Jurnal Veterinar Malaysia

ISSN 0128-2506

Vol. 31 No. 2 (Dec) 2019

Veterinary Association Malaysia

J. Vet. Malaysia (2019) 31 (2): 13-18

Journal Articles

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

L.W. Hoong[1], A.R. Yasmin[2]*, K. Mummoorthy[1], S.S. Arshad[2], A.R. Omar[2,3], P. Anand[1] and K. Kumar[2]

[1]Gasing Veterinary Hospital, 53 & 55, Jalan 5/58, Gasing Indah, Petaling Jaya, Selangor, Malaysia
[2]Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia
[3]Institute of Bioscience, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia

SUMMARY

Feline coronavirus (FCoV) infection is a very common in cat population. FCoV is further classified into two biotypes namely feline enteric coronavirus (FECV) and mutated feline infectious peritonitis virus (FIPV), in which FIPV causes a fatal immune complex disease by changing the tropism from enterocytes to monocytes. Previous studies on molecular detection of FCoV in cats were carried out in catteries but limited study investigate the presence of FCoV antigen in local pet cats. By considering this fact, this study aims to detect FCoV antigen via RT-PCR assay in local pet cats and to compare the similarity of the identified FCoV strain with previous related virus by phylogenetic analysis. By using convenience sampling, rectal swabs and buffy coat were collected from 16 clinically ill pet cats and 5 healthy pet cats. Viral RNA was extracted and subjected to one-step RT-PCR, targeting polymerase gene. Only one out of 21 fecal samples was positive for FCoV and none from buffy coat samples. Phylogenetic analysis revealed that the identified positive sample was highly homologous, up to 95%, to FCoV strain from Netherlands and South Korea on partial sequence of polymerase gene. In conclusion, this study detected FCoV antigen in local pet cats from fecal samples while negative detection from fecal and buffy coat samples could not completely rule out the possibilities of FCoV infection due to the complexity of the virus diagnosis that require multiple series of analysis.

Keywords: Feline coronavirus (FCoV), Phylogenetic analysis, Reverse transcription polymerase chain reaction (RT-PCR)

INTRODUCTION

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

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

FCoV is transmitted through fecal-oral route. High level of virus shedding occurs up to 10 months but some cats may continue to shed the virus persistently until two years while the others may become intermittent shedders or stop to shed the virus after an average of one year (Hartmann, 2005; Pedersen et la., 2008). Thus, reverse-transcriptase polymerase chain reaction (RT-PCR) can be used to detect FCoV antigen in the feces as a part of multi-cat household management (Herrewegh *et al*., 1995b). After being infected by FCoV, monocyte-associated viraemia occurs. Thus, buffy coat which is rich with leukocytes can be used to check for viraemia due to FCoV using RT-PCR (Kipar *et al*., 2010).

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

In Malaysia, the phylogenetic analysis of FCoVs on partial sequence of 3'UTR had been done around 7 years back (Sharif *et al*., 2009b; Sharif *et al*., 2010a). Thus, this study will again use phylogenetic analysis to get a glimpse into the current status of FCoVs in Malaysia. Thus, the objectives of the study are to detect FCoV antigen via RT-PCR assay in local pet cats and compare the similarity of identified positive samples with the previous related virus by phylogenetic analysis.

* *Corresponding author: Dr. Nor Yasmin Abd Rahaman (A.R. Yasmin); Phone No: +603 8609 3473; Fax No: +603 8609 1971;*
Email: noryasmin@upm.edu.my

MATERIALS AND METHODS

Animals

The research project was firstly approved by the Institutional Animal Care and Use Committee (IACUC), with reference number: UPM/IACUC/AUP – FYP.2016/FPV. A total of 21 pet cats were identified in Gasing Veterinary Hospital, Petaling Jaya, Selangor. Sixteen of them were clinically ill with various clinical signs, including gastrointestinal signs, respiratory signs, renal disease, hepatobiliary disease whereas the other 5 cats were healthy. The signalment was categorized as shown in Table 1.

Samples Collection

The cats were identified, and owners were approached for their consent with the samples collection from their cats. Approximately 1-3 mL of blood from jugular, cephalic or saphenous vein and rectal swab were collected. Blood was collected by the attending veterinarians of the Hospital while rectal swab was collected. A total of 42 samples were collected, consisting of 21 blood samples and 21 rectal swabs, from 21 pet cats. The sampling was carried out by using convenience sampling method at which only first 21 pet cats that visited to Gasing Veterinary Hospital within the first week (8 Jun – 15 Jun 2018) were selected.

Samples Transportation, Processing and Storage

The blood was kept in ethylenediaminetetraacetic acid (EDTA) collection tube, while the rectal swab was kept in a 2.0 mL microcentrifuge tube containing 0.5 mL of Phosphate Buffer Saline (PBS). The blood was centrifuged at 450 x g for 5 minutes to separate out the red blood cells, buffy coat and plasma. The buffy coat was then carefully aspirated with 23 G needle of 1.5 inches attached to a 1 mL syringe and kept in a 2.0 mL microcentrifuge tube, stored in -80 °C until further used.

RNA Extraction from Rectal Swab Samples

The RNA from each rectal swab sample was extracted by using FavorPrepTM Viral Nucleic Acid Extraction Kit I (Favorgen®, Taiwan) according to the manufacturer's instructions. The RNA was then aliquoted and stored at -80 °C until being used.

Buffy Coat Samples

The total RNA from each buffy coat sample was extracted using TRIzol® Reagent (Invitrogen, USA) according to the manufacturer's instructions. The RNA was then aliquoted and stored at -80 °C until further used.

Primers Selection

The primer pair targets the conserved polymerase gene (RNA-dependent RNA polymerase) of genus *Alphacoronavirus*, forward 5'-GTCTGGGACTATCCTAAGTGTGA-3' and reverse 5'-CCATCATCAGATAGAATCATCATA-3' which expected PCR product is 420 bp (Sharif et al., 2010).

One Step Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

One Step RT-PCR was performed by using a commercial kit, Access RT-PCR System (Promega, USA). The PCR mixture was prepared in 25 μL reaction volume containing AMV/T*fl* Reaction Buffer (5x), 25mM $MgSO_4$, dNTPs Mix, AMV Reverse Transcriptase (5U/μL) and T*fl*

Table 1: Signalment of the pet cats, classified based on different categories.

Signalment		No. of Cats
a) Health Status	Healthy	5
	Clinically Ill	16
b) Age	<2 years old	4
	≥2 years old	17
c) Gender	Intact Male	6
	Intact Female	2
	Castrated Male	9
	Spayed Female	4
d) No. of Cats in the household	Single & indoor	10
	Single & semi-roamer	4
	*Multi & indoor	4
	Multi & semi-roamer	3
e) **Vaccination Status	Up-to-date	14
	Not-up-to-date	7

Note: The asterisk represents *Multi-cat household: ≥ 2; **Not vaccinated against FCoV respectively.

DNA Polymerase (5U/µL), 0.8U $MgSO_4$(25mM), 20 pmol of each of the forward and reverse primer and 5 μL RNA template. Nuclease-free water was used to bring the mixture to its final volume of 25 μL. The target gene regions were amplified using the following conditions: reverse transcription: 45°C (45 min), initial denaturation: 95°C (2min), denaturation: 94°C (30 sec), annealing: 54°C (30 sec), extension: 72°C (2 min), 35 cycles of repeats, and final extension: 72°C (10 min). PCR product was electrophoresed using 1.5% agarose and stained with nucleic acid staining solution (RedsafeTM, iNtRON, Korea) and visualised under UV light (GeneDirectory software (Syngene, UK). The impurified PCR products were sent for purification and sequencing at First Base Laboratories Sdn. Bhd. (Malaysia) by using Sanger sequencing method.

Bioinformatics Analysis of Feline Coronavirus Polymerase Gene Sequence

The polymerase gene sequences of the related reference isolates downloaded GenBank from was aligned with positive detection in this study by using MEGA-7 software. A neighbour-joining (NJ) phylogenetic tree was constructed using MEGA-7 software. Tree reliability was accessed by using 1000 bootstrap replications. The sequence of the local FCoV strain (from the positive sample) was compared with the isolates of FCoV, CCoV, TGEV, Human Coronavirus (HCoV) and FIPV from different countries including Unite States of America, Netherlands, China, Taiwan, Australia, Japan and Korea.

RESULTS

RT-PCR Amplification

Based on the conventional RT-PCR assay only one out of 21 cats was positive for FCoV, which was 4.76% of all cases. Positive result was defined as band being expressed at the 420 bp region after running electrophoresis on 1.5% (w/v) agarose gel. This specific band at 420 bp is the amplification products of the RNA-dependent RNA polymerase gene region of alphacoronavirus. For positive control, FECV 79-1683 strain from American Type Culture Collection (ATCC) with No. VR-989™ was used, whereas for negative control, only buffer reaction without RNA template after RT-PCR was used. Figure 1a and Figure 1b showed the RT-PCR results of the rectal swab samples from first 21 cats after gel electrophoresis. Based on the conventional RT-PCR assay, none of the buffy coat samples tested was positive for feline coronavirus (FCoV) (not shown).

Sequence Editing and Assembly

Only one PCR product of FCoV from Cat-17 was positive, and it was sent for sequencing. Sequence outputs in form of electropherogram were edited and assembled using bioinformatics software. Sequence fragment of FCoV was of length of 395 bp. The sequence was assigned with an individual identification of UPM_FCoV_ollie (GenBank accession No MQ234559)

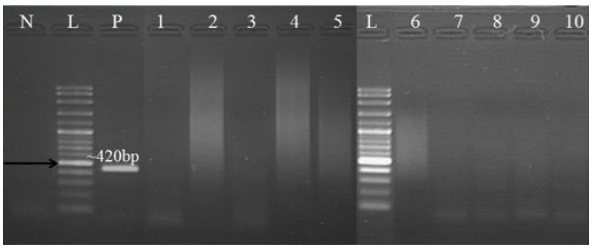

Figure 1a. RT-PCR results of rectal swabs of first 10 cats using primers targeting the conserved polymerase gene of FCoV. Lane N: Negative control; Lane L: 100 bp DNA marker; Lane P: Positive control (FECV 79-1683); Lane 1-10: Negative amplification.

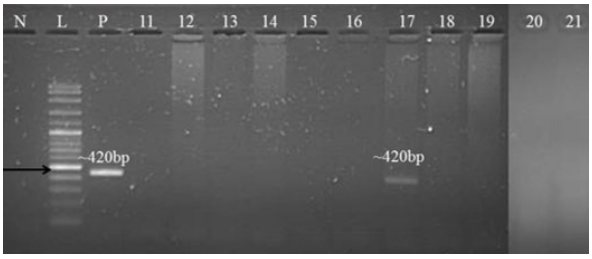

Figure 1b. RT-PCR results of rectal swabs of the remaining 11 cats using primers targeting the conserved polymerase gene of FCoV. Lane N: Negative control; Lane L: 100 bp DNA marker; Lane P: Positive control (FECV 79-1683); Lane 11-16 and 18-21: Negative amplification; Lane 17: Positive amplification.

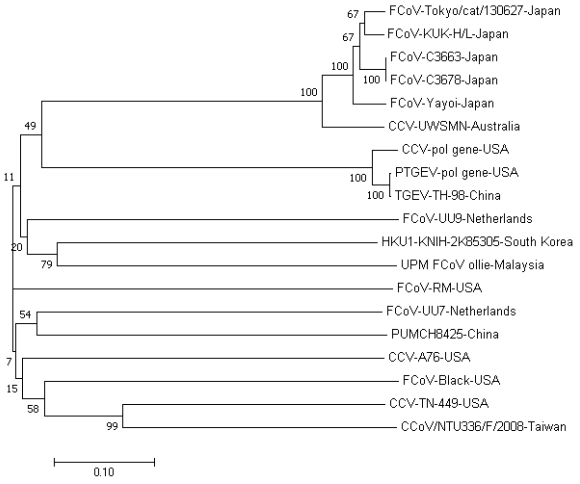

Figure 2. Neighbour-joining phylogenetic relationship of Malaysian FCoV. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. UPM_FCoV_ollie is FCoV detectec in this study. Evolutionary examines were conducted in MEGA-7.

Basic Local Alignment Search Tool (BLAST)

The results of BLAST search showed that the partial sequence of polymerase gene of the local UPM_FCoV_ollie strain (Cat-17) was highly similar (97-96%) to the reference isolates found in the GenBank® of NCBI, FCoV-UU9 (GenBank accession No. FJ938062.1) and FCoV-HLJ/HRB/2016/13 (GenBank accession No. KY566211.1).

Multiple Alignment and Pairwise Comparison

The sequence of the local UPM_FCoV_ollie was aligned with other reference isolates downloaded from Genbank® based on their sequence identity and reliability in publications. The alignment was done using MEGA V7. Table 2 shows the reference isolates of coronavirus downloaded from GenBank.

Construction of Phylogenetic Tree

The a neighbour-joining (NJ) phylogenetic tree was constructed between the local strain UPM_FCoV_ollie with the reference isolates available in GenBank® that included the coronavirus from different animal species was generated with 1000 bootstrap replicates (Figure 2). MEGA-7 software was used to construct the tree.

Based on pairwise comparison of the sequence identity matrix, the reference isolate with the closest sequence identity to local FCoV strain was the UU9 and HKU1-KNIH-2K85305 from Netherlands and South Korea respectively. The same similarity was observed on the phylogenetic tree with the FCoV_UU9 was in the same clade as the local FCoV strain (UPM_FCoV_ollie), and under the clustering of all the feline coronavirus (FCoV) isolates (Figure 2). Yet, this phylogenetic tree cannot differentiate its serotype as serotype I or II or biotype as feline enteric coronavirus (FECV) or feline infectious peritonitis virus (FIPV)

Table 2: Reference isolates of coronavirus downloaded from GenBank.

Strain	Accession No.	Country	Source	references
FCoV-UU9	FJ938062.1	Netherlands	GenBank	Spiro *et al.*, 2009
FCoV- Black	EU186072.1	USA	GenBank	Tekes *et al.*, 2008
FCoV-UU7	FJ938053.1	Netherlands	GenBank	Spiro *et al.,* 2009
FCoV-RM	FJ938051.1	USA	GenBank	Spiro *et al.,* 2009
FCoV-KUK-H/L	AB781793.1	Japan	GenBank	Terada *et al.*, 2014
FCoV-C3678	AB781796.1	Japan	GenBank	Terada *et al.*, 2014
FCoV-C3663	AB781794.1	Japan	GenBank	Terada *et al.*, 2014
FCoV-Tokyo/cat/130627	AB907634.1	Japan	GenBank	Terada *et al.,* 2014
FCoV-Yayoi	AB781795.1	Japan	GenBank	Terada *et al.,* 2014
CCV-UWSMN	AF516906.1	Australia	GenBank	Naylor *et al.,* 2002
CCV-TN-449	JQ404410.1	USA	GenBank	Thor *et al.,* 2012
CCV- pol gene	AF124986.1	USA	GenBank	Stephensen *et al.,* 1999
CCoV/NTU336/F/2008	GQ477367.1	Taiwan	GenBank	Chuang *et al.,* 2009
CCV-A76	JN856008.2	USA	GenBank	Town *et al.,* 2011
HKU1-KNIH-2K85305	JN234464.1	South Korea	GenBank	Lee *et al.,* 2013
PUMCH8425	HM130814	China	GenBank	Wang *et al.,* 2011
PTGEV- pol gene	AF124992.1	USA	GenBank	Stephensen *et al.,* 1999
TGEV-TH-98	AY437877.1	China	GenBank	Yu *et al.,* 2003
UPM_FCoV_ollie	MQ234559	Malaysia	This study	-

DISCUSSION

RT-PCR is a sensitive molecular detection method for viral RNA or DNA for various viruses. Rectal swabs sample collection was choosing for FCoV detection because it shed mainly in faeces (Hartmann, 2005). The primer pair used is targeting Pol (polymerase gene) region which is a conserved region for FCoV. Thus, it can be used to detect the circulating FCoV in the cat population (Sharif *et al.,* 2010a). Yet, it cannot differentiate biotypes or serotypes of FCoV. Besides, it can also detect another coronavirus like CCoV and TGEV.

From the RT-PCR results, only one out of 21 cats were tested positive, which was 4.76%. A previous study done by Sharif *et al.* (2009a) involved 44 cats in two catteries in Malaysia revealed that viral shedding by 84% of the cats, whereas this study utilized pet cats and at the same time 10 out of 21 of them were from single-cat household and kept strictly indoor. This group of cats had no contact with other cats, and there was no sharing of litter tray with other cats, it would definitely reduce the chances of fecal-oral transmission of FCoV. There were 4 out of 21 cats were from single-cat household but they were also semi-roamers. Semi-roaming cats used to bury feces in distinct territories which would also reduce the fecal-oral transmission of FCoV, yet there were limitations to know if the cats were in contact with other cats in the area, and the overall cat population densities in the area which could affect the virus transmission (Hartmann, 2005; Cave, 2004).

Besides, there were 7 cats from multi-cat household and the positive sample was detected from this group of cats. Multi-cat household has always been known to be a risk factor causing FCoV transmission. Most of the time, pet cats are better taken care of, with separate litter trays, and constant clean-up by the owners, this can definitely

reduce the fecal-oral route transmission of FCoV (Hartmann, 2005).

As compared to serological test, RT-PCR assay has the advantage of directly detecting FCoV viral genome which indicates current infection or shedding (if it is detected in faeces), whereas detection of antibodies indicates past infection (Sharif *et al.,* 2009a). A single negative result is not conclusive to rule out FCoV infection because a FCoV-infected cat might shed the virus intermittently towards the end of infection (Herrewegh *et al*., 1997). According to Addie and Jarret (2001), a cat has to be tested negative monthly over a 5-month period in order to be considered a non-shedder.

Originally, it was shown that FECV was only localized in enterocytes, and FIPV is mutated from FECV based on the theory of internal mutations happen at certain genes in the persistently infected cats that switches the tropism of intestinal epithelium to monocyte/macrophage (Pedersen, 2014a), thus systemic infection was a defining moment for the development of feline infectious peritonitis. But the theory was proven to be incorrect, because regardless of development of FIP, monocyte-associated viraemia does occur in FECV infection. Thus, in this study, buffy coat which is rich with leukocytes was taken for the FCoV detection indicating viraemia. Normally, viraemia occurs one week after FCoV infection, then it may not be detected afterwards, but it can recur along the course of infection in some cats (Kipar *et al.,* 2010).

In this study, there was no FCoV detected in all the buffy coat samples, indicating there was no viraemia in all 21 cats, including Cat-17 with rectal swab being positive and Cat-21 with suspected-FIP sign of recurrent peritoneal effusion. Based on RT-PCR result for Cat-17, it was postulated that cat was only affected by FECV, less likely to be FIPV, or the viraemia had subsided at the later stage of infection, even with viral shedding, as supported by study done by Kipar *et al.* (2010). Another postulation might be due to the very low number of the virus that could not be detected by rt-PCR (Pedersen, 2014b). Yet, even Cat-17 had no typical FIP signs, it cannot be confidently ruled out that FIPV may be present in the faeces, which was supported by a recent study by Wang *et al.* (2013) in Taiwan proving a horizontal transmission of FIPV via faeces, even though the earlier studies revealed that the horizontal transmission of FIPV would be very unlikely.

Based on RT-PCR result for Cat-21 with the absence of detectable viraemia or viral genome in buffy coat, it was postulated that FCoV may reside within parenchymal cells and/or resident macrophages. (Kipar *et al.,* 2010). According to Hartmann (2005), most cats with FIP will also shed virus in faeces, yet the virus load seems to reduce after a cat has developed FIP, which may explain why Cat-21 in this study showed a negative RT-PCR result for FCoV from faecal sample.

RT-PCR is a very sensitive method to detect FCoV antigen, with sensitivity and specificity are more than 90%, but the definitive diagnosis of FIP requires multiple tests due to the complexity of the diagnosis of FCoV. The examples of indirect tests that are normally used including complete blood count, with albumin, globulin and bilirubin checked, analysis of effusions, feline coronavirus antibody titers and Rivalta test. The gold standard for FIP diagnosis is immunohistochemistry on the biopsied or necropsied tissue (Pedersen, 2014b).

From the phylogenetic tree constructed based on partial polymerase gene, the local FCoV strain formed a clade with a FCoV strain from Netherlands, and from the calculation of pairwise distances, it showed similarity of sequence identity of up to 95%. It suggested that they might be from the common ancestor. However, this result was not conclusive and definitive as the sequence used for the phylogenetic analysis was only limited to partial sequence of the RNA-dependent RNA polymerase gene, which was about 395 bp. For more accurate phylogenetic analysis and identification of the local FCoV strain, a full genome of the local strain should be obtained (Sharif *et al.,* 2009b). The phylogenetic analysis of FCoV in Malaysia done back in year 2010 using primer targeted 3'UTR showed that the homology of 3'UTR sequences of Malaysia FCoV strains had a homology of about 96% and the homology decreased to 93% when compared to published sequences of FCoV from USA, Netherlands, Taiwan and UK, which was still considered to be highly homologous (Sharif *et al.,* 2010a).

CONCLUSION

This study detected feline coronavirus (FCoV) in local pet cats. Although most of the samples were negative, a single negative result could not completely rule out the possibilities of FCoV infection in the cats due to the complexity of the pathogenesis and diagnosis of the virus which requires multiple series of analysis. Phylogenetic analysis on the partial polymerase gene showed that the Malaysia FCoV strain was highly homologous to a FCoV strain from South Korea and Netherlands.

CONFLICT OF INTEREST

None of the authors of this paper has financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

ACKNOWLEDGEMENT

The authors would like to thank all cat owners and clinicians for participation in this study.

REFERENCES

Addie, D.D. and Jarett, O. (2001): Use of a reverse-transcriptase polymerase chain reaction for monitoring feline coronavirus shedding by healthy cats. Veterinary Record, 148: 649-653.

Addie, D., Belák, S., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Hartmann, K., Hosie, M.J., Lloret, A., Lutz, H. Marsilio, F., Pennisi, M.G. Radford, A.D., Thiry, E., Truyen, U. and Horzinek, M.C. (2009): Feline infectious peritonitis. ABCD guidelines on prevention and management. Journal of Feline Medicine and Surgery, 11: 594-604.

Cave, T.A., Golder, M.C., Simpson, J. and Addie, D.D. (2004): Risk factors for feline coronavirus seropositivity in cats relinquished to a UK rescue charity. Journal of Feline Medicine and Surgery, 6: 53-58.

Hartmann, K., (2005): Feline infectious peritonitis. Veterinary Clinics of North America: Small Animal Practice. 35: 39-79.

Herrewegh, A.A., De Groot, R.J., Cepica, A., Egbrinkk, H.F., Horzinek, M.C. and Rottier, P.J (1995): Detection of feline coronavirus RNA in feces, tissues, and body fluids of naturally infected cats by reverse transcriptase PCR. Journal of Clinical Microbiology. 33: 684-689.

Herrewegh, A.A., Mähler, M., Hedrich, H.J., Haagmans, B.L., Egberink, H.F., Horzinek, M.C., Rottier, P.J. and de Groot, R.J. (1997): Persistence and evolution of feline coronavirus in a closed cat-breeding colony. Virology, 234: 349-363.

Kipar, A. and Meli, M.L. (2014): Feline infectious peritonitis: still an enigma? Veterinary Pathology. 51: 505-526.

Kipar, A., Meli, M.L., Baptiste, K.E., Bowker, L.J. and Lutz, H. (2010): Sites of feline coronavirus persistence in healthy cats. Journal of General Virology. 91: 1698-1707.

Pedersen NC1, Allen CE, Lyons LA. (2008). Pathogenesis of feline enteric coronavirus infection. Journal of Feline Medicine and Surgery. 10(6):529-541.

Pedersen, N.C. (2009): A review of feline infectious peritonitis virus infection: 1963–2008. Journal of Feline Medicine and Surgery. 11: 225-258.

Pedersen, N.C. (2014a): An update on feline infectious peritonitis: virology and immunopathogenesis. The Veterinary Journal. 201: 123-132.

Pedersen, N.C. (2014b): An update on feline infectious peritonitis: diagnostics and therapeutics. The Veterinary Journal. 201: 133-141.

Sharif, S., Arshad, S. S., Hair-Bejo, M., Omar, A.R., Zeenathul, N.A. and Hafidz, M.A. (2009a): Prevalence of feline coronavirus in two cat populations in Malaysia. Journal of Feline Medicine and Surgery. 11: 1031-1034.

Sharif, S., Arshad, S.S., Hair-Bejo, M.H., Omar, A.R., Allaudin, Z.N. and Hafidz, M.A. (2009b): Phylogenetic analysis of feline coronavirus isolates from healthy cats in Malaysia. In Proceedings of the 8th Malaysia Congress on Genetics, 4-6 August 2009, Genting Highlands, Malaysia. pp. 314-318

Sharif, S., Arshad, S.S., Hair-Bejo, M., Omar, A.R., Zeenathul, N.A., Fong, L.S., Rahman, N.A., Arshad, H., Shamsudin, S. and Isa, M.K. (2010): Descriptive distribution and phylogenetic analysis of feline infectious peritonitis virus isolates of Malaysia. Acta Veterinaria Scandinavica. 52: 1.

Wang, Y.T., Su, B.L., Hsieh, L.E. and Chueh, L.L. (2013): An outbreak of feline infectious peritonitis in a Taiwanese shelter: epidemiologic and molecular evidence for horizontal transmission of a novel type II feline coronavirus. Veterinary. Research. 44: 57.