DETECTION OF BLOOD PROTOZOANS IN THE URBAN RAT POPULATIONS OF PENINSULAR MALAYSIA USING QUANTITATIVE BUFFY COAT AND GIEMSA-STAINED THIN FILM TECHNIQUES

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ABSTRACT

A total of 762 urban rats were trapped from four urban cities in Peninsular Malaysia namely Kuala Lumpur, Penang, Pahang and Malacca comprising of six urban rat species: *Rattus rattus diardii* (n=410), *Rattus norvegicus* (n=302), *Rattus exulans* (n=33), *Rattus tiomanicus* (n=8), *Rattus argentiventer* (n=7) and *Rattus annandalei* (n=2). It shows that *Rattus rattus diardii* was the highest amount to be caught which made up 53.8% of the total rats caught. The second highest to be trapped is *Rattus norvegicus*, making up 39.6% of the total rats caught. Followed by *Rattus exulans* (4.3%), *Rattus tiomanicus* (1.1%), *Rattus argentiventer* and *Rattus annandalei* with 0.9% and 0.3% respectively. Females were slightly higher at 52.8% and males at 47.2%. Adults captured were more with 502 rats (65.9%) compared to 152 sub adult rats (20.0%) and 108 juvenile rats (14.2%). Three hundred and thirty one rats were captured during dry season compared to 431 rats during wet season. Examination blood of rat from four localities of Peninsular Malaysia; Kuala Lumpur, Malacca, Pahang and Penang using Giemsa-stained Thin Film (GTF) technique and Quantitative Buffy Coat (QBC) technique resulted in identification of two blood protozoans *(Trypanosoma* sp. and *Plasmodium* sp.). Using Giemsa-stained Thin Film (GTF), a total of 456 rats (59.8%) were infected with at least one blood protozoan species from two species discovered namely; *Trypanosoma* sp. and *Plasmodium* sp. with prevalence of *Plasmodium* sp. (72.4%) higher compared to *Trypanosoma* sp. (42.1%). Only 14.5% (n =66) were found infected with both species. *Rattus rattus diardii* showed the highest prevalence of infection (62.2%), with 75.7% rats infected with *Plasmodium* sp., 38.8% infected with *Trypanosoma* sp. and 14.5% infected with both species. Using Quantitative Buffy Coat (QBC) technique, a total of 202 rats (46.8%) were infected with at least one blood protozoan species from two species discovered namely; *Trypanosoma* sp. and *Plasmodium* sp. with prevalence of *Plasmodium* sp. (61.4%)

higher compared to *Typanosoma* sp. (44.6%). Only 5.9% (n =12) were found infected with both species. *Rattus norvegicus* showed the highest prevalence of infection (46.6%), with 63.1% rats infected with *Plasmodium* sp., 44.3% infected with *Trypanosoma* sp. and 7.4% infected with both species. In this study also have the comparison between the quantitative buffy coat (QBC) technique and conventional Giemsa thin blood smear. A total of 432 blood samples from four rat species *(Rattus norvegicus*, *Rattus rattus diardii, Rattus exulans* and *Rattus argentiventer)* were screened using both techniques and successfully detected two blood protozoan species (*Trypanosoma* sp. and *Plasmodium* sp.) with *Trypanosoma* sp. predominantly infecting the population. Results showed that Giemsa-stained thin film (GTF) was the better detection method on blood parasitemia (46.7%) compared to Quantitative Buffy Coat (QBC) (38.9%) with overall detection technique sensitivity and specificity at 83.2% and 74.8% respectively. The sensitivity in detection of *Trypanosoma* sp. was 84.4% with value slightly lower for *Plasmodium* sp. infections at 76.6%. Statistical analysis proved that GTF technique was significantly more sensitive in the detection of blood protozoan infections in the rodent population compared to QBC ($p<0.05$).

ABSTRAK

Sebanyak 762 ekor tikus bandar ditangkap dari empat bandar besar di seluruh Semenanjung Malaysia iaitu Kuala Lumpur, Pulau Pinang, Melaka dan Pahang, merangkumi enam spesis tikus bandar: *Rattus rattus diardii* (n=410), *Rattus norvegicus* (n=302), *Rattus exulans* (n=33), *Rattus tiomanicus* (n=8), *Rattus argentiventer* (n=7) dan *Rattus annandalei* (n=2). Ini menunjukkan bahawa *Rattus rattus diardii* adalah spesis yang paling tinggi ditangkap iaitu sebanyak 53.8% berbanding jumlah tikus yang lain. Spesis tikus kedua tertinggi adalah *Rattus norvegicus,* 39.6% dari jumlah tangkapan. Diikuti dengan *Rattus exulans* (4.3%), *Rattus tiomanicus* (1.1%), *Rattus argentiventer* dan *Rattus annandalei* dengan masing-masing adalah 0.9% dan 0.3%. Tikus betina lebih tinggi peratusannya, 52.8% berbanding tikus jantan 47.2%. Tikus dewasa yang ditangkap adalah lebih banyak bilangannya dengan 502 ekor (65.9%) berbanding 152 ekor tikus separa dewasa (20.0%) dan 108 ekor tikus juvenile (14.2%). Tiga ratus tiga puluh satu ekor tikus telah ditangkap pada musim panas berbanding 431 ekor tikus ditangkap pada musim lembap. Pemeriksaan darah tikus dari empat kawasan di Semenangjung Malaysia iaitu Kuala Lumpur, Melaka, Pahang dan Pulau Pinang telah dijalankan bagi mengenalpasti dua jenis protozoan darah (*Trypanosoma* sp. dan *Plasmodium* sp.*)* dengan menggunakan teknik Giemsa-stained Thin Film (GTF) dan teknik Quantitative Buffy Coat (QBC). Dengan menggunakan teknik Giemsa-stained Thin Film (GTF), sejumlah 456 ekor tikus (59.8%) telah dijangkiti oleh sekurang-kurangnya satu spesis protozoan darah daripada dua spesis yang dikaji iaitu *Trypanosoma* sp. dan *Plasmodium* sp. dengan kadar kekerapannya ialah *Plasmodium* sp. (72.4%) lebih tinggi berbanding *Trypanosoma* sp. (42.1%). Hanya 14.5% (n=66) dijangkiti oleh kedua-dua spesis. *Rattus rattus diardii* menunjukkan kekerapan paling tinggi dijangkiti (62.2%), dengan 75.7% daripadanya dijangkiti dengan *Plasmodium*

sp., 38.8% dijangkiti oleh *Trypanosoma* sp. dan 14.5% dijangkiti oleh keda-duanya. Dengan menggunakan teknik Quantitative Buffy Coat (QBC), sebanyak 202 ekor tikus (46.8%) telah dijangkiti oleh sekurang-kurangnya satu jenis protozoan darah yang dikaji iaitu *Plasmodium* sp. dan *Trypanosoma* sp. dengan kekerapan bagi *Plasmodium* sp. (61.4%) lebih tinggi daripada *Trypanosoma* sp. (44.6%). Hanya 5.9% (n=12) yang dijangkiti oleh kedua-dua spesis. *Rattus norvegicus* menunjukkan kekerapan paling tinggi dijangkiti (46.6%), dengan 63.1% dijangkiti oleh *Plasmodium* sp., 44.3% dijangkiti oleh *Trypanosoma* sp. dan 7.4% dijangkiti oleh kedua-dua spesis. Dalam kajian ini juga, perbandingan antara teknik Quantitative Buffy Coat (QBC) dan teknik calitan darah nipis Giemsa yang konvensional. Sejumlah 432 sampel darah dari empat spesis tikus (*Rattus norvegicus, Rattus rattus diardii, Rattus exulans* dan *Rattus argentiventer*) dikesan dengan menggunakan kedua-dua teknik dan berjaya mengenalpasti dua spesis protozoan darah (*Plasmodium* sp. dan *Trypanosoma* sp.) dengan *Trypanosoma* sp. mendominasi dalam mengjangkiti populasi. Keputusan menunjukkan Giemsa-stained Thin Film (GTF) adalah kaedah pengesan lebih baik bagi parasitaemia darah (46.7%) berbanding Quantitative Buffy Coat (QBC) (38.9%) dengan kepekaan dan ketepatan bagi keseluruhan teknik pengesan masing-masingnya adalah 83.2% dan 74.8%. kepekaan dalam mengenalpasti *Trypanosoma* sp. adalah 84.4% dengan kadar rendah bagi jangkitan *Plasmodium* sp. pada 76.6%. Analisa statistik membuktikan secara signifikan teknik GTF adalah lebih peka dalam menentukan jangkitan protozoan darah pada populasi tikus berbanding teknik QBC ($p<0.05$).

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LIST OF SYMBOLS AND ABBREVIATIONS

n	:	sample size
etc	:	et cetera (and other things)
i,e.	:	id est (that is)
e.g.	:	gratia (for example)
km	:	kilometer
p	:	probability
QBC	:	Quantitative Buffy Coat
GTF	:	Giemsa Thin Film
PPC	:	Positive Prediction Value
NPC	:	Negative Prediction Value
%	:	percentage
µl	:	microliter
mm	:	millimeter
cm	:	centimeter
g	:	gram
min	:	minute
ml	:	milliliter
EDTA	:	Ethylenediaminetetraacetic Acid
DNA	:	Deoxyribonucleic Acid
RNA	:	Ribonucleic Acid
df	:	degree of freedom

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CHAPTER 1
INTRODUCTION

1.1 GENERAL INTRODUCTION

Rodents are mammals of the order Rodentia, with rats and mice belong to the suborder Myomorpha. Members of the Muridae family are the dominant in every region worldwide, due to their ability to adapt and exploit a wide variety of habitats and environments. Rodents particularly those belonging to the family Muridae form the largest group of mammals in Malaysia (Ow-Yang, 1971). Commensal rats and mice are rodents that live in close proximity to humans by invading our dwellings and eating human waste. There are more than 1700 species of rodents identified in the world (RatZooMan, 2006). Three species of commensal rodents are known widely distributed namely, the Norway rat, *Rattus norvegicus*; the roof rat, *Rattus rattus*; and the common mouse, *Mus musculus*.

Rodents account for about forty percent of the mammals living at the present time, and constitute more than 42% of the known mammalian species (Singleton, 2003). In many regions, however, the total number of individual rodents is probably many times greater than that of all other mammals. Nearly four million rats are born every day in the developing nations and are known to spread more than 60 diseases to humans worldwide (Hinds, 2003). Three commensal rodent species are established in most parts of the world and are spreading the risk of disease, such as plague.

People have largely categorized rodents as pest in urban and agricultural environments, despite most rodent species live in the wild with little interaction with humans. A small minority of rodent species have adapted to living in close association with humans, by using our agriculture and waste as their own food resource and

utilizing spaces in buildings as their homes. The close proximity of these rodents to human habitation means that they can spread and transmit diseases quickly. The ability of rodents to carry and transmit vector-borne diseases is facilitated by their habit which contains waste and sewage, as well as their mobility and similarity in physiology to humans. Rodents are able to vector more than 60 known diseases, and the list grows as more research on zoonosis continues (Khatoon *et al*., 2004).

There are numerous rodent-borne diseases and only the principal ones are summarized in Table 1.1. These diseases are of great social and public health importance however; their socio-economic importance has not been properly evaluated.

Table 1.1: Principal of rodent-borne diseases

Agent	Disease
Virus, Bunyaviridae	Hantavirus Pulmonary Syndrome
	Hemorrhagic Fever with renal syndrome (+ other hemorrhagic fevers)
	Nephropathia epidemica
	Crimean-Congo hemorrhagic fever
	Borna disease
Virus, Flaviviridae	Omsk hemorrhagic fever
	Kyasanur Forest Disease
	Apoi Virus Disease
	Tick-borne encephalitis
	Powassan encephalitis
Virus, Arenaviridae	Lymphocytic
	Choriomeningitis virus (LCMV)
	Lassa fever
	South American arenaviruses (Junin, Mapucho etc.)

	North American arenaviruses
Virus, Reoviridae	Colorado Tick Fever
Virus, Togaviridae	Venezuelan equine encephalitis
	Western equine encephalitis
Virus, Caliciviridae	Hepatitis E
Virus, Poxviridae	Cowpox
Virus, Picornaviridae	Contigious viral animal diseases (classical Swine
Fever, (FMD); Flaviviridae (CSF)	Foot and Mouth Diseases)
Bacteria, Spirochaetes	Leptospirosis (Weils' disease)
	Lyme disease
	Tick-borne relapsing fever
Bacteria,	Scrub typhus
Alphaproteobacteria	Murine typhus
	Sylvatic epidemic typhus
	Queensland tick typhus or spotted fever
	Rocky Mountain spotted fever
	Rickettsialpox
	Bartonella Illnesses
	Human granulocytic anaplasmosis
Bacteria,	Q-fever
Gammaproteobacteria	Salmonellosis
	Tularemia
	E.coli 0157/VTEC
	Plague (*Yersina pestis*)
Bacteria,	
Epsilonproteobacteria	Campylobacteriosis

Bacteria, Fusobacteria	Rat-bite fever and Haverhill fever
Bacteria, Bacilli	Listeriosis
Parasite, Sporozoea	Toxoplasmosis
	Babesiosis
	Crytosporidiosis
	Malaria
Parasite, Zoomastigophorea	Chagas diseases
	Leishmaniasis
	Giardiasis
Parasite, Cestoda	Taeniasis
	Rodentolepiasis
	Echinococcosis
Parasite, Trematoda	Schistosomiasis
	Human fasciolosis
	Branchylaimiasis
	Alariasis
	Echinostomiasis
Parasite, Nematoda	Trichinosis
	Capillariasis
	Angiostrongylosis
	Toxascariasis
	Baylisascariasis
	Aelurostrongylosis
Parasite, Lobosea	Amoebic dysentery
Parasite, Conoidasida	Neosporosis

In the recent years, rapid urban development and intensive agriculture is on the rise to meet the needs of the growing populations. However, many rodent species have remained more or less undisturbed in the desert and mountainous regions, but as the population expands into the rodent landscape, these animals move out into neighboring areas, and temporarily damaging crops and spreading disease. These rodents are particularly notorious for damaging growing crops like rice, sugar cane, and cocoa. The feeding habits of rodents have impacted on human economy and health worldwide (Huq *et al*., 1985). In Asia alone the amount of grain eaten by rodents in the rice fields each year would provide enough to feed 200 million Asians for a year (Singleton *et al*., 2003).

Human activities affecting ecological conditions play a significant role in the epidemiology of parasitic zoonoses. The World Health Organization defines Zoonoses (Zoonosis, singular.) as "Those diseases and infection which are naturally transmitted between vertebrate animals and man". Zoonotic disease give the impacts such as illness, monetary loss, adverse effect on morale of personnel, unfavorable publicity, medico legal implications and man-hours lost.

There is a rising concern that rodents may pose health risk to humans because of the increased movement of people between rural and urban areas and between countries. The increased clearance of the natural habitats for development further promotes rodent-human contact thus providing an ideal situation for disease transmission. Man-made activities such as establishment of land development schemes, reservoir development projects, dam constructions and development of industries are carried out on ecosystems where pathogens form a part of the biotic community and there tend to disturb the natural foci and promote new foci of the diseases (Ambu, 1996). These animals are known to transmit diseases and act as reservoir host for many zoonotic

pathogens including parasites that pose a health risk to humans (Walsh *et al*., 1993; Meyer *et al*., 1995; Singleton *et al*., 2003).

It is therefore not surprising that rodents play a major role in harboring and transmitting many diseases to other mammals, including man. In the transmission of human disease, the extent to which some rodents have become adapted to man-made environments is at least as important as the number of rodents.

Many of these rodent species are also reservoirs for pathogens that can cause debilitating diseases to human and livestock (Singleton *et al*., 2003). Rodent-borne diseases spread directly to human through bite wounds, food consumption and drinking water contaminated with rodent faeces and urine or through breathing in germs that may be present in rodent urine or droppings stirred into the air (a process known as "aerosolization"). Diseases from rodents also spread indirectly to humans via vector hosts such as ticks, mites and fleas that transmit the infection to human after feeding on infected rodents (Nadchatram *et al*. 1966; Salleh *et al*., 2003).

The impact of these diseases on human livelihoods, in both urban and agricultural communities, is poorly documented. Little is known about rodent species that are major reservoirs, life of infective life stages of bacteria, viruses, spirochaetes and helminths persisting in domestic and rural environments and how diseases are transmitted under 'natural' conditions, Also not known are prevalence of diseases in both the rodent and human populations, and the basic human epidemiology of these diseases (incidence of infection, morbidity rates, transmission rates, age and sex-related effects, effects of socioeconomic status).

Parasite infections are likely to cause some types of physical trauma to the host due to their migration within the body. Tissue destruction, tissue displacement, ulceration of the liver, and intestinal wall damage are all possible results of parasitic infection. Once

in the body, the parasites generally leach nutrients such as vitamins and essential minerals leading to nutrient loss resulting in severe vitamin deficiencies, anemia, and weight loss. Parasites also excrete toxins in the body that may lead to a number of symptoms such as swelling, heart problems, digestive disorders, blurred vision, joint pain, and sleep problems.

Hundreds of parasite species are known to infect humans. Some of these harmful organisms may live in the human body for months or even years cause no noticeable symptoms. Parasites can continue to multiply in the body, ultimately causing symptoms that mimic conditions such as diabetes, irritable bowel syndrome, heart disease and even cancer.

Parasites are generally allocated into three main categories; ectoparasites, endoparasites and blood parasites. Parasitic protozoan and helminthic infections can be diagnosed from various type of specimen depending on the sites of infection in the host. Blood parasites are usually detected in the blood, intestinal parasites in the faeces and tissue-inhabiting parasites in aspirates, biopsy specimens, sputum or urine.

Numerous laboratory captive-animal-based investigations of vector-borne pathogens (des Vignes *et al*., 2001), are used to parameterize models of disease ecology (Tompkins *et al*., 2002). However, additional factors such as socially determined behavioral interactions, differential contact rates with ectoparasites and condition-dependent susceptibility and expression of virulence are likely to influence disease dynamic within the natural population (Brown *et al*., 1999, 2003; Krasnov & Khokhlova, 2001).

Yap *et al.* (1977) investigated in small mammal populations in the Jenderak Utara FELDA Schemes, Pahang, to determine protozoan and helminth parasites of medical

health importance and concluded land development schemes not only disturb natural foci but also create new foci of the disease.

Commensal rodent species living in close association with human are potential source of parasitic transmission. Parasites transmit from one animal to human to the next via several pathways. The most common method of infection is via consumption of contaminated food and water with one of the infective stage or egg.

The house rat, *Rattus rattus diardii* (Jentink) is an urban pest and is a known reservoir host for various parasitic infections due to its close association to humans (Hira, 1975; Sinniah *et al*., 1978). This dominant rodent specie in urban cities is reported to inhabit residential homes, outbuildings, stores in Malaysia, Singapore and Indonesia (Harrison, 1952; Holz & Liem, 1965) although their habitat ranges from fields near human habitation (Lim, 1974). These furtive synanthrops play havoc in human societies by transmitting diseases (Otto and Otto, 1983; Hobson and Collier, 1984) by harbouring a number of zoonotic important ecto-and endo- parasites (El-Safi & Peters, 1991; Velez *et al*., 1995; Webster & Macdonald, 1995a; Yaghoobi & Javadian, 1996; Yasuraoka *et al*., 1996). Many parasitic diseases surveys have been carried out in Malaysia (Sandosham, 1955; Lie, 1964; 1971; Bisseru & Aziz, 1970; Dunn, 1972; Lee & Danaraj, 1972; Dissanaike *et al*., 1978) particularly parasitic infections in rats (Harrison, 1957; Sandosham, 1957; Schacher & Cheong, 1960; Balasingam, 1963; Lim *et al*, 1965, 1977; Gatha, 1966; Singh & Chee-Hock, 1971; Betterton & Lim, 1975), however limited studies detailed the distribution and prevalence of diseases. Endoparasite infections in commensal and forest rodents have been well documented in Malaysia (Mulkit & Cheong, 1971; Ow-Yang, 1971; Lim *et al*., 1976; Sinniah *et al*., 1978; Krishnasamy *et al*., 1980; Yap *et al*., 1977; Leong *et al*., 1979) with protozoan, nematode and cestode infections such as *Toxoplasma gondii,*

Eimeria muris, Spironuclues muris, Giardia muris, Cryptosporodium sp., *Encephalaprotitozoan cuniculi, Hepatozoan muris,* and *Babesia muris* in human (Nama & Parihar, 1976; Soulsby, 1982; Claveria *et al*., 2005). Gatha (1966) and Mulkit Singh and Cheong (1971) studied the incidence of nematode infections in various species of feral rats in Malaysia. While, Ow-Yang (1971), Lim and Muul (1970) and Lim *et al*, (1976) reported on nematode parasites of Malaysian rodents in oil-palm plantations and other habitats. Schacher and Cheong (1960) reported on the nematode fauna from rats in Kuala Lumpur and Singapore, and Dunn *et al*, (1968) included this species in a report on the major groups of parasites of rats in Malaysia. To date, no case of plague was ever reported in Malaysia in the past 54 years and no reported case of yellow fever has ever been recorded.

Leptospirosis is a rodent borne emerging disease among the rural communities in developing countries in Asia. The common flu-like symptom is usually often misleading until serious clinical damage occurs. If left untreated, patients can develop kidney damage, meningitis, liver failure and respiratory distress. This disease is associated to occupational hazard and appropriate precautions are rarely taken due of lack of knowledge of transmission particularly among those involved in agriculture and town workers.

Other diseases such as, filariasis is widespread in the coastal rice-field and riverine swamp forest regions while the actual endemicity of Japanese encephalitis and thyphus is not known.

Many researches were conducted on helminthic infections but very few report on the blood parasites in rodents in this country. Since ten years ago, only one such research was reported in Malaysia (Paramaswaran, S. *et al*., 2003). There are several studies on the clinical, pathologic and hematologic alterations caused by hemoparasites in

domestic animals, its diagnosis and transmission (Madruga *et al*., 2002). Blood parasites, such as *Babesia* sp. and *Hemobartonella* sp. can cause death to domestic and wild animals however rarely to humans via biological vectors and also reservoirs of blood parasites such as ticks or fleas (Daszak *et al.*, 2000; Bossi *et al*., 2002; Moreas *et al*., 2003; Rios *et al*., 2003; Quintao-Silva & Ribeiro, 2003).

Babesia sp. is an intraerythrocytic protozoan infecting a variety of domestic and wild mammals (Homer *et al*., 2000) that causes a tick borne infection known as babesiosis. A number of species infect humans however babesiosis in Asia is said to be sporadic (Li & Meng, 1984; Shih, 1997; Saito *et al*, 2000).

In Malaysia malaria and dengue/dengue haemorrhagic fevers is considered as the most important vector-borne diseases in terms of morbidity and mortality affecting both the rural and urban populations respectively. Recently extensive work on simian malaria was also carried out in Malaysia when natural infection of *Plasmodium knowlesi* was reported in a man and experimentally with *Plasmodium inui* (Wharton & Eyles, 1961; Wharton *et al*., 1962; Eyles *et al*., 1963; Cheong *et al*., 1965; Coatney, 1971). It is probable that more cases of human malaria of simian origin may occur as man encroach further deep into the jungle for development.

Malaria is highly endemic in the east Malaysian State of Sabah and in Pahang, Kelantan and Perak in Peninsular Malaysia. In 1993 about 73% of a total of 39,890 cases were reported from Sabah (VBDCP, 1993) and was associated to suspected widespread use of chloroquine-resistant falciparum malaria, the refractory habit of *Anopheles balabacensis* vector and the vast and difficult terrain where malaria is rampant. In Pahang, Kelantan and Perak high proportion of the cases occurred among the indigenous Orang Asli population who live the nomadic life and where the application of the intradomicinary residual spraying failed due to the nature of houses.

The use of larvicides such as temephos for vector control however is limited to the sub urban areas for the purpose of preventing reintroduction of malaria to areas that are under control.

Trypanosomes are unicellular organism (Phylum Protozoa) belonging to the order Kinetoplastida, family Trypanosomatidae and genus Trypanosoma. These organisms are blood tissue parasites known as haemoflagellates. The subgenus Trypanozoon and brucei group comprise of five members of which one is *Trypanosoma evansi* causing 'surra'. The parasites can be transmitted via a mechanical vector to a healthy host by the rat louse, *Polyplax spinulosa* (Khachoian & Arakelian, 1978).

Toxoplasma gondii is an intracellular and obligate coccidian parasite transmitted within and between different host species by many portals (Dubey & Beattie, 1988). Common species of commensal rats have been reported as potential chronic vectors of *Toxoplasma gondii* infection to cats and other livestock animals (Webster, 1994; Dubey and Frenkel, 1998), and humans (Hove & Dubey, 1999; Tenter *et al*., 2000; Jones *et al*., 2004).

Trypanosomiasis due to *Trypanosom evansi*, a blood protozoan, infects a variety of hosts including camels, horses, cattle, buffaloes, pigs and dogs. The parasite is transmitted mechanically by biting flies in North predominantly in Africa, the near and Far East, Central and South America, and Mauritius. *Trypanosoma evansi* has the wide geographical distribution in South America, Africa and Asia particularly in Southeast Asia (Luckins, 1988) and plays an important role on the productivity of small farm holders in livestock (Reid, 2002).

The disease known as Surra is characterized by fever, blood and anaemia. The affected animal loses body conditions and if not treated may die. The disease is fatal in

horses and pigs. Currently, surra is on the list of notifiable diseases (list B disease) of the OIE (World Organization for Animal Health) (Sarah *et al*., 2006).

Trypanosoma evansi is normally transmitted by mechanical transmission involving the bloodsucking Diptera, particularly members of the Tabanidae and Stomoxys (Lorne, 1986). Additionally, hematophagous bat plays a role as a natural host and vector in Latin America (Heitor *et al*., 2005). The most important symptom of the infection in cattle is abortion, which occurs suddenly without any clinical signs in the late stage of pregnancy (Loehr, 1986; Lun *et al*., 1993).

The damage and losses caused by rodent pests are substantial. In meeting the increasing demands as a result of rapid population growth, it is essential to minimize the damage and losses due to rodent pests. Most researches on rodent pests are centered on control method such as biological control (use of micro-organisms, parasites, predators etc.). It is evident a great deal of issues relating to rodent control and management are still unresolved. Thus calls for greater training and research input in various fields of rodent biology (systematic, ecology, physiology, behavior, genetics, reproductive biology, etc.) to enable an effective and economically sound rodent control and management program can be formulated.

These studies are need urgently if we are to be able to provide recommendations on practices for rat management and general public health to reduce the transmission and impact of rodent zoonoses. The effectiveness of DDT spraying, the bio-efficacy of biological agents, *Bacillus thuringiensis* H-14 on vector mosquitoes including *Anopheles* sp. (Foo & Yap, 1982) are some examples of applied research that were successfully carried out. Currently the use of impregnated bed nets (VBDCP 1993) is being implemented for malaria vector control by the Ministry of Health, Malaysia as this solution is considered a cheap and environment and user friendly.

At present, therefore, effort should be concentrated on the collection of epidemiological information and identification and mapping of the vector habitat. More detailed studies should be initiated to determine the parasitofauna of rats; the information obtained could be used also to ascertain the possible routes of dispersal of house rats and its zoonotic potential to human health in Malaysia (Leong *et al*., 1979). Various control strategies were applied for the control of vector-borne diseases in Malaysia.

1.2 RODENTS

Rodent to a zoologist, refers to the whole group of mammals belonging to the order Rodentia (i.e. rats, squirrels, porcupines, etc.) but to the pest control agent, it means simply rats and mice only while to the epidemiologist, rodents are of public health importance due to their host reservoir nature to many diseases.

This group of mammals is one of the more important vertebrate pests. "Pest" is derived from the Latin for plague, and any destruction and damage to property and food crops, including illness and not strictly referred to in an agricultural sense. For example, the destruction of property and loss of crop due to rodents directly affect man's environment and deprives him of certain benefits such as his income and health related issues. As such, economic loss and public health implication are two undesirable effects that can be traced to rodents.

The economic loss and health hazards posed by rodents if left unchecked can be astronomical. World Health Organization (WHO) reported that if one rat was left free to roam in a warehouse for a year, it consumes 27 pounds of food and approximately deposit 25,000 droppings spoiling more food. Worldwide, rats destroy more than 33 million tons of grains each year that can feed the population of India for the same period.

The most hazardous to man are the commensal species i.e. the house and field rats. "Commensal" refers to rodent populations living in close contact with man in his cities, or villages, or his farms. Commensal rodents are so adaptable to human environment and consume anything that humans eat (and a great deal more), and survive in any habitat from scorching desert to the freezing arctic. These mammals climb, swim and propagate with agility. The common urban species responsible for causing extensive damages are the Norway rat (*Rattus norvegicus*), Roof rat (*Rattus rattus*), and House mouse (*Mus musculus*). These species are cosmopolitan in distribution and are the most destructive pest in cities an around human habitations throughout the world.

The field rodents, on the other hand cause irreparable damage to food crops in the field. The losses inflicted can be colossal if left unchecked. In a recent survey in Thailand, it is estimated that there is an annual loss of 20% in coconut plantations, 40% sugar cane, and in some years more than 70% of rice crops. In Indonesia, rice crops loss was estimated at 15 to 20% annually, however in some areas more that 90% of rice crops are destroyed. In the Philippines, a conservative figure showed a 10% loss of the total annual crops, and in the Republic of Korea, grain losses due to field rodents was estimated at 20% of the country's production. The field rodents consist of more diversified speciation that vary as per geographical region. In Malaysia the field rodents are the Polynesian rat (*Rattus exulans*), the Ricefield rat (*Rattus argentiventer*), the wood rat (*Rattus tiomanicus*), The Greater Bandicoot (*Bandicota indica*) and The Lesser Bandicoot (*Bandicota bengalensis*).

Diseases of animals or zoonoses (from the Greek zoion-animal, nosos-diseases) are diseases only of animals and do not occur in man. However, zoonoses have the ability to pass from animals to man either directly or through a vector, for example scrub and murine typhus is referred to as being transmutable. Such transmissible diseases are

called zooanthroponoses or anthropozoonoses i.e. diseases common to animals and man, regardless how man has acquired them.

Diseases that are transmissible to man by rodents are common. The most dreaded and still potentially dangerous disease transmissible to man by rats or their arthropod ectoparasites is the plague (*Yersinia* (*Pasteurella*) *pestis*), which is spread by the Oriental rat flea (*Xenopsylla cheopis*) where it was estimated to have kill one in three Europeans in the fourteenth century. In subsequent centuries, the disease swept through India, West Asia and China arriving in Hong Kong in 1894. The disease eventually spread to Southeast Asia and today is still endemic in certain parts of Vietnam, Burma and Indonesia.

1.2.1 Urban rat species

1.2.1.1 *Rattus norvegicus* (Berkenhout, 1769)

Figure 1.1: *Rattus norvegicus* (Source: http://www.vebiexport.com)

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vertebrata

Class: Mammalia

Order: Rodentia

Family: Muridae

Subfamily: Murinae

Genus: *Rattus*

Species: *norvegicus*

Distribution: Originally a native of northern Eurasia, this rat specie spread to cities around the world due to commerce. In Southeast Asia this rat is only found indoors, however, in some countries and Pacific islands, it also occupies outdoor habitats.

Description: There are two known colour phases, the black and the brown forms. In some countries, the black form can be easily mistaken for the Greater Bandicoot (*Bandicota indice*) which is also black uniformly; the former is distinguishable from the latter by the skull ridges, and a more convenient character is the width of the incisor teeth. In the Norway rat, the width is not more than 3.5mm as compared to more than 4mm in the Greater Bandicoot.

The brown phase is a greyish brown fur on the dorsal, grey ventral, often with a white chest patch. The feet are white and the underside of the tail is paler than above, but not as sharply demarcated. The Norway rat has short ears and tail.

Size: It is a large rat. The length of adult is as follows: Head & Body length: 160-240mm; Tail length: 70-100% of Head and body length; Hind Feet length: 38-45mm; Ear length: 20-23mm. Body weight: 200-280grams. Number of mammary glands: 3+3=12.

Ecology and Behavior: It usually occupies the ground floor of buildings. In Southeast Asia, it is found mainly near seaport cities and has acquired the habit of scavenging in garbage dumps, refuse and in sewer systems. Warehouses are the favorite haunts.

In tropical countries this rat breeds all the year round. The lactating period is between 21-24 days. The number of embryos per litter varies in reports is about 8.50 $\pm$1.98 in Indonesia (Lim, 1970), 8.3$\pm$2.6, in Rangoon (Walton *et al*., 1977) and in Malaysia the range was 2.8 (Harrison, 1962).

1.2.1.2 *Rattus rattus* (Linnaeus)

Figure 1.2: *Rattus rattus* (Source: http://www.planet-mammiferes.org)

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vertebrata

Class: Mammalia

Order: Rodentia

Family: Muridae

Subfamily: Murinae

Genus: *Rattus*

Species: *rattus*

Distribution: This species is native to Asia but has a global distribution. It is by far the most widely distributed species in Southeast Asia and Asian countries. Due to its geographic variations, this rat is known by many different subspecies throughout the region.

Description: This rat is also known as a Roof rat. It is a slender built, graceful, sleek, medium-sized rat with pointed snout, large ears, and a tail as long as or usually longer than the head and body length. The fur coloration has several forms. The black form

(*Rattus rattus*), a European type house rat has been occasionally found in seaports of Asian cities. The brown phase with either a white-belly or grey-belly or yellowish-brown-belly, is the most predominant type in Southeast Asia. It is distinguished from the Norway rat by the smaller size, longer tail and ear, and a smaller brownish foot.

Size: The measurements are: Head and body length: 120-180mm; Tail length: 105-130% of Head and body length; Hind feet length: 30-38mm; Ear length: 20-27mm. Body weight: 140-180grams. Number of mammary glands: 2+3=10.

Ecology and behavior: The Southeast Asia species is more confined to the indoor of human habitations while in other Asian countries, the outdoors types are generally found. The indoor types inhabit a wide variety of human structure, and similar to the Norway rat are pests of warehouse. These rats scavenge in rubbish dumps of nearby houses while the outdoor types are found in the scrub and field habitations and are semi-arboreal.

The subspecies, *Rattus rattus diardii* or the common house rat is widely distributed near human habitation in urban, suburban and rural areas of both Malaysia and Indonesia. It has no ability to burrow and therefore live in all kinds of human structures such as warehouses in the cities. In the natural habitat, this mammal builds its nest in holes of other animals and breeds about four times a year with six to eight embryos per litter (Harrison, 1962).

1.2.1.3 *Rattus exulans* (Peale, 1848)

Figure 1.3: *Rattus exulans* (Source: http://www.planet-mammiferes.org)

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vertebrata

Class: Mammalia

Order: Rodentia

Family: Muridae

Subfamily: Murinae

Genus: *Rattus*

Species: *exulans*

Distribution: Distribution from Burma, Thailand to the Malay Archipelago and east to the Pacific islands. The mainland indoor population, *Rattus exulans* is of a duller coloration and smaller in size than the *Rattus exulans* from Fiji and Microneasia, which never enters houses.

Description: The Polynesian rat, is believed to have originated in Southeast Asia. The colour of the pelage is variable with olivaceous fur on the dorsal, grey ventrally,

brownish feet with a unicolor tail. The dorsal fur is short and smooth and spiny in some individuals.

Size: It is a small rat larger in size than *Mus musculus*. It measures Head and body length: 90-140mm; Tail length: 85-120% of Head and body length; Hind feet length: 20-26mm. Body weight: 40-80grams.

Ecology and behavior: In Malaysia, this species inhabits a diverse range of habitats from dwelling in urban and rural areas to gardens, grasslands, wasteland, coconut plantation, ricefields and forest-edges (Lim *et al*., 1977: Medway, 1983). In most of the Philippine islands, it can be found from lowlands to mountainous terrain (Barbehenn *et al*., 1973). It is a pest on stored food and household effects, but is also in ricefields in Malaysia and Indonesia (Lim, 1974). In the Pacific islands it is one of the major pests in coconut plantations (Barbehenn *et al*., 1971).

Indoors this species nests in a variety of human structures, preferably in storerooms and kitchen and mostly terrestrial in habit although climbs very well. While outdoors, it nests in piles of rubbish and occupies burrows of other rats in ricefields. It nests in the opened nuts at the bottom of the pile of discarded husks and also inside rotting coconut stumps in coconut plantations. It is nervous in nature, but in captive tame easily. It is active both during the day and night in ricefields. The female breeds approximately four to six times in a year with four to six embryos per litter.

1.2.1.4 *Rattus argentiventer* (Robinson & Klose, 1916)

Figure 1.4: *Rattus argentiventer* (Source: http://www.planet-mammiferes.org)

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vertebrata

Class: Mammalia

Order: Rodentia

Family: Muridae

Subfamily: Murinae

Genus: *Rattus*

Species: *argentiventer*

Distribution: This species is widely distributed throughout the whole of Southeast Asia but is more confined to ricefields in Malaysia.

Description: It is distinguishable from other field species by a relatively shorter tail, about 95% of the head and body length. In young specimens the tail length may be about 105%. It appearance is similar to *Rattus tiomanicus*, differing by the roughage of

the fur dorsally, the silvery coat under belly and large size feet, lighter in colour than the former.

Size: The measurements are Head and body length: 140-210mm; Tail length: 95-105% of Head and body length; Hind feet length: 34-36mm, Ear length: 19-23mm. Body weight: 90-180grams. Number of mammary glands: 3+3=12.

Ecology and behavior: *Rattus argentiventer* is essentially a ricefield rat or can be found in the lalang fringes close to oil palm plantation and also occasionally inhabit open plantations eating seedling trees (Lim, 1974). This species is far more destructive to ricefields in Southeast Asia, and also oil palms. The females breed about four to five times a year with four to twelve embryos per litter.

The knowledge in behavior and ecology of the commensal species is essential for better rodent control management particularly the nesting habits. Of the six species, *Rattus exulans* is the least problematic indoor type compared to the *Rattus norvegicus* and *Rattus rattus diardii*. Little is known of *Mus musculus* apart from, it is strictly an indoor species and due to its smaller size is more difficult to trap. Of the field rats, *Rattus argentiventer* and *Rattus tiomanicus* are the most destructive, the former in ricefield and the latter in oil palm plantations. *Bandicota indica* is also destructive, but is confined to the sugar cane plantations in the northern region of Peninsular Malaysia and to a certain extent in ricefields, while *Bandicota bengalensis* confined to Penang Island, and is similar in habit to *Rattus rattus diardii*.

1.3 SAMPLING SITES

In present study, four locations were chosen to represent urban cities with representing different geographical location namely; Kuala Lumpur representing the west, Pahang representing the east, Penang representing the north and Malacca representing the south of Peninsular Malaysia. The four cities were chosen due to its location within the heart of a modern urban city bustling with round-the-clock human activity. The trapping sites were in the vicinity of the public areas such as food court and market.

Kuala Lumpur (3°8′N 101°41′E) is the federal capital and most populous city in Malaysia. It has an area of 243 km^2 and estimated population of 1.7 million as of 2015 consisting of the city center and vicinity, managed by the Kuala Lumpur City Hall (DBKL). Greater Kuala Lumpur, also known as the Klang Valley, is an urban agglomeration of estimated 7.2 million in 2013. The west coast of Peninsular Malaysia is wider in flat land than the east coast. Kuala Lumpur is the cultural, financial and economic center of Malaysia due to its position as the capital as well as being a prime city.

Pahang (3°45′N 102°30′E) is a state located at the east coast of Peninsular Malaysia. Kuantan, the state capital of Pahang has over 500,000 inhabitants. It is the largest city on the East Coast of Peninsular Malaysia and the 9th largest city in Malaysia. It is situated near the mouth of the Kuantan River and faces the South China Sea. The National Physical Plan 2005 identified Kuantan as one of the future growth centers as a hub for trade, commerce, transportation and tourism. Kuantan is also considered as the social, economic and commercial hub for the East Coast of Peninsular Malaysia due to its strategic location. Rapid development has transformed and modernized Kuantan.

Penang (5°24′N 100°14′E) is an island state in the north region of Peninsular Malaysia. Georgetown is the capital of the Penang and is located on the north-east corner of Penang Island. The heritage city has slowly grown over the years from a small agriculture and farming area to a high-rise metropolitan city. Today with a total population of about 1.6 million as of 2015, this city is fast growing in terms of infrastructure, economy and population and also a popular tourist attraction due to its recognition as a UNESCO heritage site.

Malacca (2°12′N 102°15′E) is located in the southern region of Peninsular Malaysia, sits adjacent to the Straits of Malacca. This state covers an area of 1,664 km^2 or 1.3% area of Malaysia and third smallest state after Perlis and Penang. The population is more than three quarter million people (872,900 in 2015). In the 13^{th} to 14^{th} century Malacca was a major trading port and rich with historical and cultural interest.

Figure 1.5: The location sampling sites: Georgetown, Penang; Kuala Lumpur; Malacca and Kuantan, Pahang, in Peninsular Malaysia. (Source: Image expert from the University of Texas Library).

1.4 BLOOD PARASITES

1.4.1 Trypanosoma

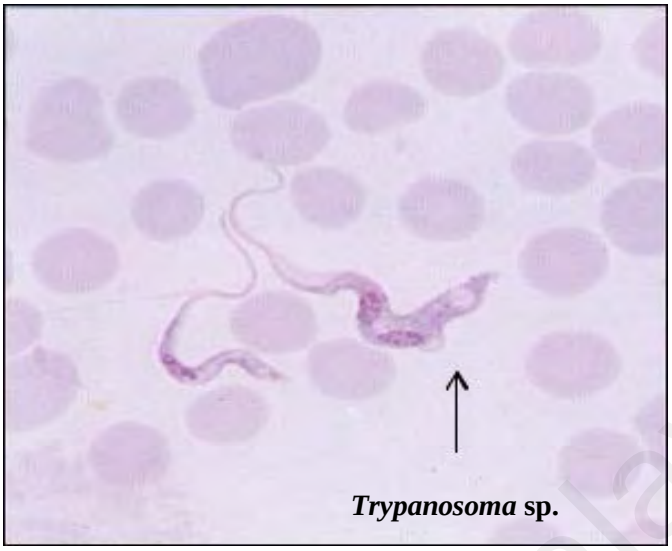

Figure 1.6: *Trypanosoma* sp.

Kingdom: Excavata

Phylum: Sarcomastigophora

Class: Kinetoplastida

Order: Trypanosomatida

Genus: *Trypanosoma*

Trypanosomes are protozoan haemoflagellates parasites that live in the blood and lymph. The single flagellum arises from the kinetoplast which is situated posterior to the nucleus. The flagellum runs the length of the body along the undulating membrane and usually beyond it as an anterior free flagellum. The flagellated form found in humans is called a trypomastigote, or trypanosome.

This parasite undergoes a series of morphological changes consisting of the trypomastigote form in the vertebrate host and/or trypomastigote or promastigote form in the gut of the invertebrate host. Intracellular life cycle stages are normally found in the amastigote form. The trypomastigote morphology is unique to species in the genus *Trypanosoma*.

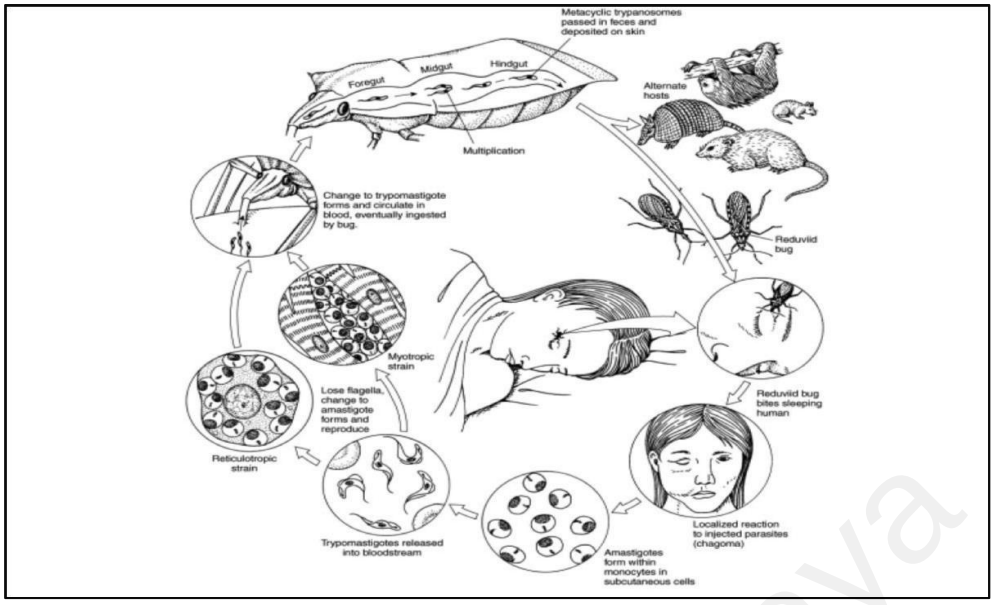

Figure 1.7: Life cycle of *Trypanosoma* sp. (Source: https://www.studyblue.com)

1.4.1.1 *Trypanosoma lewisi*

Trypanosome (*Herpetosoma*) *lewisi* parasitizes synanthropic rodents of the genus *Rattus* via the rat-flea as vectors (Pedro & Jose, 2002). Rats are infected principally by oral route, through ingestion of flea faeces or fleas. Although Herpetesoma species are considered specific to a single vertebrate host genus, they infect a relatively broad range of flea vectors (Molyneux, 1969; Linardi & Botelho, 2002; Desquesnes *et al*., 2002). *Xenopsylla cheopis, Nosopsyllus fasciatus, Ctenocephalides canis* and *Ctenocephalides felis* have been incriminated as intermediated hosts (Molyneux, 1969), *Xenopsylla cheopis* is the principal vector in tropical and subtropical areas (Hoare, 1972).

Trypanosoma lewisi infection in wild rodents is widely reported over the world i.e., among black rats in Niger, West Africa (Dobigny *et al*., 2011), *Rattus* and *Bandicota* species in Thailand (Jittapalapong *et al*., 2008), free living rats in Poland (Karbowiak & Wita, 2001), *Rattus norvegicus* in Brazil (Linardi & Botelho, 2002), rats collected from different localities in northern Iraq (Molan & Hussein, 1988), and house rats in Ibadan (Akinboede *et al*., 1981). *Trypanosoma lewisi*-like haemoflagellate was also reported in

a single *Rattus tiomanicus* during a field study of small wild mammals in Central Pahang (Yap *et al*., 1977) and *Trypanosoma conorhini* in *Rattus rattus* (Laird, 1966).

They are well adapted to their respective hosts and have a high degree of host specificity. Trypanosomes are extracellular parasites and exposed to the humeral immune response of the host at all times. Generally, the pathogenicity of mammalian trypanosomiasis is defined according to whether the infection produces recognizable disease and pathological damages or is innocuous to the host. Generally, trypanosomes are pathogenic only for hosts with immature immune system (Murray *et al*., 1982; William *et al*., 1991; Pentreath, 1995).

Trypanosoma lewisi is usually nonpathogenic to humans but can acquire the desired virulence and emerge as human pathogen causing serious disease, in the right combination of environmental, host and organism related factors. *Trypanosoma lewisi* infection has been reported in infants including a 45- day-old Thai infant displaying fever, anaemia, cough and anorexia (Sarataphan *et al*., 2007). It was also reported in a two months old infant in urban Mumbai, India (Kaur *et al*., 2007) and in a 4-month-old Malaysian infant with a 3-week history of lassitude, loss of appetite, feverish and anaemic with a heavy trypanosome infection upon admission (Johnson, 1933) although re-examination of the original specimen from the Johnson's patient after 37 years later did not support the tentative identification of the organism as *Trypanosoma lewisi* (Weinman, 1970). Dissanike *et al*. (1974) also reported two cases of trypanosome infections in the Orang Asli in west Malaysia. Recently, trypanosomes were observed in the peripheral blood smear of a 37-day-old Indian infant admitted off feeds, with fever and convulsions (Verma *et al*., 2011).

Life cycle

The life cycle consists of two hosts; a blood feeding invertebrate and a vertebrate host. An infected flea takes a blood meal from the infected rat and then defecates passing metacyclic trypanosomes in the feces. The rat ingests the flea feces allowing the trypanosomes to penetrate the rat esophagus and enter the bloodstream to multiply by binary fission, forming rosettes of epimastigote. The rosette erupts, releasing the epimastigotes which transform to trypomastigotes where it remains in the bloodstream for several weeks. When ingested by a flea during a blood meal the trypomastigotes penetrate the stomach epithelium and multiply. New trypomastigotes are produced and released back to the stomach and can re-enter the epithelial cells for further division. Eventually, the released trypomastigotes move to the rectum and transform into epimastigotes before undergo further multiplication to produce large numbers of infective metacyclic trypomanosomes.

1.4.1.2 *Trypanosoma evansi*

Trypanosoma evansi is closely related to other *Trypanozoon* species, including *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* at the genetic level. *Trypanosoma evansi* is spread by mechanical transmission of infected blood through hematophagous insects such as tabanid flies. However, the exact role of tabanids in the transmission of trypanosomiasis in the Sungai Dusun area has yet to be determined (Chan *et al*., 2004).

Surra caused by *Trypanosoma evansi* mainly infects camels, cattle, buffaloes and other wild animals (Hoare, 1972). Human infection by animal species of Trypanosome is usually impossible because of a trypanolytic factor in human serum (Prashant *et al*., 2005**).**

1.4.2 Plasmodium

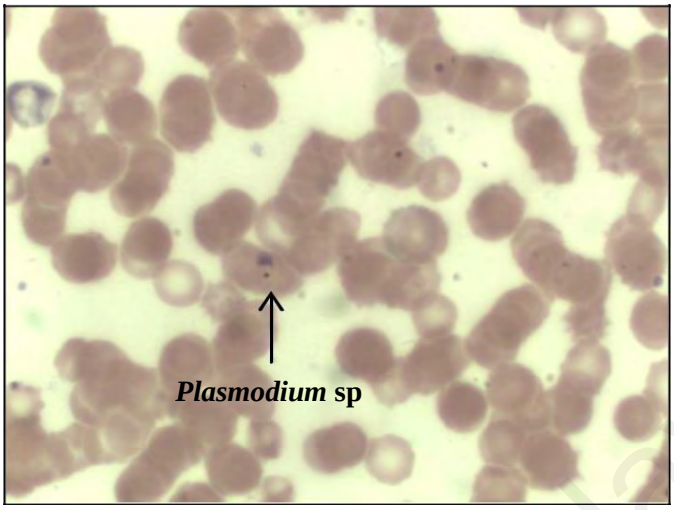

Figure 1.8: *Plasmodium* sp.

Kingdom: Chromalveolata

Superphylum: Alveolata

Phylum: Apicomplexa

Class: Aconoiasida

Order: Haemosporida

Family: Plasmodiidae

Genus: *Plasmodium*

Plasmodium is an Apicomplexan parasite and often referred to as Sporozoa. It has a complex life cycle that involves two different hosts, a mammalian host and an invertebrate host comprised of the *Anopheles* mosquito. The sexual stages of reproduction and an asexual stage of reproduction (known as sporogony), takes place in the mosquito host. In the human host, another type of asexual reproduction takes place called schizogony and results in the formation of infective stages that attack and invade the mammalian red blood cells and is responsible for the fever associated with malarial infections. Also in the red blood cells there are the sexual stages of the parasite known

as gametocytes that where taken by the mosquitoes in blood meal will produce male and female gametes when ingested by a mosquito.

Life cycle

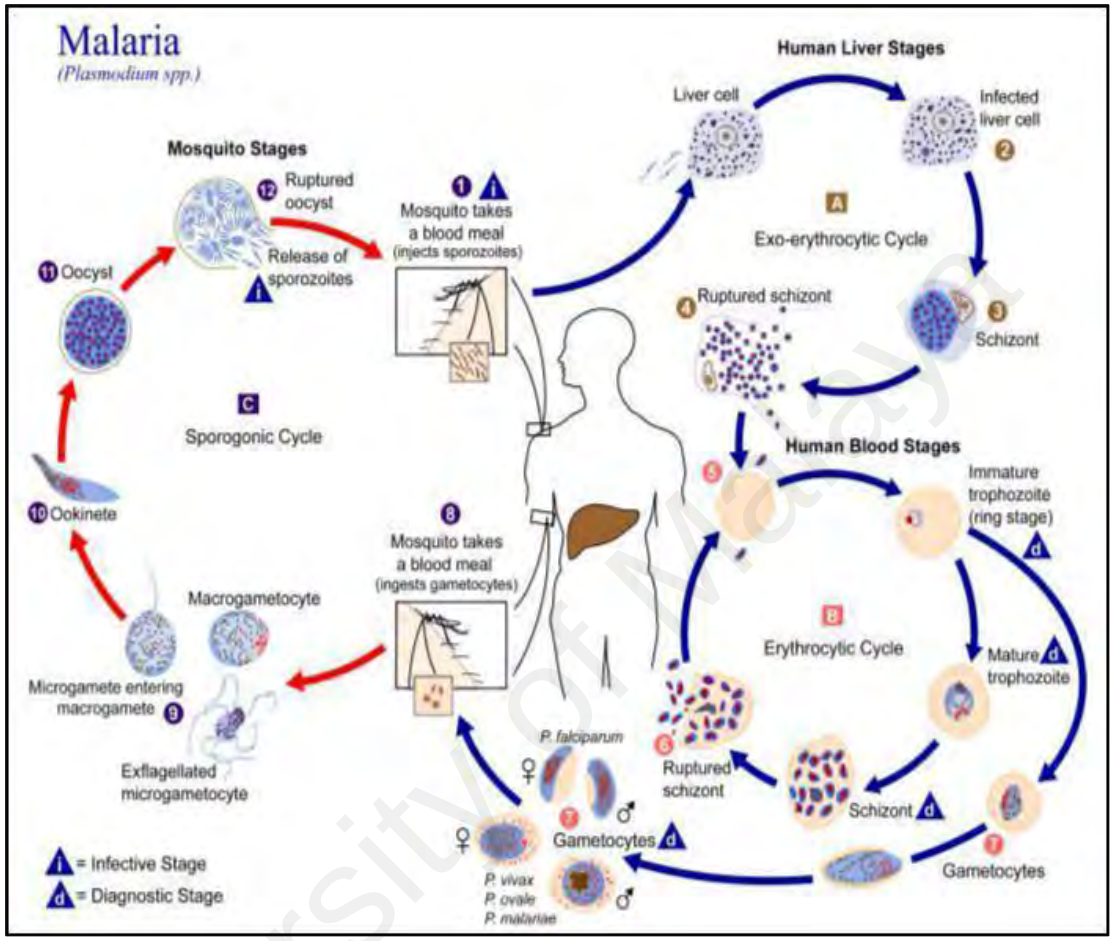

Figure 1.9: Life cycle of *Plasmodium* sp. (https://www.cdc.gov/malaria)

Approximately 200 Plasmodium species exploit a wide range of vertebrate hosts, including mammals, birds and reptiles. At least 11 species infect humans and four species infecting humans that vary considerably in their virulence (Bruce-Chwatt, 1985). Unfortunately, the consequences of natural malarial infection (i.e. in natural hosts in the wild) for other vertebrate hosts are poorly known, but evidence suggests interspecific difference in virulence (Coatney *et al*. 1971; Garnham, 1980; Landau & Boulard, 1978).

It is now recognized that malaria caused by certain species of plasmodia of nonhuman primates probably must be classified as a zoonosis (Contacos & Coatney, 1963). Surveys and ecological studies of rodents and the prevalence of parasites have been carried out in Malaysia (Adams, 1933; Audy *et al.*, 1950; Sandosham, 1953; Yeh, 1955; Dunn, 1966; Dunn *et al*. 1968). Schacher and Cheong (1960) summarized the parasites of three species of common house rats. Helminth parasite surveys in the house rats were carried out by Gatha (1966) and Mulkit Singh and Cheong (1971) in Singapore and Malaya studied the incidence of nematode infections in various species of feral rats in Malaysia. Ow-Yang (1971), Lim and Muul (1970) and Lim *et al*. (1976) reported on endoparasites of rodents in oil-palm plantations and other habitats.

Despite a considerable amount of studies having investigated the occurrence of parasitic infections in Malayan rats (Harrison, 1957; Sandosham, 1957; Schacher & Cheong, 1960; Balasingam, 1963; Lim *et al*. 1965, 1977; Gatha, 1966; Singh & Cheong, 1971; Betterton & Lim, 1975), little is known of blood parasitemia in the urban rodent population. Therefore, the general objectives of this study were to determine rodent species diversity in urban cities, host-parasites relationship and their zoonotic importance.

1.5 OBJECTIVES

The specific objectives of the present study are as follows:

- To determine the diversity of urban rodent population in Peninsular Malaysia.
- To identify and determine the prevalence of blood parasitaemia of urban rat populations in Peninsular Malaysia.
- To determine intrinsic (host sex and age) and extrinsic (location and season) factors contributing to the blood parasitaemia in the urban rat population.
- To compare the sensitivity of two methods for blood protozoan detection using Quantitative Buffy Coat (QBC) and Giemsa-stained Thin Film (GTF).

CHAPTER 2

URBAN RAT POPULATION STRUCTURE AND DISTRIBUTION IN PENINSULAR MALAYSIA

2.1 INTRODUCTION

Rodents account for about forty percent of the mammals living at the present time, and constitute more than 42% of the known mammalian species (Singleton, *et al*., 2003). In many regions, however, the total number of individual rodents is probably many times greater than that of all other mammals. Nearly four million rats are born every day in the developing nations and are known to spread more than 60 diseases to human worldwide and the list grows as more research on zoonosis continues (Khatoon *et al*., 2004; Hinds, 2003). Three commensal rodent species are known to be established in most parts of the world and spreading the risk of disease, such as plague.

Rodents do not recognize international boundaries, different human cultures, or economic standards. They are migratory and well adapted to the different environment, thus making them one of the most geographically spread animal species thus a major agricultural, urban and social pests across much of the developed and developing world. This tendency and overall geographical spread of rodents contributes to its prevalence as a zoonotic disease reservoir and hence this places importance in closely studying them.

People have largely categorized rodents as pest in urban and agricultural environment, despite most species living in the wild with little interaction with humans. A small minority of species have adapted to living in close association with humans, using our agriculture and waste as their own food resource and utilizing spaces in buildings as their homes. The close proximity means that they can spread and transmit diseases quickly. The ability of rodents to carry diseases is encouraged by their habit of

utilizing our waste and sewage, their mobility and similarity in physiology to humans. They cause numerous problems as agricultural pests but also act as reservoirs and transmitters of pathogens, which is particularly acute in developing countries (Grazt *et al*., 1997; Dalu & Feresu, 1997; Kumar *et al*., 1997; Kilonzo *et al*., 1997; Stanseth *et al*., 2003).

Therefore, concerted effort should be initiated for a long-term rodent population surveillance to determine the urban rat distribution structure and diversity as the information obtained could be used to ascertain its zoonotic potential to human health.

2.1.1 Rodent problems

Rodents have been subjected to extensive reviews (Hopf *et al*., 1976; Jackson, 1977; Taylor, 1972) and pose major problems that can be grouped into three main categories:

i) Agriculture and forestry

ii) Stored products and household effects

iii) Public health

2.1.1.1 Agriculture and forestry

Studies in Malaysia on rodents have mainly been directed as agricultural pests. The severity is illustrated in oil palm plantation where minimum direct loss as a result of rat damage is estimated to be 134 kilogram oil per hectare per year (Wood, 1994). There is approximately one million hectares of land cultivation for oil palm in Malaysia and the minimum loss amounts to millions of ringgit. Biological and physically prevention methods are currently explored in many oil palm estates to prevent rodent population spread and growth.

2.1.1.2 Stored products and household effect

In addition to depredation of foodstuffs (eaten or destroyed), commensal rodents also contaminate the food supplies with their droppings and fur resulting in further losses of foodstuff and additional expenses in replacing new material. Rodents also cause considerable damage to manufactured goods and fabric of buildings particularly damage to wire insulation creating potential fire hazard.

2.1.1.3 Public health

Rodents are vectors to many diseases to man and domestic animals that have health and economic implications. However, their socio-economic importance has not been properly evaluated.

Disease such as Hantavirus is severe and often fatal (Pini *et al*., 2003). Leptospirosis is serious bacterial illness with 6000 reported cases and death 350 in the year 2000 in Thailand. Lassa fever, a disease endemic in West Africa infects an estimated 100,000 to 300,000 people per year with approximately 5000 deaths (Centre for Disease Control, 2006).

Rodents have also been linked to spread of emerging disease like Severe Acute Respiratory Syndrome (SARS) in Asia (Lin *et al*., 2004) and transmission of babesiosis, cestodes, trematodes and nematodes (Abdel-Salam *et al*., 1994; Seong *et al*., 1995). Pathogens transmitted through the bites of rodents or the ectoparasites it carries are listed among the serious emerging infectious diseases worldwide (Childs *et al*., 1998; Randolph *et al*., 1999). An example is *Rattus norvegicus* is known to carry a number of pathogens (Meerburg *et al*., 2009) including Weil's disease, rat bites fever, cryptosporidiosis, viral hemorrhagic fever (VHF), Q fever and Hantavirus pulmonary syndrome. In the United Kingdom, *Rattus norvegicus* is an important reservoir for *Coxiella burnetii*, the bacteria that causes Q fever (Webster *et al*., 1995b).

2.1.2 Rodent controls

In order to effectively control and manage the control pests, an integrated rodent control program is essential. This should consist of the following:

i) Quantitative assessments of rodent-borne damage; this aspect received the least attention in formulating quantitative methods in assessing rodent-borne damage.

ii) Surveillance of rodent population and prediction of damage; this aspect entails a good understanding of the population dynamics of the species concerned however, not much work is done towards obtaining this basic information.

iii) Efficacy and selection of control methods; most research on rodent pests appear to be centered on control methods which may be summarized as follows:

a) Reduction of numbers of rodents by means of baits incorporating rodenticide.

b) Protection by means of mechanical barriers or chemical repellents.

c) Trapping.

d) Application of poison dusts, poisoned water and fumigation.

e) Ecological control through habitat manipulation.

f) Biological control (use of microorganisms, parasites, predators, etc.)

g) Chemosterilant.

2.2 METHODOLOGY

Fieldwork was carried out within four major cities with each location representing different unique geographical location in Peninsular Malaysia namely; Kuala Lumpur (3°8′51″N 101°41′36″E) representing the west, Pahang (3°49′00″N 103°20′00″E) representing the east, Penang (5°25′00″N 100°19′00″E) representing the north and Malacca (2°12′N 102°15′E) representing the south of Peninsular Malaysia.

2.2.1 Trapping

Trapping of rats was conducted for four days, three nights from November 2006 until November 2011. All the rats were trapped alive using specially made steel wire traps measuring 29 x 22 x 50 cm using dried fish, sweet potatoes, fruits and coconut as baits. Trapping was conducted with the cooperation of the assigned units from each municipality from each respective city. The main criteria for site selection were proximity to housing, obscuration from public view and association with drainage defects. Each day, 30 traps were placed at varying distances and different types at sites where most rat activity was expected. The sites were identified based on local peoples' observations of rodent activity, or from signs of rats, e.g. faeces, rat pathways or footprints. Traps were placed inside and around houses as well as in uncultivated surroundings.

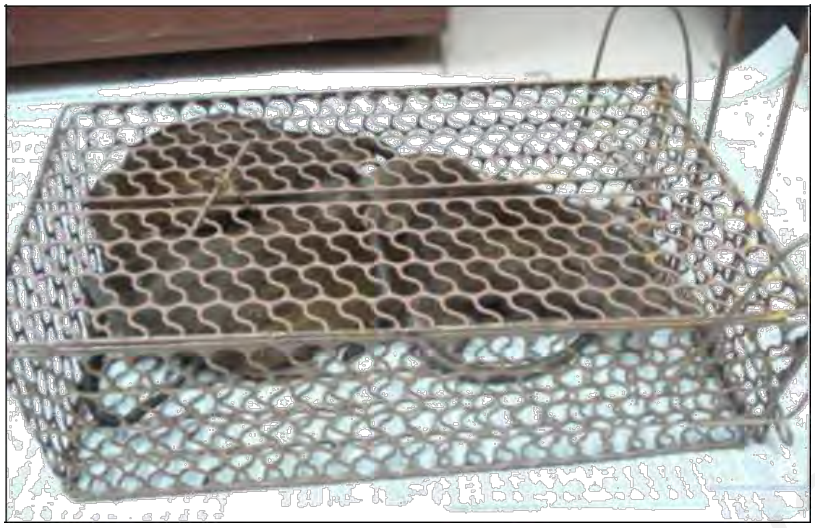

Figure 2.1: Rats trapped in steel wire traps measuring 29 x 22 x 50 cm

2.2.1.1 Kuala Lumpur

Rats trapped were part of a vector program by the Vector Control Unit of Kuala Lumpur City Hall (DBKL) from November 2006 until November 2011. Sampling was done 6 times during dry season and 6 times during wet season. Rats were trapped from the market, food court and by the shop lots from areas surrounding Kuala Lumpur such as Chow Kit, Sentul, Setapak and Bukit Bintang.

Captured rats were sent to the Institute of Biological Sciences, University of Malaya, Kuala Lumpur. The dissection process was performed at the Parasitology Lab, Institute of Biological Sciences in Faculty of Science, University of Malaya. Rats were then euthanized with chloroform and post-mortem of the rats was conducted according to standard procedures as described by Rusli (1988).

2.2.1.2 Pahang

From November 2008 to July 2009, a total of 2 trapping were conducted during wet season and 2 times during dry season in the vicinity of Kuantan. The Vector and Rodents Unit of Kuantan Municipal Council or Majlis Perbandaran Kuantan (MPK) was assisted in capture the rats. Rats were captured by using various techniques such as

thermal fogging technique, pesticides, standard metal basket traps, sweep net and self-designed traps, trapping killer rat (TKR) traps that specially designed by Kuantan Municipal Council in order to control the rat population around Kuantan. This was in addition to using conventional methods such as sweep nets at night and was considered the most effective and cost savings. Trapping sites include surrounding homes, underground holes, streets, markets, food court areas in Tanjung Api hawker centers, Sungai Kuantan and Taman Tas and markets such as Pasar Besar Kuantan and Pasar Borong Kemunting. Rats captured the night before were collected and examined immediately the following morning at Animal House Lab of Faculty of Medicine in International Islamic University Malaysia (IIUM), Kuantan.

Figure 2.2: Thermal fogging technique used by Kuantan Municipal Council to control rat population

Figure 2.3: Burrow entrance of rats at the side of a ditch

Figure 2.4: Trapping using standard metal basket traps and sweep net

Figure 2.5: Staff from Kuantan Municipal Council at work trapping the rats at nights

Figure 2.6: Trapping killer rat (TKR) traps that specially designed by Kuantan Municipal Council to trap rodents

2.2.1.3 Penang Island

Trapping in Penang was carried out between March 2008 and May 2010 with a total of 3 times during wet season and 3 times during dry season. Assistance in providing rats specimens were given by Public Health Unit, Municipal Council of Penang Island or Majlis Perbandaran Pulau Pinang (MPPP). Trapping methods include the use of standard metal wire basket traps with peanut butter and bread as bait, fogging and poisoning.

Municipal Council of Penang Island aided in trapping rats loitering in food courts and markets in the city center and surrounding areas of Georgetown such as Campbell street market. Trapping was repeated for four consecutive nights. Traps were checked and collected every morning and brought to the Parasitology Lab of School of Biological Science in University of Science Malaysia (USM) for post mortem examination.

Figure 2.7: Campbell street market

Figure 2.8: Standard metal basket trap were located at drain

Figure 2.9: Rats were caught by standard metal basket trap

Figure 2.10: *Rattus rattus dirdii* with the orange fur

2.2.1.4 Malacca

Trapping in Malacca was conducted between August 2009 to May 2010 with a total of 2 sampling times during dry season and 2 times during wet season. Rats were captured by Lilati Control Unit of Melaka Historic City Council or Majlis Bandaraya Melaka Bersejarah (MBMB). Melaka Historic City Council captured rats in area around Malacca near shop lots, markets, streets and food courts. The traps were set in the evening, and checked the following morning. Rats captured were brought to the Biology Lab of Melaka International College of Science and Technology (MiCoST) for further examination.

Figure 2.11: Rats were captured by Lilati Control Unit of Malacca Historic City Council

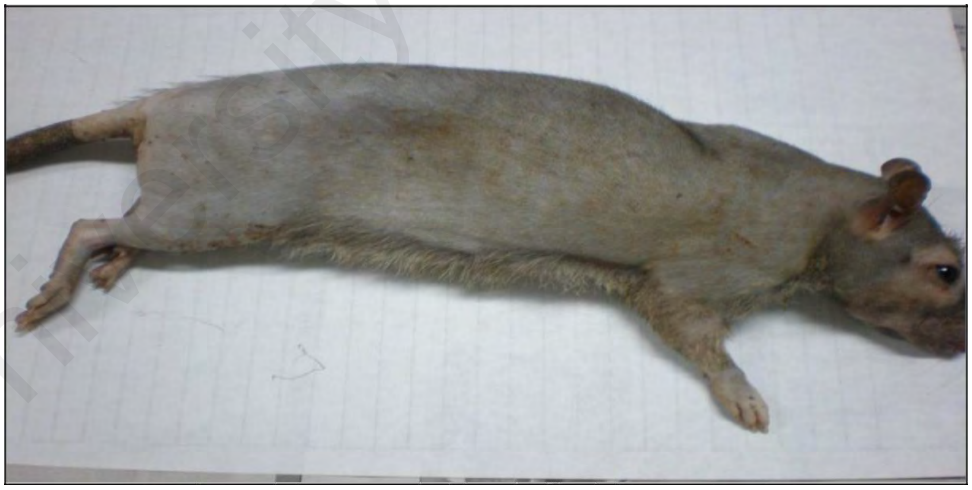

Figure 2.12: *Rattus norvegicus*

2.2.2 Morphometric examination

Trapped rats were killed humanely by placing the trapped rodent into a cloth bag containing cotton wool soaked with chloroform. Morphometric measurements of head-body, tail, ear, hind feet, body weight and physical appearances were recorded. The age, sex and species were determined through physical examination. Species identification was based on descriptions by Harrison & Quah (1962), Medway (1983) and Payne & Francis (1998).

Figure 2.13: Rat was killed humanely by placing into a cloth bag containing cotton wool soaked with chloroform

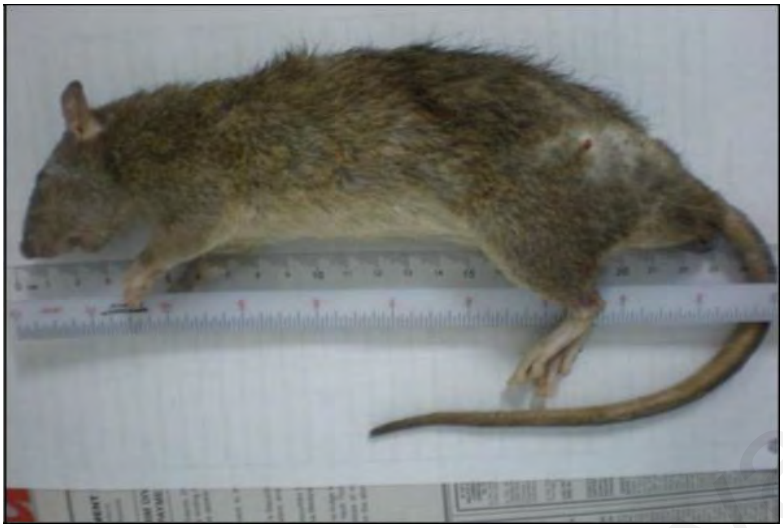

Figure 2.14: Morphometric measurement for head-body length of rat

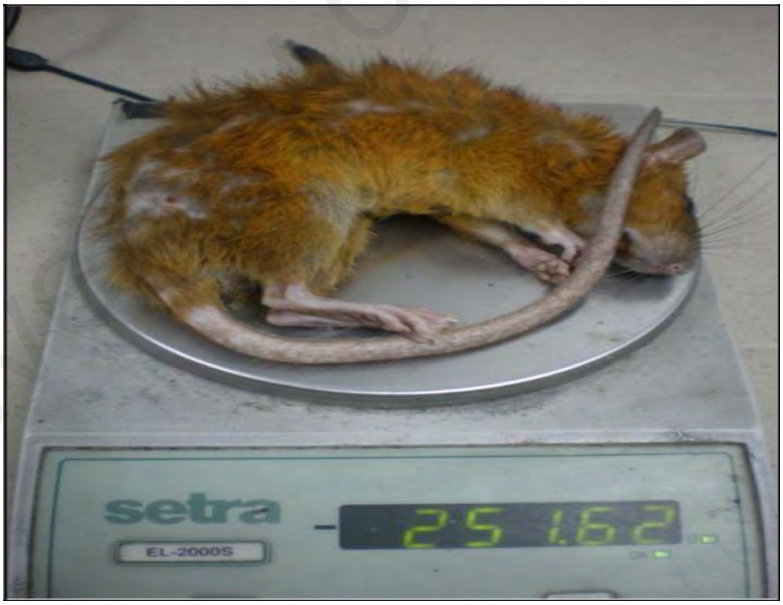

Figure 2.15: Morphometric measurement for body weight of rat

2.3 RESULT

A total of 762 urban rats were trapped from four urban cities in Peninsular Malaysia (Figure 2.1) comprised of six commensal rat species: *Rattus rattus diardii* (n=410), *Rattus norvegicus* (n=302), *Rattus exulans* (n=33), *Rattus tiomanicus* (n=8), *Rattus argentiventer* (n=7) and *Rattus annandalei* (n=2). *Rattus rattus diardii* (53.8%) was the dominant rat species captured followed by *Rattus norvegicus* (39.6%), *Rattus exulans* (4.3%), *Rattus tiomanicus* (1.1%), *Rattus argentiventer* and *Rattus annandalei* with 0.9% and 0.3% respectively (Table 2.1).

According to host-sex, more females were caught (52.8%) than males (47.2%) in Figure 2.2. Table 2.1 shows that based on host-sex, slightly more females were caught for all the six species compared to males. More females were captured compared to males with a ratio of 1.1:1.

According to host-age, total number of adults captured was higher with 502 rats (65.9%) compared to 152 sub adult rats (20.0%) and 108 juvenile rats (14.2%) with a ratio of 4.6:1.4:1 in Figure 2.3. The proportion of the total urban rats captured from all locations is according to season, host sex and age of urban rats is summarized in Table 2.1.

According to season, the overall rats captured were higher during wet season (431) compared to dry season (331) with a ratio of (1.3:1). However, the number caught in Malacca and Kuantan during dry season were higher (Table 2.1).

According to site, total number capture in Kuala Lumpur was highest with 433 rats. *Rattus rattus diardii* (327) was the most common rat collected, followed by *Rattus norvegicus* (57), *Rattus exulans* (33), *Rattus tiomanicus* (8), *Rattus argentiventer* (6) and *Rattus annandalei* (2). The urban rat populations showed more female captured compared to males with 223 females and 210 male respectively. According to host age,

there were less number of juvenile rats captured compared to adult and sub adult with 78 juveniles, 122 sub adults and 233 adults urban rats. Most of the urban rats in Kuala Lumpur were captured during wet season (241 rats) compared to dry season (192 rats) (Figure 2.4).

A total of 117 urban rats were captured from Pahang, with only one species (*Rattus novergicus*) captured in Kuantan. There were more females (61 rats) than males (56 rats). However, according to the host age, lower numbers of juveniles were captured compared to sub adults and adults with 5 juveniles, 7 sub adults and 105 adults. Higher numbers of rats were captured during dry season (63 rats) compared to wet season (54 rats) (Table 2.1).

A total of 111 rats of 3 species: *Rattus norvegicus* (75), *Rattus rattus diardii* (35) and *Rattus argentiventer* (1) were collected with 67 females and 44 males of urban wild rats in Malacca. According to host age, the numbers of juveniles (10 rats) were less compared to sub adults (15 rats) and adults (86 rats). Most of the rats were captured during dry season with 63 rats compared to wet season with 48 rats (Table 2.1).

The capture of urban rats from Penang was 101 rats. There were only 2 species captured from this north coastal site. The highest species was *Rattus norvegicus* (53) followed by *Rattus rattus diardii* (48). Ratio between male and female were almost equal with 51 females and 50 males. Meanwhile, according to the host age, there were more adults (78 rats) captured compared to juveniles (15 rats) and sub adults (8 rats). The numbers of urban rats captured during wet season (88 rats) were higher compared to dry season (13 rats) (Table 2.1).

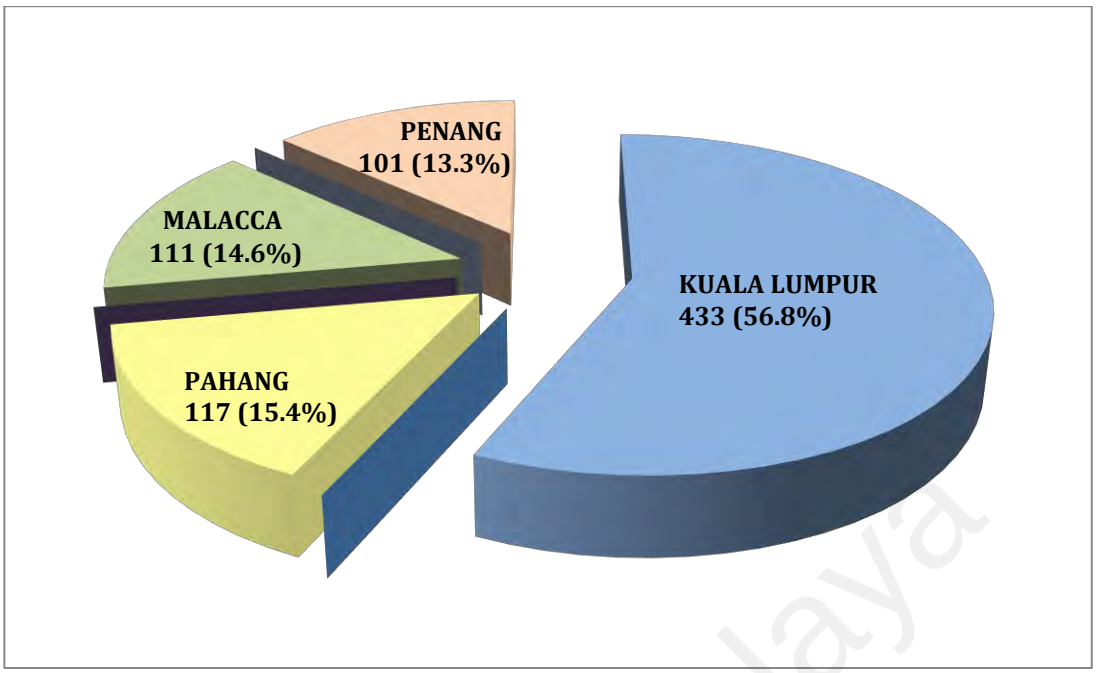

Figure 2.16: Distribution of urban rats captured in Peninsular Malaysia based on location

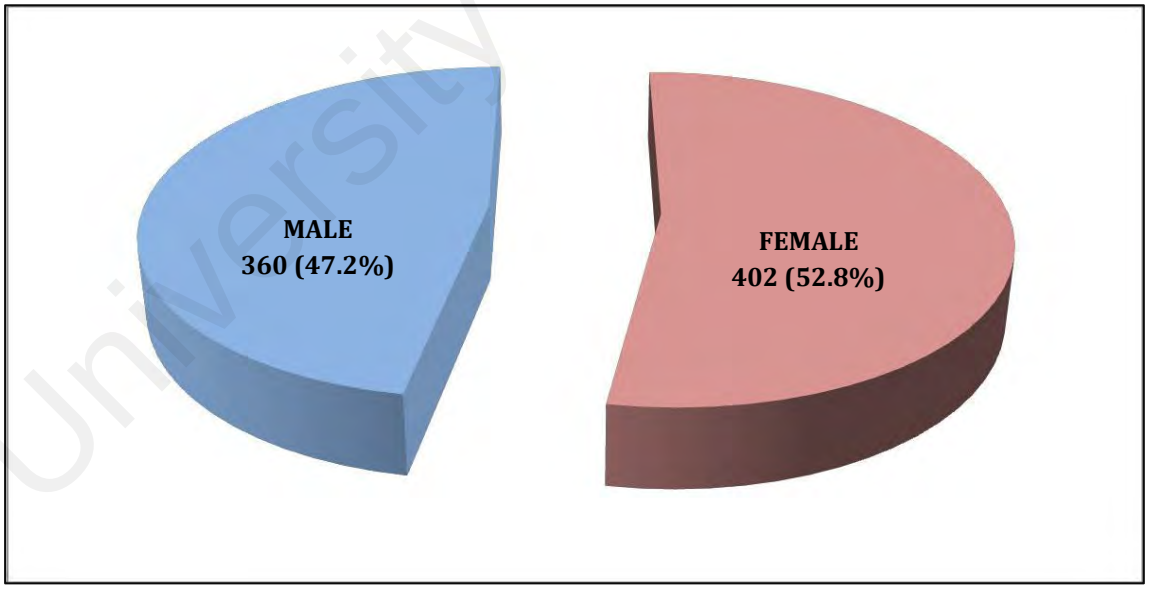

Figure 2.17: Distribution of urban rats captured in Peninsular Malaysia based on sex

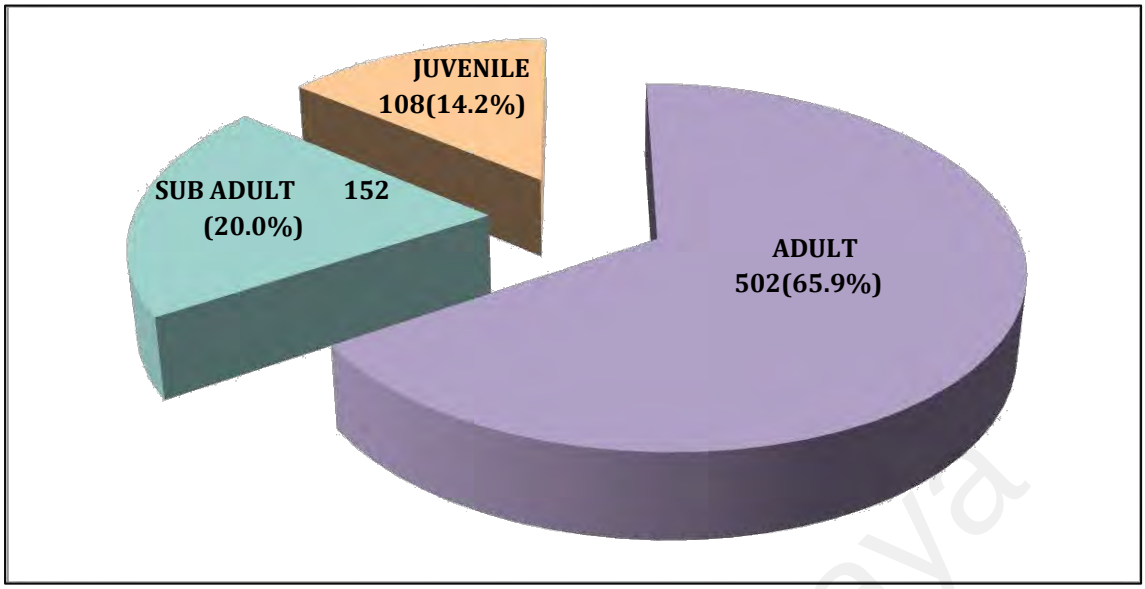

Figure 2.18: Distribution of urban rats captured in Peninsular Malaysia based on age

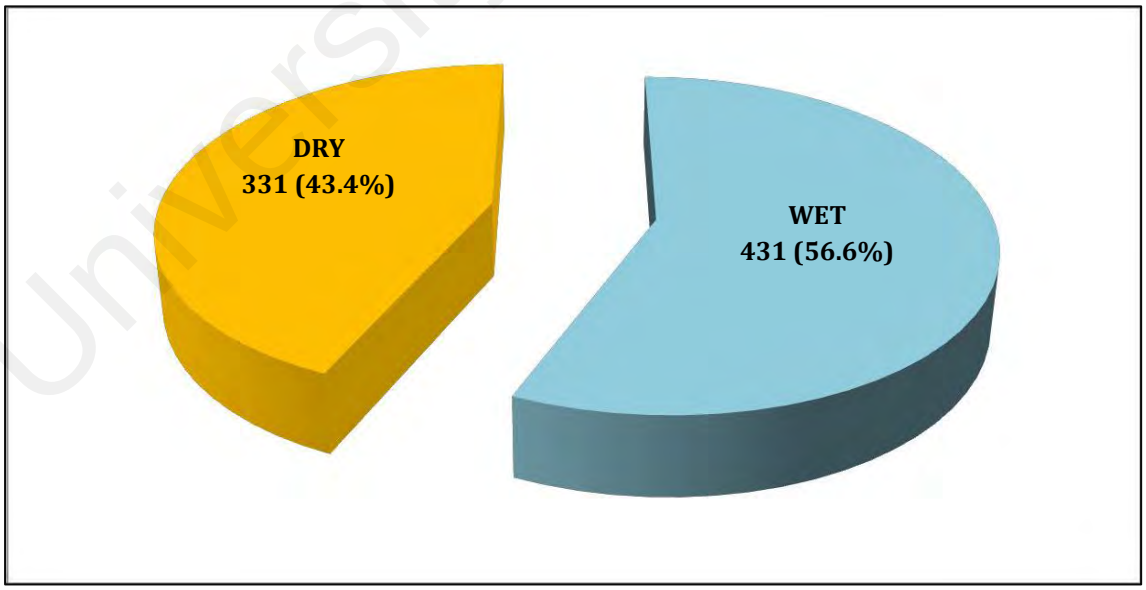

Figure 2.19: Distribution of urban rats captured in Peninsular Malaysia based on season

Table 2.1: The rat population structure relative to location, species, age, sex and season

Location		Species of Rat						Host sex		Host Age			Season	
		RRD	**RN**	**RE**	**RT**	**RA**	**RAn**	**Female**	**Male**	**A**	**SA**	**J**	**Dry**	**Wet**
Kuantan	**n**	-	117	-	-	-	-	61	56	105	7	5	63	54
	%		100.0					52.1	47.9	89.7	6.0	4.3	53.9	46.2
Malacca	**n**	35	75	-	-	1	-	67	44	86	15	10	63	48
	%	31.5	67.6			0.9		60.4	39.6	77.5	13.5	9.0	56.8	43.2
Georgetown	**n**	48	53	-	-	-	-	51	50	78	8	15	13	88
	%	47.5	52.5					50.5	49.5	77.2	7.9	14.9	12.9	87.1
Kuala Lumpur	**n**	327	57	33	8	6	2	223	210	233	122	78	192	241
	%	75.5	13.2	7.6	1.9	1.4	4.6	51.5	48.5	53.8	28.2	18.0	44.3	55.7
Total	**n**	**410**	**302**	**33**	**8**	**7**	**2**	**402**	**360**	**502**	**152**	**108**	**331**	**431**
	%	**53.8**	**39.6**	**4.3**	**1.1**	**0.9**	**0.3**	**52.8**	**47.2**	**65.9**	**20.0**	**14.2**	**43.4**	**56.6**

*RRD-*Rattus rattus diardii*; RN-*Rattus norvegicus*; RE-*Rattus exulans*; RT-*Rattus tiomanicus*; RA-*Rattus argentiventer*; RAn-*Rattus annandalei*;

A-adult; SA-Sub-adult; J-Juvenile.

Table 2.2: The rat population structure in relation to species, location, age, sex and season

Rat species		Location				Host sex		Host Age			Season	
		Kuala Lumpur	Pahang	Malacca	Penang	Female	Male	A	SA	J	Dry	Wet
Rattus rattus	**n**	327	-	35	48	127	128	141	76	38	119	136
diardii	**%**	75.5		31.5	47.5	49.8	50.2	55.3	29.8	14.9	46.7	53.3
Rattus	**n**	57	117	75	53	88	79	131	24	12	82	85
norvegicus	**%**	13.2	100	67.6	52.5	52.7	47.3	78.4	14.4	7.2	49.1	50.9
Rattus	**n**	33	-	-	-	15	8	7	5	11	16	7
exulans	**%**	7.6				65.2	34.8	30.4	21.7	47.8	69.6	30.4
Rattus	**n**	8	-	-	-	4	3	2	1	4	1	6
tiomanicus	**%**	1.8				57.1	42.9	28.6	14.3	57.1	14.3	85.7
Rattus	**n**	6	-	1	-	1	2	1	2	-	-	3
argentiventer	**%**	1.4		0.9		33.3	66.7	33.3	66.7			100
Rattus	**n**	2	-	-	-	-	1	1	-	-	-	1
annandalei	**%**	0.5					100	100				100
Total	**n**	**433**	**117**	**111**	**101**	**235**	**221**	**283**	**108**	**65**	**218**	**238**
	%	**56.8**	**15.4**	**14.6**	**13.3**	**51.5**	**48.5**	**62.1**	**23.7**	**14.3**	**47.8**	**52.2**

* A-adult; SA-Sub-adult; J-Juvenile.

Table 2.3: The rat population diversity according to host sex in each location

Rat species		Location							
		Kuala Lumpur		Pahang		Malacca		Penang	
		Female	Male	Female	Male	Female	Male	Female	Male
Rattus rattus diardii	**n**	165	162	-	-	22	13	22	26
	%	50.5	49.5			62.9	37.1	45.8	54.2
Rattus norvegicus	**n**	26	31	61	56	45	30	29	24
	%	45.6	54.4	52.1	47.9	60.0	40.0	54.7	45.3
Rattus exulans	**n**	24	9	-	-	-	-	-	-
	%	72.7	33.3						
Rattus tiomanicus	**n**	5	3	-	-	-	-	-	-
	%	62.5	37.5						
Rattus argentiventer	**n**	3	3	-	-	-	1	-	-
	%	50.0	50.0				100		
Rattus annandalei	**n**	-	2	-	-	-	-	-	-
	%		100						
Total	**n**	**223**	**210**	**61**	**56**	**67**	**44**	**51**	**50**
	%	**51.5**	**48.5**	**52.1**	**47.9**	**60.4**	**39.6**	**50.5**	**49.5**

Table 2.4: The rat population diversity according to host age in each location

Rat species		Location											
		Kuala Lumpur			Pahang			Malacca			Penang		
		A	SA	J	A	SA	J	A	SA	J	A	SA	J
Rattus rattus diardii	**n**	183	94	50	-	-	-	18	8	9	32	6	10
	%	56.0	28.7	15.3				51.4	22.9	25.7	66.7	12.5	20.8
Rattus norvegicus	**n**	31	17	9	105	7	5	67	7	1	46	2	5
	%	54.4	29.8	15.8	89.7	6.0	4.3	89.3	9.3	1.3	86.8	3.8	9.4
Rattus exulans	**n**	12	7	14	-	-	-	-	-	-	-	-	-
	%	36.4	21.2	42.4									
Rattus tiomanicus	**n**	2	1	5	-	-	-	-	-	-	-	-	-
	%	25.0	12.5	62.5									
Rattus argentiventer	**n**	3	3	-	-	-	-	1	-	-	-	-	-
	%	50.0	50.0					100					
Rattus annandalei	**n**	-	2	-	-	-	-	-	-	-	-	-	-
	%		100										
Total	**n**	**233**	**122**	**78**	**105**	**7**	**5**	**86**	**15**	**10**	**78**	**8**	**15**
	%	**53.8**	**28.2**	**18.0**	**89.7**	**6.0**	**4.3**	**77.5**	**13.5**	**9.0**	**77.2**	**7.9**	**14.9**

*A-adult; SA-sub-adult; J-juvenile

Table 2.5: Wild rats captured by location, host age, and season

Location		Season					
		Dry			Wet		
		A	SA	J	A	SA	J
Kuala Lumpur	**n**	109	51	32	124	71	46
	%	56.8	26.6	16.7	51.5	29.5	19.1
Pahang	**n**	53	5	5	52	2	-
	%	84.1	7.9	7.9	96.3	3.7	
Malacca	**n**	48	8	7	38	7	3
	%	76.2	12.7	11.1	79.2	14.6	6.2
Penang	**n**	9	2	2	69	6	13
	%	69.2	15.4	15.4	78.4	6.8	14.8
Total	**n**	**219**	**66**	**46**	**283**	**86**	**62**
	%	**66.2**	**19.9**	**13.9**	**65.7**	**20.0**	**14.4**

*A-adult; SA-sub-adult; J-juvenile

2.4 DISCUSSION

Rodents in the human environment cause great economic loss by consuming or contaminating vast quantities of food and animal feed and transmitting diseases. Therefore the study of rodent population structure is necessary to a better understanding of rodent infestation for both health and economic benefit.

A considerable number of urban rats and species of rats captured in this study has proved that the rodent population in the four urban cities was capable of supporting a fairly diverse wild rat population particularly in public spaces such as wet markets and food courts where there is excess food availability. The abundance of food in addition to the short breeding cycle and suitable living habitat for commensal rodents has caused the rodent population to thrive in the urban landscapes. This can be a potential public health hazard in the absence of a good rodent management program due to the close proximity between urban rats to human and food source pose a threat of transmission of zoonotic diseases. Therefore a long term monitoring system on the rodent population and its parasitofauna is pertinent in order to determine the risk these vectors play as potential reservoir to human infection (Jittapalapong *et al*., 2007).

Six rat species were recorded with *Rattus rattus diardii*, the dominant species with more than half of total number captured, followed by *Rattus norvegicus* (39.6%). The dominance of both of these species were not entirely surprising, as the presence of the both rat species within the city have been previously reported (Schacher & Cheong, 1960; Ow-Yang, 1971; Singh, 1995; Mohd Zain *et al.,* 2012) and are commonly known to live in human environment and normally identified as pest to human community.

Rattus rattus is widely distributed in particularly near human habitation in urban, sub urban and rural areas through the entire range of both Malaysia and Indonesia. This rodent species does not have the ability to burrow and as such live in all kinds of human

structures or in natural habitat they build nest in the holes of other animals. The adult female breeds four times a year with six to eight embryos per litter (Harrison, 1962). The dominance of *Rattus rattus diardii* has also been widely reported in previous studies (Harrison, 1957; Leong *et al*., 1979; Zahedi *et al*., 1984; Singh, 1995; Mohd Zain *et al.,* 2012) and its existence is widely believed introduced to Peninsular Malaysia (Harrison, 1957; Dhaliwal, 1961) due to the favorable ecological conditions for the permanent establishment, continuance and perhaps proliferation.

Rattus norvegicus usually occupies lower floor of buildings and structures and in tropical countries this rat breeds all year round. The relatively large number of *Rattus norvegicus* specimens from Pahang, Malacca and Penang was expected, given that the rat species were more commonly associated to coastal areas (Harrison, 1957). In Southeast Asia, this species is found mainly near cities with links to river and waterways where they are found scavenging in garbage dumps, refuse and in sewer systems and residing in warehouses and factories. It is possible less numbers were trapped due to the placement of traps which were not near their normal habitat.

Their low abundance of other species suggests that these rats live in small pocket communities within the dominant rat species population. It would therefore be of some interest to investigate how the predominant rat species affects the subsistence of these minority species.

Males rats are known be more active, roam further and are territorial compared to females but in this study both sexes are found actively scavenging for food at night. This also means that during capture, the majority of females were not nursing young and could be in their estrus stage hence their high activity thus explain their high capture numbers. Female rats are more active and aggressive in search of food when not

in littering season and spending more time in the nest/burrows to protect and feed the young.

The urban rat population structure was made primarily of adult rats compared sub adults and juvenile. The adult rats are known to also spend a lot of time out roaming further for food and mate and also require higher intake of food compared to the rest. Juveniles tend to roam closer to the nest attributing to the smaller home range. Male adults rats are also territorial thus limiting the range for juveniles to roam. It is also speculated that the disproportionate number of adult rat is generated by unbalanced social competition, restricting the overall movement of the younger rats.

In order to control the rodent population successfully, the municipal of each city plays an important role. Hygiene and sanitation is a key factor that in establishing a successful rodent control program in homes, restaurants and various other industries. This should be in combination with a variety of methods including better garbage disposal system in addition to combat rodent infestation with the use of trapping, thermal fogging and rodenticides to combat infestation in hotspot locations such as public food courts. Community awareness campaigns have also been introduced in many cities such cash award in exchange with the number of rodents caught.

2.5 CONCLUSION

The urban wild rat population structure in urban cities in Peninsular Malaysia is described and comprised of six species with *Rattus rattus diardii* the most dominant followed by *Rattus norvegicus*, *Rattus exulans*, *Rattus tiomanicus*, *Rattus argentiventer* and *Rattus annandalei*. The present observations of diversity were consistent with previous records. The high numbers of rodent in the urban cities can be due to human activities that favours the establishment and continuation of the commensal rat population. More female rats were captured compared to males meanwhile higher number adult rats captured compared to juveniles. Higher number of rats was also captured during wet season compared to dry season. The host-sex and age distribution can be explained by the rodent's inherent social behavior. This study highlights the importance of better management of rodent control and public awareness in ensuring a successful rodent control program that involves getting both the community and health authorities involved in rodent control and better understanding on diseases transmission by rodents.

CHAPTER 3

THE DETECTION OF THE BLOOD PROTOZOANS IN THE URBAN RAT POPULATION OF PENINSULAR MALAYSIA USING GIEMSA-STAINED THIN FILM

3.1 INTRODUCTION

Rodents are widely distributed throughout the world and account for about forty percent of the mammals living at the present time. In many regions, however, the total number of individual rodents is probably many times greater than that of all other mammals. It is therefore not surprising that rodents have come to play a major role in harbouring and transmitting many diseases to other mammals, including man. In the transmission of human disease, the extent to which some rodents have become adapted to man-made environments is at least as important as rodent numbers.

In the recent past, urban development and intensive agriculture have been extended to meet the needs of rapidly growing populations. During this period, many rats have remained more or less undisturbed in forest and mountainous regions, but even here they may still cause concern when they increase in number and move out into neighbouring areas, temporarily damaging crops and spreading disease.

Rodents are a key mammalian group and are highly successful in adapting to many environments throughout the world. There are more than 1700 species of rodents identified in the world (RatZooMan, 2006). Rodents particularly those belonging to the family Muridae form the largest group of mammals in Malaysia (Ow-Yang, 1971). They are known to transmit diseases and act as reservoir host for many zoonotic pathogens including parasites that pose a health risk to humans (Walsh *et al*., 1993; Meyer *et al*., 1995; Singleton *et al*., 2003; Paramasvaran *et al*., 2009b).

Many studies have been done on the prevalence of parasites among wild rats throughout the world. In Malaysia the first recorded prevalence of blood parasites in wild rats was reported by Adams in 1933 (Adams, 1933). Little is known of the epidemiology of this blood parasite in rat populations in this country as no detailed and wide-spread surveys have been done. Both rural and urban areas of Malaysia are heavily infested with rats which pose a considerable risk in relation to the transmission of these parasites to man (Sinniah *et al*., 1979).

In this chapter, the urban rat populations in four urban cities in Peninsular Malaysia was investigated in terms of the demographic structure of the rat population according to species of rats, sex (male or female, age (adult, sub adult or juvenile) and season (dry or wet). Following this, the blood parasite of the rat population was determined using the Giemsa-stained thin film technique.

3.2 METHODOLOGY

Fieldwork was carried out within four major cities with each location representing different unique geographical location in Peninsular Malaysia. Those are Kuala Lumpur (3°8′51″N 101°41′36″E) representing the west, Pahang (3°49′00″N 103°20′00″E) representing the east, Penang (5°25′00″N 100°19′00″E) representing the north and Malacca (2°12′N 102°15′E) representing the south of Peninsular Malaysia.

3.2.1 Trapping

Trapping of rats was conducted for four days, three nights from November 2006 until November 2011. All the rats were trapped alive using specially made steel wire traps measuring 29 x 22 x 50 cm using dried fish, sweet potatoes, fruits and coconut as baits. Trapping were cooperated with the assigned units from each municipality from the respective city. The main criteria for site selection were proximity to housing, obscuration from public view and association with drainage defects. Each day, 30 traps

were placed at varying distances and different types at sites where most rat activity was expected. The sites were identified based on local peoples' observations of rodent activity, or from signs of rats, e.g. faeces, rat pathways or footprints. Traps were placed inside and around houses as well as in uncultivated surroundings.

3.2.1.1 Kuala Lumpur

Rats were trapped as part of a vector programme by the Vector Control Unit of Kuala Lumpur City Hall (DBKL) from November 2006 until November 2011. Sampling was done 6 times during dry season and 6 times during wet season. Rats were trapped from the market, food court and by the shop lots from areas surrounding Kuala Lumpur such as Chow Kit, Sentul, Setapak and Bukit Bintang.

Captured rats by Kuala Lumpur City Hall were brought to the Institute of Biological Sciences, University of Malaya, Kuala Lumpur. The dissection process was performed at the Parasitology Lab, Institute of Biological Sciences in Faculty of Science, University of Malaya. Rats were then euthanized with chloroform and post-mortem of the rats was conducted according to standard procedures as described by Rusli (1988).

3.2.1.2 Pahang

From November 2008 until July 2009, trapping was done with 2 times during wet season and 2 times during dry season in the vicinity of Kuantan, the state capital of Pahang. The Vector and Rodents Unit of Kuantan Municipal Council or Majlis Perbandaran Kuantan (MPK) was responsible for providing the rats. Rats were captured by using various techniques such as thermal fogging technique, pesticides, standard metal basket traps, sweep net and self-designed traps, trapping killer rat (TKR) traps that specially made by Kuantan Municipal Council in order to control diverse species of rats' population around Kuantan. After using a variety of ways, using conventional methods such as hunting with sweep net at night by the unit's staff considered the most

effective in addition to cost savings. Some locations in the city become threat rats including surrounding homes, underground holes, streets, markets, food court areas like hawker centers Tanjung Api, Sungai Kuantan and Taman Tas. Usually trapping also done at the market such as Pasar Besar Kuantan and Pasar Borong Kemunting. These rats were trapped at the night before and were collected and examined immediately the following morning at Animal House Lab of Faculty of Medicine in International Islamic University Malaysia (IIUM), Kuantan.

Figure 3.1: Thermal fogging technique used by Kuantan Municipal Council

Figure 3.2: Trapping killer rat (TKR) traps that specially made by Kuantan Municipal Council

Figure 3.3: Rats were trapped by trapping killer rat (TKR) traps

3.2.1.3 Penang

Trapping in Penang was carried out between March 2008 and May 2010 with 3 times during wet season and 3 times during dry season. Assistance in providing rats specimens were given by Public Health Unit, Municipal Council of Penang Island or Majlis Perbandaran Pulau Pinang (MPPP). Municipal Council of Penang Island killed the rats by using certain methods including the setting up of traps with bait as peanut butter and bread, fogging and poisoning.

MPPP aided in trapping rats loitering in the food courts and markets all around Georgetown. Rat traps also were placed at Campbell street market. Trapping was repeated for four consecutive nights. Traps were checked and collected every morning. The trapped rats were brought to the Parasitology Lab of School of Biological Science in University of Science Malaysia (USM) for post mortem examination.

3.2.1.4 Malacca

Trapping in Malacca was conducted between August 2009 to May 2010 with 2 times samplings were done during dry season and 2 times during wet season. Rats were captured by Lilati Control Unit of Melaka Historic City Council or Majlis Bandaraya Melaka Bersejarah (MBMB). Melaka Historic City Council captured rats in area around Malacca by the shop houses, markets, streets and food courts. The traps were set in the evening, and checked the following morning. Rats captured were brought to the Biology Lab of Melaka International College of Science and Technology (MiCoST) for further examination.

3.2.2 Morphometric examination

Trapped rats were killed humanely by placing the trapped rodent into a cloth bag containing cotton wool soaked with chloroform. Morphometric measurements of head-body, tail, ear, hind feet, body weight and physical appearances were recorded. Each rat's age, sex and species were determined. Species identification was based on descriptions by Harrison & Quah (1962), Medway (1983) and Payne & Francis (1998).

3.2.3 Blood collection

The animal is then laid on ventral side up and the thorax moistened with 70% alcohol. A syringe is pre rinsed with 0.85% Natrium Chloride (NaCl). The beating heart can be felt most strongly to the left of the sternum between the fourth and sixth ribs (approximately in line with the middle of the sternum). The needle is inserted in at an angle of 45º and blood slowly drawn out. If the suction is too strong, the wall of the heart may be pulled against the needle, thus preventing the flow of the blood into the syringe. The animal may be exsanguinated by this method; approximately 20 ml of blood may be drawn from a 350 – 400 gram of rat (5-7% of the body weight).

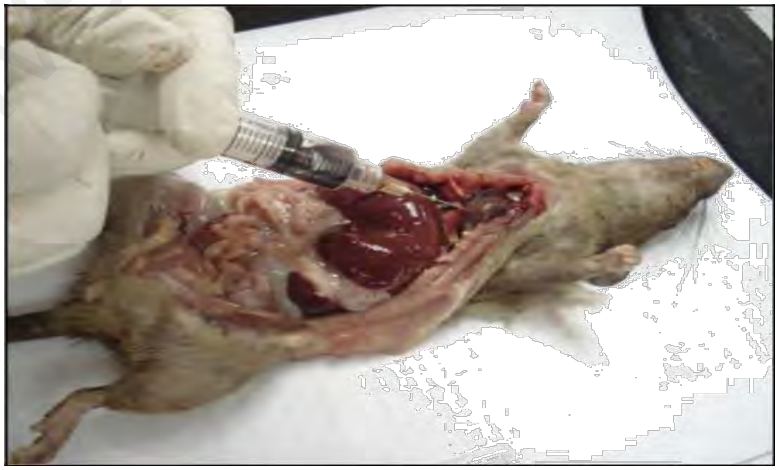

Figure 3.4: The heart puncture technique can be used by inserting the needle in a 45 degree angle directly into the heart

3.2.4 Blood thin films preparation

Slides to be used in the preparation of blood films must be clean. New slides should be alcohol-cleaned before use. If old slides are to be used, clean them first in detergent and then with 70% ethyl alcohol.

A drop of blood the size of the head of a pin is placed on one end of a microscope slide and spread the blood in an even film with another slide, tipped at an angle of about 30 - 40°. The blood spread out along the edge of contact an area of approximately 1.0-1.25 cm in diameter. A good film is thick at one end and thin at the other. Long streamers of blood indicate that the slide used as a spreader was dirty or chipped. Streaks in the film are usually caused by dust, and holes in the film indicate that grease was present on the slide. Avoid the first two drop of the blood because they are usually contaminated and too rich in platelets. The samples were dried in air dust-free area for 1 hour or longer.

3.2.5 Giemsa staining

A thin film of the specimen is fixed on a microscope slide by immersing it in pure methanol for 1 minute. Thereafter the slide is immersed to a solution of 1 part Giemsa stock to 20-30 parts of buffered water (pH 7.0 – 7.2), approximately 4% Giemsa stain solution for 20-30 minutes, then flushed with water and left to dry by air.

3.2.6 Mounting and observation

After mount the slide with neutral mountant such as DPX or Canada balsam for permanent use, the smear is examined by light microscopy under high magnification. Examine the blood films provided and try to identify gametocytes and stages of the schizogonic cycle. Red blood cells are only 7μm in diameter, and since *Plasmodium* is an intracellular parasite, need to use oil immersion and good microscopic techniques to

identify these. The analysis was performed using SPSS (Statistic Package for Social Sciences) version 12.

3.3 RESULT

3.3.1 Urban wild rat population in Peninsular Malaysia

Up to 762 wild rats were captured from 4 localities representing the west (Kuala Lumpur, n=433), east (Pahang, n=117), south (Malacca, n=111) and north (Penang, n=101). Six rat species were identified including *Rattus rattus diardii* (n=410) the most dominant rat species, followed by *Rattus norvegicus* (n=302), *Rattus exulans* (n=33), *Rattus tiomanicus* (n=8), *Rattus argentiventer* (n=7) and *Rattus annandalei* (n=2). Three hundred and thirty one rats were captured during the dry season meanwhile 431 rats during the wet season. The total number of females (n = 402 rats) outnumbered males (n = 360 rats) of which 65.9% were adults (n = 502), 20% were sub-aduts (n = 152) and 14.2% were juveniles (n = 108). The structure of the host population relative to species, age, sex and season is summarized in Table 3.1.

3.3.2 Blood parasite in urban wild rat using GTF technique

Examination blood of rat from four localities of Peninsular Malaysia using Giemsa-stained Thin Film (GTF) resulted in identification of two blood protozoans. These included *Trypanosoma* sp. and *Plasmodium* sp. from four different localities of Peninsular Malaysia i.e. Kuala Lumpur, Pahang, Malacca and Penang, are described as follows.

A total of 456 rats (59.8%) were infected with at least one blood protozoan species from two species discovered namely; *Trypanosoma* sp. and *Plasmodium* sp. with prevalence of *Plasmodium* sp. (72.4%) higher compared to *Trypanosoma* sp. (42.1%). Only 14.5% (n =66) were found infected with both species. *Rattus rattus diardii* showed the highest prevalence of infection (62.2%), with 75.7% rats infected with

Plasmodium sp., 38.8% infected with *Trypanosoma* sp. and 14.5% infected with both species (Table 3.2). According to host sex factor, the infections were more frequent in males (61.4%) compared to females (58.5%). Both sexes showed higher infections with *Plasmodium* sp. compared to *Trypanosoma* sp. with 70.1% and 74.5% respectively (Table 3.3). Relative to host-age, sub-adults were most infected (71.1%) compared to juveniles (60.2%) and adults (56.4%). All three host age group also showed higher infection with *Plasmodium* sp. compared to *Trypanosoma* sp. (Table 3.4).

From four studied locations, Kuala Lumpur (64.9%) has the highest prevalence of rats infected with blood protozoan followed by Penang (56.4%), Malacca (53.5%) and Pahang (50.4%). *Plasmodium* sp. showed higher prevalence of infection in rats compared to *Trypanosoma* sp. in all four locations studied (Table 3.5). According to the season factor, rats captured during dry season (65.9%) have the highest prevalence of infection compared to during wet season (55.2%). *Plasmodium* sp. showed higher prevalence in rats compared to *Trypanosoma* sp. during dry and wet season with 78.4% and 66.8% respectively (Table 3.6). The structure of the host infected relative to location, species, age, sex and season is summarized in Table 3.7.

3.3.3 *Trypanosoma* sp. in urban wild rat population

A total of 762 rat specimens were captured during the present study. All of these were examined for *Trypanosoma* sp. infection. Blood of each captured rat was examined by identification of various stages of *Trypanosoma* sp. using Giemsa-stained thin film technique. Of these, 192 specimens were found infected with this blood parasite; the prevalence rate was 42.1 % (Table 3.8).

In Kuala Lumpur, a total of 433 commensal rats were scanned for the presence of *Trypanosoma* sp. infection, of which 112 (39.9%) were found positive (Table 3.8). The highest (71.4%) prevalence was recorded for *Rattus rattus diardii*

followed by *Rattus norvegicus* (15.2%), *Rattus exulans* (7.1%), *Rattus tiomanicus* (3.6%), *Rattus argentiventer* (1.8%) and *Rattus annandalei* (0.9%). The prevalence rate was slightly higher in males (61.6%) than in females (38.4%). The infection rate was the highest (48.2%) in adult rats followed by sub-adult rats (32.1%) and juveniles (19.6%). Most infected rats were captured during wet season (57.1%) compared during dry season (42.9%).

Of these 117 rats were captured in Pahang, 26 (44.1%) were found positive for *Trypanosoma* sp. infection. Only one species of *Rattus norvegicus* was found. The prevalence was higher in females (57.7%) than in males (42.3%). The adult rats were more infected (88.5%) than sub-adult rats (7.7%) and juvenile rats (3.8%). 13 rats each were found positive for *Trypanosoma* sp. from wet season and dry season (Table 3.8).

The rat infection in Malacca comprised of the following species; the highest infection in *Rattus norvegicus* (78.0%) followed by *Rattus rattus diardii* (20.3%) and *Rattus argentiventer* (1.7%). More female rats were infected compared to male rats with 42 (71.2%) females and 17 (28.8%) males respectively. According to host age, there were less juveniles (n=2) infected compared to adults (n=47) and sub-adult (n=10). More rats were infected in dry season, 62.7% compared to wet season, 37.3% (Table 3.8).

From the total of 101 wild rats captured from Penang, 26 rats (45.6%) were found infected with *Trypanosoma* sp. (Table 3.8). *Rattus rattus diardii* (53.8%) was shown to have the higher infection than *Rattus norvegicus* (46.2%). More females were infected compared males with 57.7% and 42.3 % respectively. The infection rate was statistically higher in adult (65.4%) rats than in sub-adult (26.9%) rats and juvenile (7.7%). High number of rats was infected during the wet season (88.5%) compared to the dry season (11.5%).

3.3.4 *Plasmodium* sp. in urban wild rat population

Blood samples of 762 rats were examined by observation of ring stage of *Plasmodium* sp. using Giemsa-stained thin film technique. Of these, 330 were found infected; the prevalence rate was 72.4% (Table 3.9).

From a total of 433 rats captured in Kuala Lumpur, 221 (78.6%) rats were infected with *Plasmodium* sp. (Table 3.9). *Rattus rattus diardii* (77.4%) was shown to have the highest infection followed by *Rattus norvegicus* (10.0%), *Rattus exulans* (8.6%), *Rattus tiomanicus* (2.7%), *Rattus argentiventer* (0.9%) and *Rattus annandalei* (0.5%). 52.5% of the females (n = 116) and 47.5% of the males (n = 105) were infected. Most adults (51.6%) were infected followed by sub-adults (30.8%) and juveniles (17.6%). The lower infection rate of this parasite was recorded during wet season (46.2%) compared to infection rate during dry season (53.8%).

In Pahang, 40 out of 117 urban wild rats infected were *Rattus norvegicus* (Table 3.9). More rat were infected during the dry season (n=25) compared to the wet season (n=15) with more males (n=21) compared to females (n=19). The rat infection in Pahang also comprised of mainly adults (87.5%) captured compared to sub-adults (7.5%) and juveniles (10.7%).

In Malacca, 38 urban wild rats were infected with *Plasmodium* sp. *Rattus norvegicus* (76.3%) showed the highest number of rat species infected compared to *Rattus rattus diardii* (21.1%) while the lowest rat was *Rattus argentiventer* (2.6%). More females (n=27) were infected compared to males (n=11). Twenty two rats were infected during dry season and 16 rats were infected during wet season with most adult rats (81.6%) were infected compared to sub-adults (18.4%) and no juveniles were infected with this parasite (Table 3.9).

In Penang, 31 rats (54.4%) captured were found infected with *Plasmodium* sp. High number of *Rattus norvegicus* (n=17) were infected compared to *Rattus rattus diardii* (n=14). According to the host age, most adults (n=23) were infected compared to sub-adults (n=5) and juveniles (n=3). There were more males (n=18) compared to females (n=13) infected. Less rats were infected during the dry (5 rats) compared to the wet (26 rats) season (Table 3.9).

Table 3.1: The structure of the host population relative to species, age, sex and season

Location		Species of Rat						Host sex		Host Age			Season	
		RRD	**RN**	**RE**	**RT**	**RA**	**RAn**	**Female**	**Male**	**A**	**SA**	**J**	**Dry**	**Wet**
Kuantan	**n**	0	117	0	0	0	0	61	56	105	7	5	63	54
	%		100.0					52.1	47.9	89.7	6.0	4.3	53.9	46.2
Malacca	**n**	35	75	0	0	1	0	67	44	86	15	10	63	48
	%	31.5	67.6			0.9		60.4	39.6	77.5	13.5	9.0	56.8	43.2
Georgetown	**n**	48	53	0	0	0	0	51	50	78	8	15	13	88
	%	47.5	52.5					50.5	49.5	77.2	7.9	14.9	12.9	87.1
Kuala Lumpur	**n**	327	57	33	8	6	2	223	210	233	122	78	192	241
	%	75.5	13.2	7.6	1.9	1.4	4.6	51.5	48.5	53.8	28.2	18.0	44.3	55.7
Total	**n**	**410**	**302**	**33**	**8**	**7**	**2**	**402**	**360**	**502**	**152**	**108**	**331**	**431**
	%	**53.8**	**39.6**	**4.3**	**1.1**	**0.9**	**0.3**	**52.8**	**47.2**	**65.9**	**20.0**	**14.2**	**43.4**	**56.6**

*RRD-*Rattus rattus diardii*; RN-*Rattus norvegicus*; RE-*Rattus exulans*; RT-*Rattus tiomanicus*; RA-*Rattus argentiventer*; RAn-*Rattus annandalei*;

A-adult; SA-Sub-adult; J-Juvenile.

Table 3.2: Prevalence of blood protozoan in urban wild rats according to species of rats using Giemsa-stained thin film **(**GTF) technique

Species of rodent	**Number of rodents examined**	**Number of rodents positive with blood protozoan (% positive)**	**Giemsa-stained Thin Film (GTF)**		
			***Trypanosoma* sp**	***Plasmodium* sp**	**Multiple infections**
Rattus rattus diardii	410	255 (62.2%)	99 (38.8%)	193 (75.7%)	37 (14.5%)
Rattus norvegicus	302	167 (55.3%)	78 (46.7%)	108 (64.7%)	19 (11.4%)
Rattus exulans	33	23 (69.7%)	8 (34.8%)	19 (82.6%)	4 (17.4%)
Rattus tiomanicus	8	7 (87.5%)	4 (57.1%)	6 (85.7%)	3 (42.9%)
Rattus argentiventer	7	3 (42.9%)	2 (66.7%)	3 (100.0%)	2 (66.7%)
Rattus annandalei	2	1 (50.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)
Total	**762**	**456 (59.8%)**	**192 (42.1%)**	**330 (72.4%)**	**66 (14.5%)**

Table 3.3: Prevalence of blood protozoan in urban wild rats according to sex of rats using Giemsa-stained thin film **(**GTF) technique

Sex of rodent	**Number of rodents examined**	**Number of rodents positive with *blood protozoan* (% positive)**	**Giemsa-stained Thin Film (GTF)**		
			***Trypanosoma* sp**	***Plasmodium* sp**	**Multiple infections**
Female	402	235 (58.5%)	88 (37.4%)	175 (74.5%)	28 (11.9%)
Male	360	221 (61.4%)	104 (47.1%)	155 (70.1%)	38 (17.2%)
Total	**762**	**456 (59.8%)**	**192 (42.1%)**	**330 (72.4%)**	**66 (14.5%)**

Table 3.4: Prevalence of blood protozoan in urban wild rats according to age of rats using Giemsa-stained thin film **(**GTF) technique

Age of rodent	**Number of rodents examined**	**Number of rodents positive with *blood protozoan***	**Giemsa-stained Thin Film (GTF)**		
		(% positive)	***Trypanosoma* sp**	***Plasmodium* sp**	**Multiple infections**
Adult	502	283	115	203	35
		(56.4%)	(40.6%)	(71.7%)	(12.4%)
Sub Adult	152	108	45	83	20
		(71.1%)	(41.7%)	(76.9%)	(18.5%)
Juvenile	108	65	32	44	11
		(60.2%)	(49.2%)	(67.7%)	(16.9%)
Total	**762**	**456**	**192**	**330**	**66**
		(59.8%)	**(42.1%)**	**(72.4%)**	**(14.5%)**

Table 3.5: Prevalence of blood protozoan in urban wild rats according to location of rats using Giemsa-stained thin film **(**GTF) technique

Location of rodent captured	**Number of rodents examined**	**Number of rodents positive with *blood protozoan***	**Giemsa-stained Thin Film (GTF)**		
		(% positive)	***Trypanosoma* sp**	***Plasmodium* sp**	**Multiple infections**
Kuala Lumpur	433	281 (64.9%)	112 (39.9%)	221 (78.6%)	52 (18.5%)
Pahang	117	59 (50.4%)	26 (44.1%)	40 (67.8%)	7 (11.9%)
Malacca	111	59 (53.2%)	28 (47.5%)	38 (64.4%)	7 (11.9%)
Penang	101	57 (56.4%)	26 (45.6%)	31 (54.4%)	0 (0.0%)
Total	**762**	**456 (59.8%)**	**192 (42.1%)**	**330 (72.4%)**	**66 (14.5%)**

Table 3.6: Prevalence of blood protozoan in urban wild rats according to season of rats using Giemsa-stained thin film **(**GTF) technique

Season of rodent captured	**Number of rodents examined**	**Number of rodents positive with *blood protozoan***	**Giemsa-stained Thin Film (GTF)**		
		(% positive)	***Trypanosoma* sp**	***Plasmodium* sp**	**Multiple infections**
Wet	431	238	108	159	29
		(55.2%)	(45.4%)	(66.8%)	(12.2%)
Dry	331	218	84	171	37
		(65.9%)	(38.5%)	(78.4%)	(17.0%)
Total	**762**	**456**	**192**	**330**	**66**
		(59.8%)	**(42.1%)**	**(72.4%)**	**(14.5%)**

Table 3.7: The summary of results on host location, species, age, sex, season that were infected with blood protozoans as identified with GTF technique

Location		Species of Rat						Host sex		Host Age			Season	
		RRD	RN	RE	RT	RA	RAn	Female	Male	A	SA	J	Dry	Wet
Kuala Lumpur	**n**	215	33	23	7	2	1	138	143	143	87	51	140	141
	%	76.5	11.7	8.2	2.5	0.7	0.4	49.1	50.9	50.9	31.0	18.1	49.8	50.2
Pahang	**n**	-	59	-	-	-	-	27	32	53	4	2	33	26
	%		100					45.8	54.2	89.8	6.8	3.4	55.9	44.1
Malacca	**n**	12	46	-	-	1	-	42	17	47	10	2	37	22
	%	20.3	78.0			1.7		71.2	28.8	79.7	16.9	3.4	62.7	37.3
Penang	**n**	28	29	-	-	-	-	28	29	40	7	10	8	49
	%	49.1	50.9					49.1	50.9	70.2	12.3	17.5	14.0	86.0
Total	**n**	**255**	**167**	**23**	**7**	**3**	**1**	**235**	**221**	**283**	**108**	**65**	**218**	**238**
	%	**55.9**	**36.6**	**5.0**	**1.5**	**0.7**	**0.2**	**51.5**	**48.5**	**62.1**	**23.7**	**14.3**	**47.8**	**52.2**

*RRD-*Rattus rattus diardii*; RN-*Rattus norvegicus*; RE-*Rattus exulans*; RT-*Rattus tiomanicus*; RA-*Rattus argentiventer*; RAn-*Rattus annandalei*;

A-adult; SA-Sub-adult; J-Juvenile.

Table 3.8: The structure of the host infected with *Trypanosoma* sp. relative to location, species, age, sex and season using GTF technique

Location		Species of Rat						Host sex		Host Age			Season	
		RRD	RN	RE	RT	RA	RAn	Female	Male	A	SA	J	Dry	Wet
Kuala Lumpur	**n**	80	17	8	4	2	1	43	69	54	36	22	48	64
	%	71.4	15.2	7.1	3.6	1.8	0.9	38.4	61.6	48.2	32.1	19.6	42.9	57.1
Pahang	**n**	-	26	-	-	-	-	11	15	23	2	1	13	13
	%		100					42.3	57.7	88.5	7.7	3.8	50.0	50.0
Malacca	**n**	5	23	-	-	-	-	19	9	21	5	2	20	8
	%	17.9	82.1					67.9	32.1	78.0	17.9	7.1	71.4	28.6
Penang	**n**	14	12	-	-	-	-	15	11	17	2	7	3	23
	%	53.8	46.2					57.7	42.3	65.4	7.7	26.9	11.5	88.5
Total	**n**	**99**	**78**	**8**	**4**	**2**	**1**	**88**	**104**	**115**	**45**	**32**	**84**	**108**
	%	**51.6**	**40.6**	**4.2**	**2.1**	**1.0**	**0.5**	**45.8**	**54.2**	**59.9**	**23.4**	**16.7**	**43.8**	**56.2**

*RRD-*Rattus rattus diardii*; RN-*Rattus norvegicus*; RE-*Rattus exulans*; RT-*Rattus tiomanicus*; RA-*Rattus argentiventer*; RAn-*Rattus annandalei*;

A-adult; SA-Sub-adult; J-Juvenile.

Table 3.9: The structure of the host infected with *Plasmodium* sp. relative to location, species, age, sex and season using GTF technique

Location		Species of Rat						Host sex		Host Age			Season	
		RRD	**RN**	**RE**	**RT**	**RA**	**RAn**	**Female**	**Male**	**A**	**SA**	**J**	**Dry**	**Wet**
Kuala Lumpur	**n**	171	22	19	6	2	1	116	105	114	68	39	119	102
	%	77.4	10.0	8.6	2.7	0.9	0.5	52.5	47.5	51.6	30.8	17.6	53.8	46.2
Pahang	**n**	-	40	-	-	-	-	19	21	35	3	2	25	15
	%		100					47.5	52.5	87.5	7.5	5.0	62.5	37.5
Malacca	**n**	8	29	-	-	1	-	27	11	31	7	-	22	16
	%	21.1	76.3			2.6		71.1	28.9	81.6	18.4		57.9	42.1
Penang	**n**	14	17	-	-	-	-	13	18	23	5	3	5	26
	%	45.2	54.8					41.9	58.1	74.2	16.1	9.7	16.1	83.9
Total	**n**	**193**	**108**	**19**	**6**	**3**	**1**	**175**	**155**	**203**	**83**	**44**	**171**	**159**
	%	**58.5**	**32.7**	**5.8**	**1.8**	**0.9**	**0.3**	**53.0**	**47.0**	**61.5**	**25.2**	**13.3**	**51.8**	**48.2**

*RRD-*Rattus rattus diardii*; RN-*Rattus norvegicus*; RE-*Rattus exulans*; RT-*Rattus tiomanicus*; RA-*Rattus argentiventer*; RAn-*Rattus annandalei*;

A-adult; SA-Sub-adult; J-Juvenile.

Table 3.10: The structure of the host infected relative to species, age, sex and season using GTF technique

Rat species		Host sex		Host Age			Season	
		Female	**Male**	**A**	**SA**	**J**	**Dry**	**Wet**
Rattus rattus	**n**	127	128	141	76	38	119	136
diardii	**%**	49.8	50.2	55.3	29.8	14.9	46.7	53.3
Rattus	**n**	88	79	131	24	12	82	85
norvegicus	**%**	52.7	47.3	78.4	14.4	7.2	49.1	50.9
Rattus	**n**	15	8	7	5	11	16	7
exulans	**%**	65.2	34.8	30.4	21.7	47.8	69.6	30.4
Rattus	**n**	4	3	2	1	4	1	6
tiomanicus	**%**	57.1	42.9	28.6	14.3	57.1	14.3	85.7
Rattus	**n**	1	2	1	2	-	-	3
argentiventer	**%**	33.3	66.7	33.3	66.7			100.0
Rattus	**n**	-	1	1	-	-	-	1
annandalei	**%**		100.0	100.0				100.0
Total	**n**	**235**	**221**	**283**	**108**	**65**	**218**	**238**
	%	**51.5**	**48.5**	**62.1**	**23.7**	**14.3**	**47.8**	**52.2**

* A-adult; SA-Sub-adult; J-Juvenile.

3.4 DISCUSSION

Previous studies on blood protozoan from wild rats in Peninsular Malaysia were limited to one or certain location namely; in Pahang (Yap *et al*., 1977); Kuala Lumpur (Zainal-Abidin & Noor Azmi, 1999; Paramasvaran *et al*., 2003 Siti Shafiyyah *et al*., 2012 and Ipoh (Premaalatha *et al*., 2010) and generally involved smaller sampling numbers. The present study reports for the first time a study of blood protozoan infection in urban rats from four major cities namely; Kuantan, Georgetown, Malacca and Kuala Lumpur.

Two blood protozoan species were found infecting the rodent population namely; *Trypanosoma* sp. and *Plasmodium* sp. This study also records the first time, *Plasmodium* sp. infecting the rodent population unlike previous studies which only noted one specie *Trypanosoma lewisi* (Zainal-Abidin & Noor Azmi, 1999; Siti Shafiyyah *et al*., 2012), while the rest failed to detect any infection (Paramesvaran *et al*., 2003 & Premaalatha *et al*., 2010). No *Babesia* sp. infection was recorded in this study as previously also mentioned by Paramasvaran *et al*. (2003). Highest infection of blood protozoans reported from rats in Kuala Lumpur from this study. Siti Shafiyyah *et al.* (2012) also reported high prevalence of intestinal parasites from rats in the urban city of Kuala Lumpur. These situations showed the ever increasing amount of garbage collected and the rise in the number of slums in big cities contributing to the high prevalence of rats infected with parasites (Siti Shafiyyah *et al*., 2012).

Other similar studies reporting *Plasmodium* sp. infection from rats were *Plasmodium berghei* from *Rattus norvegiens* and *Rattus rattus* (Ramakrishnan & Prakash, 1950); *Plasmodium* sp. from rats (Kreier *at al.,* 1972) and *Plasmodium* sp. from small rodents of Kakamega Forest in Western Kenya (Makokha *et al*., 2011). Makokha *et al*. (2011) reported low prevalence of *Plasmodium* sp. infections with 6.78% and 3.70% in

Praomys jacksoni and *Mastomys* sp., respectively. Meanwhile, Ramakrishnan & Prakash (1950) reported on behavior and appearence of *Plasmodium berghei* in rat and Kreier *et al*. (1972) reported on the relationship between erythrocyte morphology and parasitization of *Plasmodium* sp. on rats.

Trypanosoma lewisi infections records throughout the world in *Rattus norvegicus* in Sri Lanka (Sannasuriya *et al*., 1999), *Rattus* and *Bandicota* species in Thailand (Jittapalapong *et al*., 2008), *Rattus norvegicus* in Brazil (Linardi & Botelho, 2002), black rats in Niger, West Africa (Dobigny *et al*., 2011), free living rats in Poland (Karbowiak & Wita, 2001), small rodents of Kakamega Forest in Western Kenya (Makokha *et al*., 2011), in northern Iraq (Molan & Hussein, 1988) and in Ibadan (Akinboade *et al*., 1981). A *Trypanosoma lewisi*-like haemoflagellate has also been reported in a single *Rattus tiomanicus* during a field study of small wild mammals in Central Pahang (Yap *et al*., 1977).

Trypanosoma (*Herpetosoma*) *lewisi* parasitizes synanthropic rodents of the genus *Rattus* via the rat-flea as vector (Pedro & Jose, 2002). Rats are infected principally by oral route, through ingestion of flea faeces or fleas. Although Herpetesoma species are considered specific to a single vertebrate host genus, they infect a relatively broad range of flea vectors (Molyneux, 1969; Linardi & Botelho, 2002; Desquesnes *et al*., 2002). *Xenopsylla cheopis, Nosopsyllus fasciatus, Ctenocephalides canis* and *Ctenocephalides felis* have been incriminated as intermediate hosts (Molyneux, 1969). *Trypanosome lewisi* is an animal species and is usually nonpathogenic in humans but can acquire the desired virulence and emerge as human pathogen causing serious problems in the right combination of environment, host and organism related factors. *Trypanosome lewisi* infection was previously reported in a 45- day-old Thai infant who displayed fever, anaemia, cough and anorexia (Sarataphan *et al*., 2007). It was also reported in a two

months old infant in urban Mumbai, India (Kaur *et al*., 2007) and in a 4-month-old Malaysia infant with a 3-week history of lassitude, loss of appetite, feverish and anaemic with a heavy trypanosome infection upon admission (Johnson, 1933). Dissanike *et al*. (1974) also reported two cases of trypanosome infections in the Orang Asli in west Malaysia. Recently, trypanosomes of *Trypanosoma lewisi* were observed in the peripheral blood smear of a 37-day-old Indian infant admitted with fever and convulsions (Verma *et al*., 2011).

Trypanosoma lewisi infections were found higher in male compared to female rats as also noted by Linardi & Botelho (2002) and attributed to the ecological and behavioral conditions. Male rats are territorial and with a wider home range. This behavior exposes the host to a higher risk to *Xenopsylla cheopis* infestation (Linardi *et al*. 1985a) and *Trypanosoma* infection. According to host-age, present study showed higher infections in juveniles compared to sub-adults and adults.. Similar finding also found infections higher in younger rats in Brazil (Linardi & Botelho, 2002), Norway (Eyles, 1952) and Hamakua District, Island of Hawaii (Kartman, 1954). Linardi & Botelho (2002) reported the prevalence of infection in young animals (29.3%) was similar to that of immatures (27.1%) with at least three times higher than adults (8.8%). However, his finding contradicted with Ugbomoiko (1997) which observed higher infections in adult rats.

Plasmodium causes malaria. It is also now recognized that malaria caused by certain species of plasmodia of non-human primates and can be classified as zoonotic (Contacos & Coatney, 1963). Malaria is the most important parasitic disease in the tropics with almost 300 million clinical cases of malaria occurring worldwide each year and over one million people dying (Muentener, 1999). Global death figures from malaria ranged from 1.5 to 2.7 million people, most of whom are children under 5 years of age and pregnant women (Sturchler, 1989). Malaria reduces economic productivity

due to absenteeism from school and places of work during severe attacks (Molta, 2000). Malaria is highly endemic in the east Malaysian State Sabah and in Pahang, Kelantan and Perak in Peninsular Malaysia. In 1993 about 73% of a total of 39,890 cases were reported from Sabah (VBDCP, 1993). This disease is still one of the main causes of morbidity in several rural areas particularly among the aborigine population who live nomadic life and where the application of the intra domiciliary residual spraying have not been too successful due to the nature of houses. The use of larvicides such as temephos for the control of vector however is limited to the sub urban areas for the purpose of preventing reintroduction of malaria to these areas which has been under control.

Separate syringe are used for different rats as to not cause contamination by the blood samples. The syringes used were already sterilized by the manufacturer. The blood smears should be even and thin, so that the red blood cells form the single layer. The thinness of the smear is dependent on the acuteness of angle of the smear. The quantity of the blood should be small so that the tail of the smear tapers off before reaching the end of the slide. It is also important that the edge of the spreading slide is smooth and that the spreading movement a steady in order to get an even smear. New smear should be used for all new samples.

Smear should then be kept separately and protected from contamination or flies. Sunlight and heat should be avoided. The slides should be fixed and stained fast as they will be of better quality. Old film stains do not stain as well as new ones. Fixation done by methanol gives the best result, but it takes more time. It is more use full when staining large number of smears in laboratory. Fixation is normally done for one minute and then stained with Giemsa stain that is diluted in ratio of 1:45 with distilled water. The procedure takes about 30-45 minutes.

Staining jars, in which the slides are maintained in a vertical position by groves. It is convenient to use two jars. One for methanol which can be used several times given that the methanol does not evaporate and one used for diluted Giemsa stain. After fixation with methanol, the slides are transferred to the staining jar into which the diluted stain is then poured carefully until it covers the edges of the slides entirely. When preparing the stain and during the staining process, avoid shocks and contact with metal (e.g. forceps) of the diluted Giemsa, as this may induces precipitation of the stain.

After staining, tap water is used to flush out the staining solution with its covering film consisting of fine precipitate. After washing, the slides can be put into a vertical position for drying. Drying may be accelerated by using an electrical hair drier, and this is particularly useful in a humid atmosphere, but avoids using of excessive heat. Also avoid drying smears on filter paper, as this can damage the smear and moreover result in transferring cells and parasites from the smear to the filter paper and later to another smear. Observation can then be carried out under a compound microscope. Magnifications of 40X and 100X is normally used.

It is noticeable that in the microscopic observation of fresh blood preparation can be some confusion, since *Trypanosoma lewisi* can dash through the microscopic field. In the adult form, the posterior end of the parasite is very thin, long and rigid, which is clearly seen. It is noticeable that the thin straight posterior end of the parasite retains very few stains and may not be clearly visible. Over-staining can help to make it more visible. The nucleus is anterior, the kinetoplast is far from the posterior extremity, which is very sharp and thin and the kinetoplast is oval, placed transversally to the body.

Immature forms are not very uniform and can also be confused with typical forms of another Trypanosome. Identification must be performed by examination of stained stage

of smears prepared daily from the same rat, to allow the observation of all life cycle stages of the parasite. Immature forms offer several morphological features which are epimastigote, binary dividing epimastigote, and multiple-dividing epimastigote. At these stages the stain is well retained by the cytoplasm and gives a granular aspect to the body which can be very large and contain several nuclei, amastigote and trypomastigote.

Identification of the trypanosomes was performed by microscopic observation of fresh rat blood and stained blood smears. Identification was based on morphological features of the parasites. A better distinction is given by microscopic observation of Giemsa-stained blood smears, where the parasites appear. Depending on the incubation period, parasite appeared as classical adult slender forms or as immature forms.

The etiologic agents of many serious infectious diseases utilize invertebrate hosts during a portion of their life cycle. Most of these agents are adapted to haematophagous arthropod that share their vertebrate hosts, and identification of arthropod vectors and vertebrate reservoirs is usually hint to sustained control of vector-borne diseases.

Trapping the wild rats was done in set scheduled, two weeks for one trapping and the trap was left open for three days and was replaced by other traps if the first trap used already had rat. Trapping was supposed to be done in three months was continued until five months because less amount of rat were captured in three months. The other problem happened such as raining on the day trapping should be done and need to postpone it to the next day. Less of traps provided also because the traps cannot be replaced with the unused one.

The bait used to attract the rat was salted fish, coconut and bread. The bait was replaced with a new one on the next day to maintain the freshness. Various kind of bait was used in one time to identify which bait the rats like the most. When used the salted

fish as the bait, many rats were captured rather than other bait used. This shows that rats like the salted fish as bait may be because of the smelly odour of the fish that attracted them.

The rodents were then preserved at animal house and were given food and water every day to prevent them from death or hurting themselves by acting aggressively when they are hungry. Water and food was checked twice a day (morning and evening) to make sure the quantity was enough until the day they will be dissected.

The hygiene of the traps and environment of the animal captivity should be a concerned to avoid infection that may happen in captivity and will affect the results obtained.

In rat investigation methods, anaesthetizing the animal is the most important step. Many precaution steps such as wearing a mask, gloves and lab coat is important to make sure that no ectoparasite will infect the person in charge. Anaesthetizing the animal with chloroform must be done properly to prevent any animal running with opened abdomen in the lab that will cause a panic situation.

Thrombocytes in smears often mislead novices, as in the process of drying and disintegrating they may assume various shapes, and even resemble small trypanosomes. When a thrombocyte lies across the red cell, it is sometimes taken for an intracellular haemoparasite, such as *Babesia* sp.

Stain deposit may prevent a proper examination of the smear and are sometimes mistaken for *Anaplasma* sp. or *Eperythrozoon* sp. Flies may deposit microorganism on a fresh smear, which may be mistaken for haemoparasites, and pseudoparasites may also result from yeast or algae in the water used in the staining procedure.

3.5 CONCLUSION

This chapter was aimed to study the detection of *Trypanosoma* sp. and *Plasmodium* sp. of urban rat population in Peninsular Malaysia using Giemsa-stained thin film (GTF) technique. With regards to the results obtained it is observed that the rat blood sample showed that *Plasmodium* sp. infection (72.4%) was the most prevalent, followed by *Tryapanosoma* sp. infection (42.1%). The overall infection rates are low and thus are not a health concern for drastic measures to be taken.

The presence of parasite in urban rat population is indicative of rat population as host of possible zoonotic diseases. Hence the proper maintenance of urban environment and rodent population control are important to reduce rat human contact and impact.

From the research done shows the percentage of *Trypanosome* sp. and *Plasmodium* sp. were in the fewer amounts. So it determine the population of rat at the area of studies are not dangerous to human population and will not cause human diseases by zoonotic infection but the population must be controlled because the abundance of wild rats will cause quite a mess to human population a pest.

CHAPTER 4

THE DETECTION OF BLOOD PROTOZOANS USING QUANTITATIVE BUFFY COAT (QBC) IN WILD URBAN RATS OF PENINSULAR MALAYSIA

4.1 INTRODUCTION

In the recent years, numerous quick and new techniques for malaria diagnosis have been developed, one such being the QBC (quantitative buffy coat) technique. It has been successfully applied to the diagnosis of malaria (Sodeman, 1970; Spielman *et al*., 1988; Levine *et al.,* 1989).

In 1983, a method using a capillary tube precoated with acridine orange and containing a float was developed for the rapid quantification of leucocytes in peripheral blood. This method was termed "QBC" for "Quantitative Buffy Coat Analysis". The acridine orange stains all nucleic acid containing cells and the associated fluorescence is observable under blue-violet light through a microscope. This technique was then adopted for detection of malaria parasites as the acridine orange staining permits differential colouration of green (nucleic) and red (cytoplasm) in stained parasites. Spielman *et al*. (1988) found that the QBC method is at least eight times more sensitive than Giemsa stained thick blood smears. Rickman *et al*. (1989) found that the QBC method is easier and faster to perform than the thick smear method yielding a specificity of 98.4%. In a field study, Mak *et al.* (1992) found 55.93% sensitivity and 94.92% specificity when the QBC tube method was compared with the blood smears.

Spielman *et al*. (1988) carried out his study in laboratory setting and found that the QBC could identity 10% more positives as compared to the thick blood smears. Rickman *et al.* (1989) found that the QBC could detect as few as 3 parasite/µl giving a sensitivity of 96% (when all stages of malaria were considered) and specificity of 93%

in his hospital group patients. In a laboratory setting in Thailand, Namsiripongpum *et al*. (1991) found the QBC had a sensitivity of 99.13% and a specificity of 96.76%.

The QBC technique had a sensitivity of 70% and a specificity of 98.4% (Rickman *et al*., 1989). In another study it was found the QBC to have a sensitivity of 55.93% and a specificity of 94.92% (Mak *et al*., 1992).

The acridine orange stain, originally combined with microhematocrit centrifugation using Quantitative Buffy Coat (QBC) tubes, was regarded as an alternative to the Giemsa stain (Long *et al.,* 1991; Baird *et al*., 1992). It is modified from previous uses of the hematocrit, or packed cell volume (PCV), techniques by employing a large tube (75 mm long), using acridine orange stain and a plastic float. The fact that red cells infected by parasites are less dense than non-infected ones makes it possible to separate the parasitized red blood cells and to see the parasites under ultraviolet light illumination following high speed (12,000 rpm or 14,367 g) centrifugation for five minutes. At the granulocyte/red blood cell interface, the parasitized red cells can be obscured by the fluorescing granulocyte nuclei. The parasites and granulocyte nuclei provide bright green fluorescence but can be differentiated by their size and shape.

Considerable time and experience are required for adequate preparation and interpretation of the blood smears. A less labour-intensive alternative to this conventional technique is therefore long overdue. The principal advantages of the original QBC method were sensitivity, rapidity of staining, and ease to interpret in an endemic area. The disadvantage of the method was the requirement for a fluorescent microscope with 0.3 mm or greater working distance of objective lenses, and the QBC microcentrifuge, which are relatively expensive.

In this study we evaluated the ability of the acridine orange staining of centrifuged parasites in microhematocrit tube (QBC®) to blood protozoa infections in the urban wild rat population.

4.2 METHODOLOGY

The fieldwork and sampling technique was carried out within four major cities with each location representing different unique geographical location in Peninsular Malaysia as described in chapter 3.

4.2.1 Quantitative Buffy Coat (QBC) Technique

The QBC glass capillary tube (Becton Dickinson) is 75 mm in length and 1.677 mm in diameter (Wardlaw & Levine, 1983). The tubes are internally coated with EDTA and heparin at the fill end and with acridine orange stain and potassium oxalate at the other end. Samples of blood, 55-65 µl, were transferred to the QBC tube by capillary action. The tube was then slowly tilted and rotated for about 10 seconds between fingers to dissolve the contained residues in the blood. The QBC tube was fitted with a cap and labeled. Expansion of the centrifugally separated cell layers is achieved with a 20 mm plastic float. When the plastic float is inserted into the tube, there will be a 40 µ wide space between the float exterior and the float interior. The plastic float having a specific gravity (1.055) that is midway between that of plasma (1.028) and red blood cells (1.090) is positioned. Blood filled QBC tubes were centrifuged in a QBC microhaematocrit centrifuge (Parafuge, Becton Dickinson Incorporation, Franklin Lakes, New Jersey, United State of America) at 12,000 rpm for 5 minutes. The float expanded the buffy coat, the constituents of the blood then separated into band s according to density. The QBC tubes were placed in the notched Lucide block or an acrylic holder and the area surrounding the float just beneath the buffy coat was examined under ultraviolet light-source, and a Paralens adapter which provides the ultraviolet light through a fiber cable, is connected to an Olympus light compound

microscope. Individual cells within this layer were easily seen by microscopy; the malaria parasites staining green (DNA) and orange (RNA) under blue-violet light. The entire circumference of the tube was examined systematically while moving away from the buffy coat through the erythrocyte layer. Nucleic acid and glycosaminoglycans bind to acridine orange, and fluoresce when exposed to light with a wavelength of 490 nm. The tube was examined at four quadrants by turning the tube and at least 1-4 minute was spent on each tube before it was declared negative. No attempt was made to quantify the parasitaemia in the QBC tubes.

The principle of QBC is based on the fact that on centrifugation at a high speed, the whole blood separate into plasma, buffy coat and packed red cell layer. The float gets buoyed by the packed blood cells and is automatically positioned within the buffy coat layer. Blood cells in the buffy coat layer separate according to their densities, forming visibly discrete bands, platelets remaining at the top, lymphocytes and monocytes within the middle layer and granulocytes at the bottom.

Due to acridine orange, the malarial parasite stains green (DNA; nucleus) and orange (RNA; cytoplasm). The tube is examined in the region between the red blood cells and granulocytes and within the granulocytes and mononuclear cell layer, where parasites are most abundant.

In the present study, fluorescent microscopy was through the use of a standard light microscope provided with a battery-powered light and a special blue-violet light. Red blood cells are not stained by the dye; hence remain inconspicuous under fluorescent light while the brightly fluorescent parasites are easily seen. The nuclei of the parasites emit yellowish green fluorescence whereas the cytoplasm exhibit bright red fluorescence. The tubes were examined within 3 hours of preparation. The outlines of stained parasites are well preserved and the general morphology is similar to that in

specimens stained by the Giemsa stain (Kawamoto, 1991). In positive QBC samples, the levels of infection were recorded while those without parasites were recorded as negative.

The blood is collected into the QBC tubes by capillary action. One end of the tube is filled until blood reaches the second blood fill line but before the third fill line. Ideal blood level should be between the two lines. The tube is then kept horizontally and rolled between the fingers several times to mix the blood with the anti-coagulating agent. The tube is then tilted to allow the blood to flow to the end with the orange coated stain. The tube is rolled some more to mix it with the staining agent. It is tilted again to move it away from the orange stain to create space for the inserting the tube sealant. The index finger is placed over the end of the tube nearest to the black lines.

The tube is then firmly and gently oppressed into a tray of cristoseal plastic sealants. The cristoseal plugs one end of the tube. After this is done, a float is inserted to the other open end. The tubes are then placed into slots of the centrifuge head. Ensure the sealed end of each tube is rested against the plastic gasket at the outer rim of the head. Tubes must be fixed in the groove so that the heads are balanced. The tubes are then centrifuged for 5 minutes at 12000 rpm. Once done the blood in the tubes will be separated into different layers and is ready for observation.

The blood separates into plasma, buffy coat and packed red cell layer. This separated bands go according to densities, forming visibly discrete bands, platelets remain at the top, lymphocytes and monocytes within the middle layer and granulocytes at the bottom. Nucleic acid and glycosaminoglycans bind to acridine orange, staining the malarial parasites green (DNA; nucleus) and orange (RNA; cytoplasm).

The QBC tubes are placed in a notched Lucite block or an acrylic holder and the area surrounding the float just beneath the buffy coat the examined under oil immersion. The

tube is examined under ultra violet light-source, and a Paralense adapter which provides the ultraviolet light through a fiber cable, is connected to an Olympus light compound microscope.

The region between the red blood cells and granulocytes and within the granulocytes and mononuclear cell layer is where parasites are most abundant. Blood parasite in the QBC tubes characteristically appear as green and orange fluorescing objects, within non-fluorescing red blood cells. In positive QBC samples with presence of parasites are recorded as positive while those without parasites were recorded as negative.

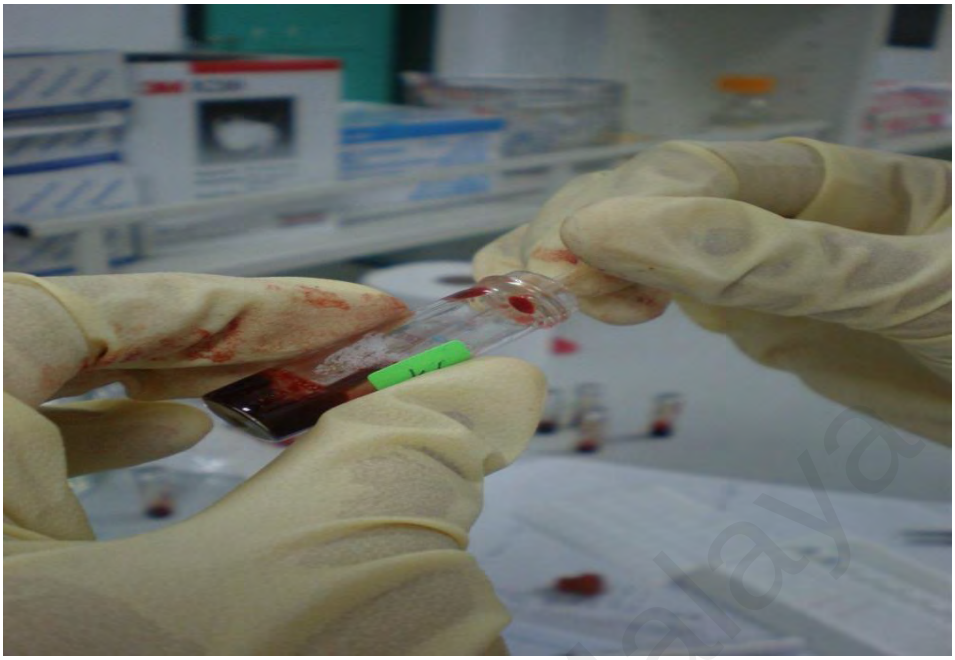

Figure 4.1: Samples of blood were transferred to the QBC tube by capillary action

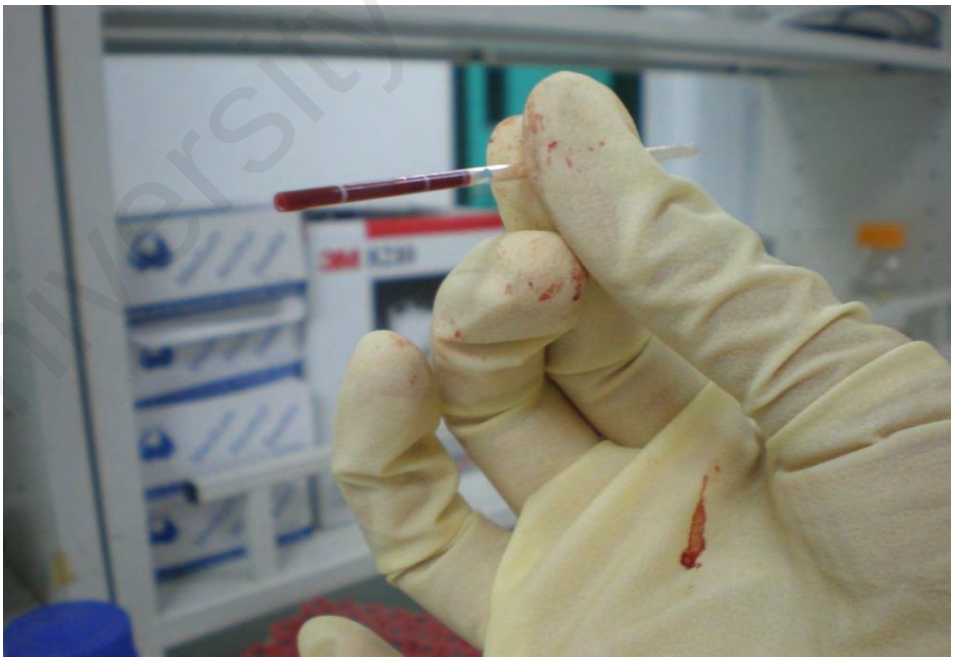

Figure 4.2: The QBC tube was slowly tilted and rotated between fingers

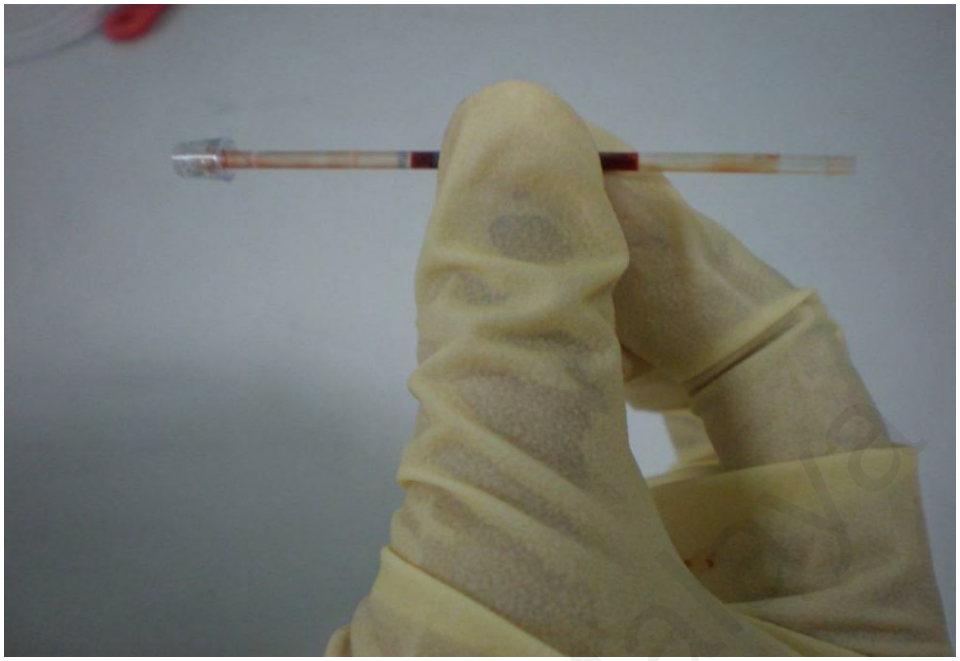

Figure 4.3: The QBC tube was fitted with a cap

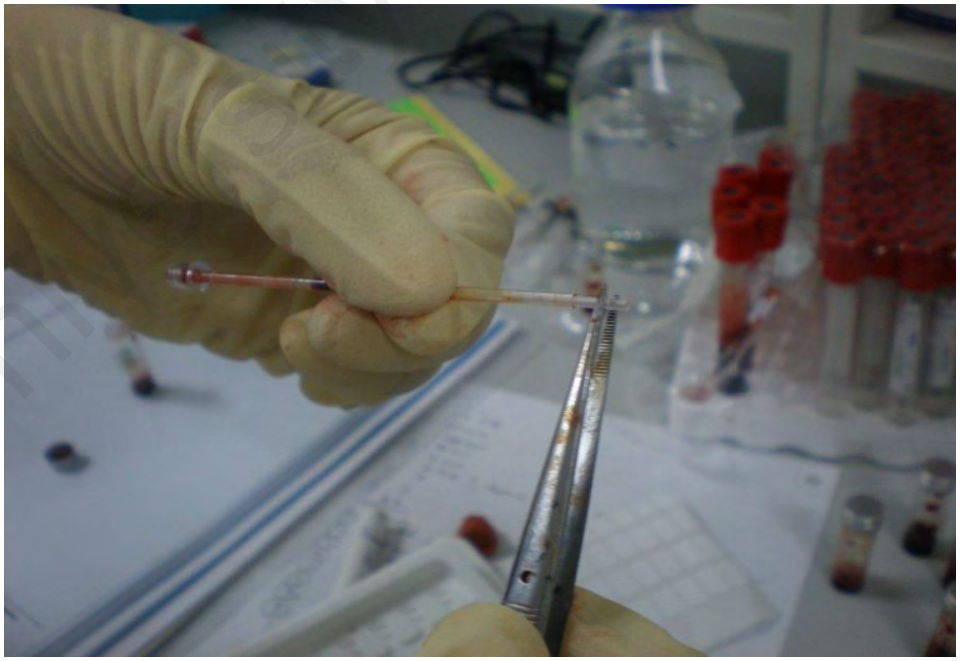

Figure 4.4: The plastic float was inserted into the QBC tube

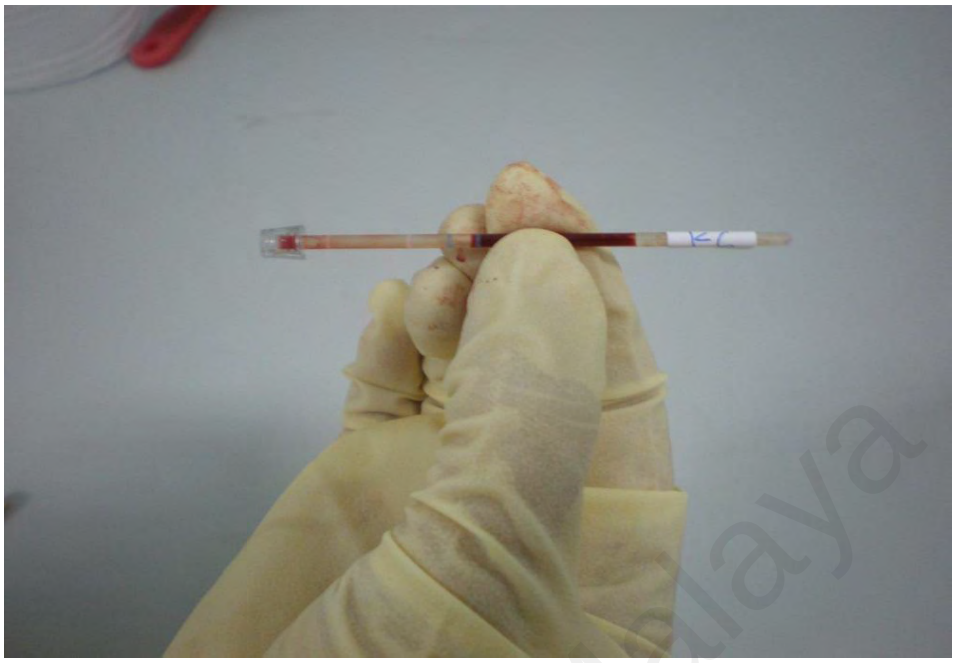

Figure 4.5: The QBC tube was labeled

Figure 4.6: The QBC tube filled with blood were centrifuged in a QBC centrifuge

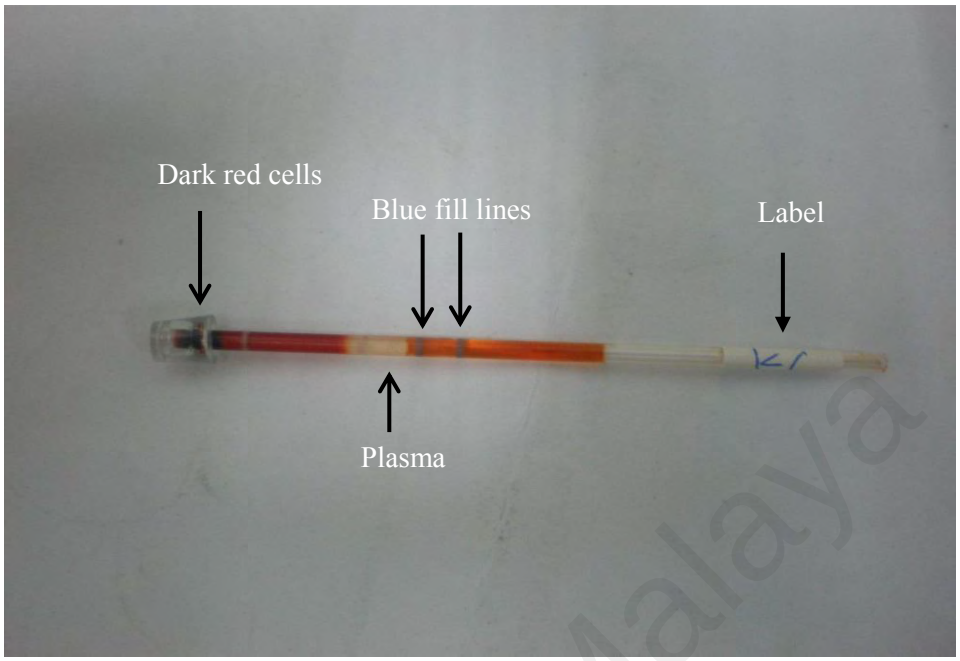

Figure 4.7: The QBC tube after centrifuging

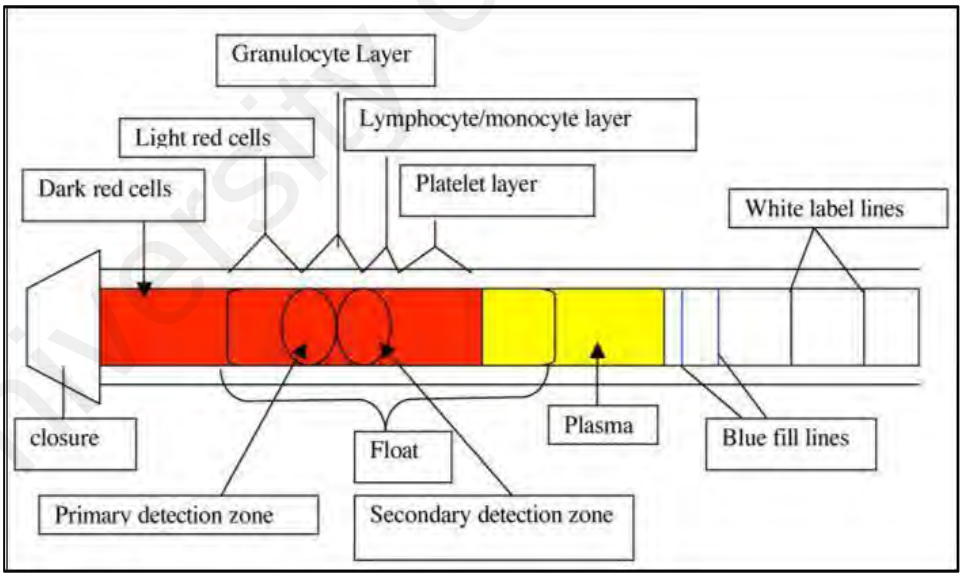

Figure 4.9: The section of blood in the QBC tube after centrifuging
(Source: http://www.cli-online.com)

4.3 RESULT

4.3.1 Blood parasite in urban wild rat using QBC technique

The examination of blood of rats from four localities of Peninsular Malaysia using Quantitative Buffy Coat (QBC) technique resulted in identification of two blood protozoans. These included *Trypanosoma* sp. and *Plasmodium* sp. from four different states of Peninsular Malaysia i.e. Kuala Lumpur, Pahang, Malacca and Penang.

A total of 202 rats (46.8%) were infected with at least one blood protozoan species from two species discovered namely; *Trypanosoma* sp. and *Plasmodium* sp. with prevalence of *Plasmodium* sp. (61.4%) higher compared to *Trypanosoma* sp. (44.6%). Only 5.9% (n =12) were found infected with both species. *Rattus norvegicus* showed the highest prevalence of infection (46.6%), with 63.1% rats infected with *Plasmodium* sp., 44.3% infected with *Trypanosoma* sp. and 7.4% infected with both species (Table 4.1). According to host sex factor, the infections were more frequent in females (47.1%) compared to males (46.4%). Both sexes showed higher infections with *Plasmodium* sp. compared to *Trypanosoma* sp. with 62.9% and 59.8% respectively (Table 4.2). Relative to host-age, juveniles were most infected (57.6%) compared to sub-adults (52.1%) and adults (44.0%). All three host age group also showed higher infection with *Plasmodium* sp. compared to *Trypanosoma* sp. (Table 4.3).

From four studied locations, Penang (48.5%) has the highest prevalence of rats infected with blood protozoan followed by Pahang (47.0%), Malacca (46.8%) and Kuala Lumpur (44.7%). *Plasmodium* sp. showed higher prevalence of infection in rats compared to *Trypanosoma* sp. in all four locations studied (Table 4.4). According to the season factor, rats captured during dry season (49.7%) have the highest prevalence of infection compared to the wet season (45.0%). *Plasmodium* sp. showed higher prevalence in rats compared to *Trypanosoma* sp. during dry and wet season with 75.0%

and 52.5% respectively (Table 4.5). The summary of the host infected relative to location, species, age, sex and season is summarized in Table 4.6.

4.3.2 *Trypanosoma* sp. in urban wild rat population

A total of 432 rat specimens were captured during the present study. All of these were examined for *Trypanosoma* sp. infection. Blood of each captured rat was examined by identification of various stages of *Trypanosoma* sp. using Quantitative Buffy Coat technique. Of these, 90 specimens were found infected with this blood parasite; the prevalence rate was 44.6% (Table 4.1-4.5).

In Kuala Lumpur, a total of 103 commensal rats were scanned for the presence of *Trypanosoma* sp. infection, of which 20 (43.5%) were found positive (Table 4.4). Only two species of rat were found with this parasite; *Rattus rattus diardii* (80.0%) and *Rattus norvegicus* (20.0%). The prevalence rate was higher in males (70.0%) than in females (30.0%). The infection rate was the highest (50.0%) in adult rats followed by sub-adult rats (35.0%) and juveniles (15.0%). Most infected rats were captured during wet season (90.0%) compare during dry season (10.0%).

Of these 117 rats were captured in Pahang, 22 of these rats were found positive for *Trypanosoma* sp. infection (Table 4.4). The prevalence was higher in males (59.1%) than in females (40.9%). The adult rats were more infected (90.9%) than sub-adult rats (9.1%) and none for juveniles. The lower infection rate of this parasite was recorded during wet season (68.2%) compared to infection rate during dry season (31.8%).

The rat infection in Malacca comprised of the following species; higher infection in *Rattus norvegicus* (72.0%) than *Rattus rattus diardii* (28.0%). More female rats were infected compared to male rats with 17 (68.0%) females and 8 (32.0%) males respectively. According to host age, there were less juveniles (n=3) infected compared

to adults (n=18) and sub-adults (n=4). More rats were infected in dry season, 56.0% compared to wet season, 44.0%.

From the total of 101 wild rats captured from Penang, 23 rats (46.9%) were found infected with *Trypanosoma* sp. (Table 4.4). *Rattus rattus diardii* (56.5%) was shown to have the higher infection than *Rattus norvegicus* (43.5%). More females were infected compared males with 52.2% and 47.8% respectively. The infection rate was higher in adult (65.2%) rats than in juvenile (26.1%) rats and sub-adults (8.7%). High number of rats was infected during the wet season (95.7%) compared to the dry season (4.3%). The species of the host infected with *Trypanosoma* sp. relative to location, species, age, sex and season is summarized in Table 4.7.

4.3.3 *Plasmodium* sp. in urban wild rat population

Blood samples of 432 rats were examined by observation of ring stage of *Plasmodium* sp. using Quantitative Buffy Coat technique. Of these, 124 were found infected; the prevalence rate was 61.4% (Table 4.1-4.6).

From a total of 103 rats captured in Kuala Lumpur, 26 (56.5%) rats were infected with *Plasmodium* sp. (Table 4.4). *Rattus rattus diardii* (57.7%) was shown to have the highest infection followed by *Rattus exulans* (23.1%) and *Rattus norvegicus* (19.2%). 53.8% of the females (n = 14) and 46.2% of the males (n = 12) were infected. Most adults (57.7%) were infected followed by juveniles (26.9%) and sub-adults (15.4%). The lower infection rate of this parasite was recorded during dry season (42.3%) compared to infection rate during wet season (57.7%).

In Pahang, 37 out of 117 urban wild rats infected were *Rattus norvegicus* (Table 4.8). More rat were infected during the dry season (n=27) compared to the wet season (n=10) with more males (n=20) compared to females (n=17). The rat infection in Pahang also

comprised of mainly adults (81.1%) captured compared to sub-adults (10.8%) and juveniles (8.1%).

In Malacca, 34 urban wild rats were infected with *Plasmodium* sp. (Table 4.4). *Rattus norvegicus* (52.9%) had the highest number of infection compared to *Rattus rattus diardii* (44.1%) while the lowest infection was *in Rattus argentiventer* (2.9%). More females (n=24) were infected compared to males (n=10). Eighteen rats caught during the dry season were infected and 16 rats caught during the wet season were infected. Most adult rats (73.5%) were infected compared to sub-adults (17.7%) and juveniles (8.8%).

In Penang, 27 rats (55.1%) captured were found infected with *Plasmodium* sp. (Table 4.4). High number of *Rattus norvegicus* (n=17) were infected compared to *Rattus rattus diardii* (n=10). According to the host age, most adults (n=20) were infected compared to sub-adults (n=2) and juveniles (n=5). There were more males (n=16) infected compared to females (n=11). Less rats were infected during the dry season (4 rats) compared to the wet (23 rats) season (Table 4.8). The results of the host infected with *Plasmodium* sp. were summarized in Table 4.8.

Table 4.1: Prevalence of blood protozoans in urban wild rats according to species of rats identified using Quantitative Buffy Coat **(**QBC) technique

Species of rodent	**Number of rodents examined**	**Number of rodents positive with blood protozoan (% positive)**	**Quantitative Buffy Coat (QBC)**		
			***Trypanosoma* sp**	***Plasmodium* sp**	**Multiple infections**
Rattus norvegicus	262	122 (46.6%)	54 (44.3%)	77 (63.1%)	9 (7.4%)
Rattus rattus diardii	159	73 (45.9%)	36 (49.3%)	40 (54.8%)	3 (4.1%)
Rattus exulans	8	6 (75.0%)	0 (0.0%)	6 (100.0%)	0 (0.0%)
Rattus argentiventer	3	1 (33.3%)	0 (0.0%)	1 (100.0%)	0 (0.0%)
Total	**432**	**202 (46.8%)**	**90 (44.6%)**	**124 (61.4%)**	**12 (5.9%)**

Table 4.2: Prevalence of blood protozoans in urban wild rats according to sex of rats identified with Quantitative Buffy Coat **(**QBC) technique

Sex of rodent	**Number of rodents examined**	**Number of rodents positive with blood protozoan (% positive)**	**Quantitative Buffy Coat (QBC)**		
			***Trypanosoma* sp**	***Plasmodium* sp**	**Multiple infections**
Female	223	105	44	66	5
		(47.1%)	(41.9%)	(62.9%)	(4.8%)
Male	209	97	46	58	7
		(46.4%)	(47.4%)	(59.8%)	(7.2%)
Total	**432**	**202**	**90**	**124**	**12**
		(46.8%)	**(44.6%)**	**(61.4%)**	**(5.9%)**

Table 4.3: Prevalence of blood protozoans in urban wild rats according to age of rats identified using Quantitative Buffy Coat **(**QBC) technique

Age of rodent	**Number of rodents examined**	**Number of rodents positive with blood protozoan (% positive)**	**Quantitative Buffy Coat (QBC)**		
			***Trypanosoma* sp**	***Plasmodium* sp**	**Multiple infections**
Adult	325	143 (44.0%)	63 (44.1%)	90 (62.9%)	10 (7.0%)
Juvenile	59	34 (57.6%)	15 (44.1%)	18 (52.9%)	0 (0.0%)
Sub Adult	48	25 (52.1%)	12 (48.0%)	16 (64.0%)	2 (8.0%)
Total	**432**	**202 (46.8%)**	**90 (44.6%)**	**124 (61.4%)**	**12 (5.9%)**

Table 4.4: Prevalence of blood protozoans in urban wild rats according to location of rats identified using Quantitative Buffy Coat **(**QBC) technique

Location of rodent captured	**Number of rodents examined**	**Number of rodents positive with *blood protozoan* (% positive)**	**Quantitative Buffy Coat (QBC)**		
			***Trypanosoma* sp**	***Plasmodium* sp**	**Multiple infections**
Pahang	117	55 (47.0%)	22 (40.0%)	37 (67.3%)	4 (7.3%)
Malacca	111	52 (46.8%)	25 (48.1%)	34 (65.4%)	7 (13.5%)
Kuala Lumpur	103	46 (44.7%)	20 (43.5%)	26 (56.5%)	0 (0.0%)
Penang	101	49 (48.5%)	23 (46.9%)	27 (55.1%)	1 (2.0%)
Total	**432**	**202 (46.8%)**	**90 (44.6%)**	**124 (61.4%)**	**12 (5.9%)**

Table 4.5: Prevalence of blood protozoans in urban wild rats according to season of rats identified using Quantitative Buffy Coat **(**QBC) technique

Season of rodent captured	**Number of rodents examined**	**Number of rodents positive with *blood protozoan* (% positive)**	**Quantitative Buffy Coat (QBC)**		
			***Trypanosoma* sp**	***Plasmodium* sp**	**Multiple infections**
Wet	271	122	66	64	8
		(45.0%)	(54.1%)	(52.5%)	(6.6%)
Dry	161	80	24	60	4
		(49.7%)	(30.0%)	(75.0%)	(5.0%)
Total	**432**	**202**	**90**	**124**	**12**
		(46.8%)	**(44.6%)**	**(61.4%)**	**(5.9%)**

Table 4.6: The structure of the host infected with both parasites relative to location, species, age, sex and season using QBC technique

Location		Species of Rat						Host sex		Host Age			Season	
		RRD	RN	RE	RT	RA	RAn	Female	Male	A	SA	J	Dry	Wet
Kuala Lumpur	**n**	31	9	6	-	-	-	20	26	25	7	14	13	33
	%	67.4	19.6	13.0				43.5	56.5	54.3	15.2	30.4	28.3	71.7
Pahang	**n**	-	55	-	-	-	-	25	30	46	6	3	33	22
	%		100					45.6	54.5	83.6	10.9	5.5	60.0	40.0
Malacca	**n**	19	32	-	-	1	-	37	15	38	8	6	29	23
	%	36.5	61.5			1.9		71.2	28.8	73	15.4	11.5	55.8	44.2
Penang	**n**	23	26	-	-	-	-	23	26	34	4	11	5	44
	%	46.9	53.1					46.9	53.1	69.4	8.2	22.4	10.2	89.8
Total	**n**	**73**	**122**	**6**	**-**	**1**	**-**	**105**	**97**	**143**	**25**	**34**	**80**	**122**
	%	**36.1**	**60.4**	**3.0**		**0.5**		**52.0**	**48.0**	**70.8**	**12.4**	**16.8**	**39.6**	**60.4**

*RRD-*Rattus rattus diardii*; RN-*Rattus norvegicus*; RE-*Rattus exulans*; RT-*Rattus tiomanicus*; RA-*Rattus argentiventer*; RAn-*Rattus annandalei*;

A-adult; SA-Sub-adult; J-Juvenile.

Table 4.7: The structure of the host infected with *Trypanosoma* sp. relative to location, species, age, sex and season using QBC technique

Location		Species of Rat						Host sex		Host Age			Season	
		RRD	**RN**	**RE**	**RT**	**RA**	**RAn**	**Female**	**Male**	**A**	**SA**	**J**	**Dry**	**Wet**
Kuala Lumpur	**n**	16	4	-	-	-	-	6	14	10	7	3	2	18
	%	80.0	20.0					30.0	70.0	50.0	35.0	15.0	10.0	90.0
Pahang	**n**	-	22	-	-	-	-	9	13	20	2	-	7	15
	%		100.0					40.9	59.1	90.9	9.1		31.8	68.2
Malacca	**n**	7	18	-	-	-	-	17	8	18	4	3	14	11
	%	28.0	72.0					68.0	32.0	72.0	16.0	12.0	56.0	44.0
Penang	**n**	13	10	-	-	-	-	12	11	15	2	6	1	22
	%	56.5	43.5					52.2	47.8	65.2	8.7	26.1	4.3	95.7
Total	**n**	**36**	**54**	**-**	**-**	**-**	**-**	**44**	**46**	**63**	**15**	**12**	**24**	**66**
	%	**40.0**	**60.0**					**48.9**	**51.1**	**70.0**	**16.7**	**13.3**	**26.7**	**73.3**

*RRD-*Rattus rattus diardii*; RN-*Rattus norvegicus*; RE-*Rattus exulans*; RT-*Rattus tiomanicus*; RA-*Rattus argentiventer*; RAn-*Rattus annandalei*;

A-adult; SA-Sub-adult; J-Juvenile.

Table 4.8: The structure of the host infected with *Plasmodium* sp. relative to location, species, age, sex and season using QBC technique

Location		Species of Rat						Host sex		Host Age			Season	
		RRD	RN	RE	RT	RA	RAn	Female	Male	A	SA	J	Dry	Wet
Kuala Lumpur	**n**	15	5	6	-	-	-	14	12	15	4	7	11	15
	%	57.7	19.2	23.1				53.8	46.2	57.7	15.4	26.9	42.3	57.7
Pahang	**n**	-	37	-	-	-	-	17	20	30	4	3	27	10
	%		100.0					45.9	54.1	81.1	10.8	8.1	73.0	27.0
Malacca	**n**	15	18	-	-	1	-	24	10	25	6	3	18	16
	%	44.1	52.9			2.9		70.6	29.4	73.5	17.7	8.8	52.9	47.1
Penang	**n**	10	17	-	-	-	-	11	16	20	2	5	4	23
	%	37.0	63.0					40.7	59.3	74.1	7.4	18.5	14.8	85.2
Total	**n**	**40**	**77**	**6**	**-**	**1**	**-**	**66**	**58**	**90**	**16**	**18**	**60**	**64**
	%	**32.3**	**62.1**	**4.8**		**0.8**		**53.2**	**46.8**	**72.6**	**12.9**	**14.5**	**48.4**	**51.6**

*RRD-*Rattus rattus diardii*; RN-*Rattus norvegicus*; RE-*Rattus exulans*; RT-*Rattus tiomanicus*; RA-*Rattus argentiventer*; RAn-*Rattus annandalei*;

A-adult; SA-Sub-adult; J-Juvenile.

4.4 DISCUSSION

The aim of this study was to detect blood protozoan parasites in the wild urban rat population using Quantitative Buffy Coat (QBC) technique. The use of this technique is well documented in many countries including Ethiopia (Spielman *et al*., 1988), Philippines (Rickman *et al*., 1989), Thailand (Pornsilapatip *et al*., 1990), Venezuela (Bosch *et al*., 1996), Nigeria (Adeoye & Nga, 2007) and Malaysia (Mak *et al*., 1992; Alias *et al*., 1996) mainly for diagnosis of malaria in humans. In Malaysia, this technique was successfully used to screen *Trypanosoma lewisi* in wild rats from Kuala Lumpur (Zainal Abidin & Noor Azmi, 1999; Zainal-Abidin & Yit-Ming 1994).

Of all the rat species, *Rattus norvigecus* was the most infected with both blood protozoan parasites, *Trypanosoma* sp. (60.0%) and *Plasmodium* sp. (62.1%), similar to findings in Sri Lanka (Sannasuriya *et al*. 1999). The relatively large number of *Rattus norvegicus* specimens from Pahang, Malacca and Penang was expected, given that the rat species were more commonly associated to coastal areas (Harrison, 1957).

In contrast to other studies, more female rats (n=105) were found infected than males (n=97). It should be males higher than females because male rats have larger home ranges and show territorial behavior are more exposed to the vector flea, *Xenopslla cheopis* than females (Linardi *et al.,* 1985), thus increasing their chances of being infected by *Trypanosoma lewisi* infections.

Rats presented significantly higher prevalence of infection in the wet season, than in the dry season. The high infection rate with *Trypanosoma lewisi* coincide with highest levels of infestation by *Xenopslla cheopis* in the municipality (Linardi *et al.* 1985).

The QBC method is shown to be a quick and effective way to detect and diagnose blood parasite cases in the field and also laboratory. This method is easier and faster to perform than the thick smear method yielding a specificity of 98% (Rickman *et al*.). In

a field study, it was found that QBC had 55.93% sensitivity and 94.92% specificity when compared with blood smears (Mak *et al*.).

However, the main disadvantage of this method was the cost as this method requires a fluorescent microscope (with 0.3 mm or greater working distance of objective lenses) and the QBC microcentrifuge, as well as limitation in estimating parasite density. It is also not suitable for field studies as it requires storage for further examination for quality control checking at a regional or central laboratory. Specimens also need careful handling during preparation and examination and prone to leakage and breakage of the capillary tubes during centrifugation if the centrifuge lid is not properly closed (Mak *et al*., 1992; Petersen & Marbiah, 1994; Alias *et al*., 1996).

4.5 CONCLUSION

Both *Trypanosoma* sp. and *Plasmodium* sp. was detected in low prevalence in the blood of urban wild rats in Peninsular Malaysia using Quantitative buffy coat (QBC) technique. *Plasmodium* sp. infection (61.4%) was the most prevalent, followed by *Tryapanosoma* sp. infection (44.6%).

The use of QBC has proven to be quite effective in the identification of rodent parasites. This method has proved to be effective, fast and efficient and a reliable method of parasite detection on the field as compared to the thin blood smear method.

The presence of blood protozoan in urban rat population is an indication that the rat population can be host of possible zoonotic diseases. Hence the proper maintenance of urban environment and rodent population control are important to reduce rat human contact and impact.

CHAPTER 5

COMPARATIVE DIAGNOSTIC TECHNIQUE STUDY BETWEEN GIEMSA-STAINED THIN FILM (GTF) AND QUANTITATIVE BUFFY COAT (QBC) IN THE DETECTION OF BLOOD PROTOZOAN INFECTIONS IN WILD RODENTS

5.1 INTRODUCTION

More recently, QBC was developed primarily for the diagnosis of malaria. This technique was developed in 1983, using a capillary tube with a float pre-coated with acridine orange for the rapid quantification of leucocytes in peripheral blood and was regarded as an alternative to the Giemsa stain (Long *et al*., 1991; Baird *et al*., 1992). It was modified from the use of the hematocrit techniques by employing a larger tube (75 mm long), using acridine orange stain and a plastic float. It works on the principle that parasitized red cells are generally less dense than non-infected ones making it possible to separate the parasitized red blood cells and viewing the parasites under ultraviolet (UV) illumination following high speed (12,000 rpm or 14,367g) centrifugation for five minutes. At the granulocyte/red blood cell interface, the parasitized red cells can be obscured by the fluorescing granulocyte nuclei. Despite both the parasites and granulocyte nuclei illuminating as bright green fluorescence, the size and shape can be differentiated. This technique was then successfully applied for the diagnosis of malaria (Sodeman, 1970; Spielman *et al*., 1988; Levine *et al*., 1989) as the acridine orange staining permits differential coloration of green (nucleic) and red (cytoplasm) in stained parasites.

Quantitative Buffy Coat (QBC) technique was developed and applied to many studies worldwide for the diagnosis of human malaria in countries such as Ethiopia (Spielman *et al*., 1988), Philippines (Rickman *et al*., 1989), Thailand (Pornsilapatip *et*

al., 1990), Venezuela (Bosch *et al*., 1996), Nigeria (Adeoye & Nga, 2007) and Malaysia (Mak *et al*., 1992; Alias *et al*., 1996).

Spielman *et al*. (1988) found that the QBC method was at least eight times more sensitive compared to Giemsa stained thick blood smears. This technique successfully detected 10% more infected samples when compared to thick blood smears (Spielman *et al*., 1988). Rickman *et al*. (1989) found that the QBC method is easier and faster to perform than the thick smear method yielding a specificity of 98.4%.

Rickman *et al*. (1989) also found that the QBC could detect as few as 3 parasite/µl giving a sensitivity of 96% (when all stages of malaria were considered) and specificity of 93% in his hospital patients. In a laboratory setting in Thailand, Namsiripongpum *et al*. (1991) found that QBC had a sensitivity of 99.13% and a specificity of 96.76%. In contrast with their field study, the QBC technique had a sensitivity of 70% and a specificity of 98.4% (Rickman *et al.*, 1989). Similar results were seen from a field study by Mak *et al.* (1992) with 55.93% sensitivity and 94.92% specificity when the QBC tube method was compared with thick blood smears.

In a comparative technique study, Jain & Kaur (2005) recorded 35% of the 200 blood samples examined positive for malaria by QBC, 31% by thick smears, 25% by thin smears and only 17% by the conventional buffy coat technique. In Kenya, QBC was compared with Giemsa-stained thick blood film (GTF) and high sensitivity and specificity values were obtained (93.6% and 91.5% respectively), but with low parasitaemia, the sensitivity dropped to 41.7% (Lowe *et al*., 1996). Higher (>90%) Positive Prediction Values (PPC), Negative Predictive Values (NPV) an concordance were recorded for QBC and GTF at two study sites in China (Wang *et al*., 1996), while lower values (<70%) were reported in Nigeria (Salako *et al*., 1999). In a Philippines

study the sensitivity of QBC for malaria diagnosis was only 70% when compared with Giemsa stained thick blood films.

Despite QBC technique was intended mainly for the diagnosis of *Plasmodium* sp. (Ferreira *et al*., 2006), Amato Neto *et al*. (1996) successfully evaluated the practical use of QBC for the parasitological diagnosis of *Trypanosoma cruzi* with regards to its sensitivity and practicality and followed by others for the diagnosis of trypanosomiasis (Bailey & Smith, 1992; Truc *et al*., 1992; Truc *et al*., 1994; Ana Marli *et al*., 2002). Zainal Abidin & Noor Azmi (1999) and Zainal Abidin & Yit Ming (1994) were the only studies which successfully adopted this method to detected *Trypanosoma lewisi* infection in blood of wild rats in Malaysia.

In contrast, diagnosis using Giemsa thick and thin blood smear technique is time consuming and requires experience for adequate preparation and interpretation. However, this technique is affordable, yet sensitive and specific in determining blood parasite in rats, parasite densities and diversity. Another advantage is that specimens can be stored, re-examined and kept for future references unlike QBC. Ross in 1903, was the first man who successfully viewed microscopically the malarial parasite using Giemsa stained blood film which is currently the gold standard technique for diagnosis of malaria. The principal advantages of QBC method were sensitivity, rapidity of staining, and ease to interpret in an endemic area. However, the disadvantages include the requirement for a fluorescent microscope with 0.3 mm or greater working distance of objective lenses, and the QBC microcentrifuge, which are relatively expensive.

Therefore the main objective of this study was to compare the sensitivity and the specificity of two diagnostic methods (Quantitative Buffer Coat and Giemsa Thin Film) in the detection of blood protozoan infection in the wild rat population of Peninsular Malaysia.

5.2 METHODOLOGY

Methodology and data collected from Chapter 3 and Chapter 4 were used in present analysis.

5.2.1 Data analyses

Data collected was analyzed using SPSS version 16.0. The results are presented as prevalence. The total data comprised of three intrinsic factors (host species, sex and age) and two variables (prevalence of infection using QBC and GTF method). In all cases significance was set at $p<0.05$. Non parametric test was performed as the data collected is nominal and non- normally distributed to determine the sensitivity between the two techniques (QBC and GTF) in the detection of blood protozoa. The analyses began with the changing of host species label into nominal data (1= *Rattus norvegicus*; 2= *Rattus rattus diardii*; 3= *Rattus exulans*; 4= *Rattus argentiventer*). Similarly, numerical values were given to the two techniques in the detection of blood protozoa with 3 levels (0= negative for all parasites, 1= positive for one parasite, 2= positive for both parasites). In all cases significance was set at $p< 0.05$.

Following this, parasitic infections were analyzed using Binary Logistic Regression to determine the effect of factors (host species, age and sex) on the prevalence of blood parasites harbored by the rodents. The dataset was grouped accordingly to two variables i.e; the detection technique and the parasite recovered. The groupings; QBCTry/ GTFTry and QBCPlasmo/ GTFPlasmo was put as the dichotomous criterion variables and the host species, age and sex were set as dichotomous predictors variables. We then coded the categorical variables (0= absent of blood parasites and 1= present of blood parasites) and species (1= *Rattus norvegicus*; 2= *Rattus rattus diardii*; 3= *Rattus exulans*; 4= *Rattus argentiventer*), sex (1=M; 2= F) and age (1=Adult, 2= Sub-adult; 3= Juvenile). The model involving all main effects and interaction combination stepwise,

and the significance of its contribution was noted. The dependent variable was analyzed in turn but each was replaced in the model before evaluating of the remainder. The model was then re-run, including those combinations which had shown significance (maximum likelihood), to locate at which significant variation resided within the factors. The probability was predicted based on the log linear analysis of contingency tables, the result were given in the text as $\chi 2$ value associated with probabilities in full logistic regression model.

For calculation of technique sensitivity and specificity, the thin blood smear count was used as standard. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and concordance were calculated as follows:

$$\text{Sensitivity} = \frac{\text{Number of specimens positive by both QBC \& thin smears}}{\text{Total number of positive thin smears}} \times 100$$

$$\text{Specificity} = \frac{\text{Number of specimens negative by both QBC \& thin smears}}{\text{Total number of negative thin smears}} \times 100$$

$$\text{PPV} = \frac{\text{Number of positives by both QBC \& GTF}}{\text{Total number of positives by QBC}}$$

$$\text{NPV} = \frac{\text{Number of negatives by both QBC \& GTF}}{\text{Total number of negatives by QBC}}$$

$$\text{Concordance} = \frac{\text{Number of positives and negatives by both QBC \& GTF}}{\text{Total no. of samples examined}}$$

5.3 RESULT

Table 5.1 showed a total of 432 wild rats of four species captured*;* *Rattus norvegicus*, *Rattus rattus diardii, Rattus exulans* and *Rattus argentiventer* in the surrounding areas of wet markets of Kuala Lumpur, Malacca, Penang and Pahang during dry and wet seasons. Among these rat species, *Rattus norvegicus* (60.6%) was the dominant followed by *Rattus rattus diardii* (36.8%), *Rattus exulans* (1.9%) and *Rattus argentiventer* (0.7%). More females were captured (51.6%) compared to males (48.4%) with higher number of adults (75.2%) followed by juveniles (13.7%) and sub-adults (11.1%). More rats were captured during the wet (62.7%) as compared to dry season (37.3%). Majority of the rat species captured were *Rattus norvegicus* and *Rattus rattus diardii*. None of the other species (*Rattus exulans* and *Rattus argentiventer*) exceeded 3% in total of the catch and will not further report in the current work. Highest number of *Rattus norvegicus* were caught in Pahang (44.6%) followed by Malacca (28.6%), Penang (20.2%) and lastly Kuala Lumpur (6.5%). For *Rattus rattus diardii*, highest number caught in Kuala Lumpur (47.8%) followed by Penang (30.2%) and Malacca (22.0%). Unexpectedly, none of these species were captured in Pahang. As a whole, many adult rats were captured in Pahang as compared to Kuala Lumpur with mostly juveniles.

Table 5.2 summarized the overall blood protozoan prevalence of infection in the rodent population. Results showed that Giemsa-stain thin film (GTF) technique recorded a total of 226 (52.3%) infected blood samples while 202 (46.8%) samples were detected by Quantitative Buffy Coat (QBC). Statistical analysis showed high significant association (p<0.05) between the techniques used for blood protozoan detection with GTF the better detection technique ($\chi 2$= 13.8, df= 1, standard residual value= 67.0), compared to QBC ($\chi 2$= 12.8, df= 1, standard residual value= 46.0).

Rodents with single infection either with *Trypanosoma* or *Plasmodium* both recorded prevalence > 10% therefore was considered further for analysis of factors affecting the prevalence and abundance of infection. Overall, 189 blood samples (43.8%) were infected with *Trypanosoma* with a marginally higher prevalence detected using GTF compared to QBC technique (Table 5.2). The minimum sufficient model confirmed significantly higher number of positive samples using GTF (n=99) than QBC (n= 90) among the rodent population ($p< 0.05$). There was an absence of interaction in the prevalence of *Trypanosoma* between host species, sex and age effect with only prevalence of adults harboring *Trypanosoma* were significantly higher ($p< 0.05$) (Table 5.4).

A total of 266 positive detections of *Plasmodium* with a significantly higher result using GTF technique as compared to QBC ($\chi2= 50.7$, df= 1, $p\leq 0.05$) (Table 5.4). The only significant effect on prevalence of parasite harbored was observed in *Rattus norvegicus* ($p\leq0.05$) where up to 17% more *Rattus norvegicus* harbored *Plasmodium* infection compared to other rat species (Table 5.4). There was also an absence of interaction for the prevalence of *Plasmodium* infection between the host species, sex and age.

Rodents with mixed infections of both *Trypanosoma* and *Plasmodium* were low and only found in 2.8% (12 samples) by QBC technique and 15 samples by GTF technique ($\chi2= 13.8$, df= 1, standard residual value= -129.0). Results also indicated that GTF was better in detection of both parasites compared to QBC ($p<0.05$).

The overall sensitivity of parasite detection was 83.2% with specificity of 74.8% (Table 5.3). However, detection of *Trypanosoma* infection was better (sensitivity: 84.4%; specificity: 93.3%) compared to *Plasmodium* (sensitivity: 76.6%; specificity: 84.7%).

Table 5.1: Positivity *Trypansoma* sp., *Plasmodium* sp. and both parasites in urban wild rats with Quantitative Buffy Coat (QBC) and Giemsa-stained thin blood Film (GTF) techniques

	Trypansoma sp.		*Plasmodium* sp.		Both	
	QBC no (%) positive	GTF no (%) positive	QBC no (%) positive	GTF no (%) positive	QBC no (%) positive	GTF no (%) positive
Species						
Rattus norvegicus	54 (20.6)	64 (24.4)	77 (29.4)	93 (35.5)	(3.4)	14 (5.3)
Rattus rattus diardii	36 (22.6)	35 (22.0)	40 (25.2)	43 (27.0)	(1.9)	(0.6)
Rattus exulans	0 (0)	0 (0)	6 (75.0)	5 (62.5)	(0)	(0)
Rattus argentiventer	0 (0)	0 (0)	1 (33.3)	1 (33.3)	(0)	(0)
Sex						
Female	44 (19.7)	50 (22.4)	66 (29.6)	79 (35.4)	(2.2)	(3.6)
Male	46 (22.0)	49 (23.4)	58 (27.8)	63 (30.1)	(3.3)	(3.3)
Age						
Adult	63 (19.4)	70 (21.5)	90 (27.7)	105 (32.3)	10 (3.1)	10 (3.1)
Juvenile	16 (27.1)	18 (30.5)	18 (30.5)	17 (28.8)	(0)	(3.4)
Sub Adult	11 (22.9)	11 (22.9)	16 (33.3)	20 (41.7)	(4.2)	(6.3)
Location						
Pahang	22 (18.8)	26 (22.2)	37 (31.6)	40 (34.2)	(3.4)	(6.0)
Malacca	25 (22.5)	28 (25.2)	34 (30.6)	38 (34.2)	(6.3)	(6.3)
Kuala Lumpur	20 (19.4)	19 (18.5)	26 (25.2)	33 (32.0)	(0)	(0.9)
Penang	23 (22.8)	26 (25.7)	27 (26.7)	31 (30.7)	(0.9)	(0)
Season						
Wet	66 (24.4)	61 (22.5)	64 (23.6)	78 (28.8)	(3.0)	(1.5)
Dry	24 (14.9)	38 (23.6)	60 (37.3)	64 (39.8)	(2.5)	11 (6.8)
Total	**90 (20.8)**	**99 (22.9)**	**124 (28.7)**	**142 (32.9)**	**12 (2.8)**	**15 (3.5)**

Table 5.2: Comparison of Quantitative Buffy Coat (QBC) an Giemsa-stained Thin blood Film (GTF) techniques in blood protozoan detection

QBC	Giemsa-stained thin film (GTF)								
	All infected no. (%)			*Trypanosoma* sp. infected no. (%)			*Plasmodium* sp. infected no. (%)		
Status	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive	168 (74.3)	58 (25.7)	226 (52.3)	76 (76.8)	23 (23.2)	99 (22.9)	95 (66.9)	47 (33.1)	142 (32.9)
Negative	34 (16.5)	172 (83.5)	206 (47.7)	14 (4.2)	319 (95.8)	333 (77.1)	29 (10.0)	261 (90.0)	290 (67.1)
Total	202 (46.8)	230 (53.2)	432 (100)	90 (20.8)	342 (79.2)	432 (100)	124 (28.7)	308 (71.3)	432 (100)

Table 5.3: Sensitivity and specificity of Quantitative Buffy Coat (QBC) and Giemsa-stained Thin blood Film (GTF)

Status	**All infected (%)**	***Trypanosoma* sp. infected (%)**	***Plasmodium* sp. infected (%)**
Sensitivity	83.2	84.4	76.6
Specificity	74.8	93.3	84.7
PPV	74.3	76.8	66.9
NPV	83.5	95.8	90.0
Concordance	78.7	91.4	82.4

*PPV- Positive Predictive Value, NPV- Negative Predictive Value

Table 5.4: Logistic regression prediction values between in the detection of blood parasitimae according to host species, sex and age

Variation	Wald	df	*p*	Exp(B)
Trypanosoma				
Species(1)	0.000	1	0.999	$5.109e^{8}$
Species(2)	0.000	1	0.999	$7.143e^{8}$
Species(3)	0.000	1	1.000	1.119
Sex(1)	0.358	1	0.550	1.146
Age(1)	3.857	1	0.050*	0.516
Age(2)	0.731	1	0.392	0.691
Plasmodium				
Species(1)	4.440	1	0.035*	0.170
Species(2)	2.480	1	0.115	0.269
Species(3)	1.589	1	0.208	0.152
Sex(1)	0.988	1	0.320	0.816
Age(1)	0.642	1	0.423	0.775
Age(2)	0.939	1	0.333	1.475

Species (1= *Rattus norvegicus*; 2= *Rattus rattus diardii*; 3= *Rattus exulans*;), sex (1=Male) and age (1=Adult, 2= Sub-adult).

*Significant $p<0.05$

5.4 DISCUSSION

As in many other cities around the globe, the rat population in Peninsular Malaysia was dominated by two main species living commensally with humans, namely *Rattus norvegicus* and *Rattus ratttus diardii,* with *Rattus norvegicus* being the dominant species. Mohd Zain *et al*. (2012) also noted this trend, but in this study *Rattus rattus diardii* was more prominently dominant than *Rattus norvegicus*.

More females were captured compared to male rats with higher number of adults compared to juveniles. Other rat epidemiology studies however, showed almost equal numbers between males and females captured, highlighting the scavenging behavior of commensal rats (Paramaswaran *et al*., 2005; 2009a; 2009b; Syed Arnez & Mohd Zain, 2006; Mohd Zain., 2008; Mohd Zain *et al.,* 2012; Alias *et* al., 2014). The lower success rate of trapping juveniles observed in the present study could be attributed to inherently limited activity and home range. It is also suggested that the greater number of adult rats generates imbalanced social competition, therefore restricting the overall movement of the juveniles. Other findings with similar observations for host age were also reported (Paramaswaran *et al*., 2005, Syed Arnez & Mohd Zain, 2006; Mohd Zain., 2008; Mohd Zain *et al.,* 2012; Alias *et al*., 2014).

The Quantitative Buffy Coat (QBC) technique have been well documented in many countries including Ethiopia (Spielman *et al*., 1988), Philippines (Rickman *et al*., 1989), Thailand (Pornsilapatip *et al*., 1990), Venezuela (Bosch *et al*., 1996), Nigeria (Adeoye & Nga, 2007) and Malaysia (Mak *et al*., 1992; Alias *et al*., 1996) mainly for diagnosis of malaria in humans. In Malaysia, this technique was successfully used to screen *Trypanosoma lewisi* in wild rats from Kuala Lumpur (Zainal Abidin & Noor Azmi, 1999; Zainal-Abidin & Yit- Ming 1994). This study reports for the first time an epidemiology study of blood protozoan in wild rat population in Peninsular Malaysia

using the quantitative buffy coat (QBC) technique as well as to compare the effectiveness of this method with the conventional GTF technique.

The sensitivity and specificity of QBC and GTF in the detection of blood protozoan infections in wild rats were 83.2% and 74.8% respectively. Sensitivity and specificity was higher for *Trypanosoma* infection (84.4% and 93.3%) compared to *Plasmodium* (76.6% and 84.7%). The sensitivity and specificity recorded in the present study was low compared to results from previous studies which ranged from 75.5% to 99.6% and 82% to 98.4% respectively (Rickman *et al*., 1989; Pornsilapatip *et al*., 1990; Wongsrichanalai *et al*., 1991; Baird *et al*., 1992; Cabezos & Bada 1993; Gay *et al*., 1994; Benito *et al*., 1994). Alias *et al.*(1996) noted lower sensitivity in the field setting due to delayed specimen processing (up to 8-10 hours after collection), as well as exposure to high ambient temperatures and humidity. Rickman *et al*. (1989) and Mak *et al.* (1992) noted that the sensitivity values were observed high only in a laboratory setting while it was low in the field.

Low sensitivity was also detected in Nigeria (Adeoye & Nga, 2007) at 55.9% and specificity was 88.8%. Their study showed low sensitivity under routine laboratory conditions and concluded that QBC was clearly unreliable at low parasitaemia level.

The advantages of the QBC technique are endless from time saving and easier technique to learn compared to the GTF technique. This method performs rapid identification of malaria infection especially in busy blood bank and outpatient clinics (Mak *et al*., 1992) as well as microfilaria infection, it has the ability to screen two parasitic diseases with a single sample of blood, particularly in areas where these diseases are endemic (Pius *et al*., 1993).

Although considerable time and experience are required for adequate preparation and interpretation of the blood smears, QBC is a less labour-intensive alternative to GTF. The principal advantages of the original QBC method were sensitivity, rapidity of staining, and ease to interpret in an endemic area.

However, the main disadvantage of this method was the cost as this method requires a fluorescent microscope (with 0.3 mm or greater working distance of objective lenses) and the QBC microcentrifuge, as well as limitation in estimating parasite density. It is also not be suitable for field studies as it requires storage for further examination for quality control checking at a regional or central laboratory. Specimens also need careful handling during preparation and examination and prone to leakage and breakage of the capillary tubes during centrifugation if the centrifuge lid is not properly closed (Mak *et al*., 1992; Petersen & Marbiah, 1994; Alias *et al*., 1996).

5.5 CONCLUSION

To conclude, this chapter was showed that Giemsa Thin Film (GTF) technique is the more reliable method in the detection of blood parasitic infections compared to Quantitative Buffy Coat (QBC) technique. QBC technique had limitations in the detection of blood protozoan than the conventional GTF technique. GTF was still the best in the overall detection of parasites and the cheapest to run. QBC technique may be very useful for quickly picking positive infections; however it still requires GTF in order to confirm the species of parasites and for quantification of parasitaemia.

CHAPTER 6
CONCLUSION

The urban wild rat population structure in four urban cities in Peninsular Malaysia is described and comprised of six species with *Rattus rattus diardii* the most dominant followed by *Rattus norvegicus*, *Rattus exulans*, *Rattus tiomanicus*, *Rattus argentiventer* and *Rattus annandalei*. The present observations of diversity were consistent with previous records. The high numbers of rodent in the urban cities were contributed by human an activity that favours the establishment and propagation of the commensal rat population. More female rats were captured compared to males meanwhile higher number adult rats captured compared to juveniles. Higher numbers of rats were also captured during wet season compared to dry season. The host-sex and age distribution can be explained by the rodent's inherent social behavior.

Low infections of *Trypanosoma* sp. and *Plasmodium* sp. were detected in the urban rat population using two techniques (Giemsa-stained Thin Film and Quantitative Buffy Coat) with *Plasmodium* sp. infection was the most prevalent, followed by *Tryapanosoma* sp. Despite low infections, in the rat population, control measures are still necessary as rodents play a role as a reservoir for many zoonotic diseases to human and cause huge economic losses.

The sensitivity and specificity of the screening methods used in this study was compared. Despite proven to be effective, fast and efficient and a reliable method of parasite detection for rodent parasites, this method has several limitation. The main disadvantages of QBC method was the cost as this method requires a fluorescent microscope (with 0.3 mm or greater working distances of objective lenses) and the QBC microcentrifuge, as well as limitation in estimating parasite density.

GTF technique is the more reliable method in the detection of blood parasitic infections compared to the QBC technique. GTF was still the best in the overall detection of parasites and the cheapest to run. QBC technique may be very useful for quickly picking positive infections; however it still requires GTF in order to confirm the species of parasites and for quantification of parasitaemia.

This study highlights the importance of better management of rodent control and public awareness in ensuring a successful rodent control program that involves getting both the community and health authorities involved in rodent control and better understanding on diseases transmission by rodents. The presence of blood parasite in urban rat population is in indicative of rat population as host of possible zoonotic diseases. Hence the proper maintenance of urban environment and rodent population control are important to reduce rat-human contact and impact.

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