JIMMA UNIVERSITY COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES DEPATMENT OF MEDICAL LABORATORY SCIENCES AND PATHOLOGY

Seroprevalence of *Toxoplasma gondii and* Associated Factors among Pregnant Women in Jimma Town, Southwest Ethiopia

By Endalew Zemene, BSc

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Advisor: Ato Ahmed Zeynudin, BSc, MSc

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Table of Contents

Page

List of Tables

page

List of figures

List of Abbreviations

ANC	Antenatal Care
CMI	Cell Mediated Immunity
ELISA	Enzyme-Linked Immunosorbent Assay
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
PCR	Polymerase Chain Reaction

Abstract

Background: *Toxoplasmosis is a very common infection in human caused by an obligate intracellular protozoan parasite Toxoplasma gondii. Humans become infected mainly by ingesting T. gondii tissue cysts present in undercooked meat, ingesting infectious oocysts present in water and garden soil contaminated by infected cat feces or through congenital transplacental transmission. About one third of primary Toxoplasmosis cases occurring during pregnancy lead to congenital Toxoplasmosis with consequent pathological effects.*

Objective: *The aim of this study is to determine the seroprevalence of T. gondii among pregnant women and assess the associated risk factors.*

Method: *A cross-sectional study was conducted from July 4 -29, 2011 among pregnant women in Jimma Town. Venous blood specimens were collected from 201 pregnant women in 5 kebeles of the town and tested for IgM and IgG anti-T. gondii antibodies by ELISA (Human Gesellschaft für Biochemica und Diagnostica mbH) according to manufacturer's instruction.*

Findings: *The overall seroprevalence of T.gondii among the pregnant women was 83.6% (95% CI, 78.5 – 88.7%). One hundred sixty three (81.1%) of the pregnant women were IgG anti -T. gondii seropositive and five of the 33 pregnant women seronegative for IgG anti-T. gondii antibody were seropositive for IgM anti-T. gondii antibody. Among the risk factors and socio-demographic characteristics assessed, age group and presence of domestic cats at home (AOR = 6.7, 95%CI =2.2 20.3) were significantly associated with anti-T. gondii seropositivity. Habit of eating raw meat, occupation, contact with soil and other socio-demographic factors did not show significant association with anti-T. gondii seropositivity.*

Recommendation: *Health information on the disease, its transmission and risk factors is recommended to be given to women of reproductive age group and policy makers should consider test for T. gondii infection during pregnancy to prevent the tragic outcome of congenital toxoplasmosis.*

Key words: Seroprevalence, Pregnancy, *T. gondii*, Jimma Town, Ethiopia

CHAPTER ONE – INTRODUCTION

1.1. Background Information

Toxoplasmosis is a disease caused by an obligate intracellular protozoan parasite *Toxoplasma gondii* (*T. gondii*). The parasite is an extremely widespread, and thus successful, protozoan with a complex life cycle centered on felines, the definitive hosts that shed infectious oocysts into the environment during infection. Oocysts, which can be stable in the environment, contaminate the food and water supply, disseminating the parasite into additional felines, as well as to other warm-blooded vertebrates [1]. The oocysts are resistant to commonly employed water treatment processes including chlorination and ozonation [2], which facilitates transmission.

Mode of transmissions of the parasite include accidental ingestion of oocyst stage of the parasite after cleaning a cat's litter box when the cat has shed Toxoplasma in its feces [3], accidental ingestion of oocysts after touching or ingesting anything that has come into contact with a cat's feces that contain *Toxoplasma* [4], accidental ingestion of oocysts in contaminated soil (e.g., not washing hands after gardening or eating unwashed fruits or vegetables from a garden) [5], drinking water contaminated with the Toxoplasma parasite [6]. In addition to becoming infected through oocysts, transmission to humans can occur through consumption of infected raw meat [7] and rarely, organ transplantation and blood transfusion [8] and mother-to-child (congenital), by the tachyzoites stage of the parasite.

The life cycle involves the feline family (both domestic and wild cats) as definitive hosts which excrete unsporulated oocyst with faeces. Humans and other warm-blooded animals act as intermediate hosts, acquiring the infection in one of the ways mentioned above. There are two asexual forms of the parasite: the tachyzoites, which can invade all types of nucleated cells and divides rapidly leading to cell death and the bradyzoites, which divides slowly and forms cysts most prominently in the muscle and brain. Ingestion of infected rodents or birds by cats keeps the sexual cycle to continue. Replication of tachyzoites replication cause acute disease, while encysted bradyzoites are long-lived, responsible for maintaining the latent infection [9].

Three distinct forms of toxoplasmosis exist. The most serious one is the congenital toxoplasmosis which often leads to serious malformations including hydrocephaly [10], intra-cerebral calcification and microcephaly [11, 12]. The second form is the acquired acute toxoplasmosis, which is characterized by the presence of *T. gondii* tachyzoites in the blood and other tissues of infected people and by a complex of clinical symptoms which varies from fever and headache to serious neurological and psychiatric malfunctions [13,]. In most cases, however, the acute toxoplasmosis is only a mild disease which is often misdiagnosed as a common bacterial or viral infection [14]. Infrequently the acute toxoplasmosis evolves into chronic disease in which the clinical symptoms as well as high titers of specific antibodies persist for many years. Mostly, all symptoms of acute disease quickly fall away, the antibody titers decrease and the toxoplasmosis evokes into the latent form of the infection. During the latent toxoplasmosis the parasite survives in the dormant form of bradyzoites mostly in the neural and muscular tissue of the host. The latent toxoplasmosis probably lasts for the whole life of infected person [15] and it can turn into acute toxoplasmosis only after serious violence of integrity of immune system [16].

The immune system, particularly the cell mediated immunity (CMI), is vital in the containment of the parasite. In fact both antibody and CMI are elicited by *T. gondii* infection, but the role of antibody in the control of the infection is secondary to the effects of the cell mediated immune response. It is involved in killing extracellular tachyzoites and is of use diagnostically. Control of the disease appears to depend on the elaboration of appropriate cytokines including interleukin (IL)-2, IL-12, and interferon-γ (INF-γ) followed by a specific cell-mediated immunity with CD8 + helper T cells apparently the most important subgroup [9, 17]. Hence, individuals with impaired immunity, particularly the cell mediated arm of immunity, and pregnant women are the risk groups of the population.

In pregnant women, primary infection during pregnancy is associated with risk of congenital transmission, which may result in tragic outcomes. Transmission to the fetus during pregnancy depends on gestational age at primary infection [18] and immune status of the pregnant woman [19], among others. Screening of pregnant women for *T. gondii* antibodies is practiced in few countries, but in most developing countries including Ethiopia it is not practiced in the routine antenatal care (ANC) service.

The diagnosis of toxoplasmosis in humans is usually made by serological detection of specific IgM and IgG antibodies by different laboratory methods. The most commonly used serologic tests to detect the presence of anti-*T. gondii* IgG and IgM antibodies are the Sabin-Feldman dye test [20], indirect fluorescent antibody (IFA) [21], and agglutination tests, or Enzyme Linked Immunosorbent Assay (ELISA) [22]. In this study, seroprevalence of *T. gondii* among the pregnant women was determined using ELISA.

1.2. Statement of the problem

T. gondii infection is widespread among humans and its prevalence varies widely from place to place. Generally, it is estimated that one third of the World's population is infected by *T. gondii*. However, seroprevalence in human populations varies greatly among countries, geographical areas within one country and even ethnic groups living in the same geographical area. Infections are found on all continents and, on the whole, more common in warm climates and in low-lying areas than in cold climates and mountainous regions [1, 23]. Seroprevalence rates of 22.5% [24] and 29.8% [25] were reported among the general population, with females usually being more at risk [26]. Often higher seroprevalence rates have been reported among one of the risk groups of the population, pregnant women.

In pregnant women several seroepidemiological studies indicate high burden of *T. gondii*. Overall seroprevalence rates as high as 92.5% [27] and 51.4% [28] have been reported. Most pregnant women infected with *T .gondii* are chronically infected while few acquire the infection during pregnancy [29]. Pregnant women with acute infection during pregnancy are at risk of congenitally transmitting the infection to the fetus.

Congenital transmission occurs as a result of primary infection of the mother and infection of the placenta. Congenital transmission as a result of primary infection during pregnancy is higher if the infection is acquired during the third trimester and is lower if the infection occurs during the first trimester. But the severity of congenital infection is substantially higher in case of congenital transmission during the first trimester which includes abortion than the infection at the third trimester [18]. There are evidences indicating a clear association between toxoplasma infection and habitual abortion [30]. Most infected newborns have no clinical signs at birth but are at risk of developing visual impairment as a result of retinochoroiditis in childhood or adolescence [31, 32], hearing loss [33], hydrocephalus and neurological disorders [32]. Congenital transmission can also occur in chronically infected pregnant women with immunosuppression.

In immucompromised pregnant women chronically infected with *T. gondii* congenital transmission can occur as a result of reactivation of latent infection, in addition to primary infection during pregnancy. As the parasite is obligate intracellular, any condition that threatens the cell mediated immunity such as HIV infection [19] can result in reactivation of the parasite in chronically infected individuals leading to congenital transmission, although there are contradicting reports in seroprevalence rate among HIV infected and non infected individuals [34 - 36]. Moreover, those receiving immunosuppressive medications are also at risk of reactivation of the parasite [37].

In immunocompetent mothers who have been immunized against toxoplasmosis before conception, immune mechanisms prevent transmission of the infection to their fetuses, except in some rare cases in which congenital transmission can take place after reinfection of a previously immune mother, perhaps as a result of overwhelmed immune response of the mother when reinfection occurs as the result of a massive inoculum, a strain with a different genetic background or a different infectious stage (oocyst vs. cysts) [38]. The infectious stage to which a person is exposed depends on the source of infection.

Several risk factors are associated with *T. gondii* infection among pregnant women. Despite inconsistency of different reports, the risk factors identified to be associated with seropositivity in pregnant women include eating undercooked sheep or goat meat, drinking unpasteurized sheep or goat milk and handling raw sheep or goat meat [39], consumption of raw meat [40] drinking well water [41], eating raw vegetables [42], contact with cats [12, 43] and occupational exposure to soil [44]. Residence has also been found to affect the infection rate [45].

Most of the above mentioned risk factors are also prevalent in Jimma Town, where this study is conducted. It is not uncommon to find raw or insufficiently cooked meat in most of the restaurants in the town [46]. In a survey carried out in 2005 most households in Jimma Town (59.5%) used open fields for waste disposal [47]. Most of the pregnant women in the town are house wives [48], who are most likely involved in household activities including washing vegetables and gardening which are potential risks for *T. gondii* infection. In addition, cats are

abundant to cause environmental contamination and the warm climate of Jimma Town [49] is ideal for sporulation and survival of oocysts of the parasite [1].

Despite the existence of risk factors for *Toxoplasma* infection and suitability of the climatic conditions favoring survival of the parasite in the study area, to our knowledge, no study has been conducted on the matter, a gap believed to be filled by this study. Even in Ethiopia, only few studies were conducted on the seroprevalence of *T. gondii;* and these studies focused on the general population [50 - 52].

Published reports on the magnitude of Toxoplasmosis in pregnant women in Ethiopia are scarce, clearly showing a deficit on data on the magnitude of the infection among pregnant women, hence, one of the reasons for undertaking this study. Besides this, serological screening of pregnant women for *T. gondii* is not undertaken as an antenatal examination in health facilities in Ethiopia.

Therefore, considering these facts, this study is aimed at determining the seroprevalence *T. gondii* and assessing the associated risk factors among pregnant women in Jimma Town, which will help in the intervention actions to be taken and serves as a base line data for further studies.

CHAPTER TWO – LITERATURE REVIEW

Published reports on Toxoplasmosis show different patterns of seropositivity for *T. gondii* depending on difference in: cat ownership, age, educational level, residence, exposure to soil, socio-economic difference, eating habit of raw or undercooked meat, source of water for drinking, eating pattern of unwashed and raw vegetables or fruits. The following attempt is made to review some of the litratures on the mentioned factors.

Age

Several studies consistently reported increasing proportion of pregnant women positive for anti-*Toxoplasma* IgG as age increases i.e. older women being more seropositive for the parasite than younger women, possibly as a result of longer exposure time. For example, in study done in Palestine in which the overall seroprevalence of IgG was found to be 27.9%, the seroprevalence rate was 17.9, 27.5 and 38.2% among pregnant women of age 16 - 22, 23 – 29 and 30 - 43 years, respectively [42]. Similarly a significant difference in seropositivity with age was reported among pregnant women in Turkey [53], Columbia [54] and women in the child-bearing age in Sudan [55].

A similarly increasing pattern of seropositivity with age was observed in the general population in a study done in Brazil, in which it was reported that each additional year of age increased the odds of being seropositive by 6% [56] and Addis Ababa [36], in which 82.1, 92.4 and 95.7% of people of age 17 -24, 25 -34 and > 35 years were seropositive.

In a study conducted in pregnant women in their first trimester in Trinidad and Tobago in which overall prevalence of 46.8% was reported, only parity was significantly associated with infection with *T. gondii*, in which case those having “3 or more” children were found to be infected more [57], perhaps related to age, with those having more children be older, similar to the studies cited above. In the study, relatively higher prevalence of primary infection (11.9%) compared to other studies was reported, suggesting new infections during pregnancy. Similarly, multiparty is reported to be associated with *T. gondii* infection among pregnant women in a study done is Saudi Arabia [28].

Socio-economy

Exposure to contaminated soil with the parasite oocysts is one source of infection as explained earlier. In a study done in northern Mexico by Esquivel C and coworkers, it was reported that significantly higher proportion of pregnant women living in a house with soil floors were found to be infected with *T. gondii* compared to those living in a house with floors made of concrete or other materials [58]. In the study, a low prevalence (6.1% of the 343 pregnant women) of IgG was reported and none of the pregnant women were positive for IgM.

Likewise, a study done by Rosso *et al* [54] in Columbia, South America, showed higher seroprevalence rate among pregnant women belonging to the lower socio-economic class as indicated by 49, 38 and 29% infection rate the lower, middle and higher socio-economic strata, suggesting that social and economic differences have a major impact on transmission of the parasite.

Similarly a study was conducted to evaluate associations between seropositivity for IgG and IgM anti-*T. gondii* antibodies and socio-economic and environmental variables in pregnant women in Brazil in which 49.2% of the 492 pregnant women included in the study were seropositive for *T. gondii*. In the study it was found that *per capita* income was significantly associated with presence of IgG antibodies, where pregnant women with low *per capita* income had greater risk of infection with *T. gondii* [12].

Cat ownership/contact

Contact with cats is often mentioned as a risk factor; however, contradicting reports were published in different literatures in the association between presence of cats in the family environment and infection with *T. gondii*. In the studies done on pregnant women in Brazil [12] and Taiwan [43], it was reported that presence of cats in the family was significantly associated with *T. gondii* infection. A similar finding was obtained in the study done in Ethiopia on the general population [51]. It is known that cats excrete millions of oocysts per day for only two weeks of their life, when they first acquire infection. Oocysts become infective one to five days after excretion, are spread by surface water, and can survive more than a year. This shows that it may be difficult to assess the association of cats and human toxoplasmosis by epidemiological surveys as soil, not cats, seems to be the main factor [59].

However, the study done in Mexico reported no association of *Toxoplasma* seropositivity with contact with cat or cleaning of cat faeces among pregnant women [58]. A similar finding with no association with cat ownership was found in the studies done in Turkey [53] and Iran [60].

Exposure to soil or gardening

Like the cat ownership, different literatures reported contradicting results in the association of exposure to soil and infection with *T. gondii*, probably due to lack of standard for the degree of exposure to soil. A retrospective analysis of serological and epidemiological data in a series of 235 pregnant women from Macedonia in which an overall prevalence of 20.4% was reported [44], exposure to soil was found to be a single infection transmission factor predictor of infection in the whole series. Similarly, studies done in Slovakia [61] and Jordan [45] to assess seroprevalence of *T. gondii* among pregnant women reported a significant association of contact with soil and *T. gondii* infection.

A similar finding was obtained among the general population in a study done elsewhere [24], in which occupations involving long-time exposure to soil were associated with higher rate of *T. gondii* seropositivity. However, no association with exposure to soil was reported in the study done in Mexico [58].

Residence

Concerning the pattern of infection associated with residence of pregnant women, a recently published study involving all pregnant women attending a maternity hospital in Saudi Arabia for 1-year period, showed rural residence was found to be a significant positive predictor for chronic *Toxoplasma* infection among others [28]. Similarly, in a study conducted in Hebron district, Palestine, involving 204 pregnant women, it was shown that more women from rural areas (36.8%) had IgG antibodies to *T. gondii* than urban women (21.4%) [42].

However, a study done on 303 women in the child-bearing age in Iran in which 26.7% were seropositive for *T. gondii*, no association of seropositivity with residence was observed [60].

Education

In a study done in Iran in which 23.4% of the 303 women of child-bearing age were seropositive for *T. gondii*, highest prevalence rate was observed in women who were illiterate (36.8%) but there was no significant association between educational status and seroprevalence of *Toxoplasma gondii* antibody [60].

Food and water related

In a multicenter case-control study done in six cities in Europe, namely Naples, Lausanne, Copenhagen, Oslo, Brussels, and Milan, it was shown that eating raw or undercooked meat was one of the risk factors predictive of acute infection with *T. gondii* in pregnant women [7]. Frequent consumption of undercooked meat [28] and raw vegetables [62] were also associated with infection with *T. gondii* in pregnant women studies done in Saudi Arabia and France respectively. Similarly, in a study done in Serbia to assess risk factors associated with toxoplasmosis in a series of 2936 women aged 15-49 years, undercooked meat consumption was found to be the single predictor of infection [63]. However, other studies [64] showed no significant association of undercooked meat consumption with *T. gondii* infection.

Outbreaks of toxoplasmosis are recognized infrequently. However, a study done by Bowie *et al* [65] associated a large community-wide outbreak of toxoplasmosis in Great Victoria to a municipal water system to be the likely source.

Several studies have been conducted on seroepidemiology of *T. gondii* in African countries and globally as discussed above, but few reports in Ethiopia have been obtained and they mainly focused on the seroprevalence of *T. gondii* on the general population [50, 51], clearly showing a literature gap. Therefore, this study will narrow the gap and puts a baseline data for further study.

Significance of the study

Seroepidemiological study of Toxoplasmososis among women of childbearing age in general and pregnant women in particular is important as it provides appropriate information to design the preventive measures. In Ethiopia few studies were conducted on the seroepidemiology of *T. gondii* and these focused on the general population. The literatures on the matter are scarce and serological screening of pregnant women for *T. gondii* is not practiced as an antenatal care in health institutions in Ethiopia. Therefore, the outcome of this study will help:

- Physicians in creating awareness on the level of seroprevalence of *T. gondii* among pregnant women
- Policy makers in designing appropriate preventive measures depending on the major risk factors
- Researchers as a base line data in identifying thematic areas on the matter for further study

Conceptual Framework

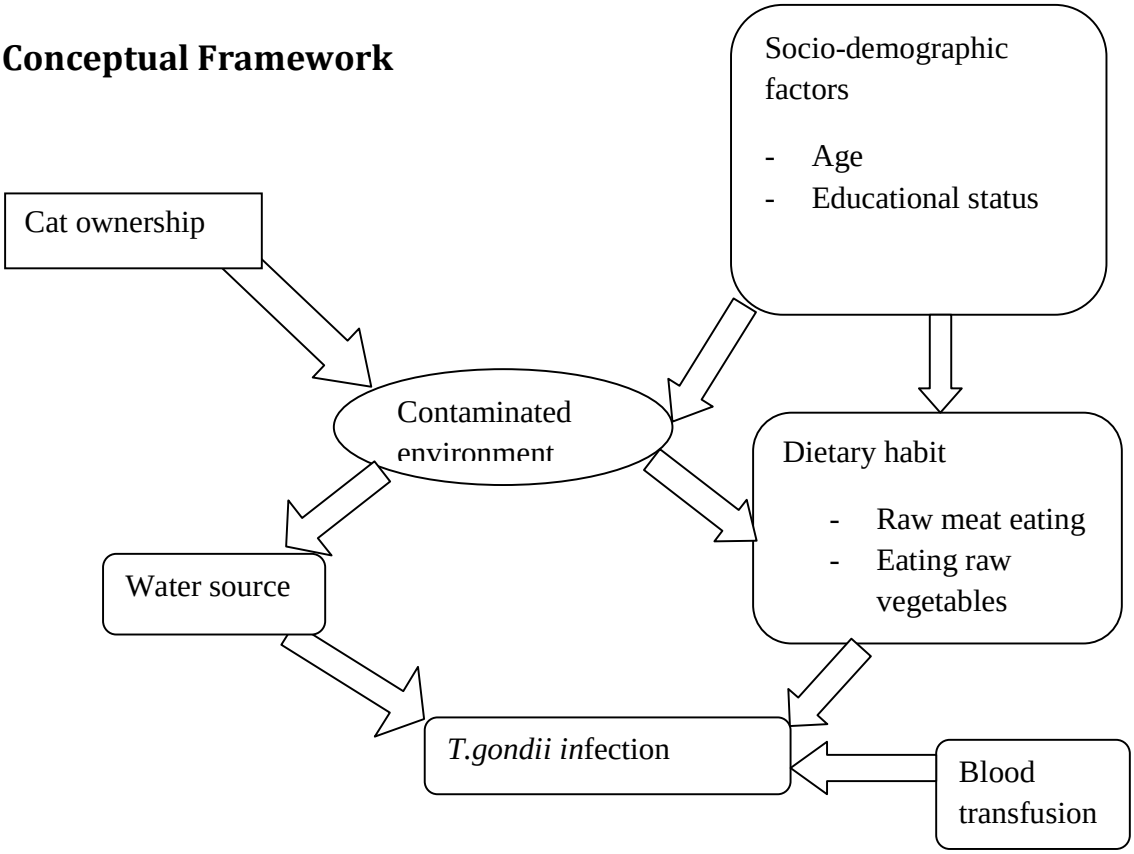


Fig. 2.1. Conceptual framework showing factors associated with *T. gondii* infection.

CHAPTER THREE
OBJECTIVE

3.1. General objective

To determine seroprevalence of *T. gondii* and assess the associated risk factors among pregnant women in Jimma Town.

3.2. Specific objectives

- To determine seroprevalence of IgG anti-*T. gondii* antibody among the pregnant women
- To determine seroprevalence of IgM anti-*T. gondii* antibody among the pregnant women
- To identify risk factors associated with infection of *T. gondii* among the pregnant women

CHAPTER FOUR
MATERIALS AND METHODS

4.1. Study area

The study was conducted in Jimma Town which is located 350 Kms South-West of Addis Ababa and has total surface area of 4,623 hectares. According to the 2007 Central Statistical Agency census report [66] the total projected population of the town was 134, 040, females constituting 49.7% of the total. The town is divided into 13 administrative kebeles. The town is generally characterized by warm climate with a mean annual maximum temperature of 30°C and a mean annual minimum temperature of 14°C. The annual rainfall ranges from 1138 mm to 1690 mm. It is located at an altitude of 1750-2000m above sea level.

Concerning health facilities in the town there are governmental and non-governmental health institutions. One governmental specialized hospital, three health centers and several private clinics, drug stores and pharmacies are found in the town.

4.2. Study design and period

A community-based cross-sectional study was conducted from July 4 - 29, 2011.

4.3. Population

4.3.1. Source population

Pregnant women in Jimma Town in the year 2011 are the source population.

4.3.2. Study population

Pregnant women selected by systematic random sampling from the selected kebeles who were voluntary to participate in the study.

4.4. Eligibility and Exclusion Criteria

4.4.1. Inclusion criteria

- Pregnant women in any of the three trimesters of pregnancy
- Pregnant women who are volunteer to participate in the study

4.4.2. Exclusion criteria

- Subjects who are seriously sick and have difficulty of responding to questions
- Subjects with problems of communication due to disability
- **Specimen exclusion** – Hemolysed and highly lipemic samples

4.5. Sample Size and sampling technique

4.5.1. Sample size

The sample size was calculated using the genera formula for a single population proportion:

$$n = \frac{(Z\ \alpha/2)^2\ p\ (1-p)}{d^2}$$

Where

n= the minimum sample size

$\frac{z\alpha}{2}$ = standard normal variable at 95% confidence level (1.96)

P = expected prevalence of *T. gondii* infection (0.9) [36]

d= margin of error (5%)

Therefore the value of n was calculated as follows:

$$n = \frac{(1.96)^2 x\ 0.9\ (1-0.9)}{(0.05)^2} \qquad n = 138$$

For the design effect, it was multiplied by 2, thus

n= 276

Since the target population is less than 10,000, population correction formula was employed as follows:

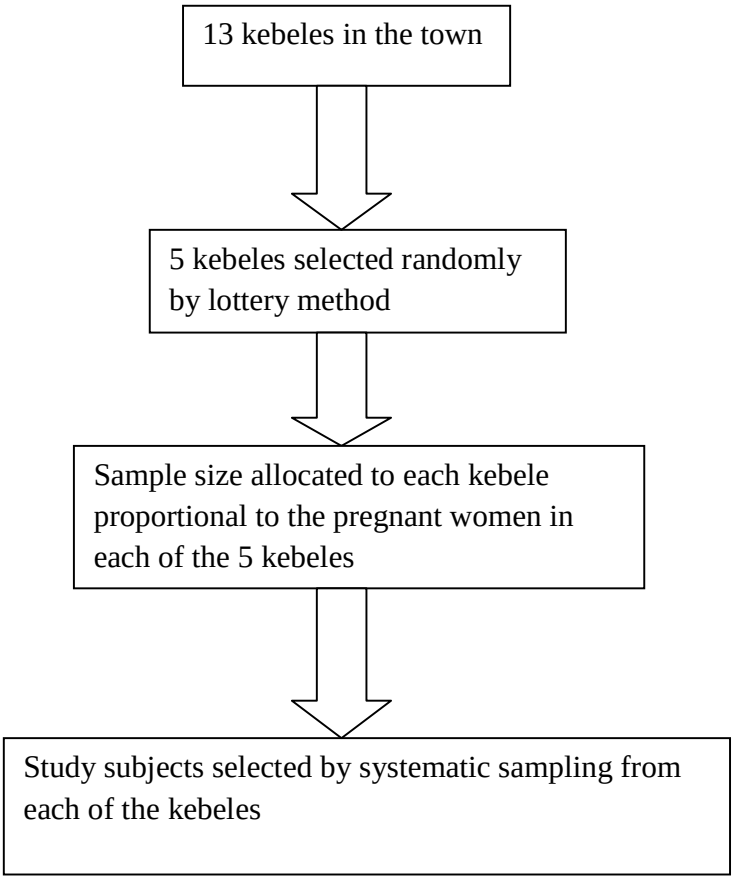
$$nf = \frac{n}{1 + n/N}$$ where N is estimated to be 800

nf = 205

Adding 10% for non-response rate, the final sample came to be 225 pregnant women.

4.5.2. Sampling techniques

The town has a total of 13 kebeles. Among these, 5 kebeles were selected randomly (38% of the total kebeles). These were Hirmata, Bosa Kito, Bocho Bore, Hirmata Merkato and Mentina. The sample size was allocated to the kebeles proportional to the total number of pregnant women in each of the kebeles. The pregnant women from each of the five kebeles were included by systematic sampling technique. The sampling interval was approximately 2, hence, the first case was selected by lottery from the two consecutive ones and every other pregnant woman from each of the kebeles was included. It can be summarized as follows:



4.6. Measurements and Data Collection

4.6.1. Study Variables

Dependent Variable

T. gondii IgG and IgM serostatus

Independent variables

- Age
- Parity
- Trimester
- Educational status
- Religion
- Occupation
- Cat ownership
- Raw meat eating habit
- History of blood transfusion
- Ethnicity

4.6.2 Data collection

Socio demographic data

Socio demographic and data on the risk factors was collected using pre-tested questionnaire (both Amharic and Afan Oromo versions as convenient) (Annex II). The data was collected by trained nurses, supervised by the principal investigator. Pregnant women were selected from each kebele and first contacted at their home. They were appointed to their respective kebeles or Higher 2 health center based on convenience to them, after which the data were collected (the questionnaire and laboratory data).

Laboratory data

Approximately 2ml venous blood was collected from each of the study participants (Annex III) by experienced personnel into clean dry test tubes following aseptic technique and transferred to the department laboratory. Serum was separated from the whole blood by centrifugation at 3000 rpm for 5 min. It was labeled and kept at -20 ^{0}C until use. Finally it was tested for anti-T. gondii IgG and IgM antibodies using ELISA test kit (*Human Gesellschaft für Biochemica und Diagnostica mbH*, Germany) following the manufacturer's instruction (Annex IV). Fresh de-ionized water used for the preparation of working WASH solution was prepared by a de-ionizer (Lonenaustauscher, Sr.No 081525, Germany) at the department of Pharmacy.

4.6.3. Operational definitions

Seropositive Human subjects who are anti- *T. gondii* IgG or IgM antibodies positive unless otherwise specified for other cases.

Eating raw meat Pregnant women who had at least once eaten raw meat in the past and/or eats at present.

Contact with soil A pregnant woman who has/had contact with soil as a result of occupation (eg. farming) and/or involved in gardening.

Illiterate Pregnant women who cannot read and write at least with one language

Presence of cats Living in a house in which domestic pet cat(s) is/are present at the time of data collection or were present at any time in the past.

4.7. Data analysis

After checking the data for completeness, missing values, and coding of the questionnaires, the data were entered to computer, processed and analyzed using SPSS for windows version 16.0 (SPSS Chicago Illinois). Bivariate and multivariate logistic regression was used for the analysis. *P* values less than or equal to 0.05 were considered statistically significant. Variables significantly associated by the bivariate analysis were candidates for the multivariate analysis and the multivariate analysis is done by the backward procedure. Finally the data is presented in tables, figures and text.

4.8. Data quality Control

- ✓ Training was given to the data collectors on the objective of the study and each item on the questionnaire.
- ✓ The questionnaire was checked regularly for completeness.
- ✓ Standard operating procedures were followed during specimen collection and processing.
- ✓ Venous blood was collected by a sterile syringe and needle in such a way that hemolysis would be avoided or minimized.
- ✓ Manufacturer's instruction was strictly followed while running the ELISA, including running controls along with the tests
- ✓ Quality of the specimen was met as per the manufacturer's specification
- ✓ The equipments used were checked for proper functioning.

4.9. Ethical considerations

- ✓ Ethical clearance was sought from Jimma University Ethical review Board and permission was obtained from Jimma Zone Health Bureau.
- ✓ During data collection all respondents were asked their permission and written informed consent was obtained before data collection.
- ✓ The right of the pregnant women to withdraw/refrain from participation in the study was maintained.
- ✓ Confidentiality of individual patients' information was maintained during data collection, analysis and interpretation using codes rather than names of the study subjects.
- ✓ The findings of the study were communicated to the health professionals in the health facilities.

4.10. Plan for dissemination

The final report was submitted to the Department of Medical Laboratory Sciences and Pathology, Jimma University, and the findings will be presented to the academics of the University. The report will also be submitted to Jimma zone health office. Moreover, the paper will be published on either national or an International Journal to communicate to the scientific community.

CHAPTER FIVE

RESULTS

5.1. Socio-demography of the study participants

In this study a total of 201 pregnant women of age ranging 17 to 35 years (mean age of 23.64 and median 23 years) have participated, with response rate of 89.3%. Most of the pregnant women were in the age range 20-24 years, accounting for 47.8 % of the total. Majority of the study participants are Oromo by ethnicity, 127 (63.2%), followed by Gurage, 19 (9.5%). Most of the pregnant women were Muslims accounting for 136 (67.7%) of the total followed by Orthodox, 50 (24.9%). Concerning their occupation, majority of them 158 (78.6%) were house wives, followed by merchants, accounting for 17 (8.5%). Nearly a fourth of the pregnant women were illiterate, who are unable to read and write.

Of the total pregnant women, 29 (14.4 %) were in their first trimester, 104 (51.7 %) in the second trimester and the remaining 68 (33.8 %) were in the third trimester. Eighty three (41.3%) of the pregnant women were primigravidae, and the remaining were multigravidae (Table 1).

Table 1: Socio-demography of the pregnant women involved for the study of seroprevalence of *T.gondii* in Jimma Town, South-West Ethiopia, July 2011

Demographic characteristics	Frequency	Percent
Age group		
15 – 19	25	12.4
20 – 24	96	47.8
25 – 29	64	31.8
30 - 35	16	8
Occupation		
Housewife	162	80.6
Merchants	17	8.5
House maids	8	4.0
Daily laborers	7	3.5
Others*	7	3.5
Ethnicity		
Oromo	127	63.2
Gurage	19	9.5
Kefa	18	9
Amhara	23	11.4
Others**	14	7
Religion		
Muslim	136	67.7
Orthodox	50	24.9
Protestant	15	7.5
Educational status		
Illiterate	49	24.4
Read and write only	15	7.5
Grade 1-4	39	19.4
Grade 5-8	51	25.4
Grade 9-12	41	20.4
12+	6	3.0
Trimester		
1st trimester	29	14.4
2nd trimester	104	51.7
3rd trimester	68	33.8

* Gov/NGO employees and students

** Yem and Tigre

5.2. Overall seroprevalence of *T. gondii*

The overall seroprevalence of *T. gondii* among the pregnant women was 83.6 % (95% CI, 78.5 – 88.7%). One hundred sixty three (81.1%) and 5 (2.5%) of the pregnant women were seropositive for IgG and IgM anti-*T.gondii* antibodies, respectively. Among all the pregnant women, 196 (97.5%) have reported to have undergone HIV screening test of which 3 responded to be HIV seropositive. However, remaining five had unknown result. All of the three pregnant women responded to be HIV seropositive were IgG anti-*T.gondii* seropositive.

5.3. Seroprevalence in relation to age and parity

Twenty five (12.4%), 97 (48.3%), 63 (31.3%) and 16 (8%) of the total study subjects were in the age range 15 -19, 20 -24, 25 – 29, 30 – 35 years, with seroprevalence rates of 64.0, 83.5, 88.9 and 83.6%, respectively. Anti-toxoplasma seroprevalence of the pregnant women showed an increasing pattern of seropositivity with increasing age group (Fig 5.1). Similarly an increasing pattern of seropositivity was observed among the pregnant women with increasing parity.

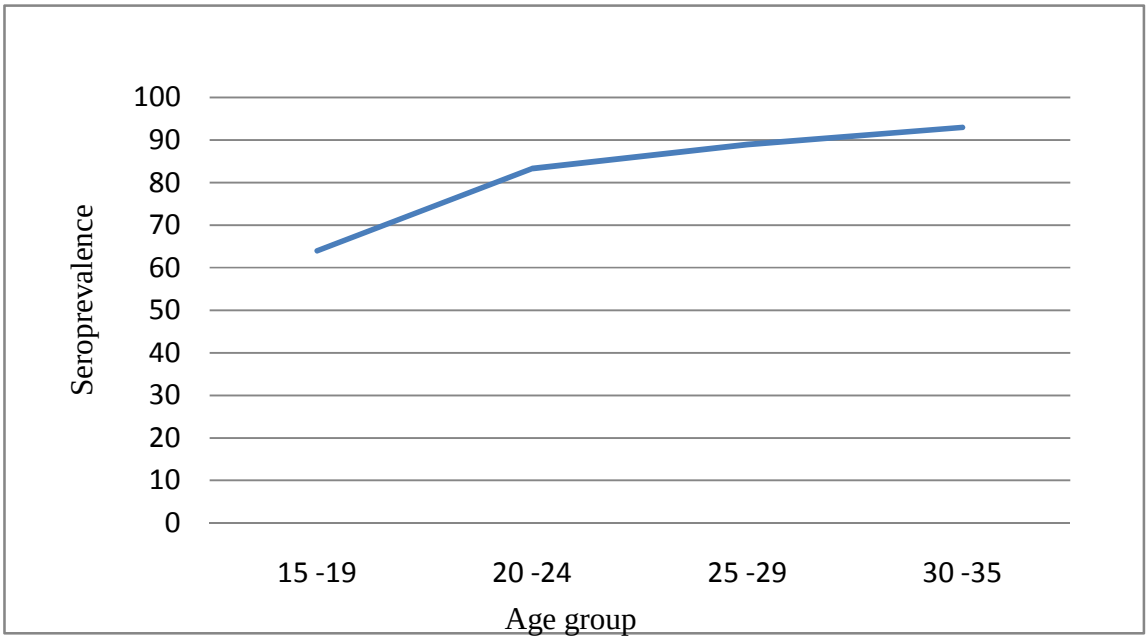


Fig. 5.1. Seroprevalence of Anti-*Toxoplasma* antibody in relation to age of the pregnant women in Jimma Town, South-West Ethiopia, July 2011

3.4. Seroprevalence in relation to other associated factors of interest

Comparable result of seropositivity was obtained among the pregnant women in the three trimesters of pregnancy (table 2), with seropositivity rate of 79.3%, 82.7% and 86.8%, respectively, which showed no statistically significant difference (P= 0.6).

Table 2. Factors associated with Toxoplasma gondii infection among pregnant women in Jimma Town, South-west Ethiopia, July 2011

Characteristics		Seroprevalence		Total	P. value
		Positive (%)	Negative (%)		
Parity (n=201)	Primiparous	63 (79.7)	16 (20.3)	79 (39.3)	
	multiparous	105 (86.1)	17(13.9)	122 (60.7)	0.238
Trimester (n=201)	1st trimester	23 (79.3)	6 (20.7)	29 (14.4)	
	2nd trimester	86 (82.7)	18 (17.3)	104 (51.7)	
	3rd trimester	59 (86.8)	9 (13.2)	68 (33.8)	0.6
Educational status (n=201)	Illiterate	38 (77.5)	11 (24.5)	49 (24.4)	
	Literate	130 (85.5)	22 (14.5)	152 (75.6)	0.19
Presence of cats (n=201)	Yes	78 (95.1)	4 (4.9)	82 (40.8)	
	No	90 (75.6)	29 (24.4)	119 (59.2)	0.006
Contact with soil (n=201)	Yes	121(81.8)	27 (18.2)	148 (73.6)	
	No	47 (88.7)	6 (11.3)	53 (26.4)	0.24
Raw meat eating habit	Yes	87 (82.9)	18 (17.1)	105 (52.2)	
	No	81 (84.4)	15 (15.6)	96 (47.8)	0.77
Source of drinking water (n=201)	Pipe	148 (84.1)	28 (15.9)	176 (87.6)	
	well	20 (80)	5 (20)	25 (12.4)	0.6

Concerning the seroprevalence in relation to educational background, 24.4% of the pregnant women were illiterate out of which 75.5 % were IgG seropositive. There is no statistically significant difference in infection rate among the illiterate and literate study participants.

Domestic cats were present in homes of 40.8% of the study participants, of which 78 (95%) were positive of anti-*T. gondii* antibody. Ninety of the 119 pregnant women (75.6%) who had no domestic cats were also seropositive for anti-*T. gondii* antibodies. The difference in seropositivity for *T. gondii* antibodies in relation to cat ownership is statistically significant (Table 2).

Majority of the pregnant women, 148 (73.6%), were involved in activities associated with soil such as gardening, out of which 121 (81.8%) were seropositive. However, there is no statistically significant difference in seropositivity rate (Table 2). Comparable proportions of the pregnant women have/had habit of eating raw meat (52.2 Vs 47.8% for those who eat and not) with no statistically significant difference in seropositivity for *T. gondii*.

Concerning the source of drinking water, 87.6 % of them use pipe water for drinking, however, the remaining use well water either as a sole source for drinking or when the pipe water is interrupted, with seroprevalence rate of 84.1 % and 80% respectively, indicating that there is no significant difference in seropositivity rate (Table 2).

After performing multiple logistic regression, only increasing age and presence of domestic cats at home of the pregnant women were found to be the predictors for anti-*T. gondii* seropositivity in the pregnant women, as shown in Table 3 below.

Table 3: Predictors of Toxoplasma infection among pregnant women in Jimma Town, South-West Ethiopia, July 2011

Variable		OR (95% CI) (Crude)	OR (95% CI) (Adjusted)
Age group	15 – 19 (n= 25)	(ref)	-
	20 – 24 (n= 96)	2.8 (1.07 - 7.56)	3.3 (1.1 - 9.3)
	25 – 29 (n= 64)	4.4(1.4 - 13.7)	5.0 (1.5 - 16.6)
	30 – 35 (n= 16)	9.0 (1.02- 79.5)	9.7 (1.03 – 90.6)
Presence of cats	No (n= 119)	(ref)	-
	Yes (n= 82)	6.3 (2.1- 18.6)	6.7 (2.2 - 20.3)

CHAPTER SIX

DISCUSSION

This study showed that the overall seroprevalence of anti- *T. gondii* antibody among pregnant women in Jimma Town is 83.6%, which is very high compared to most of the studies done in different countries. Comparable finding was reported among pregnant women in Democratic Republic of Sao Tome and Principe (75.2%) [64]. A similarly high result of seroprevalence (90.0%) was reported earlier in a study done on clients seeking immunological tests at St. Paul hospital in Addis Ababa, Ethiopia [36]. Nearly before two decades, Guebre-Xabier M *et al*., have also reported high overall seroprevalence rate (74.4%) among the general population on samples collected from different regions of Ethiopia [50].

Concerning anti- *T. gondii* IgM antibody, those pregnant women who were IgG seronegative were tested for IgM anti-*T. gondii* and five of them were IgM seropositive. Most other studies done in different countries have also reported low percentage of IgM seropositivity among pregnant women, as in the case of 1.2% in Brazil [12], 2.8% in Columbia [54], 2.4% in New Zealand [67] and 2.3% in Mexico [68].

The IgG seroprevalence of *T. gondii* obtained in this study is much higher than the one reported in Palestine, in which 27.9% of the pregnant women were IgG seropositive [42], and Saudi Arabia, where 51.4% were IgG seropositive [28]. It is also higher than the seroprevalence reported from Brazil, in which 53.03% of the pregnant women were IgG seropositive [69], neighboring Sudan (34.1%) [40] and Morocco (50.6%) [70]. It is indicated that intrauterine fetal death is significantly higher among pregnant women with IgG anti-toxoplasma antibodies than the seronegative ones [40].

A wide variability in seroprevalence of *T. gondii* among pregnant women in different regions as well as from one country to another has been documented in several studies, as indicated earlier. These variations may be attributed to differences in climatic, personal hygiene practices and feeding habits as well as difference in socio-economy and literacy status of the study population under consideration. In this study, the fact that most of the study subjects were housewives (78.6% of the total study subjects) might have contributed for the high overall seroprevalence rate of anti-*T. gondii* antibody, as house wives are frequently in contact with vegetables during preparation of food, which is prone to oocyst contamination.

In this study, the increasing seropositivity pattern of *anti-T. gondii* antibody among the pregnant women as the age increases is in agreement with most of the studies done in different countries [42, 54]. This consistent pattern of relationship of increase in anti-toxoplasma seropositivity with age is explained by the fact that older women are more likely to have been exposed to any one of the risk factors than younger women as a result of longer exposure time, hence, being seropositive, as the parasite forms cysts and establishes chronic infection [71]. In this study, it is observed that 64, 83.3, 89 and 93% of the pregnant women in the age groups 15 - 19, 20 -24, 25 – 29 and 30 - 35 years were anti-*T.gondii* seropositive, respectively.

Similarly, increasing pattern of seropositivity was observed among the pregnant women with increasing parity, in which 79.7, 81.1, 86 and 96.2% of the primiparous, gravid 2, gravid 3 and gravid 4 & above were seropositive, respectively. A similarly increasing trend of seropositivity with parity was reported in a study done elsewhere [72]. This is possibly due to the tendency to have more children as the age increases, which is also observed from this study. The younger age group mothers were more likely to be primiparous and the older ones tend to be gravid 4 and above. However, there is no statistically significant difference in seroprevalence rates in the three trimesters of pregnancy in this study.

It is observed from this study that there is a statistically significant association between *T. gondii* infection and presence of domestic cats at home. A similar finding was reported in a study done in France [62]. Contact with cats was also implicated with higher risk of Toxoplasma infection among pregnant women in Taiwan [43] and Brazil [12]. Contrary to this, some studies reported no association of seroprevalence of toxoplasma with presence of domestic cats [53, 41]. Nijem K and Al-Amleh S also reported no association of *T. gondii* seroprevalence with history of

contact with cats [42]. The way the cats' litter box is cleaned rather than the simple presence of cats may be more important for exposure to the parasite and may explain the contradiction. In addition, the prevalence of the parasite among the cats may depend on the type of cats under consideration, as to whether pets or stray cats.

In this study, increasing age and presence of domestic cats at home were found to be the predictors for infection with *T. gondii* in the pregnant women (table 9).

Contaminated drinking water is also a possible source for transmission of *T. gondii* [65]. In this study, 12.4% of the study participants use water from well for drinking either when the pipe water is interrupted or as their sole source of drinking water, out of which 80% were seropositive. With this small number of study participants using well as a source of drinking water, it seems that using of well water is not associated with *Toxoplasma* infection. However, a study done in Nigeria has indicated higher seroprevalence rates among pregnant women drinking well water compared to those using packed water [41]. It is also documented that municipal water supply can potentially be contaminated with *Toxoplasma* oocysts [6].

In the present study, it was observed that comparable proportions of the pregnant women consume raw meat with no statistically meaningful difference in infection rate. The study done in Turkey also showed no association of consumption of raw meat with anti-*T.gondii* seropositivity [53]. Similarly, the study done in Palestine reported no association of consumption of undercooked meat with toxoplamsa infection [42].

However, in the study done in Sudan, consumption of raw meat was associated with *Toxoplasma* infection [40]. Ghoneim N *et al.*, also reported a strong association of consumption of undercooked meat of sheep and goat with toxoplasmosis in Egypt [39]. Tasting of meat while cooking was also attributed to higher risk of *Toxoplasma* infection in the study done in Nigeria [41]. Similarly, consumption of undercooked meat was found to be the single predictor of infection with *T. gondii* in Serbian women aged 15 - 49 years [63].

The difference in the pattern of infection rate with *Toxoplasma* in association with consumption of raw meat could be due the difference in the prevalence rate of the parasite in the animals in the different countries as well as the type of animals consumed. For instance, in a seroepidemiological survey of toxoplamsosis conducted among domestic animals in central Ethiopia in 1989, it was reported that 22.9% the sheep, 11.6% of the goats and 6.6% of the cattle examined were seropositive [73]. In Jimma Town, from personal communication, it seems more likely that raw beef is consumed more often than raw meat of goat or sheep. According to the above figure, beef poses relatively low risk, though the toxoplasmosis seroprevalence data for these animals is not up to date. This probably indicates that raw beef may not be the major route for the transmission of the parasite in Jimma Town, which in fact needs further research. Moreover, the probability of acquiring the infection by eating raw meat is unknown.

In this study, none of the pregnant women had history of blood transfusion, which is one possible source of *Toxoplasma* infection [74].

Information on HIV serostatus of the pregnant women was collected by the questionnaire. Accordingly, three of the pregnant women responded to be HIV seropositive, all of whom were IgG anti-*T. gondii* antibody positive. Five of the pregnant women were with unknown HIV serostatus, four of whom were anti-*T.gondii* antibody positive. Amogne *et al* demonstrated that toxoplasmosis is one of the AIDS-defining events among AIDS patients in a study done at Tikur Anbessa Specialized Referral Hospital in Addis Ababa [75].

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusion

- Seroprevalence of IgG anti-*T. gondii* antibody is high among the pregnant women and still good proportion of pregnant women are susceptible to infection with *T. gondii* during pregnancy.
- The seroprevalence increased in a linear trend as the age of the pregnant women increased.
- Presence of domestic cats at home is identified as a predictor of infection with *T. gondii* among the pregnant women.

7.2. Recommendations

- Behavior Change Communication be made to pregnant women during their ANC follow up to create awareness on how to prevent the infection, particularly on how to clean cat litter.
- Policy makers and local health planners should consider prevention of Toxoplasmosis particularly during pregnancy to prevent the tragic outcomes of congenital toxoplasmosis by training the urban health extension workers as they are very close to the pregnant women in the community.
- Toxoplasmosis should be considered in HIV positive pregnant women as this increases congenital transmission of the parasite.

⇨ Future research directions

Researchers interested in the area should:

- Consider congenital transmission rate of *T. gondii*
- Preferably include IgG avidity test for better demarcation of primary infection from chronic infection.

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Annex I

Materials and reagents

- ELISA test kit
- ELISA reader
- Refrigerator
- Centrifuge
- Micro pipettes (different volume)
- Multichannel micropipette
- Timer
- Micro pipette tips
- Cotton
- Eppendorf tube
- Wash bottles
- Distilled water
- Paper towels
- Disposable syringes
- Glove
- 70% ethanol
- Gauze
- Stationary materials
- soap
- Test tubes
- hypochlorite
- de-ionized water

Annex II - Questionnaire

JIMMA UNIVERSITY

COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES AND PATHOLOGY

An interviewer guided questionnaire for risk factor assessment to *T.gondii* infection among pregnant women in Jimma Town.

Date of data collection_____

Part One - Socio-demographic information

Ind. code	Age	Ethnicity	Religion	Educational status	Occupation	Residence	Trimester	Parity

1. Age in years

2. Ethnicity 1. Oromo 3. Gurage 5. Tigre 7.Others _____

2. Amhara 4. Kaffa 6. Yem

3. Religion 1. Muslim 3. Catholic 5. Other_____

2. Orthodox 4. Protestant

4. Educational status 1. Illiterate 3. Grade (1-4) 5. Secondary (9 - 12)

2. Only able to write and read 4. Grade 5 to 8 6. University/College

5. Occupation 1. House wife 3. Merchant 5. Student

	2. Gov/NGO employee	4. Daily laborer	6. House servant	7. Others _____
6. Trimester	1. 1st trimester	2. 2nd trimester	3. 3rd trimester	
7. Parity	1. Primiparous	2. Gravid 2	3. Gravid 3	4. Gravid 4 & above

Part Two - Assessment of Risk factors

1. Habit of eating raw meat	1. Yes 2. No
2. Presence of cats	1. Yes 2. No
3. Contact with soil/ gardening	1. Yes 2. No
4. History of blood transfusion	1. Yes 2. No
5. Current HIV serostatus (if known) (**Not**e: HIV serotest not to be done for the purpose of this research)	1. unknown 2. non-reactive 3. reactive

Yuunivarsiitii Jimmaatti Kolleejii Meedikaalaafii Fayyaa Uummataa Dippaartimantii Meedikaal Laaboraatooriifii Phaatooloogii

Dhukuba tooksoopilaasmoosiis jechamu dubartoota ulfa ta'anirrati qorannoo gaggeessuuf goca qophaa'e.

Guyyaa_____

Kutaa tokkoffaa – Gaaffii waliigalaa

kodii	Umurii	saba	amantaa	Sadarkaa barnootaa	Hojii	Iddoo jireenyaa	Umurii ulfaa	Baay'ina ijoollee

101. Umurii waggaadhaan_____

102. Saba

1. Oromoo 2. Amhaara 3. Tigree 4. Guragee 5. Yam

6. Kafaa 7. Kan biraa

103. Amantaa

1. Ortoodooksii 2. Musliima 3. Kaatolikii 4. Pirooteestaantii

5. Kan biraa

104. Sadarkaa barumasaa

1. kan hin baranne 2. Dubisuuf barreessuu qofa 3. Kutaa 1-4 4. kutaa 5 – 8 5. Kutaa 9-12 6. Kolleejii/Uunivarsiitii

105. Hojii

1. kan mootumma/dhuunfaa 2. Haadha manaa 3. Daldala 4. Barattuu 5. Qonnaa 6. Hojjeettuu manaa 7. Kan biraa (ibsi)

106. Iddoo jireenyaa

1. magaalaa 2. Baadiyyaa

107. Umurii Ulfaa

1. ji’a 3 jalqabaa 2. Ji’a 3 lammaffaa 3. Ji’a 3 sadaffaa

108. Ijoollee ammayoonaa dhalatani

1. kun kan jalqabaati 2. Lammaffaakooti 3. Sadaffaakooti 4. Afurii fi isaa oli

Kutaa lammaffaa – Wanneen saaxilanu

201	Foon dheedhii ni nyaattuu?	1. Eyyee 2. Lakki
202	Adurree qabduu?	1. Eeyeee 2. Lakki
203	Ashaakiltii/qonnarratti bobaatanii beektuu?	1. Eeyyee 2. Lakki
204	Mana yaalaatii dhiigni siniikennamee beekaa?	1. Eeyyee 2. Lakki
205	Amma haalli HIV	1. hin beekamu 2. hin qabu 3. qaba

Information Sheet

Name of the Principal Investigator: Endalew Zemene

Name of the organization: Jimma University

Introduction: This information sheet is prepared by groups of researchers whose main aim is to study seroprevalence of *T.gondii* in pregnant women. The investigators include a second year Medical Parasitology graduate student, and advisor from Jimma University, college of public health and medical sciences, Department of Medical Laboratory Sciences and Pathology.

Purpose:

The purpose of this research is to determine seroprevalence of *T.gondii* antibodies among pregnant women in Jimma Town.

Infection with *T.gondii* during pregnancy or chronic infection with *T.gondii* in the presence of immunosuppression may result in transmission to the fetus, a condition known as congenital transmission, and knowing the magnitude of infection in pregnant women will help in taking appropriate preventive actions.

Several studies in different countries have reported different seroprevalence *T.gondii* in pregnant women, both high and low, and identified different risk factors associated with the infection. However, in our country, studies done on this area are currently very few and didn't address the seroprevalence in pregnant women. Therefore, considering these factors, we have planned to undertake the research among pregnant women in Jimma Town.

Procedure:

We kindly invite you to take part in this project which is aimed at determining the seroprevalence of *T.gondii* among pregnant women, as mentioned earlier. If you are willing to participate in this project, you need to understand and sign the agreement form. You will then be asked to fill some questions associated with risk factors for infection with *T.gondii*. For laboratory examination, you will provide about 2ml of venous blood, blood sample collected from the arm. The blood samples will be collected following a standard protocol. The laboratory examination results will be kept confidential using coding system whereby no one will have

access to your laboratory results. If the result of the laboratory examination shows positive, this will only be communicated to the health professional attending you for further intervention.

Risk and Discomfort

It is obvious that there is some discomfort and pain when venous blood is collected. Apart for the rare occasions where temporary bleeding may happen during or immediately after blood collection, which will be followed closely, sterile syringes will be used and there is no need to worry about acquisition of blood-borne pathogens.

Benefits

If you participate in this research, you may get direct benefit that the test result will be used for the purpose of management of health condition of the fetus as well as yourself, based on the decision of the physician in charge. In addition, your participation will help us in determining the seroprevalence of *T.gondii* in pregnant women which is an input to design control strategies of the infection, especially during pregnancy.

Incentives

You will not be provided any incentives to take part in this research.

Confidentiality:

The information that we collect from this research project will be kept confidential. Information about you that will be collected from the study will be stored in a file, which will not have your name on it, but a code number assigned to it. It will be kept under lock and key, and it will not be revealed to anyone except the principal investigator and the health professional following you.

Right to refuse or withdraw

You have full right to refuse from participating in this research if you do not wish to participate; and this will not compromise the health services you get at the health institutions in any way at any time.

Whom to contact

If you have any questions contact any of the following two individuals and you may ask at any time you want:

1. **Endalew Zemene -** Jimma University, college of public health and Medical sciences, Department of Medical Laboratory Sciences and Pathology, **Jimma**
1. **Ato Ahmed Zeynidin -** Jimma University, college of public health and Medical sciences, Department of Medical Laboratory Sciences and Pathology**, Jimma**

Fuula Odeeffannoo

Maqaa qorataa jalqabaa – Indaaloo Zammanaa

Maqaa Waajjiraa - Yuunivarsiitii Jimmaa

Kun kan qophaa'e qorattoota dhukuba tooksoopilaasmoosis jedhamu qorataniini. Dhukubni kunis kan qoratamu dubartoota ulfarratti. Qorattoonni kunis Yuunivarsiitii Jimmaatti Meedikaal Paaraasaytooloogiidhaan barataa digirii lammaffaa fi gorsaa Yuunivarsiitii Jimmaarraa dha.

Kaayyoo qorannichaa

Kaayyoon qorannoo kanaa tamsa'inni dhukkubichaa dubartoota ulfarratii akkam akka ta'e baruu dha. Dhukkuni kun yeroo ulfaa haadharraa gara mucaatti gadameessa keessatti waan darbuu danda'uuf bal'ina dhukkuba kanaa baruun barbaachisaadha. Qorannoonni biyyoota addaddaatti gageeffaman akka agarsiisanutti tamsa'inni dhukkuba kanaa sadarkaa xiqqoorraa amma guddaati. Haata'u malee Itiyoophiyaatti qorannooni dhukkuba kanaa dubartoota ulfarratti godhame hanga yoonaatti hin argamne. Waankana ta'eef qorannoo kana gaggeessuun barbaachisaa ta'ee argameera.

Haala gorrannichaa

Qorannoo kanatti hirmaachuuf yoo fedhii keessa ta'e mallattoo walii gala mallatteessitanii daqiiqaa muraasaaf gaaffiwwan gaafatamtanuuf deebii kennitu. Ittaansee harka keessanirra dhiiga miliiliitrii lama ta'u kenniitu. Kunis haala sirrii ta'een fudhatama. Laaboraatooriitti eega geessamee booda qorannichi gaggeeffama. Haala laaboraatooriirratti hundaa'uun yoo xiyyeeffannoon addaa barbaachise akka hogeessi fayyaa isin ilaalu godhama.

Qorannichaan walqabatee haalli sodaachisu

Akkuma beekamu yeroo dhiigni harkarraa fuudhamu nama dhukkubuunsaa hin oolu. Kanaan achi meeshaa qulqulluun waan fuudhamuuf sodaa dhukkuboota daddarbanuu qabaachiin sinirra hinjiru.

Waan isin argattanu

Yoo qorannoo kanarratti hirmaattani qorannoo laaboraatooriirraan ka'uun hogeessonni fayyaa haala fayyaa keessanii fi mucaa keessanii baruu nidanda'u. Tamsa'ina dhukkuba kanaa baruudhaanis haala itti to'achuun danda'amu baruuf ni fayyada.

Kaffaltii

Qorannoo kanarratti hirmaachuu keessaniin qarshiin siniikaffalamu hin jiru.

Icitii

Yaadni isin nuukennitanuu fi firiin laboraatooriirraa argamu icitiin qabama. Nama qorrannicha gaggeessuufii ogeessa fayyaa sin ilaalu qofatu beeuu danda'a.

Mirga hirmaachuudhiisuu

Qorannoo kanaratti hirmaachuun dirqama miti. Mirga hirmaachuu dhisuu qabdu. Kun ta'uunis mana wal'aansaattii tajaajilli isin yeroo kaan argattanu sinduraa hin hafu ykn addaan hin citu.

Yoo gaaffii qabaattan

Qorannoo kanaan walqabatee yoo gaaffii qabaattani namoota armaan gadii yeroo kammittuu gaafachuu ni dandeessu.

1. Indaaloo Zammanaa – Lakk. Bilbilaa. 0912071295 Yuunivarsiitii Jimmaatti Dippaartimantii Meedikaal Laaboraatorii Saayinsii fi Paatooloogii

2. Obbo Ahimad Zaynudiin – Lakk. Bilbilaa – 0911733132 Yuunivarsiitii Jimmaatti Dippaartimantii Meedikaal Laaboraatorii Saayinsiifi Pathologii

Consent Form

I have been informed about a study. For this study I have been requested to give blood sample from the arm. I have read /have been read to me all the information stated in the introductory part and I have had an opportunity to ask any ambiguous question I got satisfactory answer for all of my concerns. I have fully understood and gave my consent to give the blood specimen. It is therefore, with full understanding of the situation that I gave my informed consent and cooperate at my will in the course of the conduct of the study.

Name (participant) ----------Signature ----------Date ----------

Name (investigator) ----------Signature ----------Date ---------

If illiterate: name of the independent witness, name and signature_____,_____/_____/_____(date/month/year)

Waliigaltee

Waa'een qorannoo kanaa natti himameera. Dhiigni harkakoorraa akka fudhatamus gaafatameera. Gaaffiin ani qorannoo kanarratti qabus akkan gaafadhuuf carraan naakennamee deebii ga'aas argadheera. Haala jiru hunda baruunis fedhii guutuudhaan dhiiga koo kennee qorannoo kanatti hirmaadheera.

Maqaa hirmaattuu _____ Mallattoo_____ Guyyaa_____

Maqaa qorataa _____ Mallattoo _____Guyyaa_____

Annex III

Venous Blood collection procedure

1. Assemble the necessary materials required. Make sure the syringe is sterile and is within the expiry date.
2. Apply a soft tubing tourniquet to the upper arm of the patient to enable the veins to be seen and felt. Do not apply the tourniquet too tightly or for longer than 2 minutes. Ask the patient to make a tight fist which will make the veins more prominent.
3. Using the index finger, feel for a suitable vein, selecting a sufficiently large straight vein that does not roll and with a direction that can be felt
4. Cleanse the puncture site with 70% ethanol and allow drying. Do not re-touch the cleansed area.
5. With the thumb of the left hand holding down the skin below the puncture site, make the venepuncture with the bevel of the needle directed upwards in the line of the vein. Steadily withdraw the plunger of the syringe at the speed it is taking the vein to fill.
6. When sufficient blood has been collected, release the tourniquet and instruct the patient to open his or her fist. Remove the needle and immediately press on the puncture site with a piece of dry cotton wool. Remove the tourniquet completely. Instruct the patient to continue pressing on the puncture site until the bleeding has stopped.
7. Remove the needle from the syringe and carefully fill the container (clean, dry test tube). Discard the needle safely. *Do not* attempt to re-sheath it because this can result in needle-stick injury. Immediately label the container.
8. Check that bleeding from the venepuncture site has stopped.

Annex IV

Test principles and protocols

Principle of Toxo IgG (Classic EIA)

The HUMAN TOXO IgG ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid are coated with *Toxoplasma* antigens (TOXO-Ag) prepared from sonicated whole Toxoplasma gondii parasites (Tachyzoites). In the first incubation step corresponding specific antibodies (TOXO-IgG-Ab) present in patient specimens or controls bind to the antigens at the solid phase. At the end of the incubation unbound components are washed out. For the second incubation step anti-IgG conjugate (anti-human IgG antibodies, peroxidase conjugate) is added which binds specifically to IgG class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/substrate is added (step 3). A blue color develops changing to yellow after stopping the reaction. The intensity of the color is directly proportional to the TOXO-IgG-Ab concentration in the specimen.

The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems.

Toxo IgG Pipetting scheme

Reagents and specimens should be at room temperature before use				
Sample preparation: Dilute patient's sera1 + 100 with DIL- g 10µl serum + 1ml DIL x thoroughly Diluted samples can be stored up to 48h at 2…8 oC before testing Controls are ready for use				
Step 1	Well (µl)			
	A1 Blank	B1/C1 NC	D1/C2 CC, PC	D2…. Sample
NC in duplicate	--	100	--	--
CC, PCs in duplicate	--	--	100	--
Diluted samples	--	--	--	100
Cover with adhesive strips				
Incubate 30min, at 17…25^{0}C				
Wash 4 times	350	350	350	350
Step 2				
CON	--	100	100	100
Cover with adhesive strip				
Incubate 30min, at 17….25^{0}C				
Wash 5 times	350	350	350	350
Step 3				
SUB	100	100	100	100
Incubate 15 min at 17…..25 ^{0}C				
STOP	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader using the substrate blank in well A1				
Measure the absorbance at 450 nm as soon as possible or within 30min after termination of the reaction.				

Principle of TOXO-IgM (µ-capture assay, direct IgM detection)

The HUMAN TOXO IgM µ-capture ELISA is intended for professional use. The µ-capture ELISA for direct IgM antibody detection uses anti-human IgM antibodies (mouse) coated on microtiter wells. All IgM class antibodies if present in the patient's specimen or the controls bind to the immobilized antibodies (step 1). After the incubation unbound specimen components are removed by washing. For the second incubation step Toxoplasma antigen HRP conjugate is added, which binds specifically to the anti-Toxoplasma IgM antibodies, captured by the immobilized anti-human IgM antibodies. After a washing step to remove excess conjugate, TMB/substrate is added (step 3). A blue color develops changing to yellow after stopping the reaction. The intensity of the colors is directly proportional to the Toxoplasma IgM antibody (Toxo-IgM-Ab) concentration in the specimen.

The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems.

Toxo IgM Pipetting scheme

Reagents and specimens should be at room temperature before use				
Sample preparation: Dilute patient's sera1 + 100 with DIL, eg 10µl serum + 1ml DIL mix thoroughly(15s) Diluted samples must be used on the same day NC, CC and PC are ready for use , mix for 5 seconds				
Step 1	Well (µl)			
	A1 Blank	B1-C1 NC	D1 – G1 CC, PC	H1…. Sample
NC in duplicate	--	100	--	--
CC, PC in duplicate	--	--	100	--
Diluted samples in duplicate	--	--	--	100
Mix carefully (5s)				
Cover with adhesive strips				
Incubate 60min, at 37^0C				
Wash 4 times	300	300	300	300
Step 2				
CON	--	100	100	100
Mix carefully (5sec)				
Cover with adhesive strip				
Incubate 30min, at 37^0C				
Wash 4 times	300	300	300	300
Step 3				
SUB	100	100	100	100
Incubate 30 min at 17…..25 ^0C				
STOP	100	100	100	100
Mix carefully				
Measure absorbance at 450 nm as soon as possible or within 10min after termination of the reaction.				

Annex V

Laboratory Data Record format

Code no_____

1. Identification: Age _____
2. Date of specimen collection _____
3. Specimens: Serum ☐ plasma ☐
4. Consistency of the specimen: clear ☐ Hemolysed ☐
5. Test result: Anti-*T.gondii* IgG Positive ☐
   Negative ☐

   Anti-*T.gondii* IgM Positive ☐
   Negative ☐

Declaration

I declare that this M.Sc thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for the thesis have been duly acknowledged

Endalew Zemene (MSc Candidate) Signature _____

Date of submission 16th May 2012

Advisors

1. Ahmed Zeynudin (Assistant Professor)_____

Approved by Examiners:

1. [Name (inst.)]…… [Sign/Date]……

2. [Name (inst.)]…… [Sign/Date]…… 17th May 2012