SEROPREVALENCE OF LATENT *TOXOPLASMA GONDII* INFECTION AND ASSOCIATED RISK FACTORS AMONG PEOPLE INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS IN ARBA MINCH HOSPITAL, SOUTHERN ETHIOPIA

By:

TSEGAYE YOHANES (BSc)

A RESEARCH PAPER SUBMITTED TO JIMMA UNIVERSITY COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES, DEPARTMENT OF MEDICAL LABORATORY SCIENCES AND PATHOLOGY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL PARASITOLOGY

OCTOBER, 2013

JIMMA, ETHIOPIA

Jimma University College of Public Health and Medical Sciences
Department of Medical Laboratory Sciences and Pathology

Seroprevalence of Latent *Toxoplasma gondii* Infection and Associated Risk Factors among People Infected with Human Immunodeficiency Virus in Arba Minch Hospital, Southern Ethiopia

Advisors:

1. Endalew Zemene (BSc, MSc)
2. Serkadis Debalke (BSc, MSc)

October, 2013

Jimma, Ethiopia

I. Abstract

***Background**: Toxoplasmosis is a zoonotic disease caused by an obligate intracellular parasite known as Toxoplasma gondii (T. gondii). The parasite infects approximately half of the world's population. Latent stages of toxoplasmosis are prevalent in Human Immunodeficiency Virus (HIV) infected individuals, approximately one-third of Acquired Immunodeficiency Syndrome patients (AIDS) with an antibody to T. gondii reactivating their latent infection, resulting Cerebral Toxoplasmosis(CT) and other clinical disease. So far no documented data concerning the epidemiology of T. gondii infection in HIV infected individuals is available in the study area. This study aimed at determines the seroprevalence of latent T. gondii infection and associated factors among people infected with HIV in Arba Minch hospital, southern Ethiopia.*

***Methods**: A facility based cross sectional study design was employed. A total of 170 study participants visiting Arba Minch hospital Anti-Retroviral Therapy (ART) clinic from April 5 to June 5, 2013 were enrolled consecutively. Data regarding socio demographic and associated factors were gathered using questionnaire. Approximately two milliliters of blood sample was collected and tested for anti-T. gondii IgG antibody using Enzyme Linked Immunosorbent Assay (ELISA). Bivariate and multivariate logistic regression were used for the analysis.*

***Results**: Seroprevalence of latent T. gondii infection among the study participant was 88.2%. The seropositivity increased as age of the study participants increased. Multivariate analysis revealed that consumption of raw meat (Adjusted Odd Ratio (AOR) = 4.361;95% Confidence Interval (CI):1.409-13.496) and history of engagement in farming/gardening activities(AOR=4.051; 95% CI:1.112-14.758) were independent risk factors for Toxoplasma seropositivity.*

***Conclusion**: In the present study high prevalence of latent T. gondii infection was found among HIV infected individuals. Consumption of raw meat, engagement in farming/gardening activities were identified as main predictors of T. gondii infection. It suggests that routine screening of Toxoplasma should be considered for all HIV-infected individuals to detect latent infection. Moreover health information should be provided for HIV infected individuals, about ways to minimize exposure to the risk factors.*

***Key words**: Seroprevalence, latent T. gondii, HIV/AIDS, Arba Minch.*

II. Acknowledgements

First and foremost, I wish to thank Jimma University for providing me with financial and administrative supports to conduct this study.

I would like to express my sincere gratitude to my advisors Mr. Endalew Zemene and Mss. Serkadis Debalke for their scientific guidance, constructive comments and willingness in helping and giving me invaluable idea throughout this research work from the very beginning up to the end.

My compliment also goes to Arba Minch University for sponsoring my study program at Jimma University.

I am grateful to members of staff of Arba Minch hospital and Arba Minch regional laboratory for their help and good wishes especially to Kebede, Buzhayehu and Ashenafi Abose for their assistance and unreserved sharing of knowledge and technical skills. I also acknowledge all the study participants for their cooperation to voluntarily involve in the study.

I am indebted to my friends and co-workers Firee, Tesfa, Melaku, Senay, Tilahun and Wondowsen for unending moral support and suggestions in my academic life.

My sincere gratitude goes to my parents for their endless support and prayer. I am very appreciative.

I owe special thanks to Ato Mesfin owner of Misroy Company for importing the reagents from abroad on timely.

Finally, my compliment also goes to all those who assisted and gave me indispensable comments and suggestions during the course of this research.

III. Table of contents

IV. List of tables

V. List of figures

VI. List of Abbreviations and Acronyms

AIDS	Acquired Immuno Deficiency Syndrome
AOR	Adjusted Odd Ratio
ART	Anti-Retroviral Therapy
CC	Cut-off Control
CD	Cluster of Differentiation
CI	Confidence Interval
CT	Cerebral Toxoplasmosis
ELISA	Enzyme Linked Immuno Sorbent Assay
HAART	Highly Active Antiviral Therapy
HIV	Human Immunodeficiency Virus
IFAT	Immuno Fluorescent Antibody Test
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MCC	Mean of Cut-off Control
MNC	Mean of Negative Control
MPCM	Mean of Positive Control Medium
NC	Negative Control
OIs	Opportunistic Infections
PCH	Positive Control High
PCL	Positive Control Low
PCM	Positive Control Medium
SFDT	Sabin-Feldman Dye Test
SNNPR	Southern Nations, Nationalities' and Peoples' Region
SPSS	Statistical Package for Social Sciences
T. gondii	*Toxoplasma gondii*
TE	Toxoplasmic Encephalitis

Chapter One: Introduction

1.1 Background

T. gondii is an animal coccidian parasite which causes toxoplasmosis [1]. The parasite belongs to phylum Apicomplexa and is characterized by the presence of a polarized cell structure and two unique apical secretory organelles called micronemes and rhoptries. It is ubiquitous throughout the world and estimated to infect approximately half of the world's population [2].

There are three infectious stages of *T. gondii* to all hosts: the tachyzoites in groups or clones, bradyzoites in tissue cysts and the sporozoites in oocysts [3]. Concerning humans, the parasite can be transmitted vertically from infected mothers to the fetus transplacentally by tachyzoite stage that may also diffuse via blood transfusion, unpasteurized milk and rarely accidental inoculation. While horizontal transmission in humans may involves ingesting of environmentally sporulated oocysts in contaminated foods, unwashed fruits or vegetables and through the dormant chronic tissue cysts that are inhabitant in tissues of food animals [4, 5]. The parasite has a complex life cycle consisting of an asexual cycle in its intermediate hosts, including humans and a sexual cycle in its definitive hosts, the feline family [6].

Pathogenicity of *T. gondii* is determined by many factors including the susceptibility of the host species, virulence of the parasitic strain and the stage .The parasite invades numerous organs, infecting a broad spectrum of cell types. Tachyzoites infect macrophages and disseminated through the blood to many organs, invade, asexually multiply and cause cellular disruption leading to cell death. As the host develops resistance, tissue cysts which are called bradyzoites may form in many organs primarily in brain and muscle [7]. A chronically infected individual who develops defect in cell-mediated immunity is at risk of reactivation of latent infection [8].

Most individuals positive for *T. gondii* antibodies have no history of a clinical syndrome that was diagnosed as toxoplasmosis, leading to the supposition that majority of primary infections are asymptomatic [9]. Majority of acute infections in immunocompetent individuals go unrecognized because either it is subclinical or symptoms are nonspecific and it is falsely taken as a viral illness. The common manifestations are non tender lymphadenopathy, fatigue, headache, malaise and myalgia .The infection is usually self-limited and requires no treatment [10]. In immunocompromised patients, particularly those with AIDS, reactivation of latent

cysts in the central nervous system may cause a fatal disorder called Toxoplasmic Encephalitis (TE). The initial presentation of TE in patients with AIDS may be sub acute, patients may present with altered mental status, headache and fever associated with focal neurologic deficits. Progression of the infection can lead to confusion, speech disturbance, drowsiness, seizures and cranial nerve palsies. The eyes and lungs are the most common sites of extra cerebral manifestation of toxoplasmosis, and such manifestations may occur with or without concomitant encephalitis. Other manifestations are rare, including involvement of the gastrointestinal tract, liver, musculoskeletal system, heart, bone marrow, bladder, spinal cord, and testes [11, 12].

Generally *T. gondii* infection can be diagnosed by serologic tests, amplification of specific nucleic acid sequences, histological demonstration of the parasite or its antigens using immunoperoxidase stain and by isolation of the organism. Other rarely used methods include demonstration of antigen in serum or body fluids, toxoplasmin skin test and antigen-specific lymphocyte transformation. The initial and primary method of diagnosis involves the use of serologic tests for demonstration of specific antibody. Sabin-Feldman Dye Test (SFDT), ELISA, Immuno Fluorescent Antibody Test (IFAT) and agglutination and differential agglutination test are the most commonly used tests for the measurement of Immunoglobulin G (IgG) antibody [13,14]. All HIV infected persons should be tested for IgG antibody to detect latent *T. gondii* infection and also all HIV-infected persons, including those who lack IgG antibody to *Toxoplasma* should be counseled regarding the sources of infection [15].

Toxoplasma infection prevention should focus mainly on the three main sources of infection; meats, contaminated environment and domestic animals. General preventive measures should be applied to avoid *Toxoplasma* infection. These include proper handling and preparation of meat, vegetables and fruits. Contact with any utensils that may have been contaminated with cats' faces and cats' litter should be strictly avoided. Hands should be washed after contact with soil, dogs, cats, and before meal. Finally, all patients diagnosed with HIV should be educated about primary and secondary medical prophylaxis for *T. gondii* infection [12, 16].

1.2 Statement of the problem

Opportunistic Infections (OIs) are the most common causes of morbidity and mortality in HIV/AIDS patients globally. Toxoplasmosis is the main systemic opportunistic infection reported in HIV-infected patients and key parasitic disease which have been included in the centers for disease control and prevention case definition for AIDS [17]. The disease remains a global public health problem; its burden varies greatly from country to country [18].

The estimated seroprevalence of Latent stages of toxoplasmosis varies from <2% up to 70% in different groups of population found in Southeast Asia. High prevalence rates of the infection were reported in HIV infected patients from several countries; up to 72% from South America, 40% and above from Asian continent [17, 19].

Evidences show that toxoplasmosis is highly prevalent in HIV-infected patients, with substantial incidence of TE reported in AIDS patients. The disease is commonly encountered in clinical practices in co-existence with HIV/AIDS patients and one of the most frequent OIs, particularly in patients with full-blown AIDS. Other forms of dissemination have also been reported in AIDS patients in sites such as the eyes, lungs, heart and spinal cord [17, 19]. More than 95% of CT occurs primarily due to reactivation of latent *Toxoplasma* infection. The risk of developing CT among HIV seropositive patients is 27 times higher than those seronegatives [20]. TE usually occurs in HIV- infected patients with Cluster of Differentiation(CD) 4+ T cell counts less than 100/μl [8]. In AIDS patients, incidence of developing TE is related to *T. gondii* IgG seropositivity and low CD4+ T lymphocyte counts [21].

T. gondii infection can cause a more serious progression when accompanied with some other infection in pregnant women having HIV. Reactivation of latent *T. gondii* infections occur particularly in those who are severely immunocompromised women resulting in vertical transmission of one or both infections and sever forms of TE [22]. Furthermore, in HIV positive pregnant women, *T.gondii* infection often coexists with Hepatitis C virus and Hepatitis B virus infections and can cause serious complications leading to miscarriage, stillbirth, birth defects and enhance the mother-to-child transmission of Hepatitis B and C virus [23].

TE remains the most prevalent central nervous system disorder, accounting for one-fourth of all documented cases in both antiretroviral-treated and untreated HIV-infected persons. Even with

the widespread use of Highly Active Anti-Retroviral Therapy (HAART) the issues of patients' adherence, drug resistance, failure and cross-resistance were major risks for the development of TE. The disease is still reported in HIV-infected patients with or without prophylaxis and relapse cases after discontinuation of maintenance prophylaxis despite high CD4 + T cell count [22, 24].

Despite the availability of HAART and effective prophylaxis, TE can lead to serious morbidity and mortality, especially in resource-limited countries [25]. Decreasing rates of opportunistic diseases, including neurological infections, have been reported both in developed and developing countries with access to HAART. However, the impact of HAART seems to be lower in developing countries with access to HAART owing to delayed diagnosis of HIV infection or lack of opportunities to start treatment in patients prior to diagnosis of HIV. CT is an HIV-indicative event in 35% of patients and an AIDS-defining event in 75% of cases [26].

In African countries, high prevalence of *T. gondii* infection among HIV seropositive individuals is expected. The prevalence of *T. gondii* infection was found to be high in several African countries: 69.9% from HIV/AIDS patients in Cameroon [27]; 60% in Bamako [28]; 54% in Uganda [29]; 92.5% among pregnant women in Accra [30]; 58.4% from general population in Northern Tunisia [31]. With the advent of the HIV pandemic TE has become one of the more frequent OIs and the most commonly implicated cause of focal brain lesions, complicating the course of AIDS particularly in developing countries. In addition to these, the disease poses many diagnostic and therapeutic challenges for clinicians treating HIV-infected patients in the countries [22, 32].

Because of the great importance of *T. gondii* as a causative agent of a zoonosis, public health organizations recommend the collection of accurate epidemiological data on *T.gondii* infection. However, only few countries of the world regularly monitor toxoplasmosis in humans [5]. In Ethiopia as reviewed by Dubey and coworkers about 1 million adults are estimated to be infected with HIV with less than one-third likely receive HAART. OIs including toxoplasmosis are the leading cause of morbidity and mortality in AIDS patients [33]. There is little information concerning seroprevalence of latent *Toxoplasma* infection in HIV infected individuals in Ethiopia. Moreover, laboratory diagnosis of *Toxoplasma* infection is currently not practiced in health facilities of the country. So far no documented data concerning the epidemiology of *T. gondii* infection in HIV infected individuals is available in the study area.

Documenting seroprevalence of *T. gondii* infection in HIV/AIDS patients is of great importance for diagnostic purpose and as a baseline information from epidemiological point of view. Therefore, the present study aimed at determining the seroprevalence of latent *T. gondii* infection and its associated risk factors among people infected with HIV in Arba Minch Hospital, Southern Ethiopia.

Chapter Two: Literature Review

Seroprevalence of *Toxoplasma* infection varies in different countries of the world and among different socioeconomic groups. Different literatures documented various risk associated with *T. gondii* infection. Some of the available literatures on seroprevalence and associated risk factors of *Toxoplasma* infection are reviewed as follows.

2.1 Prevalence

A cross sectional study was conducted in Mexico, in which blood sample from HIV /AIDS patients were screened for anti-*Toxoplasma* IgG and Immunoglobulin M (IgM) antibody using ELISA. The results indicated that the prevalence of anti-*T. gondii* IgG antibody was 50.0% and IgM antibody was 1.0% [34]. Another study conducted in Cuba, showed that prevalence of *T. gondii* infection was 71.96 % on the basis of detection of IgG antibodies [35]. In a similar study carried out in Malaysia, blood sample from HIV/AIDS patients was screened for antibody against *T. gondii* by using ELISA. The study finding showed that the overall seroprevalence of *T. gondii* infection among the patients was 44.8% [36].

In another study conducted in Northern Iran, HIV infected individuals were screened for anti-*T. gondii* IgG and IgM antibody, 77.4% had serological evidence of *T. gondii* infection [37]. Lower prevalence (38.01%) of *T. gondii* infection was reported from Northeast of Iran [38]. In a study carried out in Nigeria, 54.2% of the screened patients were positive for anti-*T. gondii* IgG antibody [32].

Few studies have been conducted in Ethiopia on the seroprevalence of *T. gondii* in HIV patients. In a study done in St. Paul Hospital, high seroprevalence (93.3%) of *T. gondii* was reported [39]. Earlier 80% prevalence was documented among factory workers in Addis Ababa [40]. A recent study conducted in Bahir Dar reported seroprevalence of 87.4 % among HIV seropositive patients [41].

2.2 Factor associated with *T. gondii* infection

2.2.1 Socio-demographic factors

Age

Several studies show that prevalence of *T. gondii* infection is significantly increases with age. According to a study conducted in Benue State, Nigeria seroprevalence of *Toxoplasma*-IgG varied between 0.0%-30.3%, with the age group 13 years having the lowest seroprevalence (0.0%) and the age group 54 years having the highest, 30.3%. The finding also indicated that seroprevalence of *T. gondii* infection increased with age. Additionally, statistically significant difference was found among age groups [42]. Studies from United States [43] and Nigeria [44] also showed similar finding. An increasing pattern of seroprevalence with age was also observed in the study done in Addis Ababa [39].

Gender

An institutional based cross-sectional study carried out at Gondar University hospital among people infected with HIV showed that *T. gondii* seroprevalence was higher amongst females 54.6% than males 45.4%. However, there was no statistically significant association between gender and *Toxoplasma* seropositivity was documented [45]. Similarly, no significant relationship between gender and *T. gondii* infection among the same study group reported in Bahir Dar [41] and St. Paul hospital, Addis Ababa [39].

Place of residence

Exposure to *Toxoplasma* parasite may vary depending on residence of the individuals as the awareness on the mode of transmission may vary. However, studies reported contradicting reports. For example a seroprevalence survey of toxoplasmosis conducted in Khartoum State, Sudan among several target groups showed that there was statistically significant difference in prevalence of *T. gondii* infection between urban and rural dweller [46]. In contrary no significant differences in *T. gondii* seropositivity between urban and rural dweller was observed among HIV/AIDS patients studied in Bahir Dar, Ethiopia [41] and Gondar [45].

2.2.2 Dietary habits

Consumption of raw meat

One possible route of *Toxoplasma* transmission is consumption of raw meat infected with the parasite. However, contradicting reports exist on the prevalence of *T. gondii* with habit of eating raw meat. A study conducted in Malaysia aimed to determined possible risk factor of *T. gondii* infection and seroprevalence among HIV/AIDS patients showed that consumption of raw/ undercooked meat was not significantly associated with seroprevalence of *T. gondii* infection [47]. Similar finding were reported in other studies from Gondar [45] and Malaysia [48].

In contrast to the above consumption of raw / undercooked meat was found to be significantly associated with *T. gondii* infection in studies done in Bahir Dar [41] and Khartoum among several target groups [46].

Eating raw / unwashed vegetables or fruits

The most resent cross sectional study conducted in Bahir Dar, Ethiopia among HIV patients showed that there was no significant association between eating unwashed /raw vegetables or fruits with anti-*T. gondii* IgG antibody seropositivity [41]. However, studies done among pregnant women in Nigeria [49] and Pakistan [50] reported significant association.

2.2.3 Environmental factors

Presence of cats at home/Contact with cats

Published studies documented contradicting reports on the availability of domestic cats at home and infection with *T. gondii*. In a study carried out in Jimma town among pregnant women showed that the presence of cats was significantly associated with *T. gondii* infection [51]. On the other hand Esquivel and coworkers [52] reported no association.

Literatures also identified contact with cats as a risk factor for *Toxoplasma* infection. According to a study conducted in Malaysia, indicated that there was no statistically significant association between contact with cat and seroprevalence of *Toxoplasma* infection [47].

Another similar finding was reported in the same country [48]. On the other hand some studies showed that contact with cats was significantly associated with *T. gondii* infection. A study from Bahir Dar assessed the association between contact with cats and *T. gondii* infection. The study finding showed that contact with cats was found to be significantly associated with seropositivity [41]. Similar finding reported in Khartoum State, Sudan [46].

Farming /gardening activities

A cross sectional study conducted in Southern Brazil among blood donors showed that there was no statistical significant association between engaging in farming /gardening activities and prevalence of *T. gondii* infection [53]. Similar finding was reported in Slovakia among the general population [54]. However, studies done in Nigeria [49] and Pakistan [50] among pregnant women documented significant association of farming activity with *Toxoplasma* infection.

Source of drinking water

Drinking water sources, especially unprotected wells, may be contaminated with oocysts of *Toxoplasma* ultimately infecting human. A cross-sectional study conducted in Gondar University hospital, assessed the association between source of drinking water and seroprevalence of *T. gondii* among people infected with HIV. The study showed that source of water for drinking did not show significant association with *Toxoplasma* seropositivity [45].

2.2.4 Clinical factors

Blood transfusion

Blood transfusion is another possible source of human infection with *Toxoplasma* and serological studies documented varying prevalence of the infection among blood donors. Despite high serological evidence of the infection in blood donors, patients who had history of blood transfusion didn't have significantly high prevalence of the infection. In cross-sectional study conducted in Malaysia among HIV/AIDS patients history of blood transfusion was not associated with seropositivity of *T. gondii* [47]. Similarly finding was documented in other studies from the same country [48] and Bahir Dar [41].

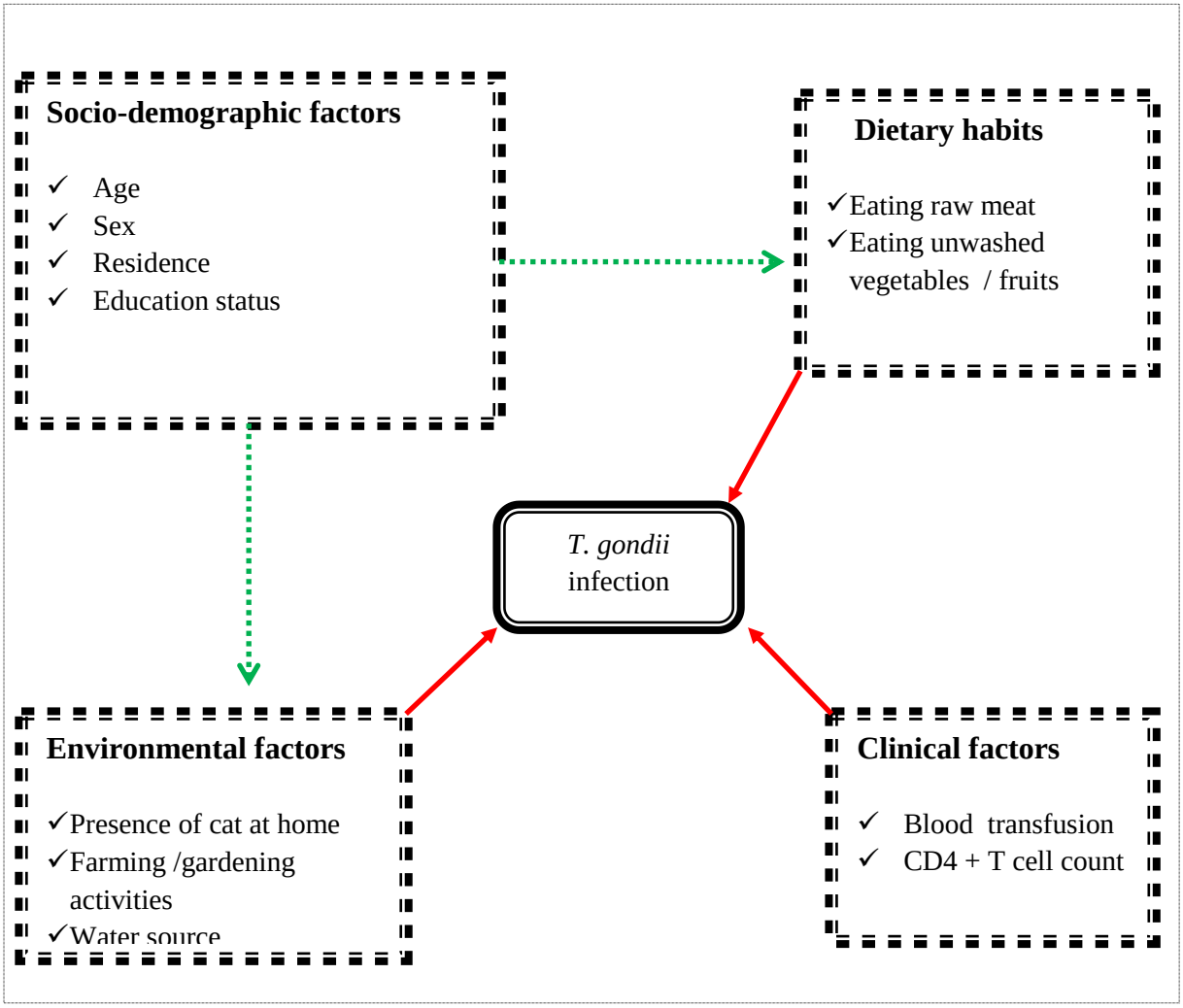
2.3 Significance of the study

Latent toxoplasmosis is prevalent in HIV infected individuals, approximately one-third of HIV/AIDS patients with antibodies to *T. gondii* reactivating their latent infection, resulting CT and other clinical disease. Determining the prevalence of latent *Toxoplasma* infection in HIV/AIDS patients would reduce the risk of treatable damage to the central nervous system and high rates of morbidity and mortality of the disease due to reactivation.

Several studies have been done on toxoplasmosis in the world, while only few study reports have been published on prevalence of *T. gondii* among HIV/ AIDs patients in Ethiopia. In the study area, so far, no study was obtained on the seroprevalence of *T. gondii* infection among people infected with HIV. Therefore the present study;

- Will determine the current prevalence of latent *T. gondii* infection and its associated factors among people infected with HIV, which serves the attending clinicians for better management of the patients.
- Will provide relevant information for government bodies, non-governmental organizations, policy makers and health planners, for future planning and interventions on toxoplasmosis in HIV infected individuals.
- Provide base line information for further studies.

2.4 Conceptual frame work



Indicator

The deactivated line indicated that there is association between the factors but not the interest of this study to see these associations

The activated line indicated that there is association between the factors and also the interest of this study to see these associations

Figure-1: Conceptual frame work for this study developed after extensive literature review on prevalence and associated risk factors of *T. gondii*.

Chapter Three: Objectives

3.1 General objective

- ✓ To assess seroprevalence of latent *T. gondii* infection and its associated risk factors among people infected with HIV in Arba Minch hospital, Southern Ethiopia, 2013.

3.2 Specific objectives

- ✓ To determine seroprevalence of latent *T. gondii* infection among people infected with HIV.

- ✓ To identify factors associated with *T. gondii* infection among people infected with HIV.

.

Chapter Four: Methods and Materials

4.1 Study area

The study was conducted at Arba Minch hospital found in Gammo Gofa zone, Southern Nations, Nationalities' and Peoples' Region (SNNPR) ; southern Ethiopia. Arba Minch town is the administrative and trading center of the Gammo Gofa zone, located at 505 km from Addis Ababa and 275 km south west of Awassa. The total area of the town is estimated about 1095 hectares and it lies at an altitude of 1300 meters above sea level, its average temperature is 29°C and the average annual rainfall is 900 mm.

Arba Minch hospital is a general hospital originally built to house 50 beds but has now expanded to 300 beds and serving a population of two million. The Hospital provides general outpatient service, surgical & obstetric emergency services, general medical and pediatrics in-patient services [55]. In Ethiopian free ART program was launched in 2005GC and Arba Minch hospital became part of this scheme, ART has been given to the patients, following the world health organization and national recommendations [56]. During the study period 1650 confirmed HIV seropositive individuals registered and actively followed in ART clinic of Arba Minch hospital [Arba Minch Hospital ART clinic data].

4.2 Study design and period

A facility based cross sectional study design was conducted from April 5 to June 5, 2013.

4.3 Population

4.3.1 Source population

The source populations were all people infected with HIV who were registered in Arba Minch hospital and on follow up in the year 2013.

4.3.2 Study population

The study population were all confirmed HIV seropositive individuals attending Arba Minch hospital ART clinic and available during the study periods.

4.3.3 Study participants

The study participants were those who attend Arba Minch hospital, ART clinic during the study period, who were fulfilled the inclusion criteria.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- All confirmed HIV seropositive individuals who were registered in the ART clinic and available during the study periods.
- Those who were willing to participate in the study were included in the study.

4.4.2 Exclusion criteria

- Severely ill patients who were unable to respond to the questionnaire.
- Those who were referred from other health centers and hospitals for a single clinical visiting in the clinic were excluded from the study.

4.5 Sample size and sampling technique.

4.5.1 Sample size determination

Sample size was determined by using single population proportion formula at 95% of confidence level and the following assumptions.

Proportion of population infected (P) 87.4%; the prevalence of toxoplasmosis 87.4 % in HIV infected individuals taken from a study conducted in Bahir Dar, Northwest Ethiopia [41].

Margin of error (d) = 5%

Non response rate =10 %

- ✓ The formula for calculating the sample size (n) is:

$$n = \frac{(z\ \alpha/_2)^2 \times P\ (1-p)}{d^2}$$

Where: n = Sample size

$Z\ (z\ \alpha/_2)^2$ = Z-score at 95 % confidence level =1.96

P = Prevalence of previous study 87.4 % = 0.874

D = Margin of error 5 % = 0.05

✓ Therefore the value of n calculated as follows

$$n = \frac{(1.96)^2 \times 0.874\ (1-0.874)}{(0.05)^2} = 169.22 \approx 169$$

✓ Since the source population <10,000 which was1650 final population correction formula is applied.

$$n' = n / (1+n/N)$$

$$n' = \frac{169}{1+1650/1650} = 154$$

✓ After application of population correction formula the minimum sample size was **154**. Considering 10 % non response rate the final sample size was 170 HIV infected individuals were included.

4.5.2 Sampling technique

Consecutive sampling technique was used; in which all study units that was available at the time of data collection and fulfill the inclusion criteria were enrolled consecutively.

4.6 Measurement

4.6.1 Study variables

4.6.1.1 Dependent variable

- Anti-*T. gondii* IgG antibody serostatus

4.6.1.2 Independent variables

- ✓ Age
- ✓ Sex
- ✓ Residence

- ✓ Education status
- ✓ Consumption of raw meat
- ✓ Consumption of raw/unwashed vegetable or fruits
- ✓ Presence of cat at home
- ✓ Farming /gardening activities
- ✓ Source of drinking water
- ✓ Blood transfusion
- ✓ CD4+ T cell count

4.6.2 Data collection instrument

A semi-structured questionnaire was used that contained questions concerning the socio-demographic, dietary, environmental and clinical information about studies participants. The questionnaire was adapted from reviewing of similar studies and prepared first in English language and was then translated into Amharic; and then pretested on 5 % subsamples for its appropriateness. The questionnaire was further modified after pretesting. Laboratory format was also used to record the laboratory test results.

Prior to conducting the actual laboratory work proper performance of all the laboratory equipments used for testing was verified and the expiry date of all reagents was checked.

4.7 Data collection process

4.7.1 Data regarding socio-demographic, associated factors and laboratory

Each patient coming to follow up clinic was interviewed about socio-demographic, dietary, environmental, clinical information and the response was filled out on the respective questionnaire. Results of serum tested for *T. gondii* IgG antibody and CD4+ T cell count were also filled on the appropriate formats. One trained nurse and a laboratory technologist were recruited during data collection and laboratory work, respectively.

4.7.2 Specimen collection and laboratory investigation

4.7.2.1 *Blood sample collection*

Venous blood specimens were collected from each study participant after written consent was obtained. Approximately 2 milliliters of venous blood was collected by needle and syringe technique aseptically from each of the study participants by laboratory technologist (principal investigator) following standard operating procedures.

4.7.2.2 *Blood sample processing*

After blood samples were properly collected, serum was separated from the other blood compartments by clotting and centrifugation at 5000 rpm for 10 minutes. Then separated serum samples were labeled and kept at -20°C till serological test was done.

4.7.2.3 *Laboratory investigation*

The stored serum samples were properly transported from Arba Minch hospital laboratory to Arba Minch regional laboratory department. Finally, it was tested for anti-*T. gondii* IgG antibody by using commercially available enzyme immunoassay test kit (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden Germany). The test was performed strictly following the manufacturer's instruction [Annex-V].

4.7.2.4 *CD4 + T cell count*

The current CD4+ T cell count of the study participants were obtained from log book following the identity number.

4.8 Data quality assurance

The following measures were undertaken so as to control the quality of the data and laboratory investigation. One day training was given to data the collector on purpose of the study, on each item in the questionnaire, how to get informed consent, and on data collection procedure. Properly designed and pre-tested data collection instrument was used. Every day the collected data was cross checked for completeness, consistency and on site correction action was made.

A standard operational procedure tools was strictly used for sample collection, transportation and storage. Special emphasis was given during coding the data sheet as well as the collected blood samples. Before used all reagents was checked being at appropriate temperature and within specified shelf life. To avoid measurement bias, internal quality control was run along with the test sample. According to the manufacturer's instruction test procedures were followed.

4.9 Administration monitoring

Validity and completeness of the overall study was supervised by advisors.

4.10 Data analysis and interpretation

Data was entered and analyzed using Statistical Package for Social Sciences (SPSS) version 17 software packages. Descriptive summaries were used to describe the study variables and summaries were presented in terms of counts and percentages. Bivariate and multivariate logistic regression were used for the analysis. Variables with a p-value < 0.25 in the bivariate analysis were entered into multivariate analysis. In multivariate analysis a p-value < 0.05 was considered as statistically significant.

4.11 Ethical Consideration

Ethical clearance was obtained from Jimma University research and ethical review committee and permission was obtained from the respective bodies Arba Minch Zonal health bureau and Arba Minch hospital. The purpose and procedures of the study were explained and a written informed consent was obtained from all study participants. Written consent was also obtained from the respective guardians for children below the age of 18 years. Privacy and confidentiality of the study participants response and laboratory test result was maintained. For those participants whose laboratory result was positive, the principal investigator was reported and consulted to the concerned physician for further management.

4.12 Operational definition

Latent infection: A lingering infection that may lie dormant, inactive or hidden in the body for a time but may become active under a certain conditions.

Consumption of raw meat: Defined as a person who have a habit of eating any kinds of uncooked meat.

Blood received: Defined as if a person has received whole blood or blood products from his/her donors.

Illiterate: A person of age 7 years or above who responded not able to read and write at least with one language.

4.13 Data dissemination plan

The final finding of the current study will be submitted to Jimma University collage of public health and medical sciences, department of medical laboratory sciences and pathology. Summary of the finding will be disseminated to Arba Minch Zonal health bureau and Arba Minch hospital. Finally publication of the study finding in a peer reviewed journals and presentation in scientific meeting will be considered.

Chapter Five: Results

5.1 Socio- demographic characteristics

A total of 170 HIV seropositive individuals who had been attending Arba Minch hospital ART clinic from April 5 to June 5, 2013 were included in the study. The mean age of the participants was (35.54 years) dominantly within the age range 35-44 years. Higher proportions of the subjects were females and married. Most respondents were resided in urban area. Majority of the participants were employed and had studied up to primary level education [Table-1].

5.2 Seroprevalence of *T. gondii* infection

Out of the total 170 HIV seropositive individuals tested for *T. gondii* -IgG antibody, 150 were found to be seropositive, giving overall prevalence rate of latent *T. gondii* infection 88.2 %.

Table-1: Prevalence of *T. gondii* infection along with socio-demographic characteristics among people infected with HIV (n=170) in Arba Minch hospital, 2013.

Socio-demographic variables	Seroprevalence		Total No (%)
	Positive No (%)	Negative No (%)	
Age group(years)			
≤ 24	11(61.1)	7(38.9)	18(10.6)
25-34	48(90.6)	5(9.4)	53(31.2)
35-44	58(90.6)	6(9.4)	64(37.6)
≥ 45	33(94.3)	2(5.7)	35(20.6)
Sex			
Male	53(86.9)	8(13.1)	61(35.9)
Female	97(89.0)	12(11.0)	109(64.1)
Place of residence			
Urban	129(87.8)	18(12.2)	147(86.5)
Rural	21(91.3)	2(8.7)	23(13.5)
Marital status			
Single	30(88.2)	4(11.8)	34(20.0)
Married	79(87.8)	11(12.2)	90(52.9)
Divorced	14(82.4)	3(17.6)	17(10.0)
Widowed	27(93.1)	2(6.9)	29(17.1)
Educational status			
Illiterate	36(83.7)	7(16.3)	43(25.3)
Primary	69(92.0)	6(8.0)	75(44.1)
Secondary	34(85.0)	6(15.0)	40(23.5)
Tertiary	11(91.7)	1(8.3)	12(7.1)
Occupational status			
Employed*	81(89.0)	10(11.0)	91(53.5)
House wife	46(90.2)	5(9.8)	51(30.0)
Others*	23(82.1)	5(17.9)	28(16.5)

* Others - include student, farmers, non employed

* Employed – include government ,private

5.3 Factors associated with *T. gondii* infection

Age group 35-44 years comprised 64 (37.6%) of the total, out of which 58 (90.6%) were seropositive. Out of 109 (64.1%) female participants 97(89.0%) were seropositive for *T. gondii*. Of the respondents 147 (86.5%) were resided in urban area, of these 129 (87.8%) were seropositive for *T. gondii*. With regard to educational status 75 (44.1%) have studied up to primary level of education of these 69 (92.0%) were seropositive [Table-1&2].

Seropositivity rate with respect to habit of eating raw meat, 134 (78.8%) were reported to had the habit of eating raw meat among these 125 (93.3%) were seropositive. Regarding the habit of eating raw/ unwashed vegetables or fruits 90 (52.9 %) were reported to had the habit of eating raw/ unwashed vegetables or fruits, of which 80 (88.9%) were seropositive. Among the total respondents 47 (27.6%) were reported the presence of cats at their home out of these 93.6% were seropositive. Seventy seven 77 (45.3%) of the study participants had reported to have a history of engagement in farming/ gardening activities, which could indicate contact with soil of these 72 (93.5%) were seropositive. Of the total respondents 17 (10 %) were reported well water use for drinking out of these 13 (76.5%) were seropositive. In the present study 11 (6.5%) individuals had history of blood transfusion, of which 10 (90.9%) were seropositive [Table-2]. Patients with CD4+ T cell count of < 200 cells/µl comprised 13 (9.6%) out of these 11(84.6%) were seropositive [Table-2&3].

Logistic regression methods were used to identify the main predictor variables associated with the infection. Variables that were entered into multivariate analysis were age, presence of cats at their home, habit of eating raw meat, previous history of engagement in farming/gardening activities and source of drinking water. Further analysis using multivariate analysis only three variables, age groups 25-34 years (AOR = 6.266; 95% CI: 1.479-26.539), 35-44years (AOR =7.176; 95% CI: 1.675 -30.748), ≥45 years (AOR = 7.205; 95% CI: 1.040-49.932), habit of eating raw meat (AOR = 4.361; 95% CI: 1.409-13.496) and previous history of engagement in farming/gardening activities (AOR = 4.051; 95% CI: 1.112-14.758) were found to be significantly associated with *T. gondii* seropositivity [Table -2].

Table-2: Bivariate & multivariate analysis of selected variables in relation to *T. gondii* infection among people infected with HIV in Arba Minch hospital 2013.

Variables	Total	Positive	p-value	COR(95%CI)	p-value	AOR(95%CI)
	No (%)	*No (%)*				
Age group(years)			0.008⊕		0.030♦	
≤ 24	18(10.6)	11(61.1)		1		1
25-34	53(31.2)	48(90.6)	0.007⊕	6.109(1.630-22.903)	0.013♦	6.266(1.479-26.539)
35-44	64(37.6)	58(90.6)	0.005⊕	6.152(1.733-21.832)	0.008♦	7.176(1.675-30.748)
≥ 45	35(20.6)	33(94.3)	0.007⊕	10.500(1.893-58.242)	0.046♦	7.205(1.040-49.932)
Sex						
Male	61(35.9)	53(86.9)	0.683	0.820(0.315-2.130)		
Female	109(64.1)	97(89.0)		1		
Place of residence						
Urban	147(86.5)	129(87.8)		1		
Rural	23(13.5)	21(91.3)	0.625	1.465(0.317-6.779)		
Educational status						
Illiterate	43(25.3)	36(83.7)	0.292	0.586 (0.217-1.582)		
Literate	127(74.7)	114(89.8)		1		
Habit of eating raw meat						
No	36(21.2)	25(69.4)		1		1
Yes	134(78.8)	125(93.3)	0.0001⊕	6.111(2.294-16.283)	0.011♦	4.361(1.409-13.496)
Eating raw/unwashed vegetable or fruits						
No	80(47.1)	70(87.5)		1		
Yes	90(52.9)	80(88.9)	0.779	1.143(0.449-2.906)		
Presence of cats at home						
No	124(72.9)	107(86.3)		1		1
Yes	46(27.1)	43(93.5)	0.207⊕	2.277(0.635-8.169)	0.115	3.417(0.743-15.717)
Farming/ gardening activity						
No	93(54.7)	78(83.9)		1		1
Yes	77(45.3)	72(93.5)	0.060⊕	2.769(0.958-8.006)	0.034♦	4.051(1.112-14.758)
Source of drinking water						
Well	17(10)	13(76.5)	0.124⊕	0.380(0.110-1.304)	0.076	0.237(0.048-1.165)
Pipe	153(90)	137(89.5)		1		1
History of blood transfusion						
No	159(93.5)	140(88.1)		1		
Yes	11(6.5)	10(90.9)	0.777	1.357(0.164-11.202)		
Current CD4 cell count						
<200	13(9.6)	11(84.6)	0.601	0.650(0.130-3.260)		
≥200	123(90.4)	110(89.4)		1		

⊕ Variables entered into multivariate analysis at p-value < 0.25

♦ Statistically significant variables at P-value < 0.05

Data on the CD4+ T cell count was available for 136 of the study participants. The range of CD4+ T cell count was from 55-1574 cells/µl. Patients with CD4 + T cell count of < 200 cells/µl had seropositivity rate of 84.6% while those of ≥ 200 cells / µl had seropositivity rate of 89.4 % [Table -3].

Table-3: Prevalence of *T. gondii* infection along with CD4 + T cell count among people infected with HIV (n=136) in Arba Minch hospital, 2013.

CD4 + T cell count (cell/ µl)	Seroprevalence		Total No (%)
	Positive No (%)	Negative No (%)	
<200	11(84.6)	2(15.4)	13(9.6)
≥200	110(89.4)	13(10.6)	123(90.4)

Age groups ≤ 24 years, 25-34 years, 35-44 years and ≥ 45 years comprised a seropositive rate of 61.1%, 90.6%, 90.6% and 94.3% respectively. Rate of *T. gondii* seropositivity was increased as the age of the study participants increased [Figure-2].

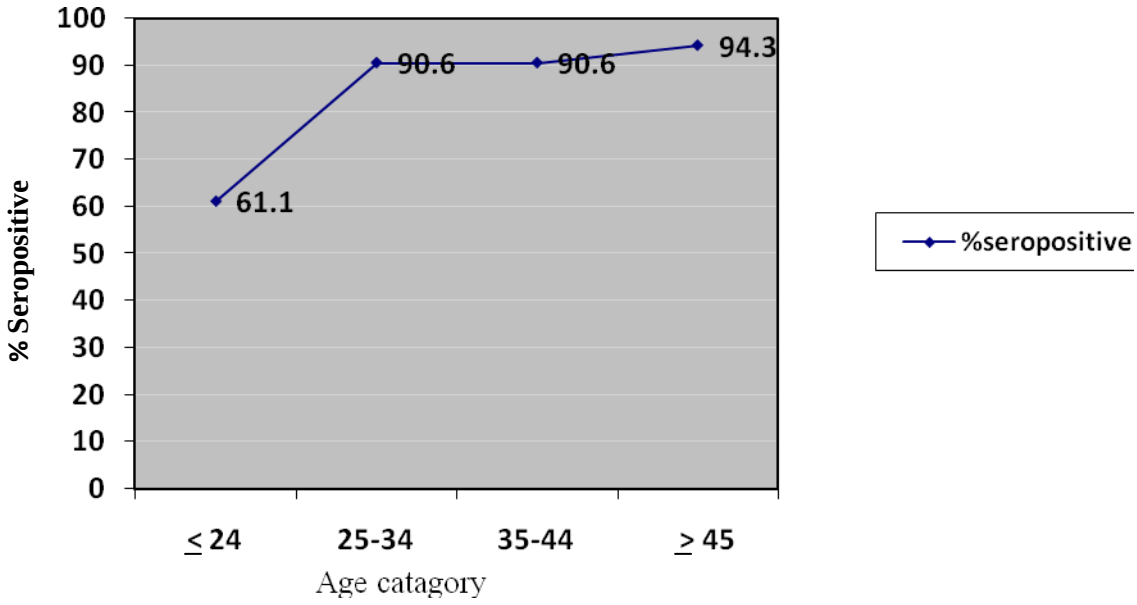


Figure-2: Rate of *Toxoplasma* Seropositivity in relation to Age among people infected with HIV in Arba Minch hospital, 2013.

Chapter Six: Discussion

In this study, seroprevalence of anti-*T. gondii* IgG antibody among the HIV-infected patients was 88.2%. This seroprevalence is consistent with previous study carried out in Bahir Dar, North West Ethiopia, in which prevalence rate of 87.4% was reported [41]. Similarly, comparable prevalence of 93.3% was reported in another study conducted in Addis Ababa [39] and Akaki town, in which 80% of HIV infected and uninfected individuals were found to be seropositive [40].

In contrast, the seroprevalence in this study is much higher than reports from Mexico [34], Cuba [35] and Malaysia [36], where prevalence rates of 50%, 71.96% and 44.8% were documented, respectively. Similarly, the seroprevalence is higher as compared to studies from Northern and Northeast Iran [37, 38] and Nigeria [32]. In these studies, seroprevalence of 77.4%, 38.01% and 54.2% were reported, respectively. The higher sero prevalence of *T. gondii* found in our study might be due to differences in geographical distribution of the parasite, which in turn is affected by difference in climatic variability. Warm climate with moist conditions are known to favor the maturation and increase survival of the oocyst of the parasite in the soil [57]. The difference in socio-economy, educational status and cultural practices of the study participants may also account for the variation.

Our finding mean that 88.2% of the study participants were seropositive for IgG antibody to *T. gondii*. In the absence of prevention strategies the study subject could be at high risk to develop toxoplasmosis reactivation. An earlier report indicates that around 47% of *T. gondii*-seropositive AIDS patients ultimately developed TE [58], especially when CD4+ T-cells count falls under 100 cells/μl [8]. Accordingly, high incidence rate of TE in our study population is expected unless preventive measures are undertaken.

In this study, seroprevalence was higher in the elder later age groups as compared to earlier. The result of this study showed that rate of *T. gondii* seropositivity increased as the age of the study participants increased [Figure-2]. The difference was also statistically significant [Table-2]. This is in agreement with previous studies [42, 43, 44]. The significant effect of age on *T. gondii* infection in this study could be explained by *Toxoplasma* infection is considered to be acquired in the early years and tends to increase with age; those who are elder are more likely to have been exposed to any one of the risk factors than younger as a result of longer exposure time.

Regarding gender and *T. gondii* seropositivity slightly higher proportion of females (89%) was seropositive compared to male (86.9%); however, the difference was not significant. This observation agrees with finding in a number of literatures [39, 41, 45].

In the present study, rate of seropositivity to *T. gondii* was not significantly differed by residence and educational status. Similar finding was reported in the study from Bahir Dar [41]. However, a study done in Sudan reported a significant difference in *Toxoplasma* seropositivity with regarding to residence [46]. This difference might be due to sample size difference, in our study small sample size was used as compared to the study done in Sudan.

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 = 4.361; 95% CI: 1.409-13.496). The rate of *T. gondii* infection was significantly higher and about four 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 Bahir Dar [41] and Khartoum, Sudan [46]. In contrast, some studies reported absence of association between *T. gondii* infection and consumption of raw meat [45, 47, 48]. The observed differences might be due to the types of meat consumed and the rate of infection in the animals.

In the present study, no significant association was observed between *T. gondii* seropositivity and eating raw/ unwashed vegetables or fruits. This finding is in agreement with study done in Bahir Dar [41]. In contrast, studies surveys done in pregnant women in Nigeria [49] and Pakistan [50] reported 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 in multivariate analysis [Table-2]. Similar findings were observed in a study done among pregnant women in Mexico [52]. On the other hand, others documented significant association of cat ownership with *T. gondii* infection [51]. The observed differences could be due to differences in the types of the cats as well as infection rates in cats.

Contamination of soil by infected cat faeces is the most common source of human exposure to the parasite. In this study, 77(45.3%) of the study participants had responded to have a history of engagement in farming/gardening activities, which could indicate contact with soil. Of these 72 (93.5%) were seropositive for *T. gondii*. Study participants who had history of engagement in farming/gardening activities showed significant association (AOR=4.051; 95%CI: 1.112-14.758) with *Toxoplasma* seropositivity [Table-2]. Similar findings were documented in studies done in Nigeria [49] and Pakistan [50]. The current finding contrasts with surveys carried among blood donors in Brazil [53] and the general population in Slovakia [54]. The difference could be attributed to differences in distribution and stage of parasite in the soil, personal hygienic practices of the study population.

Contamination of well water with oocysts from soil is likely to occur in floods or run off after rain. Oocysts can survive for long periods in water, hence, individuals who use well water may be exposed to *T. gondii* oocyst. In the current study, 10% of the study participants reported to drink well water, 76.5% of whom were *T. gondii* seropositive. There was no significant difference in seropositivity by source of drinking water (P=0.076) [Table-2].

Blood transfusion is one means of transmission of *T. gondii*. In the current study 11(6.5%) of the study participants responded previous history of blood transfusion, 90.9% of whom were *T. gondii* seropositive. In this 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 [41, 47, 48]. 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 [59], received blood is less likely to have tachyzoites stage of the parasite, infective form of the parasite by blood transfusion.

Regarding CD4 + T cell count and seropositivity to *T. gondii*, there was no significant difference in seropositivity rate between CD4 cell count < 200 and $\geq$ 200 cell/µl. This finding is consistent with a report from Malaysia [48]. In the current study, 13(9.6%) of the study participants had a CD4 + T cell count of < 200 cells/µl, of these 11 were seropositive for *T. gondii* IgG antibody [Table-3]. Patients with latent *T. gondii* infection and low CD4 + T cell count are at higher risk of reactivating latent infection. It has been observed that most cases of TE in AIDS patients are due to reactivation of latent infection and incidence of the disease is associated with *T. gondii* IgG seropositivity and low CD4+ T lymphocyte counts [21].

Strength and limitation of the study

This study sheds light on the magnitude of latent *T. gondii* infection among HIV patients and associated risk factors. The test kits utilized have high specificity and sensitivity. However, due to the expensive cost of the ELISA test kit and budget constraint, control groups could not be included, which is to be considered as a limitation.

Chapter Seven: Conclusion and Recommendations

7.1 Conclusion

In conclusion, seroprevalence of latent *T. gondii* infection was high among people infected with HIV in Arba Minch hospital. Among socio-demographic factors assessed age was significantly associated with *T. gondii* seropositivity; with seroprevalence was increasing as the age of the participants increased. Moreover consumption of raw meat and history of involving in farming /gardening activities, which could indicate contact with soil were the main predictors of acquiring *T. gondii* infection among the study participants.

7.2 Recommendations

- ✓ Considering the high prevalence of toxoplasmosis revealed by this study it is recommended to create awareness about the infection. Health information should be provided to the patients on the risk factors predisposing to *Toxoplasma* infection, particularly on preventive behavioral and personal hygienic practices such as eating well cooked meat and hand washing after outdoor activities involving soil contact.
- ✓ Laboratory screening for *T. gondii* infection of HIV patients should be integrated in the national HIV management protocol and also all HIV seropositive individuals should be screened for *T. gondii*-IgG antibody as this will contribute to early detection and management of the infection.
- ✓ In depth further study on the prevalence of CT in HIV patients is recommended in the area.

References

1. Cheesbrough M. District Laboratory Practice in Tropical Countries Part 1. 2nd ed. New York: Cambridge University Press; 2005, 300-302.
2. Romero OB, Oliveira DM, Neto VFA. Toxoplasmosis: Advances and Vaccine Perspectives. In: Morales AR, (ed.).*Current Topics in Tropical Medicine.* Croatia: InTech; 2012, 169–183.
3. Dubey JP, Lindsay DS, Speer CA. Structures of *Toxoplasma gondii* Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts. *Clinical Microbiology Reviews* 1998; 11(2):267–299.
4. Jones JL, Lopez A, Wilson M, *et al*. Congenital Toxoplasmosis: A Review. *Obstetrical and Gynecological Survey* 2001; 56(5):296–305.
5. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii* from animals to humans. *International Journal of Parasitology* 2000; 30(12-13): 1217–1258.
6. Blader IJ, Saeij JP. Communication between *Toxoplasma gondii* and its host: impact on parasite growth, development, immune evasion, and virulence. *Acta Pathologica, Microbiologica et Immunologica Scandinavica* 2009; 117: 458–476.
7. Vasan S, Tsuji M. Toxoplasmosis. In: Satoskar AR, Simon GL, Hotez PJ, Tsuji M, (eds.). *Medical Parasitology.* Texas: Landes Bioscience; 2009, 190–194.
8. George SM, Malik AK, Hilli FA. Cerebral Toxoplasmosis in an HIV Positive Patient: A Case Report and Review of Pathogenesis and Laboratory Diagnosis. *Bahrain Medical Bulletin* 2009; 31(2):1–5.
9. Schwartzman JD. Toxoplasmosis. In: Gillespie SH, Pearson RD, (eds.). *Principles and Practice of Clinical Parasitology*. England: John Wiley & Sons Ltd; 2001, 113–138.
10. Montoya JG, Liesenfeld O. Toxoplasmosis. *Lancet* 2004; 363: 1965–1976.
11. Roberts LS, Schmidt GD. Foundations of parasitology. 8th ed. New York: McGraw-Hill; 2009, pp134-139.
12. Jayawardena S, Singh S, Burzyantseva O, *et al*. Cerebral Toxoplasmosis in Adult Patients with HIV Infection. *Hospital Physician* 2008; 44(7): 17–24.
13. Macpherson CNL, Gottstein B, Geerts S. Parasitic food-borne and water-borne zoonoses. *Revue Scientifique et Technique-Office International Des Épizooties* 2000; 19 (1):240–258.
14. Montoya JG. Laboratory Diagnosis of *Toxoplasma gondii* Infection and Toxoplasmosis. *The Journal of Infectious Diseases* 2002; 185(1):73–82.

15. Centers for Disease Control and Prevention. Guidelines for Preventing Opportunistic Infections among HIV-Infected Persons -2002: Recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America. *Morbidity and Mortality Weekly Report* 2002; 51(8):1–46.
16. Bojar I, Szymańska J. Environmental exposure of pregnant women to infection with *Toxoplasma gondii* – state of the art. *Annals of Agricultural and Environmental Medicine* 2010; 17: 209–214.
17. Nissapatorn V, Sawangjaroen N. Parasitic infections in HIV infected individuals: Diagnostic & therapeutic challenges. *Indian Journal of Medical Research* 2011; 134:878–897.
18. Joynson DHM, Wreghitt TG. Toxoplasmosis: A Comprehensive Clinical Guideline. Cambridge UK: Cambridge University press; 2001. Available at: http://www.cambridge.org/9780521443289. Accessed on 2/27/2013.
19. Nissapatorn V. Toxoplasmosis: A Silent Threat in Southeast Asia. *Research Journal of Parasitology* 2010; 5 (4): 236–247.
20. Nissapatorn V, Sawangjareon N. Toxoplasmosis: IgG Avidity and Its Implication in Serodiagnosis. In: Abuelzein E, (ed.). *Trends in Immunolabelled and Related Techniques*. Croatia: InTech; 2012, 170–190.
21. Djurkovie DO, Bobie B, Vukovie D, *et al*. Risk for Toxoplasmic Encephalitis in AIDS Patients in Yugoslavia. *International Journal of Infectious Diseases* 1997; 2(2):74–78.
22. Nissapatorn V. Review toxoplasmosis in HIV/AIDS: a living legacy. *South East Asian Journal of Tropical Medicine and Public Health* 2009; 40(6): 1158–1178.
23. Simpore J, Savadogo A, Ilboudo D, *et al*. *Toxoplasma gondii*, HCV, and HBV Seroprevalence and Co-Infection Among HIV-Positive and Negative Pregnant Women in Burkina Faso. *Journal of Medical Virology* 2006; 78:730–733.
24. Sukthana Y. Toxoplasmic Encephalitis. In: Tkachev S, (ed.). *Non-Flavivirus Encephalitis*. Croatia: InTech; 2011, 281-306. Available at: http://www.intechopen.com/books/non-flavivirus-encephalitis/toxoplasmic-encephalitis. Accessed on 3/20/2013.
25. Achappa B, Mahalingam S, Shamir AR, *et al*. Clinical spectrum and outcomes for *Toxoplasma* encephalitis among AIDS patients before and during the Era of Anti-retroviral Therapy in Mangalore, India. *Journal of Clinical and Diagnostic Research* 2011; 5(7): 1397–1401.

26. Chioccola VLP, Vidal JE, Su C. *Toxoplasma gondii* infection and cerebral toxoplasmosis in HIV-infected patients. *Future Microbiology* 2009; 4(10): 1363–1379.
27. Assob JCN, Njunda AL, Nsagha DS, *et al*. *Toxoplasma* Antibodies amongst HIV/AIDS Patients Attending the University Teaching Hospital Yaoundé, In Cameroon. *African Journal of Clinical and Experimental Microbiology* 2011; 12(3): 1119–1123.
28. Maïga I, Kiemtoré P, Tounkara A. Prevalence of anti-*Toxoplasma* antibodies in patients with Acquired Immunodeficiency Syndrome and blood donors in Bamako. *Bulletin of the Exotic Pathology Society* 2001; 94(3):268–270.
29. Lindstrom I, Deogratias HK, Fred K, *et al*. Prevalence of latent and reactivated *Toxoplasma gondii* parasites in HIV-patients from Uganda. *Acta Tropica* 2006; 100(3):218–222.
30. Ayi I, Edu AAS, Apea-Kubi KA, *et al*. Sero-Epidemiology of Toxoplasmosis amongst Pregnant Women in the Greater Accra Region of Ghana. *Ghana Medical Journal* 2009; 43(3):107–114.
31. Bouratbine A, Siala E, Chahed MK, *et al*. Seroepidemiologic profile of Toxoplasmosis in Northern Tunisia. *Parasite* 2001; 8: 61–66.
32. Akanmu SA, Onwuezobe IA. Seroprevalence of *Toxoplasma gondii* IgG antibody in HIV-infected patients at the Lagos University Teaching Hospital. *HIV/AIDS - Research and Palliative Care* 2011; 3:101–105.
33. Dubey JP, Tiao N, Gebreyes WA, *et al*. A review of toxoplasmosis in humans and animals in Ethiopia. *Epidemiology and Infection* 2012; 140 (11):1935–1938.
34. Galván RML, Alvarado VV, Gutierrez GV, *et al*. Prevalence of IgG and IgM anti-*Toxoplasma* antibodies in patients with HIV and acquired immunodeficiency syndrome (AIDS). *Revista da Sociedade Brasileira de Medicina Tropical* 1997; 30:465–467.
35. Hernández IM, Izquierdo GSM. Seroprevalence of antibodies against *Toxoplasma gondii* in Cuban patients with HIV infection. *Revista Biomédica* 2004; 15:215–220.
36. Nissapatorn V, Lee C, Quek KF, *et al*. Toxoplasmosis in HIV / AIDS Patients: A Current Situation. *Japan Journal of Infectious Disease* 2004; 57:160–165.
37. Daryani A, Sharif M, Meigouni M. Seroprevalence of IgG and IgM anti-*Toxoplasma* antibodies in HIV / AIDS patients , northern Iran. *Asian Pacific Journal of Tropical Medicine* 2011; 271–274. Doi: 10.1016/S1995-7645(11)60084-9
38. Hajia M. Prevalence of IgG and IgM anti-*Toxoplasma gondii* antibodies in HIV positive patients in northeast of Iran. *Iranian Journal of Pathology* 2011; 6(2):68–72.

39. Shimelis T, Tebeje M, Tadesse E, *et al*. Seroprevalence of latent *Toxoplasma gondii* infection among HIV-infected and HIV-uninfected people in Addis Ababa, Ethiopia: A comparative cross-sectional study. *Biomedical Central Research Notes* 2009; 2:213.
40. Woldemichael T, Fontanet AL, Sahlu T, *et al* .Evaluation of the Eiken latex agglutination test for anti-*Toxoplasma* antibodies and seroprevalence of *Toxoplasma* infection among factory workers in Addis Ababa, Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1998; 92:401–403.
41. Walle F, Kebede N, Tsegaye A, *et al*. Seroprevalence and risk factors for Toxoplasmosis in HIV infected and non-infected individuals in Bahir Dar, Northwest Ethiopia. *Parasites & Vectors* 2013; 6:15. doi:10.1186/1756-3305-6-15
42. Amuta EU, Amali O, Jacob SE, *et al*. *Toxoplasma gondii* IgG antibodies in HIV/AIDS patients attending hospitals in Makurdi metropolis, Benue state, Nigeria. *International Journal of Medicine and Biomedical Research* 2012; 1(3):186–192.
43. Falusi O, French AL, Seaberg EC, *et al*. Prevalence and Predictors of *Toxoplasma* Seropositivity in Women with and at Risk for Human Immunodeficiency Virus Infection. *Clinical Infectious Diseases* 2002;35:1414–1417.
44. Uttah EC, Ajang R, Ogbeche J, *et al.* Comparative Seroprevalence and Risk Factors of Toxoplasmosis among Three Subgroups in Nigeria**.** *Journal of Natural Sciences Research* 2013; 3(8):23–28.
45. Muluye D, Wondimeneh Y, Belyhun Y, *et al*. Prevalence of *Toxoplasma gondii* and Associated Risk Factors among People Living with HIV at Gondar University Hospital, Northwest Ethiopia. *ISRN Tropical Medicine* 2013; 2013:1–5.
46. Khalil KM, Ahmed AA, Elrayah E. Seroprevalence of *Toxoplasma gondii* Infection in Humans in Khartoum State, Sudan. *International Journal of Tropical Medicine* 2012; 7 (4): 143–150.
47. Nissapatorn V, Lee CKC, Cho SM, *et al*. Toxoplasmosis in HIV /AIDS patients in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* 2003; 34(2):80–85.
48. Nissapatorn V, Kamarulzaman A, Init I, *et al*. Seroepidemiology of Toxoplasmosis among HIV- Infected Patients and Healthy Blood Donors. *Medical Journal of Malaysia* 2002; 57 (3):304–310.
49. Agboola AM, Busari OS, Osinupebi OA, *et al.* Seroprevalence of *Toxoplasma gondii* Antibodies among Pregnant Women Attending Antenatal Clinic of Federal Medical Center,

Lagos, Nigeria. *International Journal of Biological & Medical Research* 2011; 2(4): 1135–1139.

50. Khan SN, Khan S, Ayaz S, *et al*. Seroprevalance and Risk Factors of Toxoplasmosis among Pregnant Women in District Kohat, Khyber Pakhtunkhwa Pakistan. *World Applied Sciences Journal* 2011; 14 (7): 1032–1036.
51. Zemene E, Yewhalaw D, Abera S, *et al.* Seroprevalence of *Toxoplasma gondii* and associated risk factors among pregnant women in Jimma town, Southwestern Ethiopia. *Biomedical Central Infectious Diseases* 2012; 12:337.
52. Esquivel CA, Castorena AT, Liesenfeld O, *et al*. Seroepidemiology of *Toxoplasma gondii* Infection in Pregnant Women in Rural Durango, Mexico. *The Journal of Parasitology* 2009; 95(2):271–274.
53. Loges LA, Cademartori BG, Farias R. Seroprevalence and associated factors to *Toxoplasma gondii* infection in blood donors in Southern Brazil. *Revista Panamericana de Infectologia* 2012; 14(1):27–31.
54. Studenicová C, Bencaiová G, Holková R. Seroprevalence of *Toxoplasma gondii* antibodies in a healthy population from Slovakia. *European Journal of Internal Medicine* 2006; 17: 470–473.
55. CBID-Ethiopia, Belated report on Arba Minch Hospital 2011. Available at: http://cbid-ethiopia.blogspot.com/2011/08/belated-report-on-arba-minch-hospital.html. Accessed on 2/27/2013
56. Jerene D, Lindtjørn B. Disease progression among untreated HIV-infected patients in south Ethiopia: implications for patient care. *Journal of the International AIDS Society* 2005; 7(3):66.
57. Meerburg BG, Kijlstra A. Changing climate-changing pathogens: *Toxoplasma gondii* in North-Western Europe. *Parasitology Research* 2009; 105:17–24.
58. Zangerle R, Allerberger F, Pohl P, *et al*. High risk of developing toxoplasmic encephalitis in AIDS patients seropositive to *Toxoplasma gondii*. *Medical Microbiology and Immunology* 1991; 180(2):59-66.
59. Chiang TY, Hsieh HH, Kuo MC, *et al*. Seroepidemiology of *Toxoplasma gondii* Infection among Healthy Blood Donors in Taiwan. *PLoS ONE* 2012;7(10): e48139. doi:10.1371/journal.pone.0048139

Annexes

Jimma University College of Public Health and Medical Sciences, Department Of Medical Laboratory Sciences and Pathology

Annex-I: Subject information sheet

Dear participants: good morning/good afternoon?

My name is Tsegaye Yohanes and I am following second degree program at Jimma University. I am conducting a research on the parasite *Toxoplasma gondii* which cause a severed disease in an immunocompromised person. The main objective of this study is to determine the prevalence of *Toxoplasma gondii* infection and assessing associated factors among people infected with human immunodeficiency virus. You are the one; therefore you are kindly requested to participate in this study. Your participation in this study is completely on voluntary bases and you have the right to refuse from participation. If you agree to participate in this study about 2 ml blood specimen will be collected from you for laboratory test. During collection of blood you may feel some minimum discomfort, but this does not produce a series pain. I need to assure you that your response and laboratory results would be kept confidential.

I would like to inform you again your participation is very essential not only for the successful accomplishment of the study but also for producing relevant information which will be helpful to design appropriate strategies for the prevention of the disease.

Do you agree to participate in this study? Yes, continue _____No, thank you! _____

Thank you for your cooperation!!!

Subject information sheet (Amharic version)

ጅማ ዩኒቨርሲቲ የሕብረተሰብ ጤናና ህክምና ሳይንስ ኮሌጅ ሳቦራቶሪ እና ፓቶሎጂ ት/ክፍል

ስተሳታፊዎች ስለጥናቱ መረጃ መስጫ ቅፅ

ውድ የመጠይቁ/ የጥናቱ ተሳታፊ□ች እንደምን አደራችሁ/ዋላችሁ::

እኔ ስሜ ፀጋዬ ዮሃንስ እባላለሁኝ ፡ የሁለተኛ ዲግሪ ትምህርት በጅማ ዩኒቨርሲቲ እ□ተከታተልኩኝ ስሆን በአሁን ሰዓት የመመረቂያ ጥናታዊ ፅሁፍ ችለኦንላዝማ ጎንዲ በሚባል ጥገኛ ህ□ስ ላ□ እ□ስራ□ እቸለ□:: □ህም ዓቸኛ ህ□ስ □ከሽታ መከላከል አቅማቸው አነስተኛ በሆኑ ሰዎች ላይ ከፍተኛ በሽታ ያመጣል:: የጥናቱም ዋና ዓላማ የጥገኛ ህዋሱን የበሽታ ስርጭት እና ተዛማች የበሽታው መንስኤዎችን ኤችአይቪ ቫይረስ በደማቸው ውስጥ የሚገኝባቸው ሰዎች ላይ መጠኑን ለማወቅ ነው:: እናም እርስዎ የዚህ ጥናት ተሳታፊ ስንዲሆኑ እና ስለፈላጊውን መረጃ እንዲሰጡኝ ስል በታላቅ ትህትና እ□□ቃለ□:: በዚህ ጥናት ውስጥ የመሳተፍ ወይም ያለመሳተፍ ሙሉ መብትዎ የተጠበቀ ነው:: በዚህ ጥናት ለመሳተፍ ፍቃደኛ ከሆኑ ፭.00 ሚ.ሊ የሚሆን □□ም ናሙና ከእርስ□ □□ስ□ል □ህም □ሚ□ሸል□ ዓቸኛ ህዋሱ ለስ ወይም የለም የሚለውን በምርመራ ለማወቅ ነው:: ደም በሚወሰድበት ጊዜም ትንሽ የህመም ስሜት ሊሰማ□ት ይችላል ይህ ማለት ግን ስጊዜው የሚሰማዎት እንጂ በእርስዎ ላይ ችግር የሚፈጥር አይደለም ::

ከዚህ በተጨማሪ ሳረጋግጥልዎ የምወደው ነገር ቢኖር እርስዎ የሚሰጡት መረጃ እንዲሁም የደም ናሙና ውጤት በታማኝነት እንደም□ብቅ በትፋሚ ሳረፋፅዓል□ እ□□ል□::

በመፈረሜም እርስዎ የሚሰጡት መረጃ ስንዲሁም የደም ናሙና ውጤቱ ከጥናቱ መሳካት ባለፈ በስፋት በሽታ□ን ከመከላከል አንፃርም በጎ ጎኑ የላቀ መሆኑን ሳረጋግጥልዎ እ□□ለሁ::

በዚህ ጥናት ውስጥ ለመሳተፍ ፍቃደኛ ነዎት? አዎን □ቅዓ ው_____

አይደለሁም አመሰግናለሁ_____

ስትብብርዎ እ□□ በጣም አመሰግናለሁ!!

Annex-II: Informed Consent form

Jimma University College of Public Health and Medical Sciences, Department Of Medical Laboratory Sciences and Pathology

Code No _____

Age _____ Sex _____

I have been informed by Ato Tsegaye Yohanes, a postgraduate student at Jimma University, about a study that plans to investigate the prevalence of *T. gondii* infection and associated factors. For this study I have been requested to participate and to give a blood sample. The investigator has briefed me that there are no major risks associated with the procedure. The investigator also informed me that all my response and laboratory results would be kept confidential. Moreover, I have also been well informed of my right to withdraw from participating. I have been given enough time to think over before I signed this informed consent. It is therefore, with full understanding of the situation that I gave my informed consent and cooperated at my will in the course of the conduct of the study.

Name (participant) _____ Signature _____ Date _____

Name (data collector) _____Signature _____Date _____

Informed Consent form (Amharic version)

ጅማ ዩኒቨርሲቲ የሕብረተሰብ ጤናና ህክምና ሳይንስ ኮሌጅ ሳቦራቶሪ እና ፓቶሎጂ ት/ክፍል

የጥናቱ ተሳተፊ የስምምነት ቅፅ

መለ□ ቁጥር_____

እትሜ_____ ጭታ_____

እኔ □□ህ ጥናት ተሳታፊ የሆንኩ ፅሰሰብ በጅማ ዩንቨርሲቲ □ትህረ ምረቃ ተማሪ በሆኑት በስቶ ፀጋዬ ዮሐንስ ችክሶንሳዝማ ጎንዲ □ሚባል ዓ ቸኛ ህ□ስን □በሽታ·□ን ስርጭት እና ተዛማች የበሽታ·ው መንስሴዎች ስማ□ቅ በሚያደርጉት ጥናት ሳይ በቂውን መረጃ ተቀብያለሁ።

ስለዚህም በዚህ ጥናት እንድሳተፉና የደም ናሙና ስንድሰጥ ተነግሮኛል፣ በተጨማሪም የጥናቱ ባለቤት በገለፁልኝ መሠረት ከጥናቱ ጋር በተያያዘ ሁኔታ· □ሚመጣ ተዛማች ጉዳት እንደሲለሁና የምሰጠው መረጃ እና የሳቦራቶሪ ውጤት በታ·ማኝነት ሚስጥረን እንደሚጠብቁ እናም ከዚህ ጥናት ውጭ እንደማይውል ስረጋገጠሁልኛል እንዲሁም በጥናቱ ያስመሳተፍ ሙሉ መብት እንዳለኝም ችሰና□ልኛል።

እኔም በዚህ መሰረት በቂ ጊዜ ተሰችኝ ስስቤበት እናም ሁኔታ·ውን በሙሉ በመረዳት በጥናቱ ውስጥ ስመሳተፍ በመስማማት ደህን የፈቃደኝነት ማረጋቸ□ □ል □ርማ□ለሁ።

የጥናቱ ተሳተፊ ስም_____ ኛርማ_____

ቀን_____

መረጃውን ሰብሳቢ ስም_____ ኛርማ_____

ቀን_____

Annex-III: Questionnaire

Jimma University College of Public Health and Medical Sciences, Department Of Medical Laboratory Sciences and Pathology

Questionnaire will be filled by the data collector by interviewing each participant during the study period. When the participant answered from choice please thick on the answer or write in the space provided.

Code number _____ Interviewer_____Date of interview _____

I. Socio-demographic variables

1. Age in years_____

2. Sex A. Male ☐ B. Female ☐

3. Place of residence A. Urban ☐ B. Rural ☐

4. Marital status A. Single ☐ B. Married ☐ C. Divorced ☐ D. Widowed ☐

5. Your level of education? A. Illiterate ☐ B. Primary education ☐

C. Secondary education ☐ D. Tertiary education ☐

6. Occupation status A. Employed ☐ B. House wife ☐ C. Other (specify) _____

II. Other factors associated with the disease

A. Do you have a habit of eating raw meat? A. Yes ☐ B. No ☐

B. Do you eat raw vegetables /unwashed fruits? A. Yes ☐ B. No ☐

C. Do you have domestic cat(s) at home? A. Yes ☐ B. No ☐

D. Have you been involved in farming/gardening activities? A. Yes ☐ B. No ☐

E. Where do you get your drinking water? A. Well ☐ B. Pipe ☐

F. Have you ever transfused blood? A. Yes ☐ B. No ☐

Questionnaire (Amharic version)

ጅማ ዩኒቨርሲቲ የሕብረተሰብ ጤናና ህክምና ሳይንስ ኮሌጅ ሳቦራቶሪ እና ፓቶሎጂ ት/ክፍል

ስተሳታፊዎች የተዘጋጀ መጠየቅ

መ□□ቁ የሚሞላው መረጃ በሚሰበስበው ሰው ሲሆን የሚሞላውም ተሳታፊዎችን ቃስመጠይቅ በማድረግ ይሆናል። ተሳታፊ□ ሲመልስ እባክዎ መልሱ ላይ ምልክት ያድርጉ ወይም በተሰጠው ክፍት ቦታ □ሪ□።

መለ□ ቁጥር _____ □□ቂ _____ የጠየቀበት ቀን _____

I. ማህበራዊ እና ሌሎች ተያያዝነት ያላቸው መረጃዎች

1. እትሜ በ ስመት_____

2. ፆታ ሀ. ወንድ □ ለ. ሴት □

3. የሚኖሪበት ቦታ ሀ. ከተማ □ ለ. ቭ□ር □

4. የትዳር ሁኔተ ሀ. □ላቫ □ ለ. □ቭ□ □ ሐ. ስግብቶ የፈታ □ መ. □ሞተ □

5. የትምህርት ሁኔታ ሀ. □ልተማረ □ ለ. የመጀመሪያ ደረጃ ትምህርት □
ሐ.□ሁለተኛ ደረጃ ትምህርት □ መ. ሶስተኛ ደረጃ ትምህርት □

6.የስራ ሁኔታ ሀ. ሰራተኛ □ ለ. የቤት እመቤት □ ሐ. ሌላ ካለ(□ዝ ቀስ)_____

II. ከበሽታው ጋር ተያያዝነት ያላቸው መረጃዎች

ሀ. ዓይ ስፋ □መቭሱ? ሀ. አዎ □ ለ. አልመገብም □

ለ. □ልበለስ ቀ□ላቀ□ል ፣ □ልታ□በ አፉአፉ ተመልበ□ □□ቃሱ? ሀ. አዎ □ ለ. አላውቅም □

ሐ. ድመት በቤተዎ አልዎት? ሀ. አዎ □ ለ. የለኝም □

መ. በግብርና ፣በስትክልት ስፍራ ማስዋብ ስራ ላይ ይሳተፋሱ? ሀ.አዎ □ ለ. አልሳተፍም □

ሠ. ስመጠጥ የሚጠቀሙበት ውሃ ከየት □ቸሱ? ሀ.ከጉድጓት □ ለ.ከቧንቧ □

ረ. ደም ከሌላ ሰው ተሰጥቶዎት ያውቃል? ሀ. አዎ □ ለ. አያውቅም □

Annex-IV: Materials and venous blood collection procedures

Properly collected specimens represent a very important step for the laboratory and laboratory testing. The laboratory results are only as good as the specimens received for testing. Collection of quality blood specimens from subjects requires specific tools for obtaining the sample, post collection processing, handling, shipping and storage. Errors that can occur during the collection and handling of blood specimens are potentially numerous (e.g., inaccurate identification of specimens, the use of incorrect anticoagulants, the formation of hematomas and hemoconcentration). Reducing errors during blood collection will result in biologically representative specimen. The essential steps and required materials for proper collection of blood specimen listed as followed.

Materials needed

A. Needles, holder/adapter (if venipuncture collection methods used)

B. Evacuated blood collection tubes

C. Disposable, single use syringes with needle (if syringes collection methods used)

D. Tourniquet

E. Antiseptic70% isopropyl alcohol wipes

F. 2x2 Gauze or cotton balls (Gauze sponges)

G. Disposable gloves

H. Needle disposal unit

I. Adhesive bandages/tape

Procedure

1. Identify the patient correctly. The phlebotomist must ensure that the blood specimen is being drawn from the designated individual.
2. Position the patient. The patient should sit in a chair, lie down, or sit up in bed then hyperextend the patient's arm.

3. Examine the antecubital area of the arm and choose a prominent vein. The median cubital vein located in the antecubital fossa is generally the vein of choice.

4. Apply the tourniquet 3–4 inches. Do not place too tightly or leave on more than 2 minutes.

5. The patient should make a fist without pumping the hand and select the vein puncture site by palpating the area of arm vein location.

6. Prepare the patient's arm using an alcohol wipe. Cleanse in a circular fashion, beginning at the site and working outward then allow to air dry.

7. Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. The needle should form a 15–30 degree angle with the surface of the arm and should enter the skin with the bevel facing upward.

8. Swiftly, but gently insert the needle through the skin and into the lumen of the vein. If blood flow is not immediate avoid trauma and excessive probing for the vein.

9. If withdrawing with conventional disposable syringes: Using the plunger, gently withdraw 5–10 ml of whole blood from adults, 2–5ml from children, and 0.5–2 ml from infants.

10. Blood is then transferred to the appropriate tube or vial, depending on whether serum or plasma is required for the test.

11. If withdrawing with vacuum systems: Press forward on the tube to puncture the cap and allow the evacuated collection tube to fill when the last tube to be drawn is filling, remove the tourniquet; remove the needle from the patient's arm using a swift backward motion.

12. Press down on the gauze when the needle is out of the arm and then applying adequate pressure to avoid formation of a hematoma.

13. Dispose of contaminated materials and supplies using an appropriate disposal device. DO NOT RECAP NEEDLE. If breakage of a tube containing a collected sample should occur avoid all contact with exposed skin and follow proper procedures for the cleanup and disposal of infectious waste.

14. Mix the tube by inverting it several times and label all appropriate tubes at the patient's bedside or the drawing area.

15. Inspect the samples to assess the need for sample recollection and/or rejection and deliver specimens promptly to the laboratory or processing area.

N.B -Because it is often impossible to know which might be infectious, all patient blood specimens are to be treated with standard precautions. Obtaining blood from subjects may involve contacts with patients with underlying infectious diseases so it is important to follow safety and infection control procedures during collection and handling of their specimens.

Annex-V: Laboratory test protocol used for the detection of IgG Antibodies to *Toxoplasma gondii* in Human serum

TOXO-IgG

ELISA Test for the detection of IgG antibodies to *Toxoplasma gondii* in Human serum

Package size 96 Tests

REF 51209

Intended use

The Toxo-IgG ELISA is intended for the detection of IgG class antibodies *Toxoplasma gondii* in human serum.

Enzyme-Linked Immunosorbent Assay

Principle

The Human-*Toxoplasma*-IgG ELISA is based on the classical ELISA technique. The microtitre strip wells as a solid are coated with *Toxoplasma* antigens (*Toxoplasma*-Ag) prepared from sonicated whole *T. gondii* parasites. In the first incubation stage corresponding specific antibodies (*Toxoplasma*-IgG-Antibody) present in patients specimen or controls bind to the antigen at the solid phase. At the end of incubation unbound particles are washed out. For the second incubation step anti-IgG conjugate (anti-human IgG antibodies, peroxidase conjugated) 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, substrate is added then blue color formed. A blue color develops changes to yellow after stopping the reaction using stopping reagent. The intensity of the produced color is directly proportional to the *Toxoplasma*-IgG-Antibody concentration in the specimen. The absorbance of controls and specimen is determined using ELISA microplate readers at 450 nm.

Reagents and contents

1. Microtiter strips (in 1 strip holder) coated with sonicated *T. gondii* antigen
2. *Toxoplasma* IgG Negative Control(NC) green cap ready for use, amount 2.5 ml
3. *Toxoplasma* IgG Cut-off Control(CC) white cap amount 2.5 ml concentration 5.0IU/ml
4. *Toxoplasma* IgG Positive Control Low(PCL) red cap ready for use amount 2.5 ml concentration 30 IU/ml
5. *Toxoplasma* IgG Positive Control Medium(PCM) red cap read for use amount 2.5 ml concentration 100IU/ml

6. *Toxoplasma* IgG Positive Control High(PCH) red cap ready for use amount 2.5 ml, concentration 200IU/ml
7. Dilution buffer (white cap) ready for use amount100 ml
8. Anti-IgG conjugate (white cap) ready for use amount12 ml
9. Wash solution white cap amount 50 ml
10. Substrate reagent (black cap) amount 13 ml
11. Stop solution (red cap) amount 15ml
12. Adhesive strips, amount 2 pieces

Reagent preparation

Bring all reagents to room temperature (15-25^{o}C) before use. Reagents not in use should always be stored at 2-8^{o}C.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2-8^{o}c.

Specimen type

The assay should be run using serum sample. Specimens may be stored for 7 days at 2-8^{o}C or longer at -20^{o}C. Thawed specimen must be homogenized. Eliminate particulate matter by centrifugation or filtration. Do not use highly lipemic or hemolysed specimens.

Procedure

Follow the procedure exactly as described in the listed table below.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: Select the required number of microtiter strips.

P5: Run duplicates for controls. Pipette controls and specimen on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimize reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the controls in the indicated positions at half way time of the series.

P7: Avoid/remove air bubbles prior to incubation and reading of absorbance.

P8: Substrate should be incubating in the dark.

Washing solution preparation and procedure

Working wash solution

Dilute wash solution 1+20 with deionized water, e.g. 50 ml wash solution +1000 ml deionized water = 1050 ml. Stability of the working wash solution up to 60 days at 15-25^{o}C.

Washing procedure

The wash procedure is critical insufficient washing will result in poor precision or falsely high absorbance.

W1: In case of automatic washing fill and prime with working wash solution. Subsequently wash stripes respectively 4 to 5 times. Ensures the washer fills all wells completely and aspirates off efficiently after 30sec.

W2: After washing remove remaining liquid by tapping the plate upside down on tissue paper

Pipetting scheme				
Sample preparation dilutes patients' serum as 1+100 with diluents. E.g 10µ**l** serum + 1ml diluents then mix carefully				
Step-1	Well [µ**l]**			
	A1 Blank	**B1/C1** [NC]	**D1/C2** [PC]	**D2**...sample
[NC] in duplicate	---	100	---	---
[CC] in duplicate D1/E1	---	---	100	---
[PCL] in duplicate F1/G1	---	---	100	---
[PCM] in duplicate H1/A2	---	---	100	---
[PCH] in duplicate B2, C2	---	---	100	---
Diluted sample	---	---	---	100
[MIC] cover with adhesive strips and incubate 30 minutes at 17-25^{o}c				
[WASH]= wash four times	350	350	350	350
Step-2				
[CON] Conjugate		100	100	100
[MIC]= cover with adhesive strips and incubate 30 minutes at 17-25^{o}c				
[WASH] wash five times	350	350	350	350
Step-3				
[SUB] Substrate	100	100	100	100
Incubate 15 minutes at 17-25^{o}c				
[STOP] stop solution	100	100	100	100
Zero the ELISA microtiter reader using the substrate blank in well A1. Measure the absorbance at 450nm as soon as possible or within 30 minutes. Or after terminating of the reaction, using a reference wavelength of 630-690nm				
[MIC]= Microtiter [SUB]=Substrate [CON]= Conjugate [PC]= Positive Control				

Calculation of the mean of controls and Cut-off values

Mean absorbance values of NC, CC and PCL, PCM, PCH are calculated and the test run considered valid provided that the following criteria are met:

1. Substrate blank in well A1< 0.150
2. Mean of Negative Control(MNC) ≤ Mean of Cut-off Control(MCC)
3. Mean of Positive Control Medium (MPCM) ≥ 0.750
4. MPCM: MNC ≥5

Interpretation of results

A450 (patient)≥MCC+15% MCC: Anti-*Toxoplasma*-lgG-Antibody-positive

A450 (patient) <MCC-15% MCC: Anti-*Toxoplasma*-lgG-Antibody-negative

Absorbance of patients (A_P) lying between the calculated Cut-offs are equivocal.

Test performances

Diagnostic specificity = 99.2%

Diagnostic sensitivity = 96.1%

Overall agreement = 98.0%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to good laboratory practices. All materials contaminated with patient specimen or controls should be inactivated by validated procedures (autoclaving or chemical treatment).

Annex-VI: Laboratory test result recording format

Code number _____

Age _____ Sex _____

Section A: - Investigation of *Toxoplasma* IgG-serostatus

Absorbance	**Interpretation**	**Remarks**
	Positive	
	Negative	
	Equivocal	

Final test result_____

Section B: - Current CD_4 + T cell count

CD4+ T cell count: _____ cell/µl

Reported by_____

Date of reporting _____

Signature _____

Declaration

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

Name _____

Signature _____

Assurance of principal investigator

The undersigned agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the college of Public Health and Medical Science in effect at the time of grant is forwarded as the result of this application.

Name of the student: _____

Date._____ Signature _____

APPROVAL OF ADVISORS

This thesis has been submitted with my approval as University advisor.

Name of the first advisor: _____

Date._____ Signature _____

Date of submission: _____

Name of the second advisor: _____

Date._____ Signature _____

Date of submission: _____