1. INTRODUCTION

1.1 Background

Intestinal parasitic infection (IPI's) caused by pathogenic helminth and protozoan species are endemic throughout the world. They affect an estimated 3.5 billion person and causes clinical morbidity in approximately 450 million (WHO 2000). Economic burden caused by common helminth parasites (hookworm, roundworm, whipworm) infection was high (Sackey 2001). IPI have a worldwide in distribution but are most prevalent in under developed countries, while the prevalence varies with different communities of developing and developed countries. Intestinal helminth parasite infection was more prevalent in developing countries (Roberts et al. 2011).

The distribution of intestinal parasitic infection depends on many factors such as low socio-economic status (Nwizu et al. 2011, Mekonnen et al. 2014), poor sanitation and personal hygiene (Alene and Dohe 2014), lack of potable water (Wordemann et al. 2006, Amuta et al. 2011), etc which stimulate the parasite infection. There are so many factors that cause anaemia during pregnancy among them parasitic infection is major in developing countries (Roberts et al. 2011).

Anaemia is regarded as a major risk factor for an unfavorable outcome of pregnancy. It has been associated with low birth weight and Intra Uterine Growth Retardation (IUGR), (Acharya et al. 2004). Prevalence of anaemia is a major health problem worldwide and is high particularly among girls and pregnant women (Adak and Nazri 2006).

Earlier studies have shown that intestinal parasitic infection was significantly associated with anaemia in pregnant women, such as from Nepal (Dreyfuss et al. 2000, Chaudhary and Maharjan 2014, Shah and Baig 2005), Africa (Getachew et al. 2012, Rodriguez-Morales et al. 2006) etc. Some authors showed that anaemia was associated with helmith parasitic infection (Tefera 2014, Getachew et al. 2012) while other showed not only the helminth but also protozoan parasites also contribute anaemia in pregnant women (Kefiyalew et al. 2014).

1.2 Anaemia during Pregnancy

Anemia in pregnancy is a major public health problem, especially in developing countries. It is defined as a reduction in the oxygen carrying capacity of the blood which may be due to a reduced number of red blood cells, a low concentration of haemoglobin or a combination of both (Norton 2003). Anaemia is one of the most prevalent nutritional deficiency problems affecting pregnant women as haemoglobin level dropped less than 11gm/ dl. Haemoglobin level of 9.0-10.9gm/dl is considered as mild anaemia, 7.0-8.9gm/dl is moderate anaemia and less than 7gm/dl is considered as severe anaemia. It affects 41.58% of pregnant women globally, with the high prevalence (68.87%) in Nepalese women, (Shah and Gupta 2002) as well as in African women (58.9%) pregnant women (Obiakor et al. 2014).

Socio-demographic association showed that anaemia may caused due to lack of education (Singh et al. 2009, Nwizu et al. 2011), low socio economic status (Nwizu et al. 2011), lack of fruit consumption (Rakic et al. 2013), geophagy (Ansari et al. 2008) as well as excess bleeding during menstrual period (Lealem et al. 2015). During pregnancy women might suffer with anaemia during first trimester (Bardisi 2015), second trimester (Alene and Dohe, 2014) as well as third trimester (Lealem et al. 2015). Anaemia is caused due to various reasons, nutritional deficiency (Marahatta 2009) as well as parasitic infection such as hookworm infection (Dreyfuss et al. 2000), helmiths infection (Hoque et al. 2015), *T. trichura* infection (Boye et al. 2014), *A. lumbricoides* infection (Rodriguez-Morales et al. 2006) during the pregnancy.

Anaemia during pregnancy causes low birth weight (Acharya et al. 2004), premature labour and results adverse effect on fetal growth and preterm delivery (Allen 2001). Anaemia could cause retardation of fetal development (Acharya et al. 2004). Iron deficiency anaemia is a risk factor for preterm delivery, low birth weight, inferior neonatal health (WHO 1991).

Maternal anaemia in pregnancy commonly considered a risk factor for poor pregnancy outcome and can result in complication that threaten the life of both mother and foetus as well as anaemia considered as one of the major risk factor contributing Intrauterine Growth Retardation (Acharya et al. 2004).

1.3 Intestinal Parasitic Infection during Pregnancy

Intestinal parasitic infection was prevalent in developing countries (Robert et al. 2011) is considered as major public health problem. Protozoan parasites that cause adverse effect among pregnant women are *E. histolytica*, *Giardia* sp, *C. cayetanensis*, *C. parvum* etc. where helminth parasites such as *A. lumbricoides*, Hookworn and *T. trichiura* are most prevalent among pregnant women. Soil transmitted helminth infection are more prone in developing countries (Robert et al. 2011).

It has been found that most prevalent parasite among pregnant women was hookworm in case of Nepal (Dreyfuss et al. 2000, Navitsky et al. 1998) where some current data showed not only hookworm but also *A. lumbricoides* was prevalent among the pregnant women (Shah and Baig 2005, Chaudhary and Maharjan, 2014). Beside *A. lumbricoides* and Hookworm, *T. trichiura* and *S. stercoralis* were prevalent among pregnant women (Chaudhary and Maharjan, 2014) were not reported by earlier author (Dreyfuss et al. 2000, Navitsky et al. 1998, Marahatta 2009).

Helminth parasites in comparison with protozoan parasites were more common and cause adverse effect during pregnancy but sometimes only protozoan parasites were recorded from pregnant women in Rabat (Guelzim et al. 2014). Helminth parasites reported from different countries such as Thailand (Herter et al. 2007), Kenya (Robert et al. 2011, McClure et al. 2014, Wekesa et al. 2014), Ethiopia (Getachew et al. 2013, Getachew et al. 2012, Kefiyalaw et al. 2014). Similarly, *E. vermicularis* recorded from most of African countries such as Nigeria (Omordian et al. 2012), Ethiopia (Kefiyalew et al. 2014). Getachew et al. 2013) Kenya (Wekesa et al. 2014), Ghana (Ayeh-Kumi et al. 2009).

S. stercoralis is recorded from Nepalese pregnant women (Chaudhary and Maharjan 2014) as well as from Thailand (Patney et al. 2007) and African women found infected with this parasite (Boye et al. 2014, Alli et al. 2011). Cestodes parasites were collected from Nepalese pregnant women (Chaudhary and Maharjan 2014), also from other countries (Ayeh Kumi et al. 2003).

1.4 Parasitic Infection, Anaemia and Their Risk Factor

The relationship between parasitic infection and anaemia in pregnancy had established and parasites interfere with haemoglobin of host body (Getachew et al. 2012). Intestinal

parasites persistent in developing countries where parasites get favorable environmental condition to adhere being infective among people as well as in soil. Low socio-economic status (Mekonnen et al. 2014), poor sanitation (Chaudhary and Maharjan et al. 2014), unhygenic drinking water (Van Eijk et al. 2009, Chaudhary and Maharjan 2014), unavailability of deworming tablet (Gebre and Mulugeta 2015), walking barefoot (Larocque et al. 2005), all these were in favor with parasite transmission among pregnant women of developing countries. Some author revealed farming communities were more prone to parasite infection (Tandulkar et al. 2013).

Prevalence of intestinal parasitic infection is more common in developing country than developed countries where anaemia is an important health problem among the pregnant women (Marahatta 2009) and associated with low socio economic status (Ononge et al. 2014), lack of nutrious food (Bondevik et al. 2000).

Sanitation and personal hygiene is a leading factor of intestinal parasitic infection (Chaudhary and Maharjan 2014), in contrast Shah and Baig (2005) revealed most of the important leading factor was the knowledge of pregnant women regarding parasitic infection as well as the education of women (Amuta et al. 2008) where high parity and short pregnancies difference significantly associated with IPI (Nwizu et al. 2011). Some author revealed domestic animals around the habitation or home sharing with domestic animal was a leading factor of parasitic infection (Sackey 2001). Some factor like open defecation practice (Chaudhary and Maharjan 2014) as well as rural residence (Kefiyalew et al. 2014, Chaudhary and Maharjan 2014) associated with parasitic infection among pregnant women. Consumption of left over fruit was a leading factor of helminth infection (Mekonnen et al. 2014). Beside parasitic infection some factor related with anaemia were nutritional deficiency and lack of iron supplementation (Gebre and Mulugeta 2015).

1.5 Objectives

1.5.1 General Objective

To find out the association between intestinal parasitic infection and anaemia with the risk factors in pregnant women visiting Gyanaecology department of TUTH.

1.5.2 Specific Objectives

1. To determine general prevalence of the intestinal parasites in pregnant women.
2. To determine the association between anaemia and intestinal parasitic infection in pregnant women.
3. To find out the risk factors of parasitic infection and anaemia during pregnancy.

2. LITERATURE REVIEW

Anaemia is defined as a reduction in oxygen-carrying capacity of the blood which may be due to a reduced number of red blood cells, a low concentration of haemoglobin or a combination of both (Norton 2003). Hemoglobin is the oxygen-carrying component of red-blood cells is measured in gram per deciliter (gm/dl), are manufactured in bone marrow. Anaemia is the commonest nutritional deficiency worldwide with its highest prevalence among young children and pregnant women (Obiakor et al. 2014). It is especially more common in developing countries due to poor nutrition and high prevalence of parasitic infection (Dreyfuss et al 2000). Anaemia is a major contributor to maternal death in developing countries (Obiakor et al. 2014) and carries a lot of threat to the mothers as well as baby. Iron deficiency is thought to be most common cause of anaemia globally, although other condition such as foliate, vitamin B12 and vitamin A deficiency (WHO 2011), as well as chronic inflammation, parasitic infection, malignancy and inherited disorders all can cause anaemia (Marahatta 2009). There are several study results which emphasized that there is direct or indirect correlation between intestinal parasites and anaemia. Association of anaemia with intestinal parasite has been established, hence by eliminating intestinal parasite anaemia may be reduced (Obiakor et al. 2014).

The major intestinal parasite of global public health concern are the protozoan species *E. histolytica* and *G. intestinalis* and soil transmitted helminthes *A. lumblicoides, T. trichiura* and hookworm (WHO 1999, 2000). Soil-transmitted helminths (STH) infection occurs most frequently in under-developed countries. Millions of persons suffer from disease caused by them, with women bearing a particularly heavy pathological burden (WHO, 1999). The present review focused on the parasitic association with anaemia and their associated factor in different continents.

2.1 Scenario of Intestinal Parasitic Infection and Anaemia

More than 3.5 billion people are infected with intestinal parasites worldwide (WHO 2000). The large numbers of pregnant women in developing countries were infected with intestinal parasites (Roberts et al. 2011). Intestinal parasitic infection especially helminth

have been associated with anaemia in pregnant women in developing countries (Steketee et al. 2003).

Soil-transmitted helminths, *A. lumbricoides, T. trichiura* and hookworm has been shown to be an important predictor of anaemia among pregnant women of developing countries (Dreyfuss et al. 2000, Rodriguez-Morales et al. 2005, Chaudhary and Maharjan 2014). Not only in pregnant women, different author showed that *A. lumbricoides* associated with decreased hemoglobin in children (Stoltztus et al. 1997). Similarly *T. trichiura* infection also been reported as a causative factor of anaemia in pregnant women (Boye et al. 2014).

Most of the researches on the basis of intestinal parasitic infection were carried out in developing countries. Due to poor and low socio-economic status, people of developing country are in risk with intestinal parasite infection as well as IPI prevalence rate was high among them (Robert et al. 2011).

Globally STH showed 1,472 million people have roundworm infection, 1,298 million have hookworm infection and 1,049 million have whipworm infection (Crompton 1999). Most of the studies showed *A. lumbricoides* as the most prevalent intestinal parasite (Mekonnen et al. 2014, Bong-jin et al. 2003, Adegnika et al. (2010), Van Eijk et al. 2009, Rodriguez-Morales et al. 2006, Degareage et al. 2012, Rai et al. 1997)

Brooker (2008) done the investigation of soil transmitted helminth in southeast Asia and showed that land surface temperature influence the distribution of *A. lumbricoides* and *T. trichiura,* climate and topography influence the distribution of helminth parasite (Brooker 2007) where Appleton and Gouws (1996) revealed soil type, rainfall and altitude also influence the distribution of helminth parasite.

Intestinal parasitic infection is the common health problem causing large number of morbidity and mortality throughout the world particularly in Asian continent. Adolescent girls in Bangladesh has been shown to be infected with common intestinal parasites (*A. lumbricoides, T. trichiura,* Hookworm) with 33.5% prevalence rate (Banu et al. 2014) and some other author such as Sehgel et al. (2010), Kaliappan et al. (2013), Heter et al. (2007) and Bong-Jin et al. (2003) reported common intestinal parasites from population of North India, Tamil Nadu, Thailand and Philippines.

Most of the research on the basis of intestinal parasitic infection in relation with anaemia among pregnant women was carried out from African continent and intestinal parasites has been reported from different states of Africa by different researcher such as Lealem et al. (2015), Ketiyalew et al. (2014) where Wondu and Bijlsma (2012) showed that prevalence of parasitic infection among pregnant women was 13.7%, out of which 35.5% for *A. lumbricoides*, 28.65% for *T. trichiura*, 22.6% for *E. histolytica, among them 30.4% were anaemic*. Similarly Omordion et al. (2012) showed rate of intestinal parasitic infection among students and pregnant women was 12.5% and 24.4% with heavy and higher infection of *A. lumbricoides* but low co-infection occurred. Obiezue et al. (2013) showed prevalence rate of anaemia 16.3% among Nigerian pregnant women were highly infected with intestinal parasites (58.9%) had decreased haemoglobin level.

Few literatures were available regarding intestinal parasitic infection among pregnant women and other people in developed countries like Europe and Australia. Very decreased rate of intestinal parasitic infection (11.1%) has been obtained among Italian people and children (15%), (Masucci et al. 2008). In American countries some author carried out study in children and adult showed decreased rate of intestinal parasitic infection (Wordemann et al. 2006, Anderson et al. 1998, Kaminsky et al. 2014).

In national context, it has been reported that soil transmitted helminth were endemic in Nepal (William-Blangero et al. 1993) where Rai et al. (2000) revealed prevalence rate of STH in soil of Kathmandu valley found to be 37.0%. Investigation carried out in STH revealed most people in Kathmandu valley infected with STH parasites (51.4%) than people in Chitwan (17%) and Illam (11.7%), (Rai et al. 2000). IPI has been reported in Nepal by different author (Shakya et al. 2012, Yong et al. 2000, 2013, Rijal et al. 2001, Pradhan et al. 2013, Tandulkar et al. 2013, Maharjan 2013), among the parasites identified *A. lumbricoides* was dominant in soil of Kathmandu valley and *Trichostrongylus* was dominant in soil outside the valley (Rai et al. 2000).

A. lumbricoides infection as most dominant parasite infection (Parajuli et al. 2004, Shrestha and Maharjan 2013, Estevez et al. 1983, Rai and Gurung 1986, Rai et al.1995, 1999, 2000) where other author showed Hookworm as the major parasites (Kunwar et al.

2006, Dreyfuss et al. 2000, Yong et al. 2000, Marahatta 2009, Shah and Baig 2005, Alem et al. 2013, Navilsky et al. 1998).

Significant association between STH parasites (*A. lumbricoides*, *T. trichiura*, Hookworm) and decreased haemoglobin had been shown by Getachew et al. (2012) where *A. lumbricoides* found as a strong predictor of anaemia (Rodriguez-Morales et al. 2006, Kefiyalew et al. 2014, Tefera et al. 2014). Some author revealed *T. trichiura* was significantly associatioed with anaemia (Boye et al. 2014). Similarly *A. lumbricoides* and Hookworm was found associated with decreased hemoglobin level and were more prevalent among the anaemic pregnant women (Tefera 2014, Shah and Baig 2005, Chaudhary and Maharjan 2014). Similarly, another author (Obiakar et al. 2014) showed IPI rate 58.9% and anaemic rate 46.5% revealed IPI significantly associated with decreased haemoglobin level in pregnant women in Nigeria. Similarly Ghimire and Mishra (2005) found association between IPI and hemoglobin level in Nepal.

Intestinal parasitic infection alone constitute one of the major public health problem in Nepal (Rai et al. 2002), over 60% of Nepalese were infected with intestinal parasites (Estevez et al. 1983, Rai and Gurung 1986, Rai et al.2000a, 2001b) and in some rural region 90% people were infected with intestinal parasites (Estevez et al. 1983, Rai et al. 2000a).

Ayenew et al. (2014) reported less prevalence of anaemia among Ethiopian pregnant women (9.7%), while Ansari et al. (2008) showed very increased prevalence of anaemia 90.5%, among Pakistani pregnant women. Melku et al. (2014) showed prevalence of anaemia in pregnant women of Gondar was 16.6%. An investigation done among Ethiopian pregnant women showed prevalence rate of anaemia 36.1% (Gebre and Mulugeta 2015).

In national context, Sinha et al. (2012) reported that 47.25% were anaemic were mostly in second trimester. Adak and Nazri (2006) showed prevalence rate of anaemia 54% among girls and pregnant woman in Eastern Nepal where Shah and Gupta (2002) revealed 68.8% anaemic rate was higher than 65.5% investigated by Baral and Onta (2009). Similarly Bondevik et al. (2000) showed prevalence rate of anaemia among pregnant women in Kathmandu valley was 62.2% and Marahatta (2006) showed 42.6% anaemia prevalence among patients of TUTH.

Dreyfuss et al. (2000) revealed iron deficiency in pregnant women was the risk factor of anaemia (Alene and Dohe 2014) where Jufar and Zewde (2014) showed prevalence rate of anaemia 21.3% among Ethiopian pregnant women. Erhabor showed 58.3% anaemic rate among pregnant women attending antenatal care in sokoto where Neizu et al. (2011) showed prevalence rate of anaemia only 17% among Nigerian pregnant women.

A. lumbricoides causes intestinal obstruction, liver abscess, local irritation and damage with malabsorption with the infection and protein energy malnutrition (Rodriguez-Morales et al. 2006). Overall prevalence of IPI rate showed 32.8% in Uganda (Mehraj et al. 2008) where IPI rate among children in Karachi was 52.8%, *Giardia*, *Ascaris*, *H. nana* were common parasites identified. Debalke et al. (2013) showed STH infection rate in private and government school 20.9% and 53.5%.

Rai et al. (2002) showed malnutrition in rural area causes child mortality and chronic malnutrition in women. Shrestha (2001) showed IPI rate 81.94% among the children of Lalitpur with highest prevalence of *A. lumbricoides* (75.45%). Similarly Shrestha et al. (2007) reported 42.5% school children had IPI where soil contamination rate was 28.5%.

2.2 Risk Factor of Intestinal Parasitic Infection and Anaemia

There are so many risk factors which stimulate intestinal parasitic infection. Among these factors poverty is considered as main risk factor of intestinal parasitic infection. Poor socio-economic conditions are linked with higher prevalence of soil transmitted helminth. STH helminthes are wide spread in distribution in under-developed countries (Roberts et al. 2011). Other risk factor contributing to IPI includes hand washing practices (Chaudhary and Maharjan 2014), lack of knowledge about IPI (Shah and Baig 2005), type of drinking water source (Chaudhary and Maharjan 2014) and open defecation (Chaudhary and Maharjan 2014).

Population most in risk with IPI are pregnant women and children and adult in certain high risk occupation such a miners (WHO 1999). Some research showed women living in rural area were in risk with IPI than women living in urban (Larocque et al. 2005, Gebre and mulageta 2015). Some studies showed poor nutritional status associated with IPI in pregnant women (Banu et al. 2014).

Some author showed intestinal parasitic infection acquired due to poor personal hygiene and sanitation like hand washing behavior after defecation (Kaliappan et al. 2013, Yong et al. 2000, Parajuli et al. 2009, Tandulkar et al. 2013, Mbule et al. 2013) and Alene and Dohe (2014) showed intestinal parasitic infection associated with absence of toilet.

Untreated water or unhygienic water consumption might cause or stimulate intestinal parasite infection (Tandulkar et al. 2013, Shakya et al. 2012, van Eijk et al. 2009, Alene and Done 2014).

Low education status or illiteracy is also considered as leading factor of IPI. Some author showed significant association between IPI and education (Shakya et al. 2012, Banu et al. 2014, Gebre and mulugeta 2015) as well as short interval between pregnancies (Nwizu et al. 2011) found associated with intestinal parasitic infection

Some researcher showed low socio-economic status associated with intestinal parasitic infection (Mekonnen et al. 2014, Ekundyo et al. 2007, Nwizu et al. 2011). Tandulkar et al. (2013) revealed intestinal parasitic infection associated with farming profession. While Larocque et al. (2005) investigated walking bare foot was significantly associated with intestinal parasitic infection. Deworming tablet decreased the intestinal parasitic infection (Gebre and mulugela 2015). Consumption of raw vegetable and fruit associated with intestinal parasitic infection. Singh et al. (2009) showed anaemia in pregnant women was associated with education level (Mbule et al. 2013)

Anaemia was found associated with geophagy, tea consumption, low red meat consumption (Ansari et al. 2008) as well as age of pregnant women and being in 1^{st} trimester (Bardisi 2015) or being in 2^{nd} or 3^{rd} trimester (Alene and Dohe 2014).

Some author revealed lack of fruit and vegetable consumption was associated with anaemia and excess bleeding during menstrual period contribute anaemia (Lealem et al. 2015). Some author showed nutrition as a predictor of anaemia in pregnant women (Mbule et al. 2013).

3. MATERIALS AND METHODS

3.1 Study Area

Nepal is a small landlocked country in South East Asia bounded by China in North and India in South East and West. It lies between 28° 22' west to 30° 27' North latitude and 80° 4' East and 88° 12' East longitude.

Tribhuvan University Teaching Hospital lies in Maharajgunj of the Kathmandu valley. It was established in 1972 AD. The 300 bedded TUTH was completed in 1984 AD with the generous support of Japan International Cooperation Agency (JICA). Approximately 100 to 150 pregnant women attend the antenatal for regular follow up. Doctor provides health class focusing the HIV infected pregnant women but compulsory for all pregnant women coming for their antenatal care for the first time. Hospital recommend for deworming tablet and iron tablet to prevent intestinal parasitic infection and anaemia during pregnancy.

3.2 Materials

3.2.1 Equipments

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

3.2.2 Chemicals

i. 2.5% potassium dichromate
ii. Normal saline

iii. Iodine solution

iv. Soap

3.2.3 Preparation of 2.5% Potassium Dichromate

2.5 gm of potassium dichromate weighted accurately and dissolved in 100 ml of distilled water and dissolved well. This potassium dichromate solution poured in freshly collected stool sample as much as to covered the stool sample for good preservation of parasite present in the stool.

3.2.4 Preparation of Normal Saline

8.5 gm of sodium chloride dissolved in 1000 ml of distilled water and it was useful for observing the characteristic movement of the parasites present in fresh stool sample.

3.2.5 Preparation of Iodine Solution

The iodine solution used in the present study was prepared by dissolving 10 gm of potassium iodine in 100 ml distilled water and 5 gm iodine crystals (powered) are slowly added in it then filtered the solution and kept in stopper bottle. This iodine solution used for staining the intestinal parasites present in stool sample.

3.3 Study Design

The study was designed to assess the association between anaemia and intestinal parasitic infection during pregnancy. It includes determination of parasitic infection by using stool examination as well as haematological data collection for estimation of haemoglobin level.

3.3.1 Sample Size

A total of 200 pregnant women attending antenatal clinic of Tribhuvan University, Teaching Hospital during February to April (2015) were included in this study.

3.3.2 Inclusion and Exclusion Criteria

a. Inclusion Criteria

All the pregnant women coming for their antenatal care were included in the study.

b. Exclusion Criteria

Pregnant women who did not ready for stool collection were excluded in this study.

3.4 Stool Sample Collection and Examination

3.4.1 Sample Collection and Preservation

Before initiation of the study in Teaching Hospital Gynecology department, meeting with health worker and doctors was conducted which made feasible for the stool collection of pregnant women. Orientation about the proper methods of collection of stool was provided to ensure the good condition of stool sample. Stool sample 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 Teaching Hospital (photo no.1). Stool sample were collected in the same day or next day in early morning to ensure maximum load of possible parasites.

After stool collection immediately 2.5% potassium dichromate solution was poured in vials as much as to cover the stool sample Which help in maintaining the shape and size of protozoan and helminth parasites and preventing further development. The stool samples were marked or coded for identification (photo no.2). These preserved samples were transported to a parasitological laboratory of the Central Department of Zoology, Tribhuvan University.

3.4.2 Laboratory Examination

Microscopic examination of stool sample was carried out in parasitological laboratory of Central Department of Zoology.

3.4.2.1 Macroscopic Examination

Before slide preparation of stool sample, the physical characteristics of stool sample were examined.

3.4.2.2 Microscopic Examination

Preserved stool sample were collected in working laboratory with safety precautions. Both unstained and stained smear was used in the study.

a. Unstained Smear Preparation of Stool

A portion of stool sample was taken with the help of bamboo stick and emulsified with freshly prepared normal saline on a clean glass slide. A clear cover slip was placed over it and noticed the parasite present in the stool sample with the help of microscope.

b. Stained Smear Preparation of Stool

As similarly unstained smear preparation, stained smear was prepared but Iodine stained preparation was used in this purpose where Lugol's iodine solution was kept instead of normal saline solution (photo no.3). The stained smear of stool sample was observed under the microscope in 10 X 10 and 10 X 40 magnifications (photo no.4). Observation was made starting from one end of the slide to another so that whole field was examined. Identification of the intestinal parasites i.e. egg of helminth and cyst of protozoan was done with the help of supervisor.

3.5 Data Collection of Hemoglobin Level

Hemoglobin report of respective pregnant woman was recorded from Haematology Laboratory of Gynecology department in TUTH.

3.6 Questionnaire Survey

Structured questionnaire was prepared before administered among the pregnant women. A total of 200 pregnant women attending antenatal clinic in TUTH were included in questionnaire survey. The questionnaire was focused on probable factor that could relate with intestinal parasitic infection such as demographic characteristics, practices and knowledge regarding intestinal parasites among the pregnant women (photo no.5). Questionnaire was translated into Nepali language who could not understand the English language.

Photos of Hospital and Laboratory Activities.

Photo 1 : Sample collection in TUTH

Photo 4 : Observation of stool sample under microscope

Photo 2 : Marking the stool samples

Photo 5 : Questionnaire survey with pregnant women in TUTH

Photo 3: Slide preparation of stool sample

4. RESULTS

The study was conducted among 200 pregnant women attending antenatal clinic at Tribhuwan University Teaching Hospital, Kathmandu from February to April 2015. Early morning stool samples were collected in sterile vials and preserved at 2.5% potassium dichromate solution. Preserved samples were brought to the parasitology laboratory of Central Department of Zoology for microscopic examination in order to find out the parasitic infection. Along with the stool sample questionnaire survey was conducted using structured questionnaire for the assessment of KAP related risk factors.

4.1 General Prevalence of the Intestinal Parasites in Pregnant Women

A total of 200 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 35% (n =70) among the pregnant women.

Table 1: Prevalence of specific intestinal parasites in pregnant women

Parasties	**Frequency (N =200)**
Protozoan Parasite	
E. histolytica	5 (2.5%)
Nematodes	
A. lumbricoides	61 (30.5%)
T. trichiura	2 (1%)
S. stercoralis	2 (1%)
Cestode	
H. nana	6 (3%)

Pregnant women were found to be infected with five species of helminth parasites. Among them *A. lumbricoides* was the most prevalent (30.5%) followed by *H. nana* (3%), *E. histolytica* (2.5%), *T. trichiura* (1%) and *S. stercoralis* (1%), (Table 1). But none of them were infected with hookworm species.

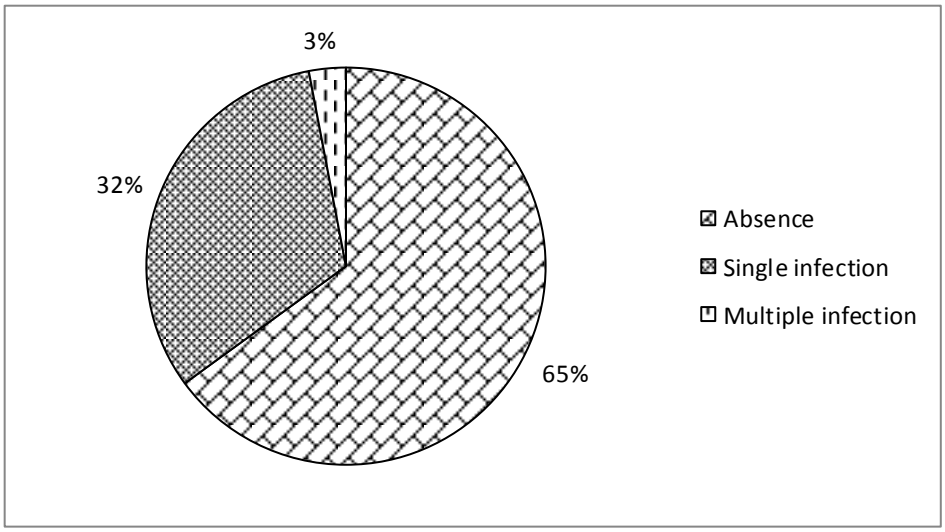

Fig 1 : Degree of parasite infection in pregnant women

Pregnant women were found to be infected with either single parasites or multiple parasites. Degree of infection indicated that, out of total positive cases maximum 32% pregnant women were infected with single parasitic infection either by protozoan parasite or by helminth parasite. While only 3% of them found infected with multiple infection (Fig 1).

Table 2 : Intensity of parasitic infection in pregnant women

S.N.	**Parasitic intensity**	**frequency (N = 200)**
1.	Light	17 (8.5%)
2.	Moderate	34 (17.0%)
3.	Heavy	19 (9.5%)

Note:

Light infection - 0-2 eggs/cysts/ larva per field

Moderate infection- 3-5 eggs/cysts/ larva per field

Heavy infection - > 6 eggs/cysts/ larva per field

Overall infection is categories as light, moderate and heavy infection. Parasitic intensity were calculated on the basis of number of eggs, cysts and larvae present in the stool sample as per microscopic field. Among the infected population maximum pregnant

women were infected with moderate infection 17% (n=34) compared to the light and heavy infection (Table 2).

All six pregnant women with multiple infection showed heavy infection. Among single infection one pregnant woman with *E. histolytica*, three pregnant women with *H. nana* and nine pregnant women with *A. lumbricoides* were heavily infected. Among the 19 heavily infected pregnant women two were non-anaemic and remaining were anaemic.

4.2 Association between Anaemia and Intestinal Parasitic Infection in Pregnant Women

Haematological data of pregnant women were collected from hospital record. On the basis of the haematological data, 100 each pregnant women with anaemic and non anaemic were enrolled in the study. Among anaemic cases 58% (n=58) had parasitic infection (Fig 2). Similarly out of 100 (50%) non anaemic cases, only 12% (n=12) were infected with intestinal parasites. The association of anemia with intestinal parasitic infection was statistically significant (χ^2=75.8, d.f=2, p = 0.000)

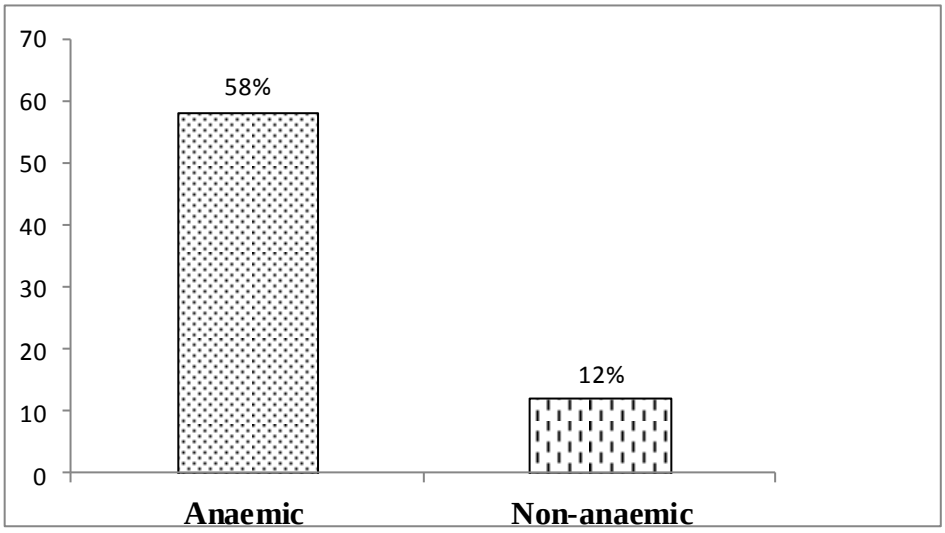

Fig 2 : Prevalence of Parasitic infection in anemic and non- anemic pregnant women

Table 3 : Median hemoglobin level of pregnant women with and without parasite infection

Parasites	Number	Median Hb (gm/dl)	P-value
Absence	130	11.66	0.000
Single infection	64	10.35	
Multiple infection	6	10.28	

The median hemoglobin level of pregnant women without any parasites was 11.66 gm/dl whereas the pregnant women having single infection was 10.35 gm/dl and those having multiple infection was 10.28 gm/dl. Pregnant women with parasitic infection either single or multiple infection falls under anaemic category with haemoglobin level below 11gm/dl. Post Hoc analysis indicated the significant association between parasitic infection and anaemia. ($\chi 2=75.8$, d.f=2, p=0.000)

Table 4 : Association between anaemia and parasitic group in pregnant women

Parasites	Anaemia (N = 100)	Non anaemia (N = 100)	Relative risk (RR)	Odd ratio (OR)	χ^2-value	P-value
Intestinal parasites	58	12	10.10	3.94*	46.505	0.000
Protozoan	3	2	1.515	1.206	0.205	0.50
Helminths	55	10	11.00	5.5*	46.15	0.000
Nematodes	49	10	8.647	2.29*	39.65	0.000

Note: OR = 1, exposure not effect odd of outcome

OR > 1, exposure associated with high odd of outcome

OR <1, exposure associated with low odd of outcome

The risk of anemia in parasitic infected population found to be higher Odd Ratio of outcome contributing 3.94 fold increased OR of maternal anemia whereas the relative risk found to be 10.10. Similarly risk of anemia in pregnant women infected with helminthes found to be 5.5 fold increased odd of maternal anemia whereas the relative risk found to be 11.00. Pregnant women infected with nematodes had 2.29 fold increased odd of anemia. Relative risk of anemia due to protozoan parasites measured 1.15 while this value for helminthes found to be 11.00 and in case of nematode it was found to be 8.647.

In general intestinal parasites particularly helminth parasites contribute three to five fold greater risk of causing anaemia in pregnant women.

Table 5: Association of specific intestinal parasite infection with anemia in pregnant women

Species	**Anaemia (N = 100)**	**Non-anaemia (N = 100)**	**χ^2-Value**	**p-value**
E. histolytica	3 (1.5%)	2(1%)	0.205	0.50
A. lumbricoides	52 (26%)	9 (4.5%)	39.65	0.000
T. trichiura	2 (1%)	-	2.02	0.156
S. stercoralis	2 (1%)	-	2.02	0.156
H. nana	5 (2.5)	1 (0.5%)	2.75	0.106

Protozoan parasitic infection due to *E. histolytica* in pregnant women showed almost similar infection (1-1.5%) which is statistically not significant. Although *H. nana* was high in anaemic pregnant women compared to non anaemic, the association was not significant. But with regard to *A. lumbricoides* infection, anaemic pregnant women showed remarkably high compared to non-anaemic with statistically significant association. But in case of *T. trichiura* and *S. stercoralis*, the association were not significant since the parasites was only recorded from anaemic pregnant women.

Along with the stool sample collection, questionnaire survey was conducted simultaneously using structured questionnaire for the assessment of KAP related risk factor to determine which factors were associated with risk for infection with intestinal parasites and anaemia in pregnant women.

4.3 Risk Factors of Parasitic Infection and Anaemia During Pregnancy

Factors which are associated with parasitic infection and anaemia in pregnant women were defined as risk factors. These factors were categorized as demographic risk factors, knowledge related risk factors and practices related risk factors.

Demographic risk factors were assessed on the basis of age of pregnant women and their current trimester, ethnic group and family size. Knowledge related risk factors were assessed on the basis of awareness of pregnant women and level of education. Similarly practices related variables included in this study were hand washing practice with soap

after defecation, house sharing with live stock, deworming, working in field, occupation, use of open source of water etc and their association with intestinal parasitic infection and anaemia was analyzed.

Table 6: Assessment of demographic risk factors of parasitic infection and anemia during pregnancy

Demographic characteristics	Prevalence of anaemia	χ^2-Value	P-value	Prevalence of intestinal parasites	χ^2-value	P-value
Age						
<20 (7)	3 (42.9%)			1 (14.3%)		
20-25 (121)	64 (52.9%)	1.056	0.78	44 (36.4%)	1.423	0.70
26-31 (52)	24 (46.2%)			18 (34.6%)		
>31 (20)	9 (45.0%)			7 (35.0%)		
Trimester						
1st (44)	30 (68.2%)			16 (36.4)		
2nd (62)	26. (41.9%)	7.814	0.020	20 (32.3)	0.29	0.862
3rd (94)	44 (46.8%)			34 (36.2%)		
Ethnic Group						
Brahmin (79)	35 (44.3%)			22 (27.8%)		
Chhetri (33)	21 (63.6%)	3.480	0.323	12 (36.4%)	3.62	0.30
vaishya (84)	42 (50.0%)			35 (41.7%)		
Sudra (4)	2 (50%)			1 (25.0%)		
Family size						
≤ 4 (132)	74 (56.1%)			48(36.4%)		
5-8 (63)	24 (38.1%)	5.711	0.58	20 (31.7%)	0.45	0.79
≥ 9 (5)	2(40.0%)			2(40.0%)		

Age of the study population has been divided into three groups. Assessment of age group showed that maximum pregnant women belonging to age group (20-25yrs) were highly anaemic compared to other age group. Anaemia was not found to be associated with age group where as parasitic infection also showed maximum in this age group with insignificant association with age group (Table 6).

Assessment of trimester to that of anaemia as well as intestinal parasitic infection revealed significant association with anaemia and insignificant association with parasitic

infection. Overall assessment showed that pregnant women at the initial phase of pregnancy i.e., first trimester were found to be more prone to anaemia compared to second and third trimester.

Pregnant women were divided into four ethnic groups according to their caste. Assessment of ethnicity showed that maximum pregnant women belonging to vaishya were highly infected with parasites. Parasitic infection was not found to be associated with ethnicity whereas anaemia showed maximum in chhetri ethnic group with insignificant association.

Whole study population has been divided into 3 different family strata i.e.≤ 4, 5-8 and ≥ 9 etc. Assessment of family size to that of anaemia as well as intestinal parasitic infection revealed insignificant association.

Table 7: Assessment of knowledge related risk factors of parasites infection and anemia during pregnancy

Knowledge Relate risk factor	**Prevalence of anaemia**	**χ^2-Value**	**P-value**	**Prevalence of intestinal parasites**	**χ^2-value**	**P-value**
Literacy						
illiterate (18)	10 (55.6%)	1.29	0.52	7(38.9%)	0.526	0.76
upto secondary level (104)	48 (46.2%)			38(36.5%)		
above secondary level (78)	42 (53.8%)			25 (32.1%)		
Awareness						
aware(104)	52 (50%)	0.000	0.55	33 (31.7%)	1.01	0.19
unaware (96)	48 (50%)			37 (38.5%)		

Literacy of pregnant women was categorized as illiterate, education upto secondary level and education above secondary level. Although pregnant women were educated, condition of anaemia and intestinal parasitic infection showed almost similar prevalence with statistically insignificant association.

Pregnant women were categorized as aware and unaware on the basis of knowledge regarding parasitic infection and found their equal involvement in this study. Similarly

the condition of anaemia and intestinal parasitic infection showed almost similar prevalence with statistically insignificant association.

Table 8: Practice related risk factor of parasitic infection and anaemia during pregnancy

Practice related risk factor	Prevalence of anemia	χ^2-Value	P-value	Prevalence of intestinal parasites	χ^2-value	P-value
Hand wash after toilet						
With soap water	96 (49.0%)	5.128	0.061	66 (33.7)	7.58	0.014
water only (196 (4))	4 (100%)			4(100%)		
House sharing with live stock						
yes (42)	22 (52.4%)	0.121	0.431	16 (88.1)	4.170	0.035
No (158)	78 (49.4%)			54 (34.2%)		
Intake of Anthelminths						
No	68 (57.6%)			48 (40.7%)		
Within 6 months	1 (20.0%)	4.88	0.02	1 (20.0%)	0.186	0.123
Beyond 6 months	31 (40.3%)			21 (27.3%)		
Working in field						
Yes (29)	20 (69.0%)	4.88	0.02	15 (51.7%)	4.17	0.035
No (171)	80 (46.8%)			55 (32.2%)		
Occupation						
House wife (107)	51 (47.7%)	0.502	0.285	71 (68.3%)	0.186	0.66
workers (93)	49 (52.7)			59 (61.5%)		
Use of water (Pond, well, river) for cooking, washing and bathing						
Yes (37)	18 (51.4%)	0.16	0.5	22 (59.5%)	11.93	0.001
No (163)	82 (49.7)			48 (29.4%)		

Hygiene and sanitation of pregnant women play important role on acquiring parasitic infection. Maximum pregnant women wash their hands with soap after defecation, assessment revealed that there was statistically significant association with intestinal parasitic infection indicating washing without soap after defecation showed important risk factor which association with anaemia revealed insignificant.

Although maximum pregnant women do not share their house with live stock showed almost similar prevalence of anaemia with statistically insignificant association but

prevalence rate of parasitic infection was found high in those who shared house with live stock with statistically significant association showing sharing house with live stock is a risk factor for parasitic infection.

Deworming tablet is important for pregnant women. Overall assessment showed that pregnant women who had taken deworming tablet within six months were least infected with parasites and had low prevalence of anaemia with statistically significant association.

Maximum pregnant women do not work in field and pregnant women who work in field were highly infected with parasites with high anaemic cases indicating statistically significant association. The result revealed that contaminated soil is a risk factor for parasitic infection.

Occupations of pregnant women were categorized as house wife and worker. Among them condition of anaemia and intestinal parasitic infection showed almost similar prevalence with statistically insignificant association.

The use of open source of water (pond, well, river) is a leading cause of parasitic infection. Those who use open source of water were highly infected then other who do not use open source of water. Assessment revealed that there was statistically significant association with intestinal parasitic infection showing use of open source of water is another risk factor.

Cyst and eggs of Intestinal Parasites in Pregnant Women

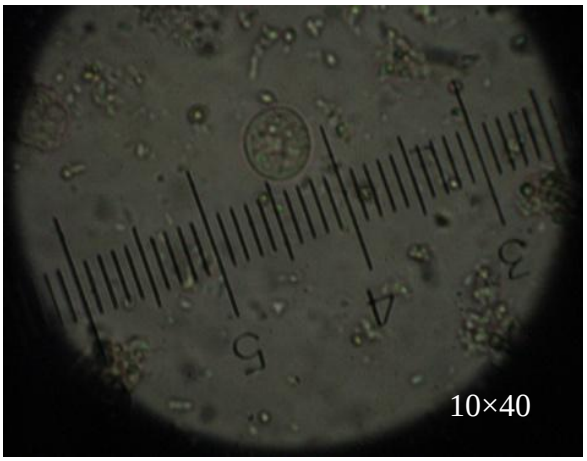

Photo 6 : Cyst of *E. histolytica*

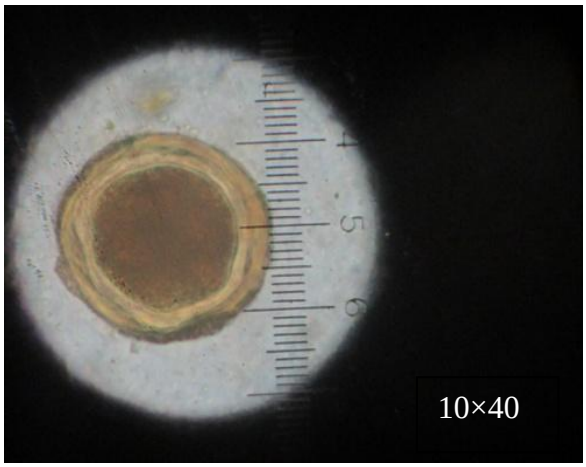

Photo 7 : Egg of *A. lumbricoides*

Photo 8 : Larva of *S. stercoralis*

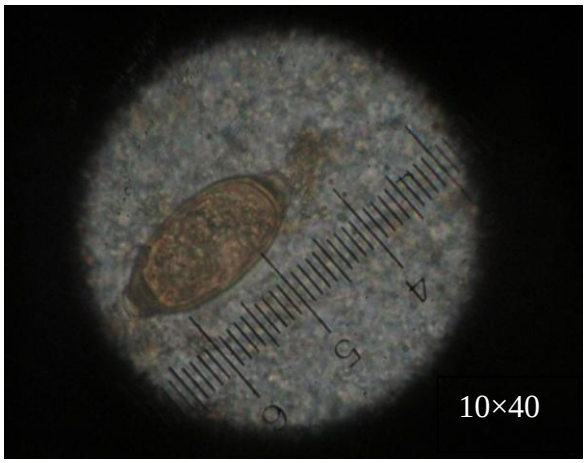

Photo 9 : Egg of *T. trichiura*

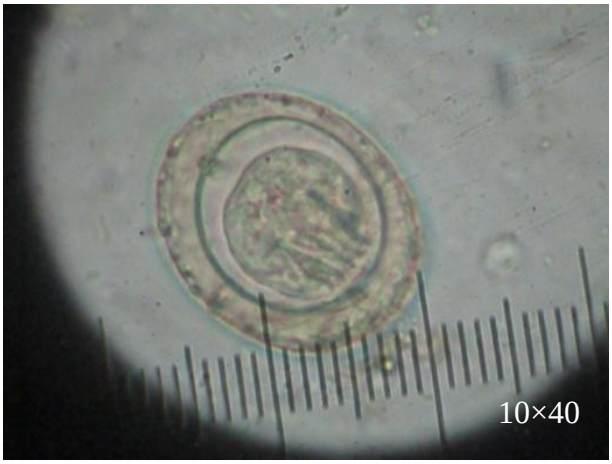

Photo 10 : Egg of *H. nana*

5. DISCUSSION

Intestinal parasitic infection is a common public health problem in most of the developing countries (Roberts et al. 2011). The main cause of intestinal parasite transmission may be contributed by socio-economic condition (education, income, occupation) and personal hygiene (habit of hand washing after defection, use of latrine, foot wear, nail clipping etc) so these infection are regarded as ‘’disease of poverty.’’ Millions of people suffer from disease caused by them, mostly women and children bearing a particularly heavy pathological burden (WHO 1999). IPI causes reduced growth (Stephenson et al. 2000), increased risk for protein energy malnutrition (Stephenson et al. 2000) as well as iron deficiency anaemia (Dreyfuss et al. 2000) and reduced cognitive development (Acharya et al. 2004). Many women in Nepal are at risk with intestinal parasite infection (Dreyfuss et al. 2000, Rai et al. 1994) as they spend a major part of their life either in pregnancy or as lactating mothers (Dreyfuss et al. 2000).

Present study was carried out among the pregnant women attending antenatal clinic at Tribhuvan University Teaching Hospital. Out of 200 pregnant women 70(35%) were infected with intestinal parasites. Among infected population, only 2.5% belonged to protozoan parasites and 32.5% infected with helminth parasites. In this study 5 different types of intestinal parasites were found which were *A. lumbricoides, H. nana, T. trichiura, E. histolytica* and *S. stercoralis.* The present study showed *A. lumbricoides* (30.5%) as the most prevalent intestinal helminth parasites followed by *H. nana* 6(3%).

Overall prevalence rate of parasitic infection among pregnant women at TUTH showed comparatively less than result revealed from Sarlahi district (Navitsky 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).

In global context some research showed comparatively higher prevalence of IPI than present study over African countries like Ethiopia (Lealem et al. 2015, Getachew et al. 2012, Tefera et al. 2014), Kenya (McClure et al. 2014), Nigeria (Obiakor et al. 2014, Omorodin et al. 2012, Obiezue et al. 2013), Morocco (Guelzim et al. 2014) etc. Some other study showed lower prevalence than present study such as research carried among

pregnant women of South America (Larocque et al. 2006), Africa (Boye et al. 2014, Baidoo et al. 2010) and Dhaka (Banu et al. 2014).

E. histolytica, G. lamblia, G. intestinalis, C. cayetanensis, C. parvum are the commonly distributed protozoan which parasites are prevalent in Nepal. Most of the diarrhea caused due to *E. histolytica* and *Giardia* species but occasionally due to *C. cayetanensis*. Among them only *E. histolytica* was reported in present study. Very decreased rate of *G. lamblia* and *E. histolytica* was obtained by Marahatta (2009) in Kathmandu, were also reported by Chaudhary and Maharjan (2014) in Biratnagar but none of the protozoan were reported by Dreyfuss et al. (2000), Shah and Baig (2005) and Navitsky et al. (1998) in pregnant women .

The common protozoan parasites were reported from different countries of the world. *E. histolytica* and *G. lamblia* were reported from Venezuela (Rodriguez-morales et al. 2006), Ghana (Ayeh-Kumi et al. 2015), Nigeria (Bassey 2009, Omorodion et al. 2012, Obiakor et al. 2014), Ethiopia (Alem et al. 2013, Jufar and Zewde 2014, Mulugeta et al. 2014) Morocco (Guelzim et al. 2014) and from India (Verweij et al. 2010).

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 , T. trichiura,* and *S. stercoralis* were identified which were also identified in pregnant women of Biratnagar including hookworm (Chaudhary and Maharjan 2014), were also reported by other researcher (Shah and Baig 2005, Navitsky et al. 1998, 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, Alli et al. 2011), Ethiopia (Kefiyalew et al. 2014, Jufar and Zewde 2014, Getachew et al. 2012, Gedefaw et al. 2015), Ghana (Fuseini et al. 2010) Kenya (Wekesa et al. 2014, McClure et al. 2014, Van Eijk et al. 2008), in 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).

E. vermicularis was commonly called pin worm parasites are generally not recorded in stool examination. Occasionally these parasites eggs are found during stool examination. This parasites infection has been reported from several African countries such as from Nigeria (Omorodian et al. 2012), Ethiopia (Kefiyalew et al. 2014, Getachew et al. 2013), Kenya (Wekesa et al. 2014), Ghana (Ayeh-Kumi et al. 2009) where *S. stercoralis* demonstrated in Thailand (Herter et al. 2007), Nigeria (Alli et al. (2011), Ghana (Boye et al. 2014). Hookworm was not recorded in Ghanian pregnant women (Boye et al. 2014). Both *S. stercoralis* and *E. vermicularis* was recorded by Ayeh-kumi et al. (2009) in Ghana.

Common cestode parasites infecting human being includes *Hymenolepis nana, Hymenolepis diminuta, Taenia solium, Taenia saginata* in national as well as in global context. Among them in present study only *H. nana* was recorded from the pregnant women attending antenatal in TUTH, was also reported by Chaudhary and Maharjan (2014) in Biratnagar but not reported by some other earlier researcher (Shah and Baig 2005, Navitsky et al. 1998, Dreyfuss et al. 2000, Marahatta 2009).

Both *Taenia* sp and *H. nana* were reported from African countries such as in Ethiopia (Alem et al. 2013, Getachew et al. 2013) and Ghana (Ayeh-Kumi et al. 2009). Some author demonstrated *H. nana* as a most common parasites in Indian pregnant women, (Sehgal et al. 2014).

Among 3 nematode parasites reported, *A. lumbricoides* was found to be highly prevalent (30.5%) as shown by Chaudhary and Maharjan (2014) also showed *A. lumbricoides* was dominant over Biratnagar. Similar result forwarded by Shah and Baig (2005) while some other earlier studied showed Hookworm as a predominant (Dreyfuss et al. 2000, Navitsky et al. 1998, Marahatta 2009). This higher rate of *A. lumbricoides* infection among pregnant women attending antenatal at TUTH might be due to high prevalence rate (36.5%) of soil contamination with egg of *A. lumbricoides* (Rai et al. 2000) and contamination of drinking water with intestinal parasites (Bajracharya and Maharjan 2015) as well as contamination of raw sewage with water and vegetables, poor sanitation and poor personal hygiene.

Roberts et al. (2011), demonstrated high prevalence rate of *A. lumbricoides* (56.2%) and Hookworm (78.8%) in case of Nepal while compared with Kenya, showed prevalent rate for *A. lumbricoides and* Hookworm in pattern 52.3% and 39.5%.

Globally most of the research showed *A. lumbricoides* as a prevalent intestinal parasites in different countries such as in Western Kenya (Van Eijk et al 2008), in Ethiopia (Mulugeta et al. 2014, Tefera et al. 2014, Getachew et al. 2012, Ketiyalew et al. 2014) in Ghana (Boye et al. 2014) in Nigeria (Omorodion et al. 2012) in Venezuela (Rodriguez-Morales et al. 2006) where Hookworm reported as most dominant parasites in Ghana (Fuseini et al. 2010) and in Nigeria (Obiezue et al. 2013). In contrast with this result, in Morocco only protozoan parasites were recorded where helminth parasites were absent (Guelzim et al. 2014).

In this study single infection occurred among 64(32%) pregnant women and co-infection recorded among 6(3%) pregnant women which is similar with result shown by Chaudhary and Maharjan (2014) with higher single infection compared to multiple infection.

In this study most of the pregnant women infected with moderate infection of intestinal parasites i.e. 34 (17%), compared to light and heavy infection. Pregnant women who had infected with much number of parasites (heavy infection) had more parasitic burden than light and moderately infected women.

Anaemia is a public health problem of both developed and developing countries. It has been identified that about 50% people in the world had anaemia (WHO 2002). Studies from South Asian countries (Bondivek et al. 2000, Dreyfuss et al. 2000) has estimated 75% prevalence of anaemia among pregnant women, the highest in the world (WHO 1992).

In national context there are limited studied done on the anaemia based on pregnancy, which are comparable to the result of present study. Marahatta showed anaemic rate 42.6% in Nepal Medical College Teaching Hospital. Adak and Nazri (2006) found prevalence rate of anaemia 54% in Birjung where Bondevik et al. (2000) showed prevalence of anaemia 62.2% in Kathmandu. Similarly Shah and Baig (2005) showed prevalence rate 58.9% in Sarlahi District and 55% in Biratnagar (Chaudhary and Maharjan 2014). While Comparing with earlier studies low prevalence rate of anaemia (50%) was obtained in present study done on Teaching hospital. The probable reason

might be that, this is a hospital based study done in Kathmandu valley where women came to antenatal were informed about health through health class to the pregnant women by doctors.

Due to persistent intestinal parasites (Hoque et al. 2009), lack of education (Darling and Rajagopal 2014) and poverty (Nwizu et al. 2011), pregnant women of African countries are more suffered by anaemia (McClure et al. 2014). Several studies showed different anaemic rate among pregnant women of Africa, such as carried out study in anaemia in African countries such as showed 32.5% anemic rate in Uganda (Ononge et al. 2014), showed 53.9% anaemic rate in Ethiopia (Getachew et al. 2012) and Jufar and Zewde (2009) showed only 21.3% anaemic rate where Kefiyalew et al. (2014) showed only 27.9% in Ethiopia. Similarly Siteti et al. (2014) showed prevalence rate of anaemia 40% in Kenya but McClure et al. (2014) recorded significantly higher rate of anemia (71%) over Kenya. Gebre and Mulagete (2015) showed 36.1% anaemic rate in Ethiopia, Boye et al. (2014) showed anaemic rate 20.5% in Ghana, Obiakor et al. (2014) showed 58.9% in Nigeria, Darling and Rajagopal (2014) showed 36% pregnant women suffering by anaemia in Libya, Tefera (2014) showed anaemic rate 51.9% in Ethiopia.

The most common cause of anaemia among pregnant women are menstrual bloodless 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).

Some author showed geophagy, tea consumption, low red meat consumption, all were associated with anaemia (Ansari et al. 2008) as well as age of pregnant women and being in 1st trimester associated with anaemia (Bardisi 2015). Some other researcher revealed lack of vegetable and fruit, being in 3rd trimester, excess bleeding during menstruation period contribute anaemia (Marahatta 2008). One of major factor associated with anaemia in developing country is consumption of plant based food containing insufficient iron (Van den Broek 2003), iron absorption stimulated by vitamin C rich food and inhibited by tea and coffee (Cook and Monsen 1997).It was revealed that iron and vitamin A supplementation during pregnancy reduce the anaemia (Dreyfuss et al. 2000).

Unlike the present study Larcoque et al. (2005) showed highest prevalence of intestinal parasitic infection (90.69%) in pregnant women in Africa and prevalence of anaemia recorded 47.31% among the infected women 47.22% have hookworm infection, 82.25% have *T. trichiura* infection but the IPI were not associated with anaemia in pregnant women but in present study association with intestinal parasite and anaemia was found statistically significant. Similar result was shown by Banu et al. (2014) in Bangladesh and in Nepal by Dreyfuss et al. (2000), Shah and Baig (2005), Navilsky et al. (1998) and Chaudhary and Maharjan (2014) all demonstrated the helminth infection significantly associated with anaemia where Hoque in Ethiopia revealed all the the intestinal protozoan and helmith equally contribute the anaemia (Kefiyalaw et al. 2014) but Hoque et al. revealed only helminth parasites associated with anaemia.

Common intestinal parasites established relation with haemoglobin and regarded as predictor of anaemia showed by Getachew et al. (2012) studied in Ethiopia. Similarly *A. lumbricoides* infection associated with decreased haemoglobin level, demonstrated in Venezuala (Rodriguez-Morales et al. 2006) where other research carried out in Ethiopia also revealed *A. lumbricoides* could cause anaemia among pregnant women (Kefiyalew et al. 2014). Another study carried out in Ghana revealed *T. trichiura* also related with anaemia (Boye et al. 2014).

Sometimes *A. lumbricoides* along with hookworm was found associated with decreased hemoglobin level and were more prevalent among the anaemic pregnant women (Tefera 2014, Shah and Baig 2005), where Chaudhary and Maharjan (2014) revealed out of 17 hookworm infected women 10 were anaemic whereas 21 women with ascariasis, 11 had decreased haemoglobin level showed *A. lumbricoides* associated with anaemia in pregnant women. Similar result forwarded by Shah and Baig showed 24 patient with Ascariasis, 14 had anaemia revealed association of *A. lumbricoides* infection with haemoglobin.

Association between socio-demographic characteristics and anaemia showed insignificant relationship. The age of pregnant women was divided into four groups, <20, 20-25, 26-31, >31. High rate of parasitic infection and anaemia found among 20-25 years, this result is somewhat similar with Awasti et al. (2003) and Dreyfuss et al. (2000). In this study age of pregnant women was not significantly associated with anaemia and IPI.

In present study there is no significant association between trimester and IPI among the pregnant women however intestinal parasitic infection as well as prevalence rate of anaemia obtained decreased in second trimester, it might be due to benefit from antihelminthic treatment (Brooker et al. 2008). In first trimester prevalence of anaemia and intestinal parasitic infection showed high, the reason behind it might be vomiting and nausea decreasing nutrient in body in other hand intestinal parasites interfere with host nutrition both causes pregnancy more complicated. Similar result obtained by Bardisi (2015) and some other showed high prevalence of anaemia in second trimester (Alene and Dohe 2014).In this study ethnicity showed no association with IPI and anaemia. Similarly family size of pregnant women also not associated with IPI and anaemia.

Knowledge related risk factor of parasitic infection and anaemia during pregnancy were assessed on the basis of literacy and awareness. In this study literacy of pregnant women showed no statistical association with intestinal parasitic infection and anaemia however illiterate pregnant women were more infected with parasites (55.6%) than literate (53.8%). Anaemia prevalence rate in illiterate pregnant women showed 38.9% where this value for educated pregnant women was 32.1%. There are several researches done on the basis of education and parasitic infection in pregnant women which has emphasized educated pregnant women were less suffered by IPI than uneducated (Shakya et al. 2012).

No association occurred between knowledge of pregnant women with IPI and anaemia in the present study while some author revealed knowledge of parasites decreases risk of intestinal parasitic infection and anaemia (Rai et al. 1998, Shah and Baig 2005).

Personal behavior and hygiene (not washing hand after defecation, defecating in open field) contribute the IPI which is indirectly related with poverty. This study showed hand washing behavior after defection significantly associated with IPI and anaemia in pregnant women. Similar result obtained from other research such as Kaliappan et al. (2013), Parajuli et al. (2009). Some author showed IPI was significantly associated with habit of open defecation or lack of toilet (Alene and Dohe 2014).

IPI in pregnant women who shared their house with domestic lives stock was higher than who did not share their house with domestic animal or pet animal, it might be due to contact with animal dung and domestic animal around home causes contamination of soil

(Sackey 2001). Significant association was obtained between IPI and sharing house with animals. No association obtained between anaemia and house sharing with live stock.

Deworming tablet decreases risk of anaemia and intestinal parasitic infection (Smith 2010). In the present study women who had taken deworming tablet were comparatively less infected than who had not taken deworming tablet but not significantly associated where association between deworming and anaemia was significantly associated.

Pregnant women in this study who work in field were highly infected with intestinal parasites as well as most of them were suffered by anaemia. IPI and anaemia were found significantly associated with pregnant women working in field. Some similar finding forwarded by Amuta et al. (2008), recorded that farmer were significantly infected with intestinal parasites and Rai et al. (2000) revealed that soil of Kathmandu valley is highly contaminated (36.5) with eggs of *A. lumbricoides*.

Occupation of pregnant women in this study categorized as housewife and worker. Present study showed insignificant association between occupation of pregnant women with IPI and anaemia but in contrast some author showed housewife were more in risk with IPI (Amuta et al. 2008) than worker.

Generally parasitic infection occurred due to consumption of unhygienic water (Wordemann et al. 2006, Hopkins 2013). Present study showed pregnant women who consume open source of water were mostly infected with intestinal parasites but anaemia was no found associated with quality of water. Similar finding obtained by Amuta et al. (2010) revealed open source of water (well, pond, river) significantly associated with IPI.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The overall prevalence of intestinal parasite among pregnant women was obtained 35%, in women visiting the antenatal care in TUTH. Out of 200 pregnant women 70 women were infected with intestinal parasites. All together five different intestinal parasites were identified such as *E. histolytica, A. lumbricoides, T. trichiura, S. stercoralis, H. nana,* among them *A. lumbricoides* identified as a most common parasite followed by *H. nana*. *S. stercoralis* and *T. trichiura* were found only in anaemic pregnant women where *A. lumbricoides* was most prevalent among anaemic pregnant women. Odd ratio shows pregnant women infected with IPI were 3.94 fold increased OR of maternal anaemia where those for helminth was 5.5 fold increased OR of anaemia and nematode contribute 2.29 fold increased OR of anemia. The analysis showed association of anaemia with intestinal parasitic infection was statistically significant.

The median hemoglobin level of the pregnant women without any parasite was with the normal range whereas pregnant women infected with intestinal parasites either single or multiple infection falls under category with hemoglobin level below 11gm/dl. Prevalence of IPI and anaemia showed significant relationship with field working and hand washing behaviour of pregnant women. Use of open source of water was associated with IPI whereas house sharing with domestic animals or other pet animals was associated with intestinal parasitic infection.

6.2 Recommendations

Based upon the discussion and conclusion derived from the present study, following recommendation for the effective control of intestinal parasitic infection and anaemia during pregnancy have been suggested.

1. All the pregnant women attending antenatal should screened for intestinal parasites.
2. Pregnant women should be encouraged for regular ANC follow up, so that early diagnosis and treatment reduce the adverse impact of anaemia both on foetus and mothers.

3. Pregnant women should be encouraged more to maintain personal hygiene and sanitation during pregnancy.
4. Since *A. lumbricoides* is associated with anaemia, deworming along with folic acid supplement must be compulsary to each pregnant woman.

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ANNEX–I

Questionnaire

Question Schedule for baseline health survey in pregnant women coming for ANC visit in TUTH

1. Serial No. :

2. OPD No.

3. Name of Patient :

4. Age :

5. Address :

6. Ethnic group
   i. Brahmin ii. Chhetri iii. Vaishya iv. Sudra

7. Religion
   i. Hindu ii. Muslim iii. Buddhist iv. Christian

8. Occupation of the women
i. House Wife ii. Worker

9. Education
   i. Illiterate ii. Upto Secondary Level iii. Above Secondary Level

10. Husband's occupation
    i. Farmer ii. Skilled Worker

11. POG ... weeks

12. Types of House
    i. Cemented ii. Muddy

13. Toilet of house
    i. Yes ii. No

14. Use of water (pond, well or river) for bathing, cooking & washing clothes

i. Yes ii. No

15. Any kind of live stock or domesticated animals of name:

i. No ii. Cattle (Goat, Cow, Buffalo) iii. Poultry (Hen, Pig) iv. Dog, v. cat

16. Food habit

i. Vegetarian ii. Non-Vegetarian

17. If non-vegetarian, which are taken frequently:

i. Mutton, Chicken, Fish, ii. Buff iii. Other (Pig)

18. Preparation of meat dishes

i. Raw ii. Cooked iii. Fried

19. Knowledge regarding effect of helminthic infection of body

i. Yes ii. No

20. Knowledge regarding prevention of infestation

i. Yes ii. No

21. Have you taken antihelminthics?

No

If yes, when

a. within 6 months b. Beyond 6 months

22. If yes, have you were treated

i. self medication ii. Hospital

iii. Ayurvedic medicine iv. No response

23. How do you wash your hands after toilet?

i. water only ii. With soap iii. with other (mud, ash cow dung)

24. Washing behaviour (after field, before eating)

i. water with soap ii. With others

25. Alcohol using during pregnancy?

i. Yes ii. No

26. Smoking during pregnancy?

i. Yes ii. No

27. Family Size

i. ≤4 members ii. 5-8 members iii. ≥ 9 members

28. Result

i. Hb% ii. Stool test iii. Parasites

29. Type of Parasites