1. INTRODUCTION

1.1 Background

Nepal is an agro-based country where 80% of the people's occupation is agriculture. Livestock is an integral part of agro-production system, which plays a vital role in national economy. This sector contributes about 18% to the national GDP and 31.5% to agricultural GDP (Poudel et al. 2012). It is an important sector contributing in employment and poverty reduction. There are various zoonotic disease associated with livestock farming such as Brucellosis, Cryptosporidiosis, Leptospirosis, Echinococcosis, Toxocariosis, Trichinosis, Tularemia, Toxoplasmosis etc.

Toxoplasmosis is one of the medically and veterinary important disease caused by an obligate intracellular protozoan parasite *Toxoplasma gondii*. It belongs to the phylum Apicomplexa, family Sarcocystidae and class Coccidian (Appendix I), and is the only species in the genus *Toxoplasma*. Coccidian are cysts forming parasites that infect their hosts through the gastrointestinal tract.

It has a worldwide distribution in human population infecting up to one third of global population and a wide range of other mammal including pigs and avian species (Sukthana 2006, McAllister 2005, Dubey and Beattie 1988 and Miller et al. 1972). The first report on *Toxoplasma* infection from Nepal appeared in 1989 (Rai et al. 1989), in a community in Eastern Nepal. Later the prevalence was reported from Mustang and Chitwan district (Rai et al. 1994) and, subsequently in the Central and Western region (Rai et al. 1996). Nearly half of Nepalese are *Toxoplasma* seropositive (Rai et al. 2011). Among meat animals, pigs showed highest prevalence (79.6%) followed by goats and sheep (Rai et al. 1996).

1.2 Toxoplasmosis in human

Infection with *T. gondii* can occur pre- or postnatal. After birth, humans are usually infected by ingestion of oocysts in soil or water that have been contaminated with cat feces, or by ingestion of tissue cysts in undercooked meat (Dubey and Beattie 1988, Bowie et al. 1997, Bahia-Oliveira et al. 2003, Jones et al. 2005 and de Moure et al. 2006). It has also been

suggested that human infection can occur after ingestion of tachyzoites in milk (Riemann 1975) and through transmission from mother to fetus, particularly when *T. gondii* is contracted during pregnancy (Tenter et al. 2000). Although infection can be transmitted via transfusion of platelets and blood cells and via organ transplants, these modes of transmission are less common than transmission via meat and oocysts. However, disseminated and other fatal toxoplasmosis may result from organ transplantation because the patients are being given treatments that are immunosuppressive.

Toxoplasmosis, a serious food-borne parasitic disease, is usually asymptomatic in immunocompetent individuals but can cause severe effect in immuno-compromised and congenitally infected individual (Dubey 2010 and Zhou et al. 2011). These days it became common cause of death in AIDS patients. Toxoplasmic encephalitis is the most common clinical presentation of toxoplasmosis among AIDS patients. It is usually the result of reactivation of latent tissue cysts (Wong et al. 1984 and Israelski et al. 1993) when persons become severely immunosuppressed, with the highest risk occurring when the CD4+ T-lymphocyte count drops below 50 cells per micro liter (Leport et al. 1996).

Unless immunosuppression occurs and the organism reactivates, postnatally acquired infections are generally asymptomatic or associated with self limited symptoms such as fever, malaise, lymphadenopathy (Montoya and Liesenfeld 2004 and Remington et al. 2006). Once infected, human are believed to remain infected for life. In rare cases, humans, previously healthy have suffered from severe and even fatal disease, including pulmonary and multivisceral involvement, possibly from more virulent types of the organism (Carme et al. 2002 and Demar et al. 2007).

1.2.1 Congenital Toxoplasmosis

Congenital toxoplasmosis is group of symptoms that occur when an unborn baby (fetus) is infected with the parasite *T. gondii* causing severe damage to fetus (Montoya and Liesenfeld 2004, Kravetz and Federman 2005). Congenital infection usually leads to a wide variety of manifestation in the fetus and infant including spontaneous abortion, still-birth, a live infant with classic signs of congenital toxoplasmosis such as hydrocephalus or microcephalus,

cerebral calcifications and retinochorioditis, an infant who fails to thrive or has CNS involvement or retinochoroiditis, or an apparently normal infant who develops retinochoroiditis or symptoms of CNS involvement later in life (McAuley et al. 1994).

Congenital disease may become apparent at birth or not until the second or third decade of life (Jones et al. 2001, Remington et al. 2000 and Liesenfeld et al. 2000). The rate of transmission to the fetus is 10-15% in the first trimester of gestation, which may increase to 68% in the third trimester (Thulliez et al. 1992). Thus maternal infection early in pregnancy are less likely to be transmitted to the fetus than infections later in pregnancy but early fetal infection are likely to have more severe consequence than late infection (Holliman 1995).

1.3 Toxoplasmosis in livestock

There is a widespread distribution of *Toxoplasma* infection in a variety of livestock, wild animals and pets. Among livestock, pigs, sheep, and goats have the highest rates of chronic *T. gondii* infection but less frequently in infected poultry, rabbits, and horses (Tenter et al. 2000). The tissue cysts of *T. gondii* contained in meat of livestock are an important source of infection for humans. Tissue cysts may develop as early as 6-7 days after infection of intermediate hosts by both oocyst and other tissue cysts (Dubey et al. 1998). Tenter et al. (2000) and Schlundt et al. (2004) estimated that the percentage of meat-borne cases was approximately 30% to 63%, depending on eating habits. *Toxoplasma* cysts in pork can persist for a long time, and has been considered an important source of infection for human (EFSA 2007). But tissue cysts are rarely present in buffalo meat or beef, and meat from these animals is considered to be low-risk for harboring viable parasites (Jones and Dubey 2012). Although tissue cysts are less resistant to environmental conditions than oocysts, they are relatively resistant to changes in temperature and remain infectious in refrigerated (1 to 4°C) carcasses or minced meat for up to 3 weeks, i.e. probably as long as the meat remains suitable for human consumption (Dubey 1988 and Tenter et al. 2000).

The prevalence of infection in meat producing animals varies widely both within and between countries (Tenter et al. 2000).Toxoplasmosis is considered to be an important cause of neonatal death and abortion in sheep, goats and pigs. In these animals symptoms include

fever, dyspnoea, loss of vision, nervous signs followed by lethargy and cough. In pigs most infection are subclinical. Clinical toxoplasmosis has been reported most often in nursing pigs. Infected pigs are born dead, sick or become sick within 3 weeks after birth, some remain clinically normal.

1.4 Immune response

Infection with *T. gondii* stimulates the development of both humoral and cellular immune responses, with cell mediated response being more prominent. When someone is exposed to *T. gondii*, their immune system responds by producing antibodies to parasite. Two types of *Toxoplasma* antibodies may be found in the blood, IgM and IgG. IgE antibodies also appear early in infection, and then their level begins to decline. IgM antibodies usually appeared within a week or two after the initial exposure. It is first to be produced by the body. IgM antibody production rises for a short time period and declines. IgG antibodies appear several weeks after infection and peak at approximately 6-8 weeks post infection. Once a person is exposed to *T. gondii* infection, they will have some amount of IgG antibodies in their blood for the rest of their life. IgG antibodies are produced by the body to provide long-term protection. Therefore, the detection of IgM indicates the acute phase of toxoplasmosis and the presence of IgG demonstrate prior infection. *T. gondii* IgG antibody testing can be used, along with IgM testing, to confirm the presence of a recent or previous *Toxoplasma* infection. In this study, commercially available ELISA kit was used for detecting *T. gondii* antibody in serum of sampled pigs and pregnant women.

1.5 Objectives of the study

1.5.1 General objectives

- To determine the seroprevalence of *Toxoplasma gondii* infection in pigs and pregnant women of Bhaktapur District.

1.5.2 Specific objectives:

- To determine prevalence of *T. gondii* in farmed pigs of different VDCs (Tathali, Chittapol, Sipadol, Bode, Duwakot and Changunarayan) of Bhaktapur district.
- To determine the association of *T. gondii* infection in pregnant women in relation to meat eating habit and their contact with cat and its feces.

1.6 Rational of study

Toxoplasma gondii is an extremely successful pathogen responsible for significance morbidity and mortality especially in congenitally infected and immunocompromised individual (Elsheikha 2008). On farms, *T. gondii* is a major cause of abortion and problems with fertility in livestock, regarding pigs and therefore a significant cause of lost profitability in livestock agriculture. In Nepal, among meat animals, pigs showed the highest prevalence i.e.79.6% (Rai et al. 1996). So, there is a need of research on epidemiology of Toxoplasmosis in pig. The raw and undercooked meat eating habit of Nepalese people also create the possibility of transmission of infection. The pet animals mostly the cats are not kept in well manage and healthy condition, this creates the threats of infection to people with oocyst secreted in cat feces even if they don't have cats.

Toxoplasmosis, one of the neglected parasitic infection, is a disease that have been targeted by CDC for public health action because relatively little attention has been devoted to their surveillance, prevention and treatment. In Nepal, only little information is available about epidemiology of *T. gondii* in pregnant women and animal, especially in Bhaktapur district. Therefore, present study sought to determine the seroprevalence and asses some risk factors associated with *Toxoplasma gondii* amongst pregnant women and farmed pigs of Bhaktapur district.

1.7 Limitations of the study

The high cost of ELISA kit makes the limitation of the sample size. However, all possible efforts have been made to fulfill the objectives of the study.

2. LITERATURE REVIEW

2.1 Discovery of pathogen

Nicolle and Manceaux in 1908, while working for Leishmaniasis, found a protozoan in tissue of *Ctenodactylus gundii* as new organism at Pasteur Institute in Tunis, South Africa. The parasite was named *Toxoplasma gondii* based on the morphology (L. toxo = arc or bow, plasma = life) and host. Alfonso Splendore identified the same organism in a rabbit in Brazil in the same year. The parasite has since been found to be capable of infecting all warm blooded animals including human making it one of the most successful parasitic organisms worldwide and perhaps the widest host range of any parasite (Dubey 2008). Its medical importance was realized only after 1939 when *T. gondii* was identified in tissues of a congenitally infected infant, and its veterinary importance was known when it caused abortion storms in sheep in 1957.

Wolf, Cowen and Paige first conclusively identified *T. gondii* in an infant girl from New York, USA (Wolf et al. 1939). The child developed seizures and chorioretinitis within few days of birth and died at the age of one month. Post mortem report demonstrated the free and intracellular *T. gondii* in lesions of encephalomyelitis and retinitis. Later, Sabin (1942) summarized details about congenital toxoplasmosis and proposed its typical clinical signs such as hydrocephalus or microphalus, intracerebral calcification and chorioretinitis.

2.2 Transmission

The mechanism of transmission of *T. gondii* remained a mystery until its life cycle was discovered in 1970. Initially transmission by arthropods was suspected, but this was never proven (Frenkel 1970 and 1973). In human, the first congenital *T. gondii* infection was described by Wolf, Cowen and Page (1939) and later found to occur in many species of animals, particularly sheep, goats and rodents. Weinman and Chandler (1954) suggested that transmission might also occur through the ingestion of undercooked meat. Jacobs et al. (1960) provided evidence to support this idea. They found that the cyst wall was dissolved by proteolytic enzymes but the released bradyzoites survived long enough to infect the host. This hypothesis was tested by Desmonts et al. (1965). They observed the transmission is

common in human in some localities where raw meat is routinely eaten. Hutchison (1965) first discovered that *T. gondii* infection is associated with cat faces.

A recent study conducted in dogs demonstrated that *T. gondii* can be transmitted sexually in that species. The male dogs infected with *T. gondii* found to have parasite in their semen. The infected semen was artificially inseminated into four uninfected female dogs. After seven days of insemination, all four female dogs had *T. gondii* antibodies (Arantes et al. 2009). The evidence of sexual transmission was also found in semen of human in Germany. Among 125 semen collected, three had evidence of *T. gondii*, of which two had blood antibodies also (Disko and Vogel 1971).

2.3 Prevalance of Toxoplasmosis in Global Context

2.3.1 In Human

Seroprevalence estimated for human population varies greatly among different countries, among different geographical areas within one country and among different ethnic groups living in the same areas (Tenter et al. 2000).

The seroprevalence of *T. gondii* infection and associated risk factors were investigated in Guangdong province, China. The overall seroprevalence of 0.46% and 7.01% were found to be positive for *T. gondii* IgM and IgG antibodies respectively. Contact with cats, consumption of raw or under-cooked wild and domestic animals or pork products were considered risk factor for infection. The study showed no relation between anti-*Toxoplasma* IgM and IgG antibodies and unwashed raw vegetables or fruits, educational level and different age group (Duan et al. 2012).

The overall seroprevalance of *T. gondii* among pregnant women in Jimma town, Southwestern Ethiopia was high (83.6%). 81.1% of the pregnant women were IgG seropositive and 2.5% were IgM seropositive. This study concluded that pregnant women having domestic cat at their home were at higher risk of *T. gondii* infection (Zemene et al. 2012).

Alvarado-Esquivel et al. (2011) conducted a case control seroprevalence study and found that *Toxoplasma* infection was associated with consumption of raw meat (OR= 5.77: 95% CI: 1.15-28.79; p= 0.03), unwashed raw fruits (OR= 2.50; 95% CI: 1.11-5.63: p= 0.02) and living in a house with soil floors (OR= 3.10: 95% CI: 1.22-7.88: p= 0.01). The seroprevalence among 200 fruit and vegetable workers was 7.5% and 7.8% among 400 control subjects. The result showed seroprevalence increased with age.

Osunkalu, et al. (2011) conducted a study to compare the pattern of seroprevalence of *T. gondii* IgG antibodies among HIV infected persons presenting with neurological complications and those without. Seroprevalence was higher (58%) in subject without neurological complication than person with neurological complications (40%). The overall seroprevalence among HIV- positive respondents was 54.2%.

Huang et al. (2010) studied *T. gondii* infection in People working on Pig Farms in China with overall seroprevalence of 12.26%. The result showed highest prevalence (23.52%) in the farm managers followed by the pig breeders (11.54%) and the farmers (7.69%).

Sroka et al. (2010) determined seroprevalance of *T. gondii* and associated risk factors among pregnant women in Fortaleza, Brazil. The seroprevalance was high in women less than 25 years and with poor socio-economic condition. Analysis showed that consumption of vegetables washed with untreated water, consumption of chicken and dog ownership were factors associated with IgG seropositivity. Seroprevalance of IgG and IgM were 68.6% and 0.5% respectively.

The serological prevalence of *T. gondii* in pregnant women in Southern Brazil was carried out by using automated immunoenzymatic assays. The result showed that 53.03% of women were positive for IgG and 3.26% were IgM positive. IgG avidity tests were carried out to discriminate past and recent infection, 28.3% presented low avidity. The seroconversion index observed in this study was 0.44% (Vaz et al. 2010).

Abu-Madi et al. (2010) carried out the study which included patients who are at high risk for TORCH pathogen such as pregnant women, their fetuses, neonates and AIDS patients. Among women of childbearing age, 35.1% and 5.2% were positive for IgG and IgM

antibodies respectively and three infants less than or equal to six months were congenitally infected. Older age, African nationality, positive cytomegalovirus (CMV) and herpes simplex virus (HSV) serostatus were considered as factors associated with *T gondii* seropositivity.

A serological survey was carried out in Tanga district of north-eastern Tanzania among occupationally-exposed groups including abattoir workers and other groups. The overall seroprevalance was 46%. The seroprevalance of *Toxoplasma* antibodies was significantly higher amongst individuals who keep livestock (52.2%) and abattoir workers (46.3%). The study summarized that consumption of raw or undercooked meat and keeping pets especially cats present more risk factors than occupational groups (Swai and Shoonman, 2009).

The serological testing for IgG/IgM anti *Toxoplasma* antibodies fail to differentiate recent and the past infection. So, Iqbal, J. and Khalid, N. screened serum samples of pregnant women in their first trimester by the Vitek Immuno Diagnostic Assay System (VIDAS) and VIDAS IgG avidity tests. On serological screening, 119 women were positive for IgG antibodies and 31 for IgM antibodies. Nine IgM positive and seven IgM negative women had low avidity antibodies indicating recent infection and 19 had high avidity indicating infection acquired in the distant past (Iqbal and Khalid 2007).

The first national serological prevalence of *T. gondii* in India was reported by Dhumne et al. (2007). The solid phase immunocapture ELISA test showed IgG antibodies in 24.3% samples and 2% had IgM antibodies. The lowest seroprevalance were detected in northern and highest in southern part of India indicating the effects of drier conditions and therefore, a negative impact on the survivability of *T. gondii* oocysts.

According to Studenicova et al. (2005), in Slovakia the possible risk for toxoplasmosis are consumption of raw meat and raw vegetables or fruits. A significant increase in toxoplasmosis with increasing age was confirmed. By using ELISA test the overall IgG antibody prevalence was 24.2% in the study population, while specific IgA and IgM antibodies was negative.

A study carried out in Hebron district, Palestine determined that the possible routes of infection in pregnant women were contaminated soil, drinking rain water and eating raw

vegetables rather than eating uncooked meat or contact with cats (Nijem and Al-Amleh 2005).

A few studies performed in Turkey found that seroprevalance of *T. gondii* in women at childbearing age ranges from 19.2% to 85% and estimated that incidence of congenital toxoplasmosis is 0.1% (Gun et al. 1993, Kara et al. 1999 and Petersen et al. 2001).

The serological survey conducted in Northern Mexico showed lower (6.1%) seroprevalence among pregnant women. This study concluded that poor housing conditions as soil floors, turkey meat consumption might contribute to acquire *T. gondii* infection (Alvarado-Esquivel et al. 2006)

In European countries, prevalence vary from 9% to 67% (Nash et al. 2005, Ertug et al. 2005, Cook et al. 2000, Herma et al. 2004, Jenum et al. 1998 and Jeannel et al. 1988).

2.3.2 In animals

Toxoplasmosis is a zoonosis arising from man's close contact with domestic cats (*Felis catus*) (Kravetz and Federman 2002 and Roman et al. 2006). Of many species of animals infected with *T. gondii* only felids (both domestic and wild) shed *T. gondii* oocysts (Miller et al. 1972). At least 17 species of wild felines have been reported to shed oocysts of *T. gondii* (Lukesova and Literak 1998). Sero-epidemiololgical studies in Pacific (Wallace 1969), Australia (Munday 1972) and United States (Dubey et al. 1997) showed an absence of *T. gondii* without cats, confirming the important role in epidemiology of infection. *T. gondii* in cats are usually asymptomatic, and vertical transmissions occur only infrequently (Dubey 1986). In domestic cats, antibodies to *T. gondii* was detected in up to 74% of adult cat populations, depending on the type of feeding and whether cats are kept indoors or outdoors (Lappin et al. 1992).

In United States, the prevalence of viable *T. gondii* in sheep tissue has been measured to be as high as 78% (Dubey et al. 2008) and a 2011 survey of goats found a seroprevalance of 53.4% (Dubey et al. 2011). The percentage of pigs harboring viable parasites has been

measured to be as high as 92.7% and as low as 0%, depending on the farm or herd (Jones and Dubey 2012).

Chadwick et al. (2013) first conducted the extensive survey of *T. gondii* in UK wildlife, Eurasian otters, a sentinel species of fresh waters. Seroprevalance of 39.5% was recorded by using Gold Standard Sabin-Feldman Dye Test with increase in prevalence with age. The relatively high prevalence of *T. gondii* suggests widespread fecal contamination of freshwater ecosystems with oocysts in UK.

According to Desksne et al. (2013) age and outdoor access were found to be the most significant factors associated with *T. gondii* infection in cats in Latvia. ELISA test of 242 serum samples of cats showed 51.6% seropositivity and microscopical examination of 80 fecal samples diagnosed *T. gondii* like oocyst in two cats.

A study was carried out in Kunming, Southwest China to estimate the seroprevalance of *T. gondii* infection in pet dogs in China. A total of 611 serum samples were collected and assayed by the indirect haemagglutination (IHA) using a commercially available kit. 21.6% pet dogs were positive for *T. gondii* antibodies. Seroprevalance of female and male dogs were 20.6% and 22.4% respectively but this was not statistically significant (Duan et al. 2012).

The seroprevalance of *T. gondii* in Yaks (*Bos grunneius*) in northwestern China was surveyed using indirect haemagglutination test. Antibody of *T. gondii* was found in 35.08% serum samples (Quan et al. 2011).

Chikweto et al. (2011) collected 750 serum samples of food animal in Grenada and Carriacou. The modified agglutination test (MAT) showed prevalence of 23.1% in pigs, 44.1% in sheep, 42.8% in goats, and 8.4% in cattle indicating that pigs, sheep and goats could be the important sources of *T. gondii* infection if their meat is consumed undercooked.

Boughattas et al. (2011) studied *Toxoplasma* infection in Tunisia in horses which revealed 17.7% seroprevalence using the Modified Agglutination Test.

The overall seroprevalance of 29.51% was reported by Shu et al. (2010) in slaughtered pigs in Sichuan, China indicating relatively high seroprevalance in pigs and consumption of pork be a risk factor for human infection with *T. gondii*.

A serological study conducted by Huang et al. (2010) estimated the prevalence of *T. gondii* infection in breeding sows in Western Fujian Province, China. By an indirect haemagglutination antibody test prevalence revealed 14.38%.

A serological study conducted in Goats in Thailand showed 27.9% seroprevalence using Commercial latex agglutination test kits. Female and Dairy goats were more likely to be seropositive than male and meat goats (Jittapalapong et al. 2005).

A serological study carried out in Brazil and Peru concluded that raw or inadequately cooked pork is an important source of infection in human. The seroprevalance was higher in pigs from Peru (32.3%) as compared to Brazil (9.6%), as detected by ELISA and Western blot method (Suarez-Aranda et al. 2000).

In Costa Rica, the overall prevalence of antibodies was 34.4% in cattle and 43.8% in swine (Arias et al. 1994).

2.4 Toxoplasmosis in Nepal

Rai et al. (2011) first reported the congenital toxoplasmosis in a 53 days old full term male baby weighing 2.6 kg delivered by caesarean section. The baby had hepato-splenomegaly and optic nerve coloboma with large scar in the right eye. The TORCH panel test showed significantly high *Toxoplasma* IgM antibody level compared with IgM antibody level against other agent. The treatment was started accordingly but did not improve and died after 6 days.

A sero study conducted by Rai et al. in 2003 in selected patients in Kathmandu showed an overall prevalence of 50.7%. This study included patients with ocular disease, malignancy, women with bad obstetric history and others (patients with fever, lymphadenitis and encephalitis). Of which 5.7% had IgM antibodies. Patients with malignancy had highest

positive rate (68.7%) followed by groups of others. Of the different groups, women with BOH had highest *Toxoplasma* IgM positive rate (25%).

High *Toxoplasma* seroprevalance associated with meat eating habits in Nepal was carried out by Rai, S.K. (1999) in local people living in Achham (n=215) and Dang (n=189) district in western Nepal. The overall seroprevalance was found to be 65.3% with no significant difference in two districts (Achham 66.9% and Dang 63.5%, p value 0.546). *Toxoplasma gondii* antibodies were detected using Micro latex Agglutination Test. The higher seroprevalance was reported in female and the Indo-Aryan ethnic group compared with their male and Tibeto-Burman.

Rai et al. (1998) tested sera from 345 pregnant Nepalese women aged 16-36 yrs and 13 women with bad obstetric history using Microlatex Agglutination and ELISA methods. Of the total 13 women with BOH 5 (38.5%) had *Toxoplasma* antibodies of which 2 (40%) were IgM seropositive. The overall seroprevalance was 53.4%. Prevalance was slightly higher (59%) in older age-group (27-36yrs) compared with younger age-group (16-26 yr) (52.2%). There was no significant difference in antibody prevalence between Tibeto-Burman (57.8%) and Indo-Aryans (52.7%) with p value greater than 0.05.

Sero-epidemiological study of *Toxoplasma* infection in Central and Western region of Nepal, carried out by Rai et al. (1996) showed prevalence rate 48% and 49% respectively. A total of 1,237 serum samples were collected from Nuwakot, Kathmandu valley and Chitwan in Central region and Mustang, Surkhet and Banke district in Western Region. Micro-latex agglutination and Enzyme linked immunosorbent assay (IgM ELISA) method showed seropositive rate of 38, 46, 64, 51, 67 and 44% respectively. Ethnically, Tibeto-Burman showed higher seropositive rate in Central Region. In contrast, Indo-Aryans showed higher seropositive rate in Western Region. Only 1% of *Toxoplasma* antibody positive samples showed *Toxoplasma* IgM antibody positivity.

Shibata et al. (1995) studied the applicability of PCR in the early diagnosis of toxoplasmosis in a murine model orally infected with *Toxoplasma gondii* (S-273). Mice blood and brains collected on various post infection days were analyzed by PCR (35 cycles) and a portion of

brain tissue from each mouse was examined for the presence of parasite cysts. This study showed *Toxoplasma* parasites can be detected earlier in the blood than in the brain during primary infection indicating blood PCR is the more useful procedure.

The overall seropositivity rate of 57.9% was found in study carried out by Rai et al. (1994) in two different geographical areas, Chitwan and Mustang district. Female in Chitwan showed significantly higher (71.2%) positive rate compared to Mustang (43.3%) ($p < 0.001$). A slight increase in positive rate with age was observed in Chitwan while decreasing trend was noticed in Mustang. Though not significant, Indo-Aryans showed higher positive rate (69.2%) compared to Tibeto-Burman (63.1%) in Chitwan while the reverse was noticed in Mustang (Tibeto-Burman 53.8% and Indo-Aryan 38.4%).

2.5 Diagnosis

Toxoplasmosis may be diagnosed by direct and indirect methods. Direct method include PCR for detection of DNA of parasite from sample fluids, mouse inoculation method, cell culture, ophthalmic testing and ratiological studies (Montoya and Liesenfeld, 2004 and Swisher et al. 1994). Indirect method include detection of anti-T gondii antibodies such as Sabin-Feldman dye test, Indirect fluorescent antibody (IFA), agglutination test and ELISA test (Sabin and Feldman 1948 and Montoya and Remington 1996).

Faragalla M. El Moghazy, Omnia M. Kandil and Raafat M. Shaapan, collected blood samples from pigs of different ages and sexes for the comparative serologic diagnosis of *T. gondii* infection. The modified agglutination test (MAT) revealed higher prevalence of toxoplasmosis (56.6%) followed by ELISA (52.2%) Indirect haemagglutination test (IHAT) (42.7%) and lowest with Methylene blue dye test (DT) (35.5%). The result of this survey recommended MAT and ELISA as more sensitive and specific serological test in diagnosis of toxoplasmosis in pigs (Moghazy et al 2011).

3. MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Bhaktapur District, located 27036' to 27044' northern latitude and 85021' to 85032' eastern longitude, covering an area of 138.46 square kilometer. Administratively, it is divided into 16 VDCs and two municipalities namely Bhaktapur and Madhyapur municipalities. Total population according to 2011 census survey was 2,25,461. Of which 49.08% were female. The town is generally warm in summer and cool in winter.

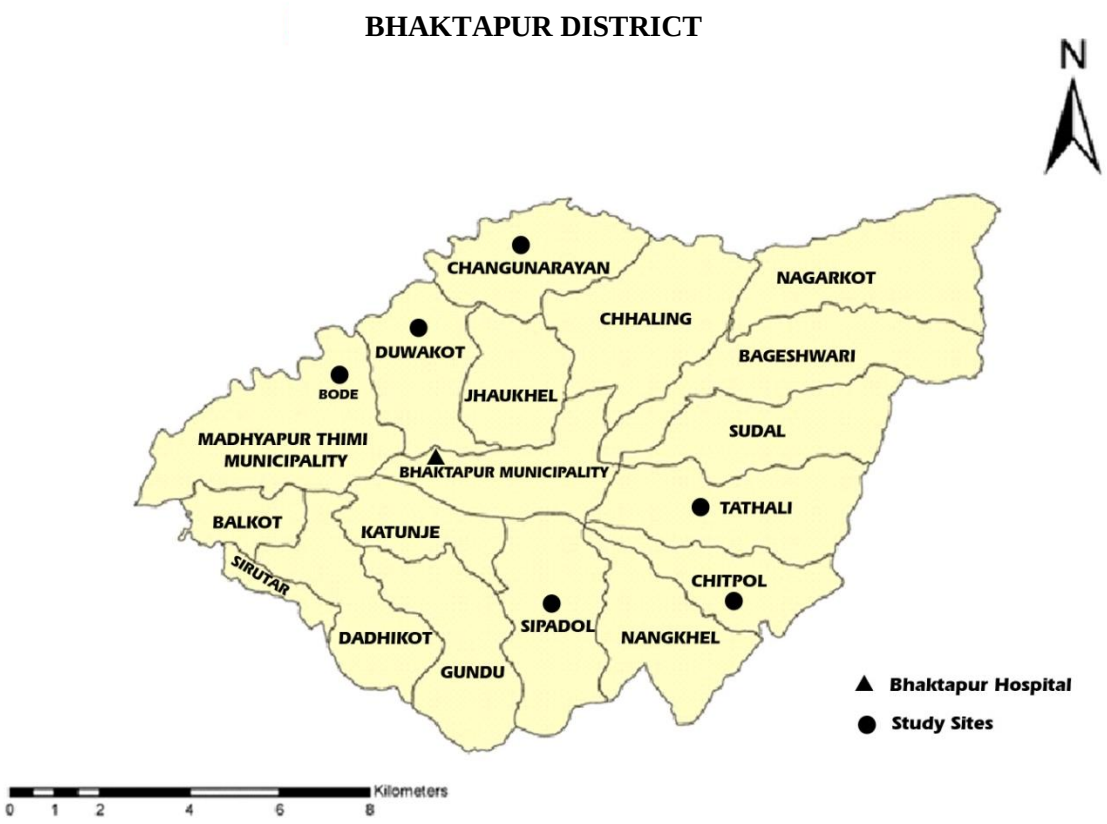


Fig 1. Study area

The blood samples of pregnant women from Bhaktapur Hospital and pig blood samples from organized pig farm of different VDCs (Tathali, Chittapol, Sipadol, Bode, Duwakot, Changunarayan) of Bhaktapur District were collected randomly.

3.2 Study Design

Cross-sectional study design was applied as the research tool in this study.

3.3 Sampling Technique and Sample size

A total of 41 blood samples of farmed pigs were collected between 3rd June 2012 to 6th June 2012 which counted 16 samples from two sites of Chittapol VDC, 13 from Tathali, 3 from Sipadol, 3 from Duwakot, 1 from Changu Narayan VDC of Bhaktapur municipality and 9 from Bode of Madhyapur municipality.

All pregnant women seeking prenatal check up from 10th July 2012 to 26th July 2012 at Bhaktapur hospital, located in Bhaktapur municipalities, was invited to participate in the study. During the study period 50 pregnant women were attended. Inclusion criteria for the study subjects were pregnant women in any of three trimesters of pregnancy and who accepted to participate in the study.

3.4 Blood collection and Serum Preparation

The Pigs blood samples were collected from back of the ear with the help of syringe and transferred to vials and kept in cold box. It was then transported to National Zoonoses and Food Hygiene Research Center, Tahachal, Kathmandu. The serum was separated from the whole blood by centrifugation at 2000 rpm for 20 min. The serum were transferred in vials and stored in deep freeze (-20℃).

Blood from pregnant women were taken by a nurse at hospital and then centrifuged to separate serum and kept at -20℃ in refrigerator. After the completion of serum collection, all the samples were transported to National Zoonoses and Food Hygiene Research Centre, Kathmandu for serological study.

Photograph 1. Collecting sample blood from pig

Photograph 2. Questionnaire with pig farmer

Photograph 3. Collecting blood from pregnant woman at Bhaktapur Hospital

Photograph 4. Questionnaire with pregnant women

3.5 Instrumentation

Different tools used in this study were as follows:

3.5.1 Questionnaire

A structured questionnaire was performed to Pig farmer and pregnant women. There were questions eliciting socio-demographic data including age, education, occupation, parity, gestational age and related risk factors including presence of cat at their home, playing habit with cat, source of drinking water, eating habit (veg or non veg), pork meat eating habit, raw meat eating habit, history of abortion (Appendix II and III).

3.5.2 Materials and chemical used in laboratory purpose

Equipments

- Centrifuging machine
- Cold box
- Refrigerator
- Micro well plate
- Eppendorf tube (1ml)
- Borosil beaker (1000ml)
- Pipette (100µl, 1000µl)
- Incubator
- Vortex
- Shaker
- Aspirator (Multiple washer)
- Absorbent paper
- Multipipette
- ELISA Reader

Chemicals

- Washing Solution
- Enzyme conjugate
- TMB-Substrate
- Stopping Reagent

3.6 Serum test (Sample Processing)

Toxoplasma gondii infection in human and animals was detected by antibody levels and the current analysis was based on the prevalence of *T. gondii* specific IgG antibodies. In this study, *T. gondii* IgG antibody testing was carried out by using commercially available "DS-EIA-ANTI-TOXO-G-FAST" ELISA kit (DSI S.r.l, Saronno, Italy) following the manufacturer's instructions (Appendix IV).

3.6.1 Determination of antibodies for *T. gondii*

3.6.1.1 Principal of the EIA test

Toxoplasma gondii antigens are fixed to the interior surface of microwells. Patient's serum is added and any antibody present to *Toxoplasma* will bind to these antigens. The microwells are washed to remove unbound serum proteins. Antibodies conjugated with Horseradish Peroxidase enzyme and directed against human IgG are added and will in turn bind to any human IgG present. The microwells are washed to remove unbound conjugate and then chromogen/substrate is added. In the presence of peroxidase enzyme the colorless substrate is hydrolyzed to a colored end-product. The color intensity is proportional to the amount of antibodies present in the patient's serum.

3.6.2 Assay Procedure

1. The 96 micro well plate was placed and marked one end of each strip for orientation.
2. The samples were diluted in the ratio of 1:100 with Sample Diluents (10μl serum to 1ml Sample Diluent) but the standards should not be diluted since they are ready for use.
3. 100μl Standards and serum specimens were pipetted in subsequent wells. The pipetting of serum specimens should not extend beyond five (5) minutes to avoid assay drift.
4. The microwells were incubated at room temperature (23-25℃) for 15 minutes.
5. Microwells were washed by inverting and flicking into a sink. It was completely filled with Washing Solution and repeated washes three times and again refilled with washing solution and soaked for 5 minutes. Wells were emptied and blotted with absorbent paper. Using an automatic washer wells were filled and aspired five times without soak.

6. 100μl Enzyme Conjugate was pipetted into each well.
7. It was incubated at room temperature (23-25℃) for 15 minutes.
8. Microwells were washed as in step 5.
9. Then 100μl TMB-Substrate was pipetted in each well.
10. Incubated at room temperature (23-25℃) for 5 minutes.
11. Finally, 100μl Stopping Reagent was pipetted into each well using the same pipetting sequence as in step 9.
12. The color intensity of the solution in each well was measured using a microwells reader with a 450 nm filter. (Before reading carefully the exterior of the wells were wiped and checked that there were no residue or scratches that may give erroneous readings).
13. By the data obtained from the microwell reader the standard curve was prepared and calculated the results.

3.6.2.1 Validity of the Assay

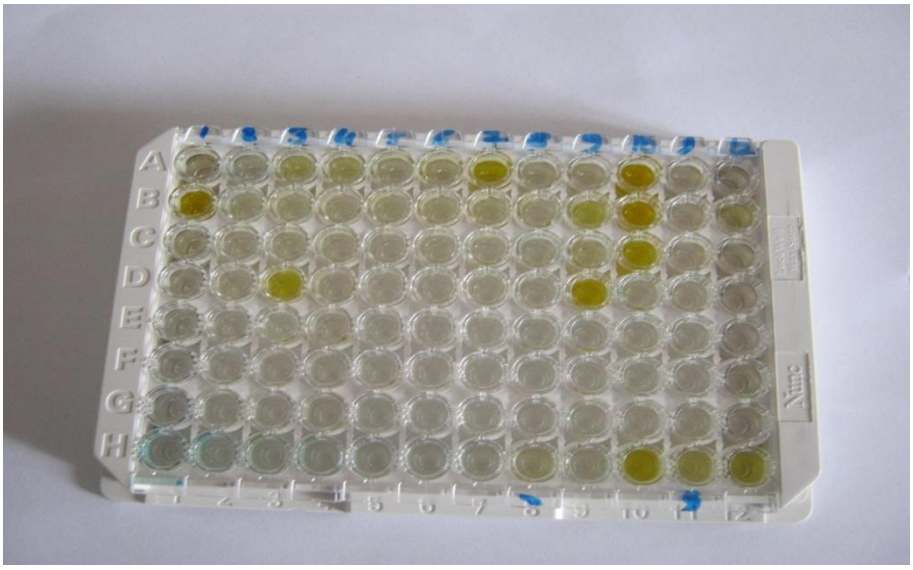
The assay was considered to be valid when,

1. The mean OD value for the 0 IU/ml standard is lower than 0.150. Higher values indicate an incorrect washing procedure. In such a case, the efficiency of the washing device should be checked.
2. The mean OD 450nm of the 1000 IU/ml standard is higher than 1.000. Lower values may indicate kit or control decay. In such a case, it should check the expiry date of the kit before repeating the assay.

3.7 Calculation

Calculation was done by plotting the standard curve on the graph paper between the OD value and IgG concentration.

Photograph 5. Testing serum samples in Lab



Photograph 6. ELISA result

3.8 Statistical analysis

Data collected were checked for completeness and consistency then analyzed using SPSS version 16.0 software package. Statistical significance was determined by Chi-square tests. P-values less than 0.05 were considered statistically significant in the analysis. Odd ratio (OR) and 95% confidence interval (CI) were also calculated.

4. RESULTS

4.1 Seroprevalence of *T. gondii*

The overall seroprevalence of anti-*T.gondii* IgG antibodies among the pregnant women was 22% indicating past infection.

Table 1. Overall seroprevalence of toxoplasmosis

Species	No. of samples tested	No. of positive samples	Prevalance %
Pregnant women	50	11	22
Pigs	41	0	0

Note: Seroprevalence in sampled pig's blood was observed nil during study period. This may be due to technical error in the assay procedure. In this study, same enzyme conjugate was used in testing serum samples of pigs and pregnant women.

General characteristic of sampled pigs:

Out of the 41 sampled pigs, 26 (63.41%) were female. Half (58.54%) of the sampled pigs were in the age range of 0-1 years, 21.95% in 1-2 years and 19.51% belong to age range greater than two year. Breed of the sampled pigs were Yorkshire, Hampshire and Crossed.

Table 2. General characteristics (age, sex and breed) of farmed pigs

Characteristics		No. of Pigs	Percentage %
Age group	0-1 yr	24	58.54
	1-2 yr	9	21.95
	> 2yr	8	19.51
Sex	Male	15	36.59
	Female	26	63.41
Breed	Yorkshire	26	63.42
	Hampshire	8	19.51
	Crossed	7	17.07

4.2 Sociodemographic description of the study population

A total of 91 blood samples, 41 from farmed pig and 50 from pregnant women seeking prenatal check up in Bhaktapur Hospital were collected. About half of the total no. of pregnant women was in the age range of 15-25 years. Majority of them (78%) were house wives and 20% were illiterate. Fourty eight percent of the pregnant women were within second trimester. Majority of pig farmer and pregnant women were unknown about toxoplasmosis.

Table 3. Distribution of *T. gondii* in pregnant women on basis of demographic characteristics

Demographic characteristics	Seroprevalence		Total no. (%)
	Positive no. (%)	Negative no. %	
Age group			
• 15-25 year	10 (29.41)	24 (70.59)	34 (68)
• 26-35 year	1 (6.25)	15 (93.75)	16 (32)
Religion			
• Hindu	9 (20.45)	35 (79.55)	44 (86.27)
• Buddhist	1 (20)	4 (80)	5 (9.81)
• Christian	1 (100)	0	1 (1.96)
Occupation			
• Housewife	8 (20.51)	31 (79.49)	39 (78)
• Office	2 (40)	3 (60)	5 (10)
• Student	0	1 (100)	1 (2)
• Business	1 (20)	4 (80)	5 (10)
Level of education			
• Illiterate	2 (20)	8 (80)	10 (20)
• Primary Level	1 (33.33)	2 (66.67)	3 (6)
• Secondary level	6 (21.43)	22 (78.57)	28 (58)
• More	0	9 (100)	9 (18)
Gestational age			
• First trimester	1 (8.33)	11 (91.67)	12 (24)
• Second trimester	5 (20.83)	19 (79.17)	24 (48)
• Third trimester	5 (35.71)	9 (64.29)	14 (28)
No. of pregnancy (Parity)			
• One	7 (25.9)	20 (74.1)	27 (54)
• Two	3 (15.8)	16 (84.2)	19 (38)
• > than two	1 (25)	3 (75)	4 (8)

4.3 Factors associated with seropositivity

In the bivariate analysis, various possible risk factors were analyzed with *T. gondii* infection.

Table 4. Factors associated with *Toxoplasma gondii* infection among the pregnant women (n=50) in Bhaktapur district

Characters	Seroprevalence		P value	OR (95% CI)
	Positive no. (%)	Negative no. (%)		
Cat ownership				
Yes	6 (20.69)	23 (79.31)	0.529	0.835 (0.217-3.212)
No	5 (23.81)	16 (76.19)		
Playing habit with cats				
Yes	1 (16.67)	5 (83.33)	0.604	0.68 (0.071-6.515)
No	10 (22.73)	34 (74.27)		
Working in garden				
Yes	7 (25.93)	20 (70.07)	0.353	1.662 (0.418-6.606)
No	4 (17.39)	19 (82.61)		
Raw meat eating habit				
Yes	2 (15.38)	11 (84.62)	0.404	0.566 (0.105-53.896)
No	9 (24.32)	28 (75.68)		
Consumption of pork				
Yes	3 (30)	7 (70)	0.382	1.714 (0.361-8.147)
No	8 (16)	32 (64)		
Drinking water				
Treated	1 (20)	4 (80)	0.699	1.143 (0.114-11.413)
Untreated	10 (22.2)	35 (77.8)		
Age group				
15-25 year	10 (29.41)	24 (70.59)	0.064	6.25 (0.725-53.896)
26-35 year	1 (6.25)	15 (93.75)		
Level of education				
Illiterate	2 (20)	8 (80)	0.618	0.861 (0.154-4.799)
Literate	9 (22.5)	31 (77.5)		
Occupation				
Housewives	9 (22.5)	31 (77.5)	0.618	1.161 (0.208-6.473)
Others	2 (20)	8 (80)		

OR = Odds ratio CI = Confidence Interval

Among 50 total participants, 6 (12%) had habit of playing with cat and only one of them were seropositive. This seroprevalance did not reach statistical significance (OR = 0.68: CI=0.071-6.515: P = 0.604). From the questionnaire survey, domestic cats were recorded from 29 (58%) study participants, of which 6 (20.69%) were positive for anti-*T. gondii* antibody. However, this prevalence did not reach statistical significance (OR =0.835: 95% CI =0.217- 3.212: p value = 0.529).

Majority of pregnant women (54%) had reported to work in garden or farming activities which indicate frequent contact with soil. Of which 7 (25.93%) were seropositive. But there was no significant difference (OR=1.662: CI=0.418-6.606: P=0.353) between seropositive rate and contact with soil.

Regarding the pork eating habit of pregnant women, 10 (20%) was found to eat pork, of which 3 (30%) were seropositive for anti-*T. gondii* antibody. But this seroprevalence of toxoplasmosis in pregnant women with pork eating habit was not statistically difference (OR=1.714: CI=0.361-8.147: P = 0.382). Twenty six percent pregnant women were found to have habit of eating raw meat. However, there was no significant association (OR=0.566: CI= 0.105-53.896: P=0.404) between *T. gondii* infection and raw meat eating habit. With regard to the association of seroprevalance with the type of drinking water, 45 (90%) pregnant women were observed to drink untreated water. Twenty two percent of them were found seropositive. Type of drinking water did not show significant association (OR=1.143: CI=0.114-11.413: P=0.699) with *T. gondii* infection.

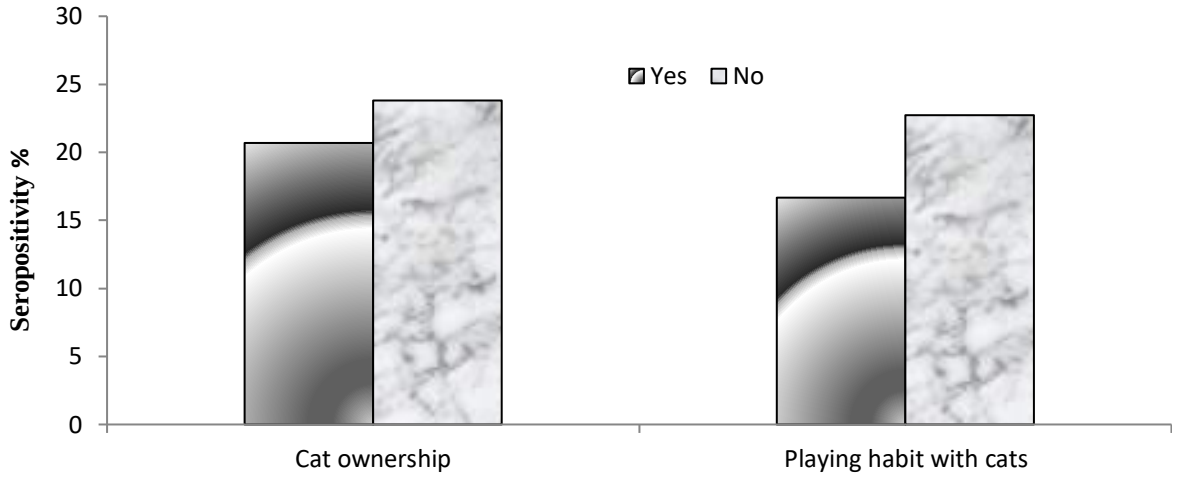


Fig 2. Toxoplasmosis based on cat ownership and playing habit with cats

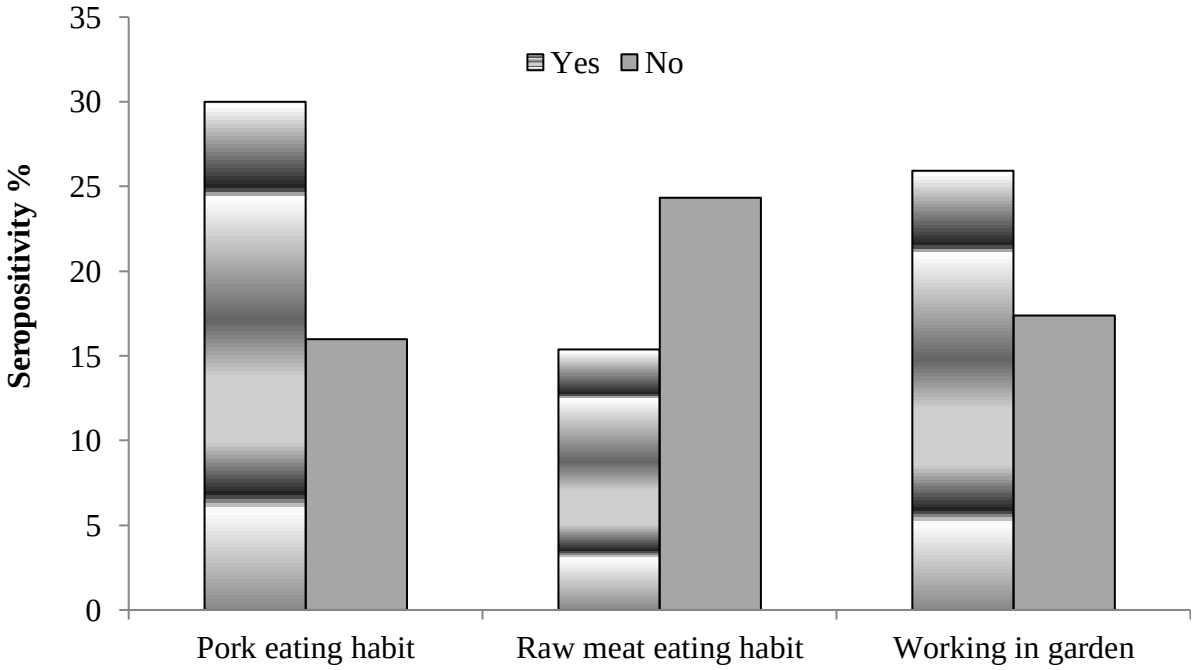


Fig 3. Distribution of *T. gondii* infection in pregnant women with different characters

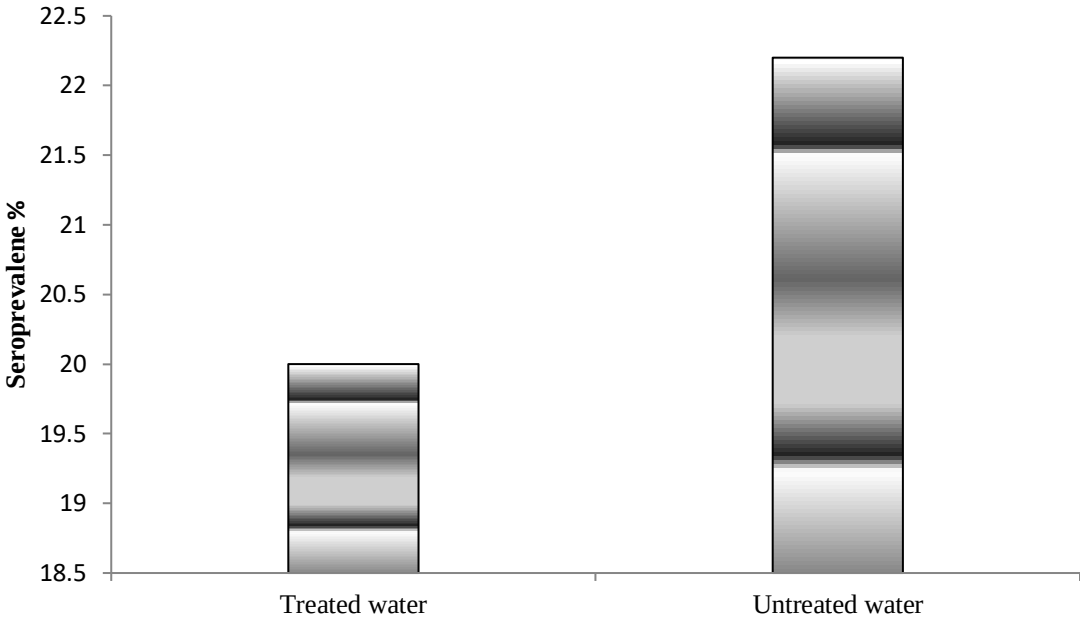


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Fig 4. Toxoplasmosis in relation to type of water

Among the 50 total respondents, the highest prevalence was found within the age range of 15-25 years that is 29.41%. But there was no statistically significant difference in the prevalence of *T. gondii* in pregnant women of different ages (OR=6.25: CI=0.725-53.896: P =0.073). Regarding the occupation of the participants, 78% were housewives. Of these, 20.51% were seropositive for *T. gondii*. There was no significant difference (OR=1.161: CI=0.208-6.473: P=0.655) in *Toxoplasma* seropositivity among individuals with different occupation. With regard to the association of seroprevalance with educational background, 10(20%) of the pregnant women were illiterate, of which 2(20%) were seropositive. However, this prevalence was not statistically significant (OR=0.861: CI=0.154-4.799: P= 0.969).

5. DISCUSSION

Toxoplasmosis is a disease usually asymptomatic in healthy adult but lead to severe consequences in an immunocompromised person such as an AIDs patient or pregnant women. It has a worldwide distribution in human population infecting up to one third of global population and a wide range of other mammals and avian species. Nearly half of Nepalese are *Toxoplasma* seropositive (Rai et al. 2011). However, the seroprevalence of *T. gondii* infection in pregnant women have been poorly studied probably due to lack of diagnostic facilities.

The present study sought to determine the seroprevalence of *Toxoplasma gondii* infection in pigs and pregnant women of Bhaktapur districts. Overall seroprevalence of 22% was found among pregnant women. This finding was lower (53.4%) than the prevalence reported from the study carried out by Rai et al. (1998) among pregnant women with bad obstetric history using micro latex agglutination (MLA) and ELISA methods. Zemene et al. (2012) with the use of ELISA methods reported overall seroprevalence of 83.6% in Ethiopia in 2012. Study carried out using similar methodology by Ayi et al. (2009), in Greater Accra region of Ghana showed 92.5% IgG seroprevalence. Lelong et al. (1995) reported a high *Toxoplasma* seroprevalence (75%) in young pregnant women in Madagascar. In Brazil, Sroka et al. (2010) using micro particle enzyme immunoassay found IgG seroprevalence of 68.6%. In contrast, present finding is higher than the prevalence reported in Korea where the Sabin-Feldman dye test (DT), latex agglutination test (LAT) and IgG enzyme linked immunosorbent assay (IgG ELISA) indicated seroprevalence of 3.7%, 3.4% and 4.0% respectively (Han et al. 2008). Alvarado-Esquivel et al. (2006) in Mexico found seroprevalence of 6.1% among pregnant women using IMx toxo IgM and IMx Toxo IgG 2.0 kits.

Seropositivity among pigs in Nepal was 79.6% (Rai et al.1996), in Grenada was 44.1% (Chikweto et al. 2011), in Massachusetts, 92.7% (Dubey et al. 2002), in Costa Rica 43.8% (Arisa et al. 2004) and in Brazil 11.5% (Frazao-Teixeira and Oliveira 2011). In contrast, in present study seroprevalence in sampled pig's blood was observed nil. This negative result

indicates that there was no prior exposure to the *Toxoplasma gondii*. These individuals are presumed to be susceptible to a primary infection.

Nijem and Al-Amleh (2005) in Palestine and Zemene et al. (2012) in Ethiopia showed increase seropositivity with increase in age of pregnant women. But in the current study, seropositivity of anti *T. gondii* antibody was observed higher (29.41%) in the age group 15-25 year that is also consistent to the study conducted by Rai et al. in 1998 and 1996. He reported that over 40% of Nepalese acquire *Toxoplasma* infection by the age of 20. Similarly Lelong et al. (1995) also reported higher seroprevalence of 75% in young pregnant women in Madagascar indicating that most of the infections occur during an early life. The observation showed younger women are more prone to risk factors in compare to older women.

Many workers have correlated prevalence of toxoplasmosis in the human population with various risk factors. Alvarado-Esquivel et al. (2011) showed that *Toxoplasma* infection was associated with consumption of raw meat (OR= 5.77; 95% CI: 1.15-28.79; P = 0.03), unwashed raw fruits (OR = 2.50; 95% CI: 1.11-5.63; P = 0.02), and living in a house with soil floors (OR = 3.10; 95% CI: 1.22-7.88; P = 0.01). Liu, et al. (2009) in China identified eating raw or undercooked meat and unwashed raw vegetables or fruit as possible risk factors of toxoplasmosis. Similarly Studenicova et al. (2005) found consumption of raw meat and unwashed raw vegetables and fruits are possible risk factors among pregnant women in Slovakia while in Korea, Han et al. (2008) found consumption of raw meat was main route of *T. gondii* infection but was not associated with the consumption of unwashed vegetables and drinking untreated water. In present study, 26% and 54% of pregnant women participated in questionnaire were found with raw meat eating habit and working in garden respectively but observed no significant association with *T. gondii* infection. Similarly, thirty percentage of pregnant women having pork meat eating habit were seropositive for toxoplasmosis but was not significantly associated which was in contrast with Dubey (2009) and Duan et al. (2012) who found pork meat as a major risk factor for infection.

Felids, being able to excrete the environmentally resistant oocysts, are the key animal species in the life cycle of *T. gondii*. Swai and Schoonman (2009), in Tanzania, reported pets keeping, especially cats were associated with toxoplasmosis. But Ertug et al. (2005), faced

difficulties to assess association between cats and human toxoplasmosis by epidemiological surveys as no oocysts found on cat fur. Oocysts are often buried in soil along with cat feces, and soil contact is universal and difficult to avoid. There is always possibility that oocysts in cats may gain infectious ability outside their body or through contact with soil. Cat ownership, playing habit with cats and working in garden or contact with soil were assessed in the current study, however, no significant association was found.

Contaminated drinking water is also a potential source of *T. gondii* infection (Bowie et al. 1997). Han et al. (2008) in Korea showed significant association between toxoplasmosis and drinking untreated water. Significant association between toxoplasmosis and drinking general supply water, well water by pregnant women were reported in Cameroon and Nigeria (Nijunda et al. 2011 and Ishaku et al. 2009). Questionnaire with pregnant women revealed 78 % (39) of them were found to drink tap water, 4 (8%) use well water and 7(14%) use jar water and only 5 (10%) of them use treated (boiled) water. However no significant association between source of drinking water and *Toxoplasma* infection was observed.

Studies conducted by Jones et al. (2001), Nash et al. (2005) and Varella et al. (2003) showed a significance decreases in seropositivity as the level of the education increases but current study found no statistical difference between *T. gondii* infection and the level of education. *T. gondii* infection and history of abortion, gestational age were observed insignificant in current study that coincide to the results of similar work by Ertug et al. (2005) and Ataeian and Tadayon (2000). *T. gondii* infection was not observed associated with religion and occupation in Bhaktapur district during study period.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The overall seroprevalence of anti- *T. gondii* IgG antibodies was found to be 22% among pregnant women during study. The present percentage of prevalence rate in pregnant women is low as compared with those reported in other regions of Nepal. The prevalence was observed higher in the age group 15-25 year indicating that most of the infections occur during an early life. Along with, present study did not found significant association of toxoplasmosis with any of the possible risk factors.

6.2 Recommendation

- Since, no significant association was observed between toxoplasmosis with either of the risk factors, showing these risk factors are not the exact source of infection. Further research is needed to find out exact source of infection in human and animals.
- Majority of pregnant women and pig farmer are unknown about toxoplasmosis. So health education and awareness should be launched to reduce the risk of infection in pregnant women

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APPENDIX I

Systematic position of *Toxoplasma gondii*

Kingdom : Alveolata

Phylun : Apicomplexa

Class : Coccidia

Subclass : Eucoccidiorida

Order : Eimeriorina

Family : Sarcocystidae

Genus : *Toxoplasma*

Species : *gondii*

(from : http//www.ncbi.nlm.gov/Taxonomy/)

Life cycle

T. gondii has a complex life cycle that includes sexual and asexual reproduction (Dubey and Beattie, 1988 and Howe and Sibley, 1995). The life cycle of parasite was describe only in 1970 when it was discovered that the definitive hosts are the member of the family felidae, including domestic cats in which sexual reproduction occur. Various warm-blooded animals in which asexual reproduction occur serve as intermediate hosts. Sexual reproduction occur within enterocytes of the cat intestine producing millions of thick walled zygote containing cysts known as oocysts. Oocysts are shed by cats into the environment and they find their way to the intermediate host via comtaminated soil, food and water. Oocyst sporulate outside the body and becomes infectious one to five days after excretion which contain ttwo sporocysts, each containing four sporozoites. Sporulated oocysts can survive and remain infective for many months in cold and dry climates (Dubey et al. 2011). In the intermediate hosts, the cyst wall is dissolved by proteolytic enzymes in the stomach and intestine freeing infectious *T. gondii* parasites to invade host cells (Dubey, 2009) where they replicate by endodyogeny (asexual reproduction) producing rapidly growing highly infective tachyzoites during acute infection (Howe and Sibley, 1995). In chronic infection, the parasites convert to slowly growing bradyzoites, which are encysted to form tissue cysts and reside in host cells

(Dubey and Frenkel, 1976). The cellular differentiation of *T. gondii* from tachyzoites to the bradyzoite tissue stage is the underlying cause of chronic toxoplasmosis. These bradyzoites reactivate and convert to tachyzoites if host immune response is compromised, such as in AIDs patients (Montoya and Liesenfeld, 2004). *T. gondii* is the only apicomplexan parasite that can transmit directly orally among intermediate host and cause infection without cycling through its definitive hosts. This is the reason for potential spread of parasite among the wide range of intermediate hosts.

APPENDIX II

Questionnaire to pregnant women

Name:

Address:

Age :

Religion:

Occupation:

Level of Education:

Parity no.:

1. Have you owned cat at your home?

a. Yes b. No

2. Do you or any member of your family enjoy playing with cat?

a. Yes b. No

3. Do you have any livestock other than cat?

a. Yes b. No

If yes, what are they?

4. What safety measures do you apply while working in the gardening and field?

a. wearing gloves

b. wearing boots

c. others

5. Are you vegetarian or non vegetarian?

a. vegetarian b. non vegetarian

6. If non vegetarian, what type of meat you usually consume?

a. Pigs b. Buffalo c. Chicken d. Others

7. What type of dishes you prefer?

a. a. Raw meat b. Well cooked

If vegetarian, do you have habit of eating raw vegetables?

a. Yes b. No

8. What are the sources of your drinking water?

a. Well b. Tap c. Mineral water d. Jar e. Others

9. Is there any history of abortion or still birth?

a. With yourself Yes/No

b. In your family Yes/No

c. Maternal home Yes/No

d. Neighbor Yes/No

If Yes, a. When ?

before 1 year before 2 yr More than 2 yr

b. Frequency

Once Twice More than twice

10. How many children do you have?

a. One b. Two c. more than 2

11. What is there physical development?

a. a. Healthy b. Weak c. Unhealthy

If unhealthy, Are they suffering from any kind of diseases?

12. What is their mental development?

a. normal b. abnormal

13. Do your child have any eye related problem?

a. Yes b. No

If Yes,

a. By Birth b. After birth

14. Have you ever been diagnosed of Toxoplasmosis before?

a. Yes b. No

15. If Yes, Have you done treatment for it? And what?

16. Have you ever heard about toxoplasmosis ?

a. Yes b. No

APPENDIX III

Questionnaire to Pig Farmers

Name:

Address:

Cast:

Religion:

Occupation:

Level of education:

Number of Pig in the farm:

1) Which breed of pigs do you have?

a. Yorkshire b. Hampshire c. Crossed d. Other

2) What kind of food do you provide them?

3) Can you see cat around the pig farm?

a. Yes b. No

4) Do cat disturb in fodder of Pigs?

a. Yes b. No

5) Do you feed your pig raw meat?

a. Yes b. No

If yes what type?

a. Buffalo b. Chicken c. Others

6) Water availability?

a. Yes b. No

If yes, what type of water supply do you have?

a. Tap water b. River c. Well d. Other

7) Is there any livestock present in or around farm?

a. Buffalo b. Sheep c. Chicken d. Ducks e. Others

8) Do you think your Pigs are healthy?

a. Yes b. No

If No,

a. Labored breathing b. Diarrhea

c. Loss of vision d. Inactivity

e. Abortion f. Others

9. Have your sows aborted any piglets?

a. Yes b. No

10. Have the piglets become sick within three weeks of birth or died soon after birth?

a. Yes b. No

11. Do you have any idea why the sows aborted?

12. How often you consult a veterinarian?

13. How often do you clean your farm?

14. Have you ever got training or taken courses about pig farming?

a. Yes b. No

If Yes, what was the training about?

a. Pig husbandry and management

b. Pig disease and infection

c. Others

15. How long have you been raising pigs?

16. Do you have any ideas about diseases transmitted by Pigs?

APPENDIX IV

Performance characteristics of 'DS-EIA-ANTI-TOXO-G-FAST' ELISA kit.

Diagnostic sensitivity: ≥ 98%

It has been calculated on 150 positive samples from different donors (samples from vaccination are acceptable) determined by the reference method.

Diagnostic specificity: ≥ 98%

It has been calculated on 100 negative samples from different donors determined by the reference method.