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MOLECULAR DETECTION AND CHARACTERISATION OF FELINE MORBILLIVIRUS IN MALAYSIA

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

MOLECULAR DETECTION AND CHARACTERISATION OF FELINE MORBILLIVIRUS IN MALAYSIA

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Feline morbillivirus (FeMV), a feline virus under the family of Paramyxoviridae was identified in several countries across the world. This virus is the largest morbilliviruses with the size of 16,050 bases and encodes eight non-structural and structural proteins, which are the N, P/V/C, M, F, H and L proteins. Phylogenetic analysis revealed that this virus shared less than 80% nucleotide identities to other known paramyxoviruses. There was also evidence of higher detection of FeMV in kidney tissue compared to other type of samples suggesting the virus is nephrotropic.

To date, no study has been conducted in Malaysia to determine the presence as well as to characterise the local FeMV isolates. Therefore, the objectives of this study were to detect and characterise FeMV from domestic cats in Malaysia. Molecular analysis utilising nested RT-PCR assay targeting the L gene of FeMV performed on either urine, blood and/or kidney samples collected from the 208 cats in this study revealed 82 (39.4%) positive cats. From these 82 samples, FeMV RNA were detected from 48 (58.5%) pet cats and 34 (27.0%) shelter cats. FeMV-positive samples were found from 63/124 (50.8%) urine samples and 20/25 (80.0%) kidney samples, while all blood samples 0/96 (0.0%) were negative for FeMV.

In this study, a new primer set was developed targeting partial N gene of FeMV by RT-PCR. Partial L and N gene sequencing of the RT-PCR-positive samples showed 85-99% identity to the previously reported FeMV and it is significantly different from all other morbilliviruses. Phylogenetic analyses of the identified Malaysian FeMVs were clustered with other Asia FeMVs. Additionally, randomly selected urine samples were cultured where out of four FeMV-

positive urine samples, two samples showed CPE which is characterised by cell clumping, cell rounding, detachment, lysis and syncytia formation at day 12. This study is focused on a preliminary investigation of the existence of feline morbillivirus in Malaysia. The detection and molecular characterisation of this virus in domestic cats in Malaysia is important in order to examine the existence and significance of this virus to the feline population.

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

PENGESANAN DAN PENCIRIAN MOLEKULAR MORBILLIVIRUS FELIN. DI MALAYSIA

Oleh

NUR HIDAYAH BINTI MOHD ISA

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Morbillivirus felin (FeMV) adalah virus kucing di bawah keluarga *Paramyxoviridae* yang dikenalpasti di beberapa negara di seluruh dunia. Virus ini adalah morbillivirus yang terbesar dengan saiz 16,050 bes dan terdiri daripada lapan protein bukan struktur dan struktur, yang merupakan protein N, P / V / C, M, F, H dan L. Analisis filogenetik mendedahkan bahawa virus ini berkongsi kurang daripada 80% identiti nukleotida kepada paramyxovirus lain yang diketahui. Terdapat juga bukti pengesanan FeMV yang lebih tinggi dalam tisu buah pinggang berbanding dengan jenis sampel lain yang menunjukkan virus ini adalah nefrotropik.

Sehingga kini, tiada kajian telah dijalankan di Malaysia untuk menentukan kehadirannya isolat FeMV tempatan serta mencirikannya. Oleh itu, objektif kajian ini adalah untuk mengesan dan mencirikan FeMV dari kucing domestik di Malaysia. Analisis molekul menggunakan ujian reaksi rantai polimerase transkriptase membalik (RT-PCR) tersarang yang mensasarkan FeMV gen L yang dilakukan pada sampel air kencing, darah dan/atau buah pinggang yang diambil dari 208 kucing dalam kajian ini menunjukkan 82 (39.4%) kucing positif. Dari 82 sampel ini, RNA FeMV dikesan dari 48 kucing peliharaan (58.5%) dan 34 kucing dari tempat berlindung (27.0%). Sampel positif FeMV didapati dari sampel air kencing 63/124 (50.8%) dan 20/25 (80.0%) buah pinggang, manakala semua sampel darah 0/96 (0.0%) adalah negatif untuk FeMV.

Dalam kajian ini, satu set primer telah dibangunkan untuk mengenalpasti sebahagian gen N FeMV menggunakan RT-PCR. Penjujukan gen separa L dan N dari sampel positif RT-PCR menunjukkan identiti 85-99% kepada FeMV

yang dilaporkan sebelum ini dan ia jauh berbeza daripada semua morbillivirus yang lain. Analisis filogenetik dari FeMV Malaysia yang dikenal pasti telah dikelompokkan dengan FeMV Asia yang lain. Di samping itu, sampel air kencing yang positif FeMV dipilih secara rawak, dua daripada empat sampel menunjukkan kesan sitopatik yang dicirikan oleh pengumpalan sel, pembulatan sel, detasmen, pemecahan sel dan pembentukan sinsitium pada hari ke 12. Kajian ini difokuskan pada penyiasatan awal tentang kewujudan FeMV di Malaysia. Pengesanan dan pencirian molekul virus ini dalam kucing domestik di Malaysia adalah penting untuk mengkaji kewujudan dan kepentingan virus ini kepada populasi kucing.

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	percentage
°C	degree celcius
µmol	micromolar
µl	microlitre
AAFP	American Association of Feline Practitioners
AAHA	American Animal Hospital Association
AGID	agar gel immunodiffusion
AKI	acute kidney injury
BLAST	Basic Local Alignment Search Tool
bp	base pair
cDNA	complementary DNA
CDV	canine distemper virus
C.I	confidence interval
CKD	chronic kidney disease
CMV	cetacean morbillivirus
CPE	cytopathic effect
CRFK	Crandell-Rees feline kidney
DMEM	Dulbecco's modified Eagle's medium
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
F	fusion
FBS	fetal bovine serum
FIP	feline infectious peritonitis
FFPE	formalin-fixed paraffin-embedded
FLUTD	feline lower urinary tract disease
FeMV	feline morbillivirus
FPaV	feline paramyxovirus
FPV	Fakulti Perubatan Veterinar
g	gram
H	haemagglutinin

HMPV	human metapneumovirus
IACUC	Institutional Animal Care and Use Committee
ICTV	International Committee on Taxonomy of Viruses
ID	identification
IFA	indirect immunofluorescence assay
IgG	immunoglobulin G
kb	kilobase
L	litre
M	matrix
MEGA	Molecular Evolutionary Genetics Analysis
min	minute
mL	mililitre
mmol	milimolar
MV	measles virus
N	nucleocapsid
NiV	Nipah virus
n	number
nm	nanometer
NCBI	National Centre for Biotechnology Information
OD	optical densities
P	phosphoprotein
PDV	phocine distemper virus
PPRV	peste des petits ruminant virus
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
rpm	revolutions per minute
RPV	rinderpest virus
SPSS	Statistical Package for the Social Sciences
ssRNA	single-stranded RNA
SLAM	species-specific signaling lymphocyte activation molecule
TAE	tris-acetate-ethylenediaminetetraacetic acid
TIN	tubulointerstitial nephritis
UK	United Kingdom

UPM	Universiti Putra Malaysia
US	United States
USG	urine specific gravity

CHAPTER I

INTRODUCTION

Morbilliviruses belong to the order of Mononegavirales and categorised under one of the six genera in the family of Paramyxoviridae. It is an enveloped single-stranded, negative sense RNA virus and encodes a single envelope-associated matrix protein (M), two glycoproteins (hemagglutinin H and fusion protein F), two RNA-polymerase associated proteins (phosphoprotein P and large protein L), and a nucleocapsid protein (N) that encapsulates the viral RNA (Sato *et al*., 2012). This group of virus caused significantly serious disease in their respective hosts, which includes measles virus (MV) in human, rinderpest virus (RPV) in cattle, phocine distemper virus (PDV) in seals, peste des petits ruminant virus (PPRV) in sheep and goats and canine distemper virus (CDV) in several species of carnivores (Rima and Duprex, 2006). Each morbilliviruses infect specific animal species due to the preferential use of species-specific signaling lymphocyte activation molecule (SLAM) and nectin 4 as receptor (Tatsuo *et al*., 2001; Park *et al*., 2014).

CDV infection has been reported in wild felidae such as lions, tigers and leopards, there is no report on CDV infection in the domestic cats. In view of this phenomenon, a molecular epidemiology study was conducted and lead to the discovery and isolation of a proposed novel feline paramyxovirus in domestic cats in Hong Kong and China (Woo *et al*., 2012). The virus was named feline morbillivirus (FeMV) which was first isolated in Hong Kong stray cats and has been identified having fewer than 80% nucleotide identities to other known paramyxoviruses. Then, followed by a few studies conducted in Japan and Europe (Italy and Germany) which detected similar virus in their pet cats population with <90% sequence similarities with the Hong Kong isolate (Furuya *et al*., 2014; Park *et al*., 2014; Sakaguchi *et al*., 2014; Lorusso *et al*., 2015; Sieg *et al*., 2015). To date, feline morbillivirus has been identified in Asia countries (China, Japan and Thailand), Europe (Germany and Italy), USA, South America, Turkey and United Kingdom.

FeMV is related to the tubulointerstitial nephritis (TIN) in domestic cats which involves primary injury to the renal and eventually leads to renal failure. The virus has been assumed to be involved in renal pathologic process as FeMV was mostly detected in the urine samples (Woo *et al*., 2012). However, a study that was conducted in Japan revealed that there was no association between the FeMV infection and TIN (Sakaguchi *et al*., 2014). Given the association of TIN with unknown aetiologies of renal disease in feline patients and the recent discovery of FeMV, the characterisation of the virus and its association with renal disease were pertinent to determine the importance of this virus infection in cats.

Currently, no prevalence study has been done to detect the presence of FeMV infection in the local cat population in Malaysia. Therefore, the detection and molecular characterisation of this virus in domestic cats in Malaysia is important in order to examine the existence and importance of this virus to the feline population.

Thus, the objectives of this study were as follows:

1. To detect feline morbillivirus from urine, blood and kidney samples of local cats by targeting the L gene using nested RT-PCR analysis.
2. To design and optimise primers for conventional RT-PCR assay targetting partial N gene of FeMV.
3. To characterise FeMV-positive samples by phylogenetic analyses and virus isolation study.

The hypotheses for this study were:

1. Feline morbillivirus is prevalent in domestic cats in Malaysia.
2. There is a significant association between renal/urinary system diseases and FeMV-positive cats.

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