

## Sequencing Analysis of Partial *N* gene of Feline *Morbillivirus* from Malaysia

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### ABSTRACT

Feline morbillivirus (FeMV) is a new emerging virus of domestic cats categorized under the genus of *Morbillivirus*, associated with chronic kidney disease (CKD). The origin of the virus is yet to be determined, and whether it is caused by a spill-over event from wildlife or domestic cats remains speculative. Recombination event has been reported in FeMV isolate found in Japan; therefore, characterization of FeMV strains isolated in Malaysia (i.e., FeMV-Malaysia isolates) may provide some insight, thus adding some information on the viral evolution of FeMV. Therefore, this study aims to conduct a phylogenetic analysis and assess any genetic changes in the *N* gene of FeMV-Malaysia isolates. Through sequencing of *N* gene of seven isolates using three overlapping primer sets, the sequences spanning approximately 1.5kb of FeMV-*N* gene were obtained. DNA sequencing, nucleotide sequences, amino acid residues alignments, and phylogenetic analysis were performed. A nucleotide sequence alignment was also performed to compare the isolates obtained from two previous studies. From the alignment mentioned above, there were 19 variable sites of which there were absence of amino acid changes except for isolate UPM210 at position 806 and isolate UPM315 at position 823. Furthermore, protein alignment was done to compare FeMV-Malaysia isolates with FeMV strains from other

countries, along with other morbillivirus-related isolates. From one of the conserved regions located within the *N* gene, similar amino acid sequences were detected across different morbilliviruses. Lastly, from the phylogenetic tree, it was illustrated that all partial FeMV-*N* gene Malaysia isolates sequenced in this study were clustered together in the same clade whereby these FeMV-*N* genes Malaysia isolates shared a

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common ancestor with isolates from Japan (SS3, MiJP003, ChJP073) and Thailand (Thai-U16, CTL16, CTL43).

*Keywords:* Feline morbillivirus, partial *N* gene, sequencing analysis

## INTRODUCTION

Feline morbillivirus (FeMV) is a non-segmented, negative-sense, single-stranded RNA morbillivirus that was first discovered in Hong Kong in 2012, and it has been speculated to cause chronic kidney disease (CKD) in domestic cats (Woo et al., 2012). It forms a distinct species under the genus *Morbillivirus*, with nucleotide identities less than 80% of the known paramyxoviruses. Similar to other paramyxoviruses and morbilliviruses, the genome organization of FeMV follows the rule of six, where it efficiently replicates when there are six nucleotides in length. In addition, the gene organization of FeMV (3'-*N-P/V/C-M-F-H-L-5'*) is similar to that of other morbilliviruses.

Nucleoprotein (N), which is translated from the *N* gene, plays a crucial role in the transcription of the virus (Sourimant & Plemper, 2016). The RNA-dependent RNA polymerase (RdRp) will synthesize the viral RNA only when encapsidated by the N protein as a template. Both polymerase-associated phosphoprotein (*P*) and large (*L*) genes, which are involved in all polymerase activities, are responsible for forming the RdRp complex and the N protein. In addition, two glycoproteins, which are the fusion (F) and haemagglutinin (H) proteins,

make up the outer layer or envelope of the virus that connects to the matrix (M) protein surrounding the ribonucleoprotein (Rima et al., 2019).

Genetic changes in a virus could be identified by performing sequences alignment of the nucleotide or amino acid residue. Genetic changes of a virus may include mutation and recombination (Fleischmann, 1996). Compared to DNA viruses, RNA viruses hold a higher mutation due to a lack of proofreading function in their replication enzymes. Recombination of FeMV within *F* and *H* genes has reported that the FeMV-Japan strains (MiJP003) could probably originate from recombination between two FeMV parental strains that are closely related to Japan (ChJP073) and Hong Kong (776U) isolates (Park et al., 2014). Hence, any genetic changes that might have occurred in the *N* gene of FeMV-Malaysia isolates could be investigated through this work.

## MATERIALS AND METHODS

### Sample for Sequencing Analysis

Samples for sequencing analysis (n = 7) were obtained as part of two studies to determine the molecular prevalence of FeMV in Malaysia and the development of TaqMan-based reverse-transcriptase polymerase chain reaction (RT-PCR) assay targeting the FeMV-*N* gene (Makhtar et al., 2021; Mohd Isa et al., 2019). Briefly, the urine sample was collected from cats either by manual compression or cystocentesis. The collected urine was then placed into a

sterile sample collection bottle and stored at 4°C prior to processing. The debris in the urine was removed by centrifugation at  $2,320 \times g$ , where the supernatant was obtained, mixed at a ratio of 1:1 with RNeasy<sup>®</sup> solution (Ambion, USA), and stored at -20°C prior to RNA extraction.

### Total RNA Extraction

Total RNA extraction for collecting urine samples was performed by using the Direct-zol<sup>™</sup> RNA MiniPrep Plus Kit according to the manufacturer's recommendation (Zymo Research, USA). Briefly, the TRI Reagent<sup>®</sup> solution was added into each sample at a ratio of 3:1 and mixed thoroughly for 5 min. Next, an equal volume of ethanol (95%–100%) was added to the mixture and vortexed thoroughly. The mixture was then transferred into a Zymo-Spin<sup>™</sup> III CG Column attached to a collection tube and centrifuged at  $12,000 \times g$  for 1 min. The flow-through was discarded, and the column was transferred into a new collection tube. Next, a volume of 400  $\mu$ L of Direct-zol<sup>™</sup> RNA PreWash was added into the column and centrifuged at  $12,000 \times g$  for 1 min, upon which the flow-through was discarded. This step was repeated, followed by the addition of 700  $\mu$ L of RNA Wash Buffer to the column and centrifugation at  $12,000 \times g$  for 2 min. The column was then transferred into an RNase-free tube, followed by the elution step repeated twice by adding a volume of 20  $\mu$ L of RNase-Free Water to the column matrix and centrifuged at  $12,000 \times g$  for 1 min.

### cDNA Synthesis

Extracted RNA was converted into cDNA prior to conventional RT-PCR assay. The cDNA synthesis was conducted using the SensiFAST<sup>™</sup> cDNA Synthesis Kit (Bioline, United Kingdom) following the suggested protocol: 25°C for 10 min in primer annealing step, 42°C and 48°C each for 15 min in reverse transcription step, and 85°C for 5 min in inactivation step. The cDNA templates were stored at -20°C prior to conventional RT-PCR analysis.

### FeMV-N Gene Amplification by RT-PCR and Sequencing of N Gene

Conventional RT-PCR was performed using MyTaq<sup>™</sup> Red Mix (Bioline, United Kingdom) on the seven FeMV-positive urine samples (UPM23, UPM52, UPM53, UPM210, UPM231, UPM305, and UPM315). RNase-free water (Promega, USA) was used as a negative control in each PCR run. Three different primers were used to obtain the full sequence of the FeMV-N gene from Malaysian isolates (Figure 1). The first primer set (1F\_16 and 1R\_8) covering the early region of the N gene was designed using the PrimerQuest tool from Integrated DNA Technologies (IDT) website based on the alignment sequences from Thailand (Thai-U16), China isolates (M252A), and Japan isolates (ChJP073, MiJP003, and SS3) (Table 1). The second (FN-2F and FN-2R) and third (FN-3F and FN-3R) primer sets targeting the middle and end regions of the N gene in this study, respectively, were published primers (Park et al. (2014)). The amplification

was performed in a thermal cycler using different annealing temperatures for each primer set (Table 2). Amplified PCR product was analyzed on 1.5% agarose gels and visualized using a UV transilluminator (Syngene, United Kingdom). The PCR

products were then purified and sequenced on an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, USA) utilizing both forward and reverse primers described in this study (Table 1).

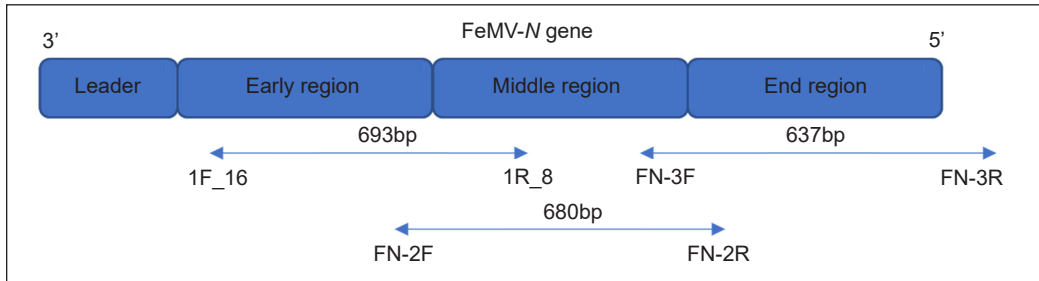


Figure 1. Schematic diagram on *N* gene primer design. Three different sets of overlapping primers were designed to obtain a ~1.5kb sequence of the *N* gene. Both primers set FN-2 (FN-2F and FN-3R) and FN-3 (FN-3F and FN-3R) were obtained from a previous study by Park et al. (2014), whereas 1F-16 and 1R-8 were newly designed primer sets

Table 1  
Primer sequences were used to amplify three different regions targeting the *N* gene

Region	Primer	Sequence (5'-3')	Product size (bp)	Source
Early region	1F_16	CTGAAATCACTTGCCGCATTTA	693	This study
	1R_8	TGCCACCATGAATCGTCTTAT		
Middle region	FN-2F	GTTAGCTTAGGATTTGAGAACCC	680	(Park et al., 2014)
	FN-2R	CACCATCTCTTGACCAAGTCT		
End region	FN-3F	GCTATGGAGTTATGCCATGGG	637	
	FN-3R	GTTGTGAACCTTGAGGTCCTAAG		

Table 2  
PCR condition for three different primer sets targeting the *N* gene

Step	Temperature	Time	Cycle
Initial denaturation	95°C	1 min	1×
Denaturation	95°C	15 sec	
Annealing	54°C(1F_16 and 1R_8)	1 min	35×
	58°C(FN-2F and FN-2R)		
	58°C(FN-3F and FN-3R)		
Extension	72°C	1 min	
Final extension	72°C	5 min	1×
Hold	12°C	∞	1×

**Phylogenetic Analysis**

The sequences from all seven isolates were aligned and trimmed using the MEGA X (version 10.0.05) software. Then, all sequences were compared with all available FeMV sequences in National Center for Biotechnology Information (NCBI) nucleotide databases using nucleotide basic local alignment search tool (BLAST). Partial sequences of N gene FeMV-Malaysia isolates were subsequently subjected to phylogenetic tree construction, along with the other FeMV-N gene sequences available in the GenBank using the MEGA

X software (Table 3). The phylogenetic tree was constructed using the Maximum Likelihood method with bootstrap values of 1,000 replicates based on the general time-reversible (GTR) model. The construction of the phylogenetic tree also included other distantly-related viruses under the genus *Morbillivirus*, which were the canine distemper virus (CDV), peste des petits ruminants virus (PPRV), rinderpest virus (RPV), dolphin morbillivirus (DMV), phocine distemper virus (PDV), and measles virus (MeV) as an outgroup comparison (Sakaguchi et al., 2014; Sieg et al., 2019).

Table 3  
*Viral strains included for phylogenetic tree construction*

Species	Isolate	Year	Location	Accession number
FeMV	761U	2012	Hong Kong	JQ411014
FeMV	776U	2012	Hong Kong	JQ411015
FeMV	M252A	2012	China	JQ411016
FeMV	OtJP001	2014	Japan	AB924120
FeMV	MiJP003	2014	Japan	AB924121
FeMV	ChJP073	2014	Japan	AB924122
FeMV	SS1	2014	Japan	AB910309
FeMV	SS2	2015	Japan	LC036586
FeMV	SS3	2015	Japan	LC036587
FeMV	US1	2015	USA	KR014147
FeMV	Thai-U16	2017	Thailand	MF627832
FeMV	PIUMA/2015	2015	Italy	KT825132
FeMV	Tremedino	2018	Italy	MK088516
FeMV	Pepito	2018	Italy	MK088517
FeMV	Capitan Harlock	2018	Italy	MK188746
FeMV	Nerina	2018	Italy	MK188747
FeMV	Pedro	2018	Italy	MK188748
FeMV	Sheryl	2018	Italy	MK188749
FeMV	Rossino	2018	Italy	MK188750
FeMV	Trezampe	2018	Italy	MK188751
FeMV	Claudio	2018	Italy	MK188752
FeMV	Tris	2018	Italy	MK188753
FeMV	Lilly	2018	Italy	MK188754

Table 3 (continue)

Species	Isolate	Year	Location	Accession number
FeMV	TV17	2017	Germany	MG563820
FeMV	Gordon	2018	Germany	MK182089
FeMV	TV25	2018	Germany	MK182090
FeMV	ZRU293/17	2018	South Africa	MH813465
FeMV	CTL16	2019	Thailand	MN164531
FeMV	CTL43	2019	Thailand	MN164532
CDV	CDV SY	2012	China	KJ466106
PPRV	SnDk11I13	2013	Africa	KM212177
RPV	Kabete O	1996	Africa	NC006296
DMV	631IMM5031711	2011	USA	KU720625
PDV	PDV/USA 2006	2006	USA	KY629928
MeV	Ichinose-B95a	1998	USA	NC001498

## RESULTS

### DNA Sequencing Analysis and Basic Local Alignment Search Tool (BLAST) of *N* Gene

BLAST search analysis of partial *N* gene of all seven FeMV-Malaysia isolates revealed high similarities with 29 FeMV isolates from other countries (81%–99%). All sequences have been submitted in the GenBank under accession numbers MN264638-MN264642, MN792827, and MN792828 (Table 4). In addition, high similarities were observed between FeMV-Malaysia isolates (98%–99%) and FeMV isolates originated from Asian countries, such as Japan (LC036587), and Thailand (MN164531) (Table 5).

### Pairwise Alignment on *N* gene of FeMV-Malaysia Isolates

Multiple alignments of FeMV-Malaysia isolates detected in this study were performed to assess any significant nucleotide difference. There were 19 variable sites observed

Table 4

Genome submission to NCBI

Sample ID	Accession number
UPM23	MN264638
UPM52	MN264639
UPM53	MN264640
UPM210	MN264641
UPM231	MN264642
UPM305	MN792827
UPM315	MN792828

out of 962 among the seven FeMV-*N* genes Malaysia isolates. However, none of these sites resulted in different amino acid residues except for isolate UPM210 and isolate UPM315 at positions 806 and 823, respectively (Figure 2). For UPM210, nucleotide change at position 806 resulted in amino acid serine compared to asparagine in other isolates. In contrast, amino acid valine was detected as a result of nucleotide change at position 823 for UPM315 isolate compared to amino acid isoleucine among the other six FeMV-Malaysia isolates.

Table 5  
Summary of BLAST search for partial FeMV-N gene sequences

Sample ID/ Accession no.	Identities similarity X/Y (%)	Accession number for reference strain	Remark for reference strain
UPM23 MN264638	1595/1608 (99%)	LC036587	Feline morbillivirus <i>N, P/V/C, M, F, H, L</i> genes for nucleocapsid protein, phosphoprotein, matrix protein, fusion protein, hemagglutinin, protein, RNA polymerase, complete CDS, strain: SS3
UPM52 MN264639	1567/1584 (99%)	LC036587	Feline morbillivirus <i>N, P/V/C, M, F, H, L</i> genes for nucleocapsid protein, phosphoprotein, matrix protein, fusion protein, hemagglutinin, protein, RNA polymerase, complete CDS, strain: SS3
UPM53 MN264640	1563/1578 (99%)	MN164531	Feline morbillivirus isolate CTL16, complete genome
UPM210 MN264641	1565/1580 (99%)	MN164531	Feline morbillivirus isolate CTL16, complete genome
UPM231 MN264642	1603/1618 (99%)	MN164531	Feline morbillivirus isolate CTL16, complete genome
UPM305 MN792827	1547/1564 (99%)	LC036587	Feline morbillivirus <i>N, P/V/C, M, F, H, L</i> genes for nucleocapsid protein, phosphoprotein, matrix protein, fusion protein, hemagglutinin, protein, RNA polymerase, complete CDS, strain: SS3
UPM315 MN792828	1587/1607 (99%)	MN164531	Feline morbillivirus isolate CTL16, complete genome

Note. X: Total nucleotide similarities between FeMV-N gene Malaysia isolates and FeMV-N gene of reference strains in GenBank; Y: Total nucleotide of FeMV-N gene of reference strains in GenBank; CDS: Coding DNA sequence

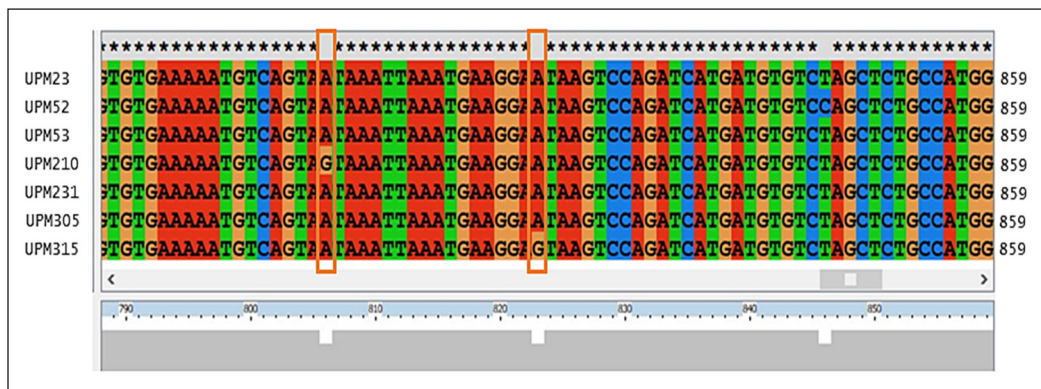


Figure 2. Multiple alignments of nucleotide detected in *N* gene from FeMV-Malaysia isolates. Boxes indicate nucleotide differences for UPM210 and UPM315 at positions 806 and 823, respectively, among the sequenced FeMV-Malaysia isolates. The position of the nucleotides is at the bottom, while the number of nucleotides for each isolate is presented to the right of each isolate. The asterisk (\*) symbol represents identical nucleotides among the isolates

### Nucleocapsid Protein Analysis

In this work, 320 amino acids were successfully encoded, compared with 519 amino acids that should be encoded when a full *N* gene is sequenced. One highly conserved sequence motif among paramyxoviruses from Sequence 3 was indicated in the red box in Figure 3. Comparison between amino acid residues for FeMV-Malaysia isolates and other FeMV isolates, along with species from the

same genus of *Morbillivirus*, demonstrated consistent conserved amino acid residues (as underlined in Figure 3) in all species included in the alignment. In addition, the amino acid residue PPRV and RPV at position 328 was detected as alanine, whereas in other isolates, the amino acid at the same position was serine. Due to the nucleotide differences, wherein PPRV and RPV, nucleotide GCC was detected, compared with other isolates whereby nucleotide TCT was detected.

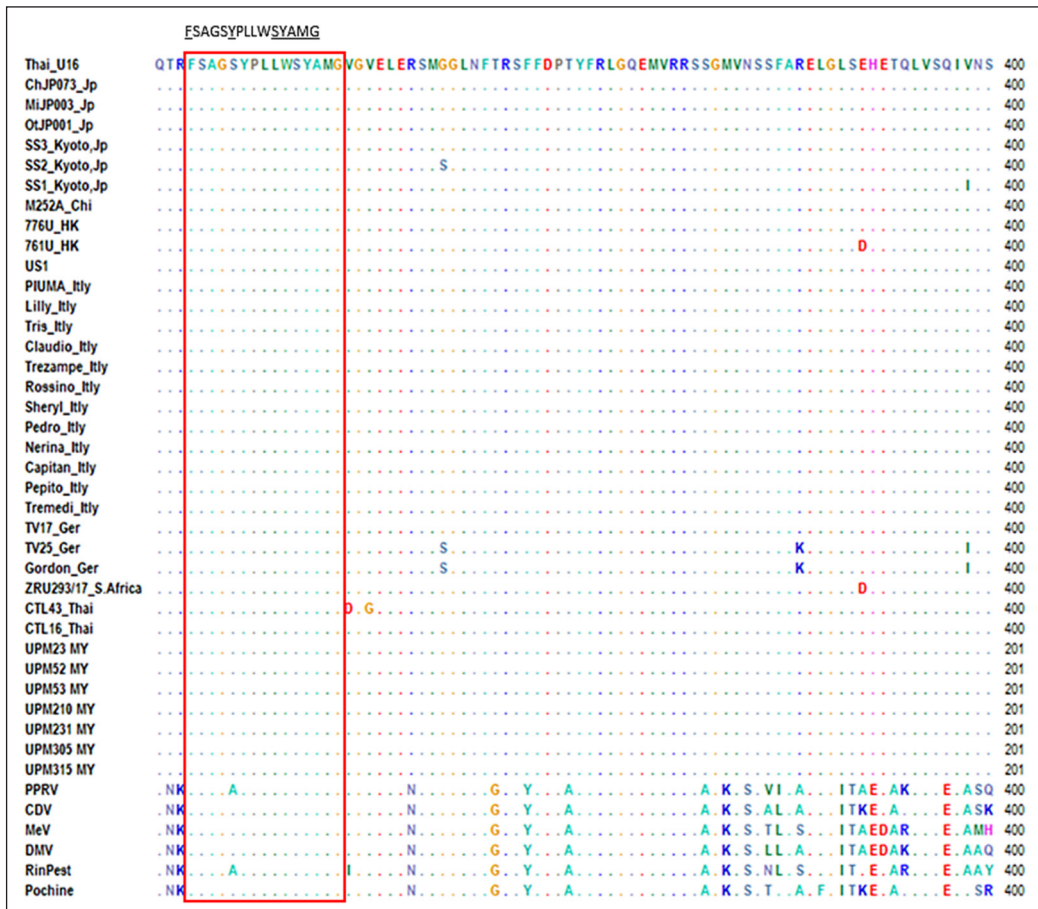


Figure 3. Multiple alignments of N protein amino acid residues of FeMV and other morbilliviruses sequences. Box indicates conserved motifs in paramyxoviruses. Amino acid residue numbers for each protein isolate are presented to the right of each isolate. Dots denote the identical amino acid residues between each protein isolate



**Phylogenetic Tree Analysis of Partial FeMV-N Gene Sequences of Malaysia Isolates**

Phylogenetic analysis was conducted to review the evolutionary relationship between isolates from different countries. All partial FeMV-N gene Malaysia isolates (UPM210, UPM231, UPM305, and UPM315) showed >99% identical sequence with the isolates

detected in the previous study: UPM23, UPM52, UPM53; thus, these seven isolates were clustered together within the same clade (Figure 4). In addition, FeMV-Malaysia isolates showed a close relationship to isolates from Thailand (Thai-U16, CTL16, and CTL43) and China (M252A) with 98% nucleotide similarities while sharing 98%–99% nucleotide similarities with three of

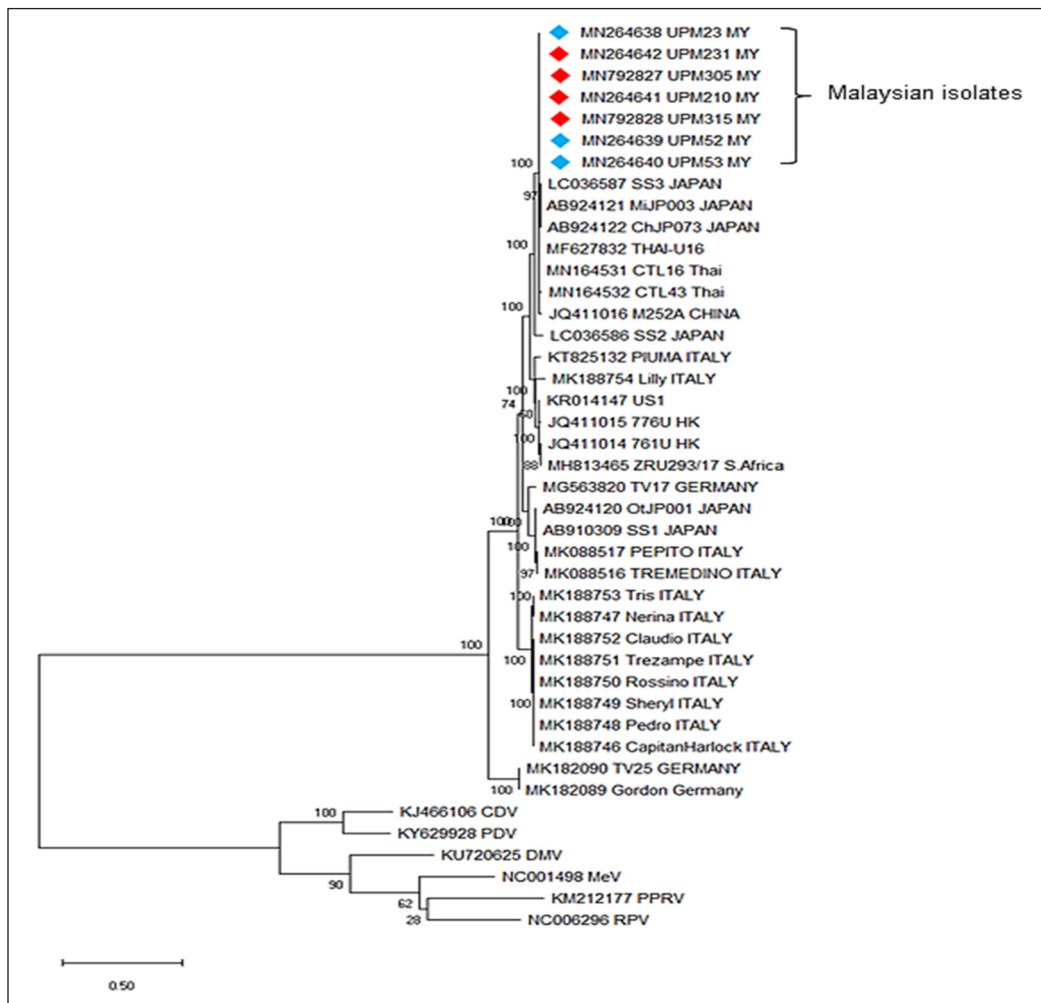


Figure 4. Phylogenetic analysis based on partial FeMV-N gene sequences of Malaysia isolates. FeMV-N gene Malaysia isolates detected in this study, which clustered together, are represented by red diamond shape (◆) (Makhtar et al., 2021) and blue diamond shape (◆) (Mohd Isa et al., 2019). The evolutionary analysis was constructed using the maximum likelihood method based on the general time-reversible model using MEGA X (version 10.0.05) software. The scale represents the number of substitutions per site

Japan isolates (SS3, MiJP00, and ChJP073). However, the SS2 isolate from Japan showed only 94% nucleotide similarities with Malaysian isolates, of which it formed a different branch within the phylogenetic tree. Furthermore, comparing with BLAST search of nucleotide similarity result, it also can be noted that isolates (SS3 and CTL16) that recorded >98% similarity with FeMV-*N* gene Malaysia isolates detected in this study were closely related to each other, illustrating a consistent finding with the outcome from BLAST search. In addition, another genotype of FeMV, FeMV genotype 2 (FeMV-GT2), established its cluster with two other isolates: TV25 and Gordon. All 29 FeMV isolates available in GenBank also formed a distinct clade separated from the outgroup isolates, comprising other members of the genus *Morbillivirus*.

## DISCUSSION

Feline morbillivirus has been implicated as the cause of interstitial nephritis associated with chronic kidney disease in cats; however, further evidence is needed to prove Koch's postulate of FeMV infection (de Luca et al., 2021). Nevertheless, many countries have reported the occurrence of FeMV among cats; therefore, the importance of this virus should not be undermined. Upon successful detection of FeMV among cats in Malaysia, further characterization of these FeMV isolates is warranted. However, an attempt to obtain a full sequence of the FeMV-*N* gene from this study had been unsuccessful, as none of the primer sets designed could amplify the leader region

of the *N* gene. Nevertheless, BLAST search and sequencing analyses of partial *N* gene were performed on seven positive samples from previous studies: UPM23, UPM52, UPM53, UPM210, UPM231, UPM305, and UPM315 (Makhtar et al., 2021; Mohd Isa et al., 2019). Furthermore, a BLAST search showed a high similarity percentage between isolates obtained from a study by Makhtar et al. (2021) with previously detected FeMV-Malaysia isolates, as the samples were collected within the same geographical area. From the BLAST result of 29 FeMV isolates from other countries, a high similarity (98%–99%) was observed with FeMV isolates from China, Japan, and Thailand, indicating that FeMV isolates from Malaysia have been circulating in the Asian region. Besides that, the factor of Asian climate may also play a role in transmission and adaptation of the virus, which could contribute to the high similarity percentage among the isolates from Asian countries, compared with that from other countries (Wu et al., 2016).

Based on the nucleotide alignment of the partial *N* gene of the seven FeMV-Malaysia isolates, two different amino acid substitution site was detected, each one within isolate UPM210 and UPM315. This amino acid difference might be due to the position of the nucleotide where it lies at the structurally variable region of the  $N_{TAIL}$  domain, which comprises approximately 120 to 150 amino acid residues (Communie et al., 2014). This finding is also in agreement with a study on FeMV-N protein by Woo et al. (2012), whereby at both positions (806 and 823), amino acid substitutions were

detected among the FeMV isolates from China and Hong Kong. Even though RNA virus does frequently undergo mutation by approximately one mutation per virus genome copy to combat the host immune response, the mutation usually would not interfere with the essential virus function, and if it does, it will result in positive or negative selection, which leads to a superior mutation that enables the virus to survive despite the replication of the host immune response (Fleischmann, 1996). On the other hand, if the mutation damages the virus's essential function, a negative selection will occur, whereby the mutation will be deleted. Nevertheless, due to the redundancy of genetic code, most mutations are neutral, demonstrating no changes towards protein function, as the amino acid remains unchanged. However, to assess any mutation for the FeMV-Malaysia isolates, whole genome sequencing should be done to compute the pairwise alignment of FeMV isolates. This study focused only on N protein, so calculating the percentage of nucleotide similarities for the other five open reading frames (ORFs) for FeMV could not be accomplished. Based on the phylogenetic analysis, even though whole genome sequencing was not done, it is noteworthy that the FeMV-Malaysia isolates formed a distinct clade with the previously introduced new genotype of FeMV, FeMV-GT2, which was detected in Germany (Sieg et al., 2019).

Nucleoprotein (N) comprises of the  $N_{CORE}$  which is the N-terminal moiety covering the first 400 amino acids of N protein and the  $N_{TAIL}$  domain which is the

C-terminal moiety (Thakkar et al., 2018).  $N_{CORE}$  is responsible for RNA encapsidation, a prerequisite for viral RNA synthesis. Amino acid analyses identified conserved motifs within the *Paramyxovirinae*, which are Region 1, 172-QxW(I,V)xxxK(A,C)xT-184; Region 2, 268-FxxT(I,L)(R,K) $\Omega$ (G,A) (L,I,V)xT-280; and Region 3, 323- FxxxxYPxx $\Omega$ S $\Omega$ AMG-339, where x indicates any amino acid residue,  $\Omega$  indicates an aromatic amino acid residue, and either one of the residues in parentheses (Morgan, 1991). On the other hand, the interaction of N and P proteins is mediated by the  $N_{TAIL}$  region of approximately 120 to 150 amino acids of the structurally variable region (Communie et al., 2014). During the initial stage of infection, the RNA-dependent RNA polymerase (RdRp) complex will only recognize and synthesize viral RNA, which is encapsidated by N protein as a template (Sourimant & Plemper, 2016). Although the N gene is quite conserved compared to the F and H genes, given the importance during early infection, the characterization of the N gene may give further insight into the newly emerging FeMV infection in cats.

However, from the three highly conserved regions of the N protein, only one conserved region was obtained in this study. The conserved sequence (amino acid residue at a position between 324–328) showed a similar amino acid residue in alignment with 29 other FeMV isolates from different countries, consistent with a previous study (Woo et al., 2012). Moreover, within the conserved Region 3, a different amino acid residue, alanine (A), was detected in RPV and PPRV at position

328, whereas other isolates resulted in serine (S) amino acid residue. However, there was little information on whether the amino acid difference played a significant role in RNA binding and forming the helical nucleocapsid; hence, there is a need for future *in vitro* study elucidating the effects of amino acid changes towards the nucleocapsid role. In addition, the full N protein sequence analysis is warranted by designing a primer set that targets the leader region.

## CONCLUSION

Sequences of ~1.5kb from seven FeMV-Malaysia isolates were obtained through conventional RT-PCR detection from selected urine samples. A BLAST search of the partial *N* gene of FeMV-Malaysia isolates (UPM23, UPM52, UPM53, UPM210, UPM231, UPM305, and UPM315) revealed to have had a high percentage of nucleotide similarities with isolates from Japan (SS3) and Thailand (CTL16). From phylogenetic analysis, the seven FeMV-Malaysia isolates are clustered together with other Asian countries, such as Thailand (Thai-U16, CTL43, CTL16) and Japan (SS3, MiJP003, ChJP073). In addition, nucleotide alignment of the seven FeMV-Malaysia isolates detected 19/962 variable sites, of which only two isolates, UPM210 and UPM315, had amino acid substitution. From the alignment of amino acid residues of FeMV-isolates and other morbilliviruses, consistent amino acid residues were observed in one of the conserved regions in morbilliviruses. Therefore, to assess for any genetic change, it

is suggested to perform sequence alignments and phylogenetic analysis from other genes, especially on *F* and *H* genes, which are more heterogeneous and prone for recombination.

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