



**UNIVERSITI PUTRA MALAYSIA**

**PREVALENCE OF GASTROINTESTINAL PROTOZOA IN PET AND  
STRAY CATS IN THE KLANG VALLEY, MALAYSIA**

**TAN LI PING**

**FPV 2019 11**



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CATS IN THE KLANG VALLEY, MALAYSIA**

By

**TAN LI PING**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the Degree of Master of  
Veterinary Science**

**May 2019**



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## DEDICATION

*I would like to dedicate this thesis to my beloved late father Mr. Tan Tai Huan for all his support and love during my education journey; my mother for her endless support, love and advices ; my supervisors for all their knowledge they have shared with me and their guidance throughout this journey*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Veterinary Science

## **PREVALENCE OF GASTROINTESTINAL PROTOZOA IN PET AND STRAY CATS IN THE KLANG VALLEY, MALAYSIA**

By

**TAN LI PING**

May 2019

**Chairman : Associate Professor Malaika Watanabe, DVM, PhD**  
**Faculty : Veterinary Medicine**

Gastrointestinal protozoa that are commonly reported to cause diarrhea in cats include *Giardia duodenalis*, *Cryptosporidium* spp., *Cystoisospora* spp., and *Tritrichomonas foetus*. Even though, *Entamoeba* spp. is rarely reported in cats, it has been recently identified in the stool samples of the feline species in Malaysia. *Toxoplasma gondii* is often reported in cats and though rarely causes diarrhea, it can be fatal to cats. Most of these protozoa carries a zoonotic risk as well. Currently, there is a lack of updated information on the current prevalence of these gastrointestinal protozoa in Malaysia. Thus, the objectives of this study were to determine the prevalence of these gastrointestinal protozoa in pet and stray cats in Klang Valley, Malaysia; to determine the seroprevalence of *Toxoplasma gondii* in pet and stray cats in Klang Valley, Malaysia; and to investigate the risk factors associated with protozoan infections. 201 fecal samples and 198 blood samples were randomly collected from pet cats presented to University Veterinary Hospital and stray cats from the local council, Dewan Bandaraya Kuala Lumpur. Among the collected fecal samples, 24 were cultured for *T. foetus* using InPouch® TF Feline, simple fecal floatation was performed on 44 samples, 58 fecal samples were subjected to *Giardia* spp. antigen chromatographic immunoassay and all 201 samples to polymerase chain reaction (PCR). PCR was performed to detect *Giardia* spp., *Cryptosporidium* spp., *Toxoplasma gondii*, *Tritrichomonas foetus* and *Entamoeba histolytica*. Indirect-ELISA was used to detect *Toxoplasma gondii* antibody in blood samples collected. Samples were then grouped according to their age, sex, breed and management to study the risk factors associated with the gastrointestinal protozoa infection. In general, the overall prevalence in the cat population in Klang Valley was 50% *Cystoisospora* spp., 7.9% *Giardia duodenalis*, 10.4% *Toxoplasma gondii* and 33% *Tritrichomonas foetus*. Seroprevalence of *Toxoplasma gondii* was 5.6% among samples collected. Both *Cryptosporidium* spp. and *Entamoeba* spp. failed to be amplified in multiple attempts in our study, thus the molecular epidemiology of these two

gastrointestinal protozoa in the cat population in Klang Valley remain unknown. Age, sex, breed and pet cat management showed no significant association with infection with *Giardia duodenalis*, *Toxoplasma gondii* and *Tritrichomonas foetus* in cats ( $P>0.05$ ). Stray cats showed a significantly higher risk for protozoan infection as compared to pet cats ( $P<0.05$ ). In conclusion, a high prevalence of gastrointestinal protozoa was detected in the cat population in the Klang Valley. This is alarming due to the clinical importance and zoonotic risk of the protozoa detected.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

## **PREVALENS PROTOZOA GASTROUSUS DI KALANGAN KUCING PELIHARAAN DAN KUCING TERBIAR DI LEMBAH KLANG**

Oleh

**TAN LI PING**

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Protozoa gastrousus di kalangan kucing yang sering dilaporkan sebagai penyebab cirit-birit termasuk *Giardia duodenalis*, *Cryptosporidium* spp., *Cystoisospora* spp., dan *Tritrichomonas foetus*. Walaupun, *Entamoeba* spp. jarang dilaporkan pada kucing tetapi, ia dikesan dalam sampel najis kucing di satu kajian di Malaysia baru-baru ini. *Toxoplasma gondii* kerap dilaporkan di kalangan kucing, walaupun ia jarang menyebabkan cirit-birit, tetapi ia boleh membawa maut kepada kucing. Kebanyakan protozoa ini juga membawa risiko zoonotik. Pada masa ini, kita tidak mempunyai informasi semasa tentang prevalens protozoa gastrousus yang disebutkan. Oleh itu, objektif kajian ini ialah untuk menentukan prevalens protozoa gastrousus di kalangan kucing perliharaan dan kucing terbiar di kawasan Lembah Klang, Malaysia; untuk menentukan seroprevalens *Toxoplasma gondii* pada kalangan kucing perliharaan dan kucing terbiar di Lembah Klang, Malaysia ; dan untuk menyiasat faktor-faktor risiko yang dikaitkan dengan infeksi protozoa. 201 sampel najis dan 198 sampel darah telah dikumpulkan daripada kucing perliharaan yang dibawa ke Hospital Veterinar UPM dan kucing terbiar di majlis tempatan, Dewan Bandaraya Kuala Lumpur secara rawak. Antara sampel najis yang dikumpulkan, 24 sampel telah dikultur dengan menggunakan InPouch® TF Feline, teknik pengapungan najis mudah telah dilaksanakan atas 44 sampel, 58 sampel najis pula diuji dengan immunoassay kromatografi antigen *Giardia* spp. dan kesemua 201 sampel diuji dengan tindak balas rantai polimerasi(PCR). PCR telah dijalankan untuk mengesan *Giardia* spp., *Cryptosporidium* spp., *Toxoplasma gondii*, *Tritrichomonas foetus* and *Entamoeba histolytica*. Teknik ELISA-tidak langsung(Indirect-ELISA) telah digunakan untuk mengesan antibodi *Toxoplasma gondii* dalam sampel darah yang dikumpul. Kesemua sampel kemudian diasingkan mengikut umur, jantina, baka dan pengurusan kucing di rumah untuk meneliti faktor risiko yang dikaitkan dengan infeksi protozoa gastrousus. Keseluruhannya, prevalens di kalangan populasi kucing di Lembah Klang terdiri daripada 52% *Cystoisospora* spp., 7.9% *Giardia*



*duodenalis*, 10.4% *Toxoplasma gondii* dan 33% *Tritrichomonas foetus*. Seroprevalens *Toxoplasma gondii* dapat dilihat dalam 5.6% sampel yang dikumpul. Kedua-dua *Cryptosporidium* spp. dan *Entamoeba* spp. gagal diimplikasikan walaupun banyak percubaan telah dilakukan, ini menyebabkan prevalens dua protozoa gastrusus di populasi kucing di Lembah Klang tidak diketahui. Umur, jantina, baka dan pengurusan kucing peliharaan di rumah menunjukkan tiada kaitan yang penting dengan infeksi *Giardia duodenalis*, *Toxoplasma gondii*, dan *Tritrichomonas foetus* dalam kucing ( $P>0.05$ ). Kucing terbiar mempunyai risiko tinggi yang ketara terhadap infeksi protozoa gastrusus jika dibandingkan dengan kucing peliharaan ( $P<0.05$ ). Konklusinya, prevalens protozoa gastrusus yang tinggi telah dikesan di kalangan populasi kucing di Lembah Klang. Situasi ini membimbangkan kerana melibatkan kepentingan klinikal dan risiko zoonotik protozoa yang dikesan dalam kajian ini.

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I certify that a Thesis Examination Committee has met on 10<sup>th</sup> of May 2019 to conduct the final examination of Tan Li Ping on her thesis entitled Prevalence of Gastrointestinal Protozoa in Pet and Stray Cats in Klang Valley, Malaysia in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Veterinary Science

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## LIST OF ABBREVIATIONS

%	Percent
<	Less than
>	More than
°C	Degree Celcius
µl	Microliter
µm	Micrometer
µM	Micromolar
AIDS	Acquired Immunodeficiency Syndrome
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
<i>C. andersoni</i>	<i>Cryptosporidium andersoni</i>
<i>C. bovis</i>	<i>Cryptosporidium bovis</i>
<i>C. canis</i>	<i>Cryptosporidium canis</i>
<i>C. fayeri</i>	<i>Cryptosporidium fayeri</i>
<i>C. felis</i>	<i>Cryptosporidium felis</i>
<i>C. hominis</i>	<i>Cryptosporidium hominis</i>
<i>C. macropodum</i>	<i>Cryptosporidium macropodum</i>
<i>C. muris</i>	<i>Cryptosporidium muris</i>
<i>C. parvum</i>	<i>Cryptosporidium parvum</i>
<i>C. rivolta</i>	<i>Cryptosporidium rivolta</i>
<i>C. ryanae</i>	<i>Cryptosporidium ryanae</i>
<i>C. suis</i>	<i>Cryptosporidium suis</i>
<i>C. wrairi</i>	<i>Cryptosporidium wrairi</i>
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate

ELISA	Enzyme-linked Immunoassay
<i>E. chattoni</i>	<i>Entamoeba chattoni</i>
<i>E. dispar</i>	<i>Entamoeba dispar</i>
<i>E. equi</i>	<i>Entamoeba equi</i>
<i>E. histolytica</i>	<i>Entamoeba histolytica</i>
<i>E. moshkovskii</i>	<i>Entamoeba moshkovskii</i>
<i>E. polecki</i>	<i>Entamoeba polecki</i>
<i>E. suis</i>	<i>Entamoeba suis</i>
g	Gram
<i>G. agilis</i>	<i>Giardia agilis</i>
<i>G. argeae</i>	<i>Giardia argeae</i>
<i>G. duodenalis</i>	<i>Giardia duodenalis</i>
<i>G. intestinalis</i>	<i>Giardia intestinalis</i>
<i>G. lamblia</i>	<i>Giardia lamblia</i>
<i>G. microti</i>	<i>Giardia microti</i>
<i>G. muris</i>	<i>Giardia muris</i>
<i>G. psittaci</i>	<i>Giardia psittaci</i>
GI protozoa	Gastrointestinal Protozoa
HIV	Human Immunodeficiency Virus
IFAT	Immunofluorescence Antibody Test
IACUC	Institutional Animal Care and Use Committee
IgG	Immunoglobulin class G
MAT	Modified Agglutination Test
min	Minute
MgCl <sub>2</sub>	Magnesium Chloride
ml	Milliliter

mm	Millimeter
mM	Millimolar
mZN	Modified-Ziehl Neelsen
NC	Negative Control
O.D	Optical Density
OR	Odd Ratio
P	Probability
PC	Positive Control
<i>P. hominis</i>	<i>Pentatrichomonas hominis</i>
PCR	Polymerase Chain Reaction
rpm	Revolution per minute
rRNA	Ribosomal Ribonucleic acid
s	Second
S/P	Sample to Positive
spp.	Species
TAE	Tris-acetate-ethylenediaminetetraacetic acid
<i>T. foetus</i>	<i>Tritrichomonas foetus</i>
<i>T. gondii</i>	<i>Toxoplasma gondii</i>
U	Unit
UPM	Universiti Putra Malaysia
USA	United State of America



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## CHAPTER 1

### INTRODUCTION

#### 1.1 Brief Overview

Gastrointestinal protozoa is one of the causes of diarrhea in cats often overlooked due to lack of sensitive diagnostic methods that are easily accessible in clinics or hospitals. The most common gastrointestinal protozoa reported in cats include *Giardia* spp., *Cryptosporidium* spp., *Toxoplasma* spp., *Cystoisospora* spp., *Tritrichomonas foetus*, and *Entamoeba histolytica* (Lappin,2005). *E. histolytica* is rarely found in cats, but a recent study in Malaysia reported the detection of *Entamoeba* spp. in cats (Ngui et al., 2014). These gastrointestinal protozoa are not just clinically important to the feline species, but some of these protozoa are zoonotic. Most of these protozoa have the potential to cause problems in immunocompromised individuals and some pose the risk of abortion in pregnant women. Among the gastrointestinal protozoa that are zoonotic, *T. gondii* is potentially the most pathogenic protozoa specially in immunocompromised individuals. The increasing number of cat being kept as pets would also impose risk of cross infection between these two species. Currently, there is a paucity of information regarding the prevalence of these gastrointestinal protozoa in both pet and stray cat population in Malaysia.

#### 1.2 Aim and Objective

There were 3 main objectives of this study.

1. To determine the current prevalence of the gastrointestinal protozoa among pet and stray cats.
2. To determine the seroprevalence of *Toxoplasma gondii* in the pet and stray cat population in the Klang Valley.
3. To investigate the risk factors associated with infection with these protozoa.

#### 1.3 Justification and Novelty

Despite the large population of cats in Malaysia, not many studies have been carried out in Malaysia on gastrointestinal protozoa of cats. The most recent study was carried out by Ngui et al. (2014) in Malaysia using microscopic detection techniques in a small sample size of 28 cats. The protozoa that were detected among the stray population in the study by Ngui et al, 2014 were *Giardia* spp. (10.7%), *Cryptosporidium* spp. (7.1%), *Entamoeba* spp. (10.7%) and *Cystoisospora* spp. (3.7%). Seroprevalence of *T. gondii* (14.7%) was



determined in a study a decade ago by Chandrawathani et al. in 2008. *T. foetus*, an emerging protozoa known to cause chronic diarrhea in cats has been recently detected showing prevalence of 6.7% using culturing technique in Malaysia in a preliminary study performed by our team in 2016. Currently, there are still no reports on molecular detection of gastrointestinal protozoa in cats in Malaysia. The paucity of updated data on the gastrointestinal protozoa of pet and stray cats in Malaysia and the use of diagnostic methods in previous studies that are lower in sensitivity warranted the importance of this study.



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Tan Li Ping (Michelle) was born on the 9<sup>th</sup> of July 1992 in Kuala Lumpur, Malaysia. She attended SJK(C) Jalan Davidson in Kuala Lumpur during primary and took her secondary education in SMK Cochrane Perkasa. Afterwards, she attended her Foundation in Foundation of Agricultural Science in Universiti Putra Malaysia in 2010. After one year of study, she then enrolled into the Faculty of Veterinary Medicine, Universiti Putra Malaysia in 2011 and completed her undergraduate degree in Doctor of Veterinary Medicine (DVM) in 2016. She did her final year research project during Bachelor degree with the title of "Detection of gastrointestinal protozoa in pet cats in the Klang Valley" During September 2016, she enrolled in the degree of Master Veterinary Science in the same Faculty in the field of Small Animal Medicine under the supervision of Associate Professor Dr. Malaika Watanabe, Dr. Reuben Sunil Kumar Sharma, Dr. Puteri Azaziah Megat Abd Rani and Dr. Sharifah Salmah Syed Hussain. Her research interest revolves around Small Animal Medicine and Parasitology. Her Master degree is a continuation of her final year research project during Bachelor degree.



## LIST OF PUBLICATIONS

### Publication :

Tan L. P., Puteri A. M. A. R., Reuben S. K. S., Sharifah S. S. H. and Malaika W. First molecular detection of *Tritrichomonas foetus* in Domestic Cats in Klang Valley, Malaysia (Accepted for publication in Tropical Biomedicine, in press)

### Conference Proceedings :

Tan L. P., Puteri A. M. A. R., Reuben S. K. S., Sharifah S. S. H. and Malaika W. Seroprevalence of *Toxoplasma gondii* in Pet and Stray Cats in Klang Valley, Malaysia. (Proceeding from 29<sup>th</sup> VAM Congress 2017 (pp. 88) (Poster presentation)

Tan L. P., Puteri A. M. A. R., Reuben S. K. S., Sharifah S. S. H. and Malaika W. Prevalence of Gastrointestinal Protozoa in Pet and Stray Cat in the Klang Valley, Malaysia. (43<sup>RD</sup> WSAVA 2018) (Poster presentation)



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