



UNIVERSITI PUTRA MALAYSIA

***ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION
OF FELINE CORONAVIRUS IN MALAYSIA***

AMER KHAZAAL SALIH AL-AZAWY

FPV 2011 5

**ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION
OF FELINE CORONAVIRUS IN MALAYSIA**

By

AMER KHAZAAL SALIH AL-AZAWY

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
In Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

December 2011

DEDICATED WITH LOVE AND GRATITUDE TO:

THE SPIRIT OF MY FATHER AND MOTHER

MY BELOVED WIFE...JINAN,

WHO WAS THE SOURCE OF INSPIRATION,

UNDERSTANDING AND ENCOURAGEMENT

THROUGHOUT MY STUDY

LOVING SONS, YESSER AND HYDER

LOVING DAUGHTERS, SARAH AND RAZ

FOR THEIR ENDLESS PATIENCE AND UNDERSTANDING

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements of the degree of Doctor of Philosophy

**ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION
OF FELINE CORONAVIRUS IN MALAYSIA**

By

AMER KHAZAAL SALIH

December 2011

Chairman: Associate Professor Siti Suri Arshad, PhD

Faculty: Faculty Veterinary Medicine

Feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV) are coronaviruses causing disease in cats. FIPV is a highly fatal and immune-mediated pyogranulomatous disease, whereas FECV is asymptomatic and a subclinical or mild enteric infection. Both FIPV and FECV are further subdivided into serotypes I and II.

Cases of FIP occur in young and adult cats with varying degree of severity. In Malaysia, Feline coronavirus (FCoV) is prevalent where 100% of cats in catteries developed antibodies against the feline coronavirus. However, 84% of cats kept in catteries and shelters, were found to have viral genome of feline coronavirus in their feces. FIP is believed to be mutated from FECV and the virus has tropism towards the macrophages.

Although many cats can be infected with FCoV, only 10% will develop FIP disease. There is no effective vaccine to control the disease because immunized cats are found to be more susceptible to infection than unvaccinated cats due to the antibody-dependent enhancement (ADE) phenomena. Although the FCoV is prevalent in Malaysia, the virus

causing the disease has not been characterized and serotype. The study was conducted to isolate, identify, characterize and type the local feline coronavirus. The local isolates and two reference strains of FCoV were compared with respect to their biological properties, morphology and genome homology. A total of 42 cats clinically suspected of having FIP disease were obtained during the 4 years period (2007-2010) and were designated as UPM1C/07 to UPM42C/10. Affected cats showing respiratory distress with distended abdominal cavity were sampled. Ascites fluid or tissues from cat dying from FIP were screened for FCoV by a one-step reverse transcriptase polymerase chain reaction (RT-PCR) assay and adapted in Crandell feline kidney (Crfk) and *Felis catus* whole foetus (Fcwf-4) cell cultures. About 95% (40/42) of them were positive by virus isolation and confirmed by RT-PCR. Upon infection into two cell lines, infected cells showed cytopathic effect (CPE) characterised by giant cells, ballooning and detachment of infected cells. Fcwf-4 cell line provides more suitable growth conditions for local isolate FCoVs. Representative of three FCoV isolates namely the UPM5C/08, UPM11C/08 and UPM24C/09 were clone purified by limiting dilution and were used for subsequent studies. Identification of the virus isolates was conducted by indirect immunofluorescent (IIF), indirect immunoperoxidase (IIP), haematoxylin and eosin staining (H&E) and transmission electron microscope (TEM). The study showed that FCoV multiply in the cytoplasm of infected cells. The virus particles possessed the characteristic of coronavirus with spherical shapes and surrounded by club shaped peplomers indicative of typical coronavirus.

The determination of the serotypes of local FCoV and their phylogenetic relatedness was performed by analyzing their S gene region using published primers. Following

analyses, 97.5% and 2.5% of local FCoV isolates belonged to serotypes I and II, respectively. Phylogenetic analysis performed on selected 12 FCoV type I local isolates on the S region showed that the homology amongst them ranged from 92.3-100% and decreased to 90% with reference viruses. Whereas the local FCoV type II has 97.5-99% homology with reference strains. Malaysian FCoV isolates fall in 3 clusters where 2 clusters comprise most of type I which are closer to the USA, Netherlands, UK, Japan and Taiwan isolates, and third cluster comprises the type II which is in the group of reference viruses of USA and Italy.

In conclusion, this is the first detailed study on isolated local feline coronavirus in an attempt to adapt the viruses in *vitro* and characterize them. About 95% (40/42) of cats that were naturally infected with feline coronavirus have the viral genome in their body excretion. The isolated viruses were adaptable in cell culture and induce of cytopathic effect. FCoV type I was highly prevalent amongst the local cats.

Key word: Feline coronavirus, Crfk, Fcwf-4, Indirect immunofluorescent, Indirect immunoperoxidase, H&E, RT-PCR, Phylogenetic analysis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**PENGASINGAN, PENGENALPASTIAN DAN PENCIRIAN MOLEKUL
KORONAVIRUS KUCING DI MALAYSIA**

Oleh

AMER KHAZAAL SALIH

Disember 2011

Pengerusi: Profesor Madya Dr Siti Suri Arshad, Ph.D.

Fakulti: Fakulti Perubatan Veterinar

Virus kucing berjangkit peritonitis (FIPV) dan koronavirus kucing enterik (FECV) adalah penyebab penyakit kucing. FIPV menyebabkan pyogranulomatous penyakit yang sangat fatal dan imun-pengantara, manakala FECV berupa asimptomatik dan jangkitan enterik subklinikal atau ringan. Kedua-dua FIPV dan FECV terbahagi kepada serotip I dan II. Kes-kes FIP berlaku pada kucing muda dan dewasa dengan pelbagai tahap keterukan. Di Malaysia, FCoV tersebar luas di mana 100% daripada kucing dalam catterri didapati mempunyai antibodi terhadap koronavirus kucing. Walau bagaimanapun, kucing di catterri dan tempat perlindungan, 84% didapati mempunyai genom virus koronavirus kucing dalam najis mereka. FIP dipercayai bermutasi dari FECV dan virus mempunyai tropisma pada sel makrofaj. Walaupun banyak kucing boleh dijangkiti dengan FCoV, hanya 10% akan mendapat penyakit FIP. Tiada vaksin yang berkesan untuk mengawal penyakit kerana kucing yang menerima imunisasi lebih terdedah

kepada jangkitan daripada kucing tak vaksin disebabkan oleh fenomena ADE. Walaupun FCoV adalah lazim di Malaysia, virus yang menyebabkan penyakit ini tidak di ciri dan serotip. Kajian ini telah dijalankan untuk mengasing, mencari dan menaip koronavirus kucing tempatan. Isolat tempatan dan dua strain rujukan daripada FCoV dibanding dengan merujuk kepada sifat-sifat morfologi biologi, dan homologi genom. Sebanyak 42 kucing berpetanda klinikal yang disyaki mempunyai penyakit FIP diperolehi semasa tempoh 4 tahun (2007-2010) dan telah diujukkan sebagai UPM1C/07 hingga UPM42/10. Kucing berpenyakit menunjukkan masalah pernafasan dengan perut buncit disampel. Cecair ascites atau tisu dari kucing yang mati sebab FIP di saring untuk FCoV dengan esei RT-PCR dan disesuaikan dalam sel kultura Crfk dan Fcwf-4. Kira-kira 95% (40/42) sampel positif dengan pengasingan virus dan disahkan oleh RT-PCR. Jangkitan kepada sel menunjukkan kesan cytopathic (CPE) yang dicirikan oleh sel gergasi, membulat dan tanggal. Fcwf-4 sel didapati lebih sesuai untuk percambahan FCoVs dalam mengasingkan isolate tempatan. Tiga FCoV klon UPM5C/08, UPM11C/08 dan UPM24C/09 yang dituliskan secara pencairan terhad telah digunakan untuk pengkajian berikutnya. Pencirian isolat virus telah dijalankan melalui teknik immunopendafloran tidak langsung (IIF), immunoperoxidase secara tidak langsung (IPP), H & E dan TEM. Kajian menunjukkan bahawa FCoV membiak di dalam sitoplasma sel yang dijangkiti. Zarah virus mempunyai pencirian koronavirus dengan bentuk sfera dan dikelilingi oleh peplomers. . Penentuan serotip FCoV tempatan dan filogenetik keberhubungan mereka dilakukan dengan menganalisis rantau gen S. Berikutan analisis filogeni, 97.5% dan 2.5% daripada FCoV tempatan adalah serotip I dan II masing-masing. Filogenetik analisis yang dilakukan ke atas 12 FCoV tempatan pada rantau S menunjukkan bahawa homologi di kalangan mereka antara 92.3-100% dan menurun

kepada 90% dengan virus rujukan. Manakala jenis II FCoV tempatan mempunyai homologi 97.5-99% dengan strain rujukan. Isolat FCoV Malaysia terbahagi dalam 3 kelompok dimana 2 kelompok terdiri daripada jenis I yang kebanyakannya berasal dari Amerika Syarikat, Belanda, UK, Jepun dan Taiwan, dan kelompok ketiga terdiri daripada jenis II yang dalam kumpulan virus rujukan Amerika syarikat dan Itali. Kesimpulannya, ini adalah kajian tempatan pertama yang terperinci mengenai coronavirus kucing dalam usaha untuk mengasingkan, penyesuaian vitro dan pencirian. Kira-kira 95% (40/42) kucing yang dijangkiti secara semulajadi mempunyai genom coronavirus kucing dalam perkumuhan badan mereka. Virus terpicil menyesuaikan diri dalam sitoplasma sel dan merangsang kesan sitopatik.

Kata kunci: coronavirus kucing, Crfk, Fcwf-4, immunofluorescent tidak langsung, immunoperoxidase tidak langsung, H & E, RT-PCR, analisis filogenetik.

ACKNOWLEDGEMENTS

I am grateful to the Almighty ALLAH, lord of all creations, is heavenly, luxuriates and blessing over my throughout my life and the period of this study in UPM.

My utmost appreciation and extreme gratitude are conveyed to my supervisor, Assoc. Prof. Dr. Siti Suri Arshad for her invaluable guidance, knowledge, constructive comments and suggestions throughout the duration of my study. Additionally, her great effort and time spent to improve the quality of my thesis is very much appreciated. I am forever indebted for her kindness, patience, support, motivation and encouragement, all of which has helped me finish my project. Her sincerity in helping me is deeply cherished, and I look up to her not only as my mentor, but also as a sister. Many thanks Dr.

I would also like to express my heartfelt thanks and appreciation to Prof. Dr. Mohd Hair Bejo and Prof. Dr Abdul Rahman Omar, my co-supervisors for their constructive suggestion and supports which were really helpful towards the completion of my study.

I am grateful to Prof. Datin Paduka Dr. Aini Ideris for her assistance in facilitates my admission to UPM.

My sincere thanks gratitude is extended to Prof. Dr Tengku-Azmi Tengku Ibrahim for assistance in electron microscope analysis as well as for editing my manuscript.

I would like to express my sincere thanks and appreciation to Dr. Viviane Benetka, University of Veterinary Medicine, Vienna, Austria for kindly provided me with reference FCoV DF2 (type II) and KU2, NW1 FCoV strains type I virus.

This work would not have been possible without the support and helping hand from the staff members of virology laboratory, in particular to Mr Mohd Kamarudin Awang, Mr Mohd Nazri Abd Hamid, Mr Shahrudin Uda Ibrahim, Mrs Norzatul Iffah Ahmad, and Ms. Noraini Said.

I am also extremely grateful to all the staff in Microscopy Unit and Molecular Biomedicine of Institute of Bioscience, particular, Mr. Rafiuz Zaman Haron, Mrs. Aminah Jusoh, Mrs. Faridah Akmal, Dr. Tan Sheau Wei, Mrs Nancy Liew Woan Charn, Mrs. Norhaszalina, and Mrs. Mastura.

I would like to offer my special thanks to Dr. Hamid Al-Tammemi for his assistance in the preparation of HIS.

I am forever grateful to my best friend, Dr. Nazrina Camalxaman (Ina). Thank you for all kind of help. My thanks also go to Yi Wan, Dr. Mayada Hasson, Dr. Nathera Mohamed, Dr. Tan, Dr. Majed Hamed, Dr. Faruk Bandi, Dr. Saeed Sharif, Dr. Ajwad Awad, Dr. Omar Emad, Dr. Hutheyfa AL-Salih, Dr. Faiz Fawzi, Dr. Hemen Othman, Dr. Abdul Rahman, Eng, Mr. Thaseen Saad and Afzal Hafidz.

I would like to express my gratitude to the Ministry of Higher Education and Scientific Research of Iraq, Universiti of Diyala, Faculty of Veterinary Medicine, for giving me the opportunity to carry out full time Ph.D. programme.

My sincere thanks also to the Malaysian Government and Universiti Putra Malaysia in particular for supporting me throughout the course of my study.

I wish to express my deepest and heartfelt appreciation to my wife JINAN, and my sons YASEER, HADER, and my daughter SARAH for being supportive, understanding, patience, their sacrifice and support throughout my study. Thanks for everything. Without my family, I would never be here and accomplishing this challenging task will not be possible.

I also express my gratitude to my brother and sisters for their encouragement and support.

My thanks also go to the Assoc Prof. Dr. Abdul Razzaq Shafiq, Dr. Karim Sadun and Dr. Adnan Hikman, for all kind of help in my study.

Finally, many thanks to all who have helped or contributed in one way or other towards the completion of this study.

I certify that a Thesis Examination Committee has met on 13 December, 2011 to conduct the final examination of Amer Khazaal Salih Alazawy on his Doctor of philosophy thesis entitled “**ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF FELINE CORONAVIRUS IN MALAYSIA**” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Y. Bhg. Professor Tengku Azmi bin Tengku Ibrahim, PhD

Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Associate Professor Dr. Hassan b Hj Mohd Daud, PhD

Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Associate Professor Dr. Jasni bin Sabri, PhD

Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Y. Bhg. Professor Peter J.M. Rottier, PhD

Department of Infections Diseases and Immunology
Veterinary University
Utrecht University
(External Examiner)
Netherlands

SEOW HENG FONG, PhD

Professor and Deputy
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

Siti Suri Arshad, PhD

Assoc. Prof
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohd Hair Bejo, PhD

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Abdul Rahman Omar, PhD

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.



AMER KHAZAAL SALIH

Date: 13 December 2011

TABLE OF CONTENTS

	Page
DEDICATION	i
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	xi
DECLARATION	xiii
LIST OF TABLES	xx
LIST OF FIGURES	xxii
LIST OF ABBREVIATIONS	xxxv
1 INTRODUCTION	1
2 LITERATURE REVIEW	8
2.1 Historical Background	8
2.2 Aetiology	9
2.2.1 Classification	9
2.2.2 Virion Morphology	12
2.2.3 Structural Proteins	14
2.2.4 Viral Genome and Non-structural Proteins	17
2.2.5 Viral Replication	19
2.2.6 Virus Cultivation	20
2.2.7 Clone Purified Feline Coronavirus	24
2.3 Epidemiology	26
2.4 Pathogenesis	27
2.5 Clinical Signs	30
2.6 Diagnosis	34
2.6.1 General Diagnosis Tests	35
2.6.2 Serology	38
2.6.3 Antibody Antigen Complex Detection	39
2.6.4 Necropsy and Histopathology of FIP Infection	39
2.6.5 Immunohistochemistry Staining	41
2.6.5.1 Indirect Immunofluorescent Test (IIF)	41
2.6.5.2 Indirect Immunoperoxidase Test (IIP)	43
2.6.6 Electron Microscopy	44
2.6.7 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Assay	46
2.7 Treatment	48

2.7.1	Treatment of FECV Infection	49
2.7.2	Treatment of FIP Infection	49
2.8	Prevention	51
3	GENERAL MATERIALS AND METHODS	
3.1	Animals	53
3.2	Sample Collection and Processing	53
3.2.1	Ascites Fluid	53
3.2.2	Tissues	54
3.3	Propagation of Cell Culture	56
3.3.1	Cell Culture	56
3.3.1.1	Crandell Feline Kidney (Crfk)	56
3.3.1.2	Felis Catus Whole Fetus (Fcfw-4)	56
3.3.2	Resuscitation of Frozen Cell Culture	57
3.3.3	Maintenance of Adherent Cell Culture	57
3.3.4	Viable Cell Counting by Hemocytometer	58
3.4	Adaptation and Propagation of Feline Coronavirus in Cell Culture	60
3.4.1	Viruses	60
3.4.1.1	Reference Viruses	60
3.4.1.2	FCoV Local Isolate	61
3.4.2	Virus Inoculation in Cell Culture	61
3.4.3	Harvesting of Viruses	61
3.5	Production of Clone purified Virus	62
3.5.1	Viruses	62
3.5.2	Plaque Purification by Limiting Dilution	62
3.6	Purification of the Virus Isolate	63
3.6.1	Viruses	63
3.6.2	Purification of the Virus by Sucrose Gradient	63
3.7	Titration of Virus Isolates	64
3.7.1	Virus Titration by Tissue Culture Infective Dose (TCID ₅₀)	65
3.7.2	Virus Titration by Plaque Forming Assay	66
3.8	Electron Microscopy Studies	66
3.8.1	Negative Contrast Electron Microscopy (NCEM)	67
3.8.2	Transmission Electron Microscopy	67
3.9	Reverse Transcriptase Polymerase Chain Reaction	69
3.9.1	RNA Extraction from Infected Cell Culture	69
3.9.2	Determination of RNA Concentration and Purity	70
3.9.3	Agarose Gel Electrophoresis	70
3.9.4	Ethidium Bromide Staining	71
4	ISOLATION OF FELINE CORONAVIRUS FROM NATURALLY INFECTED CATS	
4.1	Introduction	72
4.2	Materials and Methods	75
4.2.1	Animal and Sample	75
4.2.2	Inoculation of Virus Suspension in Cell Culture	75

4.2.3	Production of Clone Purified Virus	75
4.2.3.1	Viruses	75
4.2.3.2	Clone Purified Virus by Limiting Reduction	76
4.2.4	Virus Purification by Sucrose Gradient	76
4.2.5	Virus Titration	76
4.2.5.1	Viruses	76
4.2.5.2	Virus Titrations by TCID ₅₀	76
4.2.5.3	Virus Titration by Plaque Forming Assay	76
4.2.6	Growth Curve Study	77
4.2.7	Negative Contrast Electron Microscope	78
4.2.8	Detection of FCoV in Clinical and Post-mortem Samples by RT-PCR Assay	78
4.2.8.1	Samples	78
4.2.8.2	RNA Extraction and RT-PCR Assay	79
4.2.8.3	Determination of RNA Concentration and Purity	79
4.2.8.4	Oligonucleotide Primers	79
4.2.8.5	Reverse Transcription and PCR Reaction	79
4.2.8.6	Agarose Gel Electrophoresis	81
4.2.8.7	Ethidium Bromide Staining	81
4.2.8.8	Specificity of the RT-PCR Assay	81
4.2.8.9	Sensitivity of the RT-PCR Assay	82
4.3	Results	82
4.3.1	Detection of FCoV in Ascites Fluid and Tissue Samples using RT-PCR	82
4.3.2	Specificity of the RT-PCR Assay	85
4.3.3	Sensitivity of the RT-PCR Assay	86
4.3.4	Viable Cell Counting by Hemocytometer	87
4.3.5	Virus Adaptation and Propagation	87
4.3.6	Clone Purified Virus by Limiting Dilution	95
4.3.7	Virus Purification by Sucrose Gradient	97
4.3.8	Virus Titration	98
4.3.8.1	Titration of the Virus by TCID ₅₀	98
4.3.8.2	Titration of the Virus by Plaque Forming Assay	98
4.3.9	Virus Growth Analysis	98
4.3.10	Negative Contrast Electron Microscopy (NCEM)	102
4.4	Discussion	104
4.4.1	Detection of FCoV in Ascites Fluid and Tissue Samples Using RT-PCR	104
4.4.2	Virus Adaptation and Propagation	105
4.4.3	Clone Purified Virus by Limiting Dilution	108
4.4.4	Virus Purification by Sucrose Gradient	109
4.4.5	Morphology of FCoV	110
4.2.6	Growth Curve Study Analysis of FCOVs Clone UPM11C/08 in Comparison to Virulent and Avirulent Reference Viruses in Fcwf-4 Cell Culture	111
4.5	Conclusion	113

5 CHARACTERIZATION AND IDENTIFICATION OF LOCAL FELINE CORONAVIRUS ISOLATE

5.1	Introduction	114
5.2	Material and Method	119
5.2.1	Viruses	119
5.2.1.1	Reference Virus	119
5.2.1.2	FCoV isolates	119
5.2.2	Cell Culture	119
5.2.2.1	Crandell Feline Kidney (Crfk)	119
5.2.2.2	Felis Catus Whole Fetus (Fcwf-4)	120
5.2.3	Virus Growth in Cell Culture	120
5.2.3.1	Reference Virus Inoculation	120
5.2.3.2	Clone Purified FCoV Isolates	120
5.2.4	Production of Hyperimmune Serum	121
5.2.4.1	Virus	121
5.2.4.2	Rabbits	121
5.2.4.3	Virus Inoculation	122
5.2.4.4	Harvesting the Blood	123
5.2.4.5	Serum Processing and Purification	123
5.2.5	Serum Neutralization Test (SNT)	124
5.2.6	Transmission Electron Microscope (TEM)	124
5.2.6.1	Viruses	125
5.2.6.2	Cell Culture and Viruses Inoculation	125
5.2.7	Haematoxylin and Eosin Staining (H&E)	125
5.2.8	Immunocytochemical Assay	126
5.2.8.1	Indirect Immunofluorescent Test (IIF)	127
5.2.8.2	Indirect Immunoperoxidase Test (IIP)	128
5.3	Results	130
5.3.1	H&E Staining	130
5.3.1.1	Control Crfk and Fcwf-4 Stained with H&E	130
5.3.1.2	Virus Infected Crfk and Fcwf-4 Cell Cultures	133
5.3.2	Immunocytochemical Assay	139
5.3.2.1	Indirect Immunofluorescent Test (IIF)	139
5.3.2.2	Indirect Immunoperoxidase Test (IIP)	143
5.3.3	Transmission Electron Microscope (TEM)	146
5.3.4	Serum Neutralization Test	161
5.4	Discussion	162
5.4.1	Histopathological Change of Cell Monolayers Following FCoV Infection	162
5.4.2	Localization of Virus Antigen in Infected Cell Cultures as Detected by IIFT	163
5.4.3	Localization of Virus Antigen in Infected Cell Culture as Detected by IIPT	167
5.4.4	Transmission Electron Microscope (TEM)	169
5.5	Conclusion	177

6	MOLECULAR CHARACTERIZATION OF LOCAL FELINE CORONAVIRUS ISOLATES	
6.1	Introduction	179
6.2	Material and Method	184
6.2.1	RT-PCR Assay for Differentiation Between types I and II	184
6.2.1.1	Viruses	184
6.2.1.2	Samples	184
6.2.1.3	RNA Extra and RT-PCR Assay	184
6.2.1.4	Determination of RNA Concentration and Purity	185
6.2.1.5	Oligonucleotide Primers	185
6.2.1.6	Reverse Transcription and PCR Reaction	186
6.2.1.7	Agarose Gel Electrophoresis	189
6.2.1.8	Ethidium Bromide Staining	189
6.2.1.9	Specificity of the RT-PCR Assay	189
6.2.1.10	Sensitivity of RT-PCR Assay	189
6.2.2	Phylogenetic Analysis of FCoV Isolates	190
6.2.2.1	Study Design	190
6.2.2.2	Purification of PCR Product	190
6.2.2.3	Sequence and Phylogenetic Analysis	191
6.3	Results	195
6.3.1	RT-PCR Assay for Differentiation Between Types I and II	195
6.3.1.1	Incidence of Types I and II FCoVs	195
6.3.1.2	Specificity of the RT-PCR Assay	200
6.3.1.3	Sensitivity of the RT-PCR Assay	202
6.3.1.4	Sequence and Phylogenetic Analysis	203
6.4	Discussion	219
6.4.1	Incidence of Types I and II	219
6.4.2	Specificity and Sensitivity of Primers	221
6.4.3	Sequence and Phylogenetic Analysis	222
6.5	Conclusion	226
7	GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH	
7.1	General Discussion	227
7.2	Conclusion	234
7.3	Recommendation for Further Research	236
	BIBLIOGRAPHY	237
	APPENDICES	262
	Elaborate of Appendix:	
	A : Antibiotic and Medium	263
	A1 Antibiotic-Trypsin-Versine (ATV)	263
	A2 Antibiotic-Antimycotic and Anti PPLO agents	263
	A3 Growth Medium	264

	A4	Maintenance Medium	265
	A5	Methylcellulose Overlay Medium	265
	A6	Trypsin	266
B: Buffer			268
	B1	TNE Buffer	268
	B2	Phosphate Buffer Saline(PBS)	268
	B3	0.025 Tris Buffer Saline (pH 7.4)	269
	B4	2.5% Glutaraldehyde Cacodylate-Buffered	269
	B5	Osmium Tetraoxide Buffer	270
	B6	Resin Mixture	270
	B7	Tris-Acetate EDTA (TAE)	271
	B8	Sucrose Gradient	271
C: TCID₅₀			272
D: Cell Count by Haemocytometer			278
E: CPE Percentage			279
F: Staining			281
	F1	H&E Staining	281
	F2	Uranyl Acetate Staining	282
	F3	Lead Citrate Staining	282
	F4	Phosphotungsten Acid (PTA)	283
	F5	Trypan Blue	283
G: Plaque Assay Analysis			284
BIODATA OF STUDENT			285
LIST OF PUBLICATIONS			287

LIST OF TABLES

Table		Page
3.1	List of cats suspected of FIP sampled during the four years of study (2007-2010) which was obtained from UVH-UPM and private veterinary clinic in Selangor	55
4.1	Reaction mixture used for the RT-PCR assay for the detection of FCoV	80
4.2	Amplification program for the conventional RT-PCR assay.	81
4.3	Percentage of CPE monolayer Fcwf-4 showing CPE over time following infection with UPM11C/08	279
4.4	Percentage of CPE monolayer Crfk showing CPE over time following infection with UPM11C/08	280
4.5	List of suspected FCoV local isolates used in this study. All isolates were subjected to RT-PCR screened for conserved UTR region and propagated in cell culture	88
4.6	The summary of virus strains and their titer as determined by TCID ₅₀ according to the method of Reed & Muench (1983)	272
4.7	Calculation of infectivity titer of FCoV UPM5C/08 by TCID ₅₀ according to the method of Reed & Muench (1983)	273
4.8	Calculation of infectivity titer of FCoV UPM11C/08 by TCID ₅₀ according to the method of Reed & Muench (1983)	274
4.9	Calculation of infectivity titer of FCoV UPM24C/09 by TCID ₅₀ according to the method of Reed & Muench (1983)	275
4.10	Calculation of infectivity titer of reference FIPV 79-1146 by TCID ₅₀ according to the method of Reed & Muench (1983)	276
4.11	Calculation of infectivity titer of reference FECV 79-1683 by TCID ₅₀ according to the method of Reed & Muench (1983)	277
4.12	Plaque Assay Analysis of clone purified FCoV UPM5C/08, UPM11C/08 and UPM24C/09	284
5.1	The titer reading of anti FCoV FIPV 79-1146 HIS following SNT assay against FCoV local isolates	161

6.1	Oligonucleotide primers used in the outer and inner PCR for the detection of FCoV types I and II	185
6.2	Reaction mixture used for RT-PCR assay to differentiation between FCoV types I and II	186
6.3	Reaction mixture for RT-nPCR assay to differentiation between FCoV types I and II	187
6.4	Amplification program used to amplify type I FCoV by RT-PCR	187
6.5	Amplification program used to amplify type I FCoV by RT-nPCR.	188
6.6	List of sequence of isolates and strains included for phylogenetic analysis	192
6.7	List of corresponding reference viruses for type I FCoVs obtained from GenBank	193
6.8	List of corresponding reference viruses for type II FCoVs obtained from GenBank	194

LIST OF FIGURES

Figure		Page
2.1	A schematic diagram of a typical coronavirus structure. S, spike glycoprotein; HE hemagglutinin-esterase glycoprotein; M, membrane glycoprotein; E, small envelope protein; N nucleocapsid phosphoprotein (Source, Petit, 2005).	12
2.2	Genome organization of feline coronavirus. The gene for the polymerase polyprotein is indicated (POL1a and POL1b). The genes for the structural proteins S, E, M, and N and presumptive nonstructural proteins 3a, 3b, 3c, 7a, and 7b. The functions of most of these nonstructural proteins are widely unknown (Source, McDonagh <i>et al.</i> , 2011).	18
2.3	Photograph shows a cat suspected of FIP with effusion or wet form lesion. Copious peritoneal fluid is seen in the peritoneal cavity (Arrow) (Source: Sharif <i>et al.</i> , 2010).	32
2.4	Cross section of a kidney of a cat with non-effusive dry form FIP. Granulomatous out located on the capsular surface (A) and extending into the parenchyma of the kidney (B) (Source: Pedersen, 2009).	33
4.1	Screening of cell culture infected with ascites fluid and tissue samples of cats suspected of FIP for FCoV using RT-PCR and 3`UTR primers pair P205/P211 produced the target band of 223bp. The 1.5% agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. Lane no. 1-18 are samples ;M: 100 marker; NTC : non-template control: Positive control (FIP 79-1146); Lanes 6 and 17: uninfected cell culture; Lane 1: UPM1C/07; Lane 2: UPM2C/07; Lane 3: UPM3C/07; Lane 4: UPM4C/07; Lane 5: UPM5C/08; Lane 7; UPM7C/08; Lane 8: UPM8C/08; Lane 9: UPM9C/08; Lane 10: UPM10C/08; Lane 11: UPM11C/08; Lane 12: UPM12C/08; Lane 13: UPM13C/08; Lane 14: UPM14C/09; Lane 15: UPM15C/09; Lane 16: UPM16C/09; Lane 18: UPM18C/09.	83
4.2	Screening of cell culture infected with ascites fluid and tissue samples of cats suspected of FIP for FCoV using RT-PCR and 3`UTR primers pair P205/P211 produced the target band of 223bp. The 1.5% agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. Lane no.19-42 samples; M: 100 marker; NTC: non-template control; Lane 19: UPM19C/09; Lane 20: UPM20C/09; Lane 21: UPM21C/09; Lane 22: UPM22C/09; Lane 23: UPM23C/09; Lane 24: UPM24C/09; Lane 25: UPM25C/09; Lane 26: UPM26C/09; Lane 27: UPM27C/09; Lane 28: UPM28C/09; Lane 29: UPM29C/10; Lane 30: UPM30C/10; Lane 31: UPM31C/10; Lane 32: UPM32C/10; Lane 33: UPM33C/10; Lane 34: UPM34C/10; Lane 35: UPM35C/10; Lane 36: UPM36C/10;	84

Lane 37: UPMC37/10; Lane 38; UPM38C/10; Lane 39: UPM39C/10;
Lane 40: UPM40C/10; Lane 41: UPM41C/10; Lane 42: UPM42C/10.

- 4.3 Specificity of RT-PCR assay using 3'UTR primers pair P205/P211 to produce 223 bp. The 1.5 agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. Lane 1: NTC: non-template control; Lane 2: FIPV79-1146; Lane 3: FECV 79-1683; Lane 4: CCV; Lane 5: FeLV; Lane 6: Uninfected Crfk cells; M: 100 bp DNA marker. 85
- 4.4 Sensitivity of RT-PCR assay was performed using 3'UTR primers pair P205/P211 to produce 223 bp. The 1.5 agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. Detection limit was done by performing RT-PCR assay on ten-fold serial dilution of RNA from FIPV79-1146. Lane 3: 10 ng of RNA; Lane 2: 100 ng RNA; Lane 1: 1µg RNA. 86
- 4.5 Summary of FCoV local isolates positivity following RT-PCR assay and their adaptability in Crfk and Fcwf-4. All samples which are positive for RT-PCR are also positive in fwcf-4 cell culture. 89
- 4.6 Figures show the unstained Crfk cell culture following infection with clone UPM11C/08 at third passage. (A) Infected cell showed moderate to diffuse cell abnormality characterized by enlarged, rounded and ballooned cells (Arrow) after 72 hours PI; 20x Mag. Scale bar, 100 µm.(B) Aggregation of the infected cells that lead to detachment of the monolayer from the culture flask at 4-5 days PI leaving empty spaces (Arrow); 20x Mag. Scale bar, 100 µm. 90
- 4.7 The unstained Crfk cell culture at third passage. (A) Uninfected Crfk cells monolayer showed the confluent monolayer at 72 hours 4x Mag. Scale bar, 500µm. (B) Uninfected Crfk cell monolayers showed the confluent monolayer and appeared as spindle to stellate morphology and regular arrangement at 72 hours 20x Mag. Scale bar, 100 µm. 901
- 4.8 The unstained Fcwf-4 cell culture following infection with clone UPM11C/08 at third virus passage. (A) Infected cells showed multifocal CPE characterized initially as cells enlarged and rounding (Arrows) after 15 hours PI. 10x Mag. Scale bar, 200µm. 92
(B) Infected cells showed CPE characterized by multinuclear giant cells formation (Arrows) at 24 hours PI. 20x Mag.
- 4.9 The unstained Fcwf-4 cell culture at third passage. (A) Uninfected Fcwf-4 cells monolayer showed confluent monolayer at 72 hours. 4x Mag. Scale bar, 500µm. (B) Uninfected Fcwf-4 cell monolayers appeared regular arrangement with spindle to stellate morphology at 72 hours 20x Mag. Scale bar, 100µ. 93

- 4.10 The unstained Fcwf-4 cell culture following infection with clone UPM11C/08 at fifth passage. (A) Infected cell culture showed CPE characterized by marked multifocal to diffuse CPE (Arrows) within 2-3 days PI. 20x Mag. Scale bar, 100 μ m. (B) Infected cell culture showed CPE characterized by multinuclear giant cells formation and cell rounding (Arrows) as early as 24 hrs PI. 20x Mag. Scale bar, 100 μ m. 94
- 4.11 The plaques formed by clone purified FCoV isolates following a plaque dilution assay. The clone purified FCoV isolates was designated as clone UPM11C/08. (A) The plaques of FCoV local isolate form in Fcwf-4 cell show homogeneous plaques with size up to 3 mm in a diameter. (B) The plaques of FCoV local isolate form in Crfk cell show homogeneous plaques with size up to 1 mm in a diameter. (C) Control uninfected Fcwf-4 cell monolayers that received only sterile PBS showed no plaque formation. 96
- 4.12 A single virus band of clone purified FCoV UPM11C/08 in 20-50% sucrose gradients following overnight ultracentrifugation. 97
- 4.13 A growth curve analysis of FCoV clone UPM11C/08 in Fcwf-4 cell culture. Following virus infection, CAV and ECV are first detected at 15 hours and 24 hours PI, respectively. The CAV and ECV reached its peak titer of 5.7 log₁₀ TCID₅₀ /0.1ml and 5.5 log₁₀ TCID₅₀/0.1 ml respectively at 48 hours PI. Both CAV and ECV titer starts to drop after 48 hours PI. 99
- 4.14 A growth curve analysis of FCoV reference virus FIPV 79-1146 in Fcwf-4 cell culture. Following of virus infection, CAV and ECV are first detected at 6 hours and 12 hours PI, respectively. The CAV and ECV reached its peak titer of 5.8 log₁₀ TCID₅₀ /0.1ml and 5.5 log₁₀ TCID₅₀ /0.1ml respectively at 36 hours PI. Both CAV and ECV titer starts to drop after 48 hours PI. 100
- 4.15 A growth curve analysis of FCoV reference virus FECV 79-1683 in Fcwf-4 cell culture. Following of virus infection, CAV and ECV are first detected at 12 hours and 18 hours PI, respectively. The CAV and ECV reached its peak titer of 5.2 log₁₀ TCID₅₀ /0.1ml and 5 log₁₀ TCID₅₀ /0.1ml at 36 hours PI. Both CAV and ECV titer starts to drop after 48 hours PI. 101

- 4.16 Negative contrast electron microscopy of FCoV following staining with 2% PTA. (A) Clone purified FCoV UPM C11/08 shows typical characteristic of feline coronavirus. Virus particles are pleomorphic with many of spherical shapes. The virus particles are surrounded by knob-like spikes envelope (Arrow). Scale bar, 100nm. (B) Purified reference FIPV 79-1146 shows typical characteristic of feline coronavirus. The virion of spherical shapes is surrounded by knob-like spikes envelope (Arrow). Scale bar, 100nm. 103
- 5.1 Normal uninfected Crfk cell culture at 72 hours. H&E staining. (A) Normal cell appeared as spindle-shape and regularly arranged. 10x Mag. Scale bar, 200 μ m. (B) Normal monolayer shows majority of cells are with single nuclei and a few cells contained two nuclei (Arrow). 20x Mag. Scale bar, 100 μ m. 131
- 5.2 Normal uninfected Fcwf-4 cell culture at 72 hours. H&E staining. (A) Normal cells appeared as spindle to stellate morphology and regularly arranged. 10x Mag. Scale bar, 200 μ m. (B) Normal monolayer shows cells containing one or two nuclei (Arrow). 20x Mag. Scale bar, 100 μ m. 132
- 5.3 Infected Crfk cell culture following infection with clone purified FCoV UPM11C/08. H&E staining. Infected cells transform from normal spindle with oval shaped nuclei and regular arranged to irregular arranged of cells with extensive granulation in the cytoplasm (Black arrow). The nucleus are deeply stained and appeared rounded (White arrow) at 18 hours PI. 20x Mag. Scale bar, 100 μ m. 133
- 5.4 Infected Fcwf-4 cell culture following infection with clone purified FCoV UPM11C/08. H&E staining. Infected cells changes from spindle to stellate morphology to irregular arranged with dark nucleus (Black arrow). There is a diffused granulation of the cytoplasm around the nucleus (White arrow) at 18hours PI. 20x Mag. Scale bar, 100 μ m. 134
- 5.5 Infected Crfk cell culture following infection with clone purified FCoV UPM11C/08. H&E staining. Infected cells loss their plasma membrane and causes the nucleus to aggregate and form syncytial cells that consist of more than 20 nuclei (Arrows) at 36 hours PI. 20x Mag. Scale bar, 100 μ m. 135
- 5.6 Infected Fcwf-4 cell culture following infection with clone purified FCoV UPM11C/08. H&E staining. Infected cells loss their plasma membrane and causes the nucleus to aggregate and form syncytial cells that consist of more than 20 nuclei (Arrow) at 36 hours PI. 20x Mag. Scale bar, 100 μ m. 136

- 5.7 Infected cell cultures following infection with clone purified FCoV UPM11C/08 isolates at 72 hours PI. H&E staining. (A) Infected Crfk cells started to lose their sharp outline and showed extensive degeneration. Majority of dead cells detached and left empty spaces (Arrow). 10x Mag. Scale bar, 200µm. (B) Infected Fcwf-4 cells had lost their cytoplasmic boundary resulting in the pooling of the nuclei and aggregations of the infected cells (Arrow). 10x Mag. Scale bar, 200µm. 137
- 5.8 Infected cell cultures following infection with reference strain FIPV79-1146. H&E staining. (A) Infected Crfk cells shows CPE at 12 hours PI. Note the granulation in the cytoplasm around the nucleus. 20x Mag. Scale bar, 100µm. (B) Infected Fcwf-4 cells show syncytium which is observed as early at 36 hours PI (Arrow). 10x Mag. Scale bar, 200µm. 138
- 5.9 Immunofluorescent antibody staining of infected cell culture following infection with clone purified UPM11C/08. IIF staining. (A) The fluorescence signal appears as fine granular fluorescent material in the perinuclear region at 6 hours PI (Arrows). 40x Mag. Scale bar, 50µm. (B) The fluorescence signal appears as granulations covering larger areas within the cytoplasm at 24 hours PI (Arrow). No signal present in the nucleus. 100x Mag. Scale bar, 20µm 140
- 5.10 An immunofluorescent antibody staining of infected cell culture following infection with clone purified FCoV UPM11C/08 at 36 hours PI. The fluorescence signals occupy more areas in cytoplasm where the nucleuses are without any signals (Arrows). 100x Mag. Scale bar, 20µm. 141
- 5.11 An immunofluorescent antibody staining of Fcwf-4 cells following infection with reference FIPV 79-1146 at 6 hours PI (Arrow). The fluorescence signals appear more intense and occupy the whole cytoplasm (Arrow). 100x Mag. Scale bar, 20 µm. 142
- 5.12 Immunoperoxidase staining of Fcwf-4 cell cultures. Uninfected cell showing no specific signals at 72 hours. 40x Mag. Scale bar, 50µm. 143
- 5.13 Immunoperoxidase staining of Fcwf-4 cell cultures following infection with clone purified FCoV UPM11C/08. Infected cells show brownish discoloration in the perinuclear region at 6 hours PI (Arrow). 40x Mag. Scale bar, 50 µm. 144
- 5.14 Immunoperoxidase staining of Fcwf-4 cells culture infected with FCoV local isolate. Cells infected with clone purified UPMC11/08 between 36-48 hours PI, an obvious increased in brownish discoloration in the cytoplasm as well as in the perinuclear region (Arrow). 40x Mag. Scale bar, 50µm. 145

- 5.15 Electronmicrograph of normal uninfected Fcwf-4 cell cultures.
Note: Uninfected Fcwf-4 cell one oval nucleus (N) centrally with one nucleoli (NU). 7,700x Mag. Scale bar, 2 μ m. 146
- 5.16 Electronmicrograph of normal uninfected Fcwf-4 cell cultures at 7 days old. The nucleus (N) and the nuclear membrane (NM) are lined by two membranes (Arrows). The cytoplasm contains mitochondria (M), vacuole (V), rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER) and Golgi apparatus (not shown). 21,000x Mag. Scale bar, 1 μ m. 147
- 5.17 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 at 1-2 hours PI. (A) Numerous virus-like particles are closely apposed to the cell plasma membrane (Arrow). 27,000 Mag. Scale bar, 1 μ m. (B) A virus-like particle penetrates the cell by invaginating the plasma membrane and carried it into the cytoplasm (Arrow). Note the tri-laminar membrane it formed. 100,000x Mag. Scale bar, 0.2 μ m. 148
- 5.18 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 at 1-2 hours PI.(A) A virus-like particle invaginating the host plasma membrane is coated with double membrane to form a vacuole at the site of virus entry (Arrow). 60,000x Mag. Scale bar, 0.5 μ m. (B) Virus-like particles which are characterized by spherical shaped with rough surface electron dense envelope are present at the extracellular compartment (Arrow). 77,000x Mag. Scale bar, 0.2 μ m. 150
- 5.19 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 at 6 hours PI lead to formation of a large DMV. Insert shows the double membrane vacuoles DMV with approximately diameter ranging from 400-600 nm at 6 hours PI (Arrow). 60,000x Mag. Scale bar, 0.2 μ m. 151
- 5.20 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 at 6 hours PI. (A) This low magnification electron micrograph gives a view of double membrane vacuoles DMVs (White arrow) contained numerous viral particles. They are also vacuoles without virus nearby the vacuoles with virus in which is unknown, at this point of the study. 80,000x Mag. Scale bar, 500 nm. 152
(B) Insert shows the double membrane vacuoles DMV gives a clearer view of the structure (Arrow). 200,000x Mag. Scale bar 100 nm.
- 5.21 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 at 6 hours PI. (A) Increased number of intracytoplasmic vesicles near the Golgi apparatus (GA) (Arrow). 153

27,000 Mag. Scale bar, 1 μ m.
 (B) Swelling of Golgi sacs at perinuclear region (PN) (Arrow).
 40,000x Mag. Bar=0.5 μ m.
 (C and D) Mitochondria (M) and RER, SER are morphological normal
 and show no obvious changes. They are also vacuoles without virus
 nearby the other vacuoles adjacent to the mitochondria which is
 unknown, at this point of the study. 60,000x Mag. Scale bar, 0.2 μ m.

- 5.22 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 between 6-15 hours PI.(A) Virus-like particles are present in vacuoles of different sizes, small vacuoles containing 1-4 particles (White arrow) while larger ones containing 20-24 virus particles (Arrowhead). They are also vacuoles without virus nearby the vacuoles with virus in which is unknown. 27,000x Mag. Scale bar, 1 μ m. (B) Mature virus particles extracellular location seen in majority of the infected cells (Arrow). 40,000 Mag. Scale bar, 0.5 μ m. 155
- 5.23 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 at 24 hours PI. Many virus-like particles are present within a vacuoles in different stages of maturation with sizes ranging from 69 -149 nm. Note the smooth electron dense envelope virus-like particles (Black arrow) and the distinct rounded, intact and thick vacuole membrane (White arrow). 100,000x Mag. Scale bar, 0.2 μ m. 156
- 5.24 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 at 24-36 hours PI. Aggregations of virus-like particles within vacuoles. Some parts of the vacuoles membrane were no longer intact and causes the virus-like particles to disseminate in to the cytoplasmic matrix (arrow). 40,000x Mag. Scale bar, 0.5 μ . 157
- 5.25 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 at 48 hours PI. Virus-like particles aggregations in the cytoplasmic matrix (Arrow) and a virus-like particle in the vesicle of Golgi apparatus (Arrow head). 27,000x Mag. Scale bar, 1 μ m. 158
- 5.26 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 after 48 hours PI. Changes in the organelles at late stage of infection. Note the numerous degenerate swollen vacuoles (white arrow) consisting mostly of matured virus particles (black arrow). 25,000x Mag. Scale bar, 1 μ m. 159

- 5.27 Electronmicrograph of Fcwf-4 cell culture infected with clone purified FCoV UPM11C/08 after 48 hours PI. (A) Few matured virus particles are seen fused directly to the plasma membrane (Black arrow) and other seen free into extracellular space (White arrow). 100,000 Mag. Scale bar, 200 nm. (B) Matured virus particles being expelled through a fluke-like canal (White arrow) created adjacent to the plasma membrane for exportation of the virus particles to the extracellular spaces (Black arrows). Note: The plasma membrane 80,000 Mag. Scale bar, 500 nm. 160
- 6.1 Optimization of RT-PCR for differentiation of type I and type II FCoV in feline cell culture infected with reference viruses of FCoVs by using four sets of primer pair amplifying the S region. (A) RT-PCR test uses primer pair fecv1b to amplify the outer region of S gene and with PCR product of 275bp for type I FCoV. The 1.5 agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. M: 100 bp DNA marker; Lane 1: NTC: non-template control; Lane 2: Type I reference virus FIPV NW-1; Lane 3: Type I reference virus FIPV KU2. (B) RT-PCR test uses primer pair fecv2b to amplify the outer region of S gene and with PCR product of 232 bp for type II FCoV. The 1.5 agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. M: 100 bp DNA marker; Lane 1: NTC: non-template control; Lane 2: Type II reference virus FIPV 79-1146; Lane 3: Type 11 reference virus FIPV DF2. 196
- 6.2 Optimization of RT-PCR and RT-nPCR assay for differentiation of type I and type II FCoV in feline cell culture infected with reference viruses of FCoVs by using four sets of primer pair amplifying the S region. (A) PCR test uses primer fecv1bn to amplify the inner region of S gene and with PCR product of 183 for type I FCoV. The 1.5 agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. M: 100 bp DNA marker; Lane 1: NTC: non-template control; Lane 2: Type I reference virus FIPV NW-1; Lane 3: Type I reference virus FIPV KU2. (B) PCR test uses primer fecv2bn to amplify the inner region of S gene and with PCR product of 151 for type I FCoV. The 1.5 agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. M: 100 bp DNA marker; Lane 1: Type II reference virus FIPV 79-1146; Lane 2: Type 11 reference virus FIPV DF2; Lane 3: NTC: non-template control. 197
- 6.3 A Screening of FCoVs adapted cell culture isolates to detect FCoV type I B, C using RT-PCR assay of the S region. Primer sets fecv1bf/fecv1br detects the outer region of the S gene with product size of 275 bp. The 1.5% agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. M: 100 bp DNA marker; P: Positive control reference virus type I FIPV NW1; NTC: No-template control; Lane 198

1: UPM1C/07; Lane 2: UPM2C/07; Lane 3: UPM3C/07; Lane 4: UPM4C/07; Lane 5: UPM5C/08; Lane 6: UPM6C/08; Lane 7: UPM7C/08; Lane 8: UPM8C/08; Lane 9: UPM9C/08; Lane 10: UPM10C/08; Lane 11: UPM11C/08; Lane 12: UPM12C/08; Lane 13: UPM13C/08; Lane 14: UPM14C/09; Lane 15: UPM15C/09; Lane16: UPM16C/09; Lane17:UPM17C/09; Lane18: UPM18C/09; Lane 19: UPM19C/09; Lane 20: UPM20C/09; Lane 21: UPM21C/09 and Lane22: UPM22C/09. Note: Lane 8: Type II FCoV, negative with primers of types I.

- 6.3 D,E Screening of FCoVs adapted cell culture isolates to detect FCoV type I using RT-PCR assay of the S region. Primer sets fecv1bf/fecv1br detects the outer region of the S gene with product size of 275 bp. The 1.5% agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. M: 100 bp DNA marker; NTC: No-template control; Lane 23:UPM23C/09; Lane 24; UPM24C/09; Lane 25: UPM25C/09; Lane 26: UPM 26C/09;Lane 27: UPM27C/09; Lane 28: UPM28C/09; Lane 29: UPM29C/10; Lane 30: UPM30C/10; Lane 31: UPM31C/10; Lane 32: UPM34C/10; Lane 33: UPM33C/10; Lane 34: UPM34C/10; Lane 35: UPM357C/10; Lane 36: UPM36C/10; Lane 37: UPM37C/10; Lane 38: UPM38C/10; Lane 39: UPM391C/10 and Lane 40: UPM40C/10. 199
- 6.4 Screening of FCoVs adapted cell culture isolates to detect FCoV type II using RT-PCR assay of the S region. Primer sets fecv2bf/fecv2br detects the outer region of the S gene with product size of 232 bp. The 1.5% agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. M: 100 bp DNA marker; NTC: No-template control; Lane 1: Positive control reference virus type II FIPV DF2; Lane 2: UPM8C/08; Lane3; UPM1C/07; Lane 4: UPM2C/07; Lane 5: UPM3C/07; Lane6: UPM4C/07. 199
- 6.5 Specificity studies on the RT-PCR assay using specific primer pairs to detect FCoV type I and type II on different reference virus strains. (A) Primers pair fecv1bf, fecv1br are used to detect the S region of FCoV type I and produces PCR product of 275 bp. The 1.5 agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. M: 100 bp DNA marker; Lane 1: Reference virus type I FIPV NW-1; Lane 2: Reference virus type II FIPV 79-1146; Lane 3: Reference virus type II FECV 79-1683; Lane 4: Reference virus type II FIPV DF; Lane 5: Reference virus type I FIPV KU2; Lane 6: NTC: non-template control. (B) Primers pair fecv2bf, fecv2br are used to detect the S region of FCoV type II and produces PCR product of 232 bp. The 1.5 agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. M: 100 bp DNA marker; Lane 1: NTC: non template control; Lane 2; Reference virus type I FIPV NW1; Lane 3: Reference virus type I FIPV KU2; Lane 4: Reference virus type II FIPV 79-1146; Lane 5: 201

Reference virus type II FECV 79-1683; Lane 6: Reference virus type II FIPV DF2.

6.6 Sensitivity studies on RT-nPCR assay. (A) Using specific primer pairs fecv1bnf /fecv1bnr to detect the S region of FCoV type I. The primer pairs amplify the target region with PCR product of 183bp. Detection limit was done by performing nested PCR assay on ten-fold dilution of RNA extracted from FIPV NW1 type I reference virus. The 1.5 agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. Lane 4 NTC: Non-template control; Lane 3: 10 ng of RNA; Lane 2: 100 ng RNA; Lane 1: 1µg RNA. (B) Using specific primer pairs fecv2bnf / fecv2bnr to detect the S region of FCoV type II. The primer pairs amplify the target region with PCR product of 151bp. Detection limit was done by performing nested PCR assay on ten-fold dilution of RNA extracted from FIPV DF2 type II reference virus. The 1.5 agarose gel was electrophoresed and stained with 0.5µg/ml ethidium bromide. Lane 4: NTC: Non-template control; Lane 3: 10 ng of RNA; Lane 2: 100 ng RNA; Lane 1: 1µg RNA.

201

6.7 Sequence Identity Matrix of partial S gene of FCoV type I. Twenty seven FCoVs were compared: Local isolates UPM2C/07, UPM5C/08, UPM11C/08, UPM13C/O8, UPM14C/09, UPM22C/09, UPM24C/09, UPM25C/09, UPM26C/09, UPM27C/09, UPM28C/09, UPM35C/10 are shaded and corresponding reference sequences from GenBank: Black, UU19, RM, UU31, UU10, UCD11a, UU8, UU4, UU7, NTU2, UU34, FCoV/S, UCD13, CIJe, UCD5. Local isolates were identical amongst themselves with more 92.3-100% homologous. The degree of identity reduces to 90% when compared with reference sequences FCoV from GenBank.

205

Note: The names were truncated to maximum 5 characters. The value could be converted to percentage by multiplying it with 100. Legend: ID= identical, 1=UPM2C, 2= UPM5C, 3= UPM11C, 4= UPM13C, 5= UPM14C, 6= UPM22C, 7= UPM24C, 8= UPM25C, 9= UPM26C, 10= UPM27C, 11= UPM28C, 12= UPM35C, 13= Black, 14= UU19, 15= RM, 16= UU31, 17= UU10, 18= UCD11a, 19= UU8, 20= UU4, 21= UU7, 22= NTU2, 23= UU34, 24= FCoV/S, 25= UCD13, 26= CIJe, 27= UCD5.

6.8 Sequence nucleotide difference of partial S gene of FCoV type I. Twenty seven FCoV type I were compared: Local isolates UPM2C/07, UPM5C/08, UPM11C/08, UPM13C/O8, UPM14C/09, UPM22C/09, UPM24C/09, UPM25C/09, UPM26C/09, UPM27C/09, UPM28C/09, UPM35C/10 are shaded and corresponding reference sequences from GenBank: Black, UU19, RM, UU31, UU10, UCD11a, UU8, UU4, UU7, NTU2, UU34, FCoV/S, UCD13, CIJe, UCD5. Nucleotides differences among local FCoV type I isolates 0-21, while nucleotides

206

difference increased to 1-29 when compare with reference sequences strains FCoV. Note: The names were truncated to maximum 5 characters. The value could be converted to percentage by multiplying it with 100. Legend: ID= identical, 1=UPM2C, 2= UPM5C, 3= UPM11C, 4= UPM13C, 5= UPM14C, 6= UPM22C, 7= UPM24C, 8= UPM25C, 9= UPM26C, 10= UPM27C, 11= UPM28C, 12= UPM35C, 13= Black, 14= UU19, 15= RM, 16= UU31, 17= UU10, 18= UCD11a, 19= UU8, 20= UU4, 21= UU7, 22= NTU2, 23= UU34, 24= FCoV/S, 25= UCD13, 26= CIJe, 27= UCD5.

- 6.9 Sequence Identity Matrix of partial S gene of FCoV type II. Six FCoV were compared: Local isolate UPM8C/08 and corresponding reference sequences from GenBank, FIPV 79- 1146, FIPV complete genome (AY994055), FIPV DF2, FIPV/E2 and CCV/NTU. The local isolate show identical in the range of 97.5-100% with FIPV 79-1146, FIPV complete genome with accession number (AY994055), FIPV DF2, FIPV/E2, but show 77.6-79% identical with CCV/NTU. Note: The names were truncated to maximum 5 characters. The value could be converted to percentage by multiplying it with 100. 207
- 6.10 Sequence difference count matrix of partial S gene of FCoV type II. Six FCoV type II were compared: Nucleotides difference count matrix among local isolate FCoV type II UPM8C/08 and corresponding reference sequences from GenBank, FIPV 79- 1146, FIPV complete genome (AY994055), FIPV DF2, FIPV/E2 were (2-5 nt), while nucleotides difference count matrix increased (45-48 nt) when compared with CCV/NTU. Note: The names were truncated to maximum 5 characters. The value could be converted to percentage by multiplying it with 100. 208
- 6.11 Sequence Identity Matrix of partial S gene of type I and II FCoV. Twenty seven FCoVs were compared: Local isolates type I, UPM2C/07, UPM5C/08, UPM11C/08, UPM13C/O8, UPM14C/09, UPM22C/09, UPM24C/09, UPM25C/09, UPM26C/09, UPM27C/09, UPM28C/09, UPM35C/10 are light shaded and corresponding reference sequences from GenBank: Black, UU19, RM, UU31, UU10, UCD11a, UU7, NTU2, UU34, FCoV/S/3b, UCD13, CIJe, UCD5 compared with type II, UPM8C/08, FIP 79-1146, FIPV DF2 are thick shaded. Local isolates type I identical amongst themselves with more than 92.3-100% homologous. The degree of identity reduces to 90% when compared with reference of type I from GenBank. Whereas the homology percentage decreased to 53.6% when compared with FCoV type II. Note: The names were truncated to maximum 5 characters. The value could be converted to percentage by multiplying it with 100. 209
- Legend: ID= identical, 1=UPM2C, 2= UPM5C, 3= UPM11C, 4= UPM13C, 5= UPM14C, 6= UPM22C, 7= UPM24C, 8= UPM25C, 9= UPM26C, 10= UPM27C, 11= UPM28C, 12= UPM35C, 13= Black, 14= UU19, 15= UU31, 16= UU10, 17= UU7, 18= NTU, 19= UU34,

20= UCD11a, 21= FCoV/S/3b, 22= UCD13, 23= CIJe, 24= UCD5, 25= UPM8C/08, 26= FIPV 79-1146, 27= FIPV DF2.

6.12 Sequence nucleotide difference of partial S gene of FCoV isolates type I and II. Twenty seven FCoV were compared: Local isolate type I, UPM2C/07, UPM5C/08, UPM11C/08, UPM13C/O8, UPM14C/09, UPM22C/09, UPM24C/09, UPM25C/09, UPM26C/09, UPM27C/09, UPM28C/09, UPM35C/10 are light shaded and corresponding reference sequences from GenBank: Black, UU19, RM, UU31, UU10, UCD11a, UU7, NTU2, UU34, FCoV/S/3b, UCD13, CIJe, UCD5 were compared with type II FCoV, UPM8C/08, FIP 79-1146, FIPV DF2 are thick shaded. Nucleotides differences among local FCoV type I isolates (0-21 nt), while nucleotides difference increased to 1-29 when compare with reference sequences FCoV type 1 from GenBank. While the range increased (137-143 nt) when compared with type II FCoV. Legend: ID= identical, 1=UPM2C, 2= UPM5C, 3= UPM11C, 4= UPM13C, 5= UPM14C, 6= UPM22C, 7= UPM24C, 8= UPM25C, 9= UPM26C, 10= UPM27C, 11= UPM28C, 12= UPM35C, 13= Black, 14= UU19, 15= UU31,

16= UU10, 17= UU7, 18= NTU, 19= UU34, 20= UCD11a, 21= FCoV/S/3b, 22= UCD13, 23= CIJe, 24= UCD5, 25= UPM8C/08, 26= FIPV 79-1146, 27= FIPV DF.

6.13 Multiple nucleotide sequence alignment were performed using ClustalW Multiple alignment (Bioedit version 7.0.9) on the 221 bp fragment of the S gene from 12 type I Malaysian local FCoV isolates in comparison with some reference isolates retrieved from GenBank. Sequence identity is indicated by dots while dash indicates deletion. The Malaysian FCoV type I local isolates GenBank accession numbers are HQ206644, HM628778, HQ206646, HQ829849, HQ829850, HQ206647, HQ206648, HQ206649, HQ206650, HQ206651, and HQ829851 respectively. The reference strains type I GenBank accession numbers are EU186072, HQ392470, FJ938051, HQ012371, HQ012371, FJ938059, FJ917519, FJ938055, FJ938054, FJ938053, DQ160294, HQ012372, AB535528, FJ917523, DQ848678 and FJ917522 respectively.

6.14 Multiple nucleotide sequence alignment were performed using ClustalW Multiple alignment (Bioedit version 7.0.9) on the 200 bp fragment of the S gene from type II Malaysian local FCoV isolates UPM8C/08 accession number is JF757239. The latter isolate is compared with some reference isolates retrieved from GenBank. Sequence identity is indicated by dots while dash indicates deletion. The reference strains include FIPV 79-1146 (DQ010921), FIPV complete genome (AX994055), FIPV DF2 (DQ286389), and FIPV E2 (X06170).

- 6.15 Phylogenetic relationships determined by the S gene sequence of the local FCoV isolates type I and corresponding sequence of FCoV from GenBank. The phylogenetic trees were generated using tree top phylogenetic tree predication program (GeneBee-Molecular Biology Server) and displayed in PHYLIP format with bootstrap values. Malaysian isolates type I are located in frames A and B. Note: The names were truncated to maximum 5 characters to meet the requirement of the program. 216
- 6.16 Phylogenetic relationships determined by the S gene sequence of the local FCoV isolates type I and type II and corresponding sequence of FCoV from GenBank entries. The phylogenetic trees were generated using tree top phylogenetic tree predication program (GeneBee-Molecular Biology Server) and displayed in PHYLIP format with bootstrap values. Malaysian isolates type I and II are located in frame A, B and C. Note: The names were truncated to maximum 5 characters to meet the requirement of the program. Note: Malaysian isolates of FCoV type I grouping in frame A includes, UPM2C/07, UPM5C/08, UPM24C/09, UPM27/09, UPM28C/09, UPM26C/09, UPM11C/08, UPM13C/08, UPM14C/09, UPM35C/10, UPM22C/09 and frame B include UPM25C/09, while grouping of FCoV type II include UPM8C/08. 217
- 6.17 Unrooted Phylogenetic based on comparison of S nucleotide sequence illustrating the evolutionary relationship of FCoV types I and type II local isolates and corresponding FCoV entries from the GenBank. The tree was obtained using the neighbour-Joining algorithm on the basis of nucleotide distances. Database sequences showing the distance between type I and type II FCoV. Type II represented by one isolate UPM8C/08. While type I comprises of majority of isolates namely UPM2C/07, UPM5C/08, UPM24C/09, UPM27/09, UPM28C/09, UPM26C/09, UPM11C/08, UPM13C/08, UPM14C/09, UPM35C/10, UPM22C/09 and UPM25C/09. 218

LIST OF ABBREVIATIONS

A	Ampere
Ab	Antibody
ADE	Antibody-dependent Enhancement
aka	As know as
AIHA	Autoimmune Hemolytic Anemia
APN	Aminopeptidase-N
ATCC	American Type Culture Collection
ATV	Antibiotic Trypsin Versen
bp	Base Pairs
BSH	British Short Hair
BSA	Bovine Serum Albumin
°C	Degree Celsius
CMI	Cell-Mediated Immunity
cm	Centimeters
cm ²	Centimeter Square
CNS	Central Nervous System
CO ₂	Carbon Dioxide
CPE	Cytopathic Effect
Crfk	Crandell Reese Feline Kidney
CSF	Cerebrospinal fluid
DAB	3-3'-Diamino Benzidine Hydrochloride
dH ₂ O	Distilled Water
DMEM	Dulbecco Minimal Essential Medium
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Ttriphosphate
ds	Double Stranded
DSH	Domestic Short Hair
EDTA	Ethylen Diamine Tetra-acetate
ELISA	Enzyme-linked Immunosorbent Assay
EM	Electron Microscope
ER	Endoplasmic Reticulum
FBS	Fetal Bovine Serum
FITC	Fluorescence Isothiocyanate
FCoV	Feline Coronavirus
FECV	Feline Enteric Coronavirus
FIP	Feline Infectious Peritonitis
FIPV	Feline Infectious Peritonitis Virus
g	Gravity
gm	Gram
h	Hours
H&E	Hematoxylin and Eosin
HIS	Hyperimmune Serum

HRP	Horse-Radish Peroxidase
IgG	Immunoglobulin G
IIFT	Indirect Immunofluorescent Test
IPT	Indirect Immunoperoxidase Test
i/m	Intramuscular
i/v	Intravenous
Kbp	Kilobase pairs
kDa	Kilodalton
M	Molar
mAb	Monoclonal Antibody
MEM	Minimum Essential Medium
MHV	Murine Hepatitis Virus
min	Minute
ml	Milliliter
mM	Millimolar
mm	Millimeter
MW	Molecular Weight
MOI	Multiplicity of Infection
mRNA	Messenger Ribonucleic Acid
NaCl	Sodium Chloride
NCBI	National Center for Biotechnology Information
NCEM	Negative Contrast Electron Microscope
ng	Nanogram
nm	Nanometer
nt	Nucleotide
NTC	No Template Control
OD	Optical Density
ORF	Open Reading Frame
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PFU	Plaque Forming Unit
pH	Hydrogen-ion Concentration
PI	Post Infection
POL	Polymerase
PTA	Phosphotungstic Acid
RNA	Ribonucleic Acid
RNAase	Ribonuclease
rpm	Revolution Per Minute
RT	Reverse Transcriptase
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
S	Spike
sec	Second
SNT	Serum Neutralization Test
SPF	Specific Pathogen Free
TAE	Tris-Acetate EDTA
TC	Tissue Culture

TCID ₅₀	Tissue Culture Infectivity Dose at Fifty Percent
TE	Tris-EDTA
TEM	Transmission Electron Microscope
TGEV	Transmissible Gastroenteritis Virus
TEN	Tris-EDTA-NaCl
UK	United Kingdom
UPM	Universiti Putra Malaysia
USA	United States of America
UTR	Untranslated Region
UV	Ultraviolet
UVH	Universiti Veterinary Hospital
V	Voltage
VN	Virus Neutralisation
v/v	Volume/Volume
w/v	Weight/Volume
w/w	Weight/Weight
Vero	Cell Line Derived From Green African Monkey Kidney
µg	Microgram
µl	Microliter
µm	Micrometer
µM	Micromolar

CHAPTER I

INTRODUCTION

Feline coronavirus (FCoV) was first recognized in 1950s as a specific disease of cats (Holzworth, 1963) which came a disease named, feline infectious peritonitis (FIP). Wolfe and Griesemer (1966) was the first to show that the disease is caused by a virus. Ward (1970) recognized the close similarities of the FIP virus (FIPV) to members of the family *Coronaviridae*. Cases of FCoV infection have been reported world wide and in all known cat population known (Rottier, 1999).

FCoV is known to be prevalent and common by cause infection in cat populations, with particularly high prevalence in catteries and multiple-cat households (Addie and Jarrett, 1992; Simons *et al.*, 2005). The generic name FCoV has been loosely applied to all biotype and serotype of feline coronavirus.

Circulating antibodies against FCoV are found in 90-100% of cats in catteries or multiple-cat households and 50% in pet cats (Addie and Jarrett, 1992; Herrewegh *et al.*, 1997; Arshad *et al.*, 2004).

There are two biological types of coronavirus in cats: feline enteric coronavirus (FECV) which causes from asymptomatic infection to severe enteritis and the other is a systemic pathogen known to cause feline infectious peritonitis (FIPV) (Pedersen, 1995).

These FCoVs can infect domesticated cats and other members of the family *Felidae* (Vennema *et al.*, 1998). FECV is the common form of FCoV, which is virtually non-pathogenic that can be transmitted in nature between cat populations. It causes subclinical or mild enteric infections due to lesions located in the apical columnar

epithelium of the intestinal villi, from the caudal part of the duodenum to the cecum, and can cause mild to severe diarrhea in young kittens (Pedersen et al., 1981a; Addie and Jarrett, 1992). Unlike FECV infection, the FIP is an immune-mediated disease characterized by peritonitis and/or pleuritis with occasional central nervous system and ocular involvement. The inflammatory infiltrate consist of lymphocytes, plasma cell, and macrophages resulting in either nonsuppurative or granulomatous inflammation (Horzinek and Pedersen, 1982; Horzinek and Osterhaus, 1979). It is the most important cause of death of infectious origin in cats worldwide, and was originally thought to be an uncommon and fatal disease manifestation of single ubiquitous coronavirus affecting both domestic and wild *felids* (Holzworth, 1963; Pedersen *et al.*, 1981; Cave *et al.*, 2002).

FIPV was found to be morphologically and antigenically indistinguishable from FECV and were therefore called biotypes of feline coronaviruses. These two viruses differ only biologically (Pedersen, 1983; Corapi *et al.*, 1992; Vennema, 1999) where they are divided into serotypes I and II on the basis of cross-reactivity with canine coronavirus (CCV) in virus neutralization assays. Type I viruses show hardly any or no neutralization with anti CCV sera and grow poorly in cell culture and cause a slowly developing cytopathic effect (CPE) in many cell lines. Whereas type II FCoV are relatively easy to culture (Hohdatsu *et al.*, 1992; Motokawa *et al.*, 1996; Vennema, 1999) in many different cell lines including *felis catus* whole foetus (Fcwf-4) and Crandell feline kidney (Crfk) cells (Pedersen et al., 1984a). Based on the virus neutralisation activity and genomic analyses, serotype II FCoV strains show close antigenic and genetic relationship to CCV and transmissible gastroenteritis virus

(TGEV), respectively. They seem to have been arisen by double recombination between FCoV serotype I and CCV (Herrewegh *et al.*, 1998).

The close genetic relationship between FIP and common FECV infections was first reported by Pedersen *et al.* (1981b). Molecular studies showed that mutations in the FECV genome induce the virulent FIPV variants in infected cats and the presence of these variants coupled with an inadequate immune response lead to the development of a fatal immune-mediated clinical disease of FIP (Vennema *et al.*, 1998).

There are two forms of FIP that occur in nature. The classical effusive or wet form of the disease is the most common and easiest to diagnose, seen in approximately 75% of FIP cases. The disease is characterized by accumulation of fluid in the chest or abdomen. The proteinaceous fluid often contains fibrin clots and variable amount of cells, mostly non-degenerated neutrophils, macrophages and lymphocytes (Pedersen, 1995; Paltrinieri *et al.*, 1999; Hartmann, 2005). Dry form or noneffusive FIP result when a cell-mediated immune response dominates and form granulomas in various organs. The granulomatous lesions are usually found in the eyes, central nervous system (CNS), and parenchymatous abdominal organs, (Pedersen *et al.*, 1981; Hartmann, 2005).

Many attempts have been made to develop dependable clinical method or laboratory test to isolate the virus and to diagnose FIP (Pedersen, 1987a; Sparkes *et al.*, 1991). The diagnosis of FIP is difficult because the rare consequence of the infection where only 1-5% of seropositive cats eventually came down with FIP. The FIP occurred mainly in kittens, but sometimes also in old cats (Evermann *et al.*, 1991; Addie and Jarrett, 1992; Rottier, 1999).

The pathogenesis, viral morphology, and morphogenesis of FIPV are studied in tissues of naturally and experimentally infected cats, as well as in tissue cultures. Following *in vitro* infection in cell culture, myriad viral particles were observed both extracellularly and intracellularly (Hoshino and Scott, 1980; Pedersen and Boyle, 1980; Beesley and Hitchcock, 1982; Klumperman *et al.*, 1994; Ng *et al.*, 2003). Virus replication occurred in the cytoplasm of infected cells with no apparent nuclear involvement. Viral particles were found most frequently singularly or in groups in the cisternae of the dilated smooth and rough endoplasmic reticulum. They were observed to be present in vacuoles of various size and shapes or present freely in the cytoplasm (Hoshino and Scott, 1980; Klumperman, 1994; Lai and Cavanagh, 1997).

A definitive diagnosis of FCoV can be achieved by direct immunohistochemical examination of cells in the ascites fluid of cat suspected of FIP (Paltrinieri *et al.*, 1999). Indirect immunofluorescence test is several times more sensitive than immunoperoxidase for detection of viral antigens in natural infection with FCoV or infected cell culture (Pedersen, 2009). Although these immunohistochemical diagnostic assays are a helpful tool for FIP diagnosis, result can only be interpreted in correlation with clinical symptoms (Sparkes *et al.*, 1991), because the clinical or pathological manifestation of the infection are nonspecific and confused by those of many other diseases of cats (August, 1984).

Nowadays in all laboratories, polymerase chain reaction (PCR) assay has been used as a rapid and sensitive test to diagnose viral diseases. Compared with serological tests, PCR provides obvious advantage of direct detection of FCoV genome rather than

documenting a previous immunological activity encountered with a coronavirus (Hartmann, 2005).

FCoV disease had also been diagnosed by reverse transcriptase polymerase chain reaction (RT-PCR) assays by using primers targeted to highly conserved regions of the viral genome at the 3' untranslated region (3' UTR) (Herrewegh *et al.*, 1995b; Fehr *et al.*, 1997), or S-protein gene (Li and Scott, 1994; Gamble *et al.*, 1997). These genomic regions are common to all FCoV strains and served as a valuable tool for detection of the virus in blood, peritoneal fluid, feces and tissue samples of infected cats.

Moreover, the N-terminal domain of the S-protein gene of the spike regions allows the differentiation between type I and type II FCoVs (Posch *et al.*, 1999). Many countries have reported the occurrence of type I and type II FCoVs amongst the cats population in respective geographical region. Surveyed on the FCoV prevalence in cats have been documented in USA (Vennema, 1999), UK (Addie *et al.*, 2003), Japan (Hohdatsu *et al.*, 1992; Shiba *et al.*, 2007), Austria (Benetka *et al.*, 2004), Switzerland (Kummrow *et al.*, 2005) and Taiwan (Lin *et al.*, 2009).

The current information on the geographical distribution of FCoV indicates a high incidence of type I in many countries.

The first FIP case in Malaysia was reported in 1981 (Wong *et al.*, 1983). The preliminary study on FCoV antibody titer showed that FCoV is highly prevalent in Malaysia where 100% of cats in catteries were seropositive (Arshad *et al.*, 2004).

However, recent studies in cats kept in catteries using RT-PCR assay showed that 84% of the cats were positive (Sharif *et al.*, 2010). Based on the reported serological test and RT-PCR assay, we believed the virus is present in the local cats. Therefore, it is imperative to isolate the virus and examined their biological properties and compared with reference viruses.

The main aim of this study is to isolate the virus in sensitive feline cell cultures. Isolated cell culture adaptable virus will be further identified by examining their physical properties, morphogenesis, and locating their replication site in the cell. In addition to that, information on the serotypes and genotypes that are circulating in the region are ascertained.

Therefore, the objectives of this study were

1. to isolate FCoV from ante and post-mortem cases from naturally infected cats and cats suspected of being infected with FCoV.
2. to examine the biological characteristic of local isolates of FCoV in cell cultures.
3. to clone purified selected local virus isolates of FCoV by limiting dilution technique.
4. to detect local FCoV isolates by indirect immunofluorescent (IIF) and immunoperoxidase (IIP) techniques.
5. to observe the histopathology of FCoV infected cell culture by H&E staining.
6. to study of morphogenesis of clone purified FCoV UPM11C/08 isolate in Fcwf-4 cell.

7. to detect and differentiate FCoV type I and type II among local isolate of FCoV by RT-PCR assay.

8. to sequence the S region and examine the relatedness of Malaysian FCoV and reference strain by phylogenetic analysis.



BIBLIOGRAPHY

Addie, D.D. and Jarrett, J.O. (1990). Feline coronavirus infections. In C.E Greene. *Infectious diseases of dog and cat*. (pp.300-312). Philadelphia: WB Saunders.

Addie, D.D. and Jarrett, J.O. (1992). A study of naturally occurring feline coronavirus infection in kittens. *Vet Rec*, 130, 133-137.

Addie, D.D., Toth, S., Murray, G.D. and Jarrett, J.O. (1995). Risk of feline infectious peritonitis in cats naturally infected with feline coronavirus. *Am J Vet Res*, 56(4), 429-434.

Addie, D.D., Toth, S., Herrewegh, A. and Jarrett, J.O. (1996). Feline coronavirus in the intestinal contents of cats with feline infectious peritonitis. *Vet Rec*, 139(21), 522.

Addie, D.D. and Jarrett, J.O. (2001). Use of a reverse-transcriptase polymerase chain reaction for monitoring feline coronavirus shedding by healthy cats. *Vet Rec*. 148: 649–53.

Addie, D.D., Schaap, I., Nicolson, L. and Jarrett, J.O. (2003). Persistence and transmission of natural type I feline coronavirus infection. *J Gen Virol*, 84(10), 2735.

Addie, D.D., and Jarrett, J.O. (2006). Feline coronavirus infection. *Infectious Diseases of the Dog and Cat*. St Louis: Saunders-Elsevier, 88–102.

Addie, D.D., McLachlan, S.A., Golder, M., Ramsey, I. and Jarrett, J.O. (2004). Evaluation of an in-practice test for feline coronavirus antibodies. *J Feline Med Surg*, 6(2), 63-67.

Al-Ajeeli, K.S. 1993. Studies on Malaysian isolates of orf virus, *PhD Thesis, Universiti Putra Malaysia*.

An, S., Chen, C.J., Yu, X., Leibowitz, J.L. and Makino, S. (1999). Induction of apoptosis in murine coronavirus-infected cultured cells and demonstration of E protein as an apoptosis inducer. *J Virol*, 73(9), 7853.

Arshad, S.S. 1993. A study on two Malaysian isolates of infectious bronchitis virus, *MSc Thesis, Universiti Putra Malaysia*.

Arshad, S.S., Lee W.W., Hassan L., Kamarudin A I M., Siti-Farawahida A W. and Cheng N.A.B.Y. (2004). Serological survey of catteries for cats infected with feline coronavirus. *J Vet Malaysia*, 17, 19-22.

- Ashley, C. and Caul, E. (1982). Potassium tartrate-glycerol as a density gradient substrate for separation of small, round viruses from human feces. *J Clin Microbiol*, 16(2), 377.
- August, J.R. (1984). Feline infectious peritonitis. An immune-mediated coronaviral vasculitis. *Vet Clin North Am Small Anim Practice*, 14(5), 971-984.
- Ballesteros, M., Sanchez, C. and Enjuanes, L. (1997). Two amino acid changes at the N-terminus of transmissible gastroenteritis coronavirus spike protein result in the loss of enteric tropism. *Virology*, 227(2), 378-388.
- Barlough, J.E., Stoddart, C.A., Sorresso, G. P., Jacobson, R.H. and Scott, F.W. (1984). Experimental inoculation of cats with canine coronavirus and subsequent challenge with feline infectious peritonitis virus. *Lab Anim Sci*, 34(6), 592-597.
- Barlough, J., Jacobson, R., Scott, F., Frederick, C. and Yanow, E. (1985). Effect of recent vaccination on feline coronavirus antibody test results. *Feline Pract*, 23 (3) 14-20.
- Barlough, J. and Shacklett, B. (1994). Antiviral studies of feline infectious peritonitis virus in vitro. *Vet Rec*, 135(8), 177.
- Barr, S.C. and Bowman, D.D. (2006). *The 5-minute veterinary consult clinical companion: Canine and feline infectious diseases and parasitology* Wiley-Blackwell.
- Beesley, J. and Hitchcock, L. (1982). The ultrastructure of feline infectious peritonitis virus in feline embryonic lung cells. *J Gen Virol*, 59(1), 23.
- Bell, E., Toribio, J., White, J., Malik, R. and Norris, J. (2006). Seroprevalence study of feline coronavirus in owned and feral cats in Sydney, Australia. *Aust Vet J*, 84(3), 74-81.
- Benetka, V., Kubber-Heiss, A., Kolodziejek, J., Nowotny, N., Hofmann-Parisot, M. and Mostl, K. (2004). Prevalence of feline coronavirus types I and II in cats with histopathologically verified feline infectious peritonitis. *Vet Microbiol*, 99(1), 31-42.
- Bergman, R.L. (2006). Miscellaneous spinal diseases. In J. R. August. *Consultations in feline internal Medicine* (pp. 470-479). Missouri: Elsevier Saunders.
- Binn, L.N., Lazar, E.C., Keenan, K.P., Huxsoll, D.L., Marchwicki, R. H. and Strano, A. J. (1974). Recovery and characterization of a coronavirus from military dogs with diarrhea. *Proceedings, Annual Meeting of the United States Animal Health Association*, (78), 359-366.
- Black, J.W. (1980). Recovery and in vitro cultivation of a coronavirus from laboratory-induced cases of feline infectious peritonitis (FIP). *Vet Med Small Anim Clin*, 75: 811-814.

Boettcher, I.C., Steinberg, T., Matiasek, K., Greene, C.E., Hartmann, K. and Fischer, A. (2007). Use of anti-coronavirus antibody testing of cerebrospinal fluid for diagnosis of feline infectious peritonitis involving the central nervous system in cats. *J Am Vet Med Assoc*, 230(2), 199-205.

Bos, E., Luytjes, W. and Spaan, W. (1997). The function of the spike protein of mouse hepatitis virus strain A59 can be studied on virus-like particles: Cleavage is not required for infectivity. *J Virol*, 71(12), 9427.

Bournsnel, M., Brown, T., Foulds, I., Green, P., Tomley, F. and Binns, M. (1987). Completion of the sequence of the genome of the coronavirus avian infectious bronchitis virus. *J Gen Virol*, 68(1), 57.

Boyle, J.F., Pedersen, N.C., Evermann, J.F., McKeirnan, A.J., Ott, R.L. and Black, J.W. (1984). Plaque assay, polypeptide composition and immunochemistry of feline infectious peritonitis virus and feline enteric coronavirus isolates. *Adv Exp Med Biol*, 173, 133-147.

Breuer, W., Stahr, K., Majzoub, M. and Hermanns, W. (1998). Bone-marrow changes in infectious diseases and lymphohaemopoietic neoplasias in dogs and cats a retrospective study. *J Comp Pathol*, 119(1), 57-66.

Brockway, S.M., Clay, C.T., Lu, X.T. and Denison, M.R. (2003). Characterization of the expression, intracellular localization, and replication complex association of the putative mouse hepatitis virus RNA-dependent RNA polymerase. *J Virol*, 77(19), 10515.

Brown, M.A., Troyer, J.L., Pecon-Slattey, J., Roelke, M.E. and O'Brien, S.J. (2009). Genetics and pathogenesis of feline infectious peritonitis virus. *Emerging Infec Dis*, 15, 1445-1452.

Buddle, B.M., Dellers, R.W. and Schurig, G.G., (1984). Heterogeneity of contagious ecthyma virus isolates. *Am J Vet Res*, 45, 75-79.

Can-Sahna, K., Soydal Ataseven, V., Pinar, D. and Eigdem Oguzoglu, T. (2007). The detection of feline coronaviruses in blood samples from cats by mRNA RT-PCR. *J Feline Med Surg*, 9(5), 369-372.

Carstens, E.B., Estes, M.K., Lemon, S M., Maniloff, J., Mayo, M.A., McGeoch, D.J. (2000). Coronaviridae. In *Virus taxonomy*, ed, van Regenmortel, M. H. V., Fauquet, C. M and Bodmer, J. L, p.835-849. Academic Press, San Diego, California.

Caul, E. and Egglestone, S. (1977). Further studies on human enteric coronaviruses. *Arch Virol*, 54(1), 107-117.

Caul, E., Ashley, C. and Egglestone, S. (1978). An improved method for the routine identification of faecal viruses using ammonium sulphate precipitation. *FEMS Microbiol Lett*, 4(1), 1-4.

Cavanagh, D. (1995). The coronavirus surface glycoprotein. In ‘the Coronaviridae’ (S. G. Siddell, Ed.), pp. 73–113. Plenum, New York.

Cavanagh, D. (1997). Nidovirales: A new order comprising coronaviridae and arteriviridae. *Arch Virol*, 142(3), 629-633.

Cavanagh, D. (2005). Coronaviridae: A review of coronaviruses and toroviruses. In M.W.A. Schmidt. *Coronaviruses with Special Emphasis on First Insights Concerning SARS* (1-54). Basel: Birkhauser.

Cave, T., Thompson, H., Reid, S., Hodgson, D. and Addie, D. (2002). Kitten mortality in the United Kingdom: A retrospective analysis of 274 histopathological examinations (1986 to 2000). *Vet Rec*, 151(17), 497.

Chang, H.W., DeGroot, R.J., Egberink, H.F. and Rottier, P.J. (2010). Feline infectious peritonitis: insight into feline coronavirus pathogenesis and epidemiology based on genetic analysis of viral 3c gene. *J Gen V*, 91, 414-420.

ChaoNan, L., BiLing, S., ChengWei, W. and LingLing, C. (2009). Isolation and identification of a novel feline coronavirus from a kitten with naturally occurring feline infectious peritonitis in taiwan. *Taiwan Vet J*, 35(3), 145-152.

Cheley, S., Morris, V. L., Cupples, M. J. and Anderson, R. (1981). RNA and polypeptide homology among murine coronaviruses. *Virology*, 115(2), 310-321.

Christianson, K.K., Ingersoll, J.C., Landon, R.M., Pfeiffer, N.E. and Gerber, J.D. (1989). Characterization of temperature sensitive feline infectious peritonitis coronavirus. *Arch Virol*. 109, 185-196

Collins, A.R., Knobler, R.L., Powell, H. and Buchmeier, M.J. (1982). Monoclonal antibodies to murine hepatitis virus-4 (strain JHM) define the viral glycoprotein responsible for attachment and cell-cell fusion. *Virol*, 119(2), 358-371.

Coons, A.H., Creech, H., Jones, R. and Berliner, E. (1942). The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. *J Immunol*, 45(7), 159-170.

Corapi, W.V., Olsen, C. and Scott, F.W. (1992). Monoclonal antibody analysis of neutralization and antibody-dependent enhancement of feline infectious peritonitis virus. *J Viro*, 66(11), 6695-6705.

Corapi, W.V., Darteil, R.J., Audonnet, J.C. and Chappuis, G.E. (1995). Localization of antigenic sites of the S glycoprotein of feline infectious peritonitis virus involved in neutralization and antibody-dependent enhancement. *J Virol*, 69(5), 2858-2862.

Crandell, R.A., Fabricant, C.G. and Nelson-Rees, W.A. (1973). Development, characterization, and viral susceptibility of a feline (*Felis catus*) renal cell line (CRFK). *In Vitro Cellular and Developmental Biology-Plant*, 9(3), 176-185.

Cunningham, C.H. (1970). Avian infectious bronchitis. *Adv Vet Sci Comp Med*, 14, 105-148.

David-Ferreira, J. and Manaker, R. (1965). An electron microscope study of the development of a mouse hepatitis virus in tissue culture cells. *J Cell Bio*, 24(1), 57-78.

Davies, H.A. and Macnaughton, M. (1979). Comparison of the morphology of three coronaviruses. *Arch Viro*, 59(1), 25-33.

Dea, S., Roy, R. and Elazhary, M. (1982). Coronavirus-like particles in the feces of a cat with diarrhea. *Can Vet J*, 23(5), 153.

Dean, G.A., Olivry, T., Stanton, C. and Pedersen, N.C. (2003). In vivo cytokine response to experimental feline infectious peritonitis virus infection. *Vet Microbiol*, 97 (1-2), 1-12.

de Groot, R., Luytjes, W., Horzinek, M., Van der Zeijst, B., Spaan, W. and Lenstra, J. (1987). Evidence for a coiled-coil structure in the spike proteins of coronaviruses. *J Mol Biol*, 196(4), 963-966.

de Groot, R.J., Van Leen, R.W., Dalderup, M.J.M., Vennema, H., Horzinek, M.C. and Spaan, W.J.M. (1989). Stably expressed FIPV peplomer protein induces cell fusion and elicits neutralizing antibodies in mice. *Virology*, 171(2), 493-502.

de Haan, C. A. M. and Reggiori, F. (2008). Are Nidovirales hijacking the autophagy machinery. *Autophagy*, 4(3), 276-282.

Delmas, B., Gelfi, J. and L'Haridon, R. (1992). Aminopeptidase N is a major receptor for the enteropathogenic coronavirus TGEV. *Nature*, 357, 417-420.

Denison, M.R., Yount, B., Brockway, S.M., Graham, R.L., Sims, A.C., Lu, X.T. and Ralph, S.B. (2004). Cleavage between replicase proteins p28 and p65 of mouse hepatitis virus is not required for virus replication. *J Virol*, 78(11), 5957.

de Vries, A.A. F., Horzinek, M.C., Rottier, P.J.M. and de Groot, R.J. (1997). The genome organisation of the Nidovirales: Similarities and differences among arteri-, toro- and coronaviruses. *Semin Virology*, 8(1), 33-48.

Diaz, J.V. and Poma, R. (2009). Diagnosis and clinical signs of feline infectious peritonitis in the central nervous system. *Can Vet J*, 50(10), 1091-1093.

Duarte, A., Veiga, I. and Tavares, L. (2009). Genetic diversity and phylogenetic analysis of feline coronavirus sequences from Portugal. *Vet Microbio*, 138(1-2), 163-168.

Dye, C. and Siddell, S.G. (2005). Genomic RNA sequence of feline coronavirus strain FIPV WSU-79/1146. *J Gen Virol*, 86(8), 2249.

Dye, C., Temperton, N. and Siddell, S.G. (2007). Type I feline coronavirus spike glycoprotein fails to recognize aminopeptidase N as a functional receptor on feline cell lines. *J Gen Virol*, 88(6), 1753.

Dye, C., Helps, C.R. and Siddell, S.G. (2008). Evaluation of real-time RT-PCR for the quantification of FCoV shedding in the feces of domestic cats. *J Feline Med Surg*, 10(2), 167-174.

Eleouet, J.F., Rasschaert, D., Lambert, P., Levy, L., Vende, P. and Laude, H. (1995). Complete sequence (20 kilobases) of the polyprotein-encoding gene 1 of transmissible gastroenteritis virus. *Virology*, 206(2), 817-822.

East, M.L., Moestl, K., Benetka, V., Pitra, C., Honer, O.P. and Wachter, B. (2004). Coronavirus infection of spotted hyenas in the serengeti ecosystem. *Veterinary Microbiology*, 102(1-2), 1-9.

Evermann, J., Baumgartener, L., Ott, R., Davis, E. and McKeirnan, A. (1981). Characterization of a feline infectious peritonitis virus isolate. *Vet Pathol*, 18(2), 256-265.

Evermann, J., Burns, G., Roelke, M., McKeirnan, A. and Greenlee, A. (1983). Diagnostic features of an epizootic of feline infectious peritonitis in captive cheetahs [virus]. *Proceedings of Annual Meeting-American Association of Veterinary Laboratory Diagnosticians (USA)*.

Evermann, J.F., Heeney, J.L., McKeirnan, A.J. and O'Brien, S.J. (1989). Comparative features of a coronavirus isolated from a cheetah with feline infectious peritonitis. *Virus Res*, 13(1), 15-27.

Evermann, J.F., McKeirnan, A.J. and Ott, R.L. (1991). Perspectives on the epizootiology of feline enteric coronavirus and pathogenesis of feline infectious peritonitis. *Vet Microbiol*, 28: 243-255.

Fehr, D., Holznagel, E., Bolla, S., Hauser, B., Herrewegh, A.A. P.M., Horzinek, M.C. and Hans, L. (1997). Placebo-controlled evaluation of a modified life virus vaccine

against feline infectious peritonitis: Safety and efficacy under field conditions. *Vaccine*, 15(10), 1101-1109.

Fehr, D., Holznagel, E., Bolla, S., Hauser, B., Herrewegh, A., Horzinek, M. and Lutz, H. (1995). Evaluation of the safety and efficacy of a modified live FIPV vaccine under field conditions. *Feline Pract*, 23(3), 83-88.

Fischer, F., Stegen, C.F., Masters, P.S. and Samsonoff, W.A. (1998). Analysis of constructed E gene mutants of mouse hepatitis virus confirms a pivotal role for E protein in coronavirus assembly. *J Virol*, 72(10), 7885.

Fiscus, S.A. and Teramoto, Y.A. (1987a). Antigenic comparison of feline coronavirus isolates: Evidence for markedly different peplomer glycoproteins. *J Virol*, 61(8), 2607-2613.

Fiscus, S.A. and Teramoto, Y.A. (1987b). Functional differences in the peplomer glycoproteins of feline coronavirus isolates. *J Virol*, 61(8), 2655.

Flintoff, W.F. (1984). Replication of murine coronavirus in somatic cell hybrids formed between a mouse fibroblast cell line and either a rat schwannoma line or a rat glioma line. *Adv Exp Med Biol*, 173, 301-313.

Foley, J.E., Poland, A., Carlson, J. and Pedersen, N.C. (1997a). Patterns of feline coronavirus infection and fecal shedding from cats in multiple-cat environments. *J Am Vet Med Assoc*, 210(9), 1307-1312.

Foley, J.E., Poland, A., Carlson, J. and Pedersen, N.C. (1997b). Risk factors for feline infectious peritonitis among cats in multiple-cat environments with endemic feline enteric coronavirus. *J Am Vet Med Assoc*, 210(9), 1313-1318.

Foley, J.E., Lapointe, J.M., Koblik, P., Poland, A. and Pedersen, N.C. (1998). Diagnostic features of clinical neurologic feline infectious peritonitis. *J Intern Med* 12(6), 415-423.

Foley, J., Rand, C. and Leutenegger, C. (2003). Inflammation and changes in cytokine levels in neurological feline infectious peritonitis. *J Feline Med Surg*, 5(6), 313-322.

Frana, M., Behnke, J., Sturman, L. and Holmes, K. (1985). Proteolytic cleavage of the E2 glycoprotein of murine coronavirus: Host-dependent differences in proteolytic cleavage and cell fusion. *J Virol*, 56(3), 912

Freshney R.I. (2005). *Culture of animal cells: a manual of basic technique*. 5th Ed. New York: Wiley-Liss.

Froshauer, S., Kartenbeck, J. and Helenius, A. (1988). Alphavirus RNA replicase is located on the cytoplasmic surface of endosomes and lysosomes. *J Cell Biol*, 107(6), 2075.

Gallo, D. (1983). Uses of immunofluorescence in diagnostic virology. *Am J Med Technol*, 49(3), 157.

Gamble, D.A., Lobbiani, A., Gramegna, M., Moore, L.E. and Colucci, G. (1997). Development of a nested PCR assay for detection of feline infectious peritonitis virus in clinical specimens. *J Clin Microbiol*, 35(3), 673.

Garwes, D.J., Bountiff, L., Millson, G.C. and Elleman, C.J. (1984). Defective replication of porcine transmissible gastroenteritis virus in a continuous cell line. *Adv Exp Med Biol*, 173, 79-93.

Gentile, M. and Gelderblom, H.R. (2005). Rapid viral diagnosis: Role of electron microscopy. *Microbiol*, 28(1), 1-12.

Giori, L., Giordano, A., Giudice, C., Grieco, V. and Paltrinieri, S. (2011). Performances of different diagnostic tests for feline infectious peritonitis in challenging clinical cases. *J Small Anim Pract*, 52(3), 152-157.

Glansbeek, H.L., Haagmans, B.L., Te Lintelo, R.G. (2002). Adverse effects of feline IL-12 during DNA vaccination against feline infectious peritonitis virus. *J Gen Virol*, 83, 1-10.

Godet, M., L'Haridon, R., Vautherot, J.F. and Laude, H. (1992). TGEV corona virus ORF4 encodes a membrane protein that is incorporated into virions. *Virology*, 188(2), 666-675.

Goitsuka, R., Tsuji, M., Ohashi, T., Onda, C., Tuchiya, K., Hirota, Y., Takahashi, E. and Hasegawa, A. (1991). Characterization of feline infectious peritonitis virus isolate. *J Vet. Med Sci*, 53(2), 337-339.

Goldsmith, C.S., Tatti, K.M., Ksiazek, T.G., Rollin, P.E., Comer, J.A. and Lee, W.W. (2004). Ultrastructural characterization of SARS coronavirus. *Emerging Infect Dis*, 10(2), 320-326.

Gombold, J.L., Hingley, S.T. and Weiss, S.R. (1993). Fusion-defective mutants of mouse hepatitis virus A59 contain a mutation in the spike protein cleavage signal. *J Virol*, 67(8), 4504.

Gonzalez, J.M., Gomez-Puertas, P., Cavanagh, D., Gorbalenya, A.E. and Enjuanes, L. (2003). A comparative sequence analysis to revise the current taxonomy of the family Coronaviridae. *Arch Virol*, 148:2207-2235.

- Goodson, T.L., Randell, S.C. and Moor, L. (2009). Feline infectious peritonitis. *Compendium*, 31(10), 1-9.
- Gorbalenya, A.E., Snijder, E.J. and Spaan, W.J.M. (2004). Severe acute respiratory syndrome coronavirus phylogeny: Toward consensus. *J Virol*, 78(15), 7863.
- Gorbalenya, A.E., Enjuanes, L., Ziebuhr, J. and Snijder, E.J. (2006). Nidovirales: Evolving the largest RNA virus genome. *Virus Res*, 117(1), 17-37.
- Gosert, R., Kanjanahaluethai, A., Egger, D., Bienz, K. and Baker, S.C. (2002). RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. *J Virol*, 76(8), 3697.
- Guy, J.S., Breslin, J.J., Breuhaus, B., Vivrette, S. and Smith, L.G. (2000). Characterization of a coronavirus isolated from a diarrheic foal. *J Clin Microbiol*, 38(12), 4523.
- Harcourt, B.H., Jukneliene, D., Kanjanahaluethai, A., Bechill, J., Severson, K.M. and Smith, C.M. (2004). Identification of severe acute respiratory syndrome coronavirus replicase products and characterization of papain-like protease activity. *J Virol*, 78(24), 13600.
- Hardy, W.D. and Hurvitz, A.I. (1971). Feline infectious peritonitis: Experimental studies. *J Am Vet Med Assoc*, 158(6), 944-1002.
- Harkness, J.W. and Bracewell, C.D. (1974). Morphological variation among avian Infectious bronchitis virus strains. *Res Vet Sci*, 16(1), 128-131.
- Hartmann, K., Binder, C., Hirschberger, J., Cole, D., Reinacher, M. and Schroo, S. (2003). Comparison of different tests to diagnose feline infectious peritonitis. *J Vet Intern Med*, 17(6), 781-790.
- Hartmann, K. (2005). Feline infectious peritonitis. *Vet Clin North Am Small Anim Practice*, 35(1), 39-79.
- Hartmann, K. and Ritz, S. (2008). Treatment of cats with feline infectious peritonitis. *Vet Immunol Immunopathol*, 123(1-2), 172-175.
- Hayat, M. (1986). Basic techniques for transmission electron microscopy. *Orlando, etc.: Academic Press 411pp. Review by A.Glauert in J Microsc*, 143, 323-324.
- Hayashi, T., Goto, N., Takahashi, R. and Fujiwara, K. (1977). Systemic vascular lesions in feline infectious peritonitis. *Jpn J Vet Sci*, 39, 365-377.

Hayashi, T., Takahashi, R. and Fujiwara, K. (1978). Detection of coronavirus-like particles in a spontaneous case of feline infectious peritonitis. *J Vet Sci*, 40, 207-212.

Hayashi, T., Utsumi, F., Takahashi, R. and Fujiwara, K. (1980). Pathology of non-effusive type feline infectious peritonitis and experimental transmission. *Jpn J Vet Sci*, 42, 197-210.

Hayashi, T., Yanai, T., Tsurudome, M., Nakayama, H., Watabe, Y. and Fujiwara, K. (1981). Serodiagnosis for feline infectious peritonitis by immunofluorescence using infected suckling mouse brain sections. *Jpn J Vet Sci*, 43, 669-676.

Hayashi, T., Watabe, Y., Takenouchi, T. and Fujiwara, K. (1983). Role of circulating antibodies in feline infectious peritonitis after oral infection. *J Vet Sci*, 45, 487-494.

Hazelton, P.R. and Gelderblom, H.R. (2003). Electron microscopy for rapid diagnosis of infectious agents in emergent situations. *Emerging Infect Dis*, 9(3), 294-303.

Hegyí, A., Friebe, A., Gorbalenya, A.E. and Ziebuhr, J. (2002). Mutational analysis of the active centre of coronavirus 3C-like proteases. *J Gen Virol*, 83(3), 581.

Herrewegh, A.A.P.M., Vennema, H., Horzinek, M.C., Rottier, P.J.M. and de Groot, R.J. (1995a). The molecular genetics of feline coronaviruses: Comparative sequence analysis of the ORF7a/7b transcription unit of different biotypes. *Virol*, 212(2), 622-631.

Herrewegh, A., De Groot, R., Cepica, A., Egberink, H.F., Horzinek, M.C. and Rottier, P. (1995b). Detection of feline coronavirus RNA in feces, tissues, and body fluids of naturally infected cats by reverse transcriptase PCR. *J Clin Microbio*, 33(3), 684.

Herrewegh, A., Egberink, H., Horzinek, M., Rottier, P. and Groot, R. (1995c). Polymerase chain reaction (PCR) for the diagnosis of naturally occurring feline coronavirus infections. *Feline Pract*, 23(3), 56-60.

Herrewegh, A., Mahler, M., Hedrich, H., Haagmans, B., Egberink, H., Horzinek, M., Rottier, P.J.M. and de Groot, R.J. (1997). Persistence and evolution of feline coronavirus in a closed cat-breeding colony. *Virol*, 234(2), 349-363.

Herrewegh, A.A.P.M., Smeenk, I., Horzinek, M.C., Rottier, P.J.M. and de Groot, R.J. (1998). Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. *J Virol*, 72(5), 4508.

Hickman, M., Morris, J., Rogers, Q. and Pedersen, N. (1995). Elimination of feline coronavirus infection from a large experimental specific pathogen-free cat breeding colony by serologic testing and isolation. *Feline Pract*, 23(3), 23-28.

Hirschberger, J., Hartmann, K., Wilhelm, N., Frost, J., Lutz, H. and Kraft, W. (1995). Clinical symptoms and diagnosis of feline infectious peritonitis. *Tierarztl Praxis*, 23(1), 92-99.

Hohdatsu, T., Okada, S. and Koyama, H. (1991a). Characterization of monoclonal antibodies against feline infectious peritonitis virus type II and antigenic relationship between feline, porcine, and canine coronaviruses. *Arch Virol*, 117(1), 85-95.

Hohdatsu, T., Nakamura, M., Ishizuka, Y., Yamada, H. and Koyama, H. (1991b). A study on the mechanism of antibody-dependent enhancement of feline infectious peritonitis virus infection in feline macrophages by monoclonal antibodies. *Arch Virol*, 120(3), 207-217.

Hohdatsu, T., Sasamoto, T., Okada, S. and Koyama, H. (1991c). Antigenic analysis of feline coronaviruses with monoclonal antibodies (MAbs): Preparation of MAbs which discriminate between FIPV strain 79-1146 and FECV strain 79-1683. *Vet Microbiol*, 28(1), 13-24.

Hohdatsu, T., Okada, S., Ishizuka, Y., Yamada, H. and Koyama, H. (1992). The prevalence of types I and II feline coronavirus infections in cats. *J Vet Med Sci*, 54(3), 557-562.

Hohdatsu, T., Tatekawa, T. and Koyama, H. (1995). Enhancement of feline infectious peritonitis virus type I infection in cell cultures using low-speed centrifugation. *J Virol Methods*, 51(2-3), 357-362.

Hohdatsu, T., Izumiya, Y., Yokoyama, Y., Kida, K. and Koyama, H. (1998). Differences in virus receptor for type I and type II feline infectious peritonitis virus. *Arch Virol*, 143(5), 839-850.

Holmes, K. (1985). Replication of coronaviruses. *Fields Virology, 1st Ed.* BN Fields, M.Knipe, RM Chanock, JL Melnick, B.Roizman, and RE Shope, Eds. Raven Press, New York, 1331-1344.

Holst, B.S., Englund, L., Palacios, S., Renström, L. and Berndtsson, L.T. (2006). Prevalence of antibodies against feline coronavirus and *Chlamydia felis* in Swedish cats. *J Feline Med Sur*, 8(3), 207-211.

Holzworth, J. (1963). Some important disorders of cats. *Cornell Vet*, 53, 157-160.

Homburger, F., Smith, A., and Barthold, S. (1991). Detection of rodent coronaviruses in tissues and cell cultures by using polymerase chain reaction. *J Clin Microbiol*, 29(12), 2789.

Horsburgh, B.C., and Brierley, I. and Brown, T.D.K. (1992). Analysis of a 9.6 kb sequence from the 3' end of canine coronavirus genomic RNA. *J Gen Virol*, 73(11), 2849.

Horsburgh, B.C. and Brown, T.D. (1995). Cloning, sequencing and expression of the S protein gene from two geographically distinct strains of canine coronavirus. *Virus Res.* 39, 63–74.

Horwitz, M. and Scharff, M. (1969). The production of antiserum against viral antigens. *Fundamental Techniques in Virology. Academic Press Inc. New York*, 253-262.

Horzinek, M. and Osterhaus, A. (1979). The virology and pathogenesis of feline infectious peritonitis. *Arch Virol*, 59(1), 1-15.

Horzinek, M.C., Lutz, H. and Pedersen, N.C (1982). Antigenic relationships among homologous structural polypeptides of porcine, feline, and canine coronaviruses. *Infect Immun*, 37(3), 1148.

Horzinek, M.C., Ederveen, J. and Egberink, H. (1986). Virion polypeptide specificity of immune complexes and antibodies in cats inoculated with feline infectious peritonitis virus. *Am J Vet Res*, 47, 754-761.

Horzinek, M., Vennema, H., de Groot, R., Harbour, D., Dalderup, M., Gruffydd-Jones, T., Horzinek, M.C. and Spaan, W.J.M. (1990). Immunogenicity of recombinant feline infectious peritonitis virus spike protein in mice and kittens. *Adv Exp Med Biol*, 276, 217-222.

Horsburgh, B.C., Brierley, I. and Brown, T.D.K. (1992). Analysis of a 9.6 kb sequence from the 3'end of canine coronavirus genomic RNA. *J Gen Virol*, 73(11), 2849.

Hoshino, Y. and Scott, F.W. (1978). Replication of feline infectious peritonitis virus in organ cultures of feline tissue. *Cornell Vet*, 68: 411-417.

Hoshino, Y. and Scott, F. (1980a). Coronavirus-like particles in the feces of normal cats. *Arch Virol*, 63(2), 147-152.

Hoshino, Y. and Scott, F.W. (1980b). Immunofluorescent and electron microscopic studies of feline small intestinal organ cultures infected with feline infectious peritonitis virus. *Am J Vet Res*, 41(5), 672-681.

Hsieh, L.E., Lin, C.N., Su, B.L., Jan, T.R., Chen, C.M., Wang, C.H., Lin, D., Lin, C.T. and Chueh, L.L. (2010). Synergistic antiviral effect of galanthus nivalis agglutinin and nelfinavir against feline coronavirus. *Antiviral Res*, 88, 25-30.

Hsiung, G.D (1973). Diagnostic Virology- An illustrated Handbook, *New Haven and London, Yale University Press*.

Hsiung, G.D (1994). Hsiung's Diagnostic Virology: AS Illustrated by Light and Electron Microscopy, (4th edition). *New Haven and London: Yale University Press*.

Ibrahim, A.L. and Lai, C.M. (1981). Laboratory diagnosis of viral diseases of domestic animals at Unversiti Partanian Malaysia by TEM. *Pertanika*, 4: 137-140.

Ishida, T., Shibana, A., Tanaka, S., Uchida, K. and Mochizuki, M. (2004). Use of recombinant feline interferon and glucocorticoid in the treatment of feline infectious peritonitis. *J Feline Med Surg*, 6(2), 107-109.

Jacobse-Geels, H. and Horzinek, M. (1983). Expression of feline infectious peritonitis coronavirus antigens on the surface of feline macrophage-like cells. *J Gen Virol*, 64(9), 1859.

Jacobs, L., de Groot, R., van der Zeijst, B.A.M., Horzinek, M.C. and Spaan, W. (1987). The nucleotide sequence of the peplomer gene of porcine transmissible gastroenteritis virus (TGEV): Comparison with the sequence of the peplomer protein of feline infectious peritonitis virus (FIPV). *Virus Res*, 8(4), 363-371.

Kass, P. and Dent, T. (1995). The epidemiology of feline infectious peritonitis in catteries. *Feline Pract*, 23(3), 27-33.

Kazushige, K., Kaneda, Y., Goto, N. and Kanoe, M. (1987). Isolation and characterization of a new and high titer strain of feline infectious peritonitis virus. *J Vet Med Sci*, 49(6), 1105-1111.

Kennedy, M., Boedeker, N., Gibbs, P. and Kania, S. (2001). Deletions in the 7a ORF of feline coronavirus associated with an epidemic of feline infectious peritonitis. *Vet Microbiol*, 81(3), 227-234.

Kennedy, M.A., Abd-Eldaim, M., Zika, S.E., Mankin, J.M. and Kania, S.A. (2008). Evaluation of antibodies against feline coronavirus 7b protein for diagnosis of feline infectious peritonitis in cats. *Am J Vet Res*, 69(9), 1179-1182.

Keyaerts, E., Vijgen, L., Pannecouque, C., Van Damme, E., Peumans, W. and Egberink, H. (2007). Plant lectins are potent inhibitors of coronaviruses by interfering with two targets in the viral replication cycle. *Antiviral Res*, 75(3), 179-187.

Kipar, A., Kremendahl, J., Addie, D., Leukert, W., Grant, C. and Reinacher, M. (1998). Fatal enteritis associated with coronavirus infection in cats. *J Comp Pathol*, 119(1), 1-14.

Kipar, A., Meli, M.L., Failing, K., Euler, T., Gomes-Keller, M.A., Schwartz, D., Lutz, H. and Reinacher, M. (2006). Natural feline coronavirus infection: Differences in cytokine patterns in association with the outcome of infection. *Vet Immunol Immunopathol*, 112(3-4), 141-155.

Kirkegaard, K., Taylor, M.P. and Jackson, W.T. (2004). Cellular autophagy: Surrender, avoidance and subversion by microorganisms. *Nature Rev Microbio*, 2(4), 301-314.

Kiss, I., Kecskeméti, S., Tanyi, J., Klingeborn, B. and Belak, S. (2000). Preliminary studies on feline coronavirus distribution in naturally and experimentally infected cats. *Res Vet Sci*, 68(3), 237-242.

Klumperman, J., Locker, J.K., Meijer, A., Horzinek, M.C., Geuze, H.J. and Rottier, P. (1994). Coronavirus M proteins accumulate in the Golgi complex beyond the site of virion budding. *J Virol*, 68(10), 6523.

Kubo, H., Yamada, Y.K. and Taguchi, F. (1994). Localization of neutralizing epitopes and the receptor-binding site within the amino-terminal 330 amino acids of the murine coronavirus spike protein. *J Virol*, 68(9), 5403.

Kummrow, M., Meli, M.L., Haessig, M., Goenczi, E., Poland, A., Pedersen, N.C., Hofmann-Lehmann, R. and Lutz, H. (2005). Feline coronavirus serotypes 1 and 2: Seroprevalence and association with disease in Switzerland. *Clin Diagn Lab Immunol*, 12(10), 1209-1215.

Lai, M. and Cavanagh, D. (1997). The molecular biology of coronaviruses. *Adv Virus Res*, 48, 1-100.

Lai, M. (1998). Cellular factors in the transcription and replication of viral RNA genomes: A parallel to DNA-dependent RNA transcription 1. *Virol*, 244(1), 1-12.

Lai, M.M.C., Perlman, S. and Anderson, L.J. (2007). Coronaviridae. In: Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E. (Eds.), *Fields Virology. Lippincott Williams and Wilkins, Philadelphia, PA*, pp. 1305–1335.

Laude, H., Chapsal, J.M., Gelfi, J., Labiau, S. and Grosclaude, J. (1986). Antigenic structure of transmissible gastroenteritis virus. I. properties of monoclonal antibodies directed against virion proteins. *J Gen Virol*, 67(1), 119.

Leung, W.K., To, K., Chan, P.K.S., Chan, H.L.Y., Wu, A.K.L., Lee, N., Yuen, K.W. and Sunq, J.J.Y. (2003). Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection1. *Gastroenterology*, 125(4), 1011-1017.

Li, X. and Scott, F.W. (1994). Detection of feline coronaviruses in cell cultures and in fresh and fixed feline tissues using polymerase chain reaction. *Vet Microbio*, 42(1), 65-77.

Lin, C.N., Su, B.L., Wang, C.H., Hsieh, M.W., Chueh, T.J. and Chueh, L.L. (2009). Genetic diversity and correlation with feline infectious peritonitis of feline coronavirus type I and II: A 5-year study in Taiwan. *Vet Microbiol*, 136(3-4), 233-239.

Loa, C., Lin, T., Wu, C., Bryan, T., Thacker, H., Hooper, T. and Schrader, D. (2002). Purification of turkey coronavirus by sephacryl size-exclusion chromatography. *J Virol Methods*, 104(2), 187-194.

Lomniczi, B. (1977). Biological properties of avian coronavirus RNA. *J Gen Virol*, 36(3), 531.

Look, A.T., Ashmun, R.A., Shapiro, L.H. and Peiper, S.C. (1989). Human myeloid plasma membrane glycoprotein CD13 (gp150) is identical to aminopeptidase N. *J Clin Invest*, 83(4), 1299.

Low, R.J., Colby, E.D., Covington, H.J. and Benirschke, K. (1971). Report on the isolation of an agent from cell cultures of kidneys from domestic cats. *Vet Rec*, 88(21), 557-559.

Luby, J., Clinton, R. and Kurtz, S. (1999). Adaptation of human enteric coronavirus to growth in cell lines. *J Clin Virol*, 12(1), 43-51.

Macnaughton, M., Davies, H.A. and Nermut, M. (1978). Ribonucleoprotein-like structures from coronavirus particles. *J Gen Virol*, 39(3), 545.

Marioni - Henry, K., Vite, C.H., Newton, A.I.L. and Winkle, T.J. (2004). Prevalence of diseases of the spinal cord of cats. *J Vet Intern Med*, 18(6), 851-858.

Masters, P.S. and Sturman, L.S. (1990). Background paper. Functions of the coronavirus nucleocapsid protein. *Adv Exp Med Biol*, 276, 235-238.

Masters, P. S. (2006). The molecular biology of coronaviruses. *Adv Virus Res*, 66, 193-292.

McArdle, F., Bennett, M., Gaskell, R.M., Tennant, B., Kelly, D.F. and Gaskell, C. J. (1992). Induction and enhancement of feline infectious peritonitis by canine coronavirus. *Am J Vet Res*, 53(9), 1500-1506.

McDonagh, P., Sheehy, P.A. and Norris, J.M. (2011). In vitro inhibition of feline coronavirus replication by small interfering RNAs. *Vet Microbiol*, 150, 220-229.

McIntosh, K. (1974). Coronaviruses: A comparative review. *Curt. Top. Microbiol. Immunol*, 63, 85-129.

McIntosh, K., Peiris, J., Richman, D., Whitley, R. and Hayden, F. (2009). Coronaviruses. *Clin Virol*, (Ed. 3), 1155-1171.

McKeirnan, A., Evermann, J., Hargis, A., Miller, L. and Ott, R. (1981). Isolation of feline coronaviruses from two cats with diverse disease manifestations. *Feline Pract*, 11, 16-20.

McKeirnan, A., Evermann, J., Davis, E. and Ott, R. (1987). Comparative properties of feline coronaviruses in vitro. *Can J Vet Res*, 51(2), 212.

Mitchell, D.A.J., Lerch, T.F., Hare, J.T. and Chapman, M.S. (2010). A pseudo-plaque method for infectious particle assay and clonal isolation of adeno-associated virus. *J Virol Methods*, 170, 9-15.

Mochizuki, M., Mitsutake, Y., Miyanohara, Y., Higashihara, T., Shimizu, T. and Hohdatsu, T. (1997). Antigenic and plaque variations of serotype II feline infectious peritonitis coronaviruses. *J Vet Med Sci*, 59(4), 253-258.

Mohandas, D.V. and Dales, S. (1991). Endosomal association of a protein phosphatase with high dephosphorylating activity against a coronavirus nucleocapsid protein. *FEBS Lett*, 282 (2), 419-424.

Montali, R.J. and Strandberg, J.D (1972). Extraperitoneal lesions in feline infectious peritonitis. *Vet Pathol*. 9: 109-121.

Morgan, R.V. (2008). *Handbook of Small Animal Practice* (PP.19-1095). Missouri: Saunders Elsevier.

Motokawa, K., Hohdatsu, T., Aizawa, C., Koyama, H. and Hashimoto, H. (1995). Molecular cloning and sequence determination of the peplomer protein gene of feline infectious peritonitis virus type I. *Arch Virol*, 140(3), 469-480.

Motokawa, K., Hohdatsu, T., Hashimoto, H. and Koyama, H. (1996). Comparison of the amino acid sequence and phylogenetic analysis of the peplomer, integral membrane and nucleocapsid proteins of feline, canine and porcine coronaviruses. *Microbio Immunol*, 40(6), 425-433.

Nakane, P.K. and Pierce, G.B. (1967). Enzyme-labeled antibodies: Preparation and application for the localization of antigens. *J Histochem Cytochem*, 14(12), 929-931.

Naqi, S.A. (1990). A monoclonal antibody-based immunoperoxidase procedure for rapid detection of infectious bronchitis virus in infected tissues. *Avian Dis*, 34(4), 893-898.

Ng, M.L., Tan, S.H., See, E.E., Ooi, E.E., and Ling, A.E. (2003). Proliferative growth of SARS coronavirus in vero E6 cells. *J Gen Virol*, 84(12), 3291.

Olsen, C.W., Corapi, W.V., Jacobson, R.H., Simkins, R.A., Saif, L.J. and Scott, F.W. (1993). Identification of antigenic sites mediating antibody-dependent enhancement of feline infectious peritonitis virus infectivity. *J Gen Virol*, 74(4), 745.

Paltrinieri, S., Parodi, M.C. and Cammarata, G. (1999). In vivo diagnosis of feline infectious peritonitis by comparison of protein content, cytology, and direct immunofluorescence test on peritoneal and pleural effusions. *J Vet Diagn Invest*, 11(4), 358.

Paltrinieri, S., Grieco, V., Comazzi, S. and Cammarata Parodi, M. (2001). Laboratory profiles in cats with different pathological and immunohistochemical findings due to feline infectious peritonitis (FIP). *J Feline Med Surg*, 3(3), 149-159.

Patel, J. and Heldens, J. (2009). Review of companion animal viral diseases and immunoprophylaxis. *Vaccine*, 27(4), 491-504.

Payment, P. and Trudel, M. (1993). Titration of viruses in cell culture by cytopathic effect. In: Methods and techniques in virology. *Payment P and Trudel M (eds). Marcel Dekker inc.* pp 32-35.

Pedersen N.C. (1976a). Morphologic and physical characteristics of feline infectious peritonitis virus and its growth in autochthonous peritoneal cell cultures. *Am Vet Res*, 37, 567-572.

Pedersen, N.C. (1976b). Serologic studies of naturally occurring feline infectious peritonitis. *Am J Vet Res*, 37(12), 1449-1453.

Pedersen, N.C., Ward, J. and Mengeling, W.L. (1978). I. Antigenic relationship of the feline infectious peritonitis virus to coronaviruses of other species. *Arch Viro*, 58:45-53.

Pedersen, N.C. and Boyle, J. (1980). Immunologic phenomena in the effusive form of feline infectious peritonitis. *Am J Vet Res*, 41(6), 868-876.

Pedersen, N.C., Boyle, J.F., Floyd, K., Fudge, A. and Barker, J. (1981a). An enteric coronavirus infection of cats and its relationship to feline infectious peritonitis. *Am J Vet Res*, 42(3), 368-377.

Pedersen, N.C., Boyle, J.F. and Floyd, K. (1981b). Infection studies in kittens, using feline infectious peritonitis virus propagated in cell culture. *Am J Vet Res*, 42(3), 363-367.

Pedersen, N.C. (1983). Feline infectious peritonitis and feline enteric coronavirus infections. *Part I. Feline Pract. Plenum Press*, 1995: 294. 13: 9-13.

Pedersen, N.C., Black, J.W., Boyle, J.F., Evermann, J.F., McKeirnan, A.J. and Ott, R.L. (1984a). Pathogenic differences between various feline coronavirus isolates. *Adv Exp Med Biol*, 173, 365-380.

Pedersen, N.C., Evermann, J.F., McKeirnan, A.J. and Ott, R.L. (1984b). Pathogenicity studies of feline coronavirus isolates 79-1146 and 79-1683. *Am J Vet Res*, 45(12), 2580-2585.

Pedersen, N.C. and Floyd, K. (1985). Experimental studies with three new strains of feline infectious peritonitis virus FIPVUCD2, FIPV-UCD3, and FIPV-UCD4. *Compend Cont Educ Pract Vet*, 7, 1001-1011.

Pedersen, N.C. (1987a). Virologic and immunologic aspects of feline infectious peritonitis virus infection. *Adv Exp Med Biol*, 218, 529-550.

Pedersen, N.C. (1987b). Coronavirus diseases (coronavirus enteritis, feline infectious peritonitis). *Diseases of the Cat. The WB Saunders Co., Philadelphia*, 193-214.

Pedersen, N.C. (1995). An overview of feline enteric coronavirus and infectious peritonitis virus infections. *Feline Pract*, 23(3), 7-20.

Pedersen, N.C, Sato, R., Foley, J. and Poland, A. (2004). Common virus infections in cats, before and after being placed in shelters, with emphasis on feline enteric coronavirus. *J Feline Med Surg*, 6(2), 83-88.

Pedersen, N.C., Allen, C.E. and Lyons, L.A (2008). Pathogenesis of feline enteric coronavirus infection. *J Feline Med Surg*, 10: 529-541.

Pedersen, N.C. (2009). A review of feline infectious peritonitis virus infection: 196-2008. *J Feline Med Surg*, 11, 225-258.

Pedersen, K.W., Van Der Meer, Y., Roos, N. and Snijder, E.J. (1999). Open reading frame 1a-encoded subunits of the arterivirus replicase induce endoplasmic reticulum-derived double-membrane vesicles which carry the viral replication complex. *J Virol*, 73(3), 2016.

Perlman, S. and Netland, J. (2009). Coronaviruses post-SARS: Update on replication and pathogenesis. *Nature Rev Microbiol*, 7(6), 439-450.

Pesteanu-Somogyi, L.D., Radzai, C. and Pressler, B.M. (2006). Prevalence of feline infectious peritonitis in specific cat breeds. *J Feline Med Surg*, 8(1), 1-5.

Petit, C.M. (2005). *Genetics and Functions of the SARS Coronavirus Spike Protein*, PhD Thesis, Louisiana State, University and Agricultural and Mechanical College, USA.

Petric, M. and Szymanski, M. (2000) Electron microscopy and immunoelectron microscopy. *Clinical Virology Manual*, (Ed. 3), Washington, DC: ASM Press, 2000: 54–65.

Poland, A.M., Vennema, H., Foley, J.E. and Pedersen, N.C. (1996). Two related strains of feline infectious peritonitis virus isolated from immunocompromised cats infected with a feline enteric coronavirus. *J Clin Microbiol*, 34(12), 3180.

Posch, A., Posch, U., Kubber-Heiss, A., Seiser, M. and Moestl, K. (1999). Differentiation of feline coronaviruses type I and II strains by RT-PCR. In: *Proceedings of the WSAVA World Veterinary Congress, Lyon*.

Povey, R. and Johnson, R. (1969). A standardized serum neutralization test for feline viral rhinotracheitis: II. The virus-serum system. *J Comp Pathol*, 79(3), 387-392.

Pratelli, A., Martella, V., Decaro, N., Tinelli, A. and Camero, M. (2003). Genetic diversity of a canine coronavirus detected in pups with diarrhoea in Italy. *J Virol Methods*, 110(1), 9-17.

Prentice, E., Jerome, W.G., Yoshimori, T., Mizushima, N. and Denison, M.R. (2004). Coronavirus replication complex formation utilizes components of cellular autophagy. *J Biol Chem*, 279(11), 10136.

Pringle, C. (1999). Virus taxonomy-1999. *Arch virol*, 144,421-429.

Qin-Fen, Z., Jin-Ming, C., Xiao-Jun, H., Wei, L. and Dong-Yan, T. (2003). Morphology and morphogenesis of severe acute respiratory syndrome (SARS)-associated virus. *Acta Biochimica et Biophysica Sinica*, 35, 587-591.

Reed, L.J. and Muench, H (1938). A simple method of estimating fifty per cent endpoints. *Am. J. Hyg*, 27, 493-497.

Risco, C., Anton, I., Enjuanes, L. and Carrascosa, J. (1996). The transmissible gastroenteritis coronavirus contains a spherical core shell consisting of M and N proteins. *J Virol*, 70(7), 4773.

Robb, J.A. and Bond, C.W. (1979a). Coronaviridae. In *Comprehensive Virology*, vol. 14, pp. 193-247, ed. H. Franenkel-Conrat and R.R. Wanger. New York: Plenum Press.

Robb, J.A. and Bond, C.W. (1979b). Pathogenic murine coronaviruses I. characterization of biological behavior in vitro and virus-specific intracellular RNA of strongly neurotropic JHMV and weakly neurotropic A59V viruses. *Virology*, 94(2), 352-370.

Rohrer, C., Suter, P. and Lutz, H. (1994). The diagnosis of feline infectious peritonitis (FIP): A retrospective and prospective study. *Euro J Comp Anim Pract*, 4, 23-29.

Rossen, J., Voorhout, W., Horzinek, M., Van der Ende, A., Strous, G. and Rottier, P. (1995). MHV-A59 enters polarized murine epithelial cells through the apical surface but is released basolaterally. *Virology*, 210(1), 54-66.

Rottier, P.J.M., Horzinek, M.C. and Van Der Zeijst, B. (1981). Viral protein synthesis in mouse hepatitis virus strain A59-infected cells: Effect of tunicamycin. *J Virol*, 40(2), 350-357.

Rottier, P.J.M. (1995). The coronavirus membrane protein. In *The Coronaviridae*, ed S. G. Siddell, pp. 115-139. Plenum Press, New York, NY.

Rottier, P.J.M. (1999). The molecular dynamics of feline coronaviruses. *Vet Microbio*, 69(1-2), 117-125.

Rottier, P.J.M., Nakamura, K., Schellen, P., Volders, H. and Haijema, B.J. (2005). Acquisition of macrophage tropism during the pathogenesis of feline infectious peritonitis is determined by mutations in the feline coronavirus spike protein. *J Virol*, 79(22), 14122-14130.

Savary, K., Sellon, R. and Law, J. (2001). Chylous abdominal effusion in a cat with feline infectious peritonitis. *J Am Anim Hosp Assoc*, 37(1), 35.

Schmidt, O.W. and Kenny, G.E. (1981). Immunogenicity and antigenicity of human coronaviruses 229E and OC43. *Infect Immun*, 32(3), 1000.

Scott, F., Corapi, W. and Olsen, C. (1995). Independent evaluation of a modified live FIPV vaccine under experimental conditions (Cornell experience). *Feline Pract*, 23 (3), 74-76.

Sharif, S., Arshad, S.S., Hair-Bejo, M., Omar, A.R., Zeenathul, N.A. and Hafidz, M.A. (2009). Prevalence of feline coronavirus in two cat populations in Malaysia. *J Feline Med Surg*, 11, 1031-1034.

Sharif, S., Arshad, S.S., Hair-Bejo, M., Omar, A.R., Zeenathul, N.A., Fong, L.S., et al. (2010). Descriptive distribution and phylogenetic analysis of feline infectious peritonitis virus isolates of Malaysia. *Acta Vet Scandinavica*, 52, 1-7.

Sharpee, R.L., Mebus, C.A. and Bass, E. P. (1976). Characterization of a calf diarrheal coronavirus. *Am J Vet Res*, 37(9), 1031-1041.

Shelly, S., Scarlett-Kranz, J. and Blue, J. (1988). Protein electrophoresis on effusions from cats as a diagnostic test for feline infectious peritonitis. *J Am Anim Hosp Assoc*, 24(5) 495-500.

Shi, S.T., Schiller, J.J., Kanjanahaluethai, A., Baker, S.C., Oh, J.W. and Lai, M. (1999). Colocalization and membrane association of murine hepatitis virus gene 1 products and de novo-synthesized viral RNA in infected cells. *J Virol*, 73(7), 5957.

Shieh, C.K., Soe, L.H., Chang, M.F., Stohlman, S.A. and Lai, M. (1987). The 5'-end sequence of the murine coronavirus genome: Implications for multiple fusion sites in leader-primed transcription. *Virology*, 156(2), 321-330.

Shiba, N., Maeda, K., Kato, H., Mochizuki, M. and Iwata, H. (2007). Differentiation of feline coronavirus type I and II infections by virus neutralization test. *Vet Microbiol*, 124(3-4), 348-352.

Siddell, S., Wege, H., Barthel, A. and ter Meulen, V. (1981). Coronavirus JHM: Intracellular protein synthesis. *J Gen Virol*, 53(1), 145.

Siddell, S., Wege, H. and ter Meulen, V. (1982). The structure and replication of coronaviruses. *Curr Top Microbiol Immunol*, 99, 131-163.

Siddell, S.G., Anderson, H.D., Macnaughton, M.R., Pensaert, M., Stohlman, S.A. and Sturman, L. (1983). Coronaviridae. *Intervirology* 20: 181-189. Siddell, S. G., Wege, H and Meulen, V. The biology of coronavirus. *J Gen Virol*, 64; 761-776.

Siddell, S. G. 1995. The Coronaviridae: an introduction. In. *The Coronaviridae*, ed. S. G. Siddell, pp. 1-10. Plenum Press, New York, N.Y.

Simons, F.A., Vennema, H., Rofina, J.E., Pol, J.M., Horzinek, M.C. and Rottier, P.J.M. (2005). A mRNA PCR for the diagnosis of feline infectious peritonitis. *J Virol Methods*, 124(1-2), 111-116.

Simmons, G., Gosalia, D.N., Rennekamp, A.J., Reeves, J.D., Diamond, S.L. and Bates, P. (2005). Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proceedings of the National Academy of Sciences of the United States of America*, 102(33), 11876.

Snijder, E.J., Bredenbeek, P.J., Dobbe, J.C., Thiel, V., Ziebuhr, J. and Poon, L.L.M. (2003). Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineages. *J Mol Biol*, 331(5), 991-1004.

Snijder, E.J., Van Der Meer, Y., Zevenhoven-Dobbe, J. and Onderwater, J. J. M. (2006). Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. *J Virol*, 80(12), 5927.

Spaan, W.J.M., Rottier, P.J.M., Horzinek, M.C. and Van Der Zeijst, B.A.M. (1981). Isolation and identification of virus-specific mRNAs in cells infected with mouse hepatitis virus (MHV-A59). *Virology*, 108(2), 424-434.

Spaan, W., Cavanagh, D. and Horzinek, M.C. (1988). Coronaviruses: Structure and Genome Expression. *J. gen. Virol*, 69, 2939-2952.

Spaan, W.J.M., Brian, D., Casvanagh, D. (2004). Coronaviridae. In: Fauquet, C; Mayo, M. A; Maniloff, J; Desselberger, U; Ball, L. A, eds. *Virus Taxonomy: VIIth Report of the International Committee on Taxonomy of Viruses*. London: Elsevier Academic Press; 945-962.

Sparkes, A., Gruffydd-Jones, T. and Harbour, D. (1991). Feline infectious peritonitis: A review of clinicopathological changes in 65 cases, and a critical assessment of their diagnostic value. *Vet Rec*, 129(10), 209.

Sparkes, A.H., Gruffydd-Jones, T.J., Howard, P. and Harbour, D.A. (1992). Coronavirus serology in healthy pedigree cats. *Vet Rec*, 131:35–36.

Sparkes, A.H., Gruffydd-Jones, T.J., and Harbour, D.A. (1994). An appraisal of the value of laboratory tests in the diagnosis of feline infectious peritonitis. *J Am Anim Hosp Assoc*, 30, 345-350.

Sparkes, A.H. (2004). Feline coronavirus infection. In Chandler, ed. E.A., Gaskell, C. J and Gaskell, R.M. Feline medicine and therapeutics, PP. 623-634. Oxford: Blackwell publishing.

Stertz, S., Reichelt, M., Spiegel, M., Kuri, T., Martínez-Sobrido, L. and García-Sastre, A. (2007). The intracellular sites of early replication and budding of SARS-coronavirus. *Virology*, 361(2), 304-315.

Stoddart, M., Gaskell, R., Harbour, D. and Pearson, G. (1988). The sites of early viral replication in feline infectious peritonitis. *Vet Microbiol*, 18(3-4), 259-271.

Stoddart, C.A. and Scott, F.W. (1989). Intrinsic resistance of feline peritoneal macrophages to coronavirus infection correlates with in vivo virulence. *J Virol*, 63:436-440.

Sturman, L.S. and Takemoto, K.K. (1972). Enhanced growth of a murine coronavirus in transformed mouse cells. *Infect Immun*, 6(4), 501.

Sturman, L.S., Holmes, K. and Behnke, J. (1980). Isolation of coronavirus envelope glycoproteins and interaction with the viral nucleocapsid. *J Virol*, 33(1), 449.

Takano, T., Hohdatsu, T., Toda, A., Tanabe, M. and Koyama, H. (2007). TNF- α , produced by feline infectious peritonitis virus (FIPV)-infected macrophages, upregulates expression of type II FIPV receptor feline aminopeptidase N in feline macrophages. *Virology*, 364, 64-72.

Tammer, R., Evensen, O., Lutz, H., and Reinacher, M. (1995). Immunohistological demonstration of feline infectious peritonitis virus antigen in paraffin-embedded tissues using feline ascites or murine monoclonal antibodies. *Vet Immunol Immunopathol*, 49(1-2), 177-182.

Tekes, G., Hofmann-Lehmann, R., Stallkamp, I., Thiel, V. and Thiel, H.J. (2008). Genome organization and reverse genetic analysis of a type I feline coronavirus. *J Virol*, 82(4), 1851.

Tellier, R., Nishikawa, J., Petric, M., Specter, S., Hodinka, R. and Young, S. (2009). Electron microscopy and immunoelectron microscopy. *Clin Virol Man*, (Ed. 4), 64-76.

Tenser, R.B. (1978). Ultracentrifugal inoculation of herpes simplex virus. *Infect Immun*, 21(1), 281-285.

- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res*, 25(24), 4876-4882.
- Tooze, J., Tooze, S. and Warren, G. (1984). Replication of coronavirus MHV-A59 in sac- cells: Determination of the first site of budding of progeny virions. *European J Cell Biol*, 33(2), 281-293.
- Tooze, J. and Tooze, S.A. (1985). Infection of AtT20 murine pituitary tumor cells by mouse hepatitis virus strain A59: Virus budding is restricted to the Golgi region. *Euro J Cell Biol*, 37, 203-212.
- Tooze, S.A., Tooze, J. and Warren, G. (1988). Site of addition of N-acetyl-galactosamine to the E1 glycoprotein of mouse hepatitis virus-A59. *J Cell Biol*, 106(5), 1475-1487.
- Tresnan, D.B., Levis, R. and Holmes, K.V. (1996). Feline aminopeptidase N serves as a receptor for feline, canine, porcine, and human coronaviruses in serogroup I. *J Virol*, 70(12), 8669-8674.
- Trotman, T.K., Mauldin, E., Hoffmann, V., Del Piero, F. and Hess, R.S. (2007). Skin fragility syndrome in a cat with feline infectious peritonitis and hepatic lipidosis. *Vet Dermat*, 18(5), 365-369.
- Tseng, C., Tseng, J. and Perrone, L. (2005). Apical entry and release of severe acute respiratory syndrome-associated coronavirus in pplarized Calu-3 lung epithelial cells. *J Virol*, 79, 9470-9479.
- Tupper, G., Evermann, J., Russell, R. and Thouless, M. (1987). Antigenic and biological diversity of feline coronaviruses: Feline infectious peritonitis and feline enteritis virus. *Arch Virol*, 96(1), 29-38.
- Tusell, S.M., Schittone, S.A. and Holmes, K.V. (2007). Mutational analysis of aminopeptidase N, a receptor for several group 1 coronaviruses, identifies key determinants of viral host range. *J Virol*, 81(3), 1261-1273.
- Vennema, H., De Groot, R.J., Harbour, D.A., Horzinek, M.C. and Spaan, W.J.M. (1991). Primary structure of the membrane and nucleocapsid protein genes of feline infectious peritonitis virus and immunogenicity of recombinant vaccinia viruses in kittens. *Virol*, 181(1), 327-335.
- Vennema, H., Rossen, J., Wesseling, J., Horzinek, M. and Rottier, P. (1992). Genomic organization and expression of the 3'end of the canine and feline enteric coronaviruses. *Virol*, 191(1), 134-140.

Vennema, H., Poland, A., Hawkins, K. F. and Pedersen, N. (1995). A comparison of the genomes of FECVs and FIPVs and what they tell us about the relationships between feline coronaviruses and their evolution. *Feline Pract*, 23(3)40-44.

Vennema, H., Godeke, G., Rossen, J., Voorhout, W., Horzinek, M. and Opstelten, D. (1996). Nucleocapsid-independent assembly of coronavirus-like particles by co-expression of viral envelope protein genes. *EMBO J*, 15(8), 2020-2028.

Vennema, H., Poland, A., Foley, J. and Pedersen, N.C. (1998). Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. *Viol*, 243(1), 150-157.

Vennema, H. (1999). Genetic drift and genetic shift during feline coronavirus evolution. *Vet Microbio*, 69(1-2), 139-141.

Vijaykrishna, D., Smith, G., Zhang, J., Peiris, J., Chen, H. and Guan, Y. (2007). Evolutionary insights into the ecology of coronaviruses. *J Virol*, 81(8), 4012.

Walker, P., Bonami, J., Boonsaeng, V., Chang, P., Cowley, J. and Enjuanes, L. (2005). Family roniviridae. *Virus Taxonomy, VIIIth Report of the International Committee on Taxonomy of Viruses*, PP. 975–979. Elsevier, Academic Press, Amsterdam.

Ward, J., Munn, R., Gribble, D. and Dungworth, D. (1968). An observation of FIP. *Vet Rec*, 83, 416-417.

Ward, J.M. (1970). Morphogenesis of a virus in cats with experimental feline infectious peritonitis. *Viol*, 41(1), 191-194.

Watari, T., Kaneshima, T., Tsujimoto, H., Ono, K. and Hasegawa, A. (1998). Effect of thromboxane synthetase inhibitor on feline infectious peritonitis in cats. *J Vet Med Sci*, 60(5), 657-659.

Weber, O. and Schmidt, A. (2005). Coronavirus infections in veterinary medicine. In M.W.A. Schmidt. *Coronaviruses with Special Emphasis on First Insights Concerning SARS*, (55-69).

Weiss, M., Steck, F. and Horzinek, M.C. (1983). Purification and partial characterization of a new enveloped RNA virus (Berne virus). *J Gen Virol*, 64(9), 1849.

Weiss, R.C. and Scott, F.W. (1981). Pathogenesis of feline infectious peritonitis: Pathologic changes and immunofluorescence. *Am J Vet Res*, 42(12), 2036-2048.

Wege, H., Siddell, S. and Ter Meulen, V. (1982). The biology and pathogenesis of coronaviruses. *Curr Top Microbiol Immunol*, 99, 165.

Wesseling, J.G., Vennema, H., Godeke, G.J., Horzinek, M.C. and Rottier, P.J.M. (1994). Nucleotide sequence and expression of the spike (S) gene of canine coronavirus and comparison with the S proteins of feline and porcine coronaviruses. *J Gen Virol*, 75(7), 1789.

Weiss, S.R. and Navas-Martin, S. (2005). Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *Microbio Mol Biol Rev*, 69(4), 635.

Wentworth, D.E. and Holmes, K.V. (2001). Molecular determinants of species specificity in the coronavirus receptor aminopeptidase N (CD13): Influence of N-linked glycosylation. *J Virol*, 75(20), 9741.

Wilhelmsen, K.C., Leibowitz, J.L., Bond, C.W. and Robb, J.A. (1981). The replication of murine coronaviruses in enucleated cells. *Virol*, 110(1), 225-230

Wilkinson, M. (1993). Purification of RNA. In *Essential molecular biology*, Volume 1 A Practical Approach, ed. Brown, T. A., Oxford University Press, pp 71.

Wolfe, L.G. and Griesemer, R.D. (1966). Feline infectious peritonitis. *Vet Pathol*, 3(3), 255-270.

Wolfe, L.G. and Griesemer, R.D. (1971). Feline infectious peritonitis: Review of gross and histopathological lesions. *J Am Vet Med Assoc*, 158, 987-993.

Wong, W.T., Cheng, B.Y. and Lee, J.Y.S. (1983). Feline infectious peritonitis-two case reports. *Kajian Vet. Malaysia*. 15, 30-35.

Woods, R.D. (1978). Small plaque variant transmissible gastroenteritis virus. *J Am Vet Med Assoc*, 173(5 Pt 2), 643-647.

Woods, R.D. and Pedersen, N.C. (1979). Cross-protection studies between feline infectious peritonitis and porcine transmissible gastroenteritis viruses. *Vet Microbiol*, 4(1), 11-16.

Woods, R.D. (1982). Studies of enteric coronaviruses in a feline cell line. *Vet Microbiol*, 7(5), 427-435.

Woods, R.D. and Wesley, R.D. (1988). Cultivation techniques for animal coronaviruses: Emphasis on feline infectious peritonitis virus, canine coronavirus, transmissible gastroenteritis virus, and porcine hemagglutinating encephalomyelitis virus. *Methods Cell Sci*, 11(2), 95-100.

Woods, G.L. and Johnson, A.M. (1989). Rapid 24-well plate centrifugation assay for detection of influenza A virus in clinical specimens. *J Virol Methods*, 24(1-2), 35-42.

Yamada, A. and Imanishi, J. (1992). Detection of influenza B virus in throat swabs using the polymerase chain reaction. *Acta Virol*, 36(3), 320.

Yu, G. Y. and Lai, M. (2005). The ubiquitin-proteasome system facilitates the transfer of murine coronavirus from endosome to cytoplasm during virus entry. *J Virol*, 79 (1), 644.

Zhang, Z., Schwartz, S., Wagner, L. and Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *J Comp Biol*, 7(1-2), 203-214.

Zook, B., King, N., Robison, R. and McCombs, H. (1968). Ultrastructural evidence for the viral etiology of feline infectious peritonitis. *Vet Pathol*, 5(1), 91.

Zelus, B.D., Schickli, J.H., Blau, D.M., Weiss, S.R. and Holmes, K.V. (2003). Conformational changes in the spike glycoprotein of murine coronavirus are induced at 37 {degrees} C either by soluble murine CEACAM1 receptors or by pH 8. *J Virol*, 77(2), 830.