

**MACROPARASITE COMMUNITIES OF WILD RATS
FROM ISLAND AND COASTAL HABITATS IN
PENINSULAR MALAYSIA**

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**FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
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ABSTRACT

The occurrences of macroparasites communities in 363 wild rats of two urban coastal cities (Kuantan and Malacca) and two islands (Carey Island and Penang) of Peninsular Malaysia were recorded between May 2010 to August 2011. The wild rats from both habitats comprised of mainly commensal rat species namely; *Rattus norvegicus*, *Rattus rattus diardii*, *Rattus tiomanicus* and *Rattus argentiventer*. Overall, higher numbers of females (60.3%) were captured compared to males (39.7%) with more adults (87.9%) compared to juveniles (12.1%). The rats were infected with a minimum of one species and a maximum of six species of ectoparasites while up to five species of endoparasites were seen in each rat. In *R. norvegicus* the highest number of ecto and endoparasites infestation/infection was seen respectively.

There were no differences in the types of macroparasites recovered from both coastal and island rat populations. Overall, ectoparasites diversity index was low for all sites, although Carey Island recorded higher index compared to other locations with 8 numbers of species. Nine cosmopolitan ectoparasite species were recovered in total with 5 mite species, (*Laelaps nuttali*, *Laelaps echidninus*, *Ornithonyssus bacoti*, *Listrophoroides* sp., *Laelaps sculpturatus*) 2 lice, (*Polyplax spinulosa*, *Hoplopleura pacifica*,) one flea (*Xenopsylla cheopis*) and one tick (*Ixodes granulatus*). Host sex (male and female) and host age (adult and juvenile) did not influence ectoparasite infestation on the rat population. However, seasons played a significant role with *Ornithonyssus bacoti*, *Laelaps sculpturatus*, *Polyplax spinulosa*, *Hoplopleura pacifica* and *Ixodes granulatus* with higher infestation during wet compared to dry season.

Endoparasites infections comprised entirely of cosmopolitan species, namely *Nippostrongylus brasiliensis*, *Angiostrongylus malaysiensis*, *Capillaria hepatica*, *Mastophorus muris*, *Heterakis spumosa*, *Hepatojarakus malayae*, *Syphacia muris*,

Taenia taeniaeformis, *Hymenolepis diminuta* and *Rodentolepis* (= *Hymenolepis*) *nana*. Species diversity index was low in all locations. Host sex influenced endoparasitic infection only for *Capillaria hepatica*, *Syphacia muris* and *Rodentolepis* (= *Hymenolepis*) *nana* while other helminths (*Hepatojarakus malayae*, *Taenia taeniaeformis*, *Rodentolepis* (= *Hymenolepis*) *nana*) showed significant effects between host ages. Season also played a significant role determining diversity and prevalence for most endoparasite species with higher infection observed during the dry season.

Despite low diversity, seven ectoparasites (*Laelaps nuttali*, *Laelaps echidninus*, *Ornithonyssus bacoti*, *Polyplax spinulosa*, *Hoplopleura pacifica*, *Ixodes granulatus* and *Xenopsylla cheopis*) and 5 helminths species (*Capillaria hepatica*, *Angiostrongylus malaysiensis*, *Hymenolepis diminuta*, *Taenia taeniaeformis*, *Rodentolepis* (= *Hymenolepis*) *nana*) recovered were zoonotic. The close association between the rat populations with human may facilitate the transmission of zoonotic parasites circulating in these ecosystems. Therefore, long term monitoring of the rodent population is necessary in order to predict future disease prevalence.

ABSTRAK

Sebuah kajian mengenai kekerapan kehadiran komuniti makroparasit daripada 363 populasi tikus liar daripada bandar persisiran pantai (Kuantan dan Melaka) dan pulau (Pulau Carey dan Pulau Pinang) dalam Semenanjung Malaysia telah dijalankan pada bulan Mei 2010 hingga Ogos 2011. Populasi tikus liar daripada kedua-dua habitat adalah terdiri daripada sepsis tikus komensal; *Rattus norvegicus*, *Rattus rattus diardii*, *Rattus tiomanicus* dan *Rattus argentiventer*. Secara keseluruhannya, lebih banyak tikus betina (60.3%) telah ditangkap berbanding tikus jantan (39.7%) dengan lebih banyak tikus dewasa (87.9%) berbanding tikus muda (12.1%). Perumah telah dijangkiti dengan sekurang-kurangnya 1 spesis dan maksimum 6 spesis ektoparasit manakala sehingga lima sepsis endoparasit. Spesis tikus, *R. norvegicus* telah merekodkan jangkitan ekto dan endoparasit yang paling tinggi.

Tiada perbezaan diantara makroparasit yang dijumpai dalam populasi dikedua-dua habitat. Secara keseluruhannya, kepelbagaian indeks dalam ektoparasit adalah rendah, walau bagaimanapun indeks di Pulau Carey adalah yang paling tinggi berbanding tempat-tempat lain dengan 8 spesis ektoparasit. Sembilan cosmopolitan ektoparasit telah dijumpai dengan sejumlah 5 spesis tungau, (*Laelaps nuttali*, *Laelaps echidninus*, *Ornithonyssus bacoti*, *Listrophoroides* sp., *Laelaps sculpturatus*) 2 kutu, (*Polyplax spinulosa*, *Hoplopleura pacifica*,) satu pinjal (*Xenopsylla cheopis*) dan satu sengkenit (*Ixodes granulatus*). Jantina perumah (jantan dan betina) dan umur perumah (dewasa dan muda) tidak mempengaruhi kadar jangkitan ektoparasit dalam populasi tikus. Walau bagaimanapun, musim telah memainkan peranan penting dengan menunjukkan signifikansi dalam *Ornithonyssus bacoti*, *Laelaps sculpturatus*, *Polyplax spinulosa*, *Hoplopleura pacifica* dan *Ixodes granulatus* dengan kadar jangkitan yang tinggi pada musim lembab berbanding musim kering.

Jangkitan endoparasit terdiri daripada keseluruhannya kosmopolitan spesies iaitu; *Nippostrongylus brasiliensis*, *Angiostrongylus malaysiensis*, *Capillaria hepatica*, *Mastophorus muris*, *Heterakis spumosa*, *Hepatojarakus malayae*, *Syphacia muris*, *Taenia taeniaeformis*, *Hymenolepis diminuta* dan *Rodentolepis* (= *Hymenolepis*) *nana*. Jantina perumah mempengaruhi kadar jangkitan endoparasit hanya pada *Capillaria hepatica*, *Syphacia muris* dan *Rodentolepis* (= *Hymenolepis*) *nana* manakala bagi cacing lain (*Hepatojarakus malayae*, *Taenia taeniaeformis*, *Rodentolepis* (= *Hymenolepis*) *nana*) menunjukkan kesan signifikan diantara umur perumah. Musim juga memainkan peranan dan menunjukkan kesan signifikan bagi kepelbagaian dan kelaziman dalam spesies endoparasit yang paling banyak dengan jangkitan yang paling tinggi pada musim kering.

Selain dari kepelbagaian spesies yang rendah, 7 spesies ektoparasit (*Laelaps nuttali*, *Laelaps echidninus*, *Ornithonyssus bacoti*, *Polyplax spinulosa*, *Hoplopleura pacifica*, *Ixodes granulatus* dan *Xenopsylla cheopis*) dan 5 spesies cacing (*Capillaria hepatica*, *Angiostrongylus malaysiensis*, *Hymenolepis diminuta*, *Taenia taeniaeformis*, *Rodentolepis* (= *Hymenolepis*) *nana*) dijumpai adalah zoonotik. Kesan daripada hubungan yang rapat dalam populasi tikus dan manusia boleh menyebabkan penyebaran parasit zoonotik ini di dalam ekosistem. Oleh itu, pemerhatian jangka panjang dalam populasi rodent adalah perlu bagi meramalkan kelaziman penyakit pada masa hadapan.

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APPENDICES

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Appendix B	Brillouin Index
Appendix C	Published paper – Nur Syazana, M.T, Mohd Zain, S.N and Jeffery, J. (2013). Biodiversity and macroparasitic distribution of the wild rat population of Carey Island, Klang. <i>Tropical Biomedicine</i> , 30(2), 199-210

ABBREVIATIONS

n	sample size
k	negative binomial exponent
%	percentage
µm	micrometer
°C	degree celcius
cm	centimeter
mm	millimeter
P	prevalence
MI	mean intensity
CL	confidence limits
A	abundance

CHAPTER 1: INTRODUCTION

1.1 Wild Rats

Rats are one group of mammals that have successfully exploited a wide variety of habitat and environment throughout the world. Although people have largely categorized rats as pests in urban and agricultural environments, most rats live in the wild with little interaction with humans. Some have adapted to live in close association with humans, using our agriculture and waste as their own food resources and utilizing building structure as homes.

There are more than 1700 species of rodents identified in the world (RatZooMan, 2006). In Malaysia, the genus *Rattus* consists of nearly 200 sub-species and most species inhabit forest or island habitat. A total of 12 rodent species have been identified in urban and agricultural habitats and the well known commensal rat species found in Malaysia include *Rattus norvegicus*, *Rattus rattus diardii*, *Rattus argentiventer*, *Rattus exulans* and *Rattus tiomanicus* (Paramasvaran *et al.*, 2012).

Wild rats carry a large number of parasites that carry diseases. Due to its omnivorous eating habits, active social behavior, constant exposure to human/animal waste combined with its high reproductive rate, rats act as the perfect reservoirs for many dangerous pathogens.

As rodents live in close proximity to human habitation, it can spread and transmit diseases easily. The ability of rats to transmit diseases is also encouraged by their habits of utilizing our waste and sewage, their mobility and similar physiologies that humans and rodents share. These infections not only spread through direct contact with wild rat's body, excretions, bodily fluids, and bites, but also through food, water and soil contaminated by rats. Another mode of transmission occurs through arthropod vectors that live on wild rats that are capable of transmitting parasites to human. Rodents share

a wide variety of habitat from towns, cultivated land and forest, extending from the shore to the mountain peaks and from ground level to the forest (Parasmavaran *et al*, 2012). Different rodent species tend to be selective in their habitat, and when habitats are destroyed or disturbed either through agricultural intensification activities, deforestation, or urbanization it can bring about changes in diversity of rodent species (Walsh *et al*, 1993; RatZooMan, 2006).

There are substantial numbers of studies done on the morphology, physiology, genetics, and behaviour of rodents (Medway, 1978; Payne & Francis, 1985; Harrison, 1951; 1952; 1955; Yong, 1968; 1969; Dhaliwal, 1961; Lim, 1970). Additional morphological information on rat species with emphasis on fur structure, colour of the species including the tail was also identified in these studies (Medway & Lim, 1966; Lim, 1970). Additional to this, Dhaliwal (1962; 1963) has undertaken a detailed morphological comparison of *Rattus jalorensis* and *Rattus rattus diardii* from two Malaysian localities, and shown statistically that the two taxa distinct species.

Other studies by Harrison (1948: 1957; 1961: 1966), Medway (1969), Musser *et al*. (1983) and Payne & Francis (1985) also highlight the distinct characteristics of different species. The principle characters of *Rattus tiomanicus* collected in Selangor was described by Medway & Lim (1966) while Harrison (1956) added a new record of Bandicoot rats from Malaysia, Sumatra and Thailand.

Ecological and geographical distribution studies of *Rattus rattus* were carried out by Chasen (1933, 1940) and Harrison (1957) and described *Rattus rattus diardii* essentially a house rat and *Rattus jalorensis* the more successful species living in mangrove forest and scrub while, *Rattus argentiventer* a rat of grassland and ricefield but never found in villages adjacent to the ricefield. Ow-Yang (1971) showed different habitat preferences

between commensal rodent species (*Rattus rattus diradii*, *Rattus exulans*, *Rattus jalorensis* and *Rattus norvegicus*).

Rattus rattus diardii occurred in a much wider habitat range in Singapore Island compared to the mainland, due to the absence of *Rattus jalorensis* and *Rattus argentiventer* from the island (Dhaliwal, 1961). They also suggested that competition existed between closely related species and indicated that the concurrent presence of one species have a direct impact on other species.

1.2 Common parasites infecting rats

Human parasitic infections acquired through transmission from wild rats presents a huge problem in tropical countries like Malaysia (Traub *et al.*, 1974; Brown *et al.*, 1977; Tan, 1979; Singh, 1990). Wild rats are known vectors for a number of diseases (leptospirosis, hantavirus, typhus and plague) which are widespread and difficult to control or prevent due to the highly-adaptable and unpredictable nature of wild rats combined with climate most favorable for parasite growth. Today, through advances of our country's development in sanitation, health care and education, we have been able to manage and contain this problem, if not control it.

Many scientists have studied well the rat parasite's lifecycle (Nadchatram, 1960; Nadchatram & Ramalingam, 1974; Balasingam, 1965; Varughese, 1973; Ow-yang, 1974; Singh *et al.*, 1976), morphology (Ow-Yang *et al.*, 1983; Yeh, 1955), behaviour (Harrison, 1953; Kundin *et al.*, 1972) and medical significance (Lim *et al.*, 1976; Yap *et al.*, 1977; Ho & Krishnasamy, 1990). The study of incidence and distribution of diseases in large populations, define and explain the host and the parasite interactions, and determine the factors that affect the spread and severity of the infection. Results from epidemiological studies are often used to explain observations, identify the problem, predict and explore possible avenues of controlling the disease, and develop several control strategies.

Data gathered from such studies would enable us to understand the dynamics of parasite lifecycle, the role of wild rats as vectors and eventually predict and manage these rat-borne diseases. Efforts to control and predict parasitic transmission requires epidemiologists to continuously monitor parasite infections in wild rats.

Parasites can be divided into two categories which are; endoparasite and ectoparasite. Endoparasites are parasites that live inside the rat body and obtain nutrition and shelter

from the host. Traditionally, endoparasites are grouped into protozoans (such as *Tritrichomonas muris*, *Spiroplasma muris* and *Giardia muris*) and helminthes. More specifically, helminths are grouped into nematodes, cestodes, trematodes and pentastomides. Not all endoparasites infest or produce pathological symptoms in the rat. Parasites that live on skin or attach to hair follicles are known as ectoparasites. Rodent ectoparasites are categorized under 4 broad groups namely; mites, ticks, flea and lice and a large proportion are vectors to zoonotic diseases and play important role in transmission of arboviruses, streptococcal infections, choriomeningitis, plague, tularemia, leptospirosis and spirochetosis (Manson & Stanko, 2005).

Parasite infesting rats maybe crucial for the parasite's growth and survival, or the infection could be accidental. Rats also act as the definitive hosts for several parasites like *Angiostrongylus costaricensis*, but some function as intermediate hosts (*Rodentolepis* (= *Hymenolepis*) *nana*).

Studies of macroparasites in wild rats from Peninsular Malaysia have provided considerable data on the diversity and infection levels in the past (Audy, 1957; Kohls, 1957; Sandosham, 1957a; Schacher & Cheong, 1960; Nadchatram *et al.*, 1966; Dunn *et al.*, 1968; Singh & Cheong, 1971; Yap *et al.*, 1977; Sinniah *et al.*, 1978; Leong *et al.*, 1979; Zahedi *et al.*, 1984; Ho *et al.*, 1985; Krishnasamy *et al.*, 1981; 1980; Ambu *et al.*, 1996; Mariana *et al.*, 2005; 2011; Syed-Arnez & Mohd Zain, 2006; Mohd Zain, 2008; Paramasvaran *et al.*, 2009a; 2009b; Madinah *et al.*, 2011; Mohd Zain *et al.*, 2012). Most of these studies were conducted in urban cities, wildlife reserves and forest habitats. However, macroparasites studies done on island and coastal habitats are limited. Paramasvaran (2009b) compared macroparasites population between different habitats however solid conclusions could not be made due to the small sampling in particular for the coastal habitat.

1.2.1 Ectoparasites of wild rats

1.2.1.1 Mites

Mites are classified under the class arachnids and considered host specific. This means it can only infect specific host species however, sometimes can cross over from one species to another, if the choice of host is not available. Acariasis is infestation by mites cause severe itching and rashes. The tropical rodent mite *Ornithonyssus bacoti* is round in shape and appears dark when engorged with blood. This mite survives on fomites on bedding or litter, and can only be found on animals while feeding causing anemia and also transmit rickettsia blood parasites. It is the only mite specie that also bites other animals including humans. Infestation takes place via direct contact with an infected animal and also when the mite inhabits contaminated bedding or wood products.

Kingdom	Animalia
Phylum	Arthropoda
Class	Aracnida
Order	Mesostigmata
Family	Macronyssidae
Genus	<i>Ornithonyssus</i>
Species	<i>Ornithonyssus bacoti</i>

Table 1.1: Scientific classification of *Ornithonyssus bacoti*.

Radfordia ensifera is a fur mite that can cause dermatitis and is occasionally seen as white specks of dust on hair follicles. This mite is most commonly seen on rats and produces intense itching, leading to scabs and is most frequently seen on the shoulders, neck, and head region of the rat. The rat fur mite and mange mite do not infest humans or other animals.

Other mite species commonly found infesting wild rats include *Laelaps nuttali* and *Laelaps echidninus* (spiny rat mite). The distribution of the spiny rat mite is worldwide infesting *Rattus norvegicus* and other wild rat species (Ugbomoiko & Obiamiwe, 1991; Soliman *et al.*, 2001). The domestic rat mite (*Laelaps nuttali*) is also widely distributed, primarily on other *Rattus* sp. with occurrence mostly in tropical and warm temperate areas while *Laelaps sculpturatus* is commonly found on forest rodents.

Kingdom	Animalia
Phylum	Arthropoda
Class	Aracnida
Order	Mesostigmata
Family	Laelapidae
Genus	<i>Laelaps</i>
Species	<i>Laelaps nuttali</i> <i>Laelaps echidninus</i> <i>Laelaps sculpturatus</i>

Table 1.2: Scientific classification of *Laelaps* sp.

Listrophoroides sp. is a fur mite of Asian and African murine rodents belonging to the Atopomelidae family. This mite attaches itself permanently on small mammals. They have an elaborate organ for the attachment to the hairs of their hosts. The anterior leg embraces the hair of the host and clasps it against striated coxal membranes. This is the largest genus of the family which includes 16 genera and more than 150 species (Fain, 1981). This mite is widely distributed throughout the world (North and South America, Africa, Madagascar, the Oriental region, New Guinea and Australia) and is associated with rodents, shrews and primates.

Kingdom	Animalia
Phylum	Arthropoda
Class	Aracnida
Order	Astigmata
Family	Atopomelidae
Genus	<i>Listrophoroides</i>
Species	<i>Listrophoroides</i> sp.

Table 1.3: Scientific classification of *Listrophoroides* sp.

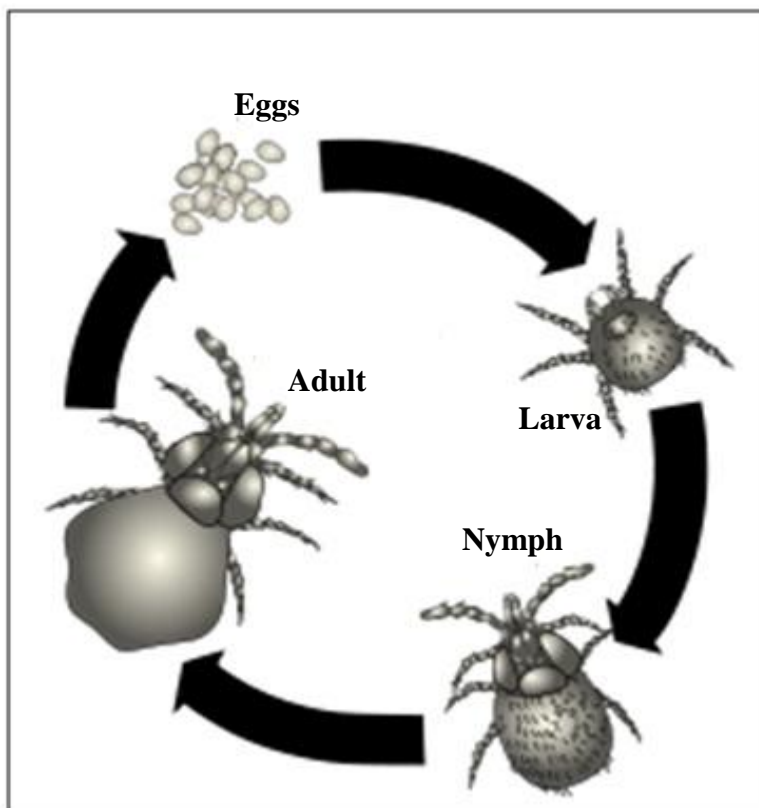


Figure 1.1: General life cycle of mites. (Source: Infection Landscape)

The length of the life cycle varies dramatically between species, ranging from a couple of months to less than a year, and is largely determined by temperature and climate. In temperate regions, this mite typically requires longer time to complete the cycle while in tropical climates the life cycle duration is usually much shorter. The time length of the life cycle also is correlated with the number of mite generations that emerges after each season. Shorter life cycle duration allows for a larger number of cycles thus, more generations of mites per season.

1.2.1.2 Ticks

Ticks are found worldwide. They are a blood-sucking, opportunistic parasite that attaches itself to the skin of a variety of vertebrate hosts. There is no segmentation on the body and is dorsal-ventrally flat with four pairs of legs.

All stages in the life cycle are blood sucking, however it is only the adult stage that causes problems to human. Human tick-associated diseases are most common during the summer months when the likelihood of contact increases during outdoor activities, usually in wooded areas. Tick bites are often painless and the presence of the tick may not be detected for some time. Ticks generally do not pose any problems to human other than causing erythematous papule and drops off after engorgement on blood. The site of attachment may itch and become painful. Secondary infections may occur, often as a result of the remaining mouthparts still attached after the tick is removed. Ticks latch on any part of the body but are frequently found near the hairline, around the ears, groin, and armpits.

Whilst the bites of most ticks are inconsequential, they can carry a number of human disease agents including viruses (Langat encephalitis), bacteria (tick typhus) and protozoa (babesiosis). These organisms cause serious and life-threatening illness to human and animals (Chul-Min *et al.*, 2006). There are two main families of ticks: the hard ticks (Ixodidae) and the soft ticks (Argasidae). The Ixodidae tick attach to the host over a prolonged period of time (several days) while the Argasidae rapidly feed and drop off. Consequently, they are frequently undetected.

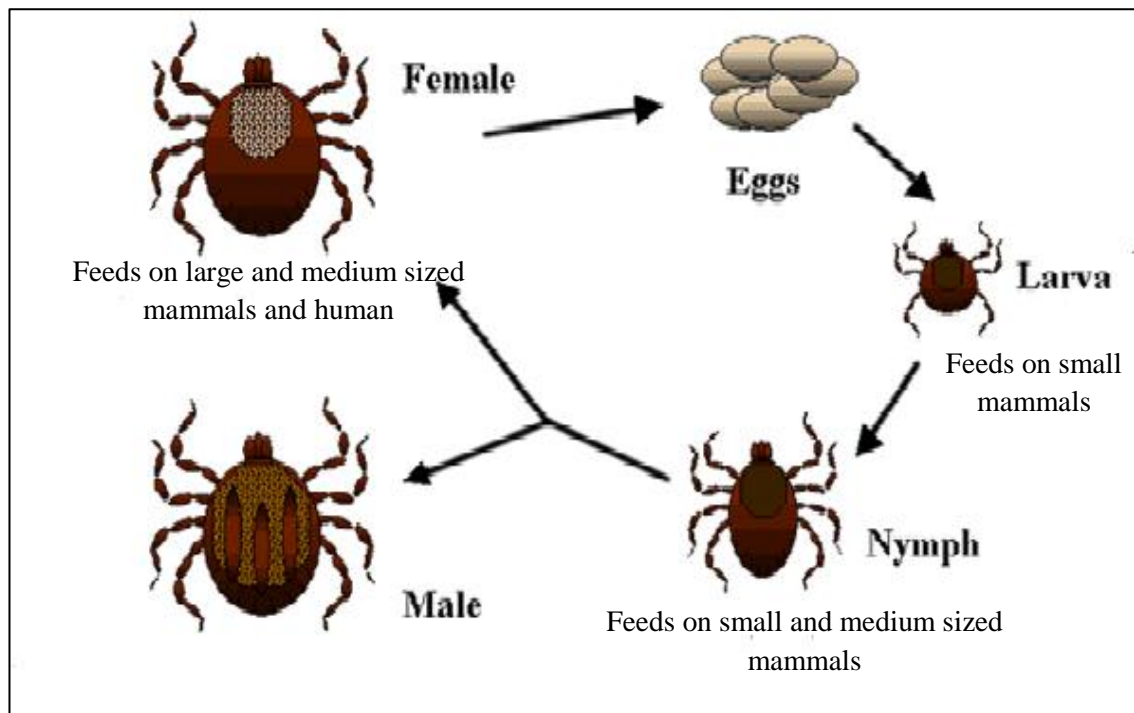


Figure 1.2: Life cycle of tick family Ixodidae. (Source: Wikimedia)

In the majority of species, especially those that feed on mammals and birds (but excluding bats), the larvae seeks the host and feed rapidly (15-30 minutes) then drops to molt in the sand, duff or cracks and crevices of the natural habitat and molt to first stage nymphs (N1) resembling miniature adults in body characteristics, especially the leathery, mammillated body cuticle, but lack genital pore and any evidence of sexual dimorphism. The N1 nymphs also attack hosts, feeding rapidly as the larvae, then retreating after their meal to molt in sheltered spaces. Nymphs molt require many moults in the life cycle before emerging as an adult. Almost all argasid ticks have multi-host feeding pattern (Hoogstraal & Aeschlimann, 1982).

The Argasidae tick goes through many nymphal stages contributing to a much longer life cycle than in the Ixodidae. In addition, many argasid ticks can persists long periods of starvation during their development enabling extension of the life cycle for many years.

Unfed females of the genus *Ixodes*, do not normally oviposit even after mating. Only following blood-feed, the mated females commence oviposition, depositing small egg clutches containing as many as several hundred eggs per batch. When oviposition is completed, the ticks remain vigorous and seek for host to feed and oviposit again. The number of gonotrophic cycles varies considerably between individuals within the species, as well as between species, although rarely exceeds 6. This pattern of repeated gonotrophic cycles is often followed by long periods of waiting between blood meals, enabling the ticks to disperse their progeny gradually over time, often across a span of many years.

Ixodes granulatus, *Amblyomma* sp. and *Dermacentor* sp. are examples of Ixodidae commonly infest rodents. In Malaysia, *Ixodes granulatus*, *Haemaphysalis* sp., *Rhipicephalus* sp. and *Ornithodoros* sp. are known to parasitize rats, squirrels and shrews in all active stages-larva, nymph and adults (Mariana *et al.*, 2005; 2008; 2011; Madinah *et al.*, 2011). *Ixodes granulatus* is medically important as the main vector for the Russian spring-summer encephalitis virus (Nadchatram *et al.*, 1966).

Kingdom	Animalia
Phylum	Arthropoda
Class	Aracnida
Order	Ixodida
Family	Ixodidae
Genus	<i>Ixodes</i> <i>Amblyomma</i> <i>Dermacentor</i>
Species	<i>Ixodes granulates</i> <i>Amblyomma</i> sp. <i>Dermacentor andersoni</i>

Table 1.4: Scientific classification of family Ixodidae.

Dermacentor andersoni are brown ticks with a light silver-gray ornamentation on the dorsal scutum, dorsal portions of the legs and on the basis capitulum (mouthpart). *D. andersoni* is known as a vector for Rocky Mountain spotted fever (*Rickettsia rickettsii*), tularaemia (*Francisella tularensis*) and Colorado tick fever (Colorado tick fever virus). The salivary glands secrete neurotoxins that cause paralysis to the host. Humans are normally attacked by the adults and will attach and feed on exposed skin areas of the neck and head region. The nymphs and larvae do not feed on humans.

1.2.1.3 Lice

The main two lice orders are Mallophaga that bite or chew, and the Anoplura (family Pediculidae) the species that suck blood. Order Anoplura commonly infest domestic animals and common in rats.

Lice are host specific and infest by direct contact or infested bedding. Lice are insects and can be seen with the naked eye. Their entire life cycle completes approximately 14 to 21 days, from egg to nymph to adult on the host. They obtain nutrition by sucking blood, causing anemia to the rat. They are also vectors to bacteria (*Hemobartonella muris*), causing a disease similar to tick fever. The louse is a small wingless primitive insect, with a flattened body, which passes through three life stages from egg to nymph and finally adult. There is no metamorphosis, the newly hatched nymph similar to the adult except in size. The egg or nit is glued to a hair or feather of the host and the insect spends its whole life on that animal unless it is removed or transferred to another similar host.

There are about 2,800 lice species worldwide. The size ranges from 0.5 to 10 mm long dorsal ventrally flattened with reduced compound eyes and no ocelli. The antennae have between, 3 to 5 segments and capitate (with a knob on the end) and recessed into the head in the Amblycera. For the Ischnocera tick, the antennae is filiform (thin and linear) and modified as a clasping organs in the male. Their mouthparts are designed for biting with no cerci. There is a suggestion that they may have evolved from the Psocoptera.

Female lice lay up to 100 eggs which are cemented to the hair or feathers of the host with a clear fast drying glue, secreted onto the hair or feather by the female immediately before laying egg. The eggs take about 3 or 4 days to hatch and the nymphs go through 3 larval instars for about 20 days before they reach maturity.

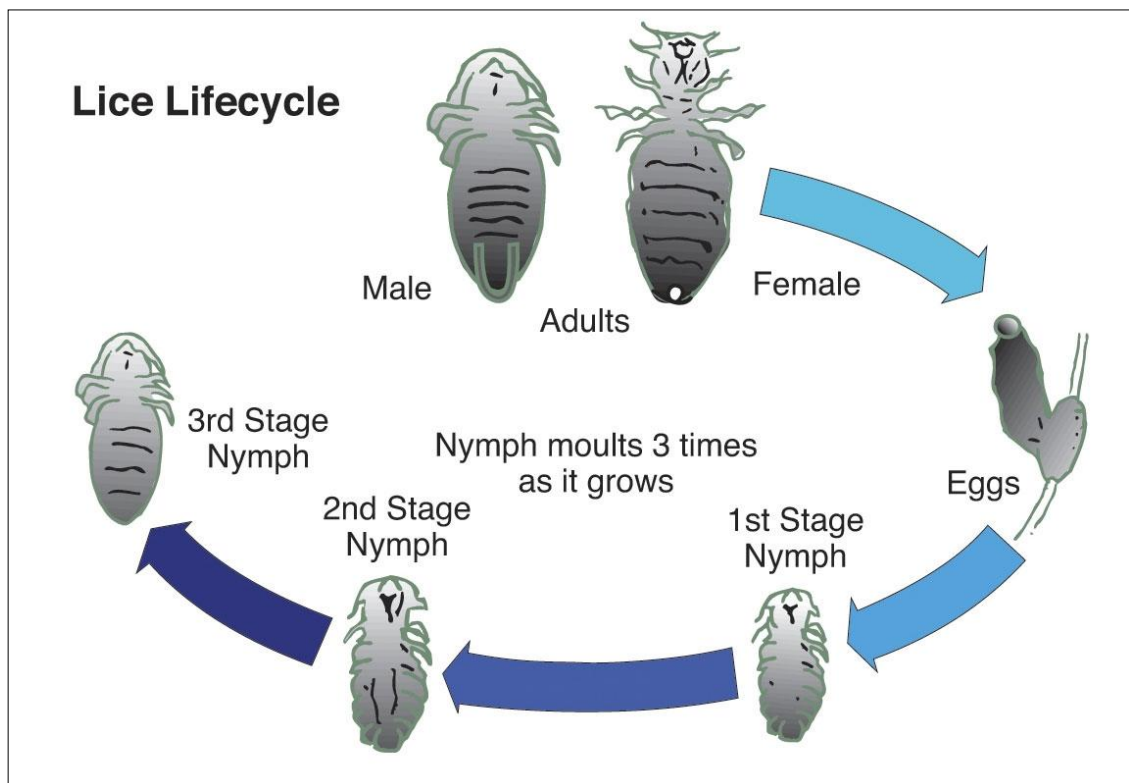


Figure 1.3: Life cycle of rat lice. (Source: Bayer animal)

One common louse species found on rats is *Polyplax spinulosa* (Burmeister, 1839) which is a known carrier vector for the bacteria, *Mycoplasma* spp. *Polyplax spinulosa* is also known as the 'spined rat louse'. It sucks on blood therefore, in addition to pruritus or itching, hair thinning and self-trauma, the infestation on mice or rats may develop anemia and become weak and lethargic. Infestation can be detected by the silvery colored nits attached to the hair. Lice can transmit blood diseases, eperythrozoonosis to mice, and haemobartonellosis to rats and also *Rickettsia typhi* that cause typhoid fever.

Two species; *Polyplax spinulosa* and *Hoplopleura pacifica* are considered to be of public health importance because they are known to harbor plague bacilli and transmit tularemia and bartonellosis to humans and play an adjunctive role in the transmission of murine typhus and plague from rat to rat (Zahedi *et al.*, 1984).

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Anoplura
Family	Polyplacidae
Genus	<i>Polyplax</i>
Species	<i>Polyplax spinulosa</i>

Table 1.5: Scientific classification of *Polyplax spinulosa*.

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Anoplura
Family	Hoplopleuridae
Genus	<i>Hoplopleura</i>
Species	<i>Hoplopleura pacifica</i>

Table 1.6: Scientific classification of *Hoplopleura pacifica*.

1.2.1.4 Flea

Thousands of fleas species have been identified worldwide and they are known to infect human and animals. The common rat flea is *Xenopsylla cheopis*. It can cause severe irritation and is responsible for flea allergy dermatitis. It is the primary vector for bubonic plague and murine typhus. Infection takes place after blood feed on an infected rodent, and following the bite, it goes through several developmental stages before becoming an adult. The adult fleas, is between 1-5mm, laterally flattened, wingless insects that infest the animal's fur. Reinfestation can occur if no further care is taken to surrounding environment of the animal. The eggs deposited on the host can fall off the host to the surrounding environment, develop and emerge as young adults either moving back to the host, or to a newly acquired host.

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Siphonaptera
Family	Pulicidae
Genus	<i>Xenopsylla</i>
Species	<i>Xenopsylla cheopis</i>

Table 1.7: Scientific classification of *Xenopsylla cheopis*.

The mouthparts have two distinct functions: one for squirting saliva or partly digested blood into the bite, the other for sucking up blood from the host. This process mechanically transmits pathogens carried by the flea. Fleas detect carbon dioxide exhaled from humans and animals and jump swiftly to the source to feed on the newly found host. The flea is wingless so it cannot fly, but can jump long distances with the

help of its small powerful legs which consists of four parts. The part that is closest to the body is the coxa followed by the femur, tibia and tarsus. A flea can jump up to 200 times its own body length and about 130 times its own height.

The flea's body is only about one tenth of an inch and is constructed to jump long distances. The body consists of three regions: head, thorax, and abdomen. The head and the thorax have rows of bristles (called combs) and the abdomen consists of eight visible segments.

There are four stages in the life cycle. Microscopic white eggs are laid by the female on the animal. However, it will dislodge itself and fall off into dust, animals bedding or crevices on the floor until it hatches between one to ten days later depending on the environment. A larva will emerge and is similar looking to a worm that is about two millimeters long. It has a small body and mouth parts but no arms or legs. At this stage, the flea do not suck blood, instead it feeds on dead skin cells, droppings, and other smaller parasites lying around them in the dust. When it matures, it will make a white silken cocoon around itself and pupates. The adult flea will emerge after a week to six months and suck the host's blood and mate with other fleas. A female flea mate only once then lays eggs every day with up to fifty eggs per day. Fleas thrive in warm environment all year round.

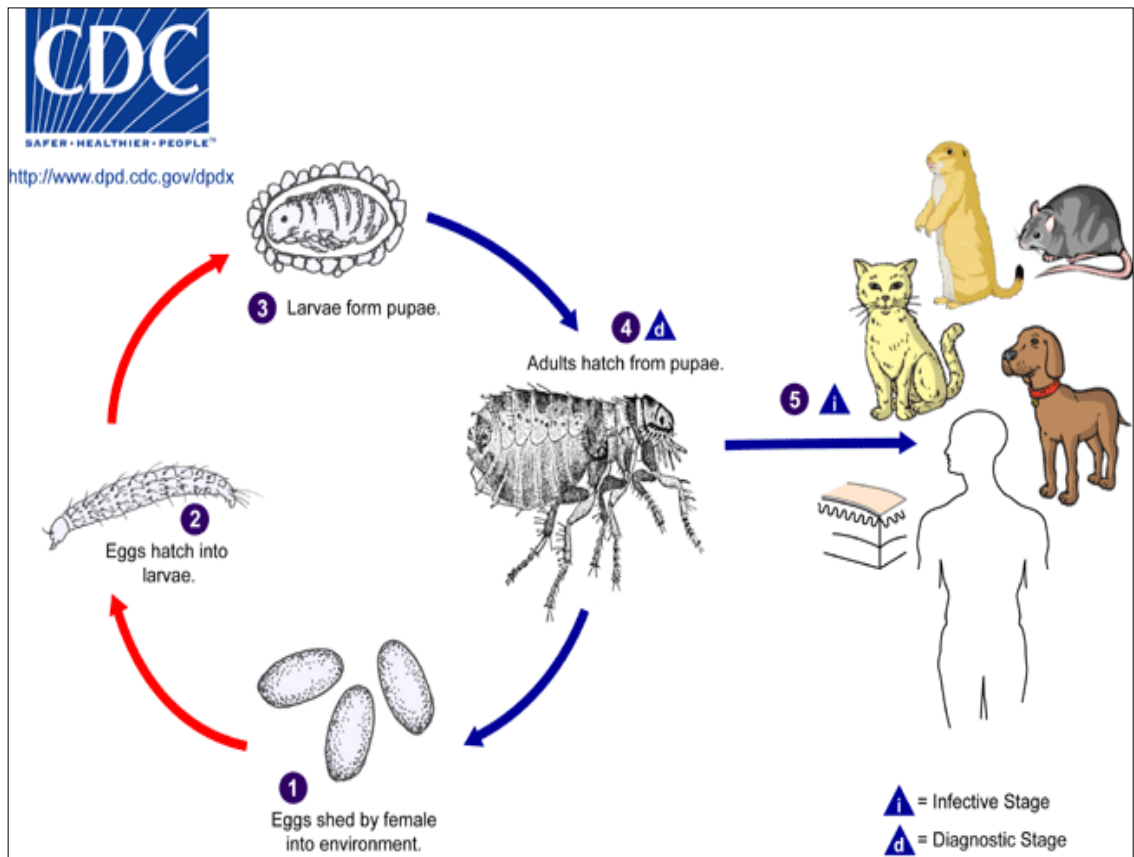


Figure 1.4: Life cycle of *Xenopsylla cheopis*. (Source: DPDx)

1.2.2 Endoparasites of wild rats

1.2.2.1 Nematode

The nematodes or roundworms are the most diverse phylum of pseudocoelomates. The majority of nematodes are free living, and found in soils most of the earth's ecosystems and some also survive in other living hosts. Nematode species are very difficult to distinguish; over 28 000 species have been discovered, of which over 16 000 are parasitic. Nematodes are recognized as important parasites of plants and animals (Bowman *et al.*, 2002). Nematodes are simple organisms possessing simple digestive, nervous, excretory and reproductive system but lack respiratory or circulatory system. The entire structure functions on the basis of a very high internal turgor pressure that is maintained in the body cavity, the pseudocoelom, in which 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). Nematodes are generally elongate and cylindrical, tapering at both ends (Cheng, 1986). Male nematodes are usually smaller than female with spicules that are paired but for some species, one (e.g., *Trichuris*) or none (e.g., *Aspicularis*).

1.2.2.1.1 *Angiostrongylus malaysiensis/cantonensis*

Angiostrongylus species is a common rodent helminth and a potential agent of eosinophilic meningitis or Angiostrongyliasis, the most common cause of eosinophilic meningitis in Southeast Asia and the Pacific Basin. Also known as the rat lungworm, this parasite resides in the pulmonary arteries of rats. Snails are the primary intermediate hosts, where the larvae develop into the infective stages. It is characterized by a tough outer cuticle, unsegmented body, and a fully developed GI tract. The order Strongylida includes all hookworms and lungworms. The family Metastrongyloidea is generally characterized as long, slender and threadlike worm that resides in the lungs of the definitive host.

Kingdom	Animalia
Phylum	Nematode
Class	Secernentea
Order	Strongylida
Family	Metastrongylidae
Genus	<i>Angiostrongylus</i>
Species	<i>Angiostrongylus malaysiensis</i> <i>Angiostrongylus cantonensis</i>

Table 1.8: Scientific classification of *Angiostrongylus* sp.

Humans are incidental hosts and become infected through ingestion of larvae from raw or undercooked snails or other vectors, or from contaminated water and vegetables. The larvae is transported via the blood to the central nervous system, causing eosinophilic meningitis, a serious condition that can lead to death or permanent brain and nerve damage. Angiostrongyliasis is on the rise as globalization aids in the geographic spread

of the disease by the transmission of this parasite via eating raw or undercooked snails and vegetables.

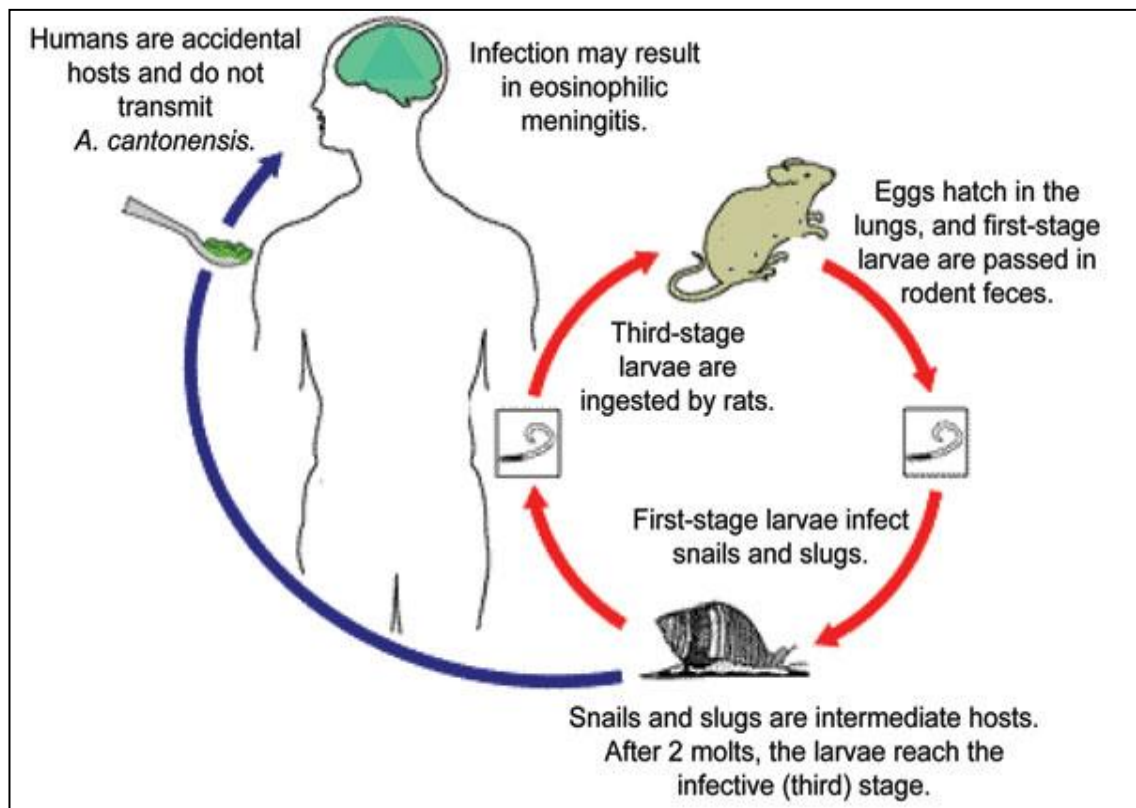


Figure 1.5: Life cycle of *Angiostrongylus cantonensis*. (Source: DPDx)

1.2.2.1.2 *Capillaria hepatica*

Bancroft (1893) first made the discovery of this nematode in the rat liver. It primarily infects rodents and is rarely found in humans. The infection is transmitted to humans either through direct or indirect consumption of rat contaminated food (Rafique *et al.*, 2009). The life cycle of *C. hepatica* is completed in a single host species. The eggs are laid in the liver however, matures outside of the host body and in the environment prior to infecting a new host. The unembryonated eggs are excreted from the feces of the host and then deposited in the soil. Ideal condition for the survival and embryonation of ova is a cool and moist environment. The eggs are oval shaped and encased in a double layer shell with many mini pores on the surface. The unembryonated eggs are approximately between 30 to 55.8µm. The adults are often found in rodent liver surrounded by connective tissues which makes recovery of intact adult specimens difficult for morphological studies. It also infects a wide variety of other wild and domestic mammals like monkey, squirrel, mole, shrew, opossum, weasel and skunk.

Kingdom	Animalia
Phylum	Nematode
Class	Adenophorea
Subclass	Enoplia
Order	Trichurida
Family	Trichinellidae
Genus	<i>Capillaria</i>
Species	<i>Capillaria hepatica</i>

Table 1.9: Scientific classification of *Capillaria hepatica*.

This nematode can cause hepatic capillariasis, which is zoonotic but is seldom described in human. The infected liver will show yellowish-white patches on the surface and in the parenchyma. The presence of *C. hepatica* is confirmed by the recovery of the typical bipolar eggs (Sinniah *et al.*, 1979).

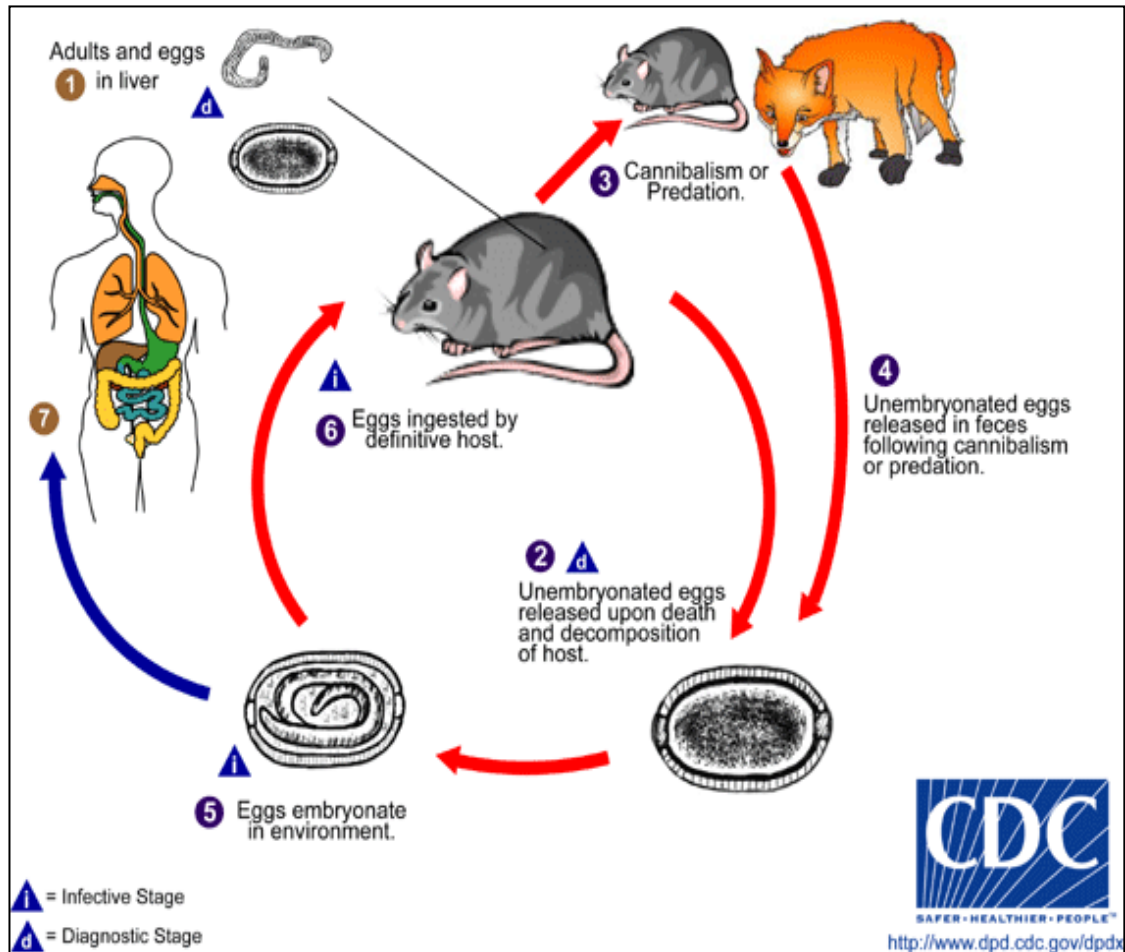


Figure 1.6: Life cycle of *Capillaria hepatica*. (Source: DPDx)

1.2.2.1.3 *Heterakis spumosa*

Heterakis spumosa was first discovered by Schneider in 1866. It is a whitish worm, cylindrical and with fine longitudinal striations throughout the body. The body is covered by a proteinaceous layer called cuticle. They are dioecious with marked sexual dimorphism. At the anterior end is the mouth with distinct lips, and the anus at the posterior. Males are shorter and smaller, with straight blunt tails while females are bigger and longer, with curved tails. They cause nodular typhlitis, diarrhoea, emaciation and death.

Kingdom	Animalia
Phylum	Nematode
Class	Secernentea
Subclass	Rhabditia
Order	Ascaridida
Family	Heterakidae
Genus	<i>Heterakis</i>
Species	<i>Heterakis spumosa</i>

Table 1.10: Scientific classification of *Heterakis spumosa*

Adult worms have three small lips with a cylindrical esophagus that swells posteriorly, ending in a distinct bulb (Baker, 2007). This species is usually found in the caecum or in the upper colon of the rodents. The colon mucosa contains soluble factors that significantly increase the release of eggs by the female in vitro. The egg release is stimulated further with the presence of colon factors from rodents harboring adult worms.

They exhibit a direct life cycle with adults inhabiting in the ceacum. Eggs are passed out through the feces and develop into L2 in the egg. Like other ascarids, the L2 is the infective larva. The infective egg can survive for years. Once the egg is eaten, the egg hatches and the L2 molts 3 times before migrating to the ceacum before finally becoming an adult.

1.2.2.1.4 *Hepatojarakus malayae*

The helminth was first described by Yeh (1955) in the biliary passages of the liver of *Rattus rattus jarak* and also in the inferior vena cava. The worms are medium-sized with the anterior ends attenuated. The cuticles have fine transverse striations. Laterally between the cuticular layers, is a mass of refractile globular granules which occupy more than half the length of the worm. The oral opening has one interesting feature as there are no lips, but it is surrounded by a rudimentary corona radiata consisting of a group of about 16 elements. The female tail is conical and terminates in a spike, while the male worm has a spicule that splits both anteriorly and posteriorly giving the appearance of two unequal spicule fused together (Yeh, 1955).

Kingdom	Animalia
Phylum	Nematode
Class	Secernentea
Order	Strongylida
Family	Molineidae
Genus	<i>Hepatojarakus</i>
Species	<i>Hepatojarakus malayae</i>

Table 1.11: Scientific classification of *Hepatojarakus malayae*.

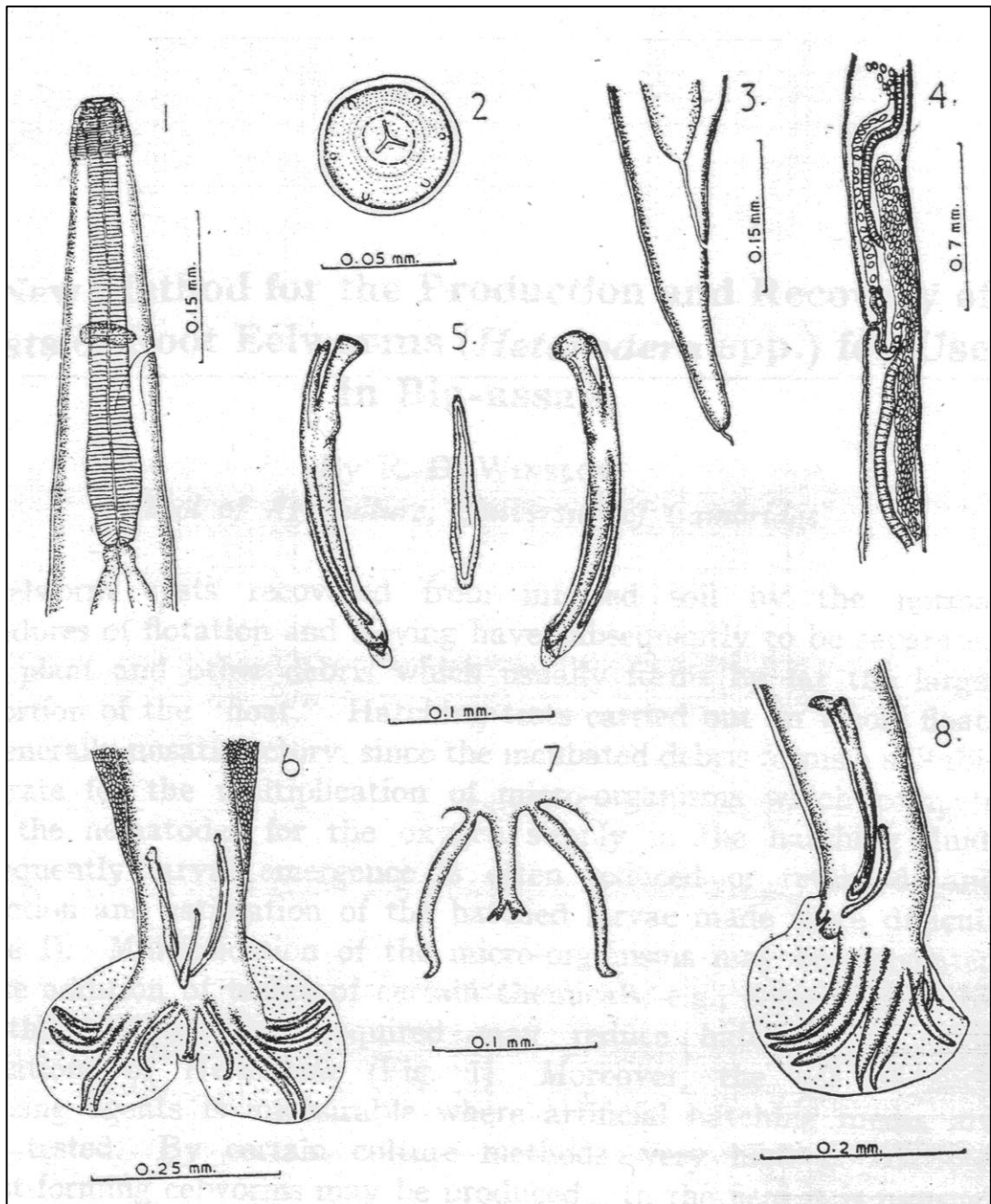


Figure 1.7: The illustration of *Hepatojarkus malayae*. (1) Anterior end of worm (2) End-on view of head (3) Tail of female (4) Vulval region of female (5) Spicules and gubernaculums (6) Dorsal view of male tail (7) Dorsal ray of bursa (8) Lateral view of male tail. (Source: Yeh, 1955).

1.2.2.1.5 *Syphacia muris*

The helminth from the family Oxyuridea parasitizing rats includes; *Syphacia muris*, *Aspiculuris tetraptera*, and *Syphacia obvelata*. The adult *S. muris* has a rounded anterior region and tapered posterior region that ends in a sharply pointed tail. The mouth is rounded with three distinct lips (Baker, 2007). *S. muris* is known to alter host physiology, for example, rats infected with *S. muris* have impaired intestinal electrolyte transport and decreased weight gain (Wagner, 1988).

The adult males of *A. tetraptera* are between 2 to 4 mm long and 120 to 190µm wide, with a short conical tail. The females are between 3 to 4 mm long and 215 to 275µm wide, with a conical tail longer than male. Infection with *A. tetraptera* is generally regarded to have no clinical significance (Baker, 2007).

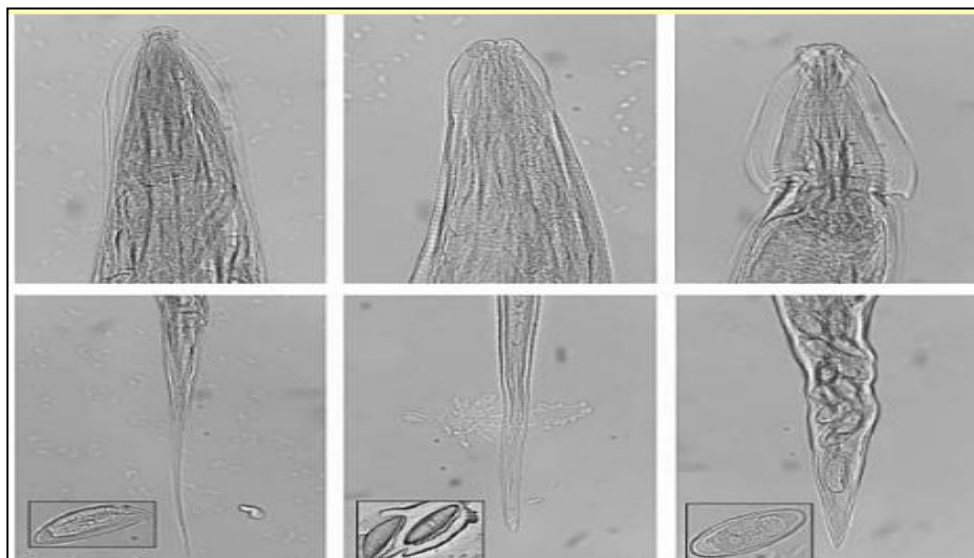


Figure 1.8: Head, tail, and eggs of the adult female pinworms *S. muris* (left), *S. obvelata* (middle), and *A. tetraptera* (right). Heads and tails photographed at 250x. Egg photographed at 400x. (Source: Baker, 2007)

Kingdom	Animalia
Phylum	Nematode
Class	Secernentea
Subclass	Spiruria
Order	Oxyurida
Family	Oxyuridae
Genus	<i>Syphacia</i>
Species	<i>Syphacia muris</i>

Table 1.12: Scientific classification of *Syphacia muris*.

The life cycle of *Syphacia muris* is direct and completed within seven days with no moulting in the egg. The two moults happen outside the egg between 24 to 40 hours after infection. The adult worms primarily inhabit the caecum. Females lay their eggs in the perianal area, and the eggs subsequently leave the host via fecal pellets. Transmission occurs when the infective egg is ingested by another host. The eggs hatch in the colon, where the larvae develop to maturity, and the cycle repeats again. The life cycle of *A. tetraptera* differs from that of *Syphacia obvelata* and *Syphacia muris* in that, it is 10-12 days longer, and the eggs of *A. tetraptera* require an additional 6 days for embryonation to the infective stage (Flynn, 1973; Wescott, 1982).

1.2.2.1.6 *Nippostrongylus brasiliensis*

Nippostrongylus brasiliensis is an intestinal nematode of rodents, closely related to the human hookworm (*Ancylostoma* and *Necator*) which infects an estimated 740 million people in the tropics. The life history and development of *N. brasiliensis* was first described by Yokogawa (1922).

Kingdom	Animalia
Phylum	Nematode
Class	Chromadorea
Suborder	Strongylida
Family	Heligmonellidae
Genus	<i>Nippostrongylus</i>
Species	<i>Nippostrongylus brasiliensis</i>

Table 1.13: Scientific classification of *Nippostrongylus brasiliensis*.

The life cycle is similar to the human hookworms, *Necator americanus* and *Ancylostoma duodenale*. The adults produce eggs in the small intestine, which then are passed out of the host in faeces. The larvae then develop into the infective L₃ stage. This stage penetrates the host skin or is ingested, then carried via circulation to the heart and lungs where they penetrate aveoli. Here they are coughed up and swallowed and then enter the intestine and finally, attaching themselves to the intestinal wall where they develop into adult producing eggs.

1.2.2.2 Cestodes

The Cestoidea or tapeworms constitute an interesting group of parasitic flatworms. Cestodes are a class of worms that is characterized by their flat, segmented bodies. They lack mouthparts and digestive tract, and their body surfaces are covered with a tegumental layer. A true tapeworm generally poses segmented bodies with a specialized holdfast organ and a scolex at the anterior end that is embedded in the intestinal mucosa of the host. They lack digestive tract and absorb nutrients through the body surface.

Most tapeworms are hermaphrodites with each proglottid having its own complete set of male and female reproductive organs. The mature proglottid that is full with eggs will separate itself from the worm. The new segments are constantly generated to replace shedded proglottids. Each proglottid is an independent functioning unit. The entire chain of segments can be up to several meters long, although some tapeworm species are only a few millimeters in length. Adult worm or their larval stages, can pose a threat to humans and companion animals. The larvae inhabit the extraintestinal tissues and produces systemic infections with the clinical effects related to the size, number, and location of cysts. Adult worms are found in the small intestine and infections are usually asymptomatic, but may cause abdominal distress, anorexia, dyspepsia, localized pain, nausea, and diarrhoea.

The life cycle involves a definitive host with one or more intermediate hosts except for the one-host cycle of *Rodentolepis* (= *Hymenolepis*) *nana*. Each stage has specialized larval form (cysticercus, cysticeroid; coenurus, hydatid; coracidium, proceroid, plerocercoid).

The hooked larva penetrates the gut wall and moves throughout the body via the blood and the lymph system in the intermediate host (mammals) or man, as an accidental host. It will develop into an infective cyst in certain organs of the intermediate host. The cyst

has a rudimentary scolex that will allow it to embed itself in the intermediate host, and then ingested by the final host (cat or dog). In the intestinal tract of the final host the scolex becomes exposed and attaches itself to the intestinal mucosa, where the tapeworm finally develops into the adult form.

The common Cestodes found in rodents are *Taenia taeniaformis*, *Hymenolepis diminuta*, and *Rodentolepis* (= *Hymenolepis*) *nana*.

1.2.2.2.1 *Taenia taeniaformis*

The Order Cyclophyllideans is associated primarily with terrestrial food chains (Georgi, 1974). *T. taeniaformis* infects mainly rodents and cats, but in unusual circumstances can also be found in human, but is extremely rare. Rodents act as the secondary intermediate hosts and when eaten by cats, the worm develops to the adult stage in the intestine of the feline.

Kingdom	Animalia
Phylum	Platyhelminthes
Class	Cestoda
Order	Cyclophyllidea
Family	Taeniidae
Genus	<i>Taenia</i>
Species	<i>Taenia taeniaformis</i>

Table 1.14: Scientific classification of *Taenia taeniaformis*

Adult *Taenia taeniaformis* can be subdivided into three body sections; scolex, neck, and strobila. The scolex is made up of 4 large suckers arranged around the sides. Behind the scolex is the neck region, and following this is the strobilus. The strobilus is made of many linearly arranged sections, a proglottid in each section. The neck region is small and produces the proglottids by way of transverse constrictions with the youngest proglottid at the anterior end (next to the neck) and subsequent sections increasing in size and maturity as they reach the posterior end of the strobila.

The life cycle of *Taenia taeniaformis* involves the small rodent as its intermediate host and infects the definitive host i.e. cat; via ingestion of the infected rodent. The larva then migrates through the intestinal wall and develops into a strobilocercus in the

rodent's liver. The strobilocercus reaches maturity prior to infecting the cat. This development phase usually takes 2 months (Singh & Rao, 1965). The patent infection develops in cats between 32 to 80 days after ingestion (Williams & Shearer, 1981).

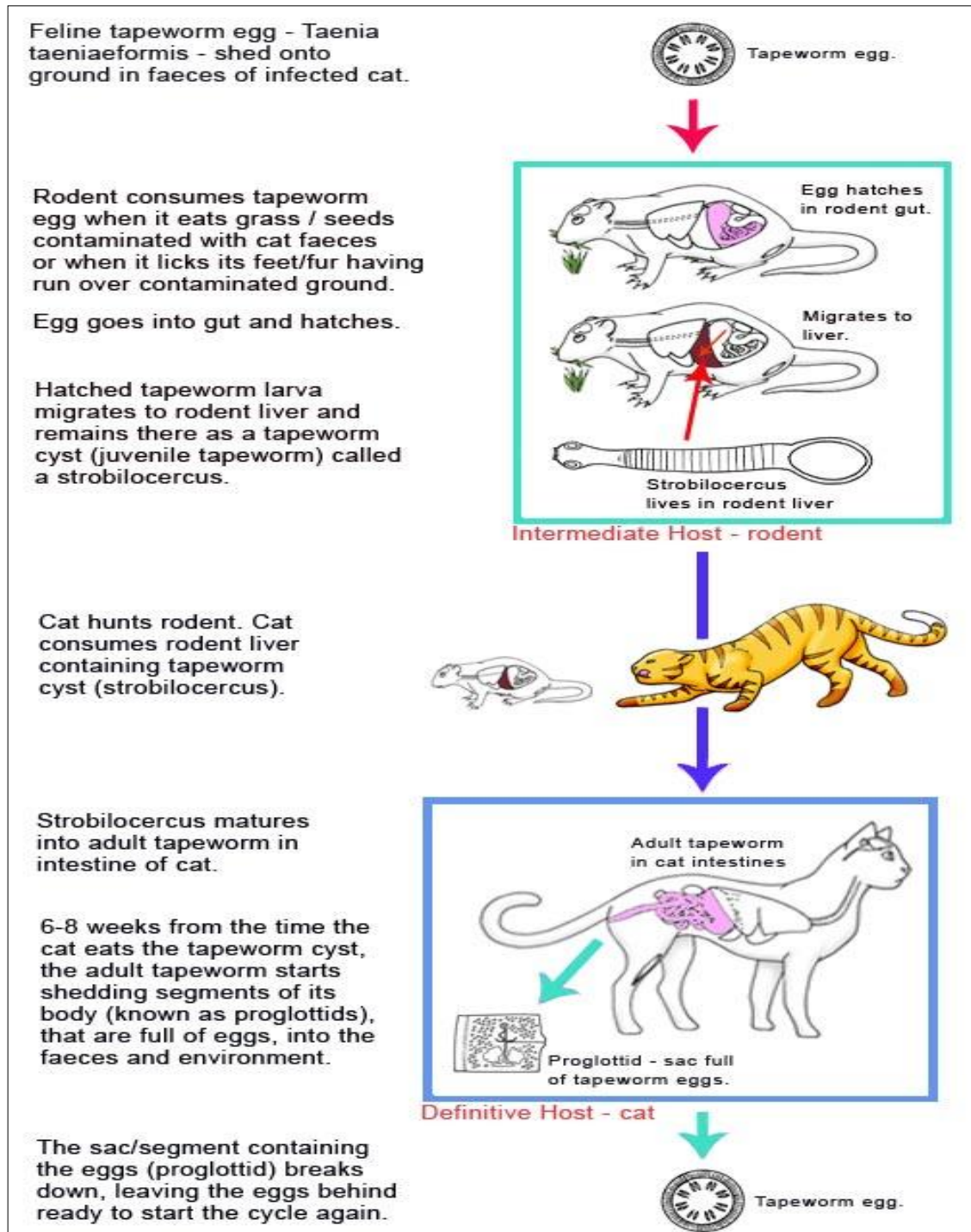


Figure 1.9: Life cycle of *Taenia taeniaeformis*. (Source: DPDx)

1.2.2.2.2 *Hymenolepis diminuta*

Hymenolepis diminuta (also known as rat tapeworm) is the member of order Cyclophellidae and family Hymenolepididae. *H. diminuta* can be found in the small intestine of wild rodents and primates including human.

Kingdom	Animalia
Phylum	Platyhelminthes
Class	Cestoda
Order	Cyclophyllidea
Family	Hymenolepididae
Genus	<i>Hymenolepis</i>
Species	<i>Hymenolepis diminuta</i>

Table 1.15: Scientific classification of *Hymenolepis diminuta*

The life cycle requires an arthropod as the intermediate host, usually the mealworm beetle (*Tenebrio molitor*), flour beetle (*Tribolium confusum*), moth, or flea (*Nosopsyllus fasciatus*) (Baker, 2007). It has a pear-shaped scolex bearing 4 deep suckers and its scolex is almost similar to the *Rodentolepis* (= *Hymenolepis*) *nana* except that *H. diminuta* bears no rostellar hooks. The mature proglottids are much wider than long and lacks any trace of digestive tract and absorbs interstitial fluid through its external covering. Human infections is possible however, requires the ingestion of the intermediate host (Baker, 2007; Dewey, 2011).

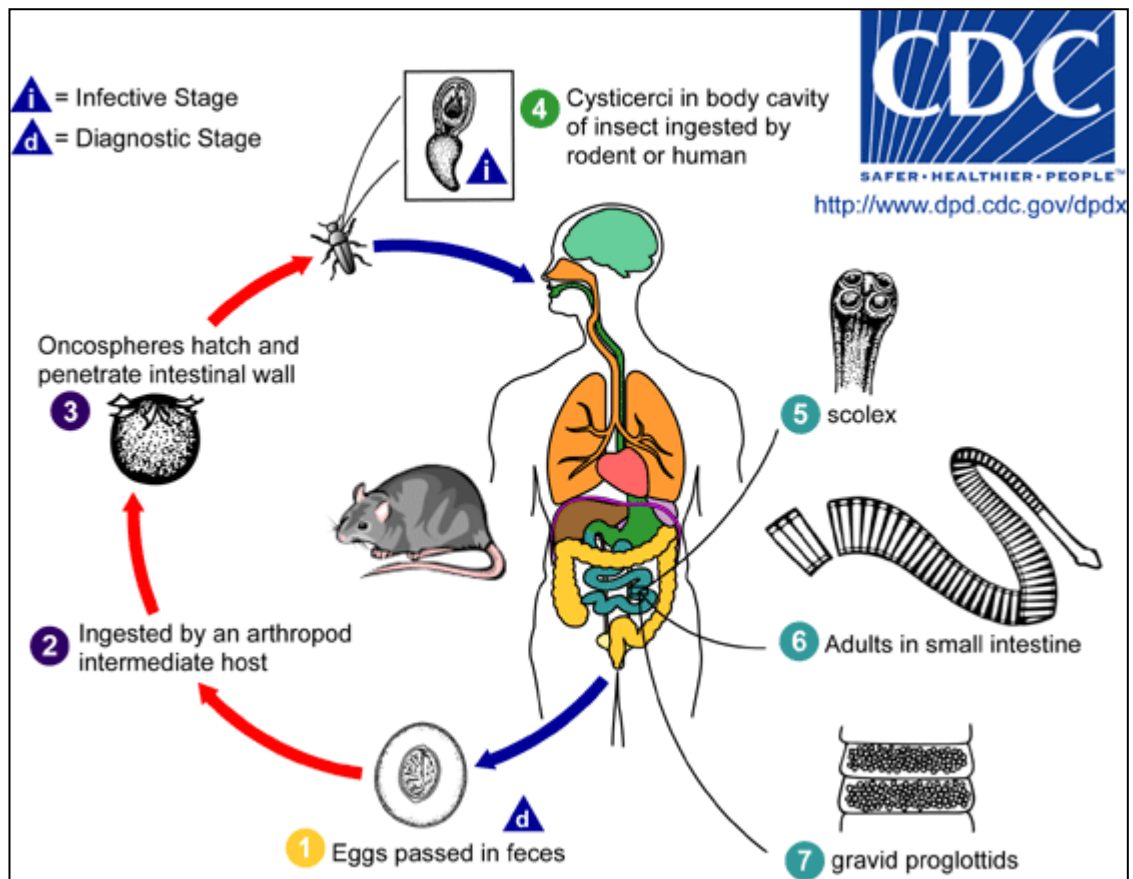


Figure 1.10: Life cycle of *Hymenolepis diminuta*. (Source: DPDx)

1.2.2.2.3 *Rodentolepis* (=Hymenolepis) *nana*

Rodentolepis nana (*Hymenolepis nana*, *Hymenolepis fraterna*, *Vampirolepis nana*), is also known as the 'dwarf tapeworm'. *Rodentolepis* (=Hymenolepis) *nana* is a slender worm, usually about 25 mm to 40 mm long and less than 1 mm wide. The scolex is knob like in shape and consists of 4 unarmed suckers with the rostellum armed with a single ring of 20 to 27 small hooklets. The segments are wider than long. The ova is spherical or ovoid measuring between 30-47µm in diameter and distinguishes it morphologically from *H. diminuta*. The worm length is affected by the host's immune status and the temperature at which the host is housed (Baker, 2007).

Eggs released from mature proglottids in the upper ileum are usually passed out through the feces. If swallowed by another human, it will develop into a hexacanth oncospheres and burrow itself into the villi of the small intestine. These oncospheres will develop into tail-less cysticercoids and migrate towards the ileum and finally attach to commence the formation of proglottids. The eggs ingested by insects (fleas, beetles or cockroaches) hatch to form tailed cysticercoids which remain unchanged as long as they are inside the insect.

Rodentolepis (=Hymenolepis) *nana* has a cosmopolitan distribution and is thought to be the most common tapeworm throughout the world. The infection is more frequently seen in children although adults can also be infected causing hymenolepiasis.

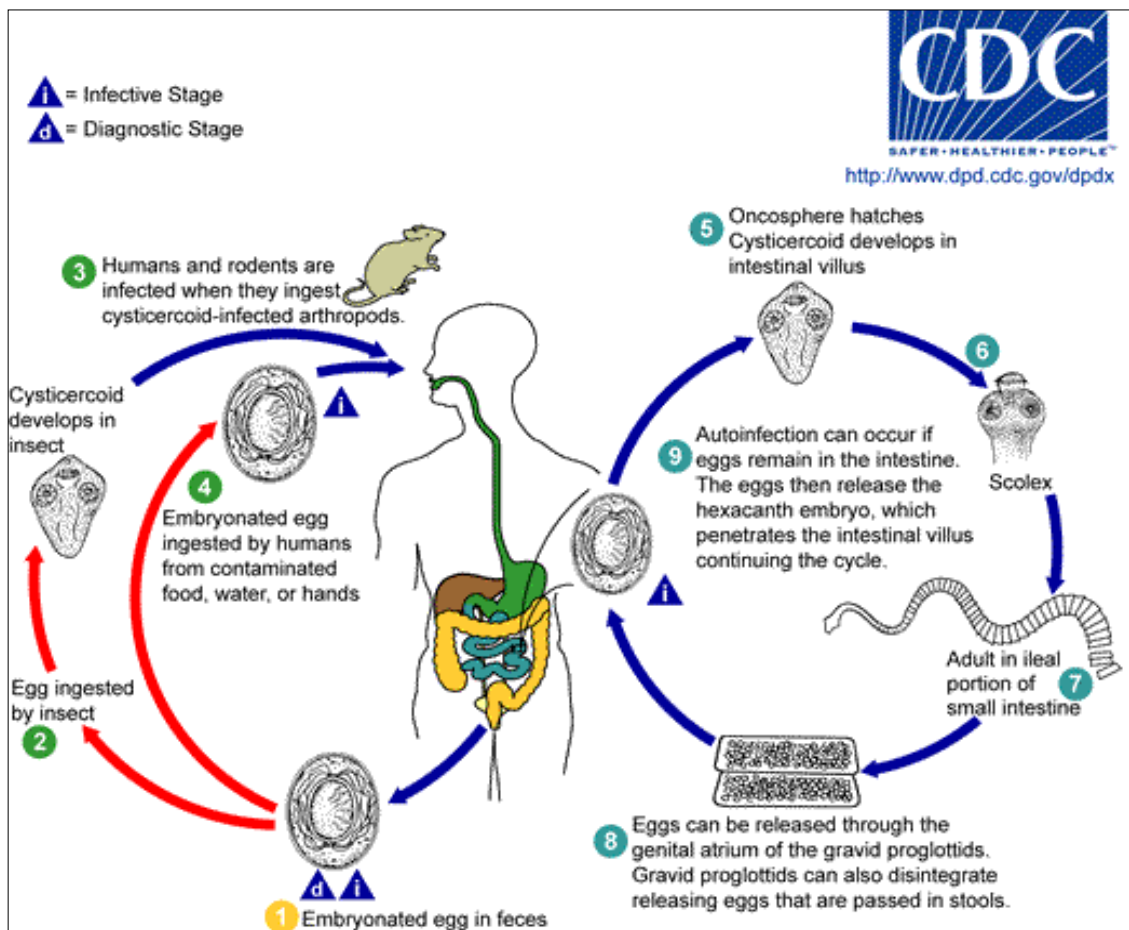


Figure 1.11: Life cycle of *Rodentolepis* (= *Hymenolepis*) *nana*. (Source: DPDx)

The lifecycle of *Rodentolepis* (= *Hymenolepis*) *nana* does not require an intermediate host and complete development occurs within the villi of a single host, resulting in a direct life cycle. It can also utilize an insect as an intermediate host.

1.3 Zoonotic diseases of rats.

Zoonoses is define as animals capable of carrying one or more infectious agents that are transmissible to humans. The most notable zoonotic diseases of rats are Rabies (*Lyssavirus*), Plague (*Yersinia pestis*), and Hantavirus. The rat ectoparasites have been long known to be capable of transmitting human diseases such as murine typhus, scrub typhus, and the plague. The potential threat to public have been one of the major justifications for many studies, as exemplified in Dohany *et al.* (1980), Ho and Krishnasamy (1990) and Chuluun *et al.* (2005). According to Zahedi *et al.* (1984), some ectoparasites (mites, lice, flea and tick) are known vectors for pathogenic organism. Therefore, infestation of ectoparasites on rodents should not be ignored as they play a role in transmission of diseases.

Endoparasites of rats play an important role in the zoonotic cycles of many diseases, some of which are more important than others, eg. Schistosomiasis, Hymenolepiasis, and Angiostrongylosis.

1.3.1 Murine typhus

Murine typhus, also known as endemic typhus is a form of typhus transmitted by *Xenopsylla cheopis*. It is caused by a type of bacteria known as *Rickettsia typhi*. The infected person does not realize that they have been bitten by fleas most of the time. Human infection occurs because of flea-fecal contamination from the bite on human skin. Symptoms of endemic typhus include headache, fever, chills, myalgia, nausea, vomiting, and cough. Murine typhus is largely considered to be uncommon despite indications that the disease is more widespread than previously thought (Audy, 1949; Brown *et al.* 1977). Endemic typhus is treatable with antibiotics. Most people recover fully, but death may occur in the elderly, severely disabled or patients with a depressed immune system. The most effective treatment used includes tetracycline and chloramphenicol.

1.3.2 Scrub typhus

Scrub typhus fever is a disease caused by bacteria called *Orientia tsutsugamushi*. Scrub typhus is transmitted through the bite of an infected chigger and is arguably one of the more well-known diseases associated with the rat-borne ectoparasites in Peninsular Malaysia. Scrub typhus epidemiology has been linked to human behaviour and occupation and is known to be associated with cultivated fields and secondary forests (Tay *et al.* 1999). It is the common cause of fever in rural populations (Brown *et al.*, 1978). Other chigger species have also been implicated in maintaining and transmitting the disease (Traub & Wisseman, 1974, Dohany *et al.*, 1980). Scrub typhus fever does not spread directly from person to person but through bite of an infected chigger. Symptom may include sore on the skin with a 'punched out' appearance (skin ulcer) at the site where the mite attached. Several days after the appearance of the ulcer, other symptoms may develop which includes fever, headache, sweating, blood-shot eyes, swollen lymph nodes, rash, lung infection, vomiting, and diarrhoea.

1.3.3 Bubonic plague

The plague is transmitted to humans through a flea bite from an infected rat seeking other sources of blood including human. The rodent plague outbreak has claimed many lives including animals throughout the centuries. Direct transmission takes place when handling infected rodents, rabbits, or wild carnivores that prey on these animals and transmit the plague bacteria through cuts on the person's skin. Pet dogs and cats can also bring plague-infected fleas into the home. Infected cats become sick and may directly transmit plague to human who handle or care for them. Inhaling droplets expelled by the coughing from a plague-infected person or animal (especially house cats) can result in plague of the lungs (plague pneumonia). Transmission of plague pneumonia from human to human is uncommon but dangerous during an epidemic and can quickly spread.

Bubonic plague causes severe infection to humans and rodents and is caused by a bacterium called *Yersinia pestis*. Plague pneumonia, or pneumonic plague, is caused by the same bacteria as bubonic plague but the victim becomes infected by inhaling infected droplets from the lungs from a person infected with the plague infection that has spread to the respiratory system. It is the most contagious form that progresses rapidly, with death usually occurring in less than three days in virtually all untreated cases.

During the Black Death, millions of people became infected with pneumonia from *Yersinia pestis* and infected each other by coughing and sneezing. According to the World Health Organization, there are between 1,000 to 3,000 reported cases of bubonic plague worldwide each year although there are no known cases in Australia or Europe. Areas with known cases include Russia, the Middle East, China, Southwest and

Southeast Asia, Madagascar, southern and eastern Africa, the Andes Mountains and Brazil.

1.3.4 Angiostrongyliasis

Angiostrongyliasis is an infection from the nematode worm, *Angiostrongylus* sp. Human symptoms includes eosinophilic meningitis or meningoencephalitis. Infection first presents with severe abdominal pain, nausea, vomiting, and weakness, which gradually lessens and progresses to fever, followed by CNS symptoms including severe headache and stiffness of the neck. Most cases occurred in Asia and the Pacific Basin (Thailand, Taiwan, mainland China, the Hawaiian Islands, and other Pacific Islands). However, cases have been reported in the Caribbean. The geographic range of the parasite has expanded, probably is facilitated by infected ship-borne rats and the diversity of snail species that act as intermediate hosts.

The adult worm resides in the pulmonary arteries of rodents, where it reproduces. The eggs hatches to larvae in the arteries followed with the larvae migrating into the pharynx then swallowed again into the intestine and finally passed out through the stool. The first stage larvae (L1) penetrates or is swallowed by the intermediate host, the snails then transforms into the second stage larvae (L2) then finally into the third stage infective larvae (L3). Humans and rats acquire the infection through ingestion of contaminated snails or paratenic hosts including prawns, crabs, and frogs, or raw vegetables containing the intermediate and paratenic hosts. After passing through the gastrointestinal tract, the larvae enters the blood circulation and migrates to the meninges and develop for about a month before migrating to the pulmonary arteries, where they fully develop into adults.

Humans are accidental hosts because the larvae cannot develop into mature adults and reproduce in humans. The larvae will develop into juvenile L4 stages in the brain and CSF however, will die quickly inciting the inflammatory reaction that causes clinical symptoms of infection.

The incubation period between the time of ingestion of the infected food to the onset of neurological symptoms varies widely from person to person and has been observed lasting between 3-36 days (Ramirez-Avila, 2009). Angiostrongyliasis can easily be prevented by educating the public the necessity of thorough cooking and washing of food and control of mollusks. Other prevention strategies may include control of the rat population.

1.4 Macroparasite studies.

1.4.1 Studies on ectoparasites infesting rats.

A considerable number of studies have focused on rat ectoparasites from Peninsular Malaysia. The majority of related works included surveys and field collection however, most studies focused on a specific ectoparasites group (Kohls, 1957; Singh *et al.*, 1995; Saleh *et al.*, 2003). In contrast overall diversity surveys were comparatively few (Audy & Harrison, 1953; Zahedi *et al.*, 1984; Shabrina *et al.*, 1989; Paramasvaran *et al.*, 2009b). Past studies focused merely on cataloging the ectoparasites host distribution and describing novel species. Majority of the past studies were also short term field observation and surveys (Kohls, 1957; Audy & Womersley, 1957; Johnson, 1964). However, there have been no attempts to investigate the effects of host's age and host's sex on ectoparasite burdens and transmission.

Among the rat-borne ectoparasites groups; rat mites have received the most attention. Rat mites are highly diverse and specialized group that are further divided into chiggers, mesostigmatidmites, listrophorids and myobiids. Major works include Domrow & Nadchatram (1963), Domrow (1962) and Saleh *et al.*, (2003). Additionally, mites have often been reported in general surveys (Shabrina, 1990; Shabrina & Salleh, 1995).

The local distributions of chiggers are of great interest. Noteworthy works include Audy (1956a; 1956b; 1956c), Nadchantram *et al.* (1966) and Nadchantram (1970). Rat ticks and fleas were only occasionally reported while the rat lice were almost entirely overlooked.

There had only been two long-term surveillance studies on vector fleas among local small mammals (Singh, 1990; Singh *et al.*, 1995). Both studies recognized *Xenopsylla cheopis* as the most prevalent rat flea species in the country. Zahedi *et al.* (1996) showed higher flea index (*Xenopsylla cheopis*) between urban rat populations compared

to the semi urban rat populations. He also reported 6% of the rats trapped in the city harbored the cat flea, *Ctenocephalides felis*. *Xenopsylla cheopis* infestation were also reported by several authors including, Traub (1950), Zahedi *et al.* (1984), Shabrina *et al.* (1989), Mariana *et al.* (2005) and Paramasvaran *et al.* (2009b).

Literature available on the rat lice also seemed somewhat limited. Studies concerning the local rat lice diversity have been strictly focused on the morphological aspect for identification and establishing phylogenic relationship (Johnson, 1964; 1972). Presently, *Hoplopleura pacifica*, *Hoplopleura dissicula*, *Hoplopleura pectinata*, *Hoplopleura malaysiana*, *Hoplopleura diaphora* and *Polyplax spinulosa* have been recorded from Peninsular Malaysia and only occasionally included in general rat ectoparasites surveys (Zahedi *et al.*, 1984; Shabrina *et al.*, 1989; Mariana *et al.*, 2005; Paramasvaran *et al.*, 2009b).

The first major study compiled a host-parasite checklist on rat tick from Peninsular Malaysia was done with special emphasis on rat hosts and a comprehensive taxonomic key (Kohls, 1957). Extensive works revealed that the infestation on the wild terrestrial rats were common and generally host for immature ticks (e.g. *Amblyomma* sp., *Haemaphysalis* sp. and *Dermacentor* sp.) while *Ixodes granulatus* was found to be the only adult tick that parasitizes rats (Kohls, 1957; Domrow & Natchatram, 1963; Natchatram *et al.*, 1966; Lim, 1972; Ho & Krishnasamy, 1990; Saleh *et al.*, 2003). Other species also included *Dermacentor* sp., *Haemaphysalis* sp., *Rhipicephalus* sp. and *Ornithodoros* sp. infested wild rats of forest habitat (Mariana *et al.*, 2005; 2008; 2011; Madinah *et al.*, 2011).

Shabrina (1990) showed low infestations (30%) from 105 small mammals (rats, tree shrew and squirrel) captured in Ulu Gombak, Selangor. The ectoparasites recovered fell under 8 broad groups (mite mesostigmata, mite trombiculid, listrophorids, myobiids,

ticks, lice, fleas and cheyletid. Several species recovered were known vectors for virus rickettsia in human and animal infections, while some species are agents for dermatitis. Meanwhile, Saleh *et al.*, (2003) determined 2 medically important ectoparasites species namely *Ixodes granulatus* and *Leptotrombidium deliense*. On the other hand, Chuluun *et al.* (2005) recovered 7 ectoparasites species in rats at Kuala Selangor Nature Park.

Collections of rat ectoparasites have been reported from various habitats throughout Peninsular Malaysia, including primary lowland forest (Ho *et al.*, 1985; Saleh *et al.*, 2003), secondary forest (Shabrina & Salleh, 1995) and wildlife reserves includes Gua Musang (Shabrina, 1991). In the most recent forest study, Madinah *et al.* (2011) recovered 14 species of ectoparasites from rodents and scandents caught in four localities of wildlife reserves in Peninsular Malaysia. Meanwhile, Mariana *et al.* (2011) study on acarine ectoparasites of forest reserve in Johore found 7 species of mesostigmatid mites, 6 of which were on rodents.

Paramasvaran *et al.* (2009b) reported the ectoparasite fauna of rodents and shrews from four habitats (forest, coastal, ricefield and urban) in Kuala Lumpur and the states of Selangor and Negeri Sembilan, Malaysia and identified a total of 20 species and incriminated the following ectoparasites as important vectors or as mechanical carriers for the transmission of zoonotic diseases: *Ixodes granulatus*, *Dermacentor* sp., *Haemaphysalis* sp., *Amblyomma* sp., *Ascoschoengastia indica*, *Leptotrombidium deliense*, *Ornithonyssus bacoti*, *L. nuttali*, *H. pacifica*, *P. spinulosa* and *X. cheopis*.

Ectoparasites studies have also been well documented worldwide. Ectoparasites survey on *Rattus rattus* from Makurdi, Nigeria showed the tick accounted for 54.9% of all the total ectoparasites (Omodu *et al.*, 2010). Meanwhile, studies in southeastern Bangladesh showed that from the 73 rodents caught a variety of ectoparasites such as mites (*Laelaps nuttali*, *Laelaps echidninus*, *Lyponissoides* sp. and *Ornithonyssus bacoti*) and fleas

(*Xenopsylla cheopis*) (Fuehrer *et al.*, 2012) were recorded. In the Philippines, *Rattus* spp. trapped from wet markets in Quiapo, Manila and Balayan, Batangas were infested with *Echinolaelaps echidninus* and *Polyplax spinulosa* (Flores *et al.*, 2005) while in Egypt, Sohail *et al.* (2001a: 2001b) recovered 7 common ectoparasites species (mites, fleas and louse) from *R. rattus* and *R. norvegicus* and indicated that sex, body size and age of rat does affect the prevalence and general indices of the ectoparasites. Rodents captured in Bandar Abbas, Iran were found to be infected with 69 ectoparasites comprised of flea, lice, mite and tick with high prevalent of *Xenopsylla* sp. (Kia *et al.*, 2009). Abu-Madi *et al.*, (2001) study showed flea (*Xenopsylla astia*) prevalence and abundance in the winter were very similar in both sexes and age group, however both parameters of infestation were markedly higher among juveniles compared to adults during summer.

In Malaysia, the effects of extrinsic factors such as rainfall and season on the ectoparasite load have only been briefly explored (Traub & Wiseman, 1974; Roberts *et al.*, 1977; Singh *et al.*, 1995; Lim, 1972)

1.4.2 Studies on Endoparasites Infecting Rats.

There have been a considerable number of studies on rat helminth from Peninsular Malaysia. Most studies on the local wild rat endoparasites had been surveys, recording and cataloging the helminth fauna from various different habitats throughout Peninsular Malaysia (Gantha, 1966; Singh & Cheong, 1971; Leong *et al.*, 1979; Sinniah, 1979; Sinniah *et al.*, 1979; Syed-Arnez & Mohd Zain, 2006; Mohd Zain, 2008; Paramasvaran *et al.*, 2009a; Mohd Zain *et al.*, 2012). Surveys focused on specific parasites have also been made by Yeh (1955), Bhaibulaya & Cross (1971), Lim *et al.* (1974; 1977a; 1977b) and Krishnasamy *et al.* (1981), among many others.

Between the various endoparasites group, nematodes have received the most attention (Schacher & Cheong, 1960; Cheong & Singh, 1965; Ow-Yang, 1971; 1974). The earliest report of nematodes from Malaysian murids was provided by Adam (1933), who erected a new genus and species of hookworm, *Cyclodontostomum purvisi*, from the large intestine of a rat from Raub. Accompanying this description were records of another hookworm, *Ancylostoma malayanum* (Alessandrini, 1905), recovered from a rat from Seremban, and the cosmopolitan oxyurid, *Syphacia obvelata* (Rudolphi, 1902), from rats from Taiping and Pahang. Ow-Yang (1971) surveyed the diversity of nematode parasites in Malaysian rodents in an attempt to assess the potential risk of parasite that are zoonotic and to contribute knowledge of the Malaysian parasitic fauna. On the other hand, Balasingam (1963) redescribed *C. purvisi* from Malayan giant rats and the possible relationship with other hookworm genera. This parasite was never reported before and no other species have thus far been assigned to the genus of *Cyclodontostomum*. Meanwhile, Singh & Cheong (1971) showed that *C. purvisi* was distributed in 7 Malaysian rat species.

The epidemiology of *Capillaria hepatica* (Bancroft, 1893) was reported by several authors from different species of rats trapped from various states in Malaysia (Audy *et al.*, 1950; Schacher & Cheong, 1960; Singh & Cheong, 1971; Sinniah, 1979).

Only several reports on the presence of filariae in Malayan rats have been noted. Singh *et al.* (1976) and Mak & Lim (1974) described the filarial worm *Breinlia booliati* which was also reported in the Malayan forest rat (Singh & Ho 1973). Dunn & Ramachandran (1962) recorded an undescribed species of filarial worm from the heart of *Rattus sabanus*. Morphological studies showed that the worm belongs to the family of Splendidofilariinae (Dipetalonematidae) which was also recorded by Sivanandan *et al.* (1965) in the heart and lungs of *Rattus sabanus*.

A new nematode species (*Gnathostoma malaysiae*) was found in the stomach of *Rattus surifer* and *Rattus tiomanicus* trapped in the mountainous area of Tioman Island, Pahang (Miyazaki & Dunn 1965). This was almost similar morphologically to *Gnathostoma doloresi* (Tubangui, 1925). However, it can be easily distinguished from this species by the shape of cuticular spines, the size of fertilized eggs, and the features of the caudal pedunculate papillae of the male.

Several studies have highlighted the endoparasites of commensal and forest rodents in different habitats in Malaysia. However, most studies focused on the primary lowland rainforest (Betterton & Lim, 1975; Singh *et al.*, 1987; Ho & Krishnasamy, 1990). Other habitat survey included cities (Schacher & Cheong, 1960; Gantha, 1966; Leong *et al.*, 1979; Paramasvaran *et al.*, 2009a; Mohd Zain *et al.*, 2012), island (Audy *et al.*, 1950; Dunn, 1966; Lim *et al.*, 1974; Khairul Anuar, 1977; Mohd Zain, 2008), oil palm estates (Lim & Muul, 1970; Krishnasamy *et al.*, 1980) and disturbed primary forest (Yap *et al.*, 1977; Betterton, 1979; Ambu *et al.*, 1996; Syed-Arnez & Mohd Zain, 2006).

Studies on forest rats recovered high diversity of endoparasites with 23 nematode species, 3 cestodes species, 2 trematode species and one pentastomide species from the Endau Rompin National Park with an overall prevalence of 90.63%, with Heligmonellidae parasites being the dominant helminth and identified new rat host of several parasites species (Syed Arnez & Mohd Zain, 2006).

Mohd Zain (2008) surveyed 2 islands on the Strait of Malacca to understand the effects of island biogeography on the diversity of rats and their parasites. However, only commensal rats with low helminth community were recovered. Meanwhile, Paramasvaran *et al.* (2009a) reported the endoparasite fauna of rodents caught in five wet markets in Kuala Lumpur and identified 11 species to be zoonotic. It was shown for the first time the association of helminth infections with the habitats the rodents inhabit.

The most recent study on helminth communities from two urban rat populations (*Rattus rattus* and *R. norvegicus*) in Kuala Lumpur (Mohd Zain *et al.*, 2012) recovered eleven species of helminth parasites comprising seven nematodes (*Heterakis spumosum*, *Mastophorus muris*, *Nippostrongylus brasiliensis*, *Syphacia muris*, *Pterygodermatitis tani/whartoni*, *Gongylonema neoplasticum*, *Angiostrongylus malaysiensis*), three cestodes (*Hymenolepis nana*, *Hymenolepis diminuta* and *Taenia taeniaeformis*) and one acanthocephalan (*Moniliformis moniliformis*). High prevalence of infection was recorded in rat populations with significant differences between host sexes in the overall abundance of helminths.

Rat endoparasites studies have extensively been documented worldwide (Kataranovski *et al.*, 2008; 2010; Coomansingh *et al.*, 2009). Endoparasite infection of rodents in Ahvaz, South West Iran reported for the first time presence *Rictularia ratti* and *Gongylonema monigi* from *R. norvegicus* and *R. rattus* in Iran. In Qatar, parasitic infections of the urban rat, *R. norvegicus* recovered only one species of helminth

(*Hymenolepis diminuta*) (Abu-Madi *et al.*, 2001) and showed intrinsic factor such as, host age attributed to the endoparasite infection where infection was observed higher in adults compared to juvenile rats. Griselda *et al.* (2005) recorded 13 species of helminth from 3 different sites in Hidalgo, Mexico and showed *N. brasiliensis* was the most prevalent. All the species collected were new geographic distribution record for Hidalgo, Mexico. New morphological details and first record of *Heterakis spumosa* and *Syphacia muris* was also noted in Argentina from *R. norvegicus* (Maria *et al.*, 2008) while in Pakistan, helminth in rodents captured from different city structures showed *H. nana* was the most prevalence (Rafique *et al.*, 2009).

1.5 Justification of study

Many works on the distribution and the prevalence of endoparasites in the wild rat population was conducted many years ago (Audy *et al.*, 1950; Dunn *et al.*, 1966; Lim *et al.*, 1974). However, there are limited numbers of studies on coastal and island habitats. Previous studies are also limited to one or certain locations and generally involved small numbers of samples in a particular location. The effects of intrinsic and extrinsic factors affecting parasite burden and transmission have not been fully explored. Development and urbanization has impacted the natural environment and in addition, climate change and increasing temperatures bring a fair share of concern with regards to transmission of diseases in the tropics. Urban cities in island and coastal sites in Peninsular Malaysia have experienced urbanization in infrastructure development and population growth which could have impacted the parasite community affecting the wild rat population and changes to the population distribution over time is also poorly understood. Therefore, there is still a need to continue to monitor diseases of rats in effort to understand the parasite distribution and to understand the outbreak of zoonotic diseases carried by these parasites.

1.6 Objectives

- To determine diversity and distribution of wild rat population from island (Penang and Carey Island) and coastal (Kuantan and Malacca) habitats in Peninsular Malaysia.
- To determine the macroparasites (ecto- and endoparasites) population from the wild rats population from island and coastal habitat in Peninsular Malaysia.
- To evaluate the role of intrinsic (host age, host sex) and extrinsic (season, location) factors in structuring the macroparasites community of the wild rats population.
- To identify parasites (ecto- and endoparasites) of public health importance and to determine potential risk to each location.

CHAPTER 2: STUDY LOCATIONS AND METHODOLOGY

2.1 Study area

Four localities were selected to represent urban coastal cities (Kuantan and Malacca) and island (Penang and Carey Island) habitats. The sites also were chosen based on locations i.e.; north (Penang), west (Carey Island), east (Kuantan) and south (Malacca). Climate here is generally uniform throughout the year with the annual variation normally was less than 3°C. The temperature recorded during study period ranged between 25.5-28.6°C (Malaysian Meteorological Department). This study was divided into two seasons; dry (January-March and June-September) and wet (April-May and October-December). Trapping commenced between May 2010 to December 2011.

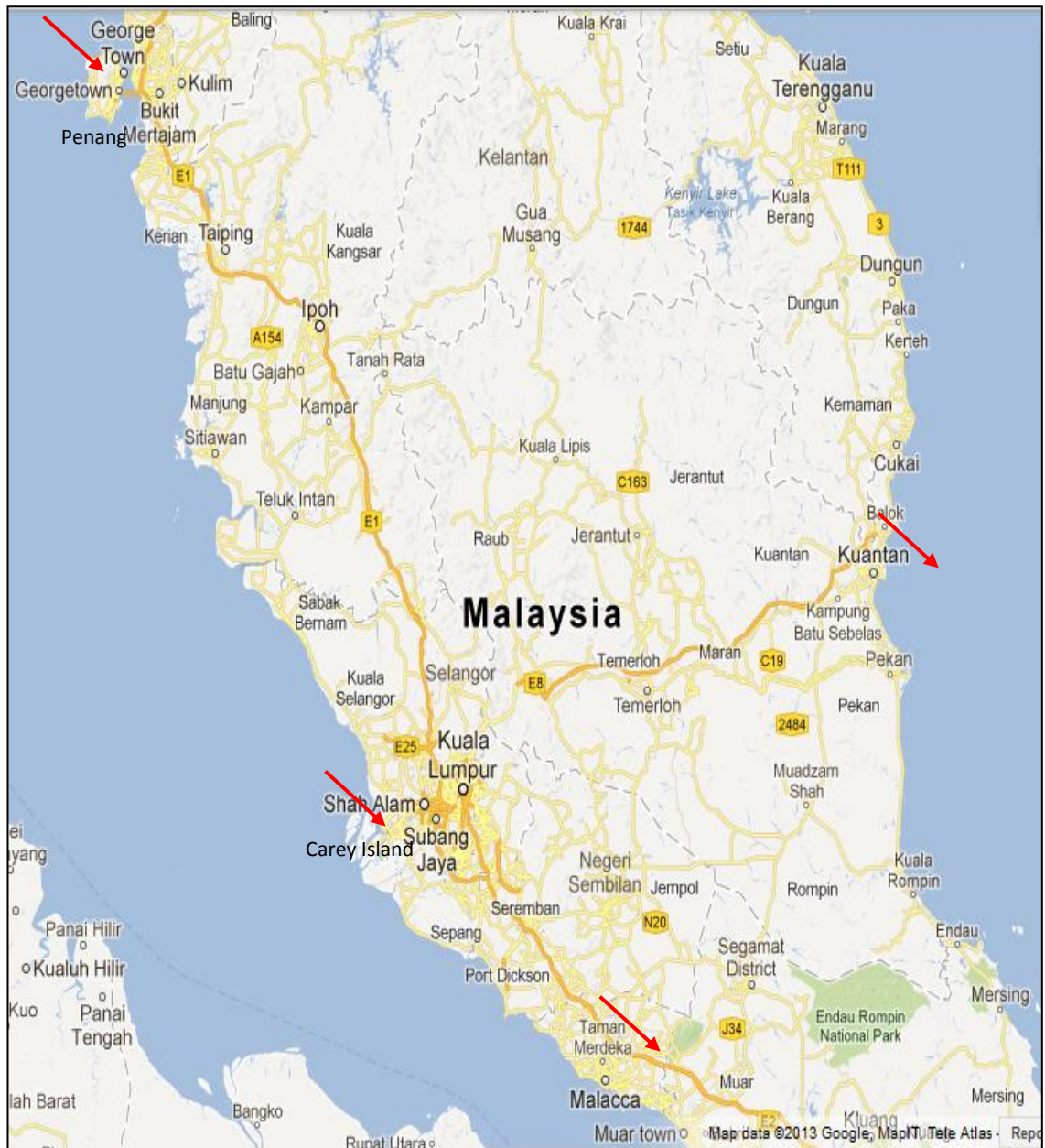


Plate 2.1: The location of study area, Penang, Carey Island, Kuantan and Malacca in Peninsular Malaysia. Image excerpt from Google Earth™ mapping service.

Carey Island (2°52'N 101°22'E), is situated on the south of Port Klang and north to Klang River in Kuala Langat District of Selangor on the west of Peninsular Malaysia. It is separated from the mainland by Langat River and connected by a bridge from Chodoi near Banting. It is an oil palm plantation, belonging to the Sime Darby Plantation. This island spans a total area of 16, 000 hectare and more than three quarters (11, 700 hectare) of the area here are cultivated. The island inhabitants include a group of *Orang*

Asli, Malay and Indian settlers that form a very significant component of the overall land use of the island. In the early 1900s, Carey Island was known as Pulau Si Alang (or Pulau Bangsar) was only inhabited by the Mah Meri tribe. Presently, this island is known as Carey Island after an Englishman planter, Edward Valentine John Carey who acquired the land to grow rubber trees. Over the past several decades, general expansion and agriculture across this area has resulted in serious ecological changes that have irrevocably altered the composition and structure of terrestrial rats as well as their parasite population.

Penang island (5°24'N 100°14'E) is located on the northwest coast of Peninsular Malaysia by The Strait of Malacca. The Island geography is separated from mainland by a channel three kilometers wide at the narrowest point. It is bordered by Kedah in the north and east, and Perak in the south. Penang is the second smallest state in area after Perlis and the island is the fourth-largest in the country with an area of 293 km². It is the most populated island in the country with an estimated population of 678,000. The island is connected with the mainland by the Penang Bridge. Highly urbanised and industrialised Penang is one of the most developed and economically important states in the country, as well as a thriving tourist destination. Georgetown is the capital of the Penang state of Malaysia and located on the north east corner of Penang Island.

Kuantan city (3°29'N 103°12'E) was chosen to represent a coastal urban city on 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 undergone several development projects to transform and modernize the city. Kuantan is now also recognized as the future centre for trade, commerce, transportation and tourism.

Malacca (2°12'N 102°15'E) is the third smallest Malaysian state, after Perlis and Penang. It is located in the southern region of the Peninsular Malaysia, on the Straits of Malacca. It borders Negeri Sembilan to the north and the state of Johor to the south. The capital is Malacca City, which is 148 km south east of Malaysia's capital city Kuala Lumpur. The modern city has expended in all directions to include infrastructure, for tourism and economy.

2.2 Rat trapping

Wild rats were captured with cooperation from the assigned units from each municipality and or with animal welfare organization from each city (apart from Carey Island). The areas selected were the public spaces such as wet market, food court, main streets and around shop lots. A total of 30 wire traps were set up in certain area such as wet market and oil palm plantation over a span of 4 days and 3 nights each time.

2.2.1 Carey Island

Trapping was divided into two phases. Phase one was conducted in June 2010 while the second phase was carried out in December 2010. A total of 4 sessions was done during the wet (2 times) and dry season (2 times). All rats were trapped using collapsible wire traps measuring 16.5'X6'X6' inches (Plate 2.2) with oil palm fruits as bait. A total of 50 traps were left overnight over a span of 4 days 3 nights in the vicinity for Mangrove Research Center and plantation including the surrounding areas of the *Orang Asli* villages. All rats captured were collected in the morning and brought to the laboratory in Mangrove Research Center for further investigation.



Plate 2.2: Collapsible wire traps (16.5" X 6" X 6").

2.2.2 Penang

In Penang, trapping was carried out in the city of Georgetown in August 2010 and November 2011. A total of 3 sessions were conducted during the wet (2 times) and dry season (1 time). Wire traps with baits were set up and placed in distinct sites located along Lebuhraya Campbell wet market and Jelutong wet market in Georgetown (Plate 2.3). Peanut butter and dried fish were used as baits and with a total of 30 wire cage traps placed around every night. Trappings were carried out in the span of four days and three nights. The traps were checked every morning and all captured rats were collected and brought to the Parasitology Lab of School of Biological Science in University of Science Malaysia (USM) for post mortem examination.



Plate 2.3: Lebu Campbell wet market, Penang.

2.2.3 Kuantan

In Kuantan, rats were trapped with the cooperation of the Vector Control Unit of the Kuantan municipal (MPK). Traps were set up in along markets and hawker stalls surrounding areas in Jalan Pasar, Teluk Chempedak and Tanjung Api and several areas around the Kuantan city and along the Sungai Pahang. Peanut butter and bread and dried fish were used as baits and with a total of 30 wire cage traps placed around every night. The trapping was conducted in July 2010 with a total of 2 sessions trapping during the dry season. Rats captured were then brought to the Animal House Lab of Faculty of Medicine in International Islamic University Malaysia (IIUM), Kuantan for further examination.

2.2.4 Malacca

In Malacca, the rats were trapped around Melaka Tengah district including Ayer Keroh, Batu Berendam and Kota Melaka area. Rats were captured with the assistance of the municipality of Melaka Tengah, Vector Control Unit of Majlis Bandaraya Melaka Bersejarah (MBMB). Trapping was conducted from May 2010 to February 2011 with a total of 3 trapping sessions during the one wet and two dry season sessions. Fish craker, coconut and dried fish were used as baits and with a total of 30 wire cage traps placed around every night. The rat traps were then brought to the Biology Lab of Melaka International College of Science and Technology (MiCoST) for further examination.

2.3 Ectoparasite collection

Wild rats successfully captured were subjected to morphometric examination. All rats were euthanized first by placing the rat into a cloth bag containing cotton wool soaked with chloroform. Rats were identified based on descriptions from Medway (1982) and Payne and Francis (1985). Morphometric measurements of the head-body, tail, ear, hind foot and weight of each rat was recorded and separated into two age-weight groups, namely, juvenile (<100g) and adult (>100g), as previously described by Brookes and Rowe (1987). However, for *R. norvegicus* rats below 140g in weight were allocated to juveniles (MacDonald & Barrett, 2005).

The fur of each specimen was combed with a fine tooth comb to dislodge any ectoparasite into a white paper. Fine forceps were used to remove ticks and mites from the skin when it was difficult to dislodge them by combing. The contents on the white paper were examined carefully with hand lens and any ectoparasites present were picked using the moistened end of sharpened wooden applicator stick and placed into specimen bottles containing 70% alcohol, except for ticks were later mounted for identification. A separate container was used for each rat. Mesostigmatid mites and lice were cleared in lactophenol and mounted with Hoyer's medium.

2.4 Post-mortem and endoparasites collection

Post-mortem examination of the rats was conducted according to standard protocol. The entire gut, from esophagus to anus was removed. Each section of the gut was placed into separate Petri dish, slit lengthwise and dissected in saline solution using a dissecting microscope. Stomach and caecum contents if excessive were filtered. All endoparasites recovered were collected, counted and preserved in 70% alcohol before further identification. Helminths were processed according to types. Nematodes recovered were fixed and cleared with lactophenol as temporary mounts and observed under microscope while cestodes were first relaxed, hydrated, stained, dehydrated and mounted in Canada balsam. The microhabitat and intensity of each endoparasite species was recorded.

2.5 Macroparasite Identification

Endoparasites and ectoparasites were examined, identified and determined using direct microscopic examination. Individual parasites were repeatedly washed in physiological saline, pH7.3. Each specimen was identified up to species level, counted and preserved and labeled. Rat ectoparasite specimens were cleared and mounted in polyvinyl lactophenol. Mounted slides were incubated at 40°C in the oven for a week before being sealed. The ectoparasite species was determined based on the morphological characteristics of the specimens. Strandmann and Mitchell (1963) and Johnson (1964) were primary references for mesostigmatid mites and lice identification.

For clearing and identification of nematodes, 5% glycerin in lactophenol was used as temporary mount. As for trematodes, cestodes and acanthocephalan worms were stained in Paracarmine stain, dehydrated in alcohol series, cleared in methyl salicylate oil and mounted in Permout or Canada balsam for identification. The helminthes were

identified using keys, illustrations and publications by Singh and Cheong (1971), Yap *et al.* (1977), Leong *et al.* (1979), Krishnasamy *et al.* (1980), Rusli (1988), Miyazaki (1991) and Ambu *et al.* (1996).

2.6 Data processing

All the ectoparasite and endoparasites recovered were analyzed using the software Quantitative Parasitology 3.0 (Reiczigel *et al.*, 2001). The prevalence, mean intensity, median intensity, with 95% confidence interval (Bush *et al.*, 1997) was calculated using the software. The mean intensity was reported to give information on the total quantity of parasites in the sample. The standard deviation (\pm SD) was not provided because for aggregated distributions frequently shown by parasites population as this may result in a wide confidence interval. Confidence interval preferably confidence level $\geq 95\%$ gave information about the uncertainty of the estimates, thus enabling a comparison at a glance (Rozsa *et al.*, 2000). The aggregation index, the exponent k of the negative binomial model (Krebs, 1989) was also calculated using the same software. This value was provided to characterize the skewness of the distribution (Rozsa *et al.*, 2000). Analysis of species diversity was done by calculating Simpson's Index and Brillouin Index from the Species Diversity and Richness IV software (Seaby & Henderson, 2006).

CHAPTER 3: WILD RAT POPULATION OF COASTAL AND ISLAND HABITATS

3.1 Introduction

As the world population rises and world's resources become scarcer, the need to protect human health and food sources from the ravages of rats have become increasingly important (Drummond, 1975). Rats are nocturnal mammals therefore are rarely seen (Hafner *et al.* 1998). Wild rat have a greater ability than most other animal species to harbour many zoonotic agents (Kataranovski *et al.*, 2008). Given their broad distribution and their close contact with different animals and humans, rats play an important role as reservoir host for vector-borne disease agent (Klimpel *et al.*, 2007). Commensal rats can cause great losses to the economy and affect both individuals and companies, particularly pre-harvest damage they cause to cereals (Stenseth *et al.*, 2003). On global scale, it was estimated that almost 280 million undernourished individuals could additionally benefit if more attention were paid to reducing pre- and post-harvest losses by rat (Meerburg *et al.*, 2009). Urbanization and rapid growth of towns have resulted in an increase in the accumulation of garbage and refuse creating favourable conditions for the proliferation of rats, and they rarely remain uninfected or harbor just a single species infection in nature (Behnke *et al.*, 2001).

Rats adapt themselves to practically any terrestrial habitat from the arctic to the tropic provided with food. Being homeothermal animals they are active even under severe climatic conditions. Therefore, rats can be described as highly adaptable and able group of animal and responsive to any given opportunities (Ryszkowski, 1975).

Some rats and mice are confined to human settlements or to disturbed vegetation modified by man. Others are strictly forest-dwellers either terrestrial or partially or

mainly arboreal. Immature rats are frequently caught and can be difficult to identify as they often differ in appearance from the adults with smaller measurement, but also with difference in colour and fur texture (Payne & Francis, 1998).

The rodent family Muridae is the largest mammalian family and contains 1,326 species, which are divided into 17 subfamilies (Klimpel *et al.* 2007). The genus *Rattus*, is one of the largest genera with as many as 78 species (Nowak, 1983). General morphological characteristics of this genera are the head and body between 80 to 300 mm with shorter or longer tail than the head-body length. The dorsal fur is black, grayish, dark brown, or yellowish or reddish brown, and the ventral region usually grayish or whitish. The rat finds shelters in a variety of locations including burrows in the ground or under rocks, under logs, and in piles of rubbish. Some are good climbers and prefer to nest in trees and other elevated positions (Walker, 1968).

There are a few studies done on wild rat populations in island and coastal habitats (Audy *et al.*, 1950; Nadchatram *et al.*, 1966; Medway, 1966; Lim *et al.*, 1974; Miyazaki & Dunn, 1965; Paramasvaran *et al.*, 2009b). However, only few studies focused on wild rat distribution on island and coastal habitats, however there is limited information on wild rat populations due to the small sample size (Mohd Zain, 2008). Most of the wild rat population studies reported were focused on commensal rat distribution in the urban cities (Zahedi *et al.*, 1996; Leong *et al.*, 1979; Singh & Cheong, 1971; Sinniah, 1979; Paramasvaran *et al.*, 2009a; 2009b; Mohd Zain *et al.*, 2012).

3.2 Common rat species

3.2.1 *Rattus rattus diardii*

R. rattus is native to Asia Minor and the Orient (Walker, 1968). In Malaysia, *R. rattus diardii* also known as the Malayan black rat is generally restricted to towns (Chasen, 1933). *R. rattus* can be identified by its finely grizzled olive brown upperparts and under parts usually slightly paler, buffy-brown with grey bases, but may be very pale brown to dull grey-brown. The tail is entirely brownish and the weight is between 100 to 200 grams. Their habitats are strictly confined to human settlements, plantations and gardens near settlement (Payne & Francis, 1998).

The black rats are prolific, but produce smaller and fewer litters than the Norway rat (*R. norvegicus*). After a gestation period of approximately 22 days, an average litter of about six young are born. It has been estimated that each adult female produces approximately 40 young per year (Linzey, 1998).

R. rattus diardii is distinguished from Malaysian field rat (*R. tiomanicus*) and Ricefield rat, (*R. argentiventer*) by coloration of the underparts and from Polynesian rat (*R. exulans*) by larger its size.

3.2.2 *Rattus norvegicus*

The Norway rat (*R. norvegicus*) is native to Japan and eastern Asia (Walker, 1968). In Malaysia, the Norway rat is usually found in coastal towns including Kuala Lumpur, Langkawi, Georgetown, Penang and Singapore city (Medway, 1983). However, *R. norvegicus* can be found also in agricultural area, natural forests and urban areas. Their dorsal region is brown, with the coarse hair but without prominent spines and the ventral region is grey or grayish brown. Its tail is dark brown on the upperside, unpigmented on the underside, without a sharp line of demarcation between the two shades (Medway, 1983). They feed on a variety of items including plants and animal materials if established around poultry houses. They can feed extensively on eggs and young chickens. They also have been known to kill lambs and young pigs. The females have 12 mammae compared to female's *R. rattus* with only 10 mammae (Payne & Francis, 1998).

R. norvegicus can breed throughout the year with the female producing up to five litters a year. The gestation period is only 21 days and litters can number up to 14, although 7 is common. They can reach sexual maturity in about five weeks and the maximum life span is up to three years. When lactating, female rats display a 24-hour rhythm of maternal behaviour and will usually spend more time attending to smaller litters than large ones (Grotta, 1969)

R. norvegicus are also known to be reservoirs to disease such as the bubonic plague which is transmitted to man by the bite of a flea, endemic typhus fever, rat-bite fever and a few other dreaded diseases. Zinsser (2008) in his book 'Rats, Lice and History' revealed that rat-borne typhus altered the human destiny more than the influence of any person in history. Rat-borne diseases are believed to have taken more human lives in the last ten centuries than all the wars and revolutions ever fought.

3.2.3 *Rattus tiomanicus*

Rattus tiomanicus or Wood Rat can be recognized by the finely grizzled olive brown upperparts, slightly darker in the midline, and hair relatively smooth without prominent spines. Its underparts is pure white or dull white and tail entirely dark brownish. Their feet are relatively broad with a distinct pattern of fine ridges on the pads of the underside. *R. tiomanicus* is usually nocturnal and lives in secondary and coastal forests, plantations, garden, scrub and grassland, but rarely in houses or tall dipterocarp forests. They build loose spherical nests of fresh green leaves, dry grass, or similar materials, or higher, e.g., in recesses in tree stumps or the crown of oil palms (Medway, 1983, Payne & Francis, 1998).

R. tiomanicus (formerly called *R. jalorensis*) was well recognised as the predominant and usually the sole rat species inhabiting oil palms in the Peninsular Malaysia (Harrison, 1957). However, distribution and ecology of *R. tiomanicus* was also observed on the small islands off the tip of Sabah was primarily in the lowlands on Borneo and extended up to altitude of 1700m on slopes of Mount Kinabalu in Sabah (Md Nor, 1996). In Peninsular Malaysia, rat damage by this species was reported as early as the 1930s (Wood & Chung, 2003).

3.2.4 *Rattus argentiventer*

Distribution of *Rattus argentiventer* or Ricefield rat is generally found in ricefields, scrub, grassland, and young plantations but absent from the forested interior. They are active mostly on the ground (burrows extensively) and nests in holes in the ground (Payne & Francis, 1998). The dorsal fur of *R. argentiventer* rat is olive brown with yellow and black hairs intermixed among the brown. The fur is rough but without prominent spines and the tail is entirely dark brownish. This rat can be distinguished by the other species by coloration (Medway, 1983). Young individuals have an orange-coloured tuft in front of each ear. They also can be found in nurseries and young oil palms (Wood, 1982).

R. argentiventer is polyestrous with an estrus cycle between 4 to 5 days. Gestation period lasts 3 weeks, with 3 to 8 young per litter and between 1 to 12 litters a year. Female rice field rats have 12 mammae, builds the nest 3 to 5 days before parturition. The young are born blind and fully furred. After 15 days, their eyes open. The young leave the nest after 3 weeks and reach sexual maturity after 3 months. All young experience maternal care and reared with their litter mates. Males play only a small part in the care of the young (Nowak, 1991, Hamilton, 1939). They live in large groups with a social hierarchy of dominant males and a few high ranking females and they tend to show territorial behaviour. They also have a vocal repertoire consisting mainly of squeals and whistles used in aggressive encounters (Lam, 1978: 1979, 1980: Nowak, 1991).

In Peninsular Malaysia, *R. argentiventer* was examined extensively from the rice-fields of Krian, northwest Perak (Chasen, 1933), where they were found feeding freely on the growing rice crops, grasses, roots and insects especially grasshoppers. The breeding

season begins from early harvest to late months of July or August and dependent on food supply.

3.3 Objectives

The objectives of present study were:

1. To determine the diversity and distribution of wild rat population in coastal (Kuantan and Malacca) and island (Penang and Carey Island) habitats of Peninsular Malaysia.
2. To determine the frequency distribution of macroparasites infection in wild rat populations in relation to rat species, locations, host sex, host age and seasons.

3.4 Result

3.4.1 Wild rat population in Peninsular Malaysia

A total of 363 wild rats were successfully captured from islands and coastal area in Peninsular Malaysia namely Kuantan, Malacca, Carey Island and Penang Island. Female rats (n=219, 60.3%) captured were higher compared to male rats (n=144, 39.7%) with more adults (87.9%) compared to juveniles (12.1%) (Table 3.1). The rat population comprised of the following four commensal rat species namely; *Rattus norvegicus*, *Rattus rattus diardii*, *Rattus tiomanicus* and *Rattus argentiventus*. *R. norvegicus* (56.7%) was the most dominant rat species followed by *R. rattus diardii* (16.3%) and *R. tiomanicus* (14.8%) (Figure 3.1). Two hundred and twenty six rats were captured during dry season and 137 during wet season from the four study sites.

Table 3.1 summarized general rat population according to host sex and host age. More females rats were captured for all rat species compared to males and more adults compared to juveniles. Also, more females were captured from all sites apart from Penang (Table 3.2). While, Table 3.3 summarized the rat population according to host age in each location. More adults were captured compared to juveniles in all sites.

The rat population in Kuantan comprised of the following species; *Rattus norvegicus* (72.8%), *Rattus rattus diardii* (20.4%) and *Rattus tiomanicus* (6.8%). More female rats were captured compared to male rats with 56 females and 47 males respectively. According to host age, there were less juveniles (n=6) captured compared to adults (n=97). All of 103 rats were captured during the dry season.

A total of 104 wild rats captured from Malacca consisted of predominantly *R. norvegicus* (55.8%) followed by *R. argentiventer* (29.8%), *R. tiomanicus* (9.6%) and *R. rattus diardii* (4.8%). There were more females (n=85) compared to males (n=19)

captured. According to the host age, less juveniles (n=16) were captured compared to adults (n=88). Less rats were captured during the wet (39 rats) compared to the dry (65 rats) season.

Two island habitats surveyed were Carey Island and Penang. In Carey Island, 81 wild rats were trapped in various locations around the oil palm plantation area. *Rattus tiomanicus* (45.7%) showed the highest number of rat species captured compared to *Rattus rattus diardii* (25.9%) and *Rattus argentiventus* (16.0%) while the lowest rat was *Rattus norvegicus* (12.3%). More females (n=47) were captured compared to males (n=34). Twenty three rats were captured during dry season and 58 rats were captured during wet season with fewer number of juveniles (n=14) compared to adult (n=67).

In Penang, 63 out of 75 wild rats captured were *Rattus norvegicus* followed by *Rattus rattus diardii* (n=12). More rat were captured during the wet season (n=40) compared to the dry season (n=35) with more males (n=44) compared to females (n=31). The rat population in Penang also comprised of mainly adults (89.3%) captured compared to juveniles (10.7%).

The number of the total wild rats captured from each area in this study according to host sex, season, and age is summarized in Figure 3.2 and Table 3.4.

3.4.2 Frequency distribution of ectoparasites in wild rat population

A total of nine ectoparasite species were recovered from this study namely; five mites species, *Laelaps nuttali*, *Laelaps echidninus*, *Ornithonyssus bacoti*, *Listrophoroides* sp., and *Laelaps sculpturatus*, two species of lice, *Polyplax spinulosa* and *Hoplopleura pacifica*, one species of tick, *Ixodes granulatus* and one species of flea, *Xenopsylla cheopis*.

From the total of 363 wild rats captured from the 4 sites, 324 rats (89.3%) were found infected with ectoparasites. Each infected rat harboured a minimum of one and a maximum of six ectoparasite species. Most rats captured harboured either single (39.1%) or 2 species (35.2%) of ectoparasites. Figure 3.3 showed the frequency distribution of ectoparasite infracommunity richness in the wild rat population from Peninsular Malaysia.

Table 3.5 showed the frequency distribution of ectoparasite infracommunity richness infesting the rat population according to species. *R. norvegicus* (90.7%) showed the highest infestation followed by *R. argentiventer* (88.6%), *R. tiomanicus* (87%) and *R. rattus diardii* (86.4%). A large proportion of *R. norvegicus* and *R. rattus diardii* harboured with a single ectoparasite specie (47.1% and 37.3% respectively) while most of *R. tiomanicus* harboured with 3 ectoparasite species (29.6%).

Table 3.6 showed the frequency distribution of ectoparasite infracommunity richness infesting the rat population according to location.

In Kuantan, 89 rats (86.4%) were found infested with ectoparasites. *R. norvegicus* (88%) was shown to have the highest infestations followed by *R. rattus diardii* (85.7%) and *R. tiomanicus* (71.4%). Each infected rat harboured a minimum of one specie and a

maximum of 3 ectoparasite species. Most rats harboured only a single specie (Table 3.7).

From a total of 104 rats captured in Malacca, 81.7% rats were infested with a minimum of one specie and a maximum of four ectoparasite species. High infestation was shown in *R. argentiventer* (85.3%) followed by *R. norvegicus* (82.8%) and *R. tiomanicus* (60%). Most rats here harboured either single or 2 ectoparasite species (Table 3.8).

In Carey Island, 76 rats (93.8%) captured were found to be infested with ectoparasites up to a maximum of six ectoparasite species. All *R. argentiventer* (100%) were infested with ectoparasites followed by *R. tiomanicus* (97.3%) and *R. rattus diardii* (81%). Most rats here harboured 2 ectoparasite species (Table 3.9).

In Penang 74 rats captured (98.7%) were found to be infested with ectoparasites with *R. norvegicus* (100%) the highest infestation compared to *R. rattus diardii* (91.7%). Each infected rat harboured a minimum of one specie and a maximum of three ectoparasite species. Most rats in Penang harboured with 2 ectoparasite species (Table 3.10).

The majority of *R. norvegicus* (90.7%) captured were infested with ectoparasites, with 93.5% having high infestation during the wet season compared to the dry season (89.1%) with most harbouring one single specie. High number of *R. rattus diardii* were infested during the wet season (95%) compared to the dry season (82.1%) with most rats harboured with either single or 2 ectoparasite species. Similarly, greater number of *R. tiomanicus* had higher infestation during the wet season (100%) compared to the dry season (74.1%). Most of *R. tiomanicus* had either 2 or 3 ectoparasite species. Table 3.11 shows the frequency distribution of ectoparasite infracommunity richness infesting the rat population according to season.

More *R. norvegicus* females were captured compared to males with almost similar ectoparasites infestation between both sexes (90.8% males and 90.7% females). Both sexes harboured only a single ectoparasite species. From 51 *R. rattus diardii* (86.4%) infested, more males (88.9%) were infested with ectoparasites compared to female rats (85.4%) with most rats harbouring either single or 2 ectoparasite species. However, more female (87.9%) *R. tiomanicus* had higher ectoparasites infestation compared to males (85.7%). *R. tiomanicus* also had between 2 or 3 ectoparasite species. Table 3.12 showed the frequency distribution of ectoparasites infracommunity richness infesting the rat population according to host sex.

High number of adult *R. norvegicus* and *R. rattus diardii* were infested with ectoparasites compared to juveniles (90.9% and 88.9% respectively). Most of these rat species harboured a single or 2 ectoparasite species. However, 92.9% of juvenile *R. tiomanicus* were infested with ectoparasites compared to adults (85%) with most rats harboured with 2 ectoparasite species. The distribution of *R. argentiventer* was not calculated due to the small sample size. Table 3.13 showed the frequency distribution of ectoparasites infracommunity richness infesting the rat population according to host age.

3.4.3 Frequency distribution of endoparasites in wild rat population

From the total of 363 rats captured in Kuantan, Malacca, Carey Island and Penang, 335 rats (92.3%) were found to be infected with endoparasites. Each infected rat harboured a minimum of one or up to a maximum of five endoparasite species. Ten species were recovered in total from this study namely; three cestodes species, *Taenia taeniaformis*, *Hymenolepis diminuta* and *Rodentolepis* (= *Hymenolepis*) *nana* and seven nematodes species, *Nippostrongylus brasiliensis*, *Angiostrongylus malaysiensis*, *Capillaria hepatica*, *Mastophorus muris*, *Heterakis spumosa*, *Hepatojarakus malayae* and *Syphacia muris*.

Figure 3.4 shows the frequency distribution of endoparasite infracommunity richness in the wild rat population from Peninsular Malaysia. Most rats captured harboured with 2 species (28.4%) followed by 3, 1, 4 and 5 endoparasite species respectively.

Result from Table 3.14 showed the frequency distribution of endoparasite infracommunity richness infecting the rat population according to species. *R. argentiventer* (95.5%) showed the highest infection followed by *R. tiomanicus* (92.6%), *R. norvegicus* (92.2%) and *R. rattus diardii* (89.8%). The three main rat species (*R. norvegicus*, *R. rattus diardii*, *R. tiomanicus*) harboured with 2 ectoparasite species (28.6%, 28.8% and 44.4% respectively) while *R. argentiventer* harboured with 3 ectoparasite species (36.4%).

Table 3.15 showed the frequency distribution of endoparasite infracommunity richness infesting the rat population according to location.

A total of 96 rats (93.2%) captured were found infected with endoparasites in Kuantan. *R. norvegicus* (96%) was shown to have the highest infections followed by *R. rattus diardii* (81%) and *R. tiomanicus* (42.9%). Each infected rats harboured a minimum of

one and a maximum of 5 endoparasite species. Most rats were found harbouring either 2 or 3 endoparasite species (Table 3.16).

From a total of 104 rats captured in Malacca, 101 (97.1%) rats were infected with a minimum of one and up to a maximum of five endoparasite species. All *R. argentiventer* and *R. tiomanicus* were infected with endoparasites while only 94.8% *R. norvegicus* were infected. Most rats harboured either a single or 2 ectoparasite species (Table 3.17).

In Carey Island, 73 rats (90.1%) captured were found infected with endoparasites with most rats harbouring either a single or 2 endoparasite species. *R. rattus diardii* (90.5%) was shown to have the highest infection followed by *R. tiomanicus* (89.2%) and *R. argentiventer* (84.6%). Each infected rats harboured a minimum of one and a maximum of 4 endoparasite species (Table 3.18).

In Penang, 65 (86.67%) rats were infected, with each rat harboured up to 4 endoparasite species. *R. norvegicus* (84.1%) had the highest infecting followed by *R. rattus diardii* (66.7%). Most rats in Penang harboured either a single or 2 endoparasite species (Table 3.19).

Similar infection (92.2%) was observed during the wet and dry season from 190 infected *R. norvegicus*. Most of this rat species harboured either a single or 3 ectoparasite species between both seasons. High number of *R. rattus diardii* was infected during the wet season (95%) compared to the dry season (87.2%) with most rats harboured with single ectoparasite specie. However, all *R. tiomanicus* captured during the dry season was infected (100%) when compared to the wet season (85%). Most of *R. tiomanicus* harboured with 2 ectoparasite species. Table 3.20 showed the frequency distribution of endoparasite infracommunity richness infecting the rat population according to season.

More female (96.3%) *R. norvegicus* were infected with endoparasites compared to males (87.8%) with both sexes harbouring either 2 or 3 endoparasite species. However for *R. rattus diardii*, more males (94.4%) were found to be infected with endoparasites compared to female rats (87.8%) with most rats harbouring either a single or 2 ectoparasite species. However, more female (93.9%) *R. tiomanicus* were infected with endoparasites compared to males (90.5%). Most of *R. tiomanicus* harboured 2 endoparasite species. Table 3.21 shows the frequency distribution of endoparasite infracommunity richness infecting the rat population according to host sex.

High numbers of adult *R. norvegicus* (93.5%) was infected with endoparasites compared to juveniles (57.1%) with most rats harbouring either a single or 2 endoparasite species. Similarly, more adult *R. rattus diardii* (91.1%) were infected with endoparasites compared to juveniles (85%) with most rats harbouring either a single or 2 endoparasite species. However, similar infection between adults and juveniles were observed in *R. tiomanicus* (93%) with most rats harbouring 2 endoparasite species. The distribution of *R. argentiventer* was not calculated due to the small sample size. Table 3.22 shows the frequency distribution of endoparasite infracommunity richness infecting the rat population according to host age.

Table 3.1: The rat population according to host sex and host age in Peninsular Malaysia

Rat species	Host sex		Host age	
	Male	Female	Adult	Juvenile
<i>R. norvegicus</i>	98	108	199	7
<i>R. rattus diardii</i>	18	41	45	14
<i>R. tiomanicus</i>	21	33	40	14
<i>R. argentiventer</i>	7	37	35	9
TOTAL	144	219	319	44

Table 3.2: The rat population according to host sex in each location.

Rat species	Location							
	Kuantan		Malacca		Carey Island		Penang	
	M	F	M	F	M	F	M	F
<i>R. norvegicus</i>	41	34	16	42	3	7	38	25
<i>R. rattus diardii</i>	3	18	0	5	9	12	6	6
<i>R. tiomanicus</i>	3	4	3	7	15	22	0	0
<i>R. argentiventer</i>	0	0	0	31	7	6	0	0
TOTAL	47	56	19	85	34	47	44	31

*M, male; F, female

Table 3.3: The rat population according to host age in each location.

Rat species	Location							
	Kuantan		Malacca		Carey Island		Penang	
	A	J	A	J	A	J	A	J
<i>R. norvegicus</i>	75	0	56	2	9	1	59	4
<i>R. rattus diardii</i>	17	4	2	3	18	3	8	4
<i>R. tiomanicus</i>	5	2	6	4	29	8	0	0
<i>R. argentiventer</i>	0	0	24	7	11	2	0	0
TOTAL	97	6	88	16	67	14	67	8

*A, adult; J, juvenile

Figure 3.1: The wild rat population from coastal and island habitats of Peninsular Malaysia according to species.

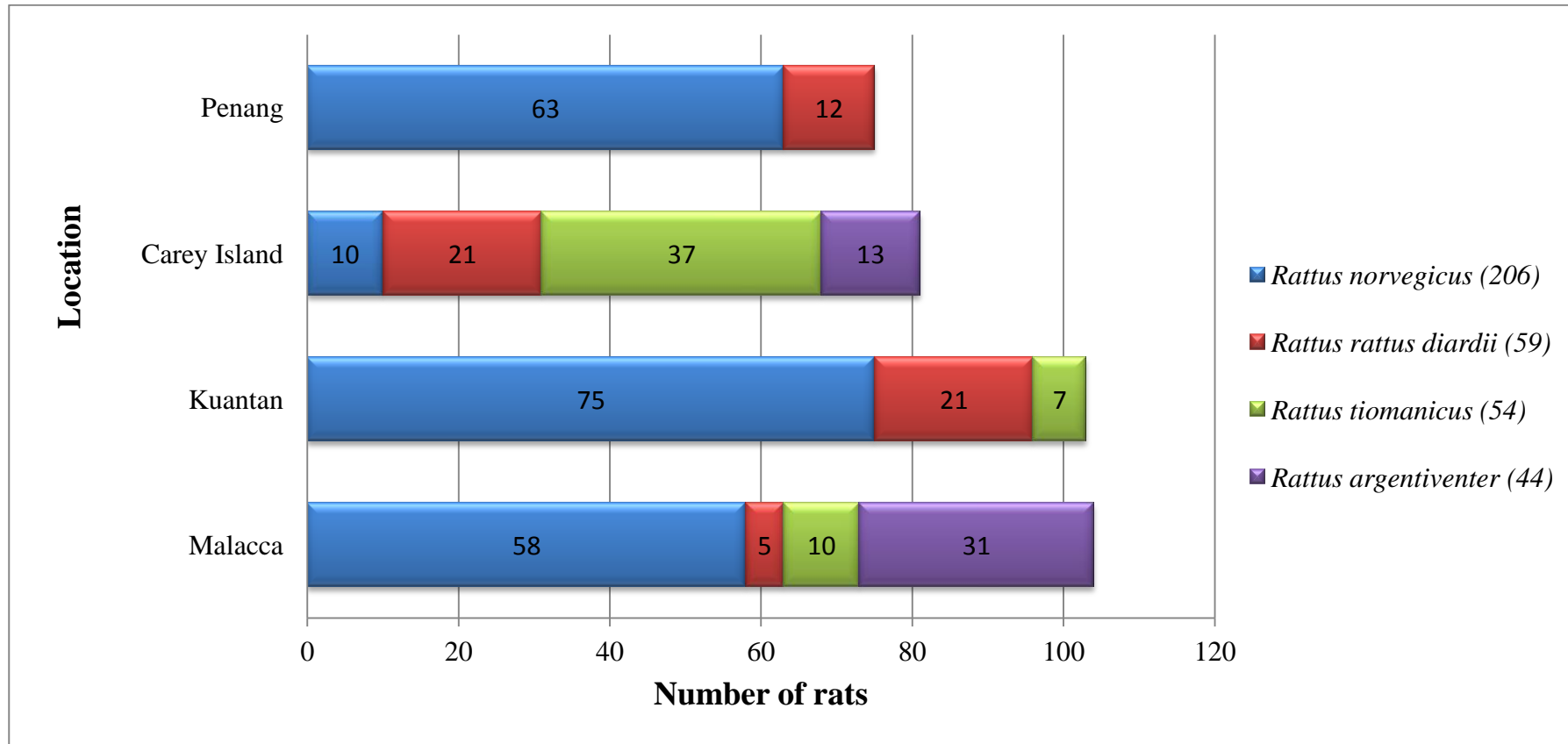


Figure 3.2: The wild rat population captured from four locations according to host sex.

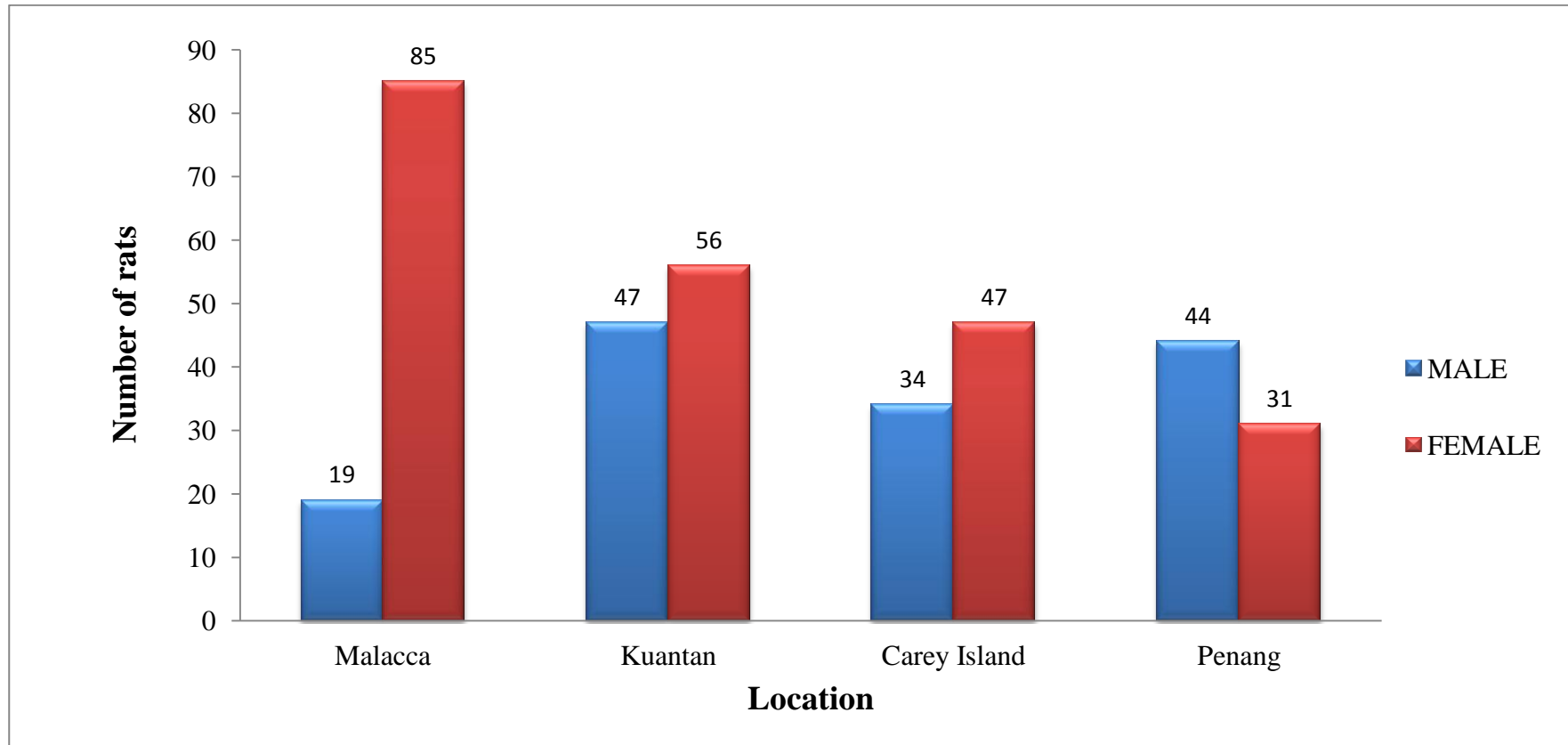


Table 3.4: Number of wild rats examined by site, host age, and season from Peninsular Malaysia.

Site	Season			
	Wet		Dry	
	Adult	Juvenile	Adult	Juvenile
Kuantan	0	0	97	6
Malacca	37	2	51	14
Carey Island	45	13	22	1
Penang	35	5	32	3
TOTAL	117	20	202	24

Figure 3.3 Frequency distribution of ectoparasite infracommunity richness in the wild rat population from Peninsular Malaysia.

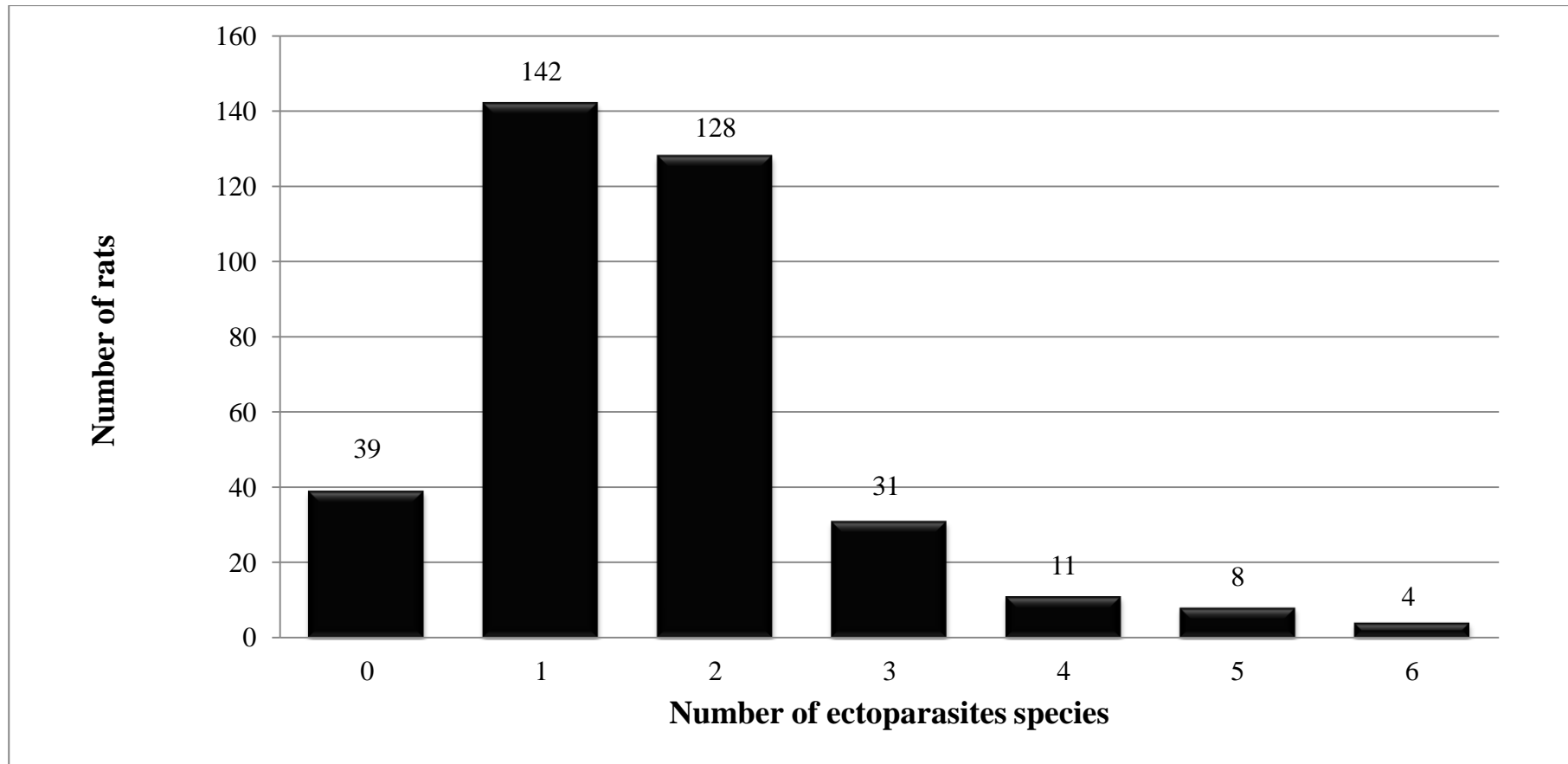


Table 3.5: The frequency distribution of ectoparasite infracommunity richness infesting the rat population according to species.

Species	No. of parasites in host								
	0	1	2	3	4	5	6	Total	
<i>R. norvegicus</i>	No. of host	19	97	68	13	5	2	2	206
	Prevalence (%)	11.3	47.1*	33.0	6.3	2.4	0.9	0.9	100
<i>R. rattus diardii</i>	No. of host	8	22	18	6	3	2	0	59
	Prevalence (%)	13.6	37.3*	30.5	10.1	5.1	3.4	0	100
<i>R. tiomanicus</i>	No. of host	7	12	12	16	3	3	1	54
	Prevalence (%)	12.9	22.2	22.2	29.6*	5.6	5.6	1.9	100
<i>R. argentiventer</i>	No. of host	5	11	25	1	0	1	1	44
	Prevalence (%)	11.4	25.0	58.8*	2.2	0	2.3	2.3	100

* Highest prevalence of infection in species

Table 3.6: The frequency distribution of ectoparasite infracommunity richness infesting the rat population according to location.

Location	No. of parasites in host								
		0	1	2	3	4	5	6	Total
Kuantan	No. of host	14	60	23	6	0	0	0	103
	Prevalence (%)	13.6	58.3*	22.3	5.8	0	0	0	100
Malacca	No. of host	19	41	42	1	1	0	0	104
	Prevalence (%)	18.3	39.4	40.4*	0.9	0.9	0	0	100
Carey Island	No. of host	5	16	25	13	10	8	4	81
	Prevalence (%)	6.2	19.7	30.9*	16.1	12.4	9.9	4.9	100
Penang	No. of host	1	25	38	11	0	0	0	75
	Prevalence (%)	1.3	33.3	50.7	14.7	0	0	0	100

* Highest prevalence of infection by site.

Table 3.7: The frequency distribution of ectoparasite infracommunity richness infesting the rat population in Kuantan.

Location		No. of parasites in host							Total
		0	1	2	3	4	5	6	
<i>Rattus norvegicus</i>	No. of host	9	43	18	5	0	0	0	75
	Prevalence (%)	12.0	57.3	24.0	6.7	0	0	0	100
<i>Rattus rattus diardii</i>	No. of host	3	13	5	0	0	0	0	21
	Prevalence (%)	14.3	61.9	23.8	0	0	0	0	100
<i>Rattus tiomanicus</i>	No. of host	2	4	1	0	0	0	0	7
	Prevalence (%)	28.6	57.1	14.3	0	0	0	0	100

Table 3.8: The frequency distribution of ectoparasite infracommunity richness infesting the rat population in Malacca.

Location		No. of parasites in host							Total
		0	1	2	3	4	5	6	
<i>Rattus norvegicus</i>	No. of host	10	31	15	1	1	0	0	58
	Prevalence (%)	17.2	53.4	25.7	1.7	1.7	0	0	100
<i>Rattus argentiventer</i>	No. of host	5	7	19	0	0	0	0	31
	Prevalence (%)	16.1	22.6	61.3	0	0	0	0	100
<i>Rattus tiomanicus</i>	No. of host	4	1	5	0	0	0	0	10
	Prevalence (%)	40.0	10.0	50.0	0	0	0	0	100

Table 3.9: The frequency distribution of ectoparasite infracommunity richness infesting the rat population in Carey Island.

Location		No. of parasites in host							
		0	1	2	3	4	5	6	Total
<i>Rattus tiomanicus</i>	No. of host	1	7	12	10	3	3	1	37
	Prevalence (%)	2.7	18.9	32.4	27.0	8.1	8.1	2.7	100
<i>Rattus rattus diardii</i>	No. of host	4	5	5	2	3	2	0	21
	Prevalence (%)	19.0	23.8	23.8	9.5	14.3	9.5	0	100
<i>Rattus argentiventer</i>	No. of host	0	4	6	1	1	1	0	13
	Prevalence (%)	0	30.8	46.2	7.7	7.7	7.7	0	100

Table 3.10: The frequency distribution of ectoparasite infracommunity richness infesting the rat population in Penang.

Location		No. of parasites in host							Total
		0	1	2	3	4	5	6	
<i>Rattus norvegicus</i>	No. of host	0	23	33	7	0	0	0	63
	Prevalence (%)	0	36.5	52.4	11.1	0	0	0	100
<i>Rattus rattus diardii</i>	No. of host	1	2	5	4	0	0	0	12
	Prevalence (%)	8.3	16.7	41.7	33.3	0	0	0	100

Table 3.11: The frequency distribution of ectoparasite infracommunity richness infesting the rat population according to season.

Location	Prevalence (%)	No. of parasites in host							
		0	1	2	3	4	5	6	Total host
<i>Rattus norvegicus</i>	Wet	6.5	46.8	28.6	6.5	6.5	2.6	2.6	77
	Dry	10.9	47.3	35.7	6.2	0	0	0	129
<i>Rattus rattus diardii</i>	Wet	5.0	10.0	40.0	25.0	10.0	10.0	0	20
	Dry	17.9	51.3	25.6	2.6	2.6	0	0	39
<i>Rattus tiomanicus</i>	Wet	0	14.8	25.9	33.3	11.1	11.1	3.7	27
	Dry	25.9	29.6	37.0	7.4	0	0	0	27

Table 3.12: The frequency distribution of ectoparasite infracommunity richness infesting the rat population according to host sex.

Location	Prevalence (%)	No. of parasites in host							Total host
		0	1	2	3	4	5	6	
<i>Rattus norvegicus</i>	Male	9.2	53.1	26.5	8.2	3.1	0	0	98
	Female	9.3	41.7	38.9	46.3	1.9	1.9	1.9	109
<i>Rattus rattus diardii</i>	Male	11.1	22.2	38.9	11.1	11.1	5.6	0	18
	Female	14.6	43.9	36.8	9.8	2.4	2.4	0	41
<i>Rattus tiomanicus</i>	Male	14.3	23.8	14.3	28.6	4.8	9.5	4.8	21
	Female	12.1	21.2	42.4	15.2	6.1	3.0	0	33

Table 3.13: The frequency distribution of ectoparasite infracommunity richness infesting the rat population according to host age.

Location	Prevalence (%)	No. of parasites in host							
		0	1	2	3	4	5	6	Total host
<i>Rattus norvegicus</i>	Adult	9.0	48.2	32.2	6.5	2.0	1.0	1.0	199
	Juvenile	14.3	14.3	57.1	14.3	0	0	0	7
<i>Rattus rattus diardii</i>	Adult	11.1	42.2	26.7	11.1	4.4	4.4	0	45
	Juvenile	21.4	21.4	42.9	7.1	7.1	0	0	14
<i>Rattus tiomanicus</i>	Adult	15.0	22.5	30.0	17.5	7.5	5.0	2.5	40
	Juvenile	7.1	21.4	35.7	28.6	0	7.1	0	14

Figure 3.4 Frequency distribution of endoparasite infracommunity richness in the wild rat population from Peninsular Malaysia.

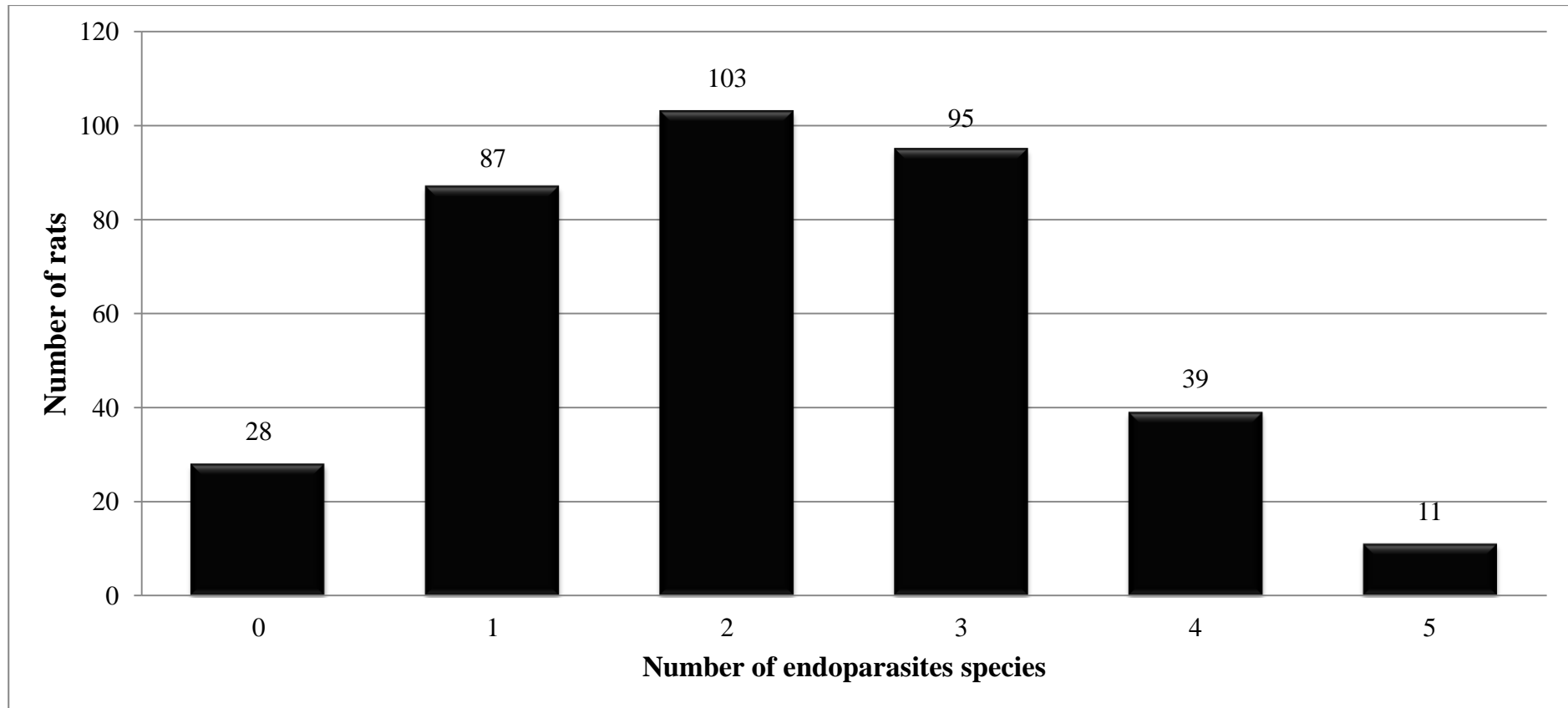


Table 3.14: The frequency distribution of endoparasite infracommunity richness infecting the rat population according to species.

Species	No. of parasites in host							Total
	0	1	2	3	4	5		
<i>R. norvegicus</i>	No. of host	16	54	59	51	22	4	206
	Prevalence (%)	7.7	26.2	28.6*	24.8	10.7	1.9	100
<i>R. rattus diardii</i>	No. of host	6	17	17	15	3	1	59
	Prevalence (%)	10.2	28.8	28.8*	25.4	5.1	1.7	100
<i>R. tiomanicus</i>	No. of host	4	11	24	13	1	1	54
	Prevalence (%)	7.4	20.4	44.4*	24.1	1.9	1.9	100
<i>R. argentiventer</i>	No. of host	2	5	3	16	13	5	44
	Prevalence (%)	4.6	11.4	6.8	36.4*	29.6	11.4	100

* Highest prevalence of infection in species.

Table 3.15: The frequency distribution of endoparasite infracommunity richness infecting the rat population according to location.

Location	No. of parasites in host							Total
	0	1	2	3	4	5		
Kuantan	No. of host	7	20	34	28	11	3	103
	Prevalence (%)	6.8	19.4	33.0*	27.1	10.7	2.9	100
Malacca	No. of host	3	20	21	33	19	8	104
	Prevalence (%)	2.9	19.2	20.2	31.7*	18.3	7.7	100
Carey Island	No. of host	8	23	26	18	6	0	81
	Prevalence (%)	9.9	28.4	32.1*	22.2	7.4	0	100
Penang	No. of host	10	24	22	16	3	0	75
	Prevalence (%)	13.3	32.0*	29.3	21.3	4.0	0	100

* Highest prevalence of infection by site.

Table 3.16: The frequency distribution of endoparasites infracommunity richness infecting the rat population in Kuantan.

Location		No. of parasites in host						Total
		0	1	2	3	4	5	
<i>Rattus norvegicus</i>	No. of host	3	13	22	25	10	2	75
	Prevalence (%)	40.0	17.3	29.3	33.3	13.3	26.7	100
<i>Rattus rattus diardii</i>	No. of host	4	3	9	3	1	1	21
	Prevalence (%)	19.0	14.3	42.9	14.3	4.8	4.8	100
<i>Rattus tiomanicus</i>	No. of host	4	3	0	0	0	0	7
	Prevalence (%)	57.1	42.9	0	0	0	0	100

Table 3.17: The frequency distribution of endoparasite infracommunity richness infecting the rat population in Malacca.

Location		No. of parasites in host						
		0	1	2	3	4	5	Total
<i>Rattus norvegicus</i>	No. of host	3	20	15	10	8	2	58
	Prevalence (%)	5.2	34.5	25.9	17.2	13.8	3.4	100
<i>Rattus argentiventer</i>	No. of host	0	0	2	14	10	5	31
	Prevalence (%)	0	0	6.5	45.2	32.3	16.1	100
<i>Rattus tiomanicus</i>	No. of host	0	0	3	6	0	1	10
	Prevalence (%)	0	0	30.0	60.0	0	10.0	100

Table 3.18: The frequency distribution of endoparasite infracommunity richness infecting the rat population in Carey Island.

Location		No. of parasites in host						Total
		0	1	2	3	4	5	
<i>Rattus tiomanicus</i>	No. of host	4	7	18	7	1	0	37
	Prevalence (%)	10.8	18.9	48.6	18.9	2.7	0	100
<i>Rattus rattus diardii</i>	No. of host	2	10	4	5	0	0	21
	Prevalence (%)	9.5	47.6	19.0	23.8	0	0	100
<i>Rattus argentiventer</i>	No. of host	2	5	1	2	3	0	13
	Prevalence (%)	15.4	38.5	7.7	15.4	23.1	0	100

Table 3.19: The frequency distribution of endoparasite infracommunity richness infecting the rat population in Penang.

Location		No. of parasites in host						
		0	1	2	3	4	5	Total
<i>Rattus norvegicus</i>	No. of host	10	20	19	12	2	0	63
	Prevalence (%)	15.9	31.7	30.2	19.0	3.2	0	100
<i>Rattus rattus diardii</i>	No. of host	4	3	4	1	0	0	12
	Prevalence (%)	33.3	25.0	33.3	8.3	0	0	100

Table 3.20: The frequency distribution of endoparasite infracommunity richness infecting the rat population according to season.

Location	Prevalence (%)	No. of parasites in host						Total host
		0	1	2	3	4	5	
<i>Rattus norvegicus</i>	Wet	7.8	35.1	33.8	19.5	3.9	0	77
	Dry	7.8	20.9	25.6	27.9	14.7	3.1	129
<i>Rattus rattus diardii</i>	Wet	5.0	35.0	25.0	30.0	5.0	0	20
	Dry	12.8	25.6	30.8	23.1	5.1	2.6	39
<i>Rattus tiomanicus</i>	Wet	14.8	11.1	48.1	22.2	3.7	0	27
	Dry	0	29.6	40.7	25.9	0	3.7	27

Table 3.21: The frequency distribution of endoparasite infracommunity richness infecting the rat population according to host sex.

Location	Prevalence (%)	No. of parasites in host						
		0	1	2	3	4	5	Total host
<i>Rattus norvegicus</i>	Male	12.2	26.5	31.6	21.4	6.1	1.0	98
	Female	3.7	25.9	25.9	26.8	14.8	2.8	108
<i>Rattus rattus diardii</i>	Male	5.6	50.0	22.2	16.7	5.6	0	18
	Female	12.2	19.5	31.7	29.3	4.9	2.4	41
<i>Rattus tiomanicus</i>	Male	9.5	23.8	38.1	23.8	4.8	0	21
	Female	6.1	18.2	48.5	24.2	0	3.0	33

Table 3.22: The frequency distribution of endoparasite infracommunity richness infecting the rat population according to host age

Location	Prevalence (%)	No. of parasites in host						Total host
		0	1	2	3	4	5	
<i>Rattus norvegicus</i>	Adult	6.5	26.1	29.1	25.1	11.1	2.0	199
	Juvenile	42.9	28.6	14.3	14.3	0	0	7
<i>Rattus rattus diardii</i>	Adult	8.9	26.7	33.3	24.4	4.4	2.2	45
	Juvenile	14.3	35.7	14.3	28.6	7.1	0	14
<i>Rattus tiomanicus</i>	Adult	7.5	20.0	42.5	27.5	2.5	0	40
	Juvenile	7.1	21.4	50.0	14.3	0	7.1	14

3.5 Discussion

Rats harbour various types of parasites of which some are zoonotic and can give severe pain to the animal and cause death to the human. Since rats live in close proximity with humans, it is very important to understand the implications the parasites harboured by these rodents can bring to human and their environment. In this study, four locations were selected to represent coastal and islands habitat. All sites apart from Carey Island were urban cities. Overall, the composition of rat population between the two environments were almost similar with 4 commensal rat species inhabiting all sites however, with species distribution differing between sites.

R. norvegicus (56.7%) was the most dominant rat species followed by *R. rattus diardii* (16.3%), *R. tiomanicus* (14.9%) and *R. argentiventer* (12.1%). *R. tiomanicus* was not present in Penang while *R. argentiventer* was only present in Carey Island and Malacca. Sinniah (1979) recorded higher number of rodents species with up to 9 species captured from various habitats including oil palm estates in Peninsular Malaysia. Paramasvaran *et al.*, (2009b) found 3 rat species namely *R. rattus diardii*, *R. norvegicus* and *R. exulans* from urban, coastal and rice field habitats. Mohd Zain, (2008) studied the rodent population from Pulau Jarak and Pulau Langkawi and recovered 3 commensal rodent species (*R. tiomanicus*, *R. rattus diardii* and *R. exulans*) inhabiting the island. This large number of commensal rats in these sites has been undoubtedly maintained by the continuous human activities, ample food supply and appropriate infrastructure in the surrounding area.

In the present study higher number of *R. norvegicus* was captured from the 3 sites; Kuantan (72.8%), Malacca (55.8%) and Penang (100%). This rat species is commonly found in coastal areas (Harrison, 1957) but in some studies it has been reported from other habitats (Dunn *et al.*, 1968); Sinniah *et al.*, 1979; Ow-Yang, 1971). However,

Zahedi *et al.* (1984) reported that *Rattus norvegicus* was the second most dominant rat species after *R. rattus diardii* found in urban and semi-urban habitats.

In Carey Island, the rat population was dominated by *R. tiomanicus* (45.7%). Dominance of *R. tiomanicus* was consistent with the study by Krishnasamy *et al.* (1980) given that this rat species is commonly associated with scrub, secondary forest and plantations, particularly oil-palm estates (Ow-Yang, 1971). In addition, Chasen (1933) and Medway (1966) had considered this rat to be common in primary forests of all altitudes.

R. rattus diardii species was the second most dominant species captured in 3 locations except Malacca. Large numbers of *R. rattus diardii* have also been reported in previous studies, particularly Harrison (1957), Leong *et al.* (1979), Zahedi *et al.* (1984), Singh *et al.* (1995), Paramasvaran *et al.* (2009b) and Mohd Zain *et al.* (2012). The rat species is widely believed to be introduced in Peninsular Malaysia, and it seems apparent that the existing ecological conditions in this study areas are suitable for the proliferation of the *R. rattus* community. However, the absence of *R. argentiventer* from Kuantan and Penang is probably because these species are less adaptable to urban environment they are usually found in ricefield, scrub and grassland. High proportion of *R. argentiventer* was captured in Malacca due to the patches of ricefield found scattered in suburban areas.

More females for 3 rats species (*R. norvegicus*, *R. rattus diardii*, *R. tiomanicus*) were captured in all locations except in Penang, suggesting that the female rats are perhaps more active and by extension would have a comparatively wider home range than the males. Similarly as reported by Krishnasamy *et al.* (1980), more female rats were captured compared to males, suggesting that females were more active in foraging for food. However, studies by Paramaswaran *et al.* (2005), Syed Arnez & Mohd Zain

(2006), Mohd Zain (2008) and Mohd Zain *et al.* (2012) showed almost equal numbers between males and females captured highlighting the scavenging behaviour of commensal rats were independent from one another.

The lower number of juvenile rats captured can also be attributed to inherently limited activity and home range. Previous studies with similar results include, Paramaswaran *et al.* (2005), Syed Arnez and Mohd Zain (2006), Mohd Zain (2008) and Mohd Zain *et al.* (2012). It is also suggested that the greater number of adult rats generates unbalance social competition, restricting the overall movement of the younger.

High percentage of wild rats from island and coastal sites was infested with ectoparasites. Overall, the ectoparasites diversity was somehow low, although consistent with previous records by Zahedi *et al.* (1984), Singh *et al.* (1995) and Paramaswaran *et al.* (2009b). The ectoparasites recovered were comprised completely of cosmopolitan species. This probably occurred because the arthropod were maintained by the predominantly commensal rat species in the habitat.

Paramaswaran *et al.* (2009b) recorded 57.7% rats were infested with at least one species of ectoparasites. However, majority of rats in this study harboured between one to two ectoparasite species. This could be because of the overwhelmingly large number of rats occupying similar location in both habitats, circuitously facilitating transmissions and multiple infestations through inter- and intraspecific interactions.

The majority of wild rats from both habitats also harboured high prevalence of endoparasites indicating the immense magnitude of helminth infections among rats living within the areas. In addition, the availability of suitable intermediate hosts, are the contributing factors to the widespread presence of cestode infections while nematodes which have direct lifecycles occur relatively easier, conceivably also in a more consistent manner (Bellocq, 2003). The relatively high rainfall and humidity in

Malaysia is suitable for the eggs to hatch before the infective larvae penetrated or eaten by the host.

Large proportion of the rat population in this study harboured between 2 to 3 species of endoparasites. Paramasvaran *et al.* (2009a) reported mainly had single helminth infections in rats caught in a wet market in Kuala Lumpur. The increase in the infracommunity richness in this study could be influenced by the food the rat consumes and lifestyle in general that would have enabled them to thrive in the area and at the same time maintaining and transmitting a distinct and highly adaptive cosmopolitan endoparasite community. Omnivorous diet and foraging behaviour of wild rats may also expose them to a variety of endoparasites resulting high prevalence and abundance observed.

The parasitic infection between host sexes showed females were infected higher with both ecto and endoparasites except for *R. rattus diardii* where males showed higher infection. Similarly, female host bias was observed previously in Wertheim's study (1963). However, studies by Mafiana *et al.* (1997), Abu Madi *et al.* (2001) and Mohd Zain (2008), reported significant differences to which males appeared to be more susceptible to infection compared to females. One possible explanation is that the male sex hormone has an immunodepressive effect on male rats (Ferrari *et al.*, 2004).

On the other hand, the host age showed significant differences with adults heavily infected compared to juveniles in all rat species except *R. tiomanicus*. Parasitic burdens between the host ages could be attributed to longer exposure time to infective stages in older rats (Krishnasamy *et al.*, 1980). Greater number of adult rats were captured compared to juveniles was also noted in this study. More juvenile bias was observed in *R. tiomanicus* for ecto and endoparasites infection suggesting that *R. tiomanicus* in

agriculture, scrubs and forest area had more chances of picking up parasites, thus increasing the level of infection.

The endoparasites infection in all rat species showed almost similar prevalence of infection with no discrimination to season. However, it was noted that higher ectoparasites infestations in wet season compared to dry season, a condition suitable for the survival of ectoparasites especially for mites and lice. Mites thrive in moist circumstances and unable to survive for more than a few days in low humidity. In his study, Abu-Madi *et al.* (2001) showed intrinsic factor such as, season can attribute to the ectoparasite infestation where infestation was observed higher during the (dry and arid) summer months in a dessert. The warm and wet climate in Malaysia with no marked contrasting seasons comparable to those experienced in temperate climate countries could be the reason why this trend was not visible here.

3.6 Conclusion

The wild rat populations captured from four locations in Peninsular Malaysia comprised of four commensal rat species namely; *Rattus norvegicus*, *Rattus rattus diardii*, *Rattus tiomanicus* and *Rattus argentiventer*. More female rats were captured compared to males meanwhile higher number of adults were captured compared to juveniles. The numbers of wild rats captured during wet season were higher when compared to the dry season except for *R. tiomanicus*. There were no distinctions between the compositions of the two rat populations in both habitats. The only difference observed is the dominant rat species in Carey Island was *R. tiomanicus*. Similar cosmopolitan ecto and endoparasite species were recovered from all locations however higher species diversity was observed in Carey Island.

Species richness analysis showed infected rats harboured a minimum of one to up to a maximum of six ectoparasite species and up to five endoparasite species with the majority harboured between one ectoparasite and two endoparasite species. Parasites distribution were normally overdispersed or aggregated within the rat population where only a few hosts harboured large number of parasites. *R. norvegicus* was infested with the highest prevalence of ectoparasite species with similar burden between host sex and season while heavier infestation in adult compared to juveniles. Similarly, significantly higher endoparasites infection was observed in *R. norvegicus* with regards to host sex and host age.

CHAPTER 4: THE ECTOPARASITES COMMUNITIES OF THE WILD RAT POPULATIONS IN PENINSULAR MALAYSIA

4.1 Introduction

Ectoparasites are a diverse and highly adapted group of animals that infest the external body surface of vertebrates (Hanafi-Bojd *et al.*, 2007). They pose a public health threat as potential disseminator of plague, scrub and murine typhus, tularaemia and bartonellosis. Ectoparasites such as ticks can cause serious and life-threatening illnesses in humans and animals (Chul-Min *et al.*, 2006). Animals and their ectoparasites play important roles in distribution of arboviruses, streptococcal infections, choriomeningitis, plague, tularemia, leptospirosis and spirochetosis (Manson & Stanko, 2005).

The local rat-borne ectoparasites from urban habitat have received little attention compared to endoparasites studies. Only a few studies on collections of small mammal ectoparasites have been reported from island and coastal habitat (Upham *et al.*, 1971; Nadchatram, 1966; Singh, 1990) but none of these focused on wild rats as a specific host.

Zahedi *et al.*, (1984) and Paramasvaran *et al.*, (2009b) studies are the only works on ectoparasites fauna in the urban rats. Though there have been other related studies from similar local habitats (i.e Traub, 1960; Singh *et al.*, 1995; Nadchatram *et al.*, 1966; Nadchatram & Ramalingam, 1974), however these studies focused on selected ectoparasites species and offered a limited perspective on the overall rat ectoparasites diversity.

There are several studies of ectoparasites in small mammals including rats from forest or wildlife reserves (Salleh Ismail *et al.*, 2003; Madinah *et al.*, 2011; Mariana *et al.*,

2011; Mariana *et al.*, 2008; Mariana *et al.*, 2005; Chulun *et al.*, 2005; Shabrina *et al.*, 1989; Shabrina, 1990, 1991; Ho *et al.*, 1985; Ho & Krishnasamy, 1990).

In this chapter, an investigation into ectoparasites fauna of wild rats from island habitats (Carey Island and Penang) and urban coastal cities (Kuantan and Malacca) in Peninsular Malaysia were reported. The objectives of this study were to update the present ectoparasites fauna in terms of prevalence of infections and abundance of each ectoparasites species in wild rat populations in relation to the habitat. This study also evaluated the role of intrinsic (host age, host sex) and extrinsic (season, location) factors in structuring the ectoparasites community of the wild rats population and identify ectoparasites species that is of known public health importance and potential risk to each location.

4.2 Result

From the 363 rats captured, a total of 319 (87.9%) wild rats were found to be infected with ectoparasites. Recovery of ectoparasites via fine tooth combing of all the rats recovered 3,412 specimens, with abundance value of 8.36 ectoparasites per infected rat. The largest ectoparasite burden in a single rat host was 345. The ectoparasites recovered consist of four major groups namely the mites, lice, tick and flea. The ectoparasites examined on rats were mainly in adult stage, however some ticks recovered were nymphs.

Overall, a total of nine species of ectoparasites were successfully identified. Most species were cosmopolitan species namely five species of mites; *Laelaps nuttali*, *Laelaps echidninus*, *Ornithonyssus bacoti*, *Listrophoroides* sp., and *Laelaps sculpturatus*, two species of lice, *Polyplax spinulosa* and *Hoplopleura pacifica*, one tick species, *Ixodes granulatus* and one flea species, *Xenopsylla cheopis*. Each rat harboured a minimum of one specie and a maximum of six species of ectoparasites. Figure 4.1 showed the distribution of all ectoparasites recovered from this study.

Results from overall analysis of all rats captured from all sites showed *Laelaps nuttali* (55.9%, CL: 50.69-61.02) was the most prevalence while *Ornithonyssus bacoti* revealed the lowest prevalence with 1.65% (CL: 0.73-3.53).

Of the 5 mite species; *Listrophoroides* sp. was found the most abundant ectoparasites with mean intensity 27.41 per infected rats meanwhile the flea, *Xenopsylla cheopis* was the least abundant with mean intensity 1.06 per infected rats. The distribution pattern recovered of more frequently occurring ectoparasites exhibited typically overdispersed or aggregated characteristic with k value ranging between 0.010-0.511. Table 4.1 summarized the quantitative analysis of ectoparasites recovered from rats of the four locations in Peninsular Malaysia.

The rat populations were dominated by 3 commensal rat species namely *Rattus norvegicus* (206) followed by *Rattus rattus diardii* (59) and *Rattus tiomanicus* (54). The 3 dominant rat species harboured ectoparasites with almost similar prevalence of all species groups with 89.3% *R. norvegicus*, 87% *R. tiomanicus* and 83.1% *R. rattus diardii* respectively. All ectoparasite species recovered were found on all rat species.

The mite, *Laelaps nuttali* was the most prevalent ectoparasites recovered from all rat species. *R. tiomanicus* showed the highest (63%) infestation meanwhile *R. norvegicus* and *R. rattus diardii* showed 55.8% and 42.4% respectively. One of the mite species, *Listrophoroides* sp. was found the most abundant ectoparasites infesting *R. norvegicus* with mean intensity 63 per infected rats.

Table 4.2 summarized the prevalence, mean intensity, abundance as well as the comparative analysis of ectoparasites in rat species. The analysis between the 3 rat species showing no significant differences in the prevalence except for *Listrophoroides* sp., *Laelaps sculpturatus*, *Hoplopleura pacifica* and *Ixodes granulatus*. *Laelaps sculpturatus*, *Hoplopleura pacifica* and *Ixodes granulatus* were observed to be of high in prevalence infesting *R. tiomanicus* with 20.4%, 29.6% and 24.1% respectively compared to other species. Prevalent of *Listrophoroides* sp. was low in all rat species is due to the small sample size of this species.

Table 4.3 showed the analysis of species diversity using Simpson Index and Brillouin Index. Overall, the Simpson Index and Brillouin Index showed low ectoparasites diversity with 5.24 and 1.78 respectively. Both indices showed similar values between male (D=4.80, HB 1.70) and females (D=4.89, HB=1.73) however, slightly higher diversity between adults (D=5.24, HB=1.78) compared to juveniles (D=4.27, HB=1.53). While higher indices during wet season (D=4.37, HB=1.64) compared to dry season

(D=4.15, HB=1.47). Both indices also showed Carey Island with the highest (D=4.93, HB=1.74) diversity compared to the other sites.

4.2.1 Ectoparasites distribution according to location

Two mite species (*Laelaps nuttali* and *Laelaps echidninus*) were found in all study sites with *L. nuttali* the most prevalent ectoparasites from all sites (55.9%). Table 4.4 summarized the prevalence of all the ectoparasites recovered in this study according to location. Statistical analysis showed significant difference in infestation between the five species; *Laelaps nuttali* ($p=0.02$), *Laelaps echidninus* ($p=0.00$), *Polyplax spinulosa* ($p=0.00$), *Hoplopleura pacifica* ($p=0.00$) and *Xenopsylla cheopis* ($p=0.01$) in the 4 sites (Table 4.4).

Table 4.5 summarized the general index of ectoparasites recovered from wild rats according to locations. *Listrophoroides* sp. (5.07) showed the highest index in Penang followed by *Polyplax spinulosa* (5.05) recovered in Carey Island. However, *Xenopsylla cheopis* showed the lowest index value from 4 locations ranging between 0.009-0.12. Carey Island also showed high index value for *Hoplopleura pacifica* (4.72).

In Kuantan, 89 rats (86.4%) out of 103 rats captured were infected with ectoparasites. Only 4 species of ectoparasites were recovered namely *Laelaps nuttali*, *Laelaps echidninus*, *Polyplax spinulosa* and *Xenopsylla cheopis*. The mite, *Laelaps nuttali* was identified as the most prevalent ectoparasite with 43.7% (CL: 34.45-53.4) wild rats infected meanwhile the flea *X. cheopis* was the least prevalent with 10.7% (CL: 5.7-18.32). However, the louse *Polyplax spinulosa* was the most abundant with mean intensity 10.55 per infected rat meanwhile the flea, *X. cheopis* the least with mean intensity 1.09 per infected rat. All ectoparasites recovered exhibited a negative binomial distribution with k value ranging between 0.097-0.464 apart from *X. cheopis* due to low

number of sample. Quantitative analysis of ectoparasites recovered from wild rats in Kuantan was summarized in Table 4.6.

Four ectoparasites species were recovered from 85 infected rats (81.73%) out of 104 wild rats captured from Malacca namely; *Laelaps nuttali*, *Laelaps echidninus*, *Hoplopleura pacifica* and *Xenopsylla cheopis*. *Laelaps nuttali* (64.4%, CL: 54.83-73.14) was the most prevalent ectoparasite meanwhile the flea *Xenopsylla cheopis* was the least prevalent with only 1% (CL: 0.05-5.13) infestation. The louse *Hoplopleura pacifica* was identified as the most abundant ectoparasite with mean intensity 4.0 per infected rat meanwhile *Xenopsylla cheopis* was found the least with mean intensity 1.0 per infected rat. All ectoparasites recovered exhibited a negative binomial distribution with k value ranging between 0.030-0.796 apart from *X. cheopis* due to low number of sample. The quantitative analysis of ectoparasite recovered from wild rats in Malacca was summarized in Table 4.7.

Highest number of ectoparasites recovered was in Carey Island with 8 species namely; *Ixodes granulatus*, *Polyplax spinulosa*, *Hoplopleura pacifica*, *Laelaps nuttali*, *Laelaps echidninus*, *Ornithonyssus bacoti*, *Listrophoroides* sp. and *Laelaps sculpturatus*. Seventy six rats (93.8%) out of 81 wild rats captured were found to be infected with ectoparasites. *Laelaps nuttali* was the most prevalent with 60.5% (CL: 49.39-70.77) out of 81 wild rats was infected while *Ornithonyssus bacoti* was the least prevalent with 7.4% (CL: 3.28-15.28) rats infected. The louse *Hoplopleura pacifica* was the most abundant with mean intensity 10.91 per infected wild rat meanwhile *Ornithonyssus bacoti* was the least with mean intensity 2.0 per infected rat. All ectoparasites recovered exhibited a negative binomial distribution with k value ranging between 0.064-0.561. The quantitative analysis of ectoparasites recovered in Carey Island was summarized in Table 4.8.

In Penang, 74 (98.7%) from 75 rats captured were found infected with ectoparasites. Six species were recovered from the infected wild rats namely *Laelaps nuttali*, *Laelaps echidninus*, *Listrophoroides sp.*, *Polyplax spinulosa*, *Hoplopleura pacifica* and *Xenopsylla cheopis*. The louse *Polyplax spinulosa* (54.7%, CL: 43.31-66.09) was found the most prevalent meanwhile *Xenopsylla cheopis* was the least prevalent with 5.3% (CL: 1.83-13.13) rats infected. However, *Listrophoroides sp.* was the most abundant with mean intensity 47.5 per infected rat meanwhile the flea, *Xenopsylla cheopis* the least with mean intensity 1.0 per infected rat. All ectoparasites recovered exhibited a negative binomial distribution with k value ranging between 0.020-0.380 apart from *X. cheopis* due low sampling number. The quantitative analysis of ectoparasite recovered from wild rats in Penang was summarized in Table 4.9.

4.2.2 Ectoparasites distribution according to host sex

The rat population captured more females (219) compared to males (144). Both sexes were found to harbor ectoparasites with higher infestation in males (90.3%) compared to females (88.6%). Overall, 9 species of ectoparasites recovered were present on both host sexes namely *Laelaps nuttali*, *Laelaps echidninus*, *Ornithonyssus bacoti*, *Listrophoroides sp.*, *Laelaps sculpturatus*, *Polyplax spinulosa*, *Hoplopleura pacifica*, *Ixodes granulatus* and *Xenopsylla cheopis*. However, *Ornithonyssus bacoti* and *Laelaps sculpturatus* were not found on male *R. norvegicus* while *Xenopsylla cheopis* was absent on female *R. tiomanicus* (Table 4.11).

Table 4.10 summarized the prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM), as well as the comparative analysis of rat ectoparasites between both host sexes. Prevalence of *Laelaps nuttali* was highest in both sexes with females (56.2%, CL: 49.3-62.9) infestation was slightly higher compared to males (55.6%, CL: 49.3-62.9) followed by the rat mite, *Laelaps echidninus*. Infestation

in males were lower (34.7%, CL: 26.98-43.1) compared to females (43.8%, CL: 37.15-50.69) significantly in terms of mean intensity ($p=0.02$) and abundance ($p=0.01$).

No significant differences were shown for prevalence, mean intensity and abundance of infections between both sexes for all ectoparasite species recovered apart for prevalence of *Polyplax spinulosa* (Table 4.10).

Table 4.11 summarized the prevalence and comparative analysis of rat's ectoparasites between both host sexes in relation to rat species. *Laelaps nuttali* was found prevalent in both sexes in all rat species. No significant difference was shown between rat species apart from *Polyplax spinulosa* and *Hoplopleura pacifica* on *R. rattus diardii* and *H. pacifica* on *R. tiomanicus*.

4.2.3 Ectoparasites distribution according to host age

Overall, the rat population were dominated by adults (319) compared to juveniles (44). Both groups harboured ectoparasites with prevalence between both age groups slightly higher in adult (89.7%) compared to juvenile rats (86.4%). *Listrophoroides* sp. was only found on adult rat in all rat species while *Ornithonyssus bacoti*, *Laelaps sculpturatus*, *Xenopsylla cheopis* were absent on juvenile *R. norvegicus* (Table 4.13).

Table 4.12 summarized the prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM), as well as the comparative analysis of rat ectoparasites between adult and juvenile wild rats. The analysis between adult and juvenile rats showed no significant differences between prevalence, mean intensity and abundance of infections between all ectoparasites species recovered.

Prevalence of the mite *Laelaps nuttali* was slightly lower in adult rats (55.5%, CL: 49.84-61.03) compared to juveniles (59.1%, CL: 43.24-73.67). Relative to host age,

there were no significant differences found in the 3 most prevalent ectoparasites species (*L. nuttali*, *L. echidninus* and *P. spinulosa*) although, higher infections were observed in juveniles. Another mite species, *Ornithonyssus bacoti* was the least prevalent with adult (1.6%, 0.51-3.62) infestation slightly lower than juveniles (2.3%, 0.05-12.03). Only adult rats were found infected with the mite, *Listrophoroides* sp. with low prevalence at 5.3% (CL: 3.13-8.4) and high value of mean intensity 27.4 per infected rat (Table 4.12).

Similarly, no significant differences were found in the 3 more prevalent ectoparasites species (*L. nuttali*, *L. echidninus* and *P. spinulosa*) in relation to the 3 dominant rat species apart from *Listrophoroides* sp. in *R. rattus diardii* ($p=0.002$) and *R. tiomanicus* ($p=0.012$) where *Listrophoroides* sp. was totally absent in juvenile hosts. *H. pacifica* was significantly higher in juveniles compared to adult in *R. tiomanicus*. Table 4.13 summarized the prevalence and comparative analysis of rat's ectoparasites between both host ages in different rat species.

4.2.4 Ectoparasites distribution according to season factors

From the total of rat population caught, 226 rats were trapped during the dry season and 137 during wet season from the four locations. Ectoparasites infested rats were lower during the dry (n=193, 85.4%) compared to wet season (n=131, 95.6%). All 9 species of ectoparasites recovered were found in both seasons except for *Ornithonyssus bacoti*, *Laelaps sculpturatus* and *Ixodes granulatus* which were present only during wet season.

From 3 most dominant ectoparasites species, *Laelaps nuttali* was most prevalent species with slightly higher infestation during dry season (57.5%, CL: 50.79-64.06) compared to the wet season (53.3%, CL: 44.57-61.86). This is followed by *Laelaps echidninus* with higher infestation during the dry season (41.6%, CL: 35.09-48.32) compared to the

wet season (38%, CL: 29.8-46.64) with significant mean intensity value ($p=0.00$) and abundance ($p=0.01$).

Table 4.14 summarized the prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM), as well as the comparative analysis of rat's ectoparasites recovered from wild rats during dry and wet season. Statistical analysis showed no significant differences according to prevalence between seasons, mean intensity and abundance of infections for all ectoparasite species recovered except for *Ornithonyssus bacoti*, *Laelaps sculpturatus*, *Polyplax spinulosa*, *Hoplopleura pacifica* and *Ixodes granulatus* observed higher during the wet season compared to dry season.

Table 4.15 summarized the prevalence and comparative analysis of rat ectoparasites during wet and dry season in different rat species. No significant differences were found for the 3 more prevalence ectoparasites species (*L. nuttali*, *L. echidninus* and *P. spinulosa*) apart from *P. spinulosa* in *R. rattus diardii* ($p=0.00$) and *R. tiomanicus* ($p=0.001$) where *P. spinulosa* infestation was higher during the wet season. Also, significant difference was found in *R. norvegicus* for *Laelaps sculpturatus*, *Hoplopleura pacifica* and *Ixodes granulatus* while *Laelaps sculpturatus*, *Hoplopleura pacifica* and *Ixodes granulatus* showed significant difference on *R. rattus diardii* and *R. tiomanicus* between both seasons.



Plate 2.4: *Haplopleura pacifica*

Magnification (4x10)



Plate 2.5: *Polyplax spinulos*

Magnification (4X10)



Plate 2.6: *Laelaps nuttali*

Magnification (4x10)



Plate 2.7: *Laelaps echidninus*

Magnification (4x10)

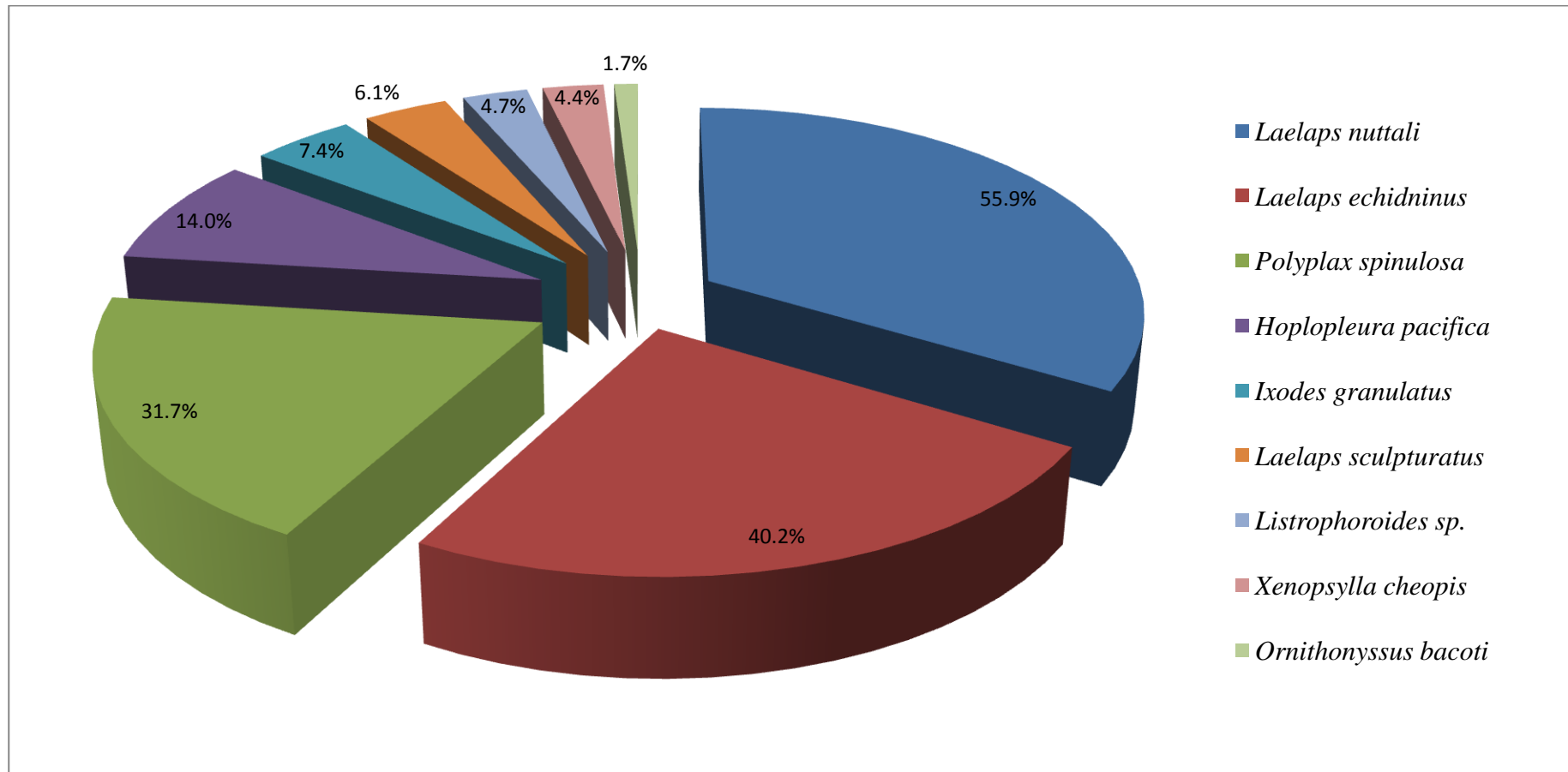


Figure 4.1: Prevalence of ectoparasite species recovered from wild rats in Peninsular Malaysia.

Table 4.1: Quantitative analysis of ectoparasites recovered from wild rats captured in Peninsular Malaysia.

Ectoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Mites						
<i>Laelaps nuttali</i>	748	55.9% (50.69-61.02)	3.68 (3.28-4.17)	2.06 (1.77-2.38)	1-16	0.511
<i>Laelaps echidninus</i>	454	40.2% (35.25-45.45)	3.11 (2.70-3.58)	1.25 (1.04-1.52)	1-17	0.334
<i>Ornithonyssus bacoti</i>	12	1.7% (0.73-3.53)	2.00 (1.00-3.67)	0.03 (0.01-0.09)	1-6	0.014
<i>Listrophoroides sp.</i>	466	4.7% (2.84-7.39)	27.41 (5.24-104.76)#	1.28 (0.22-5.06)	1-345	0.010
<i>Laelaps sculpturatus</i>	57	6.1% (3.95-9.05)	2.59 (1.82-3.86)	0.16 (0.09-0.26)	1-10	0.038
Lice						
<i>Polyplax spinulosa</i>	995	31.7% (26.98-36.64)	8.69 (6.59-11.90)	2.7 (2.02-3.85)	1-97	0.121
<i>Hoplopleura pacifica</i>	449	14.0% (10.71-18.01)	8.80 (5.55-18.71)#	1.24 (0.74-2.87)	1-144	0.046
Tick						
<i>Ixodes granulatus</i>	214	7.4% (5.05-10.70)	7.93 (5.81-11.59)	0.59 (0.36-0.96)	1-34	0.024
Flea						
<i>Xenopsylla cheopis</i>	17	4.4% (2.50-7.06)	1.06 (1.00-1.19)	0.05 (0.02-0.07)	1-2	**

- limits uncertain due to low sample size

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

Table 4.2: Prevalence, mean intensity, abundance and comparative analysis of ectoparasites between rat species in Peninsular Malaysia.

Ectoparasites species		<i>Rattus norvegicus</i>	<i>Rattus rattus diardii</i>	<i>Rattus tiomanicus</i>	<i>P</i>-value
<i>Laelaps nuttali</i>	P	55.8%	42.4%	63.0%	0.079
	MI (95% CL)	3.44 (2.95-4.04)	4.48 (3.04-6.44)	3.68 (2.76-4.82)	
	A (95% CL)	1.92 (1.57-2.33)	1.90 (1.19-3.07)	2.31 (1.59-3.24)	
<i>Laelaps echidninus</i>	P	38.8%	37.3%	31.5%	0.622
	MI (95% CL)	2.65 (2.15-3.28)	3.55 (2.55-5.77)	4.0 (2.76-5.76)	
	A (95% CL)	1.03 (0.79-1.35)	1.32 (0.78-2.25)	1.26 (0.7-2.11)	
<i>Ornithonyssus bacoti</i>	P	0.5%	3.4%	3.7%	0.056
	MI (95% CL)	6.0 (0.0-0.0)#	1.5 (1.0-1.5)#	1.0 (0.0-0.0)#	
	A (95% CL)	0.03 (0.0-0.09)	0.05 (0.0-0.15)	0.04 (0.0-0.09)	
<i>Listrophoroides</i> sp.	P	2.9%	10.2%	9.3%	0.022*
	MI (95% CL)	63 (2.33-234)	1.5 (1.0-2.17)	15.8 (3.2-45.4)	
	A (95% CL)	1.83(0.06-8.53)	0.15 (0.05-0.31)	1.46 (0.22-5.57)	
<i>Laelaps sculpturatus</i>	P	2.9%	3.4%	20.4%	0.000*
	MI (95% CL)	4.0 (1.67-7.17)	3.5 (1.0-3.50)	1.73 (1.09-2.45)	
	A (95% CL)	0.12 (0.03-0.3)	0.12 (0.0-0.44)	0.35 (0.17-0.65)	
<i>Polyplax spinulosa</i>	P	30.6%	47.5%	27.8%	0.381
	MI (95% CL)	8.21 (5.95-13.2)	6.85 (3.93-11.5)	13.3 (6.47-37.5)	
	A (95% CL)	2.51 (1.67-4.03)	3.25 (1.83-6.03)	3.7 (1.57-10.7)	

Table 4.2: Prevalence, mean intensity, abundance and comparative analysis of ectoparasites between rat species in Peninsular Malaysia. [Continued]

Ectoparasites species		<i>Rattus norvegicus</i>	<i>Rattus rattus diardii</i>	<i>Rattus tiomanicus</i>	<i>P</i>-value
<i>Ixodes granulatus</i>	P	3.9%	6.8%	24.1%	0.000*
	MI (95% CL)	8.0 (5.0-10.75)	15.3 (8.0-27.5)	6.0 (3.69-11.23)	
	A (95% CL)	0.31 (0.13-0.61)	1.03 (0.19-3.02)	1.44 (0.72-3.28)	
<i>Xenopsylla cheopis</i>	P	5.3%	6.8%	1.9%	0.572
	MI (95% CL)	1.09 (1.0-1.27)	1.0 (0.0-0.0)#	1.0 (0.0-0.0)#	
	A (95% CL)	0.06 (0.02-0.1)	0.07 (0.02-0.14)	0.02 (0.0-0.06)	
<i>Hoplopleura pacifica</i>	P	11.7%	8.5%	29.6%	0.003*
	MI (95% CL)	4.92 (3.33-7.46)	9.6 (4.0-15.2)	13.9 (4.56-48.3)	
	A (95% CL)	0.57 (0.33-0.95)	0.81 (0.19-2.07)	4.13 (1.22-15.1)	

- limits uncertain due to low sample size

* - significant

P - prevalence

MI - mean intensity

A - abundance

CL - confident

Table 4.3: Simpson's and Brillouin Index of Diversity for ectoparasite infestation in the wild rat population in Peninsular Malaysia.

Factors		Simpson's Index	Brillouin Index
Host sex	Male	4.80	1.70
	Female	4.89	1.73
Host age	Adult	5.24	1.78
	Juvenile	4.27	1.53
Seasons	Dry	4.15	1.47
	Wet	4.37	1.64
Location	Kuantan	2.38	1.02
	Malacca	2.24	0.87
	Carey Island	4.93	1.74
	Penang	3.66	1.41
Total population		5.24	1.78

Table 4.4: Prevalence of ectoparasites recovered from wild rats captured in Kuantan, Malacca, Carey Island and Penang of Peninsular Malaysia.

Ectoparasites	Kuantan (n=103)	Malacca (n=104)	Carey Island (n=81)	Penang (n=75)	<i>p</i>-value
<u>Mites</u>					
<i>Laelaps nuttali</i>	43.7%	64.4%	60.5%	53.3%	0.02*
<i>Laelaps echidninus</i>	35.0%	54.8%	25.9%	42.7%	0.00*
<i>Ornithonyssus bacoti</i>	-	-	7.4%	-	0.10
<i>Listrophoroides</i> sp.	-	-	11.1%	10.7%	0.93
<i>Laelaps sculpturatus</i>	-	-	27.2%	-	0.10
<u>Lice</u>					
<i>Polyplax spinulosa</i>	28.2%	-	54.3%	54.7%	0.00*
<i>Hoplopleura pacifica</i>	-	6.7%	43.2%	12.0%	0.00*
<u>Tick</u>					
<i>Ixodes granulatus</i>	-	-	33.3%	-	0.10
<u>Flea</u>					
<i>Xenopsylla cheopis</i>	10.7%	1.0%	-	5.3%	0.01*

* significant

Table 4.5: General ectoparasites index from wild rat populations captured in four locations of Peninsular Malaysia.

Ectoparasites	Kuantan (n=103)	Malacca (n=104)	Carey Island (n=81)	Penang (n=75)
<u>Mites</u>				
<i>Laelaps nuttali</i>	1.15	2.28	2.44	2.6
<i>Laelaps echidninus</i>	0.85	1.92	0.84	1.31
<i>Ornithonyssus bacoti</i>	-	-	0.15	-
<i>Listrophoroides</i> sp.	-	-	1.06	5.07
<i>Laelaps sculpturatus</i>	-	-	0.70	-
<u>Lice</u>				
<i>Polyplax spinulosa</i>	2.99	-	5.05	3.71
<i>Hoplopleura pacifica</i>	-	0.27	4.72	0.52
<u>Tick</u>				
<i>Ixodes granulatus</i>	-	-	2.64	-
<u>Flea</u>				
<i>Xenopsylla cheopis</i>	0.12	0.009	-	0.05

Table 4.6: Quantitative analysis of ectoparasites recovered from wild rats captured in Kuantan, Peninsular Malaysia.

Ectoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Mites						
<i>Laelaps nuttali</i>	118	43.7% (34.43-53.40)	2.53 (2.00-3.71)	1.11 (0.81-1.67)	1-16	0.464
<i>Laelaps echidninus</i>	88	35.0% (26.14-44.64)	2.44 (1.81-3.89)#	0.85 (0.57-1.45)	1-17	0.327
Lice						
<i>Polyplax spinulosa</i>	308	28.2% (20.26-37.81)	10.55 (7.00-16.31)	2.97 (1.74-5.15)	1-56	0.097
Flea						
<i>Xenopsylla cheopis</i>	12	10.7% (5.70-18.32)	1.09 (1.00-1.27)	0.12 (0.06-0.18)	1-2	**

- limits uncertain due to low sample size

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

Table 4.7: Quantitative analysis of ectoparasites recovered from wild rats captured in Malacca, Peninsular Malaysia.

Ectoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Mites						
<i>Laelaps nuttali</i>	237	64.4% (54.83-73.14)	3.54 (2.97-4.34)	2.28 (1.80-2.88)	1-13	0.796
<i>Laelaps echidninus</i>	200	54.8% (45.18-64.47)	3.51 (2.91-4.19)	1.92 (1.50-2.45)	1-12	0.562
Lice						
<i>Hoplopleura pacifica</i>	28	6.7% (3.21-13.33)	4.00 (1.71-7.71)	0.27 (0.08-0.72)	1-12	0.030
Flea						
<i>Xenopsylla cheopis</i>	1	1% (0.05-5.13)	1.00 (0.00-0.00)#	0.01 (0.00-0.03)	1-1	**

- 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 ectoparasites recovered from wild rats captured in Carey Island, Peninsular Malaysia.

Ectoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Mites						
<i>Laelaps nuttali</i>	198	60.5% (49.39-70.77)	4.04 (3.22-5.04)	2.44 (1.78-3.31)	1-16	0.221
<i>Laelaps echidninus</i>	68	25.9 (17.12-36.48)	3.24 (2.33-4.38)	0.84 (0.51-1.36)	1-9	0.033
<i>Ornithonyssus bacoti</i>	12	7.4% (3.28-15.28)	2.00 (1.00-3.67)	0.15 (0.05-0.43)	1-6	0.064
<i>Listrophoroides sp.</i>	86	11.1% (5.72-20.2)	9.56 (2.44-28.0)#	1.06 (0.22-3.70)	1-56	0.175
<i>Laelaps sculpturatus</i>	57	27.2% (18.39-38.21)	2.59 (1.82-3.77)	0.70 (0.43-1.20)	1-10	0.561
Lice						
<i>Polyplax spinulosa</i>	409	54.3% (43.18-64.87)	9.30 (6.23-17.32)#	5.05 (3.26-9.33)	1-97	0.261
<i>Hoplopleura pacifica</i>	382	43.2% (32.65-54.33)	10.91 (6.26-25.89)#	4.72 (2.49-12.17)	1-144	0.172
Tick						
<i>Ixodes granulatus</i>	214	33.3% (23.8-44.42)	7.59 (5.48-11.15)	2.44 (1.58-3.99)	1-34	0.148

- limits uncertain due to low sample size

Table 4.9: Quantitative analysis of ectoparasites recovered from wild rats captured in Penang, Peninsular Malaysia.

Ectoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	k
Mites						
<i>Laelaps nuttali</i>	195	53.3% (41.97-64.74)	4.88 (3.72-6.22)	2.60 (1.87-3.59)	1-14	0.380
<i>Laelaps echidninus</i>	98	42.7% (31.93-54.01)	3.06 (2.22-4.56)	1.31 (0.85-2.07)	1-14	0.356
<i>Listrophoroides sp.</i>	380	10.7% (5.01-19.85)	47.5 (1.75-216.0)#	5.07 (0.21-27.69)	1-345	0.020
Lice						
<i>Polyplax spinulosa</i>	278	54.7% (43.31-66.09)	6.78 (4.22-14.27)#	3.71 (2.16-7.57)	1-84	0.281
<i>Hoplopleura pacifica</i>	39	12.0% (6.19-21.21)	4.33 (2.11-9.44)	0.52 (0.19-1.31)	1-17	0.054
Flea						
<i>Xenopsylla cheopis</i>	4	5.3% (1.85-13.13)	1.00 (0.00-0.00)#	0.05 (0.01-0.11)	0-1	**

- limits uncertain due to low sample size

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

Table 4.10: Prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM) and comparative analysis of rat ectoparasites between both host sexes in Peninsular Malaysia

Parasite species	Prevalence			Mean Intensity			Abundance \pm SEM		
	Male (%)	Female (%)	<i>P</i> value	Male	Female	<i>P</i> value	Male	Female	<i>P</i> value
<i>Laelaps nuttali</i>	55.6	56.2	0.91	3.38	3.89	0.26	1.88 \pm 0.24	2.18 \pm 0.22	0.33
<i>Laelaps echidninus</i>	34.7	43.8	0.10	2.46	3.45	0.02*	0.85 \pm 0.14	1.51 \pm 0.18	0.01*
<i>Ornithonyssus bacoti</i>	2.1	1.4	0.69	2.67	1.33	0.53	0.03 \pm 0.02	0.04 \pm 0.03	0.82
<i>Listrophoroides</i> sp.	3.5	5.5	0.45	8.33	73.2	0.39	2.54 \pm 2.34	0.46 \pm 0.28	0.46
<i>Laelaps sculpturatus</i>	5.5	6.9	0.65	2.2	2.9	0.48	0.15 \pm 0.06	0.16 \pm 0.06	0.93
<i>Polyplax spinulosa</i>	40.3	26.0	0.01*	9.33	7.97	0.62	2.07 \pm 1.02	3.76 \pm 0.39	0.15
<i>Hoplopleura pacifica</i>	18.8	11.0	0.06	10.7	6.67	0.52	2.01 \pm 1.04	0.73 \pm 0.19	0.39
<i>Ixodes granulatus</i>	6.3	8.2	0.55	8.78	7.5	0.74	0.55 \pm 0.27	0.62 \pm 0.17	0.84
<i>Xenopsylla cheopis</i>	5.6	3.7	0.44	1.13	1.0	0.41	0.06 \pm 0.02	0.04 \pm 0.01	0.58

* significant

Table 4.11: Prevalence and comparative analysis of rat ectoparasites between both host sexes according to rat species from Peninsular Malaysia

Parasite species	<i>Rattus norvegicus</i>			<i>Rattus rattus diardii</i>			<i>Rattus tiomanicus</i>		
	Male (%)	Female (%)	<i>P</i> value	Male (%)	Female (%)	<i>P</i> value	Male (%)	Female (%)	<i>P</i> value
<i>Laelaps nuttali</i>	54.1	57.4	0.362	50.0	40.1	0.143	66.7	60.7	0.723
<i>Laelaps echidninus</i>	35.7	41.7	0.256	38.9	36.6	0.653	28.6	33.3	0.291
<i>Ornithonyssus bacoti</i>	0	0.92	0.067	5.6	2.4	0.287	4.7	3.0	0.192
<i>Listrophoroides</i> sp.	2.0	3.7	0.092	11.1	9.7	0.102	4.7	12.1	0.104
<i>Laelaps sculpturatus</i>	0	5.6	0.102	5.6	2.4	0.280	33.3	12.1	0.090
<i>Polyplax spinulosa</i>	32.7	28.7	0.201	66.7	39.0	0.003*	38.1	21.2	0.192
<i>Hoplopleura pacifica</i>	10.2	12.9	0.173	16.7	4.9	0.023*	42.9	21.2	0.003*
<i>Ixodes granulatus</i>	2.0	5.6	0.439	5.6	7.3	0.391	19.0	27.3	0.089
<i>Xenopsylla cheopis</i>	6.1	4.6	0.284	5.6	7.3	0.390	4.7	0	0.378

* significant

Table 4.12: Prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM) and comparative analysis of rat ectoparasites between host ages in Peninsular Malaysia

Parasite species	Prevalence			Mean Intensity			Abundance \pm SEM		
	Adult (%)	Juvenile (%)	<i>P</i> value	Adult	Juvenile	<i>P</i> value	Adult	Juvenile	<i>P</i> value
<i>Laelaps nuttali</i>	55.5	59.1	0.75	3.48	5.12	0.09	1.93 \pm 0.16	3.02 \pm 0.65	0.12
<i>Laelaps echidninus</i>	39.5	45.5	0.51	3.09	3.25	0.78	1.22 \pm 0.13	1.48 \pm 0.33	0.49
<i>Ornithonyssus bacoti</i>	1.6	2.3	0.54	2.2	1.0	1.0	0.03 \pm 0.02	0.02 \pm 0.02	0.76
<i>Listrophoroides</i> sp.	5.3	0	0.24	27.4	0	1.0	1.46 \pm 1.09	0	0.38
<i>Laelaps sculpturatus</i>	6.3	4.5	1.0	2.6	2.5	0.93	0.16 \pm 0.05	0.11 \pm 0.09	0.66
<i>Polyplax spinulosa</i>	31.7	31.8	1.0	8.98	6.29	0.24	2.84 \pm 0.52	2.0 \pm 0.65	0.32
<i>Hoplopleura pacifica</i>	12.9	22.7	0.10	9.56	5.7	0.42	1.21 \pm 0.48	1.3 \pm 0.49	0.94
<i>Ixodes granulatus</i>	6.9	11.4	0.35	8.46	5.6	0.18	0.58 \pm 0.17	0.64 \pm 0.29	0.86
<i>Xenopsylla cheopis</i>	4.7	2.3	0.70	1.07	1.0	1.0	0.05 \pm 0.01	0.02 \pm 0.02	0.37

* significant

Table 4.13: Prevalence and comparative analysis of rat ectoparasites between host ages according to rat species from Peninsular Malaysia

Parasite species	<i>Rattus norvegicus</i>			<i>Rattus rattus diardii</i>			<i>Rattus tiomanicus</i>		
	Adult (%)	Juvenile (%)	<i>P</i> value	Adult (%)	Juvenile (%)	<i>P</i> value	Adult (%)	Juvenile (%)	<i>P</i> value
<i>Laelaps nuttali</i>	55.8	57.1	0.590	40.0	50.0	0.352	65.0	57.1	0.235
<i>Laelaps echidninus</i>	38.7	42.8	0.462	35.6	42.9	0.198	32.5	28.6	0.172
<i>Ornithonyssus bacoti</i>	0.5	0	0.291	2.2	7.1	0.402	5.0	0	0.091
<i>Listrophoroides</i> sp.	3.0	0	0.108	13.3	0	0.002*	12.5	0	0.012*
<i>Laelaps sculpturatus</i>	3.0	0	0.107	4.4	0	0.278	22.5	14.3	0.071
<i>Polyplax spinulosa</i>	29.6	57.1	0.098	53.3	28.6	0.057	22.5	42.9	0.059
<i>Hoplopleura pacifica</i>	11.6	14.2	0.231	6.7	14.3	0.182	22.5	50.0	0.009*
<i>Ixodes granulatus</i>	35.2	14.2	0.930	6.7	7.1	0.273	25.0	21.4	0.321
<i>Xenopsylla cheopis</i>	5.5	0	0.192	6.7	7.1	0.276	2.5	0	0.107

* significant

Table 4.14: Prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM) and comparative analysis of rat ectoparasites between seasonal factors in Peninsular Malaysia

Parasite species	Prevalence			Mean Intensity			Abundance \pm SEM		
	Dry (%)	Wet (%)	<i>P</i> value	Dry	Wet	<i>P</i> value	Dry	Wet	<i>P</i> value
<i>Laelaps nuttali</i>	57.5	53.3	0.48	3.89	3.32	0.2	2.24 \pm 0.22	1.77 \pm 0.22	0.13
<i>Laelaps echidninus</i>	41.6	38.0	0.51	3.54	2.33	0.00*	1.47 \pm 0.18	0.88 \pm 0.14	0.01*
<i>Ornithonyssus bacoti</i>	0	4.4	0.00*	0	2.0	1.0	0	0.09 \pm 0.05	0.24
<i>Listrophoroides</i> sp.	5.3	3.6	0.61	38.17	1.6	0.39	2.03 \pm 1.55	0.06 \pm 0.03	0.40
<i>Laelaps sculpturatus</i>	0	16.1	0.00*	0	2.59	1.0	0	0.42 \pm 0.11	0.01*
<i>Polyplax spinulosa</i>	20.8	49.6	0.00*	9.0	8.41	0.82	1.88 \pm 0.42	4.18 \pm 1.01	0.08
<i>Hoplopleura pacifica</i>	4.0	30.7	0.00*	4.33	9.76	0.28	0.17 \pm 0.09	2.99 \pm 1.11	0.19
<i>Ixodes granulatus</i>	0	19.7	0.00*	0	7.93	1.0	0	1.56 \pm 0.38	0.49
<i>Xenopsylla cheopis</i>	4.9	3.6	0.79	1.09	1.0	0.43	0.05 \pm 0.02	0.04 \pm 0.02	0.46

* significant

Table 4.15: Prevalence and comparative analysis of rat ectoparasites between seasonal factors according to rat species from Peninsular Malaysia

Parasite species	<i>Rattus norvegicus</i>			<i>Rattus rattus diardii</i>			<i>Rattus tiomanicus</i>		
	Dry (%)	Wet (%)	<i>P</i> value	Dry (%)	Wet (%)	<i>P</i> value	Dry (%)	Wet (%)	<i>P</i> value
<i>Laelaps nuttali</i>	58.9	50.6	0.201	35.9	55.0	0.091	70.4	65.6	0.261
<i>Laelaps echidninus</i>	37.2	41.6	0.192	38.5	35.0	0.291	25.9	37.0	0.191
<i>Ornithonyssus bacoti</i>	0	1.2	0.391	0	10.0	0.028*	0	7.4	0.072
<i>Listrophoroides</i> sp.	2.3	3.9	0.198	10.2	10.0	0.567	18.5	0	0.058
<i>Laelaps sculpturatus</i>	0	7.7	0.021*	0	10.0	0.029*	0	40.7	0.000*
<i>Polyplax spinulosa</i>	25.6	38.9	0.281	30.8	80.0	0.000*	7.4	48.1	0.001*
<i>Hoplopleura pacifica</i>	6.9	19.5	0.045*	0	25.0	0.000*	0	59.1	0.000*
<i>Ixodes granulatus</i>	0	10.4	0.012*	0	20.0	0.000*	0	48.1	0.000*
<i>Xenopsylla cheopis</i>	6.2	3.8	0.219	5.1	10.0	0.281	3.7	0	0.106

* significant

4.3 Discussion

An exceptionally high percentage of the wild rat population from the island and coastal sites were infested with ectoparasites. However, the overall ectoparasites diversity was low, consistent with prior records by Zahedi *et al.* (1984), Nadchatram *et al.* (1966), Singh *et al.* (1995) and Paramasvaran *et al.* (2009b). The ectoparasites comprised of entirely cosmopolitan species (*Laelaps nuttali*, *Laelaps echidninus*, *Ornithonyssus bacoti*, *Listrophoroides* sp., *Laelaps sculpturatus*, *Polyplax spinulosa*, *Hoplopleura pacifica*, *Ixodes granulatus* and *Xenopsylla cheopis*) could have been introduced and maintained in site by commensal rats. Only nine species of ectoparasites were recorded in this study with concurrent infestations commonly observed. The ectoparasite species diversity was almost similar between the four locations with only 4 ectoparasites species found in Kuantan and Malacca and 6 species in Penang. This showed that the overwhelmingly large number of rats occupying the same niche circuitously facilitate the transmissions and allowed multiple infestations through inter and intraspecific interactions.

However, diversity in Carey Island was higher with 8 ectoparasites species recovered. With the marked exception of *Laelaps sculpturatus*, *Ornithonyssus bacoti* and *Ixodes granulatus*, ectoparasites present in here were similar to the other study locations. This could be due to the exploration of a wider niche in Carey Island increasing the diversity of ectoparasite species. Overall, no significant difference in ectoparasites diversity observed between urban coastal cities and agriculture island site.

All locations showed high ectoparasites infestation with Penang was the most infested (98.7%) followed by Carey Island, Kuantan and Malacca. The ectoparasites recovered in urban island sites also showed high number of specimens (994) although Carey Island recorded the highest (1,426). The high infestation of rats in Penang showed

overlapping of niche between the 2 dominant rat species (*R. norvegicus* and *R. rattus diardii*). In contrast, high diversity and intensity of ectoparasites in Carey Island was due to the wider niche present in the environment.

Overall, intrinsic factors (host age and sex) showed no significant effects in determining ectoparasites distribution in wild rat population except for rat louse (*Polyplax spinulosa*) showed significant difference between male and female rats with more male rats heavily infected with *Polyplax spinulosa* compared to females. This pattern could be due to males foraging further resulting in the increase of exposure to infestation.

However, season played a role in the prevalence of most of the ectoparasites species with the diversity and prevalence of the ectoparasites slightly higher during the wet season. It is well known that the high rainfall during wet season is suitable for the survival of the ectoparasites especially the mites and lice. Mites thrive in moist conditions and appeared not to survive for more than a few days in low humidity (Jacobs, 2006). Abu-Madi *et al.* (2001) also noted the prevalence and abundance of ectoparasites were season and host age dependent where the infestations were higher in juveniles during summer season.

R. norvegicus recorded the highest ectoparasites infestation. In addition, infestation in *R. rattus diardii* and *R. tiomanicus* were almost similar with no marked differences. Paramasvaran *et al.* (2009b) noted that *R. rattus diardii* had the highest ectoparasites infestation in urban habitat while in the coastal habitat only 2 rat species (*R. rattus diardii* and *R. exulans*) were found to be infested with ectoparasites.

According to Ho & Krishnasamy (1990), mites are common ectoparasites infesting in small mammals. The rat mites, *Laelaps nuttali* and *Laelaps echidninus* were the 2 most predominant species found infesting the rat population from all the locations. Earlier study also showed high infestations of mites from four habitats namely forest, urban

coastal and rice field (Paramasvaran *et al.*, 2009b). Studies on distribution of mites have been extensively reported in Peninsular Malaysia (Zahedi *et al.*, 1984; Nadchatram *et al.*, 1966; Shabrina, 1990, 1991; Ho *et al.*, 1985; Madinah *et al.*, 2011; Mariana *et al.*, 2011). High prevalence of *Laelaps nuttali* was observed in *R. tiomanicus* was also previously recorded by Singh (1990), Shabrina and Salleh (1995) and Mariana *et al.* (2005).

Two other mite species, *Ornithonyssus bacoti* and *Laelaps sculpturatus* were only recovered in Carey Island with lower intensity. Paramasvaran *et al.*, (2009a) also recorded both species on urban and forest rats while Zahedi *et al.*, (1996) captured only one rat with *Ornithonyssus bacoti*. *Ornithonyssus bacoti* is rarely found on Malaysian rats although it has worldwide distribution and has been incriminated to cause pruritic dermatitis in man. The first authentic case of dermatitis caused by *O. bacoti* in Malaysia was reported by Nadchatram and Ramalingam (1974).

Present study notes the first recorded observation of *Laelaps sculpturatus* on commensal rats from an agriculture based island habitat (Carey Island). High prevalence of this mite was found on *R. tiomanicus* compared to other species. *Laelaps sculpturatus* has been frequently recorded on wild rats from forest and wildlife reserves. However, this observation is consistent with the available information from previous studies (Domrow & Nadchatram, 1963; Ho & Krishnasamy, 1990; Mariana *et al.*, 2005; 2011; Paramasvaran *et al.*, 2009b; Madinah *et al.*, 2011).

On the other hand, the mite species *Listrophoroides* sp. has been recovered only from island rats (Carey Island and Penang) with the highest abundance, mean intensity and general index. Listrophorid mites are obligate parasites and live on the fur of mammals. They are also associated with rodents and widely distributed throughout the world (Fain & Bochkov, 2004). Low intensity presence of *Listrophoroides* sp. on wild rats in

Peninsular Malaysia has been previously recorded. (Shabrina, 1990; 1991; Singh, 1990; Shabrina & Salleh, 1995; Ho & Krishnasamy, 1990; Paramasvaran *et al.*, 2009b; Madinah, 2011). However, none of the mentioned studies recovered *Listrophoroides* sp. on wild rats from urban coastal city and island habitats. The listrophorids could only be identified up to the genus level due to the large number of specimens recovered. This further confirms its widespread occurrence among the commensal rat population.

The cosmopolitan lice, *Polyplax spinulosa* and *Hoplopleura pacifica* were the two common lice species observed in this study. High prevalence of *Hoplopleura pacifica* infestation was seen in *R. tiomanicus* compared to other species. Johnson (1972) marked the first observation of *Hoplopleura pacifica* in *Rattus norvegicus* from Peninsular Malaysia. Similarly, Paramasvaran *et al.* (2009b) reported this two species infesting urban rat. High prevalence of *Polyplax spinulosa* infesting *R. rattus diardii* further confirmed the common host for this louse (Zahedi *et al.*, 1984). This louse is known to be host specific and only infests certain types of rats (Shabrina, 1990). On the other hand, Chuluun *et al.* (2005) reported the presence of 3 louse species namely; *Hoplopleura pescinata*, *Polyplax spinulosa* and *Hoplopleura pacifica*. General index of *Polyplax spinulosa* was also recorded high in all locations and rat species. Similar trend was also seen in Egypt for *Polyplax spinulosa* infesting *R. rattus* and *R. norvegicus* (Sohail *et al.*, 2001a; 2001b).

The present widespread and aggressive lice infestations observed especially in Penang could be due to the environmental changes taking place here. Gradual rise in humidity and temperature levels, possibly due to uncontrolled air pollution and trapped heat, may have provided favorable conditions for rapid rat lice propagation and growth, resulting in the increase in intensity. In addition, the fur texture of commensal rats could also be a factor (Shabrina *et al.*, 1989). Lice are considered to be of public health importance because they harbour the plague bacilli and transmit tularemia and bartonellosis to

humans and also play an adjunctive role in the transmission of murine typhus and plague from rat to rat (Zahedi *et al.*, 1984).

The only tick species recovered on rats in this study was *Ixodes granulatus* and was only recovered in Carey Island. The high prevalence of *Ixodes granulatus* infesting *R. tiomanicus* is due to the ideal conditions of the habitat in Carey Island. Previous studies that also recovered this tick from *R. tiomanicus* were by Singh (1990), Shabrina & Salleh (1995) and Mariana *et al.* (2005) while others found this tick infesting other forest rodents (Salleh *et al.*, 2003; Mariana *et al.*, 2005; 2008; Madinah *et al.*, 2011). These reports confirm that the common hosts for *Ixodes granulatus* were rodents (Audy *et al.*, 1960). Distribution of this ectoparasite extends from Malaysia to Southeast Asia, eastern India and China (Nadchatram, 2008). The absence of ticks on the other rats sampled may be due to the environment being not suitable for tick survival because ticks are likely to be found in habitats such as shrubs, forest and plantation and not in urban sites. Furthermore, infestation with tick species especially in *Rattus rattus diardii* and *Rattus norvegicus* is rarely reported in the literature. However, Audy (1957) reported infestation of *Rattus rattus diardii* with adult *Ixodes granulatus* and also the immature stage. Paramasvaran *et al.* (2009b) found a single *Rattus rattus diardii* was infested with the tick *Amblyomma* sp. while Nadchantram *et al.* (1966) reported a female tick from a log of wood near a beach. Another common tick species, *Dermacentor* sp. was also previously reported on the forest rodent (Paramasvaran *et al.*, 2009b).

Ixodes granulatus is of medical importance because it is the main vector of Langkat Virus which is similar to Russian Spring-Summer Encephalitis Complex (RSSE) (Smith, 1956). This species is also known to transmit other pathogens (Marchette, 1966) and have been shown to be involved in spread of tick typhus and Q fever in the climax forest of Peninsular Malaysia (Marchette, 1966). The genus *Ixodes* is also associated

with many other tick borne zoonotic diseases in many part of the world (Roberts & John, 2001; RatZooMan, 2006).

Xenopsylla cheopis was the only flea found on all rat species in this study. However, its low overall intensity suggest limited distribution, and could be possibly due to the effective rodent and flea control measures rather than meteorological conditions during sampling. The general index for *Xenopsylla cheopis* in this study was lower compared to Paramasvaran *et al.* (2009b) which showed higher flea index and indicated strong relationship between flea infestations among urban rats. However, the prevalence of flea on these rats may not reflect the true picture of flea infestation because the rats were examined 12 hours after they were captured and during this time the fleas could have jumped off their host. High general index of *Xenopsylla cheopis* was also seen in Egypt which was observed on *R. rattus* and *R. norvegicus* (Sohail *et al.*, 2001a; 2001b).

Xenopsylla cheopis also infests the host's nest rather than on the host itself. The flea has been previously reported from Kuala Lumpur (Zahedi *et al.*, 1984; Singh *et al.*, 1995) while Paramasvaran *et al.* (2009b) recovered flea on rats from urban and coastal sites. Traub (1950) and Singh *et al.* (1995) recorded *R. norvegicus* from the local urban areas harbouring fleas. *Xenopsylla cheopis* also known as the tropical rat flea or oriental rat flea is the primary vector for bubonic plague and murine typhus. The oriental rat flea is most famous for contributing to the Black Death Europe. The rat flea has two eyes, yet it can only see very bright light. The oriental rat flea has no genal or pronotal combs. This characteristic can be used to differentiate the oriental rat flea from the cat, dog, and other fleas.

The current changes in the global climate due to the rapid environmental degradation may alter the ecology of rodents and create new foci that could promote the vector's profiliration and transmit rodent borne parasitic diseases. The ecological variations will

increase contact between human and rodents resulting in a heavier disease burden that would challenge the efficiency of the public health services.

4.4 Conclusion

Low ectoparasites species diversity was observed in the wild rat populations from all four locations even with exceptionally high infestation levels. Nearly 88% of the rats harboured ectoparasites, with an overall abundance value of 8.36 per infected rat. A total of 9 cosmopolitan ectoparasites species were recovered i.e. *Laelaps nuttali*, *Laelaps echidninus*, *Ornithonyssus bacoti*, *Listrophoroides* sp., *Laelaps sculpturatus*, *Polyplax spinulosa*, *Hoplopleura pacifica*, *Ixodes granulatus* and *Xenopsylla cheopis*. Overall *Laelaps nuttali* was the most prevalent species while *Listrophoroides* sp. was the most abundant with the highest mean intensity. All ectoparasites species recovered were vectors of zoonotic diseases with the exception of *Listrophoroides* sp. and *Laelaps sculpturatus*. Most of the ectoparasites recorded the general index value of more than 1 apart from *Xenopsylla cheopis* and *Ornithonyssus bacoti*.

Although most ectoparasites were similar between sites, ectoparasites diversity from island location (Carey Island and Penang) were more diverse compared to coastal sites (Kuantan and Malacca). It is not surprised with the fact that *Ixodes granulatus*, *Laelaps sculpturatus* and *Ornithonyssus bacoti* were only found in Carey Island which was consistent with their known habitat distribution which are shrubs, forest and plantation. No significant differences in infestation levels were observed. All sites recorded low diversity index except Carey Island, and the agriculture habitat was more diverse in ectoparasites species compared to other sites.

Infestation of ectoparasites between host sex (male and female) and host age (adult and juvenile) were almost similar for most ectoparasites (except for *Polyplax spinulosa*). However, season played a significant role in determining the ectoparasite

infracommunity in the rat populations in Peninsular Malaysia with *Ornithonyssus bacoti*, *Laelaps sculpturatus*, *Polyplax spinulosa*, *Hoplopleura pacifica* and *Ixodes granulatus* were highly infected during wet season compared to dry season. The ectoparasites thrive in climate that is high in humidity which was suitable for their growth and survival.

As part of the ongoing efforts to catalogue and describe the local rat-borne ectoparasite diversity and distribution, present observation have shed some light into selected biological, behavioural and ecology characteristics of both the ectoparasites and their host. Future studies should endeavour to investigate and incorporate information from various habitats in Peninsular Malaysia to provide a more comprehensive view of each habitat's unique rat ectoparasite community structure and distribution.

CHAPTER 5: THE ENDOPARASITES COMMUNITIES OF THE WILD RAT POPULATIONS IN PENINSULAR MALAYSIA.

5.1 Introduction

There have been considerable numbers of studies done on rat endoparasites world wide. Most general surveys included studies by Gantha (1966) in Singapore, Mafiana *et al.* (1997) in Nigeria, Na. mue & Wongsawad (1997) in Thailand, Abu-Madi *et al.* (2001) in Qatar, Kia *et al.* (2001) in Iran, Griselda *et al.* (2005) in Mexico, Coomansingh *et al.* (2009) in Grenada, and Rafique *et al.* (2009) in Pakistan.

The local wild rat endoparasites surveys include recording and cataloguing of the helminth fauna from various different habitats throughout Peninsular Malaysia. Notable examples on general surveys include Dunn *et al.* (1968), Singh & Cheong (1971), Yap *et al.* (1977), Leong *et al.* (1979), Sinniah (1979), Paramasvaran *et al.* (2005), Syed-Arnez & Mohd Zain (2006), Paramasvaran *et al.* (2009a) and Mohd Zain *et al.* (2012). Other studies focused on selected species of endoparasites and offered limited perspective on overall endoparasites community on host (Yeh, 1955; Bhaibulaya & Cross, 1971; Lim *et al.*, 1975; 1977a; 1977b; Krishnasamy *et al.*, 1981).

Studies on the distribution and the prevalence of rat endoparasite population on island and coastal environment however are limited (Audy *et al.*, 1950; Dunn *et al.*, 1966; Lim *et al.*, 1974). The most recent study was a survey on endoparasites of wild rodent population on two islands, namely Pulau Langkawi and Pulau Jarak (Mohd Zain, 2008). However, this study did not provide much information on distribution and diversity of endoparasites in wild rats due to small sampling number.

Therefore, the present study hopes to determine the current endoparasite infection of wild rats from islands (Carey Island and Penang) and coastal (Kuantan and Malacca)

sites in Peninsular Malaysia 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 wild rat population. This study will also identify the endoparasite species that is of public health importance and the risk impact to the each of the location sampled.

5.2 Result

A total of 363 wild rats were captured from four locations in Peninsular Malaysia, with high endoparasites prevalence (92.3%). Post-mortem examinations recovered 19, 912 helminths, with abundance value of 54.9 endoparasites per infected rat. The largest worm burden in a single rat host was 574. The rats harbored a minimum of one to up to a maximum of five species of endoparasites. The endoparasites recovered consisted of two major groups namely the Nematode and Cestodes.

A total of 10 species of endoparasites were identified namely seven species of Nematode; *Nippostrongylus brasiliensis*, *Angiostrongylus malaysiensis*, *Capillaria hepatica*, *Mastophorus muris*, *Heterakis spumosa*, *Hepatojarakus malayae* and *Syphacia muris*, and three Cestode species; *Taenia taeniaeformis*, *Hymenolepis diminuta* and *Rodentolepis* (= *Hymenolepis*) *nana*. Figure 5.1 showed the distribution of the endoparasites recovered from wild rats of four locations in Peninsular Malaysia.

Overall the hookworm, *Nippostrongylus brasiliensis* (71.9%, CL: 66.96-76.33) was the most prevalence endoparasite recovered while *Hepatojarakus malayae* revealed the lowest prevalent with 0.6% (CL: 0.1-2.0). *Nippostrongylus brasiliensis* was the most abundant endoparasite with mean intensity of 66.43 per infected rat meanwhile, *Hepatojarakus malayae* was the less abundant with mean intensity 1.50 per infected rat.

All endoparasites species recovered exhibited a negative binomial distribution with k value ranging between 0.021-0.258 apart from *H. malayae* due to low number of samples. Table 5.1 summarized the quantitative analysis of endoparasites recovered from rats of the four locations in Peninsular Malaysia.

Rattus norvegicus (206) was the most dominant species followed by *Rattus rattus diardii* (59) and *Rattus tiomanicus* (54) with prevalence of endoparasites infection almost similar between the rat species *R. norvegicus* (90.8%), *R. tiomanicus* (87%) and *R. rattus diardii* (86.4%).

All endoparasites species were present in the 2 rat host species (*R. norvegicus* and *R. tiomanicus*) with *Nippostrongylus brasiliensis* the most prevalent parasites infecting *R. norvegicus* (71.4%) followed by *R. tiomanicus* (70.4%) and *R. rattus diardii* (66.1%). This nematode was also the most abundant infecting *R. tiomanicus* with mean intensity 96.5 per infected rats.

Table 5.2 summarized the prevalence, mean intensity, abundance as well as the comparative analysis of endoparasites between rat species. Prevalence of endoparasite infection between all rat species was not significant except for *Angiostrongylus malaysiensis*, *Heterakis spumosa* and *Taenia taeniaeformis*. No *Angiostrongylus malaysiensis* was recovered from *R. rattus diardii*. High prevalence of *Heterakis spumosa* (33.3%) was observed in *R. tiomanicus* while *Taenia taeniaeformis* (28.6%) in *R. norvegicus*.

Table 5.3 showed the analysis of species diversity using Simpson's Index and Brillouin Index. Overall, the Simpson Index and Brillouin Index showed low diversity with 1.33 and 0.64 respectively. According to host age factor, both indices showed similar indexes between male (D=1.32, HB=0.60) and females (D=1.33, HB=0.63) however, slightly higher diversity was observed in juveniles (D=1.36, HB=0.64) compared to adult

(D=1.33, HB=0.63). Higher diversity of endoparasites was observed during dry season (D=1.37, HB=0.64) compared to wet season (D=1.30, HB=0.58). Both indices also recorded the highest diversity index in Carey Island (D=1.78, HB=0.89) followed by Malacca (D=1.34, HB=0.57), Kuantan (D=1.24, HB=0.48) and Penang (D=1.23, HB=0.43) (Table 5.3).

5.2.1 Endoparasites distribution according to location

Four helminths species were found prevalent from all sites (*Nippostrongylus brasiliensis*, *Mastophorus muris*, *Taenia taeniaeformis* and *Hymenolepis diminuta*). The nematode species; *Hepatojarakus malayae* and *Syphacia muris* were recovered only in rats from Carey Island. Table 5.4 summarized the prevalence of all helminth recovered in this study according to location.

A total of 96 of the 103 wild rats (93.2%) captured in Kuantan were infected with 6 helminthes species. Table 5.5 summarized the quantitative analysis of endoparasites recovered from rats from Kuantan. *Nippostrongylus brasiliensis* was found the most prevalent species and abundance with 66% (CL: 56.02-75.07) and mean intensity 63.35 per infected rat followed by *Capillaria hepatica* (44.7%, CL: 34.85-54.78). However, *Heterakis spumosa* was the least prevalent with 20.3% (CL: 13.08-29.47). All endoparasites recovered exhibited a negative binomial distribution with k value ranging between 0.110-0.207 apart from *Capillaria hepatica* where no intact worms were possible to retrieve.

Seven endoparasite species were recovered from wild rats in Malacca, of which 101 of 104 rats (97.1%) were found infected. Similar to Kuantan rats, *Nippostrongylus brasiliensis* was also the most prevalent species with 84.6% infecting rats and the highest abundance with mean intensity 70.08 per infected rat followed by *Capillaria*

hepatica with 75.0% (CL: 65.6-82.9). *Angiostrongylus malaysiensis* was the least prevalent with 1.0% (CL: 0.02-5.25) wild rats infected. All endoparasites recovered here exhibited a negative binomial distribution with k value ranging between 0.097-0.544 apart from *Capillaria hepatica* where no intact worms were retrieved and *Angiostrongylus malaysiensis*, due to low number of samples. Table 5.6 summarized the quantitative analysis of endoparasites recovered from wild rats in Malacca.

Table 5.7 summarized the quantitative analysis of endoparasites recovered from wild rats in Carey Island. Eight species of endoparasites were recovered from the 73 of 81 rats (90.1%) captured. The nematode, *Nippostrongylus brasiliensis* was also the most prevalent species with 66.7% rats infected and abundance with mean intensity 98.91 per infected rats. *Hepatojarkus malayae* was the least prevalent with 2.5% (CL: 0.3-8.64) wild rats infected. All endoparasites recovered exhibited a negative binomial distribution with k value ranging between 0.021-0.263 apart from *Hepatojarkus malayae* and *Taenia taeniaeformis* due low sampling numbers.

In Penang, 65 (86.7%) out of the 75 wild rats captured were found to be infected. Again, *Nippostrongylus brasiliensis* was the most prevalent species with 68.0% (CL: 56.2-78.3) followed by *Hymenolepis diminuta* at 36% infection (CL: 25.23-47.9). *Angiostrongylus malaysiensis* was the least prevalent with 2.7% (CL: 0.32-9.31) rats infected. *Nippostrongylus brasiliensis* was the most abundant with mean intensity 29.82 per infected wild rat. All endoparasites recovered exhibited a negative binomial distribution with k value ranging between 0.029-0.230 apart from *Angiostrongylus malaysiensis* due low sampling numbers. The quantitative analysis of endoparasites recovered in Penang was summarized in Table 5.8.

5.2.2 Endoparasites distribution according to host sex

The rat populations was composed of more females (219 rats) than males (144 rats) with females (94.5%) harbouring more parasites compared to males (88.9%). All 10 species were recovered from both host sexes except for *Hepatojarakus malayae*. This species only found in female rats with 0.9% (CL: 0.05-4.77) infected. The three most prevalent endoparasites recovered were *Nippostrongylus brasiliensis*, *Hymenolepis diminuta* and *Capillaria hepatica* (Table 5.9).

Table 5.9 summarized the prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM), as well as the comparative analysis of rat endoparasites between both host sexes. No significant differences were shown in the prevalence, mean intensity and abundance of infections of all species recovered between both sexes apart for prevalence of *Capillaria hepatica*. Although the prevalence of *Syphacia muris* and *Rodentolepis* (= *Hymenolepis*) *nana* ($p < 0.05$) showed a significant value, however this could be due to low sampling numbers.

Table 5.10 summarized the prevalence and comparative analysis of rat endoparasites between both sexes in relation to rat species. *Nippostrongylus brasiliensis* was found prevalent in both sexes for all rat species. No significant difference was shown between the parasites recovered and the rat species apart from *Capillaria hepatica* and *Syphacia muris* in *R. rattus diardii*.

5.2.3 Endoparasites distribution according to host age

Overall, the rat population was composed of more adults (319 rats) compared to juveniles (44 rats). Both groups harboured endoparasites with prevalence between both age groups higher in adults (93.1%) compared to juveniles (86.4%). *Hepatojarakus malayae* was only found infecting juvenile rats ($p=0.014$).

Table 5.11 summarizes the prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM), as well as the comparative analysis of rat endoparasites between both host ages. No significant difference were found in the prevalence of infection between host age except for *Hepatojarakus malayae*, *Taenia taeniaeformis* and *Rodentolepis* (= *Hymenolepis*) *nana* ($p<0.05$). Prevalence of *Nippostrongylus brasiliensis* was highest in both host-age groups with infection in adults (73.0%, CL: 67.3-81.9) slightly higher compared to juveniles (63.6%, CL: 49.3-72.9) followed by *Hymenolepis diminuta*. Infection in juveniles (27.3%, CL: 17.15-41.71) were lower compared to adults (32.3%, CL: 26.98-45.1) significantly, in terms of mean intensity ($p=0.01$) and abundance ($p=0.005$).

Similarly, no significant differences were found in the 3 more prevalent endoparasites species (*Nippostrongylus brasiliensis*, *Capillaria hepatica* and *Hymenolepis diminuta*) in relation to the 3 dominant rat species apart from *Nippostrongylus brasiliensis* and *Hymenolepis diminuta* in *R. norvegicus* where infection was significantly higher in adults compared to juveniles. Also, significant difference was observed for *Mastophorus muris* and *Taenia taeniaeformis* in *R. norvegicus* and *R. rattus diardii* while *Heterakis spumosa* and *Rodentolepis* (= *Hymenolepis*) *nana* showed significant differences in *R. rattus diardii* and *R. tiomanicus* between both age groups. Table 5.12 summarized the prevalence and comparative analysis of rat endoparasites between both host ages in different rat species.

5.2.4 Endoparasites distribution according to season

From the total of rat population caught, 226 rats were trapped during the dry season and 137 during wet season from the four locations. Endoparasites infected rats were slightly higher during the dry season (n=211, 93.4%) compared to wet season (n=124, 90.5%). All 10 species of endoparasites recovered in the present study were found in both seasons except for *Hepatojarakus malayae* and *Syphacia muris* which were present only during wet season.

Table 5.13 summarized the prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM), as well as the comparative analysis of rat endoparasites recovered. Prevalence of infection between both seasons for all endoparasite species were significantly different apart for *Nippostrongylus brasiliensis*, *Hepatojarakus malayae* and *Hymenolepis diminuta*. However, *Mastophorus muris*, *Heterakis spumosa*, *Syphacia muris* and *Taenia taeniaeformis* showed significant differences in abundance of infection ($p < 0.05$). Prevalence of infection for *Nippostrongylus brasiliensis* was high in both seasons with slightly higher infection during dry season (73.0%, CL: 67.3-81.9) compared to wet season (70.1%, CL: 49.3-72.9). This is followed by *Hymenolepis diminuta* with infection higher in the dry season (35%, CL: 17.15-41.71) compared to the wet season (26.3%, CL: 19.98-41.1).

Table 5.14 summarized the prevalence and comparative analysis of rat endoparasites during dry and wet season in different rat species. Prevalence of infection for *Nippostrongylus brasiliensis* between the 3 rat species were not significant however, *Capillaria hepatica* was significant for all rat species and *Hymenolepis diminuta* infection in *R. norvegicus* was higher during dry season compared to wet season.

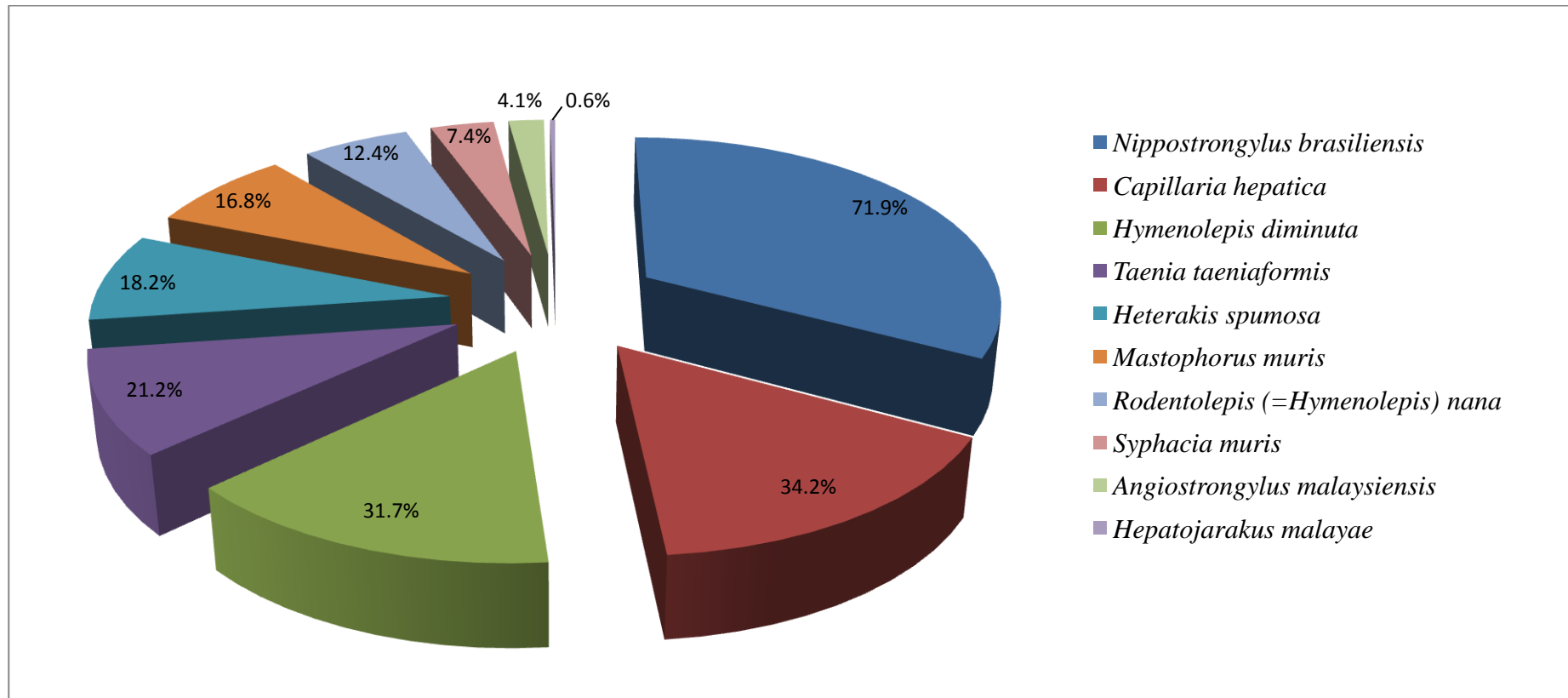


Figure 5.1: Prevalence of endoparasite species recovered from wild rats in Peninsular Malaysia.

Table 5.1: Quantitative analysis of endoparasites recovered from rats captured from all locations in Peninsular Malaysia.

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	<i>k</i>
Nematode						
<i>Nippostrongylus brasiliensis</i>	17337	71.9% (66.96 - 76.33)	66.43 (57.3 – 77.36)	47.76 (40.07 – 55.9)	1 – 574	0.258
<i>Angiostrongylus malaysiensis</i>	38	4.1% (2.43 - 6.71)	2.53 (1.67 – 4.47)	0.1 (0.06 – 0.21)	1 -11	0.026
<i>Capillaria hepatica</i>	-	34.2% (29.39 - 39.24)	-	-	-	-
<i>Mastophorus muris</i>	208	16.8% (13.19 – 21.04)	3.41 (2.79 – 4.21)	0.57 (0.42 – 0.76)	1 – 13	0.095
<i>Heterakis spumosa</i>	569	18.2% (14.43 – 22.56)	8.62 (6.48 – 13.08)	1.57 (1.5 – 2.40)	1 – 89	0.061
<i>Hepatojarakus malayae</i>	3	0.6% (0.1 – 2.0)	1.50 (1.0 – 1.5)#	0.01 (0.0 – 0.02)	1 – 2	**
<i>Syphacia muris</i>	274	7.4% (5.05 – 10.7)	10.15 (5.96 – 17.41)	0.75 (0.39 – 1.47)	1 - 57	0.021

- limits uncertain due to low sample size

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

Table 5.1: Quantitative analysis of endoparasites recovered from rats captured from all locations in Peninsular Malaysia. [Continued]

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	K
Cestode						
<i>Taenia taeniaformis</i>	153	21.2% (17.19 – 25.73)	1.99 (1.66 – 2.47)	0.42 (0.31 – 0.55)	1 – 13	0.223
<i>Hymenolepis diminuta</i>	736	31.7% (26.98 – 36.64)	6.40 (5.06 – 8.54)	2.03 (1.51 – 2.70)	1 – 61	0.412
<i>Rodentolepis</i> (= <i>Hymenolepis</i>) <i>nana</i>	594	12.4% (9.34 – 16.22)	13.20 (7.18 – 33.13)#	1.64 (0.85 – 4.15)#	1 - 214	0.034

- limits uncertain due to low sample size

‡ - sample not aggregated enough to fit the negative binomial

Table 5.2: Prevalence, mean intensity, abundance and comparative analysis of ectoparasites between rat species in Peninsular Malaysia.

Endoparasites species		<i>Rattus norvegicus</i>	<i>Rattus rattus diardii</i>	<i>Rattus tiomanicus</i>	P-value
<u>Nematode</u>					
<i>Nippostrongylus brasiliensis</i>	P	71.4%	66.1%	70.4%	0.717
	MI (95% CL)	67.2(54.8-81.6)	42.1(28.5-61.5)	96.5(62.1-147.6)	
	A (95% CL)	47.9 (38.6-59.8)	27.8(18.2-42.5)	67.9(42.9-107.6)	
<i>Angiostrongylus malaysiensis</i>	P	2.4%	-	13.0%	0.002*
	MI (95% CL)	2.2(1.0-2.6)#	-	1.86(1.14-2.43)	
	A (95% CL)	0.05(0.01-0.12)	-	0.24(0.09-0.48)	
<i>Capillaria hepatica</i>	P	31.6%	27.1%	22.2%	0.402
	MI (95% CL)	-	-	-	
	A (95% CL)	-	-	-	
<i>Mastophorus muris</i>	P	17.0%	13.6%	11.1%	0.570
	MI (95% CL)	3.4(2.69-4.2)	3.38(2.0-6.13)	3.17(1.0-6.5)	
	A (95% CL)	0.58(0.38-0.82)	0.46(0.17-1.05)	0.35(0.09-1.15)	
<i>Heterakis spumosa</i>	P	11.7%	23.7%	33.3%	0.000*
	MI (95% CL)	9.71(5.13-22.7)	6.93(4.0-11.4)	8.39(5.06-14.6)	
	A (95% CL)	1.13(0.51-2.78)	1.64(0.8-3.2)	2.8(1.46-5.80)	
<i>Hepatojarakus malayae</i>	P	0.5%	-	1.9%	0.344
	MI (95% CL)	1.0(0.0-0.0)#	-	2.0(0.0-0.1)#	
	A (95% CL)	0.0(0.0-0.1)#	-	0.04(0.0-0.11)	

Table 5.2: Prevalence, mean intensity, abundance and comparative analysis of ectoparasites between rat species in Peninsular Malaysia. [Continued]

Endoparasites species		<i>Rattus norvegicus</i>	<i>Rattus rattus diardii</i>	<i>Rattus tiomanicus</i>	P-value
<i>Syphacia muris</i>	P	7.8%	11.9%	3.7%	0.271
	MI (95% CL)	13.3(6.81-24.3)	4.43(2.43-6.43)	1.5(1.0-2.0)	
	A (95% CL)	1.03(0.44-2.25)	0.53(0.2-1.17)	0.06(0.0-0.17)	
<u>Cestode</u>					
<i>Taenia taeniaformis</i>	P	28.6%	15.3%	7.4%	0.001*
	MI (95% CL)	2.17(1.76-2.81)	1.44(1.0-1.89)	1.5(1.0-2.0)	
	A (95% CL)	0.62(0.46-0.87)	0.22(0.08-0.39)	0.11(0.02-0.28)	
<i>Hymenolepis diminuta</i>	P	35.4%	25.4%	25.9	0.217
	MI (95% CL)	7.49(5.85-10.6)	3.93(2.73-5.53)	3.07(1.71-5.64)	
	A (95% CL)	2.7(1.9-3.8)	1.0(0.54-1.68)	0.8(0.37-1.65)	
<i>Rodentolepis</i> (= <i>Hymenolepis</i>) <i>nana</i>	P	4.9%	8.5%	9.3%	0.308
	MI (95% CL)	10.1(2.7-30.7)	2.4(1.4-3.2)	11.0(6.4-14.8)	
	A (95% CL)	0.49(0.11-1.92)	0.2(0.05-0.46)	1.02(0.31-2.33)	

- limits uncertain due to low sample size

* - significant

P - prevalence

MI - mean intensity

A - abundance

CL - confident limits

Table 5.3: Simpson's and Brillouin Index values for the endoparasite infection in the wild rat population in Peninsular Malaysia.

Factors		Simpson's Index	Brillouin Index
Host sex	Male	1.32	0.60
	Female	1.33	0.63
Host age	Adult	1.33	0.63
	Juvenile	1.36	0.64
Seasons	Dry	1.37	0.64
	Wet	1.30	0.58
Location	Kuantan	1.24	0.48
	Malacca	1.34	0.57
	Carey Island	1.78	0.89
	Penang	1.23	0.43
Total population		1.33	0.64

Table 5.4: Prevalence of endoparasites recovered from rats captured in Kuantan, Malacca, Carey Island and Penang of Peninsular Malaysia.

Endoparasites	Kuantan (n=103)	Malacca (n=104)	Carey Island (n=81)	Penang (n=71)
Nematode				
<i>Nippostrongylus brasiliensis</i>	66.0%	84.6%	66.7%	68.0%
<i>Angiostrongylus malaysiensis</i>	0	1.0%	14.8%	2.7%
<i>Capillaria hepatica</i>	44.7%	75.0%	0	0
<i>Mastophorus muris</i>	25.2%	17.3%	11.1%	10.7%
<i>Heterakis spumosa</i>	20.4%	0	55.6%	0
<i>Hepatojarakus malayae</i>	0	0	2.5%	0
<i>Syphacia muris</i>	0	0	6.2%	0
Cestode				
<i>Taenia taeniaformis</i>	32.0%	16.3%	16.0%	18.7%
<i>Hymenolepis diminuta</i>	35.9%	36.5%	16.0%	36.0%
<i>Rodentolepis</i> (= <i>Hymenolepis</i>) <i>nana</i>	0	39.4%	0	5.3%

Table 5.5: Quantitative analysis of endoparasites recovered from rats captured in Kuantan of Peninsular Malaysia.

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	<i>k</i>
Nematode						
<i>Nippostrongylus brasiliensis</i>	4308	66.0% (56.02 – 75.07)	63.35 (45.75 – 88.34)	41.83 (29.36 – 59.55)	1 – 411	0.207
<i>Capillaria hepatica</i>	-	44.7% (34.85 – 54.78)	-	-	-	-
<i>Mastophorus muris</i>	96	25.2% (17.19 – 34.76)	3.69 (2.65 – 4.88)	0.93 (0.56 – 1.43)	1 – 11	0.148
<i>Heterakis spumosa</i>	81	20.4% (13.08 – 29.47)	3.86 (2.62 – 6.14)	0.79 (0.46 – 1.37)	1 – 16	0.110
Cestode						
<i>Taenia taeniaformis</i>	65	32.0% (23.18 – 41.96)	1.97 (1.55 – 2.48)	0.63 (0.44 – 0.89)	1 – 6	0.427
<i>Hymenolepis diminuta</i>	209	35.9% (26.7 – 45.98)	5.65 (4.54 – 6.92)	2.03 (1.45 – 2.78)	1 - 16	0.199

Table 5.6: Quantitative analysis of endoparasites recovered from rats captured in Malacca of Peninsular Malaysia.

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	<i>k</i>
Nematode						
<i>Nippostrongylus brasiliensis</i>	6167	84.6% (76.22 – 90.95)	70.08 (59.38 – 86.5)	59.3 (49.8 – 74.6)	1 – 332	0.544
<i>Angiostrongylus malaysiensis</i>	2	1.0% (0.02 – 5.25)	2.00 (0.00 – 0.00)#	0.02 (0.00 – 0.06)#	0 – 2	**
<i>Capillaria hepatica</i>	-	75.0% (65.60 – 82.90)	-	-	-	-
<i>Mastophorus muris</i>	63	17.3% (10.59 – 25.97)	3.50 (2.5 – 5.28)	0.61 (0.34 – 1.07)	1 -13	0.097
Cestode						
<i>Taenia taeniaformis</i>	34	16.3% (9.82 – 24.88)	2.00 (1.29 – 3.12)	0.33 (0.17 – 0.59)	1 – 7	0.156
<i>Hymenolepis diminuta</i>	254	36.5% (27.31 – 46.56)	6.68 (4.26 – 11.79)	2.44 (1.44 – 4.43)	1 -56	0.162
<i>Rodentolepis</i> (= <i>Hymenolepis</i>) <i>nana</i>	582	39.4% (29.90 – 49.50)	14.2 (7.51 – 36.7)	5.60 (2.88 – 13.72)	1 - 214	0.132

- limits uncertain due to low sample size

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

Table 5.7: Quantitative analysis of endoparasites recovered from rats captured in Carey Island of Peninsular Malaysia.

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	<i>k</i>
Nematode						
<i>Nippostrongylus brasiliensis</i>	5341	66.7% (55.31 – 76.70)	98.91 (72.85 – 138.4)	65.94 (46.4 – 94.8)	1 – 574	0.208
<i>Angiostrongylus malaysiensis</i>	30	14.8% (7.89 – 24.45)	2.50 (1.5 – 5.0)#	0.37 (0.17 – 0.88)#	1 – 11	0.106
<i>Mastophorus muris</i>	22	11.1 (5.20 – 20.05)	2.44 (1.0 – 3.89)	0.27 (0.1 – 0.57)	1 – 6	0.078
<i>Heterakis spumosa</i>	488	55.6% (44.08 – 66.60)	10.84 (7.84 – 18.07)	6.02 (4.09 – 9.86)	1 – 89	0.263
<i>Hepatojarakus malayae</i>	3	2.5% (0.3 – 8.64)	1.50 (1.0 – 1.5)#	0.04 (0.07 – 1.58)	1 – 2	**
<i>Syphacia muris</i>	33	6.2% (2.03 – 13.83)	6.6 (1.80 – 15.6)	0.41 (0.07 – 1.58)	1 – 24	0.021
Cestode						
<i>Taenia taeniaformis</i>	17	16.0% (8.83 – 25.9)	1.31 (1.08 – 1.54)	0.21 (0.11 – 0.33)	1 – 2	**
<i>Hymenolepis diminuta</i>	35	16.0% (8.83 – 25.9)	2.69 (1.62 – 4.23)	0.43 (0.21 – 0.84)	1 – 8	0.107

- limits uncertain due to low sample size

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

Table 5.8: Quantitative analysis of endoparasites recovered from rats captured in Penang of Peninsular Malaysia.

Endoparasites	Intensity	Prevalence (95% CL)	Mean Intensity (95% CL)	Abundance (95% CL)	Range	<i>k</i>
Nematode						
<i>Nippostrongylus brasiliensis</i>	1521	68.0% (56.2 – 78.3)	29.82 (17.69 – 50.31)	20.28 (12.11 – 34.23)	1 – 311	0.230
<i>Angiostrongylus malaysiensis</i>	6	2.7% (0.32 – 9.31)	3.0 (0.0 – 0.0)#	0.08 (0.0 – 0.2)#	1 – 3	**
<i>Mastophorus muris</i>	27	10.7% (4.71 – 19.95)	3.38 (2.25 – 4.5)	0.36 (0.15 – 0.69)	1 – 6	0.059
Cestode						
<i>Taenia taeniaformis</i>	37	18.7% (10.59 – 29.34)	2.64 (1.64 – 5.36)	0.49 (0.24 – 1.12)	1 – 13	0.133
<i>Hymenolepis diminuta</i>	238	36.0% (25.23 – 47.9)	8.81 (5.33 – 16.26)	3.17 (1.69 – 6.05)	1 – 61	0.146
<i>Rodentolepis</i> (= <i>Hymenolepis</i>) <i>nana</i>	12	5.3% (1.47 – 13.1)	3.0 (1.0 – 6.5)	0.16 (0.03 – 0.60)	1 – 8	0.029

- limits uncertain due to low sample size

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

Table 5.9: Prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM) and comparative analysis of rat endoparasites between both host sexes in Peninsular Malaysia.

Parasite species	Prevalence			Mean Intensity			Abundance \pm SEM		
	Male (%)	Female (%)	<i>P</i> value	Male	Female	<i>P</i> value	Male	Female	<i>P</i> value
<i>Nippostrongylus brasiliensis</i>	66.7	75.3	0.075	68.3	65.3	0.799	45.5	49.2	0.675
<i>Angiostrongylus malaysiensis</i>	2.8	5.0	0.420	1.25	3.0	0.236	0.04	0.15	0.115
<i>Capillaria hepatica</i>	20.8	42.9	0.000*	-	-	-	-	-	-
<i>Mastophorus muris</i>	12.5	19.6	0.086	3.72	3.28	0.621	0.47	0.64	0.342
<i>Heterakis spumosa</i>	17.4	18.7	0.782	10.76	7.32	0.413	1.87	1.37	0.551
<i>Hepatojarkus malayae</i>	0.0	0.9	0.52	0.0	1.5	1.0	0.0	0.01	0.303
<i>Syphacia muris</i>	12.5	4.1	0.004*	11.56	7.33	0.374	1.44	0.30	0.108
<i>Taenia taeniaformis</i>	19.4	22.4	0.6	1.89	2.04	0.699	0.37	0.46	0.437
<i>Hymenolepis diminuta</i>	30.6	32.4	0.731	8.05	5.38	0.201	2.46	1.74	0.319
<i>Rodentolepis (=Hymenolepis) nana</i>	2.8	18.7	0.000*	6.0	13.90	0.248	0.17	2.60	0.163

*significant

Table 5.10: Prevalence and comparative analysis of rat endoparasites between both host sexes according to rat species from Peninsular Malaysia.

Parasite species	<i>Rattus norvegicus</i>			<i>Rattus rattus diardii</i>			<i>Rattus tiomanicus</i>		
	Male (%)	Female (%)	<i>P</i> value	Male (%)	Female (%)	<i>P</i> value	Male (%)	Female (%)	<i>P</i> value
<i>Nippostrongylus brasiliensis</i>	70.4	72.2	0.37	55.6	70.7	0.06	66.7	72.7	0.12
<i>Angiostrongylus malaysiensis</i>	0	4.6	0.19	0	0	0.10	14.3	12.1	0.83
<i>Capillaria hepatica</i>	24.5	37.9	0.37	11.1	34.1	0.00*	19.0	24.2	0.43
<i>Mastophorus muris</i>	13.3	20.4	0.10	11.1	14.6	0.24	9.5	12.1	0.22
<i>Heterakis spumosa</i>	8.2	14.8	0.09	27.8	22.0	0.73	28.6	36.4	0.49
<i>Hepatojarkus malayae</i>	0	0.93	0.19	0	0	0.10	0	3.0	0.13
<i>Syphacia muris</i>	12.2	3.7	0.29	22.2	7.3	0.00*	4.8	3.0	0.43
<i>Taenia taeniaformis</i>	22.4	34.3	0.28	16.7	14.6	0.19	9.5	6.1	0.53
<i>Hymenolepis diminuta</i>	34.7	36.1	0.51	16.7	29.3	0.12	33.3	21.2	0.11
<i>Rodentolepis</i> (=Hymenolepis) <i>nana</i>	2.0	7.4	0.34	5.6	9.8	0.21	4.8	12.1	0.14

* significant

Table 5.11: Prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM) and comparative analysis of rat endoparasites between host ages in Peninsular Malaysia.

Parasite species	Prevalence			Mean Intensity			Abundance \pm SEM		
	Adult (%)	Juvenile (%)	<i>P</i> value	Adult	Juvenile	<i>P</i> value	Adult	Juvenile	<i>P</i> value
<i>Nippostrongylus brasiliensis</i>	73.0	63.6	0.212	66.9	62.2	0.757	48.9	39.6	0.387
<i>Angiostrongylus malaysiensis</i>	4.1	4.5	0.701	2.69	1.5	0.236	0.11	0.07	0.568
<i>Capillaria hepatica</i>	33.5	38.6	0.502	-	-	-	-	-	-
<i>Mastophorus muris</i>	17.9	9.1	0.196	4.5	3.33	0.574	0.59	0.41	0.498
<i>Heterakis spumosa</i>	18.8	13.6	0.532	8.55	9.33	0.855	1.61	1.27	0.681
<i>Hepatojarkus malayae</i>	0	4.5	0.014*	0.0	1.5	1.0	0.0	0.07	0.302
<i>Syphacia muris</i>	7.2	9.1	0.553	10.0	11.0	0.861	0.72	1.0	0.702
<i>Taenia taeniaformis</i>	23.8	2.3	0.000*	2.0	1.0	1.0	0.48	0.02	0.000*
<i>Hymenolepis diminuta</i>	32.3	27.3	0.605	6.76	3.33	0.01*	2.18	0.91	0.005*
<i>Rodentolepis (=Hymenolepis) nana</i>	9.7	31.8	0.000*	15.61	7.86	0.373	1.52	2.5	0.394

*significant

Table 5.12: Prevalence and comparative analysis of rat endoparasites between both host ages according to rat species from Peninsular Malaysia.

Parasite species	<i>Rattus norvegicus</i>			<i>Rattus rattus diardii</i>			<i>Rattus tiomanicus</i>		
	Adult (%)	Juvenile (%)	<i>P</i> value	Adult (%)	Juvenile (%)	<i>P</i> value	Adult (%)	Juvenile (%)	<i>P</i> value
<i>Nippostrongylus brasiliensis</i>	72.4	42.9	0.00*	66.7	64.3	0.32	77.5	57.1	0.12
<i>Angiostrongylus malaysiensis</i>	2.5	0	0.13	0	0	0.10	12.5	14.3	0.32
<i>Capillaria hepatica</i>	32.2	14.3	0.12	26.7	28.6	0.65	17.5	35.7	0.11
<i>Mastophorus muris</i>	17.6	0	0.00*	15.6	7.1	0.00*	10.0	14.3	0.51
<i>Heterakis spumosa</i>	11.6	14.3	0.52	28.9	7.1	0.00*	40.0	14.3	0.00*
<i>Hepatojarkus malayae</i>	0	14.3	0.14	0	0	0.10	0	7.1	0.09
<i>Syphacia muris</i>	7.5	14.3	0.20	11.1	14.3	0.23	5.0	0	0.10
<i>Taenia taeniaformis</i>	29.6	0	0.00*	20.0	0	0.00*	7.5	7.1	0.57
<i>Hymenolepis diminuta</i>	36.7	0	0.00*	22.2	35.7	0.30	27.5	21.4	0.31
<i>Rodentolepis (=Hymenolepis) nana</i>	5.0	0	0.21	4.4	21.4	0.00*	2.5	28.6	0.00*

* significant

Table 5.13: Prevalence, mean intensity, abundance of infection \pm standard error of the mean (SEM) and comparative analysis of rat endoparasites between seasonal factors in Peninsular Malaysia

Parasite species	Prevalence			Mean Intensity			Abundance \pm SEM		
	Dry (%)	Wet (%)	<i>P</i> value	Dry	Wet	<i>P</i> value	Dry	Wet	<i>P</i> value
<i>Nippostrongylus brasiliensis</i>	73.0	70.1	0.549	66.7	66.0	0.959	48.7	46.3	0.797
<i>Angiostrongylus malaysiensis</i>	1.8	8.0	0.005*	2.75	2.46	0.734	0.05	0.19	0.217
<i>Capillaria hepatica</i>	49.1	9.5	0.000*	-	-	-	-	-	-
<i>Mastophorus muris</i>	22.1	8.0	0.000*	3.6	2.55	0.164	0.79	0.20	0.000*
<i>Heterakis spumosa</i>	14.2	24.8	0.012*	4.53	12.5	0.062	0.64	3.1	0.037*
<i>Hepatojarkus malayae</i>	0.0	1.5	0.142	0.0	1.5	1.0	0.0	0.02	0.290
<i>Syphacia muris</i>	0.0	19.7	0.000*	0.0	10.1	1.0	0.0	2.0	0.046*
<i>Taenia taeniaformis</i>	26.5	12.4	0.001*	1.93	2.18	0.635	0.51	0.27	0.033*
<i>Hymenolepis diminuta</i>	35.0	26.3	0.103	5.66	8.03	0.332	1.98	2.11	0.867
<i>Rodentolepis (=Hymenolepis) nana</i>	18.1	2.9	0.000*	14.2	3.0	0.189	2.58	0.09	0.165

* significant

Table 5.14: Prevalence and comparative analysis of rat endoparasites between both season according to rat species from Peninsular Malaysia

Parasite species	<i>Rattus norvegicus</i>			<i>Rattus rattus diardii</i>			<i>Rattus tiomanicus</i>		
	Dry (%)	Wet (%)	<i>P</i> value	Dry (%)	Wet (%)	<i>P</i> value	Dry (%)	Wet (%)	<i>P</i> value
<i>Nippostrongylus brasiliensis</i>	70.5	72.7	0.32	61.5	75.0	0.27	74.1	66.7	0.31
<i>Angiostrongylus malaysiensis</i>	1.6	3.9	0.51	0	0	0.10	3.7	22.2	0.00*
<i>Capillaria hepatica</i>	40.3	16.9	0.00*	41.0	0	0.00*	44.4	0	0.00*
<i>Mastophorus muris</i>	24.8	3.9	0.00*	12.8	15.0	0.55	7.4	14.8	0.12
<i>Heterakis spumosa</i>	12.4	10.4	0.21	20.5	30.0	0.19	29.6	37.0	0.09
<i>Hepatojarakus malayae</i>	0	1.3	0.32	0	0	0.10	0	3.7	0.12
<i>Syphacia muris</i>	0	20.8	0.00*	0	35.0	0.00*	0	7.4	0.09
<i>Taenia taeniaformis</i>	35.7	16.9	0.09	23.1	0	0.00*	7.4	7.4	0.10
<i>Hymenolepis diminuta</i>	40.3	27.3	0.00*	23.1	30.0	0.34	22.2	29.6	0.35
<i>Rodentolepis (=Hymenolepis) nana</i>	6.2	2.6	0.41	7.7	10.0	0.20	18.5	0	0.00*

* significant

5.3 Discussion

The majority of wild rats in Peninsular Malaysia harbour endoparasites. This has revealed the immense magnitude of helminth infections among rats living within the same area. However, species diversity of helminth was low compared to other studies (Dunn, 1966; Singh & Cheong, 1971; Yap *et al.*, 1977; Leong *et al.*, 1979; Sinniah, 1979; Paramasvaran *et al.*, 2009a; Syed-Arnez & Mohd Zain, 2006; Mohd Zain *et al.*, 2012). Sinniah (1979) recorded 18 species of helminths infecting rats from various habitats in comparison to only ten species identified in this study. The helminth community belonged to two groups; 3 cestodes (*Taenia teniaformis*, *Hymenolepis diminuta*, *Rodentolepis* (= *Hymenolepis*) *nana*) and 7 nematodes (*Nippostrongylus brasiliensis*, *Angiostrongylus malaysiensis*, *Capillaria hepatica*, *Mastophorus muris*, *Heterakis spumosa*, *Syphacia muris* and *Hepatojarakus malayae*), all of which are cosmopolitan species suggesting that the commensal rats maintained the parasite life cycle in the habitat. Mohd Zain *et al.* (2012) also recorded similar number of helminth species however, the overall infection rate for *R. norvegicus* in this study was 51.5% which is much higher indicating an increase in infection over the years in the urban habitats.

The rat population showed dominance of *R. norvegicus* in coastal and island habitats. Therefore not surprisingly the helminth infection for this species was higher than other species since species richness is largely dependent on host density and sampling effort (Feliu *et al.*, 1997).

Diversity of helminth species was observed highest in Carey Island with 8 species recovered compared to the rest. However, Sinniah (1979) recorded 18 species of helminths infecting rats from various habitats which included an oil palm estate. Similarly, Krishnasamy *et al.* (1980) recorded the same number of helminth species

however the overall infection rate for the most dominant rat species, *R. tiomanicus* was 79.2%, much lower prevalence than his study at 94% indicating declining levels of infection over the years. This could be the result of the commensal rat species established in the habitat, as well as their high population levels acting as contributing factors for widespread and high endoparasitic infections.

All locations showed high percentage of endoparasites infections in the rat population in Malacca followed by Kuantan, Carey Island and Penang. The intensity of helminth infection in Malacca was also the highest followed by Carey Island, Kuantan and Penang. High infection levels in the urban habitats of Malacca and Kuantan suggested that existing urban environment provided a conducive condition for the transmission of the helminths among the rat population.

The nematode, *Nippostrongylus brasiliensis* was the most common species found in all locations and rat species of Peninsular Malaysia. This feature seems to be characteristic trait for this parasite as several studies have also reported similar observations (Leong *et al.*, 1979; Syed Arnez and Mohd Zain, 2006). Previous island study has recorded, high prevalence of multiple species of Heligmonellidae (100%) among the rats (Mohd Zain, 2008). Similarly, Paramasvaran *et al.* (2009a) also found *Nippostrongylus brasiliensis* in 13 rats out of 97 rats captured in five wet markets. Transmission of *N. brasiliensis* is via infective motile L3 larvae penetrating the skin. The direct lifecycle of this nematode also occurred relatively easy with the warm and wet climate in Malaysia with no marked contrasting seasons provides optimum conditions for the survival and hatching of eggs of *N. brasiliensis*. High prevalence of this species is also shown in other country namely, Mexico (Griselda *et al.*, 2005), South West Iran (Kia *et al.*, 2001) and Grenada, West Indies (Coomansingh *et al.*, 2009).

Another nematode, *Mastophorus muris* was also found in the rat population from all locations. Previous studies also recorded this species infecting wild rats from a variety of habitats in Malaysia ranging from urban, forest and coastal habitat (Leong *et al.*, 1979; Krishnansamy *et al.*, 1980; Ambu *et al.*, 1996; Paramasvaran *et al.*, 2005, 2009a; Syed Arnez & Mohd Zain, 2006; Mohd Zain *et al.*, 2012). Two nematode species, *Hepatojarakus malayae* and *Syphacia muris* were not present in the other locations but were only found infecting rats in Carey Island. The presence of *Syphacia muris* and *Hepatojarakus malayae* were previously recorded by Singh & Cheong (1971), Schacher & Cheong (1960) and Yap *et al.* (1977). The high infection of *Hepatojarakus malayae* in *R. tiomanicus* was similar to Yeh (1955). Adam (1933), recorded *Syphacia obvelata* from unidentified rats in Taiping and Pahang, but since no description was given it is not possible to say whether he could have been dealing with *Syphacia muris*. This parasite was also recorded for the first time in Argentina (Maria *et al.*, 2008) and in Hidalgo, Mexico (Griselda *et al.*, 2005). Dunn *et al.* (1968) reported the frequency of this nematode was inversely related to the proportion of plant food in the diet.

The high prevalence of *Heterakis spumosa* in *R. tiomanicus* from Carey Island indicated the ecological distribution of this parasite extended to rats inhabiting oil palm plantation. The nematode was recorded only from Kuantan and Carey Island however, was previously recorded from disturbed forest habitat (Singh and Krishnasamy, 1979) and lowland forest (Ow-Yang, 1971). High infection in rats may be due to their direct lifecycle which occurred relatively easier, conceivably also in a more consistent manner (Bellocq, 2003). This was enhanced by the relatively high rainfall and humidity in Malaysia that was suitable for the hatching of eggs prior to penetration of the infective larvae or diet consumption by the host. Maria *et al.* (2008) recorded for the first time *Heterakis spumosa* recovered from rats in Argentina.

The occurrence of *Angiostrongylus malaysiensis* in some rodents in this study indicated a diet that included intermediate host snails. In Peninsular Malaysia, *Angiostrongylus cantonensis* was first reported in *Rattus rattus diardii* and *Rattus exulans* (Schacher & Cheong, 1960) however, this parasite was absent in *R. rattus diardii*. This could be due to the small sampling size of *R. rattus diardii* from all locations. However, high prevalence of *Angiostrongylus malaysiensis* was observed in *R. tiomanicus* which was in agreement with a previous report that *Rattus argentiventer*, *Rattus exulans* and *Rattus tiomanicus* were common definitive hosts of *Angiostrongylus malaysiensis* (Lim *et al.*, 1976). Other studies on *Angiostrongylus* sp. included Lim *et al.* (1965; 1973), Lim & Heyneman (1965), Lim and Ungku Omar (1969), Heyneman & Lim (1965a), Lim (1970), Bhaibulaya & Cross (1971), Yap *et al.* (1977) and Mohd Zain *et al.* (2012).

High infection of *Capillaria hepatica* was observed in rats from Kuantan and Malacca. This might be due to the trapping of rats within limited area where the foci of infection may have been high. Infection of rats to this parasite is depended on the availability of the infective stages to the rat hosts. As most of the rats feed and live within the same area, infections were easily maintained among the population. Singh & Cheong (1971) found most rat species to be susceptible to *Capillaria hepatica* infections while Schacher & Cheong (1960) found low incidence of infection in house rats in Singapore. *Capillaria hepatica* was recorded higher in *R. norvegicus* in this study. Lim *et al.* (1977b) related the foraging and omnivorous behavior of commensal rats, as well as cannibalism, particularly *R. norvegicus* to be responsible for the pervasive nature of the infection. The distribution of this nematode was recorded throughout Malaysia and Singapore (Audy *et al.*, 1950; Gatha, 1966; Lim *et al.*, 1977b; Sinniah, 1979; Leong *et al.*, 1979; Paramasvaran *et al.*, 2005; Syed-Arnez & Mohd Zain, 2006; Paramasvaran *et al.*, 2009a).

Capillaria hepatica can be transmitted to other animals through consumption of infected liver (Lim *et al.*, 1977; Calle, 1961). The first human infection was in a soldier in India and to date twenty three cases have been reported (Sinniah *et al.*, 1979). The parasite can cause acute or subacute hepatitis with marked eosinophilia and with persistent fever. Hepatomegaly (which is non-specific on radiography or sonography) may develop, with eggs in the liver parenchyma inducing necrosis and abscess formation in humans (Miyazaki, 1991).

Only three cestodes found in the present study namely; *Taenia taeniaformis*, *Hymenolepis diminuta* and *Rodentolepis* (= *Hymenolepis*) *nana*. Both *Taenia taeniaformis* and *Hymenolepis diminuta* were found high in infection from all locations meanwhile, *Rodentolepis* (= *Hymenolepis*) *nana* was recovered with low prevalence in Malacca and Penang. Mohd Zain *et al.* (2012) also found these 3 cestodes species in wild rat from Kuala Lumpur. Paramavaran *et al.* (2009a) reported higher diversity up to six cestodes species recovered from urban rats, namely *Rodentolepis* (= *Hymenolepis*) *nana*, *Hymenolepis diminuta*, *Hymenolepis sabnema*, *Hymenolepis* sp., *Raillietina* sp. and *Taenia taeniaformis*. Other similar studies included, Dunn (1966), Yap *et al.* (1977), Sinniah (1979), Leong *et al.* (1979), Ambu *et al.* (1984), Paramasvaran *et al.* (2005), Mohd Zain (2006) and Syed-Arnez & Mohd Zain (2006) therefore, it can be concluded that these cestodes species were more commonly associated with commensal rats.

The life cycle of *Hymenolepis diminuta*, infection depended on the ingestion of the intermediate host such as fleas and cockroaches to complete its lifecycle. However, it is not always necessary require an intermediate host to complete the lifecycle. Sinniah *et al.* (1978) reported infections of both *Hymenolepis diminuta* and *Rodentolepis* (= *Hymenolepis*) *nana* from oil palm in estate workers indicating transmission of both parasites to humans. Khairul (1978) reported similar infection of a Malay man from a

fishing community in Teluk Bahang, Penang while Sandosham (1955) reported 1% of hospital patients in Singapore were infected with *Rodentolepis* (= *Hymenolepis*) *nana*. These infections were generally observed prevalent in conditions where high temperatures and sanitary conditions were poor (Miyazaki, 1991). It is estimated that more than 21 million people in the world suffer from hymenolepiasis and the majority were in the tropics and subtropics (Parija, 1990).

Taenia taeniaformis infections between the 3 rats species (*R. norvegicus*, *R. tiomanicus*, *R. rattus diardii*) was observed to be significantly high. Other similar studies worldwide included Grenada (Coomansingh *et al.*, 2009), Pakistan (Rafique *et al.*, 2009) and Mexico (Griselda *et al.*, 2005). The cestodes larvae, strobilocercus develops in or on the liver of infected cats however it utilizes rodents as an intermediate host. Infection in humans is rare and only occurs through accidental ingestion of eggs and liver cysts as reported in a child in Sri Lanka (Ekanayake *et al.*, 1999). Rat infections of *Hymenolepis diminuta* and *Rodentolepis* (= *Hymenolepis*) *nana* have been documented worldwide in Qatar (Abu-Madi *et al.*, 2001), South West Iran (Kia *et al.*, 2001) and Pakistan (Rafique *et al.*, 2009).

Overall, the intrinsic factor and host sex did not show any differences between the endoparasites species except for *Capillaria hepatica*, *Syphacia muris* and *Rodentolepis* (= *Hymenolepis*) *nana*. *Capillaria hepatica* and *Rodentolepis* (= *Hymenolepis*) *nana* infection were higher in females compared to males. This pattern could be due to females foraging further and the availability of intermediate hosts resulting in the increase of exposure to infection. The female host bias was observed previously (Wertheim, 1963). However, *Syphacia muris* infection was higher in males compared to females. Such large and widespread infection in males is thought to have been due to frequent, long- term exposure to contaminated food, water and surroundings.

There were no significant effects observed between host ages and the parasites species apart from *Hepatojarakus malayae*, *Taenia taeniaformis* and *Hymenolepis nana*. Infections were noted to be higher in juveniles compared to adults suggesting that juveniles were actively foraging, thus increasing their exposure to infection. However, the infection of *Taenia taeniaformis* was higher in adults compared to juveniles. This was likely to be linked with accumulation of parasites in adults through foraging without the host exhibiting any acquired immunity. Overall, the rats infected in this study were mostly adults and this phenomenon may be a possible explanation for high prevalence of *Nippostrongylus brasiliensis*. *Nippostrongylus brasiliensis* infection normally stimulates a T-cell mediated immune response that results in the expulsion of worms during a primary infection. However, this phenomenon failed to develop when the infection occurred in rats less than 6 weeks old and the worms persisted into adult life (Wakelin, 1996).

The diversity and prevalence for most of the endoparasite species were slightly higher during the dry season. This could be due to the environmental condition during the dry season was suitable for the survival and life cycle of the endoparasites. However, some of the endoparasites species showed high infection during wet season (*Angiostrongylus malaysiensis*, *Heterakis spumosa*, *Hepatojarakus malayae* and *Syphacia muris*). This seasonal pattern occurrence to some extent determined their annual population cycles (Mohd Zain *et al.*, 2012). A striking seasonal pattern with highest prevalence and abundance of helminth in *R. rattus* was recorded in the early autumn and winter periods on North Island of New Zealand, at a season that also coincided with the wetter part of the year (Charleston & Innes, 1980). However, since the climate in Malaysia is 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 rat population.

5.4 Conclusion

An overwhelming percentage of rats from the four locations in Peninsular Malaysia were heavily infected with endoparasites (92.3%). Endoparasite infections observed comprised entirely of cosmopolitan species, namely *Nippostrongylus brasiliensis*, *Angiostrongylus malaysiensis*, *Capillaria hepatica*, *Mastophorus muris*, *Heterakis spumosa*, *Hepatojarakus malayae*, *Syphacia muris*, *Taenia taeniaeformis*, *Hymenolepis diminuta* and *Rodentolepis* (= *Hymenolepis*) *nana*. *Nippostrongylus brasiliensis* was the most prevalent and abundant parasite found from all sites. The agriculture habitat in Carey Island showed the highest endoparasites diversity compared to the urban habitat with 8 endoparasites species recovered.

The rats from all the study locations were found to be infected with a wide range of endoparasites with zoonotic potential. Of the 10 different species of helminths identified 5 of them are zoonotic (*Capillaria hepatica*, *Angiostrongylus malaysiensis*, *Hymenolepis diminuta*, *Taenia taeniaeformis*, *Rodentolepis* (= *Hymenolepis*) *nana*). The close association of rats to human activities may facilitate the transmission of these zoonotic parasites to human. It is recommended that the present rat control measures to be reviewed by the relevant authorities and also to improve its rat-borne disease surveillance programmes.

Variation in intrinsic (host sex and host age) and extrinsic (season) factors played a significant role in determining the endoparasite infracommunity of several species in the wild rat populations in Peninsular Malaysia. The endoparasites fauna of the different sites were analogous with one another and provided a unique insight into the behaviour, diet and ecology of their hosts.

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APPENDICES

Appendix A

Simpson's Index

The statistic, C is given by:

$$C = \sum_i^{Sobs} P_i^2$$

where, $Sobs$ is the number of observations but is usually approximated as:

$$P_i^2 = \left(\frac{N_i}{N_T} \right)^2$$

where N_i is the number of individuals in the i th species and N_T the total individuals in the sample. The index is:

$$D = \frac{1}{C}$$

The larger its value the greater the diversity.

Appendix B

Brillouin Index

The Brillouin index, HB, is calculated using:

$$HB = \frac{\ln N! - \sum_{i=1}^s \ln n_i!}{N}$$

where N is the total number of individuals in the sample, n_i is the number of individuals belonging to the i th species and s the species number.

Appendix C

Published paper - Nur Syazana, M.T, Mohd Zain, S.N and Jeffery, J. (2013).

Biodiversity and macroparasitic distribution of the wild rat population of Carey Island, Klang. *Tropical Biomedicine*, 30(2), 199-210