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# Clinical Study and Rapid Detection of Feline Parvovirus in Suspected Cats by Polymerase Chain Reaction Method

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**Abstract**

The aim of this research was to detect the presence of Feline Parvovirus (FPV) in blood samples of FPV suspected cats by Polymerase Chain Reaction (PCR) method. This research used eleven fresh blood samples of FPV suspected cats which were obtained from the Internal Medicine Laboratory of Faculty of Veterinary Medicine at UGM, Yogyakarta, Clinic Pet Care in Semarang and Medania Private Clinic in Yogyakarta. The DNA was first extracted from the blood using DNA isolation kit. Then the template of DNA was used for amplification by PCR method using CPV primers to target the Viral Protein-2 (VP-2) encoding gene. The PCR products were then visualized using 1% Agarose Gel electrophoresis and UV-Transilluminator. A positive result was indicated by the appearance of a DNA fragment in size of 518 bp which can be interpreted as the presence of FPV in the obtained blood samples. PCR products were then sent for sequencing to determine the nucleotide sequence of the VP-2 gene. The sequences obtained were aligned using multiple alignment method with three FPV isolates obtained from the database of Genebank using the MEGA6.06 software program. From the eleven cat blood samples obtained, 8 samples indicated positive with FPV infection. These results showed that PCR method can be used to detect FPV in samples derived from blood specimens from FPV suspected cats. Conventional PCR method was also used to confirm the cause of the symptoms shown by the infected cats as some of the symptoms such as gastroenteritis is quite common among many other viral infection.

**Key words;** Feline Parvovirus (FPV), VP-2 gene, PCR, DNA sequencing,

## Introduction

Animal parvoviruses, belonging to the genus Parvovirus within the family Parvoviridae, are usually responsible for acute gastroenteritis and leukopenia in young animals. Within the family Parvoviridae, there are two subfamilies, Parvovirinae and Densovirinae. Parvovirus species have been identified and named from isolates, corresponding to the occurrence in specific animal hosts belonging to the mammalian orders Carnivora and Rodentia. Hence, parvoviruses have been classified according to their host range: Feline Parvovirus (FPV), Mouse Minute Virus (MMV), Mink Enteritis Virus (MEV) and Canine Parvovirus (CPV) (Hueffer and Parrish, 2003; Steinel et al., 2001).

Feline Parvovirus has been known since the 1920s, whereas CPV only emerged as a dog pathogen in the late 1970s. Evidence suggests that the original type 2 (CPV- 2) came from FPV, showing a genome homology of 98%, and gave rise to the antigenic variant CPV-2a through five or six amino acid mutations in the major capsid protein VP2 during 1979–1981. A second variant, CPV-2b, was identified in 1986, which naturally infects dogs and cats (Parrish et al., 1991). A third variant, CPV-2c, was discovered in Italy most recently in 2000, displaying an exceptional ability to spread rapidly through the canine population in that country (Buonavoglia et al., 2001; Martella et al., 2005) as well as in other European countries, Asia (Nakamura et al.,2004) and America (Kapil et al., 2007).

All viruses grouped in the family *Parvoviridae* are non-enveloped, isometric particles, 18-26 nm in diameter. The icosahedral parvovirus particles consist of a single copy of a linear, non-permuted DNA molecule, 4-6 kb in size and with a molecular weight of 1500-2000 kDa (Cassinotti et al., 1993). The negative stranded viral genome encodes for two major proteins: the non-structural protein (NS), and the viral capsid protein (VP). Together, the capsid confers much stability upon the virus particles, allowing for resistance to inactivation by pH ranges 3-9, lipid solvents, or temperatures up to 60 °C. Parvoviruses are thus among the most resistant viruses known making it difficult to eliminate their infectivity from biological materials such as blood and blood products. (Lauren et al., 1994). The terminal region of the genome consists of palindromic sequences at both the 3' and 5' ends that fold back to form "hairpin" structures that are stabilized by self-hydrogen bonding. These "hairpin" structures are critical for genome replication (Berns, 1990).

The first outbreaks of the disease in captive felids were reported in the 1930's and 1940's. The epidemiology of FPV is characterized by an acute infection with shedding of high virus titers in the feces of diseased animals. The virus is ubiquitous because of its contagious nature and capacity for persistence in the environment. Unvaccinated kittens that acquire maternally derived antibody (MDA) through colostrum are usually protected for up to 3 months of age (though longer duration of MDA to 20 weeks sufficient to interfere with vaccination has been reported). Virus shedding usually lasts only 1 to 2 days, but virus can stay infectious in the environment for weeks or even months. FPV is maintained in a population by its environmental persistence rather than prolonged viral shedding; hence, fomites play an important role in disease transmission because of prolonged survival of the virus on all sorts of contaminated surfaces, such as shoes, hands, food dishes, and bedding (Green et al., 2006).

The incubation period before the onset of clinical signs is usually 4-5 days, and the clinical course can rapidly progress to death. Due to virus properties and needs, the primary pathologic site for viral replication is within the intestinal crypts, resulting in enteritis and diarrhea due to malabsorption and increased permeability. The virus affects cells located deep in the intestinal crypts, while differentiated absorptive cells on the surface of the villi are non-dividing and are not affected. Thymic atrophy and extensive damage to all white blood cell populations and precursors, resulting in severe leukopenia are also common consequences of FPV infections (Lamm et al., 2008).

Feline Parvovirus infection is a huge problem to cats all around the world. It is an infectious disease which transmits fast from cat to cat through direct or indirect contact. The incredibly fast transmission between infected cats and susceptible cats causes a great loss financially and emotionally to both cat breeders as well as cat owners. Due to its fast transmission characteristic among highly susceptible cats, it has become very important to diagnose the virus quickly as a control measure to prevent further spread of the disease and to determine the appropriate treatment for infected cats.

Tentative diagnosis of FPV infection is done by anamnesis; questioning the patient's owner about the history of the patient as well as the onset of clinical signs and by physical examination based on the signs. However, these methods do not assure a confirmed diagnosis of FPV. The most prominent clinical sign, gastroenteritis is a very common sign shown by many other enteric diseases, such as Feline Infectious Peritonitis, Feline Leukemia Virus, parasitic infections (*Toxocara cati, Dipylidium caninum, Ancylostoma tubaeformae*), protozoan gastroenteritis (*Giardia spp*), and bacterial gastroenteritis (*Salmonella spp, E.coli, Campylobacter spp*).

Various laboratory methods can also be used to diagnose FPV in infected cats. Hematological examination, direct hemagglutination of red blood cells by virus, hemagglutination-inhibition test to determine the presence of antibody in the sample, antigen-capture enzyme immunoassay or immunofluorescence (ELISA) for the detection of antigen in tissues, and polymerase chain reaction (PCR) assay for the detection of viral DNA in tissues (Decaro et. al., 2005). Of all the methods mentioned above, PCR is widely used to detect the presence of various viruses which then gives a definite, accurate and fast diagnosis for viral

infection in suspected animals. In this research, the PCR method was used to determine the presence of FPV virus in blood samples collected from FPV suspected cats.

The high emergence of FPV infected cats in Indonesia is a problem which needs to be further examined. With a mortality rate of 90% in kittens, the virus is usually diagnosed at a very late stage of infection making it difficult to treat the infected cat (Levy et al, 2009). Most of the time, the clinical signs are mistaken for other gastroenteritis related diseases. Vaccines have been created to combat the disease but it is still very prevalent in Indonesia. There are still very few evidence suggesting that CPV emerged from FPV as a host range variant through evolution of its nucleotide sequence. So in this research, specific primer for VP-2 protein of CPV-2 is used to determine the presence of FPV through PCR method to analyze the relationship between the two viruses.

Molecular based diagnosis such as PCR has a few advantages in terms of speed, sensitivity and specificity making it appropriate for diagnosing viral infection. Thus, the objective of this research was to expand the technique of rapid diagnosis by using PCR method to detect the presence of FPV in blood samples obtained from FPV suspected cats. This research was also conducted to analyze the relationship between canine parvovirus and feline parvovirus as there has been evidence through various journals about canine parvovirus emerging from its probable ancestor of FPV.

## Materials and Methods

A total of eleven fresh blood samples of 1 ml each sample was collected from cat patients showing clinical signs of FPV infection. The eleven blood samples were obtained from three different places; five samples were obtained from the Internal Medicine Laboratory of Faculty of Veterinary Medicine at UGM, Yogyakarta, four samples were collected from Clinic Pet Care in Semarang, and two samples were collected from Medania Private Clinic in Yogyakarta.

### Preparation of DNA Template and Primer Design

DNA isolation was done using a DNA extraction Kit (Genomic DNA mini kit GeneAid (catalog number: GB100, Lot #ZL11604) which comprised of GeneAid RBC Lysis Buffer, GB buffer, Elution Buffer, absolute ethanol, WI buffer, and Wash Buffer which had ben added with ethanol. DNA amplification was done using the conventional PCR kit (KAPA2G Fast PCR Kit, with the catalog number KK5008) which comprised of 5X KAPA2G Buffer A (containing 1.5 mM of $Mg^{2+}$), dNTP Mix (10 mM) and $MgCl_2$ (25 mM), H2O (PCR grade), Eurican 6 vaccine (product from Romindo Primavet com) as positive control, and a pair of primers (forward and reverse) which can be seen in the Table 1.

For the electrophoresis, the materials used were Agarose Gel UltraPure™ (Invitrogen) with the catalog number: 16500-100, TBE buffer (Tris base-Boric acid-EDTA) 1X, Cybersafe DNA Stain from 1st BASE, Blue Loading Dye (catalog number: 10816-015) and 100 bp DNA *Ladder* (catalog number 15628-019).

### DNA Amplification

The DNA extracted from the blood samples of FPV suspected cats were used as templates in the PCR method to amplify the VP-2 gene of the FPV. The components used in PCR method were the extracted DNA from the blood samples of FPV suspected cats, a pair of CPV-2B primers (forward and reverse) with the concentration of 10 pmol/µl each, dH2O and the 5xKAPPA2G conventional PCR kit which contains Buffer A and dNTP mix. The mixture of components which were used for a single PCR process was 25 µl. The compositions of the components used were 2 µL of DNA template, 1 µL of Forward Primer, 1 µL of Reverse Primer, 12.5 µL of PCR Master mix, and 8.5 µL of H2O.

**Table 1.** Primer of VP-2 gene found in *Canine Parvovirus* (Zhang *et al*., 2010)

| Targat Gene | Primer Sequence | PCR Product |
|---|---|---|
| Viral Protein-2 (VP-2) | F:5'-TCCAGAAGGAGATTGGATTC-3'<br>R: 5'TTCTAGGTGCTAGTTGAGATT-3' | 518 bp |

The PCR process starts with the initial denaturation step at 94 ºC for 5 minutes. The amplification process was achieved by means of 40 cycles of denaturation stage which is done at 94 ºC for 45, annealing which is done at 54 ºC for 45 seconds and elongation which is done at 72 ºC for 45 seconds. Finally the PCR process ended with a final elongation step at 72 ºC for 5 minutes followed by soaking at 4 ºC to bring back down the temperature. Commercialized Canine Parvovirus (CPV) vaccine (Eurican 6) was used as the positive control while aquabidest ($dH_2O$) was used as the negative control.

**Electrophoresis of PCR Products**

1% Agar Rose Gel was used as a medium in the electrophoresis process to view the PCR products that have been amplified. The components that were used in the making of the 1% gel were 0.5 g of Agar Rose Powder and 50 ml of Tris Boric Acid EDTA (TBE) solution diluted 10x which were mixed in a 100 ml Erlenmeyer flask and covered with a plastic cling wrap. The flask was then placed in a microwave for 60 seconds and was left to cool once it was taken out. The slightly cooled agarose gel solution was then added with 4 µl of Sybersafe DNA stain using a micropipette. The gel was then poured into a 50 ml gel casting tray which has been taped at the sides and ends. The samples comb was placed into the gel casting tray carefully and it was left to cool at room temperature for an hour. Once the gel was solidified, the sample combs were carefully removed and the casting tray was then placed into the electrophoresis chamber filled with 1x TBE solution. Components that were used to load each well of the gel were 3.5 µl of aquabidest ($dH_2O$), 1.5 µl of loading dye, 5 µl of template and 5 µl of control positive and control negative each. Once the components were mixed, the 10 µl of mixture was loaded into each well in the right order using a pipette. In the first and last well, 5 µl of 100 bp ladder which acts as the marker was loaded followed by the positive control mixture in the second well and the negative control mixture in the third. Once all the template mixtures were loaded, the machine was turned on and left to run for 30 minutes with a 100 volt current running through.

The results of electrophoresis was then observed using a UV *Transilluminator* ($\lambda$= 260 nm). Positive results were shown when there were appearances of DNA bands the size of 518 bp which can be interpreted as the presence of VP-2 gene of FPV.

**DNA Sequencing**

PCR products that showed positive results (appearance of band) were then sent for sequencing at PT. Genetika Science Indonesia to determine the nucleotide sequence of the VP2 gene of the virus. The sequenced samples were then compared with the following FPV sequence retrieved from the database of Genebank (Accession number: KJ813895, KJ813894.1, KJ813893.1) by multiple alignment using the Molecular Evolution Genetics Analysis (MEGA) 6.06 software program. The nucleotide base sequences and amino acid alignments were analyzed to observe any changes in the base pair. Using the MEGA 6.06 program, a phylogeny tree was constructed using the neighbour- joining method. A bootstrap analysis with 1000 replicates was done to assess the confidence level of the branch pattern. The same program was used to calculate pairwise genetic distances by using the Kimura's two-parameter model.

## Results and Discussion

**Clinical Diagnosis and Hematological Examination**

This research used 1 ml of eleven fresh blood samples which was obtained from cat patients showing clinical signs of FPV. Patient data obtained is shown in Table 2.

Most of the symptoms shown by all eleven cat patients from which blood samples were taken from include anorexia, vomiting, bloody diarrhea, dehydration and fever. The clinical signs leads to a diagnosis inclined towards Parvovirus infection. The figure 1 showed one of the patients from which the blood sample was taken.

A routine blood test/ hematological examination was done on one of the suspected cat's blood sample (sample V2). The results obtained are compared with the normal hematological values of cats and shown in Table 3.

**Table 2.** Data of the cat patients from which blood samples were taken from.

| No | Code | Date of collection | Clinic | Signalmen & Clinical signs |
|---|---|---|---|---|
| 1. | V1 | 15/04/2014 | Internal Medicine Laboratory | Female, 2 years, vomiting, serous discharge from nose, T: 40.3ºC |
| 2. | V2 | 15/04/2014 | Internal Medicine Laboratory | Female, 8 months, inappetence, vomiting, diarrhea, T: 40 ºC |
| 3. | V3 | 15/04/2014 | Internal Medicine Laboratory | Male, 4 months, bloody diarrhea, vomiting, anorexia, T: 39.8ºC |
| 4. | V4 | 26/04/2014 | Internal Medicine Laboratory | Male, 1 year, anorexia, vomiting, dehydration, T: 40 ºC |
| 5. | V5 | 26/04/2014 | Internal Medicine Laboratory | Female, 6 months, bloody diarrhea, vomiting, dehydration, T: 39.9ºC |
| 10. | V10 | 13/06/2014 | Medania Private Clil | Female, 10 months, vomiting, |
| 11. | V11 | 23/06/2014 | Medania Private Clinic | Male, 1 year, anorexia, bloody diarrhea, vomiting, T:40.2 ºC |

**Figure 1.** One of the kitten suspected of Feline Parvovirus infection showing clinical signs such as diarrhea (indicated by yellow arrow), emaciation and weakness.

Based on the results shown above, the suspected cat can be diagnosed with leukopenia, a decrease in white blood cell count. This symptom occurs due to the damage of the hematopoietic precursor cells found in the bone marrow and lymphoproliferative tissues of the infected cat. It can also be seen that there is a drop in the neutrophil value as well as the lymphocyte value. The supply of leucocytes (especially neutrophils) is inadequate for the inflamed intestines (Goddard et al., 2008).

## Viral DNA Extraction and VP-2 Gene Amplification.

The location of the primer used for PCR amplification according to Zhang et al. (2010) based on alkaline sites are as follows: forward primer is located on-site from base 1727 to 1746,

**Table 3.** The table shows a comparison of normal feline hematological values with the blood sample of cat suspected of having FPV.

| No. | Analyte | Unit | Normal hematology | Suspected Cat (V2) |
|---|---|---|---|---|
| 1. | RBC | $x10^{12}/l$ | 5.0-10.0 | 7.3 |
| 2. | Hemoglobin | g/dl | 8-15 | 13.8 |
| 3. | Leucocytes | L | 5000-20000 | 4350 |
| 4. | Neutrophils | L | 2500-12500 | 2258 |
| 5. | Lymphocytes | L | 1500-7000 | 392 |
| 6. | Monocytes | L | 0-800 | 217 |
| 7. | MCV | Fl | 37.0-55.0 | 45 |
| 8. | MCHC | g/dl | 30.0-35.0 | 41.8 |

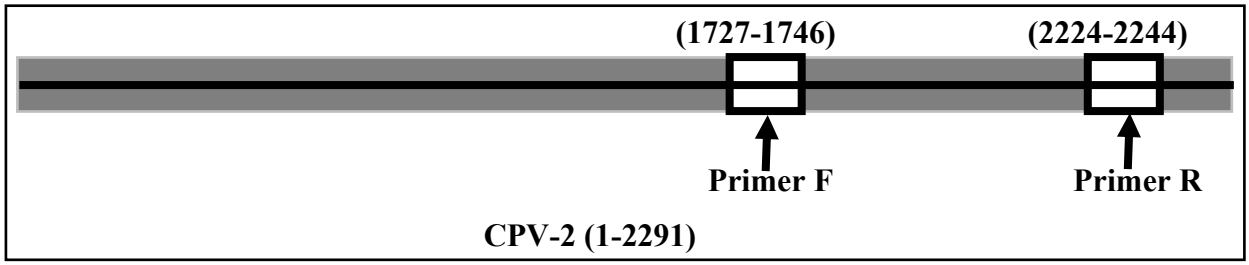


**Figure 2.** Annealing sites of VP-2 primer Forward and Reverse (Zhang et al. 2010).

while the reverse primer is located at the base site from 2224-2244. More details can be seen in Figure 2.

All 11 blood samples which were isolated using the DNA extraction kit were then amplified using the PCR method. The VP2 gene of the FPLV strains were successfully amplified using specific forward and reverse primers of CPV and the results were visualized by using 1% agarose gel electrophoresis. The obtained results can be seen in Figure 3.

The results of PCR amplification of VP-2 gene found in blood samples from patients which were clinically diagnosed with FPV which can be seen in lane 2,3,4,5,7,8,10 and 11, it can be concluded that the cats were positively infected with FPV. Lane number 1, 6 and 9 do not indicate the presence of DNA fragments sized 518 bp, so it can be concluded that the blood samples were not amplified with VP-2 gene of FPV, thus no infection occurred in the cats.

The gene targeted to be amplified in the PCR process was the gene encoding the structural protein VP-2. The VP-2 protein makes up 54-55 (out of 60) protein subunits which forms the non-enveloped capsid. With non-enveloped protein capsids of around 260 A˚ diameter, constructed in the simplest icosahedral form (T = 1), these remarkably dense and rugged particles deliver their enclosed genomes into the cell, traverse the cytoplasm, and penetrate the nucleus while still comprising a structurally intact, albeit somewhat rearranged, capsid (Farr et al., 2006; Sonntag et al., 2006). It can be concluded from the amplification of the VP2 gene done by PCR method that samples V2, V3, V4, V5, V7, V8, V10 and V11 have the VP2 gene fragment which induces the neutralizing antibody reaction from the host's immune system.

The conventional PCR method used in this research has been widely used all over the world to diagnose the causative agents of an infectious disease. Once positive results (appearance of DNA bands) are obtained from the PCR method, the next step would be to sequence the samples to compare the nucleotide base sequence of the VP-2 gene with samples obtained from the database of Genebank.

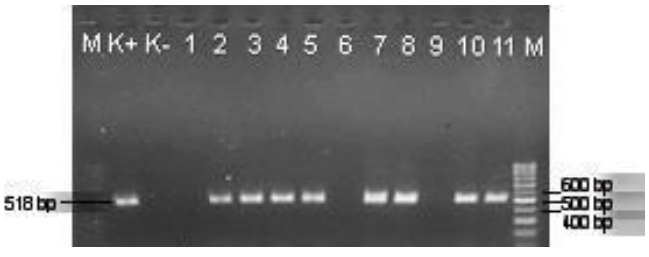


**Figure 3.** Agarose gel electrophoresis of PCR products. M = marker, K + = positive control, K- = negative control, no. 1-11: samples. DNA bands are seen in all lanes except the lane number 1, 6 and 9. The strips of DNA that appeared are of the size 518 bp. Size is determined by seeing lane M which is the Ladder marker of DNA where each band is the size of 100 bp.

**Table 4.** Recapitulation of the results of PCR amplification of the VP2 gene of FPV

| No. | Sample Code | Results of PCR Amplification |
|---|---|---|
| 1. | V1 | Negative |
| 2. | V2 | Positive |
| 3. | V3 | Positive |
| 4. | V4 | Positive |
| 5. | V5 | Positive |
| 6. | V6 | Negative |
| 7. | V7 | Positive |
| 8. | V8 | Positive |
| 9. | V9 | Negative |
| 10. | V10 | Positive |
| 11. | V11 | Positive |

The recapitulation of the results of PCR amplification of the VP2 gene of Feline Parvovirus is summarized in Table 4.

Table showed that it can be concluded that eight out of eleven samples confirmed the presence of VP-2 gene of FPV. Thus, this shows that 73% of the cats showing clinical signs of FPV infection were positively diagnosed with FPV infection.

## DNA Sequence analysis

All eight positive samples were sequenced because the DNA bands of all were amplified clear and well. The eight samples sequenced were V2, V3, V4, V5, V7, V8, V10, V11 and K. K is a positive control using vaccines Eurican 6 (Romindo Primavet.com). The full-length sequences of the VP2 gene (1755 nucleotide) of the Feline Parvovirus analyzed were obtained by assembling the nucleotide sequences using the MEGA 6.06 program. Amino acid translation confirmed that the sequences encoded a VP2 protein of 584 amino acids. The sequences obtained were aligned using multiple alignment method with 3 FPV samples obtained from the database of Genebank as shown in table 5 below.

**Table 5.** FPV samples obtained from database of Genebank with accession number

| No. | Isolate Name | Accession number |
|---|---|---|
| 1. | FPV.Racoon1 | KJ813895.1 |
| 2. | FPV.Racoon2 | KJ81 3894.1 |
| 3. | FPV.Bobcat | KJ813893.1 |

Once the sequenced samples were aligned using multiple alignment with the three samples obtained from the Genebank, the homology between them can be observed. The results show that there are 17 changes in nucleotide positions encountered by the VP-2 sequences between the isolates from the Genebank and the sequenced samples. These substitutions are called Single Nucleotide Polymorphism where a single nucleotide is replaced with another. For example, a SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T). These changes may or may not affect the sequence of amino acids in the protein produced because it depends on the codon formed after the substitution. This is because some codons still produce the same amino acid despite having different base pairs. Substitution of two nucleotides can be observed between the isolates from the Genebank which is between the first two isolates, raccoon AIV52366.1, raccoon2 AIV42365.1 and the Bobcat AIV42364.1 at position 65 with the change T→C and position 295 with the change A→G. This causes the small percentage of genetic differences between the 3rd isolate (bobcat) with the first two (raccoon) which can be seen in the Table 6.

The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Kimura 2-parameter model. The analysis involved 11 nucleotide sequences, 3 of which were FPLV isolates from the Genebank and 8 were test samples that were sequenced. There

**Table 6.** Estimates of evolutionary divergence between sequences

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. raccoon AIV42366.1 | | | | | | | | | | | |
| 2. raccoon2 AIV42365.1 | 0.000 | | | | | | | | | | |
| 3. bobcat AIV42364.1 | 0.004 | 0.004 | | | | | | | | | |
| 4. 1675960 V2 PrF | 0.014 | 0.014 | 0.010 | | | | | | | | |
| 5. 1675961 V3 PrF | 0.020 | 0.020 | 0.016 | 0.014 | | | | | | | |
| 6. 1675962 V4 PrF | 0.024 | 0.024 | 0.020 | 0.014 | 0.008 | | | | | | |
| 7. 1675963 V5 PrF | 0.020 | 0.020 | 0.016 | 0.006 | 0.020 | 0.012 | | | | | |
| 8. 1675964 V7 PrF | 0.014 | 0.014 | 0.018 | 0.020 | 0.034 | 0.026 | 0.014 | | | | |
| 9. 1675965 V8 PrF | 0.018 | 0.018 | 0.022 | 0.032 | 0.018 | 0.018 | 0.030 | 0.016 | | | |
| 10. 1675966 V10 PrF | 0.010 | 0.010 | 0.014 | 0.020 | 0.030 | 0.022 | 0.018 | 0.004 | 0.012 | | |
| 11. 1675967 V11 PrF | 0.016 | 0.016 | 0.012 | 0.006 | 0.016 | 0.008 | 0.004 | 0.018 | 0.026 | 0.014 | |

were a total of 518 nucleotide positions in the final dataset. Evolutionary analyses were conducted in MEGA 6.06 software program.

### Design of Phylogeny Tree

A phylogenetic tree was constructed from the full length VP-2 nucleotide sequence of samples and additional 3 sequences retrieved from the Genebank database.

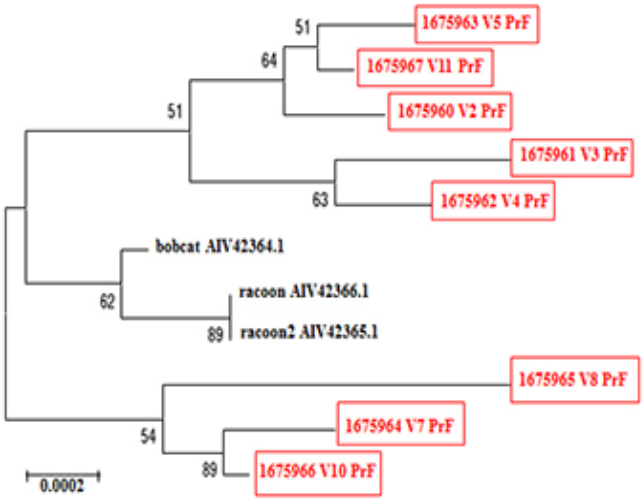


**Figure 4.** Phylogenic relationships among the different parvovirus isolates based on VP-2 nucleotide sequences.

The tree was inferred by the MEGA 6.06 software program using the neighbor-joining method. The type of phylogeny test used was Bootstrap method with 1000 replications. The samples in the green box indicates the eight positive samples that were sequenced.

### Conclusion

Conventional PCR method can be used as a solid diagnosis in determining the presence of VP-2 gene of Feline Parvovirus in fresh blood samples obtained from clinically suspected cats. Conventional PCR method is also used to confirm the cause of the symptoms shown by the infected cats as some of the symptoms such as gastroenteritis is quite common among many other viral infection. Out of the eleven samples which were obtained from FPV suspected cats that were used in this research, the eight samples which showed positive results in the PCR amplification method were V2, V3, V4, V5, V7, V8, V10 and V11 while the 3 samples which showed negative were V1, V6 and V9. The eight positive samples that were sent for sequencing and then aligned using MEGA 6.06 software program showed very minimal nucleotide substitution when compared to three FPV samples obtained from the database of Genebank. This concludes that even though there are many strains of FPV, they are still closely related genetically.

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