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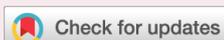
Original Article

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Expression of the VP2 protein of feline panleukopenia virus in insect cells and use thereof in a hemagglutination inhibition assay

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Feline panleukopenia virus (FPV) causes leukopenia and severe hemorrhagic diarrhea, killing 50% of naturally infected cats. Although intact FPV can serve as an antigen in the hemagglutination inhibition (HI) test, an accidental laboratory-mediated infection is concern. A non-infectious diagnostic reagent is required for the HI test. Here, we expressed the viral protein 2 (VP2) gene of the FPV strain currently prevalent in South Korea in a baculovirus expression system; VP2 protein was identified by an indirect immunofluorescence assay, electron microscopy (EM), Western blotting (WB), and a hemagglutination assay (HA). EM showed that the recombinant VP2 protein self-assembled to form virus-like particles. WB revealed that the recombinant VP2 was 65 kDa in size. The HA activity of the recombinant VP2 protein was very high at 1:2^{15}. A total of 143 cat serum samples were tested using FPV (HI-FPV test) and the recombinant VP2 protein (HI-VP2 test) as HI antigens. The sensitivity, specificity, and accuracy of the HI-VP2 test were 99.3%, 88.9%, and 99.3%, respectively, compared to the HI-FPV test. The HI-VP2 and HI-FPV results correlated significantly (r = 0.978). Thus, recombinant VP2 can substitute for intact FPV as the serological diagnostic reagent of the HI test for FPV.

Keywords: feline panleukopenia virus; VP2 protein; baculovirus expression system; hemagglutination inhibition test

Introduction

Feline panleukopenia caused by the feline panleukopenia virus (FPV) and canine parvovirus (CPV) variants 2a, 2b and 2c is the most common and contagious viral disease in cats [1,2]. The clinical signs include vomiting, a high fever, leukopenia, and hemorrhagic diarrhea, resulting in death in about 50% of animals [3]. Since FPV was first identified in cats in the 1920s, it has been isolated from cats, raccoons, monkeys, tigers, and wild carnivores worldwide [4,5]. In South Korea, although many reports have addressed CPV, few have focused on FPV [6,7], which is transmitted via direct or indirect contact with infected animals or contaminated materials. The virus is very stable in the environment, persisting for over 1 year [8].

FPV is a member of the protoparvovirus genus in the family *Parvoviridae*. The genome is a single-stranded DNA molecule about 5 kb in size that encodes viral proteins 1 and 2 (VP1 and VP2, respectively) and two non-structural proteins (NS1 and NS2) [9-11]. Anti-VP2 antibodies are neutralizing; VP2 contains B-cell

epitopes in the N-terminal and loop domains [8,12]. Therefore, the VP2 gene encoding 584 amino acids has been used for evolutionary analysis, and VP2 has been used to detect FPV antibodies after natural infection and vaccination [4,13,14]. VP2 serves as an antigen in the hemagglutination inhibition (HI) test and an FPV subunit vaccine.

Baculovirus expression systems yield high levels of recombinant proteins and virus-like particles (VLPs) [15]. Several recombinant baculoviruses expressing VP2 of CPV and FPV have been created [16,17]. The recombinant VP2 proteins form VLPs via self-assembly and serve as antigens for subunit vaccines, enzyme-linked immunosorbent assays (ELISAs) [17,18], and HI tests.

Virus neutralization (VN), HI, ELISA, and one-step immunochromatography assays have been used to detect anti-FPV and anti-CPV antibodies in animals [19,20]. HI methods are used as standard for detecting such antibodies induced by natural infection or vaccination in dog and cat sera. Although VN tests are accurate and useful for detecting anti-CPV and -FPV antibodies, cell culture and immunostaining are required because FPV does not cause cytopathic effects (CPEs) in small numbers of infected Crandell-Rees feline kidney (CRFK) cells. Thus, VN tests are not appropriate for large-scale sero-surveillance. The HI assay is preferable, because it does not require cell culture. In addition, the HI test is more economical than the VN test and commercial ELISA kits. Currently, no diagnostic reagent for HI of CPV and FPV is available in South Korea. A VP2 protein-based HI test is required for sero-epidemiological studies of FPV.

Here, we expressed recombinant FPV VP2 protein in a baculovirus system and compared the HI titers obtained using FPV and recombinant VP2 to assay cat serum samples.

Materials and Methods

Cells and viruses

CRFK (ATCC CCL-94) cells were grown in Dulbecco's modified Eagle's medium with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and an antibiotic-antimycotic cocktail (Gibco, USA). These cells were used to isolate and propagate the FPV1901 strain (KVCC number: VR2100012), which was obtained from a naturally infected Korean cat in 2019. CPV0901 strain (KVCC number: VR0900173) was propagated in A72 cells (ATCC CRL-1542), and used as a control for the detection of optimal hemagglutination assay (HA) activity in various pH condition. *Spodoptera frugiperda* (*Sf*9) (CRL-1711; ATCC, USA) cells grown in Grace's insect medium supplemented with

10% (v/v) FBS were used to propagate the recombinant baculovirus expressing the FPV VP2 protein.

Serum samples

In total, 143 sera from normal cats residing in Chungcheongnam-do and Gyeongsangbuk-do Provinces were collected from 2019 to 2020. All were inactivated at 56°C for 30 minutes prior to the HI assay. We do not know whether the normal cats had been vaccinated against FPV.

Construction of recombinant baculovirus

The full nucleotide sequence of the VP2 gene from FPV1901 was synthesized by Bioneer Co. Ltd. (Korea) with *BamH1* and *Xho1* restriction enzyme sites at the ends of the open reading frame. A plasmid containing this construct was digested with *BamH1* and *Xho1*. The insert was cloned into the multi-cloning site of the pFastBac HT-B vector to yield FPV-VP2HT, which was transformed into competent *Escherichia coli* DH10Bac cells (Invitrogen, USA). Recombinant *E. coli*, identified by polymerase chain reaction (PCR) using the pUC/M13 primer pair (Table 1), was propagated and the recombinant bacmid DNA (BacFPVVP2) was extracted for further transfection. After addition of *Sf*9 cells to a 24-well plate, mixtures of 1 μg BacFPV-VP2 DNA and 8 μL X-treme GENE HP DNA transfection reagent (Roche Diagnostics GmbH, Germany) were added to *Sf*9 cells according to the manufacturer's instructions. After incubation for 8 hours at 27°C, the transfection mixture was removed and replaced with new Grace's medium containing 10% (v/v) FBS. Cells were observed every day and harvested 5 days after transfection. The recombinant V2-expressing baculovirus (ReBac FPVVP2) was passaged three times in *Sf*9 cells. The third-passage virus served as the viral stock and was propagated to produce recombinant VP2 in *Sf*9 cells.

Identification of recombinant VP2 protein using an indirect immunofluorescence assay

An immunofluorescence assay (IFA) was used to identify recombinant VP2 using a mouse anti-CPV monoclonal antibody (APQA, Korea) and a mouse 6x-His-Tag monoclonal antibody (Thermo Fisher Scientific, USA). *Sf*9 cells infected with ReBac

Table 1. The primer sequences used to verify the presence of the feline panleukopenia virus VP2 gene in the recombinant bacmid by polymerase chain reaction

Primer	Oligonucleotide (5'to3')	Expected size
pUC/M13F	CCCAGTCACGACGTTGTAAAACG	4,230 bp
pUC/M13R	AGCGGATAACAATTTCACACAGG	

FPVVP2 were fixed in 80% (v/v) cold acetone for 15 minutes and incubated with the two antibodies at 37°C for 1 hour. The cells were washed twice with phosphate-buffered saline (PBS, pH 7.2), stained with fluorescent isothiocyanate-conjugated anti-mouse immunoglobulin (Ig) G + IgM antibodies (200-fold dilution; KPL Laboratories, USA), and examined under a 200× fluorescence microscope.

Identification of VLPs by electron microscopy

*Sf*9 cells grown in a 150-cm^2 flask were infected with ReBac FPVVP2, harvested with 5 mL of culture supernatant 4 days later and subjected to three freeze/thaw cycles. The recombinant VP2 solution was clarified by centrifugation at 3,000 × *g* for 15 minutes; the supernatant was layered onto a cesium chloride solution with a density 1.43 g/cm^2 and centrifuged at 100,000 × *g* for 2 hours using a SW-41 rotor. The band on top of the cesium chloride layer was collected and injected into a Slide-A-Lyzer dialysis tube (Thermo Fisher Scientific). After dialysis against PBS overnight at 4°C, one drop was placed on a Formvar-coated grid and negatively stained with 1% (w/v) uranyl acetate. ReBac FPVVP2 VLPs were visualized by electron microscopy (EM).

Western blotting of recombinant FPV VP2

The molecular weight of FPV VP2 was determined by Western blotting (WB). The supernatant, cell lysate, and FPV1901 samples were separated on a 12.5% (w/v) Tris-glycine gel and transferred to a nitrocellulose membrane, which was exposed to an anti-CPV VP2 monoclonal antibody (APQA) overnight at room temperature. Secondary antibody (horse radish peroxidase–conjugated goat anti-mouse IgG antibody; 1:2,000 dilution) was then added followed by incubation for 1 hour. After washing, 3′3-diaminabenzidine was added for color development.

HA and HI tests

The optimal HA activities of three antigens (FPV1901, recombinant VP2 protein, and CPV0901) were measured in Sorensen solution at pH 5.8 to 6.8. FPV1901 and recombinant VP2 protein were then subjected to serial two-fold dilution in 50 μL amounts of buffer at pH 6.0 in a 96-well (U-form) microplate; 50 μL amounts of a 0.6% (w/v) pig erythrocyte suspension with 0.1% (w/v) bovine serum albumin in Sorensen buffer was added and the plate was incubated at 4°C for 1 hour. The HA titer was the reciprocal of the highest dilution of FPV1901 or recombinant VP2 exhibiting an HA reaction. HI tests were then performed using the two antigens in the microplates. The HI

test details have been published previously [20]. The HI titers measured using FPV1901 and recombinant VP2 were compared.

Statistical analysis

Least-squares linear regression analysis was employed to determine the correlation coefficient (r value) between two HI titers. The r value was automatically calculated by Microsoft Excel 2010 software (Microsoft Corp., USA).

Results

Identification of recombinant VP2 protein

The VP2 gene of FPV1901 was cloned into the pFastBac HT-B vector and the recombinant plasmid, FPV-VP2HT, identified by *BamH1/Xho1* double digestion. BacFPVVP2 DNA was also PCR-checked using the bacmid primer pair. As shown in Fig. 1, the VP2 genes in the pFastBac HI-B vector and BacFPVVP2 DNA were 1,755 and 4,230 bp in size, respectively. *Sf*9 cells infected with ReBac FPVVP2 exhibited CPEs, such as rounding and shedding (Fig. 2A); normal *Sf*9 cells did not show CPEs. Expression of recombinant VP2 in *Sf*9 cells was verified by IFA; the addition of a monoclonal anti-CPV antibody and anti-6-histidine antibody resulted in obvious green fluorescence
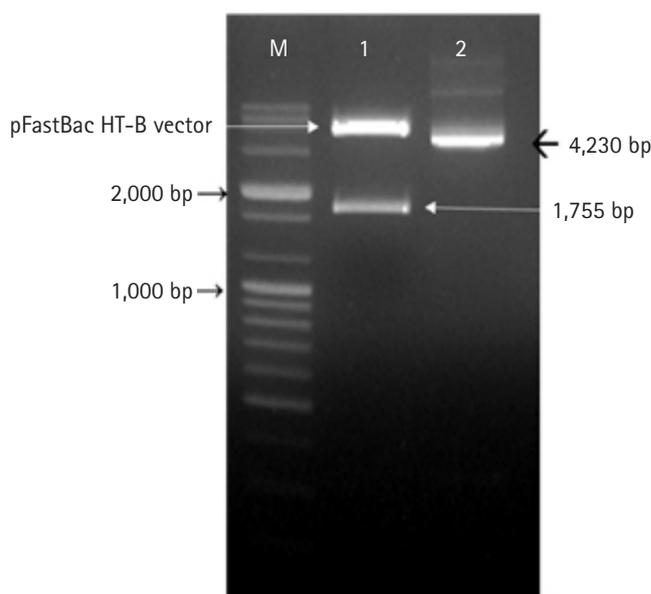

Fig. 1. Identification of the VP2 gene in the pFastBac HT–B vector and the bacmid. Insertion of the VP2 gene into the pFastBac HT-B vector was confirmed by *BamH1/Xho1* double digestion. The bacmid containing the VP2 gene was identified by polymerase chain reaction. M, 1–kb DNA ladder, lane 1, pFastFPVVP2 plasmid; lane 2, Bacmid DNA containing the VP2 gene. FPV, feline panleukopenia virus.
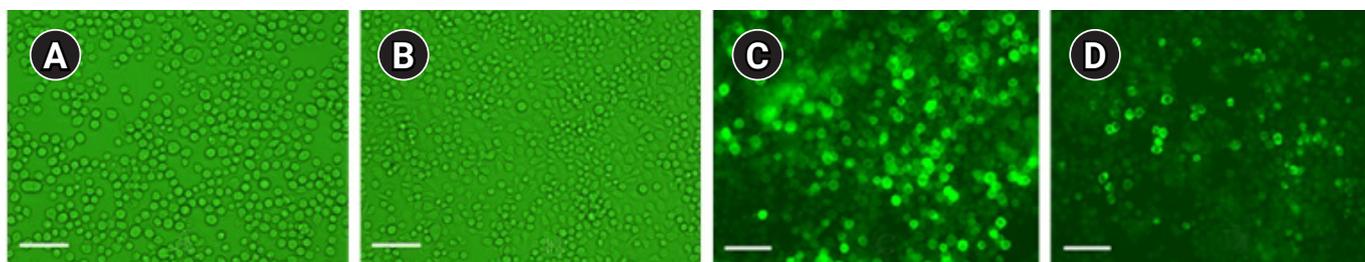
Fig. 2. Identification of recombinant feline panleukopenia virus (FPV) VP2 baculovirus. The cytopathic effects in *Spodoptera frugiperda* (*Sf*9) insect cells infected with recombinant FPV VP2 baculovirus (A); and normal *Sf*9 insect cells (B). Immunofluorescence of *Sf*9 insect cells infected with recombinant baculovirus expressing VP2, as revealed by a mouse monoclonal antibody against VP2 (C) and an anti–6–histidine antibody (D).

(Fig. 2C and D). To visualize the VLPs of ReBac FPVVP2, the virus was propagated in *Sf*9 cells and VLPs purified by cesium chloride centrifugation. EM revealed that the VLPs were about 22 nm in size, typical of the *Parvoviridae* (Fig. 3). The cell supernatant and lysate were subjected to WB; the monoclonal anti-CPV antibody identified proteins with a molecular weight of 65 kDa in both the recombinant VP2 preparation and FPV1901 (Fig. 4). The HA activities of FPV1901 and ReBac FPVVP2 were examined at various pH values. The optimal pH of Sorensen buffer, i.e., that providing the highest HA titers, was pH 6.0 (Table 2). The cell supernatant and lysate were used in the HA test; as shown in Fig. 5, the HA titer of the lysate was 1:2^{15}.

Comparison of the HI-FPV and HI-VP2 tests

The two assays were used to detect FPV HI antibodies in 143 cat serum samples. Table 3 shows that 135 and 134 were positive on the HI-FPV test (titer ≥ 1:20) and new HI-VP2 test, respectively. The HI-FPV and HI-VP2 test identified nine and eight FPV-negative samples, respectively. The sensitivity, specificity, and accuracy of the HI-VP2 test were 99.3%, 88.9%, and 99.3%, respectively, compared to those of the HI-FPV test (Table 3). Fig. 6 shows the correlation between the HI-FPV and HI-VP2 data. The r value of the HI-VP2 test was 0.978.
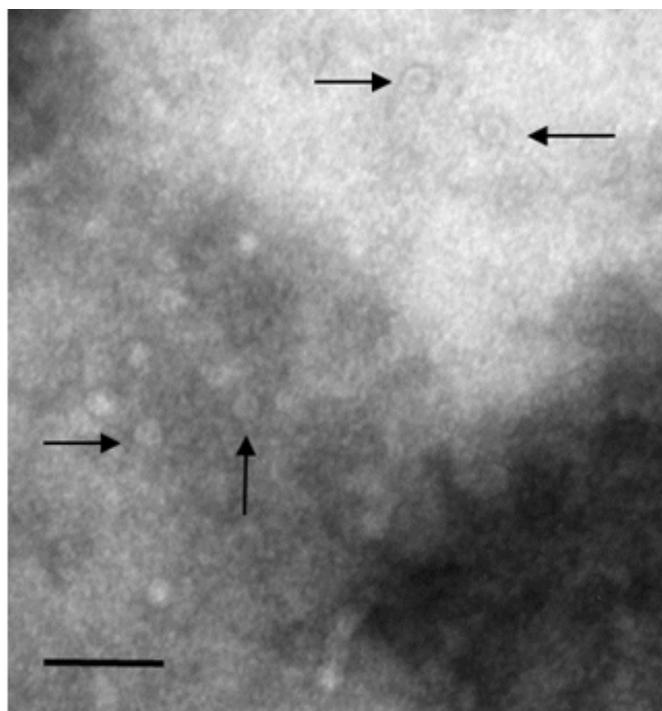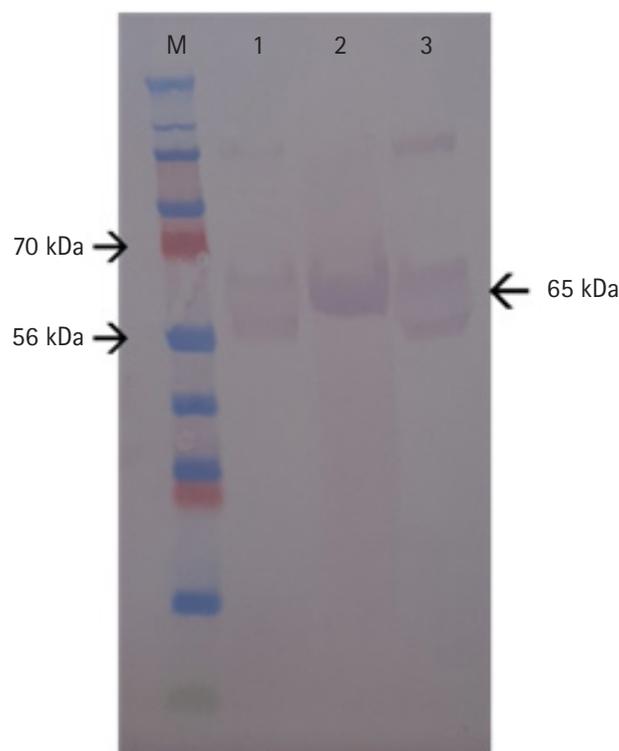

Fig. 3. Transmission electron micrograph of ReBac FPVVP2. Scale bar: 100 nm. Arrows indicate the virus-like particles of feline panleukopenia virus. FPV, feline panleukopenia virus.

Fig. 4. Western blotting of the recombinant feline panleukopenia virus VP2 protein expressed by a recombinant baculovirus using a monoclonal antibody against parvovirus. The molecular weight of recombinant VP2 was 65 kDa. M, protein ladder; lane 1, supernatant; lane 2, cell lysate; lane 3, feline panleukopenia virus.

Table 2. The HA activities of FPV1901 and the recombinant VP2 protein

HA titer using a 0.6% (w/v) pig erythrocyte suspension			
pH	FPV1901	ReBacFPVVP2	CPV0901
5.8*	9[†]	9	4
6	10	10	4
6.2	8	8	5
6.4	7	7	7
6.6	6	6	10
6.8	2	2	9

HA, hemagglutination assay; FPV, feline panleukopenia virus; CPV, canine parvovirus.
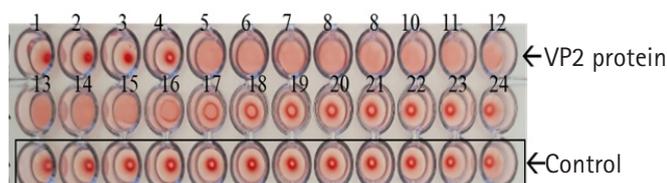*pH of Sorensen buffer solution.
[†]$\log_2$.

Fig. 5. Hemagglutination assay (HA) activity of recombinant VP2 protein of feline panleukopenia virus. The HA titer is $1:2^{15}$. The phenomenon that looks like a negative reaction in columns 1 to 4 is due to the prozone phenomenon.

Discussion

Several systems have been used to express VP2 of CPV and FPV. VP2 contains the major viral epitopes. PCR amplification of the VP2 gene has been used to detect wild FPV [21]. VP2 has been expressed in several hosts, including *E. coli* [22], insect cells [16,23,24], and a plant [25], for use as a subunit vaccine. We expressed VP2 in a baculovirus system, as revealed by IFA, WB, EM, and an HA test. Bac-to-Bac systems expressing foreign proteins have many advantages, such as appropriate post-translation modification and protein processing [23]. A recombinant VP2 protein vaccine is a safe alternative to a whole-virus vaccine; vaccination with live FPV is associated with FPV and CPV DNA in the white blood cells of asymptomatic cats [1]. A VP2 subunit vaccine could replace the traditional live vaccine [24]. Our recombinant VP2 expressed in insect cells may be a suitable replacement.

Serological FPV surveys can determine field infection and vaccination rates. Although the VN test is accurate, many veterinary laboratories prefer to use the simpler and cheaper HI and ELISA tests. Thus, we developed an HI method using recombinant VP2 protein for sero-surveillance of FPV antibodies in normal cats, and compared it to the conventional HI-FPV test.

Table 3. The sensitivity, specificity, and accuracy of the HI-VP2 test for detecting FPV antibodies in comparison with the HI-FPV1901 test

Serological test		No. of samples with HI-FPV1901		
		Positive	Negative	Sum
HI-VP2	Positive	134	0	134
	Negative	1	8	9
	Sum	135	8	143
	Sensitivity*	0.993	NA	
	Specificity[†]		0.889	
	Accuracy[‡]			0.993

HI, hemagglutination inhibition; FPV, feline panleukopenia virus; NA, not available.
*Sensitivity (%) = (number of positive results in both tests/number of positive results in the HI-FPV test) × 100.
[†]Specificity (%) = (number of negative results in both tests/number of negative results in the HI-FPV test) × 100.
[‡]Accuracy (%) = (number of both real positive and real negative results/total number of samples) × 100.
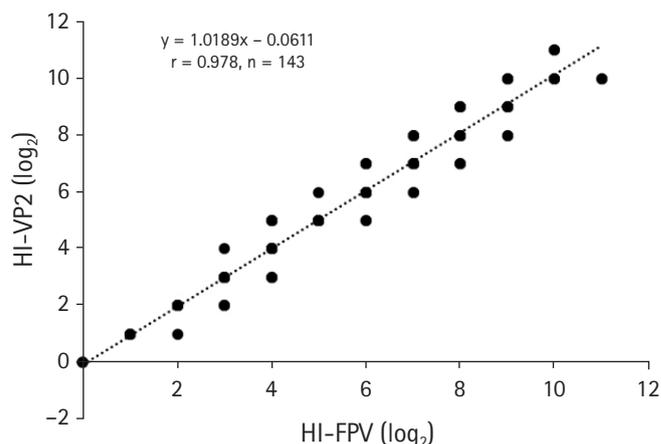

Fig. 6. Correlation between feline panleukopenia virus (FPV) antibody titers obtained using the hemagglutination inhibition (HI)-FPV and HI-VP2 tests to evaluate 143 cat serum samples. The correlation is indicated by the linear regression line.

The conventional HI method uses an infectious FPV strain to measure antibodies in sera. The HA activity of FPV propagated in CRFK cells does not exceed $1:2^{10}$. Our recombinant VP2 protein exhibited an HA activity of over $1:2^{15}$ and is non-infectious. Thus, our recombinant VP2 can serve as an antigen in the HI test.

The sensitivity, specificity, and accuracy of the HI-VP2 test, and the correlations with the results of the traditional HI test, were determined by analyzing 143 cat serum samples. The sensitivity (99.3%) of the HI-VP2 test for FPV antibody detection was significantly higher than the specificity (88.9%). Only 1 of 135 positive and 8 negative samples showed differences between by the two test methods. One of nine HI-FPV-negative samples

was missed by the HI-VP2 test (11.1% false-positive rate). This false positive reaction showing a difference of one level in HI test may reflect a non-specific reaction [26]. It is necessary to test a large number of negative samples to identify the cause of the false-positive.

The HI-VP2 and HI-FPV HI titers were well-correlated (r = 0.978); the HI-VP2 test was thus as reliable as the HI-FPV test. A new I-ELISA using purified recombinant VP2 can readily detect FPV antibodies in cat sera.

In conclusion, we expressed recombinant FPV VP2 using a baculovirus expression system. We detected viral CPEs, performed an IFA and WB, derived HA values and applied EM analysis. The recombinant VP2 protein in a cell lysate had a very high HA titer ($1:2^{15}$) and can thus serve as a new diagnostic reagent for HI tests to detect FPV.

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References

1. Balboni A, Bassi F, De Arcangeli S, Zobba R, Dedola C, Alberti A, Battilani M. Molecular analysis of carnivore Protoparvovirus detected in white blood cells of naturally infected cats. BMC Vet Res 2018;14:41.

2. Stuetzer B, Hartmann K. Feline parvovirus infection and associated diseases. Vet J 2014;201:150-155.

3. Parrish CR. Pathogenesis of feline panleukopenia virus and canine parvovirus. Baillieres Clin Haematol 1995;8:57-71.

4. Chung HC, Kim SJ, Nguyen VG, Shin S, Kim JY, Lim SK, Park YH, Park B. New genotype classification and molecular characterization of canine and feline parvoviruses. J Vet Sci 2020;21:e43.

5. Yang S, Wang S, Feng H, Zeng L, Xia Z, Zhang R, Zou X, Wang C, Liu Q, Xia X. Isolation and characterization of feline panleukopenia virus from a diarrheic monkey. Vet Microbiol 2010;143:155-159.

6. Kim SG, Lee KI, Kim HJ, Park HM. Prevalence of feline panleukopenia virus in stray and household cats in Seoul, Korea. J Vet Clin 2013;30:333-338.

7. Shin A, Lee M, Kim S, Kang SH. On-line capillary electrophoresis for enhanced detection sensitivity of feline panleukopenia virus. J Chromatogr B Analyt Technol Biomed Life Sci 2012;909:22-25.

8. Chapman MS, Rossmann MG. Structure, sequence, and function correlations among parvoviruses. Virology 1993;194: 491-508.

9. Kelly WR. An enteric disease of dogs resembling feline panleucopaenia. Aust Vet J 1978;54:593.

10. Mira F, Purpari G, Lorusso E, Di Bella S, Gucciardi F, Desario C, Macaluso G, Decaro N, Guercio A. Introduction of Asian canine parvovirus in Europe through dog importation. Transbound Emerg Dis 2018;65:16-21.

11. Shackelton LA, Hoelzer K, Parrish CR, Holmes EC. Comparative analysis reveals frequent recombination in the parvoviruses. J Gen Virol 2007;88(Pt 12):3294-3301.

12. Allison AB, Organtini LJ, Zhang S, Hafenstein SL, Holmes EC, Parrish CR. Single mutations in the VP2 300 loop region of the three-fold spike of the carnivore parvovirus capsid can determine host range. J Virol 2015;90:753-767.

13. Horiuchi M, Yamaguchi Y, Gojobori T, Mochizuki M, Nagasawa H, Toyoda Y, Ishiguro N, Shinagawa M. Differences in the evolutionary pattern of feline panleukopenia virus and canine parvovirus. Virology 1998;249:440-452.

14. Li G, Ji S, Zhai X, Zhang Y, Liu J, Zhu M, Zhou J, Su S. Evolutionary and genetic analysis of the VP2 gene of canine parvovirus. BMC Genomics 2017;18:534.

15. Su WJ, Shen WD, Li B, Wu Y, Gao G, Wang WB. A novel way to purify recombinant baculoviruses by using bacmid. Biosci Rep 2009;29:71-75.

16. Chang D, Liu Y, Chen Y, Hu X, Burov A, Puzyr A, Bondar V, Yao L. Study of the immunogenicity of the VP2 protein of canine parvovirus produced using an improved Baculovirus expression system. BMC Vet Res 2020;16:202.

17. Jiao C, Zhang H, Liu W, Jin H, Liu D, Zhao J, Feng N, Zhang C, Shi J. Construction and immunogenicity of virus-like particles of feline parvovirus from the tiger. Viruses 2020;12:315.

18. Elia G, Desario C, Pezzoni G, Camero M, Brocchi E, Decaro N, Martella V, Buonavoglia C. Recombinant ELISA using baculovirus-expressed VP2 for detection of antibodies against ca-

nine parvovirus. J Virol Methods 2012;184:98-102.

19. Abd-Eldaim M, Beall MJ, Kennedy MA. Detection of feline panleukopenia virus using a commercial ELISA for canine parvovirus. Vet Ther 2009;10:E1-E6.

20. Yang DK, Yoon SS, Byun JW, Lee KW, Oh YI, Song JY. Serological survey for canine parvovirus type 2a (CPV-2a) in the stray dogs in South Korea. J Bacteriol Virol 2010;40:77-81.

21. Cheng N, Zhao Y, Han Q, Zhang W, Xi J, Yu Y, Wang H, Li G, Gao Y, Yang S, Liu W, Xia X. Development of a reverse genetics system for a feline panleukopenia virus. Virus Genes 2019; 55:95-103.

22. Park JS, Choi BK, Vijayachandran LS, Ayyappan V, Chong CK, Lee KS, Kim SC, Choi CW. Immunodetection of canine parvovirus (CPV) in clinical samples by polyclonal antisera against CPV-VP2 protein expressed in Esherichia coli as an antigen. J Virol Methods 2007;146:281-287.

23. Chambers AC, Aksular M, Graves LP, Irons SL, Possee RD, King LA. Overview of the baculovirus expression system. Curr Protoc Protein Sci 2018 91:5.4.1-5.4.6.

24. Jin H, Xia X, Liu B, Fu Y, Chen X, Wang H, Xia Z. High-yield production of canine parvovirus virus-like particles in a baculovirus expression system. Arch Virol 2016;161:705-710.

25. Molina A, Veramendi J, Hervás-Stubbs S. Induction of neutralizing antibodies by a tobacco chloroplast-derived vaccine based on a B cell epitope from canine parvovirus. Virology 2005;342:266-275.

26. Dall'Ara P, Lauzi S, Filipe J, Caseri R, Beccaglia M, Desario C, Cavalli A, Aiudi GG, Buonavoglia C, Decaro N. Discrepancy between in-clinic and haemagglutination-inhibition tests in detecting maternally-derived antibodies against canine parvovirus in puppies. Front Vet Sci 2021;8:630809.