UNIVERSITI PUTRA MALAYSIA

MOLECULAR DETECTION OF FELINE LEUKEMIA VIRUS (FeLV) IN CLINICALLY ILL LOCAL CATS

KUNAMBIGA MUMMOORTHY

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MOLECULAR DETECTION OF FELINE LEUKEMIA VIRUS (FeLV)

IN CLINICALLY ILL LOCAL CATS

KUNAMBIGA MUMMOORTHY

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CERTIFICATION

It is hereby certified that we have read this project entitled ‘‘Molecular detection of Feline leukemia virus (FeLV) in clinically ill local cats”, by Kunambiga Mummoorthy and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course of VPD 4999-Final Year Project

DR NOR YASMIN ABD. RAHAMAN
DVM(UPM), PhD.(UPM)
Senior lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

PROFESSOR DR. ABDUL RAHMAN OMAR
DVM (UPM), PhD. (USA)
Professor of Immunology
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

ASSOC. PROFESSOR DR SITI SURI ARSHAD

DVM(UPM), MSc. (UPM), PhD. (London)

Deputy Dean of Academic and Student Affairs

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DR NURFAZILA SAULOL HAMID

DVM (UPM), PhD. (Liverpool)

Senior lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DR. PREM ANAND

BVSc.(Hons), MACVSc. (Australia), MRCVS

Director

Gasing Veterinary Hospital

(Co-supervisor)

DEDICATIONS

This project paper is dedicated to

My family

My friends

My coffee

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LIST OF ABBREVIATIONS

%	Percent
µL	Microliter
µM	Micromolar
°C	Degree Celsius
ATCC	American Type-culture Collections
BLAST	Basic Local Alignment Search Tool
bp	Base pair
CD4+	Cluster of differentiation 4
CD8+	Cluster of differentiation 8
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
Dntp	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent Assay
FeLV	Feline leukemia virus
g	Gram
IACUC	Institutional Animal Care and Use Committee
IN	Integrase
Kb	Kilobase
LTRs	Long Terminal repeats
MEGA	Molecular Evolutionary Genetics Analysis

mL	Milliliter
mg	Milligram
mg/kg	Milligram per kilogram
MgSO4	Magnesium Sulphate
Mm	Millimeter
NCBI	National Center for Biotechnology Information
NJ	Neighbour-joining
PBS	Phosphate buffer solution
RNA	Ribonucleic acid
RNase	Ribonuclease
RT	Reverse transcriptase
SU	Surface protein
RT-PCT	Reverse Transcription Polymerase Chain Reaction
TAE	Tris-acetate-ethylenediaminetetraacetic acid
UPM	Universiti Putra Malaysia
USA	United States of America
UK	United Kingdom
UV	Ultraviolet
w/v	Weight per volume
x*g*	Relative centrifugal force

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinanar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek IlmiahTahun Akhir

PENGESANAN MOLEKUL FELINE LEUKEMIA VIRUS (FeLV) DALAM KUCING TEMPATAN YANG SAKIT SECARA KLINIKAL

Oleh

Kunambiga Mummoorthy

2017

Penyelia: Dr. Nor Yasmin Abd. Rahaman

Penyelia bersama:

Professor Dr. Abd. Rahman Omar

Associate Professor Dr. Siti Suri Arshad

Dr. Nurfazila Saulol Hamid

Dr. Prem Anand

Feline leukemia virus (FeLV) tergolong di bawah genus Gammaretrovirus dan dikaitkan dengan pelbagai tanda-tanda klinikal di seluruh dunia. Setakat ini, pencirian molekul FeLV tempatan yang pertama dan satu-satunya dilakukan pada tahun 2014 telah

mendedahkan bahawa pencilan tempatan berkait rapat dengan pencilan UK. Oleh kerana liputan terhad kajian dan sifat semula jadi virus yang biasanya mengintegrasikan DNA ke dalam genom dan menjalani mutasi, kajian mengenai status semasa jangkitan FeLV adalah diperlukan. Oleh itu, kajian ini bertujuan untuk mengesan antigen FeLV pada kucing sakit secara klinikal dengan mengunakan kaedah RT-PCR dan untuk membandingkan persamaan varian yang dikenalpasti pada masa ini dengan pencilan virus terdahulu dari Malaysia dan taburan geografi yang lain. Dengan menggunakan kaedah pensampelan mudah, plasma dan air liur dikumpulkan dari 15 kucing sakit secara klinikal dan 5 kucing sihat dari Hospital Gasing Veterinar. Nukleik asid virus telah diasingkan dan tertakluk kepada One-Step RT-PCR dengan primer khusus yang mensasarkan kawasan U3LTR dan gag separa yang sangat terpelihara. Dua kucing telah diuji positif untuk antigen dari kumpulan sakit secara klinikal. Separa nukleotida penjujuk and analisis filogenetik mendedahkan bahawa varian semasa didapati 93-99% homolog kepada pencilan Malaysia sebelum ini dan masih berkait rapat dengan varian daripada UK. Menariknya, ia juga didapati berkait rapat dengan varian yang diasingkan dari Jepun. Kesimpulannya, kajian ini menonjolkan kemungkinan hubungan evolusi di antara FeLV dari Malaysia dengan FeLV dari UK dan Jepun.

Kata kunci: *Feline Leukemia Virus (FeLV), RT-PCR, Separa nukleotida, penjujukan, analisis filogenetik, varian*

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 DETECTION OF FELINE LEUKEMIA VIRUS IN CLINICALLY ILL IN LOCAL CATS

By

Kunambiga Mummoorthy

2017

Supervisor: Dr Nor Yasmin Abd. Rahaman

Co-supervisors:

Professor Dr.Abd. Rahman Omar

Associate Professor Dr.Siti Suri Arshad

Dr. Nurfazila Saulol Hamid

Dr.Prem Anand

Feline leukemia virus (FeLV) belongs under the genus Gammaretrovirus and is associated with a wide range of clinical signs worldwide. Sofar, the first and only molecular characterization of local FeLV isolates performed in 2014 revealed that local isolates to be closely related to UK isolate. Due to limited coverage of the study and the nature of the virus that typically integrates the DNA into the host genome and undergoes mutation, study on the current status of FeLV infection is necessary. Therefore, this study aim to detect FeLV antigen in clinically ill cats by RT-PCR and to compare the

currently identified variant similarity with previous related virus isolates from Malaysia and other geographical distribution. By using convenience sampling method, plasma and saliva were collected from 15 clinically ill cats and 5 healthy cats from Gasing Veterinary Hospital. Viral nucleic acid was extracted and subjected to One-Step RT-PCR with specific primer targeting the highly conserved U3LTR and partial gag regions. Two cats were tested positive for the antigen from the clinically ill group. Partial nucleotide sequencing and phylogenetic analysis revealed that the current variant are found to be 93-99% homologous to the previous Malaysian isolates and is still closely related to UK isolate. Interestingly, they were also found to be closely related to isolates from Japan. In conclusion, this study highlights the possibilities of evolutionary relations between FeLV from Malaysia with FeLV of UK and Japan.

Keywords: *Feline leukemia virus, RT-PCR, partial nucleotide sequencing, phylogenetic analysis*

1.0 INTRODUCTION

Feline leukaemia virus (FeLV) is belongs to Gammaretrovirus genus from the family of Retroviridae. It was first described in the year 1964 by Jarrett *et al*., and is a naturally occurring gammaretrovirus that infects domestic catsand sporadically wild cats (Arjona *et al.,* 2007; Torres *et al.,* 2008; Bolin *et al.,*2011). It is an enveloped, positive sense, single-stranded RNA virus (Boeke *et al*., 2011). There are currently four FeLV subgroups of clinical importance namely FeLV-A, FeLV-B, FeLV-C or FeLV-T (Chandhasin *et al*., 2005; Levy, 2008). Subgroup FeLV-A is found in all naturally infected cats meanwhile subgroup B arises from the recombination of endogenous FeLV with FeLV-A whereas FeLV subgroup C and T are the mutated forms of FeLV-A. (McCaw,2010; Eiden *et al.,* 2010). FeLV is mostly transmitted via oro-nasal route via saliva and nasal secretions through sharing of food and water dishes as well as the cat's grooming or aggressive behaviour. Occasionally, vertical transmissions could occur but is of little relative significance (Gomes-Keller *et al.,* 2006; McCaw, 2010).

FeLV related disorders are associated with the manifestation of immunosuppression, lymphoid or myeloid tumors, anaemia, reproductive problems, immune complexes, enteritis and certain other disorders (McCaw, 2010). FeLV infections are divided into four stages namely the abortive infection (regressor cats), regressive infection (transient viraemia followed by latent infection), progressive infection (persistent viraemia) and focal or atypical infection (Torres *et al*., 2005, Hofmann-Lehmann *et al*., 2007; 2008, Levy *et al*., 2008). The disease is distributed worldwide in the feline population and the

prevalence varies greatly with geographical location and risk factors such as health status, age and population density (Gleich *et al*., 2009, Bande *et al*., 2012).

In most clinics and hospitals, the diagnosis of FeLV is usually done with the detection of p27 antigen with rapid test kits (Hartmann *et al*., 2007). Conversely, the demonstration of p27 antigen is relatively difficult during the early viraemia and latent infections and studies conducted showed that FeLV viral RNA and provirus DNA are better predictors of progressive and latent infections respectively (Cattori *et al*., 2009).

To date, the first and only molecular assay investigation of the clinical status of Malaysian cats was carried out in 2014 by Bande *et al*., together with the first sequencing and phylogenetic characterizations of the Malaysian FeLV isolates that revealed Malaysian FeLV to be highly homologoues to each other and showed a possible evolutionary relationship with FeLV in UK. The hypothesis for this study proposes that the current local FeLV variant would be highly homologous to previous Malaysian isolates and would likely be closely related to the UK strain. This study was undertaken to fulfil these following objectives:

1. To detect FeLV antigen in clinically ill cats by using RT-PCR method.
2. To compare currently detected FeLV with previously detected FeLV from Malaysia and other geographical distribution

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