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Review Article

Establishment of a Viral Challenge Pig Model with Classical Swine Fever

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2. Keywords

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1. Abstract

Classical swine fever (CSF) is one of the most important viral diseases in pigs world wide. The causative virus is CSF virus (CSFV) which belongs to a small enveloped RNA virus of the genus *Pestivirus*. CSFV can infect with domestic pigs and wild boar. Since wild boars are a reservoir host, wild boars can transmit the virus sporadically to the domestic pig farms. CSFV infection in pigs mainly causes server dead in the infectious pigs, which seriously affects the economic loss of pig farmers. Vaccination is only an emergency option to control CSF. Therefore, the Research and Development (R&D) of vaccines against CSFV infection is very important. Development of a CSFV challenge pig model that complies with the development of CSF vaccines will shorten the R&D time of vaccines and accelerate the CSF vaccines into the market. It can be seen from our results of the development of CSFV challenge pig model, which the abnormal clinical symptoms in pigs was seen after the viral challenge and continued until the end of the experiment. Six pigs died after the CSFV (high virulent strain: ALD; 7.5×10^5 $FAID_{50}$) challenge with a mortality rate of 100% (6/6) within 14 days post-challenge. The examination of all pigs in the normal control group (n = 6) and the viral challenge group (n = 6), the gross lesion appearances in the viral challenge group were in the larynx, spleen, mesenteric lymph nodes, kidneys, and bladder as multiple spotted bleedings in the larynx, peripheral infarctions in the spleen, edema and hyperemia in the mesenteric lymph nodes, slight spotted bleedings in the kidneys, and mucosal bleedings in the bladder. Additionally, the ileocecal valve showed button ulcer just in some viral challenge pigs. All pigs were normal in the normal control group according to the clinical symptoms, change of body weight and body weight, and the gross examination on the body appearances and organs. According to the results of this study, the CSFV challenge pig model has been successfully established which can be provided to related units for R&D of CSF vaccines. The model will be applied in the future and promoted the development of vaccines in pigs.

3. Introduction

Classical swine fever (CSF), also named hog cholera, is a contagious viral pig disease. CSF is caused by a virus of the genus *Pestivirus* of the family *Flaviviridae*. There is only one serotype of CSF virus (CSFV) in the world. Currently, CSF is a disease listed by World Organization for Animal Health (OIE) Terrestrial Animal Health Code and must be reported to the OIE [1, 2].

Swine are the only species known to be susceptible. The most common method of CSFV transmission is through direct contact between pig groups. According to the reports, the wild boar population may play a role in the epidemiology of CSF. CSFV is shed in saliva, nasal secretions, urine, and feces to infect other healthy pigs. Staffs contact with the contaminated vehicles, pens, feed, or clothing that may spread the disease. Other animals are chronic carriers of CSF may show no clinical signs of illness but may shed CSFV in their feces. Vertical infection can be occurred and offspring of

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infected sows can be infected in the uterus [2, 3].

CSF involved the acute and chronic phases. The clinical symptoms of CSF presented range from severe, with high mortality, to mild or even unapparent. The first barrier to prevent an outbreak of the CSF is to apply strict and rigorous sanitary prophylaxis according to the OIE Terrestrial Animal Health Code. When a CSF outbreak occurs, many actions must be set in place urgently. Among of these actions for preventing CSF spread, vaccination is an effective tool to prevent CSF spread [1, 3].

According to the information as the outbreaks of CSF in vaccinated herds, epidemiological monitoring data, and molecular evolutionary analysis, CSF is still causing new outbreaks in the world [1, 2, 3]. Therefore, an effective vaccine to prevent CSFV infection and spread is a top priority for controlling CSF outbreaks and preventing economic losses. CSF is currently found in many areas of the world. In order to promote the development of CSF vaccines, the establishment of a viral challenge pig model with CSF suitable for R&D of vaccines is very important and need.

4. Materials and Methods

4.1. Experimental Reagents

Experimental reagents included as phosphate buffered saline (PBS; No. P3813, Sigma-Aldrich®), Zoletil 50 (Vibac Laboratories, Carros, France), azaperone (Stresnil®;Elanco Animal Health, USA).

4.2. Cell Line and Culture

A porcine kidney cell line used was PK-15 (ATCC® CCL-33). PK-15 cells were grown in Eagle's Minimum Essential Medium (MEM; GIBCO®) supplemented with 10% fetal bovine serum (FBS; HyClone®), 2 mM L-glutamine (Invitrogen®), 100 U/mL penicillin and 100 mg/mL streptomycin (Invitrogen®) in a humidified 5% CO_2 incubator at 37°C.

4.3. Animal Care

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Animal Technology Laboratories, Agricultural Technology Research Institute (ATRI), Miaoli, Taiwan. Twelve 12-week-old Specific Pathogen Free (SPF) pigs were ordered from ATRI, Miaoli, Taiwan (the ATRI approval No.: 107016) and experimented in the GMO veterinary building, Animal Drugs Inspection Branch (ADIB), Animal Health Research Institute, Council of Agriculture, Executive Yuan, Miaoli, Taiwan (the ADIB approval No: 107-T12). The 12 pigs were housed 6 pigs per animal room under a 12-h light/dark cycle at 22-24°C and 70-75% humidity. Normal laboratory diet (FWUSOW industry, Taichung, Taiwan) and fresh water were supplied to pigs continuously ad libitum.

4.4. Experimental Animals and Grouping

Twelve12-week-old SPF pigs (negative for antibody and antigen of CSF) were obtained from ATRI, Taiwan. All SPF pigs were randomly divided into two groups (6 pigs/group), normal control group and viral challenge group.

4.5. Viral Challenge Test

The strong virulence of CSFV (strain ALD, viral titer is 3.75×10^5 $TCID_{50}$/mL) was challenged to the viral challenge group by 2 mL intra-muscle-administration. At the each designed experimental points, the detection of clinical symptoms, survival, and detection of body weight (BW) and body temperature (BT) in each group was performed to compare the difference of these above indexes between two groups.

4.6. Monitor of Clinical Symptoms and Survival, and Detection of Body Weight and Body Temperature in Pigs

In this study, the monitor of clinical symptoms and survival, and the detection of BW and BT in each group were performed once per day. Six indexes of clinical symptoms as spirit, appetite, excretion, breathe, gait, and body appearance are used for the scoring (Table 1).

Table 1: Six indexes of clinical symptoms as spirit, appetite, excretion, breathe, gait, and body appearance for the scoring.

Score	Spirit	Appetite	Excretion	Breathe	Gait	Body appearance
1	Normal	Normal	Normal	Normal	Normal	Normal
2	Inactive / weak	Suboptimal	Atherosclerosis	Slight	Slight limp	Petechial bleeding / Scabs
3	Lying down	Unable to eat	Watery diarrhea	Severe	Severe limp	Anemia / Jaundice

4.7. Gross Pathologic Examination

At the end of the experiment, all pigs were sacrificed and dissected. Then, the collection and gross appearance examination of pig's larynx, spleen, mesenteric lymph nodes (MLN), kidneys, bladder, and ileocecal valve were performed by a senior pathologic veterinarian.

4.8. Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (one-way ANOVA), Student's *t*-test, Fisher's exact test, and Kruskal-Wallis one-way ANOVA. Survival in the group comparisons was performed using Fisher's exact test. Clinical examination in the group comparisons was performed using Kruskal-Wallis test. Others in the group comparisons was performed using ANOVA. Differences between groups were considered statistically significant at $^{*}p< 0.05$, $^{**}p< 0.01$, and $^{***}p< 0.001$.

5. Results

5.1. Changes of Body Temperature in Pigs

The BT of pigs were measured before the viral challenge. BT of all pigs is between 39.1-39.2°C. After viral challenge with CSFV, BT of two groups is measured once a day until the end of the experiment. From the next day of viral challenge (days post-challenge 1; DPC1), the average BT of the pigs in the viral challenge group continuously rise with the average BT of pigs between 40.4-41.0°C. In the normal control group, the average BT of the pigs maintained between 38.5-39.8°C. With the time of CFSV challenge, the average BT of the pigs in the viral challenge group was gradually in-

crease (higher than 40°C) until DPC 9. On DPC 10, the average BT of the pigs in the viral challenge group was gradually decrease (lower than 40°C). Unfortunately, CSFV challenge pigs began to die on DPC 10 until DPC 14.

5.2. Average Weight Gain and Percentage of Average Weight Gain in Pigs

From the beginning to the end of the experiment, the average weight gain (AWG) of the normal control group was 10.50 ± 1.37 kg and AWG of the viral challenge group was -6.73 ± 1.34 kg; the percentage of AWG in the normal control group was 44.93 ± 7.09% and that in the viral challenge group was -26.14 ± 4.32%. AWG and percentage of AWG in the normal control group was significantly higher than that in the viral challenge group ($p < 0.001$) (Table 2).

Table 2: The average weight gain and average daily weight gain of the viral challenge group and the normal control group. Data were presented as mean ± SD. $^{***}p< 0.001$.

Group	No	Average weight gain (kg)	Percentage of average weight gain (%)
Viral challenge group	6	-6.73 ± 1.34	-26.14 ± 4.32
Normal control group	6	10.50 ± 1.37***	44.93 ± 7.09***

5.3. The Mortality Rate Post Viral Challenge

On DPC 10, CFSV challenge pigs begin to die. Within DPC 10-14, all six pigs died in the viral challenge group. Six pig deaths occurred in the viral challenge group within DPC 14 with a mortality rate of 100% (6/6). Moreover, all pigs were survived in the normal control group with a mortality rate of 0% (0/6).Survival rate of pig after the viral challenge in the viral challenge group was significantly lower than that in the normal control group ($p < 0.001$).

5.4. Clinical Symptoms of Pigs Post Viral Challenge

The clinical symptoms of the pigs in the viral challenge group can be found inactive/weak in spirit index; suboptimal in appetite index; atherosclerosis in excretion index; slight breathe; slight limp in gait index; petechial bleeding/scabs in body appearance post CSFV challenge. With the time of challenge, the clinical symptoms gradually become serious. On DPC 1-7, the clinical score of the six clinical symptom indexes was 1-2. With the time of challenge, the clinical score of the six clinical symptom indexes was 2-3 on DPC 8-14. The clinical symptoms of the pigs in the viral challenge group is more serious than those in the normal control group ($p < 0.001$).

5.5. Macroscopic Lesions of Pigs Post Viral Challenge

After viral challenge, the pigs continually died (n = 6) within DPC 14 in the viral challenge group. Six pigs died after the CSFV challenge with 7.5×10^5 $FAID_{50}$ ALD strain.The examination of all pigs in the normal control group (n = 6) and the viral challenge group (n = 6), the gross lesion appearances in the viral challenge group were in the larynx, spleen, mesenteric lymph nodes, kidneys, and bladder as multiple spotted bleedings in the larynx, peripheral infarctions in the spleen, edema and hyperemia in the mesenteric lymph nodes, slight spotted bleedings in the kidneys, and mucosal bleedings in the bladder. Additionally, the ileocecal valve showed button ulcer just in some viral challenge pigs. However, all pigs were normal in the normal control group (Table 3).

Table 3: Macroscopic lesions of pigs post viral challenge. Data were presented as + and -."+"mean the positive macroscopic lesions were found in pigs."-"mean the macroscopic lesions were not found in pigs.

Group	No. of pig	Larynx	Spleen	Mesenteric lymph nodes	Kidneys	Bladder	Ileocecal valve
		Multiple spotted bleedings	Peripheral infarctions	Edema and hyperemia	Spotted bleedings	Mucosal bleedings	Button ulcer
Viral challenge group	1	+	+	+	+	+	–
	2	+	+	+	+	+	+
	3	+	+	+	+	+	–
	4	+	+	+	+	+	–
	5	+	+	+	+	+	+
	6	+	+	+	+	+	–
Normal control group	7	–	–	–	–	–	–
	8	–	–	–	–	–	–
	9	–	–	–	–	–	–
	10	–	–	–	–	–	–
	11	–	–	–	–	–	–
	12	–	–	–	–	–	–

6. Discussion

According to many reports, pigs showed typical clinical signs and thermal reaction of CSF after inoculation of cell culture adapted challenge virus. Fever was observed from DPC 2 and the highest peak of temperature was observed on DPC 6 [4, 5]. In this study, after CSFV challenge (7.5×10^5 $FAID_{50}$ ALD strain), the average BT of the pigs in the viral challenge group continuously rise with the average BT of pigs between 40.4-41.0°C on DPC 1. With the time of CFSV challenge, the average BT of the pigs in the viral challenge group was gradually increase (higher than 40°C) until DPC 9. On DPC 10, the average BT of the pigs in the viral challenge group was gradually decrease (lower than 40°C). According to thermal reaction, the thermal reaction post CSFV challenge in our viral challenge pig model was similar previous reports [4, 5].

Additionally, some reports presented that CSFV challenge animals had reduced feed intake, depression, reluctant to walk from DPC 3. CSFV challenge animals were completely off fed followed by diarrhoea, ataxia and swaying movement of the hindquarter on DPC 5. CSFV challenge animals became recumbent with posterior paresis and vigorous belly movement on DPC 7. CSFV challenge animal shad respiratory distress/dyspnoea, shivering, convulsions, purulent conjunctivitis and purple cyanotic discoloration especially at ear tips, muzzle, ventral abdomen, medial side of legs and testes as reported earlier on DPC 8-10. CSFV challenge animal died on DPC 11-12 [4, 5]. In this study, six pigs were challenged with 7.5×10^5 $FAID_{50}$ CSFV (ALD strain). The clinical symptoms of the pigs in the viral challenge group can be found the clinical score of the six clinical symptom indexes was 1-2 on DPC 1-7,. With the time of challenge, the clinical score of the six clinical symptom indexes was 2-3 on DPC 8-14. With the time of challenge, the clinical symptoms gradually become serious as inactive/weak in spirit index; suboptimal in appetite index; atherosclerosis in excretion index; slight breathe; slight limp in gait index; petechial bleeding

/ scabs in body appearance. Furthermore, CFSV challenge pigs begin to die on DPC 10. Within DPC 10-14, all six pigs died in the viral challenge group. Six pig deaths occurred in the viral challenge group within DPC 14 with a mortality rate of 100% (6/6). The dead time and mortality rate in our CSFV challenge pigs were similar with the previous reports [4, 5].

AWG of pig is a primary economic driver of health and performance in swine production [4, 5]. Reduced AWG results from loss of appetite and reduced feed in take that usually caused by high temperatures and pneumonia. In our viral challenge pig model, the reduced AWG was presented in the viral challenge pigs as a consequence of CSFV infection [6].

Under natural infection with CSFV, acute phase of CSF-infected dead pigs presented the purple cyanotic discoloration of the skin, enlarged and hemorrhagic lymph nodes, hemorrhagic tonsils, turkey egg kidney, multifocal splenic infarcts, and congested GI tract and mesenteric blood vessels [4, 7, 8]. Under artificial infection with high virulent CSFV, CSF-infected dead pigs presented the severe pyrexia and acute clinical symptoms [9-11]. In this study, six pigs were challenged with 7.5×10^5 $FAID_{50}$ CSFV (ALD strain). The gross examination of 6 pigs in the viral challenge group showed that multiple spotted bleedings in the larynx, peripheral infarctions in the spleen, edema and hyperemia in the MLN, slight spotted bleedings in the kidneys, and mucosal bleedings in the bladder. Additionally, the ileocecal valve showed button ulcer just in some viral challenge pigs. According to our results, our CSFV challenge pig model has been successfully developed.

The objective of this study was to establish a viral challenge pig model with CSF suitable for the need of R&D of CSF vaccines. According to our all results, a CSFV challenge pig model was successfully established. In the future, we hope this viral challenge animal model will be applied in the R&D of swine vaccines.

7. Conclusion

CSF is one of the most important viral diseases in pigs worldwide. Vaccination is only an emergency option to control CSF. Therefore, R&D of an effective CSF vaccine is very important for controlling CSF outbreaks and preventing economic losses. In order to promote the development of CSF vaccines, the establishment of a viral challenge pig model with CSF suitable for R&D of vaccines is very important and need. According to our results, we have successfully established a viral challenge pig model with CSF. This model will be suitable for the R&D need of CSF vaccines.

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