

**DETECTION OF INTESTINAL PARASITES AND  
*Toxoplasma gondii* IN KOTA BHARU PET CATS VIA  
MICROSCOPY AND MOLECULAR TECHNIQUES**

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*Toxoplasma gondii* IN KOTA BHARU PET CATS VIA  
MICROSCOPY AND MOLECULAR TECHNIQUES**

**by**

**NURUL AIN BINTI RUSLI**

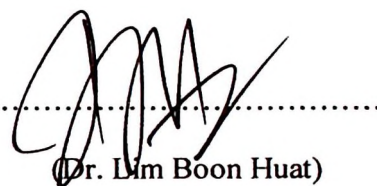
**Thesis submitted in fulfillment of the requirements  
for the degree of  
Bachelor of Health Science**

**JUNE 2014**

## CERTIFICATE

This is to certify that the dissertation entitled “Detection of Intestinal Parasites and *Toxoplasma gondii* in Kota Bharu Pet Cats via Microscopy and Molecular Techniques” is the bonafide record of research work done by Ms. Nurul Ain Binti Rusli during the period from September 2013 to May 2014 under my supervision.

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“In the name of Allah, Most Gracious, Most Merciful”

“Over the knowledgeable, Allah the Most Knowledgeable”

All praises and gratitude is to Allah, the Lord to whom every single creature in the heaven and the earth belongs to. Thanks Allah for giving me the strength and patient during this challenging time. May peace and blessings be on the leader of all creation, the prophet Muhammad S.A.W, his family and companion.

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## LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

bp	base pair
ddH <sub>2</sub> O	deionized distilled water
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
EtBr	ethidium bromide
<i>g</i>	relative centrifugal force (rcf)
g	gram
IC	internal control
kb	kilo base pair
mg	milligram
MgCl <sub>2</sub>	magnesium chloride
min	minute
mL	milliliter
mm	millimeter
PCR	polymerase chain reaction
s	second
sp	species
<i>Taq</i>	<i>Thermus aquaticus</i>
TAE	Tris-Acetate EDTA
Tris	tris (hydroxymethyl) aminomethane
V	volt
μl	microliter
μm	micrometer
μM	micromolar

**PENGESANAN PARASIT USUS DAN *Toxoplasma gondii* DALAM KUCING  
PELIHARAAN DI KOTA BHARU MENGGUNAKAN KAEDAH MIKROSKOP  
DAN MOLEKULAR**

**ABSTRAK**

*Toxoplasma gondii* ialah protozoa parasit zoonotik yang menyebabkan toxoplasmosis. Kucing adalah perumah tetap kepada *T. gondii* dan takungan terhadap penularan parasit usus yang lain. Maka, kajian ini bertujuan untuk memeriksa dan mengenal pasti parasit usus dalam kucing peliharaan dan mencipta ujian dupleks tindak balas untaian polimeras (PCR) untuk mengesan *T. gondii* dalam sampel najis kucing. Sejumlah 36 sampel najis kucing peliharaan dikutip dari kawasan Kota Bharu, Kelantan. Kesemua sampel najis disaring menggunakan kaedah asas iaitu smir basah. Seterusnya, kesemua sampel dimendapkan menggunakan kaedah pemendapan formalin eter dan diwarnakan dengan menggunakan kaedah pewarnaan tetap iaitu diubahsuai asid cepat. Ujian PCR juga telah dicipta untuk mengesan internal transcribed spacer (ITS), iaitu jujukan DNA daripada bahagian 18S rRNA gen *T. gondii*. Gen *Plasmodium falciparum* digabungkan dalam PCR sebagai kawalan dalaman untuk mengelak keputusan negatif yang salah. Keputusan menunjukkan 58% sampel kucing peliharaan di Kota Bharu adalah positif dengan satu atau lebih parasit usus. Menerusi smir basah, kehadiran telur *Toxocara cati*, *Toxascaris leonine*, *Ancylostoma* sp., *Trichuris vulpis*, *Uncinaria stenocephala*, *Trichuris trichiura*, *Ascaris* sp. dan *Capillaria* sp. telah dikesan dalam sampel najis sebanyak 5(14%), 5(14%), 3(8%), 1(3%), 1(3%), 1(3%), 1(3%) dan 1(3%) masing-masing. Teknik pemendapan menunjukkan 8(22%) sampel najis adalah positif bagi *T. cati*, 6(17%) bagi *T. leonine*, 3(8%) bagi *Ancylostoma* sp., 2(6%) bagi *U. stenocephala*, 1(3%) bagi *T. vulpis*, *T. trichiura*, *Ascaris* sp. dan *Capillaria* sp.. Walau bagaimanapun, tiada satu pun sampel tinja kucing mempunyai *T. gondii* setelah dikesan menerusi

kaedah mikroskop ataupun PCR. Ujian PCR dianggap negati yang sahih kerana saiz amplicon kawalan dalaman bersaiz 150 bp diperolehi dalam setiap asai PCR. Kesimpulannya, tiada kucing peliharaan dijangkiti oleh *T. gondii* tetapi membawa parasit usus yang lain terutamanya *T. cati*.

## DETECTION OF INTESTINAL PARASITES AND *Toxoplasma gondii* IN KOTA BHARU PET CATS VIA MICROSCOPY AND MOLECULAR TECHNIQUES

### ABSTRACT

*Toxoplasma gondii* is a zoonotic parasitic protozoon that causes toxoplasmosis. Cat is a definitive host for *T. gondii* and a reservoir for transmission of other intestinal parasites. Thus, this study was embarked upon to examine and identify intestinal parasites in pet cats and developed an in-house duplex polymerase chain reaction (PCR) assay for detection of *T. gondii* in the faecal specimen. A total of 36 pet cats' faecal samples were collected from different areas in Kota Bharu, Kelantan. All stool samples were screened using conventional saline wet mount and examined by direct microscopy. Subsequently, the samples were concentrated by formalin ether sedimentation techniques and stained with permanent modified acid-fast stain. For specific DNA detection, an in-house duplex PCR assay was developed to detect an internal transcribed spacers (ITS1) region of 18S rRNA gene of *T. gondii*. *Plasmodium falciparum* gene was incorporated into the PCR assay as the internal control to rule out false negative results. Result showed that 58% were positive with one or more parasites. For direct wet mount, eggs of *Toxocara cati*, *Toxascaris leonine*, *Ancylostoma* sp., *Trichuris vulpis*, *Uncinaria stenocephala*, *Trichuris trichiura*, *Ascaris* sp. and *Capillaria* sp. were observed in 5(14%), 5(14%), 3(8%), 1(3%) ,1(3%), 1(3%), 1(3%) and 1(3%) stool sample, respectively. In the concentration technique, 8(22%) of stool samples were positive for *T. cati*; 6(17%) for *T. leonine*, 3(8%) for *Ancylostoma* sp., 2(6%) for *U. stenocephala*, 1(3%) for *T. vulpis*, *T. trichiura*, *Ascaris* sp. and *Capillaria* sp.. Interestingly, neither oocysts nor DNA of *T. gondii* was detected by microscopy and PCR assay. The latter assay revealed that the results were all true negatives because each PCR assay produced the expected internal

control amplicon of 150 bp. In conclusion, none of the pet cats was infected with *T. gondii* but they carried other intestinal parasites, in which *T. cati* was the most common.

# CHAPTER 1

## INTRODUCTION

### 1.1 Research Background

Cats are hosts for numerous intestinal parasites, which may be zoonotic. Zoonotic diseases are infectious diseases that can be spread from animals to humans. There are many zoonotic diseases and can be caused by viruses, bacteria, parasites, and fungi. Their threat to human health is growing due to increasing global movement of people and animals and the effects of human populations expanding into previously undeveloped wildlife habitats (Salant *et al.*, 2010).

The two main types of intestinal parasites are helminths and protozoa. There are several intestinal helminths such as tapeworms, hookworm, pinworms, and roundworms. In their adult form, helminths cannot multiply in the human body. Protozoa can multiply inside the human body, which can allow serious infections to develop. Intestinal parasites are usually transmitted when someone comes in contact with infected faeces (Salant *et al.*, 2010).

*Toxoplasma gondii* is a ubiquitous, intracellular protozoan parasite that causes toxoplasmosis, a zoonotic disease (Salant *et al.*, 2010). Since its first description in the *gondii*, a rodent from North Africa, by Charles Nicolle and Louis Manceaux in 1908, the parasite was progressively recognized as the agent of a widespread zoonosis (Robert-Gangneux and Dardé, 2012).

of other intestinal parasites.(Ramos Silva *et al.*, 2001). *T. gondii* completes the sexual stage of their life cycle, producing oocysts that are excreted in the faeces. Cats excrete approximately 20 million eggs between 3-18 days after infection (Tasawar *et al.*, 2013).

Warm-blooded animals including most livestock and humans that act as intermediate hosts ingest oocysts from the contaminated environment and become infected (Tasawar *et al.*, 2013). Human usually become infected through ingestion of oocysts in contaminated soil and water; tissue cysts in undercooked meat, or congenital transmission (Innes *et al.*, 2009).

The disease is of economic importance and it has become a public health concern since it leads to abortions and neonatal complications in humans. Three groups of people are at risk of acquiring toxoplasmosis are the foetus of seronegative pregnant women, young children and immunocompromised patients (Dubey and Jones, 2008). *T. gondii* infection in cats is both of epidemiological and clinical significance. While acute infection can be fatal to young kittens, cats may be asymptomatic, increasing the likelihood of accidental infection (Elmore *et al.*, 2010).

Historically, infective cats have been identified by microscopy to detect oocysts, approximately 10–13 µm in diameter, found in their faeces. Because the sensitivity of this method is low, detection is problematic when small oocyst numbers are present (Salant *et al.*, 2010). Furthermore, oocysts of other coccidian parasites present in cat faeces may not be differentiated microscopically from *T. gondii*. Therefore, polymerase chain reaction (PCR) that is specific and sensitive was used in this study for detection of *T. gondii* oocysts in faeces of pet cats.

## **1.2 Problem Statement**

*T. gondii* is a zoonotic parasitic protozoon, where cat has been recognized as a reservoir. Thus, this study was carried out to determine the presence of *T. gondii* infection and some intestinal helminth parasites in pet cats in Kota Bharu.

## **1.3 Objective**

General Objective:

To examine intestinal parasites and *Toxoplasma gondii* in Kota Bharu pet cats using a direct microscopic stool examination technique and a molecular approach.

Specific Objectives:

1. To observe and identify the presence of *T. gondii* and intestinal parasites in pet cats via microscopy examination.
2. To developed an inhouse PCR assay to detect the presence of *T. gondii* in Kota Bharu pet cats.

## **1.4 Significance of Study**

Domestic cat has a long and mostly favourable history in its association with human beings. In modern times, cats are appreciated for their ability to provide companionship (Neidhart and Boyd, 2002) as well as for deterring rodents from invading the home. However, cats are the only definitive host of the *T. gondii*, and thus shedding of oocysts by cats can transmit toxoplasmosis. It is thought that about 1% of domestic cats are excreting oocysts at any one time (Innes *et al.*, 2009).

This pilot study aimed to throw light on *T. gondii* infection of zoonotic importance and some of intestinal parasites present in pet cats. Many of them cause economic problems

and public health hazards. Severe disease may arise as a result of congenital infection, and of infection or reactivation in people who are immunocompromised.

Currently, screening of oocyst shedding in cats cannot rely on microscopy because oocyst identification lacks sensitivity and specificity. Therefore, a rapid, sensitive and specific polymerase chain reaction (PCR) which can amplify DNA of *T. gondii* was applied in this study.

As Malaysia marches into the new millennium with aspirations to be a fully developed nation, the health and well being of its people remains a top priority in the agenda. A critical area for future research will be to determine how transmission between animals and human can be control by improving knowledge about toxoplasmosis. Thus this study is deemed important to determine the presence of *Toxoplasma* infection in pet cats, as well as to ascertain the presence of other intestinal helminth parasites in these pets in Kota Bharu.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Toxoplasma gondii

*T. gondii* is a coccidian that is ubiquitous and an obligate intracellular protozoan belongs to the phylum Apicomplexa, subclass coccidian. This is the only species in the genus *Toxoplasma*. Protozoa under the phylum Apicomplexa are absent of pseudopodia, flagella, undulating membrane or cilia, but exhibit gliding motility and so-called sporozoa (Botero-Kleiven, 2006).

They generally complete the sexual stage of their life cycles within the definitive host's intestinal tract which are feline family and causes toxoplasmosis, a zoonotic disease. Members of the phylum Apicomplexa have highly developed structures at their anterior regions called apical complexes, which facilitates the parasite attachment to and penetration of the host cell. It is the cause of toxoplasmosis, a major zoonosis (Salant *et al.*, 2010).

### 2.1.1 Taxonomy of *T. gondii*

*T. gondii* taxonomy is shown in **Error! Reference source not found.** Sporozoa belong to the phylum Apicomplexa. It contains two classes namely Aconoidasida and Conoidasida. Parasites of the class Aconoidasida either belong to the order Haemosporida containing the genus *Plasmodium* or *Piroplasmida* containing the genus *Babesia*. Parasites of the class Conoidasida either belong to the two suborders. One of the suborders is Eimeriorina containing five important genera: *Cryptosporidium*, *Cyclospora*, *Sarcocystis*, *Toxoplasma* and *Isospora*. *Toxoplasma gondii* is the only species in the genus *Toxoplasma*.

SCIENTIFIC CLASSIFICATION	
KINGDOM	Chromalveolata
PHYLUM	Apicomplexa
CLASS	Conoidasida
ORDER	Eucoccidiorida
FAMILY	Sarcocystidae
GENUS	Toxoplasma
SPECIES	<i>gondii</i>

Table 2.1: Taxonomy of *Toxoplasma gondii*  
Available from: [http://en.wikipedia.org/wiki/Toxoplasma\\_gondii](http://en.wikipedia.org/wiki/Toxoplasma_gondii)

### 2.1.2 Morphological Stages

*T. gondii* has a complex life cycle with three infectious stages; sporozoites in oocysts, tachyzoites; the actively multiplying stage, and bradyzoites; the slowly multiplying stage that are enclosed in tissue cysts. Oocysts are excreted in faeces, whereas tachyzoites and bradyzoites are found in tissues and milk (Dubey and Lappin; Dubey and Jones, 2008).

Oocysts are subspherical to ellipsoidal in shape and measure between 10-12  $\mu\text{m}$  (Dubey and Jones, 2008). Oocysts are highly environmentally resistant. Desiccation can kill oocysts (Figure 2.1). Cats excrete *T. gondii* oocysts in faeces 3 to 10 days after ingesting bradyzoites,  $\leq 18$  days after ingesting of sporulated oocysts and  $\leq 13$  days after ingesting tachyzoites (Dubey, 1998). After sporulation, which takes place between 1 and 21 days, oocysts containing two sporocysts, each containing four sporozoites are infective when ingested by mammals including human and give rise to the tachyzoite stage.

Tachyzoites (Figure 2.2) is the rapidly multiplying stages of the parasite. It measures about 6x2  $\mu\text{m}$  crescentic or oval shaped. They penetrate actively into all nucleated cells and form a cytoplasmic vacuole. After repeated replication, host cells are disrupted. Later, tachyzoites will propagate into the bloodstream and infect many tissues (Montoya and Liesenfeld, 2004).

Replication leads to cell death and rapid invasion of neighbouring cells. The tachyzoite form causes a strong inflammatory response and a tissue destruction that cause clinical manifestations of disease. Tachyzoites are transformed into bradyzoites under the pressure of the immune response to form cysts (Nissapatorn, 2013).

Bradyzoites persist inside cysts for the life of the host. They are morphologically identical to tachyzoites but multiply slowly, express stage-specific molecules, and are functionally different. Tissue cysts contain hundreds and thousands of bradyzoites (**Error! Reference source not found.**). Cysts are infective stages for intermediate and definitive hosts. They have a high affinity for neural and muscular tissues and are located mainly in the central nervous system (CNS), eye, skeletal and cardiac muscles as well as other visceral organs (Dubey, 1998). Tissue cysts break down periodically, with bradyzoites transforming into tachyzoites that reinvade host cells and again transform to bradyzoites within new tissue cysts (Montoya and Liesenfeld, 2004).



Figure 2.1: Oocysts in formalin-preserved wet mount preparation of cat feces (unstained, high power)  
Available from: <http://ruby.fgcu.edu/courses/davidb/50249/web/toxo83.htm>

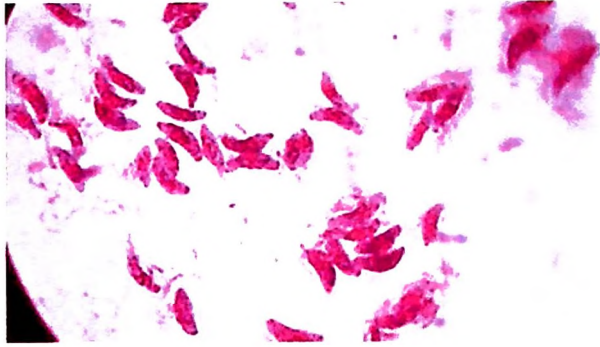


Figure 2.2: Tachyzoites from the peritoneal exudates of infected mice stained with Giemsa. Observe under 100x objectives, Toxoplasmosis.

Available from:

<http://www.oculist.net/downaton502/prof/ebook/duanes/pages/v4/v4c046.html#ref>

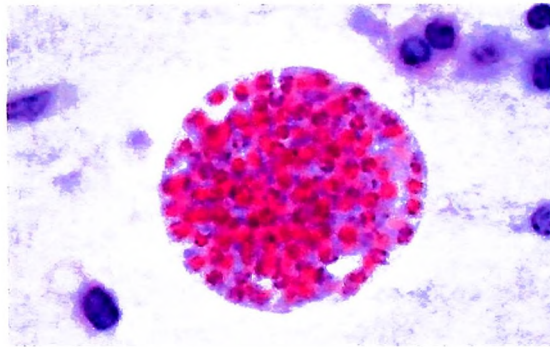


Figure 2.2: Bradyzoites of *T.gondii*