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Protective efficacy of LPC vaccine in pigs against different genotypes of classical swine fever virus

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ABSTRACT Four distinct genotypes of classical swine fever virus (CSFV), comprising one historical virus strain and three invaded strains, were found in Taiwan during 1989-2006. Two invaded viruses, genotype 2.1a and 2.1b, were selected as challenging candidates for valuating the heterotypic protection from genotype 1.1 vaccine virus. Pigs were vaccinated with various dosages (1, 1/10 and 1/100 doses) of LPC vaccine and challenged with CSFV invaded strains, genotype 2.1a or genotype 2.1b. The protective efficacy of LPC vaccine was further evaluated by these two genetically distinct CSFVs, the results showed that all pigs vaccinated with 1 and 1/10 doses were fully protected. Pigs vaccinated with 1/100 dose and challenged by genotype 2.1b virus were also protected. There were no clinical signs, virus shedding, viremia, and gross lesions among the protected pigs. However, only one of the three pigs vaccinated with 1/100 dose showed clinical signs after challenging with genotype 2.1a virus. The 12-week-old pigs could develop a higher neutralizing antibody titers than those of 8-week-old pigs after LPC vaccination. It indicated that the LPC vaccine currently used in Taiwan could provide fully protection against these two invaded CSFVs.

Keywords: *classical swine fever virus; genotype; LPC vaccine; protection*

INTRODUCTION

Classical swine fever (CSF) is a contagious and devastating disease among swine and wild boars worldwide capable of causing severe economic losses in animal husbandry. The disease often follows an acute course characterized by generalized hemorrhages, high morbidity and mortality. The causative agent, classical swine fever virus (CSFV), belongs to the genus *Pestivirus* within the family *Flaviviridae* [15]. CSFV is structurally and antigenetically closely related to the ruminant pestiviruses that cause bovine virus diarrhea and border disease [16].

CSF is the most serious threat to pig production in Taiwan with an incidence rate of 8.13% in 1947. This infectious disease has over the year caused great losses to the swine industry [13]. The attenuation of the LPC strain was originally done in Taiwan in the 1950s. The Rovac strain of CSFV has undergone about 1050 serial passages in rabbits and become a safe and effective vaccine for pigs against CSF. Beginning in 1958, the CSF control program decided to extend vaccination to the whole island of Taiwan. Through the island-wide application of the LPC virus vaccine, the CSF

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incidence rate dropped to 0.02% in 1965 [13]. Owing to the Government's compulsory vaccination policy, CSF is well control in Taiwan. However, sporadic outbreaks of the disease still occur every year.

In endemic areas, vaccination using live attenuated vaccine virus has been considered as an effective method for controlling CSFV infection [10]. The LPC vaccine is currently and compulsorily used for CSF control in Taiwan. The quality control of the LPC vaccine is examined by the Animal Drugs Inspection Branch, Animal Health Research Institute, Taiwan. The efficacy test of LPC vaccine was described below. Two piglets at approximately 8 weeks of age were intramuscularly inoculated with 1/100 dose of each new tested batch of vaccine. The immunized pigs were challenged with virulent CSFV ALD strain containing 10^4 50% minimum lethal dose (MLD_{50}) of viruses at two weeks post-vaccination. The requirement for qualified vaccine is that the challenged pigs should survive without showing any clinical signs. Challenged pigs are still protected by 1/10 dose of vaccine is the requirement for commercial vaccines taken from the markets.

Genetic analysis of CSFV isolated around the world has been tentatively divided into three major genetic groups, each with three or four subgroups: 1.1, 1.2, 1.3; 2.1, 2.2, 2.3; 3.1, 3.2, 3.3, 3.4 [5, 21]. Phylogenetic analysis of Taiwanese field isolates of CSFV showed that four virus population with distinct genotypes including one historical strain (subgroup 3.4) and three new invaded virus strains (subgroup 2.1a, 2.1b and 2.2) exist in Taiwan during 1989-2003 [20]. Sequencing analysis of 3' untranslated gene region of wild-type CSFV isolated between 2004 and 2007, all wild-type CSFV isolates belong to subgroup 2.1a [19]. Subgroup 2.1a viruses were first isolated in 1994 and predominated before 1995. However, Subgroup 3.4 viruses were prevalent in the early years, not being isolated after 1996. A dramatic switch in genotype from subgroup 3.4 to 2.1a was observed in Taiwan from the past decades [19, 20]. The genotype switch from historical to invaded strains makes the vaccine protective efficacy questionable. Fortunately, there has been no case of CSFV infection in Taiwan since 2006. The purpose of this study, therefore, is to evaluate whether the LPC vaccine can fully protect pigs against the two invaded CSFV strains, subgroup 2.1a and subgroup 2.1b.

MATERIALS AND METHODS

Vaccine

A commercial LPC vaccine originated in visceral organs of rabbits manufactured by Animal Health Research Institute (AHRI) (lot number: 2563 and 2567) was used in this study.

Challenging viruses

Two invaded virus strains of CSFV belonging to subgroup 2.1a (PT-1999) and 2.1b (YL-2001) were used for inoculation. These two CSFV field isolates were first conducted in PK-15 cell line with three passages, then was propagated in SPF pigs with two passages to recover their virulence. The challenging viruses were prepared from anticoagulated blood of the second-passage of pigs. Viral titers were determined by a serially dilution (10^0 – 10^{-8}) of the blood samples and inoculation with CSFV in PK15 cell lines followed by staining PK15 cell with indirect fluorescence antibody (IFA). Pigs were intramuscularly inoculated with 1 mL whole

blood with viral titer of 10^5 $TCID_{50}$/mL.

Experimental design

Two experiments were performed to determine the efficacy of LPC vaccine on two of CSFV invaded strains.

Experiment 1: Nine 8-week-old SPF pigs were divided into three groups. Pigs of each group (n = 3) were vaccinated intramuscularly with 1, 1/10 and 1/100 of LPC vaccine respectively. Two control pigs did not receive vaccine and were housed in different room. Each pig was challenged by intramuscular inoculation with diluted whole blood containing 10^5 $TCID_{50}$ of CSFV subgroup 2.1a (PT-1999) on the 14th day post-vaccinated (DPV)

Experiment 2: Eight 12-week-old SPF pigs were divided into two groups. Pigs of each group (n = 4) were vaccinated intramuscularly with 1/10 and 1/100 of LPC vaccine respectively. Two control pigs were not vaccinated and housed in different room. Each pig was challenged by intramuscular inoculation with diluted whole blood containing 10^5 $TCID_{50}$ of CSFV subgroup 2.1b (YL-2001) on the 14th DPV.

Clinical signs were evaluated and body temperatures were measured daily following the CSFV challenge. Whole blood samples were collected from the tested pigs for viral isolation. Serum samples were prepared for the measurement of anti-CSFV neutralization titers. Nasal swab specimens were collected for RT-PCR assays. All pigs were euthanized for post-mortem examination and viral isolation on the 21st day post-challenge (DPC).

Clinical signs and post-mortem

The clinical signs and body temperatures of the challenged pigs were recorded daily. All pigs were slaughtered and necropsy on the 21st DPC at the end of the experiments.

Sampling procedures

Blood was collected for serum and heparin blood samples from each pig on 0, 1st, 2nd, 3rd, 5th, 6th, 7th, 8th, 9th and 21st DPC. Nasal swab sample was taken daily from both nostrils from 0 to 9 DPC. The cotton tip was transferred to a vial containing 2 mL of DMEM and frozen at -20℃. Tissue samples such as tonsil, spleen and lympho-nodes were collected from all experiment pigs, which died of CSFV or slaughtered at the end of experiments for virus isolation and RT-PCR detection. All tissue specimens were prepared as a 10% (w/v) emulsion by homogenizing in Eagle's minimum essential medium (EMEM).

Virus isolation (VI) and antigen detection

The blood samples and 10% homogenate tissue samples were frozen and thawed to release the viruses for CSFV isolation. Serum samples and tissue samples were then inoculated onto PK-15 cells cultured in 96-well cell culture plates. Monolayers of PK-15 cells were incubated at 37˚C in 5% CO2 for 4 days and then cells were fixed using cold 80% aqueous acetone. CSFV was detected in fixed monolayers of PK-15 cells by IFA assay using CSFV-specific porcine polyclonal antibody and goat-anti-porcine IgG (H+L) conjugated with FITC (Jackson, Cambridgeshire, UK). The viral titer was determined and expressed as $TCID_{50}$ per milliliter.

Serum neutralizing antibody assay

Serum neutralizing antibody (SNA) titers

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were determined by fluorescent antibody virus neutralization (FAVN) test on PK-15 cells, following the standard procedures in Standards for Diagnostic Tests and Vaccines of the Office International des Epizooties (OIE) Manual [18] with slight modifications. Briefly, 50 μl of two-folded and serially diluted serum samples were incubated with equal volume of 200 $TCID_{50}$ CSFV (LPC strain) in a duplicate well of 96-wells plate at 37°C for 2 h. A total of 50 μl of 2×10^5 PK-15 cells were then added and incubated at 37°C in 5% CO_2 for 3 days. After removing the inoculum, the CSFV infected cells were fixed with 10% formalin in 0.01M phosphate buffered saline containing 0.05% (V/V) Tween 20 (PBST, pH7.2) for 15 min. After removal of the formalin, the cells were washed three times with PBST. A total of 50 μl of polyclonal antibody (AHRI, Taiwan) was added to each well and incubated at 37°C for 45 min. The cells were then washed three times with PBST. A total of 50 μl of 1:200 goat-anti-porcine IgG (H+L) conjugated with FITC (Jackson, Cambridgeshire, UK) was added and incubated at 37℃ for further 45 min. Cells were washed three-times with PBST for plaque counting under the invert fluorescent microscope. Serum neutralizing antibody titer was calculated as a reciprocal of the serum dilution value at which viral growth was inhibited.

RNA extraction and RT-PCR amplification

Viral RNA was extracted directly from 100 μL of the 10% (w/v) emulsion of tissues, organs, serum or swabs using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RT-PCR was performed to detect the presence of CSFV RNA in samples. The sequences of the primer pair, C5 and C6 which are specific for 3' non-translated region of CSFV, have been previously described. This primer pair allows 6.3 $TCID_{50}$/mL of CSFV to be detected in clinical samples [19].

RESULTS

Protective efficacy of LPC vaccine in pigs against CSFV subgroup 2.1a and 2.2 viruses.

All challenged pigs (n= 4) in the control group of the two experiments developed severe CSF symptoms such as fever, depression, anorexia, skin haemorrhages and moderate neurological signs. These clinical signs started from the 3rd DPC and lasted until the pigs died. Four control pigs and one of three pigs vaccinated with 1/100 dose of LPC vaccine died from 12 to 20 DPC.

Experiment 1: All pigs (n= 6) vaccinated with 1 and 1/10 dose of LPC vaccine were fully protected against a lethal dose of CSFV 2.1a challenge, which was based on the observation of clinical signs and the serum antibody titer. However, only one of the three pigs (No. 373) vaccinated with 1/100 dose showed apparent CSF clinical signs after the challenge of CSFV 2.1a. Other two of the three pigs (No. 371, 372) vaccinated with 1/100 dose were fully protected against CSFV 2.1a (Table 1). Body temperatures of all vaccinated pigs were under 40℃ except the No. 373 of pig.

Experiment 2: All pigs (n= 8) vaccinated with 1/10 and 1/100 dose of LPC were fully protected against the challenge of CSFV 2.1b. No clinical symptoms were observed among the vaccinated pigs after the virus challenge, and their body temperatures were under 40°C during the whole experimental period.

Analysis of anti-CSFV neutralizing antibody

Pigs' sera collected at different DPC times were detected for the SNA titer against CSFV.

Experiment 1: The CSFV-specific neutralizing antibody could be detected on the 0 DPC in the pigs vaccinated with 1 and 1/10 dose of LPC vaccine. However, no neutralizing antibody (SNA titer ≦3) could be detected in the pigs vaccinated with 1/100 dose at the same time (Table 2). A gradual increase in CSFV-SNA titer from the 0 to 21st DPC was noted in all vaccinated pigs except the one of number 373.

Experiment 2: The presence of CSFV-specific neutralizing antibody was appeared on the 0 DPC in all vaccinated pigs with 1/10 and 1/100 dose of LPC vaccine. A gradual increase in CSFV-SNA titer was found in all vaccinated pigs from the 0 to 21st DPC (Table 3).

No neutralizing antibodies could be detected in all control pigs (n = 4) at any measured time. The average SNA titers in pigs at the 0, 5th, 9th and 21st DPC in experiment 2 were higher than those in the experiment.

CSFV isolation from anticoagulated blood and organs

Viremia was noted in all control pigs (n = 4) and in one of the three pigs that have been vaccinated with 1/100 dose of LPC vaccine (No. 373, Table 1). The tonsils of all control pigs and the No. 373 of pig showed positive on IFA test. Other pigs that slaughtered at the end of experiments, however, These tonsils did not show positive on IFA test.

RT-PCR for nasal swab, organs and serum samples

RT-PCR indicated CSFV positive on nasal swab samples of all control pigs (n = 4) and the No. 373 of pig. No virus shedding was noted in the nasal secretions of other pigs. RT-PCR, moreover, showed positive on tonsil, spleen, and lympho-node samples of the five infected animals. However, those samples of other pigs that slaughtered at the end of the experiment showed negative on RT-PCR detection. No CSFV viral nucleic acid was detected by RT-PCR in all serum samples collected from vaccinated pigs except the No. 373 of pig.

Macroscopic lesions

All control pigs and the No. 373 of pig showed typical gross lesions of CSF, including spleen infarction, swollen lymph nodes, multiple petechial and ecchymotic haemorrhages on mucous membranes. However, no haemorrhage lesions were noted in kidney, lung, tonsil, lymph nodes or gut on other pigs.

DISCUSSION

Classical swine fever is a highly contagious and often fatal disease of swine. Vaccination is one of the most successful methods for preventing CSFV infection. The attenuated lapinized vaccine strains of CSFV are currently the most widely used in the world for the control of CSF. Previous studies showed that three attenuated lapinized CSF vaccine strains, C-strain, LPC, and HCLV could provide full protection against the disease [6, 7, 8, 13, 14, 17, 24]. Compulsory vaccination for CSF has been carried out in Taiwan for decades. However, sporadic outbreaks of CSF still occur every year. The CSFV genotype switching from historical to invaded strain was also observed [19, 20]. The genotype change makes us wonder whether the current LPC vaccine used in Taiwan can provide enough protection. Two invaded CSFV

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strains were employed to challenge the LPC-immunized SPF pigs. The protective efficacy of LPC vaccine was then evaluated.

One dose of LPC vaccine immunizing piglets on 3 and 6-week old was the main vaccination program against CSF used in Taiwan. Pigs were immunized with a standard dose or a lower dose of LPC vaccine and then challenged with either genotype 2.1a or genotype 2.1b viruses on the 14th DPV. The protected efficacy of LPC vaccine against these two invaded and genetically distinct CSFV strains was evaluated. The results indicated that all pigs vaccinated with 1, 1/10 and 1/100 dose were fully protected, except the No. 373 of pig which had vaccinated with 1/100 dose still showed clinical signs after genetic group 2.1a virus challenge. Survived pigs showed no clinical symptoms after challenge and the body temperatures were under 40°C during the experiment period. All control pigs show severe CSF signs in the necropsy. The vaccinated pigs except No. 373 of pig, however, displayed no internal lesions.

Concerning the development of neutralizing antibody, the results showed that most vaccinated piglets showed CSF antibody titer on the 14 DPV at the time of challenge (0 DPC). In experiment 1, the 8-week-old piglets vaccinated with 1 and 1/10 dose of LPC vaccine showed low antibody titers (1:6 to 1:23) in the early post-vaccination phase of 14th DPV. The average antibody titers of 1 dose vaccinated pigs (1:19) are higher than those of 1/10 dose vaccinated pigs (1:8). However, one pig vaccinated with 1/100 dose of LPC vaccine could not develop the neutralizing antibody from 0 DPC to death and showed clinical signs of CSF (table 2). The SNA titer of pig sera were gradually increased after CSFV challenge from 0 DPC to 21 DPC in all vaccinated pigs except the No. 373 of pig. The 12-week-old piglets vaccinated with 1/10 and 1/100 dose of LPC vaccine in experiment 2 showed higher antibody titers (1:32 to 1:91) in 0 DPV than those of the pigs in experiment 1 (1:≦3 to 1:23). That is, 12-week-old pigs could develop a higher neutralizing antibody titers than those of 8-week-old pigs after LPC vaccination

Two invaded viruses, genotype 2.1a and 2.1b, were selected as challenging candidates for valuating the heterotypic protection from genotype 1.1 vaccine virus. The genotype 3.4, historical virus strain, was prevalent before 1995. However, it disappeared from the field since 1996 and was never isolated from the field thereafter [19, 20]. Previously studies indicated that pigs vaccinated with LPC vaccine could develop enough immunity against the infection of the historical virus strains [11, 12]. The genotype 2.2 virus was only found in 1995 and resulted in small outbreaks, and never was isolated since then [20]. Therefore, the genotype 2.2 and 3.4 viruses were not selected for challenge viruses. The 2.1a viruses share approximately 94.1–95.1% identity with the 2.1b viruses in the E2 gene [4]. The nucleotide similarity between the LPC vaccine strain (subgroup 1.1) and the 2.1a (83-s106 strain) and 2.1b (84-KS1 strain) showed only 82.4% and 82.2% nucleotide identity, respectively, base on the complete envelope protein gene [20]. The ALD virulent strain is usually used as a challenge virus on the quality test of LPC vaccine. Both ALD and LPC vaccine strains belong to subgroup 1.1 and the nucleotide similarity reach to 94.3% base on the complete envelope protein gene (data

not shown). The LPC vaccine strain, thus, appears to have nice protective efficacy against ALD attack. Therefore, we chose genotype 2.1a and 2.1b viruses as candidates for challenging and tested the heterotypic protection from genotype 1.1 of LPC vaccine virus. The LPC vaccine, a commercial product used in this study, could protect pigs with 1/10 dose. Although the No. 373 of pig vaccinated with 1/100 dose of LPC vaccine suffered the disease after challenging with 2.1a virus, this vaccine still conforms to the requirement for commercial use. Therefore, we declare that the LPC vaccine currently used in Taiwan could provide fully protection against 2.1a and 2.1b viruses. Similar results were also observed on HCLV-immunized pigs that were fully protected against the challenge of genotype 2.1 and 2.2 viruses [23]. These results indicated that the pigs immunized with lapinized vaccine strains (LPC or HCLV) could resist the challenge of CSFV isolates with a great genetic diversity.

An effective vaccine combined with a proper vaccination program is very important for the efficacy of CSF immunization in the field. The level of maternal antibody against CSFV will affect the vaccine efficacy on piglets [1, 2, 3, 9]. Previous study showed that LPC vaccinated piglets with the maternal antibody titers 1:32 or below received one dose of LPC vaccine could survive after the challenge of virulent CSFV. Vaccinated piglets that possessed the maternal antibody titers between 1:32 and 1:64 could survive but showed clinical signs and histopathological reaction. However, vaccinated piglets with maternal antibody titers above 1:64 would die after the challenge of virulent CSFV [9]. Other study indicated that CSFV-specific cellular and antibody responses would be significantly inhibited in the piglets with the passive antibody titer above 1: 64 (detected by neutralizing peroxidase-linked assay method) at the time of vaccination. On the other hand, a passive titer up to 128 did not provide protection against CSFV genotype 2.2. [22]. The level of the maternal antibody at the time of vaccination is critical to the protective efficacy in the field. It is very important to vaccinate piglets at proper time to avoid the inactivity of vaccine viruses. The results of this study indicated that the conventional LPC vaccine could provide enough protection on piglets against the challenge of CSFV genotype 2.1a and 2.1b. However, the maternal antibody level of piglets at the time of vaccination will affect the protective efficacy of LPC vaccine. The vaccine failure occurrence in the field may be due to interference from the passive acquired antibody rather than the immunogenicity of the vaccine itself. The optimal age of piglets for vaccination should be decided based on the maternal antibody level to gain sufficient immunity.

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Tabe 1: Viremia and challenge results

Pig No	Dosage	Day post-challenge								Challenge results
		0	1	2	3	5	6	7	8	
362	0	－	－	－	－	2＋	2＋	3＋	3＋	D
363	0	－	－	－	＋	2＋	2＋	3＋	3＋	D
365	1	－	－	－	－	－	－	－	－	S
366	1	－	－	－	－	－	－	－	－	S
367	1	－	－	－	－	－	－	－	－	S
368	1/10	－	－	－	－	－	－	－	－	S
369	1/10	－	－	－	－	－	－	－	－	S
370	1/10	－	－	－	－	－	－	－	－	S
371	1/100	－	－	－	－	－	－	－	－	S
372	1/100	－	－	－	－	－	－	－	－	S
373	1/100	－	－	－	＋	＋	2＋	3＋	3＋	D
601	0	－	－	－	＋	2＋	2＋	3＋	3＋	D
602	0	－	－	－	＋	2＋	2＋	3＋	3＋	D
603	1/10	－	－	－	－	－	－	－	－	S
604	1/10	－	－	－	－	－	－	－	－	S
605	1/10	－	－	－	－	－	－	－	－	S
606	1/10	－	－	－	－	－	－	－	－	S
607	1/100	－	－	－	－	－	－	－	－	S
608	1/100	－	－	－	－	－	－	－	－	S
609	1/100	－	－	－	－	－	－	－	－	S
610	1/100	－	－	－	－	－	－	－	－	S

Experiment 1: pig number from 362 to 373；Experiment 2: pig number from 601 to 610
The degree of viremia represented by plaque number according to no viremia (-), mild (＋), moderate (2＋), and severe (3＋).
S: survived
D: died

Tabe 2: SN titer of LPC vaccinated pigs after challenging with PT–1999 strain (experiment 1).

Pig No	Dosage	-14 DPC	0 DPC	5 DPC	9 DPC	21 DPC
362	0	≦3	≦3	≦3	≦3	Die
363	0	≦3	≦3	≦3	≦3	Die
365	1	≦3	11	32	64	181
366	1	≦3	23	45	64	256
367	1	≦3	23	45	256	724
368	1/10	≦3	8	32	91	181
369	1/10	≦3	6	23	181	362
370	1/10	≦3	11	32	362	724
371	1/100	≦3	≦3	11	16	362
372	1/100	≦3	≦3	8	64	181
373	1/100	≦3	≦3	≦3	≦3	Die

LPC vaccine, lot no: 2563 by AHRI.
All pigs were challenged with 10^5 $TCID_{50}$ CSFV PT–1999 strain (subgroup 2.1a) 14 days post LPC vaccination.

Tabe 3: SN titer of LPC vaccinated pigs after challenging with YL–2001 strain (experiment 2).

Pig No	Dosage	-14 DPC	0 DPC	5 DPC	9 DPC	21 DPC
601	0	≦3	≦3	≦3	≦3	Die
602	0	≦3	≦3	≦3	≦3	Die
603	1/10	≦3	45	128	256	724
604	1/10	≦3	91	182	362	1024
605	1/10	≦3	45	256	512	1448
606	1/10	≦3	91	128	512	1448
607	1/100	≦3	32	128	362	1024
608	1/100	≦3	45	181	256	724
609	1/100	≦3	23	91	256	512
610	1/100	≦3	32	128	362	724

LPC vaccine, lot no: 2567 by AHRI.
All pigs were challenged with 10^5 TCID50 CSFV YL–2001 strain (subgroup 2.1b) 14 days post LPC vaccination.

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LPC 疫苗對不同基因型豬瘟病毒之保護效力

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摘要 1989–2006 年臺灣發現了 4 種不同基因型的豬瘟病毒（CSFV），包括一種傳統病毒株和三種新入侵病毒株，兩種新入侵的病毒（基因型 2.1a 和 2.1b）被選為基因型 1.1 疫苗毒株免疫後的異型保護效果評估。豬隻使用不同劑量（1、1/10 和 1/100 劑量）的 LPC 疫苗接種，並用新侵入型 CSFV 病毒株（基因型 2.1a 或基因型 2.1b）進行攻毒。通過這兩種不同基因的 CSFV 攻毒進一步評估 LPC 疫苗的保護功效，結果顯示所有接種 1 和 1/10 劑量的豬都得到了充分保護。接種 1/100 劑量疫苗並受到基因型 2.1b 病毒攻毒的豬也受到保護。受保護豬沒有出現臨床症狀、排毒現象、病毒血症和肉眼病變。然而，用 1/100 劑量接種的三頭豬中有一頭豬經過基因型 2.1a 病毒攻毒後出現臨床症狀，接種 LPC 後 12 週齡豬比 8 週齡豬產生更高的中和抗體力價。結果顯示，臺灣目前使用的 LPC 疫苗可對這兩種新入侵的 CSFV 能提供全面性保護。

關鍵詞：豬瘟病毒、基因型、兔化豬瘟疫苗、保護效力

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