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Sero-Prevalence of *Toxoplasma Gondii* and Risk Factors Associated with Pregnant Women of District Dir Lower, Pakistan

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ABSTRACT

Toxoplasmosis is worldwide distributed zoonotic disease that infects about 20% to 90% adult population of the world. The parasite is usually found all around the globe which infects all warm-blooded creatures including human beings. Therefore, the current study was conducted to determine the seroprevalence of Toxoplasma gondii and risk factors associated with pregnant women of District Dir Lower, Pakistan. Blood samples from 200 pregnant women were collected and their corresponding serums were tested for anti-toxoplasma IgM, IgG antibodies. During serological analysis, two various tests were used to screen the collected serum i.e. Rapid Diagnostic Test (RDT) and ELISA. All the samples were screened through RDT for target etiological agent and the positive cases were then confirmed using ELISA. A self-design questionnaire was used to collect the socio-demographic information of the patient. Out of total 200 samples, 34 samples were seropositive and 166 samples were seronegative for etiological agent by Rapid Diagnostic Test. Among 34 positive samples, 30 were seropositive for IgG antibody and 4 were seropositive for IgM antibody. None of the pregnant women were reactive to both T. gondii IgM and IgG antibodies. The overall seroprevalence of the infection was 17%. All the positive samples were also screened by mean of ELISA for both IgG and IgM antibodies. Of the 34 positive samples, 27 specimens were sero-positive for T. gondii IgG antibody and 3 specimens were sero-positive for T. gondii IgM antibody. The current study concluded that the infection of Toxoplasma gondii is relatively high. Having contact with domestic animals, drinking pipe water (general network) source and consumption of raw fruits/vegetables were recognized as probable risk factors associated with the infection of T. gondii. Moreover, the burden of disease was found to be high in the second trimester of pregnancy.

INTRODUCTION

Toxoplasmosis is a parasitic zoonotic disease triggered by an obligate intracellular protozoan parasite known as *Toxoplasma gondii* (Hotez and Kamath, 2009; Kijlstra and Jongert, 2009; Mazigo et al., 2013; Tenter et al., 2000). This pathogen infects all warm-blooded animals, including humans, and is globally distributed (Dubey, 2008a; Elmore et al., 2010). Toxoplasmosis is considered one of the most widespread parasitic infections, affecting approximately 20% to 90% of the global adult population (Zemene et al., 2012; Carter and Fleck, 1966). It is regarded as the third most significant food-borne infection leading to mortality, following listeriosis and salmonellosis (Scallan et al., 2011).

The global seroprevalence rate of *T. gondii* varies significantly by region, ranging from less than 10% to more than 80%. Higher prevalence rates—above 50%—are typically reported in the Middle East, Latin America, and sub-Saharan Africa (Varella et al., 2009; Lopes-Mori et al., 2013; Doudou et al., 2014; Agmas et al., 2015). Conversely, lower to moderate rates (7%–50%) are observed in developed nations, including the United States, Europe, and parts of Northeast Asia (Findal et al., 2015; Hung et al., 2015; Jones et al., 2014).

Toxoplasma gondii undergoes a complex life cycle consisting of both sexual and asexual stages. The sexual phase occurs exclusively in felids (domestic and wild cats), which are the definitive hosts, while the asexual stage takes place in nearly all warm-blooded animals, including humans, who serve as intermediate hosts (Weiss and Kim, 2011; Nissapatorn, 2011). The parasite exists in three infectious stages: the rapidly dividing tachyzoite, the slowly dividing bradyzoite within tissue cysts, and the

environmentally resistant oocyst (Weiss and Kim, 2011; Hogan et al., 1960).

Humans typically acquire toxoplasmosis through ingestion of undercooked or raw meat containing tissue cysts, or food and water contaminated with oocysts shed in the feces of infected cats (Ahmadpour et al., 2014; Elsheikha, 2008; Retmanasari et al., 2017; Gencer et al., 2014; Saki et al., 2015; Saki et al., 2016). Less commonly, infection can occur via organ transplantation or blood transfusion (Robert-Gangneux and Dardé, 2012).

Although *T. gondii* infection is often asymptomatic, it can lead to flu-like symptoms such as fever, headache, and lymphadenopathy in some individuals (Robert-Gangneux and Dardé, 2012). It poses a significant risk to immunocompromised individuals (e.g., HIV/AIDS patients or those undergoing chemotherapy), where it can result in serious ocular and neurological complications such as visual toxoplasmosis or toxoplasmic encephalitis (Minkoff et al., 1997; Lindström et al., 2006; Ahmadpour et al., 2014).

Of particular concern is toxoplasmosis during pregnancy. When primary infection occurs during pregnancy, *T. gondii* can cross the placenta and lead to congenital toxoplasmosis, potentially causing miscarriage, stillbirth, or severe birth defects such as hydrocephalus, chorioretinitis, and neurological deficits (Jones et al., 2010; Jones et al., 2003; Peyron et al., 2017). Infections acquired prior to conception generally pose little risk of congenital transmission (Saadatnia and Golkar, 2012), while the likelihood of fetal infection increases with gestational age (Foulon et al., 1999). However, infections in early pregnancy tend to result in more severe fetal damage (Foulon et al., 1999; Robert-Gangneux and Dardé, 2012).

The detection of *T. gondii* infection during pregnancy relies heavily on serological testing for IgG and IgM antibodies, as well as PCR detection of parasitic DNA in amniotic fluid (Sukthana, 2006; Singh, 2003). Countries like France and Austria have implemented routine prenatal screening programs, significantly enhancing early detection and prevention efforts (Wong and Remington, 1994; Singh, 2003; Remington et al., 2004; Breugelmans et al., 2004).

Management of congenital toxoplasmosis includes pharmacological interventions, such as the administration of spiramycin or a combination of pyrimethamine and sulfadiazine, not to cure the infection but to reduce fetal transmission and potential damage (Kijlstra and Jongert, 2009; Breugelmans et al., 2004).

In Pakistan, especially in rural and underdeveloped areas like District Dir Lower, factors such as close contact with domestic animals, poor food hygiene practices, inadequate health education, and limited access to diagnostic facilities may contribute significantly to the disease burden among pregnant women. Despite the potential severity of congenital toxoplasmosis, there is a lack of routine screening programs and awareness in such settings. Therefore, understanding the sero-prevalence and risk factors in this population is critical to devising effective public health interventions.

AIMS AND OBJECTIVES

- Identification and sero-prevalence of anti-*Toxoplasma gondii* IgM and IgG among pregnant women of district Dir lower, Pakistan.
- Meticulous investigation of socio-demographic characteristics of the pregnant women associated with Toxoplasmosis in study area.
- To determine the risk factors and mode of transmission of toxoplasmosis among the pregnant women population of Dir Lower.

MATERIALS AND METHODS

Study Area

This study was carried out at District Head Quarter (DHQ) Hospital Timargara, District Dir Lower Khyber Pakhtunkhwa, Pakistan.

Study Samples

Under aseptic conditions, initially 4 to 5 ml of blood sample was collected from T. gondii suspected women with the help of disposable sterile syringe. About 200 blood sample was collected from suspected women who were directed by the clinician for T. gondii diagnosis was used in this study. The age groups of suspected patients were range from 17 to 36 years old. A socio-demographic based questionnaire was designed for the collection of information including age, occupation, educational level, socioeconomic status and exposure to animal index from the suspected pregnant women for the study.

Inclusion Criteria

Consenting pregnant women of all stages of pregnancy as well as age group 17-36 years and who belongs to Dir lower were included in this study.

Exclusion Criteria

Non-pregnant women and those out of District Dir Lower as well as women who did not consent to participate were excluded from this study.

Methodology

Questionnaires History

An organized questionnaire was used and a complete history of each and every case was obtained to assess the potential hazard determinants for the disease. Mainly the questions were concentrated on the following conceivable hazard determinants of toxoplasmosis:

- Contact with animal.
- Milk consumption.
- Meat consumption.
- Consumption of raw fruits/vegetables.
- Drinking water sources.

Socio-demographic Characteristics

The clinical and sociodemographic details comprising age, occupation, socioeconomic position, level of education and animal catalogue exposure, were achieved from each and every participant.

Sample Collection

After previous histories collection, individuals were subjected to different laboratory diagnosis. Four to five ml of blood sample was collected from each suspected patient

through venepuncture using sterile string under aseptic condition and transferred it to sterile test tube without anticoagulant. The tubes were then labeled carefully and appropriately with the patient identity number.

Sample Processing

All samples were handled carefully according to the lab procedure. Each sample was centrifuged at 4000 rpm for 5-10 minutes to separate serum from whole blood. The serum was then transferred to Eppendorf tubes from centrifuged sample by using micro pipette and stored in freezer at 8˚C for about one week and -20˚C for further serological investigation.

Sample Screening

The samples were analyzed through serological method at Pathology laboratory of DHQ hospital Timergara. During serological analysis two different tests were performed for screening, e.g. Rapid Diagnostic test (RDT) and ELISA. All the samples were screened through Rapid Diagnostic Test (RDT) for target etiological agent and the positive cases were further verified by ELISA.

Rapid Diagnostic Test

Rapid Diagnostic Test is a lateral flow chromatographic immunoassay for the recognition and differentiation of anti-Toxoplasma IgM and IgG antibodies in plasma or serum which was performed by Onsite Toxo IgM/IgG Rapid Test Device, manufactured by CTK Biotech, Inc. USA.

Result Interpretation

- **Negative result**: Only control line (C-band) appears.
- **Positive result**:
  - C and M line = IgM positive
  - C and G line = IgG positive
  - C, M, and G lines = both IgM and IgG positive

Invalid result: No C line appears.

ELISA (Enzyme Linked Immunosorbent Assay)

The positive cases for rapid diagnostic test against *T. gondii* were further processed for IgM and IgG specific antibody by means of Enzyme Linked Immunosorbent Assay. For this purpose, commercially available anti-Toxoplasma antibodies detection ELISA kit (BioCheck, Inc. South San Francisco) was used to detect IgG and IgM antibodies from the serum samples. The ELISA kit was processed according to manufacturer's instructions.

Result Interpretation

- **Positive**: Yellow color indicates presence of IgM or IgG antibodies.
- **Negative:** No color indicates absence of antibodies.
- **Cut-off value**: Less than 1.0 = negative; greater than 1.0 = reactive or positive

Statistical analysis

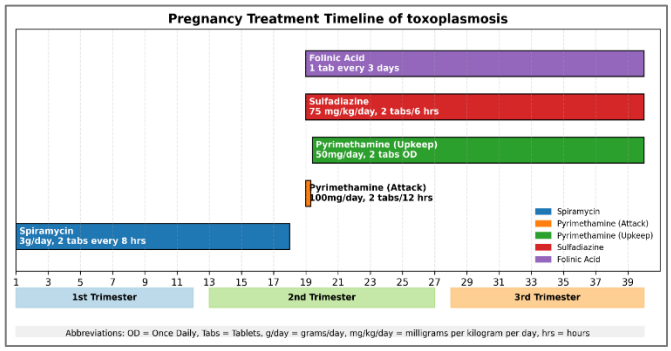
Python programming skills, tools and its libraries was used for all types of statistical data analysis

RESULTS

Therapeutic plan for toxoplasmosis during pregnancy (**Fig.1**). During 1st trimester drug Spiramycin is used, after that combination therapy consisted of sulfadiazine and pyrimethamine have been started from 18 weeks of pregnancy.

Figure 1

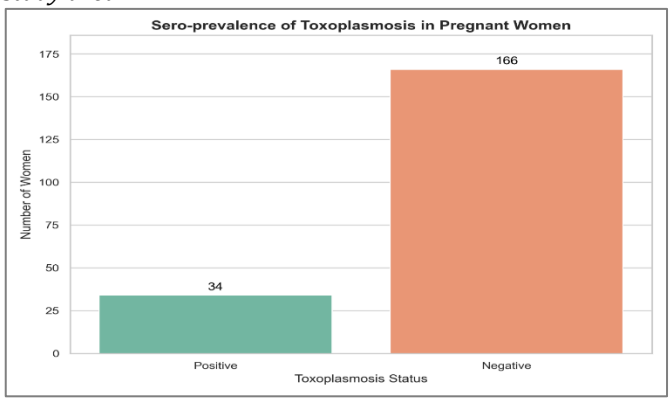
Indicating drug administration by gestational weeks, with trimester bands and treatment abbreviations.



A total of 200 pregnant women of different age groups in the present study were examined for the presence of toxoplasmosis. Out of total, 34 were confirmed by ICT as sero-positive and 166 were confirmed as sero-negative for toxoplasmosis (**Fig.2**)

Figure 2

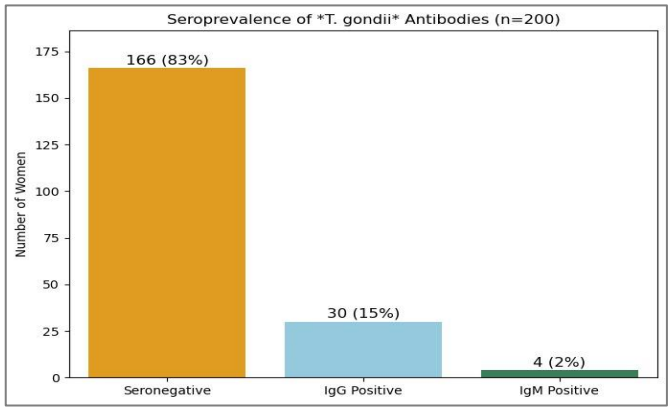
Seropositivity of T. gondii among the pregnant women in the study area.



Among 34 positive ICT cases, 30 (15%) were seropositive for antibody IgG and 4 (2%) were IgM antibody positive. None of these pregnant women were reactive to both IgM and IgG *T. gondii* antibodies. This represents the overall seroprevalence of *T. gondii* was 17% in the study area **(Fig. 3).**

Figure 3

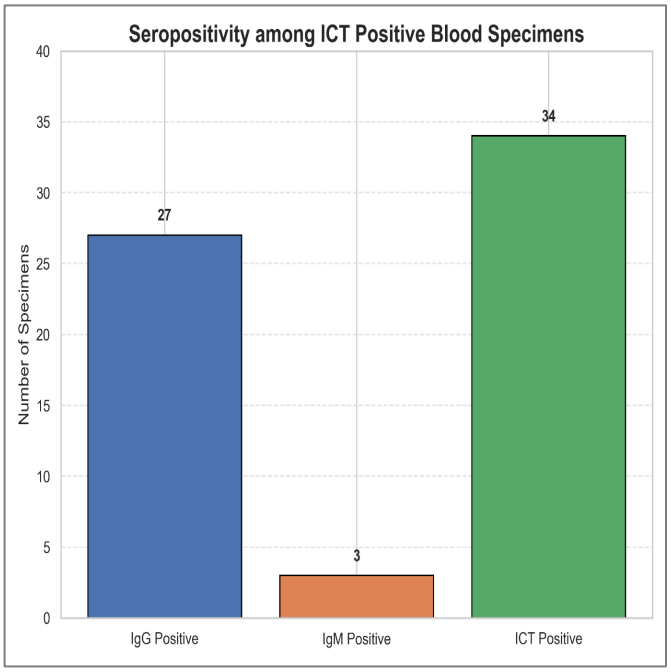
Estimation of Toxo-IgG and IgM in pregnant women by ICT in District Dir Lower



All the ICT positive blood specimens were further confirmed as positive by means of ELISA for both Toxo-IgM & IgG antibodies. Of the thirty-four ICT positive samples 27 specimens were sero-positive for *T. gondii* IgG antibody and 3 specimens were seropositive for *T. gondii* IgM antibody **(Fig. 4)**.

Figure 4

Estimation of Toxo-IgG and IgM in pregnant women by ELISA.



All the socio-demographic characteristics were asked from each of the participant with her permission via patient proforma, in which each participant was asked for her age, occupation, educational status, stages of pregnancy (trimester) and for risk factors linked with the *T. gondii* infection such as the presence of domestic animals, cats/dogs, unpasteurised milk consumption, raw meat consumption, drinking water sources and ingestion of raw fruits/vegetables.

In case of age wise distribution, the participants were divided into three age groups. Among all the age groups, the highest seropositivity (18.2 %) was observed in the age group 17-24 years, followed by the second group with (16.7%) while the 3rd age group contributed with the less number (15%) of seropositive cases.

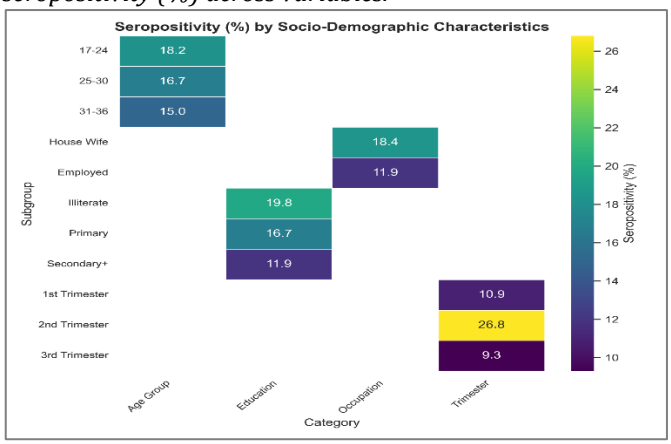
The occupational status of each participant was also examined, in which majority of the positive cases were identified in house wives with the prevalence rate of (18.4%), followed by employed women with (11.9%).

Of the participant educational status, most of the positive cases were identified in illiterate women who unable to read and write, followed by the women with primary education and secondary and above, with the positivity rate of (19.8%), (16.7%) and (11.9%) respectively.

Regarding the trimester of pregnancy, the highest prevalence rate (26.8%) was found in the second trimester, followed by first trimester (10.9%) and third trimester (9.3%) (**Fig.5**).

Figure 5

Socio-demographic characteristics of pregnant women (n=200) visited DHQ Hospital Timergara showing seropositivity (%) across variables.



As we mentioned earlier that the participants were also asked for the possible risk factors in the patient proforma. Among the participants, the highest prevalence rate (20.6%) was identified in those women who had domestic animal in their home, while (9.4%) in those who did not have any contact with the domestic animal**.**

The seroprevalence rate with respect to the presence of cats/dogs at home, the highest rate, 24% (25/104), was identified in those women who had no contact with cat/dogs in comparison to those who had in touch with cat/dogs (9.4%).

Regarding to the dietary habit of the participants, the highest rate of prevalence (18.2%) was reported in those women who did not utilize unpasteurized milk as compare to those who utilize unpasteurized milk (14.7%).

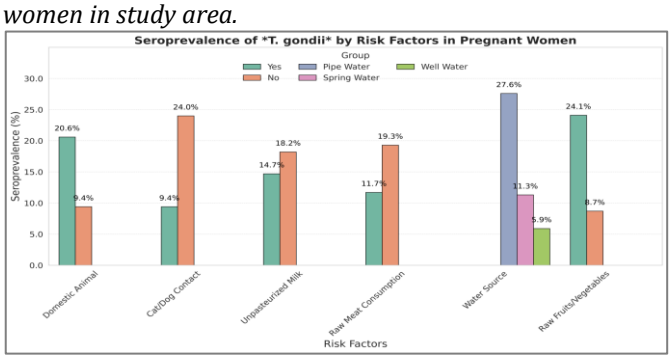
While in case of raw meat consumption, the highest prevalence rate (19.3%) was found in those who did not eat raw meat, followed by those who ate raw meat which was (11.7%).

Among the participants who utilize pipe water as drinking source had more prevalence rate (27.6%) in the study area, followed by in those who utilized spring water (11.3%) and well water (5.9%) as a drinking source.

In case of raw fruits/vegetables consumption, the highest rate of *T. gondii* (24.1%) was found in those pregnant women who consumed raw fruits/vegetables in comparison to those who did not consume raw fruits/vegetables (**Fig.6**)

Figure 6

Risk factors associated with T. gondii among the pregnant women in study area.



DISCUSSION

Toxoplasmosis is globally distributed zoonotic disease that infect approximately one third of the world's human population (Rostami *et al.*, 2017). The disease can from mother to fetus and leads to congenital toxoplasmosis during pregnancy which may cause severe obstetrics complication such as premature birth, miscarriages and abortion. On other hand in surviving babies, the infection can cause consequences such as hydrocephaly, cerebral calcification, mental retardation and even blindness to the infected new-born (Fallahi *et al.*, 2018). Therefore, the current study was conducted to determined the sero-prevalence of Toxoplasma gondii and risk factors associated with pregnant women in the study area.

In the current study, the overall sero-prevalence of *T. gondii* infection amongst pregnant women was 17%. Thirty (15%) were only IgG seropositive and 4 (2%) were IgM seropositive. None of these pregnant women were positive for both IgM and IgG. Overall, 4 (2%) pregnant women were positive for IgM antibodies. Presence of IgM antibodies amid pregnancy indicates the presence of acute *Toxoplasma* infection and a sign of higher risk of mother-fetus transmission (Tekkesin, 2012). Prior study in this regard showed that in the absence of cure the risk of congenital infection from acute *Toxoplasma* infection in gestation is around 50% (Paquet *et al.*, 2013). Initial diagnosis of infections in pregnant mothers is of great significance for first initiation of measures that decreases the transmission risk and potential consequences on the newborn. So, screening of *T. gondii* infection ought to be considered as a part of the prenatal investigation during antenatal care follow up.

The overall seroprevalence of *T. gondii* infection among pregnant females was in agreement with the study carried out in Rawalpindi–Islamabad, Pakistan, where the overall sero-prevalence was found 17% (Khan *et al.*, 2011; Tenter *et al.*, 2000), in Swabi, Pakistan, 19.25% (Faisal *et al.*, 2014). The results of current study are also in association with studies conducted at London, Singapore and Thailand where overall seroprevalence of *T. gondii* was reported 17.32%, 17.2% and 21.6%, respectively, in women of child-bearing age (Flatt and Shetty, 2012; Wong *et al.*, 2000).

In contrast to our study, higher sero-prevalence rate 48% and 63% was reported among pregnant women from Azad Kashmir and Punjab, Pakistan, respectively (Khan *et al.*, 2011), from Tanzania, 30.9% (Mwambe *et al.*, 2013), Iran, 31.42% (Mohaghegh *et al.*,

2016), Burkina Faso, 31.1% (Bamba *et al.*, 2017), Northern Tanzania, 45% (Paul and

Chilongola, 2017), Yemen, 45.4% (Al-Eryani *et al.*, 2016) and Ethiopia 85.3% (Abamecha and Awel, 2016). On other hand, the lower seroprevalence in pregnant women was reported from Taiwan 7.7% (Hung *et al.*, 2015), Norway, 9.3% (Findal *et al.*, 2015), Zambia, 5.87% (Frimpong *et al.*, 2017) and Korea 3.7% (Aqeely *et al.*, 2014). The variances in sero-prevalence of *T. gondii* found might be due to dissimilarities in geographical dissemination of the parasite, personal hygienic practices, socio-economic, nourishing habit of the study population and difference in test techniques may also account for the variation.

In this study, the seroprevalence of IgG and IgM among the study participants was 15% and 2% respectively. This rate is slightly greater than rates reported from Saudi Arabia (Alanazi *et al.*, 2017), where the sero-prevalence of IgG and IgM among pregnant women was 13.5% and 0.6% respectively. On other hand, it is lower than the study carried out in Riyadh, where IgG and IgM prevalence was 32.5% and 6.4% respectively (Alghamdi *et al.*, 2016) and Nigeria, where the sero-prevalence of IgG and IgM was 30.8 % and 3.3 % respectively (Yusuf *et al.*, 2016). Such variation in seroprevalence could be due to differences in behavioral and in climatic conditions, where higher seroprevalence is related with wetter and hotter areas, which is good for sporulation of oocysts related to less moist areas (Zemene *et al.*, 2012).

Presence of domestic animals is also mentioned as a predictor for Toxoplasmosis. The prevalence rate in our study indicates (20.6%) of pregnant women had contact with domestic animals compared to those who did not (9.4%), **(Fig.6)**. So, the result indicates that domestic animals are the risk factor of the disease. Similar risk factors are identified in some other studies (Elnahas *et al.*, 2003; Morris and Croxson, 2004) who stated greater than 34% of pregnant females have nearby interaction with household animals. Preceding studies also reported that regular contact with domestic animals was associated *T. gondii* infection (Abu-Madi *et al.*, 2010; Nijem and Al Amleh, 2009).

Cats/dogs play a major role in transmitting *T. gondii* and defecate millions of oocysts in a short time period. Though, our study showed that the presence of cats/dogs at home was not related significantly with the sero-positivity of *T. gondii* in comparison to those had no contact **(Fig.6)**. In some other studies, similar outcomes were observed in southern Brazil (Mareze *et al.*, 2019), southern Ethiopia (Jula *et al.*, 2018), and Sri Lanka (Iddawela *et al.*, 2017). In contrast, some others studies recognized significant correlation of *T. gondii* infection with the existence of cat/dogs at house (Adeniyi *et al.*, 2018; Abamecha and Awel, 2016). Variance in prevalence of parasite, the types of cats/dogs and rate of infection in cats/dog may account for observed differences.

The consumption of unpasteurized (untreated) milk could be a hazard determinant for *T. gondii* infection amid pregnancy. In this study it was observed that (14.7%) of the seropositive women had drinking habit of unprocessed cows/goats' milk **(Fig.6)**. This was in agreement with the studies conducted in Europe, which showed 6 to 17% of pregnant females in different nations devour untreated milk (Qublan *et al.*, 2002), in Rio Grande do Sul, 8.7% reported consuming untreated milk (Cademartori *et al.*, 2008), and in the State of Goiás, Brazil, 18.6% of the pregnant women used unprocessed goats milk (Avelino *et al.*, 2004) Milk has been measured to be a possible vehicle for scattering toxoplasmosis in peoples, as experimental effort has revealed that diseased animals milk comprises tachyzoites and is able to transmit the contamination to these animals' posterity (Sacks *et al.*, 1982; Hiramoto *et al.*, 2001).

There are unpredictable reports on the relationship between ingestion of raw meat with the infection of *T. gondii*. In our finding, the consumption of raw meat was less significantly associated with *T. gondii* sero-positivity as (11.7%), **(Fig.6)**. The infection rate of *T. gondii* was significantly high (19.3%) among those who did not consume raw meat compared to those who eat raw meat **(Fig.6)**. This finding is in agreement with previous studies from Southern Ethiopia (Jula *et al.*, 2018) and Iran (Eshratkhah Mohammadnejad *et al.*, 2018) who reported less or no significant association between *T. gondii* and raw meat consumption. In contrast, some other studies from northeastern China (Jiang *et al.*, 2018), Burkina Faso (Bamba *et al.*, 2017), and Egypt (Kamal *et al.*, 2015), reported a significant association of raw meat consumption with *T. gondii* infection. The observed differences might be due to differences in the prevalence of the parasite in the animals in those nations, the rate of infection in the animals, the types of meat consumed and risk factor source related with the infection of *T. gondii*.

The other predictor of *T. gondii* seropositivity in current study is raw fruits/vegetables consumption, significant association was observed between raw fruits/vegetables and *T. gondii* seropositivity in comparison who did not **(Fig.6)**. This finding is in agreement with study conducted in Northern Tanzania (Paul and Chilongola, 2017) and Southwestern Nigeria (Dairo *et al.*, 2018). In contrast, surveys carried out in pregnant women in southern Ethiopia (Jula *et al.*, 2018), Iran (Eshratkhah Mohammadnejad *et al.*, 2018), and Addis Ababa (Gelaye *et al.*, 2015), reported no significant relationship between *T. gondii* infection and the ingestion of raw or unwashed fruits/vegetables. The variation observed could be due to differences in hygienic practices of the population studied and nourishing habit.

Contaminated source of drinking water is also potential for *T. gondii* infection (Ertug *et al.*, 2005). The seroprevalence in the current study was found to be changed according to the usage of different drinking water sources. The highest prevalence 27.6% was in the pregnant women using pipe water (general network), followed by 11.3% and 5.9% using spring and well water respectively **(Fig.6)**. High seropositivity was found in pregnant women using pipe water system in Nigeria (Ishaku *et al.*, 2009), USA (Krueger *et al.*, 2014), Libya (Elsaid *et al.*, 2014), Tanzania (Mwambe *et al.*, 2013). A study conducted in Nigeria also reported a high sero-prevalence among pregnant females using well water related to those using packed water (Ishaku *et al.*, 2009). So, a high seroprevalence rate was observed in pregnant women who used untreated source of water that may account for high risk of contamination by the parasite oocysts.

In the current study, the higher prevalence 18.2% was found in age groups 17-24 years followed 16.7% and 15% in the age groups 25-30 and 31-36 years respectively. The seropositivity of *T. gondii* was not significantly diverged by the age **(Fig.5)**. This is in accord with prior studies (Gelaye *et al.*, 2015; Endris *et al.*, 2014). On the other hand, studies in Burkina Faso (Simpore *et al.*, 2006), Jimma (Zemene *et al.*, 2012) and Turkey (Ertug *et al.*, 2005) recognized significance difference by age.

In the present study, higher prevalence rate (19.8%) was recorded among pregnant women who were unable to read and write followed by (16.7%) and (11.9%) had primary and high level of education respectively **(Fig.5)**. Women who cannot read/write and low level of education were more expected to gain *T. gondii* infection than those who had high educational level. Similar finding from Brazil (Avelar *et al.*, 2018) and Ethiopia (Agmas *et al.*, 2015) reported that pregnant females with low educational level had high seroprevalence of *T. gondii* antibody in comparison to high education level. Similar results were also reported from southern Ethiopia (Jula *et al.*, 2018), and Egypt (Kamal *et al.*, 2015) that observed parental education presented a clear defensive effect for *T. gondii* positivity. This factor elucidates the observed variation among pregnant women of dissimilar occupations analyzed. In the community *Toxoplasma gondii* infection is acquired through consumption of oocyst from contaminated environments, so educational level, occupation, personal hygiene and sanitation plays a significant role in gaining infection.

In the present study, the highest seropositivity rate of infection (26.8%) was observed among pregnant women in their second trimester of pregnancy followed by 10.9% and 9.3% in the first and third trimester respectively **(Fig.5)**. This is in agreement with the studies in Egypt (Hassanain *et al.*, 2018), Ethiopia (Gelaye *et al.*, 2015), Saudi Arabia (Aqeely *et al.*, 2014), and Zambia (Babaie *et al.*, 2013) that reported highest sero-prevalence of *T. gondii* infection in the first second trimester of pregnancy. In contrast, studies from Finland (Mwambe *et al.*, 2013) and Yemen (Saif *et al.*, 2014) reported that the seroprevalence increases with increase in gestational stages, (20-70% and 23.7-45.8% respectively).

CONCLUSION

The current study revealed that infection of *Toxoplasma gondii* is present in the pregnant women of District Dir Lower that shows the seroprevalence was relatively high. Having contact with domestic animals, drinking pipeline water (general network) source and consumption of raw fruits/vegetables were recognized as probable hazard determinants linked with the infection of *T. gondii*. However, in the population of Toxoendemic regions, there is the need to improve the rapid and delicate tools accessibility and in pregnant women the use of preventive measures in order to improve case management.

Moreover, the prevalence of toxoplasmosis seems to be high in the second trimester. Therefore, the execution of routine serological analysis is essential to decrease the effects of the disease on mothers during pregnancy and on newborn babies. Additionally, there is a vital treatment and medicine requirement to reduce the risk of congenital toxoplasmosis.

Recommendations

The present study conducted in the pregnant women has the prevalence rate of 17%. Though majority of pregnant women included in the current study had never heard about toxoplasmosis. So, it is necessary to conduct a wide educational program for the entire population in District

Dir Lower illustrating the hygienic conditions in dealing with animals and the preventive methods for diseases transmitted by animals.

We recommend that similar studies should be carried out in pregnant women based on molecular level as well as further studies based on the seroprevalence of *T. gondii* in domestic animals are necessary.

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