



UNIVERSITI PUTRA MALAYSIA

***MOLECULAR PREVALENCE OF FELINE MORBILLIVIRUS IN
SHELTER CATS***

NURUL HUSNA BINTI OMAR

FPV 2016 33

**MOLECULAR PREVALENCE OF FELINE MORBILLIVIRUS IN SHELTER
CATS**

NURUL HUSNA BINTI OMAR

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfillment for the requirement of the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

43400 UPM, Serdang, Selangor DarulEhsan Malaysia

MARCH 2016

It is hereby certified that we have read this project paper entitled “Molecular Prevalence of Feline Morbillivirus in Shelter Cats”, by NurulHusnaBinti Omar and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for course VPD 4999 – Project

DR. FARINA MUSTAFFA KAMAL
DVM (UPM), PhD (UC Davis, USA)

Senior Lecturer,
Faculty of Veterinary Medicine, Universiti Putra Malaysia
(Supervisor)

DR. GAYATHRI THEVI SELVARAJAH
DVM (UPM), PhD (Utrecht, Netherlands)

Senior Lecturer,
Faculty of Veterinary Medicine, Universiti Putra Malaysia
(Co-Supervisor)

In the name of Allah, The Most Benevolent, The Most Merciful

DEDICATIONS

To my beloved parents,

Omar Bin Hj. Hassan

and

HabsahBintiSemon

For loving and making me who I am today.

To my dearest siblings,

For supporting and cheering me throughout my life.

*“A family is a place where principles are hammered
and honed on the anvil of everyday living”*

Charles R. Swindoll

ACKNOWLEDGEMENT

In the name of Allah, The Most Benevolent, The Most Merciful

Firstly, I would like to give my sincere thanks to my father, mother and siblings for their support, cheer and love. I would like to thank my best friends AinulRiza, AisyahRidzuan, HusnaAtika and 'AisyahAminuddin for their endless help and support. Deepest gratitude to my supervisor, Dr. Farina Mustaffa Kamal for your guidance and continuous help from beginning till the end. This project is a success with your help and assistance. To Dr. GayathriTheviSelvarajah, my co-supervisor, million thanks for all the help, especially for my sample collection and always willing to help me on anything. I would also like to acknowledge all the staff of PusatPerlindunganKucingPutrajaya, ISPCA and PAWS for their willingness to help and participate in this project. Special thanks to my FYP-mate AisyahAzhar and also to MrManiam, Mr. Kevin and staff of virology laboratory 3 for helping me throughout my project, especially during laboratory works. Thank you to Ms. Hidayah Isa for your guidance, help and knowledge. I would not be able to complete this project without your help. Lastly, thank you to my classmates, DVM 2016 and to all that may have contributed directly or indirectly in this project. Thank you!

CONTENTS

TITLE.....	i
CERTIFICATION	ii
DEDICATION	iii
ACKNOWLEDGEMENTS	iv
CONTENTS	v
LIST OF TABLES	viii
LIST OF FIGURES.....	ix
ABBREVIATION	x
ABSTRAK	xi
ABSTRACT	xiii
1.0 INTRODUCTION.....	1
2.0 LITERATURE REVIEW.....	3
2.1 Paramyxoviruses.....	3
2.2 Morbillivirus.....	3
2.3 Diagnosis of Morbillivirus.....	4
2.4 Feline morbillivirus.....	5
2.5 Detection rate of feline morbillivirus in other studies	6
2.6 Current finding of feline morbillivirus in Malaysia	6
3.0 MATERIALS AND METHODS.....	8
3.1 Animals	8
3.2 Sample Collection	8
3.3 Sample Processing	9
3.4 Viral RNA Extraction	9

3.5 Nested Polymerase Chain Reaction (PCR)	10
3.6 Agarose Gel Electrophoresis	11
3.7 Statistical Analysis	11
4.0 RESULTS.....	12
4.1 Number of sample taken from each association	12
4.2 Sex	13
4.3 Detection rate of feline morbillivirus for each organization	13
4.3.1 Detection rate of FmoPV in urine samples	13
4.3.2 Detection rate of FmoPV in kidney samples	14
4.4 Prevalence of feline morbillivirus.....	15
4.5 Association between sex and feline morbillivirus infection	16
5.0 DISCUSSION.....	17
5.1 Detection rate of feline morbillivirusineach association	17
5.2 Prevalence of feline morbillivirus	18
5.3 Association between sex and feline morbillivirus infection	19
6.0 CONCLUSION AND RECOMMENDATIONS.....	20
7.0 REFERENCES.....	21

8.0 APPENDICES.....	23
8.1 Instructions for RNA extraction using TRIzol® Reagent.....	23
8.2 Instruction for Promega™ GoTaq™ 2-Step RT-qPCR System kit	26
8.3 Instruction for MyTaq™ Red Mix	27
8.4 Agarose Gel representative image	29
8.5 Samples detail	30



LIST OF TABLES

	Page No.
Table 1: Number of Sample Taken From Each Association	12
Table 2: Sex categories of the cats sampled	13
Table 3: Detection rate of FmoPV in urine for each shelter organization	14
Table 4: Data analysis of association between sex and FmoPV infection	16

LIST OF FIGURES

	Page No.
Figure 1: Graph illustrating the detection rate for each shelter organization	14
Figure 2: Graph illustrating the prevalence rate of FmoPV	15



LIST OF ABBREVIATION

%	Percentage
°C	Degree celcius
cDNA	Complementary DNA
C.I	Confidence interval
DNA	Deoxyribonucleic Acid
FmoPV	Feline morbillivirus
FPV	FakultiPerubatanVeterinar
IACUC	Institutional Animal Care and Use Committee
ID	Identification
mL	Mililiters
N	Number of cats sampled
RNA	Ribonucleic Acid
PCR	Polymerase Chain Reation
TIN	Tubulointerstitial Nephritis
UPM	Universiti Putra Malaysia

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan luakursus VPD 4999 – Projek.

SARINGAN MOLEKULAR MORBILLIVIRUS FELIN DI PUSAT PERLINDUNGAN KUCING

Oleh,

NURUL HUSNA BINTI OMAR

Penyelia: Dr. Farina Mustaffa Kamal

Penyelia bersama: Dr. Gayathri Thevi Selvarajah

Morbillivirus felin (FmoPV) adalah sejenis virus negatif yang tergolong di dalam keluarga *Paramyxoviridae*. Sebelum ini, tiada virus daripada keluarga morbillivirus yang menyebabkan infeksi di dalam kucing domestik. Walaubagaimanapun, terdapat satu kajian di Hong Kong, di mana mereka menemui satu virus baru yang kemudiannya dinamakan FmoPV. Ini diikuti oleh penemuan dan pengasingan FmoPV di Jepun dan juga Eropah. Baru-baru ini, satu kajian saringan FmoPV telah dijalankan di Malaysia dan didapati kadar prevalens FmoPV ialah 48.6%. Dalam projek ini, sampel air

kencing telah diambil secara rawak dari pada 46 ekor kucing dari pada tiga buah pusat perlindungan kucing yang terlibat. Pengesanan FmoPV di dalam sampel yang telah dikumpul telah dibuat menggunakan ujian reaksi rantai polimerase (PCR) bersarang untuk membesarkan sebahagian daripada urutan L gen FmoPV untuk produk akhir yang bersaiz 401 bp. Daripada 46 sampel air kencing yang diambil, 19 daripadanya adalah positif untuk FmoPV. Kadar prevalens FmoPV untuk kucing di dalam pusat perlindungan kucing adalah 41.3%.

Kata kunci: Morbillivirus felin, kucing, ujian PCR bersarang, L gen, prevalens.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project.

MOLECULAR SCREENING OF FELINE MORBILLIVIRUS IN SHELTER

CATS

By

NURUL HUSNA BINTI OMAR

2016

Supervisor: Dr. Farina Mustaffa Kamal

Co-supervisor: Dr. GayathriTheviSelvarajah

Feline morbillivirus (FmoPV) is an enveloped virus with non-segmented negative strands RNA genomes belongs to the genus of *Morbillivirus* under the family of

Paramyxoviridae. Previously, there were no known morbillivirusinfecton in domestic cats. However, a study was done in Hong Kong, in which they discovered a novel virus that was later named as FmoPV. This was followed by discovery and isolation of FmoPV in Japan and Europe. Recently, a screening study of FmoPV was conducted in Malaysia and the prevalence rate was found to be 48.6%. In the present study, urine samples were taken from 46 cats, randomly from three participating shelter organization. The collecting samples were then subjected to nested polymerase chain reaction (PCR) assay amplifying the L gene of FmoPV for final products of 401 bp. Out of 46 samples collected, 19 samples were tested positive for the virus FmoPV. The prevalence rate of FmoPV in shelter cats in Malaysia is 41.3%.

Key words: Feline morbillivirus, shelter cats, nested PCR assay, L gene, prevalence rate

1.0 INTRODUCTION

Morbillivirus belongs to the virus family of *Paramyxoviridae*. It is an enveloped virus with non-segmented negative strand RNA genomes. Other viruses in the genus *Morbillivirus* include measles virus, rinderpest, pestes des petits ruminant virus, canine distemper virus, phocine distemper virus and cetacean morbilliviruses (Rory *et al.*, 2015).

A novel virus under the genus of morbillivirus named feline morbillivirus (FmoPV) had been reported to cause infection in domestic cats. It was first isolated in a study done in Hong Kong stray cats (Woo *et al.*, 2012). In addition, studies conducted in Japan and Europe (Italy and Germany) detected similar virus in their pet cats population with >90% sequence similarities with Hong Kong isolate (Furuya *et al.*, 2014; Lorusso *et al.*, 2015; Siegel *et al.*, 2015). To date, feline morbillivirus has been identified only in China, Japan and Europe. The newly discovered FMoPV genomes encode eight non-structural and structural proteins which are N, P/V/C, M, F, H and L proteins. In the study conducted by Woo *et al.* (2012), L protein of the virus was detected and sequenced by using RT-PCR.

Recently, a study was conducted to detect the presence of FmoPV among client-owned cats in Malaysia where the prevalence rate was found to be 48.6% (Manorajet *et al.*, 2015). Due to the fact that pet and shelter cats are exposed to different environment, it would be interesting to investigate the prevalence of FmoPV in shelter settings.

Thus, the objectives of this study were:

1. To detect the presence of feline morbillivirus among shelter cats in Malaysia.
2. To determine the prevalence of feline morbillivirus in shelter cats in Malaysia.

The hypothesis for this project includes:

1. Feline morbillivirus is present in shelter cats.
2. The prevalence of feline morbillivirus in Malaysia is higher than client-owned cats.

7.0 REFERENCES

- Anderson, E. C. (1995). Morbillivirus infections in wildlife (in relation to their population biology and disease control in domestic animals). *Veterinary Microbiology*, 44(2), 319-332.
- Audsle, M. D., & Moseley, G. W. (2013). Paramyxovirus evasion of innate immunity: Diverse strategies for common targets. *World Journal of Virology*, 2 (2), 57-70.
- Barret, T. (1999). Morbillivirus infections, with special emphasis on morbilliviruses of carnivores. *Veterinary Microbiology*, 69 (1), 3-13.
- Buczowski, H., Muniraju, M., Parida, S., Banyard, A. C. (2014). Morbillivirus vaccines: Recent successes and future hopes. *Vaccine*, 32 (26), 3155-3161.
- deVries, R. D., Duprex, W. P., & de Swart, R. L. (2015). Morbillivirus infections: an introduction. *Viruses*, 7(2), 699-706.
- Furuya, T., Sassa, Y., Omatsu, T., Nagai, M., Fukushima, R., Shibutani, M., ...& Mizutani, T. (2014). Existence of feline morbillivirus infection in Japanese cat populations. *Archives of virology*, 159(2), 371-373.
- Koide, R., Sakaguchi, S., & Miyazawa, T. (2015). Basic biological characterization of feline morbillivirus. *The Journal of Veterinary Medical Science*, 77(5), 565.
- Lorusso, A., Di Tommaso, M., Di Felice, E., Zaccaria, G., Luciani, A., Marcacci, M., ...& Savini, G. (2013). First report of feline morbillivirus in Europe. *Veterinariaitaliana*.
- Manoraj, H., Khor, K. H., Selvarajah, G. T., Mustaffa-Kamal, F., (2015). Molecular screening of feline morbillivirus. 10th Proceeding of the Seminar on Veterinary Sciences, University Putra Malaysia.

- Nguyen, D. T., de Vries, R. D., Ludlow, M., van den Hoogen, B. G., Lemon, K., van Amerongen, G., ... & Duprex, W. P. (2013). Paramyxovirus infections in ex vivo lung slice cultures of different host species. *Journal of virological methods*, 193(1), 159-165.
- Park, E. S., Suzuki, M., Kimura, M., Maruyama, K., Mizutani, H., Saito, R., ... & Morikawa, S. (2014). Identification of a natural recombination in the F and H genes of feline morbillivirus. *Virology*, 468, 524-531.
- Sakaguchi, S., Nakagawa, S., Yoshikawa, R., Kuwahara, C., Hagiwara, H., Asai, K. I., ... & Miyazawa, T. (2014). Genetic diversity of feline morbilliviruses isolated in Japan. *Journal of General Virology*, 95(7), 1464-1468.
- Sakai, K., Nagata, N., Ami, Y., Seki, F., Suzaki, Y., Iwata-Yoshikawa, N., ... & Otsuki, N. (2013). Lethal canine distemper virus outbreak in cynomolgus monkeys in Japan in 2008. *Journal of virology*, 87(2), 1105-1114.
- Sieg, M., Heenemann, K., Rückner, A., Burgener, I., Oechtering, G., & Vahlenkamp, T. W. (2015). Discovery of new feline paramyxoviruses in domestic cats with chronic kidney disease. *Virus genes*, 51(2), 294-297.
- Woo, P. C., Lau, S. K., Wong, B. H., Fan, R. Y., Wong, A. Y., Zhang, A. J., ... & Wang, M. (2012). Feline morbillivirus, a previously undescribed paramyxovirus associated with tubulointerstitial nephritis in domestic cats. *Proceedings of the National Academy of Sciences*, 109(14), 5435-5440.