Department of Medical Laboratory sciences
College of Health Sciences
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Sero burden of *Toxoplasma gondii* and associated risk factors among HIV/AIDS patients in Armed Forces Referral and Teaching Hospital, Addis Ababa, Ethiopia

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I, here by, assure that the thesis which is entitled "sero burden of *Toxoplasma gondii* and associated risk factors among HIV/AIDS patients in Armed Forces Referal and Teaching Hospital, Addis Ababa, Ethiopia" is my original work and submitted for the partial fulfillment of the requirements for the degree of master of sciences in diagnostic & public health microbiology. It complies with the regulations of the University & meets the accepted standards with respect to originality & quality. All sources of material used for the proposal has been duly acknowledged.

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List of abbreviations

Ab	Antibody
Ag	Antigen
AIDS	Acquired Immunodeficiency Syndrome
AFRTH	Armed Forces Referral & Teaching Hospital
ART	Anti retroviral therapy
BLH	Black lion Hospital
CNS	Central nervous system
CSF	Cerebrospinal fluid
ELISA	Enzyme Linked Immuno Sorbent Assay
EMLA	Ethiopian Medical Laboratory Association
HIV	Human Immunodeficiency Virus
HRP	Horseradish Peroxidase
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IU	International Unit
NHANES	National Health and Nutrition Examination Study
SOP	Standard operating procedure
SPSS	Statistical Package of Software for Social science studies
T.E	Toxoplasma encephalitis
T. gondii	*Toxoplasma gondii*
TMB	Tetramethyl benzidine
USA	United States of America
WHO	World Health Organization

Abstract

Background: Toxoplasmosis is a zoonotic disease, worldwide distribution caused by an obligate intracellular coccidian parasite, known as *Toxoplasma gondii. T. gondii* can lead to serious diseases in immuno compromised patients such as HIV/AIDS patients. In most cases, central nervous system involvement can lead to encephalitis, which is one of the most important reasons of death among patients with HIV due to reactivation of tissue cysts that remained latent after the primary infection.

Objective: This study was conducted to assess the Sero burden of *Toxoplasma gondii* infection and to identify associated risk factors among HIV/AIDS patients in Armed Forces Referral & Teaching Hospital, Addis Ababa, Ethiopia.

Methods: A cross sectional study was conducted in Armed Forces Referral & Teaching Hospital, Addis Ababa, Ethiopia from March toMay2016. The sampling method was based on convenience sampling. After getting an informed consent, pretested questionnaire were used to gather socio demographic information and data on factors predisposing to *T. gondii* infection. Serum samples from each volunteering patients were screened for the presence of anti- *T.gondii* IgG & IgM antibodies by using ELISA test kit (CTKBIOTECH, USA). Data were entered and analyzed using SPSS version 15.0.The chi-square test was used to determine the association between variables. P values were determined and taken as a level of significance when it was found less than 0.05.

Results: The study recruited a total of 174 HIV/AIDS patients, 99 (56.9%) were males and 75 (43.1%) were females. The study also included age strata ranging from 18 to 68years. Most of the sampled subjects were found in the age group of 31 to 40 years. About 154 (88.5%) were seropositive for anti-*T. gondii* IgG antibody and 3 (1.7%) were seropositivity for anti- *T. gondii* IgM antibodies. None was positive for IgM antibody alone. Of all the variables included in the study, only presence of cat depicted association with sero-burden of anti-*Toxoplasma gondii* IgG antibody (p=0.038).

Conclusion: This study revealed high sero burden of chronic toxoplasmosis in HIV/AIDS patients. HIV/ AIDS patients having domestic cat at their home were at higher risk of *T. gondii* infection. It would be important to increase public awareness about different routes of

transmission of *T. gondii.* Besides, routine screening for Toxoplasma should be undertaken for all HIV-infected patients to minimize complication related to reactivation.

1. Introduction`

1.1Background

Toxoplasma is a member of the phylum Apicomplexa, which encompasses intracellular parasites characterized by a polarized cell structure and two unique apical secretory organelles named micronemes and rhoptries [1]. *Toxoplasma gondii* is an obligate intracellular parasite. It was first discovered by Nicolle and Manceaux in 1908 in a rodent, Ctenodactylus gundi. In the same time, Splendore (1908) independently described Toxoplasma in a laboratory rabbit in Sao Paulo, Brazil [2].

The life cycle of *T. gondii* consists of two stages (asexual and sexual); the sexual stage takes place in the intestine of the definitive host; known definitive hosts are members of the feline family, predominantly domestic cats [5]. When bradyzoites or oocytes are ingested by a feline, formation of oocytes proceeds in the epithelium of the small intestine. Several million unsporulated oocytes may be released in the feces of a single cat. Under mild environmental conditions oocysts may sporulate within three weeks period, then infecting humans and other intermediate hosts [3].

The asexual stage takes place in the intermediate hosts, which are mammals or birds [1]. During this phase rapid intracellular growth of the parasite as tachyzoite takes place. The tachyzoites can infect and multiply in almost any nucleated mammalian or avian cell. Following accumulation, tachyzoites are secreted into the blood stream and spread in the body, leading to development of an acute disease [3]. Bradyzoites are the slow-growing, transmissible, and encysted forms that are dormant [4]. The normal immune response and transformation of the tachyzoite into cyst-forming bradyzoites limit the acute stage and establish a chronic infection. Bradyzoites differ from tachyzoites mainly in their extremely slow multiplication rate (their name reflects this slow process) and in the distinct set of proteins they express [3]. The cysts are formed mainly in neural and muscular tissues, especially brain, skeletal and cardiac muscles, and can persist, inactivated, in the body for a very long time [2].

After ingestion, sporozoites penetrate enterocytes, multiplying initially in cells lining the lamina propria and later the epithelium. However, infection may spread to other tissues within a short time after ingestion. Infection is disseminated to distant organs through the blood and lymphatics

[6].Unfortunately precise information on the incubation period is not available, but there are some indications from outbreaks of infections, which is 4 to 21 days, with most illness in the second week after exposure [7].

People typically become infected by three principal routes of transmission [5]. The first one is food borne transmission [8]. People become infected by eating raw/undercooked, contaminated meat (especially pork, lamb, and venison), eating food that was contaminated by knives, utensils, cutting boards, or other foods that had contact with raw, contaminated meat [9].

The second route is from animal-to-human transmission where cats play an important role in the spread of toxoplasmosis. Cats can "recycle" and amplify the infection by releasing millions of infective oocytes into the environment [5]. Sporulated oocysts survive for long periods under moderate environmental conditions. They are known to survive on fruits and vegetables, in moist soil for months to years [5]. People can be infected by accidental ingestion of oocysts during cleaning a cat's litter box, or by eating unwashed vegetables and fruit irrigated with untreated water that has been contaminated with cat feces [8].

The third one is mother-to-child (congenital) transmission by which a woman who is newly infected with Toxoplasma during pregnancy can pass the infection to her unborn child (congenital infection) [3]. Immunocompetent women infected prior to conception not transmit toxoplasmosis to the fetus, although rare exceptions have been reported [10]. There are also rare instances of transmission i.e. via tachyzoites contained in blood products, tissue transplants, or unpasteurized milk, and laboratory workers who handle infected blood can also acquire infection through accidental inoculation [4].

Infection of this parasite leads to an asymptomatic infection in immunocompetant persons. However, 10% to 20% of patients with acute infection may develop cervical lymphadenopathy and/or a flu-like illness. The clinical course is benign and self-limited; symptoms usually resolve within a few months to a year [11]. Mortality/Morbidity studies show that immunocompromised individuals and fetuses are at particularly high risk for severe sequelae and even death [12]. Immuno-deficient patients often have central nervous system disease but may have chorioretinitis, or pneumonitis [13]. In patients with AIDS, toxoplasmic encephalitis is the most common cause of intracerebral mass lesions and is thought to be caused by reactivation of chronic infection [14].

When Toxoplasma infection is acquired by a mother during pregnancy, the parasite presents a significant risk of adverse outcome to the fetus [15]. The risk of transmission from mother to fetus is lower when maternal infection is acquired in the early stages of pregnancy but the outcome in such cases can be severe or life-threatening to the fetus. Conversely, while maternal infection acquired later in pregnancy confers a higher risk of transmission to the fetus, the clinical outcome is characteristically less severe, or the child may even be born asymptomatic [16]. Latent *T. gondii* infection may be reactivated in immune-deficient individuals (such as HIV-infected women) and result in congenital transmission of the parasite [3].

Four clinical signs are thus considered as representative of congenital toxoplasmosis; hydrocephaly or microcephaly, retinochoroiditis, cerebral calcifications and neurological injury. This clinical spectrum, which represents some of the late sequelae of infection, was later enlarged with a variety of acute signs, hydrops fetalis, erythroblastosis and jaundice with hepatosplenomegaly [17].

However Toxoplasmosis in HIV-infected patients occurs usually due to reactivation of chronic infection, and it usually presents as toxoplasmic encephalitis. The initial presentation of toxoplasmic encephalitis in patients with AIDS may be sub-acute. Patients present with altered mental status (62%), headaches (59%), and fever (41%) associated with focal neurologic deficits. Progression of the infection can lead to confusion, drowsiness, seizures, hemiparesis, hemianopsia, aphasia, ataxia, and cranial nerve palsies. Motor weakness and speech disturbance are seen as the disease progresses. If not treated promptly, patients may progress to coma with in days to weeks. 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. Extra cerebral manifestations occur less frequently than cerebral toxoplasmosis [14].

1.2. Statement of the problem

It is generally assumed that approximately 25 to 30% of the world's human population is infected by Toxoplasma [20]. Actually, the prevalence varies widely between countries (from 10 to 80%) and often within a given country or between different communities in the same region [21]. Low seroprevalences (10 to 30%) have been observed in North America, in South East Asia, in Northern Europe, and in Northern countries of Africa. Moderate prevalence's (30 to 50%) have been found in countries of Central and Southern Europe, and high prevalence have been found Latin America and in tropical African countries [20].

Toxoplasmosis is a major public health concern because the disease is serious in terms of mortality or physical and/or psychological sequellae in patients with HIV disease [21]. In the majority of normal, healthy (immune competent) subjects, infection is asymptomatic and frequently results in the chronic persistence of cysts within host tissues; the cysts normally dormant, probably for life. But, in immune compromised states, such as in HIV infections, subjects are at risk of developing acute toxoplasmosis due to reactivation of the organism if their CD4+ T-cell count decreases below 200 cells/μL [15, 16].Since the pandemic of HIV infection has spread throughout the world, toxoplasmosis has been implicated as one of the most important opportunistic infections in HIV/AIDS patients. Moreover, in up to 10% of HIV infected immune competent individuals, it causes cervical lymph-adenopathy or ocular disease [22, 23 & 24].

Toxoplasma-HIV co-infected patients have a risk as high as 30% to 40% of developing Toxoplasma encephalitis, especially those with significant immunosuppressant (CD4 cell count < 200 cells/μL)[2, 3]. Thus, identification of latently infected immunocompromised patients by determining anti-Toxoplasma IgG (immunoglobulin G) antibodies becomes essential [25].Study in the USA showed that about 30% of AIDS patients previously exposed to toxoplasma and suffered from a cerebral reactivation [26]. Consequently, it may be calculated that8 % of AIDS patients in South East England will experience a life threatening episode of cerebral disease following secondary reactivation of toxoplasmosis. In addition to this, 0.5-1 % of these patients may acquire primary toxoplasmosis associated with AIDS each year reflecting the incidence of toxoplasma infection in this group [27].

Among the congenital infections, approximately 10% of congenital toxoplasmosis results in abortion or neonatal death. It is estimated that 10-13% of the babies will have visual handicap. Clinical signs of congenital Toxoplasmosis are not apparent at first in most cases but infection acquired after birth is usually asymptomatic. Intrauterine meningoencephalitis could lead to the development of the following: cerebrospinal fluid (CSF) abnormalities, hydrocephalus, microcephaly, chorioretinitis, seizures, and deafness. Some of the severely affected infants die in utero or within a few days of birth. Other signs include maculopapular rash, generalized lymphadenopathy, hepatomegaly, splenomegaly, jaundice, and thrombocytopenia [28, 29].

The risk factors associated with seropositivity to toxoplasmosis are raw or undercooked mutton consumption and the presence of cats. Individuals consuming raw or undercooked mutton were found 16.9 times more likely to be positive than those known to consume well-served mutton. Toxoplasmosis is a zoonosis arising from man's close contact with domestic cats (Felis catus). Individuals with a known history of association with cats were 5.3 times more likely to be seropositive than those with no history of such association [30].Recent studies have identified water as a potential source of infection in both humans and animals [31].

Although toxoplasmosis is a problem throughout the world, its frequency varies from one geographic area to another. Screening for *T. gondii* is not routinely performed in Ethiopia due to limited health resources and lack of awareness of the prevalence of the disease and its mode of transmission. Lack of awareness is likely to expose them to increased risk of contracting toxoplasmosis, as they might not take proper precaution. Moreover, data on Seroprevalence of *T. gondii* and associated risk factor is limited in some regions of Ethiopia, and published reports on magnitude of *T. gondii* infection in HIV-infected individuals is mainly done with rapid serological test kits which could not reflect the actual burden of Toxoplasmosis in the country. Moreover, our study site is totally different representing Military hospital and the study participants have different risk for Toxoplasmosis. To the best of my knowledge, there is no information concerning burden of *T.gondii* in Army hospital. Therefore this study was conducted to determine the burden of *T.godii* infection & associated risk factors among HIV/AIDS infected individuals and tried to address the aforementioned gaps and further to expand a new knowledge onToxoplasmosis in Ethiopia.

1.3. Significance of the study

- ✓ The study revealed the burden of toxoplasmosis& associated risk factors among HIV-infected individuals in the study area.
- ✓ Provide relevant information for health institution and policy makers to support decision making that can improve the functioning of health care system for the prevention of toxoplasmosis and to decrease the probability of death especially in HIV/AIDS patient.
- ✓ The result of this study will also be used as a source of information to other Researchers in the future.

2. Literature review

Infection with the protozoan *T. gondii* has been found worldwide in nearly one third of the human population and varies greatly among different countries, among different geographical areas within the same country, and among different ethnic groups living in the same area [5]. Sero-prevalence in Europe is high, up to 54% in Southern European countries and it decreases with increasing latitude to 5-10% in northern Sweden and Norway. The age-specific prevalence has been decreasing in Europe over the past three to four decades. The National Health and Nutrition Examination Study (NHANES, 1999-2000) and the NHANES III (1988-1994) from the USA found *T. gondii* sero-prevalence of 15.8% in the 12-49 years age-group [32].

A study conducted in Mexico showed that the prevalence of IgG and IgM anti-Toxoplasma antibodies in patients with HIV and acquired immunodeficiency syndrome (AIDS) Through the enzyme-linked immunosorbent assay (ELISA), IgG and IgM anti-Toxoplasma antibodies were found in 92 patients of which46 (50.0%) were IgG sero-positive, and only one case (1.0%) was postivefor IgM antibodies. Of the92 patients: 53 were HIV sero-positives and 39 had AIDS [33].

Retrospective analysis was done to determine the incidence of Toxoplasma encephalitis (TE) in patients with acquired immunodeficiency syndrome (AIDS) in Yugoslavia, *T.gondii*-specific IgG antibodies were detected in 127 of the 288 AIDS patients (44.1%) tested serologically (including all with neurologic abnormalities). A total of 31 patients developed TE, indicating a TE overall attack rate of 7.8%. Of these, 29 TE episodes occurred in the *T.gondii* sero-positive patients (22.8%), significantly more often than in the *T. gondii* sero-negative among which TE occurred in two (2/l 61, 1.24%). By survival analysis, the cumulative incidence of TE in the *T. gondii* sero-positive patients was 32.7% for 60 months (30% for 24 months).even though age, sex, or HIV transmission risk factor, not associated, the risk for TE increased with the decrease in theCD4+ T-cell count [34].

Across-sectional study was carried out in Malaysia. Three hundred and one sera of HIV/AIDS patients were tested for anti-*Toxoplasma* IgG antibody by ELISA technique. The sero-prevalence of toxoplasmosis was 41.2% in HIV/AIDS patients. The sero-prevalence was significantly higher in the Malay (57.9%) than the Chinese (38.7%), followed by the Indian patients (29.6%) No possible risk factor, such as contact with cats, consumption of uncooked meat, and history of blood transfusions was found to have any significant association with the presence of anti-

Toxoplasma antibody in the study sample. The association between the presence of anti-Toxoplasma antibody and CD4 cell count was determined but no statistically significant association was found during the study period, only one case of active CNS toxoplasmosis was registered [35].

In Irana across sectional survey were conducted *between* (September 2007 and October 2008), on 78 healthy and 62 HIV+/AIDS individuals. Determination of CD4+ counts was performed by flow cytometry. The serum separated from blood samples was evaluated by conventional ELISA technique to determine the presence of antibodies to *T. gondii*. Forty eight out of 62 (77.4%) HIV/AIDS serum samples were found positive for anti-*T. gondii* IgG antibody, compared with 59 among 78 (75.6%).HIV negative samples from the same area. Six out of 62 (9.7%) HIV+/AIDS patients showed anti-*T. gondii* IgM antibody in their serum samples, compared with 7 among 78 (9%) of HIV negative samples. The mean of CD4+ counts in HIV+/AIDS was (430.8±182.3) cells/μL and in control group were (871.0±243.3) % cells/ μL. CD4+ estimation in 5 (11.1%) of HIV+/AIDS patients was <200 cells/ μL [36].

Another cross sectional observational study was conducted over a period of three years from 1st January 2011 to 31st December 2013 in India, using 1181 samples, including 661 samples from HIV positive patients and 520 from HIV negative individuals. Out of The 520 HIV negative individuals, 238 were antenatal women, above 12 years .Seroprevalence among HIV infected and non-infected was found to be 21.3 % and 14.2% respectively. The difference was statistically significant. No significant gender differences were found. Seroprevalence increased from 9.1 % to 30 % with increasing age in the HIV infected patients. Only 2 (0.84 %) samples of antenatal women were positive for immunoglobulin M captures ELSIA, while one sample was equivocally reactive [25].

From March 2003 to March 2005 at the Port Moresby General Hospital, Papua New Guinea a sero-epidemiological study was done. The study aimed to determine the prevalence of Toxoplasmosis and to ascertain whether there is indeed a difference in infection rate between HIV-positive patients and HIV-negative blood donors. Of the total 301 participants, 181 were HIV antibody positive and 120 blood donors were HIV antibody negative. Enzyme-linked immune-sorbent assay (ELISA) was used for the detection of IgG *T. gondii*-specific antibodies. Anti-*T.gondii* IgG antibodies were detected in 159 (53%) of all those recruited.The

Seroprevalence in HIV-infected participants was 108 (60%) compared to 49 (41 %) in the HIV-negative group. The study further showed that exposure to cats and highlands origin were independent risk factors but Other socio-demographic and disease variables studied such as meat diet, educational levels and length of HIV infection did not demonstrate any association[37].

A cross sectional study was carried out In Nigeria to investigate the presence of *T. gondii* IgG antibodies in HIV-infected patients attending hospitals in Makurdi metropolis, The Enzyme-linked immunosorbent assay (ELISA) technique were used to determine the presence of Toxo-IgG antibodies .39 (10.8%) were screened positive for Toxo-IgG antibodies out of the 360 HIV/AIDS patients enrolled. Males (10.3%) and females (11.2%) had similar sero-prevalence of Toxo-IgG with no significant difference the presence of Toxoplasma IgG antibodies were found to be highest in the ≥ 54 years age group. A significant difference was observed in the sero-prevalence of Toxoplasma IgG among age groups. Females with CD4 T-cell count ≤ 200 cells/mm3 recorded higher sero-prevalence (73.7%) of Toxoplasma IgG. There was no significant difference in the sero-prevalence of Toxoplasma IgG in relation to CD4 T-cell counts [38].

A study carried out in Cameroon to determine the sero-prevalence of Toxoplasma antibodies (IgM and IgG) in HIV/AIDS patient ELISA technique was employed serologically to determine *Toxoplasma* antibodies. Of the 133 patients 83(62.4%) were females and 59 (37.6%) were males; ninety three (69.9%) were positive for *Toxoplasma* antibodies. Fourteen (10.8%) of the 93 of sero-positive patients presented with both IgG and IgM-antibodies in their sera while fifty six (42.1%) and 8 (6.0%) were only sero-positive for Toxoplasma IgG or IgM-antibody respectively. Seropositivity was not dependent on the patient's sex or age. The studies conclude that 64.7% of the positive cases were due to reactivated infection [39].

There is some information which shows the prevalence of toxoplasmosis among HIV infected and HIV uninfected individuals, on a general population of Ethiopia. The study showed that *T. gondii* IgG Antibody titers > 15 IU/ml were detected in 74.4 % (755/1016) of the specimens taken from six different geographical regions. The highest prevalence of *T.gondii* antibody titers were found in children and 75% of young adults had sero-converted [40]**.**

Another study which was conducted in Nazareth town, Ethiopia showed that 60% (39/65) were positive for anti-*Toxoplasma gondii* antibodies by the Modified direct agglutination test.

Consuming raw or undercooked mutton and owning of cat were found to have significant association with sero-prevalence [30].

Another cross sectional study was conducted in Ethiopia aimed to investigate the risk factors and sero-prevalence of latent *T.gondii* infection among HIV-infected and HIV-uninfected individuals. Serologic testing for anti- *T. gondii* antibodies were done using ELISA. Results showed that the cumulative prevalence of anti-Toxoplasma IgG antibody among the study subjects was 90.0% (297/330). In addition the prevalence of latent Toxoplasma infection was 93.3% (154/165) among HIV positive and 86.7% (143/165) among HIV negative participants. Age, gender and HIV serostatus were found to be significantly associated with sero-prevalence of latent toxoplasmosis [41].

A study was done in ArbaMinch south Ethiopia, aimed to investigate sero-prevalence of latent *T. gondii* infection and assess its associated factors among HIV-Infected individuals. Serologic testing for anti- *T. gondii* antibodies were done using ELISA. the result showed that out of the total 170 HIV sero-positive study Participants included in this study,150(88.2%) were IgG anti-*T. gondii* antibody sero-positive. High Proportions of sero-positive individuals (90%) within the age group 35-44. Consumption of raw and involvement in farming/gardening activities were independent predictors of *T. gondii* Seropositivity [42].

A cross sectional study in Bahirdar, Ethiopia was conducted to determine the sero-prevalence and risk factors of toxoplasmosis in HIV infected and non-infected individuals. Serologic testing for anti- *T.gondii* antibodies was done using ELISA. Of the examined HIV sero-positive individuals, 87.4% (90/103) and 10.7% (11/103) were positive for anti-*T.gondii* IgG and IgM antibodies, respectively. In HIV negative apparently healthy blood donors, prevalence of anti-*T.gondii* antibodies were 70.29% and 2.97% for IgG and IgM, respectively. Multivariate analysis showed that undercooked or raw meat Consumption independently significantly associated with anti-*T.gondii* IgG sero-positivity in both study groups, with a significantly higher number of males affected than females [43].

Across sectional study carried out to determine the prevalence of *Toxoplasmosis* and to determine risk factors associated with Seropositivity in HIV/AIDS patients at BLH, Addis Ababa Ethiopia. A total of 150 immune compromised (HIV/AIDS) patients participated in this sero-survey. About 141 (94%) of the patients were sero-positive for anti-*T. gondii* IgG antibodies, whereas anti-*T. gondii* IgM antibodies were not detected in patients' serum. Consumption of raw vegetables and not having primary information about *Toxoplasmosis* were significant association with the presence of anti-*Toxoplasma* antibody the association between the presence of anti-*Toxoplasma* antibody and CD4+ T lymphocyte cells count were not statistically significant [44].

3. Study Objective

3.1. General Objectives

- To determine the burden of *Toxoplasma gondii* infection by serological examination and to identify associated risk factors among HIV/AIDS patients in Armed Forces Referral & Teaching Hospital, Addis Ababa Ethiopia

3.2. Specific objectives

- To determine Sero burden of *Toxoplasma gondii* IgG antibodies in HIV/AIDS patients
- To determine Sero burden of *Toxoplasma gondii* IgM antibodies in HIV/AIDS patients
- To identify the possible risk factors associated with *T. gondii* infection in HIV/AIDS patients.

3.3. Hypothesis

There is no difference in the burden of *Toxoplasma gondii* infection in the current study sites with previous similar studies in BLH [44].

4. Materials and method

4.1. Study Area

The study was conducted in Armed Forces Referral and Teaching Hospital (AFRTH), which is located in Ledeta sub city, Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia. AFRTH is organized under Health Main Directorate, Ministry of Defense. It provides medical service to members of the Ethiopian defense force and their family. AFRTH has 15 wards with 600 beds. There are 378 health care professionals with different levels and field of training. Based on the 2014/2015 annual report, the hospital provides services for 96,621 outpatients and 3,334 inpatient, 1,223 deliveries as well as 21,200 ART patients. Other than patient diagnosis, AFRTH is also engaged in different activities like health teaching and research.

4. 2. Study design and study period

A hospital based cross-sectional study was conducted from March to May, 2016 in Armed forces referral and teaching hospital, Addis Ababa, Ethiopia.

4.3. Population

4.3.1. Source population

All patients who were visiting Armed Forces Referral and Teaching Hospital during the study period.

4.3.2. Study population

All adult HIV positive patients who were referred to Armed Forces Referral and Teaching hospital ART clinic for CD-4 cell count and ART monitoring and who gave informed consent and fulfilled the inclusion criteria.

4.4. Description of variables

4.4.1. Dependent variable

- Seropositivity of *T. gondii* using IgM &IgG antibodes

4.4.2. Independent variables

- Age
- Sex
- Level of education, occupation
- Residence
- Consumption of raw/un cooked meat
- Consumption of raw/un cooked vegetable
- Cat owner ship
- History of blood transfusion
- Organ transplantation
- CD4 count
- HAART status
- WHO HIV/AIDS clinical stage

4.5. Inclusion and exclusion criteria

4.5.1. Inclusion criteria

- ✓ Aged 18 years and above
- ✓ Patients agreed to participate and gave informed consent

4.5.2. Exclusion criteria

- ✓ Study subjects whose blood sample were hemolyzed or turbidity
- ✓ Gross Lipemic blood sample was excluded
- ✓ Patients who have incomplete data

4.6. Sample size determination and Sampling

4.6.1. Sample size determination

The sample size was calculated based on single sample size estimation. The value of p taken as 87% (0.87) from the previous study conducted on Seroprevalence and risk factors for

Toxoplasmosis in HIV infected and non-infected individuals in BahirDar, Northwest Ethiopia Considering 95% confidence interval, 5% margin of error and 87 proportion, the sample size was calculated using the following standard formula

$$n = \frac{(Z\alpha/2)2\ P\ 1 - P}{d2}$$

$$n = \frac{(1.96)2 \times 0.87\ (1 - 0.87\ )}{(0.05)2}$$

$$n = \frac{3.8416 \times 0.87\ (0.13)}{0.0025}$$

$$n = 174$$

Where: n = Sample size

Z (α/2)2 = at 95% confidence interval Z value (α = 0.05) = 1.96 Where

p = Proportion of occurrence of the event to be studied 87%

d = Margin of error at (5%)

A total of 174 study subjects were included in the study

4.6.2. Sampling technique

Convenience sampling technique was employed to include study participants who meet the inclusion criteria.

4.7. Data collection and specimen transportation

One medical laboratory technologist and one nurse together with the principal investigator were involved in data collection. One of the medical laboratory technologists together with principal investigator were acting as supervisor. Both of the data collectors and supervisor were trained for two days on the data collection instrument and basic Toxo IgG and IgM ELISA analysis skills. Before the actual data collection a pre-test of the instruments and the procedure was conducted. Corrective measure have been taken after getting consent from the selected patients, socio

demographic & data on factors predisposing to *T. gondii* Infection were collected from patients using structured questionnaire (Annex E).and other HIV related Clinical data such as HAART status, as well as WHO HIV/AIDS clinical stages and CD4 cell counts of study participants were taken from patient history card & documented. The patients were asked to give blood sample and if they willing then laboratory technologist collected 4mL of venous blood sample in serum separated vacutainer tube and the sample was left for 30 minutes to facilitate clotting and then the clotted blood centrifuged to separate the serum from blood. Serum was secondly aliquated in to nunc tubes and stored at -20°C until use. Repeated freezing and thawing were avoided. All regulation was strictly followed during packaging and transportation. Anti-*T.gondii* results were given to the patients during the next attendance. `

4.8. Laboratory investigation

Serologic test was employed by using commercially available ELISA test kit (CTK BIOTECH **R**ecombiLISA Toxo IgM and IgG ELISA Kit USA) as per manufacturer's instruction. Positive and negative controls were used with each series of anti *T. gondii* IgG/IgM test (Human, USA); results were obtained by comparison with a cut-off value measured at 450nm absorbance.

4.8.1. Principle of the test

4.8.1.1. Principle of the RecombiLISA Toxo IgM

RecombiLISA Toxo IgM test is a solid phase enzyme linked immunoabsorbent assay based on the principle of the IgM capture technique for the detection of IgM anti-*T.gondii* in human serum or plasma.

The RecombiLISA toxo IgM test is composed of two key components

1. Solid micro wells pre-coated with monoclonal anti human IgM antibody
2. Liquid conjugates composed of recombinant *T.gondii* antigen conjugated with horse reddish peroxidase (HRP- *T.gondii* conjugates).During the assay, the test specimen is first incubated with the coated microwells IgM anti-Toxo, if present in the specimen, binds to the antibody coated on the micowell surface. In the second incubation with the HRP-*T.gondii* conjugates, the IgM anti-*T.gondii* antibody absorbed on the surface of microwell react to the HRP- *T.gondii* conjugates, forming a complexed conjugates. Unbounded conjugates are then removed by washing .The presence of the complexed conjugates is shown by a blue color upon additional incubation with

TMB substrate. The reaction is stopped with stop solution and absorbance are read using a spectrophotometer at 450/620-690 nm.

4.8.1.2. Principle of the RecombiLISA Toxo IgG test

RecombiLISA Toxo IgG test is a solid phase enzyme linked immunoabsorbent assay based on the principle of the indirect EIA technique for the detection of IgG to *T.gondii* in human serum or plasma. The RecombiLISA toxo IgG test is composed of two key components.

1. Solid micro wells pre-coated with recombinant Toxo antigens
2. Liquid conjugates composed of mouse anti-human IgG conjugated with horse reddish peroxidase (HRP- anti-human IgG conjugated).During the assay, the test specimen is first incubated with the coated microwells. The IgG anti-Toxo, if present in the specimen, binds to the antigen coated on the microwells surface. In the second incubation with the HRP- anti-human IgG conjugates, the IgG antibodies absorbed on the surface of microwells react to the HRP- anti-human IgG conjugates, forming a complexed conjugates. Unbounded conjugates are then removed by washing .The presence of the complexed conjugates is shown by a blue color upon additional incubation with TMB substrate. The reaction is stopped with stop solution and absorbance are read using a spectrophotometer at 450/620-690 nm.

Interpretation of Result

A. set up the cut –off value

The cutoff value =0.15+N N: Mean OD of the negative control.

B. Calculation of specimen OD ratio

Calculation an OD ratio for each specimen by dividing the OD value by the Cut off value as follows: specimen OD ratio= specimen OD/cut-off value

C .Assay validation

- ✓ The Mean OD value of the toxo IgM positive controls should be $\geq$ 0.80
- ✓ The Mean OD value of the toxo IgG positive controls should be $\geq$ 1.00
- ✓ The Mean OD value of the toxo IgM/IgG Negative controls should be $\leq$ 0.10

*Note: If the above conditions are not met check the procedure and repeat the assay

Interpretation of the results

Result	Specimen OD ratio
Negative	< 1.00

Positive	≥1.00

1. The Negative result indicates that there is no detectable IgM/IgG anti *T. gondii* in the specimen

2. Results just below the cut- off value (lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimen when it is applicable).

4.8.2. Data management & Quality control

4.8.2.1. Data management

A clear explanation of the purpose and procedures of the study were informed to introduce each interview to get the right information from each participant. Special emphasis was given during coding the data sheet as well as the sample. Supervision was made by the principal investigator. Pre-testing of the instruments, training of data collectors, checking all the materials completeness and logical consistency at the end of each day, and giving prompt feedback at the spot during the data collection process were the methods employed to ensure the quality of data.

4.8.2.2. Quality control

Prior to the beginning of any data collection, all data collectors were trained by the principal investigator on an overview of the assessment and its objectives. During the entry of data it was cross checked to assure the right data was entered correctly. All specimens were collected according to the standard operating procedure of the ART laboratory of AFRTH were strictly followed. The quality of test results were maintained using the internal quality control of the test kits for ELISA method. All reagents that are used for testing checked for their shelf life, being at appropriate temperature before using them. Test procedures were done according to the manufacturer's instruction. In addition, well trained and experienced laboratory professionals participated in the laboratory analysis procedure.

4.9. Data Analysis and Interpretation

Data was entered and analyzed using the statistical soft ware SPSS version 15.0.The Seroburden for Toxoplasmosis was expressed in percentages for the entire study group and results obtained were presented in tables, figures and graphs. The chi-square test was used to determine the

association between variable .P values were determined and taken as a level of significance when found less than 0.05.

4.10. Ethical consideration

Ethical clearance was obtained from Departmental Ethics and Research committee of the department of Medical Laboratory Science, College of Health Sciences and School of Allied Health Science of Addis Ababa University and official permission to collect data was obtained from the AFRTH administrator. Subjects were recruited after they become informed about the objectives and use of the study and after they gave informed consent. Sample taken from each patient was coded and results obtained were kept confidential. The results were notified to study participants. Individuals found to be positive for anti *T.gondii* antibodies were linked to physicians for monitoring and further management.

5. Results

5.1. Socio-demographic status of study participants

Of all the study participants (174), 99 (56.9%) were male, 163 (93.7%) were urban dwellers, 64(36.8%) were government employees and 64 (36.8%) were with educational level 9 to12. The study also included age strata ranging from 18 to 68 years. Most of the study subjects were found in the age group of 31to 40 years (41.4 %) (Table 1)

Table 1: Socio-demographic status of HIV/AIDS patients attending the Armed Forces Referral & Teaching Hospital; Addis Ababa, Ethiopia, March to May, 2016

Variable with category	Frequency	Percent (%)
Sex		
Male	99	56.9
Female	75	43.1
Age groups in years		
18-30	19	10.9
31-40	72	41.4
41-50	61	35.1
>50	22	12.6
Residence		
Urban	163	93.7
Rural	11	6.3
Occupation		
Government	64	36.8
Private	62	35.6
House wife	35	20.1
Others	13	7.5
Level of education		
Read and write	12	9.2
Primary(1-8)	47	27.0
Secondary(9-12)	64	36.8
Above grade 12	51	29.3

5.2. Clinical and other contributing factors of study subjects

Among the total study participants (174), none had a history of organ transplantation. 129 (74.1%), 144(82.8%), 167 (96.0%) and 164(94.3%) participants were having cat at home , ate raw vegetable and fruit, on HAART and WHO HIV/AIDS clinical stage I, respectively (Table 2).

Table 2: Frequency of contributing factors for *T.gondii* infection among HIV patients attending the Armed Forces Referral & Teaching Hospital; Addis Ababa, Ethiopia, March to May, 2016.

Contributing factors	Category	Frequency	Percent (%)
cat owners ship	No	45	25.9
	Yes	129	74.1
Eat uncooked meat	No	115	66.1
	Yes	59	33.9
Eat raw vegetable and fruit	No	30	17.2
	Yes	144	82.8
Having history of blood transfusion	No	136	78.2
	Yes	38	21.8
HAART status	with HAART	167	96.0
	withoutHAART	7	4.0
CD4 cell count	< 200	27	15.5
	200-500	86	49.4
	>500	61	35.1
WHO stage of HIV/AIDS	Stage I	164	94.3
	Stage II- IV	10	5.7

5.3. Burden of *T.gondii* infection

From all blood samples, collected from 174 HIV/AIDS patients and tested, 154 (88.5%) were sero-positive for anti-*T. gondii* IgG antibody and 3 (1.7%) were seropositive for anti- *T. gondii* IgM antibodies. None was positive for IgM antibody alone (Figure 1).

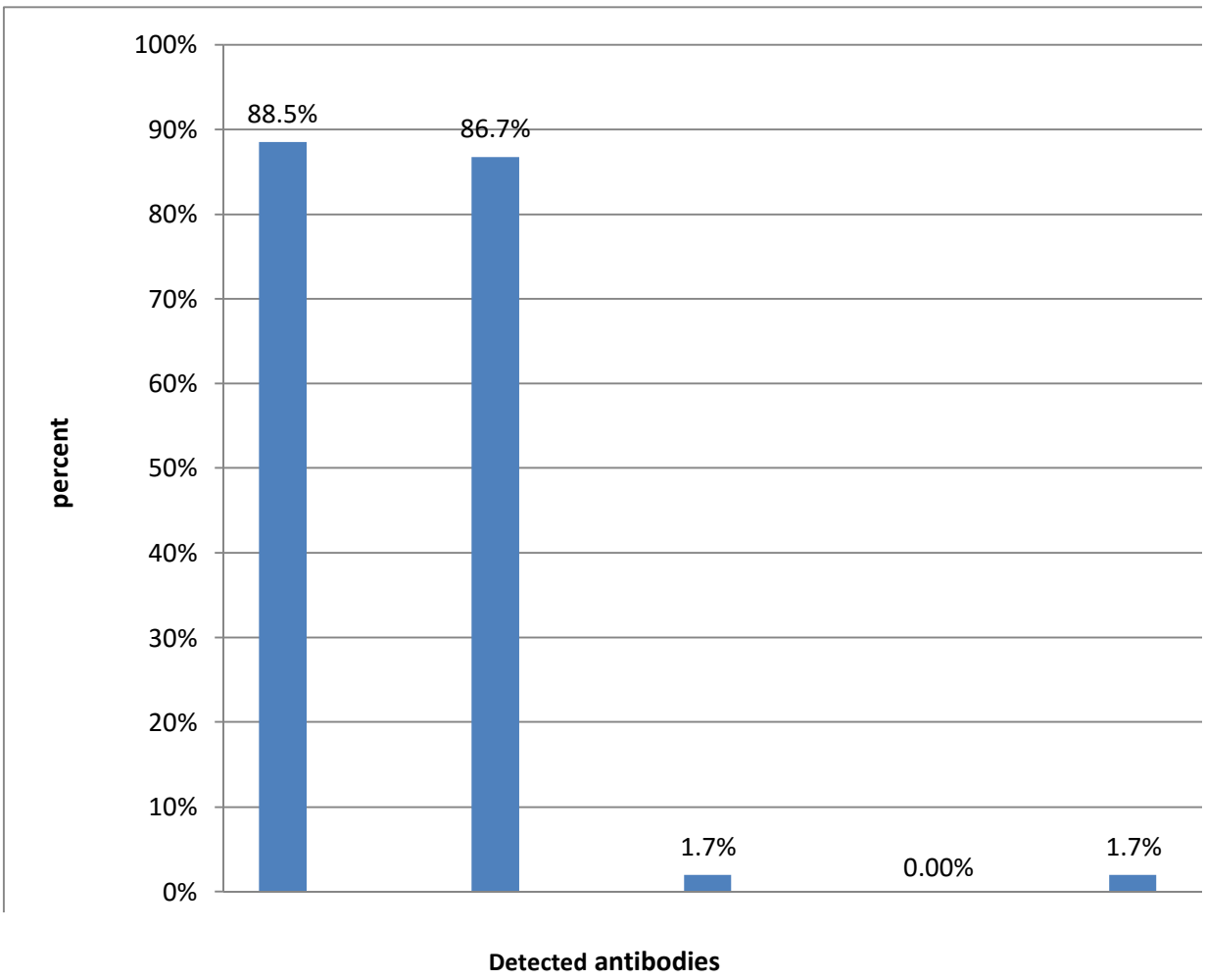

Figure 1: Sero burden of *T. gondii* infection among HIV/AIDS patients attending the Armed Forces Referral & Teaching Hospital; Addis Ababa, Ethiopia, March to May, 2016.

5.4. Association of contributing factors with *T.gondii* infection

Of all the variables included in the study, only presence of cat at home were depicted association with sero-burden of anti-*T.gondii* IgG antibody (p=0.038) as presented on (Table 3).and there was no significant difference in Toxoplasma sero-positivty to IgG in relation to HIV/AIDS related clinical data of the study participant. As shown on (Table 4).

Table 3: Association of socio-demographic and contributing factors for *T.gondii* infection among HIV/AIDS patients attending the Armed Forces Referral & Teaching Hospital; Addis Ababa, Ethiopia, March to May, 2016.

Contributing factors	Category	*T. gondii* IgG		X^2-test value	P-Value
		Positive	Negative		
Sex	Female	68	7		
	Male	86	13	0.605	0.437
Residence	Urban	144	19		
	Rural	10	1	0.071	0.790
Educational status	Reade and write	11	0		
	Elementary (1-8)	42	5	2.057	0.725
	Secondary (9-12)	54	10		
	>12	47	4		
Occupation	Government employee	54	10		
	House wife	32	3		
	Private employee	55	7	4.475	0.215
	Others	13	0		
Cat ownership	Yes	118	11		
	No	36	9	4.317	**0.038**
Eat uncooked meat	Yes	54	5		
	No	100	15	0.800	0.371
Eat raw vegetable& fruit	Yes	126	18		
	No	28	2	0.935	0.334
Had history of blood transfusion	Yes	34	4		
	No	120	16	0.046	0.831

Table 4: Association of Toxoplasma seropositivity in relation to HIV related clinical variables among HIV/AIDS patients attending the Armed Forces Referral & Teaching Hospital; Addis Ababa, Ethiopia, March to May, 2016.

Clinical Variable	Category	*T. gondii* IgG		X^2-test value	P-Value
		positive	Negative		
HAART status	with HAART	148	19		
	without HAART	6	1	0.053	0.819
CD4 cell count	<200	23	4		
	200-500	80	6	3.457	0.178
	>500	51	10		
WHO stage of HIV/AIDS	Stage I	145	19		
	Stage II - IV	9	1	1.522	0.677

6. Discussion

Among all blood samples, collected from 174 HIV/AIDS patients and tested, 154 (88.5%) were seropositive for anti-*T. gondii* IgG antibody and 3 (1.7%) seropositive for anti- *T. gondii* IgM antibodies. The finding was almost concurrent with a cross sectional study in Bahir Dar, Northwest Ethiopia, which reported 87.4% (90/103) of the HIV sero-positive individuals [43], and in ArbaMinch Hospital, south Ethiopia, in 2013 depicted 88.2% (150/170) were IgG anti-*T. gondii* antibody sero-positive out of the total that HIV sero-positive study Participants [42].

This study sero-positivity for latent anti-*T.gondii* IgG antibody prevalence is lower than the study conducted at St. Paul Hospital, Addis Ababa Ethiopia in 2009which reported latent Toxoplasma infection prevalence of 93.3% (154/165) among HIV positive patients [41] and Black Lion Hospital, Addis Ababa Ethiopia were 94% (141/150) of the HIV/AIDS patients were sero-positive for anti-*Toxoplasma gondii* IgG antibodies [44]. The possible reason for this difference could be difference in the study perioid and the use of HAART which is more practiced today than six years back.

However, our finding was higher than anti-*Toxoplasma gondii* IgG antibodies depicted by studies conducted in Nazareth town, Ethiopia in 2008 illustrated that 60 % (39/65) were positive [30].In Nigeria in 2010 among HIV-infected patients attending hospitals in Makurdi metropolis demonstrated 10.8% (39/360) positive out of the enrolled patients [38], Cameroon in 2010 revealed 52.6% (70/133) were positive [39], at the Port Moresby General Hospital, Papua New Guinea, exhibited 60% (108/181) among HIV-infected participants [37], India from 2011 to 2013 a cross sectional observational study confirmed 21.3% (141/661)[25], Iran a cross sectional survey between 2007 and 2008 exemplified 77.4% (48/62) HIV/AIDS serum samples were found positive [36].In Malaysia in 2002 reported 41.2% (124/301) positive of HIV/AIDS patients [35], Yugoslavia detected in 44.1% (127/288) of AIDS patients [34], and Mexico in 1997 explained that the prevalence were 50.0% (46/92) among HIV/AIDS infected participants [33].

The variation could be due to mainly on time of the study conducted in addition to that of the difference on method of diagnosis used at Nazareth town which was modified direct

agglutination test, geographical location and source population, methodology of study employed like the study done in India was observational type and sample size divergence.

The IgM antibody response to *Toxoplasma* infection is short-lived and it is frequently suppressed to undetectable levels in the setting of severe immunosuppression [20, 45]. In agreement with this, our study revealed lower rates of IgM seropositivity compared to IgG seropositivity which is, 3/174 (1.7%), 154/174(88.5%) respectively. Similar findings of lower rates of IgM seropositivity compared to IgG seropositivity in HIV-positive patients have also been reported by other studies from India [46], Mexico [47] and South Africa [48]. This low rate of detection of IgM antibodies in HIV-positive patients lends support to the view that the screening for this antibody in the routine diagnosis of toxoplasmosis in non-pregnant HIV-infected patients may be of limited value [20, 45].

Our present study found only presence of cat at home depicted association with sero-prevalence of anti-*Toxoplasma gondii* IgG antibody (p=0.038) of all other variables listed. This was supported by studies conducted in Nazareth town, Ethiopia, in 2008 illustrated that owning of cat were found to have significant association with sero-prevalence [30], at the Port Moresby General Hospital, Papua New Guinea, from 2003 to 2005 exhibited the exposure to cats was an independent risk factor but other socio-demographic and disease variables studied such as meat diet, educational levels and length of HIV infection did not demonstrate any association [37]; Study from Malaysia and Addis Ababa proved that CD4+ T lymphocyte cells count were not statistically associated with Toxoplasmosis [35, 38, 44].

This finding was inconsistent to that of a study in BahirDar, Nazareth town and Addis Ababa, Ethiopia reported consuming raw or undercooked mutton and vegetables were independent predictors of *T. gondii* seropositivity [30, 42, 44]; and age, gender and HIV serostatus were found to be significantly associated with sero-prevalence of latent toxoplasmosis [41]. This discrepancy could be difference in time of the study and sample size issue.

In resource-poor settings, any association between *Toxoplasma* seropositivity and HIV-related clinical variables such as CD4 cell count, ART status and HIV clinical stage may be helpful in classifying patients who may benefit from *Toxoplasma* screening or from prophylaxis against toxoplasmosis. In our study, *Toxoplasma* seropositivity was not related to all these clinical variables, suggesting that in our region screening for toxoplasmosis may not be strong predictors

for Toxoplasmosis. In agreement with our results, studies from Addis Ababa,[44] Mexico [47] and Malaysia [49] have shown no correlation between CD4 cell count and *Toxoplasma* seropositivity while another in Nairobi, Kenya [50], reported no correlation between HIV clinical staging and *Toxoplasma* seropositivity. In contrast, Belanger *et al*., [13] in France revealed that HIV patients with CD4 cell counts less than 200cells/µl were more likely to be *Toxoplasma* seropositive than those with counts greater than 200cells/µl. Probably the sample size among HIV patients with CD4 cell count below 200 could be low compared to other studies and we could not find significant association.

7. Strengths and limitations of the study

7.1. Strength of the study

- The study was carried out by senior professionals with many years of experience in ART laboratory.
- Using ELISA test kit for the analysis of *T.gondii* IgG & IgM antibodies rather than rapid test kit.

7.2. Limitation of the study

- The study recruited a small sample size limited to Armed forces Referral & Teaching hospital due to lack of enough funds to conduct the study .

8. Conclusion and Recommendation

8.1. Conclusion

The current study shows a high burden of latent *Toxoplasma gondii* infection among the study participants at Armed Forces Referral & Teaching Hospital in Addis Ababa, Ethiopia, and illustrates the current risk of developing toxoplasmic encephalitis. Thus, it may be appropriate and beneficial to include *T. gondii* screening as part of routine testing for all HIV/AIDS infected individuals. Moreover, this study shows presence of cats at home were identified as possible associated risk factors of *T.gondii* infection among HIV infected patients.

8.2. Recommendation

- Considering the relative high sero-burden of Toxoplasmosis as revealed by this study it would be important to increase public awareness about different routes of transmission of *T. gondii.*
- HIV positive individuals should limit themselves contact with cat. Cat owners particularly, HIV infected patient could take necessary preventive measures proper disposal of cat feces and keep hygiene to avoid Toxoplasma infection.
- HIV/AIDS patients should be screened for anti-*Toxoplasma* antibodies in order to minimize complication related to reactivation and or new infection.
- Follow up studies are needed to elucidate the real effect of Toxoplasmosis in HIV/AIDS patients.

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Annexes

Annex A: participant information sheet (English version)

Addis Ababa University, College of Health Sciences, School of Allied Health Science Department of Medical Laboratory Sciences, Addis Ababa, Ethiopia.

Title of the research project: Sero burden *T.gondii* and associated risk factors among HIV/AIDS patients infected in AFRTH, Addis Ababa Ethiopia

First of all we want to thank you for giving your time to undergo this conversation. Having saying this we will give you enough information about the study that we are going to do and please listen with full attention. Finally if there is any question/ ambiguity you can ask and get information.

Study

The study is on the protozoan parasite called *Toxoplasma gondii* that can infect all warm-blooded animals, including humans. however, when Toxoplasma infection is acquired by an immunodeficient patients (like peoples leave with HIV/ADIS) the parasite present significant risk of developing toxoplasmic encephalitis (TE) is the most predominant manifestation of the disease in these patients and can lead to various symptoms, ranging from headache, lethargy, in coordination, or ataxia to hemiparesis, loss of memory, dementia, or focal to major motor seizure, usually associated with fever.

Aim of study

The aim of this study is to determine the Sero burden of toxoplasmosis among peoples leaving with HIV/AIDS at Armed force Referrals and teaching Hospital and also to assess the possible risk factors associated with *T. gondii* infection.

Use of study

Studying the prevalence of toxoplasmosis among peoples leaving with HIV/AIDS in our country, Ethiopia is important to show the disease burden, to assess the possible risk factors associated with the infection. The result from this study provides base line information for future works and also to provide information for health institutions and policy makers to support

decision making that will improve the functioning of health system for the proper management of HIV/AIDS patients.

Subject's role

If they are voluntary to participate in this study, study participants required to give blood that is used only to check whether they are infected with the parasite or not. And also they will give other socio demographic information that is related with the study.

Subject's right

Study participants have the right to know their result, and if they are not interested they can out from the study at any time.

Subject's benefit

Participating in this study doesn't give any other unique benefit for study subjects. But those subjects who have positive result will be communicated to your physician and prescription of treatment and advice will be effected.

Harm

Study participants do not get any harm by participating in this study. There may feel some discomfort during blood collection but this does not produce series pain.

Confidentiality

All the data obtained from the participants including blood results will be kept strictly confidential by using only code numbers and locking the data. No one will have access to the non-coded data except the principal investigator.

Result announcement

After the study is completed study participants can get their result from the principal investigator or the place where they attend their ART follow up at any time. For those participants which have positive result they will be called by their address and know their result and also be treated.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Fewzia Mohammed (PI)

Signature: _____ Date: _____

Note: If you have any questions about this study, you should feel free to ask now or anytime throughout the study by contacting:

PI Fewzia Mohammed: Department of Medical Laboratory Sciences, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: fozia .hussien@ gmail.com Tell: 0910439776

Annex B: participant information sheet (Amharic version)

ለ ተ ሳ ታ ፊ ወ ች የ ሚ ሰ ጥ ሞ ረ ጃ

አ ዲ ስ አ በ ባ ዩ ኒ ቨ ር ስ ቲ የ ጤ ና ሳ ይ ን ስ ኮ ሌ ጅ የ ህ ክ ም ና ላ ቦ ራ ቶ ሪ ሳ ይ ን ስ ዲ ፓ ር ት መ ን ት ፣

<u>የ ጥ ና ቱ አ ር ዕ ስ ት</u>:-በ ሃ ገ ራ ች ን ኢ ት ዮ ጵ ያ በ ጦ ር ሐ ይ ሎ ች ሪ ፈ ራ ል እ ና ማ ስ ተ ማ ሪ ያ ሆ ስ ፒ ታ ል ክ ት ት ል ከ ሚ ያ ደ ር ጉ ከ ኤ ች አ ይ ቪ ጋ ር የ ሚ ኖ ሩ ሰ ወ ቺ ን ም ን ያ ክ ሉ በ ቶ ከ ሶ ፕ ላ ዝ ሞ ሲ ስ ጥ ገ ኑ ህ ዋ ስ ተ ጠ ቅ ተ ዋ ል የ ሚ ለ ው ን ለ ማ ወ ቅ እ ና በ በ ሺ ታ ው ሊ ያ ጋ ል ጫ ቸ ው ይ ች ላ ሉ ተ ብ ለ ው ከ ሚ ታ ሰ ቡ ት ነ ገ ሮ ች ም ን ያ ክ ሉ ተ ጋ ሊ ጭ ፍ ቸ ው የ ሚ ለ ው ን ለ ይ ቶ ለ ማ ወ ቅ ::

በ ቅ ድ ሚ ያ ጊ ዜ ዎ ን መ ስ ዋ ት አ ድ ር ገ ው ይ ህ ን ን ን ግ ግ ር እ ን ድ ና ደ ር ግ ስ ለ ፈ ቀ ዱ ል ን በ ጣ ም እ ና መ ሰ ገ ና ለ ን ከ ዚ ህ በ መ ቀ ጠ ል ስ ለ ም ና ካ ሂ ደ ው ጥ ና ት በ ቂ መ ረ ጃ ስ ለ ም ን ሰ ጠ ዎ ት ል ብ ብ ለ ው ያ ድ ም ጡ ፤ ያ ል ገ ባ ዎ ት ነ ገ ር ካ ለ ጥ ያ ቄ መ ጠ የ ቅ እ ና በ ቂ ማ ብ ራ ሪ ያ ማ ግ ኘ ት ይ ች ላ ሉ ::የ ሚ ካ ሄ ደ ው ጥ ና ት ማ ን ኛ ው ን ም ሰ ው ሊ ያ ጠ ቃ የ ሚ ቻ ል እ ና ከ ኤ ች አ ይ ቪ ጋ ር የ ሚ ኖ ሩ ሰ ወ ቺ ን የ በ ለ ጠ ጉ ዳ ት ሊ ያ ደ ር ስ የ ሚ ያ ሰ ች ለ ዉ ን ጥ ገ ኛ ህ ዋ ስ ሊ ይ ጥ ና ት በ ማ ካ ሔ ድ ላ ይ እ ን ገ ኛ ለ ን ::ከ ጉ ዲ ቶ ቹ ም መ ካ ከ ል ከ ፍ ተ ኛ የ አ እ ም ሮ ህ መ ም ፣ ች ግ ር እ ን ዱ ኖ ር በ ት ማ ድ ረ ግ ጥ ቂ ቶ ቹ ና ች ው

የ ጥ ና ቱ ዋ ና ዓ ላ ማ፡

በ ሃ ገ ራ ች ን ኢ ት ዮ ጵ ያ በ ጦ ር ሐ ይ ሎ ች ሪ ፈ ራ ል እ ና ማ ስ ተ ማ ሪ ያ ሆ ስ ፒ ታ ል ክ ት ት ል ከ ሚ ያ ደ ር ጉ ከ ኤ ች አ ይ ቪ ቫ ይ ረ ስ ጋ ር ከ ሚ ኖ ሩ ሰ ወ ቺ ም ን ያ ክ ሉ በ ቶ ከ ሶ ፕ ላ ዝ ሞ ሲ ስ ጥ ገ ኑ ህ ዋ ስ ተ ጠ ቅ ተ ዋ ል የ ሚ ለ ው ን ለ ማ ወ ቅ እ ና ለ በ ሺ ታ ው ሉ ያ ጋ ል ጫ ቸ ው ይ ች ላ ሉ ተ ብ ለ ው ከ ሚ ታ ሰ ቡ ት ነ ገ ሮ ች ም ን ያ ክ ሉ ተ ጋ ላ ጭ ፍ ቸ ው የ ሚ ለ ው ን ለ ይ ቶ ለ ማ ወ ቅ ነ ው ::

<u>የ ጥ ና ቱ ፋ ይ ዲ/ ጥ ቅ ም</u>

ይ ህ ጥ ና ት በ መ ጀ መ ሪ ያ በ ሃ ገ ራ ች ን ም ን ያ ክ ል ከ ኤ ች አ ይ ቪ ጋ ር የ ሚ ኖ ሩ ሰ ወ ች በ በ ሺ ታ ው ተ ጠ ቂ ና ች ው የ ሚ ለ ው ን በ ቁ ጥ ር ያ ሳ ያ ል ::በ መ ቀ ጠ ል ም ለ በ ሺ ታ ው እ ን ዲ ጋ ለ ጡ ሊ ያ ደ ር ጉ ይ ች ላ ሉ ተ ብ ለ ው የ ሚ ታ ሰ ቡ ነ ገ ሮ ች ን በ ዝ ር ዝ ር በ ማ ስ ቀ መ ጥ ለ ወ ደ ፊ ት ከ ኤ ች አ ይ ቪ ጋ ር የ ሚ ኖ ሩ ሰ ወ ች በ በ ሺ ታ ው እ ን ዳ ይ ያ ዙ እ ን ዱ ሁ ም ሊ ያ ጋ ል ጫ ቸ ው ከ ሚ ቻ ለ ነ ገ ሮ ች እ ን ዱ ር ቁ ለ ማ ድ ረ ግ ነ ው ::በ ሌ ላ በ ኩ ል ም ለ ጤ ና ተ ቋ ማ ት እ ና ህ ግ አ ው ጪ አ ካ ሊ ት የ ጤ ና አ ሰ ራ ር እ ን ዲ ሻ ሻ ል መ ረ ጃ ለ መ ስ ጠ ት ነ ው ::

<u>የ ተ ሳ ታ ፊ ዎ ች ድ ር ሻ ፣</u>

ተ ሳ ታ ፊ ዎ ች በ ጥ ና ቱ ለ መ ሳ ተ ፍ ፈ ቃ ደ ኛ ከ ሆ ኑ ጥ ገ ኛ ህ ዋ ሱ አ ለ /የ ለ ም የ ሚ ለ ው ን ለ ማ ወ ቅ እ ን ዱ ቻ ል ለ ዚ ሁ ስ ራ ብ ቻ የ ሚ ያ ገ ለ ግ ል ደ ም መ ስ ጠ ት ፣ የ ሚ ጠ የ ቁ ት ን ና ከ ጥ ና ቱ ጋ ር የ ሚ ያ ዝ መ ረ ጃ መ ስ ጠ ት ና ች ው ::

<u>የ ተ ሳ ታ ፊ ዎ ች ጥ ቅ ት</u>

፣ ተ ሳ ታ ፊ ዎ ች ው ጤ ታ ች ው ን የ ማ ወ ቅ ፣ በ ፈ ለ ጉ ጊ ዜ ና በ ማ ን ኛ ው ም ሰ ዓ ት ከ ጥ ና ቱ የ መ ው ጣ ት ጥ ቅ ት አ ል ላ ች ው ::

የ ሚያ ገ ኙ ት ጥቅ ም

ተ ሳ ታ ፊ ዎ ች እ ዚ ህ ጥ ና ት ውስ ጥ በ ሞ ካ ተ ታ ቸ ው የ ሚያ ገ ኙ ት የ ተ ለ የ ጥ ቅ ም የ ለ ም::ነ ገ ር ግ ን ጥ ገ ኛ ህ ዋ ሱ በ ደ ማ ቸ ው ውስ ጥ ከ ተ ገ ኝ ም ን ም ወ ጪ ም ድ ሃ ኒ ት በ ሞ ስ ጠ ት ይ ታ ከ ማ ለ ::

የ ሚ ደ ር ስ ባ ቸ ው ዲ ት

ተ ሳ ታ ፊ ወ ች እ ዚ ህ ጥ ና ት ውስ ጥ በ ሞ ካ ተ ታ ቸ ው የ ሚ ደ ር ስ ባ ቸ ው ዲ ት የ ለ ም ነ ገ ር ግ ን ደ ም በ ሚ ሰ ጡ በ ት ጊ ዜ አ ነ ስ ተ ኛ የ ህ ሙ ም ስ ሜ ት ሊ ሰ ማ ቸ ው ይ ች ለ ል ይ ህ ማ ት ግ ን ለ ጊ ዜ ው የ ሚ ሰ ማ ቸ ው እ ን ጂ ም ን ም ጉ ዲ ት የ ሚ ያ ስ ከ ት ል ባ ቸ ው አ ይ ደ ለ ም::

ዉጤት ና ሚረ ጃ አ ያ ያ ዝ

ከ ተ ሳ ታ ፊ ወ ች የ ሚ ገ ኝ ማ ን ኛ ው ም ሞ ረ ጃ የ ደ ም ው ጤ ት ን ጨ ም ሮ በ ሚ ስ ጥ ር የ ሚ ያ ዝ ይ ሆ ና ሌ::ይ ህ ም ተ ሳ ታ ፊ ወ ች የ ሚ ለ ዩ ት በ ስ ማ ቸ ው ሳ ይ ሆ ን በ ሞ ለ ያ ቁ ጥ ር በ መ ሆ ኑ ጥ ና ቱ ን ከ ሚ ያ ካ ሂ ደ ው ሰ ው ውጭ ሌ ለ ሰ ው የ ማ ወ ቅ እ ድ ል አ ይ ኖ ረ ው ም::

ው ጤ ታ ቸ ው ን የ ማ ሳ ወ ቅ ሂ ደ ት ጥ ና ቱ እ ን ደ ተ ጠ ና ቀ ቀ ተ ሳ ታ ፊ ወ ች የ ም ር ሞ ራ ው ን ው ጤ ት ጥ ና ቱ ን ከ ሚ ያ ካ ሂ ደ ው ሰ ው ወ ይ ም ክ ት ት ል ከ ሚ ያ ደ ር ጉ በ ት ክ ፍ ል በ ማ ን ኛ ው ም ጊ ዜ ማ ግ ኘ ት ይ ች ሊ ሉ ከ ው ጤ ቶ ቹ ም ሞ ካ ከ ል ጥ ገ ኛ ህ ዋ ሱ በ ደ ማ ቸ ው ውስ ጥ የ ሚ ገ ኙ ሰ ወ ች በ አ ድ ራ ሻ ቸ ው ተ ው ጤ ታ ቸ ው ን እ ን ዱ ያ ው ቁ እ ና እ ን ዱ ታ ከ ሞ ይ ደ ረ ጋ ል ተ ሳ ታ ፊ ወ ች ጥ ና ቱ ን በ ተ ሞ ለ ከ ተ ተ ጨ ማ ሪ ሞ ረ ጃ ማ ግ ኘ ት ከ ፈ ለ ጉ ጥ ና ቱ ን የ ም ታ ካ ሂ ደ ው ን ፈ ዉ ዚ ያ ሞ ህ ሞ ድ ብ ለ ው በ 0910439776በ ማ ን ኛ ው ም ጊ ዜ ማ ና ገ ር እ ና የ ፈ ለ ጉ ት ን ሞ ረ ጃ ማ ግ ኘ ት ይ ች ላ ሉ ::

Annex C: Consent form (English version)

I am planning to conduct a research on the parasite *Toxoplasma gondii* which cause severe complication in peoples leaving with HIV/AIDS. The objective of the study is to determine the burden of toxoplasmosis among HIV/AIDS patient and to identify possible risk factors. If you agree to participate in this study, 5 ml blood sample will be collected from you. During collection of blood you may feel some discomfort, but this does not produce series pain. All the data obtained will be kept strictly confidential by using only code numbers and locking the data. No one will have access to the non-coded data except the principal investigator. Your participation is purely voluntary, and you have the right to know your result and if it is positive you will be treated by communicating with your physician. Finally if you are voluntary to participate please put your sign and let us start our work. Participant's response:
"I am clear and agree to participate''

Participant signature ----------

Code number ----------

Date ----------

Annex D: consent form (Amharic version)

ፈቃደኛነትን ማጠየቂያ ቅጽ

እኛ ማንኛውንምሰውሊያጠቃየሚችልእናበተለይም ከኤች አይቢ ቫይረስ ጋር የሚኖሩ ሰወቺን ክፍተኝ ጉዳት የሚያደርሰዉን ጥገኛህዋስሊይጥናትበማካሔድሊይእንገኛለን:: የጥናቱዋናዓላማምበሃገራችንምንያክል ከከኤችአይቢ ቫይረስጋር ከሚኖሩ ሰወቺ ምን ያክሉ በቶከሶፕላዝሞሲስ ጥገኙህዋስ ተጠቅተዋል የሚለውንለማወቅእና ለበሺታውሊያጋልጧቸውይችላሉተብለውከሚታሰቡትነገሮችምንያክሉ

ተጋሊጭፍቸውየሚለውንለይቶለማወቅነው::

ለመሳተፍፈቃደኛከሆኑ 5mlደምከእርሰዎይወሰዲሌ::

ይህምየሚያገለግለውጥገኛህዋሱበሰውነትዎውሰጥአለወይምየለምየሚዛመዉንበደምምር መራለማረጋገጥነው::ደምየሚወሰደውከክንደዎትሊይሲሆንበዚህጊዜምትንሽየህመምስሜትሉሰማዎትይችላል:: ይህማለትግንለጊዜውየሚሰማዎትእንጅበእርሰዎሊይችግርየሚፈጥርአይደለም::የደምና መኖ ውከነገርነዎትዓሊማውጭላሌላተግባርአይውልም::ከእርሰዎየምናገኛቸውማንኛውምመረጃወችበሚስጥር የሚያዙሲሆንናመኖ ውበሚወሰድበትጊዜምሆነ ምርመራውበሚደረግበትጊዜስምዎትንአንጠቀምምማለ ትምበመለያቁጥርነውየምንጠቀመው::ተሳትፎዎትምበመለፈቃደኝነትላይየተመሰረተነው::ውጤትዎ ትንየማወቅእናበፈለጉጊዜምከጥናቱየመውጣትመብትዎምየተጠበቀነው::የደምዎውጤትየሚያሳየው ጥገኛህዋሱበሰውነትዎውስጥአለየሚልከሆነ ፣ የሚደርሰውንጉዲትለመቀነስከሃኪምዎትጋርበመነጋገርይታከማለ::እስከአሁንየተነጋገርነውበመለግልጽከሆነ ልዎትእናበጥናቱሊይ ለመሳተፍፈቃደኛከሆኑፊርማዎንእዚህጋርበማስቀመጥፈቃደኝነትዎንያረጋግጡሌን::

የተሳታፊውፈቃድመግጫ ግልጽስለሆነ ልኝለመሳተፍተስማቻለሁ!

ፊርማ.....

መለያቁጥር .....

ቀን .....

Annex E: Study data collection sheet (English version)

Instruction: In this section please encircle the number in front of the choices that exactly fits your status out of the list.

Interviewer ---------- date of interview----------

Identification

a. Code number----------

b. Address ----------

I Socio demographic characteristics &Associated risk factors

Quest No	Questions	Response	Code	Remark
01	Age	18-20 _____ 21-30_____ 31-40 _____ 41-55_____ >55_____	1 2 3 4 5	
02	Sex	Male_____ Female_____	1 2	
03	place of residence	Urban_____ Rural_____	1 2	
04	Occupation	Government House wife ---- Private ------ Other (specify)	1 2 3 4	
05	Educational status	Read and write only 1-8 grade 8-12 grade > 12+	1 2 3	
06	Do you have Cat in your house?	Yes_____ No_____	1 2	
07	Did you eat	YES_____	1	

	un cooked meat?	NO_____	2	
08	Did you eat raw or uncooked vegetable and fruit?	Yes_____ No_____	1 2	
09	Organ transplantation	Yes_____ No_____	1 2	
10	Have you Previously history of blood transfusion??	Yes_____ No_____	1 2	
11	CD4 count cells/µL	<200_____ 200-500 >500_____	1 2 3	
	HAART status	With HAART__ without HAART	1 2	
	Baseline WHO HIV/AIDS stage	Stage I_____ Stage II-IV_____	1 2	

Annex F: Study data collection sheet (Amharic version)

ለተሳታፊወችየተዘጋጀመጠይቅመጠይቁየሚሞሊውጥናቱንበሚያካሂደ ውሰውወይምመረጃበሚሰበስ በውሰውሲሆንየሚሞሊውምለተሳታፊዎቹቃለመጠይቅበማድረግይሆናል::

ጠያቂ---------- የጠየቀበትቀን----------
I. መለ ያ

ሀ. መለያቁጥር---------- ለ. አድራሻ----------

<u>ማህበራዊነክጥያቄዎችን</u>

ቁጥር	**ጥያቄ**	**መላሽ**	**ኮድ**	
01	**እድሜ**	18-20 21-30 31-40 41-50 >50	1 2 3 4 5	
02	**ጸታ**	**ወንድ** **ሴት**	1 2	
03	**የመኖሪያቦታ**	**ከተማ** **ገጠር**	1 2	
04	**የሥራአይነት**	**የመንግስትሰራተኛ** **የቤት እመቤት** **የግሉ** **ላሊ ካሀ ይጥቀ**	1 2 3 4 5	

05	የትምህርትሁኔታ	ማንበብ እና መጻፍ ብቻ ከ 1_8 ክፍሌ ከ8_12 ክፍሌ ከ12 ኛ ከፍሌ በሊይ	1 2 3 4	
06	በቤትዎድመትአለዎት?	አለኝ የለኝም	1 2	
08	ማንኛውንምያልበሰለስጋይመገባሉ?	አዎ አይደለም	1 2	
09	ያልበሰለአትክልትናፍራፍሬይመገባሉ?	አዎ አይደለም	1 2	
10	ከዚህበፊትደምተለግሰውያውቃሉ?	አዎ አይደለም	1 2	
11	የፀረ ኤች አይቪቫይረስ መድኃኒት ጀ ምረ ዎል	አዎ አይደለም	1 2	
12	የ የሲዲ ፎር ህዋሲያን መጠን	›200 ከ 200-500 ከ ‹ 500	1 2 3	
13	የደብሊውኤች(WHO) ኤች አይቪቫይረስ ክሊኒካል ደረጃ	ከ ደረጃ 1 ከ ደረጃ 2-4	1 2	

አ ስ ተ ያ የ ት----------የ ጥና ቱ ተ ቆ ጣጣሪ ፊ ር ማ ----------ቀ ን ----------

Annex G: Laboratory Techniques

G.1. Procedure for venous blood collection and processing

PURPOSE: Quality study participant care and accurate specimen results are dependent upon proper vein puncture technique, timely specimen collection, and proper processing of specimens. Common collection errors encompass incorrect identification of the study subject, hemolyzed specimens, and the use of an incorrect anticoagulant. This procedure establishes criteria for the proper collection of blood specimens by vein puncture.

MATERIALS

A. Disposable, safety, single use Syringes with needle

B. Blood collection Tubes. The vacuum tubes are designed to draw a predetermined Volume of blood. Tubes with different additives are used for collecting blood specimens for specific types of tests.

C. Disposable gloves (non-latex)

D. Tourniquet (non-latex)

E. Antiseptic. Individually packaged 70% isopropyl alcohol wipes.

F. 2x2 Gauze or cotton balls.

G. Sterile gauze pads

H. Sharp disposal Container

SAFETY

a. Apply universal (standard) safety precautions.

b. Wash hands in warm, running water with commercial foaming hand wash product before and after each study subject collection.

c. Gloves are to be worn during all specimen collection, and changed between study subjects.

d. A lab coat or gown must be worn during blood collection procedures.

e. Needles and hubs are single use and are disposed of in an appropriate 'sharps' container as one unit. Needles are never recapped after procedure.

f. Gloves are to be discarded in the appropriate container immediately after the Procedure. All other items used for the procedure must be disposed of according to proper biohazard us waste disposal policy.

g. Contaminated surfaces must be cleaned with freshly prepared 10% bleach solution. All surfaces are cleaned daily with bleach.

h. In the case of an accidental needle stick, immediately wash the area with an antibacterial soap, express blood from the wound, and contact a physician.

Venous blood collection:

1. Apply a soft tubing tourniquet to the upper arm of the patient to enable the veins to be seen and felt.
2. 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
6. Vein puncture with the l14evel of the needle directed upwards in the line of the vein.
7. When sufficient blood has been collected, release the tourniquet and instruct the patient to
8. Open his or her fist.
9. Remove the needle and immediately press on the puncture site with a piece of dry
10. Cotton.
11. Remove the tourniquet completely. Instruct the patient to continue pressing on the Puncture site until the bleeding has stopped.
12. Discard the needle safely. Do not attempt to re-sheath it because this can result in needle- Stick injury.
13. Check that bleeding from the vein puncture site has stopped.

G.2. Principle of the Assay

RecombiLISA Toxo IgM test is a solid phase enzyme linked immunoabsorbent assay based on the principle of the IgM capture technique for the detection of IgM anti-*T.gondii* in human serum or plasma. The RecombiLISA toxo IgM test is composed of two key components Solid micro wells pre-coated with monoclonal anti human IgM antibody **&** Liquid conjugates composed of recombinant *T.gondii* antigen conjugated with horse reddish peroxidase (HRP- *T.gondii* conjugates).During the assay, the test specimen is first incubated with the coated microwells. IgM anti-Toxo, if present in the specimen, binds to the antibody coated on the microwells surface. In the second incubation with the HRP-*T.gondii* conjugates, the IgM anti-*T.gondii* antibody absorbed on the surface of microwell react to the HRP- *T.gondii* conjugates, forming a complexed conjugates. Unbounded conjugates are then removed by washing .The presence of the complexed conjugates is shown by a blue color upon additional incubation with TMB substrate. The reaction is stopped with stop solution and absorbance are read using a spectrophotometer at 450/620-690 nm.

STORAGE AND STABILITY

- All reagents except the concentrated wash buffer are ready to use as supplied
- Return all reagents requiring refrigeration immediately after use
- Reseal the microwells after removing the desired number of wells
- Ensure that the reagents are brought to room temperature before opening
- All reagents are stable through the expiration date printed on the label if not opened Do not freeze the kit or expose the kit over 8ºc.

SPECIMEN COLLECTION AND PREPARATION

- RecombiLISA Toxo anti IgM test kit can be performed using serum or plasma should be prepared from whole Blood specimen obtained by acceptable vein puncture technique.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect vein puncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipoid or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing.
- Specimens should not be frozen and thawed repeatedly.

REAGENTS PREPARATION

- ✓ Bring all reagents, control to room temperature 18-28°c.
- ✓ Dilute concentrated Washing buffer 30 fold with water as following

Plate	DI water	30x wash buffer	Final volume
Full plate	580ml	20m	600ml
Half Plate	290ml	10ml	300ml
A quarter plate	145m	5ml	150ml

- ✓ Warm up the concentrated wash buffer at 37°c to dissolve the precipitate if it appears.

- ✓ Mix each reagent before adding to the test wells.
- ✓ Determine the number of microwells needed and mark on the ELISA working sheet with the appropriate information .positive and Negatives controls to be run in duplicates to ensure accuracy.

ASSAY PROCEDURE FOR IgM

1. Remove the desired number of strips and secure them in the micro well frame .Reseal un-used strips.

2. Add specimens according to the designation on the ELISA working sheet.

2.1. Blank well: Leave the blank well alone. Don't add any reagents

2.2. Control wells: Add 50 µL of Toxo IgM positive, negative control in to the designated control well respective

2.3. Test Wells: add 50 µL of test specimens in to each test well respectively.

To ensure better precision, use pipette to handle solution

3. Gently rock the wells for 20 second, and then cover the wells.

4. Incubate the wells at 37 ºC for 30 minutes.

5. Carefully remove the incubations mixture by empting the solution in to waste containers. Fill each well with diluted wash buffer and shake gently for 20-30 second .Discard the wash solution completely and tapping the plate on absorbent paper .Repeat above procedure 4 more times

6. Add 50 µl of HRP- *T.gondii* antigen conjugates in to each well except the blank well, cover the plate, and incubate at 37°c for 30 minutes.

7. Wash the pipette five times us step five described.

8. .Add 50 µl (or 1 drop) of TMB substrate A and 50 µl (or 1 drop) of TMB substrate B in to each well including the blank well.

9. Incubate at 37 ºC in dark for 10 minutes.

10. Stop the reaction by adding 50 µL (or 1 drop) of stop buffer to each well gently mix for 30 second. It is important to make sure that all the blue colure changes to Yellow color completely.

11. Set the Micro plate reader wave length at 450 nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding stop solution .a filter of 620-690 nm can be used as a reference wave length to optimize the assay result.

Interpretation of Result

A. set up the cut –off value

The cutoff value =0.15+N N: Mean OD of the negative control.

B. **Calculation of specimen OD ratio**

Calculation an OD ratio for each specimen by dividing the OD value by the Cut off value as follows: specimen OD ratio=specimen OD/cut-off value

C. Assay validation

The Mean OD value of the toxo IgM positive controls should be ≥ 0.80

The Mean OD value of the toxo IgM Negative controls should be ≤ 0.10

*Note: If the above conditions are not met check the procedure and repeat the assay

Interpretation of the results

Result	Specimen OD ratio
Negative	< 1.00
Positive	≥1.00

1. The Negative result indicates that there is no detectable IgM anti *T. gondii* in the specimen

2. Results just below the cut- off value (lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimen when it is applicable).

3. Specimen with cut of value ≥ 1.00 is initially considered to be positive by the RecombiLISA Toxo IgM Test .they should be retested in duplicate before final interpretation.

Note:

- if after re-testing of a specimen the absorbance value of the 2 duplicate are less than the cut- off value .the initial result is non repeatable and the specimen is considered to be negative with the RecombiLISA Toxo IgM Test
- if after re-testing of a specimen the absorbance value of one of the duplicates is equal or greater than the cut –off value ,the initial result is repeatable and the specimen is considered to be positive with the RecombiLISA Toxo IgM Test

Principle of the Assay RecombiLISA Toxo IgG test

RecombiLISA Toxo IgG test is a solid phase enzyme linked immunoabsorbent assay based on the principle of the indirect EIA technique for the detection of IgG to *T.gondii* in human serum or plasma. The RecombiLISA toxo IgG test is composed of two key components Solid micro wells pre-coated with recombinant Toxo antigens; &secondly Liquid conjugates composed of mouse anti-human IgG conjugated with horse reddish peroxidase (HRP- anti-human IgG conjugated).During the assay, the test specimen is first incubated with the coated microwells. The IgG anti-Toxo, if present in the specimen, binds to the antigen coated on the microwells surface. In the second incubation with the HRP- anti-human IgG conjugates, the IgG antibodies absorbed on the surface of microwells react to the HRP- anti-human IgG conjugates, forming a complexed conjugates. Unbounded conjugates are then removed by washing .The presence of the complexed conjugates is shown by a blue color upon additional incubation with TMB substrate. The reaction is stopped with stop solution and absorbance are read using a spectrophotometer at 450/620-690 nm.

STORAGE AND STABILITY

- All reagents except the concentrated wash buffer are ready to use as supplied
- Return all reagents requiring refrigeration immediately after use
- Reseal the microwells after removing the desired number of wells
- Ensure that the reagents are brought to room temperature before opening All reagents are stable through the expiration date printed on the label if not opened Do not freeze the kit or expose the kit over 8ºc.

SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma should be prepared from whole Blood specimen obtained by acceptable vein puncture technique.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect vein puncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipoid or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use.
- Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.

ASSAY PROCEDURE FOR IgG

1. Remove the desired number of strips and secure them in the micro well frame .Reseal un-used strips

2. Add specimens according to the designation on the ELISA working sheet.

2.1. Blank well: Leave the blank well alone. Don't add any reagents

2.2. Control wells: Add 50 µL of Toxo IgG positive, negative control in to the designated control well respectively.

2.3. Test Wells: add 50 µL of test specimens in to each test well respectively

To ensure better precision, use pipette to handle solution

3. Gently rock the wells for 20 sec, and then cover the wells

4. Incubate the wells at 37 ºC for 30 minutes.

5. Carefully remove the incubations mixture by empting the solution in to waste containers. Fill each well with diluted wash buffer and shake gently for 20-30 sec .Discard the wash solution completely and tapping the plate on absorbent paper .Repeat above procedure 4 more times
6. Add 100 µl (or 2 drops) of HRP- anti human IgG conjugates in to each well except the blank well, cover the plate.
7. Incubate at 37°c for 20miutes.
8. Wash the plate 5 times as step 5 described.
9. Add 50 µL (or 1 drop) of TMB substrate A and 50 µL (or 1 drop) of TMB substrate B in to each well including the blank well.
10. Incubate at 37 ºC in dark for 10 minutes.
11. Stop the reaction by adding 50 µL (or 1 drop) of stop buffer to each well gently mix for 30 second. It is important to make sure that all the blue colure changes to Yellow color completely
12. Set the Micro plate reader wave length at 450 nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding stop solution .a filter of 620-690 nm, can be used as a reference wavelength to optimize the assay result.

Interpretation of Result

A. set up the cut –off value

The cutoff value =0.15+N N: Mean OD of the negative control.

B Calculation of specimen OD ratio

Calculation an OD ratio for each specimen by dividing the OD value by the Cut off value as follows: specimen OD ratio = specimen OD / cut-off value

C .Assay validation

The Mean OD value of the Toxo IgG positive controls should be $\geq$ 1.0.

The Mean OD value of the Toxo IgG Negative controls should be $\leq$ 0.10.

*Note: If the above conditions are not met check the procedure and repeat the assay

Interpretation of the results

Result	Specimen OD ratio
Negative	< 1.00
Positive	≥1.00

- The Negative result indicates that there is no detectable IgG anti *T. gondii* in the specimen

- Results just below the cut- off value (lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimen when it is applicable).

- Specimen with cut of value ≥ 1.00 is initially considered to be positive by the RecombiLISA Toxo IgG Test .they should be retested in duplicate before final interpretation.

***Note:**

- If after re-testing of a specimen the absorbance value of the 2 duplicate are less than the cut- off value .the initial result is non repeatable and the specimen is considered to be negative with the RecombiLISA Toxo IgG Test
- If after re-testing of a specimen the absorbance value of one of the duplicates is equal or greater than the cut –off value, the initial result is repeatable and the specimen is considered to be positive with the RecombiLISA Toxo IgGTest.

Declaration

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

Name: FEWZIA MOHAMMED (BSc) Signature _____

Place: Addis Ababa University College of Health Sciences, School of Allied Health Science Department of Medical Laboratory Sciences, Addis Ababa, Ethiopia.

This thesis has been submitted with my approval as University advisor

Name_____

Signature_____

Date of submission_____