ANALYSIS OF SERUM FOR ANTIBODIES TO TOXOPLASMA GONDII IN INDIVIDUALS WITH SCHIZOPHRENIA AND BIPOLAR DISORDERS

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Analysis of Serum for Antibodies to *Toxoplasma gondii* in Individuals with Schizophrenia and Bipolar Disorders

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ABBREVIATIONS

AIDS ----------Acquired Immunodeficiency syndrome
AHRI----------Armauer Hansen Research Institute
CNS----------Central nervous system
CRP----------C Reactive Protein
ELISA----------Enzyme- linked immunosorbent assay
HIV----------Human immunodeficiency virus
HLA ----------Human Leukocyte Antigen
IDO----------Indoleamine 2, 3-dioxygenase
IFN ----------Interferon
Ig----------Immunoglobulin
IL----------Interleukin
I.U.----------International units
OR----------Odds ratio
OD----------Optical Density
SD----------Standard deviation
T. gondii ----------*Toxoplasma gondii*
TMB----------Tetra-methylbenzidine
TNF----------Tumor necrosis factor
WHO----------World Health Organization

ABSTRACT

Background: Schizophrenia and bipolar disorders are serious neuropsychiatric diseases of unknown etiology. Recent studies indicated that infectious agents such as *Toxoplasma gondii* may contribute to some cases of schizophrenia and other mental disorders. In this study we determined the prevalence of *T. gondii* infection and IgG titers against *Toxoplasma* in schizophrenia and bipolar disorder patients and control subjects which comprise first degree relatives of the patients and some individuals from neighborhoods.

Methods: 214 cases of schizophrenia, 171 patients of bipolar disorders and 363 controls recruited from Meskan and Mareko (Butajira) district for genetic study in 2001 and their stored sera were analyzed for IgG antibody against *T. gondii* by using enzyme-linked immunoassay (ELISA) for a period of March to May 2009.

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Results: Overall prevalence of IgG antibodies in all study subjects was 95.2%. The prevalence of the antibodies increased with age in cases and control subjects. The seroprevalence of *T. gondii* infection was 97.7% in patients with schizophrenia, 95.3% in bipolar disorder cases and 93.7% in control subjects. There was significantly increase in seroprevalence of IgG antibodies to *T. gondii* in schizophrenia cases compared with control groups (p=0.031) but, the difference in seroprevalence of IgG antibodies was not statistically significant between bipolar disorder cases and controls group (p=0.427). In addition, there was no significant difference in IgG titers between the cases and their close relative controls and/or non-relatives controls recruited from neighborhoods.

Conclusions and recommendations: patients with schizophrenia had a significantly higher prevalence of *T. gondii* infection than the control group. Thus, this study gives additional information to the hypothesis that exposure to *Toxoplasma* may be risk factor for schizophrenia. Further studies may be required to determine anti-*Toxoplasma* antibody level in first episode psychosis and in the same individuals in chronic course by follow up to speculate decrease in antibodies level caused by antipsychotic or mood stabilizer drugs, which might improve the clinical course in the patients.

Keywords: *Toxoplasma gondii*/ Schizophrenia/ Bipolar disorder

CHAPTER I: INTRODUCTION

1.1. Introduction

Psychotic disorders, which include schizophrenia, bipolar affective disorder are among the most devastating diseases affecting humankind (Metastatio and Bahn, 2008). The major mental illnesses have been uncertain etiology and made difficulty in diagnosis and treatment of the outcomes of psychosis (Robertson *et al*., 2006; Metastatio and Bahn, 2008).

An increased occurrence of schizophrenia and bipolar disorder in family members of affected persons suggests that genetic factors play a role in their etiology, and some candidate predisposing genes have been identified (Torrey and Yolken, 2003; Mortensen *et al*., 2003). The finding that the concordance rate of schizophrenia and bipolar disorder for monozygotic twins isn't 100% suggests that both genes and environmental factors may play a role in causation of the major mental illnesses (NIMH, 2002; Torrey *et al*., 2007).

Recently, critical interactions between genetic and infection-associated environmental factors are considered the risk factor for schizophrenia and other psychosis (Torrey and Yolken, 2003). Some epidemiological studies indicate that infectious agents may contribute to some cases of major mental illness (Torrey and Yolken, 2003). Maternal infection during pregnancy increases the risk of schizophrenia and other brain disorders of neurodevelopment origin in the offspring (Brown, 2006).

It has been proposed that factors common to the immune response to a wide variety of pathogens may be the critical link between prenatal infection and postnatal brain and behavioral pathology (Mayer *et al*., 2008). The ubiquitous protozoan parasite, *Toxoplasma gondii* (*T. gondii*) has emerged as a candidate infectious agent as a possible cause of some cases of schizophrenia and bipolar disorder as the parasite establish persistent infection within the central nervous system and can cause permanent behavioral change in experimental animals (Vyas *et al*., 2007; Webster, 2007). Recent

human studies have revealed that latent toxoplasmosis may cause personality changes and reduced psychomotor performance (Webster, 2007).

Some antipsychotic drugs used to treat psychosis have been shown to inhibit the growth of *T. gondii* in cell culture (Jones-Brando *et al*., 2003). In addition, some studies on animals have demonstrated the behavioral change induced by *T. gondii* can be partially reversed by treatment with some antipsychotic and mood-stabilizer medications (Webster *et al*., 2006). Such observation provided support for the hypothesis that the antipsychotic and mood-stabilizing activity of some medications may be achieved, or at least augmented, through their inhibition of *T. gondii* replication and/or invasion in infected individuals (Webster, 2007).

Recent studies indicate that individuals with schizophrenia have an increased prevalence of antibodies to *T. gondii* as compared with control groups (Alvarado-Esquivel *et al*., 2006; Cetinkaya *et al*., 2007; Torrey *et al*., 2007). The Meta analysis of the previous studies showed that the prevalence of antibodies to *T. gondii* in individuals with schizophrenia is significantly higher than the prevalence of antibodies in control population and suggests that *Toxoplasma* is in some way associated with a large number of cases of schizophrenia (Torrey *et al.*, 2007).

Some studies have indicated individuals with first-episode schizophrenia had significantly increased levels of antibodies to *Toxoplasma* as compared with the control subjects (Yolken *et al*., 2001; Wang *et al*., 2006). Those studies show the possible link between toxoplasmosis and schizophrenia.

Thus, to provide a more complete picture of schizophrenia and bipolar disorders causation in addition to identification of vulnerable genes, it is important to determine the role of infectious agents such as *T. gondii* that can cause prenatal infection to design more effective drugs and preventive efforts to reduce incidence and diseases associated with schizophrenia and bipolar disorders (Brown, 2006). Therefore this study was undertaken to assess the association between *Toxoplasma gondii* infection and the major mental illnesses (schizophrenia and bipolar disorder) by analyzing antibodies against this protozoan parasite from stored sera collected from patients with schizophrenia, bipolar disorders and control subjects recruited from the same geographic area.

1.2. Literature Review

1.2.1. Structure and life cycle of *Toxoplasma gondii*

Toxoplasma belongs to the phylum Apicomplexa, which consists of intracellular parasites that have a characteristically polarized cell structure and a complex cytoskeletal and organellar arrangement at their apical end (Figure 1.1). *Toxoplasma gondii* exits in three forms: tachyzoite, tissue cyst (containing bradyizoites), and oocyst (containing sporozoites).

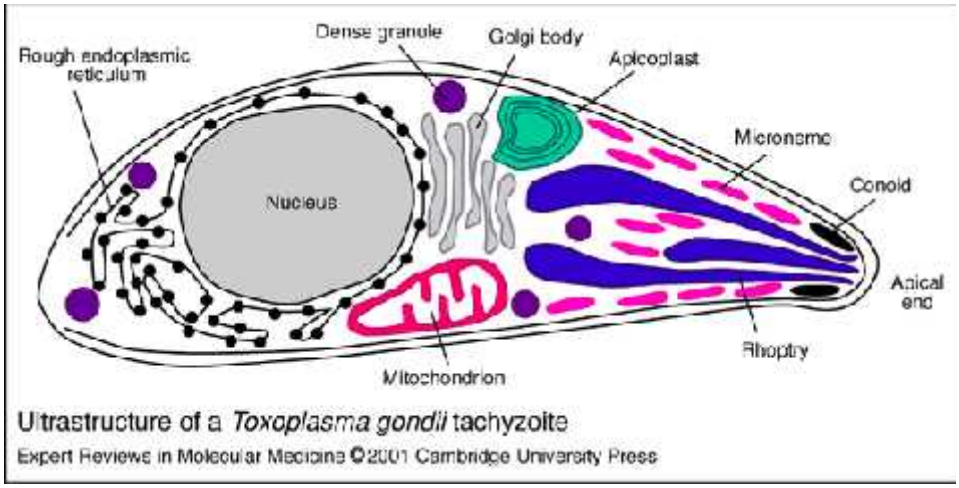

Figure1.1 Structure of *Toxoplasma gondii* tachyzoite (Adapted from Ajioka *et al*., 2001)
In the intermediate host **(**such as human beings **)**, *Toxoplasma gondii* exists in two stages: rapidly dividing tachyzoites, which are thought to be responsible for the acute infection, and bradyzoites, which are located within the cysts and are believed to persist for the life span of the host (Hammouda *et al*., 2001).

Sexual stages of *Toxoplasma gondii* occur within gut epithelial cells of the feline groups, and the products of gamete fusion, the oocysts, are shed in the feces (Black and Boothroud, 2000). Up to 10 million unsporulated oocysts per day are excreted in the feces of the feline for a period of a bout 20 days. The oocysts excreted with the feces of feline should sporulate in external environment to be infectious to other hosts (Denkers and Gazzinelli, 1998).

Unlike sexual stage of the parasite that is highly specific, occurring in no other known hosts than those of feline species, asexual stages of *Toxoplasma* lack host and tissue specificity (Denkers and Gazzinelli, 1998)(Figure 1.2**)**. In intermediate hosts, after infection of intestinal epithelial cells, the infective stages (oocysts or bradyzoites) transform into tachyzoites, and disseminate throughout the body with the help of migrating leukocytes (Channon *et al*., 2000). Tachyzoite of *T. gondii* can cross restrictive biological barriers: intestine, blood-brain barrier, blood-retina barrier and placenta (Filisetti and Candolfi, 2004; Barragan and Hitziger, 2008).

The free tachyzoites can able to infect virtually any nucleated cell they encounter (Channon *et al*., 2000), and they continue intracellular replication, spreading throughout host tissues (Denkers and Gazzinelli, 1998; Black and Boothroud, 2000). When the host develops functional immunity against *T. gondii*, the parasite is contained within tissue cysts as bradyzoites in muscles and brain tissues (Filisetti and Candolfi, 2004).

Although tissue cysts can remain dormant for the life of the intermediate host in various tissues, bradyzoites can continue their rapid division and haematogenously disseminate in the form of tachyzoite again, particularly in immune compromised, such as with the use of immunosuppressive therapy or acquired immunodeficiency syndrome (Denkers and Gazzinelli, 1998; Black and Boothroud, 2000).

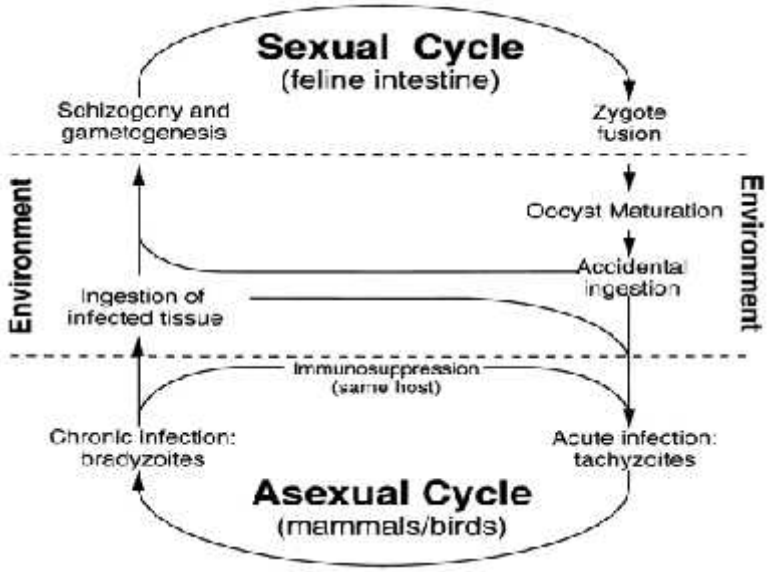

Figure 1.2. Life cycle of *Toxoplasma gondii* (Adapted from Black and Boothroud, 2000).

1.2.2. Source, route and distribution of *Toxoplasma* infection in human

Toxoplasma gondii is a protozoan parasite that infects a wide variety of warm-blooded vertebrates, including cats, livestock, and humans (Hammouda *et al.*, 2001). The two major routes of transmission of *Toxoplasma* to humans are oral and congenital (Montoya and Remington, 2008).

T. gondii infection is acquired primarily through ingestion of viable tissue cysts in infected, undercooked meat or oocysts that may contaminate soil, water, and food (Jackson and Hutchison, 1998; Spanding *et al*., 2005). Toxoplasmosis is acquired as a food-borne or waterborne pathogen, infections in agricultural animals and cats provide the reservoir for human infection (Dubey *et al*., 1998).

Acute infection with *Toxoplasma gondii* during pregnancy enables transmission of the parasite to the fetus. *T. gondii* infection can be transmitted to a fetus when a woman becomes acutely infected with the parasite during pregnancy via a placenta (vertical transmission) (Montoya and Remington, 2008). Maternal fetal transmission can also occur in immunocompromised such as HIV infected pregnant woman who are chronically infected with *T. gondii* (Dalgic, 2008). The time of maternal infection, immunological competence of the mother during parasitemia, parasite load and strain's virulence constitute the basic factors for fetal infection (Spanding *et al*., 2005; Dalgic, 2008).

The frequency of toxoplasmosis acquisition during pregnancy ranges from 1 to 4 per 1,000 pregnancies in several countries, and congenital infection has a prevalence of 0.2 to 2 per 1,000 births (Spanding *et al*., 2005). The risk of fetal infection varies depending on time as only 1 % at less than 6 weeks, 4-6 % at 6-16 weeks, 20-40 % at 16-25 weeks and 60-80 % at 36 weeks of gestation (Dalgic, 2008).

Infection caused by *Toxoplasma gondii*, toxoplasmosis, is one of the most frequent zoonoses in the world (Silva *et al*., 2008). Serological survey demonstrates prevalence of the infection from less than 10 to greater than 90% in various geographic locations (Guerrant *et al.*, 2006).

In Ethiopia, sera from 170 factory workers aged 18-45 years enrolled in a pilot study of human immunodeficiency virus 1 (HIV 1) infection in Addis Ababa were screened for anti-*Toxoplasma* IgG and the prevalence was 80% (Woldemichael *et al.,* 2003). Seroprevalence of immunoglobulin G antibody to *T. gondii* in patients with schizophrenia from Butajira, in rural part of Ethiopia, were 87.9% (Teshome *et al*., 2009).

Seropostivity prevalence may differ even in the country among populations or geographic regions. *T. gondii* infection is more common in warm climates and at lower altitude than in cold climates and mountainous regions. This distribution is probably related to conditions favoring the sporulation and survival of the oocysts (Spanding *et al*., 2005). Frequency of *Toxoplasma* infection increases with older age groups, as the probability of an individual coming in contact with one of the infective forms increases as his or her age increases (Spanding *et al*., 2005). Several authors have emphasized the influence of urban verses rural setting in toxoplasmosis. The presence of cats is relevant, as this animal has great role in the *T. gondii* life cycle (Spanding *et al*., 2005).

1.2.3. Diseases associated with *Toxoplasma gondii* infection

During acute infection, tachyzoites of *Toxoplasma* are found in all organs most prominently in muscles, including heart, liver, spleen, lymph nodes and central nervous system (Guerrant *et al*., 2006). *Toxoplasma* infection in human being is usually asymptomatic in immunocompetent adults is recently reconsidered (Flegr, 2007), as behavioral alteration in rats and mice has led some scientists to speculate that *Toxoplasma* may have similar effects in humans, even in the latent phase that had previously been considered asymptomatic (Webster, 2007).The demonstration that latent *Toxoplasma* infections can alter behavior in rodents has lead to a reconsideration of this assumption(Flegr, 2007).

In human latent *T. gondii* Infection are exerting on human personality characteristics and behaviors (Flegr *et a*l., 1996; Flegr, 2007). There was decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the parasite *Toxoplasma gondii* (Flegr *et al*., 2003). Individuals with latent

toxoplasmosis have significantly increased risk of traffic accidents than the non-infected individuals (Flegr *et al*., 2002).

Toxoplasma infection in human being usually causes disease and death in immunocompromised patients and in congenitally infected individuals (Flegr, 2007). *Toxoplasma* is one of the most opportunistic infections in immunocompromised individuals such as AIDS patients (Storch *et al*., 2005). *T. gondii* is the common opportunistic infections affecting the central nervous system (CNS) in AIDS patients (Halonen *et al*., 1996). The reactivation of latent chronic infection from cysts presents in brain tissue cause cerebral toxoplasmosis or cerebral mass lesion in AIDS patients (Colombo *et al*., 2005).

Placenta is an imperfect barrier to infection of the fetus and allows to the fetus in 30 % to 50% of infections acquired during pregnancy (Guerrant *et al.*, 2006). The rate of transplacental transmission of *Toxoplasma* and severity of disease vary with time of gestation. If maternal infection occurs during the first trimester, the risk of fetal infection is only around 10%, but disease is usually severe. Rates of congenital infection rise to about 65 % for maternal infection during the third trimester, but fetal infection in these instances is usually asymptomatic (Guerrant *et al.*, 2006).

Fetal infection by *T. gondii* can result in neurological sequel, congenital malformations and ocular disorders (Dalgic, 2008). Congenital toxoplasmosis can result in visual and hearing loss, mental and psychomotor retardation, seizures, hematological abnormalities, hepatosplenomegaly, or death (Montoya and Remington, 2008).

From congenital infected infants fewer than 15% have severe impairment of the brain or eyes, and about 80% are asymptomatic at birth, however; more than 85% of these with asymptomatic infection will develop adverse sequalae of CNS or eyes in subsequent years (Guerrant *et al.*, 2006). Some health problems caused by congenital toxoplasmosis may not become apparent until the second or third decade of life (Dalgic, 2008).

A variety of parasite and host factors determine the out come of *T. gondii* infection. The most important factors appear to be the difference in genetic susceptibility, parasite strain

difference, route of infection (ingesion of oocyst from infected cats versus tissue cysts from meat) and timing of infection (eg, in utero, early postnatal, childhood, adulthood) (Torrey *et al*., 2007).

Susceptibility and resistance to chronic *T. gondii* infection is under genetic control (Jamieson et al., 2009). HLA-DQ genes in humans are part of major histocompataibility complex that regulates the host immune responses to the parasite. HLA-DQ1 appears to be a genetic marker of resistance and HLA-DQ3 appears to be a genetic marker of susceptibility to develop cerebral toxoplasmosis in AIDS patients and congenitally infected infants (Curuthers and Suzuki, 2007).

Strains of *T. gondii* have been classified in to three major genotype (type I, II, III) based on polymorphism of their gene (Fuentes *et al*., 2001). Type II strains are most common in human toxoplasmosis and as chronic infections in animals used for human consumption. Type II is the predominate strain isolated from patients with AIDS and developed toxoplasmic encephalitis, and from these with congenital infection (Howe and Sibley, 1995). Type I strains are relatively rare in animals and common isolate from out breaks of acute toxoplasmosis, which show a tendency to cause sever ocular disease. Type III strains are assumes less pathogenic (Howe and Sibley, 1995; Curuthers and Suzuki, 2007). If congenital infection with *T. gondii* is involved in the etiology of schizophrenia, this would implicate type II strain of the parasite. The aggressiveness of type I tachyzoites might also contribute to the development of schizophrenia (Curuthers and Suzuki, 2007).

1.2.4. Diagnosis of *T. gondii* infection

Diagnosis of *Toxoplasma* infection may be established by serological tests, polymerase chain reaction (PCR), histological demonstration of the parasite and/or its antigens, or isolation of the organism (Calderaro *et al*., 2009). *Toxoplasma* may be demonstrated on stained specimens of tissue, blood, amniotic fluid, and CSF. The parasites may also be recovered from either tissue cysts or tachyzoites in tissues and body fluids by inoculation in to mice or tissue culture (Guerrant *et al*., 2006).

Polymerase chain reaction has been successfully used to diagnose toxoplasmosis in congenital and immunocompromised patients (Remington *et al*., 2004). Amplification of *T. gondii* DNA in amniotic fluid during gestation has been used successfully for prenatal diagnosis of congenital toxoplasmosis (Montoya and Remington, 2008). PCR can be used for diagnosis of *Toxoplasma* in CSF of AIDS patients suspected of *Toxoplasma* encephalitis (Storch *et al*., 2005).

The detection of *Toxoplasma*-specific antibodies is the primary diagnostic method to determine infection with *Toxoplasma* (CDC, 2009). The result of examining positive antibodies in serum sample indicates that the host has been exposed to the parasite at some time in the past (Palo Alto, 2008).

Toxoplasma specific Immunoglobulin, IgM antibodies appear after few days of the parasite infection and disappear generally after 3-5 months (Filisetti and Candolfi, 2004). But in some individuals IgM antibodies can persist for some years after infection so further tests are necessary to clarify recent and chronic *Toxoplasma* infection (Viro-Immuno Labor, 2007).

IgG antibodies usually appear within 1 to 2 weeks of the infection, peak within 1 to 2 months, fall at variable rates, and usually persist for life(Viro-Immuno Labor, 2007; Palo Alto, 2008). A single positive IgG test does not distinguish acute from chronic infection as antibodies to *Toxoplasma* persist indefinitely after acute infection, occasially in high titer. Paired sera samples taken from an individual tested at 2-4 weeks interval indicate an acute acquired infection when there is an increase in (16-fold) IgG antibody titer (Guerrant *et al.*, 2006).

Table 1.1. Determination of IgM and IgG antibodies to *Toxoplasma gondii* and Interpretation of results

IgG test result	IgM test result	Interpretation*
Negative	Negative	No serological evidence of *Toxoplasma* infection
Negative	Positive	Possible early phase of *Toxoplasma* infection
Positive	Positive	Probable recent *T. gondii* infection, reinfection or latent infection of the parasite
Positive	Negative	Infected with *Toxoplasma* for more than one year

Adopted with modification (Viro-ImmunoLabor, 2007; Montoya and Remington, 2008; CDC, 2009). * Except infants

Detection of specific IgM although is the method used all over the world to detect acute infection, persistence of IgM for long periods poses problems in distinguishing acute from chronic infection, which is of crucial importance in pregnancy (Yasodhara *et al.*, 2001). A combination of serological tests is frequently required to establish whether an individual has been more likely infected in the distant past or has been recently infected (Lappalainen and Hedman, 2004; Remington *et al.*, 2004). IgG Avidity test for *Toxoplasma* has been recently developed where timing and differentiation of primary and secondary infections (recurrent or reactivated) is crucial (Remington *et al.*, 2004). The functional affinity of specific IgG antibodies is initially low after primary antigenic challenge and increases during subsequent weeks and months (Palo Alto, 2008).

1.2.5. Mental illness

Mental illnesses represent a growing human, medical, social and economic tragedy worldwide. Psychotic disorders, which include schizophrenia, bipolar affective disorder and major depression, are among the most devastating diseases affecting humankind (Metastatio and Bahn, 2008). Psychotic disorders are common diseases, with approximately 7% of human population being affected (Metastatio and Bahn, 2008).

a) Schizophrenia

Schizophrenia is a serious, chronic, and often debilitating neuropsychiatric disease of uncertain etiology (Wang *et al*., 2006) that affects approximately 1% of the adult population world wide and is generally similar in most ethnic populations that have been studied (Robertson *et al*., 2006). The annual admission rate of psychiatric patients in Amanuel Psychiatric Hospital in Ethiopia was 4 per 100,000 population and schizophrenia was the most common discharge diagnosis (56.1%) followed by bipolar disorder (20.6%) (Fekadu *et al*., 2007). A two stage survey carried out in Butajira for onset and clinical course of schizophrenia estimated lifetime prevalence of 4.7/1000 for schizophrenia (Kebede *et al*., 2003).

Schizophrenia is a debilitating mental illness characterized by symptoms that may be positive (delusions and hallucinations, disorganized speech) and negative (affective flattening, impoverishment of speech and language, social withdrawal) in nature, as well as cognitive deficits (attention deficits, impaired executive functions such as planning, abstract thinking, rule flexibility, and inhibition of inappropriate actions and irrelevant sensory information, as well as short-term and long-term memory deficits) (Robertson *et al*., 2006).

While the incidence of the disorder is relatively low, schizophrenia is one of the major contributors to the global burden of disease. The substantial burden of disease is a reflection of two features of schizophrenia: the disorder usually has its onset in early adulthood and despite optimal treatment, approximately two-thirds of affected individuals have persisting or fluctuating symptoms (Kebede *et al*., 2004). Although antipsychotic drugs relieve the positive symptoms of schizophrenia, these drugs have limited utility in the treatment of the negative symptoms and cognitive deficits associated with this disorder (Robertson *et al*., 2006).

An increased occurrence of schizophrenia in family members of affected persons suggests that genetic factors play a role in its etiology, and some candidate predisposing genes have been identified (Torrey and Yolken, 2003). The probability of developing this disorder is elevated significantly in the relatives of people with schizophrenia

(6 % in parents) (Robertson *et al*., 2006). Schizophrenia is known to have a genetic component with concordance among monozygotic twins of 35-50 % (Torrey and Yolken, 2003; Torrey *et al*., 2007).

A large number of candidate schizophrenia susceptibility genes have been identified that encode proteins implicated in the regulation of synaptic plasticity, neurotransmission, neuronal migration, cell adherence, signal transduction, energy metabolism and neurite outgrowth(Robertson *et al*., 2006). Some of the identified genetic risk factors of schizophrenia directly involved in innate and acquired immunity include promoter polymorphisms of pro-inflammatory and anti-inflammatory cytokines, as well as human leukocyte antigens and alleles (Torrey and Yolken, 2003; Torrey *et al*., 2007).

Schizophrenia has a large genetic component, however, the significance discordance between identical twins points to the importance of environmental factors (Robertson *et al*., 2006). While a number of genetic risk factors for schizophrenia have been found, most have low odds ratios (Torrey *et al*., 2007). Many risk factors have been associated with an increased risk to develop schizophrenia, including infectious and obstetric insults, maternal malnutrition and other factors (Metastatio and Bahn, 2008).

b) Bipolar disorder

Bipolar disorder, also known as manic-depressive illness, is a brain disorder that causes unusual shifts in a person's mood, energy, and ability to function (NIMH, 2002).
The onset of full symptoms of bipolar disorder generally occurs in late adolescence or young adulthood (Wikipedia, 2009). The condition has a high rate of recurrence and if untreated, it has an approximately10–20% risk of death by suicide (Muller-Oerlinghausen *et al*., 2009). Diagnosis of bipolar disorder is based on the person's self-reported experiences, as well as observed behavior (Wikipedia, 2009).

It is well established that genetic factors are important in the etiology of bipolar affective disorder, and molecular genetic studies have identified a number of chromosomal regions of interest (Mortensen *et al*., 2003). A person's risk of developing schizophrenia or bipolar disorder is increased if the person has close relatives affected with these

diseases and that the genetic risk factor is stronger for bipolar disorder than it is for schizophrenia (Yolken and Torrey, 1995). The concordance rate of bipolar disorder for monozygotic (identical) twins is 43%; whereas it is only 6% for dizygotic (nonidentical) twins (Long, 2005).

The lifetime prevalence of bipolar disorder is 1·3 to 1·6% (Muller-Oerlinghausen *et al*., 2009). Prevalence of bipolar disorders among the adult population of Zeway islands of Ethiopia was 1.83% (Fekadu *et al*., 2004). Survey conducted to determine socio-demographic correlates of bipolar disorder in Butajira indicated the life time prevalence of bipolar disorder in this population was 0.5% (0.6% for men and 0.4% for women) (Kebede *et al*., 2005).

Bipolar disorder is the least studied among the three major psychiatric disorders of schizophrenia, major depression and bipolar disorder (Hinze-Selch, 2002). There have been relatively few studies investigating possible nongenetic risk factors for bipolar affective disorder (Mortensen *et al*., 2003).

The finding that the concordance rate of bipolar disorder for monozygotic twins (who share all the same genes) isn't 100% suggests that both genes and other factors (environmental or psychological factors) play a role in bipolar disorder causation (NIMH, 2002). The cycling clinical course of bipolar disorder has prompted psychiatrists to think of an infectious etiology of this symptomatology. Latent infections such as *T. gondii* with a relapsing-remitting course have been implicated (Hinze-Selch, 2002).

1.2.6. Infectious agents in schizophrenia and bipolar disorder

Infectious theory of psychosis, prominent early in the twentieth century, has recently received renewed scientific support. Evidence has accumulated that schizophrenia and bipolar disorder are complex diseases in which many predisposing genes interact with one or more environmental agents to cause symptoms (Yolken and Torrey, 2008).

The hypothesis, infectious cause of psychosis, is consistent with the role of neurotransmitter abnormalities in schizophrenia and bipolar disorder, because specific infectious agents have been shown to alter dopamine, serotonin, glutamate, γ-amino

butyric acid, and acetylcholine in animal models (Yolken and Torrey, 2006). The hypothesis is also consistent with neurodevelopmental models of schizophrenia and bipolar disorder. Mayer *et al*., 2008, reviewed studies conducted on animal models that show how exposure to specific infectious agents during neurodevelopment correlates with later behavioral alterations in animals (Mayer *et al*., 2008). The protozoan *Toxoplasma gondii* infections may occur early in life to cause abnormal neurodevelopment or may cause neurotransmitter abnormalities as the parasite is neurotrophic with high affinity to brain cells (Yolken and Torrey, 2008).

Another reason to look for infectious agents in schizophrenia and bipolar disorder is the well-established association between the risk of these disorders and winter-spring seasonal birth (Yolken and Torrey, 2006). Winter or early spring birth has been associated with increased schizophrenia and bipolar disorder risk (Mortensen *et al*., 2003). Many Studies have shown that individuals who later develop schizophrenia have a 5 to 15% excess of winter and spring births (Torrey *et al*., 1995). Because many infectious diseases occur seasonally, with a peak in the winter or spring, it is reasonable to postulate that fetal or newborn infection could contribute to subsequent mental illness (Torrey *et al*., 1995).

Co-infection of neurotropic pathogens may be important in behavioral changes (Yolken and Torrey, 2006). Endogenous retroviruses may be activated by infections with herpes viruses or protozoan organisms such as *Toxoplasma*, providing a potential link between infectious agents and genetic elements as causative factors in human psychiatric diseases (Oliver *et al*., 2006; Yolken and Torrey, 2006). Increased retroviral transcription in the CSF and blood of persons with recent onset psychosis supports a possible role for human endogenous retrovirus in the development of schizophrenia (Yolken and Torrey, 2006).

Recently, linkage and association studies have lead to the assumption that schizophrenia and bipolar disorders are not only genetically defined static disorders but also a dynamic process leading to dysregulation of multiple pathways (Lang *et al*., 2007). Recent studies have been indicated that schizophrenia and bipolar disorders results from aberration in neurodevelopmental processes caused by an interaction between genetic and environmental factors. However, it remains largely unknown to date how genetic and

environmental factors may interact with each other to modulate the vulnerability for these disorders (Mayer *et al*., 2008).

Recently, critical interactions between genetic and infection-associated environmental factors are considered the risk factor for schizophrenia and other psychosis (Torrey and Yolken, 2003). Early exposure to several infectious agents has been associated with later development of schizophrenia in offspring (Brown, 2006). Evidence suggests that infections known to cause congenital CNS anomalies in human, including Influenza virus, rubella virus, herpes simplex virus, cytomegalovirus and *T. gondii* might be related to the risk of schizophrenia and other psychosis (Torrey and Yolken, 2003).

Toxoplasma gondii is one of several infectious agents that might cause most cases of schizophrenia as the parasite is known to replicate within human central nervous system and alter behavior in experimental animals (Torrey and Yolken, 2003; Torrey *et al*., 2007). Epidemiological and neuropathological studies indicate that some cases of schizophrenia may be associated with environmental factors, such as exposure to the ubiquitous protozoan *Toxoplasma gondii*. Reasons for this include *T. gondii*'s ability to establish persistent infection within the central nervous system, its ability to manipulate intermediate host behavior, the occurrence of neurological and psychiatric symptoms in some infected individuals, and an association between infections with increased incidence of schizophrenia (Webster *et al*., 2006).

If *Toxoplasma* is involved in the etiology of schizophrenia and bipolar disorder, however, its synergy with genes may determine the person's brain development and immune response to other infectious agents (Torrey and Yolken, 2003). Predisposing gene makes an etiological link between toxoplasmosis and schizophrenia inherently plausible (Torrey and Yolken, 2007).

Laboratory research has found that *Toxoplasma* infected mice and rats have cognitive dysfunction and behavioral changes that make the animals easier to be captured by the parasite’s felid definitive hosts (Vyas *et al*., 2007; Webster, 2007). This is considered to be an evolutionary adaptation of the parasite; that is, *T. gondii* can facilitate the transmission from intermediate host to definitive host by changing the host animal’s

behavior and thus help it accomplish its life cycle (Vyas *et al*., 2007; Webster, 2007). Dopamine level in mice with chronic *Toxoplasma* infection was higher than in controls, which was thought to be a neurochemical mechanism of the mental or motor abnormalities found in rodent hosts, and possibly in humans (Novotna *et al*., 2005).

Recent research on humans suggests that latent infection with *T. gondii* may also alter the host's behavior, psychomotor skills, or personality (Fleger *et al*., 2002; Fleger *et al*., 2003; Flegr, 2007). Some patients with AIDS whose latent *Toxoplasma* infection has been reactivated show psychotic symptoms such as delusions and hallucinations (Torrey and Yolken, 2003). Potential link between *T. gondii* and neuropsychiatric disorders, in particular schizophrenia, has been proposed (Webster *et al*., 2006).

1.2.7. Early infection of *T. gondii* as risk factors for schizophrenia and bipolar disorder

Several maternal infections have been associated with an elevated risk of schizophrenia in offspring (Brown *et al*., 2005). Maternal infection during pregnancy increases the risk of schizophrenia and other brain disorders of neurodevelopmental origin in the offspring (Mayer *et al*., 2008). Many infectious agents seem to be involved in this association. It has been proposed that factors common to the immune response to a wide variety of pathogens may be the critical link between prenatal infection and postnatal brain and behavioral pathology (Brown *et al*., 2005; Mayer *et al*., 2008). Chemokines and cytokines are known to mediate the host response to infection and thus might explain associations between different prenatal infection and psychosis (Mayer *et al*., 2008).

It is likely that prenatal infection increase risk of schizophrenia among subgroup of vulnerable individuals, including those who are genetically predisposed or who are exposed to other environmental factors (Brown, 2006). *Toxoplasma* is transmitted to the fetus via the placenta. The proliferating tacyzoites in the fetal CNS and other organs can destroy parasitized cells and result in an inflammatory response, leading to anoxia, cell death, and tissue necrosis (Torrey and Yolken, 2003; Brown *et al*., 2005).

Fetal exposure to *T. gondii* could contribute to schizophrenia risk through early sensitization of the immune system. This might produce an imbalance of immune response found in some patients with schizophrenia; this immune constellation, in turn, could lead to the accumulation of kynurenic acid in the CNS, which is inhibitor of *N*-methyl-D-aspartate (NMDA) receptor and could lead to cognitive dysfunction and psychotic symptoms (Mortesen *et al*., 2007).

Much of the research associating infectious agents with schizophrenia or other mental illness has focused on exposure during gestation or early infancy (Torrey and Yolken, 2003). Birth cohort studies in north California indicated that second trimester maternal levels of the chemokine IL-8 were nearly twice as high for offspring who later developed schizophrenia compared with controls (Brown, 2006). In a smaller sample positive association was demonstrated between maternal TNF-α and psychotic disorder among offspring. These finding have provided the strongest evidence that prenatal infection contributes to risk for schizophrenia (Brown, 2006).

Maternal exposure to *T. gondii* is the risk factor for development of schizophrenia in adult offspring. In one birth cohort study, 63 individuals who developed schizophrenia spectrum disorders, maternal sera obtained during pregnancy showed an increased risk (OR= 2.61) of having IgG antibodies to *T. gondii* (Brown *et al*., 2005). One study in Denmark showed that newborn babies with high levels of an antibody, IgG specific to *T. gondii*, had an increased risk (1.79 times greater) of developing schizophrenia. These high levels of the antibodies were not seen in the babies that later developed other schizophrenia-like disorders or affective disorders. The investigators of this study discuss that either the mother's own antibodies to the parasitic infection may be the cause of the baby's later development of schizophrenia by affecting the fetus' developing central nervous system, or the cause might be the effect of the infection itself in the developing baby (Mortesen *et al*., 2007).

Some prospective studies also support a possible role of postnatal *T. gondii* infections in some cases of schizophrenia and bipolar disorder. Central nervous system infections during childhood increased risk of adult onset schizophrenia or other psychoses (Rantakallio *et al*., 1997). Two studies have reported greater childhood exposure to cats

among persons with schizophrenia and bipolar disorder than among controls (Torrey and Yolken, 2003). Thus potential effects of transmission of *Toxoplasma* in early childhood or later in life should thus be considered (Torrey and Yolken, 2003).

1.2.8. Effects of *Toxoplasm gondii* infection on the brain

T. gondii is neurotrophic with high affinity to neurons and glia cells (Halonen *et al*., 1996). After proliferation of tachyzoites in neuron and glia cells during acute stage, the parasite forms cysts preferentially in the brain cells and establish a chronic infection, which is a balance between host immunity and the parasites evasion of the immune response. IFN-γ is the essential mediator of the immune response to maintain the latency of chronic infection (Filisetti and Candolfi, 2004).

In brain, L-trytophan is metabolized to 5-hydroxytryptamine and other idoleamines rather than via the kynurenine pathway. Consequently, kynurenine metabolites are normally lower in brain. If immune activation is within CNS, kynurenine intermediates such as quinolinic and kynurienic acids, increase in association with large local induction in indoleamine 2, 3-dioxygenase (IDO) activity in microglia, astrocytes and invading macrophages in the brain (Heyes *et al.*, 1997).

IFN-γ induced antitoxoplasma activity in human cells, such as macrophages, fibroblasts, glioblastoma cells, and epithelial cells depends on the induction of IDO, which is the rate limiting enzyme of L-trypthophan-L-kynurenine pathway. IFN-γ induced IDO enzyme results in strong antitoxoplasmic effects through the depletion of intracellular pools of tryptophan (Fujigki *et al*., 2002). This enzyme degrades the tryptophan that is needed for the tachyzoite phase of *T. gondii*. Consequently, activated parasites die by tryptophan depletion (Hinze-selch *et al*., 2007).

The trytophan degradation products that accumulate via the kynurenine pathway may result in excess dopaminergic tone in brain. Thus, the host defense system might produce a lack of serotonin and accumulation of dopaminergic activity. Psychiatrically, this suggests depressive and psychotic syndromes (Hinze-selch *et al*., 2007).

Several studies suggest that IDO induction by IFN-γ exerts antimicrobial and antiproliferative effects possibly by depletion of L-trytophan. However, local accumulation of kynurenine metabolites, in particular, quinolinic acid, is a potent excitotoxin, and its overproduction has been linked to neuronal damage occurring in brain inflammation and initiation of lipid per oxidation. It is possible that increased levels of quinolinic acid and other kynurenine pathway metabolites play a detrimental role following *T. gondii* infection (Fujigki *et al*., 2002).

T. gondii infection, by activating astrocytes, increases kynurenic acid formation in the brain. kynurenic acid , a metabolite of the kynurenine pathway of tryptophan degradation, when it present at levels slightly above endogenous brain concentrations, this metabolite can inhibit both *N*-methyl-D-aspartate (NMDA) and 7 nicotinic acetylcholine (7nACh) receptors. These two receptors are widely purported to have causative links to cognitive processes. Thus, abnormally high kynurenic acid levels may contribute to the patients' cognitive impairment seen in schizophrenia (Schwarcz and Hunter, 2007). This concept is supported by studies in rodents, which show that experimental elevation of cerebral kynurenic acid levels results in impaired sensory gating (Schwarcz and Hunter, 2007).

Neuromodulation may represent an ideal mechanism where by *T. gondii* can influence, at least in part, the expression of host behavior (Webster, 2007). *Toxoplasma* increases levels of homovanillic acid and dopamine in CNS, which are implicated in the pathogenesis of schizophrenia (Torrey and Yolken, 2003). Significance differences in levels of homovanillic acid, norepinephrine, and in particular, dopamine have been observed between *T. gondii* infected and uninfected mice, all are substances that mediate, among others, locomotors activity, mood, learning, memory, and cerebral blood flow (Flegr *et al*., 2005; Webster, 2007).

The mechanism of the dopamine increase is not known but may involve the inflammatory release of dopamine by increasing cytokines such as IL-2 (Flegr, 2007). The dopamine imbalance between the mesolimbic and mesocortical regions in the brain is suspected to play a role in the development of schizophrenia, which could explain the observed association between schizophrenia and toxoplasmosis (Flegr, 2007). Bipolar

disorder is associated with abnormal brain levels of serotonin, norepinephrine, and dopamine (Long, 2005).

It has been also suggested that *Toxoplasma* may result in congenital CNS abnormalities without direct transmission of the parasite to the fetus. Maternal immune activation caused by *T. gondii* may cross the placenta and impair fetal neurodevelopment (Brown *et al.*, 2005; Mayer *et al*., 2008). Cytokines are involved in normal CNS development as well as in the pathogenesis of many neuropsychiatric disorders, acting directly on neuronal cells or modulating neurotransmitters and neuropeptide system (Boin *et al*., 2001).

1.2.9. Antibodies to *T. gondii* in patients with schizophrenia and bipolar disorder

Given *T. gondii's* neurotropism and association with congenital brain dysfunction, there has been long- standing interest in investigating a possible association between exposure to this parasite and the development of severe psychiatric disorders (Torrey *et al*., 2007). The availability of serological assays has allowed for the testing of exposure to *T. gondii* in large numbers of individuals (Guerrant *et al.*, 2006).

The first study of *T. gondii* antibodies in psychiatric patients was published in 1953 by Kozar in Poland. Since then, 42 additional studies carried out in 17 countries over 5 decades were identified; 23 of these subjected to a Meta analysis to define the association between *Toxoplasma* exposure and risk of schizophrenia. The results of the Meta analysis (OR=2.73) suggest that individuals with schizophrenia have an increased prevalence of antibodies to *T. gondii* when compared to health controls (Torrey *et al*., 2007). The OR of 2.73 exceeds that for genetic or other environmental factors identified to date and suggests that *Toxoplasma* is in some way associated with a large number of cases of schizophrenia (Torrey and Yolken, 2007; Torrey *et al*., 2007).

Given the high prevalence of *Toxoplasma* infection in healthy individuals, *Toxoplasma* seropostivity has a relatively low predictive value for the development of schizophrenia and other psychosis, and most individuals who are *Toxoplasma* seropositive do not have manifestations of this disorder (Torrey *et al*., 2007). Some individuals who are exposed

to *T. gondii* are expected to develop psychosis , as the outcome of the infection differs based on genetic susceptibility, parasite strain difference, route of infection, and the time of exposure (Torrey and Yolken, 2007; Curuthers and Suzuki 2007).

Individuals with first-episode schizophrenia had significantly increased levels of IgG, class antibodies to *Toxoplasma*, as compared with the control subjects (Halonen *et al*., 1996). In first-episode schizophrenia patients there was association of high antibody titers to *T. gondii* and increased CRP levels and numbers of leukocytes as markers of inflammatory activity, which indicates special condition of the patients, and might supported the more intense immune response of the host to *T. gondii* infection (Hinze-selch *et al*., 2007).

Untreated individuals with recent onset schizophrenia had significantly increased levels of serum and CSF IgG antibody to cytomegalovirus and *Toxoplasma gondii* as compared to controls (Leweke *et al*., 2004). This may suggest an involvement of this parasite in the etiology of schizophrenia (Curuthers and Suzuki, 2007).

The antibody levels for treated group were lower than the never treated group, suggesting that antipsychotic medication may have decreased the antibody levels (Torrey and Yolken, 2003). Immunomodulating antipsychotic drugs are known to cause low antibody titer in schizophrenic patients, as they modulate host cytokine secretion for immunoregulator effects or might involve in host defense against *Toxoplasma* infection (Hinze-Selch *et al*., 2007).

1.2.10. *T. gondii* and antipsychotic and mood stabilizing drugs

Elevated serum level of inflammatory cytokines such as IL-1ß has been detected in individuals with acute schizophrenia, but not chronic schizophrenia. Tachyzoites induce more pronounced inflammatory cytokine responses in host cells than do bradyzoites, and proliferation of tachyzoites in the brain may be related to the onset of schizophrenia (Curuthers and Suzuki, 2007). There where no difference in inflammatory cytokines (IL-1ßor IL-10) in serum or CSF levels in medicated patients compared with a control group (Curuthers and Suzuki, 2007).

Individuals with schizophrenia with acute symptomatology display increased T_H1 associated cytokine responses, returning to control levels during successful antipsychotic treatment. Therefore, an infection such as *T. gondii* might recurrently induce a T_H1 response that modulates the serotonin and dopamine neurotransmitter systems, leading to respective psychotic symptomatology (Hinze-Selch *et al*., 2007). Antipsychotic treatment that reorganizes these neurotransmitter systems reduces the symptoms by modulating T_H1 associated cytokine responses (Hinze-Selch *et al*., 2007).

Many antipsychotic drugs commonly used in the treatment of schizophrenia and bipolar disorder inhibit the replication of *T. gondii* tachyzoites in cell culture (Jones-Brando *et al*., 2003; Webster, 2007). The antipsychotic haloperidol and the mood stabilizer valproic acid most effectively inhibit *Toxoplasma* growth in vitro. Valproic acid inhibited the parasite at a concentration below that found in the cerebrospinal fluid and blood of individuals being treated with this medication and displayed synergistic activity with haloperidol and with trimethoprim, an antibiotic commonly used to treat *Toxoplasma* infections (Jones-Brando *et al*., 2003). Medication typically used in patients with schizophrenia and bipolar disorder has immunomodulatory and anti-infectious properties (Hinze-Selch, 2002; Hinze-Selch *et al*., 2007).

Behavioral change induced by *T. gondii* on experimental animals is partially reversed by treatment with some antipsychotic and mood-stabilizer medication. Such observation provided support for the hypothesis that the antipsychotic and mood-stabilizing activity of some medications may be achieved, or at least augmented, through their inhibition of *T. gondii* replication and/or invasion in infected individuals (Webster, 2007). Perhaps the reason that some antipsychotics and mood stabilizers can reverse some of the neurological damage associated with schizophrenia and bipolar disorder is because they are killing off the causative agents and allowing the brain to repair itself (Webster *et al*., 2006).

1.2.11. Plausibility of *T. gondii* in etiology of schizophrenia and bipolar disorders

Although clinical and epidemiological aspects of schizophrenia and bipolar disorder are consistent with a possible infectious etiology, yet there are no studies that provide a definite link between an infectious agent and these diseases (Yolken and Torrey, 1995). Increased rates of exposure to *T. gondii* have been found in individuals with schizophrenia as compared with control groups, but the correlates of *Toxoplasma* exposure in schizophrenia have not been defined (Dickerson *et al*., 2007). The causal linking mechanism between *T. gondii* and schizophrenia are at present speculative (Torrey *et al*., 2007). Some major considerations with the plausibility of *T. gondii* being etiological linked to schizophrenia and bipolar disorders are:

1. Schizophrenia and bipolar disorder are known to have genetic component. Genes are also known to influence the susceptibility of animals to *T. gondii* (Torrey *et al*., 2007). Susceptibility and resistance to chronic *T. gondii* infection in the brain is under genetic control in both human and mice. HLA-DQ genes in humans are part of the major histocompatibility complex that regulates the immune response to *T. gondii* infection. The regulation of the immune responses by these genes appears to be important to determine the susceptibility/resistance of the host to development of cerebral toxoplasmosis and congenitally infected infants (Curuthers and Suzuki, 2007).
2. Schizophrenia and bipolar disorders are known to include abnormality of neurotransmitters; animal studies have demonstrated an effect of *T. gondii* on dopamine and serotonin (Torrey *et al*., 2007; Webster, 2007; Long, 2005).
3. Schizophrenia is widely believed to be a disease of neurodevelopment; this is consistent with *T. gondii* known ability to cause prenatal infection and then remain latent for many years before becoming reactivated (Torrey *et al*., 2007; Brown *et al*., 2006).
4. An association between *Toxoplasma* infections and schizophrenia is consistent with animal's models indicating persistence behavioral changes in *Toxoplasma* infected animals (Torrey *et al*., 2007; Torrey and Yolken, 2007).

5. *T. gondii* is neurotropic with special affinity for glia, now thought to be centrally involved in the schizophrenia disease process (Torrey *et al*., 2007; Halonen *et al.*, 1996).
6. Some antipsychotic drugs used to treat schizophrenia and bipolar disorders have been shown to inhibit the growth of *T. gondii* in cell culture (Jones-Brando *et al*., 2003; Webster, 2007).
7. Individuals who develop schizophrenia and bipolar disorder are known to have had an excess number of winter and spring births; toxoplasmosis, like many infectious disease, also occurs more commonly in the winter and spring months (Yolken and Torrey, 2006).

Despite many attractive aspects with the plausibility of *T. gondii* being etiologically linked to schizophrenia, there are some major problems:

1. If one or more infectious agents do cause schizophrenia and/or bipolar disorder, there are several reasons why this may be difficult to prove. The agent may cause infection in utero or in the early postnatal period and then disappear or remain in chronic form. It would therefore be very difficult to directly detect the agent when the disease manifested itself in greater or nearly 20 years later. The original infection may initiate neurochemical damage without visible pathological change (Yolken and Torrey, 1995).
2. Most studies used to determine association between *T. gondii* infections in psychiatric patients were serological in nature and are not based on the direct detection of *Toxoplasma* organisms or DNA in infected body fluids. This is an inherent limitation of studies in *Toxoplasma* biology as the organism is difficult to detect in immunocompetent individuals (Torrey *et al*., 2007).
3. Epidemiologically seropositivity rate of *T. gondii* is very high in countries such as France and Ethiopia, where undercooked or raw meat is regularly consumed, yet schizophrenia and other psychosis have not been found to be unusually prevalent in these countries (Torrey *et al*., 2007). Possible explanations include the fact that transmission by eating tissue cysts in undercooked meat is a more benign mode of infection, thus it may pose less of

a risk for the development of schizophrenia than the consumption of oocysts shed by cats (Torrey *et al*., 2007; Curuthers and Suzuki, 2007).

4. The majority of individuals with schizophrenia do not have measurable antibodies to *T. gondii*. This fact may be related to the relative insensitivity of available serological assays or to the heterogeneity of disease pathogenesis (Torrey *et al*., 2007). It is also possible that other environmental factors may initiate neuropathogenic pathways to those employed by *T. gondii* (Yolken and Torrey, 2006).

1.2.12. Importance of determining role of *Toxoplasma* in schizophrenia and bipolar disorder diseases

With further advances in research at the interface between psychiatry and infectious disease, may eventually provide the key to proving the connection between infection and mental disturbance, and pave the way for pharmacologic treatment specifically targeted to that causative infectious organism (Yolken and Torrey, 2006). Proving a causative role for infectious agents in schizophrenia and bipolar disorder would open the door to new treatments and disease prevention strategies. In the future, it might even be possible to develop a vaccine to protect children against possible infections that contribute to these mental illnesses (Yolken and Torrey, 2008).

To improve diagnosis and treatment of schizophrenia it is necessary to gain a better understanding of the disease etiology (Metastatio and Bahn, 2008). Identification of infectious agents associated with the etiopathogenesis of schizophrenia might lead to new methods for the diagnosis, treatment and prevention of the major mental disorders (Yolken and Torrey, 2008).

If the association between *Toxoplasma* and major mental illness has been etiological, this may have important public health implication as this parasitic infection can be effectively treated and prevented (Brown, 2006). Systematic education and serological screening of pregnant women are the most reliable and currently available strategies for the prevention, diagnosis, and early treatment of the infection in the offspring (Montoya and Remington, 2008).

Toxoplasma infection can be prevented by cooking meats at 70 degrees Celsius for 10 minutes to kill the tissue cyst of the parasite (Jackson and Hutchison, 1998). It can also prevented by washing hands after handling uncooked meat, gardening or other contact with soil, wash fruits and vegetables well before eating them, avoid handling litter of cats and avoid handling stray cats (Jackson and Hutchison, 1998).

1.2.13. Significance of the study

One of the infectious agents of the central nervous system that is assumed to be a cause for schizophrenia and other mental illness is infection by *Toxoplasma gondii*. Recent studies have indicated that infections with *T. gondii* may contribute to the symptoms of schizophrenia in some individuals. These include: increased levels of antibodies of *T. gondii* have found in individuals with recent onset of schizophrenia in different study populations; increased levels of IgG antibodies to *T. gondii* measured in mothers of infants who develop schizophrenia later in life. Individuals with schizophrenia who have serological evidence of *Toxoplasma* infection do not have evidence of acute encephalitis and do not have macroscopic cysts in their brain tissue. Possible mechanisms by which *Toxoplasma* might contribute to schizophrenia would include the stimulation of cytokines within the brain, activation of endogenous retroviruses, Impair early brain neurodevelopment during prenatal infection and increase kynurenine metabolites that can damge neuron cells and Inhibit some receptors in the brain.

A recent study reviewed the serum antibody status in about 23 different studies since 1953 that compared schizophrenia patient groups and healthy controls and reported a possible etiological association between *Toxoplasma gondii* and schizophrenia with the OR much greater than for environmental and genetic causes of the disease. Therefore, the present study was undertaken to show the possible association between *Toxoplasma gondii* infection, schizophrenia or bipolar disorder.

1.3. OBJECTIVES OF THE STUDY

General Objective

- To determine the magnitude of *Toxoplasma gondii* infection in patients with schizophrenia, bipolar disorder, control subjects (healthy controls and other close relatives who are mostly parents and siblings) by using serologic diagnostic methods.

Specific Objectives

- To detect the presence of IgG antibodies against *Toxoplasma gondii* using ELISA methods in order to know the association between *Toxoplasma gondii* and schizophrenia
- To detect the presence of IgG antibodies against *Toxoplasma gondii* using ELISA methods in order to know the association between *Toxoplasma gondii* and bipolar disorders
- To compare the level of IgG antibodies titres between schizophrenia and control subjects
- To compare the level of IgG antibodies titres between bipolar disorder and control subjects

CHAPTER II: MATERIALS AND METHODS

2.1. Study design

Case-control study

2.2. Study population and area

The study populations were 214 cases of schizophrenia, 171 bipolar disorder patients, 71 healthy controls and 292 other close relatives who are mostly parents and siblings. The study subjects were recruited from Meskan and Mareko (Butajira) district, 135 km south of Addis Ababa. The district had a population of 227,135 during the 1994 censes. Forty five percent of the population, over 100,000, belongs to the age group between 15 and 49 years (OPHCC, 1994). Demographic data and other relevant information about the study participants were retrieved from stored information from genetic study (Appendix I).

Working definition

Schizophrenia: Neuropsychiatric disease characterized by symptoms such as delusions, hallucination, disorganized speech and cognitive deficits.

Bipolar disorder: A brain disorder that causes shifts in a persons mood, energy, and ability to function.

Close relatives: Healthy individuals who are mostly parents and siblings of patients with schizophrenia or bipolar disorder.

Healthy controls: Healthy individuals who live in neighborhood, non-relative, to patients with schizophrenia or bipolar disorder.

2.3. Sample collection and handling

No new blood samples were collected in this study. Frozen sera (n=748) were used for analysis of antibodies to *T. gondii* which were obtained from each study subjects for genetic study in 2001, aliquoted and stored at -20^{o}C at Armauer Hansen Research

Institute(AHRI) laboratory. The stored sera were kept frozen at AHRI where generator backup is automatically used when the usual source of light seldom switch-off. The stored sera were thawed for the first time in this study. The genetic study is on progress at Institute of Psychiatry, London (not part of this study). Serological assay for *T. gondii* was performed at AHRI where the sera were kept frozen during a period of March to May 2009.

2.4 Serum analysis for *Toxoplasma gondii* infection

VIR-ELISA ANTI-TOXO-IgG ® test kits (Viro-Immuno Labor-Diagnostica GmbH, Germany) were used for qualitative and quantitative determination of IgG antibodies to *Toxoplasma gondii* in sera collected from the study subjects. The kits had 99.1% specificity and 100% sensitivity to the IgG antibodies against *T. gondii*. Each kit contains standard anti-*Toxoplasma* IgG positive (calibrator one, 10 I.U/ml; calibrator two, 50 I.U/ml; calibrator three, 100 I.U/ml; Calibrator four, 200 I.U/ml) and negative control.
Each serum sample was analyzed at a dilution of 1:101 (10µl of sera sample to 1ml of sample dilute). Each serum sample, negative and positive controls were tested in parallel in the microtiter plates.

Principle of the assay
The detection of IgG antibodies was based on the principle of an enzyme-linked immunosorbent assay. Pre coated microtiterstrips with Purified, homogeneous antigen were used according to the manufacturer's instruction. Any specific antibodies present in the study subject's sample were bound during the first incubation (for 60 minute at 37 ^{o}C). After removing unbounded material by washing, the presence of specific antibodies was detected using Anti-human IgG conjugate during the second incubation (for 30 minute at 37 ^{o}C). Excess peroxidase conjugate was then removed by washing and TMB substrate was added and incubated for 15 minute in dark place at room temperature, resulting in the development of a blue color. The enzyme reaction was terminated by the addition of a stop solution (0.95N H_2SO4). Finally, the optical density was measured at 450 nm. The intensity of the yellow color developed was proportional to the concentration of IgG antibodies in the sample.

2.5. Calculation and interpretation of results

Qualitative and quantitative calculations of sera IgG were based on VIR-ELISA ANTI-TOXO- IgG manufactures instruction.

I. Qualitative calculation

Cut-off value=OD value of calibrator one

Cut-off range=cut-off value ±10%

Table 2.1 Qualitative determination of IgG to *T. gondii* in sera samples of schizophrenia, bipolar disorders and control subjects.

Sample IgG status to *T. gondii*	**Definition**
Negative	OD value of sample < Cut-off value -10%
Equivocal	OD value of sample ≥ Cut-off value -10% OD value of sample ≤ Cut-off value +10%
Positive	OD value of sample > Cut-off value +10%

OD value of the blank was subtracted from OD values of negative control, positive controls and each sera sample.

During analysis of the sera four samples result were equivocal. During retesting of these samples one sample became negative; one sample positive, and two samples remain equivocal. The two samples which remained equivocal were considered positive as equivocal results were defined as positive for calculation of the specificity and sensitivity of the kits we used for the analysis of IgG antibodies against *Toxoplasma*.

II. Quantitative calculation

The positive optical density values for anti-*T. gondii* IgG antibodies were converted to International units by using quantitative spreadsheet sent with the kits (see appendix VI). IgG titers in sera samples were calculated by using OD measured and labeled IgG titer value of the positive standards. OD values of calibrator one, calibrator two, calibrator

three and calibrator four was used as y-axis and their labeled IgG titers in international units/ml as x-axis. The standard curve was produced by drawing a point to point curve between OD value measured and international units/ml of the positive controls. Each study subjects IgG titers in I.U. /ml was read from the curve. IgG antibody levels >10 I.U/ml were considered to be positive. The quantitative test result (I.U/ml) of the samples were based on the WHO "international standard for anti-*Toxoplasma* serum (3^{rd} International Standard Preparation)" (Viro-Immuno Labor, 2007).

2.5. Ethical consideration

Informed consent to use the stored serum had been obtained by field workers from the study subjects in whom blood sample were collected in 2001 (Appendix II and III). For those study subjects who could not consent, because of mental disorder or age consent taken from their parents or guardians (Appendix IV and V). There was not any direct contact between the study subjects and investigator. Results of this analysis have not been linked to the study subjects' identification in any form (Appendix I). The ethical clearance for using the stored serum was already obtained from the Faculty Research Publication Committee-I (FRPC-I), Faculty of Medicine, Addis Ababa University (December 14, 2007), AHRI/ALERT Ethical Review Committee and (December 21, 2007) and the National Ethical Clearance Committee (NERC) from Ethiopian Science and Technology Agency (Ref no. RDHE/15-84/2008, 29 September 2008) (Appendix VII). The current M.Sc. research project proposal was approved by the Department Research and Ethical Review Committee, DMIP and subsequently by Institutional Review Board , Faculty of Medicine, Addis Ababa University (March 04, 2009) (Appendix VII) as a formal procedure.

2.6.Statistical analysis

Demographic and laboratory data were entered and analyzed using SPSS version 15.0 windows soft ware. For comparison of the frequencies among the groups, the chi-square and the Yates corrected test were used. A P-value of <0.05 was considered as significant.

CHAPTER III: RESULTS

3.1. Demographic characteristic of study subjects

The study populations were 214 cases of schizophrenia, 171 bipolar disorder patients, 71 healthy controls and 292 other close relatives who are mostly parents and siblings. Out of the 748 study subjects, 452/748 (60.4%) were males and 296/748 (39.6 %) were females (Table 3.1). Majority of the study subjects 450/748 (60.2%) had age distribution within 26-47 years as shown in Figure 3.1.

Most individuals with schizophrenia 108/214 (50.5%) had age distribution with in 26-36 years of the age categories (Table 3.1). There were more males than females among patients with schizophrenia in which the proportion of male subjects was 170/214 (79.4%) (Table 3.1).

Most patients with bipolar disorder 77/171 (45.0%) had age distribution with in 26-36 years of the age categories (Table 3.1). Sex distribution was nearly similar among individuals with bipolar disorder in which the proportion of male subjects was 93/171 (54.4 %) (Table 3.1). Age distribution among the control group (n=363) was almost similar in most of the age categories and the proportion of male subjects was 189/363 (52.1%) (Table 3.1).

The mean age for schizophrenia cases is 32.7 years (SD= 7.9), patients with bipolar disorder is 31.6 years (SD=7.9) and for relatives and non relatives controls is 42.1 years (SD= 15.1) (Table 3.1). There was statistically significant age difference between patients and control groups. Difference in age was significant between patients with schizophrenia and control group (95% CI: 7.2-11.6, p=0.00). Similarly, there was difference in age between patients with bipolar disorder and control subjects (95% CI: 8.1-13, p=0.00).

There was difference in sex distribution between patients with schizophrenia and control group (χ^2=42.9, P= 0.00) in which male to female sex ratio was 3.86 and 1.1 in schizophrenia patients and control group respectively. No difference in sex distribution between patients with bipolar disorder and control group (χ^2=0.254, P=0.614).

Table 3.1. Demographic characteristics of the study subjects recruited from Meskan and Butajira district and participated in analysis of sera for *T. gondii* from March to May 2009

	Study subjects		
Indexes	Schizophrenia (n= 214)	Bipolar disorder (n= 171)	Controls (n=363)
Sex (male/female)	170/44	93/78	189/174
Age (years, mean ±SD)	32.79 ± 7.89	31.62 ± 7.9	42.1 ± 15.1
Age categories			
15-25	38 (17.7%)	44 (25.7%)	59 (16.3%)
26-36	108(50.5%)	77(45%)	85(23.4%)
37-47	59(27.6%)	46(27%)	75(20.7%)
48-58	9(4.2%)	4 (2.3%)	88(24.2%)
59-69	0	0	39(10.7%)
70-80	0	0	17(4.7%)
All	**214**	**171**	**363**

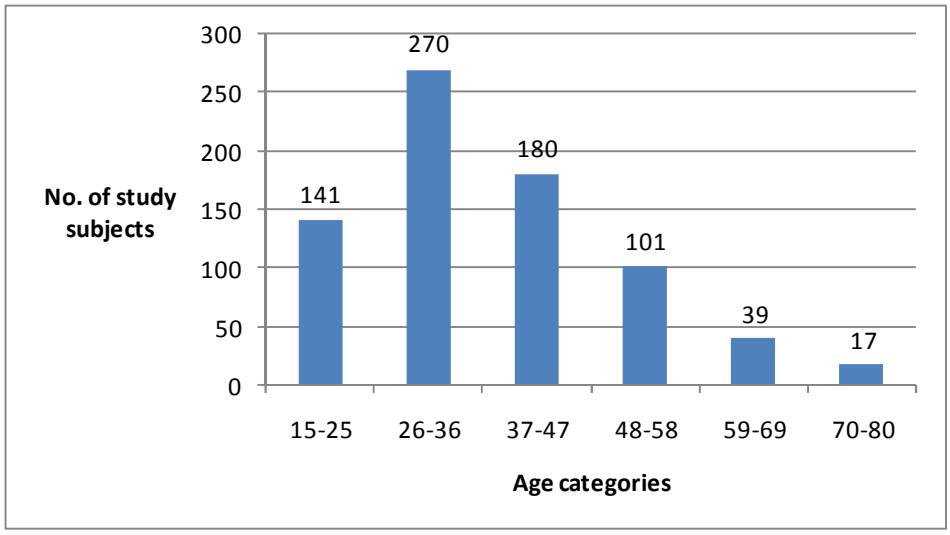

Figure 3.1. Age distribution of overall study subjects recruited from Meskan and Butajira district and participated in analysis of sera for *T. gondii* from March to May 2009

3.2. Seroprevalence of *T. gondii* infection

3.2.1 Seroprevalence of *T. gondii* infection in study subjects

From a total of 748 sera analyzed, 712/748(95.2 %) showed the presence of the specific IgG antibodies to *T. gondii* (Table 3.2 and 3.3). Seropositivity rates among male and female study subjects were 437/452 (96.7%) and 275/296(92.9%), respectively (Table 3.6). There was difference in seroprevalence of IgG antibodies between male and female in study subjects (χ^2=5.57, P=0.018).

The seroprevalence of *T. gondii* infection in patients with schizophrenia was 209/214 (97.7%), in bipolar disorders 163/171 (95.3%) and controls 340/363 (93.7%) as shown in Tables 3.2 and 3.3. The prevalence of IgG antibodies against *T. gondii* was relatively higher in patients with schizophrenia than control groups (healthy controls and close relatives) (OR=2.83; 95% CI: 1.06-7.53) (Table 3.2). Seroprevalence of *T. gondii* infection was relatively higher (although not statistically significant) in patients with bipolar disorder compared with control subjects (healthy controls and close relatives) (OR=1.38; 95% CI: 0.6-3.14) (Table 3.6).

Table 3.2. Seroprevalence of *T. gondii* infection in schizophrenia compared with control subjects

IgG status against *T. gondii*	Study subjects			OR	95% CI of OR
	Schizophrenia (n=214)	Controls (n=363)			
		Close relatives (n=292)	Healthy controls (n=71)		
IgG+	209 (97.6%)	278 (95.2%)	62 (87.3%)	2.83	(1.06-7.53)
IgG-	5 (2.4%)	14 (4.8%)	9 (12.7%)		
Total	**214 (100%)**	**292 (100%)**	**71 (100%)**		

Table 3.3. Seroprevalence of *T. gondii* infection in bipolar disorder cases compared with control subjects

IgG status against *T. gondii*	Study subjects			OR	95% CI of OR
	Bipolar disorder (n=171)	Controls (n=363)			
		Close relatives (n=292)	Healthy controls (n=71)		
IgG+	163 (95.3%)	278 (95.2%)	62 (87.3%)	1.38	(0.6-3.14)
IgG-	8 (4.7%)	14 (4.8)	9 (12.7%)		
Total	**171 (100%)**	**292 (100%)**	**71 (100%)**		

3.2.2. Seroprevalence in different age groups

Seroprevalence of IgG antibodies to *Toxoplasma* in patients and controls increased with age. Patients with schizophrenia(Table 3.4) and bipolar disorder(Table 3.5) in most age groups showed higher rates of seroprevalence, however; in most age groups the difference in individual age groups did not reach statistical significance(all p>0.05). Seroprevalence difference in all age groups taken together was significantly increased in patients with schizophrenia compared with control group (χ^2=4.66, P= 0.031), but the difference was not significant between patients with bipolar disorder compared with control subjects (χ^2=0.584, p=0.445).

Table 3.4. Seroprevalence of *T. gondii* infection in different age groups (schizophrenia compared to control subjects)

	Study subjects						**p- value**
	Schizophrenia			**Controls**			
Age groups	**No. tested**	**No. IgG+**	**% IgG+**	**No. tested**	**No. IgG+**	**% IgG+**	
15-25	38	36	94.73	59	49	83.1	0.16
26-36	108	105	97.2	85	79	92.94	0.29
37-47	59	59	100	75	72	96 .0	0.334
48-58	9	9	100	88	86	97.72	0.44
59-69	0	0		39	37	94.87	
70-80	0	0		17	17	100.0	
All	**214**	**209**	**97.66**	**363**	**340**	**93.66**	**0.031**

Table 3.5. Seroprevalence of *T. gondii* infection in different age groups (bipolar disorder compared to control subjects)

	Study subjects						p-value
	Bipolar disorder			Controls			
Age groups	**No. tested**	**No.IgG+**	**% IgG+**	**No. tested**	**No. IgG+**	**% IgG+**	
15-25	44	38	86.36	59	49	83.1	0.646
26-36	77	76	98.7	85	79	92.94	0.157
37-47	46	45	97.8	75	72	96	0.986
48-58	4	4	100	88	86	97.72	0.151
59-69	0	0		39	37	94.87	
70-80	0	0		17	17	100	
All	**171**	**163**	**95.32**	**363**	**340**	**93.66**	**0.427**

3.2.3. Seroprevalence in male and female study subjects

The prevalence of IgG antibodies to *T. gondii* did not differ significantly (all p >0.05) between males and females within each study population (Table 3.6). No difference in seroprevalence in sex in patients with schizophrenia (χ^2=0.28, p=0.6). There was no difference in prevalence of IgG antibodies to *T. gondii* between males and females in patients with bipolar disorder (χ^2=1.81, P=0.178). Similarly, no difference in seroprevalence in sex in control group (χ^2=1.67, p=0.196). Seroprevalence difference in all study subjects taken together was significantly increased in males compared with females (χ^2=5.57, P= 0.018) (Table 3.6).

Table 3.6. Seroprevalance of *T. gondii* infection in male and female study subjects

Study subjects	**Sex**						**P-value**
	Male			**female**			
	No. tested	**No.IgG+**	**% IgG+**	**No. tested**	**No. IgG+**	**% IgG+**	
Schizophrenia	170	166	97.64	44	43	97.72	0.596
Bipolar disorder	93	91	97. 85	78	72	92.3	0.178
Control	189	180	95. 23	174	160	91.95	0.196
Total	**452**	**437**	**96.66**	**296**	**275**	**92.91**	**0.018**

3.3. IgG titers to *T. gondii* in study population

a. IgG titer in cases vs. controls

Based on the quantitative determination of IgG antibodies against *T. gondii*, sera samples with IgG titers <10 I.U/ml are seronegative to *T. gondii* infection and sera having IgG titers > 10 I.U/ml are considered to be seropostive to *T. gondii* infection. Majority of study subjects (95.2%) their sera had titers of IgG antibodies against *T. gondii* greater than 10 I.U/ml (Table 3.7).

Table 3.7. IgG antibodies titer to *T. gondii* in cases and control groups

	Titer categories of IgG antibodies to *T. gondii* in IU/ml					
Study subjects	<10	10-50	51-100	101-200	>200	Total
Schizophrenia	5	46	76	52	35	214
Bipolar disorder	8	38	51	41	33	171
Control	23	79	109	92	60	363
Total	**36**	**163**	**236**	**184**	**129**	**748**

The chronic schizophrenia and bipolar disorder cases involved in our study had no increased levels of antibodies to *T. gondii* compared with control group (Table 3.7) (Figure 3.1). There was no statistically significant difference in serum level of IgG antibodies to *T. gondii* between seropostive patients with schizophrenia and controls group (χ^2=1.112, p=0.78). Similarly, no difference in level IgG antibodies to *T. gondii* between individuals with bipolar disorder and control group (χ^2=0.574, p=0.9).

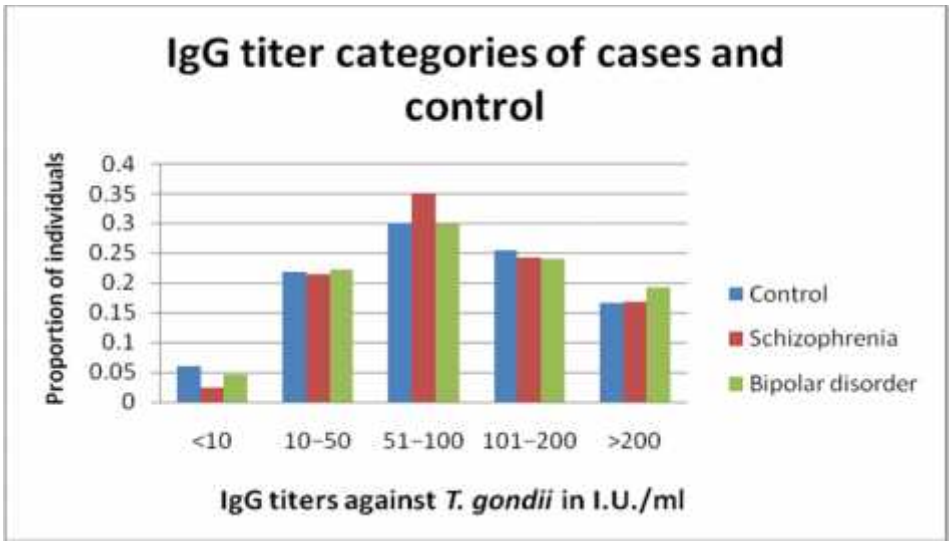

Figure 3.2. Level of serum IgG antibodies to *T. gondii* in chronic schizophrenia and bipolar disorder cases compared to control group

b. IgG titer (schizophrenia vs. their close relatives & healthy controls)

No difference in IgG antibody titers to *T. gondii* in patients with schizophrenia compared with their close relatives or healthy controls (Table 3.8). No statistically significant difference in IgG titers between seropostive schizophrenia cases and their close relative control (χ^2=3.3, p=0.35). Similarly, no difference in IgG titers between seropositive patients with schizophrenia compared to healthy controls (χ^2=0.437, p=0.93).

Table 3.8. Serointensity of IgG antibodies to *T. gondii* in individuals with schizophrenia compared with schizophrenia close relatives and healthy controls

	Titer categories of IgG antibodies to *T. gondii* in IU/ml				
Study subjects	10-50	51-100	101-200	>200	Total
Schizophrenia	46	76	52	35	209
Close relatives control	45	49	50	33	177
Healthy control	13	21	18	10	62

c. IgG titer (bipolar disorder vs. their close relatives and healthy controls)

There was no difference in IgG titers in patients with bipolar disorder compared with their close relatives or healthy controls (Table 3.9). IgG titers to *T. gondii* did not differ significantly between seropostive patients with bipolar disorder and their close relatives (χ^2=1.6, p=0.66). In addition, no difference in IgG titers between seropostive bipolar disorder cases compared to healthy controls (χ^2=0.86, p=0.83).

Table 3.9. IgG titers against *T. gondii* between seropositive bipolar disorder cases and their close relatives and healthy controls.

	Titer categories of IgG antibodies to *T. gondii*				
Study subjects	10-50	51-100	101- 200	>200	Total
Bipolar disorders	38	51	41	33	163
Close relatives controls	21	39	24	17	107
Healthy controls	13	21	18	10	62

d. Comparison of IgG titers in age categories between schizophrenia cases and control group

There was no significant difference in IgG serotiter in age categories between schizophrenia cases and control group (all P> 0.05) (Table 3.10).

Table 3.10. Comparison of IgG titers to *T. gondii* with age groups (schizophrenia cases vs. controls)

A. IgG titer: 10-50 I.U/ml

Age groups	Schizophrenia		Controls		χ^2	P-value
	No. tested	No. (%)	No. tested	No. (%)		
15-25	38	12(31.6)	59	12((20.3)	1.57	0.21
26-36	108	19(17.6)	85	19(22.4)	0.73	0.39
37-47	59	13(22)	75	15(20)	0.083	0.77
48-58	9	2(22.2)	88	20(22.7)	0.001	0.97
59-69	0	0	39	9		
70-80	0	0	17	4		

A. IgG titer: 51-100 I.U/ml

Age groups	Schizophrenia		Controls		χ^2	P-value
	No. tested	No. (%)	No. tested	No. (%)		
15-25	38	12(31.6)	59	18((30.5)	0.012	0.91
26-36	108	39(36.1)	85	25(29.4)	0.96	0.33
37-47	59	21(35.6)	75	22(29.3)	0.59	0.44
48-58	9	4(44.4)	88	24(27.2)	0.48	0.49
59-69	0	0	39	15		
70-80	0	0	17	5		

B. IgG titer: 101-200 I.U/ml

Age groups	Schizophrenia		Controls		χ^2	P-value
	No. tested	No. (%)	No. tested	No. (%)		
15-25	38	8(21)	59	11((18.6)	0.085	0.77
26-36	108	26(24.1)	85	18(21.1)	0.23	0.63
37-47	59	15(25.4)	75	23(30.7)	0.45	0.5
48-58	9	2(22.2)	88	24(27.2)	0.005	0.94
59-69	0	0	39	9		
70-80	0	0	17	7		

C. IgG titer: > 200 I.U./ml

Age groups	Schizophrenia		Controls		χ^2	P-value
	No. tested	No. (%)	No. tested	No. (%)		
15-25	38	4(10.5)	59	8((13.6)	0.2	0.66
26-36	108	21(19.4)	85	17(20)	0.009	0.92
37-47	59	9(15.3)	75	12(16)	0.014	0.9
48-58	9	1(11.1)	88	18(20.4)	0.054	0.82
59-69	0	0	39	4		
70-80	0	0	17	1		

e. Comparison of IgG titers in age categories between bipolar disorder cases and control subjects

No difference in serotiters of IgG antibodies to *T. gondii* in age categories between patients with bipolar disorder and control group (all P>0.005) (Table 3.11).

Table 3.11. Comparison IgG serotiters with age categories (bipolar disorder vs. controls)

A. IgG titer: 10-50 IU/ml

Age groups	Bipolar disorder		Controls		χ^2	P-value
	No. tested	No. (%)	No. tested	No. (%)		
15-25	44	12(27.3)	59	12((20.3)	0.68	0.41
26-36	77	18(23.4)	85	19(22.3)	0.02	0.88
37-47	46	7(15.2)	75	15(20)	0.44	0.5
48-58	4	1(25)	88	20(22.7)	0.25	0.62
59-69	0	0	39			
70-80	0	0	17			

B. IgG titer: 51-100 IU/ml

Age groups	Bipolar disorder		Controls		χ^2	P-value
	No. tested	No. (%)	No. tested	No. (%)		
15-25	44	9(20.4)	59	18((30.5)	1.32	0.25
26-36	77	29(37.7)	85	25(29.4)	1.24	0.27
37-47	46	13(28.3)	75	22(29.3)	0.02	0.9
48-58	4	0	88	24(27.3)	0.4	0.53
59-69	0	0	39			
70-80	0	0	17			

C. IgG titer: 101-200 I.U/ml

Age groups	Bipolar disorder		Controls		χ^2	P-value
	No. tested	No. (%)	No. tested	No. (%)		
15-25	44	7(16)	59	11((18.6)	0.13	0.72
26-36	77	20(26)	85	18(21.2)	0.52	0.47
37-47	46	12(26.1)	75	23(30.7)	0.29	0.59
48-58	4	2(50)	88	24(27.3)	0.18	0.67
59-69	0	0	39			
70-80	0	0	17			

D. IgG titer: > 200 I.U./ml

Age groups	Bipolar disorder		Controls		χ^2	P-value
	No. tested	No. (%)	No. tested	No. (%)		
15-25	44	10(22.7)	59	8((13.6)	1.45	0.23
26-36	77	9(11.7)	85	17(20)	2.1	0.15
37-47	46	13(28.3)	75	12(16)	2.62	0.1
48-58	4	1(25)	88	18(20.4)	0.17	0.68
59-69	0	0	39			
70-80	0	0	17			

CHAPTER IV: DISCUSSION

The ubiquitous protozoan parasite, *Toxoplasma gondii* has emerged as a candidate infectious agent as a possible cause of some cases of schizophrenia or other mental illnesses as the parasite establish persistent infection within the central nervous system and can cause prenatal infection. This study was undertaken to prove the association between *Toxoplasma gondii* infection and schizophrenia and bipolar disorder diseases.

Most of patients with schizophrenia or bipolar disorder involved in this study had age distribution in age categories of 26-36 years (Table 3.1). This is in line with the common age of onset of these diseases (late adolescence or early adulthood) (Wikipedia, 2009; MedicineNet.com, 2009).

In our study population high prevalence (95.2%) of IgG antibodies to *T. gondii* was demonstrated (Table 3.2 and 3.3). This prevalence was higher than some of the previous studies done in Ethiopia to determine seroprevalence of *T. gondii* infection (Guebre-Xabier *et al*., 1993; Eshete, 1994; Woldemichael *et al.,* 2003; Negash *et al*., 2008). The relative increase of seroprevalence in our study population might associate with habit of having raw meat in the study area or the relative low altitude of Meskan and Mareko (Butajira) district might favor the sporulation and survival of the oocysts that can increase transmission of the parasite to human. The prevalence in our overall study subjects was also comparable to some seroprevalence studies in Ethiopia (Teshome *et al*., 2009; Shimelis *et al*., 2009).

Seroprevalence of *T. gondii* in our study population was higher than some of the prevalence studies conducted in different countries (Jones *et al*., 2001**;** Fan *et al*., 2002; Rosso *et al*., 2008). The prevalence of *T. gondii* infection is expected to be very high in Ethiopia in which the risk factors associated to *Toxoplasma* infection such as raw or undercooked meat consumption and presence of cats are common (Negash *et al*.,2008).
Although *T. gondii* infection is high in Ethiopia, schizophrenia or other psychosis has not been found to be unusually prevalent in Ethiopia. Some possible explanations are: Given the high prevalence of *Toxoplasma* infection, *Toxoplasma* seropostivity has a relatively low predictive value for the development of schizophrenia, and most individuals who are

Toxoplasma seropostive do not have manifestation of this disorder (Torrey *et al*., 2007). Some individuals who are exposed to *T. gondii* are expected to develop schizophrenia or other mental illnesses as the outcome of *T. gondii* infection differs based on genetic susceptibility, parasite strain difference, route of infection, and timing of infection (e.g. in utero, early postnatal, childhood, adulthood) (Caruthers and Suzuki, 2007).

In this study the seroprevalance of IgG antibodies against *Toxoplasma* increases as the age of individual's increases in both cases and controls (Table 3.4 and Table 3.5**)**. This is consistent with other findings indicated that the frequency of *Toxoplasma* infection increases with older age groups, as chance of individuals exposed to one of the infective form of the parasite increase as their age increases (Spanding *et al*., 2005; Rosso *et al*., 2008).

Among *Toxoplasma* seropostive cases and controls involved in this study, there was no significant difference in seroprevalence of IgG antibodies to *T. gondii* in both sexes within each group of the study subjects (Table 3.6). This is in contrast to the finding reported by Dickerson *et al*. 2007 that showed an increase *Toxoplasma* seropostivity in females than males in schizophrenia cases.

An increase in seroprevalence in male compared to female in all study subjects taken together in this study was in agreement with Shimelis *et al*., 2009 study conducted in Ethiopia. This finding in our study was in contrast to some population based studies (Fan *et al*., 2002; Silva *et al*., 2008) that have indicated no difference in seroprevalence between the sexes. Thus, further studies may require determining the risk factors associated to *Toxoplasma* infection between males and females in Ethiopia.

In this study prevalence of IgG antibodies to *T. gondii* in individuals with schizophrenia was higher than the control group (Table 3.4). This suggests that infection with *T. gondii* may confer a risk factor for schizophrenia. Thus, some individuals exposed to *T. gondii* may have more likely to get schizophrenia than individuals not exposed to the parasite although the outcome of the infection could differs based on genetic susceptibility, parasite strain difference, route of infection, and timing of infection (Torrey *et al*.,2007; Caruthers and Suzuki, 2007).

An increase in prevalence of antibodies to *Toxoplasma* in individuals with schizophrenia in our study is consistent with previous studies conducted in some countries and summarized in the Meta-analysis (Torrey *et al*., 2007). An increase in seroprevalence of *T. gondii* in schizophrenia cases in this study was also in agreement with studies conducted in Turkey (Cetinkaya *et al*., 2007), Iran (Mokhtari and Mokhtari, 2006) and Northern Mexico City (Alvarado-Esquivel *et al*., 2006). An increase in prevalence of IgG antibodies to *T. gondii* in patients with schizophrenia compared with control group in our study is also consistent with other studies linking *Toxoplasma* to schizophrenia. Increased maternal exposure to toxoplasmosis (OR=2.61) and risk of schizophrenia in adult offspring (Brown *et al*., 2005) and a study on new born sera for exposure to *T. gondii* (OR=1.79) who later developed schizophrenia spectrum disorder (Mortensen *et al*., 2007).

In this study, patients with bipolar disorder had slight increase in prevalence of IgG antibodies to *T. gondii* than the control group (Table 3.5). However, the difference in seroprevalence did not show statistically significant difference. Thus, this finding indicates that *Toxoplasma* infection might not be a risk factor for bipolar disorder disease. Reports on the prevalence of *T. gondii* infection in patients with bipolar disorder are scarce. Absence of seroprevalence difference in IgG antibodies to *T. gondii* in patients with bipolar disorder compared to control subjects is consistent with study done in a Northern Mexico City (Alvarado-Esquivel *et al*. 2006).

Patients with schizophrenia and bipolar disorder involved in our study did not have significant difference in IgG titers to *T. gondii* compared with their close relatives control and/ or non relative healthy control recruited from neighborhoods (Figure 3.2 and Table 3.8). In addition, schizophrenia and bipolar disorder cases did not differ in their IgG serotiters in different age group compared with control subjects. Absence of significant difference in IgG serotiters between patients with schizophrenia and control subjects in this study is in contrast to some studies that showed patients with first onset schizophrenia had significantly increased levels of antibodies to *T. gondii* compared with control subjects (Yolken *et al*., 2001; Wang *et al.*, 2006; Hinze-Selch *et al*., 2007).

Patients with schizophrenia and bipolar disorder involved in our study are participant of Butajira study on the course and outcome of schizophrenia and bipolar disorders and they have been treated with antipsychotic and mood stabilizing drugs for variable period of time before they gave sera for the genetic study. Thus, absence of association in level of antibodies to *T. gondii* in our study participants might be because of the patients have been in antipsychotic treatment for some period of time before they were enrolled in the genetic study.

There was difference in level of antibodies to *T. gondii* in antipsychotic untreated first-episode schizophrenia cases and these who had received some treatment. The antibody levels for treated group were lower than the never treated group, suggesting that antipsychotic medication may have decreased the antibody levels (Torrey and Yolken, 2003). There was an association between the use of immunomodulating antipsychotics and low titers of antibodies against *Toxoplasma* (Hinze-Selch *et al*., 2007).

In study of Leweke *et al*. (2004) untreated individuals with recent onset schizophrenia had significantly increased levels of serum and CSF IgG antibody to *Toxoplasma gondii* compared to controls. They also found that treatment status had a major effect on the levels of antibodies in their study population. Individuals who were receiving treatment had lower levels of antibodies to *Toxoplasma gondii*. The level of antibodies to *Toxoplasma* measured in treated individuals did not differ from the levels measured in controls. No evidence of increased levels of antibodies to *T. gondii* in populations of individuals with chronic forms of schizophrenia compared to control group, which indicates that differences between patients and control subjects may be most marked at the beginning of the illness (Yolken *et al*., 2001).

Individuals with schizophrenia and bipolar disorder involved in our study have the likelihood of becoming infected with *T. gondii* either prior to or after onset of the diseases. The problem to determine the exact timing of the parasite infection has been one draw back in defining the relationship between *T. gondii* and neuropsychiatric diseases.

In our study, post-onset *T. gondii* infection in our study cases seems unlikely in view of similar findings in first episode schizophrenia patients who had not had previous

hospitalization and were unlikely to have undergone unusual environmental exposures after illness onset (Torrey *et al*., 2007). Post-onset *T. gondii* infection may also seem unlikely that similar finding reported from maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring (Brown *et al*., 2005) and increased antibodies to *Toxoplasma* in new born sera and risk of schizophrenia in adulthood (Mortensen *et al*.,2007).

LIMITATIONS OF THE STUDY

- The study subjects were on antipsychotic treatment for variable period of time which might decrease the level of antibody against the parasite.
- Study subjects were not age and sex matched.
- Study subjects were not screened for their HIV status, presence of the virus in some individuals might decrease the level of IgG antibody to *Toxoplasma*.
- Shortage of ANTI-TOXO-IgG test kits.

CONCLUSIONS

In the present study, individuals with schizophrenia had a significantly higher prevalence of *T. gondii* infection than the control group. Thus, this study gives further weight to the hypothesis that exposure to *Toxoplasma* may be risk factor for schizophrenia.

Individuals with bipolar disorder had no statistically significant difference in prevalence of IgG antibodies to *T. gondii* compared with control population. Hence, this study indicates *T. gondii* infection might not be a risk factor for bipolar disorder disease. No significant difference in IgG titers to *Toxoplasma* between the chronic schizophrenia patients and control subjects. Similarly, no difference in IgG titers to *Toxoplasma* between the chronic bipolar disorder cases and control population. Antipsychotic and mood stabilizing drugs might decrease IgG titers in our study population of the chronic schizophrenia and bipolar disorder cases.

RECOMMENDATIONS

It is important to determine

- ✓ anti-*Toxoplasma* antibodies level in first episode psychosis and in the same individuals in chronic course by follow up to speculate decrease in antibodies level caused by antipsychotic or mood stabilizer drugs might improve the clinical course in the patients.

- ✓ seroprevalence difference in sex and risk factors for exposure to *T. gondii* in population based studies in Ethiopia.

- ✓ the age at which most people get *T. gondii* infection.

- ✓ types of *T. gondii* strains found in Ethiopia.

REFERENCES

Ajioka JW, Fitzpatrick JM, Reitter CP (2001). Ultrastructure of a *Toxoplasma gondii* tachyzoite. http://www-ermm.cbcu.cam.ac.uk. 17 February 2009.

Alvarado-Esquivel C, Alanis-Quinones OP, Arreola-Valenzuela MA, Rodriguez-Briones A, Piedra-Nevarez LJ, Duran-Morales E, Estrada-Martinez S, Martinez- rcia A and Liesenfeld (2006). Seroepidemiology of *Toxoplasma gondii* infection in psychiatric inpatients in a northern Mexican city. *BioMed Central* **6:**178-184.

Barragan A, Hitziger N (2008). Transepithelial migration by *Toxoplasma*. *Bioscience and Springer Science* **47**:198-207.

Boin F, Zanardini R, Pioli R, Altamura CA, Maes M, Gennarelli L (2001). Association between G 308 tumor necrosis factor alpha genes polymorphism and schizophrenia. *Mol Psychiatry* **6**:79-82.

Black MW, Boothroyd JC (2000). Lytic cycle of *Toxoplasma gondii. Microbiol. Mol. Biol. Rev.* **64**:607-623.

Brown AS, Catherine A, Schaefer, Quesenberry CP, Liu L, Babulas VP, Susser ES (2005). Maternal exposure to toxoplasmosis and risk of Schizophrenia in adult offspring. *Am J Psychiatry* **162**:767- 773.

Brown AS (2006). Prenatal infection as a risk factor for schizophrenia. *Schizophr Bull*. **32**:200-202.

Calderaro A, Peruzzi S, Piccolo G, Gorrini C, Montecchini S, Rossi S, Chezzi C, Dettori G (2009). Laboratory diagnosis of *Toxoplasma gondii* infection. *Int J Med Sci.* **6**:135-136.

Caruthers VB, Suzuki Y (2007). Effects of *Toxoplasma gondii* infection on the brain. *Schizophr Bull*. **33**:745-751.

CDC, Centers for Disease Control and Prevention (2009).*Toxoplasma gondii* laboratory diagnosis. http:/www.cdc.gov/toxoplasmosis/diagnosis.html. 5 March 2009.

Cetinkaya Z, Yazar S, Gecici O, Namli MN (2007). Anti-*Toxoplasma gondii* antibodies in patients with schizophrenia preliminary findings in a Turkish simple. *Schizophr Bull*.doi:10.1093/schbul/sbm021.

Channon JY, Seguin RM, Lloyd H. Kasper LH (2000). Differential Infectivity and Division of *Toxoplasma gondii* in Human Peripheral Blood Leukocytes. *Infect Immun* 68: 4822-4826.

Colombo FA, Vidal JE, Augesto C, Oliverra PD, Hernandez AV, Bonasser-Filho F,Nogueira RS, Focaccia R, Pereira-Chioccola VL (2005). Diagnosis of cerebral toxoplasmosis in AIDS patients in Brazil. *J Clin Microbiol* **43**:5044-5047.

Dalgic N (2008). Congenital *Toxoplasma gondii* infection. *Marmara Medical Journal* **21**:089-101

Denkers EY, Gazzinelli RT (1998). Regulation and function of T-cell-mediated immunity during *Toxoplasma gondii* infection. *Clin Microbiol Rev* **11**:569-588.

Dickerson F, Boronow J, Stallings C, Origoni A, Yolken RH (2007). *Toxoplasma gondii* in individuals with schizophrenia: Association with clinical and demographic factors and with mortality. *Schizophr Bull* **33**:737-740.

Dubey JP, Lindsay DS, Speer CA (1998). Structures of *Toxoplasma gondii* Tachyzoites, Bradyzoites, and Sporozoites and Biology and Development of Tissue Cysts. *Clin Microbiol Rev* **11**: 267-299.

Eshete H (1994). Some notes on toxoplasmosis in pregnant women in Addis Ababa. *Ethiopian Med J* **32**:135-136.

Fan CK, Su KE, Wu GH, Chiou HY (2002). Seroepidemiology of *Toxoplasma gondii* infection among two mountain Aboriginal populations and Southeast Asian laborers in Taiwan. *J Parasitol* **88 (2)**:411-414.

Fekadu A ,Shibre T, Alem A, Kebede D, Kebrab S, Negash A, Owen M (2004). High prevalence of bipolar disorders among an isolated population of the Zeway Island in Ethiopia. *J Affect Disord*. **80**:1-10

Fekadu A, Desta M, Alem A, Prince M (2007). A descriptive analysis of admissions to Amanuel Psychiatric Hospital in Ethiopia. *Ethiop J Health Dev.* **21(2)**:173-178.

Filisetti D, Candolfi E (2004). Immune response to *Toxoplasma gondii*. *Ann Ist Super Sanità* **40**:71-80.

Flegr J, Zitkova S, Kodym P, Frynta D (1996). Induction of changes in human behavior by the parasitic protozoan *Toxoplasma gondii*. *Parastiol* **113**:49-54.

Flegr J, Havlicek J, Kodym P, Maly M, Smahel Z(2002). Increased risk of traffic accidents in subjects with latent toxoplasmosis: a reterospective case-control study. *BMC Infect Dis* **2**:1-6.

Flegr J, Preiss M, Klose J, Havlı´cˇek J, kova VM, Kodym P (2003). Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite *Toxoplasma gondii*. Dopamine, a missing link between schizophrenia and toxoplasmosis. *Biol Psychol* **63**: 253- 268.

Flegr J (2005). Probable Neuroimmunological link between *Toxoplasma* and *Cytomegalovirus* infections and personality changes in the human host. *BMC Infect Dis* **5**:54-63.

Flegr J (2007). Effect of *Toxopalsma* on human behavior. *Schizophr Bull.* **33**:757-760.

Fuentes I, Rubio JM, Ramírez C, Alvar J (2001). Genotypic Characterization of *Toxoplasma gondii* strains associated with human toxoplasmosis in Spain: Direct analysis from clinical Samples. *Clin Microbiol*. **39**(**4**): 1566–1570.

Fujigaki S, Saito K, Takemura M, Maekawa N, Yamada Y, Wada H, Seishima M (2002). L-Tryptophan-L-kynurenine pathway metabolism accelerated by *Toxoplasma* g*ondii*. *Infect Immun*. **70**: 779-786.

Guebre-Xabier M, Nurilign A, Gebre-Hiwot A, Hailu A, Sissay Y, Getachew E, Frommel D (1993). Sero-epidemiological survey of *Toxoplasma gondii* infection in Ethiopia. *Ethiop Med J*. **31(3)**:201-8.

Guerrant RL, Walker DH, Weller PF (2006). *Tropical infectious disease*. **In**: Systemic coccidia (Toxoplasmosis), Schwartzman JD and Maguire JH 2nd edn, Vol 2, Elsevier Inc, pp. 1141-1148.

Halonen SK, Lyman WD, Chiu FC (1996). Growth and development of *Toxoplasma gondii* in human neurons and astrocytes. *J Neuropathol Exp Neurol.* **55(11)**:1150-6.

Hammouda NA, Ibrahim IR, Elkerdany ED, Negm AY, Allam SR (2001). Experimental conversion of virulent RH *Toxoplasma gondii* tachyzoites in vitro. *East Mediterr Health J* **7:**181-188.

Heyes MP, Chen CY, Major EO, Salto K (1997). Different kynurenine pathway enzymes limit quinolinic acid formation by various human cells. *Biochem J*. **326**:351- 356.

Hinze-Selch D (2002). Infection, treatment and immune response in patients with bipolar disorder versus patients with major depression, schizophrenia or healthy controls. *Bipolar Disord.* **4**: 81–83.

Hinze-Selch D, Daubener W, Eggert L, Erdag S, Stoltenberg R, wilms SA (2007). Controlled prospective study of *Toxoplasma gondii* infection in Individuals with schizophrenia: Beyond seroprevalence. *Schizophr Bull.* **33**:782-788.

Howe DK, Sibley LD (1995). *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *J Infect Dis.* **172**: 1561-1566.

Jackson MH, Hutchison WM (1998). The prevalence and source of *Toxoplasma* infection in the environment. *Adv Parasitol* **28**:55- 36.

Jamieson SE, Cordell H, Petersen E, McLeod R, Gilbert RE, Blackwell JM (2009). Host genetic and epigenetic factors in toxoplasmosis. *Mem Inst Oswaldo Cruz* **104**(**2**): 162-169.

Jones-Brando L, Torrey EF, Yolken R (2003). Drugs used in the treatment of schizophrenia and bipolar disorder inhibit the replication of *Toxoplasma gondii. Schizophr Res* **62**:237-244.

Jones JF, Kruszon-Moran D, Wilson M, McQuillan G, Navin T, McAuley JB. (2001). *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. *Am J Epidemiol.* **15**:154(4):357-65.

Kebede D, Alem A, Shlbre T, Negase A, Deyassa N, Beyro T (2004). Sociodemographic correlates of schizophrenia in a rural population of Ethiopia. *J Affect Disord.* **69**:133-141.

Kebede D, Alem A, Shibre T., Negash A, Fekadu D, Deyassa N, Jacobson L, Kullgren G (2003). Onset and clinical course of schizophrenia in Butajir Ethiopia: A community based study. *Soc Psychiatry Psychiatric Epidemiol* **38**:625-631

Kebede D, Alem A, Shibre T, Negash A, Deyassa N, Beyero T (2005). Socio-Demographic correlates of bipolar disorder in Butajira, rural Ethiopia. *East Africa Med J*. **82**:34-39

Lang E, Puls I, Mller DJ, Selebohm NS, Gallinat J (2007). Molecular mechanisms of schizophrenia. *Cell physiol biochem*. **20**:687-702.

Lappalainen M, Hedman K (2004). Serodiagnosis of toxoplasmosis. The impact of measurement of IgG avidity.*Ann Ist Super Sanità* **40**:81-88.

Leweke FM, Gerth CW, Koethe D, Klosterkötter J, Ruslanova I, Krivogorsky B, Torrey EF, Yolken RH (2004). Antibodies to infectious agents in individuals with recent onset schizophrenia. *Eur Arch Psychiatry Clin Neurosci*. 254(1):4-8.

Long PW (2005). Bipolar I disorder. http://www.mentalhealth.com/dis/p20-md02.html. 19 March 2009.

Mayer U, Feldon J, Yee BK (2008). A review of the fetal brain cytokine imbalance hypothesis of schizophrenia. *Schizophr Bull*. Schbul/sbn022.

Metastatio A, Bahn S (2008).Utility of biomarkers to improve the diagnosis and treatment of schizophrenia. *Future Neurol*.**3** (**6**):619-622.

MedicineNet.com(2009). Schizophrenia symptoms, causes, diagnosis and treatment. http://www.medicinenet.com/schizophrenia/index.htm

Müller-Oerlinghausen B, Berghöfer A, Bauer M (2009). Bipolar disorder. *Lancet* **359**: 241-247.

Mokhtari M and Mokhtari M (2006).*Toxoplasma gondii* and schizophrenia. *Ann Gen Psychiatry* **5**:S169-S170.

Mortesen PB, Nørgaard-Pedersen B, Berit L, Waltoft, Sorensen TL, Hougaard D, Yolken RH (2007). *Toxoplasma gondii* as a Risk Factor for Early-Onset Schizophrenia: Analysis of Filter Paper Blood Samples Obtained at Birth. *Biol Psychiatry* **61:** 688-693.

Mortensen PB, Pedersen CB, Melbye M, Mors O, Ewald H *(*2003). Individual and Familial Risk Factors for Bipolar Affective Disorders in Denmark**.** *Arch Gen Psychiatry* **60**:1209-1215.

Montoya JG, Remington JS (2008)**.** Management ⑥ *Toxoplasma gondii* infection during Pregnancy. *Clin Infect Dis* **47**:554–566.

National Institute of Mental Health (NIMH) (2002). http://www.nimh.nih.gov. 10 March 2009.

Negash T, Tilahun G, Medhin G (2008). Seroprevalence of *Toxoplasma gondii* in Nazaret town, Ethiopia. *East Afr Med J* **5***:*211-214.

Novotna M, Hanusova J, Klose J, Preiss M, Havlicek J, Roubalova K, and Flegr J (2005). Probable Neuroimmunological link between *Toxoplasma* and *Cytomegalovirus* infections and personality changes in the human host. *BMC Infect Dis.***5**:54-63.

Oliver F, Lorraine JB, Christine LM, Robert Y, Wolfgang S (2006). Altered transcriptional activity of human endogenous retroviruses in neuroepithelial cells after infection with *Toxoplasma gondii*. *J Infect Dis* **194**:1447-1449.

OPHCC, Office of Population and Housing Census Commission (1994). The 1994 Population and Housing Census of Ethiopia. Results for Southern Nationalities and Peoples' Region. Volume 1: Part V.A bridged Statistical Report. Addis Ababa.

Palo Alto Medical Foundation (2008). Laboratory Tests for the Diagnosis of Toxoplasmosis: A Guide for clinicians. www.pamf.org/serology/clinianguide.html. 8 March 2009.

Rantakallio P, Jones P, Moring J, Von Wendt L (1997). Association between central nervous system infections during childhood and adult onset schizophrenia and other psychoses: a 28-year follow-up. *Int J Epidemiol.* **26**(**4**):837-43.

Remington JS, Thulliez P, Jose G. Montoya JG (2004). Recent Developments for Diagnosis of Toxoplasmosis. *J Clin Microbiol* **42**: 941-945.

Robertson GS, Hori SE, Powell KJ (2006). Schizophrenia: an integrative approach to modelling a complex disorder. *J Psychiatry Neurosci*. **31**: 157–167.

Rosso F, Les JT, Agudelo A, Villalobos C, Chaves JA, Tunubala GA, Messa A, Remington JS, Montoya JG (2008). Prevalence of Infection with *Toxoplasma gondii* among Pregnant Women in Cali, Colombia, South America. *Am. J. Trop. Med. Hyg.*, **78(3):**504-508

Schwarcz R, Hunter CA (2007). *Toxoplasma gondii* and schizophrenia: Linkage through Astrocyte-derived kynurenic acid. *Schizophr Bull*. **33**:652-653.

Silva CSP, Neves ED, Benchimol EI, Moraes DRD (2008). Postnatal acquired toxoplasmosis patients in an infectious diseases reference center. Braz J Infect Dis. doi: 10.1590/S1413-86702008000500016.

Shimelis T, Tebeje M, Tadesse E, Tegbaru B, Terefe A (2009). Sero-prevalence of latent *Toxoplasma gondii* infection among HIV-infected and HIV-uninfected people in Addis Ababa, Ethiopia: A comparative cross-sectional study. *BMC Res* **2:**1-5.

Spanding SM, Reis Amedoeira MR, Klein CH, Ribeiro LC (2005). Serological screening and toxoplasmosis exposure factors among pregnant women in south of Brazil. *Rev Soc Bras Med Trop*. **38**:1-15.

Storch GA, Clifford DB, Sibley LD (2005). Genotyping of *Toxoplasma gondii* Strains from Immunocompromised Patients Reveals High Prevalence of Type I Strains. *J Clin Microbiol* **43**: 5881–5887.

Teshome S, Atalay A, Abdulreshid A, Mesfin A, Teferra B, Girmay M, Negusse D, Alemayehu N, Abebaw F (2009). Trimethoprim as Adjuvant Treatment in Schizophrenia: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial. *Schizophr Bull.* doi:10.1093/schbul/sbn191.

Torrey EF, Miller J, Rawlings R, Yolken RH (1995). Seasonality of births in schizophrenia and bipolar disorder: a review of the literature. *Schizophr Res* **28**:1-38.

Torrey EF, Yolken RH (2003). *Toxoplasma gondii* and schizophrenia. *Emerg Infect Dis*. **9**:1375-1380.

Torrey EF, J. Bartko J, Lun ZR, Yolken RH (2007). Antibodies to *Toxoplasma gondii* in patient with schizophrenia: A Meta -analysis. *Schizophr Bull*. **33**:729-736.

Torrey EF, Yolken RH (2007). Schizophrenia and Toxoplasmosis. *Schizophr Bull.* **33**:727-728.

Viro-Immuno Labor-Diagnostika GmbH (2007). VIR-ELISA Anti-Toxo-IgG. Http:www.viro-immun.de/ 25 February 2009.

Vays A, Kim SK, Giacomini N, Boothroyd JC, Sapolsky RM (2007). Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc Natl Acad Sci USA* 104: 6442-6447.

Wang HL, Wang GH, Li QY, Shu C, Jiang MS, Guo Y (2006). Prevalence of *Toxoplasma* infection in first-episode schizophrenia and comparison between *Toxoplasma*-seropositive and *Toxoplasma*-seronegative schizophrenia. *Acta Psychiatr scand*. **114**:40–48.

Webster JP, Lamberton PHL, Donnelly CA, Torrey EF (2006). Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood- stabilizer and anti-parasite medication on *Toxoplasma gondii*'s ability to alter host behavior. *Proc Biol Sci*. **273**(**1589**): 1023–1030.

Webster JP (2007). The effect of *Toxopasma gondii* on animal behavior: Playing cat and Mouse. *Schizophr Bull*. **33**:752-756.

Wikipedia, the free encyclopedia (2009). Bipolar disorder. "http://en.wikipedia.org/wiki/Bipolar_disorder" 13 March 2009.

Woldemichal T, Fontanet A, Shalu T, Gilis H, Messele T, Dewit T, Yenench H, Coutinho R, Gool TY (2003).Evaluation of the Eiken latex agglutination test for anti-*Toxoplasma* antibodies and seroprevalence of *Toxoplasma* infection among factory workers in Addis Ababa Ethiopia. *Trans R Soc Trop Med Hyg* **92**:401- 403.

Yasodhara P, Ramalakshmi BA, Sarma M KJ (2001). A new approach to differentiate recent vs chronic *Toxoplasma* infection: Avidity ELISA in *Toxoplasma* serology.*Indian J Med Microbiol* **19**:145-148.

Yolken RH, Torrey EF (1995). Viruses, Schizophrenia, and Bipolar Disorder. *Clin Microbiol Rev* **8:** 131–145.

Yolken RH, Bachmann S, Rouslanova I, Glen Ford EL, Torrey EF, Schroeder J (2001). Antibodies to *Toxoplasma gondii* in Individuals with First-Episode Schizophrenia. *Clin Infect Dis* **32**:842–4.

Yolken RH, Torrey EF (2006). Infectious Agents in Schizophrenia and bipolar disorder. *Psychiatric Times* **23**:1-2.

Yolken RH, Torrey EF (2008). Are some cases of psychosis caused by microbial agents? A review of the evidence. *Mol Psychiatry* **13**: 470–479.

Appendix I: Data Collection Format for Investigation of Toxoplasmosis from Stored Sera Collected from Cases of Schizophrenia, Bipolar disorders and Control subjects

I.	General Information
001.	Age _____ (yrs)
002.	Sex _____
003.	Address _____
II.	Clinical Data
004.	History of mental illness 1. Yes 2. No
If yes	
005.	Date of diagnosis of mental illness (DD/MM/YY)_____(E.C.)
006.	Diagnosis of mental illness 1. Schizophrenia 2. Bipolar disorders 3. Others
007.	Duration of antipsychotic treatment_____(months)
III.	Laboratory Data
008.	ELISA-Serology for anti-*Toxoplasma gondii* IgG antibody 1. Positive 2. Negative 3. Borderline/Intermediate 4. OD measured—————— 5. Titer _____

Appendix II: Study subject's Information Sheet (English version)

It is remembered that you volunteered to participate in a genetic study about 6 years ago. Part of the blood you gave during that study was stored at AHRI laboratory in Addis Ababa. In this particular study we would like to use the serum, the liquid/watery part of the blood you gave during that study for an etiological research. The serum has been stored for so long because your consent to use it for this purpose was so important.

a. **Purpose:** The purpose of this study is look for possible infectious etiological agents.

b. **Duration:** We will use the serum only once for this particular study and will not use for anything else.

c. **Procedures to be carried on:**

We will do an investigation for toxoplasmosis at AHRI laboratory

d. **Risks associated with the study:** There is no risk of using the serum to you.

e. **Benefits of the study:** There will be no financial or other direct benefit to you. But your consent for us to use the stored serum sample to test for body defense elements against the diseases mentioned above will help us better understand if the diseases have any relationship to schizophrenia and bipolar disorders.

f. **Compensations:** There will be no compensation for using this serum.

g. **Confidentiality of your information**: The results of the lab findings will be kept confidential and could only be accessed by the researchers. There will be no personal information to be attached to your data.

h. **Termination of the study**: We will respect your decision if you later on change your mind and inform us not to use your sample for the test. Your withdrawal of consent will not affect your right to receive medication you used to get free from the project or continue in the cohort.

I would also like to inform you that this study was approved by the FRPC-I of the Medical Faculty, Addis Ababa University and the Ethiopian Science and Technology Agency, Ethical Review Committees. Their address is:

1. Faculty of Medicine Addis Ababa University
   Office of Associate Dean, Postgraduate Programs and Research
   P.O. Box 9086. Addis Ababa, Ethiopia
   Tel. 251-011-551-28-765

2. Ethiopian Science and Technology Agency
P.O.Box. 2490
Tel. 251-011-5511344
e-mail:estc@ethionet.et

The address of the principal investigator:

Geletta Taddele
Department of Microbiology, Immunology and Parasitology
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P.O.Box. 9086, Addis Ababa, Ethiopia
Tel: 0911098594
e-mail: geletta98@ yahoo. com

Appendix II: Study Subjects Information Sheet (Amharic version)

የጥናቱ ተሳታፊዎች የመረጃ መስጪያ ቅፅ

ከ 6 ዓመት በፊት ለዘር ሐረግ ጥናት ለመሳተፍ ፈቃደኛ ሆነው የደም ናሙና የለገሱ መሆኑ ይታወቃል፡፡ በወቅቱ ከወሰድነው የደም ናሙና ጥቂቱን አስቀርተን በአዲስ አበባ አለርት ሆስፒታል ላቦራቶሪ አኑረነዋል፡፡ በዚህ በአሁኑ ጥናት የደም ናሙናዎን ፈሳሽ ወይም ውሃማ ክፍል የአእምሮ ሕመም መነሻ ምክንያት ጥናት ልናደርግበት አስበናል፡፡

ሀ. የጥናቱ አላማ፡-ይህ ጥናት ለአእምሮ ሕመም ምክንያት ሊሆኑ የሚችሉ ኢንፌክሽኖች ካሉ ለማወቅ ይረዳናል፡፡

ለ. የሚፈጀው ጊዜ፡- ይህንን ናሙና ለአንድ ጊዜ ብቻ ለዚህ ጥናት እናውለዋለን፡፡ ከዚህ የተለዬ ለምንም ጉዳይ አንጠቀምበትም፡፡

ሐ. አጠቃቀሙም፡- ቶክሶፕላዝማ ፣**ሳይቶመጋሎ** እና ሔርፐስ የተባሉ ቫይረስ ለማግኘት የሚረዳ የላቦራቶሪ ምርመራ እናደርግበታለን፡፡

መ. ሊደርስ የሚችል አደጋ፡-ይህንን ናሙና በመጠቀማችን በርሶዎ ላይ የሚደርስ ምንም ዓይነት አደጋ የለም፡፡

ሠ. የሚያገኙት ጥቅም፡- ይህንን ናሙና በመጠቀማችን የምስጥዎ ምንም አይነት የገንዘብና ቁሳቁስ ጥቅም አይኖርም፡፡ ይሁን እንጂ በላቦራቶር ተቀምጦ የሚገኘው የደም ናሙናዎን ፈሳሽ ወይም ውሃማ ክፍል ላይ የሰውነት መከላከያ አካል የሆኑ ንጥረ ነገሮች እንዳሉ ለማየት የርሶ ፈቃድ አስፈላጊ ከመሆኑም በላይ ሺዞፍሬኒያና ባይፖላር ዲስፆርደር የተባሉ የአእምሮ ህመም አይነቶች ከኢንፌክሽኖች ጋር ያላቸውን ግንኙነት ለማጥናት ይረዳናል

ረ. ሚስጥራዊነት፡- ከላቦራቶር የሚገኘው ውጤት በሚስጥረ የተጠበቀ ይሆናል፡፡ የግልዎ የሆነ ምንም አይነት መረጃ ከውጤቱ ጋር አይያያዝም፡፡

ሰ. ፈቃደኛነትዎን ስለማቋረጥ፡- ከዚህ ጥናት ራስዎን የማግለልና ናሙናዎን እንዳንጠቀም ፈቃደኝነትዎን የመሠረዝ መብትዎና የተጠበቀ ነው፡፡ በዚህ ጥናት ላይ ፈቃደኛ አለመሆንዎ ከዚህ ቀደም በምንሰጥዎ የህክምና ክትትል አገልግሎት ላይ ምንም ተፅዕኖ አይኖረውም፡፡

ይህ ጥናት በህክምና ፋኪልቲና በኢትዮጵያ ሳይንስና ቴክኖሎጂ ኤጀንሲ የምርምር ኮሚቴዎች ታይቶ የፀደቀ ነው፡፡

አድራሻ ማወቅ ካስፈለገዎ

1. **ህክምና ፋከሊቲ፤አዲስ አበባ** ዩንቨርስቲ

ድህረ ምረቃ ፕሮግራምና ምርምር የተባባሪ ዲን ቢሮ

የመ.ሳ.ቁ. 9086 አዲስ አበባ

ስልክ 251-011-551-28-765

2. **ኢትዮጵያ ሳይንስ ቴክኖሎጂ ኤጀንሲ**

የመ.ሳ.ቁ. 2490 አዲስ አበባ

ስልክ 251-011-551-13-44

ኢሜል estc@ethionet.et

3. **የዋናው ተመራማሪ አድራሻ**

አዲስ አበባ ዩንቨርስቲ ማይክሮባይሎጂ ኢሚኖሎጂና ፓራሲቶሎጂ ት/ ክፍል

የመ.ሳ.ቁ. 9086 አዲስ አበባ

ስልክ 251-91-1408208

ኢሜል geletta98@yahoo.com

AppIendix III: Study subject's Consent form (English version)

Mr/Mrs/ Miss----------

My name is _____

Having read/heard the information about the purpose of this study I would like to ask for your consent to participate in this study – entitled "Analysis of sera for antibodies to *Toxoplasm gondii* in individuals with schizophrenia and bipolar disorders" I would like that you confirm your agreement by signing your name if you agree.

Signature of Study subjects _____Date_____

Signature of the researcher_____ Date _____

Signature of witnesses 1. _____Date _____

2———————————————Date———————

Appendix III: Consent form for study subjects (Amharic Version)

<u>የጥናቱ ተሳታፊዎች የፈቃደኝነት መጠየቂያ ቅጽ</u>

አቶ/ወ/ሮ ----------

እኔ ስሜ ---------- ይባላል፡፡

የሰጠንዎ መረጃ አንብበዎል ወይም ሰምተዎል፡፡ በመሆኑም "የደም ናሙናን ቶክሶፕላዝማ ፣ሳይቶመጋሎ እና ሔርፐስ የተባሉ ህዋሳት ምርምር ጥናት„ ለማዋል ፈቃደኝነትዎን እንጠይቃለን፡፡

ፈቃደኛ ከሆኑ ደግሞ ለጥናቱ የተስማሙ መሆንዎን በፊርማዎ እንዲያረጋግጡልን እንፈልጋለን፡፡

የጥናቱ ተሳታፊ ፊርማ ----------ቀን----------

የተመራማሪው ፊርማ ---------- ቀን ----------

የምስክሮች ፊርማ 1. **----------ቀን ----**

2. **---------- ቀን ----**

Appendix IV: Information Sheet for parents/guardians (English version)

It is remembered that your son/daughter volunteered to participate in a genetic study about 6 years ago. Part of the blood he/she gave during that study was stored at AHRI laboratory in Addis Ababa. In this particular study we would like to use the serum, the liquid/watery part of the blood he/she gave during that study for an etiological research. The serum has been stored for so long because your consent to use it for this purpose was so important.

a. **Purpose**: The purpose of this study is look for possible infectious etiological agents.

b. **Duration**: We will use the serum only once for this particular study and will not use for anything else.

c. Procedures to be carried on: We will do an investigation for toxoplasmosis, at AHRI laboratory

d. **Risks associated with the study**: There is no risk of using the serum to your son/daughter.

e. **Benefits of the study**: There will be no financial or other direct benefit to your son/daughter. But your consent for us to use the stored serum sample to test for body defense elements against the diseases mentioned above will help us better understand if the diseases have any relationship to schizophrenia and bipolar disorders.

f. **Compensations**: There will be no compensation for using this serum.

g. **Confidentiality of your information**: The results of the lab findings will be kept confidential and could only be accessed by the researchers. There will be no personal information to be attached to study subject's data.

h. **Termination of the study**: We will respect your decision if you later on change your mind and inform us not to use the sample for the test. Your withdrawal of consent will not affect the right of the study subject to receive medication he/she used to get free from the project or continue in the cohort.

I would also like to inform you that this study is approved by the FRPC-I of the Medical Faculty, Addis Ababa University and the Ethiopian Science and Technology Agency, Ethical Review Committees. Their address is :

1. Faculty of Medicine Addis Ababa University
   Office of Associate Dean, Postgraduate Programs and Research

P.O. Box 9086. Addis Ababa, Ethiopia

Tel. 251-011-551-28-765

2. Ethiopian Science and Technology Agency

P.O.Box. 2490

Tel. 251-011-5511344

e-mail:estc@ethionet.et

The address of the principal investigator is: Geletta Taddele

Department of Microbiology, Immunology and Parasitology

Faculty of Medicine, Addis Ababa University

P.O.Box. 9086, Addis Ababa, Ethiopia

Tel: 0911098594

e-mail: geletta98@ yahoo .com

Appendix IV: Information Sheet for parents/guardians (Amharic version)

<u>የመረጃ መስጪያ ቅፅ</u>

ከ 6 ዓመት በፊት ለዘር ሐረግ ጥናት ለመሳተፍ ፈቃደኛ ሆነው ልጅዎ የደም ናሙና የሰጠ/ች መሆኑ ይታወቃል፡፡ በወቅቱ ከተወሰደው የደም ናሙና ላይ ፈሳሻማውን ክፍል በአዲስ አበባ አለርት ሆስፒታል ላቦራቶሪ አኑረነዋል፡፡ በዚህ በአሁኑ ጥናት የደም ናሙናውን ፈሳሽ ወይም ውሃማ ክፍል የአእምሮ ሕመም መነሻ ምክንያት ጥናት ልናደርግበት አስበናል፡፡

ሀ. <u>የጥናቱ አላማ</u>፡-ይህ ጥናት ለአእምሮ ሕመም ምክንያት ሊሆኑ የሚችሉ ኢንፌክሽኖች ካሉ ለማወቅ ይረዳናል፡፡

ለ. <u>የሚፈጀው ጊዜ</u>፡- ይህንን ናሙና ለአንድ ጊዜ ብቻ ለዚህ ጥናት እናውለዋለን፡፡ ከዚህ የተለዬ ለምንም ጉዳይ አንጠቀምበትም፡፡

ሐ. <u>አጠቃቀሙም</u>፡- ቶክሶፕላዝማ ፣ሳይቶመጋሎ እና ሔርፐስ የተባሉ ቫይረስ ለማግኘት የሚረዳ የላቦራቶሪ ምርመራ እናደርግበታለን፡፡

መ. <u>ሊደርስ የሚችል አደጋ</u>፡-ይህንን ናሙና በመጠቀማችን በልጅዎ ላይ የሚደርስ ምንም ዓይነት አደጋ የለም፡፡

ሠ. <u>የሚያገኙት ጥቅም</u>፡- ይህንን ናሙና በመጠቀማችን ለልጅዎ የምንሰጠው/የምንሰጣት ምንም አይነት የገንዘብና ቁሳቁስ ጥቅም አይኖርም፡፡ ይሁን እንጂ በላቦራቶር ተቀምጦ የሚገኘው የደም ናሙናዎን ፈሳሽ ወይም ውሃማ ክፍል ላይ የሰውነት መከላከያ አካል የሆኑ ንጥረ ነገሮች እንዳሉ ለማየት የርሶ ፈቃድ አስፈላጊ ከመሆኑም በላይ ሺዞፍሬኒያና ባይፖላር ዲስኦርደር የተባሉ የአእምሮ ህመም አይነቶች ከኢንፌክሽኖች ጋር ያላቸውን ግንኙነት ለማጥናት ይረዳናል

ረ. <u>ሚስጥራዊነት</u>፡- ከላቦራቶር የሚገኘው ውጤት በሚስጥረ የተጠበቀ ይሆናል፡፡ የጥናቱ ተሳታፊዎች የሆነ ምንም አይነት መረጃ ከውጤቱ ጋር አይያያዝም፡፡

ሰ. <u>ፈቃደኛነትዎን ስለማቋረጥ</u>፡- ከዚህ ጥናት ልጅዎን የማግለልና ናሙናውን እንዳንጠቀም ፈቃደኝነትዎን የመሠረዝ መብትዎና የተጠበቀ ነው፡፡ በዚህ ጥናት ላይ ፈቃደኛ አለመሆንዎ ከዚህ ቀደም ለልጅዎ በምንሰጠው/ጣት የህክምና ክትትል አገልግሎት ላይ ምንም ተፅዕኖ አይኖረውም፡፡

ይህ ጥናት በህክምና ፋኪልቲና በኢትዮጵያ ሣይንስና ቴክኖሎጂ ኤጀንሲ የምርምር ኮሚቴዎች ታይቶ የፀደቀ ነው፡፡

አድራሻ ማወቅ ካስፈለገዎ

1. **ህክምና ፋከሊቲ፤አዲስ አበባ** ዩንቨርስቲ

ድህረ ምረቃ ፕሮግራምና ምርምር የተባባሪ ዲን ቢሮ

የመ.ሳ.ቁ. 9086 አዲስ አበባ

ስልክ 251-011-551-28-765

2. ኢትዮጵያ ሳይንስ ቴክኖሎጂ ኤጀንሲ

የመ.ሳ.ቁ. 2490 አዲስ አበባ

ስልክ 251-011-551-13-44

ኢሜል estc@ethionet.et

3. የዋናው ተመራማሪ አድራሻ

ገለታ ታደለ

አዲስ አበባ ዩንቨርስቲ ማይክሮባይሎጂ ኢሚኖሎጂና ፓራሲቶሎጂ ት/ክፍል

የመ.ሳ.ቁ. 9086 አዲስ አበባ

ስልክ 251-0911098594

ኢሜል geletta98@yahoo.com

Appendix V. Consent form for parents/guardians (English version)

Mr/Mrs/ Miss----------

My name is _____

Having read/heard the information about the purpose of this study I would like to ask for your consent to allow your daughter/son to participate in this study – entitled "Analysis of sera for antibodies to *Toxoplasm gondii* in individuals with schizophrenia and bipolar disorders"

I would like that you confirm your agreement by signing your name if you agree.

Signature of parent/guardian _____Date_____

Signature of the researcher_____ Date _____

Signature of witnesses 1. _____Date _____

2.————————————Date————————

Appendix V: Consent form for Parents/ guardians (Amharic Version)

<u>የወላጅ (ጠባቂ) የፈቃደኝነት መጠየቂያ ቅጽ</u>

አቶ/ወ/ሮ ----------

እኔ ስሜ ---------- ይባላል፡፡

የሰጠንዎ መረጃ አንብበዎል ወይም ሰምተዎል፡፡ በመሆኑም የልጅዎን "የደም ናሙናን ቶክሶፕላዝማ ፣ሳይቶመጋሎ እና ሔርፐስ የተባሉ ህዋሳት ምርምር ጥናት„ ለማዋል ፈቃደኝነትዎን እንጠይቃለን፡፡

ፈቃደኛ ከሆኑ ደግሞ ልጅዎ በጥናቱ እንድትሳተፍ/እንዲሳተፍ የተስማሙ መሆንዎን በፊርማዎ እንዲያረጋግጡልን እንፈልጋለን፡፡

የወላጅ (ጠባቂ) ፊርማ ---------- ቀን----------

የተመራማሪው ፊርማ ----------ቀን ----------

የምስክሮች ፊርማ 1. **----------ቀን ---------**

2. **----------ቀን -----**