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DETECTION OF Platynosomum sp. IN CATS IN THE KLANG VALLEY, MALAYSIA

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DETECTION OF *Platynosomum* sp. IN CATS IN THE KLANG VALLEY, MALAYSIA

By

NUR AMALINA NASRUDDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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July 2021

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Platynosomum sp. is a hepatic trematode causing platynosomiasis or lizard poisoning in cats through consumption or accidental ingestion of lizard, the second intermediate host of this parasite. There is little information on the detection of fluke eggs in the fecal sample and previous work only focused on stray cats with identification through post-mortem and no available information on pet cats. In general, this disease is often overlooked by veterinary practitioners due to lack of awareness and difficulty in diagnosis although the severe cases of platynosomiasis could be fatal. Therefore, the aim of this study is to determine the incidence of cat liver fluke in shelter and pet cats through fecal examination and detection in stray cats through post-mortem examination in Klang Valley, Malaysia. This study will also reveal the molecular characterization of the cat liver fluke. A total of 119 fecal samples collected from eight shelters and 82 fecal samples from veterinary clinics in Klang Valley were subjected to simple floatation and formalin-ether sedimentation technique for ova detection. From this study, *Platynosomum* sp. ova were identified in three fecal samples obtained from shelters (2.52%) and veterinary clinics (3.66%) respectively by coproscopic analysis. A total of 51 cats were obtained from city councils and subjected to post-mortem examination. The bile was examined for ova detection and the liver was sliced and the presence of adult flukes were observed. Twelve stray cats (23.53%) were found positive for *Platynosomum* sp. infections. The study observed several histopathology signs associated with platynosomiasis including hepatic steatosis, distended biliary duct, biliary duct hyperplasia and inflammation with the presence of inflammatory cells. For molecular characterization, one adult fluke per positive cats (n = 12) were selected and subjected for DNA extraction, amplification of ITS1 and *cox1* gene and sequencing. Based on phylogenetic analysis, most of the sequence of ITS1 and *cox1* gene were similar to each other and were grouped in a clade. More variation was observed in the *cox1* sequence compared to the ITS1 sequence based on haplotype and nucleotide diversity. Based on the sequence analysis, the *P. fastosum* Malaysian, Vietnam and *P. illiciens* Brazilian isolates most likely are conspecific. The findings obtained from this study presented the detection of *P. fastosum* among client-owned, shelters and stray cats in Klang Valley,

Malaysia, as well as providing additional molecular knowledge on *P. fastosum* as a reference for future molecular characterization studies.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGESANAN *Platynosomum* sp. DALAM KUCING DI LEMBAH KLANG, MALAYSIA

Oleh

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Platynosomum sp. adalah trematod hati yang menyebabkan platinosomiasis atau keracunan cicak pada kucing melalui pemakanan atau dengan tidak sengaja termakan cicak, hos pertengahan kedua parasit ini. Ada sedikit maklumat tentang pengesanan telur parasit ini di dalam sampel najis dan kajian yang telah dijalankan sebelum ini hanya fokus pada kucing liar melalui bedah siasat dan tidak ada maklumat pada kucing peliharaan. Secara umum, penyakit ini sering diabaikan oleh pegawai veterinar kerana kurangnya kesedaran dan kesukaran untuk didiagnosis walaupun pada kes-kes yang teruk, platinosomiasis boleh membawa maut. Oleh itu, tujuan kajian ini adalah untuk menentukan kejadian trematod hati kucing pada kucing di pusat perlindungan haiwan dan kucing peliharaan melalui sampel najis dan pengesanan parasit ini di dalam kucing liar melalui bedah siasat di Lembah Klang, Malaysia. Kajian ini juga akan mendedahkan ciri molekul parasit hati kucing. Sejumlah 119 sampel najis yang diambil dari lapan tempat perlindungan haiwan dan 82 sampel najis dari klinik haiwan di Lembah Klang telah melalui teknik 'centrifugal fecal sedimentation' menggunakan cecair formalin-ether dan 'simple floatation'. Melalui kajian ini, ova *Platynosomum* sp. telah dikenalpasti dalam tiga (2.52%) sampel najis yang diperoleh dari tempat perlindungan dan klinik veterinar (3.66%) masing-masing melalui analisis koproskopik. Sebanyak 51 ekor kucing telah diperoleh dari majlis perbandaran dan bedah siasat telah dijalankan. Melalui bedah siasat tersebut, cecair hempedu telah diperiksa untuk mengesan ova dan hati telah dihiris dan kehadiran cacing dewasa diperhatikan. Dua belas kucing liar (23.53%) ditemukan positif bagi *P. fastosum*. Melalui kajian ini, beberapa tanda-tanda histopatologi yang berkaitan dengan platinosomiasis termasuk steatosis pada hati, saluran hempedu membengkak, hiperplasia pada saluran hempedu dan keradangan dengan kehadiran sel-sel radang. Untuk karakter molekular, setiap satu sampel cacing dewasa daripada kucing yang positif (n = 12) telah dipilih untuk pengekstrakan DNA, amplikasi gen ITS1 dan *cox1* dan penjujukan. Berdasarkan analisis filogenetik, kebanyakan jujukan gen ITS1 dan *cox1* adalah sama antara satu sama lain dan telah dikumpulkan dalam satu klad. Lebih banyak variasi dilihat pada jujukan *cox1* berbanding ITS1 berdasarkan kepelbagaian haplotip dan nukleotida. Berdasarkan analisis jujukan, *P. fastosum* Malaysia, Vietnam dan *P. illiciens* Brazil adalah berkemungkinan spesis

yang sama. Melalui kajian ini, *P. fastosum* telah dikesan pada kucing peliharaan, tempat perlindungan dan kucing liar di Lembah Klang, Malaysia, serta telah memberikan pengetahuan tambahan mengenai karakter molekular *P. fastosum* sebagai panduan untuk masa depan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
°C	degree celsius
µL	Microliter
µm	Micrometer
ALP	Alkaline phoshatase
ALT	Alanine transaminase
AST	Aspartate transaminase
BC	Before century
BCS	Body condition score
BLAST	Basic Local Alignment Search Tool
bp	Base pair
CI	Confidence interval
cox1	Cytochrome c oxidase 1
DCSF	Double centrifugation with Sheater's sugar floatation
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
EDTA	Ethylenediaminetetraacetic acid
FE	Formalin-ether
FNA	Fine-needle aspiration
g	Gram
h	Haplotype diversity
H&E	Hematoxylin and eosin
HT	High-dosage treatment
IACUC	Institutional Animal Care and Use Committee

IM	Intramuscular
ITS1	Internal transcribed spacer 1
kg	Kilogram
L	Litre
LT	Low-dosage treatment
mg	milligram
mg/dL	Milligram per decilitre
$MgCl_2$	Magnesium chloride
ML	Maximum likelihood
mL	mililitre
mm	Milimeter
mtDNA	Mitochondrial DNA
n	Subtotal population
NaCl	Sodium chloride
NCBI	National Centre for Biotechnology Information
P. fastosum	*Platynosomum fastosum*
P. illiciens	*Platynosomum illiciens*
P.	*Platynosomum*
PACU	Pest and Animal Control Unit
PCR	Polymerase chain reaction
rDNA	Ribosomal DNA
rpm	Rotation per minute
SEA	Southeast Asia
spp., sp.	Species
TAE	Tris-acetate-EDTA
U/S	ultrasonography

UPM	University Putra Malaysia
USA	United State of America
V	Voltage
W	Watt
WI	Wisconsin
л	Nucleotide diversity

CHAPTER 1

INTRODUCTION

1.1 Background

Cat (*Felis catus*) is a small carnivorous mammal and is the only domesticated species in the Felidae family. *Felis catus* is often referred to as domestic cats to differentiate it from other wild Felidae members. The species was believed to be first domesticated around 7500BC. Domestic cats are cherished by human for their companionship and their hunting ability. Companion animals especially cats have become popular among Malaysian households and it represents a biggest segment of pet population in Malaysia. Most cat owners considered their pets as part of a family, therefore, highlight the importance of proper healthcare and disease control for their pet cats.

The arising number of cat owners in Malaysia, as well as the knowledge of transmissible zoonotic disease from the cat, trigger interest among researchers to study diseases carried by these animals. *Platynosomum fastosum (P. fastosum)* is an etiological agent for platynosomosis or platynosomiasis in cats. The disease is generally known as 'lizard poisoning' because the common house lizard (*Hemidactylus frenatus*) is believed to be the source of *P. fastosum* infection through the hunting nature of domestic cats (Basu and Charles, 2014). This trematode affects the liver of infected animals. The fluke develops in the gall bladder and bile duct of cats and causes severe hepato-biliary diseases. The *P. fastosum* infections were previously reported in tropical and subtropical countries where the intermediate host of this trematode is found to be abundant (as reviewed by Basu and Charles, 2014). Condition in Malaysia is similar to Southeast Asian (SEA) countries, exhibits high temperature and humidity, which is suitable for the development of intermediate hosts of this parasite.

Although *P. fastosum* is not zoonotic, the infection of this liver trematode causes many significant clinical effects like anorexia, lethargy, vomiting, abdominal ascites, hepatomegaly and the infection can eventually lead to the death of the infected cat. The significance of platynosomosis in cats is still controversial due to most of cats will be having very mild clinical signs or no obvious symptoms due to the low burden of the parasite in their body. However, in a higher burden fluke infection, where there is a presence of damage to the liver cell as well as thickening of the bile duct which further lead to jaundice and death of the animal.

Nevertheless, the cat liver fluke infection has been previously reported in a jaundiced cat with dehydration, icterus, alopecia, anorexia, prostration, abdominal enlargement and slightly hyperthermic with normal heart and respiratory rate (Ikhwan-Saufi et al., 2020). In Malaysia, further investigation and exploration related to *P. fastosum* is needed, however, fundamental studies on prevalence and epidemiology of the fluke in Malaysia is important in creating awareness among veterinary practitioners of this fatal disease. Role of the intermediate host and wildlife in disease transmission are required to be

explored and might be beneficial in understanding this feline hepatic trematode in terms of treatment and control of the disease.

1.2 Problem statement

The prevalence works on *P. fastosum* in Malaysia were conducted more than a decade ago (Retnasabapathy and Prathap, 1971). Since then, most of the cat liver fluke discovery were accidental and by survey and this warrant the need to investigate the current status of *P. fastosum* infection among cats in Malaysia. Despite the significant effect the parasite could have on cats, most of the previous work and discovery of this trematode was performed by post-mortem examination on stray cats. Currently, there is no available data on the prevalence of *P. fastosum* infection in client-owned cats. Therefore, this study will provide preliminary data on the prevalence of platynosomiasis infection through coproparasitology analysis and post-mortem examination technique on stray, shelter and pet cats which will helps veterinarians in diagnosing and controlling this infection. Moreover, with the revelation on the current prevalence of *P. fastosum* infections in client-owned cats, veterinarians should include platynosomiasis as a possible differential diagnosis when diagnosing patients with the associated clinical signs. Furthermore, there is no available data on the molecular characterization of *Platynosomum sp.* in Malaysia. The study aims to identify the species of the fluke infecting cats in Malaysia molecularly and to observe the closely related species for this parasite. By molecular characterization, a better understanding regarding the genetic features for the parasite could be obtained thus provide an essential information as a target for diagnosing the disease using molecular method which is currently scarce in *Platynosomum sp.*

1.3 Objectives

i. To determine the incidence of *P. fastosum* infection among client-owned and shelter cats with the fecal examinations and the association of infections with the management.

ii. To determine the prevalence of *P. fastosum* infections in stray cats through post-mortem examinations.

iii. To determine the molecular phylogenetic characteristic of *P. fastosum* from Klang Valley.

1.4 Hypothesis

i. Low prevalence of *P. fastosum* infections among client-owned and shelter cats with fecal examination will be revealed and the infections are associated with management.

ii. There is a high prevalence of *P. fastosum* infections in stray cats through post-mortem examinations.

iii. The molecular phylogenetic characteristics of *P. fastosum* in Klang Valley will be determined.

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