UNIVERSITI PUTRA MALAYSIA

PARASITES OF WILD MALAYSIAN PLANTAIN SQUIRREL (CALLOSCIURUS NOTATUS), GREY-BELLIED SQUIRREL (CALLOSCIURUS CANICEPS) AND TREE SHREW (TUPAIA GLIS)

NORMAN AFFENDI OSMAN

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NORMAN AFFENDI OSMAN

FACULTY OF VETERINARY MEDICINE,
UNIVERSITI PUTRA MALAYSIA,
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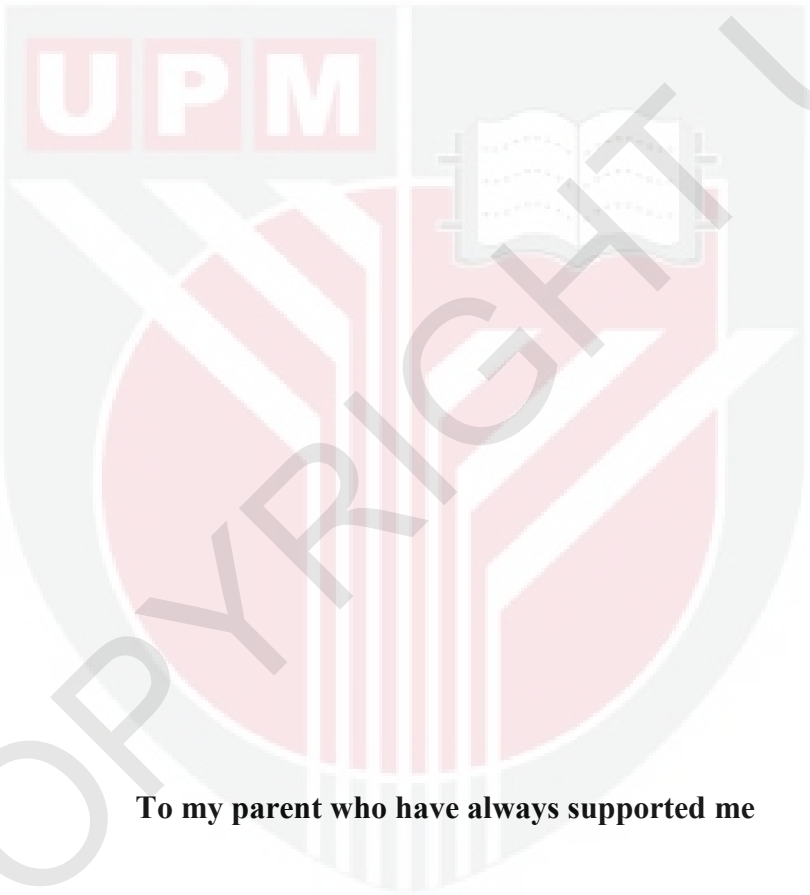
In the name of Allah, the Most Merciful and the Most Gracious

All the praises and thanks be to Allah, the Lord of the 'Alamin



To my parent who have always supported me

To my wife who have always encouraged me

And all of my family members

Without whom none of my success would be possible

It is hereby certified that we have read this project paper entitled "**PARASITES OF WILD MALAYSIAN PLANTAIN SQUIRREL (Callosciurus notatus), GREY-BELLIED SQUIRREL (Callosciurus caniceps) AND TREE SHREW (Tupaia glis)**" by Norman Affendi Osman and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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ASSOC. PROF. DR. SHAIK MOHAMED AMIN BIN HJ S. M. BAJBEE

BVSc. (Punjab), MSc (UK), PhD (Australia)

Associate Professor

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

_____

DR. TENGKU RINALFI PUTRA BIN TENGKU AZIZAN

B.App.Sc (Hons) (Kustem), M.Sc (UKM), PhD (Canterbury)

Senior Lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir.

PARASIT-PARASIT PADA TUPAI PINANG (*CALLOSCIURUS NOTATUS*), TUPAI PERUT KELABU (*CALLOSCIURUS CANICEPS*) DAN TUPAI TANAH (*TUPAIA GLIS)* LIAR DI MALAYSIA

oleh

NORMAN AFFENDI OSMAN

2015

Penyelia: Prof. Madya Dr. Shaikh Mohamed Amin Babjee

Penyelia bersama : Dr. Tengku Rinalfi Putra Tengku Azizan

Tupai pinang dan tupai perut kelabu merupakan roden yang tergolong dalam famili Sciuridae, manakala tupai tanah (order Scandentia) bukan roden dan ia tergolong dalam famili Tupaidae. Tupai tanah hampir serupa dengan tupai dari segi penampilan fizikal dan tabiat dan selalunya disalahertikan sebagai tupai. Kebiasaannya perkataan 'tupai' dalam Bahasa Melayu merujuk kepada kedua-dua jenis haiwan tersebut walaupun tupai tanah bukanlah tupai yang tergolong dalam famili roden. Ketiga-tiga

spesies mamalia kecil ini mudah ditemui dan kebanyakkannya mendiami di kawasam hutan tanah pamah, ladang-ladang kelapa sawit, dan juga di kawasan bandar di Malaysia. Haiwan-haiwan ini amat mudah menyesuaikan diri dalam apa jua persekitaran dan kadangkala ia juga dianggap sebagai makhluk perosak di kalangan para petani dan penanam buah-buahan. Setakat ini, bilangan tupai pinang, tupai perut kelabu dan tupai tanah memang banyak dan populasinya masih tidak terancam. Walaupun haiwan-haiwan ini memainkan peranan penting dalam menyebarkan benih serta mengekalkan keseimbangan ekosistem tetapi besar kemungkinan ia juga boleh menjadi pembawa kepada agen jangkitan penyakit seperti virus, bakteria rickettsia dan parasit yang boleh menyebabkan penyakit pada manusia dan juga pada haiwan lain seperti kucing, arnab dan anjing. Oleh yang demikian, kajian ini telah dijalankan untuk menentukan samada haiwan-haiwan ini boleh mendatangkan penyakit parasit kepada spesies haiwan lain melalui ektoparasit dan endoparasit yang terdapat padanya. Selain daripada itu kajian ini juga dilakukan dengan tujuan untuk menentukan jenis-jenis parasit yang terdapat padanya dan kesannya terhadap kesihatan umum. Sebanyak 9 ekor haiwan yang terdiri daripada 3 spesies yang telah disebut tadi ditangkap di sekitar kawasan pinggir bandar di Senawang, Negeri Sembilan dan diperiksa untuk ektoparasit, endoparasit dan parasit darah. Hos-hos yang diuji adalah *Callosciurus notatus*, *C. caniceps* dan *Tupaia glis*. Diantara hos-hos tersebut, 2 genera ektoparasit dari keluarga hama telah ditemui pada *T. glis* dan *C. caniceps* iaitu dari genus *Laelaps* dan berkemungkinan dari genus *Haemolaelaps*, 1 nematoda dari genus *Spirura* dan 1 cestoda dari genus *Tupaiataenia* telah dikenalpasti dari *T. glis* dan

tiada parasit darah ditemui dari palitan darah dari ketiga-tiga spesies. Di sini boleh disimpulkan bahawa kebanyakan spesimen yang telah ditangkap dan diperiksa adalah sihat, ini adalah kerana terdapat terlalu sedikit jumlah parasit dijumpai. Ini mungkin disebabkan oleh bilangan sampel yang diperolehi agak kecil. Oleh yang demikian kajian yang lebih terperinci dan penggunaan bilangan sampel yang banyak adalah dicadangkan pada masa akan datang untuk mengumpulkan dokumen yang lebih lengkap untuk menentukan kelaziman parasit dalam terdapat pada ketiga-tiga spesies mamalia kecil tersebut.

Kata kunci:Tupai, tupai tanah, ektoparasit, endoparasit, parasit darah

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 – Final Year Project.

PARASITES OF THE WILD MALAYSIAN PLANTAIN SQUIRREL (*CALLOSCIURUS NOTATUS*), GREY-BELLIED SQUIRREL (*CALLOSCIURUS CANICEPS*) and TREE SHREW (*TUPAIA GLIS*)

By

NORMAN AFFENDI OSMAN

2015

Supervisor: Prof. Madya Dr. Shaikh Mohamed Amin Babjee

Co-supervisor: Dr. Tengku Rinalfi Putra Tengku Azizan

Plantain and Grey-bellied squirrels are rodents that belong to *Sciuridae* family, whereas the common tree shrews are not rodents (order Scandentia) and they belong to *Tupaidae* family. Tree shrews are similar to squirrels in their appearance and habits and are usually mistaken as squirrels, the Malay word 'tupai' is used for both tree shrews and squirrels. These three species are widely spread and commonly found in

lowland forests, oil palm plantations, and urban environment in Malaysia. They are very adaptable to all kinds of habitats and are also regarded as pests by farmers and fruit growers. Plantain squirrels, Grey-bellied squirrels and common tree shrews are abundant throughout their range and are adaptable to changing habitats and the populations are not currently threatened. Even though they play an important role in dispersing seeds and maintaining the ecosystem but they may also be a source of infection for various viral, rickettsial and bacterial pathogens that may cause diseases in humans as well as in other domesticated animals such as cats, rabbits and dogs. Therefore, this study was carried out to determine the possible transmission of parasitic diseases to other animals species via ectoparasites and endoparasites harboured by these small mammals and to determine what are the significant family of parasites that are found in them and their impacts to public health. A total of 9 animals comprising 3 species of hosts were caught around suburban area in Senawang, Negeri Sembilan and examined for ectoparasites, endoparasites and hemoparasites. The hosts examined were *Callosciurus notatus*, *C. caniceps* and *Tupaia glis*. Of these hosts, 2 genera of ectoparasites were found in *T. glis and C. caniceps* which were mesostigmatid mites (*Laelaps* sp. and possibly *Haemolaelaps* sp.), 1 genus of nematode (*Spirura* sp.) and 1 genus of cestode (*Tupaiataenia* sp.) were identified from *T. glis* and there were no blood parasites observed from the thin blood films and wet blood mounts of these three species. It can be concluded that most of the specimens caught and examined were healthy because there were very few parasites found. This is probably due to the number of samples caught that were quite low. Therefore

longer period of study is proposed in the future to gather complete documentation of parasite prevalence in these three species.

Keywords: Squirrels, tree shrews, ectoparasites, endoparasites, blood parasites,

INTRODUCTION

Plantain and Grey-bellied squirrels are rodents that belong to Sciuridae family,whereas the tree shrews are not rodents (order Scandentia) and they belong to Tupaidae family. Tree shrews are similar to squirrels in their physical appearance and habits and always mistaken as squirrels, the Malay word 'tupai' is used for both tree shrews and squirrels. Both of these three species are widely spread and commonly found in lowland forests, oil palm plantations, and urban environment in Malaysia. They are very adaptable to all kinds of habitats and are also regarded as pests by farmers and fruit growers. Plantain, Grey-bellied squirrels and tree shrews are abundant throughout their range and are adaptable to changing habitats and populations are not currently threatened (Duckworth et *al*. 2008).

In order to identify the ectoparasite and endoparasite species of both of the squirrels, samples will be collected by setting the rat traps (collapsible cage traps) in housing estates adjacent to an oil palm plantation, fruit orchards and parks where they are usually seen and observed. In studies conducted by Faculty of Agriculture ,Yamaguchi University, *Strongyloides callosciureus*, a new species of rhabditoid nematode had been isolated from plantain squirrels which were imported from Malaysia as pets some decades ago (Sato et *al.*, 2007).

There were several of studies conducted pertaining to ectoparasites and endoparasites on these rodents species in Malaysia previously but only few research done on the blood parasites of the squirrels. There have been various studies

conducted on the parasite infesting squirrels, yet there has not been much research on the parasitic status of *C. notatus* especially with regards to helminths, blood protozoa and coccidia (Watanabe, 2000). Even though they play an important role in dispersing seeds and maintaining the ecosystem but they may also be a source of infection for various viral, rickettsial and bacterial pathogens that may cause diseases in humans as well as in other domesticated animals such as cats, rabbits and dogs. Many of such diseases are transmitted by arthropod vectors infesting rodent reservoirs (Woodhouse et *al*., 2001; Thanee et *al*.,2009)

The objective of this study are one, to identify the common ectoparasites, endoparasites and blood parasites of both squirrel species (*C. caniceps* and *C. notatus)* and in the tree shrews *(T. glis*) and two, to enumerate each of the species of parasites found in both the squirrel species and in the tree shrews.

Habitat, diet and behaviour also play important roles in determination of prevalence of the parasites in these animals. When habitats are disturbed and the composition of the small mammal community changes, ectoparasites may encounter different hosts near their nest microhabitat, and transfer may occur (Gettinger et *al*.,1995; Thanee et *al*., 2009)

LITERATURE REVIEW

Plantain and Grey-bellied Squirrels

The Plantain squirrel (*C. notatus*) and the Grey-bellied squirrel (*C. caniceps*) are species of rodent in the Sciuridae family. Plantain squirrel is also called Oriental or Tricoloured Squirrel, mean while the Grey-bellied squirrel is also referred as Golden-backed Squirrel (Duckworth et *al*. 2008). These two species of rodent are found in Indonesia, Malaysia, Singapore and Thailand (Baker, 2015). They are found in a wide range of habitats such as forests, mangroves, parks, gardens, agricultural and urban areas (Constantine, 2006). The plantain squirrels are important vertebrate pests of many plantation crops in Malaysia (Kamarudin-Lee, 1981; Hafidzi, 1992). Paramavaran et *a*l (2009) mentioned that, rodents especially those that live in close proximity with humans (synanthropic rodents) play a significant role in the transmission of diseases to humans and animals while others act as reservoir host.

Tree shrews

Acorrding to UFAW Handbook of Tree Shrews described in detail by Fuchs-Sohle (2009), tree shrews (*T. glis*) are not rodent, they belong to Tupaiidae family. They are similar to squirrels in their physical appearance and habits and always mistaken as squirrels, the Malay word 'tupai' is used for both tree shrews and squirrels. Tree shrew is also called 'tupai tanah' or ground squirrel. Despite their name, tree shrews have nothing to do with real shrews (family Soricidae) and most species of tree shrews are much more active on the ground than in trees. Tree shrew range from predominantly arboreal (e.g. *Dendrogale, T. Minor, Ptilocercus)* to the predominantly

terrestrial (e.g. *Lyonogale, Urogale*), but most tree shrews are semi arboreal and usually forage on the ground.

The Importance of Parasites

Parasites are organisms that live on or in host organisms and they totally depend on the hosts for survival of their species. There are three main classes of parasites that can cause disease in humans as well as in animals namely protozoa, helminths, and ectoparasites.Animals and their ectoparasites play important roles in distribution of arboviruses, strep-tococcal infections, choriomeningitis, plague, tularemia, leptospirosis and spi-rochetosis (Manson & Stanko, 2005: Madinah et *al*., 2011).

The arthropod ectoparasites of rodents are important vectors of pathogenic microorganisms and for parasitic zoonoses diseases (Singleton et *al*., 2003; Paramasvaran et *al*., 2009). The rapid environmental degradation resulting in the current changes in the global climate may alter the ecology of rodents and create new foci that would promote the proliferation of vectors that will transmit rodent borne parasitic diseases. These ecological changes will increase contact between human and rodents resulting in a heavier disease burden that would challenge the efficiency of the public health services. (Paramasvaran et *al*., 2009).

According to a study of the ectoparasites by Paramasvaran et *al*. (2009), the prevalence of the ectoparasites according to the species of wild rodents was reported as shown in ***Table 1.***

Table 1. Ectoparasites recovered from wild (forest) rodents

Rodent host	Mites	Chiggers	Ticks
Maxomys rajah	*Listrophoridae* *Laelaps sanguisugus* *Laelaps sculpturata* *Longolaelas longulus* *Laelaps aingworthae*	*Gahrlepia fletcheri*	*Amblyomma sp* *Dermacentor sp* *Ixodes granulatus* *Haemaphysalis*
Leopoldamys sabanus	*Listrophoridae* *Laelaps aingworthae*	*Walchiella oudemansi*	*Dermacentor sp* *Ixodes granulatus* *Haemaphysalis*
Sundamys muelleri	*Listrophoridae* *Laelaps aingworthae*	*Gahrlepia fletcheri*	*Dermacentor sp* *Ixodes granulatus* *Haemaphysalis*
Rattus bowersi	*Listrophoridae* *Laelaps sculpturata* *Laelaps aingworthae*	-	*Dermacentor sp* *Ixodes granulatus* *Haemaphysalis*
Maxomys whiteheadi	*Listrophoridae* *Laelaps sculpturata* *Laelaps aigworthae*	-	-
Tupaia glis	*Listrophoridae* *Laelaps sculpturata* *Laelaps aigworthae*	-	*Dermacentor sp*
Lariscus insignis	-	-	*Ixodes granulatus*

MATERIALS AND METHODS

This study was conducted at suburban housing estate adjacent to oil palm plantation in Senawang, Negeri Sembilan over a period of three weeks. The animals were trapped using collapsible cage traps and strong scented fruits such as bananas were used as bait. Usually cage-traps, may be chosen because of their low cost (compared with commercial ones) and because of their availability (Habreteau et *al*., 2011). The traps were set and placed in several areas on the ground and on the canopy of oil palm trees where the animals were usually observed.

One grey-bellied squirrel two plantain squirrels and six tree shrews of mixed sexes were trapped alive. Each of the captured animals species was then anaesthetized with chloroform for ectoparasites and blood collection then they were humanely euthanized for collection of gastrointestinal parasites. For ectoparasites collection, each of the anaesthesized animals, the ectoparasites were removed from fur, ears and tail with a fine comb, forceps and then stored in 70% alcohol for enumeration and identification (Ramli et *al*., 2009). The collected ectoparasites were observed using stereo and compound microscopes for identification of species. The identified ectoparasites were stained and mounted on Hoyer's medium for further confirmation of the species (Kranz, 1978; Chatbunjong et *al*., 2010). Blood samples were collected from cardiac puncture using a 23-25 gauge needle and placed directly on slides and several thin blood films were carried out on the spot as well as a direct wet blood mount. The direct wet blood mounts were examined and observed under a compound

microscope under objective x 10 to look for active parasites (e.g. *Trypanosoma* sp.). Thin blood films were air-dried, fixed in absolute methanol, and stained with Giemsa stains and observed under compound microscope with x 1000 magnification for any blood parasites (Watanabe, 2000)

The euthanized animals were dissected and the gastrointestinal tracts were isolated, cut and flushed with normal saline and then were placed in a petri dish containing isotonic solution to prevent the parasite from shrinking. The intestines were dissected or cut opened to expose the lumen and then stirred in the petri dish containing isotonic solution to separate the intestinal parasites and observed under microscope for species identification. The stomach contents were carefully examined for helminths. The helminths were examined under the compound microscope, objective x10 and x40. All the identification of ectoparasites, endoparasites and blood parasites was made by Mr. John Jeffery of the Parasitology Laboratory, Faculty of Veterinary Medicine, UPM.

RESULTS

The results of ectoparasites and endoparasites of this study are illustrated in Table 2.

Table 2:

Host Species	Mites	Chiggers	Ticks
C. caniceps	*Haemolaelaps* sp.	-	-
C. notatus	-	-	-
T. glis	*Laelaps* sp.	-	-

Host Species	Nematodes	Cestodes	Trematodes
C. caniceps	-.	-	-
C. notatus	-	-	-
T. glis	*Strongylus* sp.	*Taenia* sp.	-

Host Species	Nematodes	Cestodes	Trematodes
C. caniceps	-	-	-
C. notatus	-	-	-
T. glis	4	1	-

Ectoparasites

The mite of family Listrophoridae was found in the grey-bellied squirrel (the one and only species captured) and in one of six tree shrews examined, both mites were isolated from the ear swabs and ventral part of the body. No lice and ticks were found in the nine animals of the three species caught.

Blood parasites

All three species of the animals were negative for blood parasites following examination on both the thin blood films and wet blood mounts.

Endoparasites

Out of six tree shrews only one showed presence of the nematode *Strongylu*s sp. while another showed the presence of cestode *Taenia* sp., while neither grey-bellied nor plaintain squirrels were positive for the presence of cestodes and nematodes.

Habitat

The locations in which the animals were trapped consisted of oil palm trees and shrubs. Most of the animals were trapped around the oil palm trees where the source of feed were abundance.

Diet

The diet of these three species are mainly ripened palm oil seed and sweet-smelling fruits. Sometimes it was also observed that the tree shrews eating the insects, small reptiles and the left overs of the cat feed from the housing areas adjacent to the palm oil plantation. It was rarely seen that these animals scavenging for feed from the trash bins.

Behaviour

The tree shrews are mainly terrestrial whereas the plantain and grey-bellied squirrels are mainly arboreal that occasionally come to the ground. Most of the time the squirrels spend their times on the tree canopy, but sometimes they come to the ground to share the fallen palm oil seed or intraspecies competition for territorial. Even though these three species share the same habitat but their niche is totally different from one another. There is no evidence of interspecies competition for feed and territory.

DISCUSSION

The mite infestation of these animals were very low based on the ectoparasite evaluation done on these three species. Only grey-bellied squirrel and one out of six tree shrews were found to have mites isolated from the ears of the squirrel and the ventral part of the body of the tree shrew. No mites were found in plantain squirrel. The mesostigmatid mites from Listophoridae family were found on both animals. The identified genera of the mites were *Laelaps sp.* and *Haemolaelaps sp.* for the grey-bellied. In term of ectoparasites the arboreal species (plantain and grey-bellied squirrels) tended to have lower infestations of ectoparasites than did the terrestrial species (tree shrews). In term of public health importance none of the ectoparasites found has a major potential health risk to human.

Thanee et *al*. (2009) also mentioned that *Microfilaria* spp., *Anaplasma* spp. and *Grahamella* spp. were recovered from tree shrews but there were no evidence of these three species of animals may act as carriers or reservoirs of blood borned diseases due to the absence of blood parasites following the examination of thin blood films and wet blood mounts done in this studies. There were no blood parasites from the animals examined. This could be due to the small number of specimen (9) examined or it could have been missed during the examinations due to light infections. A similar blood parasite study conducted on small mammals in the forest fringes of Bukit Komondol in Selangor, Malaysia did not show any blood parasites either (Paramasvaran et *al*., 2009).

Endoparasite infestations in the wild animals are common scenario but it can be said that the prevalence of infestation is closely related to the habitat, diet and behaviour of the animals. Destruction of the natural habitat, introduction of exotic species of animal are the main culprits which may contribute to the increasing case of endoparasites infestation of the wild animals, for example, habitat loss will cause loss of natural feed resources hence the wild animals have to find other source of feed by means of scavenging any feed available even though it was not meant for them for example kitchen scraps. In term of public health importance nematodes and cestodes can be found in human, therefore there is a risk of infection of helminths if these animals are consumed as food.

Sharing microhabitat may also increase the possibility of ectoparasites and endoparasites transmission between two different hosts, for example when the source of feed is limited in a particular area, these wild animals will gather together to share the feed thus direct and indirect contact will occur and the possibility of transmission of the parasites may occur as well.

According to Pearse (1929), certain physical qualities, habitats and habitat preferences were related to infestation with ectoparasites. It can be said that the species most likely to be infested were those terrestrial as compared to arboreal. Competition for feed may occur but there are no evidence of territorial acts between the three species observed.

CONCLUSION

The study revealed that only two animals from three different species (grey-bellied squirrel and tree shrew) were not heavily infested with mesostigmatid mites and two species of helminths from different classes namely nematode and cestode were recovered in two out of six tree shrews. No ectoparasites and endoparasite were found in plantain squirrels.

The significance of the study, is that due to the abundance source of feed with less human interference of the ecology, it can be said that these 3 species of animals are quite healthy due to very few parasites found in the study, thus there is no need to worry about the possible transmission of diseases from these animals to human or to other animals.

RECOMMENDATIONS

Recommendations for future studies include, longer period of study is proposed in order to gather complete documentation of parasite prevalence in these three species and to develop a more comprehensive inventory of ectoparasites and endoparasites in different locations and habitats which can improve knowledge regarding host-parasite relationships, biology and ecology.

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APPENDICES

Plate 1. Collapsible cage trap with bait

Plate 2: One of the study subject captured, tree shrew (*T.glis)*

Plate 3: Withdrawal blood via cardiac puncture

Plate 4: Skin scraping for ectoparasite examination

Plate 5: Isolating the gastrointestinal tract for endoparasites examination

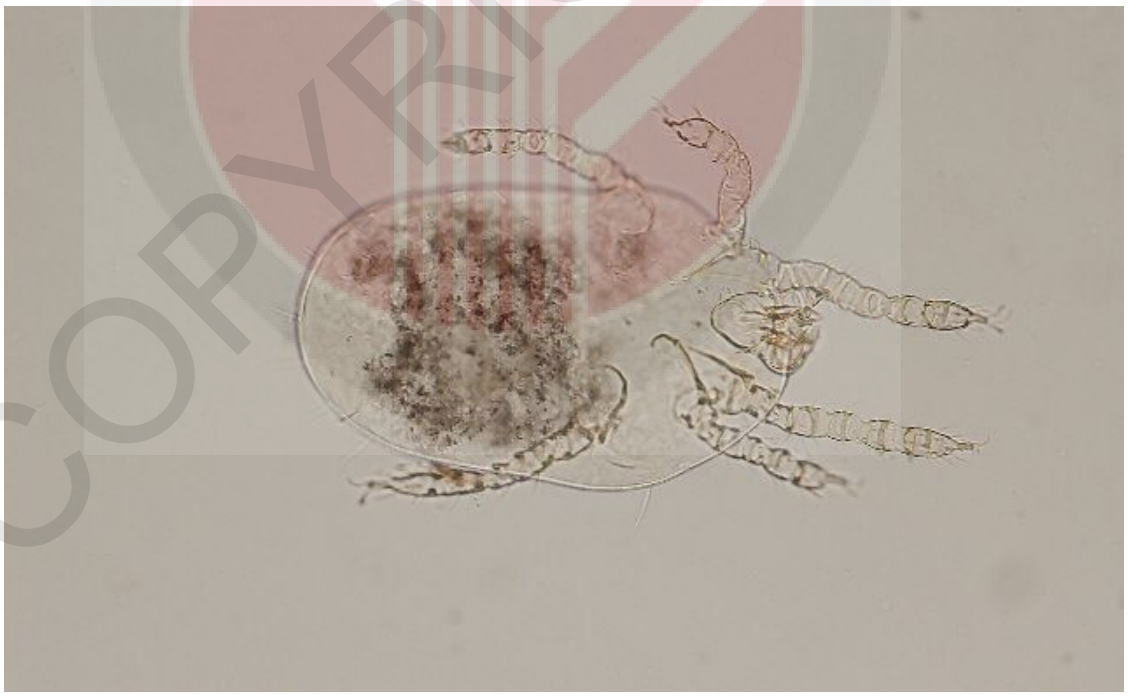

Plate 6: Mesostigmatid mite larva found on *T. glis*

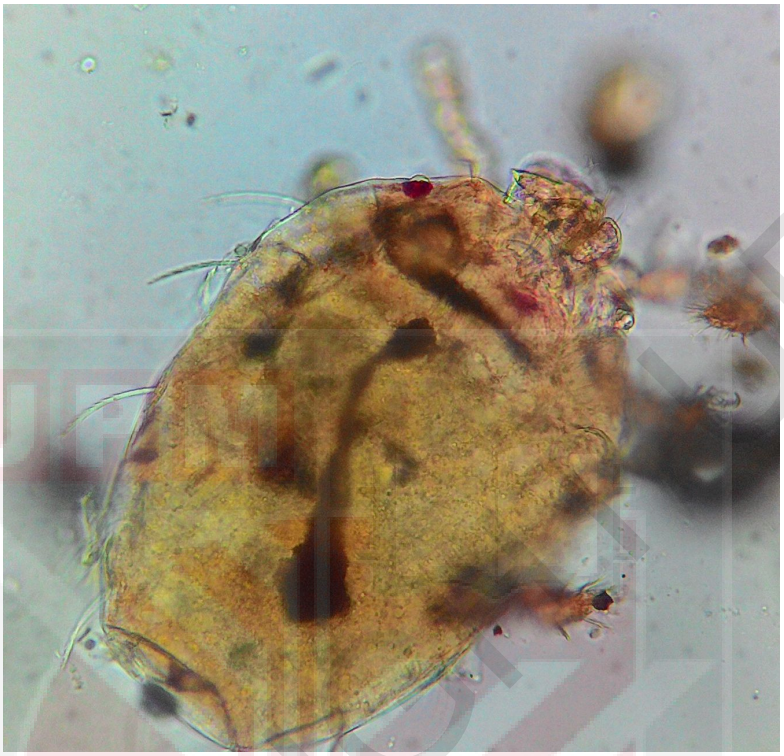

Plate 6: Mesostigmatid mite larva found on *C. caniceps*

Plate 7. *Taenia sp* recovered from *T.glis*

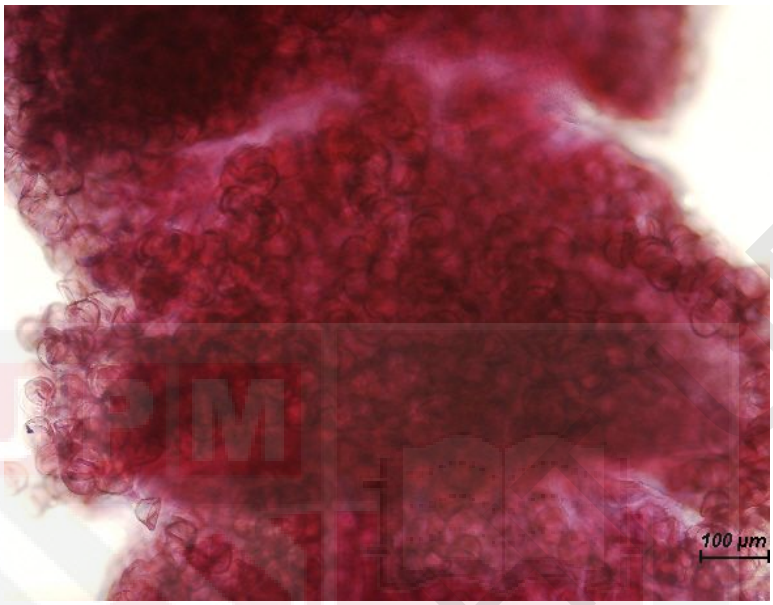

Plate 8: Proglottid of the cestode contain vast number of ova

Plate 10: Nematode recovered from *T.glis* with enlarged photo of the nematode aesophagus