CHAPTER 1

INTRODUCTION

1.1 The host

The domestic cat *Felis catus* originated from an ancestral wild species, *Felis silvestris*, the European and African Wild Cats. Domestic cats first arrived in North America with European colonists several hundred years ago and since that time cats have multiplied and thrived as pets, unwanted strays and semi-wild predators (Coleman *et al*., 1997).

Cats are the most popular pet in the world (Driscoll *et al.,* 2009) and have been associated with humans for at least 9,500 years (Figure 1.1). In United States, the combined total of pets and free-ranging cats is probably more than 100 million. Approximately, 30% of households have cats nationwide (Coleman *et al*., 1997). Due of their close association with humans, most of these cats are concentrated in areas where people live rather than in remote undeveloped areas (Coleman et al., 1997).

Domestic cats can live between 15 to 17 years as pets. However for strays, they live an estimated between 4 to 5 years (Ogan & Jurek, 1997) only. Warner (1985) studied the survival rate of a cat and observed a farm cat in Illinois showed the survival rate was 1.5 kittens per female per year. Domestic cats reach reproductive maturity at age between 7 to 12 months. A breeding female cat normally called a queen can be in estrus as frequent as five times per year. The gestation period lasts between 63 to 65 days. The female cats usually produce two litters per year and the average litter is four kittens (Nowak & Paradiso, 1983).

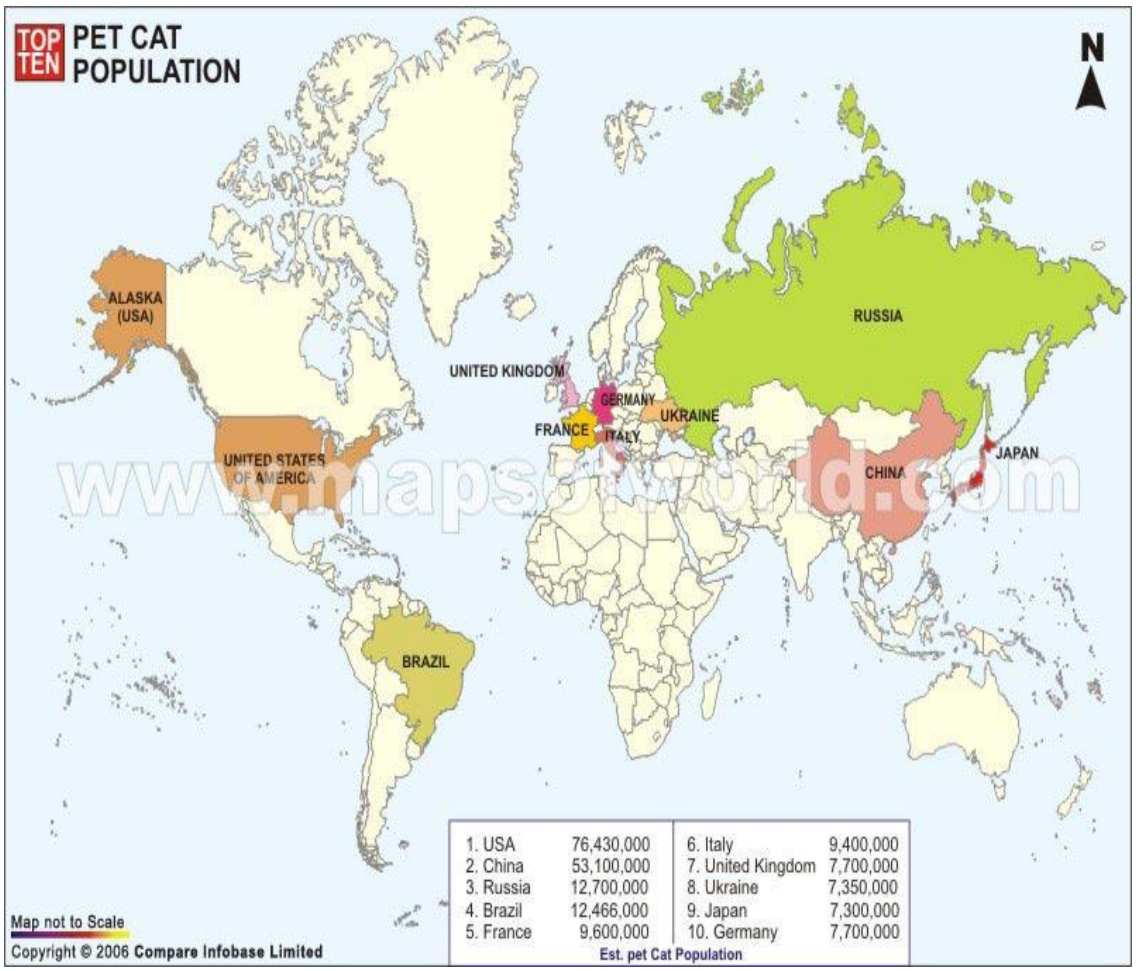

Figure 1.1: Top ten Countries with most pet cat population. Source: Maps of World (Inc, 2006).

1.2 Stray cats

Stray cats are cats that wander outdoors and can be found on the streets, food courts, markets and have no home. Cats can also be free roaming domestic pet but return to human habitation after foraging for food (Ogan & Jurek, 1997). A stray cat could also one that escaped home and unable to find way back or an abandoned cat that seeks its own shelter. The offspring of stray cats can be considered feral if born in the wild (Holton, 2007). Stray cats are quite different from feral cats. Feral cats are the free-

roaming offspring of the domestic cat. They generally fall into two categories: 1) domestic animals adapted to living on their own in rural and urban areas; or 2) homeless, lost, or abandoned pets that live on their own (Roberto, 1995).

Cats have the tendency to breed very quickly thus over-population of cats is a worldwide problem. Coleman *et al.* (1997) advocated neutering cats as a control measure of this problem. By controlling the breeding of cats will decrease the number of cat population eventually decreasing the number of cats abandoned. This method also controls the cat's behaviour by eliminating the habit of urine spraying for male cats, fighting and acting aggressively and reduces diseases such as reproductive cancer.

Stray cats are usually found in close contact with human and can easily be found in areas where food is abundant such as markets, food courts, by the streets and hawker stalls. These cats are exposed to many types of diseases and may harbour various parasites that are transmittable to human such as; toxoplasmosis, toxocariasis, rabies, opisthorchiidosis and others. Most parasites can potentially cause harm to the animal and the human's health but rarely can causes serious harm and death.

1.3 Common parasites infecting cats and its distribution

Stray cats get infected with parasites when they wander outdoors foraging for foods and are exposed to accidental ingestion of parasites. Parasites infecting cats fall under two categories namely, ectoparasite and endoparasite. Ectoparasites are parasites that live on the surface of a host. Common ectoparasites include fleas, lice, ticks and mites.

Endoparasites are parasites that live in the internal organs or tissues of its host. Common endoparasites include nematode, cestode, trematode and protozoa.

Research on feline parasitic infections from many develop countries have been systematically carried out over the last two decades and highlighted its significant hazards to human health i.e USA (Schantz, 1999; Hill *et al*., 2000; Anderson *et al*., 2003; Cheryl & Dennis, 2003) Australia (Coman, 1972; Wilson-Hanson & Prescott, 1982; Shaw *et al*., 1983; Thompson *et al*., 1993; McGlade *et al*., 2003), and Europe (Lewis, 1927; Christensen *et al*., 1946; Niak, 1972; Cowper, 1978; McColm & Hutchinson, 1980; Nichol *et al*., 1981a,b; Thienpoint *et al*., 1981; Stoichev *et al*., 1982; Engbaek *et al*., 1984; Van Beeck *et al*., 1985, Calvete *et al*., 1998; Barutzki & Schaper, 2003; Coati *et al*., 2003). Several reports also of helminth from feline populations in the Middle East have also been published (Morsy *et al*., 1980) in Jordan, (Hasslinger *et al*., 1988) in Egypt and most recently (Abu-Madi *et al*., 2008; 2010) in Qatar. However, each report from each study differs in the spectrum of helminth species infecting the local cat population.

In the past, studies of parasites infecting the stray cat population from Peninsular Malaysia have provided considerable data on the diversity and infection levels (Rohde, 1962; Zamirdin *et al.*, 1967; Mustaffa-Babjee, 1969; Retnasabapathy & Khoo, 1970; Retnasabapathy & Prathap, 1971; Yoshida *et al.*, 1973; Nagendram & Rajamanickam, 1976; Amin-Babjee, 1978; Shanta *et al.*, 1980; Zamri Saad *et al*., 1984 and Lee *et al*., 1993).

1.3.1 Cat ectoparasites

1.3.1.1 Flea

The most common and important cat flea is *Ctenocephalides felis*. It is a cosmopolitan species and a pest in the urban environment. This species are ubiquitous throughout the world wherever suitable host reside. In the United States, this flea are present in all areas except the mid- to north- Rocky mountain area (Hubbard, 1968; Roberts & Janovy, 2000; Swan & Papp, 1972).

Table 1.1: Scientific classification of *Ctenocephalides felis*

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Siphonaptera
Family	Pulicidae
Genus	*Ctenocephalides*
Species	*Ctenocephalides felis*

Ctenocephalides felis is laterally compressed, wingless, about 2 mm long and reddish-brown to black colour. It has sloping forehead, hind tibia which lacks an outer apical tooth and combs which differentiate it with other species. The male *Ctenocephalides felis* is slightly smaller than the female and has complex, snail-shaped genitalia.

Ctenocephalides felis developes into four stages namely; egg, larva, pupa and adult. Rust and Dryden (1997) reviewed in detail the biology of the cat's flea. Adult flea can

produce about 15 eggs that fall into the soil or the carpet. The eggs then hatch and produce larvae that crawl in the environment to feed. The larva undergoes two molts before becoming the third-stage larva before developing into a silken cocoon. It remains in the pupal case for about 15 days before the adult flea emerges spontaneously from the pupal case when they sense the presence of an appropriate host (Figure 1.2).

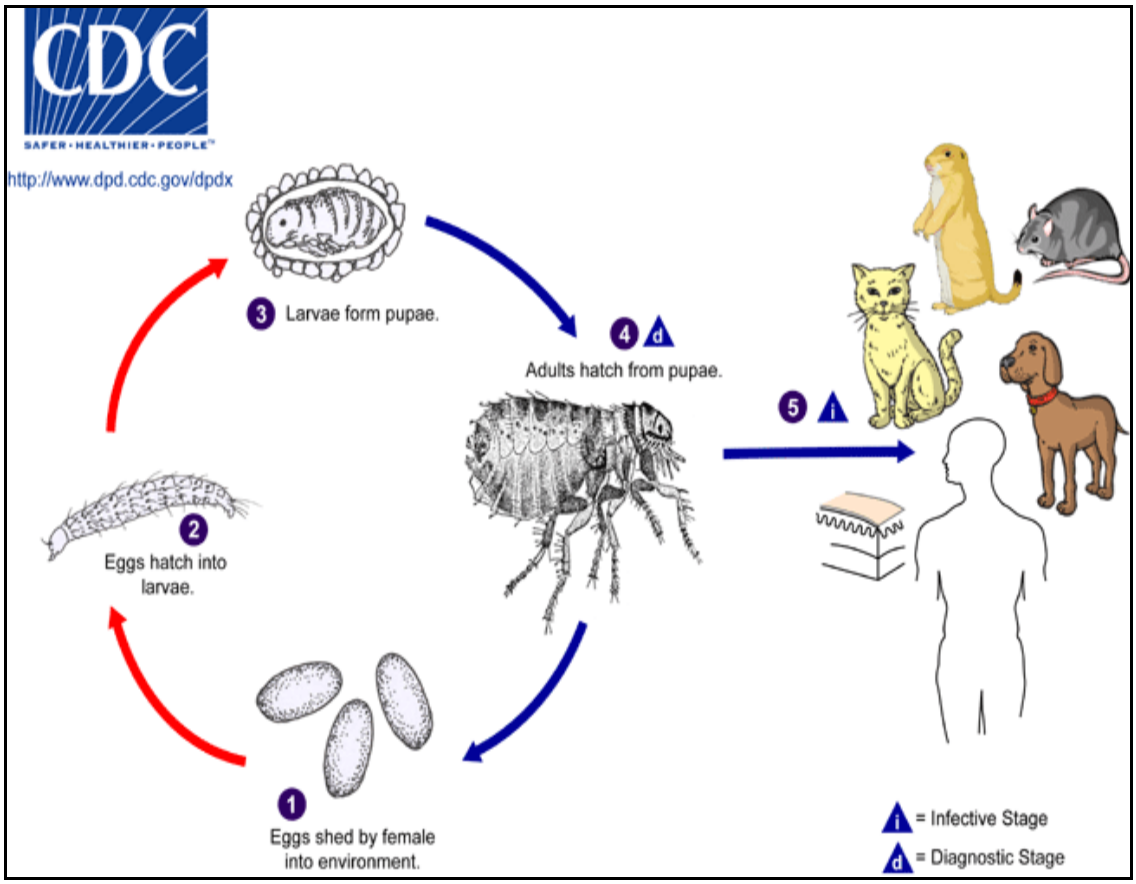

Figure 1.2: Life cycle of the cat's flea, *Ctenocephalides felis*. Source: DPDx (DPDM, 2009).

Fleas can cause skin irritation, allergies and annoyance to the human (Noor Hayati *et al*., 2002). The adult sucks on blood from the feline hosts and is capable of taking enough blood to cause severe anemia in kittens and induce cardiomegaly (King, 1997;

Yaphe *et al*., 1993). The blood imbibed by the flea pass directly through their system (Bowman *et al*., 2002).

1.3.1.2 Louse

Felicola subrostratus is the only parasitic louse species that occurs on cats. It is highly host specific (Timm & Price, 1994) and infection is not common but present in the cat population. *Felicola subrostratus* is known to occur in cats all around the world. *Felicola subrostratus* has been reported throughout the world with reports from Europe (Trotti *et al*., 1990), Asia (Mustaffa-Babjee, 1969; Amin-Babjee, 1978; Shanta *et al*., 1980), the Philippines (Eduardo *et al*., 1977), Australia (Coman *et al*., 1981), South America (Santa Cruz & Lombardero, 1987) and North America. It has a triangular head pointed forward and a groove on the ventral side of the head that assists on attaching itself on the cat's fur (Ewing, 1929).

Table 1.2: Scientific classification of *Felicola subrostratus*

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Pthiraptera
Family	Trichodectidae
Genus	*Felicola*
Species	*Felicola subrostratus*

The biology of *Felicola subrostratus* is very poorly known. Adult females lay their egg cases within the animal's hair by gluing the operculate eggs to the hair of the feline host (Burrows, 2009) (Figure 1.3). Cats that have long-haired, aged and sickly have higher potential to be infested with this parasite. Grooming can help to control the numbers of lice from developing (Bowman *et al*., 2002).

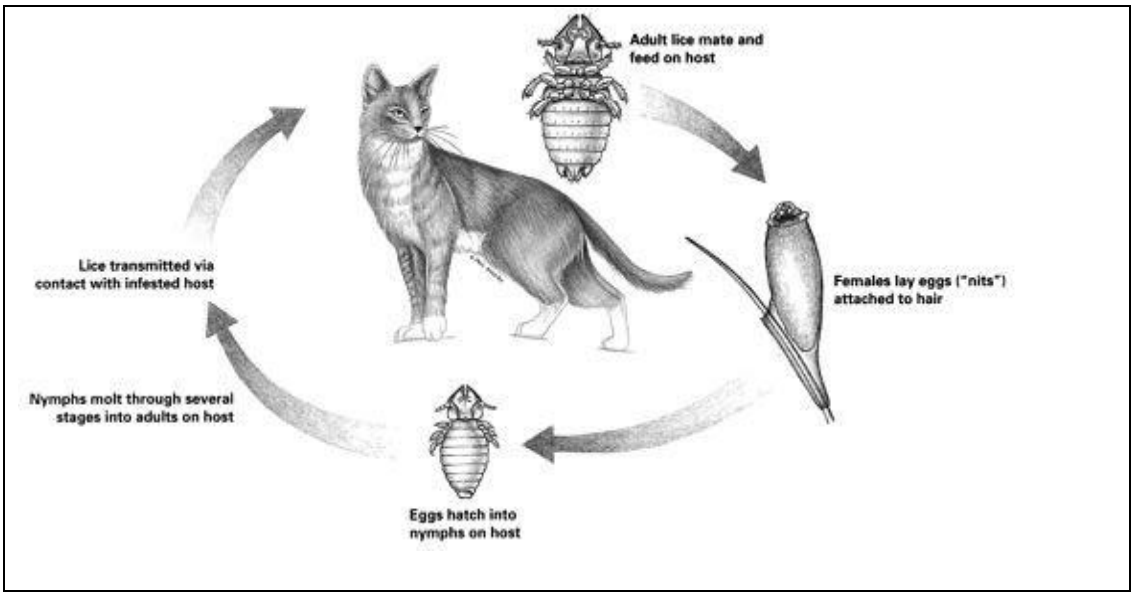

Figure 1.3: Life-cycle of the cat's louse, *Felicola subrostratus*. Source: Lecture 7 - Parasitic skin diseases – Insects (Yaphй, n.d.)

1.3.1.3 Ticks and mites

Ticks and mites are classified into four orders namely; Metastigmata, Mesostigmata, Prostigmata and Astigmata which most are free-living (Table 1.3). Metastigmata is further divided into two; the hard tick (Ixodidae) and the soft tick (Argasidae).

Careful examination of the animal's skin and ears can help detect this pest. Ticks imbed its mouthparts into the animal's skin and feed on the blood. Ticks can cause anemia and are known carriers too many serious diseases, including Lyme disease and Ehrlichia.

The common hard ticks on cats are also found on dogs and human. *Ixodes, Dermacentor, Rhipicephalus*, *Haemaphysalis* and *Amblyomma* are some of the common genera found.

Mites are much smaller in size than ticks and are difficult or impossible to see without magnification. Symptoms of cats with mite infestation vary depending on the type of mite can include itching, irritation, and hair loss. Ear mite, *Otodectes cynotis* appears as small white organism that moves within the ears. Skin mites usually require a skin scraping test. The mites live in by burrowing under the epidermis of the cat's skin. Cats infested with *Notoedres cati* typically present with lichenification of the skin on the ear tips, face and distal extremities (Foley, 1991a, b).

Mites that infest cats include *Demodex cati* cause feline demodicosis. *Demodex cati* have been described from North America, Europe, Australia, New Caledonia, Africa and India (Beugnet & Chardonnet, 1993; Chesney, 1989; Yathiraj *et al*., 1994). *Otodectes cynotis* is found around the world from different areas including the America (Foley, 1991a,b), Europe (Raschka *et al.,* 1994; Trotti *et al.*, 1990), Asia (Fukase *et al*., 1991; Tacal & Sison, 1969), Middle East (Ismail *et al*., 1982) and Australia (Coman *et al*., 1981). *Notoedres cati* has been reported in cats in Europe (Bigler *et al*., 1984; Fabbrini, 1994; Hartmannova & Mouka, 1990; Svalastoga *et al*., 1980; Tudury & Lorenzoni, 1987), the Middle East (Rak, 1972), India (Yathiraj *et al*., 1994), Africa (Zumpf, 1961), Japan (Ogata *et al*., 1980), Indonesia (Sangvaranond, 1979), Australia (Wilson-Hanson & Prescott, 1982), North America (Foley, 1991a) and South America (Larsson, 1989). Infestation with another mite species *Lynxacarus radovskyi* have been reported in the United States in cats from southern Texas (Craig *et al*., 1993), southern

Florida (Greve & Gerrish, 1981) and the Florida Keys (Foley, 1991a). This mite also reported from Puerto Rico (Fox, 1977), Hawaii (Tenorio, 1974), Fiji (Munro & Munro, 1979) and Australia (Bowman & Domrow, 1978).

The life cycle of this ectoparasite begins with an egg then a larvae stage (with three pairs of legs) followed by a nymphal stage and finally into adult then adults are characterized by having four pairs of legs and a fused anterior and posterior body that appears to lack segmentation (Bowman *et al*., 2002).

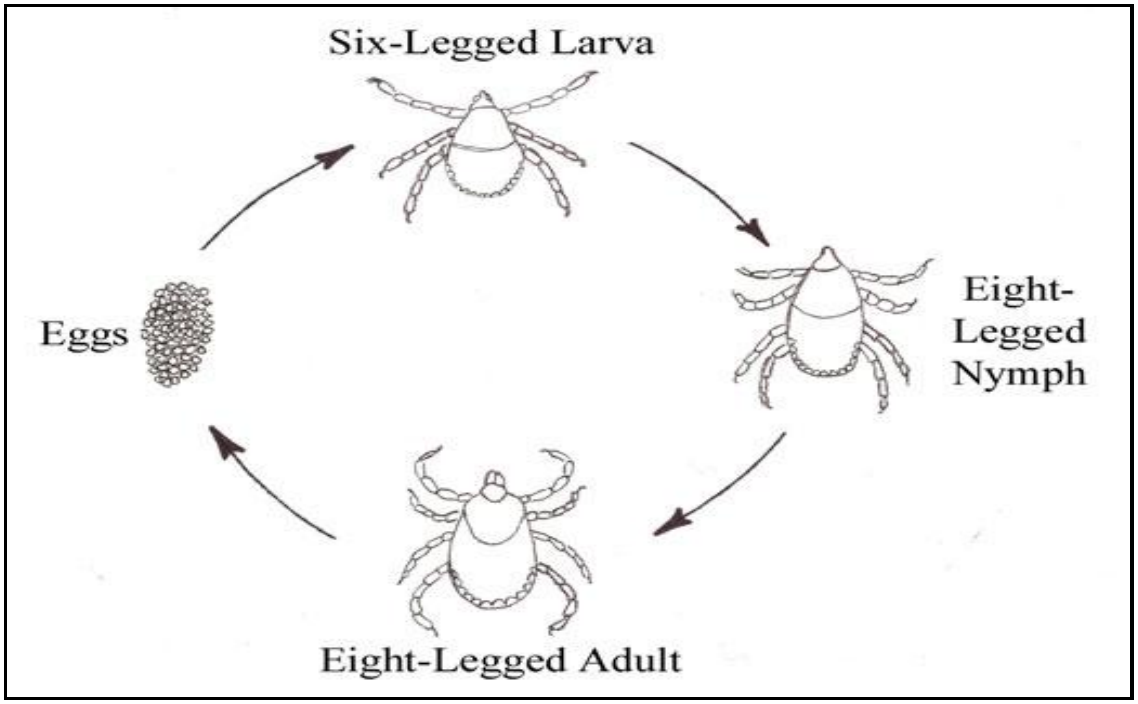

Figure 1.4: Life cycle of tick. Source: Ticks (District, 2004).

Table 1.3: Classification of ticks and mites.

Kingdom	Animalia
Phylum	Arthropoda
Class	Arachnida
Order	

Metastigmata		**Mesostigmata**		**Prostigmata**		**Astigmata**	
Family	**Ixodidae**(Hard tick) Genus: *Ixodes sp.*, *Dermacentor sp.*, *Rhipicephalus sp.*, *Haemaphysalis sp.* & *Amblyomma sp.*	Family	**Dermanyssidae** (Fowl mite) Genus: *Dermanyssus sp.*	Family	**Demodicidae** (Follicular mites) Genus: *Demodex sp.*	Family	**Sarcoptidae** (Burrowing mite) Species: *Sarcoptes scabiei* & *Notoedres cati*
	Argasidae(Soft tick) Genus: *Ornithodorous sp.* & *Argas sp.*		**Macronyssidae** (Fowl mite) Genus: *Ornithonyssus sp.*		**Cheyletidae** (Hair-clasping mite) Genus: *Cheyletiella sp.*		**Psoroptidae** (Ear mite) Species: *Otodectes cynotis*
							Listrophoridae (Hair-clasping mite) Species:*Lynxacarus radovskyi*

1.3.2 Cat Endoparasites

1.3.2.1 Phylum Nematode

Nematodes are the most numerous multicellular animal on earth. Majority are free living, can be found in soils of most of the earth's ecosystems and some parasitize on other living hosts. Nematodes are recognized as an important parasite to plants and animals (Bowman *et al.*, 2002). Nematodes possess a simple digestive, nervous, excretory and reproductive system but lack respiratory or circulatory system. The entire structure of nematodes functions on the basis of a very high internal turgor pressure that is maintained in the body cavity called the pseudocoelom, in which the different tubes of the body are suspended (Bowman *et al*., 2002). Nutrients are transported throughout the body via fluid in the pseudocoelom. The muscles of nematodes are all longitudinal and their contraction produces a thrashing motion (Campbell & Reece, 2002). Nematode populations are generally denser and more prevalent in the world's warmer regions, where longer growing seasons extend feeding periods and increase reproductive rates (Dropkin, 1980).

1.3.2.1.1 *Toxocara spp.*

Toxocara spp. is the most common intestinal parasites found in cats. These parasites are of public health importance because they cause diseases to human. *Toxocara spp.* can be found worldwide and probably the most common encountered parasites in cats (Bowman *et al*., 2003). *Toxocara cati* and *Toxascaris leonine* have been reported from many countries.

Table 1.4: Scientific classification of *Toxocara spp.*

Kingdom	Animalia
Phylum	Nematoda
Class	Secernentea
Order	Ascaridida
Family	Toxocaridae
Genus	*Toxocara, Toxascaris*
Species	*Toxocara cati* *Toxocara malaysiensis* *Toxascaris leonine*

Toxocara cati is a cosmopolitan parasite of the domestic cat and found throughout the world. This species have been reported in different countries include Germany, 45% of 155 cats (Schuster *et al*., 1997); France, 31% of 129 cats (Petithory *et al*., 1996); Tasmania, 89% of 39 cats (Milstein & Goldsmid, 1997); Taipei, 42% of 95 cats (Fei & Mo, 1997); Japan, 18.2% of 1,064 cats (Oikawa *et al*., 1991); Somalia, 28% of 50 cats (Gadale *et al*., 1988-89); South Africa, 11% of 1,502 cats (Baker *et al.,* 1989); Nothern Territory of Australia, 1% of 188 feral cats (O'Callaghan & Beveridge, 1996).

Toxascaris leonine had been reported in cats from North America with 1% to 5%, 2% to 20.5% from Europe and 11% from Ceylon (Sprent & Barrett, 1964). This parasite also have been reported from farm cats in Oxfordshire in United Kingdom with 82% infected (Yamaguchi *et al*., 1996); 1.1% of 92 feral cats in London (Nichol et al., 1981b); 4.2% of 72 stray cats in Scotland (McColm & Hutchison, 1980); 1.1% of 567 stray cats in Moscow (Vereta, 1986). Fecal sample examination also detected the eggs

of *Toxascaris leonina* from 60% of 30 stray cats in Belgium (Vanparijs *et al*., 1991) and 3.7% of 376 cats in Australia (Moore & O'Callaghan, 1985).

Toxocara spp. is under the order Ascaridida. The ascarids are cream colored, robust and the length as long as from 1 to more than 10 cm. Other characteristics of these ascarids are the presence of three large fleshy lips on the anterior end of the adult worms and the eggshells produced by the species typically are thick shelled and very resistant to environmental extremes.

Cats acquire infection by three routes; by ingestion of the infective eggs or by ingestion of infected transport, through intermediate host or through transmission of larvae through milk from mother to the kittens. Neonatal infection of *Toxocara cati* commonly occurs via the mammary glands and milk is an important route of infection for kittens (Swerczek *et al*., 1971). Detailed description of the development of *Toxocara cati* was reported by Sprent in 1956. He infected kittens via oral inoculation with infective eggs. Sprent found that the larvae of infected kitten via oral inoculation migrated away from the alimentary tract and commenced development liver-lung migration before finally reaching the stomach wall via the trachea (Sprent, 1956). Meanwhile kittens fed with infected mice, all larvae found completed their development without undergo liver-lung migration. Paratenic hosts are probably routinely involved in the life cycle of *Toxocara cati* (Bowman *et al*., 2003).

Kittens have higher potential to be infected with *Toxocara* worms compared to adult cats. Infection with this parasite is highest in kittens till up to age 6 months (Visco *et al*., 1978). Most kittens become susceptible to the infection because of their weak and under

developed immune system. The adult worms live in the small intestine of the host and take up nutrients from food that cats eat causing malnutrition and intestinal obstruction. Kittens with mild infestation may not show any symptoms. Cats with severe infestation will develop pot-bellied appearance, lose weight and have dull coats. Some cats become anemic, diarrhea, constipation and death due to the obstruction of the small intestine. After the first 6 months of life, cats can expel the parasites from their intestine recover completely (Acha & Szyfres, 1991).

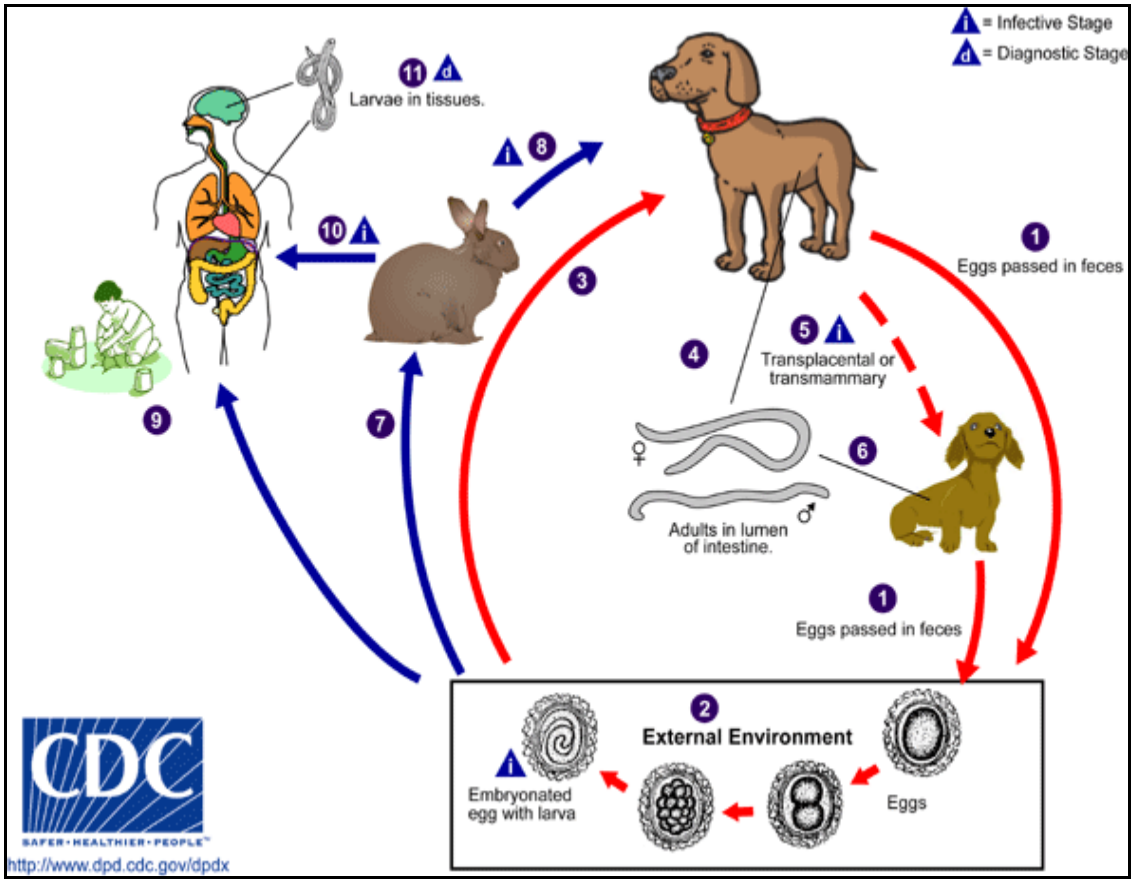

Figure 1.5: Life cycle of *Toxocara* species. Source: DPDx (DPDM, 2009).

1.3.2.1.2 *Ancylostoma spp*.

There are several species that are commonly found in cats namely *Ancylostoma tubaeforme, Ancylostoma braziliense, Ancylostoma ceylanicum* and *Uncinaria stenocephala* (Provic, 1998). *Ancylostoma spp* is classified under the family Ancylostomatidae. The adults are found in the small intestine and mostly in the jejunum (Baker *et al*., 1989) attached to the mucosa intestinal to feed on host's blood (Monti *et al*., 1998).

Table 1.5: Scientific classification of *Ancylostoma spp.*

Kingdom	Animalia
Phylum	Nematoda
Class	Chromadorea
Order	Rhabditida
Family	Ancylostomatidae
Genus	*Ancylostoma*
Species	*Ancylostoma braziliense* *Ancylostoma ceylanicum* *Ancylostoma tubaeforme*

Ancylostoma braziliense was identified from Africa and South America (Biocca, 1951). This parasite also has been reported from Central and North America. Yoshida *et al.* (1974) reported the occurrence of this nematode from Japan and mix infections of *Ancylostoma ceylanicum* and *Ancylostoma braziliense* reported from Indonesia

(Soeripto *et al*., 1978) and Malaysia (Yoshida *et al*., 1973; Amin-Babjee, 1978; Shanta *et al*., 1980).

In India, Malaysia, Taiwan and Japan, high rates of infection by *Ancylostoma ceylanicum* have been found in dogs and cats. The distributions of *Ancylostoma ceylanicum* extend south from India (Chowdhury & Schad, 1972; Ray *et al*., 1972) down the eastern coast of Africa to Madagascar and South Africa (Baker *et al*., 1989; Yoshida, 1971a; Yoshida *et al*., 1973). The distributions also extend east from India into Indonesia, Singapore, Malaysia and Thailand (Rohde, 1962; Amin-Babjee, 1978; Shanta *et al*., 1980; Setasuban *et al*., 1976; Soeripto *et al*., 1978; Yoshida, 1971a,b; Yoshida *et al*., 1973). *Ancylostoma ceylanicum* also reported from some Pacific islands including Taiwan and Okinawa (Yokogawa & Hsieh, 1961), Phillipines (Arambulo *et al.,* 1970), Sri Lanka (Dissanaike, 1961) British Solomon Islands (Haydon & Bearup, 1963), Fiji (Yoshida, 1971a) and Japan (Yoshida & Okamoto, 1972).

They are characterized by the possession of a copulatory bursa, a large dorsally flexed buccal cavity that is armed on its anterior edge with either teeth or cutting plates and the length of these nematodes about 1 to 3 cm. They have teeth-like structure or cutting plates which allows them to attach themselves to the wall of the intestine and feed on animal's blood. The eggs of the different *Ancylostoma* species found in the feces are indistinguishable from each other (Bowman *et al*., 2002)

Life cycles of different species are similar (Acha & Szyfres, 1991). Cats get infected in two routes. Firstly is via larvae penetration of the skin and secondly by larvae ingestion in the environment or the paratenic hosts (Bowman *et al*., 2003). If infection is through

the skin, the larva migrates through the lungs, up to the trachea then swallowed into the esophagus and down to the intestine where they finally mature. Infection via oral ingestion, the larvae enters the intestinal mucosa and mature. The larva is more likely to develop to the adult stage after oral inoculation rather than skin penetration (Yoshida *et al*., 1974). *Ancylostoma braziliense* may live about four to eight month and a single female worm can produce between 200 to 6000 eggs per day (Sarles, 1929).

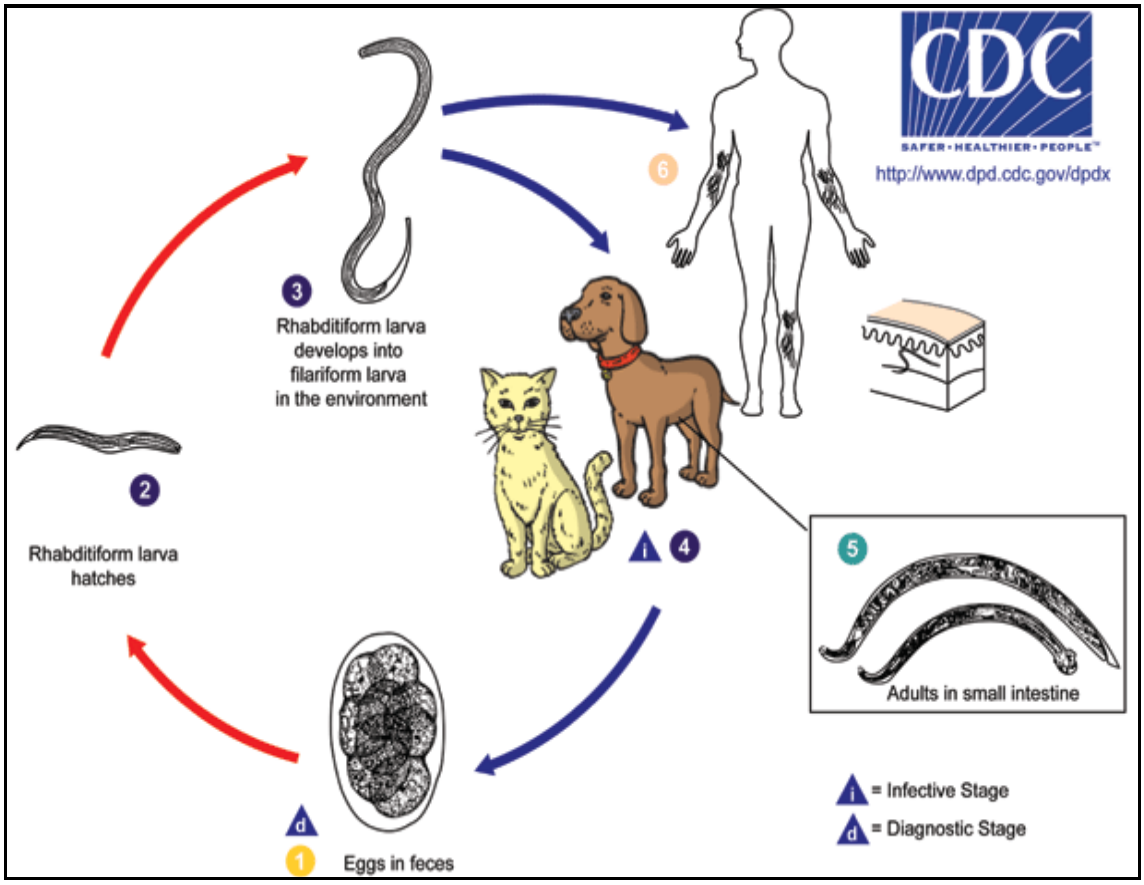

Figure 1.6: Life cycle of *Ancylostoma* species. Source: DPDx (DPDM, 2009).

Hookworms causes severe anemia due to the blood loss, malnutrition and weakness. Untreated cats may die. Cats over 6 months old harbored heavier burden meanwhile cats less than a month old were lightly infected (Amin-Babjee, 1978). Invasion of the larvae

parasites through the skin may cause dermatitis and the lesions are limited to the body parts of the animal that come in contact with soil. However, infection is short duration and disappears after 5 days (Acha & Szyfres, 1991).

1.3.2.1.3 *Strongyloides spp.*

The genus *Strongyloides* has about 50 species of obligate gastrointestinal parasites of vertebrates (Speare, 1989). This small worm penetrates the mucosa of the duodenum and jejunum of cats. It is only about 2 mm long and 0.0035 wide. The reproduction is parthenogenetic and the male worms are not found in the parasitic phase of the life cycle.

Table 1.6: Scientific classification of *Strongyloides spp.*

Kingdom	Animalia
Phylum	Nematoda
Class	Secernentea
Order	Rhabditida
Family	Strongyloididae
Genus	*Strongyloides*
Species	*Strongyloides planiceps* *Strongyloides felis* *Strongyloides tumefaciens*

Three known species of *Strongyloides spp.* are; *Strongyloides planiceps*, *Strongyloides felis* and *Strongyloides tumefaciens* (Bowman *et al*., 2003) infect cats. *Strongyloides planiceps* was originally described from Malaya (Rogers, 1943) and observed in wild

carnivores and domestic cats in Japan (Horie *et al*., 1981; Fukase *et al*., 1985). However, this species has not been observed in the United States. *Strongyloides tumefaciens* was found in domestic cats from the south-eastern United States in Louisiana, Florida, Texas and Georgia (Price & Dikmans, 1941; Malone *et al*., 1977; Lindsay *et al*., 1987). *Strongyloides felis* was observed in India and Australia.

The female species can be differentiated into two major groups. *Strongyloides planiceps* has hair-pin turn to the ovaries and produces eggs that hatch before they leave the intestine (Rogers, 1943) and *Strongyloides felis* have spiral ovaries (Chandler, 1925a) and produces eggs out through the feces (Little, 1966). Both species are found in the small intestine.

Strongyloides planiceps infects cats via oral ingestion of infective larvae or via skin penetration (Rogers, 1939). There is no description of clinical signs for cats infected with *Strongyloides planiceps* and *Strongyloides felis* (Speare & Tinsley, 1986). Infections can be detected by fecal examination for eggs or larvae or post-mortem examination of the small intestine of the host for parasitic females. Cats and dogs also can be infected by *Strongyloides stercoralis*. This species is similar in both morphologically and physiologically to the human parasite (Acha & Szyfres, 1991). *Strongyloides stercoralis* normally infects the young, as the larva has more difficulty penetrating the thick skin of adults. The first signs to appear in infected kittens and puppies are loss of appetite, purulent conjunctivitis, coughing and bronchopneumonia. The infection becomes serious in the intestinal phase causing diarrhea, abdominal pains, vomiting, dehydration and anemia.

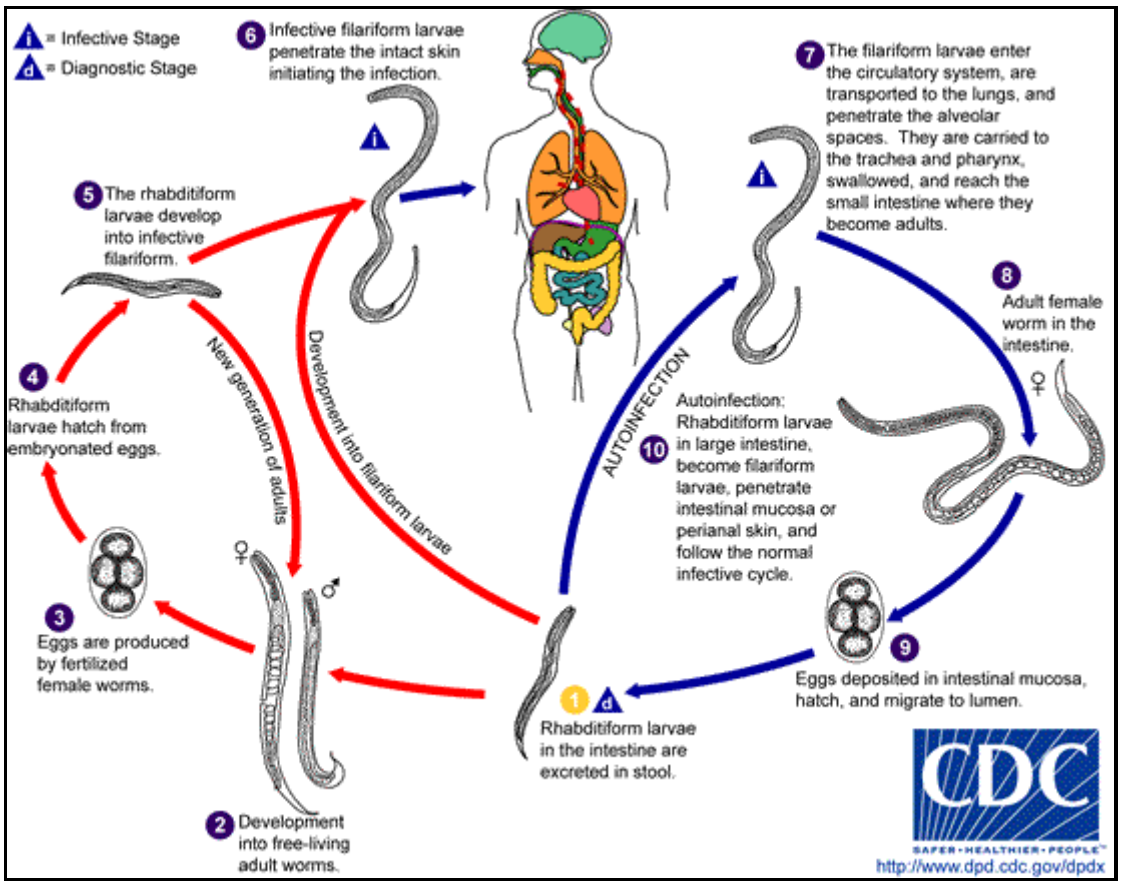

Figure 1.7: Life cycle of *Strongyloides* species. Source: DPDx (DPDM, 2009).

1.3.2.1.4 *Physaloptera praeputialis*

Physaloptera praeputialis is a stomach worm from the family Physalopteridae. This parasite is characterized by a cuticular sheath covering posterior end of the body in both sexes that appears prepuce-like. *The* adult tends to be white to pinkish and is about 3-6 cm in length. The egg is a thick, clear shell and contains a fully formed larva when passes out the feces (Bowman *et al*., 2002).

Physaloptera praeputialis have been reported from cats around the world. This parasite have been reported in America including the United States (Gustafson, 1995; Levine, 1968) and the Bahamas (Clarkson & Owen, 1959), Mexico (Zarate-Ramos *et al*., 1991),

Venezuela (Power, 1964), Argentina (Santa-Cruz & Lombardero, 1987), and Brazil (Campos *et al.*, 1974; Ogassawara *et al*., 1986). From Southeast Asia and South Pacific, Physaloptera praeputialis has been reported from cats in Hawaii (Ash, 1962), the Philippines (Tongson & San Pablo, 1979), Japan (Hayasaki *et al.*, 1982), Malaysia (Retnasabapathy & San, 1970) and Australia (Barton & McEwan, 1993). From Asia and the Middle East, this parasite has been reported from cats in Iraq (Daoud *et al.*, 1988), Iran (Mirzayans, 1972), Turkey (Burgu *et al*., 1985), Turkmenia in the former USSR (Velikanov & Sharpillo, 1984) and India (Gill, 1972). From Europe, this parasite was reported from Greece (Haralampides, 1978) meanwhile from Africa, there is only one report from South Africa (Baker *et al*., 1989).

Table 1.7: Scientific classification of *Physaloptera praeputialis*

Kingdom	Animalia
Phylum	Nematoda
Class	Secernentea
Order	Spirurida
Family	Physalopteridae
Genus	*Physaloptera*
Species	*Physaloptera praeputialis*

The life cycle of this parasite is still not clearly known but the infective larval stage develops in cockroaches and beetles (Petri & Ameel, 1950). Cats get infected by ingestion of infected insects or paratenic hosts. Cats are more likely to be infected via the ingestion of the paratenic hosts than insects (Bowman *et al*., 2003). The prepatent

period ingestion of infected insect is between 131-156 days. Cats fed with the third stage larvae from infected lizard develop patent infection after 60 days post infection (Velikanov & Sharpilo, 1984).

Cats with *Physaloptera praeputialis* infestation may vomit and the vomitus may contain one or more worms (Bowman *et al*., 2003). Gufstafson (1995) reported two infected cats developing anemia, eosinophilia and melena with intermittent vomitting for several months had adult worms in the vomitus. Humans rarely get infected and this can only occur via ingestion of the intermediate and paratenic hosts (Bowman *et al*., 2002).

1.3.2.2 Class Cestoda

Cestodes are tapeworms classified under phylum Platyhelminthes. Tapeworms can be acquired by ingestion of an intermediate host infected with larval stages of the tapeworm. All vertebrates can be parasitized with at least one species of tapeworm. There are 14 orders of cestodes currently recognized (Khalil *et al*., 1994).

Cestodes are made up of two parts namely, the scolex and the strobila. The strobila consists of a series of connected proglottides with the most posterior gravid segments equipped with both female and male reproductive parts. The structure of the scolex varies among the different species but generally it consists of four peripheral suckers surrounding a centrally placed called rostellum.

1.3.2.2.1 *Dipyllidium caninum*

Dipylidium caninum is one of the most common parasites of the domesticated dog and cat. This parasite infects cats, dogs and wild carnivores as the definitive host meanwhile, humans as occasional host (Molina *et al*., 2003).

Table 1.8: Scientific classification of *Dipylidium caninum*

Kingdom	Animalia
Phylum	Platyhlminthes
Class	Cestoda
Order	Cyclophyllidea
Family	Dipylidiidae
Genus	*Dipylidium*
Species	*Dipylidium caninum*

Dipylidium caninum is the most common tapeworm including North America (Flick, 1973; Hitchcock, 1953; Lillis, 1967) and around the world (Arundel, 1970; Baker *et al*., 1989; Bearup, 1960; Boreham & Boreham, 1990; Chandler, 1925; Clarkson & Owen, 1959; Collins, 1973; Coman, 1972; Coman *et al.*, 1981; Cowper, 1978; Dubey, 1960; Engbaek *et al*., 1984; Esle *et al*., 1977; Gadale *et al*., 1988-1989; Gregory & Munday, 1976; Hutchison, 1957; Kelly & Ng, 1975; Lewis, 1927a,b; McColm & Hutchison, 1980; Mirzayans, 1971; Moore & O'Callaghan, 1985; Niak, 1972; Nichol *et al*., 1981a,b; Poglayen *et al*., 1985; Ryan, 1976; Umeche & Ima, 1988).

The scolex of adult *Dipylidium caninum* is tiny with diameter less than 0.5 mm. The rostellum is a dome-shaped projection at the apex of the scolex with four to seven rows of tiny, backward facing and rose-thorn like hooks. The scolex possesses four muscular suckers for attachment and locomotion. The adult parasite is composed of 60 to 175 segments (Boreham & Boreham, 1990) with each proglottid containing two sets of male and two sets of female reproductive organ.

The life cycle of *Dipylidium caninum* involves the larval stages of the cat's flea (*Ctenocephalides felis)* as it intermediate host. Larvae of *Pulex irritans, Ctenocephalides canis* and *Trichodectes canis* are also capable becoming the intermediate host of *Dipylidium caninum* (Bowman *et al*., 2003). An infected flea has an average of 10 cysticercoids and infection takes place by ingestion of infected flea while grooming (Georgi & Georgi, 1992). The adult parasite can be found in the small intestine and the gravid terminal segments are passed out through the feces (Griffiths, 1978).

Adult tapeworm causes little harm to the cats and some display no symptoms at all. At most infected cats may lose weight or display poor coat quality. Owners may notice tapeworm segments passing out in the feces or gets stuck to their cat's fur. Cats with severe infestation may have convulsion and epileptiform seizures occurring (Boreham & Boreham, 1990). Heavy infestation in young animals can cause diarrhea, constipation, pot-bellied and unthrifty appearance (Bowman *et al*., 2003).

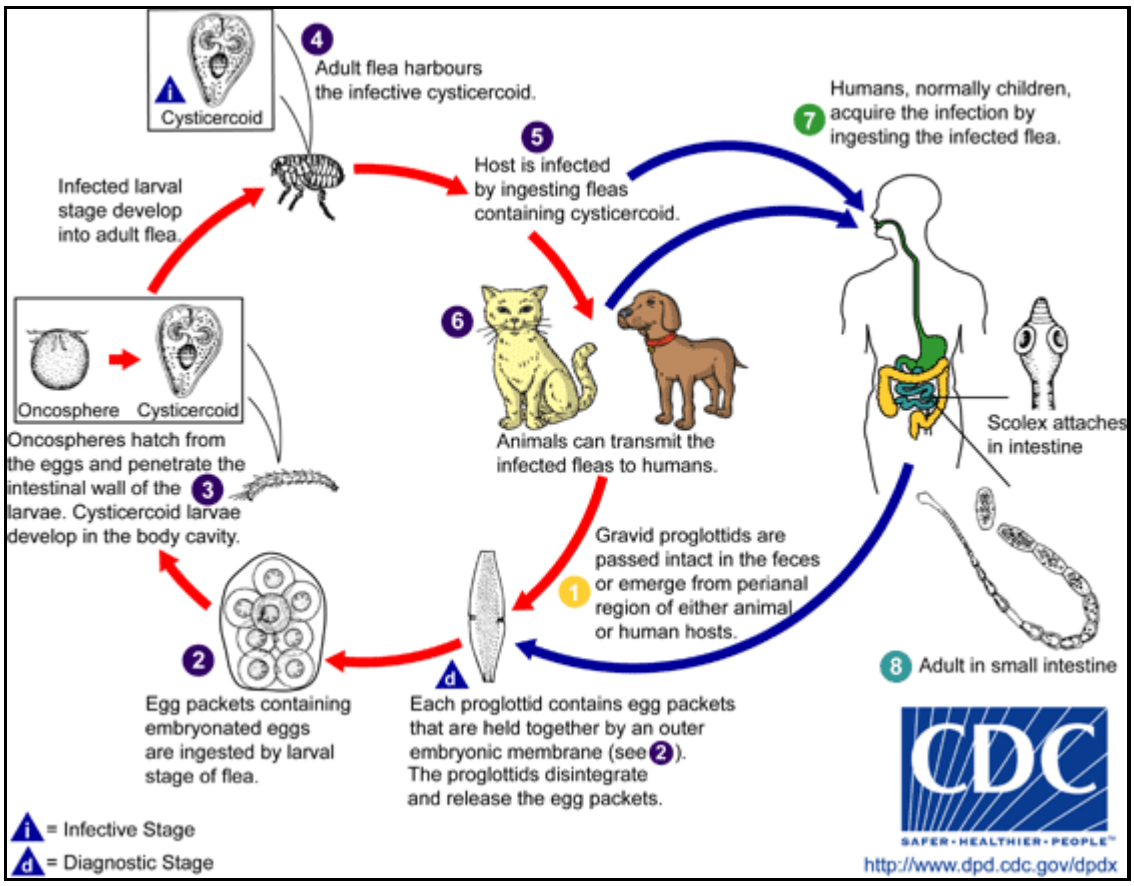

Figure 1.8: Life cycle of *Dipylidium caninum.* Source: DPDx (DPDM, 2009)

1.3.2.2.2 *Joyeuxiella pasqualei*

Joyeuxiella pasqualei is a parasite that is structurally similar with *Dipylidium caninum*. *Joyeuxiella* is distinguished from *Dipylidium* by the egg capsules. *Joyeuxiella* each contain only a single hexacanth embryo covered by uterine material while the egg capsules of *Dipylidium* contain several embryos (Bowman *et al*., 2002). The species of *Joyeuxiella* is distinguished by the shape of the rostellum, the location of the egg capsules relative to the longitudinal excretory vessels and the location of the testes relative to the vas deferens (Jones, 1983).

Joyeuxiella pasqualei have been reported from cats in Southern Europe (Austria, Spain, Italy, Hungary and Southern Russia), the Middle East, northern Africa and India (Agrawal & Pande, 1979; Jones, 1983; Supperer & Hinaidy, 1986; Witenberg, 1932), Malaysia (Shanta *et al.*, 1980) and New Guinea (Talbot, 1970). This tapeworm also have been reported in the United States in cats that have traveled to foreign countries; in a cat that was born in Nigeria (Linquist & Austin, 1981) and in a cat that resided for some time in Saudi Arabia (Blagburn & Todd, 1986).

Table 1.9: Scientific classification of *Joyeuxiella pasqualei*

Kingdom	Animalia
Phylum	Platyhlminthes
Class	Cestoda
Order	Cyclophyllidea
Family	Dipylidiidae
Genus	*Joyeuxiella*
Species	*Joyeuxiella pasqualei*

Adult *Joyeuxiella pasqualei* is found anchored to the mucosa just distal to the duodenum and at the intervals throughout the small intestine (Blagburn & Todd, 1986). The first intermediate host of this parasite has not been determined (Witenberg, 1932; Ortlepp, 1933). The second intermediate host is a small infected reptile that containing a small (0.6 x 0.75 mm) solid-bodied cysticercoids larva found in the peritoneal cavity, liver, muscles or under the skin (Witenberg, 1932). The cat becomes infested after

ingested the second intermediate host reptile. The matured cestodes 90 days post infection are between 16 and 28 cm long and have a total of 200 to 300 segments (Agrawal & Pande, 1979). Proglottids of the cestode shed through the feces (Linquist & Austin, 1981; Blagburn & Todd, 1986).

The infection of this parasite is not found to be very harmful to the cats. However, a considerable mucosal damage with necrosis of surrounding villi was found at the site of attachment of the scolex of *Joyeuxiella pasqualei* to the mucosa (Agrawal & Pande, 1979).

1.3.2.2.3 *Taenia taeniaeformis*

Taenia taeniaeformis is the most robust tapeworm parasite and the only species of Taenia reported around the world from domestic cat (Bowman *et al.* 2003). This tapeworm have been reported including the Americas (Alcaino *et al.*, 1992; Esterre & Maitre, 1985; Nolan & Smith, 1995; Ogassawara *et al*., 1986), Europe (Hinaidy, 1991), Northern Africa and the Middle East (Hasslinger *et al*., 1988; Ismail *et al*., 1983), southern Africa (Verster, 1969), India (Singh & Rao, 1965), southeast Asia (Andrews, 1937; Tanaka *et al.*, 1985), southern Pacific (Ng & Kelly, 1975; Tongson & San Pablo, 1979; Gregory & Munday, 1976).

The adult tapeworm inhabits the small intestine. The worms tend to be white, thick bodied and ranging between 15 to 60 cm in length. The scolex has two rows of hooks that have the typical claw-hammer shape of the Taeniidae and can be found within the first one-half of the small intestine (Miller, 1932). The scolex is often found attached to the mucosa and several attachment sites in the intestine.

Table 1.10: Scientific classification of *Taenia taeniaeformis*

Kingdom	Animalia
Phylum	Platyhelminthes
Class	Cestoda
Order	Cyclophyllidea
Family	Taeniidae
Genus	*Taenia*
Species	*Taenia taeniaeformis*

The life cycle of *Taenia taeniaeformis* involves a small rodent as its intermediate host and cats get infected by eating the infected rodent. The larva then migrates through the intestinal wall and develops to a strobilocercus in the rodent's liver. The strobilocercus needs to reach maturity before it can infect the cat. This development phase usually takes 2 months (Singh & Rao, 1965). The patent infection develops in cats between 32 to 80 days after strobilocerci ingestion (Williams & Shearer, 1981). Cats produce about three to four segments each day with the majority of segments contain only 500 eggs or less, and up to a maximum of 12,180 eggs (Bowman *et al*., 2002).

Taenia taeniaeformis infests mainly rodents and cats and it is extremely rare in human. Infection in cats can be detected by looking for the distinctive segments in the feces or by egg floatation (Bowman *et al*., 2002). *Taenia taeniaeformis* in cats is asymptomatic with no clinical signs with this infection (Bowman *et al*., 2003).

1.3.2.3 Class Trematoda

Trematode or fluke is classified under the phylum Platyhelminthes. This worm is flat, leaf-shaped and unsegmented with two suckers attach to host, hooks or clamp-like appendages which they attach themselves to the host's organs. The parasite that infests cats and dogs vary in size from ½ millimeters which is less than 2/100 of an inch to almost ½ inch. Eggs of trematodes have a unique characteristic with a trap door called an operculum. The life cycle of a trematode is indirect and involves intermediate hosts.

1.3.2.3.1 *Platynosomum fastosum*

The most common trematode found in cats is *Platynosomum fastosum*. This parasite has been reported in tropical countries (Taylor & Perri, 1977). *Platynosomum fastosum* is the common trematode found in cats. This parasite have been reported in the Tropics including Malaysia, Hawaii, West Africa, the Carribean, and areas surrounding the Gulf of Mexico, including the southeastern United States and the Florida Keys (Bielsa & Greiner, 1985).

Platynosomum fastosum is found normally in the bile ducts and lizards are well-known as their intermediate hosts. They also infect opossums and civet (Bowman *et al*., 2002). Cats become infected when ingested the metacercaria in the infected lizard. The metacercaria excysts and the infective stage migrates to the bile ducts and gall bladder and mature to adult within 8 to 12 weeks (Taylor & Perri, 1977). Adult *Platynosomum fastosum* are about 5 mm long and 2 mm wide with suckers that are about equal in size with the ventral sucker being about one-fourth of the body length from the anterior end.

Table 1.11: Scientific classification of *Platynosomum fastosum*

Kingdom	Animalia
Phylum	Platyhlminthes
Class	Trematoda
Order	Plagiorchiida
Family	Dicrocoeliidae
Genus	*Platynosomum*
Species	*Platynosomum fastosum*

Heavily infested cats may suffer greatly due to blockage of the biliary system (Robinson & Ehrenford, 1962). High infestation normally showed enlargement and abnormalities of the livers. In severe infestation, it is associated with emaciation, listlessness, anorexia and hepatomegaly (Ikede *et al*., 1971; Leam & Walker, 1963). Infestation may also cause jaundice, diarrhea, vomiting and death for severe cases due to respiratory difficulty cause by severe hydrothorax (Zamri Saad *et al*., 1984). Cats with light infestation show no clinical symptoms (Ikede *et al*., 1971).

1.3.2.3.2 *Clonorchis sinensis*

Clonorchis sinensis is the Chinese liver fluke member of the genus Opisthorchis. This fluke is widely distributed in regions where residents eat raw or undercooked fish (Crompton, 1999). This parasite is a fluke that normally the infection present in freshwater fish surrounding the areas bordering on the China Sea including in Japan, Korea, Taiwan, China and North Korea.

This parasite can be found in the gallbladder and bile ducts; occasionally in the pancreatic duct and small intestine (Bowman *et al*. 2002). The adult of *Clonorchis sinensis* are about 10–25 mm in length and 3–5 mm in width (Liu & Chen, 1998; Sripa *et al*., 2007). The eggs are yellowish-brow with distinct operculum and average 29 μm long by 17 μm wide (Liu & Chen, 1998).

Table 1.12: Scientific classification of *Clonorchis sinensis*

Kingdom	Animalia
Phylum	Platyhlminthes
Class	Trematoda
Order	Opisthorchiida
Family	Opisthorchiidae
Genus	*Clonorchis*
Species	*Clonorchis sinensis*

*Clonorchis sinens*is is transmitted from snails to freshwater fish and then to other fish-eating mammals and human. Cats were first observed infected in Japan (Ijima, 1887). After an animal ingested an infected fish, the fluke migrate to the bile duct through the ampulla of Vater. The trematode takes about 1 month to reach maturity and may live as long as 12 years and 3 months in a cat (Miyazaki, 1991). There are up to one-third of the feline population in some areas of China and Vietnam shedding this parasite's eggs in their feces (Bowman *et al*, 2002). The severe diseases of this parasite are related to the number of parasites. When a few parasites are present, the infection is asymptomatic and do not show any clinical symptoms. A mild infection is defined as 100 or less eggs

per gram of feces, moderate infection is 100 to 1000 and massive infection is over 1000 (Manson-Bahr & Apted, 1982).

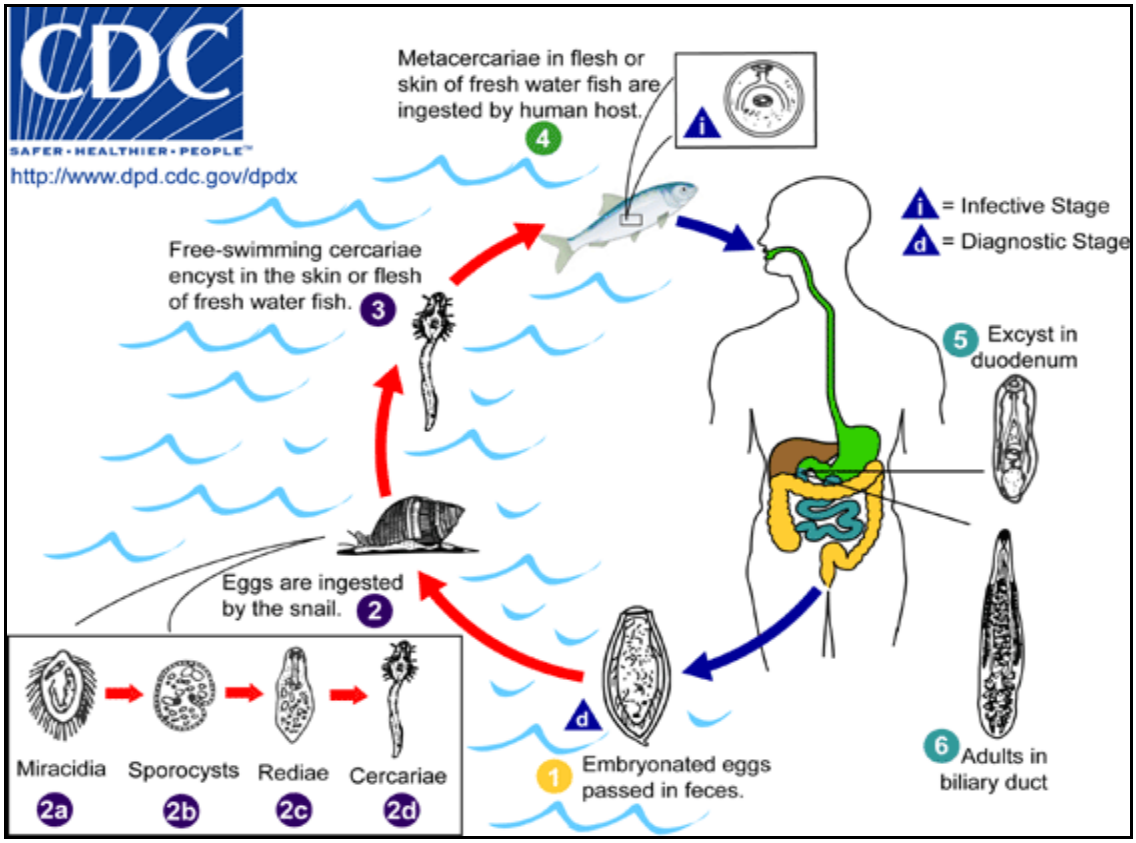

Figure 1.9: Life cycle of *Clonorchis sinensis*. Source: DPDx (DPDM, 2009).

1.4 Zoonotic diseases of cats

Cats have been reported to harbour various parasites, of which some are zoonotic cause severe pain to animals and death to humans. Stray cats are also important as the potential reservoir hosts to a variety of parasites of medical and veterinary importance (Woon-Mork *et al*., 2005; Schantz, 1994; Overgaauw, 1997; Markel *et al*., 2006) with potential health risks being in close contact with humans (Angulo *et al*., 1994; Schantz, 1994). Stray cats in urban and rural area tend to discharge helminthes eggs, larvae and protozoan cysts into the general environment (Jamshidi *et al*.,2002; Bahadori *et al*.,2004; Sharif *et al*., 2007) that are transmittable to human (Sharif *et al*.,2007). Those at risk include small children, pregnant women, the elderly and the immunocompromised (Juckett, 1997).

Based on studies conducted previously in Peninsular Malaysia (Rohde (1962), Zamirdin *et al.* (1967), Mustaffa-Babjee (1969), Retnasabapathy & Khoo (1970), Retnasabapathy & Prathap (1971), Yoshida *et al.* (1973), Nagendram & Rajamanickam (1976), Amin-Babjee (1978), Shanta *et al.* (1980), M. Zamri Saad *et al*. (1984) and Lee *et al.* (1993)), a few macroparasites species recovered have the zoonotic potential (Table 1.13).

Table 1.13: Macroparasites previously recorded in cat population from Peninsular Malaysia.

Ecto- and endoparasites (nematode, cestode and trematode) from cats	
Ectoparasites	Cestodes
**Ctenocephalides felis*	**Dipylidium caninum*
Ctenocephalides felis orientis	*Joyeuxiella pasqualei*
Felicola subrostratus	*Dipylidium sexcoronatum*
Haemaphysalis bispinosa	*Taenia taeniaeformis*
Notoedres cati	*Spirometra mansoni*
Sarcoptes scabiei	*Spirometra spp.*
Demodex felis	
Xenopsylla cheopis	
Nematodes	Trematode
**Ancylostoma ceylanicum*	*Platynosomum fastosum*
**Ancylostoma braziliense*	*Opistorchis viverrini*
**Toxocara cati*	*Haplorchis sp.*
Toxocara malaysiensis	**Clonorchis sinensis*
Toxascaris leonine	*Heterophyes sp.*
Physaloptera praeputialis	*Stictodora sawakinensis*
Dirofilaria immitis	*Haplorchis pumilio*
Gnathostoma spinigerum	*Haplorchis taichui*
Syphacia obvelata	*Haplorchis yokogawai*

* Zoonotic

1.4.1 Toxocariasis

Toxocariasis is a zoonotic disease in human cause by nematode from the genus *Toxocara*. The species affecting cats is *Toxocara cati* and the dogs; *Toxocara canis*. Toxocariasis is one of the most common zoonotic helminth infection reported in the world. The clinical disease has been diagnosed in more than 1,900 human cases from 48 different countries (Glickman & Schantz, 1981). In Western countries, seroprevalence surveys reported 2 to 5 percent adults in urban areas were positive compared to 14.2 to 37 percent from rural areas (Magnaval *et al*., 1994a). The seroprevalence was also higher in tropical countries with 63.2% in Bali (Chomel *et al.*, 1993), 86% of children in Saint-Lucia, West Indies (Thompson *et al*., 1986) and 92.8% of adults in La Reunion (French Oversea Territories, Indian Ocean) (Magnaval *et al*., 1994b).

Human can be infected by accidental ingestion of the eggs. Soil contamination with helminth eggs becomes an important source of infection especially for children since the eggs of *Toxocara spp.* have high resistance to physical and chemical factors (Acha & Szyfres, 1991). Children are vulnerable to the infection since they commonly play with soil and put contaminated objects in their mouth (Overgaauw, 1997). Adults with poor hygiene also have high potential to be infected (Acha & Szyfres, 1991). Two forms of diseases had been described in human namely; visceral larva migrans (VLM) (Beaver, 1956) and ocular larva migrans (OLM) (Molk, 1983).

The acute signs of visceral larva migrans (VLM) is associated with hepatic and pulmonary larval migration that cause abdominal pain, decreased appetite, restlessness, fever, coughing, wheezing, asthma and hepatomegaly (Magnaval *et al*., 2001). The

larvae also migrate to the other visceral organs such as muscles and brain, which can lead to seizures or behavioral changes (Huntley *et al*., 1965). VLM was first described in 1952 in children with an enlarged liver and hypereosinophilia (Beaver *et al*., 1952).

Ocular larva migrans (OLM) can cause visual impairment over a period of days to weeks. Funduscopy and biomicroscopic examination reveals uveitis, endophthalmitis, papilitis (Gass & Braunstein, 1983), retinal granulomatous lesions (Gillespie *et al*., 1993) or inflammatory masses in the peripheral vitreous (Tran *et a*l., 1999). OLM tends to occur in children with an average age of onset 8 years (Schantz, 1989). OLM apparently is an endemic disease in some areas with an estimated 1 per 100,000 persons detected in Alabama in the United States (Maetz *et al*., 1987).

1.4.2 Cutaneous larva migrans

Ancylostoma spp. can cause cutaneous larva migrans and classical hookworm disease. Cutaneous larva migrans is common syndrome in humans (Schantz, 1991) with *Ancylostoma braziliense* probably the major cause of human cases. Only rare cases have been reported from infection of *Ancylostoma tubaeform* and *Ancylostoma ceylanicum* (Davies *et al*., 1993). Cutaneous larva migrans is usually found in tropical and subtropical countries including Argentina, Uruguay, southern Brazil, Mexico (especially the Gulf coast), the Caribbean islands, the southeastern United States, several countries in Europe, South Africa, Australia, India and the Philippines (Acha & Szyfres, 1991).

The larvae upon entering an individual will undergo a somatic migration that may cause severe pneumonitis that may require hospitalization (Beaver *et al*., 1984). The lesions

are mostly located on the feet, legs, hands and any part of the skin that was exposed to the contaminated soil (Acha & Szyfres, 1991) from cats or dogs feces. Humid soils are the most favorable and suitable in the development of the larvae. The larvae invade the human through oral ingestion or penetration through the skin and cause papular eruptions at the sits of larval penetration (Wijers & Smit, 1966). Infection of this parasite poses risks to workers in contact with soil such as farmers, gardeners and construction workers. Children also have higher potential to be infected since they love to play with soil.

1.4.3 Dipylidiasis

Dipylidiasis in human is caused by the infective cysticercoid of a cat or dog tapeworm *Dipylidium caninum*. The intermediate hosts of *Dipylidium caninum* are cat's flea (*Ctenocephalides felis*) or dog's flea (*Ctenocephalides canis*). Dipylidiasis is a problem in most countries with the majority in Europe and the United States. This disease also has been observed in Chile, Argentina, Uruguay, Brazil, Venezuela, Guatemala, Mexico and Puerto Rico (Acha & Szyfres, 1991). Human get infected by the ingestion of the fleas containing the infective cysticercoids. The infection in human commonly occurs in children in close contact with family pets with flea infestation (Bowman *et al*., 2002). Symptoms include digestive disorders such as diarrhea and colic, irritability, erratic appetite and insomnia. Abdominal distension also may occur in infected patient (Belmar, 1963). Diagnosis of this parasite in human is by locating the characteristic proglottids in the feces or in the perianal area (Bowman *et al*., 2002).

1.4.4 Strongyloidiasis

Strongyloidiasis caused by the nematode, *Strongyloides stercoralis*. This parasite is a common disease intercommunicable between man, dog and cat. It was estimated in 1947, that approximately 35 million people were infected including 21 million in Asia, 8.5 million in tropical America and 400,000 in the United States. The infection rate can reach as high as 85% among poor socioeconomic groups in the hot and humid regions of the tropics (Acha & Szyfres, 1991). The infection is transmitted from one species to another by contaminated soil or feces and infection is more common where sanitation and hygiene is poor. The route of infection is commonly cutaneous (rarely oral) when host in contact with the third stage or filariform larvae and this parasite can live outside the body for up to 3 weeks.

Humans with mild infection of Strongyloidiasis showed no symptoms. Penetration through the skin by the filariform larvae only produces a small papule at the invasion site. However in some patient, uticaria occur coinciding with attacks of diarrhea and reappearance of larvae in the feces (Acha & Szyfres, 1991). Other symptoms also may occur such as abdominal pain, nausea, vomiting, cough, wheezing, respiration distress, fever and skin lesion (Farthing *et al.*, 2004). Symptoms over years and decades if they are not treated and can spread to other parts of the body and cause serious health problems.

1.4.5 Clonorchiasis

Clonorchiasis is caused by the trematode, *Clonorchis sinensis*. Clonorchiasis is a major zoonotic parasite in parts of Asia including China, Korea, Japan, Taiwan and Vietnam (Chai *et al.*, 2005). Cases of clonorchiasis is also found in other parts of the world especially for members of indigenous population who eat raw fish imported from these endemic areas (Acha & Szyfres, 1991) or inadequately cooked freshwater fish containing the infective metacercaria. Cases have been recorded in the Americas from Asian immigrant and those lived in endemic areas in the past. The infection rate was 26% from 150 Chinese migrants in New York City and 15.5% from 400 persons examined in Montreal, Canada (Sun, 1980). Surveys in Korea and Vietnam showed more than 15% are infected with this parasite (Chung *et al.*, 1991; Kieu *et al*., 1992).

Frequent symptoms of human clonorchiasis are a dull epigastric fullness or pain, mild fever, loss of appetite, diarrhea, and jaundice. Damage to the liver function is related to the number of parasites and the occurrence of successive reinfections. The disease becomes serious when the parasite burden is large and cause obstruction of the bile ducts, consequent portal cirrhosis, catarrhal cholangitis, ascites and progressive edemas. In the chronic phase, there may be cholecystitis and hepatitis (Acha & Szyfres, 1991). Epidemiological studies suggest that humans do not develop any resistance to reinfection or superinfection by *Clonorchis sinensis,* and that reinfection readily occurs upon re-exposure throughout life in those accustomed to consuming undercooked fish in endemic areas (Seo *et al*., 1981; Hong *et al*., 1994).

1.5 Justification of study

The last macroparasite study of stray cats from an urban city in Peninsular Malaysia conducted almost three decades ago by Shanta *et al.* (1980). Findings of previous studies are limited to one or certain location and generally a small number of samples in particular location (Rohde, 1962; Zamirdin *et al.*, 1967; Retnasabapathy & Khoo, 1970; Retnasabapathy & Prathap, 1971; Yoshida *et al.*, 1973; Nagendram & Rajamanickam, 1976; Amin-Babjee, 1978; Shanta *et al.*, 1980; Lee *et al.*, 1993). These studies also only focused on selected macroparasites species (Zamirdin *et al.*, 1967; Mustaffa-Babjee, 1969; Retnasabapathy & Khoo, 1970; Retnasabapathy & Prathap, 1971; Yoshida *et al.*, 1973; Nagendram & Rajamanickam, 1976; Zamri Saad *et al.*, 1984; Lee *et al.*, 1993). Data from these studies may have also been misrepresented after Zhu *et al.* (1998) discovered that an ascarid previously reported as *Toxocara canis* in cats is now represented as a new species, *Toxocara malaysiensis*. Over the past three decades, many cities in Peninsular Malaysia have also experienced urbanization in infrastructure development and population growth. This may have impacted the parasites infecting the population of stray cats as well as factors contributing to the structuring of the parasite community in stray cat population. Therefore, there is still a need to continue investigating diseases brought by cats but using larger samples examined over a longer and continuous period. This would undoubtedly provide appropriate data for analyzing the impact of season and climate change on infection levels. In addition this study would significantly further our understanding of the potential threat of transmission of zoonotic infections from feline hosts to the human population in urban cities of Malaysia.

1.6 Objectives

The objectives of present study are as follows:

- To determine the diversity and infection levels of macroparasites (ecto- and endoparasites) infections of the stray cat population in the urban cities of Peninsular Malaysia.

- To evaluate the role of intrinsic (host age, host sex) and extrinsic (season, location) factors in structuring the macroparasites community of the stray cat population.

- To determine the co-occurrence of interaction between parasite species in the stray cat population.

- To amplify the ITS1 and ITS2 regions of *Toxocara malaysiensis* rDNA by using universal PCR primers.

CHAPTER 2

STRAY CAT POPULATION AND THE MACROPARASITIC INFECTIONS IN URBAN CITIES OF PENINSULAR MALAYSIA

2.1 Introduction

Most households in Malaysia keep cats as a pet and it is common that most families own more than one cat (Amin-Babjee, 1978). In Malaysia, the numbers of cat population grows each year (Bedi, 2011). The Society of Prevention of Cruelty to Animals (SPCA) Malaysia reported on average animals received monthly is between 600-700 animals with only 90-150 animals get adopted within the same period. In 2010, a total of 8,299 numbers of animals were brought in to the shelter with only 1,790 adopted leaving many animals in the shelters to be put down.

Cats have the tendency to breed very quickly in the event their breeding is not control effectively. In Malaysia, the Noah's Ark Natural Animal Sanctuary (NANAS), a welfare organisation introduced The Strays Management Programme to curb the growing numbers of strays in a humane and effective way by sterilization of the male cat and spaying females (Wee, 2010).

In this chapter, the stray cat population in four urban cities in Peninsular Malaysia was investigated in terms of the demographic structure of the cat population according to sex (male or female), age (adult or juvenile) and season (dry or wet). Following this, the macroparasitic species richness of the cat population was determined.

2.2 Methodology

2.2.1 Study area

Four urban cities in Peninsular Malaysia were chosen as study sites with each location representing a unique geographical location. The cities selected were Kuala Lumpur representing the west, Georgetown representing the north, Kuantan representing the east and Malacca representing the south area of Peninsular Malaysia.

Kuala Lumpur (3°8′51″N, 101°41′36″E) is the capital city of Malaysia and located in the centre of Selangor state. Kuala Lumpur is fast developing in population and economy. The city is also a tourist attraction and has become the host to many international political, cultural and sporting events such as Formula One and Commonwealth Games.

Georgetown (5°25′00″N, 100°19′00″E) is an island urban city located in the north region of Peninsular Malaysia. Island geography separated from mainland by a channel three kilometers wide at the narrowest point. Georgetown is also one of the tourist attractions and fast growing in urbanize the city in terms of infrastructure, economy and population. Georgetown is the capital of the Penang state of Malaysia and located on the north east corner of Penang Island.

Kuantan town (3°49′00″N, 103°20′00″E) is a port city located at the east coast of Peninsular Malaysia. Kuantan is the capital of Pahang, the largest state in Peninsular Malaysia. The city situated near the Kuantan River and faces the South China Sea. Kuantan has had many development projects to transform and modernized the city.

Kuantan is also recognized as the future growth centre for trade, commerce, transportation and tourism.

Malacca town (2°11′20″N, 102°23′4″E) is located at the south region of Peninsular Malaysia. Malacca Town is the capital city of the state of Malacca and situated close to the Straits of Malacca. The modern city has expanded in many areas including the infrastructure, tourism and economy.

The trapping sites were in the vicinity of the public areas such as food court and market. Four major cities in Peninsular Malaysia were chosen also as they represent high human habitation. Stray cats thrive in this environment because they live in direct contact with human food for them to be able to survive.

2.2.1.1 Climate of study area

Temperature recorded throughout the year in Malaysia is generally uniform. The annual variation normally was less than 3°C. The temperature recorded by Malaysian Meteorological Department during sample collection ranged between 25.5-28.6°C monthly. Rainfall distribution in Peninsular Malaysia has seasonal variation. From the temperature and rainfall analysis, this study divided months into two season groups; dry (January-March and June-September) and wet (April-May and October-December) season. Trapping was carried out during the dry and wet season of May 2007 to August 2010 with a total of 11 times during dry season and 10 times during wet season from four locations in Peninsular Malaysia.

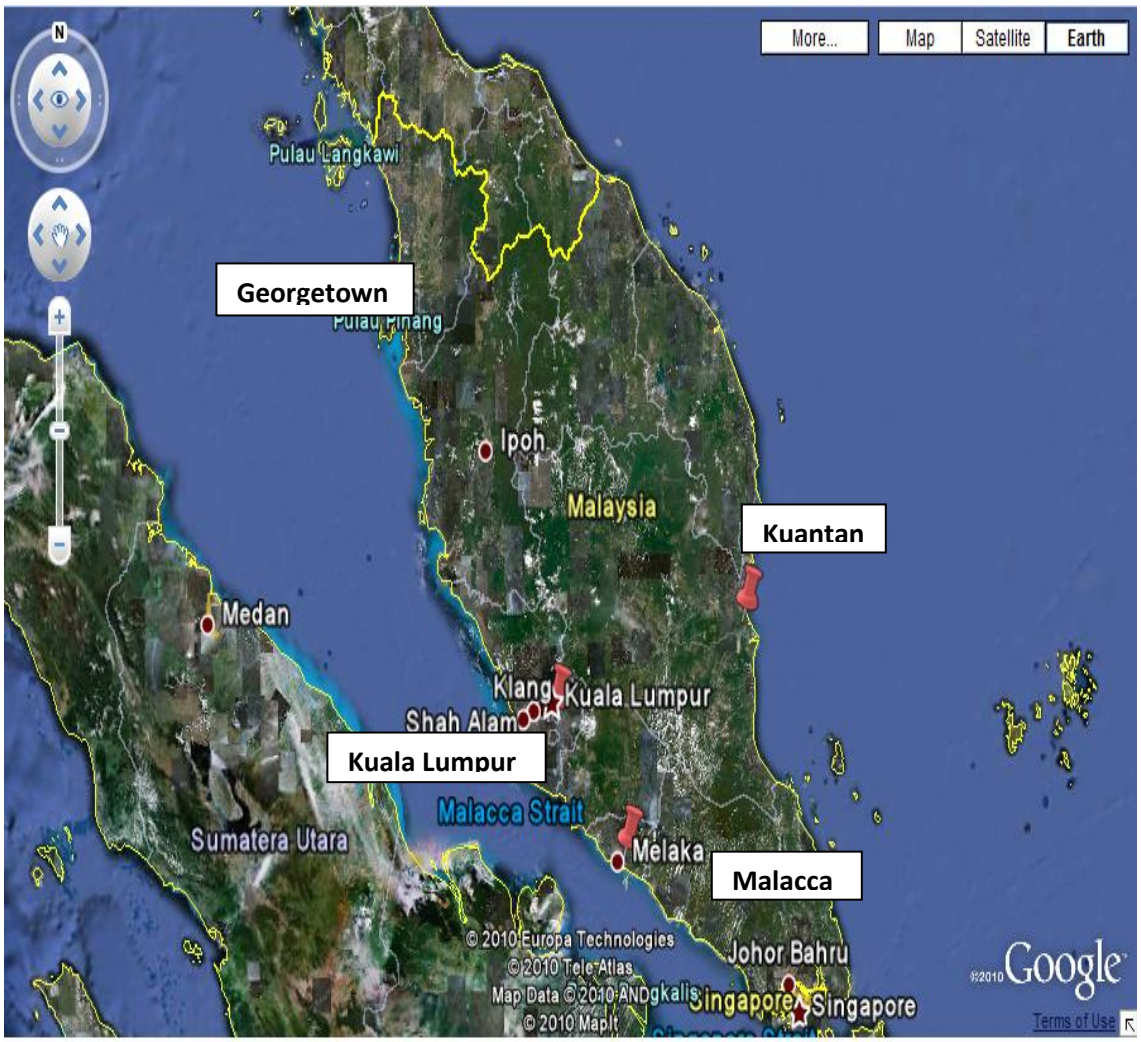

Figure 2.1: The location of study area, Kuala Lumpur, Georgetown, Kuantan and Malacca in Peninsular Malaysia. Image excerpt from Google Earth ™ mapping service

2.2.2 Sample collection and euthanization of stray cats

Stray cats were captured with cooperation with the assigned units from each municipality or animal welfare organization from the respective city. Stray cats captured were then euthanized humanely by the veterinary unit from the municipal or veterinary clinic. The euthanasia of cats was according to the method provided by the World Society for the Protection of Animals (WSPA) to ensure euthanization was truly humane and administered by responsible and appropriately by trained individuals.

2.2.2.1 Kuala Lumpur

Stray cats were captured as part of a vector control programme by the Vector Control Unit of Kuala Lumpur City Hall (DBKL) between May 2007 to June 2010 with a total of 3 times sampling was done during dry season and 3 times during wet season. Stray cats were caught live manually by hand and baited them with fish or Whiskas (food for cats) from the markets, food courts and by the shop lots from areas surrounding Kuala Lumpur especially Chow Kit and Setapak.

Stray cats also were provided by the respective animal shelters; Society for the Prevention of Cruelty to Animal (SPCA) and PAWS Animal Welfare Society Malaysia (PAWS). SPCA is a non-profit organization that campaign for animal welfare for abused or abandoned animal, help animal to get adopted also help and protects stray animals. PAWS is an organization of a small group of local and expatriate volunteers that care after stray cats and dogs and re-home them if possible. Cats from SPCA and PAWS were usually brought in from various places within Klang Valley by the public and volunteers. Sickly and aged cats were put down humanely.

All the stray cats captured by Kuala Lumpur City Hall were then euthanized using Dolethal solution containing Pentobarbitone sodium (0.5ml/kg) intravenously meanwhile, SPCA and PAWS put down the animals by inhalation of chloroform. All the euthanized stray cats were brought to the Parasitology Lab, Institute of Biological Sciences in Faculty of Science, University of Malaya (UM) immediately for post mortem examination.

2.2.2.2 Georgetown

In Georgetown, samplings of stray cats were done between March 2008 to August 2010 with 3 times during dry season and 3 times during wet season. Assistance in providing stray cats specimens were given by two organizations; The Society for the Prevention of Cruelty to Animal (SPCA), Penang and Public Health Unit, Majlis Perbandaran Pulau Pinang (MPPP).

Both organizations helped to capture stray cats loitering food courts and markets all around Georgetown. Similarly, stray cats captured by MPPP were put down humanely using Dolethal solution containing Pentobarbitone sodium (0.5ml/kg) intravenously meanwhile SPCA used chloroform. The carcasses were then brought to the Parasitology Lab of School of Biological Science in University of Science Malaysia (USM) for post mortem examination.

2.2.2.3 Kuantan

Stray cats from Kuantan were captured between the months of November 2008 to July 2010 with a total of 3 times trapping were done during dry season and 2 times during wet season. Stray cats were trapped by the municipal of Kuantan, Vector Unit of Majlis Perbandaran Kuantan (MPK) from the markets and hawker stalls surrounding areas in Jalan Pasar, Teluk Chempedak and Tanjung Api. All the cats captured were then sent to Veterinary Clinic of Department of Veterinary Services, Negeri Pahang and euthanized using Dolethal solution containing Pentobarbitone sodium (0.5ml/kg) intravenously. The carcasses were then brought to the Animal House Lab of Faculty of Medicine in International Islamic University Malaysia (IIUM), Kuantan for further examination.

2.2.2.4 Malacca

Trapping in Malacca was conducted between August 2009 to May 2010 with 2 times samplings were done during dry season and 2 times during wet season. Stray cats were captured by the municipal of Melaka Tengah, Dog's Unit of Majlis Bandaraya Melaka Bersejarah (MBMB). MBMB captured stray cats in area around Malacca by the markets, streets and food courts. Stray cats captured were then euthanized by Veterinary Unit MBMB and humanely put down using Dolethal solution containing Pentobarbitone sodium (0.5ml/kg) intravenously. The carcasses were brought to the Biology Lab of Melaka International College of Science and Technology (MiCoST) immediately for further examination.

2.2.3 Physical examination

Prior to post-mortem examination, each stray cat was subjected to physical examinations. The date of trapping, gender (male or female), age (adult or juvenile), weight and dental development (presence or absent of the canine tooth) were recorded. Cats were classified into two age groups: adult (≥1.5kg) and juvenile (<1.5kg), as described by Sharif *et al.* (2007).

Parasite species richness was analysed using the Simpson's and Brillouin diversity index from the Species Diversity and Richness IV software (Seaby & Henderson, 2006).

2.3 Results

2.3.1 Population of Stray Cats

A total of 543 stray cats were captured from four urban cities in Peninsular Malaysia namely Kuala Lumpur, Georgetown, Kuantan and Malacca (Figure 2.2). Three hundred and seventy cats were captured during dry season compared to 173 cats during wet season. From the total population, the number of females (n=343, 63.2%) were higher compared to males (n=200, 36.8%). Adults captured were slightly higher with 314 cats (57.8%) compared to 229 juvenile cats (42.2%) (Table 2.1). The proportion of the total stray cats captured in this study from all locations according to season, host sex and age of stray cats is summarized in Figure 2.3.

More cats were captured during dry season compared to wet with a ratio of (2.1:1). More females were captured compared to males with a ratio of (1.7:1) meanwhile more adults captured compare to juveniles with ratio of (1.4:1).

Total capture in Kuala Lumpur was the highest with 241 stray cats. The stray cat population showed more females captured compared to males with 188 females and 53 males respectively. According to host age, there were less number of juvenile cats captured compared to adult cats with 93 juveniles and 148 adult stray cats. Most of the stray cats in Kuala Lumpur were captured during dry season (160 cats) compared to wet season (81 cats) (Table 2.1).

The number of stray cats captured from Georgetown was 102 cats. There were more males (56 cats) than females (46 cats) meanwhile, according to the host age, there were

more juveniles (69 cats) captured compared to adults (33 cats). The numbers of stray cats captured during dry season (73 cats) were higher compared to wet season (29 cats) (Table 2.1).

A total of 100 stray cats were captured from Kuantan. Ratio between male and female were almost equal with 49 males and 51 female stray cats. However, according to the host age, lower numbers of juveniles were captured compared to adult stray cats with 37 juveniles and 63 adult stray cats. Higher numbers of cats were captured during dry season (79 cats) compared to wet season (21 cats) (Table 2.1).

Similar numbers of stray cats were captured from Kuantan and Malacca (100 cats). The numbers of female stray cats captured from Malacca were slightly higher compared to male stray cats with 58 females and 42 males. According to host age, the number of juveniles (30 cats) captured were less compared to adults (70 cats). Most of the cats were captured during dry season with 58 cats compared to wet season with 42 cats (Table 2.1).

2.3.2 Macroparasite species richness in stray cats' population

From the total of 543 stray cats captured, 468 stray cats (86.19%) were found infected with macroparasites (ecto- and endoparasites). The cats harboured a minimum of one species and a maximum of six species of macroparasites. Figure 2.4 showed the frequency distribution of infracommunity richness of parasites in stray cats from Peninsular Malaysia. Most of the cats were found harboured between one (31.1%) to two (29.5%) species of macroparasite and only 1 cat (0.18%) was found harboured up to six macroparasite species.

In Kuala Lumpur, 216 (89.63%) of the 241 stray cats captured were found infected with macroparasites. Females (89.36%) and males (90.57%) cats had similar infection with males just slightly higher than females were infected. Infections between adult and juvenile cats were quite similar too with prevalence of infection in juveniles (91.4%) higher than adults (88.5%). Higher numbers of cats were infected with macroparasites during the dry season (93%) compared to the wet season (82.7%).

From the total of 102 stray cats captured in Georgetown, 86.27% stray cats were infected with macroparasites. Similar levels of infections were found between male and female stray cats with infections in females slightly higher (89.1%) compared to males (83.9%). Although the numbers of juvenile stray cats are higher compared to adult cats, prevalence of infections in adults (87.9%) and juveniles (85.5%) were almost similar. Stray cats infected with macroparasites was higher during dry season (90.4%) compared to the wet season (75.9%).

In Kuantan, 88 stray cats (88%) out of 100 stray cats captured were found infected with macroparasites. Host sex differences in infection between male and female cats were quite similar with females (90.2%) slightly higher compared to males (85.7%). Adult and juvenile stray cats also had similar infection with 88.9% adults and 86.5% juveniles infected with macroparasites. The numbers of stray cats infected during dry season (89.9%) were slightly higher compared to the wet season (81%).

From the total of 100 stray cats captured in Malacca, 76% stray cats were found infected with macroparasites. Male cats (80.95%) had higher infection compared to females (72.4%). Host age differences in infections showed higher infections in adults (81.4%)

compared to juveniles (63.3%). Higher numbers of cats were also infected with macroparasites during the dry season (81%) compared to the wet season (69%).

Table 2.2 showed the prevalence (%) of frequency distribution of infracommunity richness of parasites in stray cats according to location. The majority of stray cats in this study harboured between one to two species of macroparasites. According to the season factor, most of the cats harboured two species (33.2%) of macroparasites during dry season meanwhile during wet season, most of the cats harboured one species (35.8%) of macroparasite (Table 2.3).

According to host sex, the majority of female cats harboured two species (30.3%) compared to male cats harboured with only one species (33.5%) of macroparasites (Table 2.4). According to host age factor, most of the adults harboured two species (27.1%) of macroparasites compared to juveniles with majority harboured one species (41.05%) of macroparasite (Table 2.5).

Table 2.6 showed the analysis of species diversity using Simpson's Index. According to the host age factor, the diversity was almost similar between males (D=5.672) and females (D=5.512) however high diversity was observed in adults (D=4.906) compared to juveniles (D=4.119). In seasonal factor, high diversity of ectoparasites and low diversity of endoparasites observed during dry season compared to species diversity during wet season. Analysis also showed Georgetown with the highest diversity index followed by Malacca, Kuala Lumpur and Kuantan (Table 2.6).

Overall, Brillouin index showed the diversity of ectoparasite species were low (all values were lower than 1) except for Georgetown. The diversity index was almost similar between males (HB=1.861) and females (HB=1.994) meanwhile high diversity was observed in adults (HB=1.888) compared to juveniles (HB=1.677). The diversity of ectoparasite species was higher during dry season compared to wet season and the opposite was observed in the endoparasite species (Table 2.7).

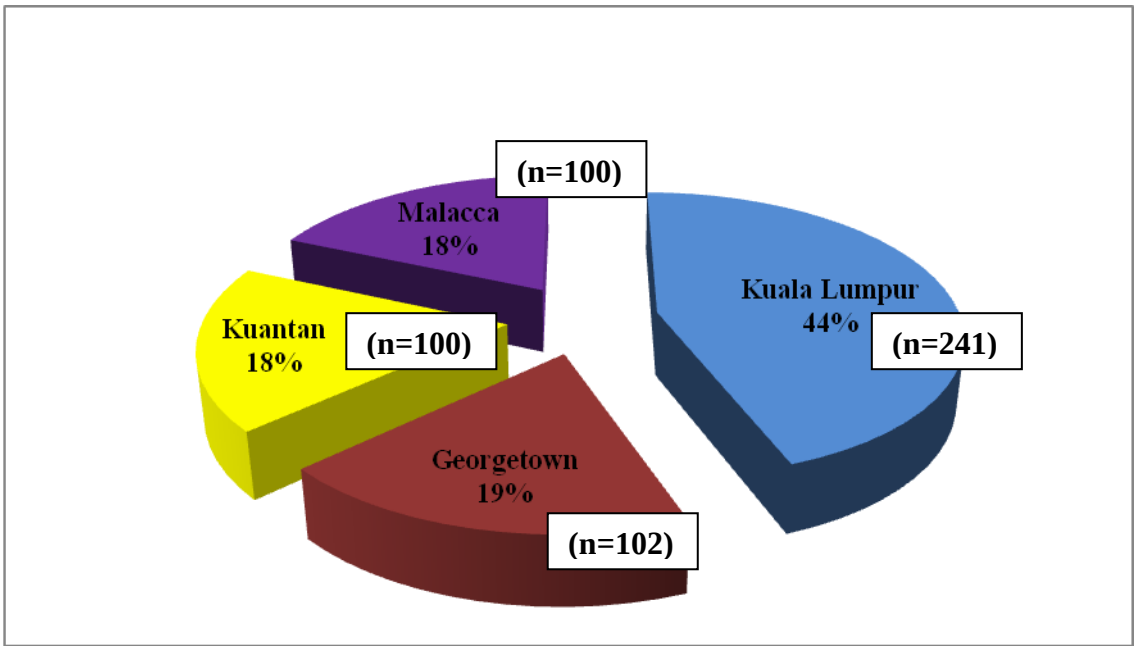

Figure 2.2: Distribution of stray cats captured in Peninsular Malaysia

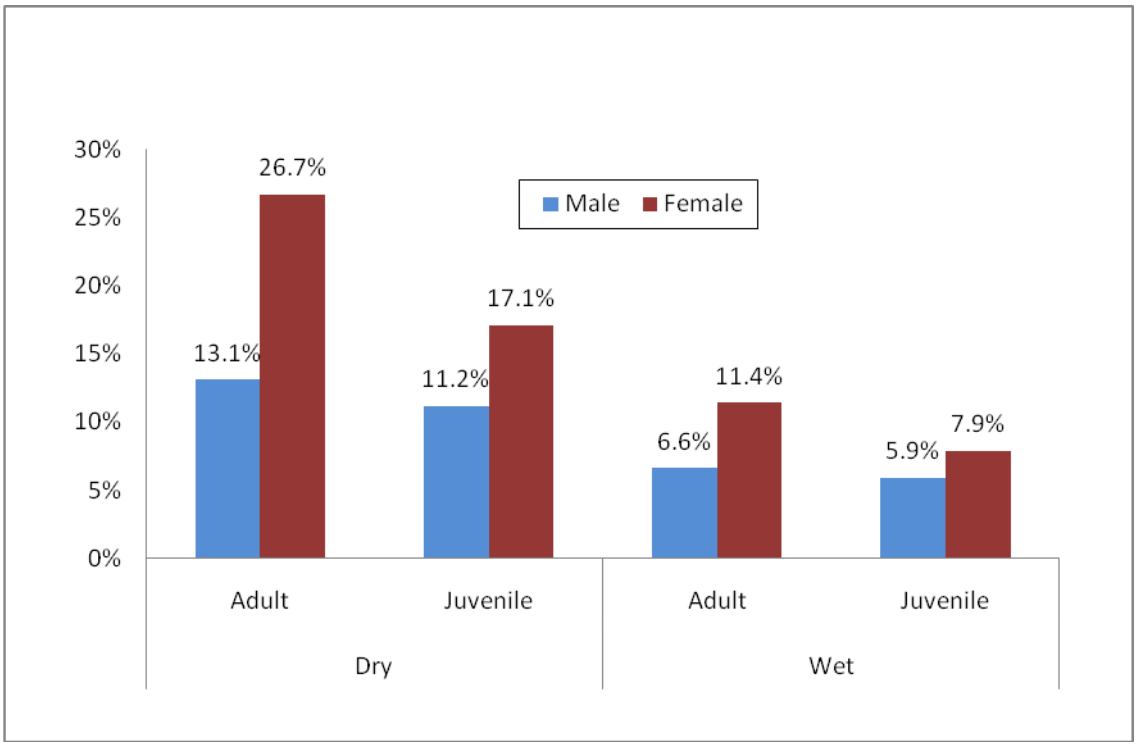

Figure 2.3: Distribution of stray cats captured by season, host age and sex from Peninsular Malaysia

Table 2.1: Number of stray cats examined by site, host age, sex and season from Peninsular Malaysia.

	Season							
	Dry				Wet			
	Female		Male		Female		Male	
Site	**A**	**J**	**A**	**J**	**A**	**J**	**A**	**J**
Kuala Lumpur	71	59	24	6	33	25	20	3
Georgetown	11	22	13	27	6	7	3	13
Kuantan	32	9	18	20	8	2	5	6
Malacca	31	3	16	8	15	9	8	10
Total	145	93	71	61	62	43	36	32

*A, adult; J, juvenile

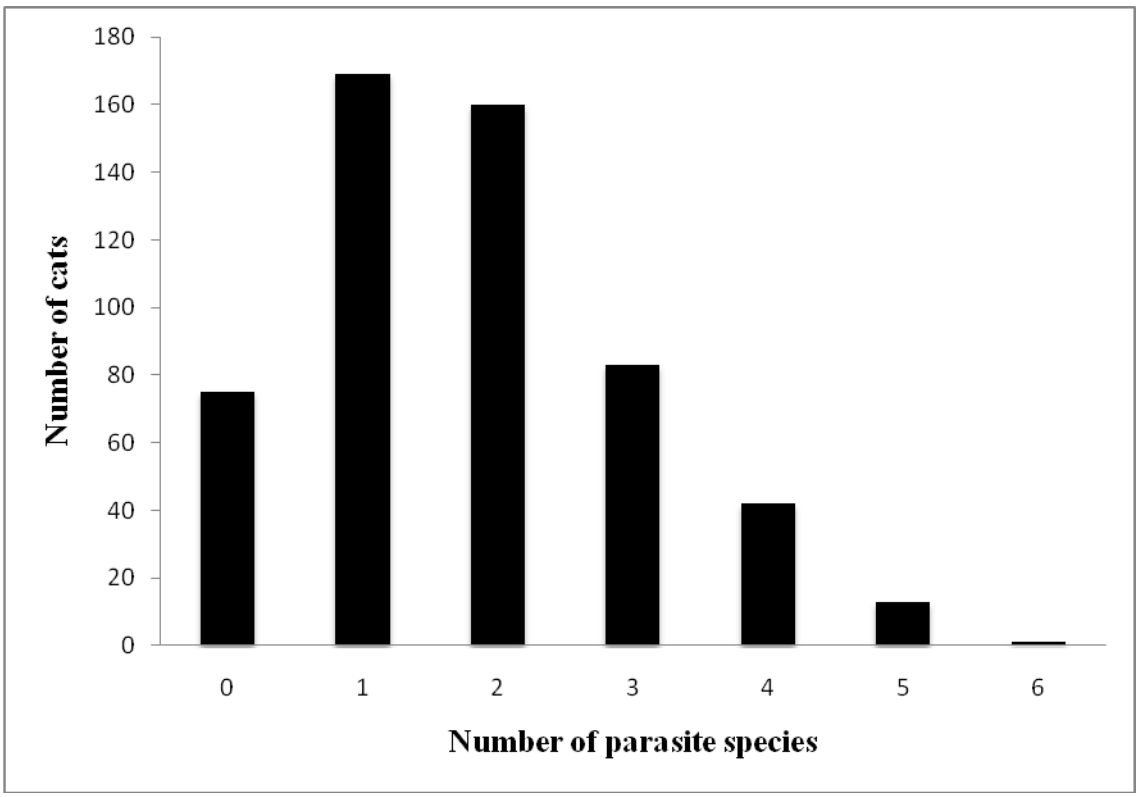

Figure 2.4: Frequency distribution of infracommunity richness of parasites in the stray cat population from Peninsular Malaysia.

Table 2.2: The prevalence (%) of frequency distribution of infracommunity richness of parasites in stray cats according to location.

Location		No. of parasites in host							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Kuala Lumpur	**No. of host**	25	74	71	33	26	11	1	241
	Prevalence (%)	**10.37**	**30.71***	**29.46**	**13.69**	**10.79**	**4.56**	**0.41**	
Georgetown	**No. of host**	14	34	36	12	5	1	0	102
	Prevalence (%)	**13.73**	**33.33**	**35.29***	**11.76**	**4.9**	**0.98**	**0**	
Kuantan	**No. of host**	12	30	27	24	6	1	0	100
	Prevalence (%)	**12**	**30***	**27**	**24**	**6**	**1**	**0**	
Malacca	**No. of host**	24	31	26	14	5	0	0	100
	Prevalence (%)	**24**	**31***	**26**	**14**	**5**	**0**	**0**	

* Highest prevalence of infection by site

Table 2.3: The prevalence (%) of frequency distribution of infracommunity richness of parasites in stray cats according to season.

Location	Prevalence	No. of parasites in host							
	(%)	0	1	2	3	4	5	6	Total host
Kuala Lumpur	**Dry**	6.88	28.75	33.75*	15.00	10.63	4.38	0.63	160
	Wet	17.28	34.57*	20.99	11.11	11.11	4.94	0	81
Georgetown	**Dry**	9.59	27.40	41.10*	15.07	6.85	0	0	73
	Wet	24.14	48.28*	20.69	3.45	0	3.45	0	29
Kuantan	**Dry**	10.13	32.91*	24.05	26.58	6.33	0	0	79
	Wet	19.05	19.05	38.10*	14.29	4.76	4.76	0	21
Malacca	**Dry**	18.97	25.86	34.48*	13.79	6.90	0	0	58
	Wet	30.95	38.10*	14.29	14.29	2.38	0	0	42

* Highest prevalence of infection by season and site

Table 2.4: The prevalence (%) of frequency distribution of infracommunity richness of parasites in stray cats according to host sex.

Location	Prevalence	No. of parasites in host							
	(%)	0	1	2	3	4	5	6	Total host
Kuala Lumpur	**Male**	9.43	37.74*	26.42	11.32	13.21	1.89	0	53
	Female	10.64	28.72	30.32*	14.36	10.11	5.32	0.53	188
Georgetown	**Male**	16.07	41.07*	26.79	12.5	3.57	0	0	56
	Female	10.87	23.91	45.65*	10.87	6.52	2.17	0	46
Kuantan	**Male**	14.29	26.53*	26.53*	24.49	6.12	2.04	0	49
	Female	9.8	33.33*	27.45	23.53	5.88	0	0	51
Malacca	**Male**	19.05	26.19	33.33*	16.67	4.76	0	0	42
	Female	27.59	34.48*	20.69	12.07	5.17	0	0	58

* Highest prevalence of infection by host sex and site

Table 2.5: The prevalence (%) of frequency distribution of infracommunity richness of parasites in stray cats according to host age.

Location	Prevalence	No. of parasites in host							
	(%)	0	1	2	3	4	5	6	Total host
Kuala Lumpur	**Adult**	11.49	22.97	25*	16.22	16.89	6.76	0.68	148
	Juvenile	8.60	43.01*	36.56	9.68	1.08	1.08	0	93
Georgetown	**Adult**	12.12	21.21	42.42*	15.15	6.06	3.03	0	33
	Juvenile	14.49	39.13*	31.88	10.14	4.35	0	0	69
Kuantan	**Adult**	11.11	22.22	23.81*	33.33	7.94	1.59	0	63
	Juvenile	13.51	43.24*	32.43	8.11	2.70	0	0	37
Malacca	**Adult**	18.57	28.57*	27.14	18.57	7.14	0	0	70
	Juvenile	36.67	36.67*	23.33	3.33	0	0	0	30

* Highest prevalence of infection by host age and site

Table 2.6: Simpson's Index values for the macroparasite infection in the stray cat population in Peninsular Malaysia.

Factors		All	Ectoparasites	Endoparasites
Host Sex	**Male**	5.672*	1.865	3.805
	Female	5.512	1.923*	4.183*
Host Age	**Adult**	4.906*	2.063*	3.702*
	Juvenile	4.119	1.933	2.317
Seasonal	**Dry**	5.678	2.197*	3.868
	Wet	6.333*	1.218	5.316*
Location	**Kuala Lumpur**	4.684	1.291	3.752*
	Georgetown	5.747*	2.9*	2.845
	Kuantan	4.275	1.637	3.296
	Malacca	4.915	1.702	3.72

***** Higher diversity by factors

Table 2.7: Brillouin Index values for the macroparasite infection in the stray cat population in Peninsular Malaysia.

Factors		All	Ectoparasites	Endoparasites
Host Sex	**Male**	1.861	0.6917	1.493
	Female	1.994*	0.8617*	1.685*
Host Age	**Adult**	1.888*	0.9082*	1.555*
	Juvenile	1.677	0.8803	1.103
Seasonal	**Dry**	2.014*	0.9772*	1.619
	Wet	2.000	0.3544	1.811*
Location	**Kuala Lumpur**	1.831	0.4648	1.588*
	Georgetown	1.887*	1.137*	1.254
	Kuantan	1.696	0.563	1.404
	Malacca	1.830	0.6032	1.541

***** Higher diversity by factors

2.4 Discussion

A considerable number of stray cats were captured from four urban cities with over 543 cats studied. The location of urban cities in the vicinity of public areas and easy access to the foods has contributed to the increasing of stray cat population. Extent to the population growth, the tendency of cat to breed very quickly has become a concern to the public of the close proximity between stray cats to human and food source and the potential of transmission of zoonotic diseases. Therefore, it is very important to study the stray population in metropolitan areas in order to determine the potential reservoir of infection for humans (Jittapalapong *et al*., 2007). Presently, among the steps taken by municipality of each city was implementing a vector control programme to manage the stray problem in each city. In addition, public awareness programme was carried out to encourage the public to control the over population by neutering the cats. Animal shelters in Kuala Lumpur and Penang have also provided a refuge for strays for adoption meanwhile, sickly and aged cats were put down humanely.

The present study involved a large scale study to have a better understanding of effects of parasitism in the stray cat population in Peninsular Malaysia. Previous studies reported only a small number of stray cats were studied. Rohde (1962) examined 62 cats where 30 cats from Kuala Lumpur, 8 from Kepong, 15 from Kota Bharu and 9 from Kampongs in Kuantan. Other previous studies were confined to a particular location such as, 111 cats (Retnasabapathy & Khoo, 1970), 304 cats from Selangor and Perak (Retnasabapathy & Prathap, 1971), 100 cats from Selangor (Amin-Babjee, 1978), 200 cats from Ipoh (Shanta *et al.*, 1980) and 55 cats from Kuala Lumpur (Lee *et al*., 1993).

The numbers of females captured in this study were higher compared to males as they were captured randomly in the public areas. Similar with previous study by Shanta *et al*. (1980), higher number of females (128 cats) captured compared to males (72 cats). More female cats were found wandering in the vicinity of public areas in the four study sites foraging foods as foraging behaviour a nature for them and their youngs to survive. A study of wood ducks (*Aix sponsa*) by Drobney & Fredrickson (1985) showed females increase their food intake during the breeding season to supply the protein for their eggs. Similarly, the numbers of adults captured were higher compared to juveniles as adults were frequently found in the public areas foraging foods for them and their youngs.

The stray cat population in Peninsular Malaysia were found to be highly infected with macroparasites (ecto- and endoparasites). Less than half of the stray cats harboured ectoparasites while around 75% of them were found to be infected with endoparasites. The infection levels of stray cats in this study concurred with reports from the previous studies such as Amin-Babjee (1978) and Shanta *et al.* (1980). However, Shanta *et al*. (1980) reported higher prevalence (92.5%) of cats infected with *Ancylostoma spp*. and 59.5% were infected with *Ctenocephalides felis.* There is a decline in the prevalence of infected cats and this decline could have been contributed by urbanization of the cities such as sanitary facilities and proper garbage disposal which decreases the number of places for the cat to forage and defecate in the urban areas.

Each infected stray cats in this study harboured a minimum of one species and a maximum of six species of macroparasites. Infracommunity species richness of parasites in stray cats by location, season, host age and host sex showed the majority of

stray cats harboured between one to two species of macroparasites. Parasites are normally overdispersed or aggregated within the cat population where a few hosts harbour large number of parasites. Higher species richness value reported by Amin-Babjee (1978) in Selangor with majority of cats harboured 3 species of parasites. This pattern could have been influenced by host and parasite behavior, climatic condition, the densities of host and infective stage, as well as their respective spatial distribution and the differences in the ability of individual hosts to mount effective immunological responses to parasite invasion (Cox, 2001).

This study also showed high diversity index of parasite species in adult stray cats compared to juveniles. This may be attributed to the habits and behaviour of the adults that frequent sources of infections where the helminthes parasite thrive, for example, the waste or rotting foods in restaurants or markets. Parasites may take advantage of inevitable stray cat's behaviour such as foraging behaviour and social behaviour to maximize their transmission. Foraging for food is thought to represent optimizations for maximizing survival and reproductive success of parasites (Stephens & Krebs, 1986). Stray cats may also have to accept food choices with lower nutritional value if they seek alternative foods to avoid parasite exposure (Lozano, 1991). High diversity of endoparasites species during wet season compared to dry showed that moist soils with tropical temperatures enhance hatching of eggs or survival and development of larval stages of nematodes for effective transmission (Rep, 1965).

2.5 Conclusion

The stray cats population captured from four urban cities in Peninsular Malaysia showed more female cats were captured compared to males meanwhile higher number of adult cats captured compared to juveniles. The numbers of stray cats captured during dry season were higher compared to wet season.

Species richness analysis showed each infected stray cats harboured a minimum of one species and a maximum of six species of macroparasites. The majority of stray cats captured harboured one to two species of macroparasites. Georgetown has the highest diversity followed by Malacca, Kuala Lumpur and Kuantan. The species diversity was similar between males and females meanwhile high diversity of species richness was observed in adults compared to juveniles. High diversity of ectoparasites and low diversity of endoparasites observed during dry season compared to species diversity during wet season.

CHAPTER 3

DIVERSITY OF ECTOPARASITES OF URBAN STRAY CATS IN PENINSULAR MALAYSIA

3.1 Introduction

Ectoparasite infestation is a common problem in cats and infected cats may infect human when foraging in public places (Jittapalapong *et al.*, 2003). Cat ectoparasites cause skin disease and also transmitting diseases to cats. It can cause from allergic reactions to human to transmit diseases such as Lyme disease, Powassan encephalitis, plague, Rocky Mountain spotted fever, trypanosomiasis, and tularemia (Nelder & Reeves, 2005). Multiple spesies infestation are frequently found in both dogs and cats in developing countries, particularly to the poor due to high cost of living and inability to give better animal care (Jittapalapong *et al.*, 2008).

Several reports of ectoparasites infecting domestic cats have been conducted in the past in Peninsular Malaysia (Zamirdin *et al.*, 1967; Mustaffa-Babjee, 1969; Nagendram & Rajamanickam, 1976; Amin-Babjee, 1978 and Shanta *et al.*, 1980) however most studies focused on selected ectoparasites species such as *Otodectes cynotis* (Zamirdin *et al.*, 1967; Nagendram & Rajamanickam, 1976) and *Felicola subrostratus* (Mustaffa-Babjee, 1969). Nagendram & Rajamanickam (1976) found 31 cases of clinically affected cats with *Otodectes cynotis* within a study period of 5 months.

Amin-Babjee (1978) produced a more comprehensive study reporting infestation of ectoparasites from the domestic cat in Selangor. The study found the cat's flea,

Ctenocephalides felis felis infected 50% cats out of 100 cats examined in Selangor. This study also showed that *Felicola subrostratus* was the only louse species found in cats however, showed low intensity of infestation. Meanwhile, *Haemaphysalis bispinosa* was found in 4% of the cat population and *Notoedres cati* causing mange lesions on the face and ears were seen in 7% of the cats' population.

In Ipoh, Shanta *et al.* (1980) recorded 8 species of ectoparasites recovered namely, *Ctenocephalides felis felis, Ctenocephalides felis orientis, Felicola subrostrata, Notoedres cati, Haemaphysalis bispinosa, Sarcoptes scabiei, Demodex felis* and *Xenopsylla cheopis*. The flea, *Ctenocephalides felis felis* showed higher prevalence with 59.5% and she concluded that the ectoparasites recovered in the study showed low degree of parasitism and did not affect the health of the animal.

In this chapter, ectoparasites of stray cats from four urban cities namely Kuala Lumpur, Georgetown, Kuantan and Malacca are reported. The main objective of this study is to determine the diversity of ectoparasites from stray cats in terms of prevalence of infections and abundance of each species. This study also determined the role of intrinsic (host age, host sex) and extrinsic (season, location) factors in structuring of the ectoparasite community in the stray cat population.

3.2 Methodology

Euthanized specimens were placed on a dissecting pan with a white background and examined for macroparasites by firstly examining the external surface for ectoparasites, followed by recovering the ectoparasites by combing the fur from all parts of the body

using fine tooth-comb. The combing was done for about 5 minutes (Zakson *et al.*, 1995) and all samples recovered were counted and placed in 70% ethanol. Each specimen was also inspected thoroughly for the presence of ticks. If present, the ticks were carefully removed with a fine forceps to ensure that the mouthparts remained intact. All ticks recovered were placed in 70% ethanol. Deep skin scrapes and deep ear swab of stray cats was not performed in this study.

For identification purposes, each ectoparasite recovered was examined microscopically to determine the specimen up to species level. Each specimen was cleared and temporary mounted on a drop of lactophenol solution on a clean glass slide for identification before finally mounted permanently in polyvinyl lactophenol. All fleas and ticks recovered were counted and identified at 40x microscopically meanwhile lice and mites were counted at 40x and identified at 100x microscopically. Further attempts were made to identify the ectoparasites up to species level where possible. Undetermined specimens were sent to Mr. John Jeffery from Department of Parasitology, University of Malaya for confirmation of species. Identification to species level was done by observation of morphological characteristics and referring to Pictorial Keys to Arthropods, Reptiles, Birds and Mammals of Public Health Significance by US Department of Health, Education and Welfare and keys by Kohls (1957) and Price & Graham (1997).

The data compiled was then analyzed using the software Quantitative Parasitology 3.0 (Reiczigel *et al.*, 2000) for prevalence, mean intensity, abundance, with 95% confidence interval (Margolis *et al.*, 1982) of parasites. Data was further analysed to determine if intrinsic and extrinsic factors affected the parasite burden. Intrinsic factors included host

sex (male or female), host age (adult or juvenile), and extrinsic factors such as season (dry or wet) and site (Kuala Lumpur, Georgetown, Kuantan and Malacca). The prevalence was compared using Fisher's Exact Test, while mean intensities and abundances were compared using Bootstrap Test, as proposed by Rozsa *et al*. (2000). Both tests were provided in Quantitative Parasitology 3.0 (Reiczigel & Rozsa, 2001).

3.3 Results

A total of 251 (46.22%) stray cats harboured ectoparasites. Post-mortem examinations recovered 2,338 specimens, with abundance value of 9.31 ectoparasites per infected cat. The largest ectoparasite burden in a single cat host was 166. The ectoparasites recovered consist of four major groups namely the flea, lice, tick and mite. The ectoparasites found on the stray cats were mainly in adult stage however some ticks recovered were nymphs.

Overall, a total of five species of ectoparasites were successfully identified namely one flea species, *Ctenocephalides felis*, two species of lice, *Felicola subrostrata* and *Heterodoxus spiniger*, one species of tick, *Haemaphysalis bispinosa* and one species of mite, *Lynxacarus radovskyi* (Plate 3.1- 3.5). Each stray cat harboured a minimum of one species and a maximum of three species of ectoparasites. Figure 3.1 showed the distribution of all ectoparasites recovered from this study.

Overall analysis of all cats captured from all sites showed *Ctenocephalides felis* (41.8%, 37.61-46.09) was the most prevalent meanwhile accidental record of common dog

louse, *Heterodoxus spiniger* showed the lowest prevalence with 0.4% stray cats infected (0.4-1.3) (Norhidayu *et al.*, 2012).

The mite, *Lynxacarus radovskyi* was found the most abundant ectoparasites with mean intensity 40.25 per infected stray cats meanwhile the tick, *Haemaphysalis bispinosa* was the least abundant with mean intensity 1.08 per infected stray cats. The mite *Lynxacarus radovskyi* is reported for the first time on domestic cats from Peninsular Malaysia (Jeffery *et al*., 2012). From all the 5 species of ectoparasites recovered, the frequently occurring ectoparasite species *Ctenocephalides felis* dispersion pattern exhibited a negative binomial distribution with a *k* value of 0.19. Table 3.1 summarized the quantitative analysis of ectoparasites recovered from stray cats of the urban cities in Peninsular Malaysia.

3.3.1 Ectoparasites distribution according to host sex

The total cat population showed more females (343) captured compared to males (200). Both sexes were found to harbor ectoparasites with 91 (45.5%) male stray cats compared to 160 female cats (46.6%). All 5 species of ectoparasites recovered were present on female stray cats namely *Ctenocephalides felis*, *Felicola subrostratus*, *Heterodoxus spiniger, Haemaphysalis bispinosa* and *Lynxacarus radovskyi*. All males were also found infested with all ectoparasite species with the exception of the cat mite; *Lynxacarus radovskyi.*

Table 3.2 summarized the prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM), as well as the comparative analysis of cat's ectoparasites between both host sexes. *Ctenocephalides felis* was found prevalence in both sexes with

infestation in males (39.5%, 32.67 - 46.64) slightly lower compared to females (43.1%, 37.84 - 48.58). Following this, the cat's louse, *Felicola subrostratus* was also found on both host sexes with 8.5% (5.02 - 13.27) males infested compared to females, 7.0% (4.53 - 10.24). Only female stray cats were found infected with the mite, *Lynxacarus radovskyi* with prevalence 2.3% (1.01 - 4.55) and high value of mean intensity 40.25 per infected cat (Table 3.2).

Statistical analysis showed no significant differences were found in the prevalence, mean intensity and abundance of infections between both sexes for all ectoparasite species recovered apart from *Lynxacarus radovskyi* showing a significant value (Table 3.2). However, this could be due to low sample size infected.

3.3.2 Ectoparasites distribution according to host age

The cat populations were mainly adults (314) compared to juveniles (229). Both groups harboured ectoparasites with prevalence of both age groups almost similar with 140 (44.6%) adults and 111 (48.5%) juvenile cats infested. All ectoparasite species recovered in this study were found on juveniles and adult cats (except for the louse, *Heterodoxus spiniger*).

Table 3.3 summarized the prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM), as well as the comparative analysis of cat's ectoparasites between adult and juvenile stray cats. Analysis between adult and juvenile stray cats, no significant differences were found in the prevalence, mean intensity and abundance of infections in all species recovered.

Ctenocephalides felis was found prevalent in both host age. Adult cats showed 38.5% (33.12 - 44.17) infestation with this flea meanwhile for juvenile stray cats showed 46.3% (39.69 - 52.98). The cat's louse, *Felicola subrostratus* was also found on both adult (8.3%, 5.48 - 11.9) and juvenile (6.6%, 3.71 - 10.58) stray cats however with low prevalences. *Heterodoxus spiniger* commonly found on dogs was first reported here on juvenile stray cats with prevalence 0.9% (0.1 - 3.12) (Table 3.3).

3.3.3 Ectoparasites distribution according to season factors

From the total cat population were caught, 370 cats were captured during dry season and 173 cats during wet season from the four study sites. Ectoparasites infesting cats were slightly higher during the dry season (n=177, 47.8%) compared to wet season (n=74, 42.8%). All 5 species of ectoparasites recovered in the present study were found in both seasons except for *Heterodoxus spiniger* present during wet season only.

Table 3.4 summarized the prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM), as well as the comparative analysis of cat's ectoparasites recovered from stray cats during dry and wet season. Statistic analysis showed no significant differences for prevalence for season, mean intensity and abundance of infections for all ectoparasite species recovered except for abundance of the cat flea where higher abundance was observed during dry season compared to wet season.

From all 5 species recovered, *Ctenocephalides felis* was found prevalent for both seasons with slightly higher infections during dry season, 42.4% stray cats (37.43-47.56) compared to the wet season 40.5% (33.08-48.18). The louse, *Heterodoxus*

spiniger was only observed during the dry season with only 0.5% infected with intensity 9.5 per infected cat.

3.3.4 Ectoparasites distribution according to location

The cat's flea, *Ctenocephalides felis* was also found prevalent from all study sites. Table 3.5 summarized the prevalence of all ectoparasites recovered in this study according to location. Statistical analysis showed infestation of two species; *Ctenocephalides felis* (p=0.00) and *Felicola subrostratus* (p=0.03) significant at the four study sites (Table 3.5).

In Kuala Lumpur, 144 stray cats (59.75%) were infected with ectoparasites out of 241 stray cats captured. Only 4 species of ectoparasites were recovered namely *Ctenocephalides felis, Felicola subrostrata, Haemaphysalis bispinosa* and *Lynxacarus radovskyi*. The flea *Ctenocephalides felis* was identified as the most prevalent ectoparasites with 57.7% stray cats infected (51.16 - 64) meanwhile the mite *Lynxacarus radovskyi* was the least prevalent with 2.5% stray cats infected (0.9 - 5.34). However, *Lynxacarus radovskyi* was the most abundant with mean intensity 13.83 per infected stray cat meanwhile the tick, *Haemaphysalis sp.* the least with mean intensity 1.13 per infected stray cat. *Ctenocephalides felis,* exhibited a negative binomial distribution with k value of 0.34. Quantitative analysis of ectoparasites recovered from stray cats in Kuala Lumpur was summarized in Table 3.6.

All five species of ectoparasites were recovered from 47 infected stray cats (46.09%) out of 102 stray cats captured from Georgetown namely *Ctenocephalides felis, Felicola subrostrata, Haemaphysalis bispinosa, Heterodoxus spiniger* and *Lynxacarus*

radovskyi. *Ctenocephalides felis* (42.2%, 32.44 - 52.34) was the most prevalent ectoparasite meanwhile the louse *Heterodoxus spiniger* and mite *Lynxacarus radovskyi* were the least prevalent with only 2% of stray cats infected (0.23 - 6.91). *Lynxacarus radovskyi* was also identified as the most abundant ectoparasite with mean intensity 119.50 per infected cat meanwhile *Haemaphysalis bispinosa* was found the least with mean intensity 1.0 per infected stray cat. *Ctenocephalides felis* exhibited a negative binomial distribution with *k* value of 0.16. The quantitative analysis of ectoparasite recovered from stray cats in Georgetown was summarized in Table 3.7.

In Kuantan, 35 stray cats (35%) out of 100 stray cats captured were infected with ectoparasites. Only two species; *Ctenocephalides felis* and *Felicola subrostrata* were found. *Ctenocephalides felis* was the most prevalence with 29% out of 100 stray cats was infected. *Felicola subrostrata* was the most abundant with mean intensity 8.14 per infected stray cats. The dispersion patterns of *Ctenocephalides felis* exhibited negative binomial distribution with *k* value of 0.13. The quantitative analysis of ectoparasites recovered in Kuantan was summarized in Table 3.8.

Twenty five stray cats (25%) were found infected with ectoparasites from 100 stray cats captured all around Malacca. Three species were recovered from the infected stray cats namely *Ctenocephalides felis*, *Felicola subrostrata* and *Haemaphysalis bispinosa* .The flea was found the most prevalence and most abundant ectoparasite found in Malacca with 16% stray cats infected and mean intensity 6.19 per infected stray cat. Only one cat was found with *Haemaphysalis bispinosa*. Table 3.9 summarized the quantitative analysis of ectoparasites recovered from stray cats in Malacca.

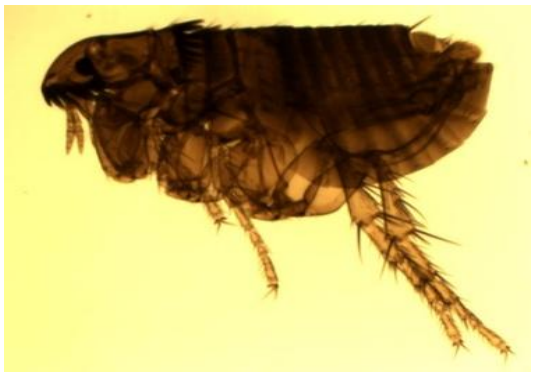

Figure 3.1: *Ctenocephalides felis*

(Magnification : 4 x 10)

Figure 3.2: *Felicola subrostratus*

(Magnification: 4 x 10)

Figure 3.3: *Heterodoxus spiniger*

(Magnification: 4 x 10)

Figure 3.4: *Haemaphysalis bispinosa*

(Magnification: 4 x 10)

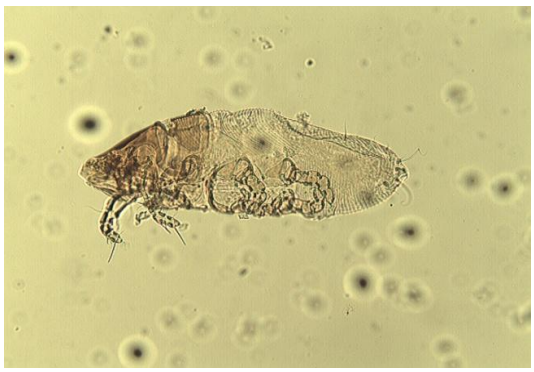

Figure 3.5: *Lynxacarus radovskyi*

(Magnification: 10 x 10)

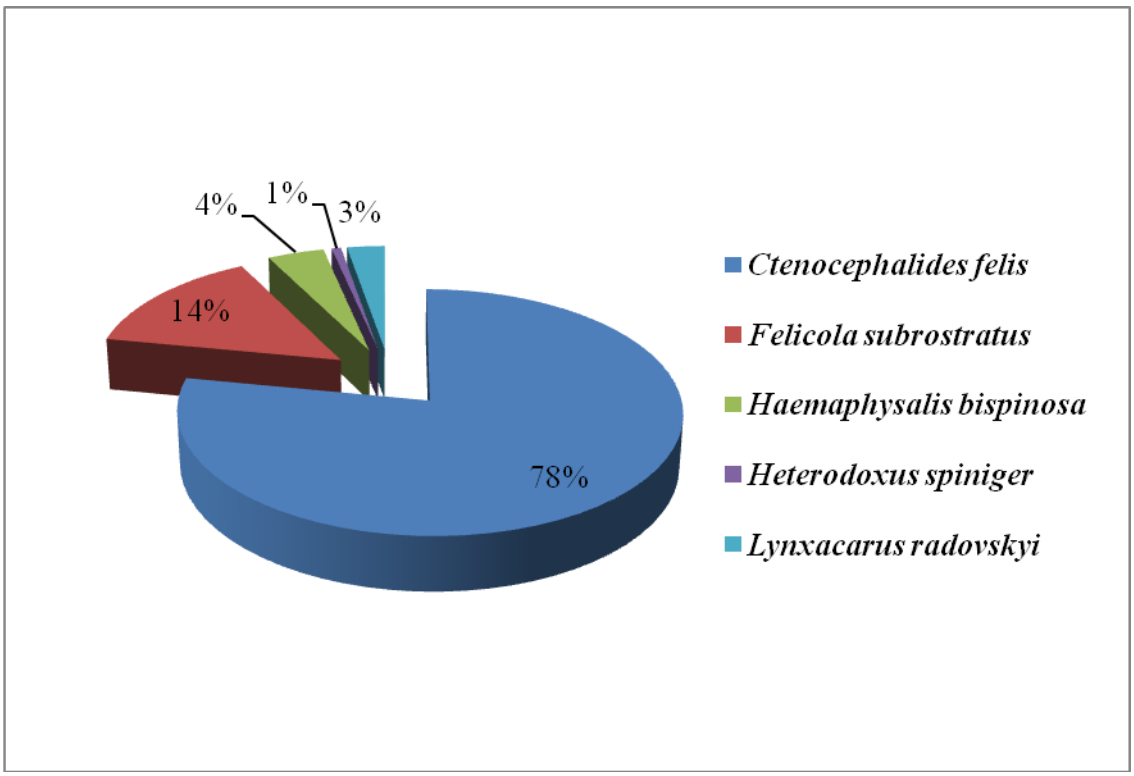

Figure 3.6: Prevalence of ectoparasite species recovered from stray cats in Peninsular Malaysia.

Table 3.1: Quantitative analysis of ectoparasites recovered from stray cats captured in Peninsular Malaysia.

Ectoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Flea						
Ctenocephalides felis	1556	41.8% (37.61-46.09)	6.85 (5.52-8.47)	2.87 (2.28-3.60)	1-102	0.190
Lice						
Felicola subrostrata	428	7.7% (5.63-10.32)	10.57 (5.57-21.33)	0.79 (0.39-1.61)	1-112	0.022
Heterodoxus spiniger	19	0.4% (0.04-1.33)	9.50 (5.00-9.50) #	0.03 (0.00-0.11)	5-14	**
Tick						
Haemaphysalis bispinosa	13	2.2% (1.14-3.83)	1.08 (1.0-1.25)	0.02 (0.01-0.04)	1-2	**
Mite						
Lynxacarus radovskyi	322	1.5% (0.63-2.89)	40.25 (12.88-98.50)	0.59 (0.15-1.69)#	1-163	0.002

- limits uncertain due to low sample size

** - fit to the negative binomial cannot be tested due to low categories

Table 3.2: Prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM) and comparative analysis of cat's ectoparasites between both host sexes in Peninsular Malaysia.

	Prevalence			Mean Intensity			Abundance ± SEM		
Parasite species	Male (%)	Female (%)	*P* value	Male	Female	*P* value	Male	Female	*P* value
Ctenocephalides felis	39.5	43.1	0.42	7.9	6.3	0.39	3.1 ± 0.7	2.7 ± 0.4	0.68
Feicola subrostratus	8.5	7.0	0.61	19.5	4.0	0.14	1.7 ± 0.8	0.3 ± 0.08	0.15
Heterodoxus spiniger	0.5	0.3	1.00	5.0	14.0	1.00	0.03 ± 0.03	0.04 ± 0.04	0.82
Haemaphysalis bispinosa	2.0	2.3	1.00	1.0	1.1	0.41	0.02 ± 0.01	0.03 ± 0.01	0.64
Lynxacarus radovskyi	0	2.3	0.03*	0	40.25	1.00	0	0.9 ± 0.6	0.19

* significant

Table 3.3: Prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM) and comparative analysis of cat's ectoparasites between host ages in Peninsular Malaysia.

	Prevalence			Mean Intensity			Abundance ± SEM		
Parasite species	Adult (%)	Juvenile (%)	*P* value	Adult	Juvenile	*P* value	Adult	Juvenile	*P* value
Ctenocephalides felis	38.5	46.3	0.46	7.5	6.2	0.40	2.9 ± 0.5	2.9 ± 0.4	0.89
Feicola subrostratus	8.3	6.6	0.51	8.6	13.6	0.54	0.7 ± 0.4	0.9 ± 0.5	0.81
Heterodoxus spiniger	0	0.9	0.18	0	9.5	1.0	0	0.08 ± 0.06	0.30
Haemaphysalis bispinosa	2.9	1.3	0.26	1.1	1.0	0.43	0.03 ± 0.01	0.01 ± 0.008	0.17
Lynxacarus radovskyi	1.6	1.3	1.0	48.6	26.3	0.61	0.8 ± 0.6	0.3 ± 0.3	0.58

Table 3.4: Prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM) and comparative analysis of cat's ectoparasites between seasonal factors in Peninsular Malaysia

	Prevalence			Mean Intensity			Abundance ± SEM		
Parasite species	Dry (%)	Wet (%)	*P* value	Dry	Wet	*P* value	Dry	Wet	*P* value
Ctenocephalides felis	42.4	40.5	0.71	7.9	4.5	0.01*	3.4 ± 0.5	1.8 ± 0.4	0.01*
Feicola subrostratus	8.1	6.4	0.60	13.3	2.6	0.10	1.1 ± 0.4	0.2 ± 0.07	0.10
Heterodoxus spiniger	0.5	0	1.00	9.5	0	1.00	0.05 ± 0.04	0	0.32
Haemaphysalis bispinosa	2.7	1.2	0.36	1.1	1.0	0.42	0.03 ± 0.01	0.01 ± 0.01	0.15
Lynxacarus radovskyi	1.6	1.2	1.00	53.2	1.5	0.12	0.9 ± 0.5	0.02 ± 0.01	0.20

* significant

Table 3.5: Prevalence of ectoparasites recovered from stray cats captured in Kuala Lumpur, Georgetown, Kuantan and Malacca Town of Peninsular Malaysia

Ectoparasites	Kuala Lumpur (n=241)	Georgetown (n=102)	Kuantan (n=100)	Malacca (n=100)	*p*-value
Flea					
Ctenocephalides felis	57.7%	42.2%	29%	16%	0.00*
Lice					
Felicola subrostratus	4.1%	15.7%	7%	9%	0.03*
Heterodoxus spiniger	-	2%	-	-	0.10
Tick					
Haemaphysalis bispinosa	3.3%	2.9%	-	1%	0.21
Mite					
Lynxacarus radovskyi	2.5%	2%	-	-	0.23

* significant

Table 3.6: Quantitative analysis of ectoparasites recovered from stray cats captured in Kuala Lumpur, Peninsular Malaysia.

Ectoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Flea						
Ctenocephalides felis	829	57.7% (51.16-64.00)	5.96 (4.70-8.05)	3.44 (2.59-4.6)	1-83	0.335
Lice						
Felicola subrostrata	26	4.6% (2.0-7.5)	2.3 (1.5-3.5)	0.11 (0.05-0.2)	1-6	0.029
Tick						
Haemaphysalis bispinosa	9	3.3% (1.44-6.44)	1.13 (1.00-1.38)	0.04 (0.01-0.07)	1-2	**
Mite						
Lynxacarus radovskyi	83	2.5% (0.91-5.34)	13.83 (1.33-39.17) #	0.34 (0.02-1.46)#	1-58	0.006

- limits uncertain due to low sample size

** - fit to the negative binomial cannot be tested due to low categories

Table 3.7: Quantitative analysis of ectoparasites recovered from stray cats captured in Georgetown, Peninsular Malaysia.

Ectoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Flea						
Ctenocephalides felis	468	42.2% (32.44-52.34)	10.88 (6.91-18.63)	4.59 (2.87-8.1)	1-102	0.157
Lice						
Felicola subrostrata	307	13.7% (9.24-24.23)	20.38 (8.50-45.50)	3.01 (1.01-7.35)	1-112	0.037
Heterodoxus spiniger	19	2.0% (0.23-6.91)	9.50 (5.00-9.50) #	0.19 (0.0-0.65)#	5-14	**
Tick						
Haemaphysalis bispinosa	3	2.9% (0.61-8.36)	1.00 #	0.03 (0.0-0.07)	1-1	‡
Mite						
Lynxacarus radovskyi	239	2.0% (0.23-6.91)	119.50 (76.00-119.50) #	2.34 (0.0-7.14)	76-163	**

- limits uncertain due to low sample size

** - fit to the negative binomial cannot be tested due to low categories

‡ - sample not aggregated enough to fit the negative binomial

Table 3.8: Quantitative analysis of ectoparasites recovered from stray cats captured in Kuantan, Peninsular Malaysia.

Ectoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Flea						
Ctenocephalides felis	160	29.0% (20.35-38.93)	5.52 (3.41-11.14) #	1.6 (0.88-3.31)#	1-50	0.131
Lice						
Felicola subrostrata	57	7.0% (2.86-13.90)	8.14 (5.43-12.43)	0.57 (0.22-1.18)	3-18	0.022

- limits uncertain due to low sample size

Table 3.9: Quantitative analysis of ectoparasites recovered from stray cats captured in Malacca, Peninsular Malaysia.

Ectoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Flea						
Ctenocephalides felis	99	16.0% (9.43-24.68)	6.19 (3.44-10.00)	0.99 (0.46-1.89)	1-24	0.061
Lice						
Felicola subrostrata	38	9.0% (4.19-16.40)	4.22 (2.67-6.33)	0.38 (0.16-0.77)	1-10	0.041
Tick						
Haemaphysalis bispinosa	1	1.0% (0.02-5.45)	1.00 #	0.01 (0.00-0.03)	1-1	‡

- limits uncertain due to low sample size

‡ - sample not aggregated enough to fit the negative binomial

3.4 Discussion

The overall ectoparasite diversity of the stray cat population in Peninsular Malaysia showed low diversity compared to prior records in Peninsular Malaysia (Amin-Babjee, 1978; Shanta *et al.*, 1980). Amin-Babjee (1978) and Shanta *et al.* (1980) recorded four and eight species respectively. Only five species of ectoparasites were recorded in this study. Intensity values of the flea, *Ctenocephalides felis* showed that ectoparasite infestations on the cat population of urban areas were high and the frequency distribution of this flea to be overdispersed.

Both intrinsic (host age and sex) and extrinsic (location and season) factors showed no significant effects in determining the ectoparasites distribution in stray cats population. However, the diversity and prevalence of the ectoparasites slightly higher during dry season. This could be due to the condition of the wet fur of the cats because of the rain during wet season do not suitable for the survival of the ectoparasites especially the flea.

The cat flea, *Ctenocephalides felis* was the most prevalent ectoparasite infesting the cat population with high intensities from all four locations. Previous studies also recorded this trend (Amin-Babjee, 1978; Shanta *et al.*, 1980). *Ctenocephalides felis* is a known biological vector to many pathogens in humans (Nelder & Reeves, 2005) and also plays a role as an intermediate host for other more important parasite namely *Dipylidium caninum*.

The cat population in Malaysia is also a host to the only a single louse species namely *Felicola subrostratus.* This parasite was the next most prevalent ectoparasite as also

showed in the previous studies (Mustaffa-Babjee, 1969; Amin-Babjee, 1978; Shanta *et al*., 1980). Infested cats with this parasite also recorded throughout the world including Europe (Trotti *et al*., 1990), Asia (Eduardo *et al*., 1977; Jittapalapong *et al*., 2008), Australia (Coman *et al*., 1981), South America (Santa Cruz & Lombardero, 1987) and North America. Although infection is not common but this parasite present in the cat population.

Present study also reports the first accidental infection of *Heterodoxus spiniger* on stray cats in Peninsular Malaysia (Norhidayu *et al*., 2012). This parasite is a common louse of dogs also on canines on all continents except Antartica and Europe (Amin & Madbouly, 1973; Price & Graham, 1997). *Heterodoxus spiniger* was found on two cats from Georgetown. *Heterodoxus spiniger* is a host specific and known only feed on dogs and a few other members of family Canidae (Price & Graham, 1997). This parasite also does not typically infest felines. Only one other case reported found *Heterodoxus spiniger* infesting a litter of kittens (Colless, 1959). *Heterodoxus spiniger* is the intermediate host to several helminth parasites of dogs including the tapeworm, *Dipylidium caninum* and the filarid nematode, *Dipetalonema reconditum* (Price & Graham, 1997). The presence of this ectoparasite on cats could be due to the close relationship between the cats and dogs influencing the parasites infection (Jittapalapong *et al*., 2008).

Only one tick species *Haemaphysalis bispinosa* was found in the cat population in Peninsular Malaysia. Generally only one tick was found attached to the tip of the ear. However, for one cat from Kuala Lumpur, two ticks were found. *Haemaphysalis bispinosa* was also recorded in previous studies (Amin-Babjee, 1978 and Shanta *et al*., 1980) with low infection. Ticks are rarely found in the urban cities as they prefer moist

and humid environment such as shade trees, woods and shrubs. Only two species of *Haemaphysalis sp*. were found in the United States namely *Haemaphysalis leporipalustris* and *Haemaphysalis chordeilis* (Bowman *et al*., 2002).

The cat mite, *Lynxacarus radovskyi* was found on cats from two sites; Kuala Lumpur and Georgetown and reported for the first time on domestic cats from Peninsular Malaysia (Jeffery *et al*., 2012). This mite also reported on two pet cats (5%) from Kampong Menteri in Taiping, Perak. Infestation with *Lynxacarus radovskyi* have been reported in many countries; United States (Craig *et al*., 1993; Greve & Gerrish, 1981; Foley, 1991a; Fox, 1977), Hawaii (Tenorio, 1974), Fiji (Munro & Munro, 1979) and Australia (Bowman & Domrow, 1978). Cats infected with large number of these mites showed a dry, dull and rust colored haircoat. Some cats may have gastrointestinal disturbances, gingivitis, anorexia, restlessness, fever and weight loss (Foley, 1991a). From the previous studies in Peninsular Malaysia, other mite species were found namely, *Otodectes cynotis* (Nagendram & Rajaminickam, 1976), *Notoedres cati* (Amin-Babje, 1978; Shanta *et al*., 1980), *Sarcoptes scabiei* and *Demodex felis* (Shanta *et al*., 1980). Unfortunately, there were no records for these ectoparasites in this study.

Ectoparasites have the potential to transmit zoonotic disease. The zoonotic character of some parasites found must serve as an alert to public health agencies, veterinarians and pet owners (Labarthe *et al*., 2004). Some zoonotic diseases only cause severe pain to human but some may cause serious disease and even death. *Ctenocephalides felis* was the most prevalent in this study and causes itchiness and small red lesion at the biting site and play a role as the intermediate host of the cat tapeworm, *Dipylidium caninum*. Human may be infected with this tapeworm by accidental ingestion of the infective

fleas. Another ectoparasite found in this study that may affect human is the mite, *Lynxacarus radovskyi*. This species was reported on one patient with a heavily parasitized cat, developed a papular forearm rash that cleared after the infestation of the cat was treated (Foley, 1991b).

Tick paralysis is a well-known syndrome that occurs in many vertebrate hosts throughout the world and induced by at least 46 species of ticks (Stone, 1988). However it is uncertain if the tick found in this study, *Haemaphysalis bispinosa* can cause tick paralysis in human. The only tick known to cause paralysis in human, notably infants is *Ixodes holocyclus*. The symptoms of tick paralysis in human being are similar to those observed in animals include ascending paralysis characterized by unsteadiness in walking or lethargy, weakness in upper limbs, difficulty in swallowing, respiratory distress and even death in the absence of treatment (Bowman *et al*., 2002).

Ectoparasitic infestations in the present study were relatively low and most species have previously been reported by Amin-Babjee (1978) and Shanta *et al.* (1980), particularly the cat flea *Ctenocephalides felis*. Two species reported here are of zoonotic in nature such as *Ctenocephalides felis* and *Lynxacarus radovskyi*. Public and pet owners should be educated of the diseases brought by cats to human. Education plays a vital role reducing the prevalence of infections with potentially zoonotic parasites in pets and their owners (Jittapalapong *et al*., 2008). Veterinarian also play an important role in the care and health of domestic cats as well as in educating pet owners regarding the transmission of zoonotic parasites and the means by which zoonotic transmission can be prevented or minimized (McGlade *et al*., 2003). It is important that methods for

prevention and control of the parasites be implemented and executed in order to reduce the environmental contamination with infective eggs and larvae (Labarthe *et al*., 2004).

3.5 Conclusion

Ectoparasites diversity of the stray cats in the four localities studied were found to be generally low with infestations slightly higher than 40% of stray cats were found infected. Five species of ectoparasites were found namely *Ctenocephalides felis*, *Felicola subrostratus*, *Heterodoxus spiniger*, *Haemaphysalis bispinosa* and *Lynxacarus radovskyi*. A louse species commonly found on dogs, *Heterodoxus spiniger*, was recovered from stray cats captured in Georgetown. The presence of this parasite on stray cat is the first reported in Peninsular Malaysia. The cat fur mite, *Lynxacarus radovskyi* also reported for the first time on domestic cats from Peninsular Malaysia.

Infestation of ectoparasites between host sex (male and female) and host age (adult and juvenile) were almost similar although not significant. Similarly, season and location did not contribute in structuring the ectoparasites community of the stray cat population in Peninsular Malaysia where infestations of ectoparasites from four locations (Kuala Lumpur, Georgetown, Kuantan and Malacca) during dry and wet season appear similar.

CHAPTER 4

DIVERSITY OF ENDOPARASITES OF URBAN STRAY CATS IN PENINSULAR MALAYSIA

4.1 Introduction

Cats pick up parasites while wandering freely outdoor or while foraging for foods. Previous epidemiological studies worldwide demonstrated high frequency of parasites in the stray cat population (McColm & Hutchison, 1980; Coman *et al*., 1981; Nichol *et al*., 1981a; Calvete *et al*., 1998).

Spain *et al.* (2001) observed prevalence of parasite infestation in stray cats was higher when compared with cats with owners. Stray cats have been found to be reservoir of many zoonotic infestations from hookworm to ascariasis (Labarthe *et al.,* 2004). These parasites can be detrimental to their health and to the humans in the vicinity (Krecek *et al*., 2010). Gastrointestinal parasitism is one of the main causes of morbidity in domestic dogs and cats and represents public health risk worldwide (Hendrix & Blagburn, 1983).

Stray cats also provide a potential reservoir of helminthic parasites to domestic cat especially in rural areas (Calvete *et al*., 1998). Excretion of faeces in public places poses health hazard especially to young children (Changizi *et al*., 2007). Jittapalapong *et al*. (2007) reported the importance of studying the stray population from Metropolitan areas in order to determine the potential reservoir of infection for humans.

Previous studies on endoparasites from cats in Peninsular Malaysia have been reported (Retnasabapathy & Khoo, 1970; Retnasabapathy & Prathap, 1971; Yoshida *et al.*,, 1973; Zamri Saad *et al*., 1984 and Lee *et al*., 1993). However, most studies focused on specific parasites of the cat and several comprehensive studies were conducted to ascertain diversity parasite infection in cats from different parts of Malaysia (Kuala Lumpur, Kepong, villages south of Kuantan and Kota Bharu) by Rhode (1962), in Selangor (Amin-Babjee, 1978) and in Ipoh (Shanta *et al*., 1980).

Earlier studies showed, the most common nematode species infecting cats were *Ancylostoma spp.* Purvis (1930) reported the occurrence of *Ancylostoma braziliense* in the domestic cat. Gordon (1922) reported *Ancylostoma ceylanicum* and *Ancylostoma braziliense* as the same species. However in 1951, Biocca stated *Ancylostoma ceylanicum* and *Ancylostoma braziliense* were two different species after a detailed morphological study. Following this study, Yoshida (1971a,b) added new morphological differences between these two species in both adult and infective larval stage.

High prevalence of *Ancylostoma spp* was shown in several states in Peninsular Malaysia. Rohde (1962) surveyed helminthes of cats from various parts of Peninsular Malaysia showed 77.8% were infected with *Ancylostoma ceylanicum* and *Ancylostoma caninum*. Yoshida *et al*. (1973) studied the occurrence between *Ancylostoma braziliense* and *Ancylostoma ceylanicum* in Malaysia found 90.6% domestic and wild cats were infected with *Ancylostoma ceylanicum* and 9.4% were infected with *Ancylostoma braziliense*. However, Amin-Babjee (1978) reported 78% of 100 cats in Selangor were

infected with mixed infection of *Ancylostoma ceylanicum* and *Ancylostoma caninum* while Shanta *et al.* (1980) found 92.5% of 200 cats around Ipoh.

Second most prevalent helminth species was *Toxocara spp.* Infection in cats for *Toxocara canis* and *Toxocara cati* were as high as 41.9% of cat's autopsied from various parts of Peninsular Malaysia (Rohde, 1962). Amin-Babjee (1978) in his study cited 27% *Toxocara cati* infecting 100 cats examined while Shanta *et al.* (1980) reported *Toxocara cati* in 14.5 % of 200 cats. Several references reported that *Toxocara canis* is found in dogs and foxes (Georgi, 1974; Soulsby, 1968) or dogs alone (Alicata, 1964; Shanta, 1982; Mustaffa- Babjee, 1984), while *Toxocara cati* only occurs in cats (Amin-Babjee, 1978; Shanta *et al*., 1980). Lee *et al.* (1993) reported an autopsy of 55 stray cats from in and around Kuala Lumpur showed prevalence of 32.7% *Toxocara spp*. with *Toxocara cati* (21.8%), *Toxocara canis* (5.5%) and three cats (5.5%) had both *Toxocara cati* and *Toxocara canis*.

In 2001, *Toxocara malaysiensis,* a new species was described in Malaysia. Molecular studies on the resultant material indicated that *Toxocara malaysiensis* represented a separate species from *Toxocara canis* and *Toxocara cati* (Zhu *et al.*, 1998). Detailed morphological examination (Gibbons *et al*., 2001) found that this species differed from *Toxocara canis* by the shape of the cervical alae, spicule length and the lip structure. *Toxocara malaysiensis* is also distinct from other species from the genus *Toxocara*. Molecular studies on the resultant material indicated that *Toxocara malaysiensis* represented a separate species from *Toxocara canis* and *Toxocara cati* (Zhu *et al.*, 1998).

Following this study, examination of an immature male specimen (Gibbons *et al*., 2001) revealed inconsistencies with published descriptions of *Toxocara canis*, and a collection of ascaridoids from cats in Kuala Lumpur. Furthermore, comparisons of aligned ITS-2 sequences of the Malaysian nematode with *Toxocara cati* and *Toxocara canis* only showed 89% and 76% homology respectively (Jacobs, 1997).

Another common parasite to cats is the nematode *Physaloptera praeputialis.* It is a parasite pink worm that found in the stomach of cats with the anterior end attached to the mucosa. *Physaloptera praeputialis* was found previously in cats in Kedah (Purvis (1931); Pillers (1931); Lancaster (1938)). In 1933, Adams also reported the occurrence of this species in Taiping and Seremban. Rohde (1962) found this nematode in 17.7% cats examined from various parts of Peninsular Malaysia. Reports by Retnasabapathy & Khoo in 1970 from Malacca, Petaling Jaya, Klang, Jinjang, Kepong and Sentul recorded 18% cats infected with this nematode. In Selangor, Amin-Babjee (1978) reported 11% cats infected while in 1980, Shanta *et al.* (1980) recorded 5% cats infected with *Physaloptera praeputialis* from 200 cats studied in Ipoh.

The two most common cestodes that infect Malaysian cats are *Dipyllidium caninum* followed by *Taenia taeniaeformis.* Purvis (1932) and Lancaster (1957) listed the occurrence of *Diphyllobothrium spp., Dipylidium caninum, Dipylidium sexcoronatum, Joyeuxiella pasqualei* and *Taenia taeniaformis*. However, Rohde (1962) only found *Dipylidium caninum* and *Joyeuxiella pasqualei* in 15 cats, *Diphyllobothrium spp*. in 14 cats and *Taenia taeniaformis* in 14 cats out of 63 cats from the various parts in Peninsular Malaysia. Amin-Babjee (1978) reported 22% of the cats were found infected with 1 or 2 species of cestodes with *Taenia taeniaeformis* the highest with 14% cats

infected in Selangor. Shanta *et al.* (1980) reported *Dipyllidium caninum* (15%), *Joyeuxiella pasqualei* (12.5%), *Taenia taeniaeformis* (11.5%) and *Spirometra mansoni* (6%) from the studied of 200 cats in Ipoh.

The most common trematode found in the locat cat is *Platynosomum fastosum* (Purvis, 1931; Orr, 1937 and Lancaster, 1938). Rohde (1962) studied cats from various parts of Malaysia found 22.6% were infected this species with average 100 flukes per cat. Retnasabapathy & Prathap (1971) found 73% of cats infected with this helminth. Amin-Babjee (1978) recorded 37% of the cat population were infected with *Platynosomum fastosum* while only one cats was infected with *Opisthorchis sinensis* in Selangor. Shanta *et al.* (1980) showed the highest occurrence of the trematode with 75.5% cats infected in Ipoh. Zamri Saad *et al.* (1984) studied on a case report on clinical, pathological and parasitological findings of cats that have *Platynosomum fastosum* infestation.

In this chapter, the current endoparasite infection of stray cat from four urban cities namely; Kuala Lumpur, Georgetown, Kuantan and Malacca were reported. The main objective of this study was to determine the diversity of endoparasites from stray cats in relation to prevalence of infections and to determine the role of intrinsic (host age, host sex) and extrinsic (season, location) factors in structuring the endoparasite community in the stray cat population.

4.2 Methodology

Post-mortem was conducted on cats after euthanasia. The stomach, small intestine, caecum, liver, bile duct, heart and lungs were removed and placed in physiological saline and was slit opened and placed in Petri dishes containing physiological saline. The contents of bile duct and gallbladder was examined for the presence of parasites. The gastrointestinal tract was slit opened along its entire length and examined for the presence of helminthes in the contents of the gut and in scraping of the mucosa (Changizi *et al*., 2007). All obtained parasites were carefully removed and preserved in 70% alcohol. Microhabitat of each parasite collected was recorded. No attempts were made to collect blood sample.

All recovered nematodes were temporarily mounted and cleared in lactophenol for examination before mounted permanently in polyvinyl lactophenol. All nematodes recovered were counted and identified at 40x microscopically except for *Ancylostoma spp.* and *Strongyloides sp*. *Ancylostoma spp.* and *Strongyloides sp.* were counted at 40x and identified at 100x microscopically. The number of individuals of each species was recorded. All nematodes were identified using the CIH Keys to the Nematode Parasites of Vertebrates (No 1- 8) (1980).

Trematodes and cestodes were stained for 10-15 minutes in Borax carmine, dehydrated in ethanol and cleared with methyl salicylate before mounted in Canada Balsam. Slides were then dried in the oven before sealing and appropriately labeled. All parasites were counted and identified at 40x microscopically. The number of cestodes was calculated

according to the number of scolexes. The number of individuals for each species was recorded. The trematodes and cestodes were identified using the key by Soulsby (1968).

Verification and detailed identification of the endoparasites were done up to species level where possible. Confirmation to species level was conducted at the Parasitology Lab of Veterinary Research Unit in Ipoh. Identification of specimens was made through morphological characteristics as well as their microhabitat.

The data compiled is then analyzed using the software Quantitative Parasitology 3.0 (Reiczigel *et al.*, 2000) for prevalence, mean intensity and abundance with 95% confidence interval (Margolis *et al*., 1982). The factors affecting parasite burden including host sex (male or female), host age (adult or juvenile), season (dry or wet) and site (Kuala Lumpur, Georgetown, Kuantan and Malacca) were compared. The mean intensity value gives information on the total quantity of parasites in the sample. The abundance value the mean number of parasites found in all hosts, involving the zero values of uninfected hosts as well. The standard deviation (±SD) was not provided in this analysis because it is useless for aggregated distributions exhibited by parasites. The prevalence was compared using Fisher's Exact Test, while mean intensities and abundances were compared using Bootstrap Test, as proposed by Rozsa *et al*. (2000). Both tests were provided in Quantitative Parasitology 3.0 (Reiczigel & Rozsa, 2001).

4.3 Results

Of the total of 543 stray cats captured from four locations in Peninsular Malaysia, 405 stray cats (74.59%) were found infected with endoparasites. Post-mortem examinations recovered 9,057 helminths, with abundance value of 22.36 endoparasites per infected cat. The largest worm burden in a single cat host was 567. Each stray cats infected with endoparasites harbored a minimum of one to up to a maximum of five species of endoparasites.

A total of 9 species of endoparasites were identified consists of three major groups of six Nematode species (*Toxocara malaysiensis, Toxocara cati, Ancylostoma braziliensis, Ancylostoma ceylanicum, Strongyloides sp., Physaloptera praeputialis*) two Cestode species (*Taenia taeniaeformis, Dipyllidium caninum)* and one Trematode species (*Platynosomum fastosum)* (Plate 4.1-4.6). Figure 4.1 showed the distribution of all endoparasites recovered from stray cats of four urban cities in Peninsular Malaysia. Most of the endoparasites were recovered from the gastrointestinal tract except for the fluke, *Platynosomum fastosum*, was found in the bile duct. The nematode, *Physaloptera praeputialis* was the only parasite found only in the stomach meanwhile both *Toxocara spp.* were found in the stomach and small intestines.

Overall result showed the hookworms *Ancylostoma ceylanicum* (31.5%) and *Ancylostoma braziliense* (30.8%) and the ascarid nematodes *Toxocara malaysiensis* (28.4%) were the most prevalent helminth species. *Strongyloides sp.* was found the most abundant endoparasite with mean intensity 160.83 per infected stray cat meanwhile the cestode, *Taenia taeniaeformis* found the less abundant with mean

intensity 2.05 per infected stray cat. In the three most frequently occurring helminth species, *Ancylostoma braziliense, Ancylostoma ceylanicum* and *Toxocara malaysiensis*, dispersion patterns exhibited negative binomial characteristics with respective *k* value of 0.10, 0.09 and 0.11. Table 4.1 summarized the quantitative analysis of endoparasites recovered from stray cats of the urban cities in Peninsular Malaysia.

4.3.1 Endoparasites distribution according to host sex

From the total stray cat population, more females (343 cats) were captured than males (200 cats). Both sexes harboured endoparasites with infection in 75.8% females (260 cats) slightly higher compared to 72.5% males (145 cats).

Table 4.2 summarized the prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM), as well as the comparative analysis of cat's endoparasites between both host sexes. No significant differences were shown in the prevalence, mean intensity and abundance of infections of all species recovered between male and female stray cats. Although the prevalence of *Dipylidium caninum* and *Platynosomum fastosum* ($p < 0.05$) showed a significant value, however this value could be due to low sample size infected.

All 9 species of endoparasites recovered in this study were present in male and female stray cats except for the *Strongyloides sp.* This species only found in female stray cats with 1.7% (0.64-3.77) infected. The three most prevalence endoparasite recovered namely *Ancylostoma braziliense, Ancylostoma ceylanicum* and *Toxocara malaysiensis* were found prevalence in both male and female stray cats (Table 4.2).

4.3.2 Endoparasites distribution according to host age

The results from this study showed more adults (314 cats) were captured compared to juveniles (229) with both hosts harboring endoparasites. Of the total, 77.1% adults (242 cats) compared to 71.2% juveniles (163 cats) were found to be infected with endoparasites.

Table 4.3 summarizes the prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM), as well as the comparative analysis of cat's endoparasites between both host ages. Significant difference occurred in the prevalence of infection between host age with most of the parasites recovered. Higher prevalence values were shown for adults compared to juveniles for all species except for *Toxocara spp*. Both *Toxocara malaysiensis* and *Toxocara cati* showed higher prevalence and abundance in juveniles compared to adult stray cats. In this analysis, only adult cats were found to harbor *Strongyloides sp.* with 1.9% (0.70-4.12) infected. (Table 4.3).

4.3.3 Endoparasites distribution according to season

The total population showed cats captured during dry season (370) were higher compared to wet season (173). Of the total, 78.1% (289) stray cats infected with endoparasites during dry season higher than 67.1% (116 cats) during wet season. All of the 9 species of endoparasites recovered in this study were present in stray cats during both seasons.

Table 4.4 summarized the prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM), as well as the comparative analysis of cat's endoparasites recovered. Significant differences occurred in the prevalence of four helminth species;

Ancylostoma braziliense, *Toxocara malaysiensis* and *Toxocara cati* are highly infected during dry season meanwhile the cestode, *Dipylidium caninum* highly infected during wet season.

4.3.4 Endoparasites distribution according to location

All endoparasites recovered were found in all urban cities except for two nematodes. *Strongyloides sp.* was only found present in stray cats from Kuala Lumpur and *Physaloptera praeputialis* was found infecting cats in all locations except for Kuala Lumpur. Table 4.5 showed the prevalence of infection with endoparasites of stray cats from four locations in this study.

In Kuala Lumpur, 181 of 241 stray cats (75.1%) were found infected with endoparasites with 8 helminthes recovered. Table 4.6 summarized the quantitative analysis of endoparasites recovered from stray cats in Kuala Lumpur. *Ancylostoma ceylanicum* was found the most prevalence with 35.3% stray cats infected meanwhile *Strongyloides sp.* was found the most abundant endoparasite with mean intensity 160.83 per infected stray cat. The dispersion patterns of *Ancylostoma braziliense*, *Ancylostoma ceylanicum* and *Toxocara malaysiensis* exhibited negative binomial distribution with *k* value of 0.10, 0.10 and 0.11 respectively.

Table 4.7 showed the summarized of quantitative analysis of endoparasites recovered from stray cats in Georgetown. Eight species of endoparasites were recovered with 73 of 102 stray cats (71.6%) captured have endoparasites infestation. In Georgetown, *Toxocara malaysiensis* was found the most prevalence with 37.3% stray cats infected

and the most abundant endoparasite with mean intensity 13.89 per infected stray cats. *Toxocara malaysiensis* exhibited a negative binomial distribution with *k* value of 0.13.

In Kuantan, 83 of 100 stray cats (83%) captured were found infected with endoparasites. Eight species of endoparasites were found consists of 8 helminthes. *Toxocara malaysiensis* was found the most prevalent and abundant endoparasites with 42% stray cats were infected and mean intensity was 13.89 per infected stray cat. The dispersion patterns of *Ancylostoma braziliense*, *Ancylostoma ceylanicum* and *Toxocara malaysiensis* exhibited negative binomial distribution with *k* value of 0.13, 0.13 and 0.24 respectively. Quantitative analysis of endoparasites recovered fom stray cats in Kuantan was summarized in Table 4.8.

Eight species of endoparasites were recovered from stray cats in Malacca with 68 of 100 stray cats (68%) found infected. *Ancylostoma ceylanicum* was found the most prevalence endoparasite with 33.0% stray cats infected meanwhile the cestode, *Dipylidium caninum* showed abundant intensity with 10 worms in the infected stray cat. Another parasite that found most abundant was *Ancylostoma braziliense* with 8.19 per infected stray cat. *Ancylostoma braziliense* and *Ancylostoma ceylanicum* exhibited a negative binomial distribution with *k* value of 0.12 and 0.13 respectively. Table 4.9 summarized the quantitative analysis of endoparasites recovered from stray cats in Malacca.

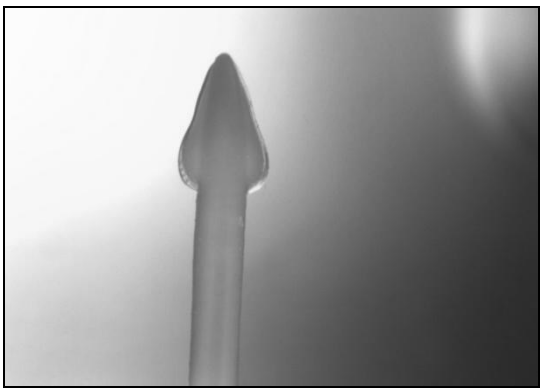

Figure 4.1: *Toxocara cati*
(Magnification: 4 x 10)

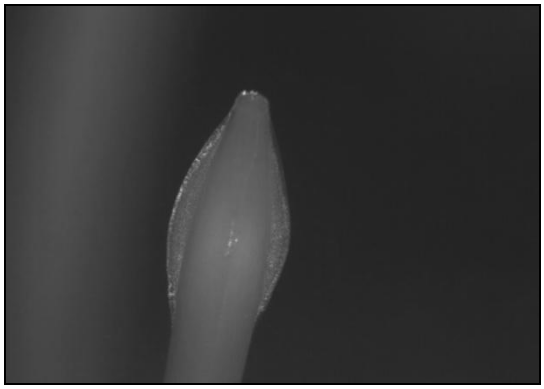

Figure 4.2: *Toxocara malaysiensis*
(Magnification: 4 x 10)

Figure 4.3: *Physaloptera praeputialis*
(Magnification: 4 x 10)

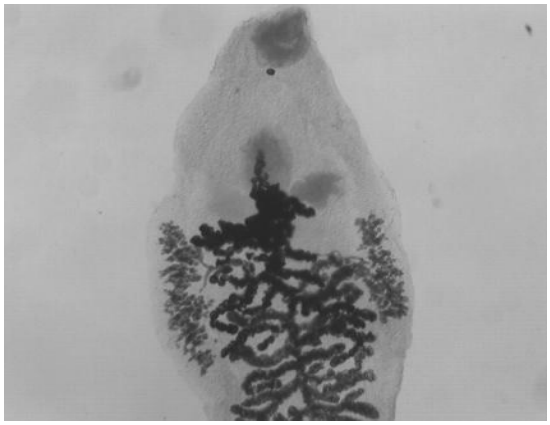

Figure 4.4: *Platynosomum fastosum*
(Magnification: 10 x 10)

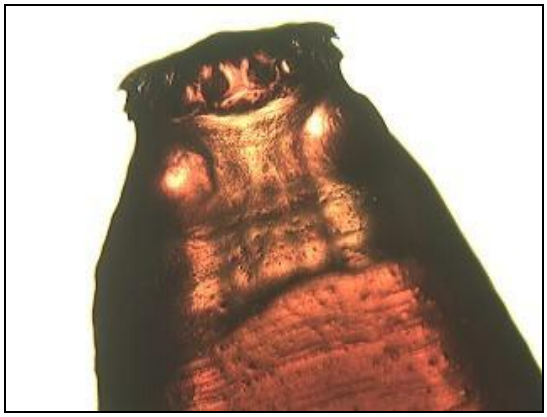

Figure 4.5: *Taenia taeniaeformis*
(Magnification: 4 x 10)

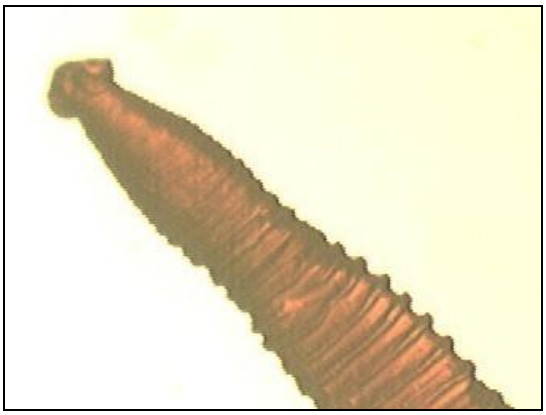

Figure 4.6: *Dipylidium caninum*
(Magnification: 4 x 10)

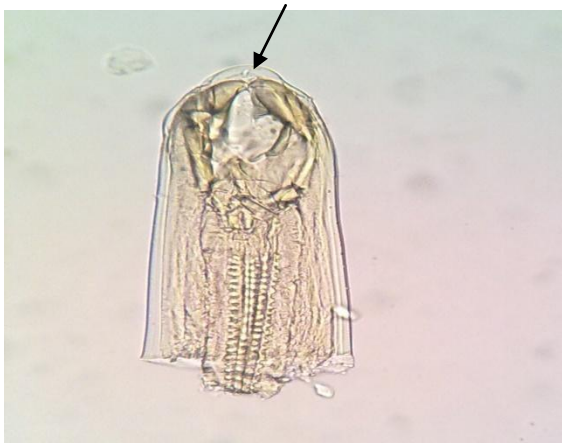

Figure 4.7: Mouth part of *A.braziliense* (10 x 10)

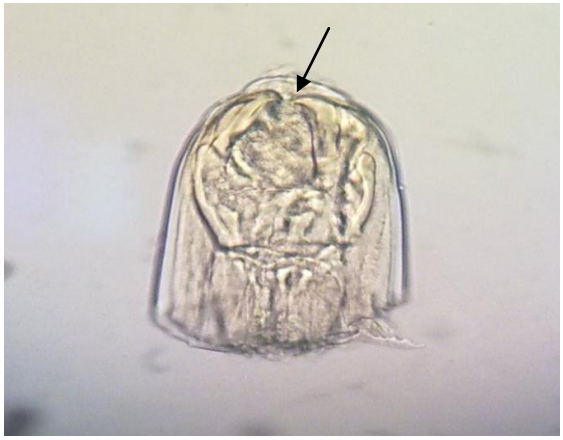

Figure 4.8: Mouth part of *A.ceylanicum* (10 x 10)

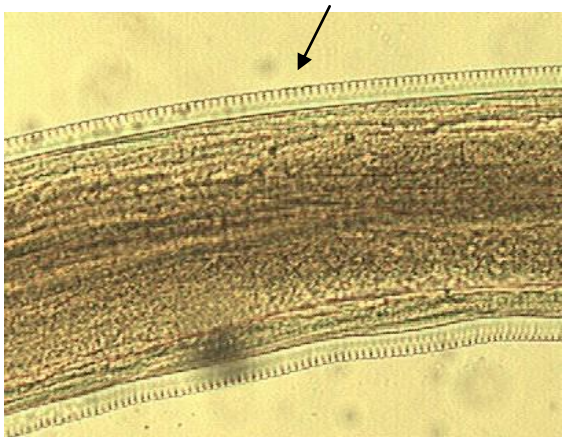

Figure 4.9: Transverse striation of *A.braziliense* (10 x 10)

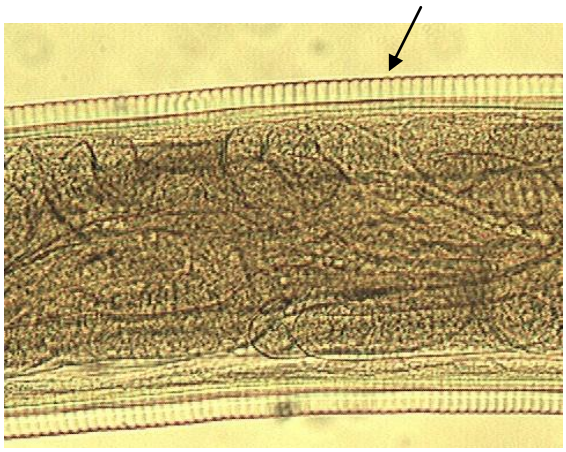

Figure 4.10: Transverse striation of *A.ceylanicum* (10 x 10)

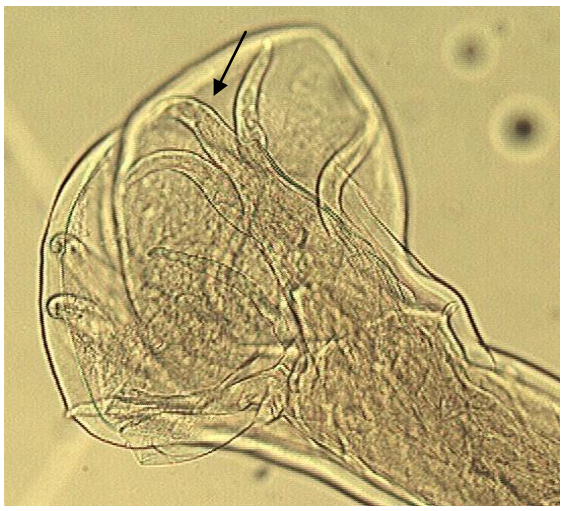

Figure 4.11: Bursa copulatrix of *A.braziliense* (10 x 10)

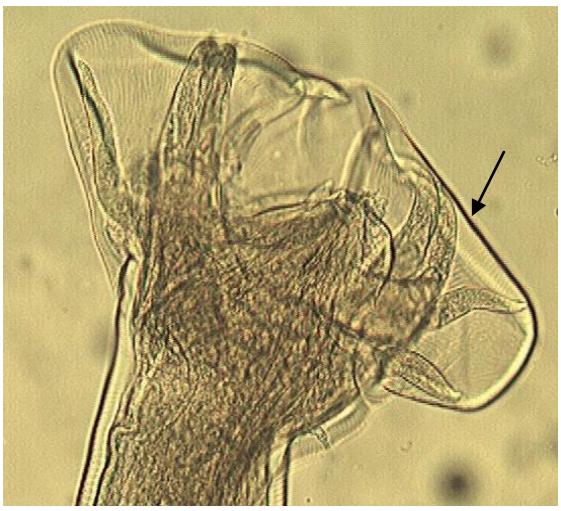

Figure 4.12: Bursa copulatrix of *A.ceylanicum* (10 x 10)

The inner teeth of *Ancylostoma braziliense* (Plate 4.7) are hooklike and smaller than those of *Ancylostoma ceylanicum* (Plate 4.8) situated behind the margin of the outer teeth. The distance between the transverse striations of *Ancylostoma braziliense* (Plate 4.9) is much smaller than that of *Ancylostoma ceylanicum* (Plate 4.10). The three lateral rays of *Ancylostoma braziliense* (Plate 4.11) are divergent from each other, while *Ancylostoma ceylanicum* (Plate 4.12) showed externo-lateral ray is widely separated from the other two rays whose points lie close together.

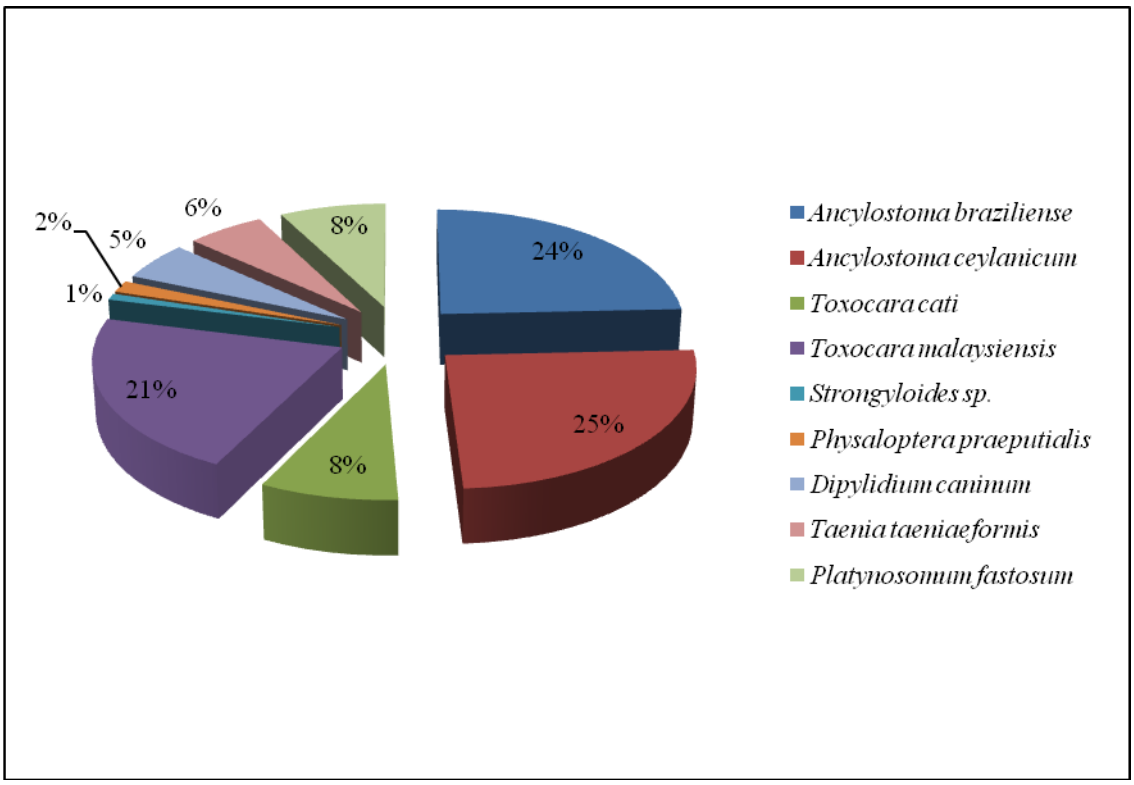

Figure 4.13: Prevalence of endoparasite species recovered from stray cats in Peninsular Malaysia.

Table 4.1: Quantitative analysis of endoparasites recovered from stray cats captured from all locations in Peninsular Malaysia.

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	K
Nematode						
Ancylostoma braziliense	1984	30.8% (26.89-34.83)	11.88 (9.24-15.35)	3.65 (2.76-4.91)	1-124	0.10
Ancylostoma ceylanicum	3487	31.5% (27.60-35.59)	20.39 (15.74-27.08)	6.42 (4.83-8.50)	1-270	0.09
Toxocara cati	191	10.3% (7.88-13.19)	3.41 (2.54-4.63)	0.35 (0.24-0.52)	1-18	0.05
Toxocara malaysiensis	1132	27.3% (23.55-31.22)	7.65 (6.18-9.42)	2.08 (1.62-2.63)	1-57	0.11
Strongyloides sp.	965	1.1% (0.40-2.39)	160.83 (21.50-361.33)	1.78 (0.15-5.22) #	2-477	0.001
Physaloptera praeputialis	67	2.0% (1.01-3.60)	6.09 (3.55-11.64)	0.12 (0.05-0.28) #	1-24	0.007

- limits uncertain due to low sample size

Table 4.1: Quantitative analysis of endoparasites recovered from stray cats captured from all locations in Peninsular Malaysia. [Continued]

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	K
Cestode						
Dipylidium caninum	483	6.6% (4.68-9.07)	13.42 (6.64-38.44) #	0.89 (0.41-2.82) #	1-229	0.02‡
Taenia taeniaeformis	80	7.2% (5.15-9.69)	2.05 (1.49-3.41) #	0.15 (0.09-0.24)	1-16	0.06
Trematode						
Platynosomum fastosum	668	9.8% (7.39-12.58)	12.60 (7.74-24.08)	1.23 (0.71-2.32)	1-171	0.03

- limits uncertain due to low sample size

‡ - sample not aggregated enough to fit the negative binomial

Table 4.2: Prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM) and comparative analysis of cat's endoparasites between both host sexes in Peninsular Malaysia

	Prevalence			Mean Intensity			Abundance ± SEM		
Parasite species	Male (%)	Female (%)	*P* value	Male	Female	*P* value	Male	Female	*P* value
Ancylostoma braziliense	29.5	31.5	0.73	10.9	12.4	0.65	3.2 ± 0.9	3.9 ± 0.7	0.94
Ancylostoma ceylanicum	27.5	33.8	0.61	11.1	24.8	0.06	3.0 ± 0.8	8.4 ± 1.5	0.47
Toxocara malaysiensis	33.0	23.9	0.47	7.1	8.1	0.51	2.3 ± 0.4	1.9 ± 0.4	0.92
Toxocara cati	12.0	9.3	0.65	4.0	2.9	0.34	0.5 ± 0.1	0.3 ± 0.08	0.47
Strongyloides sp.	0	1.7	0.09	0	160.8	1.00	0	2.8 ± 1.8	0.14
Physaloptera praeputialis	1.0	2.6	0.34	1.5	7.1	0.15	0.02 ± 0.01	0.2 ± 0.08	0.12
Taenia taeniaeformis	8.0	6.7	0.61	1.7	2.3	0.45	0.1 ± 0.04	0.2 ± 0.06	0.77
Dipylidium caninum	3.5	8.5	0.03*	8.4	14.6	0.54	0.3 ± 0.2	1.2 ± 0.7	0.38
Platynosomum fastosum	5.5	12.2	0.01*	5.8	14.4	0.14	0.3 ± 0.1	1.8 ± 0.6	0.09

*significant

Table 4.3: Prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM) and comparative analysis of cat's endoparasites between host ages in Peninsular Malaysia

	Prevalence			Mean Intensity			Abundance ± SEM		
Parasite species	Adult (%)	Juvenile (%)	*P* value	Adult	Juvenile	*P* value	Adult	Juvenile	*P* value
Ancylostoma braziliense	47.5	7.9	0.00*	12.8	4.3	0.00*	6.1 ± 0.9	0.3 ± 0.1	0.006*
Ancylostoma ceylanicum	46.5	10.9	0.00*	21.1	16.1	0.53	9.8 ± 1.6	1.8 ± 0.8	0.08*
Toxocara malaysiensis	12.7	47.2	0.05*	3.2	9.3	0.00*	0.4 ± 0.09	4.4 ± 0.6	0.04*
Toxocara cati	7.3	14.4	0.09	2.3	4.2	0.06	0.2 ± 0.05	0.6 ± 0.2	0.06
Strongyloides sp.	1.9	0	0.04*	160.8	0	1.00	3.1 ± 2.0	0	0.15
Physaloptera praeputialis	3.2	0.4	0.03*	6.5	2.0	1.00	0.2 ± 0.09	0.009 ± 0.009	0.12
Taenia taeniaeformis	11.1	1.7	0.00*	2.0	2.5	0.67	0.2 ± 0.06	0.04 ± 0.03	0.02*
Dipylidium caninum	8.6	3.9	0.04*	16.8	3.2	0.36	1.4 ± 0.7	0.1 ± 0.06	0.36
Platynosomum fastosum	16.2	0.9	0.00*	13.1	1.0	0.07	2.1 ± 0.7	0.009 ± 0.006	0.06

* Significant

Table 4.4: Prevalence, mean intensity, abundance of infection ± standard error of the mean (SEM) and comparative analysis of cat's endoparasites between seasonal factors in Peninsular Malaysia

Parasite species	Prevalence			Mean Intensity			Abundance ± SEM		
	Dry (%)	Wet (%)	*P* value	Dry	Wet	*P* value	Dry	Wet	*P* value
Ancylostoma braziliense	34.1	24.3	0.02*	11.5	13.0	0.72	3.9 ± 0.7	3.1 ± 1.0	0.55
Ancylostoma ceylanicum	34.1	26.0	0.07	21.4	17.6	0.47	7.3 ± 1.4	4.6 ± 1.0	0.12
Toxocara malaysiensis	30.8	19.7	0.007*	7.7	7.8	0.94	2.4 ± 0.3	1.5 ± 0.5	0.16
Toxocara cati	12.7	5.2	0.006*	3.2	4.7	0.49	0.4 ± 0.09	0.2 ± 0.1	0.31
Strongyloides sp.	1.4	0.6	0.67	112.2	404.0	1.00	1.5 ± 1.3	2.3 ± 2.3	0.82
Physaloptera praeputialis	1.6	2.9	0.34	6.8	5.2	0.68	0.1 ± 0.07	0.2 ± 0.08	0.73
Taenia taeniaeformis	7.8	5.8	0.48	2.0	2.1	0.94	0.2 ± 0.05	0.1 ± 0.05	0.61
Dipylidium caninum	4.6	11.0	0.009*	9.8	16.7	0.59	0.4 ± 0.1	1.8 ± 1.3	0.42
Platynosomum fastosum	9.7	9.8	1.00	12.2	13.4	0.87	1.2 ± 0.5	1.3 ± 0.6	0.88

* Significant

Table 4.5: Prevalence of endoparasites recovered from stray cats captured in Kuala Lumpur, Georgetown, Kuantan and Malacca Town of Peninsular Malaysia.

Endoparasites	Kuala Lumpur (n=241)	Georgetown (n=102)	Kuantan (n=100)	Malacca Town (n=100)
Nematode				
Ancylostoma braziliense	30.7%	22.5%	39.0%	31.0%
Ancylostoma ceylanicum	35.3%	19.6%	33.0%	33.0%
Toxocara cati	8.3%	13.7%	17.0%	5.0%
Toxocara malaysiensis	24.1%	37.3%	42.0%	10.0%
Strongyloides sp.	2.5%	-	-	-
Physaloptera praeputialis	-	1.0%	1.0%	9.0%
Cestode				
Dipylidium caninum	11.6%	2.0%	5.0%	1.0%
Taenia taeniaeformis	5.4%	2.9%	7.0%	16.0%
Trematode				
Platynosomum fastosum	13.3%	2.0%	5.0%	14.0%

Table 4.6: Quantitative analysis of endoparasites recovered from stray cats captured in Kuala Lumpur of Peninsular Malaysia.

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	K
Nematode						
Ancylostoma braziliense	978	30.7% (24.94-36.95)	13.22 (9.23-20.01)	4.06 (2.61-6.04)	1-124	0.10
Ancylostoma ceylanicum	2701	35.3% (29.24-41.67)	31.78 (23.76-46.62)	11.21 (7.78-16.02)	1-270	0.10
Toxocara cati	60	8.3% (5.14-12.53)	3.0 (1.90-4.60)	0.25 (0.13-0.44)	1-11	0.05
Toxocara malaysiensis	319	24.1% (18.81-29.98)	5.50 (4.07-7.66)	1.32 (0.90-1.95)	1-35	0.11
Strongyloides sp.	965	2.5% (0.91-5.34)	160.83 (19.5-324.5)	4.00 (0.41-12.46) #	2-477	0.003
Cestode						
Dipylidium caninum	416	11.6% (7.86-16.36)	14.86 (6.14-47.29) #	1.73 (0.67-5.61) #	1-229	0.03
Taenia taeniaeformis	45	5.4% (2.90-9.05)	3.46 (1.92-6.54)	0.19 (0.08-0.43) #	1-16	0.03
Trematode						
Platynosomum fastosum	578	13.3% (9.26-18.23)	18.06 (10.00-34.41)	2.40 (1.25-4.94)	1-171	0.03

- limits uncertain due to low sample size

Table 4.7: Quantitative analysis of endoparasites recovered from stray cats captured in Georgetown of Peninsular Malaysia.

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Nematode						
Ancylostoma braziliense	173	22.5% (14.86-31.89)	7.52 (5.26-10.39)	1.70 (1.02-2.70)	1-22	0.09
Ancylostoma ceylanicum	254	19.6% (12.40-28.65)	12.70 (7.90-20.55)	2.49 (1.33-4.61)	1-56	0.06
Toxocara cati	48	13.7% (7.71-21.96)	3.43 (2.00-7.50) #	0.47 (0.22-1.09) #	1-18	0.07
Toxocara malaysiensis	528	37.3% (27.88-47.39)	13.89 (9.63-19.34)	5.18 (3.38-7.79)	1-57	0.13
Physaloptera praeputialis	6	1.0% (0.02-5.35)	6.00 #	0.06 (0.00-0.18)	6-6	**
Cestode						
Dipylidium caninum	10	2.0% (0.23-6.91)	5.00 #	0.10 (0.00-0.25)	5-5	**
Taenia taeniaeformis	4	2.9% (0.61-8.36)	1.33 (1.0-1.67)	0.04 (0.00-0.10)	1-2	**
Trematode						
Platynosomum fastosum	10	2.0% (0.23-6.91)	5.00 (1.00-5.00) #	0.10 (0.00-0.38) #	1-9	**

- limits uncertain due to low sample size

** - fit to the negative binomial cannot be tested due to low categories

Table 4.8: Quantitative analysis of endoparasites recovered from stray cats captured in Kuantan of Peninsular Malaysia.

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	K
Nematode						
Ancylostoma braziliense	579	39.0% (29.40-49.27)	14.85 (9.08-24.90)	5.79 (3.47-10.12)	1-104	0.13
Ancylostoma ceylanicum	289	33.0% (23.91-43.12)	8.76 (5.91-12.36)	2.89 (1.82-4.52)	1-33	0.13
Toxocara cati	69	17.0% (10.22-25.82)	4.06 (2.24-7.29)	0.69 (0.32-1.46)	1-18	0.08
Toxocara malaysiensis	238	42.0% (32.19-52.29)	5.67 (4.36-7.55)	2.38 (1.70-3.38)	1-24	0.24
Physaloptera praeputialis	2	1.0% (0.02-5.45)	1.00 #	0.01 (0.00-0.03)	2-2	‡
Cestode						
Dipylidium caninum	47	5.0% (1.64-11.29)	9.40 (3.80-18.20)	0.47 (0.12-1.28) #	2-24	0.02
Taenia taeniaeformis	7	7.0% (2.86-13.90)	1.00 #	0.07 (0.02-0.12)	1-1	‡
Trematode						
Platynosomum fastosum	30	5.0% (1.64-11.29)	6.00 (1.80-12.00)	0.30 (0.05-0.95) #	1-17	0.02

- limits uncertain due to low sample size

‡ - sample not aggregated enough to fit the negative binomial

Table 4.9: Quantitative analysis of endoparasites recovered from stray cats captured in Malacca of Peninsular Malaysia.

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	K
Nematode						
Ancylostoma braziliense	254	31.0% (22.12-41.04)	8.19 (4.0-20.68) #	2.54 (1.17-6.14)	1-102	0.12
Ancylostoma ceylanicum	243	33.0% (23.91-43.12)	7.36 (4.39-12.73)	2.43 (1.33-4.45)	1-51	0.13
Toxocara cati	14	5.0% (1.64-11.29)	2.80 (1.40-4.00)	0.14 (0.03-0.35)	1-5	0.03
Toxocara malaysiensis	47	10.0% (4.90-17.63)	4.70 (1.20-17.60) #	0.47 (0.10-1.84) #	1-34	0.04
Physaloptera praeputialis	59	9.0% (4.19-16.40)	6.56 (3.44-13.33)	0.59 (0.21-1.49) #	1-24	0.03
Cestode						
Dipylidium caninum	10	1.0% (0.02-5.45)	10.00 #	0.10 (0.00-0.30)	10-10	**
Taenia taeniaeformis	24	16.0% (9.43-24.68)	1.50 (1.13-2.13)	0.24 (0.13-0.40)	1-4	0.28
Trematode						
Platynosomum fastosum	50	14.0% (7.87-22.38)	3.57 (1.86-5.86)	0.50 (0.24-1.00)	1-11	0.07

- limits uncertain due to low sample size

** - fit to the negative binomial cannot be tested due to low categories

4.4 Discussion

Levels of parasitism in the stray cat population in Peninsular Malaysia were moderately high however, species diversity was low compared to prior studies (Rohde, 1962; Amin-Babjee, 1978 and Shanta *et al.*, 1980). Three endoparasite species were found most prevalent in all urban cities namely *Ancylostoma ceylanicum*, *Ancylostoma braziliense* and *Toxocara malaysiensis* with results showing the frequency distributions of these three species were overdispersed.

Variation in intrinsic (host age) factor was shown to play a significant role in determining the endoparasite infracommunity in the stray cats populations with most parasites were highly infected in adults compared with juveniles except for *Toxocara spp*. This was likely to be linked with adult cats accumulating parasites species during foraging, without exhibiting any acquired immunity. Juvenile cats showed significantly higher in the levels of infection with *Toxocara malaysiensis* and *Toxocara cati*, following successful transplacental transmission from adult female to the young as well as transmammary transmission to the kittens.

The main effect of season was linked with differences in the density of the host population between seasons, with twice as many cats being caught in the dry season, during a period active foraging. Such a large decrease in host density in the wet season was reflected in lower prevalence and abundance values of the parasite species, despite the fact favored moist environmental conditions for the survival and infectivity of both eggs and larvae of the nematode species. However, since the climate in Malaysia generally warm and humid throughout the year, the differences between dry and wet

season may not affect the survival and infectivity of eggs and larvae infecting the stray cat population.

Overall, the stray cat population in this study were infected with six nematode species, two cestode species and one species of trematode. Stray cats get infected with cestodes and trematodes via ingestion of the intermediate hosts. Lower cestodes and trematodes infection observed in this study could be due to availability of the intermediate host. However, for hookworms transmission was depended on infected cats excreting eggs through their faeces that are left on moist ground for 2-8 days and upon a susceptible host either ingesting larvae or being actively infected through the skin (Changizi *et al.*, 2007). Meanwhile for the nematode *Toxocara spp.* produces large numbers of eggs that can survive for a long time in the environment. Transmission of this parasite is via transmammary to newborn kittens (Labarthe *et al.,* 2004).

From our study, *Ancylostoma ceylanicum* was the most prevalent endoparasite found and also the most prevalent in Kuala Lumpur and Malacca. This parasite was also reported prevalent in the previous studies (Rohde, 1962; Yoshida *et al*., 1973; Amin-Babjee, 1978 and Shanta *et al.,* 1980) however with higher values. This decline might be influenced by changes such as urbanization of the cities over the past two decades as well as, climatic changes. However, the more commonly reported hookworm worldwide, *Ancylostoma tubaeform* was not recorded in this study. This could be due to the ecological and climatic differences between the environments (Labarthe *et al*., 2004). Presently, *Ancylostoma ceylanicum* infected more adult cats compared to juveniles. This was likely to be linked with adult accumulating hookworm burdens during foraging, without exhibiting any acquired immunity.

Another hookworm found prevalent in the present study was *Ancylostoma braziliense*. Prevalence of *Ancylostoma braziliense* was observed higher compared to *Ancylostoma ceylanicum* in two locations in Peninsular Malaysia, i.e. Georgetown and Kuantan. The cats from the coastal cities (Kuantan) and island (Georgetown) have higher *Ancylostoma braziliense* could be due to the hookworms favoring sandy soils found in coastal areas (Bowman *et al*., 2002) and the ability to withstand more readily the higher levels of salt found in coastal soils (Bowman *et al*., 2002). Rhode (1962), Yoshida *et al.* (1973), Amin-Babjee (1978) and Shanta *et al*. (1980) also reported high prevalence of this helminth. *Ancylostoma braziliense* also showed higher prevalence in adults compared to juveniles. This could be due to acquired infections whilst foraging without exhibiting any acquired immunity.

Present study also reported the prevalence of *Toxocara malaysiensis* in the urban cities of Peninsular Malaysia especially, for Georgetown and Kuantan. The presence of a thick protective layer on the egg surface serves as protection to the harsh environment. Higher infections observed in juveniles compared to adult hosts were possible due to successful transplacental transmission from adult females to the foetus as well as, transmammary transmission to the kitten during suckling. Previous studies also noted this trend. However, both studies reported this nematode as *Toxocara canis* (Rohde, 1962; Lee *et al.*, 1980). Only years later, morphological (Gibbons *et al.*, 2001) and molecular (Zhu *et al*., 1998) evidences converged to indicate that *Toxocara malaysiensis* is a valid new species (Gibbons *et al*., 2001). Besides Malaysia, *Toxocara malaysiensis* also reported from cats in China (Li *et al*., 2006). The occurrence of this species from China was the first report of *Toxocara malaysiensis* in cats outside from Malaysia.

The second *Toxocara* species, *Toxocara cati* was also reported in the present study as reported in the last three previous studies in Peninsular Malaysia (Rohde, 1962; Amin-Babjee, 1978; Shanta *et al.*, 1980) with similar infection rates. Similarly, *Toxocara cati* were highly infected in juveniles compared to adults due to successful transmission from the mother to the kittens during suckling and transmammary transmission. *Toxocara cati* is implied as a causative agent of visceral larva migrans in human beings (Fisher, 2003).

Another nematode recovered included *Physaloptera praeputialis* was reported in all study areas except Kuala Lumpur as reported in the prior studies (Rohde, 1962; Retnasabapathy and Khoo, 1970; Amin-Babjee, 1978 and Shanta *et al*., 1980). The parasite infection was generally low and confined to the stomach. The paratenic hosts probably played an essential role in the infection of cats (Bowman *et al*., 2002). Lower infections observed compared to previous studies could be due to availability of the intermediate hosts such as cockroaches and beetles to the hosts (Retnasabapathy & Khoo, 1970).

Only two cestodes found in the present study, *Dipylidium caninum* and *Taenia taeniaeformis*. Both species were found in all four cities with the infestation of *Taenia taeniaeformis* higher compared to *Dipylidium caninum*. Both species also reported on previously (Rohde, 1962; Amin-Babjee, 1978 and Shanta *et al.*, 1980). However, the diversity of cestode species found in the present study was lower. Rohde (1962) reported four cestode species (*Dipylidium caninum, Joyeuxiella pasqualei, Diphyllobothrium spp., Taenia taeniaformis*) meanwhile Shanta *et al.* (1980) reported

three species infecting cats, *Dipyllidium caninum, Joyeuxiella pasqualei, Taenia taeniaeformis* and *Spirometra mansoni*.

Dipylidium caninum is one of the most common parasites of domesticated dogs and cats (Bowman *et al*., 2002). This parasite has been reported worldwide and can also infect foxes, dingoes, hyenas, wild cats, jungle cats, Indian palm cats, civet cats and wild dogs (Boreham & Boreham, 1990). *Taenia taeniaeformis* is the only species from the genus Taenia typically reported from the domestic cat worldwide. The decline of the cestode diversity over the three decades could be influenced by the availability of the intermediate host or in the change of the cat's diet. The diet change is thought to be due to the abundance of leftover cooked food available to the cat in food courts or the public feeding leftovers to the strays.

Platynosomum fastosum is the only parasite from the class trematode found in the present study in the bile ducts of stray cats as previously reported (Rohde, 1962; Amin-Babjee, 1978; Shanta *et al.*, 1980; Zamri Saad *et al*., 1984). However, the prevalence of *Platynosomum fastosum* found in this study was lower compared to previous studies. Cats are known to acquire this parasite by the ingestion of lizards (Zamri Saad *et al*., 1984). The decline in prevalence of this parasite could be due to change of eating habit of the host. This parasite was also reported in Hawaii, West Africa, South America, the Caribbean and areas surrounding the Gulf of Mexico, including the southeastern United States and the Florida Keys (Bielsa & Greiner, 1985).

Of all, only three helminths were zoonotic namely, *Ancylostoma braziliense*, *Ancylostoma ceylanicum* and *Toxocara cati*. The hookworms, *Ancylostoma braziliense*

and *Ancylostoma ceylanicum* can cause cutaneous larva migrans on humans by penetrating the skin and causes popular eruptions at the sites of larval penetration (Wijers & Smit, 1966).

Toxocara cati causes toxocariasis in human. Toxocariasis is one of the most commonly reported zoonotic helminth infections in the world (Magnaval *et al*., 2001). Two forms of diseases had been described in human namely visceral larva migrans (VLM) and ocular larva migrans (OLM). Bisseru (1968) reported test with toxocara antigen amongst 199 apparently healthy individuals showed 5.52% reacted positively.

The zoonotic role these parasites found in this study should be highlighted to the public especially by health agencies and veterinarians especially to those living in close contact with these animals (McGlade *et al*., 2003, Jittapalapong *et al*., 2008). Educators and veterinarians also play an important role to educate especially pet owners, the importance of regular deworming of pets to reduce the prevalence of zoonotic helminth infections and practice of feces disposal of pets especially in public areas such as parks (Labarthe *et al*., 2004) to prevent contamination of the enviroment.

4.5 Conclusion

Endoparasites infestation in the stray cat population was high (74.59%). Nine helminths comprised six nematode species (*Toxocara malaysiensis, Toxocara cati, Ancylostoma braziliensis, Ancylostoma ceylanicum, Strongyloides sp., Physaloptera praeputialis*) two cestode species (*Taenia taeniaeformis, Dipylidium caninum)* and one trematode species (*Platynosomum fastosum)* were found in Kuala Lumpur, Georgetown, Kuantan and Malacca. Most helminth parasites were present in all study sites except for the sole presence of *Strongyloides sp.* and the absence of *Physaloptera praeputialis* in Kuala Lumpur.

Variation in intrinsic (host age) and extrinsic (season) factors have been shown to play a significant role in determining the endoparasite infracommunity in the stray cats populations in Peninsular Malaysia. Adult cats were found infected with higher number parasites compared to juveniles. However, juvenile cats showed significantly higher prevalence and abundance levels of infection to *Toxocara malaysiensis* and *Toxocara cati.* For season, infection of *Ancylostoma braziliense, Toxocara malaysiensis* and *Toxocara cati* were higher during the dry season meanwhile the cestode, *Dipylidium caninum* was higher during the wet season.

CHAPTER 5

CO-OCCURRENCE RELATIONSHIPS BETWEEN INTESTINAL HELMINTH SPECIES IN THE STRAY CAT POPULATION

5.1 Introduction

Interactions in the species community exist among plants, herbivore consumers, and predators. There also exists interaction between parasite species (Johnson & Buller, 2011) within a host where two or more species of parasite sharing the same niche in the same host individual more often than expected by chance (Bush *et al*., 2001). Positive parasite interactions indicate that as one parasite species increases, the other species increases; as one species decreases the other species decreases. Meanwhile in negative parasite interactions indicate that as one parasite species increases, the other species decreases, and vice-versa.

There is a possibility of co-occurring species do not interact, either because they are not abundance enough to exert mutual pressure on one another or because they differ in resource use and their fundamental niches do not overlap (Poulin, 2001). Furthermore, the reproductive success of helminths may vary as a function of each worm's position in the gut (Sukhdeo, 1991).

There have been many interests in investigating the patterns of species co-occurrence however most studies focus on single-host and single-pathogen interactions and there are evidences suggesting that interaction co-occurring between parasites can influence host pathology, parasite transmission, and the evolution of virulence (Cox, 2001;

Pedersen & Fenton, 2007; Lello *et al.*, 2008; Telfer *et al*., 2008; Lively, 2009). These investigations also have led to the development of numerous methods to determine patterns occurring by random chance (Mackenzie *et al*., 2004).

Abu-Madi *et al*. (2008) observed positive co-occurrences between two cestode species; *Diplopylidium sp*. and *Taenia taeniaeformis* and also between *Taenia taeniaeformis* and the hookworm, *Ancylostoma tubaeforme* in the stray cat population in Qatar. Positive interaction was observed between cestode and nematode species in helminths sharing similar niches along the alimentary tract.

Higher abundance level of parasites may result in alteration of intestinal sites within the host (Abu-Madi *et al*., 2008). However, a study of interactions between helminth parasites of wood mice in the south of England showed that interactions between parasites were unlikely to play a dominant role in structuring the component community of helminth (Behnke *et al*., 2005).

In this chapter, interaction between parasite species in intestinal tract of the stray cat population in Peninsular Malaysia is reported for the first time in Malaysia. The main objective of this study is to investigate the co-occurrences between the four intestinal helminth species prevalent in this study (*Ancylostoma braziliense*, *Ancylostoma ceylanicum*, *Toxocara malaysiensis* and *Toxocara cati*) sharing a similar niche in the small intestine. Secondly, the objective is to examine relationships that may exist in the trend of mutual proliferation of endoparasite species between the hookworm varieties (*Ancylostoma braziliense* and *Ancylostoma ceylanicum*) and the ascarids (*Toxocara cati* and *Toxocara malaysiensis*).

5.2 Methodology

Four helminth species were chosen as they shared similar niche of the intestinal tract of the stray cat. Database of four helminth parasites include two hookworm species; *Ancylostoma braziliense* and *Ancylostoma ceylanicum* and two ascarid species; *Toxocara malaysiensis* and *Toxocara cati* were analysed to determine the interactions between the species.

Interactions between parasite species were evaluated using the bivariate Pearson's product-moment correlation technique on the abundance of parasites, relative to host gender, season and site. This correlation was able to determine relationships in terms of mutual proliferation of endoparasite species. Analysis was done for the two hookworm species; *Ancylostoma ceylanicum* and *Ancylostoma braziliense*, the two ascarid species; *Toxocara malaysiensis* and *Toxocara cati*, as well as between the four prevalent species in this study (*Ancylostoma braziliense*, *Ancylostoma ceylanicum*, *Toxocara malaysiensis* and *Toxocara cati*).

The values generated from a Pearson's ranged from -1.0 to +1.0. The closer the value was to +1 or -1, the more closely the two variables were related. If the value was close to 0, meant that there was no relationship between the variables. Positive values meant that as one variable gets larger the other gets larger and if the value was negative, as one gets larger, the other gets smaller (which is often called an "inverse" correlated). Correlation values above 0.80 are considered high.

5.3 Results

5.3.1 Relationship in the mutual interaction between *Ancylostoma spp*.

Ancylostoma spp was the most prevalent endoparasite recovered in the stray cat population with 210 (38.67%) from 543 stray cats were infested. The species *Ancylostoma ceylanicum* (31.5%) infections were slightly higher compared to *Ancylostoma braziliense* (30.8%). The prevalence and abundance values showed the distribution of stray cats infected with single infection of *Ancylostoma braziliense* only, *Ancylostoma ceylanicum* only and mix infection of both *Ancylostoma braziliense* and *Ancylostoma ceylanicum* (Table 5.1 & Table 5.2).

From the analysis, mixed infection of both *Ancylostoma spp.* (23.57%) showed higher prevalence compared to single infection of *Ancylostoma braziliense* (7.18%) and *Ancylostoma ceylanicum* (7.92%) from all cities (Table 5.1). Similarly, higher abundance values of infection observed in the mixed infection (8.88±1.27) compared to single infection of *Ancylostoma braziliense* (0.30±0.07) and *Ancylostoma ceylanicum* (0.90±0.24) (Table 5.2).

Further investigation was carried out to examine these relationships that exist in the trend of mutual proliferation of endoparasite species of the hookworm variety. After applying Pearson's correlation to the prevalence and abundance values from four sites, the correlation coefficients generated between *Ancylostoma braziliense* and *A*ncylostoma *ceylanicum* ranged from moderate ($p=0.772$) to strong ($p=0.926$) showing that as *Ancylostoma braziliense* increased, *Ancylostoma ceylanicum* followed similarly.

5.3.2 Relationship in the mutual interaction between *Toxocara spp.*

Toxocara spp. was recorded the second most prevalent helminth species with 195 (35.91%) from 543 stray cats were infected. The infections of *Toxocara malaysiensis* (27.3%) was higher compared to infection of *Toxocara cati* (10.3%). The distribution of stray cats infected with single infection of *Toxocara cati* only, *Toxocara malaysiensis* only and mix infection of both *Toxocara cati* and *Toxocara malaysiensis* were analyzed by prevalence (Table 5.3) and abundance (Table 5.4).

Prevalence and abundance values showed mixed infections between both species was lower compared to single infection from all urban cities. Higher prevalence of single infection *Toxocara malaysiensis* (25.60%) compared to single infection of *Toxocara cati* (8.66%) and mixed infection of both species (1.66%). Similarly, higher abundance was observed in single infection of *Toxocara malaysiensis* (2.02±0.27) compared to single infection of *Toxocara cati* (0.32±0.07) and mix infection of both species (0.10±0.04).

Further analyses to examine any relationship existing in terms of mutual proliferation of endoparasite species of the ascarids variety was done by applying Pearson's correlation of the data from the four sites. The correlation coefficients between *Toxocara cati* and *Toxocara malaysiensis* were strong with p=0.982 indicating that as the value of *Toxocara cati* increases or decrease, similar situation happened to *Toxocara malaysiensis*.

5.3.3 Co-occurrence between parasite species

Of the 14 species of parasites were recovered in present study, only five species played a more prominent role which included one ectoparasite species, *Ctenocephalides felis* and four endoparasite species namely *Ancylostoma braziliense*, *Ancylostoma ceylanicum*, *Toxocara malaysiensis* and *Toxocara cati*. Analyses were carried out to determine the co-occurrence between the four intestinal nematode species by calculating the Pearson's product moment correlation coefficients.

Results the analysis showed strong correlation coefficient occurred between *Toxocara cati* and *Toxocara malaysiensis* (p=0.982). There were also strong correlation coefficient in a mix infection between *Toxocara cati – Toxocara malaysiensis* and *Toxocara cati – Ancylostoma ceylanicum* (p=0.994) and between *Toxocara cati – Ancylostoma braziliense* and *Toxocara cati – Ancylostoma ceylanicum* (p=0.919). The full coefficient matrix is summarized in Table 5.5.

Table 5.1: The prevalence (%) distribution of stray cats infected with single infection of *Ancylostoma braziliense* only, *Ancylostoma ceylanicum* only and mixed infection of both *Ancylostoma braziliense* and *Ancylostoma ceylanicum*.

		Single infection *Ancylostoma braziliense*	Single infection *Ancylostoma ceylanicum*	Mix infection *Ancylostoma braziliense-Ancylostoma ceylanicum*
Kuala Lumpur	Total infected (n=241)	12	23	62
	Prevalence (%)	4.98	9.54	25.73*
Georgetown	Total infected (n=102)	9	6	14
	Prevalence (%)	8.82	5.88	13.73*
Kuantan	Total infected (n=100)	10	4	29
	Prevalence (%)	10.0	4.0	29.0*
Malacca	Total infected (n=100)	8	10	23
	Prevalence (%)	8.0	10.0	23.0*
All Locations	Total infected (n=543)	39	43	128
	Prevalence (%)	7.18	7.92	23.57*

* Highest prevalence by location and infection

Table 5.2: The abundance distribution of stray cats infected with single infection of *Ancylostoma braziliense* only, *Ancylostoma ceylanicum* only and mixed infection of both *Ancylostoma braziliense* and *Ancylostoma ceylanicum*.

		Single infection *Ancylostoma braziliense*	Single infection *Ancylostoma ceylanicum*	Mix infection *Ancylostoma braziliense-Ancylostoma ceylanicum*
Kuala Lumpur	Intensity (n=241)	35	322	3322
	Abundance	0.15±0.06	1.34±0.48	13.78±2.60*
Georgetown	Intensity (n=102)	30	73	324
	Abundance	0.29±0.13	0.72±0.40	3.18±1.00*
Kuantan	Intensity (n=100)	75	10	783
	Abundance	0.75±0.32	0.10±0.06	7.83±2.09*
Malacca	Intensity (n=100)	21	81	395
	Abundance	0.21±0.09	0.81±0.36	3.95±1.34*
All Locations	Intensity (n=543)	161	486	4824
	Abundance	0.30±0.07	0.90±0.24	8.88±1.27*

* Highest abundance by location and infection

Table 5.3: The prevalence (%) distribution of stray cats infected with single infection of *Toxocara cati* only, *Toxocara malaysiensis* only and mixed infection of both *Toxocara cati* and *Toxocara malaysiensis*.

		Single infection *Toxocara cati*	Single infection *Toxocara malaysiensis*	Mix infection *Toxocara cati-Toxocara malaysiensis*
Kuala Lumpur	Total infected (n=241)	19	57	1
	Prevalence (%)	7.88	23.65*	0.41
Georgetown	Total infected (n=102)	13	37	1
	Prevalence (%)	12.75	36.27*	0.98
Kuantan	Total infected (n=100)	11	36	6
	Prevalence (%)	11.0	36.0*	6.0
Malacca	Total infected (n=100)	4	9	1
	Prevalence (%)	4.0	9.0	1.0
All Locations	Total infected (n=543)	47	139	9
	Prevalence (%)	8.66	25.60	1.66

* Highest prevalence by location and infection

Table 5.4: The abundance distribution of stray cats infected with single infection of *Toxocara cati* only, *Toxocara malaysiensis* only and mixed infection of both *Toxocara cati* and *Toxocara malaysiensis*.

		Single infection *Toxocara cati*	Single infection *Toxocara malaysiensis*	Mix infection *Toxocara cati-Toxocara malaysiensis*
Kuala Lumpur	Intensity (n=241)	59	317	3
	Abundance	0.24±0.08	1.32±0.26*	0.01±0.01
Georgetown	Intensity (n=102)	47	524	5
	Abundance	0.46±0.20	5.14±1.13*	0.05±0.05
Kuantan	Intensity (n=100)	53	210	44
	Abundance	0.53±0.24	2.10±0.42*	0.44±0.23
Malacca	Intensity (n=100)	13	45	3
	Abundance	0.13±0.07	0.45±0.34*	0.03±0.03
All Locations	Intensity (n=543)	172	1096	55
	Abundance	0.32±0.07	2.02±0.27*	0.10±0.04

* Highest abundance by location and infection

Table 5.5: Pearson's product-moment correlation coefficients between intestinal helminthes species in stray cats from Peninsular Malaysia.

		T.cati	*T.malaysiensis*	Both *Toxocara spp*	*T.cati-A.braziliense*	*T.cati- A.ceylanicum*
T.cati	Pearson Correlation	1	0.982*	0.277	0.531	0.356
	Sig. (2tailed)		0.018	0.723	0.469	0.644
	N	4	4	4	4	4
T.malaysiensis	Pearson Correlation	0.982*	1	0.410	0.676	0.492
	Sig. (2tailed)	0.018		0.590	0.324	0.508
	N	4	4	4	4	4
Both *Toxocara spp*.	Pearson Correlation	0.277	0.410	1	0.873	0.994**
	Sig. (2tailed)	0.723	0.590		0.127	0.006
	N	4	4	4	4	4
T.cati-A.braziliense	Pearson Correlation	0.531	0.676	0.873	1	0.919
	Sig. (2tailed)	0.469	0.324	0.127		0.081
	N	4	4	4	4	4
T.cati-A.ceylanicum	Pearson Correlation	0.356	0.492	0.994**	0.919	1
	Sig. (2tailed)	0.644	0.508	0.006	0.081	
	N	4	4	4	4	4

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

5.4 Discussion

From the present study, the prevalence and abundance of mixed infections of *Ancylostoma braziliense* and *Ancylostoma ceylanicum* were more prevalent compared to single infections. Positive co-occurrence was observed between *Ancylostoma braziliense* and *Ancylostoma ceylanicum* ranging between moderate (0.772) to strong (0.926) relationship indicating that one species proliferates by suppressing the other which may be a consequence of the density dependent process. As one species increases, the other species also will increase and if one decrease, the other decrease too.

For the ascarid worms, mixed infection of *Toxocara cati* and *Toxocara malaysiensis* showed lower prevalence compared to single infection. Relationship occurred in the similar niche in the mixed infections of both species showed positive co-occurrence correlation (0.982). This result also indicates that as one species increases, the other species increases meanwhile if one species decreases, the other species decreases too.

Using Pearson's product-moment correlation coefficient analysis, significant positive co-occurrences were identified between the ascarid nematodes *Toxocara cati* and *Toxocara malaysiensis*, and also between *Toxocara cati* and the hookworm *Ancylostoma ceylanicum*. The latter species also showed a significant co-occurrence with *Ancylostoma braziliense* occupying a similar niche in the alimentary tract. These significant results are surprising in view of the relatively low abundance levels shown by all four nematode species (Table 5.3) whereby mean worm burdens ranged from 0.2±0.05 in *Toxocara cati* to 9.8±1.6 in *Ancylostoma ceylanicum*.

Similar significant co-occurrences were observed by Abu- Madi *et al.* (2008) between the two intestinal cestodes, *Taenia taeniaeformis* and *Dipylidium sp.*, in cat populations in Qatar, but the abundance values were considerably higher at 25.1 and 13.7 respectively. It is likely that a larger degree of worm establishment within the intestine as occurred in the latter cat population may result in a stronger interaction between parasite species, followed by an alteration of intestinal niches within the host. They concluded that further studies were needed to confirm whether or not quantitative interactions between intestinal helminths in cats were density–dependent, thereby influencing fecundity and the structuring of helminth communities. On the other hand, Behnke *et al.* (2005) showed that interactions between parasites were unlikely to play a significant role in structuring the component community of helminths at least in wood mice.

Nevertheless, apart from parasite interactions in feline host, further investigations were necessary to study the ecology and epidemiology of parasites in cats in Peninsular Malaysia by expending the samples examined over a longer and continuous period. This would undoubtedly provide appropriate data for analyzing the impact of season and climate change on infection levels in addition to significantly contributing to the potential threat of transmission of zoonotic infections from feline hosts to the human population in urban cities of Malaysia.

5.5 Conclusion

In this study, mixed infection of *Ancylostoma braziliense* and *Ancylostoma ceylanicum* were found prevalent compared to single infection in the prevalence and abundance value. Meanwhile, mixed infection of *Toxocara cati* and *Toxocara malaysiensis* showed the lowest prevalence compared to single infections. Both interactions showed positive co-occurrence with strong correlation value.

Positive co-occurrences have been shown to occur between the four endoparasite species (*Ancylostoma braziliense, Ancylostoma ceylanicum, Toxocara malaysiensis and Toxocara cati)* occupying similar niches within the alimentary tract of cats, but whether or not quantitative interactions occur between these parasite species requires further investigation.

CHAPTER 6

MOLECULAR CHARACTERIZATION OF *Toxocara malaysiensis*

6.1 Introduction

There are many ways to identify different parasitic-intestinal nematode species and one method is by molecular characterization of species using a DNA-based identification. This method is essential to complement the morphological studies.

In developing a PCR based diagnostic technique, the part of ribosomal DNA cistron (ITS region) was targeted. This ITS region (Internal Transcribed Spacer) contain both conserved (18S, 5.8S, 28S, and variable ITS 1 and ITS 2). Different species can be characterized with distinct DNA sequence. The Internal Transcribed Spacer (ITS) is sequence of RNA in a primary transcript that lies between precursor ribosomal subunits. These sequences are coded by ribosomal DNA. Eukaryotic organisms have two internal transcribed spacer which is ITS1 and ITS2. These ITS located between the repeating array of nuclear 18S and 28S ribosomal DNA genes. Other than ITS1 and ITS2, the rDNA cistron also contains external transcribed spacers ETS1 and ETS2 and non-transcribed spacers NTS region (Figure 6.1).

ITS data had been used in constructing phylogenetic trees, estimating genetic population structures, evaluating population-level evolutionary processes and determining taxonomic identity. ITS region was reported to be variable and heterogeneous between parasitic nematode genera and this is useful as an identification tool. The advantage of using this ITS region includes the high copy number of rDNA,

about 30,000 per cell enable the easy amplification of ITS region (Dubouzet & Shinoda, 1999).

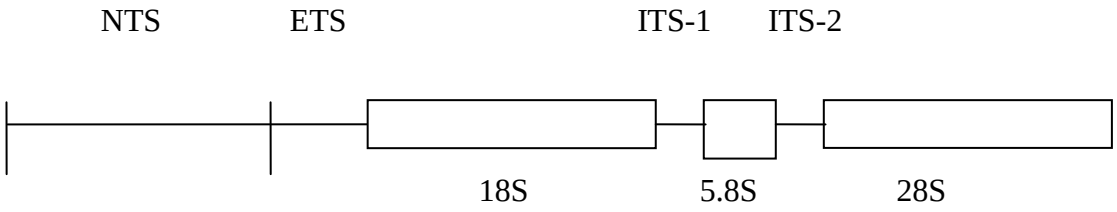

Figure 6.1: Diagram of the ribosomal DNA gene family. The regions coding for the 5.8S, 18S and 28S subunits of rRNA are shown by bars; NTS= non-transcribed spacer, ETS= external transcribed spacer, ITS= internal transcribed spacer regions.

Apart from this, ITS region consists of universal primer binding sites (Kaplan *et al*., 2000). This allows most of the species to be analyzed based on the same primers. The single PCR can decrease the diagnostic time since this technique as relatively easy to operate and more accurate. Moreover, the most important advantage is it allows identification of most species easily if compare to traditional identification methods that based on differences in morphological and morphometric characters.

Of all intestinal helminths recovered in this study, *Toxocara spp.* were one of the major health significance for cats especially kitten. Besides, toxocariasis in humans and animals caused by zoonotic transmission of *Toxocara cati* and *Toxocara canis*. In this chapter, the molecular characterization of *Toxocara malaysiensis* from some of this species recovered from this study was reported. The objective of this study was to amplify the ITS1 and ITS2 regions of *Toxocara malaysiensis* rDNA by using universal PCR primers.

6.2 Methodology

6.2.1 DNA Extraction

Identified nematode was transferred into fresh 1.5 ml microcentrifuge tube filled with 50µl of sterile distilled water. The nematode species was labeled on the tube. Total genomic DNA of the nematodes was extracted by using DNA Extraction method (Madani *et al*., 2005). The nematode was transferred into Eppendorf fuse containing 20 µl dH2O. The tissue sample was frozen in liquid nitrogen and was grounded to a fine powder with a mortar and pestle. 12 µl of Proteinase K (600 µg/ml) was added in 1X PCR buffer in a final volume 132 µl. Proteinase K was added to the mixture to break polypeptides down and followed by incubation for 2 hours at 65°C. After the incubation, the sample was incubated in 95°C for 15 minutes. The DNA sample was then left in 4°C overnight and stored in -20°C for future used.

6.2.2 Polymerase Chain Reaction (PCR)

PCR was performed in a 50µl reaction mixture in a 0.2 ml tube. One PCR reaction mixture consists of distilled water, PCR buffer, $MgCl_2$, dNTPs, primers, enzyme Taq Polymerase and the DNA. To prepare a master mix for 1 volume reaction, 26 µl of dH2O, 5 µl of PCR buffer, 2 µl of MgCl2, 8 µl of dNTPs, 2.5 µl of forward primer, 2.5 µl of reverse primer and 2 µl of Taq (enzyme) were mixed together in 1.5 ml microcentrifuge tube. Then the master mix was vortexed. 48 µl of master mix was put into each 0.2ml microcentrifuge tubes including for negative sample. About 2 µl of DNA was added into each tube except for negative sample. For negative sample, 2 µl of dH_2O was added. All the tubes were vortexed and spun followed by putting in the PCR machine (Thermal Cycler). Negative sample is a control as it will determine whether

there is a contamination in the mixture or not. If there is a band in the negative when was viewed under the UV light, that means the mixture was contaminated. The DNA amplification profile consisted of denaturation step at 94°C for three minutes, following by 38 cycles of one minute at 94°C, one minute at 55°C, one minute at 72°C and final elongation step for five minutes at 72°C. PCR products obtained were then subjected to agarose gel electrophoresis.

The primers used were;

18S Forward Primer (Fallas *et al*., 1996): 5' TTGATTACGTCCCTGCCCTTT 3'

28S Reverse Primer (Kaplan *et al*., 2000): 5' TATACGAATTCAAGTCGC 3'

Table 6.1: Components for PCR

Component	**Concentration master mix (µl) 1X**
Buffer (10X)	5
MgCl2 (50 µM)	2
dNTP	8
Primer-forward (20 µM)	2.5
Primer- reverse (20 µM)	2.5
DNA(50ng/ µl)	2
Taq polymerase (1u/ µl)	2
dH2O	26
Total	**50.0 µl reaction**

6.2.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out to determine successful DNA amplification and the specificity of PCR reaction. Agarose gel (1%) was prepared by boiling agarose powder in 1X Tris Borate EDTA (TBE). An amount of 1 µl of Ethidium Bromide was

added to the gel mixture and was allowed to cool sufficiently before being poured into the tray. A comb was placed to form wells for loading.

PCR products were mixed with loading dye before being loaded into the wells. A 100bp ladder (Forever, SeeGene South Korea) was used as molecular weight references marker to ensure the correct size of PCR product. Electrophoresis was performed at 120V for 30 minutes, the gel was then visualized under UV light (Alpha Imager) and a photograph was taken to document the results.

6.2.4 Gel Extraction (GE)

Gel Extraction was done using QIAquick Gel Extraction Kit Protocol. This protocol was designed to extract and purify DNA of 70bp to 10kb from standard or low-melt agarose gels in TAE or TBE buffer. First, the DNA fragment from the agarose gel was excise with a clean and sharp scalpel. The sliced gel was weighed in a colourless tube.

3 volumes of Buffer QG were added to 1 volume of gel (100mg ~ 100µl). After that, the gel was incubated at 50°C for 1 minute or until the gel slice has completely dissolved. To help gel dissolved, mix by vortexing the tube every 2-3 minutes during the incubation.

After the sliced gel has dissolved completely, the colour of the mixture was check to become yellow which is similar to Buffer QG without dissolved agarose. After that, 1 gel volume of isopropanol was added to the sample and mix. QIAquick spin column was placed in a provided 2ml collection tube.

To bind DNA, the sample was apply to the QIAquick column and centrifuged for 1 minute. The flow-through was discarded and QIAquick column was placed back in the same collection tube. 0.5ml Buffer QG was added to QIAquick column and centrifuge for 1 minute. This step was optional where it will remove all traces of agarose.

To wash, 0.75ml of Buffer PE was added to QIAquick column and centrifuge for 1minute. The flow-through was discarded and the QIAquick column for an additional 1 minute at 13,000rpm (~17,900 x g). Then, QIAquick column was placed into a clean 1.5ml microcentrifuge tube.

To elute DNA, 50µl of Buffer EB (10mM Tris.Cl, pH 8.5) or H2O was added to the centre of the QIAquick membrane and the column was centrifuged for 1 minute. Alternatively for increased DNA concentration, 30µl elution buffer was added to the centre of the QIAquick membrane, the column was stand for 1 minute and then the column was centrifuge for 1 minute.

After gel extraction, a 1% agarose gel was prepared for post GE. 1 µl of 6X loading dye, glycerol and 5 µl of DNA was mixed. 2 µl of Forever 100bp ladder was used as a marker. The electrophoresis was carried out at 120V for 30 minutes. The gel was then visualized under UV illumination and a photograph was taken to document the result. Post GE was done to ensure the quality of DNA was good for the next step i.e. DNA ligation step for cloning. Bands that were thin and bright showed that the amount of DNA was small.

6.2.5 Cloning

The PCR product was cloned by using Promega pGEM ®-T Easy Vector System (Promega, USA).

6.2.5.1 Preparation of Luria Bertani (LB) Agar plates

LB agar plate was used in culturing *Escherichia coli* bacteria for blue white colony selection. LB agar powder (Invitrogen, California) was added to the distilled water. A total of 6.4g of LB powder was mixed with 200ml of distilled water in a Scott bottle. The mixture was stirred by magnetic stirrer until a homogenous mixture was obtained. The mixture was then sterilized by autoclaving for 15minutes at 121°C. After the sterilization process completed, the mixture was cooled down under running tap water. 100 µl of ampicilin 100mg/ml, 1000 µl IPTG (Isopropyl-beta-thio galactopyranoside) 0.1M and 320 µl X-gal (5-Bromo-4-chloro-Indoly-β-D-Galactoside) 50mg/ml (Promega, USA) were added respectively.

These components were crucial in cloning as ampicilin allows the detection of tranformant and the IPTG induce the activity β-galactosidase. On the other hand, X-gal, a lactose analogue will be broken down by β-galactosidase to form a deep blue coloured product that aids the identification of recombinant plasmids. The mixture was then mixed and poured into each plate. These agars were allowed to solidify at room temperature before storing them in 4°C for future used.

6.2.5.2 Preparation of LB Broth Medium

LB Broth was used to propagate the plasmid to a higher volume. 5g of Luria Broth Base (Gibco-BRL, Scotland) was added to 200ml of distilled water to prepare 200ml of LB

broth medium. The mixture was then stirred by a magnetic stirrer until the powder was completely dissolved. Following this, 100ml of LB broth was transferred into approximately 20 universal bottles and were autoclaved at 121°C for 15minutes. Finally, the LB broths prepared were labeled and stored at room temperature for future used.

6.2.5.3 DNA Ligation

This ligation step using pGEM®-T and pGEM®-T Easy Vector and 2X Rapid Ligation Buffer. pGEM®-T Easy Vector and Control Insert DNA tubes were centrifuged to collect contents at the bottom of the tube. A 0.5 ml tube was used and 2X Rapid Ligation Buffer were vortexes. For a full reaction, 5.0 µl 2X Rapid Ligation Buffer was mixed together with 1.0 µl pGEM®-T Easy Vector, 3.0 µl PCR product and 1.0 µl T4 DNA Ligase. The reaction was further incubated at room temperature for 1 hour or incubated at 4°C overnight. The brightness of the band that we got from the strand will determine whether full reaction or half reaction was to be used.

6.2.5.4 Transformation

The ligation reaction was centrifuged and all content at the bottom of the tube was collected. Then, 2 µl aliquot of ligation reaction was transferred into a new sterile 1.5ml microcentrifuge tube on ice. The competent cell was taken out from the -70°C and was put on ice until about just thawed (approximately 5 minutes). About 100 µl of competent cells were carefully transferred into the sterile 1.5 ml microcentrifuge tube. The tube was gently flicked to mix content and followed by placing in ice for 20 minutes. The cells were heat shocked for 45-50 seconds in water bath at exactly 42°C. The tubes then were immediately returned to ice for 2 minutes. After that, 950 µl LB

Broth medium at room temperature was added to the tubes containing cells transformed with ligation reactions followed by incubating for 1.5 hours at 37°C with shaking (≈150 rpm). Then, 100 µl of each transformation culture were plated onto duplicate LB plate. The bunsen burner is left opened while doing this step to keep the condition sterile. Lastly, the plates were incubated overnight (16-24 hours) at 37°C.

6.2.5.5 Selection of Transformants and Recombinants

White colonies showing the presence of an insert has been successfully ligated in the vectors. A mini library (6X6=36) of successfully cloned product was made by transferring each of the selected colony onto a fresh gridded plate. The rest of the colony was placed into a sterile 0.5ml microcentrifuge tube containing 30 µl of distilled water. The tubes then were heated at the heat block at 99°C for 10 minutes followed by centrifugation for 15 seconds. About 2 µl of the mixture will be used for PCR using M13 Forward and Reverse primer. This will enable identification of real insert via comparison with the expected product size. Then, the colony PCR was done by using Buffer B, dNTPs, M13 Forward and Reverse primer, Taq and dH2O.

6.2.5.6 Plasmid Extraction.

Plasmid extraction procedure was conducted in order to extract the plasmid out from bacteria cell. The colonies were cultured first prior to extraction of plasmid. About 5µl of 100 mg/ml (stock) Ampicilin was put into LB Broth bottle. The colonies were transferred from mini library into LB Broth bottle. The colonies were cultured overnight for 16hours at water bath with shaker (220 rpm, 37°C).

Next day, the culture was spun at 6000 rpm for 15 minutes and the supernatant was discarded and the pellet was resuspended with 200 µl solution I (0°C) followed by vortexing and transferring the suspension to 1.5 ml centrifuge tubes. After resuspending the pellet, 200 µl of solution II was added and was mixed gently at room temperature for 4 minutes. About 200 µl of solution III (0°C) was added and was gently mixed on ice for 15 minutes. Then, all the tubes were spun at 13,000 rpm for 10 minutes. The supernatant was transferred to fresh 1.5 ml centrifuge tube and 10 µl of RNase was added. Then, the tubes were incubated at 37°C for 3 hours. 1 volume of phenol was added followed by vortexing for 2-3 minutes and spinning for 5 minutes. The supernatant was transferred to fresh 1.5 ml centrifuge tube. After adding 1 volume of chloroform, the tubes were vortexed for 2-3 minutes and were spun for 5 minutes. Then the upper layer was transferred.

This was followed by adding 0.1 volumes of 5 M NaCl and 2.5 volume of isopropanol and was left in ice for 20 minutes. Then, the tubes were spun at 13,000 rpm for 15minutes. The supernatant was then discarded and the tubes were inverted on paper towel to drain. About 1000 µl of 70% ethanol was added and was spun for 5 minutes at 13,000 rpm. The supernatant was discarded and the pellet (pure DNA) was collected followed by air drying in speed vacuum for 10 minutes. Lastly, 30 µl of autoclaved distilled water was added and was kept in 4°C for further use. Quantitation and qualitation of plasmids was carried out with OD reading. It was conducted using spectrophotometer that required setting and it was conducted according to manufacturer’s instructions.

6.2.5.7 Restriction Enzyme Digestion

Restriction enzyme digested was carried out to confirm the present of the cloned gene. As the gene was cloned in between two EcoR1 restriction sites, EcoR1 restriction enzyme was used to cut once in vector and once in order to cut out the cloned gene.

A total of 4.0 µl of Buffer (5X), 0.2 µl of BSA, 0.5 µl of EcoR1 (500U/ µl), 2.0 µl of DNA (5 µg) and 13.3 µl of dH2O were mixed together in microcentrifuge tube. It was vortexed and spun down. Then it was incubated at 37°C for 3 hours. The amount of DNA was based on the OD reading. 1U is defined as the amount of enzyme that required digesting 1µg of DNA in 1 hour at 37°C in 50 µl of assay buffer. After the incubation, the mixture was incubated at 65°C at heat block for 10 minutes as to inactivate the enzyme. Then 1% agarose gel was prepared and 1 µl of 2X loading buffer was mixed with 5 µl of the DNA (plasmid). 2 µl of Forever 100bp ladder was used as marker for the length of plasmids. The electrophoresis was carried out at 120V for about 30 minutes. After electrophoresis, the gel was visualized under UV illumination for detection of stained bands.

6.2.6 Sequence Analysis

All the DNAs were sent for sequence conformation. DNA sequence were edited with Chromas 2.31 (©1998-2005 Technelysium Pty Ltd) and subjected for comparison of homology using BLASTn program with reference sequences obtain from the National Center for Biotechnology (NCBI). The nucleotide sequences were aligned by using ClustalX V 1.83 (Thompson *et al*., 1997) software to compare and analyses the occurrence of the heterogeneity among the cloned sequences.

6.3 Results

6.3.1 Polymerase Chain Reaction (PCR)

Extracted DNAs were subjected to PCR. Amplification of ITS 1 and ITS 2 regions using Fallas-Kaplan (Fallas *et al*., 1996; Kaplan *et al*., 2000) primer pair yielded expected PCR products from the DNA of *Toxocara malaysiensis* obtained. Samples of *Toxocara malaysiensis* to be cloned were TM7 and TM8.

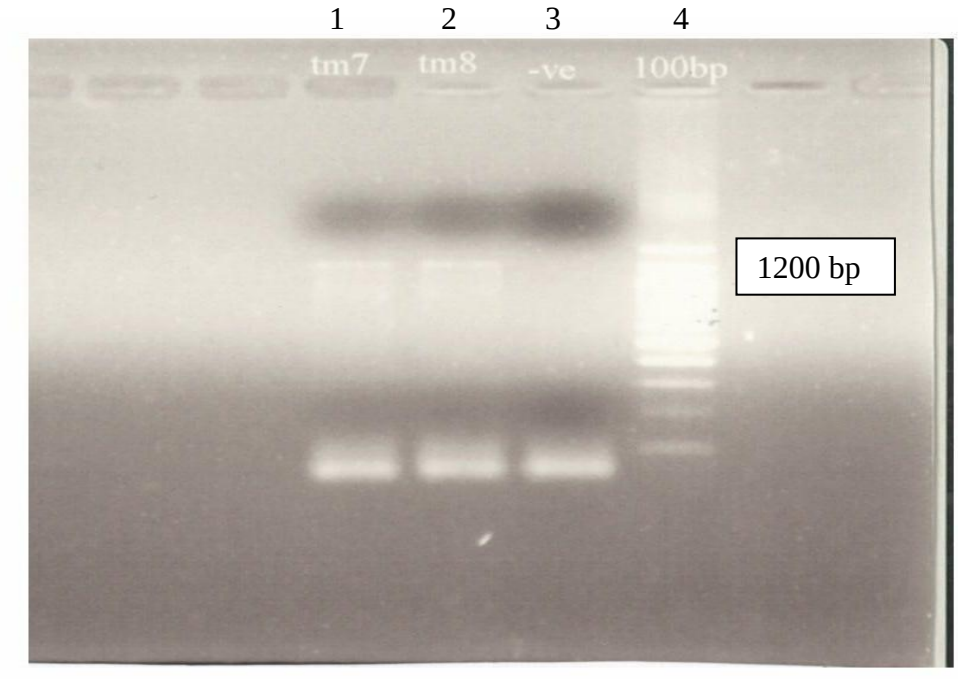

Figure 6.2: Gel Electrophoresis of *Toxocara malaysiensis* PCR products using Fallas and Kaplan primer pair.

Lane 1: TM7

Lane 2: TM8

Lane 3: Negative control

Lane 4: 100bp ladder (Forever, SeeGene South Korea)

Successfully amplified ITS 1 and ITS 2 regions from *Toxocara malaysiensis* (Lane 1 = TM7 and Lane 2 = TM8) showed expected bands amplified by universal primers with the size approximately 1200bp. Initial results suggested that the size of the amplified products can determine the species of nematodes from which the DNA was used as a template. Nevertheless, all the PCR products were conducted for the gel extraction and cloning in order to obtain a reliable DNA sequences that were further manipulated for sequence analysis.

6.3.2 Gel Extraction

Gel electrophoresis was conducted before the gel extraction step (pre GE) to excise the gel at the amplified size. Gel extraction was done using QIAquick Gel Extraction Kit to extract and purify DNA (Figure 6.3).

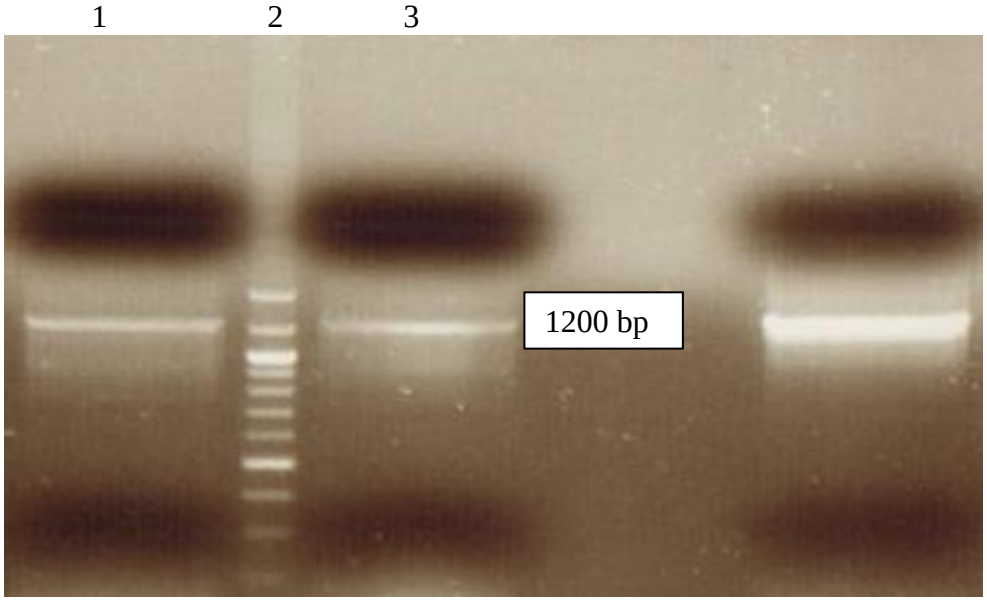

Figure 6.3: Agarose gel electrophoresis of PCR products before gel extraction.(pre-GE)

Lane 1: TM7

Lane 2: 100bp ladder (Forever, SeeGene South Korea)

Lane 3: TM8

After the gel extraction step, gel electrophoresis was conducted to show that the products still retain its initially amplified size. Lane 1 (TM7) and lane 3 (TM8) gave the expected length which approximately 1200bp (Figure 6.4). Gel extracted PCR products were ligated into pGEM-T Easy Vector (Promega USA) and transformed into JM109 *Escherichia coli* strain (competent cell).

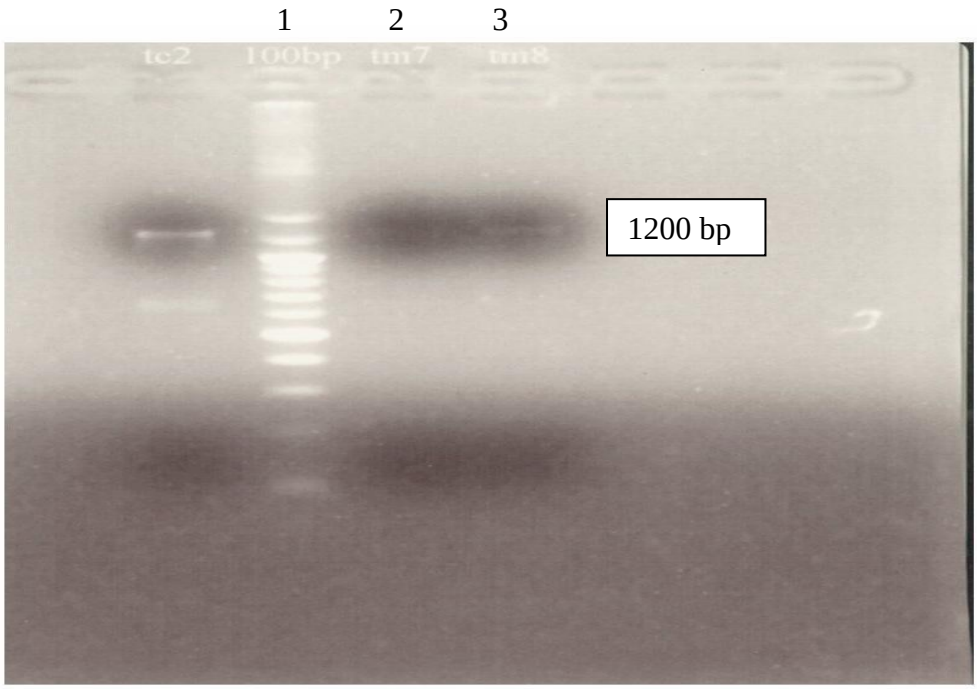

Figure 6.4: Agarose gel electrophoresis of PCR products after gel extraction. (post-GE)

Lane 1: 100bp ladder (Forever, SeeGene South Korea)

Lane 2: TM7

Lane 3: TM8

6.3.3 Colony Screening

6.3.3.1 Colony Library

The colony library was used to grow the selected colonies on the LB agar plate with 4 X 4 grids for a longer period. Because there is a possibility that a white colony will turn to blue one after a longer period, the library can be used as a guideline for the selection of the acquired colony before the plasmid extraction. Normally, the white colony indicated the presence of inserted fragment while the blue colonies indicated the absent of the inserted fragment. Nevertheless, PCR screening with M13 primers was needed to confirm the correct insert (Figure 6.5). The colonies that contained the correct insert were produced to plasmid extraction.

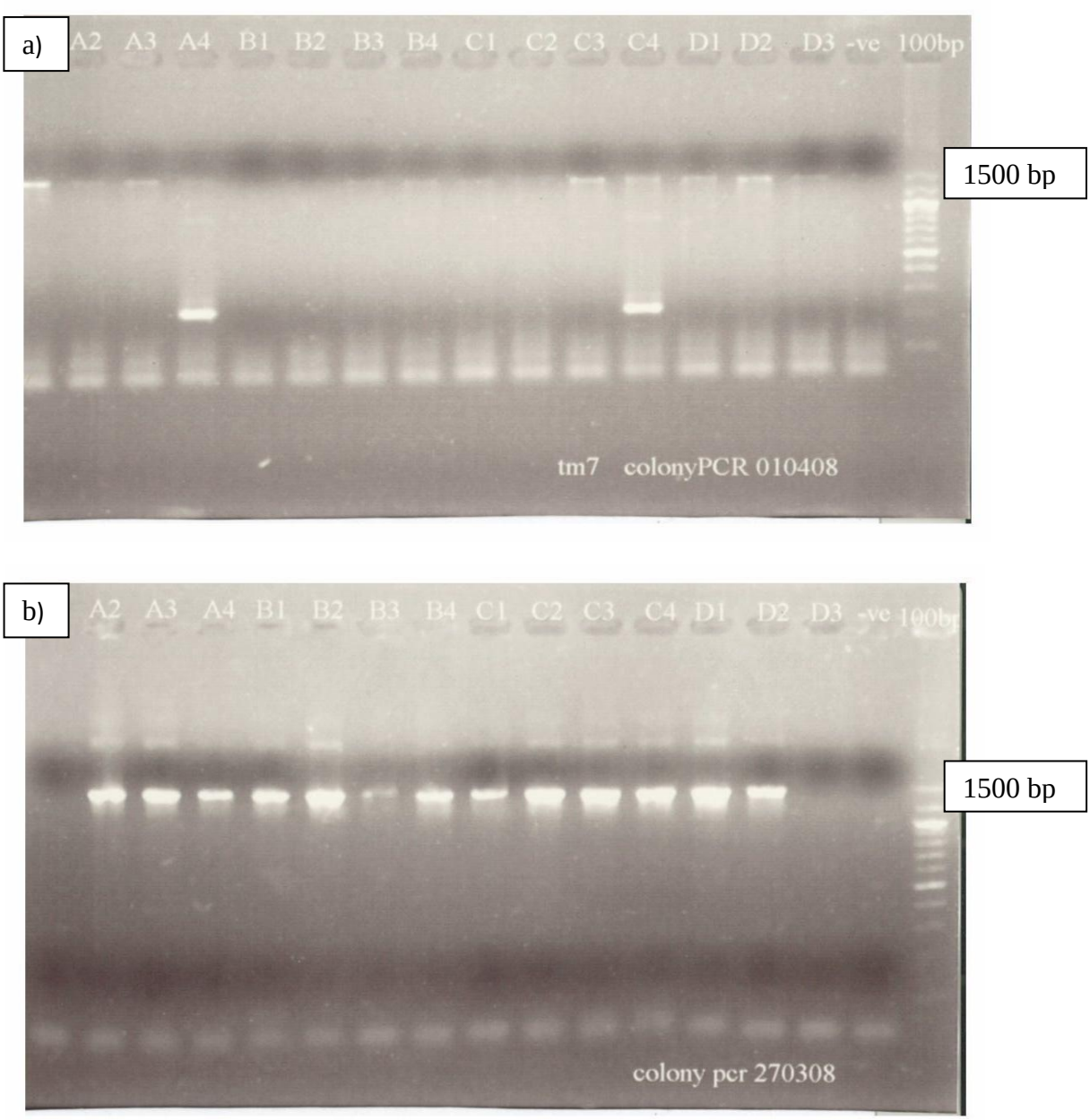

Figure 6.5: Colony screening of a) *Toxocara malaysiensis* (TM7) and b) *Toxocara malaysiensis* (TM8) by using M13 primers. Consistent PCR products of 1500bp in length were stained for those clones that had desired fragments.

6.3.3.2 Restriction Enzyme Digestion

Restriction enzyme digestion was carried out to confirm the present of the cloned gene using EcoR1 digestion enzyme. Figure 6.6 showed all digested clones produced similar result except for Figure 6.6b, all clones were homogenous except TM8C4 clone was heterogeneous.

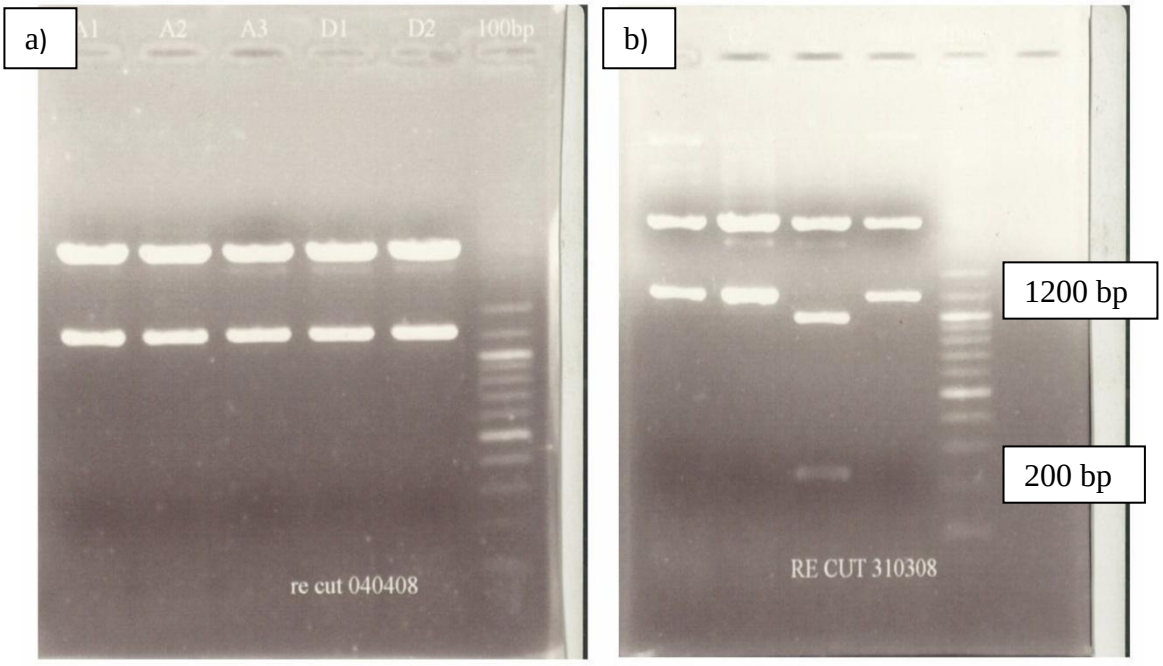

Figure 6.6: a) *Toxocara malaysiensis* (TM7) and b) *Toxocara malaysiensis* (TM8) clones after the restriction digestion by EcoR1. All cloned gene were cut at the correct size and the product length is 1200bp except TM8C4 showed two bands at 1000bp and 200bp.

6.3.4 Sequence Analysis

Cloned sequences obtained were submitted for BLAST search (www.ncbi.nlm.nih.gov). Sequence homology search was conducted by using BLASTn program. From homology search conducted for *Toxocara malaysiensis*, 99% homology obtained between the cloned sequences and the sequence in the database at ITS1 and ITS2 region of *Toxocara sp. cf. canis.* However, 97% homology was obtained between the cloned sequence and the sequence in the database at the ITS2 region of *Toxocara malaysiensis*. As *Toxocara malaysiensis n. sp.* was previously assumed to be *Toxocara sp. cf. canis*, there are quite similarities in the results of sequences homology of the sequence in the database. However, the query coverage showed a very low value with 39% and 27% respectively (Table 6.2).

Table 6.2: Sequence analysis of *Toxocara malaysiensis* using the BLASTn program

Species	**Clone**	**Homology**	**Accesion No.**	**Scores (bits)**	**E-value**	**Identities (%)**
Toxocara malaysiensis	TM7A1 TM7A2 TM7A3 TM7D1 TM7D2 TM8C2	*Toxocara sp. cf. canis* ITS 1	AJ002438.1	861	0.0	99%
		Toxocara sp. cf. canis ITS 2	AJ002440.1	604	2e-169	99%
		Toxocara malaysiensis ITS 2	AM231609.1	569	8e-159	97%

6.4 Discussion

Specific identification of nematodes of dogs and cats in every life stages are prerequisite in the study of their epidemiology, population biology and systematic and also diagnose and control of the diseases cause by them (Li *et al*., 2007). One of the best ways is by molecular characterization. Molecular methods could provide powerful and valuable tools for studying the life cycle, transmission patterns and zoonotic significance of parasite species (Zhu *et al*., 1998).

Amplification of ITS 1 and ITS 2 regions of *Toxocara malaysiensis* rDNA was successfully carried out for two isolated individuals. Amplification using Fallas-Kaplan (Fallas *et al*., 1996; Kaplan *et al*., 2000) primer yielded expected PCR products. The size of the amplified ITS products obtained were consistent and the estimated product length is 1200bp. This result was corroborated by Kaplan *et al*., (2000) stating that highly conserved nematode sequence enabled the construction of universal primer that will amplify the ITS rDNA.

The DNA of all the isolated *Toxocara malaysiensis* individuals was extracted from single nematodes. Theoretically, ITS region in one nematode individual should be homogenous. However, it was hypothesized that ITS region could vary in one nematode individual (Powers *et al*., 1997). Thus the amplified PCR fragments were cloned in order to get individual ITS sequences. This is because plasmid usually took up only one fragment of DNA molecules and subsequently gives rise to a single colony that contains the DNA sequences.

Although sequence homology conducted by BLASTn program showed high maximal identity value for *Toxocara malaysiensis* ranging between 87% to 99% however the query coverage value for both species were low with less than 40%. Query coverage showed the length coverage of the input query sequence by different high scoring pairs from the same database sequence (Tao & Moris, 2007).

Toxocara malaysiensis previously identified as *Toxocara canis* was characterized by molecular approach and confirmed that the species is neither *Toxocara canis* nor *Toxocara cati* (Zhu et al., 1998). In Zhu *et al.* (1998), the sequence showed that *Toxocara malaysiensis* was genetically more similar to *Toxocara cati* compared to *Toxocara canis* in the ITS-1, 5.8S and ITS-2. Moreover, morphological study (Gibbons *et al*., 2001) has confirmed that *Toxocara malaysiensis* differs from *Toxocara canis* in the shape of the cervical alae in cross section, spicule length, and the lip structure (Gibbons *et al*., 2001)

Toxocara malaysiensis also reported in cats from China which confirmed by sequence-based analyses of ribosomal DNA (Li *et al*., 2006). The occurrence of this species from China was the first report of *Toxocara malaysiensis* in cats outside from Malaysia and this showed that this species has broader geographical distribution (Li *et al*., 2006). Furthermore, specific forward primers were designed in the ITS-1 or ITS-2 for each *Toxocara canis, Toxocara cati, Toxocara malaysiensis* and *Toxascaris leonina* from dogs and cats in China, Australia, Malaysia, England and the Netherlands which can provide useful tools for the diagnosis and molecular epidemiological investigations of toxocariasis in humans and animals (Li *et al.*, 2007).

6.5 Conclusion

Amplification of ITS 1 and ITS 2 regions of *Toxocara malaysiensis* rDNA was successful using Fallas-Kaplan (Fallas *et al*., 1996; Kaplan *et al*., 2000) primer with estimated product length 1200bp. However, the query coverage value for both species were very low compared to maximal identity showed high percentage value. Hence, further analysis should be carried out in the future in order to corroborate present results obtained.