



***EFFECTS OF MALAYSIAN STRAINS OF *Toxoplasma gondii* ON  
DOPAMINE AND KYNURENIC ACID GENES AND THEIR POSSIBLE  
RISK IN SCHIZOPHRENIA-LIKE RAT MODEL***

**MOHAMMED NASIRU WANA**

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By

**MOHAMMED NASIRU WANA**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**June 2020**

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## **DEDICATION**

This work is dedicated to my late father, to my beloved mother, my wife and children:

Late Mal Muhammad Mekaniki, Hajiya Zara Muhammad, Mrs Balkisu Muhammad, Almustapha Muhammad Nasir, Muhammad Ammar Nasir and Annour'Aisha Muhammad Nasir



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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**Chairman : Roslaini Abd.Majid, PhD**  
**Faculty : Medicine and Health Sciences**

Schizophrenia is a complex brain disorder that is the cause of neuropathological changes with an unknown source. The pathological condition associated with the schizophrenic individual is the brain of the infected host that results into changes associated with the neurotransmitters. Neuropathological and epidemiological data have also shown that some causes of schizophrenia may be strongly related to environmental agents, such as exposure to infectious microorganism. The protozoan parasite *Toxoplasma gondii* (*T. gondii*) have been reported in the brain of rodents and also diagnosed in the schizophrenic individual which is implicated as the cause of behaviour deficits. The present study collected faeces of free-roaming cats (FRC) and pet cats (PC) in Klang Valley, Malaysia to detect and genotype *T. gondii* and further tested their effects on rats' behaviour. Wistar albino rats were injected subcutaneously with MK-801 (dizocilpine) 0.6 mg/kg twice a day for seven days before the commencement of the behaviour test to established rat model of schizophrenia. Three experimental groups of rats were inoculated from a single strain of type I, type II and type III respectively, while another group was inoculated with phosphate buffered saline (PBS) as control. Rats were tested serologically two weeks post infection (pi) to confirmed acute *T. gondii* infection. Behavioral assessment of open field test (OFT), fatal feline attraction test (FFAT) and elevated plus maze (EPM) were evaluated at nine weeks pi, while another behavioural test of Morris water maze (MWM) was carried out at 10 and 11 weeks pi. Subsequently, at the end of the behavioral test, rats were euthanized and whole brain assessed for *T. gondii* tissue cysts distribution using haematoxylin and eosin staining. Additionally, molecular analysis of a neurotransmitter gene expression of dopamine (dopamine receptor; DRD) and kynurenic acid (indoleamine-2, 3 dioxygenase; IDO) genes were evaluated. Overall, the results revealed 17 (8.5%) were *T. gondii* positive samples in both FRC and PC in Malaysia within the study area. More *T. gondii* positive faecal samples were found in FRC 13 (13.0%) compared with PC 4 (4.0%). Four *T. gondii* genotype strain of type

I, II, III and mixed infection were identified as clonal with genotype type I as the predominant. The result of the serological test of toxoplasma-IgM found at least six or more experimental rats in *T. gondii* infected groups were positive, while the control group of rat inoculated with PBS and MK-801 (model of schizophrenia) administered group of the rat were negative. The infected rats present with increased in locomotor activity, less aversion to cat urine scented areas, decrease anxiety, and poor memory retention. The results of the haematoxylin and eosin histological staining of the *T. gondii* infected rat brain coronal section revealed tissue cysts distribution. *T. gondii* tissue cysts were found in some of the major brain domain without tropism to a particular area. The present study on the brain gene expression of *T. gondii* infected rats indicated changes associated with the dopamine receptors (DRD) and indoleamine-2,3-dioxygenase (IDO). There is a reduction in the level of DRD which is involved in the dopamine pathway, while the level of IDO was elevated which is part of the kynurenic acid pathway. Further, the behavioural test coupled with DRD and IDO abnormalities support the data of cognitive deficits in *T. gondii* infected rats, while the effect of type I, type II and type III were comparable. Therefore these findings suggest that Malaysian species of *T. gondii* are implicated in some causes of behaviour changes that are responsible for schizophrenic conditions.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KESAN *Toxoplasma gondii* STRAIN MALAYSIA KE ATAS DOPAMINE DAN KYNURENIC ASID DAN KEBARANGKALIAN RESIKO SEAKAN-  
AKAN SKIZOPRENIA DI DALAM MODEL TIKUS**

Oleh

**MOHAMMED NASIRU WANA**

**Jun 2020**

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**Fakulti : Perubatan dan Sains Kesihatan**

Skizofrenia merupakan masalah gangguan fungsi otak yang kompleks yang memberi kesan ke atas perubahan neuropatologi otak yang berpunca daripada sumber yang tidak dapat dikenalpasti sehingga kini. Kesan patologi ke atas individu penghidap skizofrenia mendapati sel-sel otak pesakit-pesakit ini telah menunjukkan perubahan berkaitan dengan fungsi neurotransmitter otak. Data neuropatologi dan epidemiologi juga menunjukkan bahawa sesetengah penyebab skizofrenia adalah berpunca daripada alam sekitar, seperti pendedahan kepada jangkitan mikroorganisma. Parasit protozoa *Toxoplasma gondii* (*T. gondii*) telah dilaporkan di temui di dalam sel otak tikus dan juga individu yang di diagnosis menghidapi skizofrenia yang telah dikenalpasti daripada perubahan tingkah laku. Kajian ini telah dijalankan dengan melalui proses persampelan ke atas kucing-kucing jalanan dan juga kucing-kucing peliharaan di Malaysia bagi mengesan genotip *T. gondii* dan seterusnya menguji kesannya terhadap tikus-tikus eksperimen. Tikus putih Albino Wistar telah disuntik dengan agen MK-801 (dizocilpine) 0.6 mg/kg melalui suntikan subkutaneus dua kali sehari selama tujuh hari sebelum ujian tingkah laku ke atas model tikus skizofrenia dijalankan. Tiga kumpulan tikus-tikus eksperimen telah diinokulasi mengikut klasifikasi tunggal jenis I, jenis II, dan jenis III masing-masing, manakala kumpulan kawalan telah disuntik dengan larutan saline. Ujian serologi ke atas kumpulan tikus-tikus ini telah dijalankan selepas dua minggu daripada tarikh jangkitan (pi), bagi pengesanan jangkitan *T. gondii* akut. Manakala penilaian ke atas ujian-ujian perubahan tingkahlaku seperti ujian lapangan terbuka (OFT), empat ujian bau pilihan (FFAT) dan ujian tambahan maze (EPM) telah dijalankan pada minggu ke sembilan (pi), sementara ujian perilaku lain seperti Morris water maze (MWM) dijalankan pada minggu ke 10 dan 11 (pi). Seterusnya, pada pengakhiran ujian tingkah laku, tikus-tikus ini telah diethanisia dan keseluruhan otak telah dikeluarkan untuk penilaian sebaran sista *T. gondii* dengan menggunakan kaedah perwarnaan haemotoxylene dan eosine. Di samping itu, analisis molekul untuk ekspresi pernyataan gen-gen seperti neurotransmitter dopamine (reseptor dopamine;

DRD) dan asid kynurenic (indoleamine-2,3 dioxygenase; IDO) juga telah dijalankan. Hasil kajian ini telah menunjukkan sejumlah 17 sampel adalah positif (8.5%) *T. gondii*, dengan 13 (13.0%) daripadanya adalah sampel yang diperolehi daripada kucing-kucing jalanan berbanding 4 (4.0%) daripada kucing-kucing peliharaan. Ujian pengenalpastian genotip ke atas *T. gondii* jenis I, II, III dan jangkitan campuran telah dijalankan dan dari hasil kajian ini telah mengenalpasti bahawa klonal jenis 1 merupakan klonal jangkitan utama. Hasil ujian serologi IgM toksoplasma ke atas kumpulan-kumpulan tikus sebagai model kepada skizofrenia telah menunjukkan terdapat sekurang-kurangnya enam atau lebih dari tikus-tikus ini adalah positif. Manakala kumpulan kawalan yang terdiri daripada kumpulan tikus diinokulasi dengan larutan saline dan kumpulan tikus yang diinokulasi dengan MK-801 (model positif bagi skizofrenia) telah menunjukkan hasil negatif ke atas ke dua-dua kumpulan ini. Tikus-tikus yang telah dijangkiti menunjukkan tahap peningkatan aktiviti lokomotor berserta pengurangan fungsi aversi terhadap bau air kencing kucing, rendah keseimbangan, dan pengekal daya ingatan yang lemah. Keputusan ujian haematoxyline dan perwarnaan histologi eosin ke atas bahagian kronal otak tikus yang dijangkiti oleh *T. gondii* menunjukkan terdapat sebaran tisu sista di beberapa bahagian domain otak tanpa menunjukkan kesan tropisme di bahagian-bahagian tertentu. Kajian ekspresi gen ke atas sel otak tikus-tikus yang dijangkiti oleh *T. gondii* menunjukkan perubahan sekutuan berkaitan dengan reseptor dopamine (DRD) dan indoleamin-2, 3-dioksigenase (IDO). Kajian ini juga menunjukkan terdapat pengurangan tahap DRD yang melibatkan laluan dopamine dan terdapat peningkatan tahap IDO yang melibatkan sebahagian daripada laluan asid kynurenic. Kajian lanjutan bagi menilai perubahan tingkah laku berserta keabnormalan DRD dan IDO bagi menyokong data defisit kognitif ke atas tikus-tikus yang dijangkiti oleh *T. gondii* telah dijalankan. Hasil kajian ini menunjukkan jenis I, jenis II dan jenis III adalah sebanding. Hasil penemuan ini juga mencadangkan bahawa spesies *T. gondii* di Malaysia telah menyumbang kepada sebahagian perubahan tingkah laku yang menyebabkan skizofrenia.



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## LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BBB	Blood brain barrier
BLAST	Basic local alignment search tool
BSA	Bovine Serum Albumin
cDNA	Complementary deoxyribonucleic acid
°C	Degree Celsius
CRP	C-reactive protein
CT	Cycles threshold
DA	Dopamine
DAT	Dopamine transporter
DEPC	Diethylpyrocarbonate
DPX	Dibutyl phthalatexylene
DRD1	Dopamine receptor D1 gene
DRD2	Dopamine receptor D2 gene
DNA	Deoxyribonucleic acid
DOPA	Dihydroxyphenylalanine
DSM5	Diagnostic and statistical manual of mental disorders (5th edition)
dNTP	Deoxynucleotide
EPM	Elevated plus maze
ESA	Excretory secretory antigen
ELISA	Enzyme linked immunosorbent assay
FCORT	Four choice odour response test
FFAT	Fatal feline attraction test
FRC	Free roaming cat
GRA	Granular antigen
HIV	Human immunodeficiency virus
IDO1	Indoleamine-2, 3-dioxygenase IDO1 gene
IDO2	Indoleamine-2, 3-dioxygenase IDO2 gene
IFAT	Indirect fluorecent antibody test

IgG	Immunoglobulin G
IgM	Immunoglobulin M
KYNA	Kynurenic acid
LA	Latex agglutination
MEGA	Molecular evolutionary genetics analysis
MWM	Morris water maze
MLE	Multilocus enzyme electrophoresis
MOH	Ministry of health
MRI	Magenetic resonance imaging
mRNA	Mitochondrial ribosomal nucleic acid
NCBI	National centre for biotechnology information
NMHR	National mental health registry
nPCR	Nested polymerase chain reaction
OD	Optical density
OFT	Open field test
PBS	Phosphate buffered saline
PC	Pet cat
PCR	Polymerase Chain Reaction
PI	Post inoculation
RC	Reference gene
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RT-PCR	Real time polymerase chain reaction
SAG	Surface antigen
SEM	Standard error of mean
SN	Substantia niagra
<i>T. gondii</i>	<i>Toxoplasma gondii</i>
TBE	Tris Borate EDTA
TE	Toxoplasmic encephalitis
UVH	Universiti veterinary hospital
VTA	Ventral tagmental area

# CHAPTER 1

## INTRODUCTION

### 1.1 Background

Schizophrenia is a complex brain disorder that is the cause of neuropathological changes with unknown aetiology (Esshili et al. 2016). The disease is a complex process involving series of interaction between mental and organic factors to alter the working condition of the brain over a long period (Khan et al., 2014; Wang et al., 2013). In the beginning, the schizophrenic disease is not noticeable, but the effect of the disease takes it full control at early adulthood or late adolescent as the disease progresses (Ayhan et al., 2009). The disease approximately affects 1% of the human population worldwide (Torrey, Bartko, & Yolken, 2012). Schizophrenia is ranked as the ninth most prevalent cause of disability worldwide (Henriquez et al., 2009). The presence of schizophrenia among members of the affected individual indicates that some genetic factors play a significant role in its development (Ayhan et al. 2009). Neuropathological and epidemiological data have also shown that some causes of schizophrenia may be strongly related to environmental agents, such as exposure to infectious microorganism. Infectious agents, which includes, herpes simplex, varicella-zoster virus, rubella, polio and *Toxoplasma gondii* have been implicated as the neurotrophic cause of schizophrenia (Yolken, Dickerson, and Torrey 2009). The pathological changes associated with the schizophrenic individual brain of the infected host are changes associated with the neurotransmitters and neuro-immune modulatory mechanisms (Gatkowska et al., 2013; Wang et al., 2019). Schizophrenia is a multifaceted disease which is measured by the level of dopamine receptors (DRD) imbalance as a neurotransmitter and indoleamine-2,3-dioxygenase (Ido) which is an enzyme in the kynurenic acid pathway as part of immune-modulation (Murakami et al., 2012, 2012; Silva et al., 2002).

*Toxoplasma gondii* (*T. gondii*) is a unicellular, eukaryotic an intracellular obligate protozoan parasite of all warm-blooded animals including rodents and humans (Dubey & Jones, 2008; Hill, Chirukandoth & Dubey, 2005). *T. gondii* has been reported worldwide and found in the tropical and subtropical regions such as in Europe, in South America, in North America, in Africa and in Asia (Yan et al., 2016). The family Felidae, which include cats are the definitive host, while all other animals serve as the intermediate host (Dubey, 2006). The cat is the definitive host infected with *T. gondii* are the only known animal to excrete resistant oocysts together with their faeces into the environment (Dubey, 2006). The oocysts in the outside environment may contaminate soil, water and vegetables which can be easily ingested and this represents the largest source of infection (Aubert & Villena, 2009; Du et al., 2012; Dubey, 2006; Lass et al., 2009). Other routes of transmission include consumption of tissue cysts in undercooked meat of an infected animal, congenital via the placenta, organ transplantation and blood transfusion (Aspinall et al., 2002; Kijlstra & Jongert, 2008; Torgerson & Mastroiacovo, 2013). *T. gondii* preferred to reside in the brain of its intermediate host (Al-Qassab et al., 2009; Carruthers & Suzuki, 2007; McConkey et

al., 2013), where tissue cysts can persist for the entire life of the organism (Bezerra et al., 2012; Schares et al., 2017). The disease progresses from acute infection to the chronic or latent stage which is highly regulated by the immune status of the host (Lew, et al., 2018). In immunocompromised individuals, such as HIV patients, the disease may be life-threatening (Nimir et al., 2010), while in pregnant women, it can cause abortion (Nissapatorn, et al., 2011). In all healthy individuals, the infection is subclinical with no obvious sign and symptoms (Mohamed & Hajissa 2016), but persistent of tissue cysts in the brain is the major concern for the behavioural changes ( da Silva & Langoni, 2009; Webster, et al., 2006). Further, the effects of *T. gondii* in rats (Daniels, Sestito, & Rouse, 2015; Kannan & Pletnikov, 2012) and presence of tissue cysts in the brain of rats (Berenreiterová et al., 2011; Gatkowska, et al., 2012) have been widely reported.

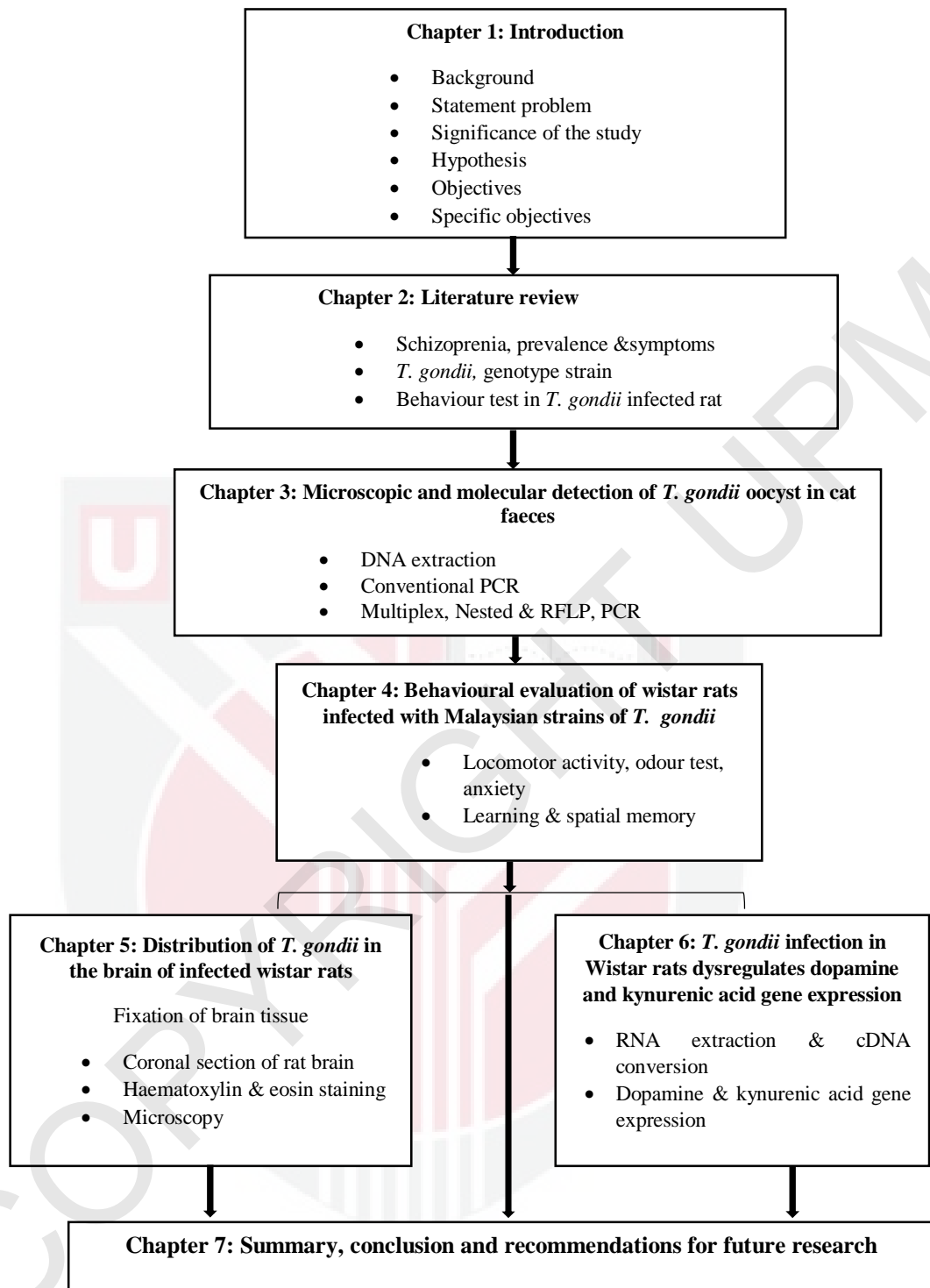
## 1.2 Statement of the problem

Schizophrenia is a multifaceted mental disorder with an unknown cause, affecting approximately 1% of the world population with full-blown episodes occurring at midst-twenties or early adulthood (Arias et al. 2012; Keefe 2008). In Malaysia, available data revealed between 2003 and 2005 when National Mental Health Registry (NMHR) for schizophrenia was created, a total of 7,351 cases were recorded (MOH, 2007). According to a survey conducted by the National Health Survey in Malaysia in the year 2015, the prevalence rate of mental illness was recorded at 29.2% (MOH, 2015). It appears in some that the cause of their mental illness is not known, while others are kept in silence for fear of victimization (Arling et al. 2009; Fekadu, Shibre, & Cleare 2010). Both schizophrenia and *T. gondii* have a worldwide distribution and are found in tropical and temperate regions of the world. It was estimated worldwide that about 2 billion people suffer from brain related illness (Elsheikha, Büsselberg, & Zhu 2016). *T. gondii* had been diagnosed in patients suffering from schizophrenia over a long period (Goodwin, Strobl, & Lindsay 2010; Henriquez et al. 2009). In Southeast Asia and Malaysia in particular, there is a substantial group of vulnerable individuals who are susceptible to the *T. gondii* infection (Mohamed & Hajissa 2016). The seroprevalence of *T. gondii* in Malaysia among schizophrenic patients was more than 50.0% and indicated an association between *T. gondii* and schizophrenia (Juanah, et al., 2013; Omar et al., 2015). The recent report of seroprevalence of *T. gondii* among migrant workers stand at 57.4% (Sahamin, 2017). Few studies were done on schizophrenia and seroprevalence of *T. gondii* in both humans and domestic animals, indicated the need to investigate the biological implication of the toxoplasmosis in mental illness (Yoon & Abdul Aziz 2014). Cats as companion animals poses a great risk factors associated with the transmission of *T. gondii* and are frequently available in the study area (Cantlay, Ingram, & Meredith, 2017; Chemoh et al., 2015; Mahdy et al., 2017). Therefore, these problems led to the conceptualization of the present research in order to evaluate the possible effects of Malaysian species of *T. gondii* on rat behaviour.

### 1.3 Significance of the study

The healthcare system in Malaysia is facing challenges with an increasing number of people requiring medical attention, both in the rural and urban communities (MOH, 2016). As the number of mental illness increases, the healthcare system must be prepared for this group of people as they are suffering in silence different from that of their healthy counterparts. The Malaysian population is known to keep cats as companion animals which are the definitive host for the protozoan parasite *T. gondii* (Afonso et al. 2013). With the increase in exposure to both pet and free roaming cats population, while undertaking normal routine activities, potential mental disorder such as schizophrenia is more likely to occur (Elsheikha et al. 2016). This mental disorder can also lead to ultimate risk-taking behaviours with dire consequences such as stigmatization, victimization, tendency to commit suicide, drug misuse, reckless driving, loss of job and finally death (Hsu, Groer, & Beckie 2014). *T. gondii* is a protozoan parasite that is found in all tropical and temperate regions of the world that can infect every available birds and mammals, including humans (Meireles, et al., 2004). In immunocompromised patients, the infection is severe and can lead to abortion in pregnant women and brain damage in HIV patient, while in an immunocompetent healthy individual, the infection is asymptomatic but the *T. gondii* tissue cysts persist in the brain indefinitely (Tyebji et al. 2019). This development of *T. gondii* tissue cysts in the brain of the healthy individual has been reported to be associated with mental disorders/psychiatric disorders such as suicide, bipolar and schizophrenia disease. In addition, it has been reported that *T. gondii* antibodies were found in both healthy and psychiatric patients in Malaysia (Ahmad, et al., 2014; Juanah et al., 2013; Sahimin et al., 2017). The outcome reported from this study provides information on *T. gondii* to assist the Malaysian healthcare system in identifying factors contributing to the spread of mental illness, particularly schizophrenia among the Malaysian population. An outlined of the thesis chapters is shown in Fig. 1.1.





\* *T. gondii*; *Toxoplasma gondii*, PCR; Polymerase chain reaction, RFLP; Restriction fragment length polymorphism, DNA; Dioxyribonucleic acid, RNA; Ribonucleic acid; cDNA; Cyclic diriboxynucleic acid

**Figure 1.1 : Organization of thesis chapters**

#### **1.4 Research Hypothesis**

There is an effect of Malaysian strains of *T. gondii* on dopamine receptors and kynurenic acid gene expression leading to behaviour changes in rats.

#### **1.5 Research questions**

1. What are the possible genetic diversity and genotype strains of *T. gondii* circulating in Klang Valley, Malaysia?
2. Does the *T. gondii* genotype strains affect locomotor activity, olfactophobia, anxiety and learning and memory capacity of rats?
3. What is the distribution of *T. gondii* genotype strains in the brains of infected rats?
4. Does gene expression levels of dopamine and kynurenic acid differ between the infected and control groups of rats?

#### **1.6 Objective of the research**

The main idea is to investigate the possible effects of Malaysian strains of *T. gondii* on rat behaviour.

#### **1.7 Specific objectives**

1. To detect and characterize the genotypes strains of *T. gondii* from faecal samples of pet cats (PC) and free-roaming cats (FRC) around Klang Valley, Malaysia.
2. To assess the behavioural changes associated with *T. gondii* infections in rats.
3. To determine the distribution of *T. gondii* tissue cyst in the brain of infected rats.
4. To determine the gene expression levels of dopamine receptors and kynurenic acid metabolites in the brain tissue of rats.

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