pre and postpartum malnutrition due to iron deficiency. The inability to obtain iron-rich and iron-absorbing food, particularly during the reproductive age or pregnancy, is one of the main causes of pregnancy in women over 35 years of age, with iron deficiency anemia (Larsson et al., 2017). As a result, the WHO and UNICEF advise all pregnant women in most of Asia and Russia, where anemia is highly prevalent, to take iron supplements.

Entamoeba histolytica and *Giardia lamblia* are the two most frequently encountered protozoa. Their naturally high infectivity makes them more pathogenic inside the host and is a common cause of dysentery. Infants are at risk of transmission during

breastfeeding in areas with a high prevalence of HIV when pregnant women have asymptomatic *Entamoeba histolytica* infections (Nhidza et al., 2020). Giardiasis in pregnancy can be a debilitating disease that endangers both the mother's and the fetus's health. In pregnancy, the standard therapeutic agents are contraindicated (Kreutner et al., 1981). *Giardia* infection during pregnancy has negative effects on the unborn baby due to the associated diarrhea, fluid and electrolyte loss, and malabsorption. Pregnant women face minimal risk from whipworm infection. *Toxoplasma gondii* poses a high risk of complications because it can infect embryonic tissues and pass through the placental barrier. Infections in the first and second trimesters are linked to less severe complications like chorioretinitis, hydrocephalus, low birth weight, and central nervous system (CNS) abnormalities, whereas infections in the third trimester are linked to late congenital complications and developmental delay. It is listed as a possible miscarriage risk factor (Valladares-Garrido et al., 2022). Downgrading of mother's nutritional status and possibilities of it in the unborn child's health are among the global effects of these parasites during pregnancy. The severity of the effects, however, varies depending on several variables, including the parasite load and species, the pregnant woman's immune system, and the presence of co-occurring diseases.

Physically, physiologically, and immunologically draining is pregnancy. All pregnancies are uncomfortable, and while most pregnancies and births are difficult, about 15% of pregnant women will experience situations that could be life-threatening, and some of them will need significant obstetrical intervention to survive (Lampinen et al., 2009). When parasite infection is present, this burden is exacerbated. Pregnancy-related intestinal parasitic infections have been linked to severe negative effects on both the mother and the fetus (Tsoka et al., 2023). However, dedicated agents are scarce compared with the antibiotics available for pregnant women, and no specific vaccine is available (Boitel & Desoubeaux, 2020). It is very challenging to successfully control parasitic infections with dedicated drugs while protecting the fetus from the harmful effects of these medications. The embryo- and feto-toxic effects of each medication should be known before using them (Boitel & Desoubeaux, 2020).

The rationale of this study is to know about the prevalence rate of helminths and protozoans among pregnant women and the risk factors associated with pregnancy to overcome maternal mortality rate, miscarriage, low-weight births, and so on with proper use of medication knowing their embryo-feto-toxic effects on pregnant women and developing fetus.

1.2 Objectives

1.2.1 General objective

- Prevalence of intestinal parasitic infection and associated risk factors among pregnant women Hetauda, Nepal.

1.2.1 Specific objectives

- To study the age-wise distribution of gastrointestinal parasites.
- To determine knowledge, attitude, and practices regarding the health and sanitation of pregnant women.

1.3 Significance of the Study

Nepal, a landlocked country has most of the parts rural and undeveloped, containing unhygienic health habits leading to various parasitic infections. The pregnant women living in marginalized areas of the Hetauda area are still behind in terms of health, education, and other aspects. This study will be very effective for developing knowledge about intestinal parasites among pregnant women of Hetauda, Makawanpur, Bagmati Province, Nepal. This type of study has not been programmed before in this area. This study will be useful in understanding the prevalence of intestinal parasitic infections and their associated risk factors among pregnant women. This study may guide early treatment before devastating effects of the diseases occurs in pregnant women because of intestinal parasites. Moreover, the present study will help future researchers to enhance their knowledge and to program the investigation of pregnant women about intestinal parasites and their infections.

2. LITERATURE REVIEW

Human beings act as a host for approximately about 70 species of protozoan and 300 species of helminthic worms observed from our primate ancestors and some from domesticated animals (Ashford & Crewe, 1998). There are still 90 common species of parasites that can cause some of the most important diseases. Humans get infected by many parasites among which intestinal parasites cause severe effects, sometimes. Intestinal parasites are those organisms that live in the gastrointestinal tract of animals including humans. Two types of intestinal parasite that is helminths and protozoans are present in human that causes infection. Before the 17th century, knowledge about parasites was limited to some external parasites like lice, and fleas and some internal parasites like tapeworms, pinworms, and guinea worms (Chandler & Read, 1961). During half of the 17th century, Leeuwenhoek discovered *Giardia* by examining his stool (Chandler & Read, 1961). *Ascaris lumbricoides* were first discovered by Linnaeus in 1758. *Trichiuria trichiura* was also discovered by Linnaeus in 1771 whose complete life cycle was studied by Grassi in 1887 and Fulleborn in 1923. Dubini discovered human hookworm in 1782. *Schistosoma haematobium* was discovered by German parasitologist Theodar Bilharz and Karl Theodar Ernst Von Siebold in 1851. *Entamoeba histolytica* was discovered by Lambl in 1859. The life history of *T.solium* was examined by Kuchemuister in 1855 and Leuckart in 1856. In 1876 *Strongyloides stercoralis* was first reported by Normand. *Cryptosporidium parvum* was discovered by Current and Uptom in 1985.

Parasitic infection is a major public health problem. Approximately 3.5 billion people get infected by intestinal parasites and around 450 children were ill due to these infections (Chongbang et al., 2016). Around 60 % of the world's population is infected with intestinal parasites (Ragunathan et al., 2010). An outbreak of *Ascaris lumbricoides* and Hookworm causes hematological disorders and decreases the nutritional level of the body respectively which may lead to severe complications during pregnancy and also the low birth weight in infants (Zapardiel et al., 2010). *Ascaris lumbricoides* are associated with earlier first births and shorter inter-birth intervals (Blackwell et al., 2015). Regarding *Ascaris lumbricoides*, much research has been conducted in the past to study its

prevalence and reported with higher among other parasites. Further, the study among pregnant women in Venezuela (Rodríguez-Morales et al., 2006), the study in western Ethopia (Yesuf et al., 2019), as well as study in in three urban areas of Bogotá, Colombia, shows higher prevalence of *Ascaris lumbricoides* (Espinosa Aranzales et al., 2018). Yet another helminths parasites, Hookworm is also reported in pregnant women. Hookworm infection in humans is brought on by infection with the parasitic helminths *Necator americanus* and *Ancylostoma duodenale*. Hookworm is reported in several countries on different continents like Ethopia (Asrat et al., 2011) and Kenya (Chege, 2020). A study of risk factors associated with anemia, iron deficiency, and iron deficiency anemia in rural Nepali pregnant women shows a higher prevalence of hookworm (Makhoul et al., 2012) which increases the risk of developing severe anemia. A similar prevalence was also known by the study done in eastern Nepal (Shah & Baig, 2005).

Giardia lamblia and *Entamoeba histolytica* are the most common protozoan parasites found in humans. *Giardia lamblia* and *Entamoeba histolytica* has been reported from countries from different continents like Sudan (Abdel-Aziz et al., 2010; Suliman et al., 2019), India (Dongre et al., 2007; Sehgal et al., 2010), Bangladesh (Hossain et al., 2019).

In the case of South American countries, (Espinosa Aranzales et al., 2018) examined the common IPI among pregnant women who live in poor conditions in three urban areas of Bogotá, Colombia by using a common combination of microscopy techniques, including direct wet mount and formal-ether concentration detected 41% of prevalence with different taxa like as *Blastocystis hominis, Endolimax nana, Entamoeba coli, Iodamoeba butschlii, Entamoeba histolytica/dispar, Ascaris lumbricoides,* and *Giardia lamblia.* Results were confirmed by additional quantitative polymerase chain reaction (qPCR) analysis of some samples. Further, (Cook et al., 2009) reported a higher prevalence of 52.9% with the following parasites: *Ascaris lumbricoides, Giardia lamblia, Entamoeba histolytica, Hymenolepis nana,* and *Blastocystis hominis.* On the other hand, (Rodríguez-Morales et al., 2006) recorded a slightly higher prevalence (73.9%) from the pregnant woman in Venezuela with a high prevalence of *Ascaris lumbricoides,* followed by *T. trichiura, G. lamblia, E. histolytica, N. americanus, E. vermicularis, S. stercoralis*.

In case of African countries, (Derso et al., 2016) found 31.5% prevalence in Felege Hiwot Referral Hospital, Bahir Dar city, Northwest Ethopia with highest prevalence of *Giardia lamblia* followed by *E. histolytica*, Hookworm, *Ascaris lumbricoides*, *Schistosoma mansoni*, *Strongyloides stercoralis*, *Taenia* spp. and *H. nana*. further, Alula et al., (2021) reported 36.7% prevalence at Shahura Primary Hospital, Northwest Ethopia with the most prevalent intestinal protozoan parasite *Entamoeba histolytica*/dispar followed by *Giardia intestinalis* and predominant helminths parasite *Ascaris lumbricoides*, followed by Hookworm, *Taenia* spp., *Strongyloides stercoralis*, and *Schistosoma mansoni*. In addition, (Alli et al., 2011) found (43.4%) prevalence at the University College Hospital in Ibadan, Nigeria using the wet preparation method, and the two concentration methods of brine (floatation) and formal-ether (sedimentation) with the highest prevalence of Hookworm and *Ascaris lumbricoides* whereas *Trichuris trichiura, Strongyloides stercoralis,* and *Enterobius vermicularis* are least prevalent. Along with this, they also found, the second trimester has a higher prevalence as Compared to other pregnancy trimesters. Likewise, another study conducted in Northwest Ethopia, a community-based cross-sectional study among pregnant women also revealed the prevalence of 43.5%with the highest prevalence by helminths followed by protozoan parasites (Aschale et al., 2022). A few years before in 2016, (Kumera et al., 2018) a study conducted in the same country reported a slightly lesser prevalence 27.4% where Hookworm and *Entamoeba histolytica* are most prevalent. They also advised avoiding drinking coffee with meals and lowering the coffee intake for expectant mothers. In addition, (Dagnaw et al., 2021) revealed a 27.7% prevalence of intestinal parasites with the highest prevalent parasites being *G. lamblia* and *S. mansoni*. They also stated that "to ensure a safe pregnancy, public health measures on the environment and water sanitation, personal hygiene education, and early deworming are essential". Similarly, (Hailu et al., 2020) a study to determine the Prevalence and Factors Associated with Intestinal Parasitic Infections among Pregnant Women in West Gojjam Zone, Northwest Ethiopia by using the formal ether technique detected 37.3% prevalence where the prevalence of hookworm was leading cause of intestinal parasitosis followed by *E. histolytica/dispar*. (Damtie & Liyih, 2021) reported with 53.4% which is the highest among all other studies conducted in Northeast, Ethopia. The most common parasite was *Taenia* species (18.1%),

followed by *Giardia lamblia, Entamoeba histolytica/dispar,* hookworms, *Ascaris lumbricoides, Schistosoma mansoni, Hymenolepis nana, Strongyloides stercoralis*, and *Enterobius vermicularis.*

In addition, the prevalence of intestinal parasitic infection in Western Ethopia (Yesuf et al., 2019) at 43.8% is higher than that of Northwest Ethopia with only two types of intestinal parasitic that is Hookworm (33.7%) followed by *Ascaris lumbricoides*. Further (Gedefaw et al., 2015) detected 19.01% prevalence by using the formal ether concentration method with *Ascaris lumbricoides* accounting for the largest percentage, followed by *Trichuris trichura, Giardia lamblia,* Hookworm, *Entamoeba histolytica, Enterobius vermicularis,* and *Schistosoma mansoni*.

In a central African country, (Adegnika et al., 2010) reported the epidemiologic data of parasitic infections and co-infections in pregnant women with a 64% prevalence of intestinal helminths and urinary schistosomiasis. This study demonstrates that pregnant women in a Central African region have a high burden of parasitic infections with significant parasitic co-infections. Whereas (Muhangi et al., 2007) in an East African country, Uganda found a prevalence of 100% with the presence of hookworm, *Mansonella perstans, S. mansoni, Strongyloides stercoralis, T. trichiura, Ascaris lumbricoides, Trichostrongylus sp*., and *Hymenolepis nana*. (Abaka-Yawson et al., 2020) examined the prevalence of intestinal parasitic infections and associated factors among pregnant women attending antenatal care in Kasoa Polyclinic by using formal ether sedimentation technique and by wet mount reported 14.3% of prevalence with different taxa like *A. lumbricoids, G. lamblia, T. trichiura, Schistosomamansoni*, Hookworm, *Hymenolepisnana,* and *Isospora Belli*. In addition, (Omorodion et al., 2012) found a prevalence of 23.74% with an infestation of *A. lumbricoides,* hookworm, *T. trichiura, Enterobious vermicularis, E. histolytica,* and *G. lamblia.*

In Asian countries, there is not sufficient research has been conducted to know the prevalence of gastrointestinal parasites and their associated risk factor among pregnant women (Nurdiati et al., 2001) also reported the occurrence of intestinal helminth infection with 69.7%. *Trichuris trichiura, Necator americanus* (hookworm), and *Ascaris lumbricoides* were the three most frequent helminths found.

Several pieces of research have been conducted in Nepal. Nepal is a developing country where the intestinal parasitic infection is a major public health problem. Most of the studies were done based on intestinal parasitic infections from different places in Nepal. In a study conducted in 2005 intestinal parasites are responsible for morbidity and mortality associated with poor nutritional status, anemia, and other factors (Rai et al., 2005).

Among them, the study conducted among pregnant women to determine the prevalence, detection, and identification of intestinal parasites and their associated risk factors shows a prevalence of 42% with *E. histolytica* as predominant followed by *G. lamblia,* Hookworm, and *Ascaris lumbricoides* (Yadav et al., 2020). Further, a study conducted in the Eastern region of Nepal (Baral et al., 2017) shows that 5.72% were positive for protozoa and 2.45% for helminths. *Giardia intestinalis* was found to be the most prevalent followed by *Entamoeba histolytica* and Hookworm. Similarly, a study done in the same year (Sapkota & Maharjan, 2017) in research entitled "Anemia association with intestinal parasitic infection in Pregnant women attending antenatal clinic at Tribhuvan University teaching hospital" mentioned that during pregnancy, there is a significant correlation between intestinal parasites and anemia with the most prevalent intestinal parasite *A. lumbricoids* followed by *H. nana* with 35% prevalence of intestinal parasites. This study also found the levels of parasitic infection to be 17(8.8%), 34 (17.0%), and 17 (9.5%) for light, moderate, and heavy infection. In addition, study done among pregnant women on their first consultation with antenatal services in Janakpur Zonal Hospital (Kayastha, 2018) reported a prevalence of 17.82% by using the direct smear technique where *Ascaris lumbricoides* with the highest prevalence followed by Hookworm and *Giardia lamblia.* Further, (Makhoul et al., 2012) investigate the risk factors associated with severe anemia and poor iron status among Nepali pregnant women showing the highest prevalence of *Ascaris lumbricoides* (*A. lumbricoides*) and *T. trichiura.* This study also shows that Hookworm infection increased the risk of developing severe anemia. Similarly, a study done in 2013 shows a very great prevalence of helminths especially *Ascaris lumbricoides* and *Trichuris trichiura* compared to the protozoan observed (Tandukar et al., 2013). Likewise, a study conducted in Saktikhor in Chitwan district of Nepal (Bhattachan et al., 2015) with prevalence of 23.3% by formal ether sedimentation

technique where *Taenia* spp being most common found followed by *Entamoeba coli, Giardia lamblia, Endolimax nana, Ascaris lumbricoides, Entamoeba histolytica/dispar, Trichuris trichiura, Hymenolepis nana, Blastocystis hominis,* and Hookworm. A few years later, a study carried out in Devdaha Municipality of Rupandehi district (Subedi et al., 2020) shows a prevalence rate of 18.66% with *Ascaris lumbricoides* having the highest prevalence whereas *Taenia sp* had the lowest prevalence. Similarly, a study done in Baglung (Thapa et al., 2021) reported a 31.32% prevalence showing the highest prevalence of *Trichuris trichura* followed by *Hymenolepis nana*. Furthermore, a study conducted in the Saptari district shows a prevalence rate of 44.2% ones with a high prevalence of *Giardia lamblia* followed by *Entamoeba histolytica*, E. coli, *Ascaris lumbricoides* (Gupta et al., 2020). In addition, (Dreyfuss et al., 2000) in their study "Hookworms, Malaria, and Vitamin A Deficiency Contribute to Anemia and Iron Deficiency among Pregnant Women in the Plains of Nepal" reported that 74.2% were infected with hookworms among 336 pregnant women in the plains of Nepal where hookworm infection intensity was the strongest predictor of iron status, especially of depleted iron stores. Furthermore, (Chaudhary & Maharjan, 2014) conducted a study to determine the "Association of anemia with parasitic infection in pregnant women attending antenatal clinic at koshi zonal Hospital" reported overall parasitic prevalence of 29% where *A. lumbricoides* as the most predominant followed by Hookworm (26.1%), *Giardia lamblia*, *Entamoeba histolytica, Trichuris trichiura*, *Strongyloides stercoralis* and *Hymenolepis nana.*

3. MATERIALS AND METHODS

3.1 Study Area

Hetauda is a sub-metropolitan city in the Makawanpur District of Bagmati Province in central Nepal. It is the administrative headquarters of the Makawanpur District and the capital of Bagmati Province. It is one of the largest cities in Nepal. It lies in the 27°25' N latitude and 85°02' E longitude and is situated at a level of 300-390m above sea level. The total area of the city is 261 km^2 with a population of 2 466 138. Hetauda shares boundaries with Chhatiwan on the east, Manahari, Haandikhola, and Sarikhet on the west, Naamtar, Bhainse, and Makawanpurgadhi on north and Bara and Parsa district on the south. The study was carried out in the Rural-Urban Healthcare centers of different wards of the Hetauda sub-metropolitan city. Ward numbers two, four, five, ten, and 11 of Hetauda were randomly selected for this study.

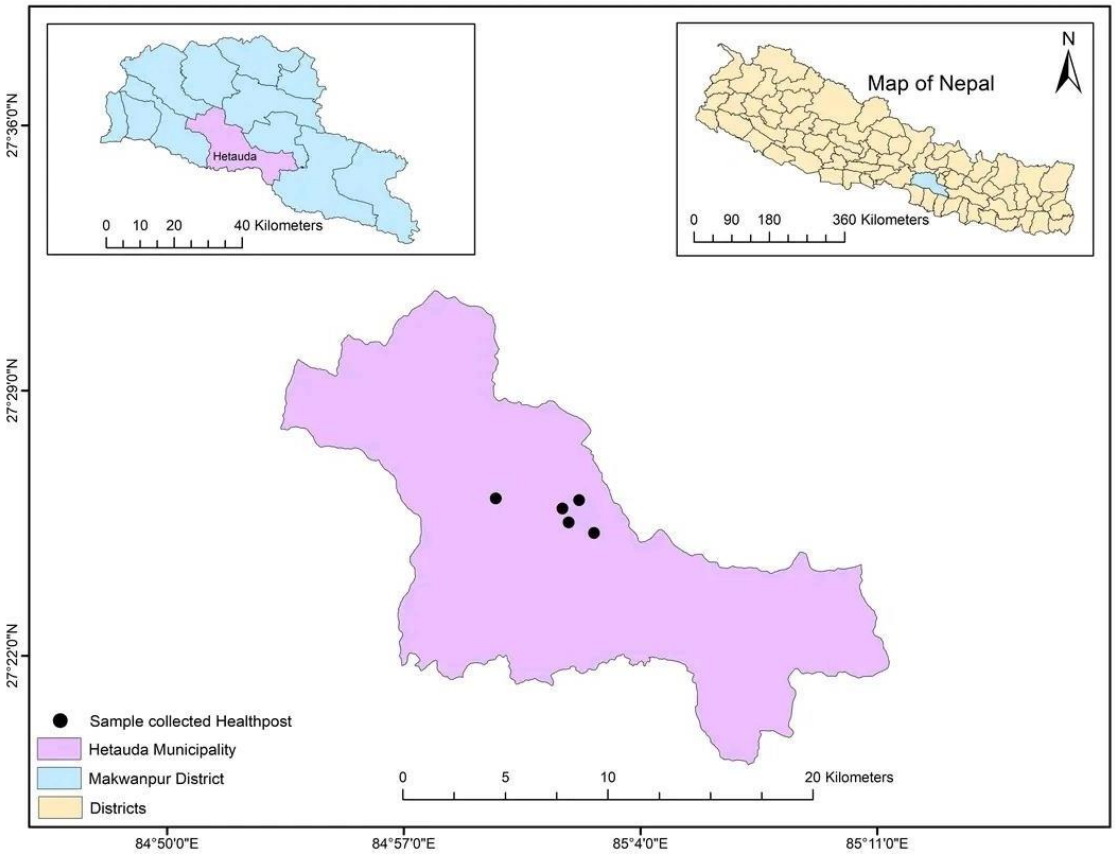

Figure1: Map showing the study area

3.2 Study design

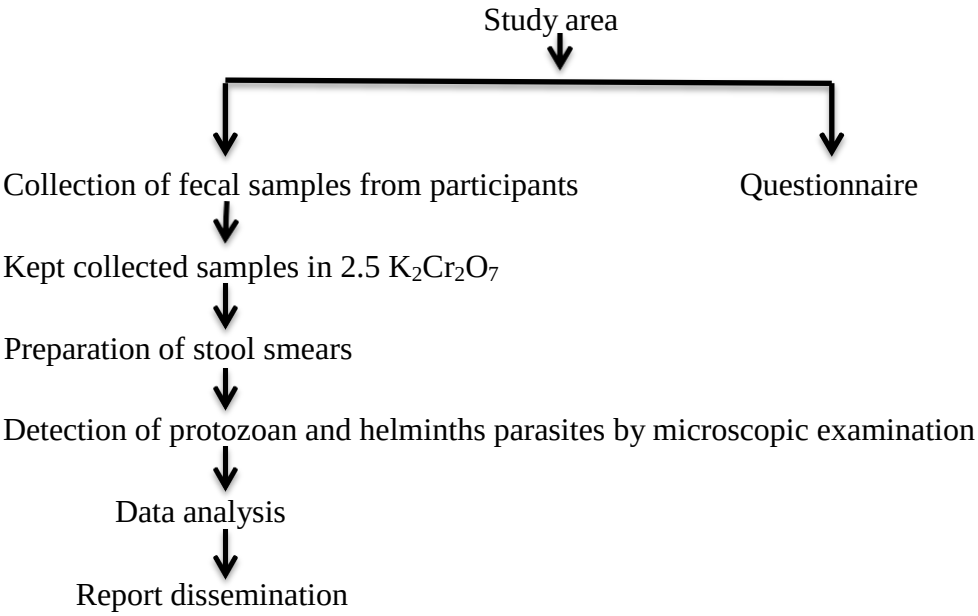

Figure 2: Flow chart showing study design

3.2.1 Pilot Field survey

A pilot survey was conducted initially to know the population and ANC visit times of pregnant lady. Necessary information about pregnant women and their visiting time was collected by discussion with authorities of Rural Urban Health care centers of the wards: two, four, five, ten and 11. Finally those wards were selected as the study area to carry out this research.

3.2.2 Sample Collection and Storage

A total of 100 pregnant women attending the antenatal care center of 5 different wards from September 2022 to February 2023 were included in this study. Pregnant women who did not ready for stool collection were excluded. Orientation about the proper methods of collection of stool was provided to ensure the good condition of stool samples. Stool samples were collected in the morning time and pregnant were instructed to avoid urine or other dust contamination of the stool sample. Pregnant women were provided collecting vials with a bamboo stick for stool collection. After stool collection immediately 2.5% potassium dichromate solution was poured in vials as much as to cover the stool sample, which help in maintaining the shape and size of protozoan and helminth parasites and preventing further development. The stool samples were marked or coded

for identification. Then it was brought to the Parasitology Laboratory of the Central Department of Zoology for further investigation of intestinal parasites. The samples were preserved in the refrigerator at 4℃.

3.2.3 Sample size

A total of 100 pregnant were randomly selected from the study area by visiting ANC.

3.4 Materials

3.4.1 Equipment

I. Compound microscope
II. Filter paper, strainer
III. Gloves, toothpick
IV. Centrifuge machine
V. Centrifuge tube
VI. Measuring cylinder
VII. Vials for sample collection
VIII. Ocular and stage micrometer

3.4.2 Chemicals

2.5% of potassium dichromate,70% alcohol, Methylene blue, Iodine solution, Sodium Chloride, Distilled water, 10% formalin, normal saline, ethyl-acetate.

3.5 Laboratory work

3.5.1 Unstained Preparation of Stool Smear

A minute portion of stool was taken with the help of a small stick and emulsified with normal saline (0.5) and a drop of it was taken on a clean glass slide. Then a coverslip was placed gently over it to spread out the emulsion into a thin, uniform, and transparent layer. After that, an excess amount of fluid was removed with the help of cotton (Zajac et al., 2021).

3.5.2 Stained Preparation of Stool Smear

Stained preparation was required for the identification and study of nuclear characteristics of protozoan cysts or dead specimens of trophozoites. The iodine-stained preparation was used for this purpose which was diluted in a ratio of 1:5 with the diluted ration (Zajac et al., 2021).

3.5.3 Differential Floatation Technique

About 3-4gm of fecal sample preserved at 2.5% $K_2Cr_2O_7$ solution was crushed in the mortar with a few milliliters of 0.9% NaCl and filtered over a tea strainer into the 14ml centrifuge tubes fitted in the test tube stand tightly. Additional 0.9% NaCl was added into the tube to make 14ml. The mixture was centrifuged for 5 min at 1200 rpm at room temperature. The supernatant was discarded immediately after the completion of centrifugation. Further, concentrated NaCl solution was poured into the centrifuge tube and made a final level of 14ml, and the centrifugation process was repeated. Immediately after the completion of centrifugation, the centrifuge tube was kept in the test tube stand tightly and the concentrated solution of NaCl was added to develop a convex surface at the top of the tube. The tube was covered by the coverslip to avoid any air bubbles being trapped and was left undisturbed for about 15-20 minutes. After 15–20 min, a coverslip was removed and kept on glass slides. The slide was examined under the microscope at 10X and 40X. Photographs of reported parasites were taken and identified based on morphology (Arora & Arora, 2014).

3.5.4 **Fo*rmalin-ethyl acetate (FEA) sedimentation***

About 2 grams of the fecal sample were thoroughly mixed in 12 ml of 0.9% w/v NaCl in a 15 ml centrifuge tube. The sample was centrifuged (1200 rpm×5 minutes) and the supernatant was discarded. Then, 10 ml of 10% formalin and 3 ml of ethyl acetate were added to the tube for subsequent centrifugation (1200 rpm×5 minutes). Finally, the supernatant was discarded, and the sediments were examined under a microscope at a total magnification of 100× and 400× with or without Gram's iodine (Adhikari et al., 2021; Zajac et al., 2021).

3.6 Identification of oocysts, eggs, and larvae of parasites

Oocysts, eggs, and larvae were identified based on morphological characters (shape and size) with the help of published and unpublished articles, and internet sources under expert supervision. The calibration obtained using an ocular and stage micrometer was used to measure the length and breadth of eggs and oocysts. Obtained calibration factor was 2.4$_{\mathscr{M}\mathrm{m}}$. All the samples were observed under a compound microscope (Swift Microscope).

3.7 Questionnaire survey

The structured questionnaire was prepared related to socio-behavioral aspects along with knowledge, attitude, and practices regarding gastrointestinal parasites and interviewed participating, pregnant women. The obtained information was used for statistical analysis. Where 1, 2, 3 and 4 of rate of health indicates excellent, very good, good, and bad health condition of participants respectively. Pregnant women who take shower daily were considered into always and those who take shower twice within a week were considered as nearly always in maintenance of health during pregnancy. Financial situation was categorized into; good/excellent were those who can easily afford every facilities/ health priorities that needs during pregnancy period, fair were those who can manage and poor were those who were struggling to meet health priorities who do not have proper health habits, and clean latrine systems.

3.8 Data analysis

Since, the study was focused on the identification of different intestinal parasites, the data were analyzed by using MS-Excel 2007 and statistical analysis was performed using "R",

version 3.5.2 with a chi-squared test. In all cases, a 95% confidence interval (CI) and $P<0.05$ was considered for the statistically significant association.

3.9 Ethical Approval and Consent to Participate

Ethical approval was applied at the Institutional Review Committee (IRC). The required permission for the collection of the fecal samples was issued by the Ward office of two, four, five, ten, and 11 wards of Hetauda sub-metropolitan city (Permission number: 153/079/080, 282/079/080, 808/079/080, 389/079/080 and 275/075/080 respectively). Before the survey, the study`s detailed purpose and procedures were explained verbally to the participants in the Nepali language. Verbal consent was before asking questionnaire and sample collection. No experimental infection was established during this research work.

4. RESULTS

The present study was carried out among 100 pregnant women attending the antenatal care center of five different wards of the Hetauda sub-metropolitan city, over 6 months, from September 2022 to February 2023. The pregnant women attending antenatal care (ANC) were interviewed using the structured questionnaire and stool samples were examined in the laboratory of the Central Department of Zoology, Kritipur.

4.1 General Prevalence of intestinal parasites in pregnant women

A total of 100 pregnant women were enrolled in the study and their stool samples were examined microscopically. The result revealed that the prevalence of intestinal parasitic infection was found to be 19% among pregnant women (Figure 3).

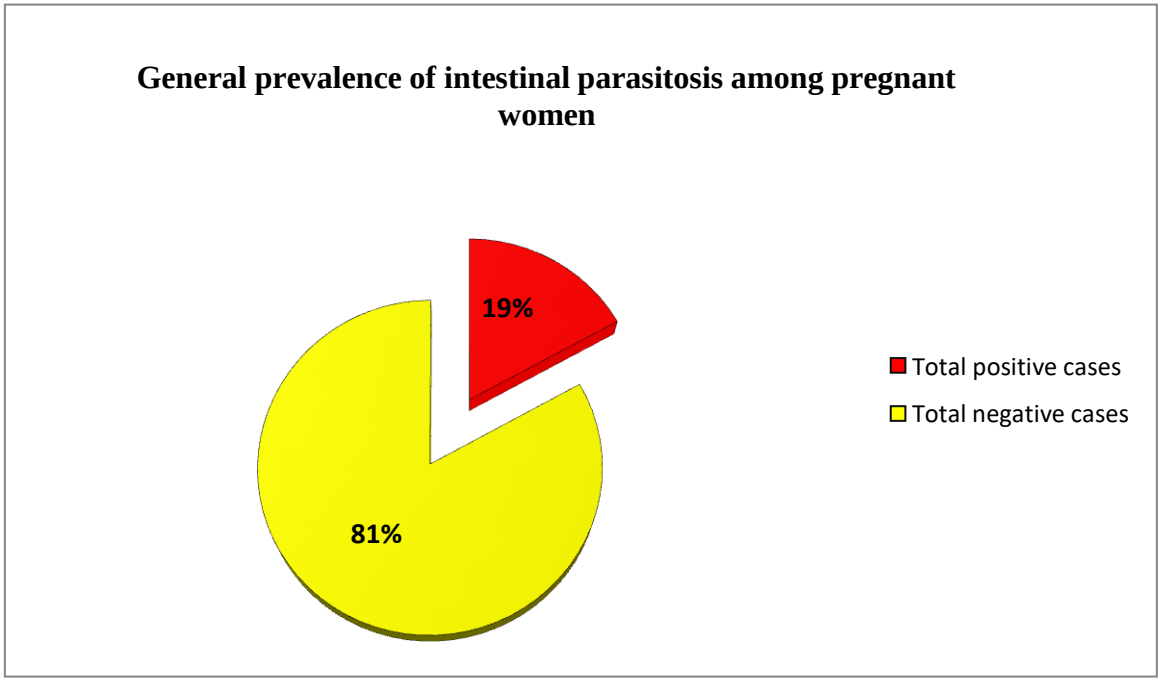

Figure 3: Pie-chart of general prevalence of intestinal parasitosis among pregnant women

4.2 Class wise prevalence of gastrointestinal parasites

Out of the 100 samples examined, five genera of parasites including one protozoan, one cestode and three nematodes were identified as gastrointestinal parasites. Prevalence of nematode was found to be 13%, cestode 2% and protozoan 4%.

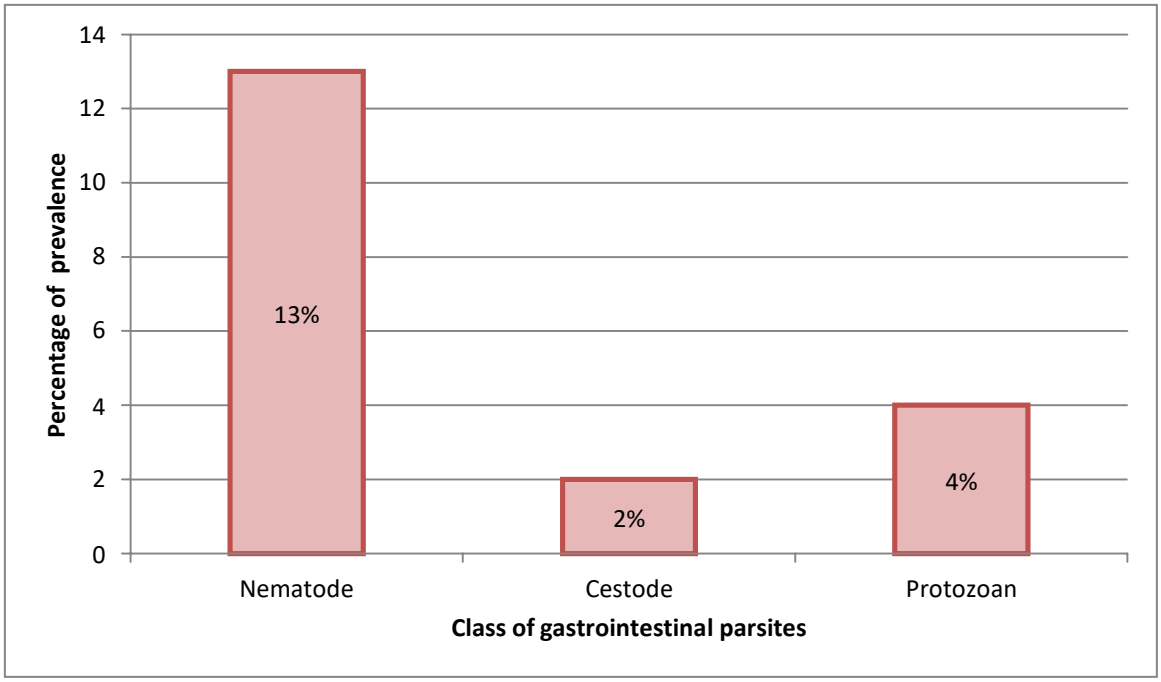

Figure 4: Column diagram of the class-wise prevalence of GI parasites

4.3 Genera-wise prevalence of gastrointestinal parasites

Out of 100 samples collected from pregnant women, the prevalence of *Ascaris lumbricoids* detected at a maximum of 8% whereas *Trichuris trichiura and Hymenolepis nana* detected a minimum of 2% (Table 1). Among 19 positive cases, shows *Ascaris lumbricoids* (42.10%) and *Entamoeba histolytica* (21.05%) are more prevalent followed by *Strongyloides stercoralis* (15.79%), *Trichuris trichiura* (10.53%) and *Hymenolepis nana* (10.53%).

Table 1 Prevalence of gastrointestinal parasites based on genera

S.N	Categories	Genera	No. of infected samples	Prevalence % (infestation with in total)	Prevalence% (infestation within positive cases)
1	Nematodes	*Strongyloides stercoralis*	3	3%	15.79%
		Ascaris lumbricoids	8	8%	42.10%
		Trichuris trichiura	2	2%	10.53%
2	Cestodes	*Hymenolepis nana*	2	2%	10.53%
3	Protozoan	*Entamoeba histolytica*	4	4%	21.05%

4.4 The pattern of infection in pregnant women

Figure 5, shows two different types of intestinal parasitic infection among the study population. 31.57% of single types of parasites were detected and 68.42% of multiple types of parasites were detected which can cause single and multiple infections.

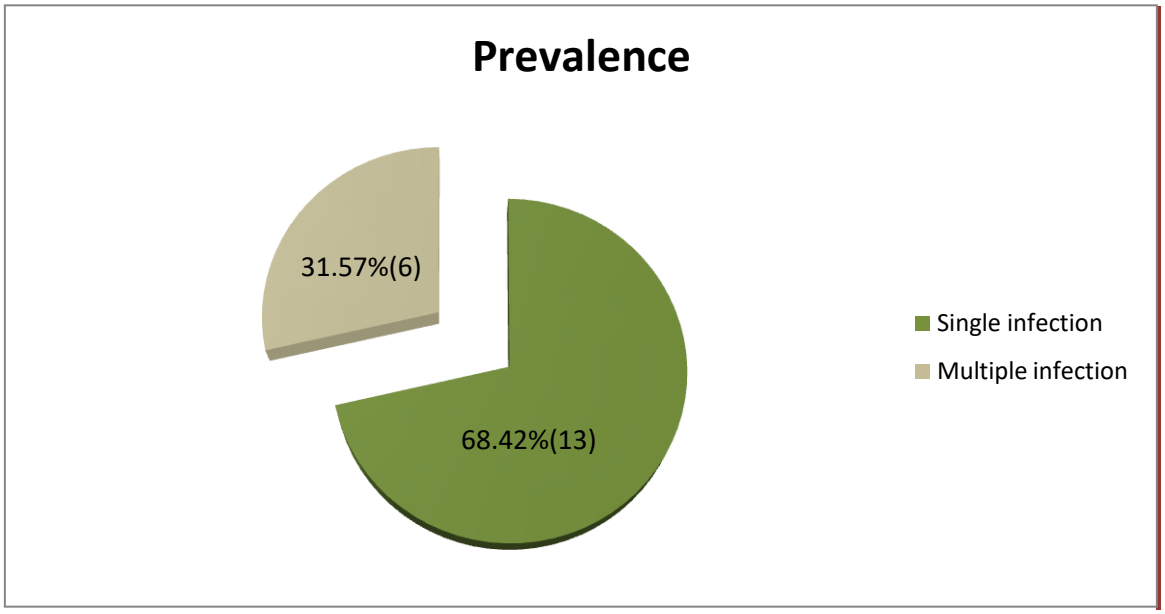

Figure 5: Pie-chart of pattern of infection

Table 2 shows that, among of a single infections *A. lumbricoides* comes at highest with 53.84% followed by *E. histolytica* with 23.07%, *T. trichura*, *H. nana*, *S. stercoralis* was with 7.69%.

Table 2: Intensity of a single infection

S.n	Parasitic infestation	no	No. positive cases (%)
1	*T. trichiura*	1	7.69
2	*A. lumbricoides*	7	53.84
3	*H. nana*	1	7.69
4	*S. stercoralis*	1	7.69
5	*E. histolytica*	3	23.07
	Total	13	100

Table 3 shows the intensity of multiple infections where *S. stercoralis + E. histolytica, T. trichiura + S. stercoralis and A. lumbricoides + H. nana* comes with same number of positive cases that is 16.67%.

Table 3: Intensity of multiple infections

S.n	Parasites	no	No. positive cases (%)(n=6)
1	*S. stercoralis + E. histolytica*	1	16.67
2	*T. trichiura + S. stercoralis*	1	16.67
3	*A. lumbricoides + H. nana*	1	16.67

4.5 Age-wise prevalence of gastrointestinal parasites

All samples were categorized into, 15-19 years old, 20-24 years old, 25-29 years old, 30-34 years old, and 35-40 years old age groups. The overall age-wise prevalence of gastrointestinal parasites was found the highest among 25-29 years and lowest among the age group of 15-19, 30-34, and 35-40 (Table 4).

Table 4: Prevalence of gastrointestinal parasites based on age and its association

AGE	No of the sample examined	Total positive (%)	Prevalence% (within groups)	P-value, p<0.05 (chi-square test)
15-19 years	4	2 (10.53%)	50%	P=0.5028
20-24 years	34	6 (31.57%)	17.65%	
25-29 years	37	7 (36.84%)	18.92%	
30-34 years	18	2 (10.53%)	11.11%	
35-40 years	7	2 (10.54%)	28.57%	

4.6 Trimesters-wise prevalence of gastrointestinal parasites

Out of 100 samples collected 14 were in 1st trimester, 76 were in 2nd trimester and 10 were in 3rd trimester out of which 2nd trimester is highly positive for gastrointestinal parasites.

Table 5: Prevalence of gastrointestinal parasites based on trimesters and their associations

Trimester	Total number (%)	Positive cases%	P-value (p<0.05)
1st	14	4(21.05)	P=0.1991
2nd	76	12(63.15)	
3rd	10	2(10.53)	
Grand Total	**100**	**19**	

4.7 Photos of eggs/oocysts/larvae of parasites

Photo 1: *Strongyloides stercoralis* (79 x 22.5 µm) at (10X x 40X)

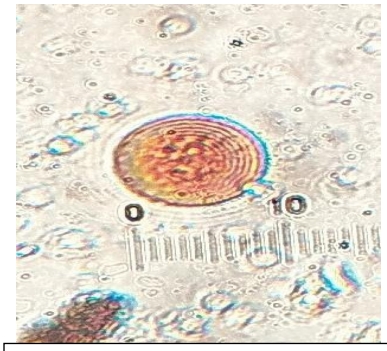

Photo 2: *Hymenolepis nana* (42.34µm) at (10X x 40X)

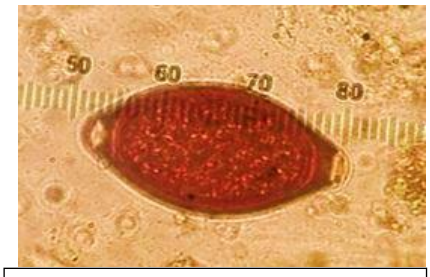

Photo 3: *Trichuris trichiura* (77 x 40 µm) at (10X x 40X)

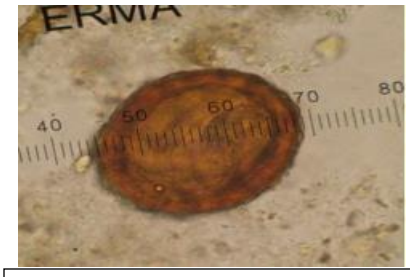

Photo 4: *Ascaris lumbricoides* (61.92 x 56.76µm) at (10X x 40X)

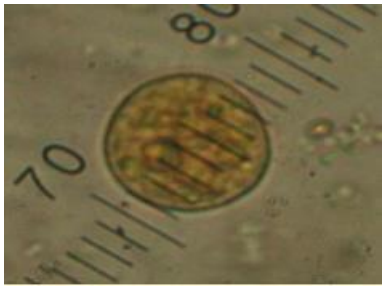

PHOTO 5: *Entameoba histolytica* (18.06μm) at (10X x40X)

4.8 Socioeconomic, demographic and behavioral characteristics of pregnant women

A total of 100 pregnant women were included in the study and a 100% response rate was obtained in filling out the questionnaires. Out of 17 different factors evaluated, the prevalence rate of GI parasites was statistically different in people with different financial statuses (p=0.0084). Yet, an unbalanced sample size because of a small number of good and poor versus fair (n=22, 20 vs 58) with high (19%) overall prevalence should be considered for careful interpretation of the finding (Table 6).

Table 6; Gastrointestinal parasitic infection by demographic, socioeconomic, and behavioral characteristics among pregnant women.

S.n.	Demographic characteristics	Subgroups	Total persons(N)	Infected person(n)	Prevalence% (100n/N)	P-values, P<0.005 (chi-square test)
1.	Rate of health	1	8	2	25	ns
		2	30	8	26.67	
		3	47	7	14.89	
		4	15	2	13.33	
2.	Prefer drinking water	Tap	66	11	16.67	ns
		Filtered	22	3	13.64	
		Boiled	12	5	41.67	
3.	Education	Literate	94	17	18.08	ns
		Illiterate	6	2	66.67	
4.	Maintenance of hygiene	Always	40	7	17.5	ns
		Nearly always	56	12	21.42	

5.	Financial status	Fair	58	12	20.69	P=0.0084
		Good	22	4	18.18	
		Poor	20	3	15	
6.	Hand washing with soap before a meal	Yes	55	8	14.55	ns
		No, with water	40	9	22.5	
		Sometimes	3	2	66.67	
7.	Cutting and cleaning of Nails	Yes	70	13	18.57	ns
		No	22	3	13.64	
		Sometimes	8	3	37.5	
8.	Eating fruits and vegetables without washing	Yes	22	2	9.09	ns
		No	71	14	19.72	
		Sometimes	8	3	37.5	
9.	Covering of food from flies	Yes	96	16	16.66	ns
		No	2	2	100	
		Sometimes	2	1	50	
10.	Eating Fallen food	Yes	3	0	0	ns
		No	84	15	17.86	
		Sometimes	13	4	30.77	
11.	Bite Fingernails	No	98	18	18.37	ns
		Sometimes	2	1	50	
12.	Shoe wearing habit	Yes	47	12	25.53	ns
		No	36	4	11.11	
		Sometimes not	17	3	17.65	
13.	Consumption of Anti-helminthic drug	Yes	95	18	18.95	ns
		No	5	1	20	
14.	Consumption of meat in the diet	Once or Twice a week	53	12	22.64	ns
		Thrice	11	2	18.18	
		None	26	5	19.23	
15.	Consumption of fruits in the diet	Everyday	31	4	12.90	ns
		Once a week	36	8	22.22	
		Once a month	14	4	28.57	
		Twice a week	19	3	15.79	
16.	Rearing Free ranging pets	Yes	28	6	21.43	ns
		No	72	13	18.06	

5. DISCUSSION

The current study indicates the prevalence, diversity, and associated risk factors for GI infections among pregnant women in central Nepal. The overall prevalence (19%) in the current study was slightly higher than the findings from pregnant women of Janakpur zonal hospital (17.82%, N=202) (Kayastha, 2018) and slightly lower than the other findings conducted in Nepal (29-49%, N=200-264) (Chaudhary & Maharjan, 2014; Sapkota & Maharjan, 2017; Yadav et al., 2020). Similarly, comparing our result with the global pregnant women`s population, the current prevalence rate following findings from Southern Ethopia (19%) (Gedefaw et al., 2015) which were higher than Ghana (14.3%, N=300) (Abaka-Yawson et al., 2020) and lower than reported from Columbia (41%) (Espinosa Aranzales et al., 2018), Venezuela (73.9%) (Rodríguez-Morales et al., 2006), Northwest Ethopia (23.4-53.4%, N=) (Alli et al., 2011; Alula et al., 2021; Aschale et al., 2022; Dagnaw et al., 2021; Damtie & Liyih, 2021; Derso et al., 2016; Hailu et al., 2020; Kumera et al., 2018), Western Ethopia (48.8%, N=315) (Yesuf et al., 2019), Gabon(64%, N=388) (Adegnika et al., 2010), Uganda(100%) (Muhangi et al., 2007), Indonesia(69.7%, N=442) (Nurdiati et al., 2001). The difference in these results might be because of different sampling geographies and their climatic conditions, different socioeconomic conditions, and behavioral practices by various pregnant women, and the different laboratory techniques used in the fecal analysis. This study has been carried out a sampling from some underdeveloped parts where some of the studied population are poor, illiterate, and far from development activities whereas some from developed parts where many of the studied pregnant women are rich, literate, and have all the access from developmental activities. In this study direct-wet mount, sedimentation, and flotation techniques were used in each fecal sample. All these factors might have favored the lower parasitic prevalence in our study.

Regarding protozoa, only one protozoan parasite *E. histolytica* was reported in this study. The prevalence of *E. histolytica* was 4%. This finding was higher from the findings from Nepal (2.5%)(Sapkota & Maharjan, 2017), Columbia (1.5%) (Espinosa Aranzales et al., 2018) and lower from the findings from Ghana (5%) (Abaka-Yawson et al., 2020), Northwest Ethopia (5.5-40.6%) (Alula et al., 2021; Aschale et al., 2022; Damtie & Liyih,

2021; Hailu et al., 2020; Kumera et al., 2018), Nigeria(10.9%) (Omorodion et al., 2012), Southwest Ethopia (8.69%) (Gedefaw et al., 2015), Venezuela (12.0%) (Rodríguez-Morales et al., 2006). These data suggest that similar to global pregnant women, *E. histolytica* is important in the current study. These parasites are known to contribute to bleeding episodes and may lead to adverse pregnancy outcomes that can be life-threatening.

It was interesting that *A. lumbricoides* was dominated nematode detected with a prevalence rate of 8%. The rate was slightly lower than the findings from Nepal (11.1-32.3%) (Chaudhary & Maharjan, 2014; Kayastha, 2018; Sapkota & Maharjan, 2017; Yadav et al., 2020), Gabon (33%) (Adegnika et al., 2010), Nigeria (65%) (Omorodion et al., 2012), Northwest Ethopia (55.5%) (Alli et al., 2011), Southwest Ethopia (28%) (Gedefaw et al., 2015), Venezuela (57%) (Rodríguez-Morales et al., 2006), higher than the findings from Ghana (4.3%) (Abaka-Yawson et al., 2020), Northwest Ethopia (2.9-8.6%)(Alula et al., 2021; Damtie & Liyih, 2021; Derso et al., 2016; Yesuf et al., 2019) and Uganda (2.3%) (Muhangi et al., 2007). The higher prevalence rate in the study area indicates the possibility of cross-transmission of *Ascaris* from domestic animals due to poor observance of personal hygiene. As we all know that the infective stages of *A. lumbricoids*, have an enormous capacity in withstanding environmental extremes. Furthermore, Ascaris eggs are coated with muco-polysaccharide substance which makes these eggs adhesive to different body surfaces and shows adhesiveness to door handles, dust, fruits and vegetables, paper money, and coins (Omorodion et al., 2012).

Strongyloides stercoralis is another nematode that had a prevalence rate of 3%. This prevalence rate was following findings from Venezuela (3.3%), lower than the findings from Uganda (12.3%), and higher than that of findings from Nepal (1-1.5%), (Chaudhary & Maharjan, 2014; Sapkota & Maharjan, 2017), Northwest Ethopia (0.4-2.3%) (Alli et al., 2011; Alula et al., 2021; Damtie & Liyih, 2021; Derso et al., 2016). *T .trichiuria* the intestinal nematode, was reported in 2% of pregnant women`s. The current prevalence rate was accordance with findings from Nepal (2%) (Sapkota & Maharjan, 2017), Northwest Ethopia (2.9%) (Alli et al., 2011), and higher than findings from Ghana (1.3%) (Abaka-Yawson et al., 2020), lower than the findings from Gabon (24%) (Adegnika et

al., 2010), Nigeria (13.08%) (Omorodion et al., 2012), Southern Ethopia (20.29%) (Gedefaw et al., 2015), Uganda (9.1%) (Muhangi et al., 2007), and Venezuela (36%) (Rodríguez-Morales et al., 2006). The presence of this nematode is decisive because of its enormous potential to infect a large human population.

In the current study, this study have reported the eggs of *H. nana*, a cestode, at a prevalence rate of 2% which was slightly lower than that reported from Nepal (3%) (Sapkota & Maharjan, 2017), and higher than those from Ghana (0.3%) (Abaka-Yawson et al., 2020), Northwest Ethopia(0.3-0.7%) (Damtie & Liyih, 2021; Derso et al., 2016), Nepal (1.5%) (Chaudhary & Maharjan, 2014) and Uganda (0.2%) (Muhangi et al., 2007). Lower prevalence was observed in this study which may be due to rare transmission occurring from the ingestion of food contaminated with fleas harboring the cysticercoid larvae.

It is widely accepted that people's socioeconomic status and behavioral tendencies influence their propensity to become parasitic (Adhikari et al., 2021). Most demographic, socioeconomic, occupational, and behavioral variables remained insignificant due to the small sample size (lack of knowledge about statistics). However, the trend of overall GI infection was higher among most pregnant women who lived in mud-built houses with large family sizes accompanied by overcrowding. Also, high prevalence rates of the GI parasites were observed in the farmers, who worked in fields, in the people with the habit of open defecation, in the people who never or occasionally wore shoes/sandals, in the people who drank water from unsafe sources without treatment, and in the people who did not practice hygienic hand-washing practices. Generally, pregnant women living in rural areas had poor personal and environmental sanitation practices, low socioeconomic status, lack of awareness, and illiteracy. As a result, there is a high likelihood of contracting an intestinal parasite infection. It suggests that these elements play a key role in the susceptibility and spread of GI parasites. Most gastrointestinal parasites are spread through the mouth while consuming tainted food or water or through the skin while walking barefoot, and notably, the abovementioned behavioral factors are fitting well (Fung & Cairncross, 2009; Yesuf et al., 2019)

In this study, there is no significant difference between socioeconomic and demographic variables other than financial status which shows similarity with a study done in Northwest Ethopia (Derso et al., 2016; Hailu et al., 2020) whereas a study done in Western Ethopia shows being a farmer, walking barefooted and absence of appropriate hand washing habit after latrine significantly increases intestinal parasitic infection (Yesuf et al., 2019). This study shows a significant difference in the financial condition in a prevalence rate, a 20.69% fair, 18.18% good, and 15% of poor who do not have proper health habits, and clean latrine systems. Financial conditions directly or indirectly affect the healthy habit of a person which plays a prompt role in a parasitic infestation.

The stage of pregnancy/gestational age was also found to be not associated with intestinal parasitic infections. This observation is consistent with previous findings where, Espinosa Aranzales and her colleagues have reported that the stage of pregnancy had no association with intestinal parasitic infections(Espinosa Aranzales et al., 2018). In contrast to these findings, the late trimesters (second and third trimesters) were associated with increased odds of intestinal parasitic infections among women (Obiakor-Okeke et al., 2014). Pregnancy necessitates an increase in nutrients, particularly iron, and results in "physiological anemia" due to hemodilution (Derso et al., 2016). According to the findings of Hailu, a high intake of green leafy vegetables was prone to intestinal parasitic infection (Hailu et al., 2020). The association between the total population eating vegetables and fruits without washing was statistically insignificant. This might be due to a lack of awareness and education. Also, it may be due to the pregnant women involved in agriculture and cultivation has limited knowledge about how and when intestinal parasites are transmitted. As a result, eating raw vegetables, open defecation, living in polluted environment, and food with soil during pregnancy plays a key role for parasitic infection.

Several limitations of the study should be considered. The methodological limitation of the study is smear preparation and examination, which might not be accommodated day to day. A second limitation is the sample size (n=100), which may affect the risk of type II error. Thirdly, the possible effect of sampling bias caused by the convenient selection of the participants might limit the findings' generability. Since participants were selected

on a first-come-first-serve basis, who visit their nearest governmental ANC service provider, there could be a chance that pregnant women who do not visit ANC would be more likely to be included. Finally, given the study`s cross-sectional nature, we are unable to identify the precise reasoning behind the linkages that observed.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, this study can serve as baseline data for evaluating and planning effective mechanisms to control and prevent GI parasitic diseases. This study shows that pregnant women have greater diversity and concomitant patterns of parasitic species. In this context, many socio-economics and behavioral factors directly or indirectly play a role in increasing parasite transmission. Notably, these GI parasites may have economic significance some are zoonotic and can be transmitted among domestic animals and humans. For effective control of these parasites, it is important to further determine the infection dynamics of these parasites. Also, immediate intervention strategies, such as screening of the women for intestinal parasites and provision of health education during their ANC visit to prevent the adverse effects on maternal and fetal health from these infections.

6.2 Recommendations

i. Pregnant women who visit antenatal care should be screened for IPI on their first visit.
ii. For early diagnosis and treatment, pregnant women should be encouraged to have regular ANC follow-ups at least four times during the pregnancy period.
iii. To prevent the recurrence of the parasites, pregnant women should be encouraged to have a healthy hygienic environment.

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PHOTOGRAPHS

Photo 6: Questionnaire survey

Photo 7: Distribution of vials

Photo 8: Microscopic Examination

Photo 9: sample packed for transportation

Photo 10: Sample after sedimentation

Photo 11: Centrifuge of samples

Photo 12: Rural-urban health care centers of ward two, three, four, five, ten and 11 respectively

Annex 1

Baseline Questionnaires related to demographic, socioeconomic, and behavioral factors

Participant Code/ Name:

Age:

Education:

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

☐1 ☐2 ☐3 ☐4

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?

☐Everyday ☐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 the financial situation of your family?

☐Poor ☐Fair ☐ Good/Excellent

8. How frequently do you maintain your personal hygiene?

☐Always ☐Nearly always ☐Nearly never ☐Never

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

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

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

☐ Yes ☐No ☐sometimes

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

☐Yes ☐No ☐sometimes

12. Do you wear foot ware while outdoor?

☐Yes ☐No ☐sometimes not

13. How many family members are in House?

14. Do you cover food from flies?

☐Yes ☐No ☐sometimes

15. Do you bite fingernails?

☐Yes ☐ No ☐sometimes

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

☐Yes ☐No ☐sometimes

17. Do you drink Boiled water?

☐Yes ☐No ☐sometimes

18. Do you know at least a way to prevent intestinal helminths and protozoans?

19. Did you consume any medication for intestinal helminths parasite after conception?

☐ Yes ☐ No

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

☐ Yes ☐ No

21. Did you notice any worms in your stool?

☐ Yes ☐ No

22. Do you ever consume raw meat?

☐ Yes ☐No ☐something