UNIVERSITI PUTRA MALAYSIA

INVESTIGATION OF DIROFILARIASIS AMONG DOG POPULATION IN DOG SHELTER, PEKAN NANAS

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INVESTIGATION OF DIROFILARIASIS AMONG DOG POPULATION IN DOG SHELTER, PEKAN NANAS

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DEDICATIONS

To God Almighty

My beloved family,

Mummy,

Weenith,

Razzy,

And pets,

Quincy, Mei Mei and Lulu

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Firstly, I would like to thank my almighty God for giving me strength and guide me throughout the whole veterinary course journey.

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LIST OF ABBREVIATIONS

KCT	Knott Concentration Test
ELISA	Enzyme-linked Immune Sorbent Assay
PCR	Polymerase Chain Reaction
EDTA	Ethylene-Diamine-Tetra-Acetic Acid
PBS	Phosphate Buffer Saline
µ	Micron
ml	Milliliter
^{o}C	Degree celcius
µl	Microliter
ITS-2	Internal Transcribe Spacer 2
Mm	Millimolar
µg	Microgram
mg	Milligram
kg	Kilogram
DNA	Deoxyribonucleic Acid
ASV	Association of Shelter Veterinarian
AVMA	American Veterinary Medical Association

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901 – Projek.

SIASATAN DIROFILARIASIS DALAM KALANGAN POPULASI ANJING DALAM PUSAT PERLINDING ANJING, PEKAN NANAS

Oleh

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2017

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Oleh sebab globalisasi dan perubahan iklim, *Dirofilaria immitis* dan *Dirofilaria repens* telah menjadi spesies nematode filarial yang paling biasa dijumpai pada anjing. Dirofilariasis merupakan penyakit zoonotik yang berpotensi untuk berjangkit kepada

manusia. Namun, terdapat kajian terhad dijalankan mengenai dirofilariasis dalam populasi anjing di Malaysia. Oleh itu, kajian ini telah dijalankan untuk mengesan parasite filarial, iaitu *Dirofilaria immitis*, *Dirofilaria repens* dan *Brugia malayi* dalam pusat perlindungan anjing yang terletak di Pekan Nanas, Johor. "Single-step multiple polymerase chain reaction (PCR)" telah digunakan dengan mensasarkan pada "internal transcribed spacer-2 (ITS-2)" DNA untuk mengesan dan membezakan antara ketiga-tiga spesies parasite filarial. Dalam kajian ini, sebanyak 40 sample darah anjing telah dikumpul dari tempat perlindungan. 20/40 anjing telah diberi oral Ivermectin setiap bulan dan Doxycycline manakala 20 ekor anjing lagi tidak diberi mana-mana ubat pencegahan cacing jantung. Dengan itu, prevalens dirofilariasis dalam empat puluh sampel adalah 17.5% (7/40). Semua sampel yang menunujukan positif menandakan *Dirofilaria immitis* dan empat daripadanya adalah dari anjing betina manakala tiga lagi adalah dari anjing jantan. "Sequencing" di ITS-2 adalah disyorkan dalam kajian ini untuk mengesahkan genotip filarial yang berjaya dikesan. Semua anjing-anjing yang terbabit ditemui dalam kumpulan yang tidak menerima sebarang pencegahan cacing jantung. Oleh itu, pelaksanaan pencegahan cacing jantung amat penting dalam menguruskan pusat perlindingan anjing.

Katakunci: dirofilariasis, zoonotic, polymerase chain reaction, prevalens

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901 – project

INVESTIGATION OF DIROFILARIASIS AMONG DOG POPULATION IN A DOG SHELTER

by

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2017

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As a result of globalization and climate change, *Dirofilaria immitis and Dirofilaria repens* are the most common species of filarial nematodes found in dogs. Dirofilariasis is a potential zoonotic disease that may be transmitted to human. There is limited study conducted regarding canine dirofilariasis in dog population in Malaysia. Hence, this study is to detect filarial parasites, i.e. *Dirofilaria immitis, Dirofilaria repens* and *Brugia malayi* in shelter dogs that located in Pekan Nanas, Johor.

A single-step multiplex polymerase chain reaction (PCR) was performed by targeting at amplifying the internal transcribed spacer-2 (ITS-2) region of extracted DNA to detect and differentiate between the three species of filarial parasites. Blood sample from 40 dogs were collected from a shelter. 20/40 dogs were given monthly oral Ivermectin and doxycycline and another 20 dogs were not on any heartworm prevention. The prevalence of dirofilariasis in forty dogs was 17.5% (7/40). All positive samples were suggestive of *Dirofilaria immitis* with four female and three male dogs respectively. Further sequencing at the (ITS-2) region is recommended to confirm the filarial genotype. All the affected dogs were found in the group that did not receive any heartworm prevention. Implementation of heartworm prevention is important in shelter management.

Keywords: dirofilariasis, zoonotic, polymerase chain reaction, prevalence

1.0 INTRODUCTION

Dirofilariasis is a disease which affects canine and zoonotic disease that may spreads world widely. There are several species of filarids that may cause filariasis in dog (Megat Abd Rani *et al*., 2010). *Dirofilaria immitis* (Heartworm) is considered as the most pathogenic canine filarids that cause heartworm disease in dogs (Megat Abd Rani *et al*., 2010). Nowadays, dirofilariasis is alarming due to its growing prevalence in both veterinary and human patients in the world (McCall *et al*., 2008)

This vector borne parasite will cause patent infection in various animals such as dogs, cats, wild canidae, ferrets and sea lions (Dillon, 2000).. The dead end host of this parasite involved horses, bears and primates which include humans and orang utans (Dillon, 2000). They typically inhabits the right ventricle and pulmonary arteries of the dogs (Simsek *et al*., 2011). Besides, *Dirofilaria immitis* will also produce microfilariae that presence in dog blood circulation (Simsek *et al*., 2011).

Mosquitos are the intermediate host for dirofilariasis. There are more than 70 species of mosquito that has been recorded to transmit *Dirofilaria immitis* (Ng, 2011). The common species of mosquito in Malaysia are *Armigeres sp., Culex sp., Aedes sp*. and *Anopheles sp*. (Ng, 2011). Most of the dogs that are infected do not show obvious clinical signs, regardless of worm burden and duration of infection (Hoch, 2008). This is often an incidental finding during routine health screening unless in dogs with very high worm burdens or complication due to heartworm infection (Hoch, 2008). This may include

pneumonitis, pulmonary endarteritis, pulmonary hypertension, pulmonary thromboembolism and cor pulmonale (Hoch, 2008).

There are altogether seven studies that have been conducted on dirofilariasis prevalence in dogs in Malaysia (Mullin, 1970; Retnasabapathy and Khoo, 1976; Dhaliwal, 1987; Toh, 2002; Yap, 2006; Yap and Ong, 2008; and Ng, 2011). The rate of prevalence range from 0.68% to 33.34% in these studies that have been conducted. The common methods used for diagnosis included direct wet blood mount, modified Knott's concentration test (KCT) and necropsy findings. There are four studies conducted; one (Dhaliwal, 1987) used the enzyme-linked immune sorbent assay (ELISA) method, two (Yap, 2006; Yap and Ong, 2008) used immunoassay and another one (Ng, 2011) used heartworm antigen test kits (IDEXX Canine SNAP® 4Dx and RapiGEN®).

Limited information is available in the literature regarding the occurrence and prevalence of heartworm disease in Johor especially study of dirofilariasis in animal shelter. Yap and Ong (2008) had conducted a study on dirofilariasis in Peninsular Malaysia however none of *Dirofilaria spp*. was detected from Johor. Besides that, Ng (2011) has reported the prevalence of dirofilariasis in owned dogs and stray dogs were 1% and 2% respectively.

This study is conducted to gain a better understanding on the status of canine dirofilariasis among dog population in the particular animal shelter and to identify the different canine filarial species in the infected dog population. This is because

dirofilariasis is a potential zoonotic disease that may be transmitted to human. Hence, the objectives of this study were:

1. To investigate the presence of canine dirofilariasis among dog population in animal shelter in Pekan Nanas, Johor

2. To identify the different *Dirofilaria* species in the particular dog population

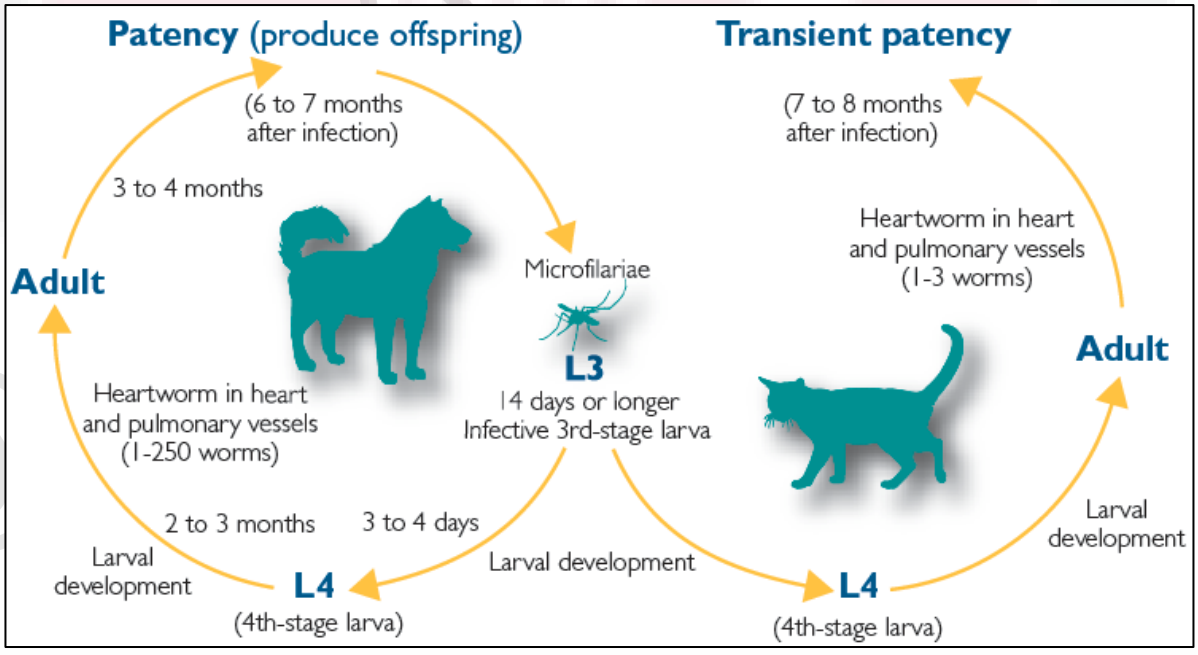
2.1 Life Cycle

Heartworm infection in dog caused by *Dirofilaria immitis* has been recognized as increasingly problem around the globe as well as in Malaysia (Ng, 2011). The life cycle of *Dirofilaria immitis* is complex. The infection depends on the intermediate host to transmit to the final host (Dhaliwal, 1988).

The life cycle of *Dirofilaria immitis* has been well documented by Hoch and Strickland (2008). The heartworm life cycle has five larval staged, which is from L1 to L5. Adult heartworms typically live in the pulmonary arteries of the host. However in cases of heavy infestations, it may invade the right ventricle, right atrium and caudal vena cava. Female worms will release microfilariae (L1) into host's bloodstream after mating. Mosquito becomes infected with microfilariae when they obtain blood meal from the infected animals. In the mosquito, the larvae molt two times from L1 to L3 over an 8 to 17 day period. The time needed for the microfilariae to molt is temperature dependent. At temperature of 27 ºC and 80% relative humidity takes about 10-14 days of development (American Heartworm Society, 2012). The L3 stage which is also known as infective stage of the heartworm is transmitted to a new host when the vector mosquito feeds. The L3 larvae will then molt into L4 stage in the host's subcutaneous, adipose or skeletal muscle tissue 1 to 12 days post infection. The final molt from the L4 stage to L5 immature adult occurs 50 to 68 days after the initial infection. The immature adults will then enter the vascular system and start to migrate to the heart and pulmonary arteries. The first worms entering the pulmonary vasculature are 1 to 1.5inches in length (American Heartworm

Society, 2012). Immature adults mature into adult heartworm on the next 99 to 152 days. As they grow and increase in size, they gradually occupy larger and larger arteries until they become completely mature (American Heartworm Society, 2007). Adult male heartworms can grow to be 6 to 7 (15 to 18cm) inches in length; adult females can reach 10 to 12 inches (25 to 30cm). The entire life cycle takes about184 to 210 days under ideal condition. Microfilariae can be seen in the blood approximately 6 to 7 months after exposure to infective L3 (Lobeck and Latimer, 2008) as only mature adults are capable of reproduction (Hoch and Strickland, 2008). A dog that develops adult worms can maintain a patent infection for up to 7.5 years following a single infection from mosquito (Abraham, 1988).

Figure 1: *Dirofilaria immitis* life cycle in dogs and cats (American Heartworm Society, 2012)



2.2 Morphology

Adult male worms range from a length of 12 to 20 cm and 0.7 to 0.9mm width whereas female length range from 25 to 31 cm with 1.0 – 1.3mm width (Schwan and Durand, 2002). The adult worms are long and slender and possess a thick cuticle (Dhaliwal, 1988). The males can be differentiate from the females by the length and males possess a distinct spiral tail (Dhaliwal, 1988).

When the parasite reaches maturity, females will produce microfilariae that are unsheathed and measure 218 to 240 µm by 4 to 7 µm width (Ludlam et al. 1970). The microfilariae of *Dirofilaria immitis* can be differentiated from *Acanthocheilonema reconditum* by the former has a tapered head and a straight tail, compare to the latter one with a blunt anterior end and a button hooked tail (Lindsey, 1965). However, these characteristics are not always recognized easily and always lead to a misdiagnosis (Dhaliwal, 1988). The species can also be differentiated by using acid phosphatase staining method via precipitation of red azo dye (Schwan and Durand, 2002). *D. immitis* will show two spots of bright stains which indicates typical enzyme activity at the anal and excretory pores whereas *A. reconditum* shows uniform staining of entire body that shows enzyme activity throughout the body (Schwan and Durand, 2002)

2.3 Prevalence of *Dirofilaria immitis* in dogs in Malaysia

Six studies on detection of dirofilariasis based on circulating microfilaria (Knott concentration test) and antigen testing in Malaysia has been carried out (Table 1). In these

studies showed that prevalence of dirofilariasis in stray dog was ranged from 25.% to 42% whereas owned dogs was from 4.5% to 10%.

Table 1: Prevalence of Canine *Dirofilaria immitis* in Malaysia

Authors	Year	Status of dogs	Prevalence	Methods	Location
Retnasabapathy and Khoo	1976	Stray (N=764)	25.8 %	Direct wet mount, KCT, Necropsy	Kuala Lumpur and Selangor
Kan *et al.*	1977	Pure bred (N=158) Mixed bred (N=212)	Pure bred 39.2% Mixed bred 27.4%	Direct wet mount, slight modification of counting chamber method, milipore filter method	Seremban
Dhaliwal	1987	Stray (N=200) Owned (N=206)	42% 10%	Direct wet mount, KCT, Necropsy, ELISA	Kuala Lumpur

Toh	2002	Stray (N=60)	31.7%	Direct wet mount, KCT, Necropsy	Selangor
Yap	2006	Stray (N=132)	33.34%	Direct wet mount, KCT, Necropsy, Immunochromatographic test	Kuala Lumpur
Ng	2006	Stray (N=50) Owned (N=100)	2% 1%	KCT, Canine antigen test kit	Johor Bharu
Yap & Ong	2008	Owned (N=336)	4.5%	Immunochromatographic test	Malaysia

2.4 Host risk factors

In a study conducted by Kan *et al*. (1977), the prevalence rate of *Dirofilaria immitis* among pedigree and non-pedigree dog in Seremban were compared. Of a total of 370 dogs were examined, pedigree dogs showed a slightly higher incidence of infection (39.2%) compared to non-pedigree dogs were less frequently infected (27.4%). Among the pedigree dogs, short hair pedigree dogs such as Boxers, Dobermans, Dachshunds and Bulldogs were more frequently infected compare to longer haired pedigree such as Alsatians, Spaniels and Terriers. Another study reported by Kan *et al*. (1977) showing

that there was no significant difference in distribution of infections among dogs with different age and sex groups. However, Noor and Lee (1981) studies showed that frequency of microfilariae in male dogs were significantly higher. A survey conducted by Byeon *et al*. (2007) revealed the status of *Dirofilaria immitis* infection in pet dogs of Busan, Korea. A total of 294 pet dogs that are older than 6 months old were examined by using SnapKit® and direct microscopic examinations method, outdoor dogs showed higher incidence of microfilariae infection (18.2%) compared to indoor dogs which were less infected (2.3%). The prevalence in males was also 2 times higher than that of females in the study. However, the difference in gender and breed were not significant when the level of outdoor activity and age factors were considered (Grieve *et al*., 1983).

American heartworm society (2012) also revealed that environmental changes, natural climatic condition as well as animal movement have increased heartworm infection potential. Commercial and residential real estate development of non-endemic areas and areas of low incidence has led to increase in prevalence by changing drainage system of undeveloped land and by providing water sources in new urban site. In the western United States, irrigation and planting of trees has expanded the habitat for *Aedes sierrensis*, the primary vector for transmission of heartworm in those states.

The prevalence of infection with *Dirofilaria immitis* was reported to increase with the age of the host (Stewart *et al*., 1979). This finding was also supported by Almeida *et al*. (2016) showing that older dogs (> 5 years old) was significantly infected.

2.5 Diagnostic test

According to American Heartworm Society (2012), the earliest that heartworm antigen and microfilariae can be detected is about 5 to 6 months post infection. Antigenemia may come first, but sometimes delays the present of microfilariae by few weeks. In a dog with very low worm burden might never be able to detect antigen in the dog. Furthermore, antigenemia may be suppressed until about 9 months post infection in infected dog that was gave macrocyclic lactone chemoprophylaxis. To obtain an optimal test result, a pre-detection period should be added to the approximate date which infection may be occur.

Microfilariae that circulate in dogs' blood can be diagnosed through careful morphological examination of circulating microfilariae, detection of circulating antigens, histochemical or immmuno-histochemical staining of circulating microfilariae or a more recent molecular approach (Simsek *et al.*, 2011).

Morphological examination of circulating microfilariae include wet blood smear or direct wet mount, Knott's concentration test and filter test (Kittleson and Kienle, 1998). Direct wet mount is good for detecting cell movement based on the motility of microfilariae (Yap, 2006). However, this method is less sensitive in examining blood with low numbers (50-100/ml) of microfilariae (Reifur *et al.*, 2004). It is also not always easy and is potentially misleading when identifying the morphology of circulating microfilariae (Rishniw *et al*., 2006) Wet blood smear is less sensitive compared to modified Knott's

concentration and filter test and should not be used as definitive diagnosis of heartworm in dogs (Kittleson and Kienle, 1998).

According to McCall *et al.* (2004), modified Knott's test is the preferred method in observing morphology as well as in differentiating *Dirofilaria immitis* from others non pathological filarial species such as *A. reconditum* by measuring the body dimension. Modified Knott's test is also found less expensive but consume more time compared to the filter test. However, serological and microfilariae examination should be applied together in screening of heartworm infestation (Simsek *et al*., 2011)

Commercial rapid test kits are also available currently in detecting antigens. However, the rapid test kits only able to detect adult female *Dirofilaria immitis* only (McCall *et al*., 2004). In the study of *Dirofilaria immitis* by Yap (2006), the two test kits used are able to detect all female adult worms regardless of the worm burden. The sensitivity and specificity of Canine SNAP® 4Dx test was 99.2% and 100%. Another test kit RapiGEN® Canine Heartworm Ag test reported to have 92.8% sensitivity and 100% specificity.

ELISA and immunochromatographic test are available in detecting circulating heartworm antigen. The differences in sensitivity presence in cases of dogs with low worm burdens. However, the specificity is nearly 100% as it can identify most occult infection consisting of at least one mature female worm. Strict compliance with manufacturer's instructions is a must in performing test in order to obtain accurate results.

False negative may be occurred when the infection is light, immature female worms or only male worms are present in the infection (American Heartworm Society, 2012).

Direct PCR method is the most sensitive method in detecting peripheral microfilaria (Simsek *et al*., 2011). The prevalence of *Dirofilaria immitis* in the study was 8.1% by PCR and microfilaria burdens of *Dirofilaria immitis* is 4.8% (Simsek *et al*., 2011). This method has also been reported by Vakalis *et al*. (1999). The statistical comparison between PCR method and microscopic examination method shown that PCR method was highly sensitive and specific in identifying dog blood (Vakalis *et al*., 1999).

3.0 MATERIALS AND METHODS

A total of 40 blood samples were collected from animal shelter that is located in Pekan Nanas, Johor. The shelter has altogether 3000 dogs. The criteria for selection were dogs that are more than six months old.

3.1 Sampling

Approximately three ml of blood were collected by cephalic venipuncture method. The blood samples collected were then transferred into an EDTA (ethylene-diamine-tetra-acetic acid). The samples were then stored in freezer (-20 ^{o}C) prior to analysis.

All methods described below were used for the detection of dirofilariasis in this study.

3.2 Polymerase Chain Reaction

3.2.1 DNA extraction of adult D. immitis and blood from QIAGEN® kit

Approximately 70 µl of blood which is anticoagulant- treated from each animal was mixed with 20 µl of proteinase K and 130 µl of PBS (Phosphate Buffer Saline) were added into a 1.5 ml microcentrifuge tube to lyse the sample. Another 200 µl of Buffer AL was added into the tube and mixed thoroughly by vortexing. The blood samples were incubated at 56^{o}C for 10 minutes. 200 µl 99.8% ethanol was added in the sample and mixed thoroughly again by vortexing. The mixture is the pipetted into a DNeasy Mini spin column placed in a 2 ml collection tube and centrifuge at 8000 rpm for 1 minute. The flow through and the collection tube were discarded and replace with a new 2 ml collection

tube. 500 µl Buffer AW1 was added into the spin column and centrifuge at 8000 rpm for 1 minute. The flow though and collection tube were then discarded and replaces with a new 2 ml collection tube. 500 µl Buffer AW2 was added, and centrifuge at 14,000 rpm for 3 minutes. Discard the flow through and collection tube. The spin column was then transferred to a new 1.5 ml microcentrifuge tube. The DNA was eluted by adding 200 µl Buffer AE to the center of the spin column membrane and incubate for 1 minute at room temperature (15 – 25°C). Following centrifugation at 8000 rpm for 1 minute to obtain the DNA pellet. The DNA pellet was then stored in -20°C freezer before analysis.

3.2.2 PCR assays

In this study, a single-step multiplex PCR targeted at amplifying the internal transcribed spacer- 2 (ITS-2) region of ribosomal DNA from Rishniw *et al.* (2006) was utilized to screen the canine filarial species in blood. Pan-filarial primers were used, forward: DIDR – F1 5'-AGT GCG AAT TGC AGA CGC ATT GAG-3' and reverse: DIDR-R1 5'-AGC GGG TAA TCA CGA CTG AGT TGA-3' to amplify and differentiate among six species of canine microfilaria include *D. immitis, D. repens*, *B. malayi, B. pahangi, A. reconditum, and A. dracunculoides* .

The PCR steps were run according to Rishniw *et al*. (2006). The PCR reaction mixture were prepared as stated in Table 2. After that, the PCR mixture was vortex and centrifuged briefly before loading in DNA template. The PCR was then run with initial activation step at 94 °C for 2 min and followed by 30 cycles of amplification (94°C for 30 s for denaturing process, 56°C for 30 s for annealing process and 72°C for 30 s for

elongation process). The final extension step was run in 72°C for 7 min. Then, 3 µl of ladder, 3 µl of positive controls, 6 µl of PCR products and 6 µl of negative control were loaded on 2% agarose gel in 1 x TAE buffer and run at 70V for 1 hour and were visualized by using illuminator (Syngene) and Geldoc (Biorad). The anticipated product sized of each different species of canine microfilaria are stated in Table 3.

Table 2: The prepared PCR reaction mixture of total 25 µl

Reagent	Volume/reaction (µl)
1. 5x Green Go Taq® Flexi Buffer	5 µl
2. 25Mm MgCl2 solution	5 µl
3. PCR Nucleotide Mix, 10Mm	1 µl
4. Forward DIDR Primer	1 µl
5. Reverse DIDR Primer	1 µl
6. GoTaq® DNA Polymerase (5µ / µl)	0.3 µl
7. Template DNA	4 µl
8. Sterile Distilled Water	7.7 µl
Total	25 µl

Table 3: Product size of different species of canine microfilaria

Primer Pair	Primer Sequence	Gene Target	Product Origin	Product size (base-pair)
DIDR – F1	AGT GCG AAT TGC AGA CGC ATT GAG	5.8S-ITS2-28S	*D. immitis*	542
			D. repens	484
DIDR –R1	AGC GGG TAA TCA CGA CTG AGT TGA		*B. malayi*	615
			B. pahangi	664
			A. reconditum	578
			A. dracunculoides	584

4.0 RESULTS

In this study, a total number of forty dogs were selected out of 3000 dog population in the animal shelter. From all the forty dogs, 50% of them are resident dogs with up-to-date vaccination programme and heartworm prevention programme. The heartworm prevention programme include monthly oral Ivermectin (6µg/kg) and Doxycycline (10mg/kg) for one month once the animals enter the shelter. Another 50% of the sampled group are newcomers that have not been given any vaccination and heartworm prevention programme. Out of the forty samples, 58% of them are female dogs and 42% of them are male dogs. The age of the dogs are difficult to estimate as most of them are rescued from the streets. 100% of the sampled dogs are local breed.

Figure 2: The percentage of status of the 40 sampled animals in the animal shelter

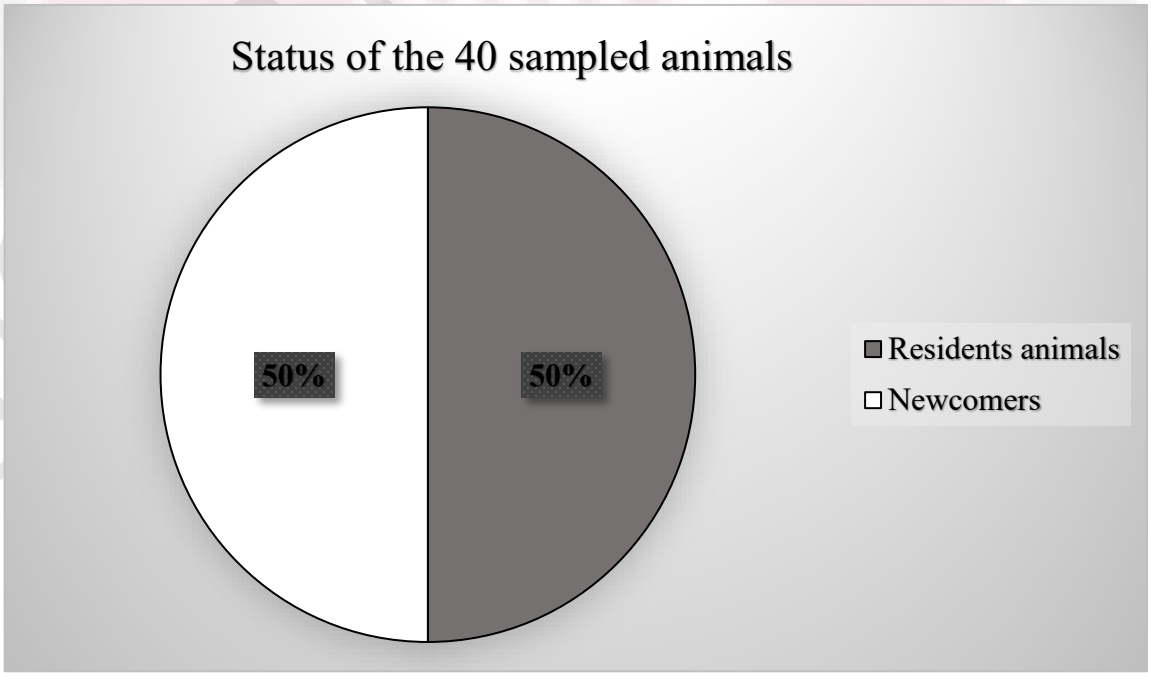
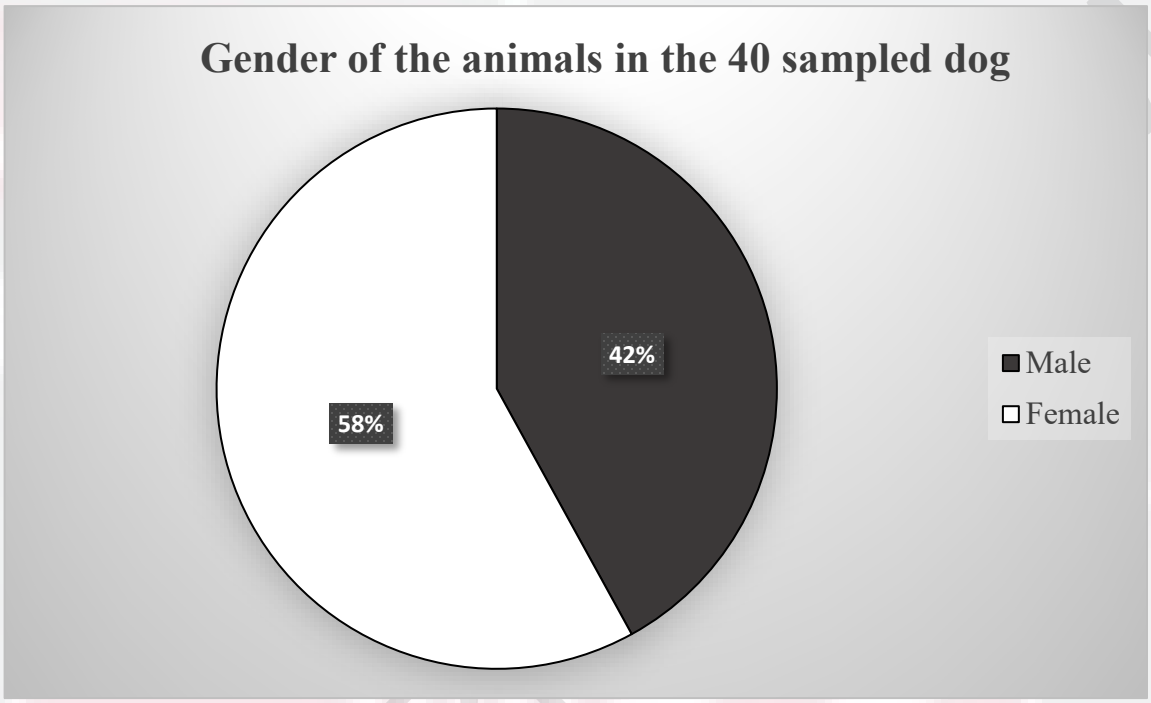


Figure 3: The percentage of the gender of the animals in the 40 sampled dog



4.1 Polymerase Chain Reaction (PCR) Assay Result

A single step multiplex PCR assay was carried out target to amplify the internal transcribed spacer – 2 region of the ribosomal DNA to detect and differentiate between the different species of the canine filarids. In total, 40 blood samples obtained from the animals in the shelter, 7 of them were tested positive with base pair between 500 to 600 and suggestive of *Dirofilaria immitis.* (Figure 4). Hence, the prevalence of dirofilariasis in forty dogs was 17.5%. Neither *Dirofilaria repens* nor *Brugia malayi* infection were detected. Besides, from all the positive results, four of them are female (57%) and three of them are male (43%). All the infected animals are from the group of newcomers, which

is 35% of the group that are non-vaccinated and not given heartworm prevention programme. (Figure 5, Figure 6 and Figure 7)

Figure 4: Molecular detection of dirofilariasis for 40 dog blood samples in the animal shelter by using PCR Assay

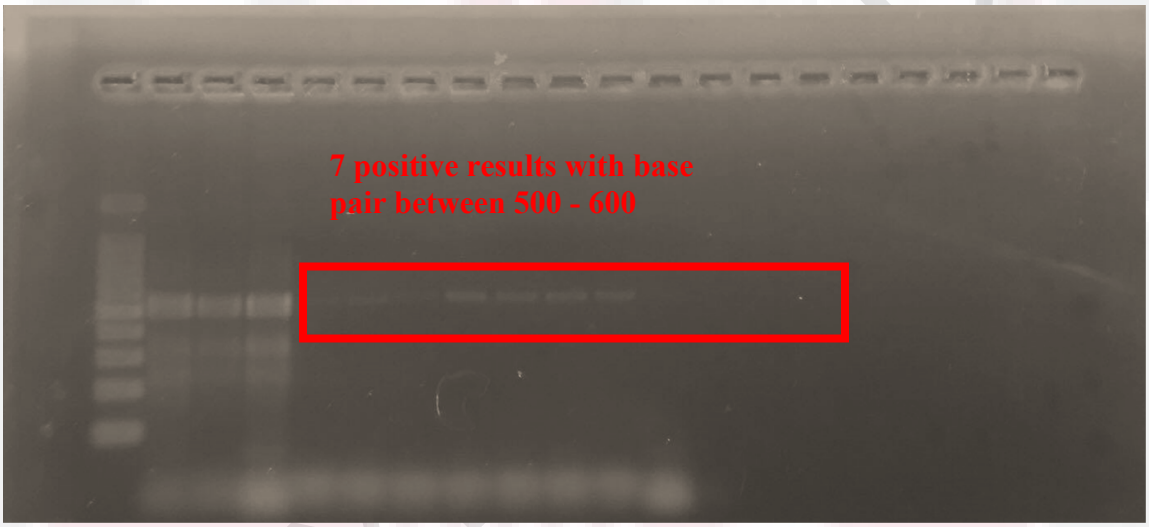


Figure 5: Percentage of dirofilariasis among 40 dog samples in animal shelter

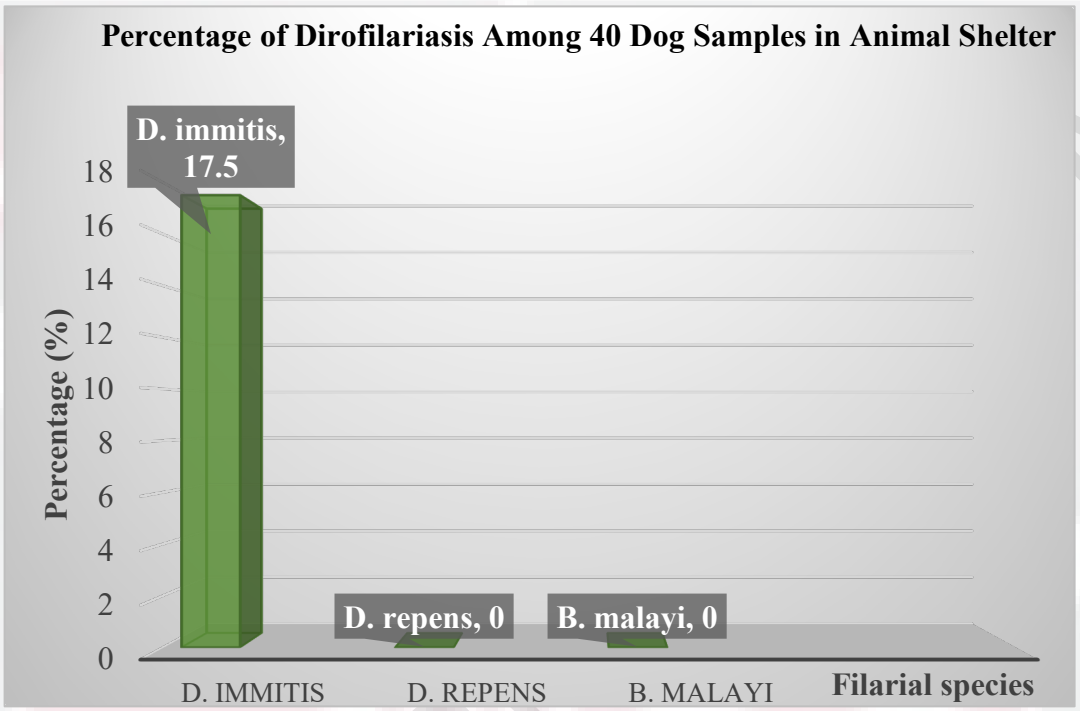


Figure 6: Percentage of the animal gender that is infected with dirofilariasis

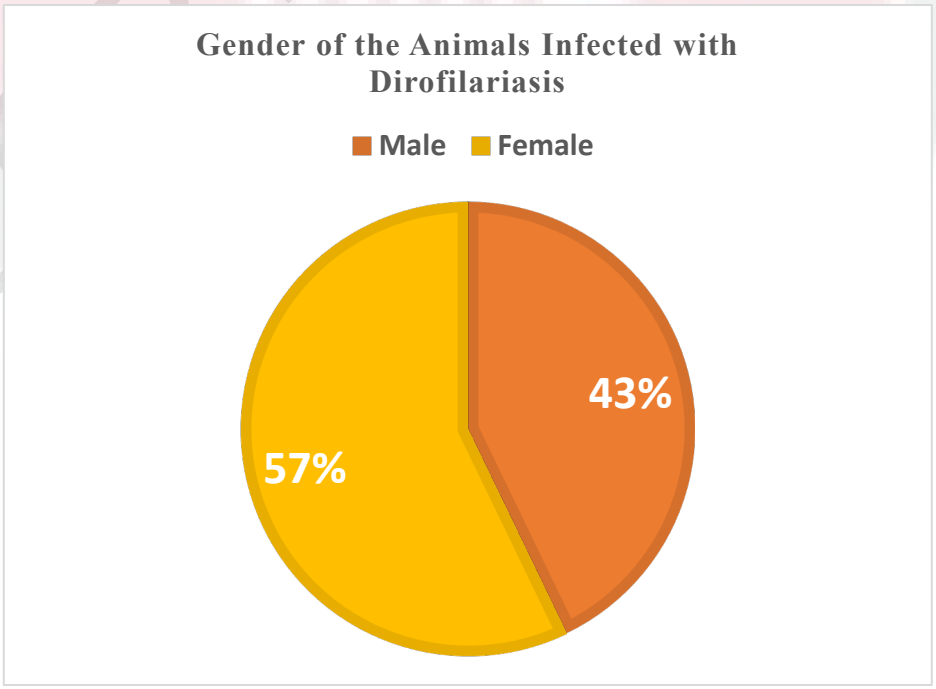
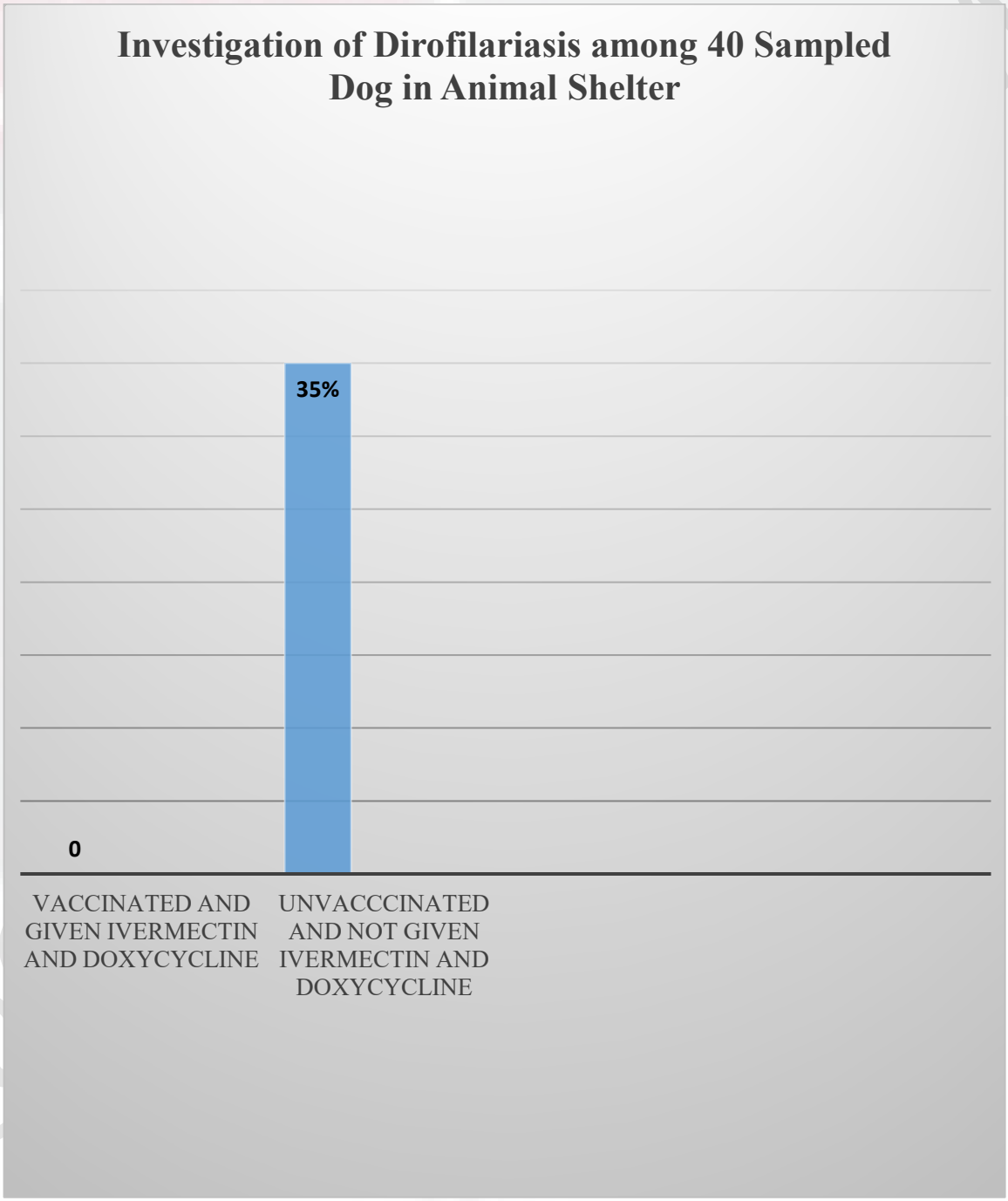


Figure 7: Percentage of infected animals from unvaccinated and not given heartworm prevention programme group



5.0 DISCUSSION

5.1 Factors affecting different prevalence of dirofilariasis

The prevalence of dirofilariasis in the animal shelter in this study was 17.5%. This is the first record regarding the prevalence of dirofilariasis in animal shelter in Pekan Nanas, Johor. Ng (2011) has conducted a study on heartworm prevalence in owned and stray dogs and the study showed prevalence of 1% and 2% respectively. Studies on heartworm antigenemia in the southern states of Penisular Malaysia (Negeri Sembilan, Melaka and Johor) was conducted by Yap and Ong (2008) and the study revealed only two positive cases in 129 samples collected and none positive samples was obtained from dogs in Johor.

No studies were conducted specifically for the prevalence of dirofilariasis in animal shelter in Johor prior to the present study. From the study conducted, seven out of forty dogs were detected positive for *Dirofilaria immitis*. The positive detection of heartworm shows that it is prevalent in the particular animal shelter. The prevalence rate is higher compare to the previous studies that were conducted in Johor and Klang Valley. This may due to a number of reasons. Firstly, it could be due to the environmental condition as all the dogs were kept in the open shelter and the risk of dogs to be bitten by infected mosquitos were high. Haddock (1987) states that yard dogs are at greater risk of exposure to mosquito as opposed to house dogs.

Secondly, it most likely could be due to the interrelationship factors that involving the host, agent and vectors. Most of the dogs in the shelter are stray dogs formerly that

have been rescued by the volunteers that worked in the animal shelter. A study conducted by Mullin (1970) in Kuala Lumpur and Petaling Jaya shows a 30.4% prevalence rate; Retnasabapathy & Khoo (1976) recorded the prevalence in Malaysia to be 25.8%; Dhaliwal & Sani (1993) shows a prevalence of 42% in Kuala Lumpur studies; Toh (2002) shows 31.7% of prevalence in stray dogs in Selangor and Yap (2006) recorded a prevalence of 33.34% in Kuala Lumpur. In their studies they have concluded that stray dogs had slightly greater prevalence compared to owned fog as they were exposed to mosquito which is compatible with this study.

Although the prevalence of this study is lower compared to the previous study that have been conducted on stray dogs in Malaysia, however the prevalence is higher if compared to owned dogs in Malaysia. This can be due to the efficacy of heartworm programme that have been conducted by the shelter. According to the volunteers that work in the shelter, all the animals are given monthly oral Ivermectin with the dosage of 6µg/kg and all the new coming dogs were treated with doxycycline (10mg/kg) for one month. According to American Heartworm Society (2012), macrocyclic lactones such as Ivermection, Milbermycin oxime, Moxidectin and Selamectin able to halt the migrating larvae that are less than two months old. McCall *et al.* (2011) also conducted a study on effect of doxycycline on early infections of *Dirofilaria immitis* in dogs and the study revealed doxycycline able to reduce *Wolbachia* that harbor in the heartworm by blocking its embryogenesis and suppressed microfilaremia progressively. These are fit to the result in this study as all the positive samples are from the group of animals that are not given any heartworm prevention programme. All the dogs that were on heartworm prevention

programme in the animal shelter are negatively tested. Study conducted by Grandi *et al*. (2010) shows that combination of doxycycline (10mg/kg twice daily for 30 days) and Ivermectin (6µg/kg – 14mg/kg once every 15 days for 6 months) is adulticide in dogs that infected with *D. immitis*. Administration of weekly prophylactic doses of Ivermectin along with intermittent doses of doxycycline over 8.5 to 9 months able to kill 78.3% of adult heartworms effectively (McCall *et al*., 2008).

5.2 Differences in findings of prevalence of dirofilariasis in animal shelter

There are few studies on prevalence of dirofilariasis in animal shelter conducted world widely. Studies conducted in Korea by Kim *et al*. and Na *et al.* with prevalence 9.8-10%; Streitel *et al.* (1977) show prevalence of 4.8% in Ohio only detected *Dirofilaria immiti*s in their studies. This is similar to the study conducted as only *Dirofilaria immitis* were found in all the seven positive samples. Study by Yabsley *et al.* (2004) at Northwester South Carolina USA showed prevalence of 18.7% with *Dirofilaria immitis* 11.1% and *Acanthocheilonema reconditum* (8.7%) and Veksin *et al.* (2014) detected *Dirofilaria repens* with 15.8% prevalence rate in Latvia. However, further sequencing of all positive PCR products at internal transcribe spacer region 2 (ITS-2) to confirm the filarial genotype in this study.

Besides that, there are also no gender significant in the study conducted. From all the seven positive samples, four positive samples are from female and another three positive samples are from male. The result is opposite with the survey conducted by Byeon *et al*. (2007) that shows males dogs were two times higher in prevalence rate compare to

the female. Study by Simsek *et al*. (2011) also showed that male dogs have higher infection rates compared to female by using direct blood smear and PCR method. However, Kan *et al.* (1976) reported that there was no significant difference in distribution of infections among dog with different age and sex group.

5.3 Diagnostic method in detecting filarial infection

In the present study, PCR method was used in identifying different species of canine filarial include *D. immitis, D. repens*, *B. malayi, B. pahangi, A. reconditum and A. dracunculoides*. Other diagnostic methods that are available include isolation of adult worms, histochemical staining of circulating mircrofilariae and by detecting the circulating antigen. However, PCR method is the most sensitive method to detect peripheral microfilarial (Vakalis *et al*., 1999). Studies by Simsek *et al*. (2011) also found that direct blood smear and PCR test are more sensitive than antibody detecting indirect ELISA which may due to the low level of antibodies in the infected dogs. Gioia *et al*. (2010) stated that microfilariae identification microscopically are uncertain sometimes and low in sensitivity when the amount of blood to be examined is in small amount.

5.4 Animal shelter management

In Malaysia, little studies have been conducted in the management of animal in the shelter. The present study was conducted in an animal shelter which is located in Pekan Nanas, Johor. There are altogether 3000 dogs that are managed in group housing. The Association of Shelter Veterinarian (ASV) (2010) discussed that group housing in shelter is good to provide healthy social contact and companionship with other animals to enhance

animal welfare. All the new coming stray dogs were given doxycycline 10mg/kg for one month. Besides, the shelter also practices annual vaccination and monthly oral Ivermectin as heartworm prevention for all the dogs. In managing a shelter, good sanitation is an integral part to reduce the risk of disease transmission to both human and animals (Cherry, 2004). Besides, a good veterinary relationship and record keeping is also vital. Shelter animals can suffer and die unnecessarily without a proper medical care (Humane Society of United States, 2007). According to Fowler (1993), preventive healthcare that is appropriate should be included in a shelter protocols to strengthen the disease resistance and minimize the exposure to pathogen. However, the animal shelter in this study is a shelter that does not practice euthanasia. American Veterinary Medical Association (AVMA) (2007) supports euthanasia in the paper of "Guidelines on Euthanasia" and stressed that the method that are carried out must be reliable, irreversible and compatible with the species, age and health status of the animals. Lastly, the shelter does not practice thorough neutering programme due to funding issue but Looney (2008) described that animal shelters should require dogs and cats to be spayed. Hart (1973, 1977) and Johnston (1991) conclude that practice neutering programme in animal shelter is strongly recommended as it able to reduce spraying, marking, fighting, heat behavior and pregnancy. Therefore, a small step of management change in the animal shelter able to maximize the quality of life and enhance the lifesaving capacity of the animals.

6.0 CONCLUSION

In conclusion, the investigation of the percentage of canine dirofilariasis among dog population in animal shelter located in Pekan Nanas, Johor was 17.5% (N=40), and the filarial species detected were suggestive of *Dirofilaria immitis*. This study shows that mosquitoes are the main vector that transmit the disease to the dog population. Besides, environmental factors and crowded dog population are the main factors for the disease transmission. Hence, implementation of a thorough heartworm prevention programme is important in managing a shelter.

7.0 RECOMMENDATION

In future study, performing sequencing at the ITS-2 region of all the positive PCR products should be recommended to confirm the filarial genotype. More dogs' samples are suggested to collect for a thorough PCR assay and dogs that are positively tested should be given prompt treatment. Lastly, the preventive health care such as annual vaccination and heartworm prevention should be improved in order to maximize the quality of life of the dogs in the shelter.

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