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Epidemiology of blood parasitic infections in the urban rat population in peninsular Malaysia

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Abstract. A total of 719 wild rats were captured from four localities representing the west (Kuala Lumpur), east (Kuantan), north (Georgetown) and south (Malacca) to determine the diversity of blood protozoan from the urban wild rat population in peninsular Malaysia. Five rat species were recovered with *Rattus rattus diardii* being the most dominant species, followed by *Rattus norvegicus*, *Rattus exulans*, *Rattus annandalei* and *Rattus argentiventer*. Two blood protozoan species were found infecting the rodent population namely, *Plasmodium* sp. (42.1%) and *Trypanosoma lewisi* (25.0%). This study reports the presence of *Plasmodium* sp. for the first time in the rodent population in Malaysia. Two main intrinsic factors were identified affecting the parasitic infections. *Trypanosoma lewisi* infections were influenced by host age and sex with infections observed higher in male and juvenile rats meanwhile *Plasmodium* sp. infections were observed almost similar in both sexes. However, infections were higher in sub-adult rats.

INTRODUCTION

Rodents particularly those belonging to the family Muridae form the largest group of mammals in Malaysia (Ow-Yang, 1971). Commensal rats and mice live at the expense of humans, invade their dwelling, eat their food and upset their comfort. They also transmit diseases to human (Otto & Burns, 1983; Hobson & Collier, 1984) as they harbour wide range of ecto- and endo- parasites with great zoonotic importance (El-Safi & Peters, 1991; Velez *et al*., 1995; Webster & Macdonald, 1995; Yaghoobi & Javadian, 1996; Yasuraoka *et al*., 1996). *Toxoplasma gondii*, *Eimeria muris*, *Spironuclues muris*, *Giardia muris*, *Cryptosporodium* spp., *Encephalaprotitozoan cuniculi*, *Hepatozoan muris*, and *Babesia muris* are a few examples infecting humans (Nama & Parihar, 1976; Soulsby, 1982; and Claveria *et al*., 2005).

Many studies have been conducted in various parts of the world to determine the prevalence of parasitic infections in the wild rat population from Taichung, Taiwan (Tung *et al*., 2009), to Baltimore, Maryland, USA (Easterbrook *et al*., 2008) and Egypt (Elshazly *et al*., 2008). The first record in Malaysia was as early as in the 1930's (Adam, 1933) and subsequently, more published works followed (Harrison, 1957; Sandosham, 1957; Schacher & Cheong, 1960; Balasingam, 1963; Lim *et al*., 1965, 1974; Gatha, 1966; Mulkit & Cheong, 1971; Ow-Yang, 1971; Singh & Cheong, 1971; Betterton & Lim, 1975; Yap *et al*., 1977; Leong *et al*., 1979; Sinniah *et al*., 1979; Krishnasamy *et al*., 1980; Paramasvaran *et al*., 2005; 2009; Mohd Zain *et al*., 2012; Benacer *et al*., 2013).

There is a paucity of information on the epidemiology of blood parasite infecting the rat population in Malaysia with most studies confined to small localities with very few rats examined. Paramasvaran *et al*. (2003) screened 27 rats in an Orang Asli village in Bukit Kemandol, Selangor

however, failed to detect any blood protozoan. Similarly, Premaalatha *et al.*, 2010, screened the blood of 10 rats from the surroundings of Veterinary Research Institute (VRI) Ipoh which were all free from any infection. Zainal-Abidin & Noor Azmi (1999) recorded *Trypanosoma lewisi* in 23 wild rats from Kuala Lumpur and noted prevalence of infection at 21.7%. The most recent study by Siti Shafiyyah *et al.* (2012) recorded low *T. lewisi* prevalence (1.5%) from 137 wild rats caught in Kuala Lumpur.

Therefore, the present study aims to investigate the diversity, infection levels of blood parasitaemia in wild rat population from four urban cities in peninsular Malaysia relative to host-age, sex and season.

MATERIALS AND METHODS

Study sites

Four major cities were selected 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 states of peninsular Malaysia. All sites were characterized by a tropical climate and high humidity throughout the year with temperatures ranging between 30°C and 36°C with heavy rainfall coinciding with the monsoon season. For this purpose, season is divided into wet and dry seasons for each year with dry months falling between January–March and June–September and wet months between April–May and October–December.

Sample collection and examination

Trapping was conducted between November 2006 and November 2011 with the assistance of the municipality from each city as part of the vector control programme. The main criteria for site selection were proximity to housing, obscuration from public view and association with drainage defects. Two rodent species, *Rattus argentiventer* and *R. annandalei* were captured close to the forest fringe area at the University of Malaya, Kuala Lumpur campus. All the rats were trapped alive using custom made steel wire traps measuring 29 x 22 x 50 cm using dried fish, sweet potatoes, fruits and coconut as baits. 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 rat faeces, rat pathways or footprints. 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 foot, weight and physical appearances were recorded. Host age (adult, sub-adult and juvenile) sex (male and female) and species for all rats captured were determined based on descriptions by Harrison & Quah (1962), Medway (1983) and Payne & Francis (1998). Blood was collected from the heart using a needle and syringe and thin blood smear was prepared with a drop of blood. The blood smear was fixed on to a glass slide by immersing in pure methanol for 1 minute. Thereafter the slide was immersed in a solution of 1 part Giemsa stock to 20-30 parts of buffered water (pH 7.0 – 7.2), 20-30% Giemsa stain solution for 20-30 minutes, then finally flushed with water and left to air dry. The slide was mounted permanently with Depex or Canada balsam and examined under light microscopy at 400x magnification to screen for parasites and 1000x magnification under oil immersion for identification. Each slide was examined for gametocytes and schizogonic cycle stages. Data collected was analyzed using SPSS (Statistic Package for Social Sciences) version 12.

RESULTS

Up to 719 wild rats were captured from 4 localities representing the west (Kuala Lumpur, n=391), east (Kuantan, n=117), north (Georgetown, n=101) and south (Malacca, n=110). Five rat species were recorded with *Rattus rattus diardii* (n=410) being the most dominant species, followed by *Rattus norvergicus* (n=302), *Rattus exulans* (n=4), *Rattus annandalei* (n=2) and *Rattus argentiventer* (n=1). Three hundred and twelve rats were captured during dry season and 407 rats during wet season. The total number of females (n = 387 cats) outnumbered males (n = 332 cats) of which 67.3% were adults (n = 484), 18.9% were sub-adults (n = 136) and 13.8% were juveniles (n = 99). The host population structure relative to species, age, sex and season is summarized in Table 1.

A total of 425 rats (59.1%) were infected with at least one blood protozoan species from the two species recovered namely; *T. lewisi* (Figure 1) and *Plasmodium* sp. (Figure 2). The infection with *Plasmodium* sp. (42.1%) was higher compared to *T. lewisi* (25.0%). Only 16% (n = 68) were found infected with both species. Infection was highest in *R. rattus diardii* (62.2%), with *Plasmodium* sp. (47.1%) infection being almost two-folds compared to T. *lewisi* (24.2%) (Table 2).

According to host sex, more males (61.4%) were infected compared to females (57.1%). *Plasmodium* sp. infections were similar between both sexes (male: 43.1%; female: 41.3%) while *T. lewisi* infections were slightly higher in males (28.9%) compared to females (21.7%) (Table 3). Relative to host-age factor, more sub-adults were infected (71.3%) compared to adults (55.4%) and juveniles (60.6%) with higher *Plasmodium* sp. infections in all age groups (Table 4).

In relation to study location, more than half of the rat population in Kuala Lumpur (63.9%), Georgetown (56.4%), Malacca (53.6%) and Kuantan (50.4%) were infected, with *Plasmodium* sp. being more prevalent compared to *T. lewisi* (Table 5). According to the season, greater infections were observed during the dry (66.0%) compared to the wet season (53.8%) including *T. lewisi* infections. Similarly, *Plasmodium* sp. infections also peaked during dry compared to the wet season at 51.6% and 34.9% respectively (Table 6).

Table 1. The rat community structure relative to species, age, sex and season

Location		Rat Species					Hot-sex		Host-Age			Season	
		RRD	RN	RE	RA	RAn	Female	Male	A	SA	J	Dry	Wet
Kuantan	n	0	117	0	0	0	61	56	105	7	5	63	54
	%		100.0				52.1	47.9	89.7	6.0	4.3	53.8	46.2
Malacca	n	35	75	0	0	0	66	44	86	14	10	62	48
	%	31.8	68.2				60.0	40.0	78.2	12.7	9.1	56.4	43.6
Georgetown	n	48	53	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	4	1	2	209	182	215	107	69	174	217
	%	83.6	14.6	1.0	0.3	5.1	51.5	48.5	53.8	28.2	18.0	44.3	55.7
Total	n	410	302	4	1	2	387	332	484	136	99	312	407
	%	57.0	42.0	0.6	0.1	0.3	53.8	46.2	67.3	18.9	13.8	43.4	56.6

*RRD – *Rattus rattus diardii*; RN – *Rattus norvergicus*; RE – *Rattus exulans*; RA – *Rattus argentiventer*; RAn – *Rattus annnandalei*; A – adult; SA – Sub-adult; J – Juvenile.

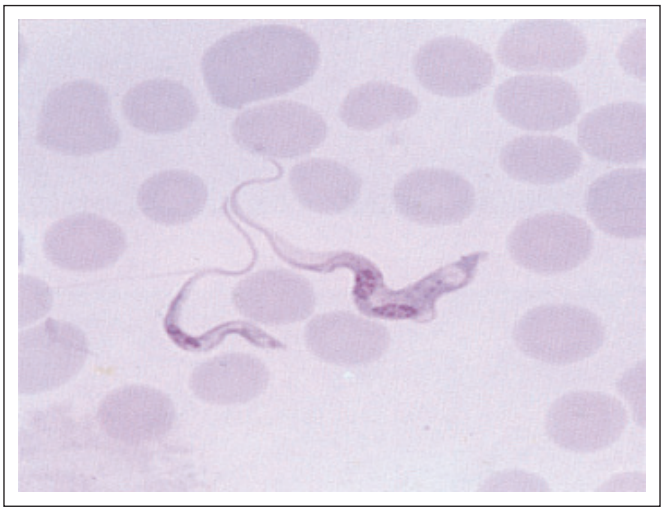

Figure 1. *Trypanosoma lewisi* (magnification: 400x)

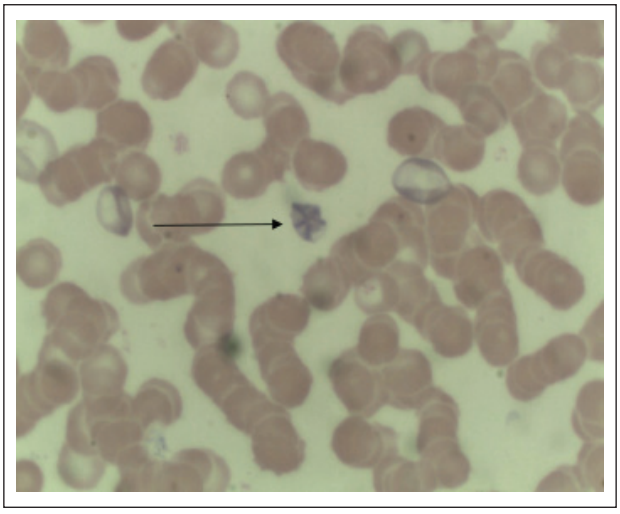

Figure 2. *Plasmodium* sp. – schizont stage
(Magnification: 400x)

Table 2. Prevalence of blood protozoan in urban wild rodents according to rodent species

Species of rodent	Number of rodents examined	Number of rodents positive with blood protozoan (% positive)	Giemsa-stained Thin Film (GTF)	
			TRY	*PLAS*
Rattus rattus diardii	410	255 (62.2%)	99 (24.2%)	193 (47.1%)
Rattus norvergicus	302	167 (55.3%)	78 (25.8%)	108 (35.8%)
Rattus exulans	4	2 (50.0%)	2 (50.0%)	1 (25.0%)
Rattus argentiventer	1	0	0	0
Rattus annandalei	2	1 (50.0%)	1 (50.0%)	1 (50.0%)
Total	719	425 (59.1%)	180 (25.0%)	303 (42.1%)

** TRY: Trypanosoma lewisi; PLAS: Plasmodium sp.*

Table 3. Prevalence of blood protozoan in urban wild rodents according to host sex

Sex	Number of rodents examined	Number of rodents positive with blood protozoan (% positive)	Giemsa Thin Film (GTF)	
			TRY	*PLAS*
♀	387	221 (57.1%)	84 (21.7%)	160 (41.3%)
♂	332	204 (61.4%)	96 (28.9%)	143 (43.1%)
Total	719	425 (59.1%)	180 (25.0%)	303 (42.1%)

**TRY: Trypanosoma lewisi; PLAS: Plasmodium sp.*

Table 4. Prevalence of blood protozoan in urban wild rodents according to host-age

Age	Number of rodents examined	Number of rodents positive with blood protozoan (% positive)	Giemsa Thin Film (GTF)	
			TRY	*PLAS*
Adult	484	268 (55.4%)	111 (22.9%)	196 (40.5%)
Sub-Adult	136	97 (71.3%)	39 (28.7%)	74 (54.4%)
Juvenile	99	60 (60.6%)	30 (30.3%)	33 (33.3%)
Total	719	425 (59.1%)	180 (25.0%)	303 (42.1%)

** TRY: Trypanosoma lewisi; PLAS: Plasmodium sp.*

Table 5. Prevalence of blood protozoan in urban wild rodents according to sampling location

Location	Number of rodents examined	Number of rodents positive with blood protozoan (% positive)	Giemsa Thin Film (GTF)	
			TRY	*PLAS*
Kuantan	117	59 (50.4%)	26 (22.2%)	40 (34.2%)
Malacca	110	59 (53.6%)	28 (25.5%)	38 (34.5%)
Georgetown	101	57 (56.4%)	26 (25.7%)	31 (30.1%)
Kuala Lumpur	391	250 (63.9%)	100 (25.6%)	194 (49.6%)
Total	719	425 (59.1%)	180 (25.0%)	303 (42.1%)

** TRY: Trypanosoma lewisi; PLAS: Plasmodium sp.*

Table 6. Prevalence of blood protozoan in urban rodents according to season

Season of rodent captured	Number of rodents examined	Number of rodents positive with blood protozoan (% positive)	Giemsa Thin Film (GTF)	
			TRY	*PLAS*
Dry	312	206 (66.0%)	79 (25.3%)	161 (51.6%)
Wet	407	219 (53.8%)	101 (24.8%)	142 (34.9%)
Total	719	425 (59.1%)	180 (25.0%)	303 (42.1%)

** TRY: Trypanosoma lewisi; PLAS: Plasmodium sp.*

DISCUSSION

Previous blood protozoan studies from wild rats in Peninsular Malaysia were limited to one locality 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 nationwide study of blood protozoan infection from urban rats from four major cities namely; Kuantan, Georgetown, Malacca and Kuala Lumpur.

Two blood protozoan species were found infecting the rodent population namely; *T. lewisi* and *Plasmodium* sp. with more than half the population being predominantly infected with a single infection. This study also records for the first time the presence of *Plasmodium* sp. infecting the rodent population. Prior to this study, only *T. lewisi* was noted (Zainal-Abidin & Noor Azmi, 1999; Siti Shafiyyah *et al*., 2012), while the rest failed to detect any infection (Paramasvaran *et al*., 2003 & Premaalatha *et al*., 2010). No *Babesia* sp. was recorded in this study as previously also mentioned by Paramasvaran *et al*., 2003).

Plasmodium sp. infection in rats occur following the infective bite by infected *Anopheles* mosquito meanwhile *T. lewisi* infections is transmitted by fleas to rat by oral route, through ingestion of flea faeces or fleas.

Trypanosoma lewisi infections have been recorded throughout the world infecting rats i.e; *R. norvegicus* in Sri Lanka (Sannasuriya *et al*., 1999), *Rattus* and *Bandicota* species in Thailand (Jittapalapong *et al*., 2008), *R. novegicus* 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 *T. 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).

The presence of *Plasmodium* was previously also reported in rats (Kreier *et al*., 1972; Makokha *et al*., 2011). Makokha *et al*. (2011) reported low prevalence of *Plasmodium* sp. infections with 6.8% and 3.7% in *Praomys jacksoni* and *Mastomys sp*., respectively. Using the current method, this study was only able to determine *Plasmodium* to genus level. Ramakrishnan & Prakash (1950) identified *Plasmodium berghei* as the species infecting *R. norvegicus* and *R. rattus* and noted the morphological characteristics of *P. berghei* in rats while Krier *et al*. (1972) reported on the relationship between erythrocyte morphology and parasitization of *Plasmodium* sp. on rats. Therefore, molecular approaches are now required to further identify this protozoan to species level.

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

Trypanosome (*Herpetosoma*) *lewisi* parasitizes synanthropic rodents of the genus *Rattus* via the rat-flea as vector (Pedro & Jose, 2002). Although *Herpetesoma* species are considered specific to a single vertebrate host genus, it could infect a wide range of flea vectors (Molyneux, 1969;

Linardi & Botelho, 2002; Desquesnes *et al*., 2002). *Xenopsylla cheopis*, *Nosopsyllus fasciatus*, *Ctenocephalides canis* and *C. felis* have been incriminated as intermediate hosts (Molyneux, 1969). *Trypansoma lewisi* parasitizes mostly animals and is usually nonpathogenic to humans however can acquire the desired virulence and emerge as human pathogen and cause serious problems in the right combination of environmental, host and organism related factors. *Trypanasoma lewisi* infection was previously reported in a 45-day-old Thai infant displayed with 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 (Aborigine) in west Malaysia. Recently, trypanosomes of *T. lewisi* were observed in the peripheral blood smear of a 37-day-old Indian infant admitted with fever and convulsions (Verma *et al*., 2011).

There are more than 100 species of *Plasmodium*, which can infect many animal species such as reptiles, birds, and various mammals and cause malaria. However, only several species have long been recognized to infect humans in nature including *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Symptoms of malaria include fever, flu-like illness, chills, headache, muscle aches, tiredness, nausea, vomiting and diarrhea. Symptoms usually appear between 10 and 15 days after the mosquito bite. Infections in particular *P. falciparum*, if not promptly treated, may cause kidney failure, seizures, mental confusion, coma, and death.

Rodents living in high density and in close proximity allowed the vectors to transmit infection to the population very quickly. This was shown with more than half of the rats screened particularly in Kuala Lumpur were positive for blood protozoan followed by Georgetown, Malacca and Kuantan.

The close proximity between human with rats in housing areas also have been identified to contribute significantly to the spread of many zoonotic diseases. Rats being closely associated with human serve as high potential for zoonotic infections to human (Siti Shafiyyah *et al*., 2012). The urban environment in big towns in Peninsular Malaysia is fast changing therefore there is an urgent need to be prepared for these emerging zoonoses (Kaur *et al*., 2007). Education and environmental hygiene play an important role in the care, prevention and control of diseases from rats to human.

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