UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF ECTO- AND ENDO-PARASITES OF PET DOGS PRESENTED TO SELECTED VETERINARY CLINICS IN KLANG VALLEY, MALAYSIA

CHONG BAN LEE

lp
FPV 2018 18

PREVALENCE OF ECTO- AND ENDO-PARASITES OF PET DOGS PRESENTED TO SELECTED VETERINARY CLINICS IN KLANG VALLEY, MALAYSIA

CHONG BAN LEE

A project paper submitted to the

Faculty of Veterinary Medicine, University Putra Malaysia

In partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

University Putra Malaysia Serdang, Selangor Darul Ehsan

MARCH 2018

CERTIFICATION

It is hereby certified that we have read this project paper entitled "Prevalence of Ecto- and Endo-parasites of Pet Dogs Presented to Selected Veterinary Clinics in Klang Valley" by Chong Ban Lee, and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project

ASSOC. PROF. DR MALAIKA WATANABE
DVM (UPM), PhD (Yamaguchi University)
Associate Professor,
Department of Companion Animal Medicine and Surgery,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

DR. PUTERI AZAZIAH MEGAT ABDUL RANI

DVM (UPM), PhD (University of Queensland)

Senior Lecturer,

Department of Companion Animal Medicine and Surgery,

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DEDICATION

This report is dedicated to

My parents Chong Ah Meng and Seh Siew Hooi,

&

Family members

For supporting me throughout these years.

ACKNOWLEDGEMENTS

First and foremost, I wish to thank my supervisor, Associate Professor Dr Malaika Watanabe and Dr Puteri Azaziah Megat Abdul Rani for their guidance and support throughout the project. I would like to thank Dr Sanny Ong, representative from Rhone Ma in providing me guidance and advice for the project, and Boehringer Ingelheim for sponsoring the project.

I would like to acknowledge and thank the staff of parasitology laboratory, Mrs. Maizatul Akmal, Mr Rashid, staff and veterinarians from University Veterinary Hospital for all the technical help and time.

Special thanks to Dr Prem Anand, Dr Mellissa Aw and fellow veterinarians in Gasing Veterinary Hospital for their assistance during sample collection in completion of this final year project.

Last but not least, I would like to thanks my dearest family and friends for their help, moral support and encouragement throughout the project. Beloved DVM class 2018, Dr Michelle Tan Lee Ping, June Chung Sing Yee, Chew Xuan-Yee, Evelyn Tie Yie Yie, Lim Set Thing, Choong Jia Jie, Joel Edward, Samuel Loi De Yun.

CONTENTS

TITLE……..i

CERTIFICATION ii

ACKNOWLEDGEMENTS v

LIST OF TABLES viii

LIST OF FIGURES viii

LIST OF ABBREVIATIONS ix

ABSTRAK x

ABSTRACT xii

1.0 INTRODUCTION 1

2.0 LITERATURE REVIEW

2.1 Ectoparasites.....3

2.2 Intestinal Helminths 4

2.3 Vector-Borne Pathogens.....6

3.0 MATERIALS AND METHODS

3.1 Recruiting Subjects.....9

3.2 Sample Collection 9

3.3 Storage and Process of Examination 10

3.4 Data Analysis 10

4.0 RESULTS

4.1 Ectoparasites....16

4.2 Intestinal Helminths....17

4.3 Vector-Borne Pathogens....18

5.0 DISCUSSION

5.1 Ectoparasites....19

5.2 Intestinal Helminths....19

5.3 Vector-Borne Pathogens....20

5.4 Overview....21

6.0 CONCLUSION....22

7.0 RECOMMENDATIONS....22

8.0 REFERENCES....23

9.0 APPENDICES

Appendix I: Baermann-Wetzel Technique....26

Appendix II: Fecal Floatation Technique....26

Appendix III: Fecal Sedimentation Technique....27

Appendix IV: Snap 4Dx Plus Test....27

Appendix V: Snap Leishmania Test....28

LIST OF TABLES

Table 1: Age, breed and sex of dogs sampled …….....12

Table 2: Overall results for each sample…….……15

Table 3: General prevalence for positive dogs……..……..……..…..16

LIST OF FIGURES

Figure 1: Age of pet dogs……13

Figure 2: Sex of pet dogs…….……13

Figure 3: Breeds of pet dogs…….……...14

Figure 4: *Ctenocephalides felis* observed under the stereo microscope…….….16

Figure 5: *Rhipicephalus sanguineus* observed under the stereo microscope…..17

Figure 6: *Ancylostoma* sp. ova detected via fecal floatation technique observed under the compound microscope……..17

Figure 7: *Trichuris* sp. ova detected via fecal floatation technique observed under the compound microscope……..……..…..18

LIST OF ABBREVIATIONS

EDTA	Ethylenediaminetetraacetic acid
NM	Neutered male
IM	Intact male
NF	Neutered female
IF	Intact female

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakultin Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Ilmiah Tahun Akhir

PREVALENS EKTOPARASIT DAN ENDOPARASIT DALAM KALANGAN ANJING PELIHARAAN YANG DIBAWA KE KLINIK VETERINAR YANG TERTENTU DI KAWASAN LEMBAH KLANG

Oleh,

CHONG BAN LEE

2018

Penyelia: Profesor Madya Dr Malaika Watanabe

Penyelia bersama: Dr Puteri Azaziah Megat Abdul Rani

Malaysia merupakan negara tropical yang mempunyai cuaca yang panas dan lembab sepanjang tahun, di mana persekitaran ini sesuai untuk kehidupan ekto- dan endo-parasit. Ujian skrining telah dijalankan atas anjing peliharaan yang berada di Lembah Klang (n=30), dan telah memenuhi kriteria pemilihan. Sampel ektoparasit, najis dan darah telah dikumpul untuk analisis. Sampek ektoparasit

tertakluk kepada pengenalan morfologi. Sampel najis tertakluk kepada Teknik Baermann-Wetzel, pengapungan dan pemendapan, untuk mengesan helmin paru-paru dan perut. Sampel darah tertakluk kepada Snap 4Dx Plus, yang digunakan untuk mengesan antibodi terhadap *Ehrlichia* spp., *Anaplasma* spp. *dan Borrelia burgdorferi*, dan antigen *Dirofilaria immitis*. Kit ujian Snap Leishmania digunakan untuk mengesan antibodi terhadap *Leishmania* spp. Ektoparasit dikesan positif dalam empat anjing, di mana dua anjing mempunyai *Ctenocephalides felis* (6.67%) dan dua anjing yang lain mempunyai *Rhipicephalus* sp. (6.67%). Lima anjing dikesan positif helmin, daripada yang positif, semua mempunyai *Ancylostoma* sp. (16.67%), manakala salah satu anjing yang positif *Ancylostoma* sp. juga dijumpai positif *Trichuris* sp. (3.33%). Tujuh sampel darah dijumpai positif dengan menggunakan Snap 4Dx, mendapat kadar prevalens 10% (3/30), untuk *Anaplasma platys*, 6.67% (2/30) untuk *Dirofilaria immitis* dan 6.67% (2/30) untuk *Ehrlichia canis*. Semua sampel didapati negatif terhadap antibodi *Borrelia burgdorferi* dan antibodi *Leishmania infantum*. Dalam kajian ini, kalaziman keseluruhan untuk parasit didapati rendah atas anjing perliharaan. Keadaan tersebut mungkin disebabkan oleh cara permeliharaan yang bagus, terurus, dan berada di persekitaran yang tidak dicemar oleh parasit. Walau bagaimanapun, anjing perliharaan masih menpunyai risiko parasit zoonotic, yang memerlukan kawalan parasit secara rutin.

Kata kunci: anjing peliharaan, Lembah Klang, ektoparasit, helmin usus, patogen bawaan vektor.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the Course VPD 4999 – Final Year Project

PREVALENCE OF ECTO- AND ENDO-PARASITES OF PET DOGS PRESENTED TO SELECTED VETERINARY CLINICS IN KLANG VALLEY, MALAYSIA

By

Chong Ban Lee

2018

Supervisor: Associate Professor Dr Malaika Watanabe

Co-supervisor: Dr Puteri Azaziah Megat Abdul Rani

Malaysia is a tropical country with hot and humid weather all year round that provides a suitable environment for the survival of ecto- and endo-parasites. A screening test was performed on pet dogs in Klang Valley (n=30), that met the selection criteria. Ectoparasites, feces and blood were collected for analysis. Ectoparasites obtained were subjected to morphological identification. Fecal samples were subjected to Baermann-Wetzel, floatation and sedimentation techniques for the detection of lungworms and intestinal helminths. The blood samples were used to conduct the Snap 4Dx Plus, that is used to detect antibodies against *Ehrlichia* spp., *Anaplasma* spp. and *Borrelia burgdorferi* and *Dirofilaria*

immitis antigen. The Snap Leishmania test was used to detect antibodies against *Leishmania* spp. Ectoparasites were detected on four dogs, there were two dogs infested with *Ctenocephalides felis* (6.67%) and two infested with *Rhipicephalus sanguineus* (6.67%). Five dogs were positive for intestinal helminths, all of which were identified as *Ancylostoma* sp. (16.67%), and one of them had mixed infection with *Trichuris* sp. (3.33%). Seven blood samples tested positive on the Snap 4Dx, with prevalence rates of 10% (3/30) for *Anaplasma platys*, 6.67% (2/30) for *Dirofilaria immitis* and 6.67% (2/30) for *Ehrlichia canis*. All samples were negative for both *Borrelia burgdorferi* antibodies and *Leishmania infantum* antibodies. In this study, there was generally a low prevalence of parasites in pet dogs most likely because they are well taken care of and managed with low environmental contamination. However, infected pet dogs still pose a zoonotic risk that necessitates routine parasitic control.

Keywords: Pet dogs, Klang Valley, ectoparasites, intestinal helminths, vector-borne pathogens.

1.0 INTRODUCTION

Malaysia is one of the countries that makes up Southeast Asia, and is located north of the equator, lying in tropical latitudes, with uniform temperature, high humidity and copious rainfall throughout the year. This climate provides a suitable environment for the multiplication and persistence of ecto- and endo-parasites all year round and is a problem for both medical and veterinary practitioners.

Dogs are known to be man's best friend, and were among the earliest to be domesticated. Dogs were initially domesticated for various purposes such as hunting, guarding, and herding but now more than ever, they are companions to people and are thought of as family members. With this paradigm shift, dogs are now kept as pet within house compounds. Pet dog ownership in Malaysia has increased exponentially due to higher incomes and purchasing power of the population.

With the greater number of dogs sharing the same parks and gardens as humans, not only does it provide the opportunity for parasites to have a constant source to complete their life cycle, but at the same time it poses a zoonotic risk to humans especially children and immunocompromised individuals.

There are several studies looking at the prevalence of intestinal helminths in household dogs (Chong, 2015) and blood parasites in pet dogs (Koh, 2013) conducted in Perak, but not much have been carried out in Klang Valley. Klang Valley comprises of Kuala Lumpur and Selangor, the most heavily populated and

affluent cities of Malaysia and therefore it was important to determine the current status of ecto- and endo-parasites status of pet dogs in Klang Valley.

This study was carried out to determine the ecto- and endo-parasites found in pet dogs are presented to selected veterinary clinics in Klang Valley, and to identify the possible zoonotic parasitic diseases that could be carried by pet dogs. Screening was conducted to identify ectoparasites, intestinal helminths and vector-borne pathogens in the pet dogs meeting specific criterion. This study aimed to provide information to veterinarians and owners on the prevalence of parasitic infestations among pet dogs in the Klang Valley, any zoonotic risks and to emphasize the need for appropriate parasitic control and prevention.

The objectives of this study were to screen, detect and study prevalence of (I) Ectoparasites, (II) Intestinal helminths, and (III) Vector-borne pathogens in pet dogs presented to selected veterinary clinics in Klang Valley.

2.0 LITERATURE REVIEW

2.1 Ectoparasites

Rhipicephalus sanguineus is the most common tick found on dogs worldwide, in both rural and urban areas, and active throughout the year (Dantas-Torres, 2010). A survey was done by Erwanas *et al.* (2014) in Ipoh, on ectoparasites of stray and pet dogs. The results showed that the most prevalent species in stray dogs was *Rhipicephalus sanguineus* (24.1%), followed by *Ctenocephalides canis* (13.8%), *Rhipicephalus microplus* (3.4%) and *Demodex canis* (3.4%); the only species found on pet dogs was *Demodex canis* (2.6%).

According to a study done by Hadi, Soviana and Pratomo (2016), morphological identification of tick infested dogs in Depok, Bogor, Jakarta and Bandung, Indonesia was found to be the same species which was *Rhipicephalus sanguineus*.

The incidence of ectoparasitic infestation was conducted by Chee *et al.* (2008), in 103 stray dogs collected in the Animal Shelter of Gwang-ju City, China, from year 2003 to 2005, the macroscopic ectoparasites found were *Ctenocephalides canis* (6.8%) and *Trichodectes canis* (1.0%). According to a summary of prevalence of flea infestation in dog populations of various countries by Dobler & Pfeffer (2011), *Ctenocephalides felis* is known to be more commonly found on domestic dogs than *Ctenocephalides canis*.

2.2 Intestinal Helminths

In Malaysia, there are a few studies conducted in various states. There was a study done by Chong (2015) on prevalence of intestinal helminths of household dogs in Ipoh, Perak. The survey was done on 62 household dogs, where samples were tested by fecal floatation technique. Intestinal helminths detected in the study were *Ancylostoma* spp. (27.4%), *Toxocara* spp. (8.1%), *Trichuris vulpis* (3.2%) and mixed infection of *Ancylostoma* spp. and *Toxocara* spp. (3.2%). There was also another study done in Ipoh by Erwanas *et al.* (2014), for ecto- and endo-parasites in stray and pet dogs, where there was higher percentage of infected stray dogs (76%) as compared to pet dogs (16%). The intestinal helminths detected in stray dogs by fecal floatation technique were *Ancylostoma* spp. (24.1%) and *Toxocara canis* (3.4%); while pet dogs were infected with *Ascaris* sp. (7.9%) and *Ancylostoma* sp. (5.3%) (Erwanas *et al.*, 2014).

In a study by Ngui *et al.* (2014) on 109 fresh fecal samples of rural dogs from Selangor and Pahang, tested by formalin ether concentration technique, the most prevalent intestinal helminth was *Ancylostoma* spp. (71.4%), followed by *Toxocara* spp. (28.6%), *Trichuris vulpis* (24.7%), *Spirometra* spp. (10.4%), *Toxascaris leonine* (5.2%), *Dipylidium caninum* (3.9%), *Ascaris* spp. (1.3%) and *Hymenolepis diminuta* (1.3%).

A study in Universiti Putra Malaysia (UPM) by Lim (1999), on prevalence of intestinal helminths which screened fecal samples collected from 50 household dogs presented to a clinic in Kuching revealed three species of intestinal helminths:

Ancylostoma spp. (24%), *Toxocara* spp. (16%) and *Trichuris vulpis* (2%) upon fecal smear and fecal floatation.

Prevalence of gastrointestinal parasites in Selangor, was done by Francis (2002) on 60 dogs from an animal shelter that were positive for *Ancylostoma ceylanicum* (63.8%), *Ancylostoma caninum* (55.0%), *Ancylostoma brazieliense* (20%), *Trichuris vulpis* (2%), *Toxocara canis* (6.7%), *Spirocerca lupi* (3.3%) and *Dipylidium caninum* (3.3%).

Canine hookworm infections are the most common intestinal helminthiasis, which is endemic in Southeast Asian countries (Mahdy *et al*., 2012). The prevalence of canine hookworms was conducted by Mahdy *et al*. (2012), in fecal samples of 221 dogs originating from three sources: urban areas, rural areas and animal shelters of Selangor, by formal-ether concentration technique. The overall prevalence was 48%, with highest prevalence in rural stray dogs (71.4%), followed by urban stray dogs (48%) and dogs in shelters (28.7%). Molecular identification found two species; *Ancylostoma ceylanicum* and *Ancylostoma caninum*. Canine hookworm carries zoonotic risk where there was a case of human infection with *Ancylostoma ceylanicum* reported by Ngui *et al.* (2014), where the infected lady was living in rural settings with overpopulation of stray dogs living in close proximity to human.

Toxocara sp. is one of the common intestinal helminths found in rural dogs of Selangor and Pahang with prevalence of 28.6%, where it was ranked second most prevalent after *Ancylostoma* sp. (71.4%) (Ngui *et al.*, 2014). Toxocariasis is also an important zoonoses that may cause visceral larva migrans or ocular larva migrans in humans (Fan, Liao & Cheng, 2013).

2.3 Vector-Borne Pathogens

In Malaysia it is common to encounter cases of vector-borne diseases caused by *Ehrlichia canis*, *Anaplasma platys* and *Dirofilaria immitis.* This is mainly because their vectors, *Rhipicephalus sanguineus* and mosquitoes are widespread in Malaysia.

From a screening done by Jamnah *et al*. (2016), on 103 blood samples collected from dogs presented to veterinary clinics in Ipoh, by microscopic examination of Giemsa stained blood smears, they found *Ehrlichia canis* (13.6%) and microfilaria of *Dirofilaria immitis* (0.97%).

Another serological study using SNAP 4Dx test kit conducted by Koh, Panchadcharam and Tay (2015) on 43 blood samples of dogs from an animal shelter in Klang Valley, *Ehrlichia* spp. (39.5%) and *Anaplasma* spp. (9.3%) were detected; none tested positive for *Borrelia burgdorferi* and *Dirofilaria immitis*.

In UPM, Lim (1999) also screened for vector-borne pathogens in 50 household dogs presented to a clinic in Kuching by microscopic examination that revealed *Babesia gibsoni* (12%), *Dirofilaria immitis* (12%), *Hepatozoon canis* (6%) and *Dirofilaria repens* (2%).

Ehrlichia spp. are obligate intracellular gram negative bacteria under the order Rickettsiales that affect monocytes, granulocytes and platelets, causing canine monocytic ehrlichiosis (Wen, Cao & Pan, 2003). 500 blood samples were collected from veterinary clinics and dog shelters of Peninsular Malaysia from February 2009 to February 2010, which were tested by polymerase chain reaction (PCR)

that reported a prevalence rate of 2% for *Ehrlichia canis* (Nazari *et al*., 2013). *Anaplasma* spp. are obligate intracellular bacteria of the family Anaplasmataceae which causes infectious canine cyclic thrombocytopenia in dogs (Waner *et al.*, 2001; Mokhtar, Lim & Tay, 2013). Molecular detection was conducted on 30 blood samples from an animal shelter of Selangor that detected *Anaplasma platys* (13.3%) and *Babesia gibsoni* (3.3%) (Mokhtar *et al*., 2013).

Dirofilaria immitis also known as heartworm is transmitted by mosquitoes to other susceptible hosts during a blood meal. In Malaysia, the common species are *Armigeres* sp., *Culex* sp. and *Aedes* sp. (Vythilingam *et al.*, 2005). There was a low prevalence detected in a study by Ng, Lee & Sani (2012), where the prevalence rate of *Dirofilaria immitis* was only 1.3% in owned and stray dogs from Johor Bahru, via thick blood smear.

Borrelia burgdorferi is a zoonotic tick-borne bacterium causing Lyme disease in Northern America and Europe, with Ixodid ticks as the vector (Fritz, 2009). Dogs are susceptible but only 5 to 10% of the dogs exposed to infected ticks develop clinical borreliosis, with mainly mild clinical signs and often self-limiting. There is no data found or reported in Malaysia yet.

Leishmaniasis is a vector-borne disease caused by protozoa of the genus *Leishmania*, which is also a possible emerging zoonotic disease that requires further investigation. Transmission requires sandflies of the genus *Phlebotomus*, that is found in the old world (Pigott *et a*l., 2014). According to Moreno and Alvar (2002), canine leishmaniasis is due to *Leishmania infantum* that is zoonotic, where

dogs serve as the reservoir host, causing human visceral leishmaniasis. Prevalence is also higher in rural areas that have greater exposure to sandflies than urban cities.

3.0 MATERIALS AND METHODS

This study was carried out in selected veterinary clinics in Klang Valley over a period of 4 weeks. A total of 30 pet dogs were recruited for sampling.

3.1 Recruiting Subjects

Flyers with basic information about the project were given out at veterinary clinics and through social media, with contact number provided. Only pet dogs that met selection criterions of having regular outdoor access, no helminthic treatment or prevention for two months, and no ectoparasites treatment or prevention for a month were selected. A brief introduction of the project and the procedures to be conducted were informed to the owner.

Pet dogs that met the project criteria were arranged to visit veterinary clinics for sample collection by appointment basis. Reminder to owner on the fecal sample collection was done before their appointment, and bring along upon visit.

3.2 Sample Collection

This study was conducted following approval from the Institutional Animal Care and Use Committee (IACUC) (UPM/ACUC/AUPU014/2018) and after obtaining owner consent.

Physical examination was conducted on all dogs, and then combed for 5 minutes to examine for any presence of ticks, fleas and/or lice. Degree of infestation was categorized into low (<5), medium (<10) or high (>10). Ectoparasites detected upon examination were stored in microcentrifuge tubes in 70% ethanol to be examined under the stereomicroscope for identification.

The dogs were manually restrained for cephalic venipuncture, where about 1ml of blood was withdrawn, and placed into EDTA tubes. The age, sex and breed of pet dogs were recorded. Fecal samples were collected at home by the owner, and brought in during the visit.

3.3 Storage and Process of Examination

Ectoparasites found were examined under the stereomicroscope for identification.

The fresh fecal samples were subjected to Baermann-Wetzel technique (Appendix I) while the rest of the fecal and blood samples were kept at 4°C until further use. The remaining fecal samples were subjected to the fecal floatation technique (Appendix II) and fecal sedimentation (Appendix III) technique; while blood samples were used to conduct the SNAP 4Dx Plus Test (IDEXX) and SNAP Leishmania Test (IDEXX) (Appendix IV). Both samples were processed within 24 hours of collection.

3.4 Data Analysis

The data collected were recorded and tabulated in Microsoft Excel. The prevalence was computed and calculated using the following formula:

$$General\ Prevalence\ (\%) = \frac{Number\ of\ positive\ samples\ for\ one\ section}{Total\ number\ of\ samples\ tested} \times 100$$

4.0 RESULTS

In this study, a total of thirty (n=30) pet dogs were sampled. The dogs were divided into two age group with 13.33% (4/30) of the dogs less than one-year old and 86.67% (26/30) more than one year of age. There were 53.3% (16/30) female and 46.67% (14/30) male dogs. The breeds of the dogs were Mixed (15/30), Shih Tzu (4/30), Golden Retriever (2/30), Toy Poodle (2/30), Siberian Husky (2/30), Chow Chow (1/30), Miniature Pinscher (1/30), Pomeranian (1/30), Bulldog (1/30) and Dalmatian (1/30) (Figure 3).

Basic information of dogs:

ID	AGE	SEX	BREED
001	10 years	IM	Mixed
002	10 years	NF	Golden Retriever
003	6 years	NF	Toy Poodle
004	12 years	NF	Dalmatian
005	18 years	IF	Shih Tzu
006	12 years	IM	Shih Tzu
007	1 year	IM	Mixed
008	8 years	NM	Shih Tzu
009	3 years	IM	Siberian Husky
010	1 year	IF	Pomeranian
011	9 years	IM	Golden Retriever
012	1 year	NM	Chow Chow
013	10 years	IM	Mixed
014	2 years	NM	Mixed
015	3 years	IM	Mixed
016	6 months	IF	Bull dog
017	4 months	IF	Toy Poodle
018	10 years	NF	Mixed
019	9 years	IM	Siberian Husky
020	10 years	IF	Mixed
021	2 years	NF	Mixed
022	5 years	IF	Shih Tzu
023	7 years	IF	Miniature Pinscher
024	10 months	NF	Mixed
025	3 years	NM	Mixed
026	8 years	IM	Mixed
027	3 years	IM	Mixed
028	9 months	NF	Mixed
029	1 year	NF	Mixed
030	1 year	NF	Mixed

Table 1: Age, breed and sex of dogs sampled

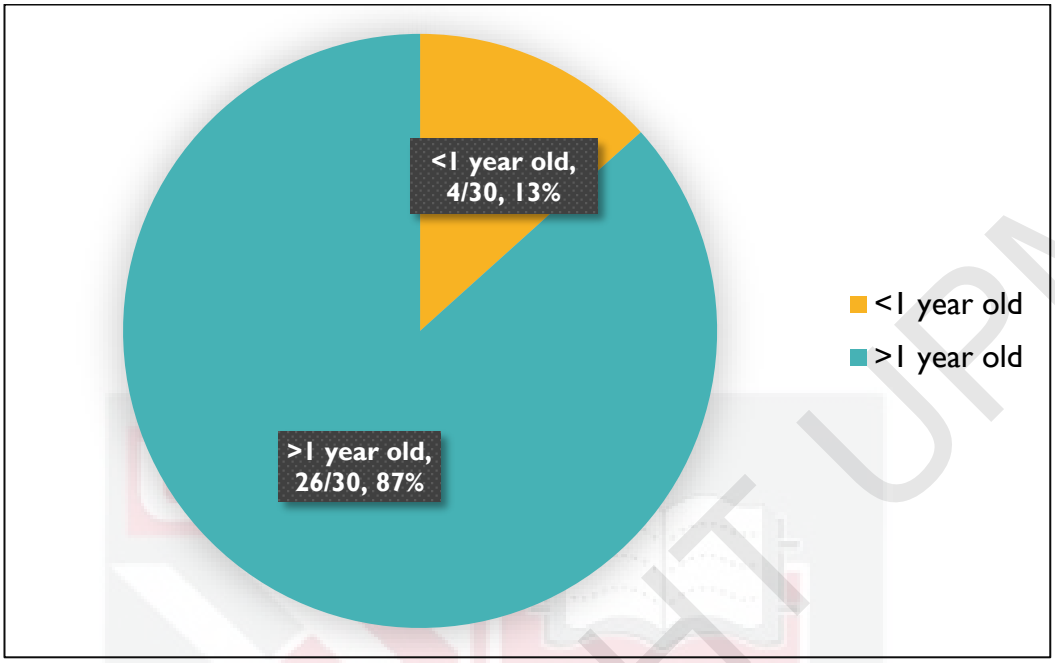

Figure 1: Age of pet dogs

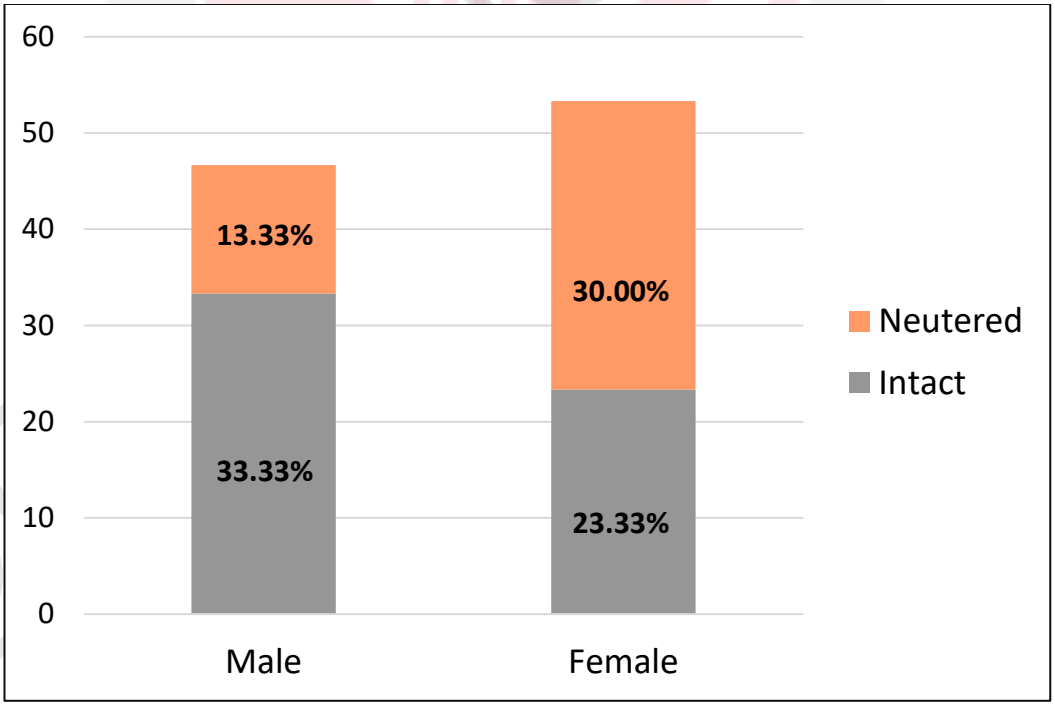

Figure 2: Sex of pet dogs

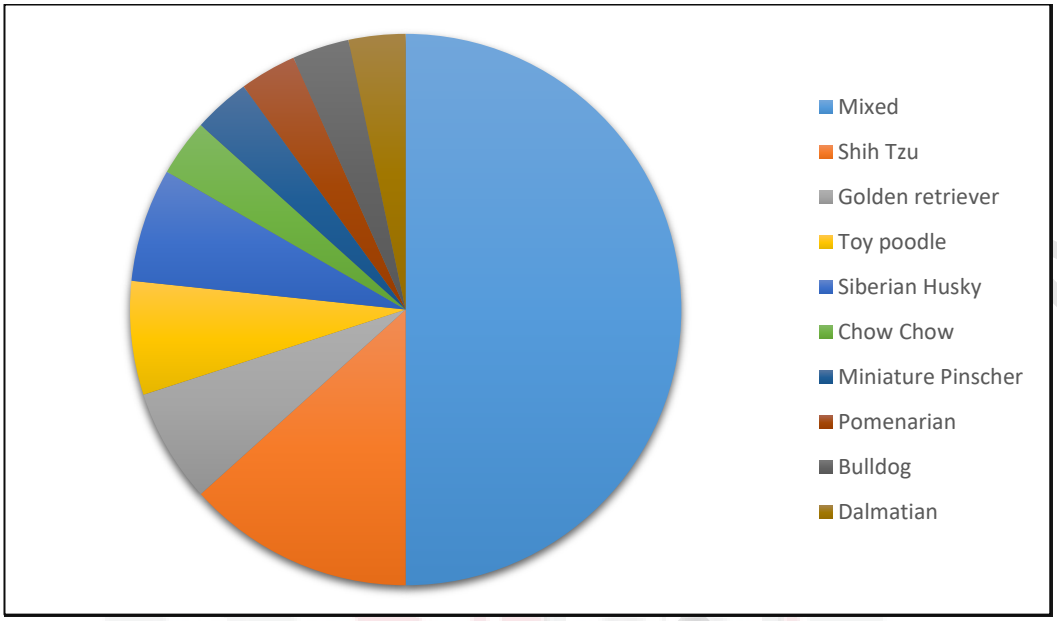

Figure 3: Breeds of pet dogs

ID	Ectoparasites	Intestinal Helminths	4Dx Snap Test	Leishmania Snap Test
001	Negative	Negative	Negative	Negative
002	Negative	Negative	Negative	Negative
003	Negative	Negative	Negative	Negative
004	Negative	Negative	Negative	Negative
005	*Ctenocephalides felis*	Negative	Negative	Negative
006	*Ctenocephalides felis*	Negative	Negative	Negative
007	Negative	*Acylostoma* sp. (F)	Negative	Negative
008	Negative	Negative	Negative	Negative
009	Negative	*Acylostoma* sp. (F,S)	*Anaplasma platys*	Negative
010	Negative	Negative	Negative	Negative
011	*Rhipicephalus sanguineus*	*Acylostoma* sp. (F)	*Dirofilaria immitis*	Negative
012	Negative	Negative	Negative	Negative
013	Negative	Negative	Negative	Negative
014	Negative	*Acylostoma* sp. (F)	Negative	Negative
015	Negative	Negative	Negative	Negative
016	Negative	Negative	Negative	Negative
017	Negative	Negative	*Anaplasma platys*	Negative
018	*Rhipicephalus sanguineus*	Negative	*Ehrlichia canis*	Negative
019	Negative	*Trichuris* sp. (F,S), *Ancylostoma* sp. (F, B)	*Dirofilaria immitis*	Negative
020	Negative	Negative	Negative	Negative
021	Negative	Negative	Negative	Negative
022	Negative	Negative	Negative	Negative
023	Negative	Negative	*Ehrlichia canis*	Negative
024	Negative	Negative	Negative	Negative
025	Negative	Negative	*Anaplasma platys*	Negative
026	Negative	Negative	Negative	Negative
027	Negative	Negative	Negative	Negative
028	Negative	Negative	Negative	Negative
029	Negative	Negative	Negative	Negative
030	Negative	Negative	Negative	Negative

*F: Fecal Floatation Technique
S: Sedimentation Technique
B: Baermann-Wetzel Technique

Table 2: Overall results for each sample

Parasites	Number of Positive Samples	General Prevalence (n=30) (%)
Ectoparasites		
Ctenocephalides felis	2	6.67
Rhipicephalus sanguineus	2	6.67
Intestinal Helminths		
Ancylostoma spp.	5	16.67
Trichuris sp.	1	3.33
Monoparasitism	4	13.33
Polyparasitism	1	3.33
Vector-Borne Pathogens		
Dirofilaria immitis antigens	2	6.67
Anaplasma platys antibodies	3	10.00
Ehrlichia canis antibodies	2	6.67
Borrelia burgdorferi antibodies	0	0.00
Leishmania infantum antibodies	0	0.00

* General prevalence estimated in relation to total number of samples analysed

Table 3: General prevalence for positive dogs

4.1 Ectoparasites

Of 30 dogs examined, only four dogs were positive for ectoparasites, with a prevalence of 6.67% (2/30) for *Ctenocephalides felis* (Figure 4) and 6.67% (2/30) for *Rhipicephalus sanguineus* (Figure 5).

Figure 4: *Ctenocephalides felis* observed under the stereo microscope

Figure 5: *Rhipicephalus sanguineus* observed under the stereo microscope

4.2 Intestinal Helminths

From fecal examination, 5 dogs were found to be positive for intestinal helminths by detection of ova. Only two genus of intestinal helminths ova were detected, which were identified as *Ancylostoma* sp. (Figure 6) and *Trichuris* sp. (Figure 7) with a general prevalence of 16.67% (5/30) and 3.33% (1/30) respectively for each genus. Out of the five dogs that were positive, four of them were infected with only *Ancylostoma* sp., while the other dog had a mixed infestation of both *Ancylostoma* sp. and *Trichuris* sp.

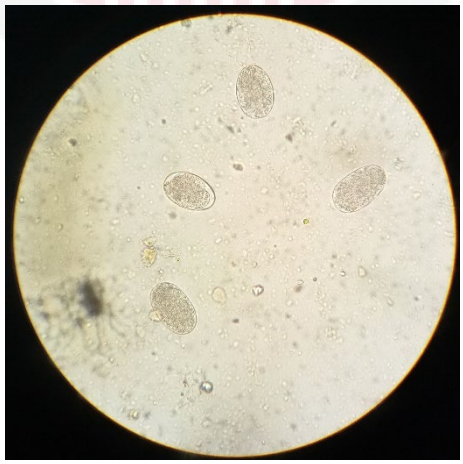

Figure 6: *Ancylostoma* sp. ova detected via fecal floatation technique observed under the compound microscope

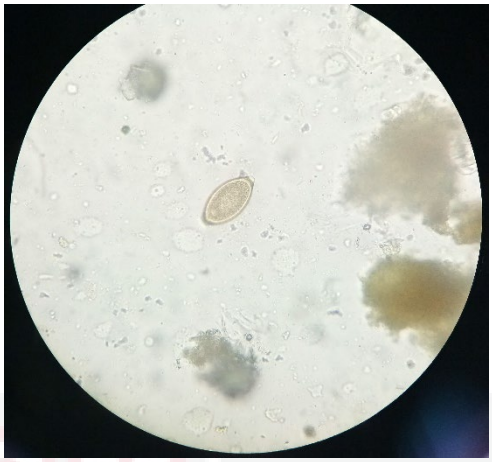

Figure 7: *Trichuris* sp. ova detected via fecal floatation technique observed under the compound microscope

4.3 Vector-Borne Pathogens

Seven dogs were positive for vector-borne pathogens with an overall prevalence of 23.33%. Among the positive results, *Anaplasma platys* had the highest prevalence of 10% (3/30), followed by *Dirofilaria immitis* 6.67% (2/30) and *Ehrlichia canis* 6.67% (2/30). All the blood samples were negative for *Borrelia burgdorferi* antibodies and *Leishmania infantum* antibodies.

5.0 DISCUSSION

5.1 Ectoparasites

In this study, four dogs were positive for ectoparasites with a prevalence rate of 13.33%. According to Dobler (2011), the most common flea species found on dogs globally is the cat flea, *Ctenocephalides felis*; while the dog flea *Ctenocephalides canis* is less frequently found. As shown in this study, only *Ctenocephalides felis* was found on the two positive dogs, with no *Ctenocephalides canis* identified in the project.

Rhipicephalus sanguineus is the most common tick found on dogs worldwide (Dantas-Torres, 2010), and among stray dogs in Ipoh with a prevalence of 53.9% (7/13) (Erwanas, 2014). In the project, the only tick species detected was *Rhipicephalus sanguineus*, therefore supporting the previous study that it is the most common tick found on dogs.

5.2 Intestinal Helminths

The prevalence rate for intestinal helminths was 16.67% (5/30), and the ova were identified as *Ancylostoma* sp. and *Trichuris* sp. All dogs were positive for *Ancylostoma* sp., and one of them was also positive for *Trichuris* sp.

Ancylostoma sp. was the most prevalent intestinal helminth found in the study, which poses a zoonotic risk of cutaneous larva migrans and eosinophilic enteritis in humans, by percutaneous route or oral ingestion of infective larva.

In a previous study, *Toxocara* sp. was one of the most common intestinal helminths found in rural dogs of Selangor and Pahang with a prevalence of 28.6%, where it

was ranked the most prevalent after *Ancylostoma* sp. (71.4%) (Ngui *et al*., 2014). However, no *Toxocara* sp. ova was detected in any of the dogs from this study. The possible reason for this is the age of dogs sampled, where 87% of dogs sampled were more than one year of age, while *Toxocara* sp. helminths are more commonly found in dogs less than 6 months old (Gates & Nolan, 2009). As dogs grow older, they develop immunity and resistance towards patent infection, thus the ova are less likely to be recovered from older dogs (Overgaauw, 1997). *Dipylidium* sp. ova was not detected in the project which might be due to the lack of intermediate hosts that is required for successful transmission, which is further supported by the low prevalence of *Ctenocephalides felis* infestations in this study. *Angiostrongylus* sp. was also not detected, thus Klang Valley might possibly be free at this point, but more samples should be tested to support the statement.

None of the dogs had diarrhea at the point of fecal sample collection, indicating that the helminthiasis may have been asymptomatic and not necessarily associated with diarrhea.

5.3 Vector-Borne Pathogens

The prevalence rate for vector-borne pathogens as determined in this study were as follows: *Anaplasma platys* (10%), *Ehrlichia canis* (6.67%) and *Dirofilaria immitis* (6.67%). The vector for both *Anaplasma platys* and *Ehrlichia canis* is *Rhipicephalus sanguineus*, which is known to be widespread in Malaysia. In addition *Dirofilaria immitis* is transmitted by mosquitoes, which are ubiquitous in the tropics and thus also posing an increased risk for dogs that have access to the

outdoors (Byeon *et al*., 2007). Therefore, the positive results obtained from this study were expected.

All samples were negative for *Borrelia burgdorferi* and *Leishmania infantum* antibodies. The main reason could be the lack of the main vector of *Borrelia burgdorferi*, Ixodes ticks. For the negative *Leishmania infantum* results, we still cannot conclude that Klang Valley is free from this disease, as positive results can only be obtained when there are high levels of antibodies associated with clinical and severe parasitism (Solano-Gallego *et al*., 2009).

There were five dogs that were positive for *Anaplasma platys* and *Ehrlichia canis* antibodies, and out of the five dogs, only one was found to be infested with *Rhipicephalus sanguineus*, and the same dog was pyrexic.

5.4 Overview

There was generally a low prevalence of parasites detected in this study. This could be due to a number of factors: pet dogs are owned, well taken care of, and provided good nutrition and kept clean. For ectoparasites that can be seen by the naked eye, owners are most likely vigilant and removed them once noticed, and also follow through with any necessary treatment or prevention. Pet dogs are usually kept in house compounds that have lower environmental contamination compared to areas frequented by stray dogs, thus less exposure to parasites in general.

6.0 CONCLUSION

In conclusion, the ecto- and endo-parasites that were found on pet dogs in the Klang Valley were: *Ctenocephalides felis* and *Rhipicephalus sanguineus;* Intestinal helminths found were *Ancylostoma* sp. and *Trichuris* sp.; Dogs were positive for *Dirofilaria immitis* antigen, *Ehrlichia canis* and *Anaplasma platys* antibodies.

Though the prevalence of the various parasites was low, this study highlights that these pathogens still exist within the pet dog population and veterinarians must be aware of this when educating client about wellness and prevention programs for their pets.

7.0 RECOMMENDATIONS

In future study, the sampling areas should be broadened to cover other geographical areas as well, to have better picture of prevalence of ecto- and endo-parasites in whole Malaysia. Increasing the sample size to be enough to represent the population, and more reliable data. Molecular detection is also recommended as it has higher sensitivity results and ability to detect up to species level.

8.0 REFERENCES

Azian, M. Y., Sakhone, L., Hakim, S. L., Yusri, M. Y., Nurulsyamzawaty, Y., Zuhaizam, A. H., ... & Maslawaty, M. N. (2008). Detection of helminth infections in dogs and soil contamination in rural and urban areas. *Southeast Asian Journal of Tropical Medicine and Public Health*, 39(2).

Bowman, D. D., Montgomery, S. P., Zajac, A. M., Eberhard, M. L., & Kazacos, K. R. (2010). Hookworms of dogs and cats as agents of cutaneous larva migrans. *Trends in parasitology*, 26(4), 162-167.

Byeon, K. H., Kim, B. J., Kim, S. M., Yu, H. S., Jeong, H. J., & Ock, M. S. (2007). A serological survey of *Dirofilaria immitis* infection in pet dogs of Busan, Korea, and effects of chemoprophylaxis. *The Korean journal of parasitology*, 45(1), 27.

Chee, J. H., Kwon, J. K., Cho, H. S., Cho, K. O., Lee, Y. J., El-Aty, A. A., & Shin, S. S. (2008). A survey of ectoparasite infestations in stray dogs of Gwang-ju City, Republic of Korea. *The Korean journal of parasitology*, *46*(1), 23.

Chong, K. L. (2015). *Prevalence of Intestinal Helminths of Household Dogs and Cats and Perception of Related Zoonoses by Owners in Ipoh, Perak, Malaysia* (DVM thesis, Faculty of Veterinary Medicine, Universiti Putra Malaysia).

Dantas-Torres, F. (2010). Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. *Parasites & Vectors*, *3*(1), 26.

Dobler, G., & Pfeffer, M. (2011). Fleas as parasites of the family Canidae. *Parasites & vectors*, *4*(1), 139.

Erwanas, A. I., Chandrawathani, P., Premaalatha, B., Zaini, C. M., Lily, R. M., Jamnah, O., ... & Rajandran, K. (2014). Parasitic Infections Found In Pet And Stray Dogs In Ipoh, Malaysia. *Malaysian Journal Of Veterinary Research,* 5(1), 27-34.

Fan, C. K., Liao, C. W., & Cheng, Y. C. (2013). Factors affecting disease manifestation of toxocarosis in humans: genetics and environment. *Veterinary parasitology*, *193*(4), 342-352.

Francis, J. J. (2002). *Gastrointestinal Parasites in Dogs from a Local Animal Shelter* (DVM thesis, Faculty of Veterinary Medicine, Universiti Putra Malaysia).

Fritz, C. L. (2009). Emerging tick-borne diseases. *Veterinary Clinics: Small Animal Practice*, *39*(2), 265-278.

Gates, M. C., & Nolan, T. J. (2009). Endoparasite prevalence and recurrence across different age groups of dogs and cats. *Veterinary parasitology*, *166*(1-2), 153-158.

Hadi, U. K., Soviana, S., & Pratomo, I. R. C. (2016). Prevalence of ticks and tick-borne diseases in Indonesian dogs. *Journal of Veterinary Science and Technology*, *7*(3).

Heukelbach, J., & Feldmeier, H. (2008). Epidemiological and clinical characteristics of hookworm-related cutaneous larva migrans. *The Lancet Infectious Diseases*, *8*(5), 302–309. Retrieved from: https://doi.org/10.1016/S1473-3099(08)70098-7 on 3rd February 2018

Jamnah, O., Chandrawathani, P., Premaalatha, B., Zaini, C.M., Sheikh Abdul Muizz, S.I., Tharshini, J. & Naheed Hb, M. (2016). Blood Protozoa Findings In Pet Dogs Screened In Ipoh, Malaysia. *Malaysian Journal Of Veterinary Research,* 7(1), 9-14.

Koh, F. X., Panchadcharam, C., & Tay, S. T. (2015). Vector-borne diseases in stray dogs in peninsular Malaysia and molecular detection of *Anaplasma* and *Ehrlichia* spp. from *Rhipicephalus sanguineus* (Acari: Ixodidae) ticks. *Journal of medical entomology*, *53*(1), 183-187.

Landmann, J. K., & Prociv, P. (2003). Experimental human infection with the dog hookworm, *Ancylostoma caninum*. *Medical journal of Australia*, *178*(2), 69-71.

Lim, A. L. L. (1999). *Prevalence of Blood and Intestinal Parasites in Dogs* (DVM thesis, Faculty of Veterinary Medicine, Universiti Putra Malaysia).

Mahdy, M. A., Lim, Y. A., Ngui, R., Fatimah, M. S., Choy, S. H., Yap, N. J., ... & Surin, J. (2012). Prevalence and zoonotic potential of canine hookworms in Malaysia. Parasites & vectors, 5(1), 88.

Mokhtar, A. S., Lim, S. F., & Tay, S. T. (2013). Research Note Molecular detection of Anaplasma platys and Babesia gibsoni in dogs in Malaysia. *Trop Biomed*, *30*(2), 345-8.

Moreno, J., & Alvar, J. (2002). Canine leishmaniasis: epidemiological risk and the experimental model. *Trends in parasitology*, *18*(9), 399-405.

Nazari, M., Lim, S. Y., Watanabe, M., Sharma, R. S., Cheng, N. A., & Watanabe, M. (2013). Molecular detection of Ehrlichia canis in dogs in Malaysia. *PLoS neglected tropical diseases*, *7*(1), e1982.

Ng, K. L., Lee, E. L., & Sani, R. A. (2012). Research Note Low prevalence of *Dirofilaria immitis* in dogs in Johor Bahru, Malaysia as a reflection of vector availability?. *Tropical biomedicine*, *29*(1), 187-190.

Ngui, R., Lee, S., Yap, N., Tan, T., Aidil, R., Chua, K., ... & Lian, Y. (2014). Gastrointestinal parasites in rural dogs and cats in Selangor and Pahang states in Peninsular Malaysia. *Acta Parasitologica*, *59*(4), 737-744.

Overgaauw, P. A., & Nederland, V. (1997). Aspects of *Toxocara* epidemiology: toxocarosis in dogs and cats. *Critical reviews in microbiology*, *23*(3), 233-251.

Pigott, D. M., Bhatt, S., Golding, N., Duda, K. A., Battle, K. E., Brady, O. J., ... & Brownstein, J. S. (2014). Global distribution maps of the leishmaniases. *Elife*, *3*.

Solano-Gallego, L., Koutinas, A., Miró, G., Cardoso, L., Pennisi, M. G., Ferrer, L., ... & Baneth, G. (2009). Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. *Veterinary parasitology*, 165(1-2), 1-18.

Vythilingam, I., Mooto, P., Jeffery, J., & Parameswaran, M. S. (2005). Potential mosquito (Diptera: Culicidae) vectors of Dirofilaria immitis (Filariidae: Onchocercidae) in two urban areas of Kuala Lumpur and its prevalence in stray dogs. In *Fifth International Conference on Urban Pests, Singapore, 11-13 July 2005*, 393-398.

Waner, T., Harrus, S., Jongejan, F., Bark, H., Keysary, A., & Cornelissen, A. W. (2001). Significance of serological testing for ehrlichial diseases in dogs with special emphasis on the diagnosis of canine monocytic ehrlichiosis caused by Ehrlichia canis. *Veterinary parasitology*, *95*(1), 1-15.

Wen, B., Cao, W., & Pan, H. (2003). Ehrlichiae and ehrlichial diseases in China. *Annals of the New York Academy of Sciences*, 990(1), 45-53.

9.0 APPENDICES

Appendix I: Baermann-Wetzel Technique

1. 1g of fecal sample was placed on double layered gauze and tied with rubber band.
2. The sample was then placed into Baermann funnel and 50ml warm water was added until the sample was fully suspended.
3. The setting was left for 12hours.
4. The water in the funnel was placed in 50ml centrifuge tube and was centrifuged at 2500rpm for 5minutes.
5. Supernatant was removed and 2drops of Lugol's solution was added.
6. The sediment was placed in a glass slide with pipette, and examined under microscope.

Appendix II: Fecal Floatation Technique

1. 1 to 3g of fecal sample was placed in a cup.
2. 20ml of saturated salt solution was added into the cup.
3. The sample was mixed until homogenized and slurry.
4. The mixture was filtered with tea strainer into a beaker.
5. Saturated salt solution was added until 40ml.
6. The mixture was pour into shell vial.
7. A coverslip was put on top and left for 10 to 20minutes.
8. The coverslip was applied on a glass slide and examined under microscope.

Appendix III: Fecal Sedimentation Technique

1. 1 to 3g of fecal sample was placed in a paper cup and 100ml of water was added in.
2. The sample was mixed until homogenized.
3. The mixture was filtered using tea strainer into a beaker.
4. The mixture was left to sit for 10 to 20 minutes.
5. About 70% of supernatant was decanted and fresh water was refilled into the beaker.
6. The mixture was left to sit for another 10 to 20 minutes.
7. About 90% of the supernatant was decanted, without disturbing the sediment.
8. A pipette was used to mix the sediment.
9. A few drops of the sediment were placed on a glass slide and coverslip was applied. Slide was examined under microscope.

Appendix IV: Snap 4Dx Plus Test

1. 3 drops of blood sample were placed into a sample tube.
2. 4 drops of conjugate were added into the sample tube.
3. The sample tube was capped and mixed by inverting it 3 to 5 times.
4. The device was placed in horizontal surface.
5. The entire mixture of the sample tube was added to the sample well of device.

6. The activator of the device was pushed until fully depressed when the color first appeared in the activation circle.
7. The test result was read after 8 minutes.

Appendix V: Snap Leishmania Test

1. 2 drops of blood sample were placed into a sample tube.
2. 6 drops of conjugate were added into the sample tube.
3. The sample tube was capped and mixed by inverting it 3 to 5 times.
4. The device was placed in horizontal surface.
5. The entire mixture of the sample tube was added to the sample well of device.
6. The activator of the device was pushed until fully depressed when the color first appeared in the activation circle.
7. The test result was read after 6 minutes.