Sys Rev Pharm 2020;11(11):1527-1533
A multifaceted review journal in the field of pharmacy

Characterization of Viral Gene Proteins Panleukopenia Local Isolate as a Vaccine Candidate

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ABSTRACT

The purpose of this study was to determine the genetic characteristics of local FPV isolates in Indonesia as a basis for determining the type of FPV to be used as a panleukopenia vaccine material in Indonesia. This research was conducted in March - September 2019. Rectal swab or stool samples were taken, performed PCR and Sequencing, data were analyzed using the BioEdit program, homology analysis and phylogenetic trees using MEGA software. The results of this study found FPV isolates using PCR. In addition, based on the alignment results, the matching results of the sample sequences V1 and V2, respectively, are the Felocell® 3 vaccine and Purevax RCP. Whereas FPV virus field isolates namely V3 - V8 samples showed some differences in sequences namely in sequences 5, 95, 150, 173, and 425. Differences in nucleotide bases in several strains, vaccines and field isolates also showed a clear genetic difference in phylogenetic trees. Alignment of DNA sequences obtained homogeneous in several strains of FPV according to the NCBI database, which is precisely in the VP1 protein, VP2 FPV. The results of sequencing from eight samples showed a match on eight strains of FPV virus in a database with identities ranging from 99.2% - 99.8%. The conclusion from the results of this study is that FPV isolates were obtained and could be used as vaccine candidates for FPV in Indonesia. In addition, further studies on experimental animals are needed.

Keywords: Characteristics, FPV, cats, local isolates, vaccines

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BACKGROUND

Feline Panleukopenia (FPL) caused by Feline Parvovirus or better known as Feline Panleukopenia Virus (FPV) is still a serious disease problem in cats both in Indonesia and in the world. Feline Panleukopenia (FPL) is an infectious disease that causes high mortality (> 90%) with nerve symptoms.[1] Handling this disease in a period of more than 40 years still uses vaccinations developed in the era of the 60s.[2] While FPV continues to adapt and evolve, so there will be an inability of the vaccine to protect cats from the virus attack. This is thought to be due to mutations in the FPV.[3] There are still many cases of cats infected with FPV after vaccination in Indonesia, but the number of events is not recorded, besides that the vaccination failure factor is still based on the assumption that cats are in an unhealthy condition at the time of vaccination. This can occur because of the suspected genetic differences or incompatibility of vaccine virus strains with field viruses. Vaccine that does not protect will create problems and losses because FPV can survive in the environment for many years and will be disinfectant resistant.[4,5]

Based on the facts above that to reduce losses due to post-vaccination FPV infection, research needs to be done by looking at and analyzing the structure of the nucleotide bases and amino acids making up the gene in the FPV so that it can determine the genetic characteristics of local FPV isolates in Indonesia as a basis for determining the type of FPV to be used as a panleukopenia vaccine material in Indonesia.

RESEARCH METHODS

Time and Place of Research

This research was conducted in March - September 2019. Rectal swab or stool samples were taken in East Java. The study was conducted at the Laboratory of Biosciences, Universitas Brawijaya Malang for primary ordering, the ADD (Animal Diagnostic Desease) Laboratory of the Faculty of Veterinary Medicine, Universitas Brawijaya for conducting research.

Rectal Swab Cat Sampling

Rectal swab sampling is performed after the handling procedure is performed. The location of the sample is taken in the rectum of the cat. Samples were taken using a sterile cotton swab and then swabed on the cat's rectum. Samples that have been taken are inserted into a viral transport medium (VTM) tube and labeled according to the individual's name. Samples that have been in the VTM tube are then stored in the freezer. Samples that have been frozen in the freezer are put into the ice box.

Polymerase Chain Reaction

DNA samples from cats infected with the panleukopenia virus were amplified using the PCR method. The pairs of primers used are forward primers (FPV_F) 5'- GCT TAC GCT TCT CTT CT-3 GCT 'and reverse (FPV_R) 5'- GCA TCA ACC AAT GAC CAA GG-3'. Amplification was started by mixing 3 µL DNA, 1.5 µL forward primer 10pmol, 1.5 µL reverse primer 10pmol, 15 µL PCR mix and 9 µL ddH20 into a 200 µL microtube (PCR tube). The amplification step starts from predenaturating 940C for four minutes, denaturating 940C for 30 seconds, then annealing at 550C for 30 seconds. Extension at 720C for 1 minute and post extension at 720C for 7 minutes. The process will repeat for 35 cycles.

DNA sequencing

PCR product sequencing from two directions is done by using the primary FPV_F and FPV_R to see the amplified sequences using the Sanger method. The PCR product shipped is a PCR product that has not been purified with an amount of 40 µL and the concentration of each primer

is 10 pmol. Sequencing results in the form of a graph stating the content of adenine, thymine, guanine, and cytosine contained in DNA fragments that have been labeled by ddNTPs.

Data analysis

The sequencing data in the form of ABI file was edited manually using the BioEdit program, homology analysis and phylogenetic tree using MEGA software.

RESULTS AND DISCUSSION

FPV is a non-zoonotic infectious disease that attacks cats in all ages and has a high mortality rate of 75%. The highest mortality and morbidity occur in cats aged one to twelve months. In the case of acute Panleukopenia the mortality ranges from 25-90% and 100% in silver infection. FPV can cause systemic infections and the most common enteric disease in kittens. The severity of FPV infection depends on age, immunity status, and the presence of secondary infection. Disease can be in the form of sub-clinical infections and per-acute syndromes that can cause sudden death.[6] There are still many cases of cats infected with FPV after vaccination in Indonesia, but the number of events is not recorded, besides that the vaccination failure factor is still based on the assumption that cats are in an unhealthy condition at the time of vaccination. This can occur because of the suspected genetic differences or incompatibility of vaccine virus strains with field viruses. According to Horiuchi[7] and Stuetzer & Hartmann[8] there have been changes in nonstructural protein 1 (NS1) and capsid 2 (VP2) genes based on time, then Garcia R, Castro TX & Miranda[9] and Lin [10] stated that antigenic variations of parvo viruses that attack dogs are identified by the names 2a, 2b, and 2c. Feline Panleukopenia Viruses have been found to infect several hosts with mutations in their capsid proteins.[11,12] Protein capsid mutations in FPV result in changes in the amino acid cysteine into serine at position 273 which can cause infection in new hosts.[13,14]

Table 1. Individual information on Panleukopenia Virus Samples in Cats

No	Name	Age	Gender	Origin	Symbol
1	*Felocell 3* Vaccine	-	-	-	V1
2	*Purevax RCP* Vaccine	-	-	-	V2
3	Lilo	1 year	Female	East Java	V3
4	Happy	5 months	Male	East Java	V4
5	Miko	4 months	Male	East Java	V5
6	Samba	1 year	Male	East Java	V6
7	Molly	5 months	Female	East Java	V7
8	Bona	7 months	Male	East Java	V8

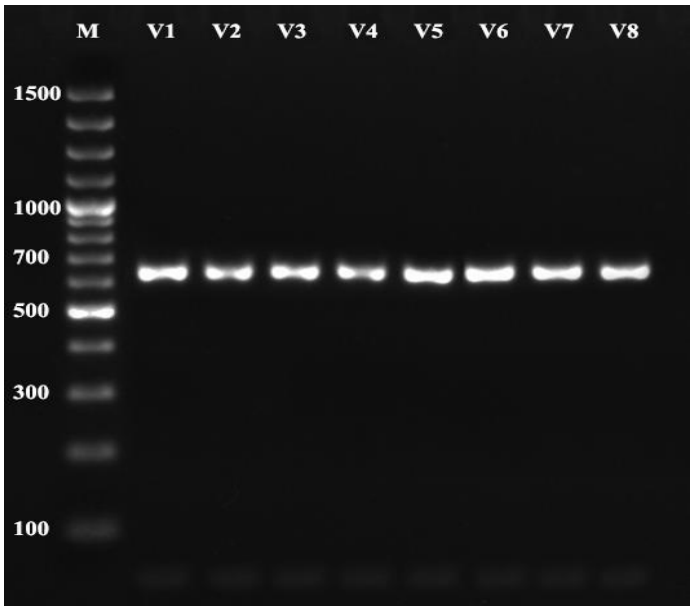

Figure 1: PCR results show a single band of ± 630 bp in the VP1 and VP2 genes according to the specifications of a pair of primers designed. M: 100bp Marker; V1: Felocell® 3 vaccine; V2: Purevac RCP vaccine; V3: Patient Lilo; V5: Happy Patient; V6: Samba patient; V7: Patient Molly; V8: Patient Bona

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                                        10        20        30        40        50        60        70        80
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FPV_Strain Philips Roxane       ATTTCTCGCCAGCAGATCAACGCTTTATAGATCAAACTAAGGACGCTAAAGATTGGGGGGGGAAAATAGGACATTATTTT
FPV_Strain FPV-BJ05             ...............................................C................................
FPV_Strain FPV-BJ04             ....T..........................A...........C................................
FPV_Strain IZSSI 3201 1 15      ................................................................................
FPV_Strain IZSSI 42807 15       ....T...........................................................................
FPV_Strain XJ-1                 ................................................................................
FPV_Strain FPV/Raccoon/RC6/BC_  --..T...........................................................................
FPV_Strain FPV/Raccoon/RC18/BC  --..T...........................................................................
FPV_V1 (Felocell 3)             ................................................................................
FPV_V2 (Purevax RCP)            ................................................................................
FPV_V3                          ....T...........................................................................
FPV_V4                          ....T...........................................................................
FPV_V5                          ....T...........................................................................
FPV_V6                          ....T...........................................................................
FPV_V7                          ....T...........................................................................
FPV_V8                          ....T...........................................................................

                                        90       100       110       120       130       140       150       160
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FPV_Strain Philips Roxane       TTTAGAGCTAAAAAAGCAATTGCTCCAGTATTAACTGATACACCAGATCATCCATCAACATCAAGACCAGCAAAACCAAC
FPV_Strain FPV-BJ05             ...............................................................................A........
FPV_Strain FPV-BJ04             ...............................................................................A........
FPV_Strain IZSSI 3201 1 15      ..............G................................................................A........
FPV_Strain IZSSI 42807 15       ..............G................................................................A........
FPV_Strain XJ-1                 ..............G................................................................A........
FPV_Strain FPV/Raccoon/RC6/BC_  ...............................................................................A........
FPV_Strain FPV/Raccoon/RC18/BC  .....G.........................................................................A........
FPV_V1 (Felocell 3)             ................................................................................
FPV_V2 (Purevax RCP)            ................................................................................
FPV_V3                          ..............G................................................................A........
FPV_V4                          ..............G................................................................A........
FPV_V5                          ..............G................................................................A........
FPV_V6                          ..............G................................................................A........
FPV_V7                          ..............G................................................................A........
FPV_V8                          ..............G................................................................A........

                                       170       180       190       200       210       220       230       240
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FPV_Strain Philips Roxane       TAAAAGAAGTAAACCACCACCTCATATTTTCATCAATCTTGCAAAAAAAAAAAAAAGCCGGTGCAGGACAAGTAAAAAGAG
FPV_Strain FPV-BJ05             ................................................................................
FPV_Strain FPV-BJ04             ................................................................................
FPV_Strain IZSSI 3201 1 15      ................................................................................
FPV_Strain IZSSI 42807 15       ................................................................................
FPV_Strain XJ-1                 ................................................................................
FPV_Strain FPV/Raccoon/RC6/BC_  ................................................................................
FPV_Strain FPV/Raccoon/RC18/BC  ..............G.................................................................
FPV_V1 (Felocell 3)             ................................................................................
FPV_V2 (Purevax RCP)            ................................................................................
FPV_V3                          ...........G....................................................................
FPV_V4                          ...........G....................................................................
FPV_V5                          ...........G....................................................................
FPV_V6                          ...........G....................................................................
FPV_V7                          ...........G....................................................................
FPV_V8                          ...........G....................................................................

                                       250       260       270       280       290       300       310       320
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FPV_Strain Philips Roxane       ACAATCTTGCACCGATGAGTGATGGAGCAGTTCAACCAGACGGTGGTCAACCTGCTGTCAGAAATGAAAGAGCTACAGGA
FPV_Strain FPV-BJ05             ...........A....................................................................
FPV_Strain FPV-BJ04             ...........A....................................................................
FPV_Strain IZSSI 3201 1 15      ...........A....................................................................
FPV_Strain IZSSI 42807 15       ...........A....................................................................
FPV_Strain XJ-1                 ...........A....................................................................
FPV_Strain FPV/Raccoon/RC6/BC_  ...........A....................................................................
FPV_Strain FPV/Raccoon/RC18/BC  ...........A....................................................................
FPV_V1 (Felocell 3)             ...........A....................................................................
FPV_V2 (Purevax RCP)            ...........A....................................................................
FPV_V3                          ...........A....................................................................
FPV_V4                          ...........A....................................................................
FPV_V5                          ...........A....................................................................
FPV_V6                          ...........A....................................................................
FPV_V7                          ...........A....................................................................
FPV_V8                          ...........A....................................................................
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                                330       340       350       360       370       380       390       400
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FPV_Strain Philips Roxane       TCTGGGAACGGGTCTGGAGGCGGGGGTGGTGGTGGTTCTGGGGGTGTGGGGATTTCTACGGGTACTTTCAATAATCAGAC
FPV_Strain FPV-BJ05             ........................................................................................
FPV_Strain FPV-BJ04             ........................................................................................
FPV_Strain IZSSI 3201 1 15      ........................................................................................
FPV_Strain IZSSI 42807 15       ........................................................................................
FPV_Strain XJ-1                 ........................................................................................
FPV_Strain FPV/Raccoon/RC6/BC_  ........................................................................................
FPV_Strain FPV/Raccoon/RC18/BC  ........................................................................................
FPV_V1 (Felocell 3)             ........................................................................................
FPV_V2 (Purevax RCP)            ........................................................................................
FPV_V3                          ........................................................................................
FPV_V4                          ........................................................................................
FPV_V5                          ........................................................................................
FPV_V6                          ........................................................................................
FPV_V7                          ........................................................................................
FPV_V8                          ..........................T.............................................................

                                410       420       430       440       450       460       470       480
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
FPV_Strain Philips Roxane       GGAATTTAAATTTTTGGAAAACGGATGGGTGGAAATCACAGCAAACTCAAGCAGACTTGTACATTTAAATATGCCAGAAA
FPV_Strain FPV-BJ05             ........................................................................................
FPV_Strain FPV-BJ04             ........................................................................................
FPV_Strain IZSSI 3201 1 15      ........................................................................................
FPV_Strain IZSSI 42807 15       ........................................................................................
FPV_Strain XJ-1                 ........................................................................................
FPV_Strain FPV/Raccoon/RC6/BC_  ......................G.................................................................
FPV_Strain FPV/Raccoon/RC18/BC  ......................G.................................................................
FPV_V1 (Felocell 3)             ........................................................................................
FPV_V2 (Purevax RCP)            ........................................................................................
FPV_V3                          ......................G.................................................................
FPV_V4                          ......................G.................................................................
FPV_V5                          ........................................................................................
FPV_V6                          ........................................................................................
FPV_V7                          ........................................................................................
FPV_V8                          ........................................................................................

                                490       500       510       520       530       540
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|.
FPV_Strain Philips Roxane       GTGAAAATTATAAAAGAGTAGTTGTAAATAATATGGATAAAACTGCAGTTAAAGGAAACATGGCTT
FPV_Strain FPV-BJ05             ...........................................................................
FPV_Strain FPV-BJ04             ...........................................................................
FPV_Strain IZSSI 3201 1 15      ...........................................................................
FPV_Strain IZSSI 42807 15       ...........................................................................
FPV_Strain XJ-1                 ...........................................................................
FPV_Strain FPV/Raccoon/RC6/BC_  ...........................................................................
FPV_Strain FPV/Raccoon/RC18/BC  ...........................................................................
FPV_V1 (Felocell 3)             ...........................................................................
FPV_V2 (Purevax RCP)            ...........................................................................
FPV_V3                          ...........................................................................
FPV_V4                          ...........................................................................
FPV_V5                          ...........................................................................
FPV_V6                          ...........................................................................
FPV_V7                          ...........................................................................
FPV_V8                          ...........................................................................
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Figure 2: Alignment results of PCR product sequences of VP1, VP2 FPV genes to database of FPV strains according to NCBI

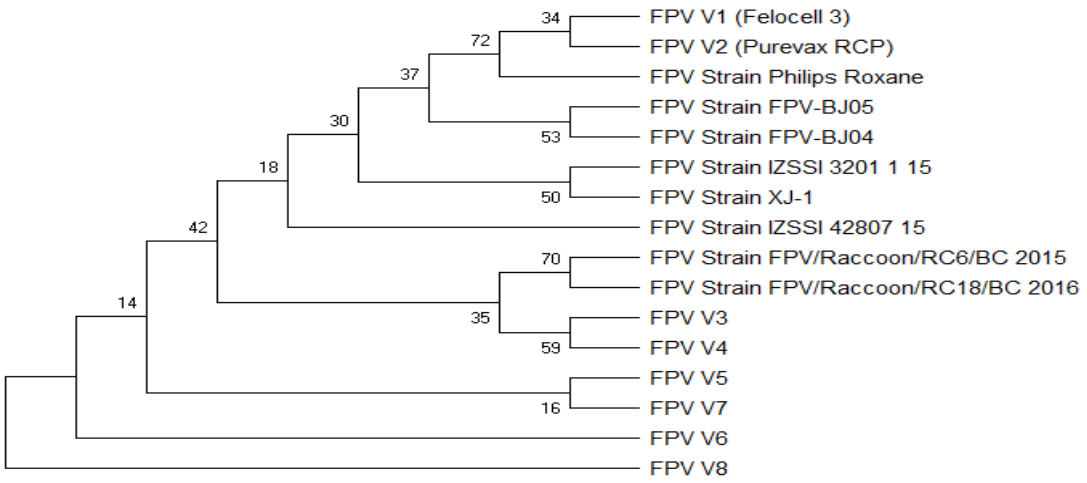

Figure 3: Genetic distances in viral DNA sequencing results based on virus strains in the database, vaccines and field isolates

FPV_Strain_Philips_Roxane															
FPV_Strain_FPV-BJ05	0.0055														
FPV_Strain_FPV-BJ04	0.0092	0.0037													
FPV_Strain_IZSSI_3201_1_15	0.0055	0.0037	0.0074												
FPV_Strain_IZSSI_42807_15	0.0074	0.0055	0.0055	0.0018											
FPV_Strain_XJ-1	0.0055	0.0037	0.0074	0.0000	0.0018										
FPV_Strain_FPV/Raccoon/RC6/BC_2015	0.0074	0.0055	0.0055	0.0055	0.0037	0.0055									
FPV_Strain_FPV/Raccoon/RC18/BC_2016	0.0112	0.0093	0.0093	0.0093	0.0074	0.0093	0.0037								
FPV_V1_(Felocell_3)	0.0018	0.0037	0.0074	0.0037	0.0055	0.0037	0.0055	0.0093							
FPV_V2_(Purevax_RCP)	0.0018	0.0037	0.0074	0.0037	0.0055	0.0037	0.0055	0.0093	0.0000						
FPV_V3	0.0111	0.0092	0.0092	0.0055	0.0037	0.0055	0.0037	0.0074	0.0092	0.0092					
FPV_V4	0.0111	0.0092	0.0092	0.0055	0.0037	0.0055	0.0037	0.0074	0.0092	0.0092	0.0000				
FPV_V5	0.0092	0.0074	0.0074	0.0037	0.0018	0.0037	0.0055	0.0093	0.0074	0.0074	0.0018	0.0018			
FPV_V6	0.0092	0.0074	0.0074	0.0037	0.0018	0.0037	0.0055	0.0093	0.0074	0.0074	0.0018	0.0018	0.0000		
FPV_V7	0.0092	0.0074	0.0074	0.0037	0.0018	0.0037	0.0055	0.0093	0.0074	0.0074	0.0018	0.0018	0.0000	0.0000	
FPV_V8	0.0111	0.0092	0.0092	0.0055	0.0037	0.0055	0.0074	0.0111	0.0092	0.0092	0.0037	0.0037	0.0018	0.0018	0.0018

Figure 4: Results of genetic distance between FPV strains with vaccines and field isolates using Pairwise Distance with Kimura-2 Parameter modeling

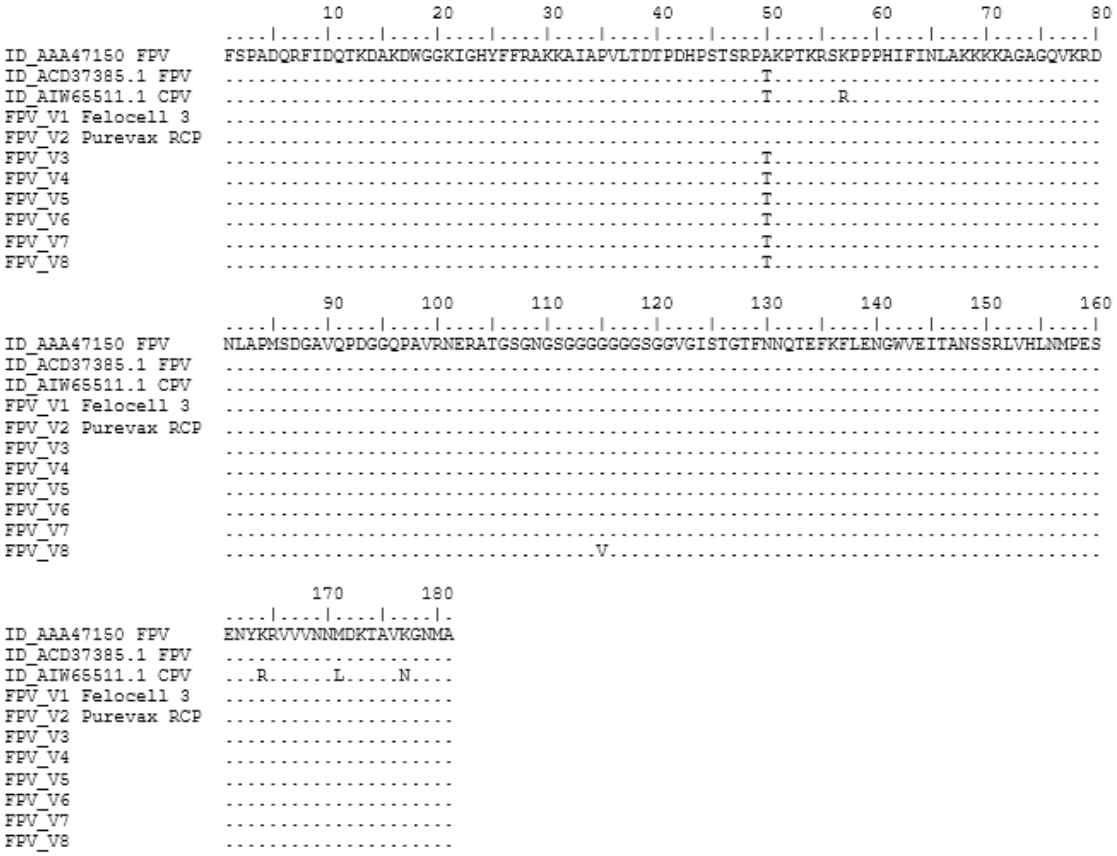

Figure 5. Analysis of Amino Acid in FPV and other isolates

Based on the alignment results, the results of the sample sequences of V1 and V2 were matched, respectively the Felocell® 3 vaccine and Purevax RCP. Whereas FPV virus field isolates namely V3 - V8 samples showed some differences in sequences namely in sequences 5, 95, 150, 173, and 425 (Figure 5). Differences in nucleotide bases in some strains, vaccines and field isolates also show a clear genetic difference in phylogenetic trees. Alignment of DNA sequences obtained homogeneous in several strains of FPV according to the NCBI database, which is precisely in the VP1 protein, VP2 FPV. The results of sequencing from eight samples showed a match on eight strains of FPV virus in a database with identities ranging from 99.2% - 99.8% (Figures 2.3 and 4).

The predominant vaccine against FPV is a modified live virus vaccine (MLV) which has been repeatedly dis- cussed on feline and mink cell lines. Inactivated vaccines have been widely used in the past but provide low levels of protective antibodies, which have relatively short lives.[15,16] Several studies have reported the occurrence of sero-negative kittens at the age of 15 weeks who have been vaccinated once or twice, and parvovirus disease in some vaccinated kittens shows that vaccination may not always be successful despite using standard protocols.[17,18]

CONCLUSION

From the results of the research conducted, FPV isolates were obtained and could be used as vaccine candidates for FPV in Indonesia.

ACKNOWLEDGEMENT

The authors acknowledge Dean Faculty of Veterinary Medicine, Universitas Airlangga for providing support to carry out this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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