BIODIVERSITY AND EPIDEMIOLOGY STUDY OF MACROPARASITES FROM STRAY CATS IN PENINSULAR MALAYSIA

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ABSTRACT

The occurrences of macroparasites from 543 stray cats were studied in four urban cities from west (Kuala Lumpur), east (Kuantan), north (Georgetown) and south (Malacca) of Peninsular Malaysia between May 2007 to August 2010. The hosts were infested with a minimum of one species and a maximum of six species of macroparasites. Of all four locations, Georgetown had the highest species diversity followed by Malacca, Kuala Lumpur and Kuantan.

Five ectoparasites species were recovered namely, *Ctenocephalides felis, Felicola subrostratus, Haemaphysalis bispinosa, Heterodoxus spiniger and Lynxacarus radovskyi.* This study also recorded the dog louse, *Heterodoxus spiniger* for the first time in Malaysia from two cats in Georgetown. The cat fur mite, *Lynxacarus radovskyi* also reported for the first time on domestic cats from Peninsular Malaysia. Overall, species diversity of ectoparasites was low compared to previous studies. Similar infestations were observed between males and females meanwhile higher diversity of species richness was observed in adults compared to juveniles. Results showed no significant effect determining the ectoparasites distribution in stray cats population for any of the factors investigated.

Up to nine species of helminthes were recovered with overall high prevalences of infection in Kuantan (83%), Kuala Lumpur (75.1%), Georgetown (71.6%) and lastly Malacca (68%). The nine helminthes comprised of six nematode species (*Toxocara malaysiensis, Toxocara cati, Ancylostoma braziliensis, Ancylostoma ceylanicum, Strongyloides sp., Physaloptera praeputialis*), two cestode *(Taenia taeniaformis,*

Dipylidium caninum) and one trematode species (*Playtnosomum fastosum*). Most helminthes were present in all study sites except for the sole presence of *Strongyloides sp.* and the absence of *Physaloptera praeputialis* in Kuala Lumpur. Variation in host age was observed playing a significant role especially for *Ancylostoma braziliense* and *Ancylostoma ceylanicum*. Adults were significantly higher compared to juvenile cats, but reversely for *Toxocara malaysiensis* and *Toxocara cati*. No significant difference occurred between host sexes meanwhile the season affect was linked with differences in the host population between seasons, with twice as many cats being caught in the dry season, during a period of more active foraging.

Pearson's product-moment correlation coefficient analysis showed positive co-occurrences have been shown to occur between the four endoparasite species (*Ancylostoma braziliense, Ancylostoma ceylanicum, Toxocara malaysiensis and Toxocara cati)* occupying similar niches within the alimentary tract of cats. Strong correlation were observed between *Toxocara cati* and *Toxocara malaysiensis* (p=0.982), *Toxocara cati – Toxocara malaysiensis* and *Toxocara cati – Ancylostoma ceylanicum* (p=0.994) and between *Toxocara cati – Ancylostoma braziliense* and *Toxocara cati – Ancylostoma ceylanicum* (p=0.919).

In the molecular characterization study, amplification of ITS 1 and ITS 2 regions of *Toxocara malaysiensis* rDNA was successful using universal Fallas-Kaplan primer with estimated product length 1200bp. Hence, further analysis should be carried out in the future in order to corroborate present results obtained.

Lastly, the zoonotic potential of three endoparasite species namely *Ancylostoma braziliense*, *Ancylostoma ceylanicum* and *Toxocara cati* in this study underscores the role of stray cats in Peninsular Malaysia as reservoir host for zoonotic disease. This study also provided a reliable basis for an ongoing monitoring, comparison and assessment of the local cat-borne endoparasites in Peninsular Malaysia.

ABSTRAK

Sebuah kajian mengenai kekerapan kehadiran makroparasit daripada 543 ekor kucing terbiar telah dianalisa di empat bandar maju di Malaysia dari bahagian Barat (Kuala Lumpur), Timur (Kuantan), Utara (Geoergetown), dan Selatan (Melaka) dalam satu tempoh waktu iaitu dari Mei 2007 hingga Ogos 2010. Didapati perumah (kucing) telah dijangkiti sekurang-kurangnya satu spesies makroparasit dan 6 spesies makroparasit adalah yang paling banyak. Antara semua kawasan, Georgetown mempunyai bilangan spesies tertinggi dari segi kepelbagaian dan ia diikuti dengan Melaka, Kuala Lumpur dan akhir sekali Kuantan.

Terdapat lima spesies ektoparasit dan kesemuanya dinamakan *Ctenocephalides felis, Felicola subrostratus, Haemaphysalis bispinosa, Heterodoxus spiniger dan Lynxacarus radovskyi.* Kajian ini turut mengambilkira kutu anjing iaitu *Heterodoxus spiniger* dimana ia merupakan penemuan pertama di Malaysia dan ia didapati pada dua ekor kucing di Georgetown. Selain itu, hama pada bulu kucing yang dinamakan *Lynxacarus radovskyi* turut dikenalpasti buat pertama kalinya pada kucing-kucing sekitar kawasan Semenanjung Malaysia. Secara keseluruhannya, kepelbagaian spesies ektoparasit adalah lebih rendah berbanding dengan kajian terdahulu. Tambahan pula, persamaan jangkitan dapat dilihat diantara jantan dan betina sementara kepelbagaian kekayaan spesies didapati lebih banyak pada kucing matang berbanding kucing muda. Hasil keputusan

menunjukkan tiada perbandingan besar terhadap pembahagian ektoparasit pada populasi kucing terbiar bagi setiap faktor yang disiasat.

Sembilan spesies helminth telah dijumpai dengan keseluruhan sebaran sebanyak 83% di Kuantan, 75.1% di Kuala Lumpur, 71.6% di Georgetown dan 68% di Melaka. Sembilan helminth tersebut terdiri daripada 6 spesies nematoda (*Toxocara malaysiensis, Toxocara cati, Ancylostoma braziliensis, Ancylostoma ceylanicum, Strongyloides sp., Physaloptera praeputialis),* dua spesies cestoda *(Taenia taeniaformis, Dipylidium caninum)* dan satu spesies trematoda (*Playtnosomum fastosum*). Hampir kesemua helmint telah didapati dalam semua kawasan kajian kecuali dengan kehadiran *Strongyloides sp*. dan ketiadaan *Physaloptera praeputialis* di Kuala Lumpur. Perbezaan bagi umur perumah yang telah dipantau memberi peranan penting terutamanya bagi *Ancylostoma braziliense* dan *Ancylostoma ceylanicum*. Kucing yang matang lebih banyak dijangkiti berbanding kucing muda. Namun ia bertentangan bagi *Toxocara malaysiensis* and *Toxocara cati*. Tiada perbezaan yang ketara berlaku di antara jantina perumah sementara perubahan cuaca menunjukkan perbezaan pada populasi perumah antara musim, dengan tangkapan 2 kali ganda lebih banyak pada musim panas.

Analisis ‘Pearson’s product-moment correlation coefficient’ menunjukkan kekerapan positif antara empat spesies endoparasit iaitu (*Ancylostoma braziliense, Ancylostoma ceylanicum, Toxocara malaysiensis and Toxocara cati*) malah menduduki tempat yang sama dalam saluran pencernaan kucing. Hubung kait yang kuat telah diperhatikan dan diteliti antara *Toxocara cati* dan *Toxocara malaysiensis* (p=0.982), *Toxocara cati – Toxocara malaysiensis* dan *Toxocara cati – Ancylostoma ceylanicum* (p=0.994) dan

diantara *Toxocara cati – Ancylostoma braziliense* dan *Toxocara cati – Ancylostoma ceylanicum* (p=0.919).

Di dalam kajian pencirian molekul, amplifikasi ITS 1 dan rantau ITS 2 bagi rDNA *Toxocara malaysiensis* telah berjaya dengan menggunakan primer *Fallas-Kaplan* dengan anggaran 1200bp produk panjang. Oleh itu, analisis lanjut perlu dijalankan pada masa akan datang untuk menyokong keputusan kajian yang deperoleh ini.

Akhir sekali, tiga spesies endoparasit iaitu *Ancylostoma braziliense*, *Ancylostoma ceylanicum* and *Toxocara cati* yang terdapat pada kucing terbiar di Semenanjung Malaysia menunjukkan potensi penyakit zoonotik. Kajian ini juga menunjukkan asas bagi kawalan berterusan, perbandingan dan penilaian terhadap endoparasit bawaan kucing di Semenanjung Malaysia

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ABBREVIATIONS

n	sample size
k	negative binomial exponent
%	percentage
µg/µl	microgram per microliter
µl	microliter
°C	degree celcius
bp	base pair
cm	centimeter
dH_2O	distilled water
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
EDTA	ethylenediaminetetraacetic acid
EtBr	ethidium bromide
g	gram
GE	gel extraction
ITS	Internal Transcribed Spacer
kb	kilo base pair
LB	Luria Bertani
$MgCl_2$	Magnesium Chloride
min	minute
ml	milliliter
mM	miliMolar
mm	millimeter
NaOH	Natrium Hydroxide

ng	nanogram
nm	nanometer
OD	Optical Density
PCR	Polymerase Chain Reaction
rDNA	ribosomal DNA
Taq	*Thermus aquaticus*
TBE	Tris Borate EDTA
TE	Tris-EDTA
TM	*Toxocara malaysiensis*
U	Unit
UV	Ultraviolet
V	Volt
x	Times