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Acarine ectoparasites of Panti Forest Reserve in Johore, Malaysia

Mariana A[1*], Mohd Kulaimi B[1], Halimaton I[1], Suhaili ZA[1], Shahrul-Anuar MS[2], Nor Zalipah M[3], Ho TM[1]

[1]*Acarology Unit, Infectious Diseases Research Centre, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia*
[2]*School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia*
[3]*Department of Biological Sciences, Faculty of Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia*

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ABSTRACT

Objective: To identify the presence of acarine ectoparasites and determine whether there is any potential public health risk in Panti Forest Reserve, Johore, Malaysia. **Methods:** Trapping of animals and avifauna was conducted simultaneously along 5 expedition trails using 150 wire traps, 10 harp traps and 30 mist nets for 6 consecutive nights. A total of 140 animals consisting of 7 species of birds, 19 species of bats, 6 species of rodents and 1 species of tree-shrew as well as 8 myriapods were examined. **Results:** Infestation rates of ticks, mesostigmatid mites and chiggers on animals examined were 24.3%, 28.6% and 27.9%, respectively. Infestation on bats was low (1.5%) and none occurred on birds. Majority of ticks extracted were at immature stages (78.9%). Genera of ticks on animals were *Amblyomma*, *Dermacentor*, *Haemaphysalis* and *Ixodes*. *Ixodes granulatus* was the only species of ticks identified from the animals. Examination of ticks under vegetation revealed 54% adults leading to identification of 3 species of ticks. A total of 7 species of mesostigmatid mites were found. 6 species were on rodent, *Maxomys surifer* and another one species, *Laelaps nuttalli* was found only on *Leopoldamys sabanus*. *Laelaps sanguisugus* was the only mesostigmatid found infesting tree-shrews. Seven genera of chiggers were identified. From this, 5 genera were on rodents, 4 genera on tree-shrews and 1 genus on a bat. **Conclusions:** A total of 16 genera, 2 sub-genus and 14 species of acarine ectoparasites were found in this area. Findings of the survey demonstrate the presence of three spesies of acarine ectoparasites which have potential health risk *i.e. Ixodes granulatus*, *Laelaps nuttalli* and *Leptotrombidium deliense*.

1. Introduction

Panti Forest Reserve (PFR), which covers an area of approximately 13 410 ha is a permanent forest reserve located in south-east of the state of Johore (1°51′N, 103°53′E), Malaysia. PFR falls near the heart of the 'Sundaland Biodiversity Hotspot' which is widely recognized as the richest region for biodiversity and ranked second in the world after the Tropical Andes for combined species diversity and endemism[1]. It is a rich assemblage of lowland and hill dipterocarp forests, freshwater swamp forest, sub-montane forest, heath forest and riparian ecosystem of rivers and streams. PFR was gazetted in 1929 as a forest reserve and is listed as an International Union for Conservation of Nature and Natural Resources (IUCN) Category 1a (Strict Nature Reserve) for protection of water and conservation of the rich forest biodiversity[2].

PFR is one of the most well-known destinations for forest bird-watching in Malaysia. There are a number of trails for nature lovers to explore. A famous trail in this area is 'Bunker Trail' aptly named because it starts from a portion of the Mersing trunk road where 2 concrete bunkers demarcate both sides of the road. These bunkers were used in World War II during the Japanese occupation of Malaysia. Since the trail has long time been a favorite birding spot for local and international birders, measures have been taken by the state government to gazette the area as a bird sanctuary.

Besides bird-watching, conservation of nature and natural resources, PFR has also become an attractive destination for researchers, scientists and tourists due to the 'Bigfoot

*Corresponding author: Mariana A, Acarology Unit, Infectious Diseases Research Centre, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia.
E-mail: mariana@imr.gov.my

(man-like beasts) Phenomenon' at the end of 2005 and the whole year of 2006. Moreover, the area has been introduced and promoted as an eco-tourism destination by the Big Foot Research Organization (BFRO), an international organization based in the United States of America[3].

Acarine (ticks and mites) ectoparasites of small animals are common in forests such as PFR and some are of known public health importance. No survey of acarine ectoparasite has been conducted in PFR in the past[4,5]. Therefore, the study was aimed to identify the presence of acarine ectoparasites which are of known public health importance and thus determine whether there is any potential public health risk in the area.

2. Materials and methods

2.1. Trapping

Small mammals and avifauna were trapped along 5 expedition trails in PFR, *i.e.*, Panti Eco Park, Sungai Sisek, Sungai Pelepah Kiri, Sungai Pak Kenit and Sungai Dohol (Figure 1). PFR is under the jurisdiction of the Forestry Department and these were the only trails made available during the expedition. Trapping of animals and avifauna was conducted simultaneously using 150 wire traps, 10 harp traps and 30 mist nets for 6 consecutive nights. Traps were checked once daily for non-volant small mammals and twice daily at early dawn and at dusk for avifauna.

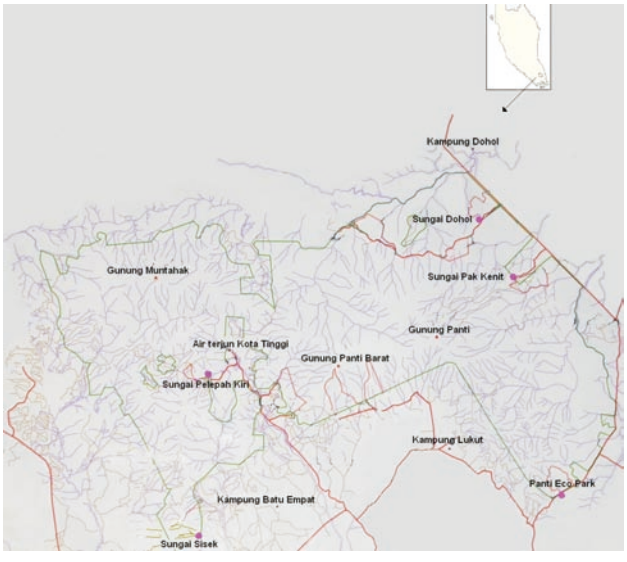

Figure 1. Map of the expedition site in the state of Johore, Malaysia.

2.2. Processing animals

The animals caught were transported in cloth bags back to the laboratory at base-camp. Those bags were turned inside-out and their contents shaken onto a white enamel tray and examined for ectoparasites. Non-volant animals were identified individually. Animals that were not protected species were killed using chloroform in a killing jar. For protected species, steps were taken to anaesthetize the animal with Zoletil® (active chemical compounds are tiletamine and zolazepam, both as hydrochloride; tiletamine as a major tranquilizer and zolezepam as muscle relaxant) which ensures a general anaesthesia with a short induction time, very few side effects and maximum safety. The dead or anaesthetized animal was then removed from the bag, placed on an enamel tray and combed thoroughly with a fine tooth comb so that dislodged materials were dropped onto the tray. The dislodged materials were examined under a dissecting microscope and ectoparasites seen were picked up with a sharpened applicator stick. Each animal was then examined in detail under 20× magnification and any ectoparasites found around the eyes, ears, nose, nasal cavity, snout and any other parts of the body were picked up with a pair of fine forceps. Nasal passages of rodents were dissected to look for chiggers (larval trombiculid mites).

Killing of avifauna was not allowed in this study and thus only a general screening of live avifauna was performed. Special attention was given to the wing membranes, eye-lids, ear lobes and nose of bats. The body fur was parted with a forcep and searched for ectoparasites. A general examination for ticks, mesostigmatid mites and chiggers was made on the skin, primary and secondary feathers of birds. Skin and feathers under both wings and the anal portion of the bird were given priority.

Myriapods (centipedes and millipedes) were hand-caught by researchers from World Wildlife Fund (WWF) Malaysia and a local university, Universiti Kebangsaan Malaysia. The areas underneath and in between body segments were screened for ectoparasites. Ticks were also collected by flagging or dragging white towels on vegetation and by examination of edges and undersides of leaves.

2.3. Preservation, mounting and identification of ectoparasites

All ectoparasites found were preserved in 70% ethanol and except for ticks, were later mounted for identification. Chiggers were directly mounted. Mesostigmatid and astigmatid mites were first cleared in lactophenol and lactic acid, respectively. All mites were mounted in Hoyer's medium. Mounted slides were incubated at 40 ℃ for a week and cover-slips ringed with paint. Wherever possible, adult ticks and other ectoparasites were identified to the species level.

3. Results

A total of 140 animals consisting of 7 species of birds [*Arachnothera longirostra* (2), *Criniger phaeo* (1), *Hypogramma hypogrammicum* (1), *Prionochilus maculates* (3), *Pycnonotus brunneus* (1), *Pycnonotus erythropthalmos* (1), *Stachyris maculate* (6)], 19 species of bats [*Balionycteris maculate* (15), *Cynopterus brachyotis* (1), *Hipposideros bicolor* (2), *Hipposideros cervinus* (4), *Hipposideros diadema* (16), *Hipposideros larvatus* (1), *Kerinoula hardwickii* (2), *Kerinoula intermedia* (1), *Kerinoula minuta* (1), *Kerinoula papillosa* (1), *Murina suilla* (2), *Myotis ridleyi* (5), *Nycteris tagata* (1), *Rhinolopus affinis* (1), *Rhinolopus rifulgens* (2), *Rhinolopus robinsoni* (1), *Rhinolopus sedulous* (1), *Rhinolopus trifoliatus*

(5), *Penthetor lucasi* (1)], 6 species of rodents and 1 species of tree-shrew as well as 8 myriapods were examined for ectoparasites. The species of avifauna and small mammals caught as well as the infestation rates were shown in Table 1.

Table 1

Ectoparasitic infestation rates on avifauna and small animals in Panti Forest Reserve, Johore (2-8 August 2006) [*n* (%)].

Host species	No. caught	No. of host infested		
		Ticks	Mesostigmatids	Chiggers
Rodents				
Leopoldamys sabanus	3	1	2	1
Maxomys rajah	1	1	0	1
M. surifer	12	9	12	6
M. whiteheadi	4	1	0	1
Total	20	12 (60.0)	14 (70.0)	9 (45.0)
Squirrels				
C. notatus	2	1	2	1
L. insignis	12	9	9	8
Total	14	10 (71.4)	11 (78.6)	9 (64.3)
Tree-shrew				
T. glis	23	12 (52.2)	6 (26.1)	21 (91.3)
Grand total	140	34 (24.3)	40 (28.6)	39 (27.9)

3.1. Ticks

A total of 34 ticks from 3 genera were extracted from animals such as rodents (60.0%) and tree-shrews (52.2%). All were *Ixodid* ticks belonging to genera *Amblyomma, Dermacentor, Haemaphysalis and Ixodes*. All ticks found on animals were at immature stages *i.e.* 42% were larvae and 58% were nymphs. Generally, attachment sites on animals were the ears, eyes, scrotum and body. Only 1 species, *Ixodes granulatus* (*I. granulatus*), is a known vector of disease[6-8] and was found on 1 species of squirrels *i.e.* *Lariscus insignis* (*L. insignis*) and 1 species of rodents *i.e.* *Maxomys surifer* (*M. surifer*) in the area.

The maximum number of ticks on a single host was 32. 25 of them were nymphs and 7 were adults of *Dermacentor* spp feeding on a squirrel, *Callosciurus notatus* (*C. notatus*). Attachment sites were inside the ear, under armpits and all over the body (Figure 2). Among these ticks, 3 pairs of males and females were found mating with only the females' mouthparts attached to the hosts (Figure 3). Each male has its mouthparts embedded into the female's body. A closer observation on one of the bloated females has found two male ticks; one tick was bigger than the other. All ticks on the squirrel varied from fully to partially fed.

Most ticks found from flagging vegetation were adults (21.1%) and can be identified to species level. Four species of ticks recovered were *Amblyomma testudinarium, Dermacentor atrosignatus, Dermacentor compactus* and *I. granulatus*. Female ticks (86.7%) were more commonly found from vegetation compared with the males (13.3%).

3.2. Mesostigmatid mites

Highest infestation was on rodents (73.5%), followed by

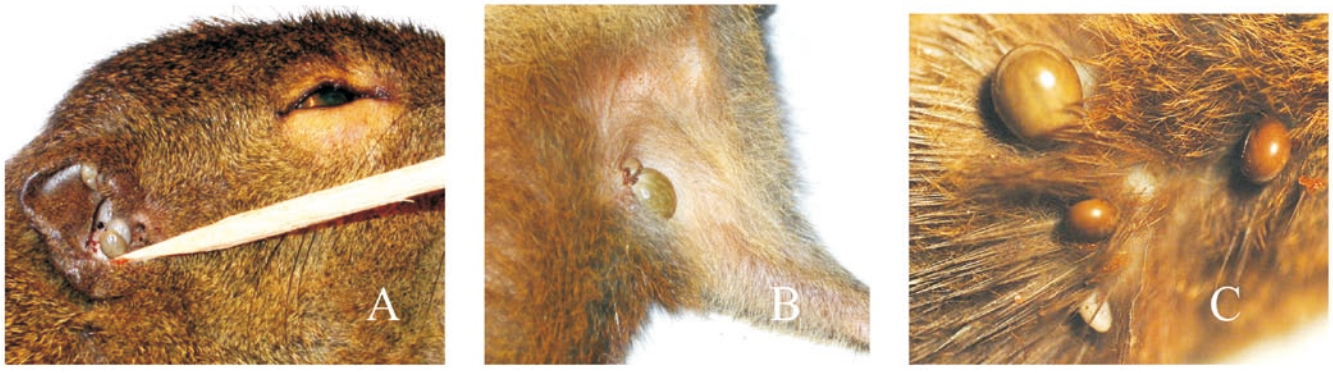

Figure 2. Attachment of ticks inside ear (A), under armpit (B) and all over body (C) of *C. notatus*.

Figure 3. Three pairs of dermacentor ticks were found mating on different squirrel, *C. notatus*.

Table 2
Species of ectoparasites found on bats, small animals and myriapods in Panti Forest Reserve, Johore (2 – 8 August 2006).

Host species	Ticks	Mesostigmatids	Chiggers
Bats			
Hipposideros diadema	–	–	*Trombigastia* spp.
Rodents			
Leopoldamys sabanus	*Ixodes* spp.	*L. nuttali*	–
Maxomys rajah	*Haemaphysalis* spp.	–	*Doloisia* spp.
M. surifer	*Dermacentor* spp	*Echinonyssus nasutus*	*Gahrliepia (Walchia)* spp.
	Haemaphysalis spp.	*Laelaps aingworthae*	*Gahrliepia (Gahrliepia)* spp.
	I. granulatus	*L. sanguisugus*	*L. deliense*
	Ixodes spp.	*Laelaps sculpturatus*	*Leptotrombidium* spp.
		Longolaelaps longulus	
		Longolaelaps whartonii	
M. whiteheadi	*Amblyomma* spp.	–	*L. deliense*
			Leptotrombidium spp.
Squirrels			
C. notatus	*Dermacentor* spp.	*Laelaps aingworthae*	*Ascoschoengastia* spp.
			L. deliense
			Leptotrombidium spp.
			Walchiella spp.
L. insignis	*Amblyomma* spp.	*Laelaps aingworthae*	*Gahrliepia (Gahrliepia)* spp.
	Dermacentor spp.	*L. sanguisugus*	*Gahrliepia (Walchia)* spp.
	I. granulatus		*Leptotrombidium* spp
	Haemaphysalis spp.		*Walchiella oudemansi*
			Walchiella spp.
Tree-shrew			
T. glis	*Amblyomma* spp.	*L. sanguisugus*	*Gahrliepia (Gahrliepia)* spp.
	Dermacentor spp.		*Gahrliepia (Walchia)* spp.
	Haemaphysalis spp.		*L. deliense*
	Ixodes spp.		*Leptotrombidium* spp.
			Siseca rara
			Siseca spp.
			Walchiella oudemansi
			Walchiella spp.
Others			
Myriapods		*Julolaelaps* spp.	*Julolaelaps* spp.

myriapods (62.5%) and tree-shrews (26.1%). A total of 4 genera in 3 families (*i.e. Laelapidae, Hirstionyssidae* and *Spinturnicidae*) were identified (Table 2). The families *Laelapidae* and *Spinturnicidae* were predominantly found on rodents and bats, respectively. A mix of these 2 families was common on tree-shrews. Four genera of laelapid mites (*Echinonyssus, Julolaelaps, Laelaps* and *Longolaelaps*) were recovered from small animals inhabiting this area. Seven species of mesostigmatids were identified. The species were *Echinonysus nasutus* (*E. nasutus*), *Laelaps aingworthae* (*L. aingworthae*), *Laelaps nuttalli* (*L. nuttalli*), *Laelaps sanguisugus* (*L. sanguisugus*), *Laelaps sculpturatus* (*L. sculpturatus*), *Longolaelaps whartonii* (*L. whartonii*) and *Langolaelaps longulus* (*L. longulus*). Six of those species were found on *Maxomys surifer* (*M. surifer*). *L. nuttalli* that is known to bite man and causing irritation[9], was only found on *Leopoldamys sabanus* (*L. sabanus*). *L. sanguisugus* was the only mesostigmatid found on *Tupaia glis* (*T. glis*).

3.3. Chiggers

The highest infestation rate was on tree-shrews (91.3%), followed by rodents (52.9%) and bats (1.5%). A total of 7 genera, 3 species and 2 sub-genus were identified in this area (Table 2). The genera were *Ascoschoengastia, Doloisia, Gahrliepia, Leptotrombidium, Siseca, Trombigastia* and *Walchiella*. Species that can be identified were *Leptotrombidium deliense* (*L. deliense*), *Siseca rara* and *Walchiella oudemansi*. The former was found on 3 species of rodents [*C. notatus*, *M. surifer*, *Maxomys whiteheadi* (*M. whiteheadi*)] and a species of tree-shrew, *T. glis*. Most of chiggers were attached to the ear-lobes, eye lids and scrotum of rodents and tree-shrews. The common attachment sites on bats were the wing membranes. *Trombigastia* spp. was the only chigger infesting the bats.

Four genera and 2 sub-genus of chiggers were found on tree-shrews. *Ascoschoengastia* and *Doloisia* were the 2 genera found in the nasal cavity of *C. notatus* and *M. rajah*, respectively. Two sub-genus of *Gahrliepia* were recovered *i.e. Gahrliepia (Gahrliepia)* and *Gahrliepia (Walchia)*. In PFR, both sub-genus were found to infest most rodents, *L. insignis* and *M. surifer* caught. However, these 2 sub-genus

were also recovered from *T. glis* caught in Sungai Sisek and Sungai Pak Kenit trails. An important vector of scrub typhus in Peninsular Malaysia, *L. deliense*[10], was found on *T. glis*, *C. notatus*, *M. surifer* and *M. whiteheadi*.

4. Discussion

High number of ticks was observed on an animal in this area. However, higher numbers had been observed in Bukit Belata Forest Reserve, Selangor where an attachment of 69 ticks (53 larva *Dermacentor* spp + 16 larva *Haemaphysalis* spp) and 45 larval *Haemaphysalis* spp. were found on an individual *M. rajah* and *T. glis*, respectively[11]. This case of a single genus of ticks dominating an animal host has also been reported else where[12].

The chigger, *L. deliense* is one of the main vectors of scrub typhus (*Orientia tsutsugamushi)* in Peninsular Malaysia[13]. Scrub typhus remains a public health problem with an estimated annual attack rate of 18.5%[14]. Seroprevalence of *O. tsutsugamushi* amongst febrile patients and rubber estate workers in rural areas of Malaysia was 24.9% and 15.3%, respectively[15]. The presence of this vector species is an indication of the potential risk for scrub typhus infection for humans that are bitten by the chiggers. To establish actual risk, there is a need to determine whether those chiggers found in PFR are infected with the rickettsia, *O. tsutsugamushi*; that however was not done in the current study. It is recommended that researchers look into this matter in the future.

Findings of the survey demonstrate the presence of three species of acarine ectoparasites which have potential health risk in PFR *i.e. I. granulatus*, *L. nuttalli* and *L. deliense*. Two of them, *I. granulatus* and *L. deliense* are known vectors of diseases. Visitors to PFR should therefore be advised of the necessary steps to prevent or reduce contact with those ectoparasites of public health importance. As part of the nation's preparedness for emerging and re-emerging infections, there is a need to document information of pathogens in local ticks and mites (acarines). Acarines have been used as epidemiological tools to detect the presence of a pathogen in a specific area[16]. In Malaysia, investigations of pathogens in local ticks and chiggers have only been recently initiated.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Mittermeier RA, Myers N, Mittermeier CG. *Hotspots: earth's biologically richest and most endangered terrestrial ecoregions*. Mexico: Conservation International; 1999.

[2] Bird Life International. *BirdLife's online world bird database: the site for bird conservation. Version 2.1*. Cambridge: Bird Life International; 2008. [Online] Available from: http://www.birdlife.org [Accessed on 26 Jun, 2008].

[3] Sittamparam R. BFRO promotes Malaysia tourism. Media Article # 499, April 2004. *New Straits Times* 2006.

[4] Putz FE. *A survey of virgin jungle reserves in Peninsular Malaysia.* Peninsular Malaysia: The Forestry Department; 1978, p. 89.

[5] Yong DL. Preliminary list of larger vertebrates in the Panti Forest Reserve, South Johore (2002-2006). 2007 [Online] Available from: wildbirdsingapore.nss.org.sg/indexF.htm1.

[6] Marchette NJ. Riketsioses (Tick typhus, Q-fever, Urban typhus) in Malaya. *J Med Entomol* 1966; **4**(2): 339-371.

[7] Hoogstraal H. Ticks in relation to human diseases caused by viruses. *Ann Rev Entomol* 1966; **11**: 261-308.

[8] Hoogstraal H, Lim BL, Nadchatram M, Anastos G. Ticks (*Ixodidae*) of *Gunong benom* and their altitudinal distribution, hosts and medical relationships. *Bull British Museum Natural History Zoology* 1972; **23**(27): 167-186.

[9] Azad AF. *Vector control series training and information guide: XIII. Mites of public health importance and their control*. Geneva: World Health Organization, Vector Biology and Control Division, WHO/VBC/86.931; 1986, p. 52.

[10] Nadchatram M, De Witt GF. Ticks, mites and health. *Perak Planters J* 1976: 79-84.

[11] Mariana A, Mohd Kulaimi B, Halimaton I, Zuraidawati Z, Ho TM, Saleh I, et al. Tinjauan ektoparasit akarin di Hutan Simpan Bukit Belata. *Siri Kepelbagaian Biologi Hutan* 2008; **9**: 161-166.

[12] Mariana A, Zuraidawati Z, Ho TM, Mohd Kulaimi B, Saleh I, Shukor MN, et al. A survey of ectoparasites in Gunung Stong Forest Reserve, Kelantan, Malaysia. *Southeast Asian J Trop Med Public Health* 2005; **36**(5): 1125-1131.

[13] Oaks SC, Ridgway RL, Shirai A, Twartz JC. Scrub typhus. *Institute Medl Res Bull* 1983; **21**: 98.

[14] Tay ST, Ho TM, Rohani My, Shamala D. Antibody prevalence of *Orientia tsutsugamushi, Rickettsia typhi* and TT118 spotted fever group rickettsiae among febrile patients in rural areas of Malaysia. *Trans R Soc Trop Med Hyg* 2000; **94**: 280-284.

[15] Tay ST, Kamalanathan M, Koay AS, Siew SC, Rohani MY, Shamala D, et al. Seroepidemiologic survey of *Orientia tsutsugamushi, Rickettsia typhi* and TT118 spotted fever group rickettsiae in rubber estate workers in Malaysia. *Am J Trop Med Hyg* 1999; **61**: 73-77.

[16] Parola P, Raoult D. Ticks and tick-borne bacterial human diseases, an emerging infectious threat. *Clin Infect Dis* 2001; **32**: 897-928.