

1. INTRODUCTION

1.1 Background

The health of a women have significant role in the development of a society and nation. A healthy mother brings 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 diseases, which have 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 to be 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, 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 on 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 haemoglobin level of less than 11gm%. Haemoglobin level of 9.0-10.9gm% is mild anemia, 7.0-8.9gm% is moderate anaemia and less than 7gm% is called severe anaemia. Anaemia is a huge medical problem in the developing world. Getting pregnant at a 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 complication 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 anaemia 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 cause 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 regions 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 complementary measures, anemia 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 relatively few studies done in the eastern region of the country. Studies done on 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%-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).

Keeping all this in view, this study was undertaken to find out the prevalence of intestinal parasitic infection with other related risk factors and its association with anaemia, though it does not reveal presence of anaemia in different aspect in pregnant women attending antenatal care in Koshi Zonal Hospital, Biratnagar.

1.2 Public health importance of intestinal parasitic infections

Parasites are those organisms, which receive nourishment and shelter from another organism where they live and hosts are the organisms which harbor the parasites. Intestinal parasitic type ranges from virus, bacteria, and protozoa to helminthes. But commonly prevalent 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 affect 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, WHO 2000). Amoebiasis, Acariasis, Hookworm infection and Trichuriasis are among the ten most common infections in the world (WHO 1987). The incidence and prevalence of these parasitic pathogens varies 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 practices 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 Whipworm 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 their first trimester (Bethony et al. 2006). Where the prevalence of Hookworm is more than 20% to 30%, the World Health Organization recommend that pregnant women should receive anthelmintic treatment (mebendazole, albendazole, levamisole or pyrantel) after their first trimester (Gyorkos 2006). Regardless of these suggestions, 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-Palomo1986). The complications of invasive amoebiasis are potentially fatal and giardiasis may cause mal-absorption in children.

Entamoeba histolytica (Lambl 1859, 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 48 million new cases and 70,000 deaths due to *Entamoeba histolytica* in 1997 (WHO 1998). The transmission of the parasite is through faecal 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 infections are reported to occur 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. Disease caused by *Entamoeba histolytica* is called amoebiasis. Asymptomatic infection with *Entamoeba histolytica* is characterized by the presence of cysts in stools in the absence of colitis or extraintestinal infection. These healthy carriers may pass millions of cysts in the stool per day as the trophozoites multiply in the intestinal lumen (Petri and Sing 1999). Clinical symptoms of acute intestinal amoebiasis include diarrhea, bloody stool that may contain necrotic mucous, abdominal pain, tenderness and fever (Petri and Sing 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 Sing 1999). In many regions, amoebiasis 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 especially 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 & Upcroft 2001). Giardiasis is caused by *Giardia lamblia*. Clinical symptoms of giardiasis include diarrhea, steatorrhea, epigastric pain, wasting, hypoalbuminemia and impaired 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 animals. They belong to the 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 host. 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 helminth 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 Hookworms infect 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 percent of moderate or severe cases of anaemia among pregnant women (haemoglobin level, < 9g per deciliter). The association between Hookworm infection and anaemia is greatest in multigravidas (Peter et al. 2004). Ancylostomiasis is caused by *Ancylostoma duodenale*, commonly called the Hookworm. Infection occurs by the entry of infective stage, filariform larva, through the penetration of skin. It can also infect man successfully by oral, transmammary and (probably) transplacental 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 0.1-0.2 ml of blood per day have been reported for

Ancylostoma duodenale (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, loss of appetite, weight loss, fatigue and anaemia. Patients appear weak they complain of dizziness, ringing in the ears 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 temperate regions. The global prevalence of *Strongyloides* is unknown, but experts estimate that there are between 30–100 million infected persons worldwide (CDC-web). *Strongyloides stercoralis* is the fourth most important intestinal nematode infection which causes Strongyloidosis, 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 and vomiting being the common gastrointestinal symptoms (Milder et al. 1981). The adult worm is largely localized in the duodeno-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 in sputum etc. While establishment inside intestine produce symptoms like nausea, vomiting, anorexia, abdominal pain, diarrhea with mucous, blood and emaciation.

1.3 Objectives of the Study

General objective:

To determine the association between intestinal parasitic infection and other factors to that of anaemia in pregnant women of Koshi Zonal Hospital, Biratnagar.

Specific objectives:

- To determine the prevalence of intestinal parasitic infection in pregnant women.
- To find out the association between anaemia and intestinal parasitic infection in pregnant women.
- To assess the parasitic infection and anaemia in relation to Knowledge, Attitude and Practices (KAP).

1.4 Limitation of the Study

Research studies face many problems, so obviously have limitations to the study. The present study no doubt bears the following limitations.

1. As this study is done for the partial fulfilment of the requirements for the Master's degree, it was completed within limited time and resources.
2. Small sample size and use of a single stool specimen to assess infection status.
3. The study is based up on non-randomized, uncontrolled trial method.
4. This study includes only the pregnant women coming for routine check-up in KZH. Hence the result cannot be generalized for particular area.
5. Haemoglobin report of respective pregnant women was collected from haematology department of KZH.
6. Nutritional status of the pregnant women was not included in this study.

1.5 Significance of the Study

Among the many health problems prevalent in Nepal, intestinal parasitic infection and anaemia in pregnancy constitutes a major health problem which is associated with water, poor sanitation, and lack of knowledge, nutritional deficiency, chronic diseases, and poverty of the country. This study done in Koshi zonal Hospital has been undertaken to find the prevalence of intestinal parasitic infection on the basis of KAP and its association with anaemia. And it will help us to know the intestinal parasitic infection among pregnant women of study area and explore the prevalence rate of helminth parasites. Thus, the study has got a great importance and significant.

2. LITERATURE REVIEW

2.1 History of parasitology

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 parasites 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 later half of 17th century Francesco Redi, Grandfather of parasitology stated that maggots developed from eggs of flies. 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).

2.2 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 co-relation 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 diseases and malnutrition in Nepal by Rai et al. (2002 a) showed due to outbreaks of diseases like diarrhea, dysentery, cholera, 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 parasitosis

(Rai et al. 2007, Bondevik et al. 2000, Dreyfuss et al. 2000). Women in the age group of 15-49 years have been found to be suffering from chronic energy deficiency (NMSS 1998). Nutritional anaemia has been found in almost 68% of women and 75% of pregnant women were anaemic (NMSS 1998, NDHS 2001).

Willian-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 helminthiasis 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 parasitic infection scenario of Nepal for 16 years (from 1979 to 1995) showed that 50% of people were infected by helminth (Chhetri 1997).

In another study conducted by Rai et al. (2000) has stated overall soil contamination rate in and outside Kathmandu valley was 36.5%. The higher soil contamination rate with parasitic helminth 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, Rai et al. 1995). The intestinal helminth parasites *Ascaris lumbricoides* (Willian-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, Raghav et al. 2008), *T. trichiura* (Willian-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 (Willian-Blangero et al. 1993, Rai et al. 1994, Nauvilsky et al. 1998, Dreyfuss et al. 2000, Parajuli et al. 2004, Gimire et al. 2005, Shah and Baig 2005, Kunwar et al. 2006, Raghav et al. 2008), *S. stercoralis* (Parajuli et al. 2004, Raghav et al. 2007) are considered as the 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, Raghav et al. 2008) and *E. histolytica* (Rai et al. 1994, Parajuli at al. 2004, 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%, 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 it other protozoan parasites *G. lamblia* were the most prevalent parasite followed by *E. histolytica* and helminth parasites respectively (Khanal et al. 2011, Magar et al. 2011).

Several report indicated that the Hookworm infection and anaemia is well co-related. Study conducted by Shah and Baig (2005) in Dhankuta District Hospital showed the high prevalence of 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. Hookworms were the stronger predictors of anaemia in pregnant women (Navilsky et al. 1998, Bondevik et al. 2000, 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. 2002 a, Jiang et al. 2005, Adak and Nazri 2006, Chandyo et al. 2006). Some of the previous studies have suggested Hookworm to be the most abundant helminth in pregnant women (Dreyfuss et al. 2000, Young et al. 2000, Marahatta 2009,), while Rai et al. (2002 a) in an overview of infectious diseases and malnutrition have mentioned Hookworm re-emerged in Nepal in recent years. Likewise in study done by Ghimire and mishra (2005) to evaluate the type of intestinal parasites and haemoglobin concentration in the people of chitwan District showed prevalence of intestinal parasites in female was 46.7%, where low concentration of haemoglobin was statistically significant in the helminth 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, Rai et al. 2002 a) 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 a, 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 bare foot when outdoor) (Parajuli et al. 2009) was associated with the prevalence

of IPI while unhealthy habit of food (less consumption of green leafy vegetable 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 anaemia in pregnant women.

2.3 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, Ndyomugenyi et al. 2008, Van Eijk et al. 2009, Alli et al. 2011, 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 *Nector 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 very decreased prevalence rate of helminth infection (11.8%) in Nigeria, with *Ascaris lumbricoides* (8.7%) and *Trichuris trichiura* (3.1%), likewise study conducted by Alli 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, 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%) than 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 infections 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, Rodriguez- Morales et al. 2005) while others showed Hookworm to be the most prevalent helminth followed by the *Ascaris lumbricoides* (Alli et al. 2011, Amuta et al. 2011). Hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*, *Giardia lamblia*, *Entamoeba histolytica*, *Nector americanus*, *Enterobius vermicularis*, *Strongyloides stercoralis*, *Taenia* species, *Entamoeba coli*, *Plasmodium*, *Schistosoma mansoni* and *P. falciparum* 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 reinfected 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, Ndyomugenyi et al. 2008, and Fuseini et al. 2010). In study done by Ndyomugenyi et al. (2008) based in endemic areas have measured hemoglobin (Hb), serum ferritin and erythrocyte protoporphyrin (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 haemoglobin concentrations below 110g/l among study population while 11%, 8% harbored *P.falciparum* and Hookworm respectively.

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

Over 200 million women become pregnant each year, most of 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 combined 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 2002, Belachew and Yosef 2006, Biradar et al. 2012). Age, educational status, economic position, antenatal care and different parasitic infections (Stephenson et al. 2000, Guyatt and Snow 2001, WHO 2002, Bentley and Griffiths 2003, Bechuram et al. 2006), iron deficiency (Engmann 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 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 Pasricha et al. (2008) in Northwest Vietnam showed that 37.53% anaemic 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 infection between anaemic and non-anaemic women. Consumption of meat at least three times a week was more common in non-anaemic women.

According to WHO (2001), the recent guidelines regarding prevention and control of iron deficiency anaemia, are the most effective ways needed to decrease them (Milman et al. 2005, Seck and Jackson 2010, Abd Elhameed et al. 2012). Seck and Jackson (2010) showed the improved in antenatal program in sengal 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 haemoglobin level.

3. MATERIALS AND METHODS

3.1 Study Area.

Nepal is situated in the central part of Asia in between 28⁰ 22' to 30⁰ 27' North latitude 80⁰ 4' to 88⁰ 12' east longitude. It is approximately 885km in length and its mean width is 193km with a total land area of 1, 47,181 sq.km. Nepal is a landlocked Himalayan country in south Asia, bordering the People's Republic of China to the north and India to the south, east and west. Nepal is one of the least developed nations in the world. Poverty, ignorance and diseases characterize life in Nepal like in the most of the Third world countries.

Biratnagar is a Sub-Metropolitan City located in southeast Nepal, the country's second largest after Kathmandu with total area of 58.48 km² and population around 201,125 with an additional half a million people living in its suburbs, thus making its total population to over 700,000 (CBS, Nepal 2013). The study was done in Koshi Zonal Hospital, which lie in the Morang district, Biratnagar.

3.2 Materials Required

3.2.1 Equipments

- i. Compound microscope
- ii. Collecting vials
- iii. Gloves
- iv. Wooden Applicator
- v. Glass slides
- vi. Cover slips
- vii. Forceps / pins
- viii. Dustbin
- ix. Cotton
- x. Camera

- xi. Filter paper

3.2.2 Chemicals 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 the parasites. This solution was prepared by dissolving 8.5gm of sodium chloride in 1000 ml 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 helminth 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 a stopper bottle of amber colour. 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.

3.3 Inclusion and Exclusion Criteria

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 Koshi Zonal Hospital during their regular check up. 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 sample 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 2 hours to 2 days of collection.

3.4.2. Laboratory examination.

Laboratory work was done in the laboratory of Koshi Zonal Hospital of Biratnagar.

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 equipments 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 trophozoites. The iodine stained preparation was used 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 colour marking on the surface of the egg shell.

3.5 Data Collection of Haemoglobin Level.

Haemoglobin report of respective pregnant women was recorded from record file of haematology department of Koshi 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 Koshi 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 connivance to take proper information.

3.7 Statistical Analysis

The data obtained from the study was analyzed by using SPSS 16.

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

4. RESULTS

The present study was carried out among 200 pregnant women attending antenatal clinic at Koshi zonal hospital, Biratnagar, over the period of five months, from April 2012 to August 2012. The pregnant women attending antenatal care (ANC) were interviewed using structured questionnaire and stool samples were examined in the laboratory department of zonal hospital, while their blood report were collected from record file of haematology department of KZH.

4.1. General prevalence of the intestinal parasites in pregnant women

Two hundred 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 29% (n=58) among the pregnant women (Figure 1). Among infected population, 51 women had single infection with either helminthes or protozoan parasites, whereas seven had more than one parasitic infection.

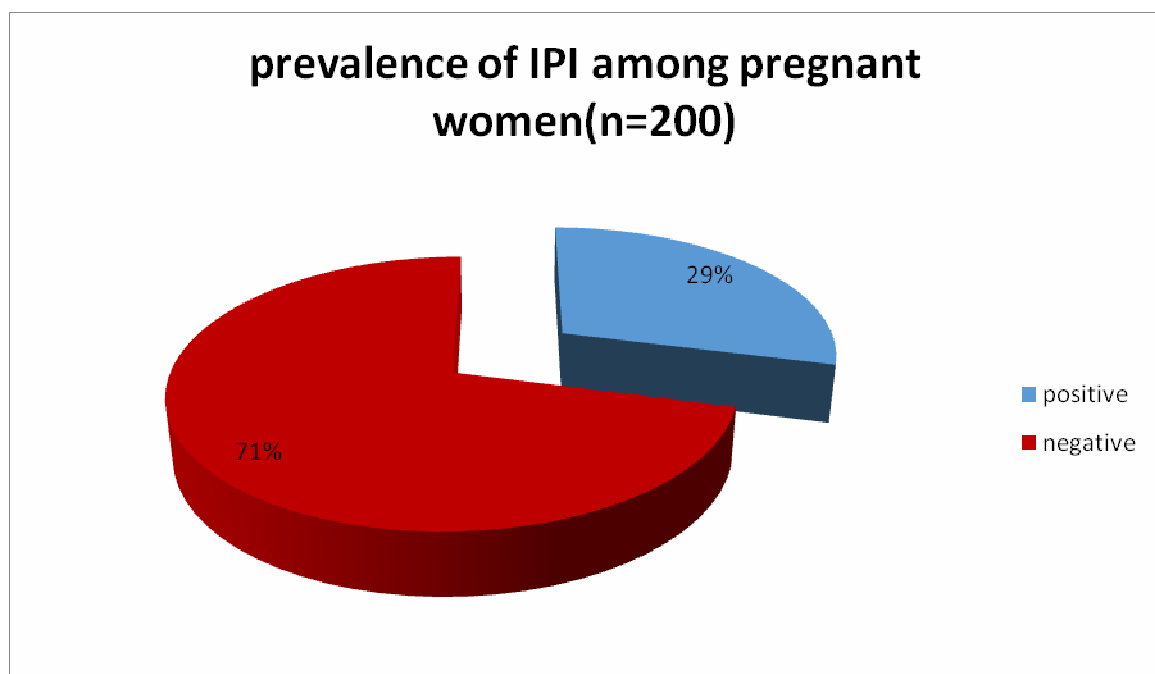


Fig. 1: General prevalence of the intestinal parasites in pregnant women.

Among infected samples, almost two-third of the parasites belonged to helminthes parasites 67.7% (n=44) whereas protozoa amounted in one-third of parasite 32.3% (n=21).

Table 1: Prevalence of specific intestinal parasitic infection in pregnant women (N=58).

Parasites	Frequency	Percentage
Protozoan parasites		
<i>Giardia lamblia</i>	14	21.5%
<i>Entamoeba histolytica</i>	7	10.7%
Nematodes		
<i>Ascaris lumbricoides</i>	21	32.3%
Hookworm	17	26.1%
<i>Trichuris trichiura</i> ,	4	6.15%
<i>Strongyloides stercoralis</i>	1	1.5%
Cestodes		
<i>Hymenolepis nana</i>	1	1.5%

Pregnant women were found to be infected with two species of protozoan parasites, four species of nematode and one species of cestode parasites. The prevalence of specific intestinal parasitic infection found in positive stool samples showed that *Ascaris lumbricoides* was the most predominant 21 (32.3%). This was followed by Hookworm 17 (26.1%), *Giardia lamblia* 14 (21.5%), *Entamoeba histolytica* 7 (10.7%), *Trichuris trichiura* 4 (6.15%), *Strongyloides stercoralis* 1 (1.5%) and *Hymenolepis nana* 1 (1.5%). Table 1 also shows the frequency of multiple infections in 58 positive stool samples.

Infection with single parasite in an individual was more common than multiple infections. Out of 58 positive cases, 51 cases (88%) included infection with single parasite, and 7 (12%) cases were infected by two species of parasites. The occurrence of *Ascaris* and Hookworm infection was more prevalent i.e. 4 (57.1%) than infection of *Ascaris lumbricoides* and *Trichuris trichiura*, *Ascaris lumbricoides* and *H. nana*, and *Entamoeba histolytica* and *Giardia lamblia* in which one case was observed in each (Figure 2).

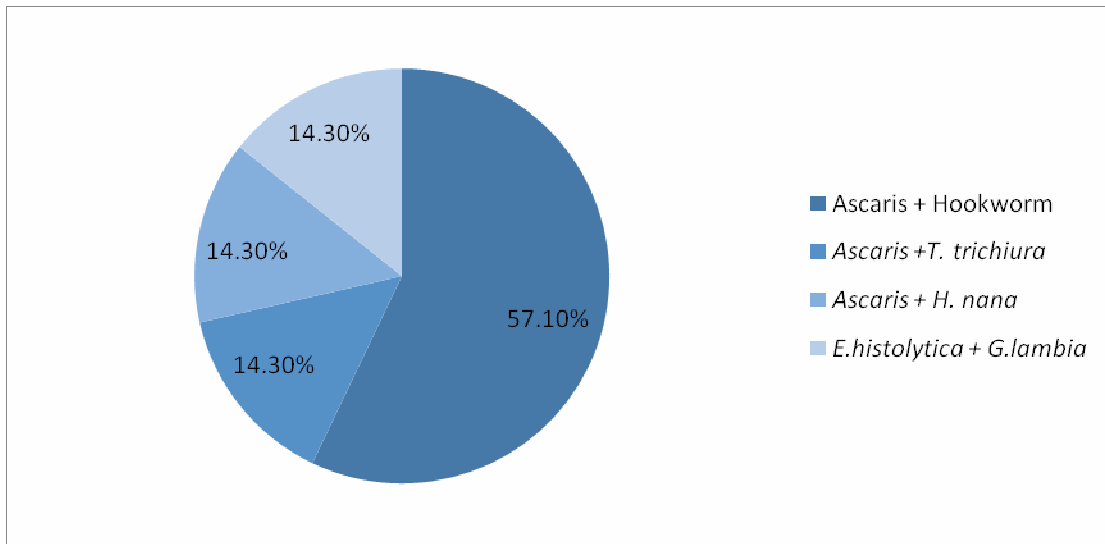


Fig. 2: Distribution of multiple parasitic infection.

4.2 Association between anaemia and intestinal parasitic infection in pregnant women

Hematological evaluation showed that 55% (n=110) of the study population had anaemia. Among these anaemic cases, 36.3% (n=40) of them had parasitic infection (Figure 3). Similarly out of 90 (45%) non-anaemic cases, only 20% (n=18) of them were infected with intestinal parasites. The association of anaemia with intestinal parasite was statistically significant ($p = 0.008$).

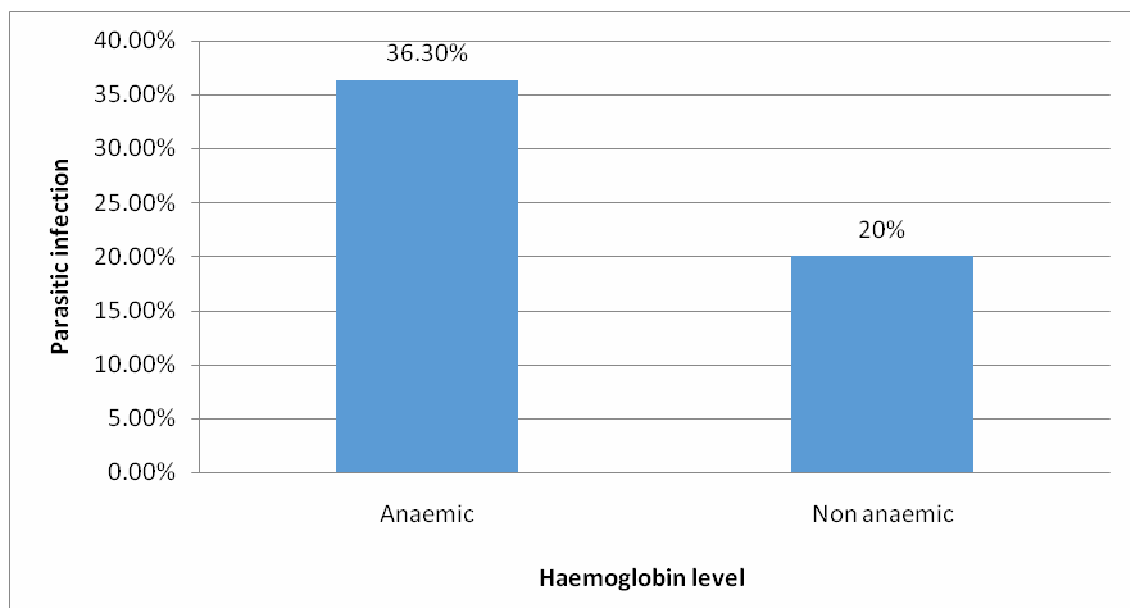


Fig. 3: Prevalence of parasitic infection in anaemic and non anaemic pregnant women.

Table 2: One way ANOVA test show mean haemoglobin level of pregnant women (n=200) with and without parasite infection.

Parasites	Number	Mean Hb \pm SD(gm/dl)	P value
No parasites	142	11.1 \pm 1.5	0.008
With parasite	58	10.3 \pm 1.7	
Single infection	51	10.4 \pm 1.80	
Multiple infection	7	9.81 \pm 0.84	
Mean total	200	10.9 \pm 1.6	

The mean haemoglobin of the study group was found to be 10.9 \pm 1.63 gm/dl (Table 2). However, the mean Hb level of pregnant women without any parasites was 11.1 \pm 1.5 gm/dl whereas pregnant women having multiple infections recorded the lowest mean Hb levels of 9.81 \pm 0.84 gm/dl (moderate anaemia). The Hb levels of 51 pregnant women who were infected with single parasite were reported to be 10.4 \pm 1.80 gm/dl and are said to have mild anaemia. Using the one-way ANOVA test, there was a significant difference within the groups ($p < 0.008$).

Table 3: Prevalence of anaemia within different specific species of intestinal parasites

Species	Haemoglobin level	
	Anaemia	Non anaemia
<i>Giardia lamblia</i>	9 (69.2 %)	4 (30.8%)
<i>E. histolytica</i>	1 (16.7%)	5 (83.3%)
<i>Ascaris lumbricoides</i>	11 (73.3%)	4 (26.7%)
Hookworm	10 (76.9%)	3 (23.1%)
<i>Trichuris trichiura</i>	2 (66.7%)	1 (33.3%)
<i>Strongyloides stercoralis</i>	1 (100%)	-
Multiple-infection	6 (85.7%)	1 (14.3%)

The prevalence of anaemia within the different specific species of Intestinal Parasites is shown in table 3. Among those infected with Hookworm, 76.9% (n=10) of them were anaemic. Similarly, in case of pregnant women who were infected with *Ascaris lumbricoides*, *Giardia lamblia*, *Trichuris trichiura*, and *Strongyloides stercoralis*, the prevalence of anaemia was found to be high i.e. 73.3% (n=11), 69.2% (n=9), 66.7% (n=2) and 100% (n=1) respectively. Additionally, 85.7% (n=6) of dual infection was affected with anaemia. In contrast to those infected with *Entamoeba histolytica*, 83.3% (n=5) were non anaemia.

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 36 years. Though majority of study population were of age group lesser than 25 year (167/200), 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, 29.3 % (n=49) cases had IPI whereas > 25 yr age group had IPI rate of 27.3% (n=9). There was no statistically significant association (P=0.5). The prevalence of anaemia in the age group up to 25 years was 55% (n=92), among them 34 cases (36.9%) was infected with parasites while from 54.5% (n=18) anaemic pregnant women above 25years, 33.3% (n=6) was infected with parasitic infection. In both the age group nematodes were the leading dominant parasites in anaemic cases (Table 4).

The urban population constituted 63.5% (127/200) of the total population, while the rural population constituted 36.5% (73/200) of the total population. Among urban population, 22.8% (n=29) had parasitic infection while prevalence among rural population was found to be 39.7% (n=29). Statistically, there was significant difference in the IPI prevalence rate among rural and urban population (p<0.009) (Table 4). The prevalence of anaemia in pregnant women whose residency was in urban and rural area was 57.4% (n=73) and 50.6% (n=37) respectively. Among those anaemic women 31.5% (n=23) from urban

residency and 45.9% (n=17) from rural residency were infected with parasitic infection (Table 4).

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

Socio demographic characteristic	Prevalence of Intestinal parasite	P-value	Prevalence of anaemia
Age group			
≤ 25yrs (N=167)	29.3% (n=49)	P = 0.5	55.% (n=92)
>25y (N=33)	27.3% (n=9)		54.5% (n=18)
Residency			
Urban (N=127)	22.8% (n=29)	P < 0.009	57.4% (n=73)
Rural (N=73)	39.7% (n=29)		50.6% (n=37)
Ethnic group			
Dalit (N=16)	12.5% (n=2)	P = 0.08	56.2% (n=9)
Tibeto- Burman (N=16)	12.5% (n=2)		43.7% (n=7)
Indo-Aryan (N=168)	32.1% (n=54)		55.9% (n=94)
House type			
Katcha (N=120)	34.2% (n=41)	P = 0.04	59.1% (n=71)
Pukka (N=80)	21.2% (n=17)		48.7% (n=39)
Family size			
≤ 4 member (N=80)	25% (n=20)	P = 0.3	45% (n=36)
5-8 member(N=100)	30% (n=30)		56% (n=56)
≥ 9 member (N=20)	40% (n=8)		90% (n=18)

The majority of the study population were from the indo-Aryan ethnic group (n=168/200). Among them, 32.1% (n=54) were infected with intestinal parasites whereas

the pregnant women from Dalit (n=16) and Tibeto-Burman ethnic group (n=16) had infection rate of 12.5% each. However no statistical association between parasitic infection and ethnicity was found (p=0.08). The prevalence of anaemia in Dalit, Tibeto-Burman and Indo-Aryan ethnic group was 56.2% (n=9), 43.7% (n=7) and 55.9% (n=94) respectively. From Dalit anaemic cases (n=2) 22.2%, Tibeto-Burman anaemic women 14.2% (n=1) and Indo-Aryan anaemic women (n=37) 39.3% were infected with intestinal parasitic infection (Table 4).

Almost 60% (n=120) of the study population was residing in Katcha type of house. Among them, 34.2% (n=41) were infected with intestinal parasite. However those who were resident in Pukka house, only 21.2% (n=17) were infected with parasitic infection. The statistical analysis showed that inhabitant of Katcha type house had significantly association with intestinal parasites (p=0.04). Women residing in Katcha house 59.1% (n=71) and 48.7% (n=39) women residing in Pukka house were anaemic pregnant women along with 39.4% (n=28) and 30.7% (n=12) were with positive sample within those anaemic cases (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 trending high as family size increases. Prevalence of IPI rate was found to be 25%, 30% and 40% among pregnant women who had family size of ≤ 4 , 5-8, and ≥ 9 respectively. Though there was no significant association (p=0.3). The prevalence of anaemia in pregnant women was 45% (n=36), 56% (n=56), 90% (n=18) in family size of ≤ 4 , 5-8, and ≥ 9 respectively, with 30.5% (n=11), 37.5% (n=21), 44.4% (n=8) infected with parasites from respective anaemic groups respectively (Table 4).

4.3.2 Assessment of intestinal parasite and anaemia in relation to knowledge

The illiterate pregnant women (n=17) had the highest IPI rate of 41.2% (n=7), whereas pregnant women with education level up to secondary level (n=151) and above secondary level (n=32) had IPI rate of 28.5% (n=43) and 25% (n=8) respectively. The infection rate was found highest in illiterate pregnant women whereas pregnant women with education level up to secondary level and above secondary level had infection rate respectively. Our study showed that the education status of pregnant women is not statistically associated with parasitic infection (p=0.4). In the same way the prevalence of anaemia in illiterate

pregnant women had the highest rate 70.5% (n=12) where as women with education level up to secondary level and above secondary level had rate of 54.9% (n=83) and 46.8% (n=15) respectively. Forty percent (n=6) of anaemic pregnant women with above secondary level education were infected with parasitic infection where 33% (n=4) of anaemic illiterate and 36.1% (n=30) of anaemic women with secondary level education were infected with parasites (Table 5).

Table 5: Assessment of intestinal parasites and anaemia in relation to their knowledge.

Knowledge related variables	Prevalence of Intestinal parasite	P-value	Prevalence of anaemia
Literacy			
Illiterate (N=17)	41.2% (n=7)	P = 0.4	70.5% (n=12)
Upto secondary level (N=151)	28.5% (n=43)		54.9% (n=83)
Above secondary level (N=32)	25% (n=8)		46.8% (n=15)
Awareness			
Aware (N=159)	27% (n=43)	P = 0.2	51.5% (n=82)
Unaware (N=41)	36.6% (n=15)		68.2% (n=28)
Occupation			
Housewife (N=186)	30.6% (n=57)	P = 0.06	56.9% (n=106)
Worker (N=14)	7.1% (n=1)		28.5% (n=4)

This study had tried to explore the impact of participant's Knowledge of transmission, infection and prevention of parasitic infection on its prevalence. Among pregnant women who were aware of transmission and prevention of parasitic infection, 27% had infection. But those who were not aware of its transmission and prevention, 36.6% had infection with intestinal parasites. Hence, not aware pregnant women were infected more with intestinal parasites than aware pregnant women. Statistically, there was no significant difference in parasitic infection rate among the participants in terms of their knowledge of transmission of infection and its prevention (p=0.2). Similarly the prevalence of anaemia in not aware women was 68.2% (n=28) in compared to that of aware women 51.5%

(n=82). While there was no major difference in parasitic infection in anaemic women i.e. 39.2% (n=11) and 35.3% (n=29) in unaware and aware women respectively (Table 5).

Majority of the study population were housewife i.e. 93% (186/200), only 7% (14/200) were from working occupation. The parasitic infection rate among housewife was found to be 30.6 % (n=57) in compared to 7.1% (n=1) among pregnant women who were working. No significant association between infection rate and occupation was found (p=0.06). There was high difference in prevalence of anaemia and anaemic women with positive cases according to their occupation. 56.9% (n=106) of housewife with 28.5% (n=1) women of working occupation were anaemic. Among those anaemic women about 36.7% (n=39) housewife and 25% (n=1) working pregnant women were infected with parasites (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. 52.5% (105/200) while 26% (52/200) and 21.5% (43/200) belongs to women from third and first trimester simuntansally. The parasitic infection rate among pregnant women who were in first, second and third trimester of their pregnancy was 18.6% (n=8), 33.3% (n=35) and 28.8% (n=15) respectively. No significant association was found between parasitic infection and different trimester of pregnancy (p=0.2). Prevalence of anaemia in pregnant women was highest 67.3% (n=35) in third trimester of pregnancy while 58% (n=61) and 32.5% (n=14) of anaemic pregnant women were found in second and first trimester of pregnancy. Among those anaemic women, 21.4% (n=3) from 1st trimester, 39.3% (n=24) from 2nd trimester and 37.1% (n=13) from 3rd trimester were infected with parasitic infection (Table 6).

Among pregnant women who didn't shared their houses with domestic live stocks, 23.7% (n=22) had intestinal parasitic infection, in compared to pregnant women who shared with live stocks had increased prevalence of rate of parasitic infection i.e. 33.6% (n=36). Though statically there was no significant association (p=0.1). The prevalence of anaemia was also closed to each other i.e. 57.9% (n=62) and 51.6% (n=48) in those pregnant women who share their life with and without livestock respectively. While 40.3% (n=25) and 31.2% (n=15) of anaemic women were also infected with parasitic infection (Table 6).

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

Practices related variables	Prevalence of Intestinal parasite	P-value	Prevalence of anaemia
Trimester of pregnancy			
1 st trimester (N=43)	18.6% (n=8)	P = 0.2	32.5% (n=14)
2 nd trimester(N=105)	33.3% (n=35)		58% (n=61)
3 rd trimester (N=52)	28.8% (n=15)		67.3% (n=35)
House sharing with live stock			
Yes (N=107)	33.6% (n=36)	P= 0.1	57.9% (n=62)
No (N=93)	23.7% (n=22)		51.6% (n=48)
Practice of toilet use			
Closed area(N=139)	22.3% (n=31)	P=0.002	51.7% (n=72)
Open area (N=61)	44.3% (n=27)		62.2% (n=38)
Hand wash after use of toilet			
With soap water(N=195)	27.2% (n=53)	P= 0.002	54.3% (n=106)
Without soap	100% (n=5)		80%(n=4)
Water (N=5)			
Intake of antihelminthes			
No (N=168)	28.6% (n=48)	P = 0.7	54.1% (n=91)
Within 6 month(N=1)	-		--
Beyond 6 month(N=31)	32.3% (n=10)		58% (n=18)
Source of drinking water			
Open source (N=18)	55.6% (n=10)	P = 0.009	38.8% (n=7)
Tap water (N=182)	26.4% (n=48)		56.5% (n=103)

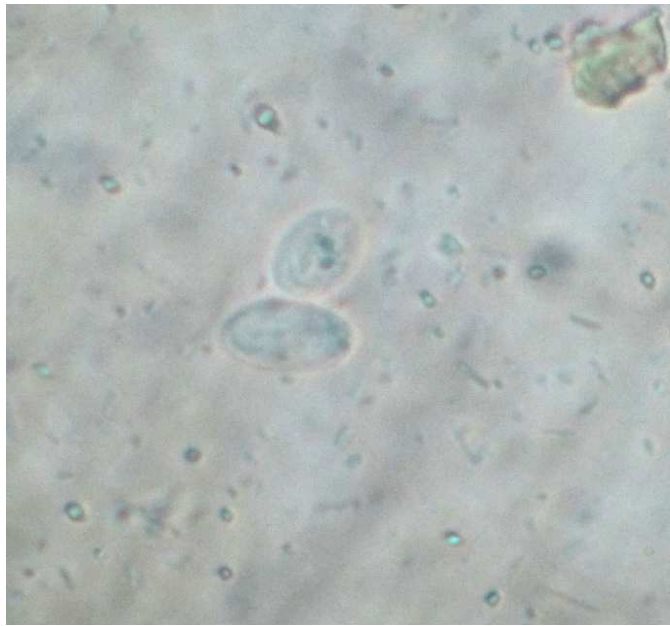
Majority of study population had habit of using toilet in house i.e. 69.5% (n=139/200), while 30.5% (61/200) belongs to those category who does not use toilet. Women who had habit of using toilet 22.3% (n=31) had parasitic infection in compared to 44.3% (n=27) of

those who were not using toilet at home. The absence of toilet at home was significantly associated with parasitic infection ($P=0.002$). Though the prevalence of anaemia in closed and open type of toilet user was 51.7% ($n=72$) and 62.2% ($n=38$) closer to each other, but prevalence of parasitic infection among anaemic women was with great difference. i.e. 29.1% ($n=21$) and 50% ($n=19$) respectively (Table 6).

There was significant association of hand washing habit after toilet use with soap water prevalence. While majority of the study population practiced soap water hand wash after toilet (195/200), only 27.2% ($n=53$) acquired IPI. Those who didn't practiced soap water habit of hand wash, there was 100% risk of parasitic infection, and was statistically significant ($p=0.002$). The prevalence of anaemia i.e. 54.3% ($n=106$) and 80% ($n=4$) was present in the group in which use of soap water was used for hand wash purpose and in group which has habit of hand wash without soap water respectively. With 33.9% ($n=36$) and 100% ($n=4$) IPI from those anaemic women (Table 6).

Out of 200 pregnant women 31 had taken deworming tablet beyond six months during the time of sample collection, where as 168 had not taken the drug while only one patient had taken it within 6 months. Parasitic infection rate among pregnant women supplemented with anti-helminthic drug beyond six month was 32.3% ($n=10$) in compared to 28.6% ($n=48$) among those who didn't take drug ($p=0.7$). Only one woman has taken albendazole within 6 month which was neither with IPI nor anaemic case. Prevalence of anaemia in both the group was very closed i.e. 58% ($n=18$) and 54.1% ($n=91$) with 38.8% ($n=7$) and 36.2% ($n=33$) IPI among those anaemic pregnant women respectively (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 significantly associated with parasitic infection among pregnant women ($p=0.009$). Among study population who used open source of water, 55.6% ($n=10$) had parasitic infection whereas only 26.4% ($n=48$) of pregnant women who didn't use open source water were infected with parasitic infection. The prevalence of anaemia was 38.8% ($n=7$) with 71.4% ($n=5$) infected positive case in study population who use open source water for daily use purposes, while 56.5% ($n=103$) were anaemic with 33.9% ($n=35$) with positive case among those anaemic population who used tap water for daily use purposes (Table 6).



Photograph 1: Cyst of *Giardia lamblia* (10X × 40X)



Photograph 2: Egg of *Ascaris lumbricoides*
(10X × 40X) (Decorticated)



Photograph 3: Egg of *Ascaris lumbricoides*
(10X × 10X) (Corticated)



Photograph 4: Egg of *Hymenolepis nana*
(10X × 40X)



Photograph 5: Egg of Hookworm
(10X × 40X)



Photograph 6: Egg of *Trichuris trichiura*
(10X × 40X)



Photograph 7: Larva of *S. stercoralis*
(10X × 40X)



Photograph 8: Observation of stool sample under microscope



Photograph 9: Researcher doing questionnaire survey

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5. DISCUSSION

Intestinal parasites are worldwide in distribution. Among them Soil Transmitted Helminthes (STHs) and other helminth parasites pose serious threat in the physical well being of human. Poverty, illiteracy and different aspects of culture may play leading role in increasing rate of prevalence of such parasites. Women communities compared to the men communities are more prone to different parasitic diseases, hence early diagnosis of Intestinal Parasitic Infection in pregnant women and in women of reproductive age should be more emphasized in order to reduce its related risk factors and its adverse outcomes.

The present study indicated that the prevalence of intestinal parasites in pregnant women is remarkable. Out of 200 pregnant women, 58 (29%) were found to be infected by at least one type of intestinal parasite. Among infected samples, 67.7% belonged to helminthic parasites whereas 32.3% to protozoan parasite. This high rate of prevalence among pregnant women may be associated with unsanitary living style, poor socio-economic condition, usual contact with soil and consumption of vegetables, fruit and water contaminated with infected faeces, walking bare foot in faecal contaminated open area, use of contaminated mud as fertilizer which was not considered in this study. Overall prevalence rate among pregnant women showed comparatively less than several studies which showed higher rate (Nauvilsky et al. 1998, Vaidya and Acharya 1998, Dreyfuss et al. 2000, Rodriguez-Morales et al. 2005, Shah and Baig 2005). Comparable prevalence of IPI was, however, reported in some other studies (Ozumba et al. 2005, Fuseini et al. 2010 and Sehgal et al. 2010). This might be due to the difference in geographical location and economic status. Rai et al. (1994) concluded that the annual rate of positivity for STH ranged from 18.0-36.6%. The factors influencing soil transmitted helminth infection includes natural factors such as temperature, humidity and socio-ecological factors, structure of dwelling, life style, and habits of food consumption.

In this study seven different types of specific intestinal parasites were found which were *Ascaris lumbricoides* (32.3%), Hookworm (26.1%), *Strongyloides stercoralis* (1.5%), *Trichuris trichiura* (6.15%), *Giardia lamblia* (21.5%), *Entamoeba histolytica* (10.7%), *H. nana* (1.51). *Ascaris lumbricoides* was the leading parasite followed by Hookworm. All these parasites had also been reported from Terai area Sarlahi District (Nauvilsky et al. 1998), in hilly area i.e. Dhankuta District (Shah and Baig 2005), in Venezuela (Rodriguez-Morales et al. 2005). The occurrence of helminthic infection at high rates among pregnant women is an indicative of faecal pollution of soil, domestic water supply around homes due to poor sanitation, ignorance of the mode of transmission of these worms and improper utilization of latrine and poor personal hygiene among the study population.

The present study had shown, *Ascaris lumbricoides* (32.3%) as the most prevalent intestinal helminth parasite followed by Hookworm (26.1%). This result is in agreement with that reported previously (Rai et al. 1994, Abebe et al. 2008, Van Eijik et al. 2009) which also showed *Ascaris lumbricoides* as the most prevalent helminth followed by Hookworm.

The result of this study was in favour 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 (26.1%) was relatively the second most common parasite species identified in this study. This prevalence rate is low when compared with the value from other studies in various parts of the country both now and in the past. Navilsky et al. (1998) reported infection rate of 78.8% in rural plain of Nepal, Dreyfuss et al. (2000) reported 74.2% in the plain of Nepal, and Kunwar et al. (2006) reported 53% in Himalayan region of Nepal. Comparable prevalence of Hookworm was reported in Vidya and Acharya (1998) study only 15.7% in Maternity Hospital, Kathmandu. Hookworm infections occur by skin penetration of the 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). However, the high prevalence of intestinal nematode recorded in this study indicates high level of unhygienic practices which enhanced transmission in the community.

This study had shown 110 (55%) pregnant women attending antenatal care at KZH were anaemic patient, among those anaemic pregnant women 40 (36.3%) had parasitic infection, while out of 200 pregnant women 90 (45%) were non-anaemic with 18 (20%) among them with parasitic infection. The prevalence rate shows the association of anaemia with intestinal parasite was statistically significant ($p=0.008$). The mean Hb level of pregnant women without any parasites was 11.1 ± 1.5 gm/dl whereas pregnant women having multiple infections recorded the lowest mean Hb levels of 9.81 ± 0.84 gm/dl (moderate anemia). The mean Hb levels of 51 pregnant women who were infected with single parasite were reported to be 10.4 ± 1.80 gm/dl (mild anaemia).

There are many studies on anaemia, both hospitals based and community based in pregnancy in Nepal, which are comparable to the result of this study. In hospital based study done by Bonevik et al. (2000) showed prevalence of anemia 62.2% in Kathmandu, while Adak and Nazri in 2006 showed 54.6% in another study conducted in young girls and pregnant women of Birgunj, and study conducted by Marahatta in 2009 showed 42.6% in NMCTH. Similarly high prevalence (50% - 60%) (Ulstein et al. 1988) of anaemia were noted in various community based studies particularly important studied carried out by Shah and Gupta (2002) showed that prevalence of anaemia in adolescent girls in Dharan, a town in eastern region of the country was 68.8%. The probable reason for low proportion of women with anemia in this study may be due to the fact that this study is hospital based study which is located in the city. The women who come for antenatal care to the hospital are slightly better informed with good health awareness. Although we cannot generalize the results of this study to the overall prevalence of anemia in this region but can envisage that the real prevalence of anemia in this area could be much higher.

Anaemia during pregnancy is major public health problem which contributes to low birth-weight and stillbirths. Anaemia in pregnancy is a socioeconomic as well as a medical problem. Preventive measures need to be addressed at community level as well as during clinical level. Many previous studies showed significant association between anaemia and presence of STH, iron deficiency, use of hormonal contraception, grandgravid mothers, aborted mothers, current irregular ANC visit, increasing gestation period, less birth interval, unhealthy habit of nutrition, micronutrient deficiency, parity index, abnormal vaginal discharge, presence of malaria, poor intake of iron, etc. At the community level, long term measures such as poverty reduction and improved nutritional

status are needed to trackle, while in clinical level, early diagnosis and treatment, health education and provision of iron supplements will help to reduce the prevalence of anaemia. 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 Broek 2003). Not all people with iron deficiency are anemic and not all people with anemia are iron deficient. Iron absorption is enhanced when consumed with foods high in vitamin C such as orange juice but substances in coffee and tea inhibit iron absorption (Cook and Mosen 1997). Anemia is caused by either a low production of Red Blood Cells or by destruction or shortened lifespan of Red Blood Cells. Malaria parasites also destroy Red Blood Cells and suppress Red Blood Cell production (WHO 2002). Dreyfuss et al. (2000) reported that 19.8% of pregnant women living in the rural plains of Nepal had *Plasmodium vivax* (*P. vivax*) malaria parasitemia. Treatment of malaria during pregnancy has been shown to be effective in reducing anemia and risk of LBW infants (Steketee 2003). Also, iron supplementation, and possibly vitamin A should be coupled with antimalarial treatment for anemia and LBW prevention during pregnancy (Dreyfuss et. al. 2000, Mahomed 2000, Mahomed 2006). *Helicobacter pylori* can cause anemia by increasing blood loss and reducing stomach acid, resulting in poor iron absorption. In addition, bacterial diarrhea may also cause anemia when chronic and characterized by bloody stools. Chronic diarrhea causes mal-absorption and under nutrition, lowering Red Blood Cell production.

Several species of worms contribute to anemia in developing countries, with Hookworms and *Schistosomes* being the most common. Both cause significant blood loss in the host, which leads to iron deficiency and anemia. For example, Hookworm infection was identified as the strongest predictor of iron status in pregnant women in a study conducted in rural Nepal, and anemia prevalence increased as intensity of Hookworm infection increased (Brooker et al. 2004). The mechanism by which Hookworm contribute to IDA is chronic blood loss. Another study also showed a significant association between hookworm infection and anaemia (Belachew and Yosef 2006). Infections causing chronic blood loss such as parasitic infection with Hookworm, increase iron requirement. Hookworm infection is described to be one of the principal causes of iron deficiency anaemia. This finding indicates the need for strengthening of interventions related to screening intestinal parasitic infection for pregnant women during their first antenatal care service.

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 yr, 29.3 % had parasitic infection whereas > 25 yr age group, 27.3% had parasitic infection. However, the result of this study was in favor to that of Awasthi et al. 2003, prevalence of STH were related to age of the host, and was decreased in >25 yrs group. Similarly, the prevalence of anaemia in both the age group was similar rate i.e. 55% and 54.5% respectively.

Among urban population, 22.8% had parasitic infection while prevalence among rural population was found to be 39.7%. 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 programmes 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 dunks as manure, use of dry dunks as fuel, use of natural resources water for daily life purposes. Though the STH occur predominantly in rural areas, the social and environmental conditions are ideal for the persistence of *Ascaris lumbricoides* (Crompton and Savioli 1993). The prevalence of anaemia in pregnant women whose residency was in urban and rural area was 57.4% and 50.6% respectively. The presence of anaemia according to their residency in this present study has been found contrast to that of previous study, although overall prevalence of anaemia remains the same. The prevalence of anaemia in the rural women was higher than prevalence of anaemia in urban women (Tadios 1996, Hyder et al.1998, Gebremedin 2004). The prevalence of overall anaemia obtained in this study (55%) 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 walking barefoot were two times likely to be anaemic. Most rural pregnant women attending antenatal care walk barefoot; even those women who have shoe do not wear regularly. They wear shoe when they come to town for antenatal care and for marketing. Walking barefoot may predispose to Hookworm infection and the consequence will result iron anaemia especially in pregnant women.

From Indo-Aryan ethnic group 32.1% were infected with intestinal parasites whereas the pregnant women from Dalit and Tibeto-Burman ethnic group, infection rate was of 12.5% each. In spite of relatively low literacy rates, unhygienic habits and the low socio-economic status of Dalits, the prevalence rate of IPI was observed lowest in this study in compared to the 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 b) higher positive rate among Dalits compared with others in a rural hilly community. This further supports the wide distribution of intestinal parasites in this study area. The prevalence of anaemia in Dalit, Tibeto-Burman and Indo-Aryan ethnic group was 56.2%, 43.7% and 55.9% 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. Thirty-Four percentages of pregnant women who had Katcha type house were infected with intestinal parasites, however those who were resident in Pukka type house only 21.2% were infected with parasitic infection. Increased prevalence of parasitic infection in former case might be result of frequent maintenance required for mudded house, regular swiping with animal dunks and might have got contaminated with infected soil, mud, water. Maintaining the house is almost harvesting parasites in the house as helminth parasites remain viable for long time in soil (Rai et al. 2000). Women residing in Katcha type house 59.1% and 48.7% women residing in pukka type house were anaemic pregnant women.

The prevalence of intestinal parasitic infection and anaemia among different strata of family size was trending 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, Hidayah et al. 1997). Prevalence of IPI rate was found to be 25%, 30% and 40% among pregnant women who had family size of ≤ 4 , 5-8, and ≥ 9 respectively. Though there was no significant association. And the prevalence of anaemia in pregnant women was 45%, 56%, and 90% in family size of ≤ 4 , 5-8, and ≥ 9 respectively.

The illiterate pregnant women was seen with the highest IPI rate of 41.2%, whereas pregnant women with education level up to secondary level and above secondary level had IPI rate of 28.5% and 25% respectively. This difference of prevalence in IPI may be as literate mothers possibly gained more benefit from the existing health and nutrition education programmes channeled through interpersonal communications or through mass media, while illiterate cannot read and understand health message in pamphlets, posters, newspapers or booklets. In the same way the prevalence of anaemia in illiterate pregnant women had the highest rate 70.5% where as women with education level up to secondary level and above secondary level had rate of 54.9% and 46.8% respectively. Several previous study conducted by Hyder et al. (1998), Bondevik et al. (2000), Shah and Baig

(2005), Seck and Jackson (2010) has shown that the higher education is associated with lower risk of anaemia.

While trying to explore the impact of participant's knowledge of transmission, infection and prevention of parasitic infection on its prevalence, the IPI infection rate among pregnant women who were aware of transmission and prevention of parasitic infection, had 27% and those who were not aware of its transmission and prevention had 36.6%. Not aware pregnant women were infected more with intestinal parasites than aware pregnant women. In one of the study done by Rai et al. (1998) showed that the level of awareness of intestinal parasites was very low and revealed that more than 90% of the studied population did not have any awareness other than Roundworm. Similarly the prevalence of anaemia in not-aware women was 68.2% in compared to that of aware women 51.5%. There is great difference between both knowledge and knowledge- related practice in our society. Abd. Elhameed et al. (2012) has already shown that the implementation of the NEG helped pregnant women to identify different sources of iron rich foods, and regulate their time to take iron supplementation.

Majority of the study population were housewife i.e. 93%, only 7% were from working occupation. The parasitic infection rate among Housewife pregnant women were found to be 30.6 % in compared to that pregnant women who were working 7.1%. There was high difference in prevalence of IPI and anaemia in pregnant women according to their occupation. Sixty percentages of housewife women and 28.5% women who were working were anaemic. Specific occupations, household clustering and behaviors influence the prevalence and intensity of helminth infection (Bethony et al. 2001), particularly for Hookworm in which the highest intensities occur among adults (Brooker et al. 2004).

Present study shows the prevalence of intestinal parasites higher at second trimester 33.3%, with 28.8% and 18.6% respectively in third and first trimester. The higher prevalence of IPI at the second trimester support the suggestions of some authorities that women in the second and third trimester could benefit from periodic antihelminthic treatment (Brooker et al. 2008). While the Prevalence of anaemia was in increasing order from first to third trimester i.e. 32.5%, 58%, 67.3% respectively. High anaemia in the third trimester may be due to improper follow up of pregnancy starting from the first trimester to the third trimester, previous history of pregnant women among aborted mothers, with grandgravidia mothers, with increasing gestation period, less birth interval, while previous studies have already shown regular ANC attendant pregnant women had

low prevalence of anaemia. Iron requirements vary by trimester of pregnancy and are increased greatly especially after the first trimester which results to iron deficiency anaemia. In special locations, such as Nepal and other developing countries, iron intake must cover extra losses due to parasitic infections.

IPI in women who shared their houses with domestic live stocks was higher i.e. 33.6%, in compared to pregnant women who didn't shared their house with any kind of domestic live stock i.e. 23.7%. Statically there was no significant association. 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 livestock, use of animal dung as manure, use of dry dung for fuel purposes, and frequent visit of domestic animals to open field area for grazing purpose which increases the chance of exposure to parasites and infect people. The result of prevalence of anaemia was also close, in both the groups i.e. 57.9% and 51.6% in those pregnant women who share their life with and without livestock respectively.

Twenty-two percent women who had habit of using toilet had parasitic infection in compared to 44.3% of those who had habit of open defecation. Likewise the prevalence of anaemia i.e. 51.7% and 62.2% was present in both the group (in closed and open defecation) respectively. Increase of households having a pit latrine and improvements in sanitation and hygiene in eastern Nepal, (Rai et al. 1997), result of this study was not in favor to that, with significant impact on the reduction of intestinal helminth infection. 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 practiced hand wash with soap water after toilet, where 27.2% acquired IPI. Those who didn't practiced soap water habit of hand wash; there were 100% 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. According to Olsen et al. (2001), households without soap had a 2.6 times higher risk of being infected with parasites. The prevalence of anaemia i.e. 54.3% and 80% was present in the group in which use of soap water was used for hand

wash purpose and in group which had habit of hand wash without soap water respectively.

IPI in pregnant women who had not taken any antihelminthic medicine was 28.6%, and 32.3% in women who had taken it beyond 6 months. Only one woman had taken albendazole within 6 month which was with no IPI nor anaemic case. IPI prevalence might have been under controlled by administering drug treatment in the community using different strategies i.e. universal treatment, targeted treatment, and selective treatment and spreading importance of antihelminthic treatment. Prevalence of anaemia in both the group was very closed i.e. 58% and 54.1% in pregnant women 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 55.6% in those women who used open sources water followed by 26.4% in those peoples who used tap or hand pump water. Statistically, the prevalence of intestinal parasites in the pregnant women was significant. This high rate of prevalence intestinal infection may be associated with unsanitary living style, poor socio-economic conditions, consumption of vegetables, fruits and water contaminated with infected faeces, Unhygienic feeding behaviour, and conservative or 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 38.8% and 56.5% in pregnant women who used open source water and tap or hand pump water for daily use purpose respectively.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The present study was carried out to determine the prevalence of intestinal parasites and its association with anaemia in pregnant women coming for antenatal care in KZH. Morning stool samples were collected in a clean vial from all those pregnant women whose orientation was done a day before, with fulfill structured questionnaire regarding their knowledge, attitude of practices in relation to the intestinal parasitic infection. And the haemoglobin report of respective pregnant women was collected from the record file of haematology department of KZH.

Out of 200 stool samples examined in KZH of pregnant women coming for their antenatal care, the prevalence rate of intestinal parasites were found to be 29% while the prevalence of anaemia was 55% among those total population. Regarding the intestinal parasites, the prevalence rate of *Ascaris lumbricoides* was found to be maximum in pregnant women followed by Hookworm, *Giardia lamblia*, *Entamoeba histolytica*, *Trichuris trichiura*, *Strongyloides stercoralis* and *H. nana* in total population, while the prevalence rate of Hookworm, *Ascaris lumbricoides* was most prevalent in anaemic pregnant women, with multiple infection (6 out of 7), while *Entamoeba histolytica* only (1 out of 6) was found in anaemic pregnant women. The association of anaemia with intestinal parasite was statistically significant ($p = 0.008$).

The mean Hb level of pregnant women without any parasites was with the normal range whereas pregnant women having multiple infection showed moderate anemia and the pregnant women who were infected with single parasite was with mild anaemia.

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 and anaemia, both was increased as the family size of pregnant women increased and the literacy status decreases. The highest prevalence rate of IPI (100%) was seen in those pregnant women who didn't use soap water for hand washing purpose, while lowest prevalence rate of IPI (7.1%) 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 helminth 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 KZH.**

[1] Serial no. :

[2] OPD no. :

[3] Name of patient:

[4] Age:

[5] Address:

(i) Rural (ii) urban

[6] Ethnic group:

(i) Dalit (ii) Tibeto – Burman (iii) Indo - Aryan

[7] Religion:

(i) Hindu (ii) Muslim (iii) Buddhist (iv) Christian

[8] Education:

(i) Illiterate (ii) Upto Secondary level
(iii) Above secondary level

[9] Occupation of women:

(1) House-wife (2) Skilled worker (3) Unskilled worker

[10] Husband's occupation:

(1) Farmer (2) Skilled worker (3) Unskilled worker

[11]. POG: -----wks

[12] Type of house:

(i) Katcha (ii) Pukka [cemented]

[13] Toilet in house:

- (i) Yes (ii) No

[A] If No, habit of bare foot

- (i) Yes (ii) No

[14] Type of water source:

- (i) Tap water (ii) Hand-pump
(iii) Well (iv) others [mineral water]

[15] Use of drinking water:

- (i) Direct water (ii) Boiled (iii) Filtered (iv) Both 2 & 3

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

- (i)Yes (ii) No

[17] Any kind of live stock or domesticated animals at home:

- (i) No (ii) cattle [goat,cow,buffalow]
(iii) poultry [hen,pig] (iv)Dog (v) Cat

[18] Food habit:

- (i) Vegetarian (ii) Non-vegetarian

[19] If non-vegetarian, which one taken frequently:

- (i) Mutton, chicken, fish (ii) Buff (iii) others [pig,]

[20] Preparation of meat dishes:

- (i) Raw (ii) cooked (iii) Fried

[21] Knowledge regarding transmission of worm:

- (i) Yes (ii) No

[22] Knowledge regarding effect of helminthic infestation on body:

- (i) Yes (ii) No

[23] Knowledge regarding prevention of infestation:

- (i) Yes (ii) No

[24] Have you taken antihelminthics?

(i) No (ii) If yes, when

(a) Within 6 month (b) Beyond 6 month

[25] If yes, how you were treated:

(i) Self medication (ii) Hospital

(iii) Ayurvedic medicine (iv) No responses

[26] How do you wash your hands after toilet?

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

[27] Washing behaviour [after fieldwork, before eating]:

(i) Water with soap (ii) with others

[28] Alcohol user during pregnancy:

(i) Yes (ii) No

[29] Smoking during pregnancy:

(i) Yes (ii) No

[30] Family size:

(i) ≤ 4 members (iii) 5 - 8 members (4) ≥ 9 members

[31] Result:

(i) Hb % -

(ii) Stool test –

(iii) Parasites -

[32] Type of parasite: