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Seroprevalence and Associated Factors of *Toxoplasma gondii* Infection among Pregnant Women Attending in Antenatal Clinic of Arba Minch Hospital, South Ethiopia: Cross Sectional Study

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Abstract

Background: *Toxoplasma gondii* (*T. gondii*) is a ubiquitous, coccidian intracellular protozoan parasite that causes toxoplasmosis. *T. gondii* infection acquired during pregnancy may result in severe damage or death of the fetus and long-term sequelae in offspring. So far, no documented data concerning the seroprevalence of *T. gondii* infection among pregnant women is available in the study area. Therefore, this study was aimed at determining of *T. gondii* sero-prevalence and associated factors among pregnant women attending in ante natal clinic (ANC) of Arba Minch hospital, southern Ethiopia.

Methods: A facility based cross sectional study design was employed. A total of 232 pregnant women visiting Arba Minch hospital ANC from February to April, 2015 were enrolled by using systematic sampling technique. Data regarding Socio-demographic and associated factors were gathered using pretest questionnaire. Approximately 2 milliliters of blood specimen was collected and serum samples were tested for anti-*T. gondii* IgG and IgM antibody using Enzyme Linked Immunosorbent Assay. Data were analyzed using SPSS version 20.

Results: Out of the total 232 pregnant women tested, 184 were found to be seropositive, giving overall seroprevalence rate of *T. gondii* infection 79.3% (95%CI; 73.7-84.5). On multivariate analysis showed that consumption of raw meat (AOR=3.211; 95% CI: 1.592-6.477) and habit of eating raw vegetables or fruit (AOR=2.669; 95% CI: 1.264-5.639) were significantly associated with *T. gondii* infection.

Conclusion: The overall seroprevalence of *T. gondii* infection among pregnant women was high and serological evidence of primary infection was observed. Therefore, screening of *Toxoplasma* infection should be considered during ANC follow up. Moreover, health information about ways to minimize exposure to the risk

factors should be provided with main focus on not eats raw meat and raw vegetables.

Keywords: Seroprevalence; *T. gondii*; Pregnant women; Ethiopia

Introduction

Toxoplasma gondii (*T. gondii*) is a causative agent of Toxoplasmosis disease; it is an intercellular parasite infected humans and other warm blooded animals. Globally approximately a half of the world populations are infected [1,2]. Bradyzoites, tachyzoites and the sporozoites in oocysts are the three infectious stages of *T. gondii* parasite to all hosts including human being [3]. Humans usually acquire *T. gondii* infection through consumption of raw or undercooked meat. It is also contracted through the consumption of improperly washed vegetables and fruits as well as drinking of water which contains oocysts. Moreover, trans placentally tachyzoites infect fetus in pregnant women [4,5].

Toxoplasmosis is normally asymptomatic in healthy individuals but can cause maternal-fetal transmission in women who acquire primary toxoplasma infection during pregnancy [6,7]. The risk of vertical transmission and associated problems are determined by the gestational age at which the primary infection is acquired. Transmission to the fetus increases from the first trimester (10% to 24%) to the third trimester (60% to 90%), but the potential of congenital defect is more severe with earlier infections [8,9].

Diagnosis of toxoplasmosis in humans is performed using different techniques. Acute and latent *T. gondii* infections during pregnancy are mostly diagnosed by serological tests including detection of anti-*T. gondii*-specific IgM and IgG antibodies [10]. Latex agglutination (LA) testing, enzyme-linked immunosorbent assay (ELISA), and/or indirect fluorescent antibody testing (IFAT) are some of the tests that used to detect the antibodies [11].

Prevalence of *T. gondii* infection in pregnant women varies greatly among different countries. Seroprevalence of 10.3% and 69% reported from Japan [12] and Northeastern Brazil [13]. In Africa, data on *T. gondii* infection during pregnancy is scant and also the burden of *T. gondii* infection in pregnant women is an under-estimated public health concern. In these countries, higher up to 92.5% seroprevalence has been reported. Pregnant women are not routinely investigated for *T. gondii* during pregnancy, and follow-up does not exist [14,15]. In Ethiopia, toxoplasmosis is a cause of multiple diseases in humans [16]. Seroprevalence of 83.6%, 81.4% and 88.2% were also reported among pregnant women, in women of child-bearing age and HIV infected individuals from Jimma [17] Central Ethiopia [18] and Arba Minch [19] respectively.

Prevalence of *T. gondii* infection and the relative contribution of the various routes of transmission in humans have not been adequately studied in Ethiopia [20]. There is also little information concerning seroprevalence of *Toxoplasma* infection in general and in pregnant women particularly. Moreover, laboratory diagnosis of *Toxoplasma* infection is currently not practiced in all health facilities of the country. To our knowledge, the seroprevalence of *T. gondii* infection in pregnant women particularly have not previously been reported in the study area. Therefore, the present study was aimed to determine sero-prevalence of *T. gondii* infection and to identify associated risk factors among pregnant women attending ANC in Arba Minch hospital, South Ethiopia.

Methods

Study design and setting

A facility based cross sectional study was conducted in Arba Minch Hospital ANC clinics. Arba Minch hospital is found in Arba Minch Town located 505 kilometers in the southern part from Addis Ababa, capital city of Ethiopia and serves more than 2 million people in South Nations Nationalities and Peoples Regional State in Ethiopia. The hospital provides services such as Out Patient Department, Inpatients (medical, surgical, obstetrics and gynecology), ophthalmology, MCH, pharmacy, laboratory and ART and TB clinics. The study was conducted from February to April, 2015.

Sample size and sampling technique

Sample size was determined using single population proportion formula to estimate the prevalence of *T. gondii* and HBV infection among pregnant mothers attending ANC in Arba Minch general hospital (large project). The sample size is calculated based on the following assumptions: prevalence of *T. gondii* infection among pregnant women 83.6% in Jimma [17] and prevalence of HBV in Mekelle (8.1%) [21]; 95% level of confidence, 5% margin of error and 10% non-response. Considering all the above constants the calculated sample size for *T. gondii* infection and HBV was 232 and 126. Finally, the larger sample size (232) was taken. Systematic sampling technique was used to recruit pregnant mothers in the study. A total of 696 pregnant women attended the ANC clinic during

the past three months before study was initiated. This number was divided for the sample size to get the sample interval (k value) which is 3. Therefore, every 3rd mother attending the clinic was enrolled in to the study until the calculated sample size was achieved within three months of data collection.

Data collection

A pre-tested questionnaire was used to collect information on socio-demographic and other predisposing factors to *T. gondii* infection. Nurses working in ANC clinic of the hospital was required, trained and collected the data by face-to-face interview. Following the interview, approximately 2 ml of venous blood was collected aseptically from each consenting study participant. Then serum was separated and stored at -20°C prior to assay. Finally, the serum was tested for anti-*T. gondii* IgM and IgG antibody using Enzyme Linked Immunosorbent Assay test kit (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden Germany) at Arba Minch National blood bank center laboratory, strictly following the manufacturer's instruction.

Data analysis

All collected data from questionnaire and laboratory were checked for completeness and consistency, and then the data was entered into computer, cleaned and analyzed using SPSS version 20.0 software package. Descriptive statistics was performed to describe demographic profile of the study participants. Bivariate and multivariate logistic regressions were used to assess the association between potential risk factors considered and *T. gondii* infection. Variables with p-value <0.25 by the bivariate analysis were entered into multivariate model. At multivariate logistic regression, p-value <0.05 was considered as statistically significant for all variables.

Ethical considerations

Before study is conducted, ethical clearance letter was obtained from Arba Minch University, College of Medicine and Health Science Research Ethical Review Committee. Permission to conduct the research was obtained from Arba Minch Zonal Health Bureau and Arba Minch Hospital. Moreover, written informed consent was obtained from all study participants prior to interview and blood collection. Confidentiality of the collected information and laboratory test results was maintained. Individual test results were communicated with the attending physician for further management of the cases.

Results

Seroprevalence of *T. gondii* infection

A total of 232 pregnant women of age between 15 to 38 years (mean 25.98 years) who attended Arba Minch Hospital ANC clinic were included and tested for anti-*T. gondii* IgG and IgM antibody, 184 were found to be seropositive, giving an

overall prevalence of 79.3% (95%CI; 73.7-84.5). Out of the total seropositive 175 (75.43%) were only IgG seropositive, 9(3.9%) were IgM seropositive and 2 of the 9 pregnant women were positive for both IgG and IgM.

In this study majority of pregnant women found within the age group 25-29 years which comprised 101 (43.5%) of the

total, out of which 79 (78.2%) were seropositive. Two hundred fourteen (92.2%) of the respondents were resided in urban area, of these 169 (79%) were seropositive for *T. gondii*. Regarding to trimester 124 (53.4%) pregnant women were found within second trimester, of which 98 (79%) were seropositive for *T. gondii* (Table 1).

Table 1 Socio-demographic characteristics of pregnant women (n=232) attending Antenatal clinic at Arba Minch Hospital, 2015.

| Variables | Seroprevalence | | Total No (%) |
|--------------------------------|----------------|----------------|--------------|
| | Positive n (%) | Negative n (%) | |
| Age group (years) | | | |
| 15-19 | 12(75.0%) | 4(25.0%) | 16(6.9%) |
| 20-24 | 59(85.5%) | 10(14.5%) | 69(29.7%) |
| 25-29 | 79(78.2%) | 22(21.8%) | 101(43.5%) |
| 30-34 | 24(68.6%) | 11(31.4%) | 35(15.1%) |
| 35-39 | 10(90.9%) | 1(9.1%) | 11(4.7%) |
| Residence | | | |
| Urban | 169(79.0%) | 45(21.0%) | 214(92.2%) |
| Rural | 15(83.3%) | 3(16.7%) | 18(7.8) |
| Marital status | | | |
| Married | 177(79.0%) | 47(21.0%) | 224(96.6%) |
| Single | 5(83.3%) | 1(16.7%) | 6(2.6%) |
| Divorced | 0(0.0%) | 0(0.0%) | 0(0.0%) |
| Widowed | 2(100%) | 0(0.0%) | 2(0.9%) |
| Educational status | | | |
| Unable to read and write | 34(72.3%) | 13(27.7%) | 47(20.3%) |
| Primary | 62(78.5%) | 17(21.5%) | 79(34.1%) |
| Secondary | 59(83.1%) | 12(16.9%) | 71(30.6%) |
| Tertiary | 29(82.9%) | 6(17.1%) | 35 (15.1%) |
| Occupation | | | |
| Government | 52(77.6%) | 15(22.4%) | 67(28.9%) |
| Housewife | 115(82.1%) | 25(17.9%) | 140(60.3%) |
| Others * | 17(68%) | 8(32.0%) | 25(10.8%) |
| Trimesters | | | |
| First (<14weeks) | 32(82.1%) | 7(17.9%) | 39(16.8%) |
| Second (14-28weeks) | 98(79.0%) | 26(21.0%) | 124(53.4%) |
| Third (>28weeks) | 54(78.3%) | 15(21.7%) | 69(29.7%) |
| Gravidity | | | |
| Primigravidae | 67(78.8%) | 18(21.2%) | 85(36.6%) |
| Multigravidae | 117(79.6%) | 30(20.4%) | 147(63.4%) |
| *include merchants and farmers | | | |

Among the total respondents, 153 (65.9%) and 176 (75.9%) were reported to had the habit of eating raw meat and unwashed/raw vegetables or fruits, respectively. The respective prevalence of *T. gondii* infection among pregnant women was 86.9% (n=133) and 83.5% (n=147) (Table 2).

Table 2 Bivariate and multivariate analyses of factors associated with *T. gondii* infection among pregnant women, Arba Minch Hospital, 2015.

| Variables | Sero-prevalence | | COR (95%CI) | AOR (95%CI) |
|--|-----------------|----------------|-----------------------|-----------------------|
| | Positive n (%) | Negative n (%) | | |
| Age group (years) | | | | |
| 15-19 | 12(75.0%) | 4(25.0%) | 0.835 (0.245-2.848) ⊕ | 1.107 (0.288-4.246) |
| 20-24 | 59(85.5%) | 10(14.5%) | 1.643 (0.724-3.731) ⊕ | 1.785 (0.747-4.267) |
| 25-29 | 79(78.2%) | 22(21.8%) | 1 | 1 |
| 30-34 | 24(68.6%) | 11(31.4%) | 0.608(0.258-1.430) ⊕ | 0.453(0.175-1.173) |
| 35-39 | 10(90.9%) | 1(9.1%) | 2.785(0.338-22.952) ⊕ | 2.516(0.286-22.101) |
| Residence | | | | |
| Urban | 169(79.0%) | 45(21.0%) | 1 | |
| Rural | 15(83.3%) | 3(16.7%) | 1.331 (0.369-4.800) | |
| Educational status | | | | |
| Unable to read and write | 34(72.3%) | 13(27.7%) | 0.717 (0.311-1.652) | |
| Primary | 62(78.5%) | 17(21.5%) | 1 | |
| Secondary | 59(83.1%) | 12(16.9%) | 1.348 (0.593-3.062) | |
| Tertiary | 29(82.9%) | 6(17.1%) | 1.325 (0.473-3.712) | |
| Trimesters | | | | |
| First (<14weeks) | 32(82.1%) | 7(17.9%) | 1.213 (0.481-3.059) | |
| Second (14-28weeks) | 98(79.0%) | 26(21.0%) | 1 | |
| Third (>28weeks) | 54(78.3%) | 15(21.7%) | 0.955 (0.466-1.956) | |
| Gravidity | | | | |
| Primigravidae | 67(78.8%) | 18(21.2%) | 0.954 (0.495-1.841) | |
| Multigravidae | 117(79.6%) | 30(20.4%) | 1 | |
| Habit of eating raw meat | | | | |
| no | 51(64.6%) | 28(35.4%) | 1 | 1 |
| yes | 133(86.9%) | 20(13.1%) | 3.651 (1.890-7.053) ⊕ | 3.211 (1.592-6.477) ⊕ |
| Habit of eating raw vegetable or fruits | | | | |
| no | 37(66.1%) | 19(33.9%) | 1 | 1 |
| yes | 147(83.5%) | 29(16.5%) | 2.603 (1.317-5.146) ⊕ | 2.669 (1.264-5.639) ⊕ |
| Presence of domestic animal(s) at home | | | | |
| no | 129(75.9%) | 41(24.1%) | 1 | 1 |
| yes | 55(88.7%) | 7(11.3%) | 2.497(1.055-5.910) ⊕ | 3.06 (0.827-11.325) |
| Presence of cat(s) at homes | | | | |
| no | 155(77.9%) | 44(22.1%) | 1 | 1 |
| yes | 29(87.9%) | 4(12.1%) | 2.058 (0.687-6.168) ⊕ | 0.778 (0.145-4.171) |

| Farming/gardening activity | | | | |
|--|------------|-----------|---------------------|--|
| no | 152(79.2%) | 40(20.8%) | 1 | |
| yes | 32(80.0%) | 8(20.0%) | 1.053 (0.450-2.461) | |
| Source of drinking water | | | | |
| well | 8(80.0%) | 2(20.0%) | 1.045 (0.215-5.091) | |
| pipe | 176(79.3%) | 46(20.7%) | 1 | |
| History of blood transfusion | | | | |
| no | 172(79.6%) | 44(20.4%) | 1 | |
| yes | 12(75.0%) | 4(25.0%) | 0.767 (0.236-2.495) | |
| Mud house condition | | | | |
| no | 136(80.5%) | 33(19.5%) | 1 | |
| yes | 48(76.2%) | 15(23.8%) | 0.776 (0.388-1.553) | |
| Habit of eating soil | | | | |
| no | 163(80.3%) | 40(19.7%) | 1 | |
| yes | 21(72.4%) | 8(27.6%) | 0.644 (0.266-1.560) | |
| ⊕ Candidate variable for multivariate analysis at <0.25; ▣ Variable significant by the multivariate analysis at p<0.05; COR: Crude Odds Ratio, AOR: Adjusted Odds Ratio, CI: Confidence Interval | | | | |

Seropositivity rate with respect to the presence of cats at home, 33 (14.2%) reported cat's presence, of which 87.9% (n=29) were found to be seropositive. In the present study 16 (6.9%) pregnant women had history of blood transfusion, of which 12 (75%) were seropositive. Forty (17.2%) of the study participants had reported to have a history of engagement in farming/gardening activities, which could indicate contact with soil and 32 (80%) of them were seropositive (**Table 2**).

Factors associated with *T. gondii* infection

Logistic regression methods were used to identify the main predictor variables associated with *T. gondii* infection. Variables that were entered into multivariate analysis were age, presence of pet animals at home, presence of cats at their home, habit of eating raw meat, habit of eating raw/unwashed vegetables and fruits. Further analysis using multivariate analysis only two variables, habit of eating raw meat (AOR=3.211; 95% CI: 1.592-6.477) and habit of eating raw vegetables or fruits (AOR=2.669; 95% CI: 1.264-5.639) were found to be significantly associated with *T. gondii* seropositivity among pregnant women attending ANC in Arba Minch hospital (**Table 2**).

Discussion

In the current study, the overall seroprevalence of *T. gondii* infection among pregnant women was 79.3% (95%CI; 73.7-84.5). One hundred seventy five (75.43%) were only IgG seropositive, 9 (3.9%) were IgM sero-positive and two of the nine pregnant women were positive for both IgG and IgM. Overall 9 (3.9%) pregnant women were positive for IgM antibodies. Presence of IgM antibodies during pregnancy

shows the presence of acute *T. gondii* infection and an indication of higher risk of maternal-fetal transmission [22]. Previous study in this regard indicated that in the absence of treatment the risk of congenital infection from acute *T. gondii* infection during pregnancy is about 50% [23]. Early diagnosis of infections in pregnant mothers is of great importance for early initiation of measures that reduces the risk of transmission and possible sequels on the newborn. Therefore, screening of *Toxoplasma* infection should be considered as a part of the antenatal investigation during ANC follow up.

The overall seroprevalence of *T. gondii* infection among pregnant women was consistent with studies carried out in Jimma (83.6%) [17], Central Ethiopia (81.4%) [18] and Congo (80.3%) [24]. In contrast to our study, higher seroprevalence was reported among pregnant women in Addis Ababa [25], Gondar (88.6%) [26], Accra (92.5%) [14], and in the same study area among HIV infected individuals [19]. On the other hand, lower seroprevalence was reported from Nigeria (40.2%) [27], Tobago (39.3%) [28], Tanzania (30.9%) [29] and Debre Tabor (68.4%) [30]. The variation in seroprevalence of *T. gondii* found might be due to differences in geographical distribution of the parasite, socio-economic, personal hygienic practices, feeding habit of the study participants and deference in test methods may also account for the variation.

There are inconsistent reports on the association between consumption of raw meat with *T. gondii* infection. In the current study, consumption of raw meat was found to be significantly associated with *T. gondii* seropositivity (AOR=3.211; 95% CI: 1.592-6.477). The rate of *T. gondii* infection was significantly higher and about three times more likely to occur in those eating raw meat compared to those who did not. This finding is in agreement with previous studies

reported from Northwest Ethiopia [31], Khartoum [32] and Egypt [33]. In contrast, some studies reported absence of association between *T. gondii* infection and consumption of raw meat [17,34,35]. The observed differences might be due to the types of meat consumed and the rate of infection in the animals.

The other predictor of *T. gondii* seropositivity in this study is consumption of raw vegetables or fruits, significant association was observed between *T. gondii* seropositivity and eating raw vegetables or fruits. This finding is in agreement with study done in Central Ethiopia [18] and Nigeria [27]. In contrast, studies surveys done in pregnant women in South china [36], Addis Ababa [25] and Felege Hiwot Hospital [31] reported no significant association between the habit of eating raw/unwashed vegetables and fruits and *T. gondii* infection. The observed differences might be due to differences in feeding habit and hygienic practices of the studied population.

Cats excrete millions of oocysts within a short period of time and play a major role in transmitting *T. gondii*. However, the current study showed that the presence of cats at home was not significantly associated with *T. gondii* seropositivity (Table 2). Similar findings were observed in Saudi Arabia [34], Tobago [28] and Turkey [35]. On the other hand, others documented significant association of cat ownership with *T. gondii* infection [17,30,36]. Differences in the types of cats as well as infection rates in cats may account for the observed differences.

In the current study 16 (6.5%) of the study participants responded previous history of blood transfusion and 75% of whom were *T. gondii* seropositive. study indicated that blood transfusion is one means of transmission of *T. gondii* infection but, in our study, there was no significant difference in *T. gondii* seropositivity between those who had previous history of blood transfusion and those not having. This finding is in agreement with previous reports [37,25]. It is known that only blood donors with acute infection with circulating *T. gondii* parasite in the blood transmit the infection. Since the prevalence of acute infection in blood donors is often very low [38] and [39] received blood is less likely to have tachyzoites stage of the parasite, infective form of the parasite by blood transfusion.

In this study, seroprevalence was found to be higher in age groups [35-38] and *T. gondii* seropositivity was not significantly differed by age (Table 2). This is an agreement with previous studies [25,26]. In contrast, studies in Jimma [17], Turkey [35] and Burkina Faso [40] documented significance difference by age. In summary, all socio-demographic and obstetric (trimester, gravidity) factors assessed none were significantly associated with *T. gondii* seropositivity [41].

Conclusion

Similar to most studies the overall seroprevalence of *T. gondii* infection was high among pregnant women and serological evidence of primary infection was observed. The high prevalence and presences of primary infection in pregnant women alert the requirement and the need of routine screening of the infection. Therefore, screening of

Toxoplasma infection should be considered during ANC follow up. Consumption of raw meat and raw vegetable or fruits was the main predictors of acquiring *T. gondii* infection among the study participants. Therefore, Health information should be provided to all pregnant women and women who are considering become pregnant on the risk factors predisposing to *Toxoplasma* infection. Moreover, further studies are recommended to determine incidence of Congenital Toxoplasmosis in the study area.

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