THE ASSOCIATION BETWEEN *Toxoplasma gondii* INFECTION AND PSYCHIATRIC DISORDERS AMONG PSYCHIATRIC PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

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by

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LIST OF SYMBOLS AND UNITS

-	Negative
+	Positive
%	Percentage
>	More than
≤	Less than or equal to
≥	More than or equal to
µl	Microliter
bp	Base pair
IU/ml	International unit per millilitre
min	Minutes
sec	Seconds
ml	Millilitre
n	Sample size
℃	Degree Celcius
V	Voltage
x g	Gravitational force
µM	Micromole
nmol	Nanomole
ng	Nanogram
mM	Millimolar
□2	Chi square

LIST OF ABBREVIATIONS

AI	Avidity index
AIDS	Acquired immunodeficiency syndrome
bp	Base pair
BD	Bipolar disorders
BPRS	Brief Psychiatric Rating Scale
CDC	Centres for Disease Control and Prevention
DNAs	Deoxyribonucleic acids
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
HAM-D	Hamilton Rating Scale for Depressive disorder
IDO	Indoleamine 2,3-dioxygenase
IFN-ɤ	Interferon-gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IgA	Immunoglobulin A
MDD	Major depressive disorder
MINI	Mini International Neuropsychiatric Interview
mPCR	Multiplex polymerase chain reaction
NHANES	National Health and Nutrition Survey
NHMS	National Health Morbidity Survey
OD	Optical density

OR	Odd ratio
PCR	Polymerase chain reaction
SPSS	Statistical Package for the Social Sciences
TBE	Tris-Borate EDTA
UKMMC	Universiti Kebangsaan Malaysia Medical Centre
UMMC	University of Malaya Medical Centre
USM	Universiti Sains Malaysia
WHO	World Health Organization
YMRS	Young Mania Rating Scale

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HUBUNGAN ANTARA JANGKITAN *Toxoplasma gondii* DAN KECELARUAN PSIKIATRI DALAM KALANGAN PESAKIT PSIKIATRIK DI HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRAK

Jangkitan *Toxoplasma*, yang disebabkan oleh protozoon *Toxoplasma gondii*, adalah salah satu parasit zoonosis yang paling biasa di seluruh dunia. Organisma polisenik heteroksenik fakultatif ini telah dikenalpasti memainkan peranan dalam etiologi pelbagai kecelaruan psikiatri. Kajian keratan rentas komparatif ini bertujuan untuk menyiasat hubungan antara jangkitan *Toxoplasma* dan kecelaruan psikiatri pada pesakit di Hospital Universiti Sains Malaysia, Kelantan. Lima puluh empat pesakit kecelaruan psikiatri daripada setiap kategori (skizofrenia, kecelaruan bipolar, dan kecelaruan kemurungan) dan 54 individu yang sihat telah dianalisa oleh kaedah asai imunoserapan terangkai enzim (ELISA) yang mengesan anti-*T. gondii* immunoglobulin G (IgG). Selepas itu, semua sampel plasma yang positif IgG dianalisis untuk IgM khusus *Toxoplasma*. Aviditi IgG ELISA telah diuji untuk sampel positif *T. gondii* IgM. Kehadiran gen *Toxoplasma* B1 dan kawasan ITS-1 dalam semua asid deoksiribonukleik (DNA) yang diekstrak daripada keseluruhan darah setiap sampel telah dikenal pasti dengan menggunakan tindak balas rantai polimerase (PCR). Maklumat sosiodemografi, manifestasi klinikal, dan faktor tingkah laku pesakit psikiatri dan individu yang sihat telah diperolehi menggunakan borang pengumpulan data. Daripada 54 pesakit dengan kecelaruan kemurungan, 24/54 (44.4%) adalah IgG+/IgM-, dan empat (16.7%) adalah IgG+/IgM+. Indeks aviditi tinggi yang menggambarkan jangkitan lampau selama lebih daripada 20 minggu dilaporkan dalam separuh daripada sampel (50.0%) dan separuh lagi (50.0%) memberikan keputusan indeks aviditi rendah yang menunjukkan kemungkinan jangkitan baru-baru ini dalam

tempoh 20 minggu. Selain itu, 30 (55.6%) pesakit dengan kecelaruan bipolar adalah IgG+/IgM-, lima (16.7%) adalah IgG+/IgM+, dan empat daripada mereka mempunyai indeks aviditi tinggi dan seorang menunjukkan indeks aviditi rendah. Manakala, 29 (53.7%) pesakit skizofrenia adalah IgG+/IgM, 2 (6.9%) adalah IgG+/IgM+, seorang daripada mereka mempunyai indeks aviditi tinggi, dan satu indeks aviditi rendah. Daripada 54 individu yang sihat, 37.0% (20/54) adalah seropositif (IgG+/IgM-) untuk antibodi *T. gondii*. Dalam kajian ini, tiada keputusan positif ditemui untuk kehadiran gen *Toxoplasma* B1 dan kawasan ITS-1. Chi-Square dan regresi logistik berganda membuktikan umur (p=0.031), status perkahwinan (p=0.007), dan pekerjaan (p=0.012) dikaitkan dengan kadar seropositif *Toxoplasma* pada pesakit yang mengalami kecelaruan psikiatri. Hubungan rapat dengan kucing/haiwan peliharaan (p=0.033) dan sentuhan dengan tanah (p=0.012) juga dikaitkan dengan kadar seropositif *Toxoplasma* pada pesakit. Kesimpulannya, penemuan mendedahkan kadar seropositiviti yang berbeza-beza, menunjukkan bahawa individu yang mengalami gangguan psikiatri seperti skizofrenia, kecelaruan bipolar, dan kecelaruan kemurungan mungkin pernah mengalami jangkitan lampau atau baru dengan *T. gondii*. Walau bagaimanapun, kajian tambahan diperlukan untuk menjelaskan hubungan sebab akibat dan mekanisme asas. Ini penting untuk pembangunan intervensi yang disasarkan dan strategi pencegahan, dengan potensi untuk meningkatkan kesihatan mental keseluruhan bagi mereka yang terjejas.

THE ASSOCIATION BETWEEN *Toxoplasma gondii* INFECTION AND PSYCHIATRIC DISORDERS AMONG PSYCHIATRIC PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRACT

Toxoplasma infection, caused by the protozoon *Toxoplasma gondii*, is one of the most common parasitic zoonoses globally. This facultative heteroxenic, polyxenic organism has been identified as playing a role in the etiology of various psychiatric disorders. The present comparative cross-sectional study purposed to investigate the association between *Toxoplasma* infection and psychiatric disorders in patients at Hospital Universiti Sains Malaysia, Kelantan. Fifty-four psychiatric disorder patients from each category (schizophrenia, bipolar disorder, and depressive disorder) and 54 healthy individuals were analyzed by an enzyme-linked immunosorbent assay (ELISA) that detects anti-*T. gondii* immunoglobulin G (IgG). Subsequently, all IgG-positive plasma samples were analyzed for *Toxoplasma*-specific IgM. IgG avidity ELISA was tested for the positive samples of *T. gondii* IgM. The presence of the *Toxoplasma* B1 gene and ITS-1 region was determined in all extracted deoxyribonucleic acids (DNAs) from the whole blood using the polymerase chain reaction (PCR). The sociodemographic, clinical manifestations, and behavioural factors of the psychiatric patients and healthy individuals were assessed using a data collection form. Out of 54 patients with depressive disorder, 24/54 (44.4%) were IgG+/IgM-, and four (16.7%) were IgG+/IgM+. A high avidity index that described a past infection for more than 20 weeks was reported in half of the sample (50.0%) and the other half (50.0%) showed a contradicting result that indicated a possible recent infection within 20 weeks. Moreover, 30/54 (55.6%) patients with bipolar disorder were IgG+/IgM-, five (16.7%) were IgG+/IgM+, and four of them had high avidity

index and one showed a low avidity index. Meanwhile, 29/54 (53.7%) patients with schizophrenia were IgG+/IgM-, 2 (6.9%) were IgG+/IgM+, one of them had a high avidity index, and one low avidity index. Out of 54 healthy individuals, 37.0% (20/54) were seropositive for *T. gondii* antibodies. In this study, no positive results were found for the presence of the *Toxoplasma* B1 gene and ITS-1 region. A Chi-Square and multiple logistic regression proved age (p=0.031), marital status (p=0.007), and employment (p=0.012) were significantly associated with *Toxoplasma* seropositivity rate in patients with psychiatric disorders. Close contact with cats/pets (p=0.033) and contact with soil (p=0.012) also were significantly associated with *Toxoplasma* seropositivity rate in the patients. In conclusion, the findings revealed varying seropositivity rates, indicating that individuals with psychiatric disorders such as schizophrenia, bipolar disorder, and depressive disorder may have experienced both past and potentially recent infections with *T. gondii*. However, additional research is needed to elucidate the causal relationships and underlying mechanisms. This is essential for the development of targeted interventions and preventive strategies, with the potential to enhance overall mental health outcomes for those affected.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Toxoplasma infection is one of the most universal parasite zoonoses. Its causative agent which is *Toxoplasma gondii* is an optionally heteroxenic, polyxenic protozoon. It can infect warm-blooded animals such as humans (Parlog *et al.,* 2015; Bay-richter *et al.,* 2019). *T. gondii* is in the phylum Apicomplexa which is an obligate intracellular eukaryotic protozoan (Tyebji *et al.,* 2019). *T. gondii* can multiply in almost every body tissue (acute phase). During the latent or chronic stage, long-lived cysts in the muscle, liver tissue, and nerve cells will be formed (Lyons *et al.,* 2002). It has been proved that *Toxoplasma* infection is associated with multiple neuropsychiatric conditions, posing interesting questions about the potential role of parasite biology and its relationships between the brain and the immune system that contributes to mental health disorders (Tyebji *et al.,* 2019).

Psychiatric disorders are increasing in developing nations like Malaysia. Based on the National Health and Morbidity Survey (NHMS) 2011, psychiatric disorder affects 12% of Malaysians aged between 18 and 60. The incidence of mental health issues among adolescents showed an increment from 10.7% to 29.2% between 1996 to 2015 (Ahmad *et al.,* 2015).

World Health Organization (WHO) predicted that depressive disorder will cause disease burden by 2030 (Ahmad *et al.,* 2015). It is estimated that there were 350 million individuals affected globally (Ahmad *et al.,* 2015). According to multiple

studies, depressive disorder is the major factor contributing to the burden of chronic disease worldwide. Personality changes and multiple mental disorders which are suicidal tendencies, depressive disorder, bipolar disorder, and schizophrenia were associated with *T. gondii* seropositivity (Henriquez *et al.*, 2009).

Bipolar disorder (BD), which is a chronic disorder, is the main cause of death and disability (Whiteford *et al.,* 2013). Both manic and depressed episodes are prominent in BD. It involves elevated mood and increased energy, resulting in over-activity, difficulty in speaking, and reduced desire for sleep (Marcus *et al*., 2012). A study among psychiatric patients with a primary diagnosis of schizophrenia, depressive disorder, and BD found that they were associated with *T. gondii* infection significantly (Hinze-selch *et al.*, 2010). Another study found a high prevalence of *T. gondii* seropositivity in patients with bipolar disorder history but did not find the same relationship in patients with a history of unipolar depressive disorder (Pearce *et al.*, 2012). However, the relationship between bipolar disorders and depressive disorder with *T. gondii* exposure has not been fully investigated.

The term "schizophrenia" relates to a group of mental disabilities characterized by perception, thinking, affectivity, and behavioural changes. Its etiology is unknown and healing treatments are not available (Tardy *et al.,* 2014). Schizophrenia is a mental disorder commonly found in late adolescence or early adulthood.

1.2 Rationale of the study

In Malaysia, the study of risk factors and the correlation between *T. gondii* infection in psychiatric patients is limited. Most of them are based on exposure to *T. gondii* and the emergence of psychiatric disorders, particularly schizophrenia (Omar *et al.,* 2015; Sutterland *et al.*, 2019). There are few studies on the connection between anti-*T. gondii* IgG titers levels and the possibility of developing other psychiatric disorders (Alvarado-Esquivel *et al.,* 2019; Alvarado-Esquivel *et al.,* 2016; Arling *et al.,* 2009; Gutiérrez-Fernández *et al.,* 2015; Khademvatan *et al.,* 2013; Torrey *et al.,* 2007).

Universiti Kebangsaan Malaysia (UKM) conducted a cross-sectional study to correlate toxoplasmosis and schizophrenia. Analysis of demographic data from schizophrenic (n=144) and psychiatrically healthy volunteers (n=144) were analysed. Comparison between schizophrenic patients (61.1%) to psychiatrically healthy volunteers (40.8%) showed a higher serointensity rate of anti-*T. gondii* IgG antibody (>60IU/mL) (Emelia *et al.,* 2012). Another cross-sectional case-control study involved 101 schizophrenic patients and 55 healthy subjects at the University of Malaya Medical Center (UMMC) and Sungai Buloh Hospital, Selangor, Malaysia. They reported 32.67% of *Toxoplasma* DNA and 51.5% of *T. gondii*-IgG antibodies detected in schizophrenia patients as compared to 3.64% of *Toxoplasma* DNA and 18.2% of IgG antibody in controls (Omar *et al.*, 2015).

Nevertheless, both studies focused on patients with schizophrenia, and no comparison was performed with other kinds of psychosis. Hence, another research is required

to determine the relationship between *T. gondii* infection and various types of psychiatric disorders, i.e., depressive disorder, bipolar disorder, and schizophrenia. It is also important to investigate the relationship between risk factors and *T. gondii* infection among these groups of patients. Thus, a cross-sectional study was carried out to identify the prevalence of *T. gondii* infection among patients with psychiatric disorders at Hospital Universiti Sains Malaysia, Kelantan, and to determine the association between sociodemographic, clinical manifestations, and behavioural factors of the seropositive patients with psychiatric disorders. The findings would also assist in understanding the etiology of schizophrenia, bipolar disorder, and depressive disorder, which in turn would add to preventive policies and approaches to therapy.

1.3 Objective(s) of the study

1.3.1 General objective

To determine the association between *Toxoplasma* infection and psychiatric disorders in patients at Hospital Universiti Sains Malaysia, Kelantan.

1.3.2 Specific objectives

i. To compare the seroprevalence and serointensity rates of anti-*T. gondii* IgG antibody between patients with schizophrenia, bipolar disorders, depressive disorder, and healthy volunteers.

ii. To compare the sociodemographic, clinical manifestations, and behavioural factors of seropositive and seronegative patients with psychiatric disorders.

iii. To compare the sociodemographic, clinical manifestations, and behavioural factors of psychiatric disorders patients with low and high anti-*T. gondii* IgG antibody titers.

iv. To determine the prevalence of *T. gondii* infection between patients with schizophrenia, bipolar disorders, depressive disorder, and psychiatrically healthy volunteers by PCR.

CHAPTER 2

LITERATURE REVIEW

2.1 Toxoplasmosis

One-third of an individual in the world was infected with *Toxoplasma*, with marked variations in geography (Montoya & Liesenfeld, 2004). It has established several transmission paths within and between various host species. Human infections can be asymptomatic or symptomatic. Once infected, humans are considered remaining infected for life. *T. gondii* infection may be infected pre-natally or post-natally. Typically, humans were infected with *T. gondii* by consuming oocysts in cat feces contaminating soil and water, consuming tissue cysts in undercooked meat, transfusion using blood from an acutely infected person, or transplantation an organ from infected individuals (Garcia Bahia-Oliveira *et al.,* 2003; Jones *et al.,* 2005; Reynolds, 2017). Most infections are asymptomatic; however, the organism may reactivate upon immunosuppression (Dubey & Jones, 2008). A minority of healthy individuals associated with *T. gondii* develop symptoms that are typically mild and develop symptoms such as lymphadenopathy, malaise, and fever (Montoya & Liesenfeld, 2004). In rare cases, individuals who were previously healthy might develop severe and often fatal diseases, likely from highly infectious types of the organism, which involve pulmonary and multi-visceral organs (Carme *et al.,* 2002; Demar *et al*., 2007).

2.1.1 *Toxoplasma gondii*

One of the most polyxenous parasites known to date is tissue cyst-forming coccidium *T. gondii*. Warm-blooded animals such as mammals, birds, and humans can

be infected by the facultative heteroxenous life cycle. *T. gondii* may cause congenital disease or abortion in its intermediate hosts. Prenatal infection of the fetus may occur if a pregnant woman accidentally ingests oocysts. *T. gondii* has been the most intensively studied coccidia due to its importance as a causative agent of zoonosis. After the first mention of its asexual stages in intermediate hosts, the *T. gondii* life cycle takes more than 60 years to complete the study. The asexual phase develops in various tissues of omnivorous or herbivorous intermediate hosts. The development of the sexual phase occurs in the carnivorous definitive host's intestine (Tenter *et al.*, 2000).

2.1.2 Routes of transmission

The life cycle of *T. gondii* is shown in Figure 2.1. The *Toxoplasma* oocysts are discarded widely into the environment by the Felidae family and domestic cats (Dubey *et al.*, 1970; Hill & Dubey, 2002; Webster, 2010). Domestic cats are the major source of infection as they produce the highest number of oocysts. The cats may excrete millions of oocysts after the ingestion of only one tissue cyst that contains bradyzoites.

Unsporulated oocysts can be found in the cat’s feces. The oocysts' sporulation process occurs between 1 to 5 days in the surrounding, they may be infective sporulated oocysts (sporocysts), with each sporocyst containing four sporozoites. Intermediate hosts such as humans, rodents, sheep, cattle, pigs, and birds ingest the infective sporulated oocysts in contaminated soil, plants, and water. The excystation process, in which the sporulated oocysts turn into sporozoites, occurs in the intestinal lumen of the intermediate hosts. The sporozoites invade the mucosa and transform into

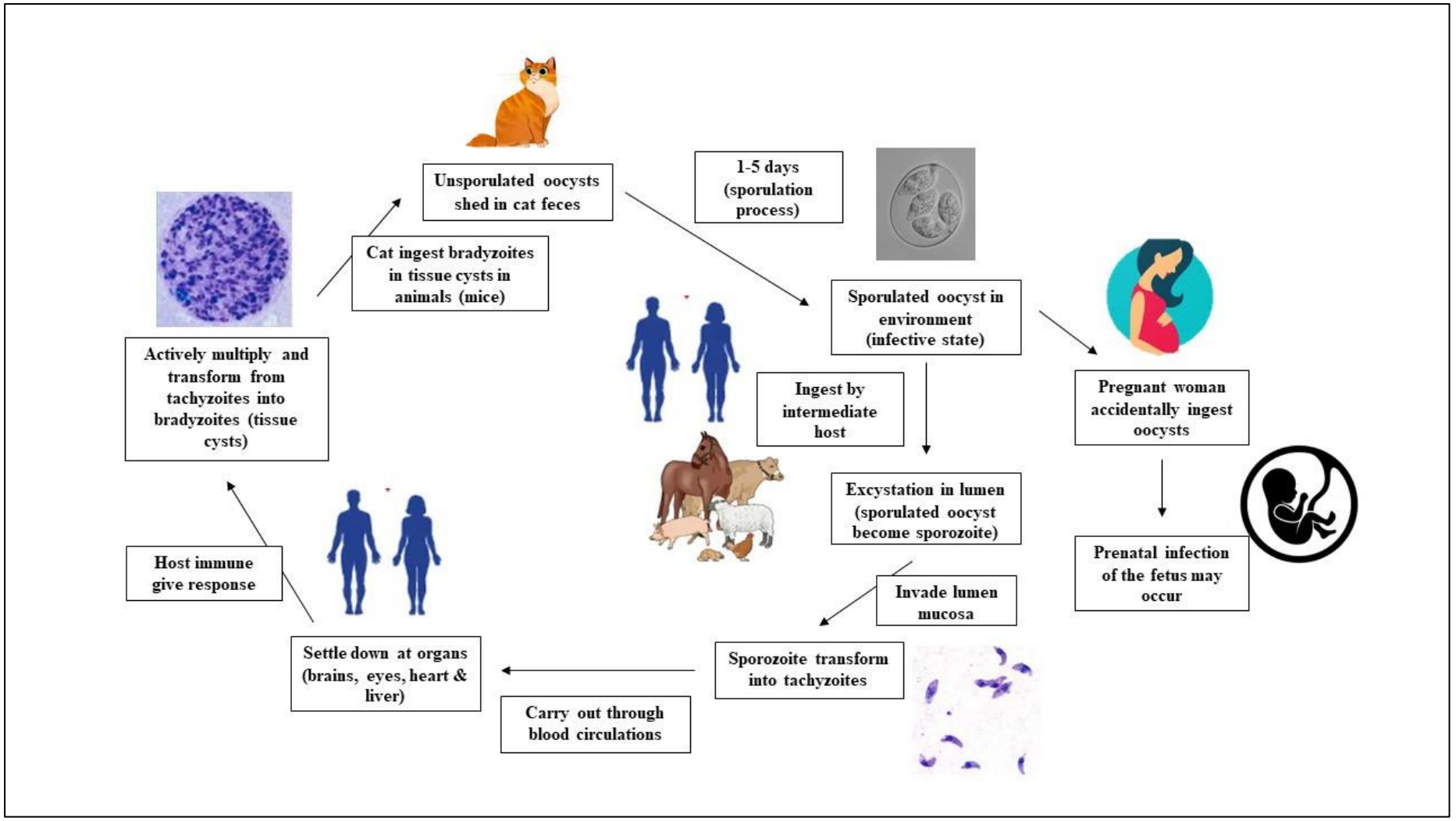

Figure 2.1 Life cycle of *Toxoplasma gondii* (Attias *et al.*, 2020)

tachyzoites. Tachyzoites are carried by the blood circulations and settle in organs of the intermediate hosts (brain, eye, heart, and liver). They actively multiply and convert into bradyzoites when challenged by the hosts' immune system. Bradyzoites or tissue cysts persist in tissues (especially muscle and brain) for many years and are known as latent infections. Reactivation of the latent infection poses a major problem in immunocompromised hosts (Khan *et al.*, 2019; Dalimi & Abdoli, 2012; Robert-Gangneux & Dardé, 2012).

2.1.3 Epidemiology of toxoplasmosis

Seroprevalence of *Toxoplasma* infection varies among countries and correlates with various sociodemographic and risk factors, such as age, ethnicity, residential area, having pets, water supply, and pregnancy (Achaw *et al.,* 2019; Hokelek, 2020; Muflikhah *et al.,* 2018). The seroprevalence rates vary according to different geographical area, Asia (13.3-85.3%), Europe (40-76%), Africa (21.74-74.8%), and North America (7.3-26.5%) (Abdollahian *et al.,* 2017; Minbaeva *et al.,* 2013; Wilking *et al.,* 2016).

A study conducted at the University of Malaya, Malaysia, reported a *T. gondii* seropositivity rate of 19.9% (n=62) among people who have a close contact with animals, with 18.3% IgG+, 1.0% IgM+, and 0.6% IgG+, IgM+ (Brandon-Mong *et al.,* 2015). The prevalence of *T. gondii* infection had been underestimated since most people are not aware of the risk factors of acquiring it, such as lifestyle and eating habits, and its association with low educational level and psychotic syndromes such as mental retardation and schizophrenia (Muflikhah *et al.,* 2018). There are no clinical manifestations or serious illness that have been caused by *T. gondii* in adults. However, the congenital infection may cause blindness and mental retardation in children. For those with depressed immunity, the disease may become more severe than others (Hamidinejat *et al.,* 2010).

2.1.4 Clinical manifestations

T. gondii initially multiplies in almost all tissues during acute phase and formed long-lived cysts in muscle, liver, and nerve cells during the latent phase of infection (Lyons *et al.,* 2002). *Toxoplasma* infection is mostly subacute, with focal neurological symptoms that occur along with altered headache, mental state, and fever. Cortical or

subcortical brain lesions were found in more than half of patients with cerebral toxoplasmosis, leading to speech, walking difficulties, and hemiparesis (Luft *et al.,* 2012).

The role of *T. gondii* in the pathogenesis of several psychiatric disorders has been highlighted in epidemiology studies, including individuals who cannot interpret details that typically correlate with cognitive levels and depressive illness, such as mental retardation and schizophrenia (Del Grande *et al.*, 2017; Muflikhah *et al.,* 2018). Poland was the first country to investigate the association between *T. gondii* and psychiatric patients, as reported in 1953 (Kozar, 1953). Nowadays, the relationship between *Toxoplasma* infection and multiple neuropsychiatric conditions creates some interesting questions about the associations between the brain and the immune system that could contribute to mental health disorders (Tyebji *et al.,* 2019).

2.1.5 Detection of toxoplasmosis

In 1948, by using a serological method, which is the Sabin-Feldman dye test, *T. gondii* was first explored. In serological tests for the detection of *Toxoplasma* infection, ELISA is widely used (Abdoli *et al.*, 2019).

2.1.5(a) Serological detection

For routine laboratory diagnosis of *Toxoplasma* infection, serological tests are the main method. Many studies use enzyme-linked immunoassays (ELISAs) to detect specific IgA, IgG, and IgM antibodies to *T. gondii* (Bellatreche *et al.,* 2022; Liyanage *et al.,* 2021; Rahumatullah *et al.,* 2012). Like other immunoassay methods, an ELISA uses highly specific antibody-antigen interactions to detect a target antigen.

Additionally, the information on whether the infection was a past or probable recent infection can be provided by IgG avidity ELISA (Suresh *et al.,* 2012). ELISA is commonly used in clinical laboratories as a typical screening test due to its best diagnostic efficacy. (Ghazy, Shaapan, & Abdel-Rahman, 2007; Robert-Gangneux & Dardé, 2012).

In this study, Platelia Toxo IgG was used. It was an indirect ELISA immunoassay for the quantitative determination of IgG antibodies to *Toxoplasma gondii* in humans. It was using an indirect ELISA immuno-enzymatic method. *T. gondii* antigen is used for coating the microplate while, a monoclonal antibody labeled with peroxydase which is specific for human gamma chains (anti IgG) is used as the conjugate. The presence and quantity of IgG antibodies to *T. gondii* in the test sample was determined by comparing the optical density (OD) of the test sample to a standard range. The Platelia Toxo IgG assay is standardized to WHO International Standard TOX-M. However, certain discrepancies of titres may be observed for the same sample when it is tested by different serological techniques. This discrepancy is because the *T. gondii* used in these various techniques contains variable proportions of the soluble membrane antigen.

Moreover, Platelia Toxo IgM had been used in this study. It was a qualitative test for detection of IgM antibodies to *T. gondii* in human by enzyme immunoassay with capture of the IgM on the solid phase. Anti-human µ-chains antibodies were coated on the solid phase (wells of the microplate). A mixture of the *T. gondii* antigen and the monoclonal anti *T. gondii* antigen antibody labeled with peroxydase was used as the conjugate. The Cut-Off value (CO) corresponds to the mean value of the optical densities (OD) of the cut-off Control duplicates (R4): CO = mean of OD R4. Sample

result is expressed by Ratio using the following formula: Sample Ratio = Sample OD/CO.

Platelia Toxo IgG avidity was used to measure the IgG avidity of the samples. The principle of this method relies on the measurement of the avidity of the IgG antibodies to *T. gondii*. The use of an agent (as urea) dissociating the link antigen/antibody in parallel with the usual technique of IgG antibodies measurement allows comparison of the optical density (OD) obtained after dissociating agent action and OD obtained without dissociating agent action. The avidity was considered low when the antigen/antibody link was easily dissociated. The avidity was considered high when the antigen/antibody link was not easily dissociated. This test has to be used only on positive samples that demonstrate an anti-*T. gondii* IgG concentration higher than or equal to 9 IU/ml with Platelia Toxo IgG. The Avidity Index (AI) of a sample is the ratio of optical densities (OD) measured with the Dissociating Solution (R13) and the Control Solution (R12):

AI = OD Sample R13 / OD Sample R12

2.1.5(b) Molecular detection

During the early phase of the infection, serological tests may be unsuccessful in detecting specific anti-*Toxoplasma* antibodies because the antibodies may not be developed for several weeks until parasitemia has elapsed (Gross *et al.,* 2000). In immunocompromised patients, the tests also cannot be used to detect the *T. gondii* infection because IgG or IgM antibody titers may not be produced in them (Contini *et al.,* 2006). To detect *T. gondii* DNA, the use of molecular approaches is a useful laboratory method, especially during early acute infections and in

immunosuppressed patients (Bellatreche *et al.,* 2022; Liyanage *et al.,* 2021; Rahumatullah *et al.,* 2012). Many studies use PCR for the detection of *T. gondii*. However, the detection of *T. gondii* using PCR-based techniques suffers from a lack of optimization in different laboratories with variation performance because of many in-house PCR techniques (Ferreira *et al.,* 2008; Kaiser *et al.,* 2007; Nagy *et al.,* 2006; Nowakowska *et al.,* 2006).

A targeted sequence within a large size and a high complexity DNA can be amplified rapidly and selectively using PCR, producing millions of copies in a quasi-exponential chain reaction. The outcome is straightforward, affordable, and easy to set up; only some knowledge about the target's nucleotide sequences is needed. PCR is not only straightforward but also reliable, quick, flexible, and sensitive (Green and Sambrook, 2020). The PCR is carried out in a reaction mixture which includes the primers, Taq polymerase, extracted DNA (template DNA), and four deoxyribonucleotide triphosphates (dNTPs) in excess in a buffer solution. Using a thermal cycler machine, the tubes containing the mixture reaction are subjected to repetitive temperature cycles, which include three steps: denaturation of the DNA template, primer annealing, and DNA molecule extension. These steps are repeated for 30-40 cycles (Green and Sambrook, 2020; Gupta, 2019; Kadri, 2019).

These days, a broad spectrum of PCR types are accessible to everyone, including multiplex PCR, nested PCR, and conventional PCR. A conventional PCR technique known as multiplex PCR uses many primers to identify numerous targets at once. Reagents can be saved by performing three PCRs in one mixing, which is an appropriate decision for repeating reactions. Furthermore, this technique can be

adapted in molecular diagnosis of any infectious disease, yielding prompt outcomes with minimal error margin (Gonçalves-de-Albuquerque *et al.*, 2014). The PCR assay gives an advantage in terms of detection of recent and recurrent infections as a sign or indicator of *T. gondii* parasitemia (Bakre, 2016). A modified PCR known as nested PCR aims to reduce the nonspecific binding of products that might occur from the amplification of unexpected primer-binding sites (Gupta, 2019). *T. gondii* can be identified rapidly, highly sensitive, and specifically using conventional PCR. In conjunction with a serological test, it can differentiate between acute, reactivated, and chronic toxoplasmosis (Castillo-Morales *et al*., 2012; Rahumatullah *et al*., 2012). PCR can be used to determine the time of seroconversion or to diagnose or rule out acute toxoplasmosis (Berredjem *et al*., 2017).

2.1.6 Treatment of *T. gondii*

There are not many options for treatment of *T. gondii*. A combination of two antimicrobial drugs that target the folate pathway is considered being the gold standard when treating *Toxoplasma* infections (Shammaa *et al*., 2021). Presently, the treatments only target the acute infection caused by the tachyzoite form of the parasite *Toxoplasma gondii*; they do not treat the underlying tissue cyst form of chronic infection (Shammaa *et al*., 2021).

Moreover, a study conducted on animals and in vitro has explored the role of oxidative stress in the pathophysiology and treatment of *T. gondii* infection (Szewczyk-Golec *et al*., 2021). They found that chemicals capable of altering redox status have the potential to be anti-*Toxoplasma* drugs, as they can decrease the viability of the parasite. Besides, host cells also may suffer certain negative effects

from oxidative stress induced by the inflammatory response. However, it was shown that taking antioxidant supplements encouraged the rise in parasitemia, leading to a milder course of the illness (Szewczyk-Golec *et al*., 2021).

2.2 Psychiatric disorders

2.2.1 Epidemiology of psychiatric disorders

Previously, the latent form of *T. gondii* infection does not result in any significant sequelae, and only reactivation of the infection poses an actual threat (McConkey *et al*., 2013). However, a growing body of evidence suggests that persistent and latent infection may be responsible for various neurologic and psychiatric symptoms (Henriquez *et al.,* 2009). Most of the studies found that *T. gondii* seropositivity is associated with multiple mental disorders and neurobehavioral disorder, such as depressive disorder, schizophrenia, bipolar disorder, and suicidal tendencies (Daher *et al*., 2021; Virus *et al*., 2021; Achaw *et al*., 2019). Infectious agents, including protozoa, viruses, and spirochetes, may cause psychosis. The microbe-specific immune response will release the neurons after being triggered by the brain structures directly or indirectly (Bergink *et al.,* 2014).

2.2.2 Depressive disorder

2.2.2(a) Epidemiology of depressive disorder

Depressive disorder is the prevalent affective disorder and the main cause of the global burden of chronic disease. Today, an estimated 350 million individuals are affected by depressive disorder (Ahmad *et al*., 2015). One in 20 people experienced an episode of depression, according to a study by Global Mental Health Survey conducted in 17 countries. Depressive symptoms frequently start at a young age and

sometimes can recur (Marcus *et al.*, 2012). The median occurrence of depressive disorder was about 40 years of age and is common in females. But this disorder can occur in people of any socio-economic background at any age (Marcus *et al.*, 2012).

A depressive disorder is manifested by decreased motivation, poor concentration, a lack of desire or enjoyment, disturbed sleep or appetite, and feelings of guilt or low self-worth. Moreover, depressive disorder is also correlated with signs of anxiety. These conditions may become persistent or recurring and contribute to serious impairments in the ability of a person to look after his or her everyday obligations. At its worst, depressive disorder can lead to suicide (Ahmad *et al.*, 2015; Marcus *et al.*, 2012).

Many animal and human studies have postulated an inflammation-associated depressive disorder model. The hypothesis states that serotonin and glutamate biosynthesis are altered by immune-mediated cytokines and lead to depressive disorder or suicidal behaviour (Dalimi & Abdoli, 2012; Webster & Mcconkey, 2010; Müller & Schwarz, 2007). Inflammation occurs in humans with infectious illnesses and is associated with mood disorders. Microbial pathogens like *T. gondii* and cytomegalovirus have been reported to cause cases of actual psychosis (Daher *et al.*, 2021; Yolken & Torrey, 2008).

2.2.2(b) Association between *T. gondii* infection and depressive disorder

The host immune response which has been triggered by *T. gondii* may lead to the development of the depressive disorder. Proinflammatory cytokines, including IL-6 and TNF, will be produced by *T. gondii*. The activated cells will secrete interferon gamma (IFN-γ) that triggers the activation of an enzyme, indoleamine 2,3-

dioxygenase (IDO). IDO blocks the *T. gondii* growth and causes tryptophan depletion, resulting in a decreased serotonin production of the brain that leads to depressive disorder (Carruthers & Suzuki, 2007; Dalimi & Abdoli, 2012; Webster & Mcconkey, 2010). Another study revealed that two *T. gondii* genes encoding phenylalanine hydroxylases and tyrosine hydroxylases. They act to catalyse phenylalanine to tyrosine. Tyrosine transforms into L dopa, which is the precursor of dopamine that alters the human behaviour (Gaskell *et al.,* 2009; Henriquez *et al.,* 2009).

The Centers for Disease Control and Prevention (CDC), United States of America (USA) reported a worldwide *T. gondii* seroprevalence rate of 33% (Hsu *et al.,* 2014). Studies have shown that patients with *T. gondii* infection are more likely to get depressive disorder than those without the infection (Henriquez *et al.,* 2009; Hinze-Selch *et al.,* 2007; Okusaga *et al.,* 2011; Tedla *et al.,* 2011; Zhang *et al.,* 2012). A significant difference (p=0.001) in anti-*T. gondii* IgG antibodies between individuals with depressive disorder (25.64%, n=39) and healthy controls (14.55%, n=55) was reported in a cross-sectional study conducted in the Mecca Region, Saudi Arabia (Al-Hussainy *et al.,* 2015). Another case-control study conducted in Durango, Mexico, reported a significantly higher seroprevalence of anti-*T. gondii* IgG antibodies in major depressive disorder (MDD) patients (12.4%, n=11) compared to control (6.2%, n=22), (OR=2.14; 95% CI=1.00–4.59; p=0.04). They found that patients with depressive episodes and recurrent depressive disorders had similar IgG *T. gondii* seroprevalence rates. However, there were no significant differences in the seropositivity of anti-*T. gondii* IgM antibodies between the two groups (p=0.27) (Alvarado-Esquivel *et al.,* 2016).

Another case-control study conducted in Mexico among 65 mixed anxiety and depressive disorder patients found a statistically significant increase in anti-*T. gondii* IgG seroprevalence in MDD compared to controls (OR=4.03; 95% CI=1.90-8.53; $p<0.001$) (Alvarado-Esquivel *et al.*, 2016). They found that it was a relationship with latent infection (IgG seropositive) than acute infection (IgM seropositive). They concluded that *T. gondii* infection may be related to a mild depressive disorder more than to severe depressive disorder (Alvarado-Esquivel *et al.*, 2016).

However, several studies reported a non-significant difference in anti-*T. gondii* IgG antibodies between depressive disorder and healthy control subjects (Arling *et al.*, 2009; Gale *et al*., 2014; Naglaa *et al*., 2016). The study of the association between *T. gondii* infection and suicidal behaviour showed that depressed individuals, n= 99 (OR=0.51; 0.42-0.61) had increased levels of anti-*T. gondii* IgG antibodies than normal individuals, n=39 (OR=0.40; 0.30-0.54) with an adjusted geometric mean titer (p=0.19, 95% CI) (Arling *et al.*, 2009). However, they did not find a significant relationship between depressed individuals and *T. gondii* seroprevalence. A study in Egypt reported *T. gondii* IgG seroprevalence of 20.3% and 11.7% among depressive disorder (24 out of 118) and control groups (7 out of 60), respectively, which was not statistically significant (Naglaa *et al.*, 2016). In another study, the *T. gondii* IgG level was also not significantly related to depressive disorder (Gale *et al.*, 2014). The findings may be due to several limitations in interpreting results associated with a cross-sectional design. Another cross-sectional study in the USA reported no association between depressive disorder and latent toxoplasmosis regardless of whether the latent toxoplasmosis was analysed as a binary (i.e., presence/absence) (OR=1.01, 95% CI=0.81–1.25; p=0.944) or continuous result (i.e.,

titers levels) (OR=1.00, 95% CI=0.96–1.06; *p*=0.868) (Gale *et al.,* 2016). In Finland, there was also no significant association of anti-*T. gondii* IgG antibodies between MDD patients and controls (CI=3.8-6.6; *p*=0.75) (Suvisaari *et al.,* 2017).

It is also possible that pre-existing MDDs are associated with *T. gondii* due to raw meat consumption or exposure to the soil in certain individuals, and that has surpassed any positive association between *T. gondii* and MDD in others (Pearce *et al.,* 2012). On the other hand, the differences in severity of depressive disorder among the studies correlated with the differences in the findings (Alvarado-Esquivel *et al.,* 2016).

2.2.3 Bipolar disorder

2.2.3(a) Epidemiology of bipolar disorder

Bipolar disorder (BD) affects 2.4% of the world's population (Merikangas *et al.,* 2011). BD etiology is multifactorial, originating from a complex relationship between environment possibility factors and genetic variation (Dakkak, 2017; Kerner, 2014). The individual has difficulties in employment, social activities, interpersonal relationships, and other significant areas. The disorder can lead to irreparable harm in various aspects of a person's well-being and socio-economic achievements (Marcus *et al.,* 2012). The intervention of contagious agents, particularly *T. gondii,* has recently created a higher awareness of major psychosis development (Bay-richter *et al.,* 2019; Torrey *et al.,* 2007; Yolken & Torrey, 2008). A study among psychiatric patients with a diagnosis of schizophrenia, depressive disorder, and BD showed that they were significantly related to *T. gondii* infection (Hinze-selch *et al.,* 2010).

2.2.3(b) Association between *T. gondii* infection and bipolar disorder

T. gondii is a neutropic organism that can encyst in the neuron, glial cells, and astrocytes of the brain (Carruthers & Suzuki, 2007). Bipolar disorder may result from damage to the brain due to the many enlarged necrosis of foci and microglia nodules. In an infant, the obstruction of the aqueduct of Sylvius or foramen of Monro can cause periaqueductal, periventricular vasculitis, and hydrocephalus. It results in more intense necrosis that could occur in the cortex and basal ganglia and sometimes in the periventricular areas. Besides, toxoplasmic encephalitis is manifested as multiple brain abscesses occur (Fekadu *et al.,* 2010; Montoya & Liesenfeld, 2004). Therefore, *T. gondii* has the potential to change human behaviour due to its direct impact on the brain tissue.

In a study in France, *T. gondii* IgG seropositivity in BD patients (76.9%, n=80/110) was significantly higher than in controls (48.2%, n=41/106) (Hamdani *et al.,* 2013). National Health and Nutrition Survey (NHANES III), United States of America (USA) reported a high prevalence of *T. gondii* seropositivity (n=41; adjusted odds ratio, 2.4; 95% CI; 1.2-4.8; $p<0.05$) in patients with BD history but did not find the same association in patients with a history of unipolar depressive disorder (Pearce *et al.,* 2012). However, 171 BD and 80 controls were investigated in a case-control study in Ethiopia. They found that the seroprevalence of *T. gondii* IgG antibodies in individuals with BD (adjusted OR=3.0; 95% CI=1.1-8.6) higher than in unaffected controls (Tedla *et al.,* 2011). Moreover, a study reported a significant difference in anti-*T. gondii* IgM between BD, which is greater than or equal to the 50^{th} and 75^{th} levels of the control group by using multivariate linear and regression analyses

(Dickerson *et al.*, 2014). It significantly elevated the odds of a low total cognitive score (Dickerson *et al.*, 2014).

However, a study in Golestan educational hospital in Ahvaz, Iran found no significant difference between the *Toxoplasma* IgG level in BD (31.6%, n=37/117) and healthy individuals (26.5%, n=53/200) (Khademvatan *et al.*, 2013). A similar finding was also reported in the northern Mexican city of Durango (odd ratio=1.7; 95% CI; 0.66–4.36; p=0.26) (Alvarado-Esquivel *et al.*, 2019). The varying findings from different studies could be related to different *T. gondii* genotypes (Khademvatan *et al.*, 2013). *T. gondii* genotypes vary in their virulence and their replication also may differ from each other (Saraei-Sahnesaraei *et al.*, 2009).

2.2.4 Schizophrenia

2.2.4(a) Epidemiology of schizophrenia

A study has shown that schizophrenic people were more exposed to cats during their youth than adults (Dubey, 2008). *Toxoplasma* can infect the perinatal brain and is associated with this schizophrenia pathogenesis (Torrey & Yolken, 2003). The rationale for some individuals infected with *T. gondii* developing schizophrenia is unsure. Possibilities such as differences in the timing of infection (utero, childhood, or adulthood), mode of infection (tissue or oocyst), and genetic susceptibility. Additionally, behavioural traits are also related to schizophrenia that can cause the infection of *Toxoplasma* (Henriquez *et al.*, 2009).

There are four major considerations regarding how *T. gondii* contributes to the etiology of schizophrenia. First is the concordance among monozygotic twins of 35%-

50% that correlated with a genetic component (Torrey *et al.,* 2007). Genes can also affect susceptibility to *T. gondii* in animals, including mice (Johnson *et al.,* 2002; Suzuki, 2002). Second, abnormalities of neurotransmitters correlate with schizophrenia. Studies of animals have shown the effect of *T. gondii* on dopamine and serotonin (Stibbs, 1985). Third, neurodevelopment is related to schizophrenia, thus can cause prenatal infection of *T. gondii* and making it remain latent for many years (Torrey *et al.,* 2007). Fourth, the relationship between *Toxoplasma* infection and schizophrenia is consistent with the studies using models of animals that indicate action changes in *Toxoplasma*-infected animals (Webster, 2001).

2.2.4(b) Association between *T. gondii* infection and schizophrenia

In 1993, the increment of *T. gondii* antibody titer in patients with schizophrenia was reported (Hsu *et al.,* 2014). Recently, the relationship between *T. gondii* infection and schizophrenia had been found in more epidemiological studies (Yolken & Torrey, 2008). Besides, patients with schizophrenia are also increasingly seropositive for *T. gondii* (Torrey *et al.,* 2007). Another study found higher *T. gondii* IgG titers in suicidal and schizophrenia patients (Okusaga *et al.,* 2011). A meta-analysis of 23 studies on schizophrenia patients showed an enhanced incidence of *T. gondii* antibodies. It indicated that a big proportion of schizophrenia cases correlate with *T. gondii* infection with modest odds ratio 2.73 (Hinze-Selch *et al.,* 2007).

In Iran, 72.5% (58/80) of schizophrenia patients and 61.6% (61/99) healthy control individuals were seropositive for anti-*T. gondii* IgG or IgM antibodies (Daryani *et al.,* 2010). Moreover, a significantly higher seroprevalence of anti-*T. gondii* IgG antibodies in schizophrenic patients (20%, n=10/50) than in controls (5.3%, n=8/150)

(odd ratio=4.44; 95% CI; 1.49-13.37; *p*=0.003) that matched by ethnic group, age, residence place, and gender found in a case-control study among the Mexican population (Alvarado-Esquivel *et al.,* 2011). Increased *T. gondii* IgG titer was also found in schizophrenia patients (n=950) in the Munich area of Germany with a 1.59 odds ratio (95% CI 1.06 to 2.40), *p*=0.03 (Okusaga *et al.,* 2011). A cross-sectional, prospective study in Germany among major depressive disorder (n=465), schizophrenia (n=277), and healthy volunteers (n=214), in which groups were adjusted for the geographic home region and age, demonstrated that *T. gondii* serointensity was significantly increased in patients, but serofrequency was comparable among the groups. Furthermore, the routes of infection also appeared to be different between patients and healthy volunteers. They suggested that the *T. gondii* infection is particularly related to schizophrenia patients due to the interactions among psychiatric vulnerability, immunomodulation, neurotransmitter systems, and genetic background (Hinze-Selch *et al.*, 2007).

2.2.5 Other related studies

Patients with psychosis had significantly increased levels of *T. gondii* IgG antibodies compared to controls (Achaw *et al.,* 2019; Alvarado-Esquivel *et al.,* 2011; Tamer *et al.,* 2008). A study conducted at the University of Gondar Hospital, Northwest Ethiopia reported that *T. gondii* IgG seroprevalence was significantly higher in psychotic patients (33.6%, n=51/152, *p*=0.001) than in controls (16.4%, n=25/152) (Achaw *et al.,* 2019).

A similar result was also reported by a study in Weihai, Eastern China, whereby a significantly higher prevalence of *T. gondii* infection among psychiatric