UNIVERSITI PUTRA MALAYSIA

MOLECULAR INVESTIGATION OF FELINE CORONAVIRUS (FCOV) IN LOCAL PET CATS

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MOLECULAR INVESTIGATION OF FELINE CORONAVIRUS (FCoV) IN LOCAL PET CATS

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Molecular Investigation of Feline Coronavirus (FCoV) in Local Pet Cats", by LiewWuanHoong 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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LIST OF ABBREVIATIONS

%	Percent
°C	Degree Celsius
µL	Microliter
µM	Micromolar
3’ UTR	3’ Untranslated region
5’ UTR	5’ Untranslated region
AMV	Avian Myeloblastosis Virus
APN	Aminopeptidase-N
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
bp	Basepair
CCoV	Canine coronavirus
cDNA	Complementary deoxyribonucleic acid
CNS	Central Nervous System
CoV	Coronavirus
DNA	Deoxyribonucleic acid
Dntp	Deoxynucleotide
E	Envelope
EDTA	Ethylenediaminetetraacetic acid
ERGIC	Endoplasmic reticulum-to-Golgi intermediate compartment

FCoV	Feline coronavirus
FECV	Feline enteric coronavirus
FIP	Feline infectious peritonitis
FIPV	Feline infectious peritonis virus
FPV	Fakulti Perubatan Veterinar
FYP	Final Year Project
G	Gauge
g	Relative Centrifugal Force
HCoV	Human coronavirus
HE	Hemagglutinin-esterase
IACUC	Institutional Animal Care and Use Committee
IFN	Interferon
kb	Kilobase
kDa	Kilodalton
M	Membrane
$MgSO_4$	Magnesium Sulphate
mL	Milliliter
mm	Millimetre
mM	Millimolar
mRNA	Messenger ribonucleic acid
N	Nucleocapsid

NCBI	National Centre for Biotechnology Information
nm	Nanometer
ORF	Open reading frame
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
pH	Potential of Hydrogen
POL	Polymerase gene
RER	Rough endoplasmic reticulum
RNA	Ribonucleic acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
S gene	Spike protein gene
TAE	Tris-acetate-ethylenediaminetetraacetic acid
TGEV	Transmissible gastroenteritis virus
UPM	Universiti Putra Malaysia
V	Voltage
w/v	Weight/volume percent

ABSTRAK

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

PENYIASATAN MOLEKUL *FELINE CORONAVIRUS* (FCoV) DALAM KUCING KESAYANGAN TEMPATAN

oleh

Liew Wuan Hoong

2017

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Jangkitan *feline coronavirus* (FCoV) adalah sangat biasa didalam populasi kucing. FCoV diklasifikasikan kepada dua biotip iaitu *feline enteric coronavirus* (FECV) dan *feline infectious peritonitis virus* (FIPV), di mana FIPV menyebabkan penyakit kompleksimun yang membawa maut dengan menukarkan tropisme dari enterosit kepada monosit. Kajian-kajian sebelum ini

pada pengesanan molekul FCoV didalam kucing dijalankan di kateri, tetapi kajian tentang penyiasatan kehadiran antigen FCoV di dalam kucing kesayangan tempatan agak terhad. Dengan mengambil kira fakta ini, kajian ini bertujuan untuk mengesan antigen FCoV melalui assai RT-PCR di dalam kucing tempatan dan membandingkan persamaan strain FCoV yang dikenalpasti dengan virus terdahulu melalui analisis filogenetik. Dengan menggunakan pensampelan mudah, swab rectum dan lapisan buf dikumpulkan daripada 16 ekor kucing kesayangan yang sakit secara klinikal dan 5 ekor kucing kesayangan yang sihat. RNA diekstrak dan tertakluk kepada *one-step RT-PCR,* yang menyasarkan gen polymerase. Daripada 21 sampel najis hanya terdapat satu yang positif bagi FCoV dan tidak ada lapisan buf yang positif. Analisis filogenetik mendedahkan bahawa sample yang dikenalpasti positif adalah sangat homologi, sehingga 95%, dengan strain FCoV dari Netherlands pada penjujuk separa gen polymerase. Kesimpulannya, kajian ini telah mengesan antigen FCoV dalam kucing kesyangan tempatan dan pengesanan negative tidak boleh secara keseluruhannya menolak kemungkinan jangkitan FCoV kerana diagnosis virus ini sangat kompleks yang memerlukan beberapa siri analisis.

Kata kunci: feline coronavirus, feline enteric coronavirus, feline infectious peritonitis virus, RT-PCR, analisis filogenetik

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999- Final Year Project

MOLECULAR INVESTIGATION OF FELINE CORONAVIRUS (FCoV) IN LOCAL PETCATS

by

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2017

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Feline coronavirus (FCoV) infection is very common in cat population. FCoV is further classified into two biotypes namely feline enteric coronavirus (FECV) and mutated feline infectious peritonitis virus (FIPV), in which FIPV causes a fatal

immune complex disease by changing the tropism from enterocytes to monocytes. Previous studieson molecular detection of FCoVin cats were carried out in catteriesbut there is limited study on investigation of the presence of FCoV antigen in local pet cats. By considering this fact, this study aims to detect FCoVantigen via RT-PCR assay in local pet cats and to compare the similarity of the identifiedFCoV strain with previous related virus by phylogenetic analysis. By using convenience sampling, rectal swabs and buffy coat were collected from 16 clinically ill pet cats and 5 healthy pet cats. Viral RNA was extracted and subjected to one-step RT-PCR, targeting polymerase gene. Only 1 out of 21fecal samples was positive for FCoVand none for buffy coat. Phylogenetic analysis revealed that the identified positive samplewas highly homologous, up to 95%, to aFCoV strain from Netherlands on partial sequence of polymerase gene. In conclusion, this study detected FCoV antigen in local pet cats and negative detection could not completely rule out the possibilities of FCoV infection due to the complexity of the virus diagnosis that require multiple series of analysis.

Keywords: feline coronavirus, feline enteric coronavirus, feline infectious peritonitis virus, RT-PCR, phylogenetic analysis

1.0 INTRODUCTION

Feline coronavirus (FCoV) is a subspecies of *Alphacoronavirus 1*, from genus *Alphacoronavirus* classified within the subfamily of *Coronavirinae*.(Kipar & Meli, 2014).FCoV infection is ubiquitous and distributed worldwide in household and wild catsespecially in crowded environment like catteries and shelters.

There are two biotypes of FCoV in cats, namelyFeline Enteric Coronavirus (FECV) and Feline Infectious Peritonitis Virus (FIPV) (Addie*et al.*, 2009; Sharif *et al.*, 2010a).Each biotype has two serotypes, I and II mainly based on their antigenic relationship to canine coronavirus. FIPV is believed to be mutated from FECV within the body of a persistently FCoV-infected cat(Pedersen, 2014a). These two biotypes are morphologically and serologically similar, but causing different clinical signs, with FECV causes a transient gastroenteritis or asymptomatic infection whereas FIPVcausesa fatal immune-mediated disease, feline infectious peritonitis (FIP).The peak age for FIP development is between 6 months to 2 years old (Hartmann, 2005).FIP is categorized into wet and dry forms accordingly to the clinical signs manifested. Wet form is characterized by peritonitis and/or pleuritis caused by complement-mediated vasculitis, leading the inflammatory fluid leaking into body cavities whereasdry form is involved with partial cell-mediated immunity, characterized by granuloma formation in various organs like central nervous system and ocular system (Pedersen, 2009)

FCoV is transmitted through fecal-oral route. Virus shedding occurs intermittently and some cats can shed virus up to 10 months (Hartmann, 2005; Sharif *etal.*,

2009b).Thus, reverse-transcriptase polymerase chain reaction (RT-PCR) can be used to detect FCoV antigen in the feces as a part of multi-cat household management (Herrewegh *et al.,* 1995b).After being infected by FCoV, monocyte-associated viraemia occurs. Thus, buffy coat which is rich with leukocytes can be used to check for viraemia due to FCoV using RT-PCR (Kipar *et al.,* 2010).

There are numerous studies have been carried on molecular detection of FCoV antigen in cats from catteries and shelters, but there is limited study on investigation of the presence of FCoV antigen in local pet cats. The hypothesis of the study proposes that FCoV antigen will be detected via RT-PCR in local pet cats. This studywill add up the information on FCoV for the future. Other than that, FCoV causes FIP which is a very fatal disease in cat, even though there have been lots of studies on FCoV, there are still issues related to FCoV waiting to be solved.

In Malaysia, the phylogenetic analysis of FCoVs on partial sequence of 3'UTR had been done around 7 years back (Sharif *et al.*,2009b; Sharif *et al.,* 2010a). Thus, this study will again use phylogenetic analysis to get a glimpse into the current status of FCoVs in Malaysia.

Thus, the objectives of the study are:

1. To detect FCoV antigen via RT-PCR assay in local pet cats.

2. To compare the similarity of identified positive samples with the previous related virus by phylogenetic analysis.

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