ANAEMIA ASSOCIATION WITH INTESTINAL PARASITIC INFECTION IN PREGNANT WOMEN ATTENDING NTENATAL CLINIC AT JANAKPUR ZONAL HOSPITAL, NEPAL

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Submitted to:

Central Department of Zoology

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Kirtipur, Kathmandu

Nepal

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DECLARATION

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

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RECOMMENDATION

This is to recommend that the thesis entitled "**Anaemia Association with Intestinal Parasitic Infection in Pregnant Women Attending Antenatal Clinic at Janakpur Zonal Hospital"** has been carried out by **Kanchan Kayastha** for the partial fulfilment of Master's Degree of Science in Zoology with special paper **Parasitology.** This is her original work and has been carried out under my supervision. To the best of my knowledge, this 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 "**Mahendra Maharjan"** this thesis submitted by **Kanchan Kayastha** entitled **" Anaemia Association with Intestinal Parasitic Infection in Pregnant Women Attending Antenatal Clinic at Janakpur Zonal Hospital"** 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 **Kanchan Kayastha** entitled **"Anaemia Association with Intestinal Parasitic Infection in Pregnant Women Attending Antenatal Clinic at Janakpur Zonal Hospital"** has been accepted 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 abbreviations
ANC	Antenatal Care
ANOVA	Analysis of Variance
CBS	Central Bureau of statistics
CDC	Centers for Disease Control
ELISA	Enzyme Linked Immune Serbent Assay
Gm/dl	Grams per deciliter
Hb	Haemoglobin
HIB	Human immunodeficiency virus
IPI	Intestinal Parasitic Infection
KAP	Knowledge, Attitude and Practices
JZH	Janakpur Zonal Hospital
NDHS	National Demographic Health Survey
NEG	Nutritional Educational Guideline
NMCTH	Nepal Medical College Teaching Hospital
NMSS	Nepal Micronutrient Status Survey
LBW	Low Birth Weight
P-Value	Probability Value
RBC	Red Blood Cell
STH	Soil Transmitted Helminthes
SPSS	Statistical Package for Social Science
WHO	World Health Organization

ABSTRACT

Anaemia during pregnancy cause direct impact on the health of both mother and faetus. Among the various reasons, intestinal parasitic infection has been considered as one of them. In order to find out the status of parasitic infection and anaemia during pregnancy, a total of 202 stool samples from pregnant women on their first consultation to antenatal services in Janakpur Zonal hospital were collected from October 2017 to April 2018.The stool sample were examined for intestinal parasite by direct smear technique , while hemoglobin level of pregnant women were collected from laboratory record of the hospital, Out of the 34 anaemic pregnant women 35.29% had parasitic infection , while from non-anaemic pregnant women, 70% of them were infected with intestinal parasite. The association of anaemia with intestinal parasite was statistically significant (p=0.005). The prevalence of *Ascaris lumbricoides* (13.86%) was most prevalent followed by Hookworm (3.46%) in anaemic pregnant women. The hemoglobin levels of 36 pregnant women who were infected with parasitic infection was reported to be 10.3 mg/dl (mild anemia). However, the overall prevalence of the parasitic infection among pregnant women was 17.82%. *Ascaris lumbricoides* 13.86% was the most predominant followed by Hookworm (3.46%) and *Giardia lamblia* (0.49%). The prevalence of the intestinal parasite showed no significance association with their residency area (p=0.18) and source of water (p=0.4) but showed stastically significant with habit of use of toilet (p=0.000). The prevalence rate of IPI was higher among larger family size of pregnant women and also illiterate/limited literacy group of population. The designing and implementation of parasite control programme should be a public health priority at local and regional level to reduce the degree of anaemia during pregnancy.

1. INTRODUCTION

1.1Background

The health of women has significance role in the development of society and nation. A healthy mother bring forth a healthy child, hence in any community the women's health must constitute the priority group. Women communities compared to the man communities are more prone to different disease, which has become a great challenge in developing countries like Nepal due to several reasons such as difference in socio-economic conditions, lifestyles and health seeking behaviors across different cultures.

Many women in Nepal seems vulnerable to the effect produced by soil transmitted helminthes (STH) as they spend a major part of their life either in pregnancy or as lactating mothers (Dreyfuss, *et al.,* 2000 and Rai, *et al.,* 1994). Hence association of anaemia with helminthes infestation is seen in the world, and by eliminating it, anaemia may be reduced with positive effect in maternal outcome. Anaemia is strictly defined as a decrease in Red Blood Cell (RBC) mass. According to WHO (1992) anaemia is one of the most prevalent nutritional deficiency problem affecting pregnant women as hemoglobin level of less than 11gm%. Hemoglobin level of 9.0-10.9gm% is mild anaemia, 7.0-8.9 gm% is moderate anaemia and less than 7gm% is called severe anaemia. Anaemia is a huge medical problem in the developing world. Getting pregnant at young age combined with high parity, bad sanitary conditions and a bad diet, contributes to give women's bad health. Maternal anaemia in pregnancy is commonly considered a risk factor for poor pregnancy outcome and can result in complications that threatens the life of both mother and fetus (WHO 1991). It has been estimated that, at any one time in developing countries, half of the population (mainly children and women of reproductive age) is affected by anaemia (Hercberg and Galan, 1992). This is why early diagnosis and treatment of anemia is very important in pregnant women.

Globally, the most common cause of anaemia is believed to be iron deficiency due to inadequate dietary iron intake, physiologic demands of pregnancy and rapid growth and iron losses due to parasitic infections. However, iron deficiency is not the only causes of anaemia, other prevalent causes of anaemia include malaria, chronic infections, nutritional deficiencies of vitamin A, foliate and vitamin B-12 (Dreyfuss,

et al., 2000). Anaemia is the most common nutritional problem worldwide with its highest prevalence among young children and pregnant women. It is especially more common in developing countries like Nepal because of poor nutrition and high prevalence of parasitic infestation. Prevalence of anaemia among pregnant women in developing countries averages 56% with a range of 35% -100% among various region of the world (WHO, 1992). Studies from South- Asian countries (Bondevik, *et al.,* 2000, Dreyfuss, *et al.,* 2000) has estimated 75% prevalence of anaemia among pregnant women, the highest in the world (WHO, 1998). According to the Demographic Health Survey in 2006 of Nepal, national coverage of iron supplementation has increased from 23% to 59%. Because of these improvements and other complimentary measures, anaemia in pregnant women has been reduced from 75% to 42%. Though there are many studies on anaemia in pregnancy in Nepal showing high prevalence, there are few studies done in the eastern region of the country. Studies done in anaemia in pregnancy in Nepal, by Bondevik, et *al.,* (2000) showed prevalence of anaemia 62.2% in a study done in Kathmandu, Nepal. Similarly high prevalence 50.0% to 60.0% of anaemia were noted in various studies (Ulstein, *et al.,* 1998) particularly in study carried out by shah and Gupta showed that prevalence of anaemia in adolescent girl in Dharan was 68.8% (shah and Gupta, 2002). Among the helminth most of author showed association between Hookworm and anaemia (Dreyfuss, *et al.,* 2000, Brooker, *et al.,* 2008) in pregnant women while some author showed not only Hookworm but also *A. lumbricoides, T. trichiura* and *S. stercoralis* were prevalent among the anaemic pregnant women (Shah and Baig, 20005; Chaudhary and Maharjan, 2014).

In order to determine the status of parasitic infection and anaemia during pregnancy, the present study was designed. It includes determination of parasitic infection by using stool examination as well as hematological data collection for estimation of haemoglobin level and analyzing statistical association of correlation.

1.1 Public health importance of intestinal parasitic infection

Parasites are those organism, which receive nourishment and shelter from another organism where they live and hosts are the organism which harbor the parasites. Intestinal parasitic type ranges from virus, bacteria, and protozoa to helminthes. But commonly prelevant and endemic types of intestinal parasites are protozoan and

helminthes. Intestinal parasitic infection (IPI's) caused by pathogenic helminthes and protozoan species are endemic throughout the world. They effect an estimated 3.5 billion persons and cause clinical morbidity in approximately 450 million (WHO 2000). The major IPI's of global public health concern are the protozoan species *Entamoeba histolytica* and *Giardia intestinalis* and the soil transmitted helminthes *Ascaris lumbricoides*, *Trichuris trichiura* and Hookworm (WHO, 1999 and WHO, 2000).

Amoebiasis, Ascriasis, hookworm infection and Trichuriasis are among the ten most common infections in the world (WHO, 1987). The incidence and prevalence of these parasitic pathogens both between and within countries. The majority of infections are associated with poverty conditions such as reduced access to safe drinking water, adequate sanitation and hygiene, housing and inadequate access to health care (Mata 1982, Montresor, *et al.,* 1998). They are also affected by poor family and community hygiene and sanitation parasites and prevailing climatic and environmental conditions (Jemaneh, 1998). The condition lay the stage for the continuous transmission of IPI's (Mata, 1982; Montresor, *et al.,* 1998; Crompton, 1999). The economic burden caused by Hookworm, Roundworm, and whip worm infection is high. This was estimated by Stephenson and colleagues (2000) to cost 39.0 million disability-adjusted life years (DALYs).The WHO has even recommended that infected pregnant women should be treated after the first trimester (Bethony, *et al.,* 2006). Where the prevalence of Hookworm is more than 20% to 30%, the World Health Organization recommended that pregnant women should receive anthelmintic treatment (mebendazole, albendazole, levamisole or pyrantel) after their first trimester (Gyorkos, 2006). Regardless of thesesuggestions, only Madagascar, Nepal and Sri Lanka have added deworming to their antenatal care programs (Brooker, *et al.,* 2008).

1.2.1. Protozoan Parasites

Protozoan parasites are morphologically and functionally complete single cell organism. Most commonly found protozoans are *Entamoeba histolytica* and *Giardia lamblia*. Their characteristic high infectivity enhances their pathogenicity within the host (Katz, *et al.,* 1989, Neva and Brown, 1994). Infections of human intestinal tract with the pathogenic protozoa *Entamoeba histolytica* and *Giardia lamblia* are common cause of diarrhea, dysentery and have worldwide distribution (WHO 1985, Martinez-

Palomol, 1986). The complication of invasive Amoebiasis are potentially fatal and giardiasis may cause mal absorption in children.

***Entamoeba histolytica* (Lambl, 1859 and Losch, 1875)**

In 1994, WHO considered the disease caused by *Entamoeba* as world's second most killer disease. The World Health Organization (WHO) estimates that there were 48million new causes and 70,000 deaths due to *Entamoeba histolytica* in 1997 (WHO, 1998). The transmission of the parasite is through fecal oral route. The parasite normally inhabits the large intestine but is also capable of invading other organs such as the liver, brain and spleen (Petri and Singh, 1999). The majority of amoebic infection is considered to be occurred in Central America, South America, Africa and Asia. These are often associated with poor water and food hygiene and sanitation practices (Petri and Singh 1999). *Entamoeba histolytica* is a potent pathogen secreting proteinases that dissolve host tissues; killing host cells on contact and engulfing RBCs. Diseases caused by *Entamoeba histolytica* is called amoebasis. A symptomatic infection with *Entamoeba histolytica* is characterized by the presence of cysts in stool in the absence of colitis or extra intestinal infection. These healthy carriers may pass millions of cysts in the stool per day as the trophozoites multiply in the intestinal lumen (Petri and Singh, 1999). Clinical symptoms of acute intestinal amoebiasis include diarrhea, bloody stool that may contain necrotic mucous, abdominal pain, tenderness and fever (Petri And Singh, 1999). Symptoms of amoebic liver abscess usually involve fever, right upper abdominal tenderness, pain, weight loss and colitis (Katz,*et al.,* 1989; Neva and Brown, 1994; Petri and Singh, 1999). In many regions, amoebasis is an important cause of diarrhea and dysentery. Amoebiasis may be more severe during pregnancy and lactation, and in person with immunodeficiency, homosexual, immigrants from certain tropical countries, and travelers are also liable to infection (WHO, 1987). The natural host range of *Entamoeba histolytica* is limited; humans and some non-human primates are the known natural hosts (Stanley, 2003).

***Giardia lamblia* (Eeuwenhoek, 1681)**

Giardia lamblia has world –wide distribution with an incidence of 1-30%. The global incidence of giardiasis is estimated to be 5, 00,000 new cases in 1997 (WHO, 1998). Although large outbreaks have been through contaminated water, the major source of infection is through faecal-oral route (Upcroft and Upcroft, 2001).Giardiasis is caused by *Giardia lamblia*. Clinical symptoms of giardiasis include diarrhea, steatorrhea, and epigastria pain wasting hypo albuminemia and impair absorption of foliate and vitamin B12 (Solomons, 1982; Neva and Brown, 1994).

1.2.2. Intestinal helminthes parasites

The helminthes parasites are multicellular, bilaterally symmetrical, triploblastic animal. They belong to phyla Platyhelminthes and nematohelminthes. They are endoparasites of intestine and blood of human body and cause different diseases. Most helminthes parasites come under the heading of intestinal infection. Many parasitic helminthes require one or two intermediate hosts. Helminthes differ from protozoa in their inability to multiply within the body of hosts. In case of helminthes, except with some exception i.e. *Hymenolepis nana* as they cannot multiply within the human body so that the single infection generally does not lead to disease condition even heavy infection and can cause only morbidity not death of patient. Methods of diagnosis of helminthic parasite include microscopic examination of stool, urine and sputum. Nowadays ELISA test and other technique are also applied to detect their presence.

***Ancylostoma duodenale* (Angelo Dubini 1838, Looss 1898)**

Recent estimates indicated that Hookworm infects approximately 1.3 billion people worldwide, and 96 million suffer from associated morbidity, including also insidious effects on nutritional status and on physical and intellectual development (Alboniko, 1997) while another report showed an estimated 44 million pregnant women are infected with Hookworm worldwide (Peter, *et al.,* 2004). Severe iron deficiency anaemia during pregnancy has been linked to increased maternal mortality, impaired lactation prematurity and low birth weight, while its infection is considered a major health threat to adolescent girls and women of reproductive age, with adverse effects on the outcome of pregnancy (Peter, *et al.,* 2004). Estimates in Nepal suggested that

Hookworm infection causes 41% of moderate severe cause of anemia among pregnant women (hemoglobin level, <9gm/dl). The association between Hookworm infection and anemia is greatest in multigravidas (Peter *et al.*, 2004). Ancylostomiasisis caused by *Ancylostoma duodenal*, commonly called the Hookworm. Infection occurs by the entry of ineffective stage, filariform larva, through the penetration of skin. It can also infect man successfully by oral, Trans mammary and (Probably) Tran's placental routes (Smyth, 1996). They suck blood lymph, bites of mucous membrane and tissues fluid from the lining of the intestinal wall. Average blood losses of 0.1 to 0.2 ml of blood per day have been reported for Ancylostoma duodenal (Roche and Layassi, 1966). The characteristic symptoms of Ancylostomiasis are gastro-intestinal disturbances, anaemia and nervous disorders. A person with a heavy infection may experience abdominal pain, diarrhea, and loss of appetite, weight loss, fatigue and anaemia. Patients appear weak and they complain dizziness, ringing in the ear and headache. Nausea and vomiting are frequent.

***Strongyloides stercoralis* (Bavay, 1876; Stiles and Hassall, 1902)**

Strongyloides is known to exist on all continents except for Antarctica, but it is most common in the tropics, subtropics, and in warm temperature regions. The global prevalence of *Strongyloides* is unknown, but expert estimates that there are between 30-100 million infected persons worldwide (CDC-web). *Strongyloides stercoralis* is the fourth most important intestinal nematode infection which causes *Strongyloides,* but its impact is much less widely appreciated than those of *Ascaris, Trichuris or* Hookworm infections. With the exception of *Strongyloides stercoralis*, helminthes do not replicate within the human host. *Strongyloides stercoralis*is symptomatic in around 50% of cases, with diarrhea, abdominal pain, Nausea vomiting being the common gastrointestinal symptoms (Milder, et *al.,* 1981). The adult worm is largely localized in the duodenal –jejunal region. Infection occurs by the entry of filariform larvae which penetrate directly through skin, when coming in contact with soil. *Strongyloides* can undergo 'autoinfection'; this infection has been reported to last more than 30 years in untreated human. The infection with these parasites can also transmit via breast milk (Stephenson, *et al.,* 2000). If the parasite invade lung it produces symptoms like pneumonia i.e., fever, cough, blood sputum etc. while establishment inside intestine produce symptoms like Nausea, vomiting, anorexia, abdominal pain, diarrhea with mucous, blood and emaciation.

1.2 Objective of the study

General objective:

To determine the status of intestinal parasitic infection and anaemia in pregnant women attending antenatal clinic at Janakpur Zonal Hospital, Janakpur.

Specific objective:

- To determine the status of intestinal parasitic infection and anaemia among the pregnant women attended Janakpur Zonal Hospital.
- To find out the association between anaemia and intestinal parasitic infection in pregnant women.
- To assess the parasitic infection, anaemia, HIV and blood sugar in relation to Knowledge, Attitude and Practices (KAP).

1.3 Significance of the study

This study was conducted with the objective to determine the prevalence of parasitic infections and anaemia. The knowledge, attitude and practices of the pregnant women was collected and related with the intestinal infection and anaemia. The collected information are useful to know the status of intestinal infections among the pregnant women of the study area and determine the prevalence rate of helminthes parasite. This study will significantly contributes to address importance of helminthes parasitic infection among pregnant women to cause anaemia.

2. LITERATURE REVIEW

The knowledge of parasitology up to the middle of seventeenth century was limited to recognition of the existence of a few common external parasites such as lice, fleas, and few internal parasite like Tape worms. *Ascaris*, Pinworms and guinea worms. However, they were considered as natural products of human bodies. Even Rudolphi and Bremser also supported this idea (Chandler and Read, 1961).

During the latter half of 17thcentury Francesco Redi, Grandfather of parasitology started that maggots developed from eggs of fleas. At the same time, Leeuwenhoek perfected microscopes and discovered *Giardia* in his own stool and other protozoan in rain water, saliva etc. (Chandler and Read, 1961).

From the middle of twentieth century, the works on parasites regarding different aspects, that is distribution, life-cycle, pathogenesis, treatments and controls become fast and went wide spread. For this especially World War I and II were responsible that accelerated interest in parasitology especially the therapeutic aspects (Parajuli, 2003).

2.1. Intestinal parasites and anaemia in National context

Worldwide as well as in National context large number of studies has been carried out in prevalence of intestinal parasitic infection and its correlation to that of the anaemia, particularly among the pregnant women because of large incidence of mother and child mortality due to anaemia. There are several study results which emphasized that there is direct or indirect co-relation between hookworm infection and anaemia. Besides Hookworm, other intestinal parasite has also been reported among different age group people including child bearing women.

In an overview of infectious disease and malnutrition in Nepal by Rai, *et al.,* (2002 a) showed due to outbreaks of diseases like diarrhea, dysentery, chlorea, enteric fever, jaundice occur frequently and are attributed to a contaminated drinking water supply, which may increases prevalence of IPI through contaminated water supply. Vitamin A deficiency was found more common in the eastern part (particularly in the plain areas) of the country (NMSS, 1998), it has been found to be associated with intestinal

parasiotosis (Rai, *et al.,* 2007 and Bondvik, *et al.,* 2000).Women in the age group of 15-49 years has been found to be suffering from chronic energy deficiency (NMSS, 1998). Nutritional anaemia has been found in almost 685 of women and 75% of pregnant women were anaemic (NMSS, 1998; NDHS, 2001).William-Blangero *et al.,* (1993) reported that Roundworm, whipworm and Hookworm were endemic in Nepal and were the major health problem for the population. While in the review of status of soil-transmitted helminthes infection in Nepal done by Rai *et al.,* (1994) showed that, the annual rate of positivity for soil-transmitted helminthes ranged from 18.0 to 36.6% evaluated. It further concluded that the annual incidence decreased every successive calendar year in both adults and children, irrespective of sex. In an analysis of parasiticinfection scenario of Nepal for 16 years (from 1979 to 1995) showed that 50% of people were infected by helminthes (Chhetri, 1997).

In another study conducted by Rai *et al.,* (2000) has stated overall soil contaminated rate in and outside Kathmandu valley was 36.5%. The higher soil contamination rate with parasitic helminthes eggs of both human and animal origin in Nepal was associated with the high prevalence of STH infections among Nepalese (Rai and Gurung, 1986; Rai *et al.,* 1994 and Rai *et al.,* 1995). The intestinal helminthes parasites *Ascaris lumbricoides* (William-Blangero *et al.,* 1993; Rai *et al.,*1994; Nauvilsky*et al.,* 1998; Dreyfuss *et al.,* 2000; Shah and Baig, 2001; Parajuli *et al.,* 2004; Kunwar *et al.,* 2006 and Raghav *et al.,* 2008), *T. trichiura* (William-Blangero, *et al.,* 1993; Nauvilsky, *et al.,* 1998; Kunwar, *et al.,* 2006; Young, *et al.* 2000, Parajuli, *et al.,* 2004, Shah and Baig, 2005, Raghav, *et al.,* 2008), Hookworm (William-Bangero, *et al.,* 1993; Rai *et al.,* 1994; Nauvilsky, *et al.,* 1998; Dreyfuss, *et al.* 2000, Parajuli, *et al.,* 2004, Ghimire, *et al.,* 2005, Shah and Baig, 2005, Kunwar *et al.,* 2006, Raghav, *et al.,* 2008) and *S. stercoralis* (Parajuli, *et al.,* 2004; Raghav, *et al.,* 2007) are considered as Soil Transmitted Helminthes (STH) which were commonly abundant in stool sample of pregnant women as well as in women of reproductive age group. And from protozoan parasites *G. lamblia* (Rai, *et al.,* 1994; Parajuli, *et al.,* 2004 and Raghav, *et al.,* 2008) and *E. histolytica* (Rai, *et al.,* 1994; Parajuli,*et al.,* 2004 and Raghav, *et al.,* 2008) were the abundant parasites.

Nauvilsky, *et al.,* (1998) in Sarlahi district showed the prevalence of helminthes infection among pregnant women was 78.8%, 56.2%, and 7.9% for Hookworm, *Ascaris lumbricoides and T. trichiura* respectively. It also showed prevalence of

Hookworm the most predominant followed by *Ascaris lumbricoides*, and *T. trichiura* respectively (Kunwar, *et al.,* 2006), while in study done by Rai, *et al.,* (1994), Parajuli, *et al.,* (2004), Raghav, *et al.,* (2008) found *Ascaris lumbricoides* most prevalent helminth parasite followed by Hookworm. Apart from its other protozoan parasite *G. lamblia* were the most prevalent parasite followed by *E. histolytica* and helminth parasites respectively (Khanal, *et al.,* 2011 and Magar, *et al.,* 2011)

Several report indicated that Hookworm infection and anaemia is well co-related. Study conducted by Shah and Baig (2005) in Dhankuta District Hospital showed the high prevalence helminthic infestation 46.5% where 58.9% were anaemic women from total population. Out of 18 women that had hookworm infestation, 16 (88.9%) were anaemic. Anaemia was significantly related to Hookworm infestation. Hookworm were strong predictors of anaemia in pregnant women (Navilsky, *et al.,* 1998; Bondevik, *et al.,* 2000 and Dreyfuss, *et al.,* 2000). Other risk factors that contributes anaemia were *P. vivax* (Dreyfuss, *et al.,* 2000), Nutritional deficiency (Bondevik, *et al.,* 2000; Dreyfuss, *et al.,* 2000; Rai, *et al.,* 2000; Jiang, *et al.,* 2005; Azak and Nadri, 2006 and Chandyo, *et al*., 2006). Some of the previous studies have suggested Hookworm to be the most abundant helminthes in pregnant women (Dreyfuss, *et al.*, 2000; Young, *et al.,* 2000 and Marahatta, 2009), while Rai, *et al.,* (2002a) in an overview of infectious disease and malnutrition have mentioned Hookworm re-emerged in Nepal in recent years. Likewise in study done by Ghimire and Mishra (2005) to evaluate the types of intestinal parasites and hemoglobin concentration in the people of Chitwan District showed prevalence of intestinal parasite in female was 46.7% where the low concentration of hemoglobin was statistically significant in the helminthic and protozoa infected females.

In another community based study in plain of Nepal conducted by Dreyfuss *et al.,* (2000) showed overall, 72.6% women were anaemic, and 88%case of anaemic were associated with iron deficiency, while 74.2% infected with Hookworms, 19.8% had *plasmodium vivax* malaria. Result of many such reports have suggested in addition to the present routine iron and foliate supplementation to pregnant Nepali women, Vitamin A-Iron deficiency (Bondevik, *et al.,* 2000; Dreyfuss, *et al.,* 2000 and Rai, *et al.,*2002a) supplementation needed to be considered, besides prevention and treatment of infection should together with dietary advice Bondevik, *et al.,* 2000, Dreyfuss, *et*

al., 2000, Rai, *et al.,* 2002, Shah and Baig, 2005; Adak and Nazri, 2006) be emphasized more strongly in the antenatal care.

Few studies done in Nepal shows unhygienic behavior (not using soap for hand washing, walking barefoot when outdoor) (Parajuli, *et al.,* 2009) was associated with the prevalence of IPI while unhealthy habit of food (less consumption of green leafy vegetables or habit of post meal tea/ coffee drinking or smaller intake of citrus fruits) (Adak and Nazri, 2006), increased with gestation, ethnic group farmers, (Bondevik, *et al.,* 2000) were associated with the increased with the anaemia in pregnant women.

2.2 Intestinal Parasites and Anaemia in Global Context

With increasing interest in the world's Neglected Tropical Diseases, many previous research articles, reports have shown the co-relation between parasitic infections and overall health in pregnancy (Hyder, *et al.,* 1998; Brooker, *et al.,* 2008; Ndyomugyenyi, *et al.,* 2008; Van Eijk, *et al.,* 2009; Alli, *et al.,* 2011 and Abd Elhameed, *et al.,* 2012). Many aspects that can stimulate the transmission of infections such as nutrition, economic status and sanitation have also been reviewed. Climate and topography are crucial determinants of the distribution of helminth infections (Brooker, 2007). Soil-transmitted helminthes are highly affected by surface temperature (Brooker, 2003), altitude, soil type, and rainfall (Appleton and Gouws, 1996).

Study carried out by Rodriguez-Morales, *et al.,* (2005) in Venezuela showed that the prevalence of parasitic infection among pregnant women 73.9%; 57% for *Ascaris lumbricoides,* 36% for *Trichuris trichiura,* 14.1% for *Giardia lamblia*, 12% for *Entamoeba histolytica,* 8.1%for N. *americanus,* 6.3% for *Enterobius vermicularis,* 3.3% for *Strongyloides stercoralis,* Hematological evaluation showed the presence of 65.1% of anaemia in pregnant women. *Ascaris lumbricoides* was the most prevalent helminth parasite followed by *Trichuris trichiura* (Bong- Jin, *et al.,* 2003, Oziumba,*et al.,* 2005), while the report prepared by Bong-Jin, *et al.,* (2003) in Roxus city, Philippines showed overall positive rate 64.5% and that of male and female were 56.6% and 72.5% respectively where the multiple infections was 29.6% and double infection with *Ascaris lumbricoides and Trichuris trichiura* were common.Oziumba *et al.,* (2005) showed with much decreased prevalence rate of helminth infection (11.8%) in Nigeria, with *Ascaris lumbricoides* (8.7%) and *Trichuris trichiura* (3.1%),

likewise study conducted by Alliei, *et al.,* (2011) among pregnant women attending antenatal clinic at the University College Hospital, Nigeria, shows 43.4% with parasitic infection. The helminthes identified were Hookworm (35.8%), *Ascaris lumbricoides* (55.5%), *Enterobius vermicularis*(3.5%), *Trichuris trichiura* (2.9%), and *Strongyloides stercoralis* (2.3%). Hookworm and *Ascaris lumbricoides* had the highest prevalence respectively. Also, an overall prevalence of co-infection was 13.8%, of which co-infection of Hookworm + *Ascaris lumbricoides* was most predominant (85.7%). This was followed by *Ascaris lumbricoides*+ *T. trichiura* (9.5%) and Hookworm+ *T. trichiura* (4.8%).

Sehgal, *et al.,* (2010) in North India showed contrast to reports in other parts of the world, (Rodriguez-Morales, *et al.,* 2005 and Van Eijik, *et al.,* 2009). The total prevalence of intestinal parasitic infection was 35.6% in pregnant women, where protozoan parasitic infection was significantly higher (81.2%) then the intestinal helminthic infection (18.8%). Van Eijik, *et al.,* (2009) in Kenya showed 76.2% were infected with at least one geohelminth; 52.3% with *A. lumbricoides*, 39.5% with Hookworm and 29% with *T. trichiura*. Geohelminth infection in pregnancy have been associated with iron deficiency, maternal anaemia and impaired nutritional status (WHO, 2002).

In the global context, some studies suggested the *Ascaris lumbricoides* to be most prevalent helminth followed by *Trichuris trichiura* (Bong-jin, *et al.,* 2003, Ozumba, *et al.,* 2005, and Rodriguez- Morales *et al.,* 2005) while others showed Hookworm to be the most prevalent helminth followed by the *Ascaris lumbricioides* (Alli *et al.,* 2011, Amuta, *et al.,* 2011). Hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*, *Giardia lamblia*, *Entamoeba histolytica*, *N. americanus*, *Enterobius vermicularis*, *Strongyloides stercoralis*, *Taenia* species are the common intestinal parasites seen in pregnant women worldwide.

Study of intestinal Parasitic Infection in Nigeria of different reproductive period specific and intensity of IPI of women shows the higher prevalence rate with 72.8% and 63.9% for pre-menstrual and post menstrual period, with Hookworm (4.8%), *Ascaris lumbricoides* (9.3%), *Taenia* species (2.1%), *E. histolytica* (18.9%) and *E. coli* (21.6%) (Amuta, *et al.,* 2011). But no significant difference in prevalence was

observed between women at different reproductive stage and women infected by different parasites.

Many pregnant women in sub-Saharan Africa showed the habit of geophagy (continuous and purposeful consumption of soil). A longitudinal study conducted in western Kenya for the re-infection rate in pregnant and Lactating women with intestinal parasites who eat on different earth mounds. Results showed that 19.6% of the women were infected with at least one of the geohelminth parasites, 11.2% of those infections were Hookworms (Luoba, *et al.*, 2005). In Tanzania, HIV positive expecting mothers who consumed soil regularly were also tested for helminthic infections. The findings of this study showed an association between geophagy and Roundworms infection (Kawai, *et al.*, 2009).

One effect of Hookworm infection is the increased risk of maternal anaemia (Ayoya, *et al.*, 2006; Ndyomugyenyi, *et al.*, 2008, and Fusini, *et al.*, 2010). In study done by Ndyomugyenyi, *et al.*, (2008) based in endemic areas have measured hemoglobin (Hb),serum ferritin and erythrocyte protopropphyrin (EP) levels in pregnant women alongside parasitic infection in Uganda, it showed a 10%increase in anaemia for women infected with parasites and 8% increase specific to Hookworm. Study carried out by Ayoya, *et al.*, (2006) conveys that 47% had hemoglobin concentrations below 110g/I among study population while 11%,8% harbored P. falciparum and Hookworm respectively.

Pregnant women infected with one or two of the helminthes were with mean hemoglobin range, women without parasites was within the normal range while mother with co-infections were within the moderate anemic range (Fuseini,*et al.*, 2010). In study done in Northern Ghana by Fuseini, *et al.*, (2010) showed 23% of pregnant women were anaemic, whereas *Plasmodium* and *S.mansoni* infections alone cause mild anaemia, Hookworm infection, alone cause moderate anaemia.

Over 200 million women become pregnant each year, most them in developing countries (WHO, 1997). Many of these women suffer from ongoing nutritional deficiencies (Mora and Nestel, 2000), repeated infections (wu, *et al.*, 2004). Another factor that contributes to under nutrition during pregnancy is a reduction in the dietary intake below the habitual level; and if I combine with increased physical activity maternal nutritional status. The presence of perinatal factors like nausea, vomiting,

heartburn, bloating, constipation and diarrhea, all these gastrointestinal disturbances have a negative effect on overall nutrient intake (Dundas and Taylor, 2002).

Anaemia in pregnancy is related to different socio-demographic factors (bethony, 2000; Belachew and Yosef, 2006 and Biradar, *et al.*, 2012). Age, educational status, economic position, antenatal care and different parasitic infections (Stephenson, *et al.*, 2000; Guyut and Snow, 2001; WHO 2002, Bentley and Griffiths, 2003 and Bechuram *et al.*, 2006), iron deficiency (Engman, *et al.*, 2008, Pasricha, *et al.*, 2008, Seck and Jackson, 2010), and Nutritional factors (Bentley and Griffiths, 2003)are seen related to the presence of anaemia in pregnant women. Engmann, *et al.*, (2008) showed the prevalence of anaemia in pregnant women in Ghana urban areas was 34%, iron deficiency 16% and the iron deficiency anaemia 7.5%respectively, it was less than previously reported.

Iron deficiency is often nutritional in origin. One of the major contributory factors in developing countries is consumption of plant based food containing insufficient iron, especially insufficient available haem iron from meat (Van den Brock, 2003; Seck and Jackson, 2010). In a community based study done by Parischa, *et al.*, (2008) in Northwest Vietnam showed that 37.53% anemic reproductive age women, with 23.10% iron deficient, while 78.15% were with Hookworm infection. Though there was no evidence of a difference in prevalence of Hookworm infections between anaemic and non-anaemic women. Consumption of meat at least three time a week was more common in non-anaemic women.

According to WHO (2001), the recent guidelines regarding prevention and controlof iron deficiency anaemia, are the most effective ways needed to decrease them (Milman, *et al.*, 2005; Seck and Jackson, 2010 and Abd Elhameed, *et al.*, 2012). Seck and Jackson (2010) showed the improved in antenatal program in Senegal and similar West African countries, with 39% of the women with anaemia and only 12-13% had parasitic infection. Similarly Abd Elhameed, *et al.*, (2012) showed 24% of them who were treated had normal hemoglobin level.

3. MATERIALS AND METHODS

3.1 Study Area

The study was carried out in the Janakpur Zonal Hospital, Janakpur. A total of 202 pregnant women attending antenatal clinic of Janakpur Zonal Hospital during October 2017 to April 2018 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 morning time and pregnant were instructed to avoid urine or other dust contamination of the stool sample. Pregnant women provided collecting vials with bamboo stick for stool collection and stool sample were collected in parasitological department of Zonal hospital. After stool collection immediately 2.5% potassium dichromate solution was poured in vials as much as to cover stool sample, which help in maintaining the shape and size of protozoan and helminthes parasites and preventing further development. The stool samples were marked or coded for identification. Hemoglobin report of respective pregnant women was recorded from Hematology laboratory of gynecology department in JZH.

3.2 Materials Required

3.2.1 Equipment's

i. Compound microscope
ii. Collecting vials
iii. Gloves
iv. Wooden applicator
v. Glass slides
vi. Cover slips
vii. Forceps
Viii. Dustbin
ix. Cotton
x. Camera
xi. Filter paper

3.2.2 Chemical required

i. 2.5% potassium dichromate
ii. Normal saline
iii. Iodine solution
iv. Soap

3.2.3 Preparation of 2.5% Potassium Dichromate

2.5gm of potassium dichromate was weighed accurately by the help of electric balance and dissolved in 100ml of distilled water and dissolved well. This solution was used for the preservation of parasite found in the stool.

3.2.4 Preparation of Normal Saline

This is useful for observing the characteristic movement of parasites. This solution was prepared by dissolving 8.5gm of sodium chloride in 1000ml of distilled water. Normal saline was used in unstained preparation.

3.2.5 Preparation of Iodine Solution

For studying the internal characters of identification of the species of protozoan parasites as well as helminthes egg, a stained preparation is required. For this purpose Iodine solution was used. The solution used in the present study was prepared by dissolving 10gm of potassium iodide in 100ml distilled water and 5gm iodine crystals (powered) are slowly added in it. The solution was then filtered and kept in stopper bottle of amber color. As the Lugol's iodine solution is too strong, it was diluted about 5 times with distilled water before putting it on a slide to mix with the stool materials.

3.3InclusionandExclusionCriteria

3.3.1 Inclusion Criteria:

All pregnant women coming for their antenatal care for the first time.

3.3.2 Exclusion Criteria:

Pregnant women, who were already on iron supplement,or who had been dewormed by anti-helminthic drug.

3.4 Stool Sample Collection and Examination.

3.4.1 Sample collection and preservation.

The stool samples were collected from the pregnant women who came to Janakpur Zonal Hospital during their regular checkup. During the study period a total of 200 stool samples were collected purposively from the pregnant women. Orientation about the proper methods of collection of stool was provided to ensure good condition of stool sample. They were oriented to collect approximately 5gm of stool early in the morning with the help of clean stick provided with clean vial. They were also instructed to avoid contamination of stool sample with urine or dust soil. To ensure good condition of stool sample following precautions were taken.

1. Each sampling vials were distributed without preservatives, individually and the samples were collected in the same day or in the next day early in the morning to ensure the maximum load of possible parasites.
2. Immediately after collection 2.5% potassium dichromate solution was put in the vials containing stool for preservation of the parasites present in the stool.
3. The stool samples were marked, coded, and processed for parasitological examination. All stool samples were processed within two hours to 2 days of collection.

3.4.1 Laboratory examination

Laboratory work was done in the laboratory of Janakpur Zonal Hospital of Janakpur.

3.4.2.1. Macroscopic examination

First of all, stool samples were examined by naked eye for adult parasites and parasite segments as well as colour and nature of stool.

3.4.2.2 Microscopic examination

All the necessary equipment's and materials were collected in working table with safety precautions. Both unstained and stained was used in the study.

Unstained smear preparation of stool

A portion of stool sample was picked up with a wooden applicator and emulsified with freshly prepared normal saline on a clean glass-slide. A clear cover slip was placed over it and excess of fluid was removed with the help of filter paper.

Stained smear preparation of stool

Stained preparation was required for identification and the study of nuclear characters of protozoan cysts and tropozoites. The iodine stained preparation was use for this purpose. Stained smear was prepared in the similar manner as prepared in unstained smear preparation. Here Lugol's iodine solution was kept instead of normal saline solution.

Both stained and unstained smear preparations were first examined under the low power (10X objective) of microscope. Observation was made starting from one end of the slide to another so that whole field was examined. When required, objects were examined under high power (40X objective) of the microscope for detailed diagnosis. Identification was done on the basis of medical laboratory manual and with the help of supervisor. During the identification of egg of helminthes and cyst of protozoa, an attention was paid on shape, size, and color marking on the surface of the egg shell.

3.5 Data Collection of Hemoglobin Level.

Hemoglobin report of respective pregnant women was recorded from record file of hematology department of Janakpur Zonal Hospital.

3.6 Questionnaire Survey.

The structured questionnaire was prepared pretested among the colleagues and tested among the sample population of pregnant women before administered among the study population. All the pregnant women visited to Janakpur Zonal Hospital who had provided the stool sample were included in the study. The questionnaire was basically focused to understand the knowledge, attitude of practices regarding the intestinal parasitic infection among the pregnant women. It was also translated in Nepali and Maithili language as per their convenience to take proper information.

3.7 Statistical Analysis

The data obtained from study was analyzed using SPSS 16.

Analysis was also done by representing with the table and bar diagram.

4. RESULTS

The present study was carried out among 202 pregnant women attending antenatal clinic at Janakpur Zonal Hospital, Janakpurdham, over the period of 6 months, from October 2017 to April 2018. The pregnant women attending antenatal care (ANC) were interviewed using the structured questionnaire and stool samples were examined in the laboratory of Zonal Hospital, while their blood report were collected from record file of hematology department of JZH**.**

4.1 General prevalence of the intestinal parasites in pregnant women

A total of 202 pregnant women were enrolled in the study and their stool samples were examined microscopically. The result revealed that the prevalence of intestinal parasitic infection (IPI) was found to be 17.8% among the pregnant women (Table1).

Table 1: prevalence of specific intestinal parasitic infection in pregnant women

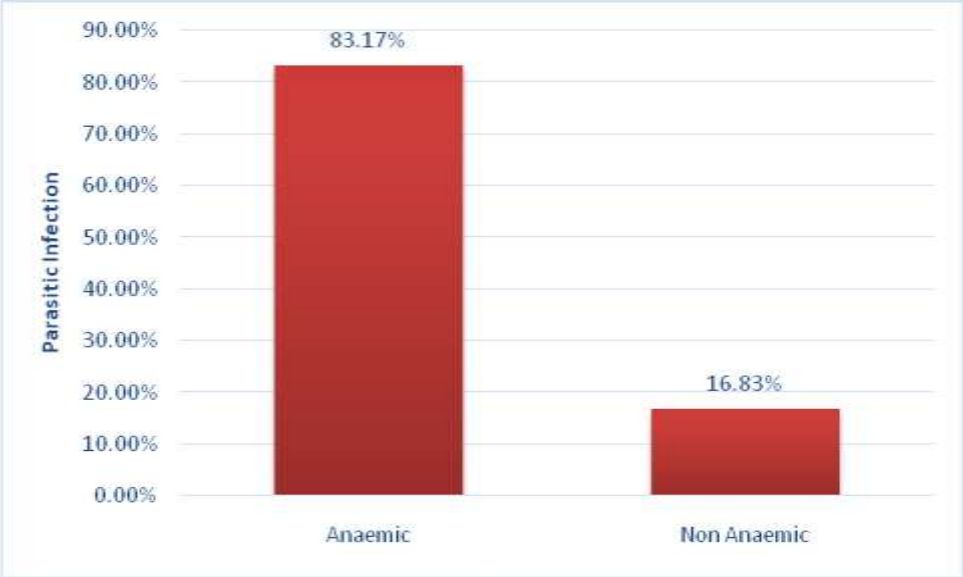
Parasites	**Frequency (N= 202)**
Protozoan parasites	
Giardia lamblia	1(0.49%)
Nematodes	
Ascaris lumbricoides	28(13.86%)
Hookworm	7(3.46%)

Pregnant women were found to be infected with three species intestinal parasites. Among them *A. lumbricoides* was the most prevalent (13.86%) compared to other intestinal parasites. This was followed by Hookworm (3.46%) and *Giardia lamblia* (0.49%) (Table 1).

4.2 Association between anaemia and intestinal parasitic infection in pregnant women

Hematological evaluation showed that the pregnant women who had anaemia, 83.17% pregnant women were infected with parasitic infection similarly in non anaemic pregnant women only 16.83% of them were infected with intestinal parasites. The parasitic infection rate was found to be significantly higher in anaemic pregnant women compared to the non-anaemic pregnant women as shown in (Fig 1).

.**Fig 1:** Prevalence of parasitic infection in anaemic and non-anaemic pregnant women.



The haemoglobin level of pregnant women is the indicative of the normal featus development. All pregnant women must have the haemoglobin level above 11gm/dl, level below this is the indicative of the anaemia. The present result showed that pregnant women with parasitic infection were found to be higher in anaemic pregnant women compared to that of non anaemic. The association of anaemia with intestinal parasite was statistically significant (P=0.005) (Table 2).

Table 2: Median haemoglobin level of pregnant women with and without parasitic infection.

Parasites	Number	Median Hb(gm./dl)	P value
No parasites	166	10.4	0.005
With parasites	36	10.3	

The median haemoglobin level of pregnant women without any parasites was 10.4 gm/dl whereas the median haemoglobin level of pregnant women having parasitic infection was recorded to be 10.3 mg/dl (Table 2). Using one way ANOVA test, there was stastiscally significant within the groups. (P=0.005).

Table.3: prevalence of anemia with different specific species of intestinal parasites

	Anaemia	Non anaemia
Giardia lamblia	-	1(0.49%)
Ascaris lumbricoides	7(3.4%)	21(10.3%)
Hookworm	5(2.4%)	2(0.99%)

The prevalence of anaemia within the different specific species of intestinal parasites is shown in table 3. Among those infected with *Ascaris lumbricoides*, 3.4 %of them were anemic. Similarly, in case of pregnant women who were infected with Hookworm, the prevalence of anaemia was found to be 2.4%. In contrast to those infected with *Giardia lamblia* 0.99% of pregnant women were non anemic.

4.3 Assessment of parasitic infection and anaemia in relation to knowledge, attitude and practices (KAP).

4.3.1 Prevalence of intestinal parasite and anaemia in relation to socio-demographic characteristics.

The age of the study population was divided into two sub-groups with cut-off point of 25 year. The lowest age of pregnant women was 16 year while the highest age was 32years. Though majority of study population were of age group lesser then 25year (156/202), the analysis showed that both the age group had similar rate of parasitic

infection as well as prevalence of anaemia. Among the age group ≥ 25 yr, 17.3% cases had IPI whereas. 25yr age group had IPI rate of 19.5%. There was no statistically significant association with age group (p=0.389). The prevalence of anaemia in the age group up to 25 years was 17.3% , among the 29 cases 16.7% was infected with parasites while from 15.2% anaemic pregnant women was above 25 years, 19.5% was infected with parasitic infection. In both the age group nematodes were the leading dominant parasites in anemic cases (Table 4).

The urban population constituted 25.7% (52/202) of the total population, while the rural population constituted 74.3% (150/202) of the total population. Among urban population, 3.8% had parasitic infection while prevalence among rural population was found to be 22.6%. Statistically, there was no significant association with residency area (p=0.18) .The prevalence of anaemia in pregnant women whose residency was in urban and rural area was 11.5% and 16.1% respectively. (Table4).

The prevalence of HIV in pregnant women whose residency was in Urban and Rural area was 0.6 % and 2.1 % respectively and prevalence of Sugar was 1.9% in urban pregnant women and 15.2 %(n=7) in rural women respectively. (Table 4)

The majority of the study population were from the Indo-Aryan ethnic group (n=173/202). Among them, 14.4% were infected with intestinal parasites whereas the pregnant women from Dalit 37.9% were infected with IPI. However no statistical association between parasitic infection and ethnicity was found (p=0.113). The prevalence of anemia, HIV and sugar in Dalit was 17.2 %, 3.4 and 10.3% and in Indo-Aryan ethnic group anaemia HIV and sugar was (n=29), 0.5 % and 4% respectively (Table 4).

Almost (n=46) all of the population were residing in katcha type of house. Among them, 50% were infected with intestinal parasite. However those who were resident in pukka house, only 8.3 % were infected with parasitic infection. The statistical analysis showed that inhabitant of Katcha type of house has significant association with intestinal parasite (p=0.00).Women residing in Katcha house 16.7% had anaemia and 6.5% had sugar and women residing in Pukka house 17.3 had anaemia, 1.2 % had HIV and 4.4% had sugar respectively (Table 4).

The prevalence of intestinal parasitic infection, presence of anaemia as well as anaemic cases with positive sample among different strata of family size was high as family size increase. The prevalence of parasitic infection, in pregnant women whose family size of < 4members, 5-8 and >9 were 7.8 %, 15 % and 27.5% respectively. The prevalence of anaemia,HIV and sugar among pregnant women who had family size of <4, 5-8 and >9 was 78%, 17.9% and 20.6% had anaemia, 2.6%, 0.9% had HIV and 5.2%, 5.6% and 3.4% had sugar respectively.(Table 4).

Table 4: Prevalence of intestinal parasites and anaemia in relation to their socio-demographic characteristics.

Socio demographic characteristics	Prevalence of parasites	Prevalence of anaemia	Prevalence of HIV	Prevalence of Sugar
Age				
<25years(N=156)	17.3%	17.3%	0.6%	1.9%
>25years(N=46)	19.5%	15.2%	2.1%	15.2%
Chi square value	0.276	0.111	0.852	0.22
P-value	0.389	0.468	0.404	0.001
Residency				
Urban(N=52)	3.8%	11.5%	_	3.8%
Rural(N=150)	22.6%	16.1%	1.3%	5.33%
Chi square value	4.906	1.402	0.700	0.181
P-value	0.18	0.167	0.550	0.501
Ethnic group				
Dalit(N=29)	37.9%	17.2%	3.4%	10.3%
Indo-Aryan(N=173)	14.4%	16.76	0.5%	4%
Chi square value	2.204	0.04	0.339	2.094
P-value	0.113	0.564	0.733	0.159
House type				
Katcha(N=46)	50%	16.7%	_	6.5%
Pukka(N=156)	8.3%	17.3%	1.2%	4.4%
Chi square value	42.112	0.013	0.596	0.313
P- value	0.00	0.532	0.596	0.407
Family size				
<4members(N=38)	7.8%	78%	2.6%	5.2%
5-8members(N=106)	15%	17.9%	0.9%	5.6
>9members(N=58)	27.5%	20.6%	_	3.4
Chi square value	7.111	3.572	1.793	0.355
P-value	0.68	0.246	0.465	0.936

4.3.2. Assessment of intestinal parasite and anaemia in relation to knowledge

The illiterate pregnant women were infected with the highest IPI rate of 52%, whereas pregnant women with education level up to secondary and above secondary level were infected with IPI rate of 13.2% and 11.1% respectively. The infection rate was found highest in illiterate pregnant women. Our study showed that the education status of pregnant women was statistically associated with parasitic infection (p=0.001). In the same way the prevalence of anaemia, HIV and sugar in illiterate pregnant women had 16 %, (n=0) and (n=0) respectively where as women with education level above secondary level had rate of 18.1%, 0.6% and5.5 % respectively. Whereas education level above secondary level had 11.1 %, 2.7 % and 5.4 % respectively. (Table 5).

Table 5: Assessment of intestinal parasites, anaemia, HIV and Sugar in relation to their knowledge

Knowledge related variables	Prevalence of intestinal parasite	Prevalence of anaemia	Prevalence of HIV	Prevalence of Sugar
Literacy				
Illiterate (N=23)	52%	16%	_	_
Upto secondary level (N=143)	13.2%	18.1%	0.6%	5.5%
Above secondary level (N=36)	11.1%	11.1%	2.7%	5.5%
Chi square value	21.77	1.035	3.87	1.934
P-value	0.001	0.771	0.405	0.397
Occupation				
Housewife (N=169)	18.9%	17.1%	0.5%	4.7%
Worker (N=33)	12.1%	15.1%	3.0%	6%
Chi square value	0.192	0.80	1.675	0.103
P-value	0.439	0.505	0.301	0.508

Majority of the study population were housewife i.e. 83% (169/202), only 16.33% (33/202) were from working occupation. The parasitic infection rate among

housewife was found to be infected with 18.9% of parasitic infection compared to working women i.e. 12.1%. No significant association between infection rate and occupation was found (p=0.0439). There was high difference in prevalence of anaemia and anaemic women with positive cases according to their occupation. 17.1% housewife were found to be anaemic and12.1% women of working occupation were anaemic. Prevalence of HIV and sugar was 3%and 6%. It was found to be highest in working pregnant women in compared to house wife 0.5%and 4.7%. (Table 5)

4.3.3 Assessment of intestinal parasite and anaemia in relation to practices

Majority of pregnant women among study population was from second trimester i.e. 50% (101/202) while 27.22% (55/202) and 22.77% (46/202) belongs to women from third and first trimester simultaneously. The parasitic infection rate among pregnant women who were in first, second and third trimester of their pregnancy were 13% 15.8% and 25.4% respectively. No significant association was found between parasitic infection and different trimester of pregnancy (p=0.716). Prevalence of anaemia in pregnant women was highest 19.5%, in first trimester of pregnancy while 15.8 and 16.3% of anaemic pregnant women were found in second and third trimester of pregnancy respectively. (Table 6)

Among pregnant women who didn't shared their houses with domestic live stocks, 3.2% had intestinal parasitic infection, in compared to pregnant women who shared their housewith live stocks had increased prevalence rate of parasitic infection i.e. 24.2%. There was statistically significant association house sharing with livestock (p=0.1). The prevalence of anaemia was 15 % and 20.9% in those pregnant women who share their house with and without livestock respectively. While prevalence of HIV and sugar in pregnant women who share and do not share their house with livestock was 0.7 %, 1.6 and 5.7 %, 3.2 % respectively. (Table 6).

Table 6: Assessment of intestinal parasites and anaemia in relation to practices

Practices related variables	Prevalence of intestinal parasite	Prevalence of anaemia	Prevalence of HIV	Prevalence of Sugar
Trimester of pregnancy				
1st trimester(N=46)	13%	19.5%	_	2.1%
2nd trimester(N=101)	15.8%	15.8%	0.9%	5.9%
3rd trimester (N=55)	25.4%	16.3%	1.8%	5.4%
Chi square value	1.211	0.520	0.879	1.058
P-value	0.716	0.879	0.738	0.733
House sharing with live stock				
Yes (N= 140)	24.2%	15%	0.7%	5.7%
No (N=62)	3.2%	20.9%	1.6%	3.2%
Chi square value	10.293	1.093	0.354	0.378
P-value	0.001	0.119	0.932	0.697
Practice of toilet use				
Open area **(N=26)**	80.7%	19.2%	_	3.8%
Closed area **(N=176**	8.5%	16.4%	1.1%	5.1%
Chi square value	72.503	0.491	0.569	0.131
P-value	0.000	0.322	0.741	0.584
Hand wash after use of toilet				
With soap and water (N=197)	16.7%	16.2%	1%	5%
Without soap & water (N=5)	60%	40%	_	_
Chi square value	3.103	0.714	0.73	0.378
P- value	0.109	0.335	0.932	0.697
Source of drinking water				
Open source **(**N=26 **)**	60%	10%	_	_
Tap water **(**N= 176)	15.6%	17.1%	1%	5.2%
Chi square value	0.439	2.129	8.712	0.45
P- value	0.440	0.15	0.97	0.405

Majority of population using toilet in house were87.12 %(176/202) while 12.87 %(26/202) belongs to those categories who does not use toilet. Women who have habit of using toilet 8.5% had parasitic infection compared to 80.7% of those who were not

using toilet at home. The absence of toilet at home was significantly associated with parasitic infection (p=0.000). Though the prevalence of anaemia in closed and open type of toilet user was 16.4% and 19.2% were closer to each other, and prevalence of HIV and sugar who have habit of toilet use was (n=0) and1.1% and those who were not using toilet was 3.8 % and 5.1 % respectively (Table 6).

The study population practiced soap water hand wash after toilet were infected with 16.7% IPI. Those who didn't practiced soap water habit of hand wash, 60% of them were infected with parasitic infection, and was no statistically significant (p=0.109) The prevalence of anaemia i.e. 16.2% and 40% was present in the group of population using soap water for hand wash purpose and group in which has habit of hand wash without soap water respectively. With 33.9% and 100% IPI from those anemic women (Table 6).

This study had also found that the use of open source of water (river, well water) for the purpose of bathing, swimming, laundering cooking was not significantly associated with parasitic infection among pregnant women (p=0.440). Among study population who used open source of water, 60% had parasitic infection whereas only 15.6% of pregnant women who didn't use open source water were infected with parasitic infection. The prevalence of anaemia was 17.1% in study population who use open source water for daily use purposes (Table 6).

Photograph 1: Egg of *Ascaris lumbricoides* (10X ×10X)(Corticated)

Photograph 2: Cyst of *Giardia lamblia* (10X × 40X)

Photograph 3: Egg ofHookworm (10X × 40X)

Photograph 4: Observation of stool sample under microscope

5. Discussion

Present study was carried out among the pregnant women attending antenatal clinic at Janakpur Zonal Hospital. Out of 202 pregnant women 36(17.82%) were infected with intestinal parasites. Among infected population, only 0.5% belonged to protozoan parasites and 17.32% infected with helminth parasites. In this study 3 different types of intestinal parasites were found which were *A. lumbricoides*, Hookworms *and Giardia lamblia.* The present study showed *A. lumbricoides* (13.86%) as the most prevalent intestinal helminth parasites followed by Hookworms 7(3.46%).

Overall prevalence rate of parasitic infection among pregnant women at JZH showed comparatively less than result revealed from Sarlahi district (Navilsky, *et al.,* 1998, Dreyfuss, *et al.,* 2000) and Dhankuta district (Shah and Baig, 2005) whereas some research showed comparatively lower IPI rate than present study as shown among pregnant women of Biratnagar (Chaudhary and Maharjan, 2014) and Kathmandu (Marahatta, 2009).

The common nematode parasites infecting human includes *A. lumbricoides, T. trichiura, A. duodenale, N. americanus, E. vermicularis, S. stercoralis, Trichostrongylus sp* etc. In the present study *A. lumbricoides* and Hookworm were identified which were also identified in pregnant women of Biratnagar (Chaudhary and Maharjan, 2014), and also reported by other researcher (Shah and Baig, 2005; Navitsky, *et al.,* 1998 and Marahatta, 2009) except *S. stercoralis, A. lumbricoides* and hookworm were most common nematode in Nepal (Robert, *et al.,* 2011).

In global context, common nematode including *A. lumbricoides, T. trichiura* hookworm were reported from African countries such as Nigeria (Obiezue, *et al.,* 2013; Omorodian, *et al.,* 2012 and Alli, *et al.,* 2011), Ethiopia (Kefiyalew, *et al.,* 2014; Jufar and Zewde, 2014; Getachew, *et al.,* 2012 and Lealem, *et al.,* 2015), Ghana (Fuseini,*et al.,* 2010), Kenya (Wekesa, *et al.,* 2014, McClure, *et al.,* 2014, Van Eijk,*et al.,* 2008), Indonesia (Nurdia, *et al.,* 2001). Robert, *et al.,* (2011) showed *A. lumbricoides* and hookworm were dominant in Kenya where *A. lumbricoides* and *T. trichiura* were common in Gabon. *Trichostrongylus* sp *was* recorded from Ghana (Fuseini, *et al.,* 2010).

The present study shown that *Ascaris lumbricoides* (13.86%) as the most prevalent intestinal helminthic parasite followed by Hookworm (3.46%). The finding of this study similar with the previous study of Rai *et al*., 1994, Abebe, *et al*., 2008, Van Eijik, *et al*., 2009. It was also showed *Ascaris lumbricoides* as the most prevalent helminth followed by Hookworm.

The result of this study was in favors with Navilsky, *et al.,* (1998) and Dreyfuss, et *al.,* (2000) that Hookworms were the stronger predictors of anaemia in pregnant women and Hookworm as the most prevalent helminth parasite in anaemic pregnant women (Nauvilsky, *et al*., 1998, Shah and Baig, 2005). General specific prevalence of Hookworm (3.46%) was relatively the second most common parasite species identified in this study. The prevalence rate is low in compare with the value of other studies in various part of the country. Navilsky, *et al.* (1998) reported infection rate 78.8% in rural plain of Nepal, Dreyfuss, *et al.* reported 74.2% in the plain of Nepal, and Kunwar, *et al*. (2006) reported 53% in Himalayan region of Nepal. Hookworm infections occur by skin penetration of L3 stage infective larvae. Poor sanitary disposal of human faeces and indiscriminate defecation are the principal factors in the etiology of Hookworm infection (Mordi and Ngwodo, 2007).

The most common cause of anaemia among pregnant women are menstrual blood loss, iron deficiency, malnutrition and parasitic infection (Marahatta, 2009). Intestinal parasitic infection interfere with food intake, absorption, storage and use of many nutrients such as iron, vitamin A, vitamin B12, vitamin C, folic acid etc. contribute to anaemia (WHO, 2011). The prevalence of anaemia is high in developing countries due to poverty, inadequate diet, unhygienic drinking water, risky and high frequency of pregnancy and lactation and poor access to health (WHO, 2011).

The present study shows that the pregnant women who had anaemia, 83.17% were infected with parasitic infection similarly in non anaemic pregnant women only 16.83% of them were infected with intestinal parasites. The prevalence rate shows that the association of anaemia with intestinal parasite was statistically significant ($p=0.005$). The result of current investigation was supported by Chaudhary and Maharjan (2014); Bondvik, *et al.,* (2000).Anaemia during pregnancy is major public health problem which contributes to low birth weight and stillbirths.

This study showed that the both age group had similar rate of parasitic infection as well as prevalence of anaemia. Among the age group ≤25 yrs., 17.3% had parasitic infection whereas >25 yrs. age group, 19.5% had parasitic infection. However the result of this study was in favor of that of Awasthi, *et al.,* 2003, prevalence of STH were related to age of the host, and was decreased in >25 yrs. Similarly, the prevalence of anaemia in both the age group was similar rate i.e. 17.3% and 15.2% respectively. The parasitic infection among pregnant women were not significantly associated with age group (p=0.389).

Among the urban population, 3.8% had parasitic infection while prevalence among rural population was found to be 22.6%. Lower prevalence of parasitic infection in urban resident pregnant women in compare to that of rural residency may be due to active participation in deworming programs organized by schools, governments, use of latrines hygienic behavior. IPI higher in rural areas pregnant women might be due to adaption of some traditional cultures like use of animal dung as manure, use of dry dung as fuel, use of open water resources for daily life purposes. Though the STH occur predominantly in rural areas, the social and environmental conditions are ideal for the persistence of *Ascaris lumbriciodes (*Crompton and Savioli, 1993). The prevalence of anaemia in pregnant women whose residency was in urban and rural areas was 11.5% and 16.1% respectively. The presence of anaemia according to their residency in this present study has been found contrast to that of previous study, although the overall prevalence of anaemia remains the same. The prevalence of anaemia in rural women was higher than prevalence of anaemia in urban women (Tadios, 1996; Hyder *et al.,* 1998 and Gebremedin, 2004). The prevalence rate of anaemia were obtained in this study (16.83%) was almost consistent with the report of WHO (1993) 40-60% in developing countries of pregnant women. Similarly a study conducted by Belachew and Yosef (2006) showed pregnant women walk barefoot were two times likely to be anaemic. Most rural pregnant attending antenatal care walk barefoot. They wear shoes when they come to town or antenatal care and for shopping. Walking barefoot may predispose to hookworm infection and the consequences will result anaemia especially in pregnant women.

From Indo-Aryan ethnic group 37.9% were infected with intestinal parasites whereas the pregnant women from Dalit ethnic group, infection rate was of 14.4 %. In spite of

relatively low literacy rate, unhygienic habits and low socio-economic status of Dalits, the prevalence rate of IPI was observed lowest in this study in compared to other ethnic group which was also in support to the result shown by Sharma *et al.,* 2004, but opposite to that result shown by Rai, *et al.,* (2002) higher positive rate among Dalits compared with others in rural hilly community. This further supports the wide distribution of intestinal parasites in this study area. The prevalence of anemia in Dalits, and Indo-Aryan ethnic group was 17.2% and 16.76% respectively. In both the cases the prevalence of IPI and anaemia was found to be higher in women whose residency was in Katcha type of houses. Fifty percent of pregnant women who had Katcha type of house were infected with intestinal parasites, however those who were residents of pukka type of house only 8.3% were infected with parasitic infection. Increased prevalence of parasitic infection in former cases might be result of frequent maintenance required for mudded house, regularly swiping with animal dunks and might have got contaminated with infected soil, mud water. Maintaining the house is almost harvesting the parasite in the house as helminthic parasites remain viable for long time in soil (Rai, *et al.,* 2000). Women residing in Katcha type house 16.7% and 17.3% women residing in pukka type house were aneamic pregnant women.

The prevalence of intestinal parasitic infection and anaemia among different strata of family size was high as family size increases in this study; this might be due to poor household hygienic score, poverty, and lack of knowledge, which has already shown their association in several previous studies (Rajeswari, *et al.,* 1994 and Hidayah, *et al.,* 1997). Prevalence of IPI rate was found to be 7.8%, 15% and 27.5%among pregnant women who had family size of ≤4, 5-8 and ≥9 respectively.

The parasitic infection rate was found to be higher (52%) in illiterate pregnant women compared to that with education level up to secondary (13.2%) and above secondary level (11.1%) respectively. The parasitic infection among pregnant women were significantly associated with literacy rate. In the same way the prevalence rate of anaemia was found to be higher in pregnant women of education level upto secondary level i.e. 18.1% in compared to that of illiterate and above secondary i.e.16% and 11.1% respectively. Several previous study conducted by Hyder, *et al.,*(1998);

Bondvik, *et al.,* (2000); Shah and Baig (2005) and Seck and Jackson (2010) has shown that the higher education is related to lower risk of anaemia.

Majority of the study population were housewife i.e. 83.7%, and only 16.3% were working population. The parasitic infection rate among Housewife pregnant women were found to be 18.9% compared to that of pregnant women who were working 12.1%. The parasitic infection among pregnant women was not significantly associated with occupation. Seventeen percentages of housewife and 15.1% of working women were anaemic. Specific occupations, household clustering and the behaviors influence the prevalence and intensity of helminthic infection (Bethony, *et al.*, 2001), particularly for hookworm in which the highest intensities occur in the adults (Brooker, *et al.*, 2004).

Present study shows that the prevalence of intestinal parasites was higher at third trimester 25.5%, compared with second and first trimester i.e. 15.8% and 13% respectively. The higher prevalence of IPI at the third trimester support the suggestions of some authorities that women in the third and second trimester could benefit from periodic anthelminthic treatment (Booker, et *al.,* 2008). High anemia in the third trimester may be due to improper follow up of pregnancy starting from the first trimester to the third trimester.

IPI in women who shared their houses with livestock was higher i.e. 24.2%, compared to that of pregnant women who didn't shared their houses with any kind of domestic live stocks i.e. 3.2%. Statically there was significantly association with livestock. The higher prevalence of IPI in former case may be due to increase mobility, more workload in the open field to fulfill their duty towards live stocks, use of animal dung as manure, use of dry dung to fuel proposes, and frequent visit of domestic animals to open field area for grazing purpose which increase the chance of exposure to parasites and infect people. The rate of prevalence of anaemia was in both the groups i.e. 15% and 20.9% in pregnant women who share their house with and without live stocks respectively.

In case of toilet using practices, the prevalence of parasitic infection rate was higher among pregnant women who have habit of open defecation i.e. 80.7% compared to that of using toilet i.e. 8.5%. Likewise prevalence of anaemia among both the

population group (in closed and open defecation) was 19.2% and 16.4% respectively. The parasitic infection among pregnant women were significantly associated with toilet using practices (P=0.000). STH may be decreased in toilet user group by reducing contamination of soil and water by promoting the use of latrines and hygienic behavior. Without a change in defecation habits, periodic deworming cannot attain a stable reduction in transmission, Yong, *et al.,* (2000) had already shown use of latrines contribute to prevent IPI.

Majority of the study population having practices of hand wash with soap water after toilet, where 16.7% acquired IPI. Those who didn't practiced soap water habit of hand wash; there were 60% of parasitic infection. Hygienic behavior, hand wash with soap water and walking without bare foot when outdoor contribute to prevent IPI, has already shown by Hyder, *et al.,* (1998); Yong, *et al.,* (2000) and Parajuli, *et al.,* (2009). Hand washing was done regularly but not with soap, so the result showed that there was higher infection in those who did not use soap for hand washing. According to Olsen, *et al.,* (2001), hand wash without soap water were 2.6 times higher risk of being infected with parasites. The prevalence of anaemia was higher among pregnant women who practices hand wash without soap water i.e. 40% compared to hand wash with soap water i.e. 16.2%respectively.

The present study had also tried to know the habit of using open source water for bathing, swimming, laundering, cooking purpose by respondents. The prevalence of parasitic infection was found to be maximum 60% among pregnant women who used open source of water followed by 15.6% in those peoples who used tap and hand pump water. Statistically, the prevalence of intestinal parasites in the pregnant women was not statistically significant with source of drinking water. This high rate of prevalence of intestinal infection may be associated with unsanitary living style, poor socio-economic conditions, consumption of raw vegetables, fruits and water contaminated with infected feces, Unhygienic feeding behavior, and traditional culture of use of river, pond water. Study done by Van Eijik, *et al.,* (2009) and Sehgal, *et al.,* (2010) have also shown the prevalence of IPI as water born infection. Similarly the prevalence of anaemia was 10% and 17.1% in pregnant women who used open source of water and tap or hand pump water for daily use purpose respectively.

Acquired immune deficiency syndrome (AIDS), caused by human immune deficiency virus (HIV), has been one of the biggest pandemics and and global health challenges. Worldwide, 35 million people were living with HIV at the end of 2013 according to WHO reports. And according to WHO 347 million people worldwide suffer from diabetes. In the year 2013, an estimated 5.1 million people died from consequences of high fasting blood sugar. Present study shows that majority of population were infected with blood sugar i.e. 4.9% compared to that of HIV i.e. 0.99%.

The major source of HIV among housewives of Nepal including pregnant women were due to their husbands who works particularly in India. The history of the two pregnant women in present study has also shown to be due to similar source of infection.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The present study was designed to determine the status of parasitic infection and anaemia in pregnant women attending antenatal clinic at JZH. Stool samples were collected in clean vial from all those pregnant women whose orientation was done a day before, with administration of structured questionnaire regarding their knowledge, attitude of practices in relation to the parasitic infection. The hemoglobin reports of respective pregnant women were collected from the record file of hematology department of JZH.

Out of 202 stool samples examined in JZH of pregnant women coming for their antenatal care, the prevalence rate of intestinal parasites were found to be 17.82% while the prevalence of anaemia was 16.83% among those total population. Regarding the intestinal parasites, the prevalence rate of *Ascaris lumbricoides* was found to be maximum in anaemic pregnant women followed by Hookworm and *Giardia lamblia*in total population, while the prevalence rate of Hookworm, *Ascaris lumbricoides* was most prevalent in anaemic pregnant women. The association of anaemia with intestinal parasite was statistically significant (p=0.005)

The prevalence of IPI in pregnant women in relation to their residency area (i.e. Urban and Rural area), habit of use of latrine and source of water used was statistically significant. And the prevalence rate of IPI (60%) was seen in those pregnant women who didn't use soap water for hand washing purpose, while lowest prevalence rate of IPI (12.1%) was seen in working pregnant women.

6.2 Recommendations

1. All pregnant women attending antenatal care should be screened for STH and IPI at their first visit.

2. To prevent recurrence parasitic infection, pregnant women and community should be encouraged to use latrine, use footwear, and improve sanitation and personal hygiene.

3. Expand prevention and treatment of helminthic infections to women of child bearing age through sanitation control programs and anti-helminthic therapy should be conducted in community level as well as in schools also.

4. Pregnant women should be encouraged more to have regular ANC follow up at least four times during pregnancy so that early diagnosis and treatment will help to reduce the prevalence of anaemia.

5. NEG for all pregnant women by a dietitian or a nutrition advisor should become part of routine services offered at antenatal clinics.

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ANNEX- I

QUESTIONNAIRE

Question schedule for baseline health survey in pregnant women coming for ANC visit in JZH.

[1] Serial no. :

[2] OPD no. :

[3]Name of patient: ……

[4] Age:

[5] Address:

(i) Rural (ii) urban

[6] Ethnic group:

(i) Dalit (ii) Indo- Aryan

[8] Education

(i) Illiterate (ii) Up to Secondary level

(iii) Above Secondary level

[9] Occupation of women:

(i) House – wife (ii) Worker

[12] Type of house:

(i) Katcha (ii) Pukka [cemented]

[13] Toilet in house:

(i) Yes (ii) No

[14] How do you wash your hand after toilet?

(i) Water only (ii) with soap (iii) with others [mud, ash]

[15] Where do you get drinking water from?

(i) Tap (ii) Well

[18] Use of pond, well or river water for bathing, cooking and washing clothes purposes.

(i) Yes (ii) No

[19] Any kind of Livestock or domesticated animals at home:

(i) No (ii) cattle [goat, cow, buffalo]

[20] Alcohol user during pregnancy:

(I)Yes (ii) No

[21] Smoking during pregnancy:

(i) Yes (ii) No

[22] Family size:

(i) <4members (ii) 5-8 members (iii) > 9 members

[23] Knowledge Regarding prevention of infestation:

(i) Yes (ii) No