A PRELIMINARY SURVEY OF ECTOPARASITES OF SMALL MAMMALS IN PANGKOR ISLAND, PERAK, MALAYSIA

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ABSTRACT

Ectoparasite host a wide range of zoonotic pathogens and are significant source of disease that affected human and animals. However, little study has been conducted on island habitat. Pangkor Island is a virgin jungle reserve and one of the most popular tourist attraction located at Perak, Malaysia. This preliminary survey was conducted to determine the prevalence of ectoparasites on small mammals residing in this island's forests. Small mammals were trapped by deploying 50 cage traps at two different sites which were Pangkor Selatan and Sg. Pinang forests. Ectoparasites were extracted from the captured individuals. Identification of the ectoparasite species was performed based on morphological features and molecular approach using *COI* (cytochrome oxidase subunit I) genes. A total of 13 individuals of small mammals belonging to 4 species (*Maxomys surifer, Rattus tiomanicus, Maxomys rajah, Callosciurus notatus*) were captured in the study areas. From these, 5 individuals from 2 small mammal species were infested with mites only, identified as *Laelaps* sp. The most infested host species was *Maxomys rajah*. Ectoparasites load in *M. rajah* was higher with 27 individual's mites collected, compared to *Rattus tiomanicus* with 7. This study provides information on ectoparasites present on small mammal hosts within the study areas. Our findings suggest that the resident and tourist of the island area were exposed to mite bites and potentially infected by mite-borne disease, therefore, precaution should be taken to avoid contact with small mammal hosts by improving the cleanliness of the island.

Keywords: Mites, Rodents, Pangkor Island, Infectious diseases, mites-borne diseases.

ABSTRAK

Ektoparasit merupakan perumah kepada patogen zoonotik yang pelbagai dan merupakan sumber penyakit yang signifikan yang boleh menjangkiti manusia dan haiwan. Walaubagaimanapun, hanya sedikit kajian telah dijalankan di habitat pulau. Pulau Pangkor ialah hutan simpan dara dan merupakan tempat tarikan pelancong yang terletak di Perak, Malaysia. Kajian awal ini di jalankan untuk melihat kehadiran ektoparasit pada mamalia kecil yang menghuni pulau tersebut. Mamalia kecil di tangkap menggunakan 50 perangkap sangkar di dua kawasan berbeza, iaitu Pangkor Selatan dan Hutan Sg. Pinang. Ektoparasit telah diekstrak dari spesimen yang ditangkap. Pengecaman ektoparasit telah dijalankan menggunakan ciri morfologi dan kaedah molekular menggunakan gen *COI* (cytochrome

oxidase subunit I). Sejumlah 13 individu mamalia kecil dari 4 spesies (*Maxomys surifer, Rattus tiomanicus, Maxomys rajah, Callosciurus notatus*) telah disampel dalam kajian ini. Dari jumlah ini, 5 individu telah di infestasi oleh hama sahaja, di kenalpasti sebagai *Laelaps* sp. Spesies yang paling timggi diinfestasi ialah *Maxomys rajah.* Ektoparasit yang terdapat pada *Maxomys rajah* adalah tertinggi dengan 27 individu hama dikumpulkan, berbanding dengan *Rattus tiomanicus,* iaitu hanya 7. Kajian ini menyediakan maklumat tentang ektoparasit yang hadir pada perumah mamalia kecil di kawasan kajian. Hasil mendapati penduduk dan pelancong di pulau ini terdedah kepada gigitan hama dan berpotensi di infestasi oleh penyakit bawaan hama, dan oleh itu, langkah berjaga-jaga perlu di ambil untuk mengelakkan hubungan dengan mamalia kecil melalui peningkatan kebersihan di sekitar pulau.

Kata kunci: Hama, Roden, Pulau Pangkor, Penyakit berjangkit, Penyakit bawaan hama.

INTRODUCTION

Pangkor Island (Pulau Pangkor) is an island off the west coast of Perak, Peninsular Malaysia. It is located in Manjung District, Perak, in the Straits of Malacca, about 3.8 nautical miles from Lumut. Pangkor Island with an area of 2,274.78 hectares, is the biggest among the eight islands in its clusters, namely the Mentangor Island, Pangkor Laut Island, Giam Island, Tukun Terindak Island, Pelandok Island, Simpan Island and Dua Island. Pangkor Island is a foremost tourist attraction in Perak and is currently being well-known as a world-class tourism centre (Mapjabil 2015). Geographically, 40% of the island is covered by forest. This island comprised of three forest reserves namely Pangkor Utara and Pangkor Selatan on the northern and southern tip of the island respectively, and Sungai Pinang forest situated at the central region of the island (Onn et al. 2010). The landscape of Pangkor Island consists of sandy beaches with mangrove swamps lining parts of its coasts, while its interior is composed of mountainous terrain covered with large expanses of lowland and hill dipterocarp forests dominated by the rattan species (*Calamus castaneus*) and palm species (*Eugeissona tristis*) (Lee et al. 2006), and strewn outcroppings of granite boulders. The forest in Pangkor is special for its virgin jungle reserve (VJR), which refers to undisturbed, sample of natural forest located in commercially productive forest (Wyatt-Smith 1950). Thus, explaining that Pangkor to become inhabitants to a great variety of animals including small mammals. However, there were very limited published reports on small mammals in Pangkor Island.

Ectoparasites are miscellaneous organisms and highly adapted animal's group that infest the external body surface of vertebrates (Hanafi-Bojd et al. 2007). They infest the host's skin, for instance, the most external orifices, especially the ears, nares and orbit, hair and sebaceous glands (Diaz 2015), from which they derive their sustenance (Meredith & Ulrich 2013). The prevalence of the ectoparasitic species can serve as an indicator to measure the risk of ectoparasitic-borne disease transmission to human that are associated with the hosts (Heukelbach et al. 2012). Some ectoparasites' species are found infesting small mammals in Malaysia are medically crucial as they act as the vector for serious disease and ectoparasitism. Ectoparasitism refers to an infestation of the host by Acarids group of the genera *Ixodes, Ophionyssus, Aponomma, Agrasidae, Hyalomma, Haemaphysalis, Amblyomma* and *Ornithodoros* (Mayer & Donnelly 2013). Infested small mammals will serve as vector for the diseases before transmitting to humans. The mode of transmission correlated to the life cycles of the parasites such as spores, eggs, cysts and juveniles (Roberts & Janovy 2006; Priscilla et al. 2015). Mites are small arthropods belonging to the class Arachnida and the subclass Acari (Acarina). They are highly diverse and specified group that can be further divided into mesostigmatid mites, chiggers, myobiids and listrophorids (Mohd Zain et al.

2015). Most types of mites are free-living; they are not parasitic but a few types become specialized to infest or feed on animal (Walter & Proctor 2013).

Small mammals are mammalian animals weighted ranges from 2 gram to 5 kg and below in adult (Bourliere 1975). It includes 10 out of 16 mammalian orders in the world in which 90% from 3900 species in the world known to have a weight less than 5 kg. Peninsular Malaysia inhabits a total of 9 orders, 25 families and 95 genera of small mammals. Non-volant small mammals were categorized in Order Rodentia, Scandentia and Insectivora. Rodentia composed of 55 species represented by the largest family which are Sciuridae and Muridae. Sciuridae is made up of squirrels which are active during the day and the sub-family Petauristinae which is nocturnal. Muridae family represented by rats which usually active at night (Medway 1978). Small mammals are simply infected by several parasitic agents because of their lifestyles; habitats and contact with other animals. Consequently, they can be a major source of internal and external parasitic infections for animals and humans (Vazirianzadeh & Rahdar 2013).

There have been no ectoparasite surveys and information found on small mammals conducted in this area. Thus, the aim of this study was to identify the ectoparasite's found on small mammals in this island, as well as to determine their prevalence in different small mammals' species. In general, this study can help to gain a better understanding about ectoparasites' infestation in the eco-tourism Island of Peninsular Malaysia, thus help in the management of hosts species. Information on host parasite-pathogen interaction is important as it is related to management of zoonotic diseases (Madinah et al. 2014). Furthermore, the prevalence of mites' infestation among these small mammals' hosts could provide the evidence on which host species is prone to mites' infestation, thus the specific approaches in controlling mites' population can be made.

MATERIALS AND METHODS

Study Areas

The study was conducted at Pulau Pangkor, Perak, Malaysia. We have chosen Pangkor Selatan and Sungai Pinang Forest reserve as survey sites. Sampling was conducted in Pangkor Selatan near Pasir Bogak and Sungai Pinang forest reserve. Both these forests were gazetted as virgin jungle reserve. Table 1 below summarizes the characteristics of these forests.

Table 1 Characteristics of selected VJR in Pangkor Island

Forest reserve	Compartment	Area of gazetted (ha)	Type of forest	Total forest area (ha)
Pangkor Selatan	3	104	Strand, heath, Hill dipterocarp forest (coastal), heath	311
Sg. Pinang	5	42	Hill dipterocarp forest and Low dipterocarp forest	987

Small Mammals Sampling

Fifty cage traps were used to capture small mammals at Pangkor Selatan and Sungai Pinang Forests. The survey involved trapping using cage traps (20x20x30 cm) at respective sites throughout the course of the study. A total of 50 cage traps were placed randomly along the stream or forest trails, and baited with oil palm fruits, sweet potatoes with peanut butter, salted

fish or a special type of aromatic banana to attract small mammals such as rodents, squirrels and tree shrews. The cage traps were inspected twice daily in the morning ca. 1000 and 1800 hours. Trapped animals were brought to the research station prior to sample collection. Morphological measurements were taken and identification from physical appearance was based on Francis (2008). Before handling, all animals were anaesthetized with an intramuscular injection of Zoletil® 50 (0.5 – 0.8 ml) as previously described (Rivas *et al*. 2015) and after gaining consciousness, they were released back at their captured sites. Before releasing, the animals were marked with numbered ear tags. Trapping and handling procedures for small mammals have been approved by the animal research ethics committee at Universiti Kebangsaan Malaysia (FST/2016/ SHUKOR/18-MAY/750-MAY-2016-SEPT.-2018-AR-CAT2).

Ectoparasite Sampling and Identification

Comb was used to collecting the mites which present throughout the entire body in between the animal's fur. Mites ware collected using fine forceps and preserved in labelled cryo-vials containing 70% alcohol for preservation. Morphological examination of mite samples identified as unknown due to lack of morphological features. In order to confirm the identities of mite species in Pulau Pangkor, a molecular approach was used to validate morphological identification. Selected mite samples from each host individual were used in the molecular method. First, mite samples were washed thrice in 70% ethanol followed by sterile deionized water (ddH_20) to remove environmental debris and for surface disinfectant (Capri et al. 2011). The extraction of DNA was performed using the MN-NucleoSpin® Tissue kit (MN Germany). Polymerase chain reaction (PCR) was performed to amplify the partial *COI* (cytochrome oxidase subunit I) genes as described by Folmer et al. (1994) for the confirmation of mite species.

Data Analysis

In an epidemiological study, prevalence is a measurement of all individuals affected by the disease at a time (Shields & Twycross 2003). Prevalence gives a figure for a factor at a single point in time (Jekel et al. 2001). In this study, the overall prevalence of infested small mammals and prevalence of infested small mammals with ectoparasites was calculated. Prevalence of infested host was calculated by using the formula below:

$$\text{Prevalence} = \frac{\text{Total number of infested small mammals}}{\text{Total number of small mammals captured}} \times 100\ \%$$

RESULTS AND DISCUSSION

A total of 13 individuals from four species and two families of small mammals were recorded during the small mammals sampling in the Pangkor island. Family Muridae was the most abundant with 12 individuals dominated by the Malaysian Wood Rat (*Rattus tiomanicus*), followed by Brown Spiny Rat (*Maxomys rajah*). Only one individual from Family Sciuridae was recorded which is the Plantain Squirrel (*Callosciurus notatus*).

From 13 individuals captured, only mites were found, which consists of 34 individual mites' altogether. The results of this survey are presented in Table 1 and 2. In total, five individual host comprising of two different species were infested by mites in this study. All mites collected were identified as *Laelaps* sp. (Figure 1 and 2) based on morphology examination through stereomicroscope and molecular analysis. Molecular analysis identified only one genus (*Laelaps*). Morphological analysis was unable to be performed due to lack of morphological features and keys. The molecular sequence has been deposited in the NCBI Genebank.

Table 1 list the host individuals as well as the mites collected from them. This study shows that prevalence of *Laelaps* sp. was higher in *Rattus tiomanicus* compared to *Maxomys rajah*. However, mites load was higher in *M. rajah* with 27 mites individuals (9 mites/host), compared to *R. tiomanicus* with 7 mites individuals (3.5 mites/host) (Table 2).

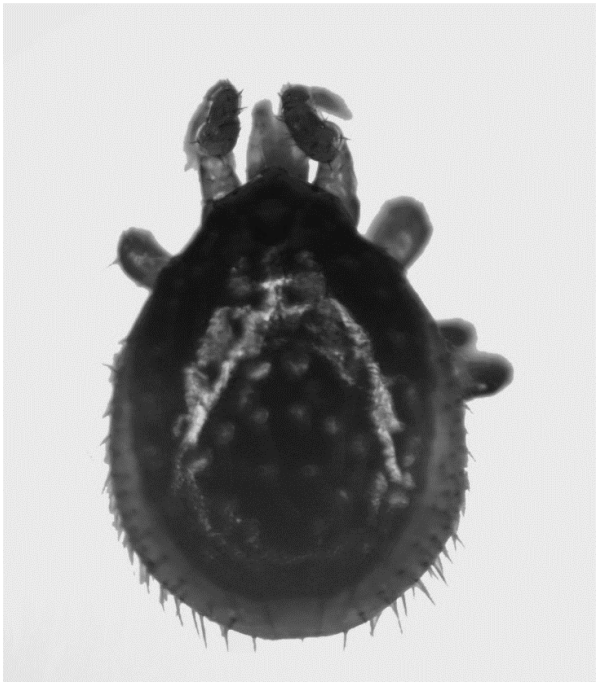

Figure 1 The photos of *Laelaps* sp. in dorsal view (under stereomicroscope: 10×40).

Figure 2 The photos of *Laelaps* sp. in ventral view (under stereomicroscope: 10×40).

Table 1 Prevalence of mites in each small mammal's host species in the study area.

Family	Species	Examined	Infested	Prevalence (%)	Mites Load
Muridae	*Maxomys rajah*	8	3	37.5	27
	Rattus tiomanicus	3	2	66.7	7
	Maxomys surifer	1	-	-	-
Sciuridae	*Callosciurus notatus*	1	-	-	-
Total		**13**	**5**	**38.5**	**34**

(-) means no ectoparasites were collected from that particular host.

Table 2 Total number of individual mites in each infested host species and mite species.

ID Code	Host species	Sex	No. of individual mites	Mites species
PP03	*Rattus tiomanicus*	F	6	*Laelaps* sp.
PP04	*Rattus tiomanicus*	M	1	*Laelaps* sp.
PP06	*Maxomys rajah*	F	9	*Laelaps* sp.
PP07	*Maxomys rajah*	M	5	*Laelaps* sp.
PP013	*Maxomys rajah*	M	13	*Laelaps* sp.
Total			**34**	

It has been documented that host density is important for determining the presence of ectoparasite species (Gómez-Rodríguez et al. 2015). Limited sampling effort restricted our preliminary survey data to conclude the relation between small mammal species and mite infestation. Rodents are often among the most abundant groups of wild animals around human settlements and the most common reservoir hosts of zoonotic infectious agents that live in close association to humans and they are known reservoirs of several pathogens of public health and veterinary importance, causing serious diseases (Yonas et al. 2011). In addition, rodents brought diseases to humans by increasing the chances of exposure to potentially infected ticks, mites and fleas, and together, they play vital roles as amplifying hosts and transporters of rickettsiae (Tay et al. 2014). However, this report on rodent–ectoparasite associations from the Pangkor Island is the first of its kind.

From this finding, only two host species *Rattus tiomanicus* and *Maxomys rajah* were infested with mites from the genus *Laelaps* sp. This mite is common species that can infest small mammals, wild animals or even human. For instance, *L. echidna,* a rat mite, was reported to be transmitted to human, periodically during building constructions or renovations, which cause disturbance to the commensal rodents colonies, thus has the potential to transmit zoonotic disease (Watson 2018). On another instance, *L. sanguineus* was found to be a vector of *Rickettsia akari* (human rickettsialpox) (Saini et. al. 2004) and *Coxiella burnettii* (Q fever) (Zemskaya 1968). Razali et. al. (2018) suggested that *Laelaps* sp. tend to be rodent generalist mites, as found in their study in *Maxomys rajah, M. whiteheadi, M. surifer* and *Leopoldamys sabanus*. This was also was supported by Madinah et al. (2011), who recorded 7 species of mesostigmatid mites in rodent's species, namely *M. rajah* and *M. whiteheadii*. Similar to this, Chuluun *et al.* (2005) recorded two *Laelaps* species from *L. nuttalli*, and *L. echidninus* in *M. whiteheadi* and *R. exulans* hosts from Kuala Selangor Nature Park. *Laelaps* sp. were reported have the highest infestation of mite on rodents at the highland of Tigray at northern Ethiopia (Yonas et al. 2011), supported our preliminary data. Infestations of ectoparasites can cause considerable discomfort to affected animals and capable of causing systemic disease that can lead to life threatening conditions, such as anaemia in young or debilitated animals (Salant et al. 2014). Little information is known about ectoparasite habitat-host relationships at the macrohabitat scale (Gómez-Rodríguez et al. 2015).

CONCLUSION

This study provides preliminary information on mites infestation among small mammals in the Pangkor island. A more comprehensive study needs to be carried out, especially to detect any potential mite-borne pathogen and diseases in this area, thus specific action can be implemented to avoid cases of the disease transmission to tourists and the local residents.

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