Original Article

A Rabbit model for evaluating the efficacy of recombinant adenovirus vaccine expressing E0-E2 antigens of classical swine fever virus

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Abstract

This study investigated the efficacy of a recombinant adenovirus vaccine expressing E0-E2 antigens (rAd-E0-E2) of the classical swine fever virus (CSFV) and determined its minimum effective dose in rabbits. Forty rabbits were randomly divided into five groups. Three groups were immunized intramuscularly with rAd-E0-E2 at doses of $10^{7.0}$, $10^{6.0}$ and $10^{5.0}$ IFU, respectively. CSFV C strain was administered to one group and saline to another group (negative control). Fourteen days after immunization, the animals were challenged intramuscularly with CSFV C strain. Stereotypical thermal responses were monitored to assess the protective efficacy of the vaccine. No typical thermal response was observed in either the $10^{7.0}$ IFU or CSFV C strain groups. Mean spleen weight to body weight ratios in these groups were 0.055% and 0.05%, respectively, no evidence of hyperemia was observed in the splenic tissue, and no CSFV C strain was detected in the spleens by either RT-PCR or indirect fluorescent antibody (IFA) test. In the $10^{6.0}$ IFU group, 6/8 rabbits showed typical thermal response and the spleen weight to body weight ratio was 0.074%. Further, in 6/8 rabbits, the splenic tissues showed hyperemia and virus was detected by IFA. In both $10^{5.0}$ IFU and control groups, all animals developed fever and the spleen weight to body weight ratios were 0.099% and 0.102%, respectively. Severe hyperemia was observed and the virus was detected in the splenic tissue by RT-PCR and IFA. In conclusion, rAd-E0-E2 confers protection against CSFV C strain infection, and a rabbit model for evaluating the efficacy of the vaccine has been successfully established. The minimum effective dose in rabbits in this study was $10^{7.0}$ IFU.

Keywords: classical swine fever virus, E0-E2 antigens, recombinant adenovirus vaccine, rabbit model, efficacy, minimal protective dose

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Background

Classical swine fever (CSF) is a highly contagious disease of pigs caused by classical swine fever virus (CSFV), which belongs to the genus *Pestivirus* within the family *Flaviviridae*. Owing to the high mortality rate, disease outbreaks among pigs tend to have severe economic repercussions for the pig industry worldwide. Vaccination is effective in the prevention of infection and has been shown to reduce morbidity and mortality from the disease (Ning, 2008). Currently available types of CSF vaccines include inactivated, attenuated, and genetically engineered vaccines (Straw et al., 2006). Of these, attenuated vaccine is the most widely used around the world. The attenuated vaccine CSFV C strain, a virulent strain which induces high fever (48-96 h after the incubation period, the body temperature of rabbits increases by 1°C compared to that of normal animals, and lasts for 12-36 h), used in China is developed at the Institute of Chinese Veterinary Drug Control (S. Q. Sun et al., 2013). Wider use of the CSFV attenuated vaccine (C strain) has led to a variety of clinical manifestations in swine fever virus-infected pigs, such as atypical classical swine fever and recessive swine fever. This phenomenon has led to chronic, latent, and other atypical forms of infection (Ning, 2008). It is often difficult to distinguish immunized pigs from wild type virus-infected pigs, which renders the outbreaks difficult to control (Everett et al., 2014; Leifer et al., 2010). Development of safer, more effective vaccines could have important implications for the swine industry worldwide.

Envelop antigen E0 of CSFV is the only glycoprotein in CSFV-infected cells which is amenable to secretion into the culture supernatant. It is a relatively conserved protein located on the surface of the virus which induces the production of neutralizing antibodies and confers immunity against CSF. Mutation of the E2 gene of CSFV has been commonly reported. The glycoprotein encoded by E2 gene is a major internal structural protein that induces a protective response (Randrianarison-Jewtoukoff and Perricaudet, 1995).

Currently, both DNA and RNA viruses are extensively utilized in vaccine development. Most viruses used in vector development (e.g. adenoviruses and pox viruses) have medium to fairly large genomes that are relatively easy to manipulate and are capable of infecting mammalian cells. This renders them ideal for use in veterinary and human vaccine development (Erdman et al., 2010; Gabitzsch et al., 2009; Geisbert et al., 2011; Pardo et al., 1997). Some relatively smaller viruses, e.g. adeno-associated viruses, have been used in vaccine development as well (Kuck et al., 2006).

The use of non-replicating human type 5 adenovirus as a vaccine vector confers several advantages. Firstly, it is non-pathogenic and does not replicate in most of the host cells. However, it can produce complete progeny virus after serial passage in HEK293 cells. Secondly, it has a wide range of hosts. Non-replication-defective adenovirus can infect a wide variety of cells, including non-dividing and dividing cells. Thirdly, the virus is stable and seldom mutates (Ndi et al., 2013). The virus can be easily generated in large quantities and high titers of the virus can be easily purified. Lastly, owing to the detailed structural and functional characterization of the adenoviral genome, the virus can be easily manipulated at the genetic level (Randrianarison-Jewtoukoff and Perricaudet, 1995).

In our previous studies, recombinant human type 5 adenovirus replication-defective (rAd-E0-E2) containing CSFV E0 and E2 genes was generated (Y. Sun et al., 2013). The genes coding E0, E2 of CSFV were amplified, and recombinant adenovirus shuttle plasmid pAd Track-E0, pAd Track-E2 were generated in our laboratory (Y. Sun et al., 2010; Y. K. Sun, 2007; Y. K. Sun et al., 2007; Tuboly and Nagy, 2001). Tandem of E0 and E2 genes was obtained using PET-32a vector, forming PET-E0-E2. Subsequently, E0-E2 was cloned into adenovirus shuttle and recombinant adenovirus shuttle plasmid pAd Track-E0-E2 was produced. The recombinant adenovirus backbone vector pAd Easy-E0-E2 was obtained, which was then transfected to human embryonic kidney cells (HEK293). rAd-E0-E2 can only proliferate in the HEK293 cells with replication-defective characteristics, and is capable of inducing anti-CSFV E2 protein-specific protective antibodies in pigs (Y. Sun, et al., 2013).

In this study, the protection of rAd-E0-E2 vaccine expressing CSF virus E0-E2 gene in rabbits was investigated, relying on the stereotypic thermal response (temperature is 1°C above normal and lasts for 12-36 h) which occurred after CSF virus infection in the rabbit. Further, the association of rabbit thermal response with the spleen weight to body weight ratio, results of reverse-transcriptase polymerase chain reaction (RT-PCR) and indirect fluorescent antibody (IFA) test, and histopathological changes in spleens was assessed. The feasibility for immunogenicity analysis for rAd-E0-E2 in rabbits was assessed using these assays.

Results were confirmed with the above five methods which also served to avoid shortcomings associated with the traditional methods, which include the influence of rabbits and environmental factors on the results of body temperature test. These advantages significantly enhanced the reliability and repeatability of the tests. These studies not only established a rabbit model to evaluate the protective efficacy of rAd-E0-E2 vaccine, but also allowed determination of the minimum effective dose that confers immune protection. These findings have significant implications for testing of the vaccine produced on a large scale with bright prospects for high market value in the future.

Materials and Methods

Virus, vaccines, cells and animals: Lyophilized rAd-E0-E2 ($\geq 2 \times 10^{7.0}$ infectious units [IFU]/mL) was obtained from Qingdao-YBIO Biological Engineering Co., Ltd. (State Key Laboratory of Animal Gene Engineering Vaccines, Qingdao City) (Randrianarison-Jewtoukoff and Perricaudet, 1995), and the virus titers were determined by IFA for detecting E0 and E2 proteins expressed by rAd-E0-E2 (Chen et al., 2013; Zhang et al., 2015). Live CSF vaccine (CSFV C strain) raised in rabbits was provided by the Qingdao-State Biological Engineering Co. and used as a positive

control in this study. Swine testis (ST) cells obtained from American Type Culture Collection (ATCC) were kept in liquid nitrogen. Healthy specific-pathogen-free (SPF) Japanese big-ear rabbits (male and female, 70-80 days old, 1.5-3.0 kg) were purchased from Qingdao Kangda Rabbit Development Co. The animals were fed *ad libitum* and maintained in cages (1 animal per cage). All experiments involving animals were conducted with the approval of the Institute Animal Ethics Committee (IAEC) (certificate No: SYXK2011-0017).

Body temperature: Forty rabbits were randomly and evenly divided into five groups (8 rabbits per group). Rectal temperature was measured with a veterinary infrared thermometer (Western Instruments Technology Co., Ltd, Type: ZHR-HRQ-S60) in the morning and afternoon, starting three days after arrival of the animals. The immunization schedule is summarized in Table 1. After vaccination, the temperature was measured once in the morning and once in the afternoon for 3 successive days. Fourteen days after immunization, the animals were challenged with 1 mL intravenous infusion of CSFV C strain diluted to 1:150 in sterile saline administered via the marginal ear vein. Temperature was measured every 6 hours for 4 consecutive days.

Table 1 Immunization protocol by study group

Groups	Vaccine	No. of rabbits	Dose (in 1 mL saline)	Injection routes
1	rAd-E0-E2	8	$10^{7.0}$ IFU	i.m.
2	rAd-E0-E2	8	$10^{6.0}$ IFU	i.m.
3	rAd-E0-E2	8	$10^{5.0}$ IFU	i.m.
4	CSFV C strain	8	1:150 dilution	i.m.
5	Saline	8	None	i.m.

IFU, infectious units; CSFV, classical swine fever virus

Detection of neutralizing antibody titers after vaccination and challenge: Serum samples were collected from all groups and neutralizing antibody titers were determined with CSFV ELISA antibody titer kit (IDEXX) on 0 d (prior to vaccination) and on 3 d, 7 d, 10 d, 14 d, 16 d and 18 d (after vaccination and challenge).

Spleen weight to body weight ratio after challenge with CSFV C strain: When the body temperature returned to normal after challenge, the rabbits were sacrificed humanely and both spleen and body weights were measured. The spleen weight to body mass index ratio (%) was then calculated.

Histochemistry analysis: The spleen was removed, and 1 cm^3 (1 cm × 1 cm × 1 cm) pieces were cut, fixed in 10% formalin, embedded in paraffin, and sectioned. After hematoxylin and eosin (HE) staining, pathological changes in the spleen were examined by microscopy.

Detection of CSFV RNA by RT-PCR: The spleens were thoroughly homogenized and RNA was extracted according to animal RNAout pillar extraction kit (QIAGEN). RT-PCR (Veriti 96 PCR instrument from Applied Biosystems products) was performed to amplify fragments between 2646 to 2751 of the whole genome of CSFV using the two-step RT-PCR kit and Taq PCR Master Mix (both TaRaKa products) with specific primers as follows:

CSFV F: CAGGTATGCGATCTCGTCAACCA, and CSFV R: GGGCACAGCCCAAATCCGAAGT (Tm = 56°C).

The PCR protocol was as follows: 95°C for 5 min for denaturation, followed by 35 cycles with each cycle consisting of template denaturation at 95°C for 30 s, annealing at 56°C for 30 s and elongation at 72°C for 2 min. The PCR product was subjected to electrophoresis (BIO-RAD Gel Doc XR + gel imaging system) in 2% agarose gels to confirm the size of the amplicons (288 bp) (Chen et al., 2013).

Identification of CSFV by IFA assay: The rabbit spleens were thoroughly homogenized in phosphate buffer saline (PBS, containing penicillin 100 U/mL and streptomycin 0.1 mg/mL) at low temperature. The tissue suspension was centrifuged and filtered through 0.22 μm filter (Millipore Express®). Meanwhile, the ST cells were cultured in 6-well plates and incubated in a CO_2 incubator (Vendor/Type:REVCO/RCO3000T-5-ABC) until 80% confluence. The filtered suspension was used to infect the ST cells and blindly passed for three generations. The ST cells were then fixed with 80% cold acetone for 30 min at 2-8°C. After washing with 1×PBS for 3 times, pig CSFV antibody (1:300, IDEXX) was added to the ST cells and incubated at 37°C for 1 h. After washing with 1×PBS for 3 times, fluorescein isothiocyanate (FITC)-labeled rabbit anti-pig IgG antibodies (1:500, Sigma) were added and incubated at 37°C for 1 h. After washing with 1×PBS for 3 times, 50% glycerol was added and the plate was observed under fluorescence microscopy (OLYMPUS CKX41).

Statistical analysis: All statistical analyses were performed using GraphPad Prism 6 (USA). The unpaired, two-tailed Student's *t*-test was used. Inter-group differences with associated p value < 0.05 were considered statistically significant.

Results

Temperature measurement before and after immunization: As shown in Figure 1, only minor fluctuations in temperature were observed in each group of rabbits before immunization. After immunization with rAd-E0-E2 at different doses, the body temperatures of immunized rabbits were within the normal range, while the rabbits immunized with

CSFV C strain vaccine developed fever (≥ 40.5°C), which is consistent with results obtained from the use of conventional swine fever vaccine (He et al., 2010).

Protection after immunization with rAd-E0-E2: Fourteen days after immunization, the rabbits were challenged with CSFV C strain. As shown in Figure 2, the body temperatures of immunized animals in each group were in the normal range (< 40.5°C) 2 days before challenge. After challenge, normal temperature was observed in both $10^{7.0}$ IFU (0/8) and CSFV C strain groups (0/8). Stereotypical thermal response was observed in both $10^{5.0}$ (8/8) and $10^{6.0}$ IFU groups (6/8), and in the control group (8/8) (Y. Sun et al., 2010).

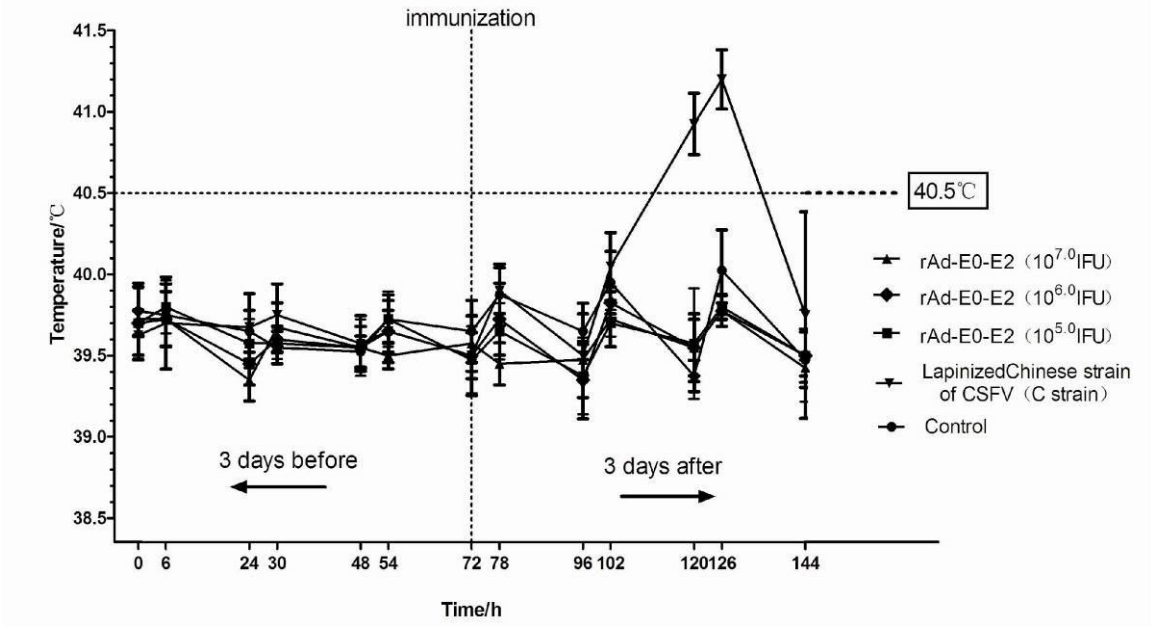

Figure 1 Actual temperature before and after immunization
Only minor fluctuations in temperature were observed in each group of rabbits before immunization. After immunization with rAd-E0-E2 at different doses ($10^{7.0}$, $10^{6.0}$, $10^{5.0}$ IFU) and saline, the body temperatures of immunized rabbits were within the normal range, while the rabbits immunized with CSFV C strain vaccine developed fever (≥ 40.5°C).

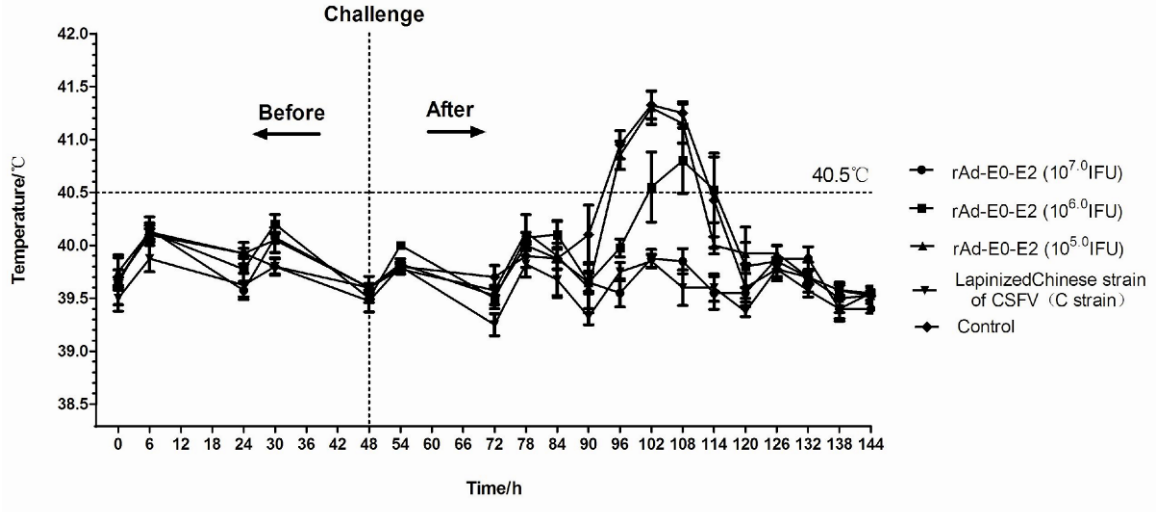

Figure 2 Thermal response in immunized rabbits following CSFV C strain challenge
Fourteen days after immunization, the rabbits were challenged with CSFV C strain. The body temperatures of immunized animals in each group were in the normal range (< 40.5°C) 2 days before challenge. After challenge, normal temperature was observed in both $10^{7.0}$ IFU (0/8) and CSFV C strain groups (0/8). Stereotypical thermal response was observed in both $10^{5.0}$ (8/8) and $10^{6.0}$ IFU groups (6/8), and in the control group (8/8).

Neutralizing antibody titers after vaccination and challenge: The results of neutralizing antibody titers before and after vaccination and challenge are shown in Figure 3. Antibody blocking rates were negative (S/P < 0.3) in all groups on 0 d (before immunization). After immunization with rAd-E0-E2 at different doses ($10^{7.0}$, $10^{6.0}$, $10^{5.0}$ IFU) and CSFV C strain vaccine (1/150 dilution), the antibody blocking rates increased gradually; the rAd-E0-E2 ($10^{7.0}$ IFU) and CSFV C strain vaccine (1/150 dilution) were positive (S/P ≥ 0.4), the rAd-E0-E2 ($10^{6.0}$ IFU) was suspicious (0.3 < S/P < 0.4), and the rAd-E0-E2 ($10^{5.0}$ IFU) and saline were negative (S/P < 0.3) on 14 d. After challenge, the antibody blocking rates increased in all rabbit groups.

Spleen weight to body weight ratio after challenge: Specimens of the splenic tissue were collected on normalization of body temperature after the challenge.

As shown in Figure 4, the index for the $10^{7.0}$ IFU group was significantly different from that of both $10^{5.0}$ IFU and control groups ($p < 0.05$ for both). The CSFV C strain group showed significant difference from that of $10^{5.0}$ IFU and control groups ($p < 0.05$ for both). However, no significant differences were observed between the $10^{7.0}$ and $10^{6.0}$ IFU groups ($p > 0.05$), between the $10^{7.0}$ IFU and CSFV C strain groups ($p > 0.05$), between the $10^{6.0}$ and $10^{5.0}$ IFU groups ($p > 0.05$), between the $10^{6.0}$ IFU and control groups ($p > 0.05$), and between the $10^{5.0}$ IFU and control groups ($p > 0.05$).

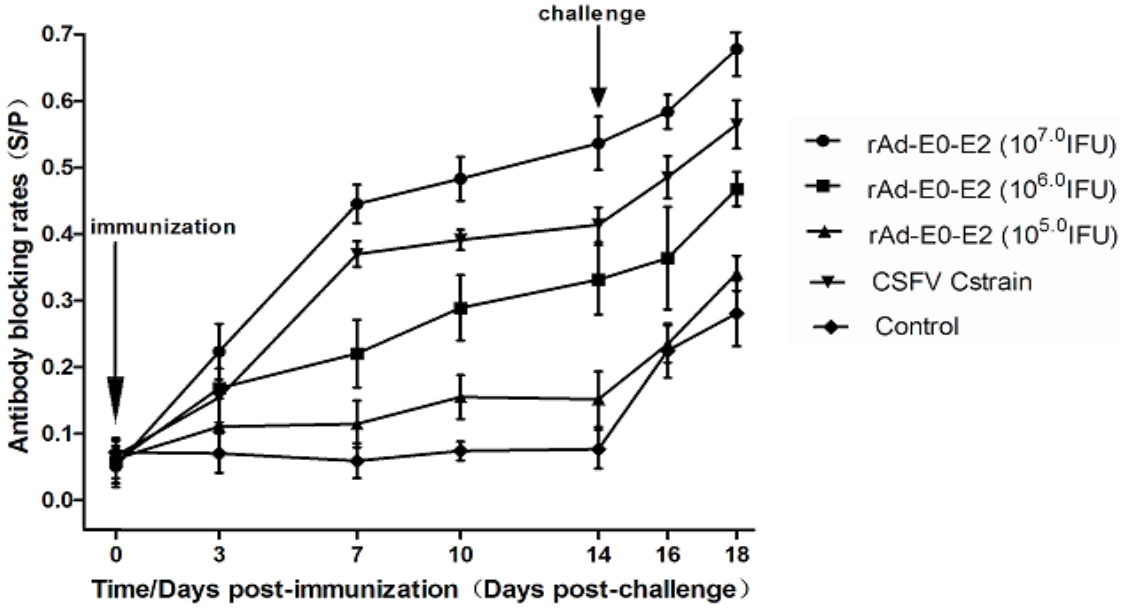

Figure 3 Neutralizing antibody titers after vaccination and challenge
Antibody blocking rates were negative in all groups ($S/P < 0.3$) on 0 d (before immunization). After immunization with rAd-E0-E2 at different doses ($10^{7.0}$, $10^{6.0}$, $10^{5.0}$ IFU) and CSFV C strain vaccine (1/150 dilution) challenge, the antibody blocking rates increased gradually; the rAd-E0-E2 ($10^{7.0}$ IFU) and CSFV C strain vaccine (1/150 dilution) were positive ($S/P \geq 0.4$), the rAd-E0-E2 ($10^{6.0}$ IFU) was suspicious ($0.3 < S/P < 0.4$), and the rAd-E0-E2 ($10^{5.0}$ IFU) and saline were negative ($S/P < 0.3$) on 14 d. After challenge, the antibody blocking rates increased in all groups.

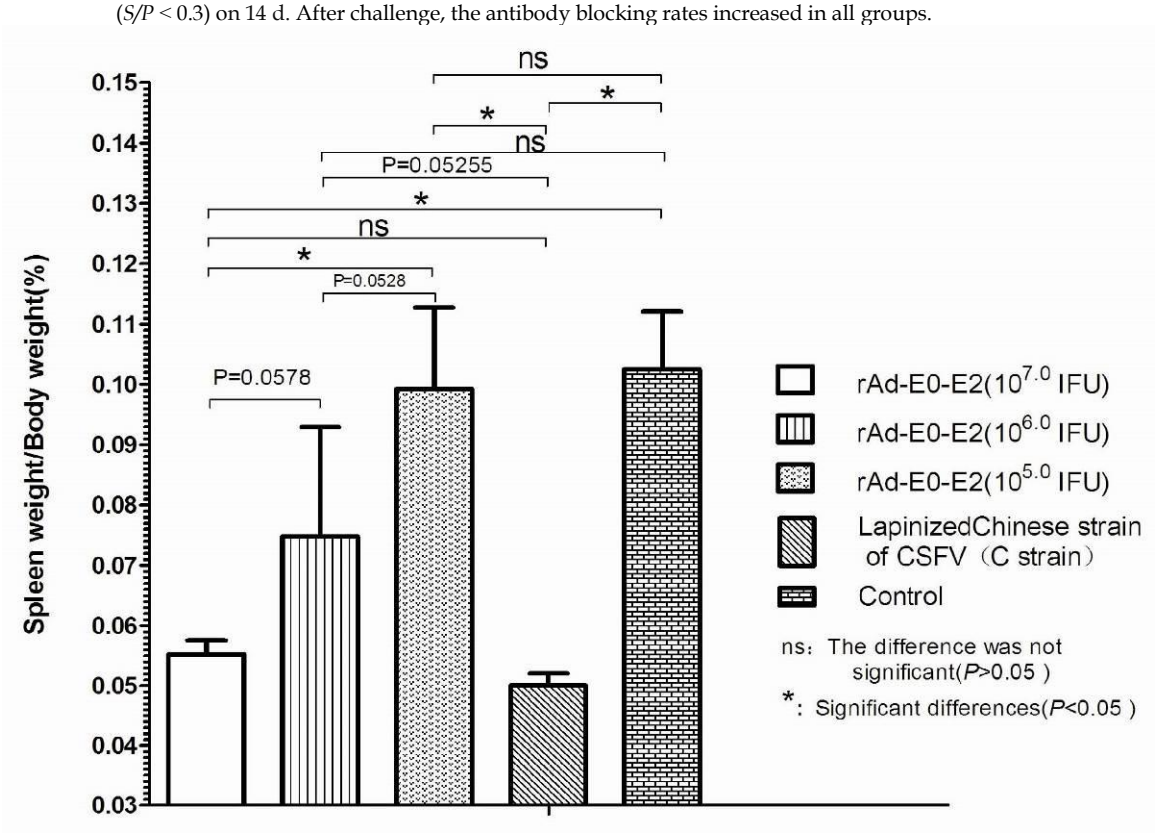

Figure 4 Percentages of spleen weight to body weight ratio
Spleens of rabbits were collected after body temperature returned to normal levels after challenge, and the ratios of spleen weight to body weight were determined.

Histopathological examination of spleen: The paraffin sections of spleen were observed under microscope for pathological changes (Figure 5). No congestion was observed in the spleens of rabbits in the $10^{7.0}$ IFU group (Figure 5A). Two different results were observed in the $10^{6.0}$ IFU group: 1) no signs of congestion evident in the splenic tissue sections (Figure 5B) and 2) severe congestion in spleen (Figure 5C). In the $10^{5.0}$ IFU group (Figure 5D), severe congestion was also found in the spleens. No signs of congestion were evident in the CSF group (Figure 5E), while signs of severe congestion were evident in the control group (Figure 5F).

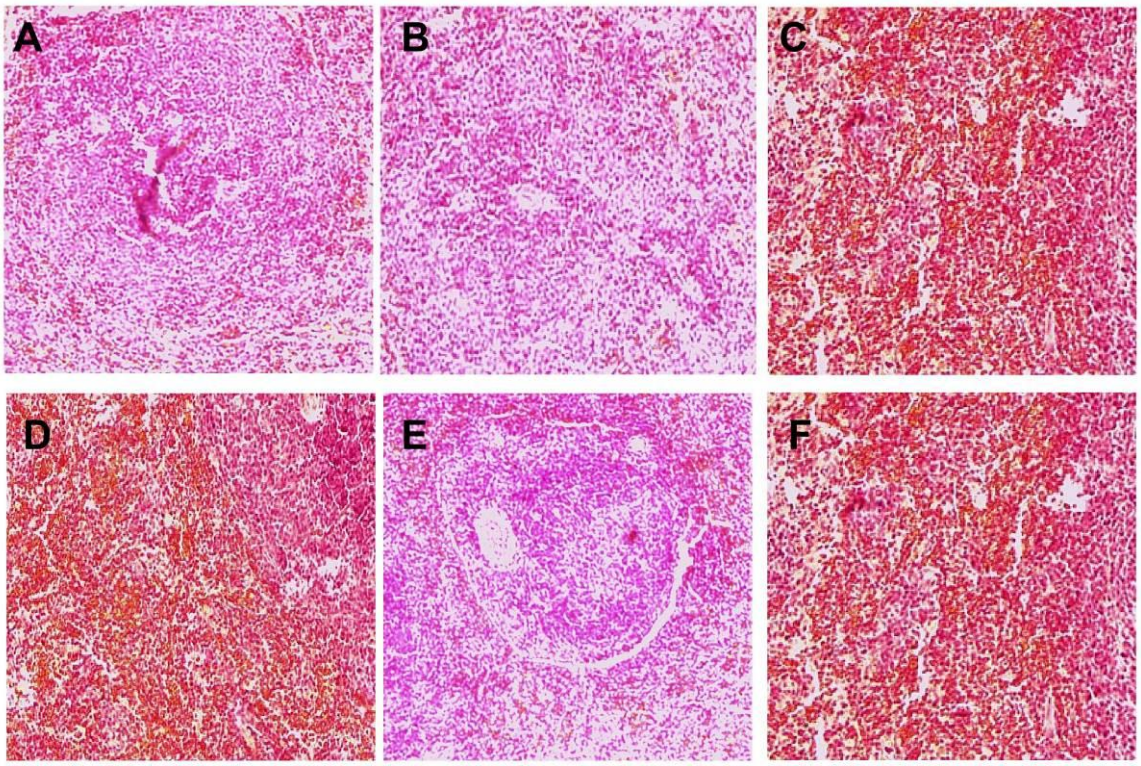

Figure 5 Histopathological examination of H&E-stained sections of splenic tissue in different study groups
A) $10^{7.0}$ IFU
B) $10^{6.0}$ IFU (protected)
C) $10^{6.0}$ IFU (unprotected)
D) $10^{5.0}$ IFU
E) CSFV C strain
F) PBS control
H&E, Hematoxylin and eosin; IFU, infectious units; CSF, classical swine fever

Post-challenge RT-PCR for CSFV C strain in immunized rabbits: Splenocytes were harvested and RT-PCR for CSFV C strain was performed with specific primers. As shown in Table 2, CSFV C strain was not detected after challenge in the $10^{7.0}$ IFU group (0/8), while the virus was found in all the other groups, including in the control (8/8), $10^{6.0}$ IFU (6/8), and $10^{5.0}$ IFU groups (8/8).

Detection of CSFV C strain in spleens by IFA: No specific green fluorescence was observed in the $10^{7.0}$ IFU (Figure 6A) and CSFV C strain vaccine groups (Figure 6E) (0/8 for both), while it was found in the control group (Figure 6F) (8/8). In the $10^{6.0}$ IFU group, specific green fluorescence was not detected in the animals which were protected (2/8) after challenge (Figure 6B), while fluorescence was observed in the unprotected animals (6/8, Figure 6C). In the $10^{5.0}$ IFU group, specific green fluorescence was detected in all animals (8/8, Figure 6D).

Discussion

In recent years, there is a growing interest in the adenovirus vector, which is the E1 and E3 region deleted replication-defective form of human type 5 virus. Recombinant adenoviruses allow concurrent expression of multiple genes, and can faithfully modify the expressed products at the post-translational level. Recombinant live vaccines using adenoviral vectors are superior to DNA vaccines and inactivated vaccines for induction of humoral immunity. There are no concerns about the interference with maternal antibodies since there are no human adenovirus antibodies in animals (Bangari and Mittal, 2006).

Adenovirus has been used as a vector for major protective antigen of CSFV. Hammond et al. (2000) developed a recombinant adenovirus expressing CSFV E2 (rpAdv) which was shown to confer complete protection from a highly virulent strain of CSFV. Later, the same group (J.M. Hammond et al., 2001) also demonstrated protection of pigs against a lethal CSFV strain after immunization with

plasmid DNA containing E2 gene first, followed by a recombinant adenovirus expressing E2 gene.

In this study, a replication-deficient human type 5 adenovirus vector system was used to develop a recombinant adenovirus vaccine rAd-E0-E2 with expression of CSFV E0-E2 antigens (Pardo et al., 1997). The protective efficacy of the vaccine was tested in pigs. The immunogenicity and protective efficacy of vaccine in rabbits were assessed by a combined analysis of stereotypic thermal reaction, spleen weight to body mass index ratio, RT-PCR, IFA, and histopathological findings. Our results demonstrated that rAd-E0-E2 induced immune protection against CSFV C strain challenge. The minimal protective dose was $10^{7.0}$ IFU. The results correlated well with the stereotypic thermal response. Our study provides a rabbit model to evaluate the protective efficacy of rAd-E0-E2 vaccine based on the consistency between the results of the four assays.

Table 2 RT-PCR for detection of CSF spleen virus

Groups	Number	Vaccine	Dose (1 mL)	CSF spleen virus	Protection ratio
1	1#	rAd-E0-E2	$10^{7.0}$ IFU	-	8/8
	2#			-	
	3#			-	
	4#			-	
	5#			-	
	6#			-	
	7#			-	
	8#			-	
2	9#	rAd-E0-E2	$10^{6.0}$ IFU	+	2/8
	10#			+	
	11#			+	
	12#			-	
	13#			-	
	14#			+	
	15#			+	
	16#			+	
3	17#	rAd-E0-E2	$10^{5.0}$ IFU	+	0/8
	18#			+	
	19#			+	
	20#			+	
	21#			+	
	22#			+	
	23#			+	
	24#			+	
4	25#	CSFV C strain	1/150 dilution	-	8/8
	26#			-	
	27#			-	
	28#			-	
	29#			-	
	30#			-	
	31#			-	
	32#			-	
5	33#	Saline	None	+	0/8
	34#			+	
	35#			+	
	36#			+	
	37#			+	
	38#			+	
	39#			+	
	40#			+	

According to Veterinary Biologics Regulations of People's Republic of China (2000 Edition) and People's Republic of China Veterinary Pharmacopoeia (2010 edition), the protective efficacy of the attenuated CSFV C strain mainly relies on experiments on animals such as pigs and rabbits (The Fourth Committee of Veterinary Biological Products, Ministry of Agriculture; Veterinary Pharmacopoeia of the People's Republic of China). However, the use of stereotypical thermal response in rabbits is associated with some disadvantages such as being more time-consuming and complicated with poor reproducibility and generally low sensitivity of results. Furthermore, results are liable to be influenced by inter-individual differences and environmental factors. Therefore, the present study compared the relationship of rabbit thermal response with spleen weight to body mass index ratio, RT-PCR, IFA, and histopathological changes, and determined the feasibility of immunogenicity analysis for rAd-E0-E2 in rabbits with these assays. A rabbit model was established for determining the protective efficacy of rAd-E0-E2 and

the results correlated with those obtained from the above-mentioned four methods and the shortcomings of traditional methods were avoided, including the effect of animals and environmental factors on the results of body temperature test. This serves to significantly improve the reliability and repeatability of results.

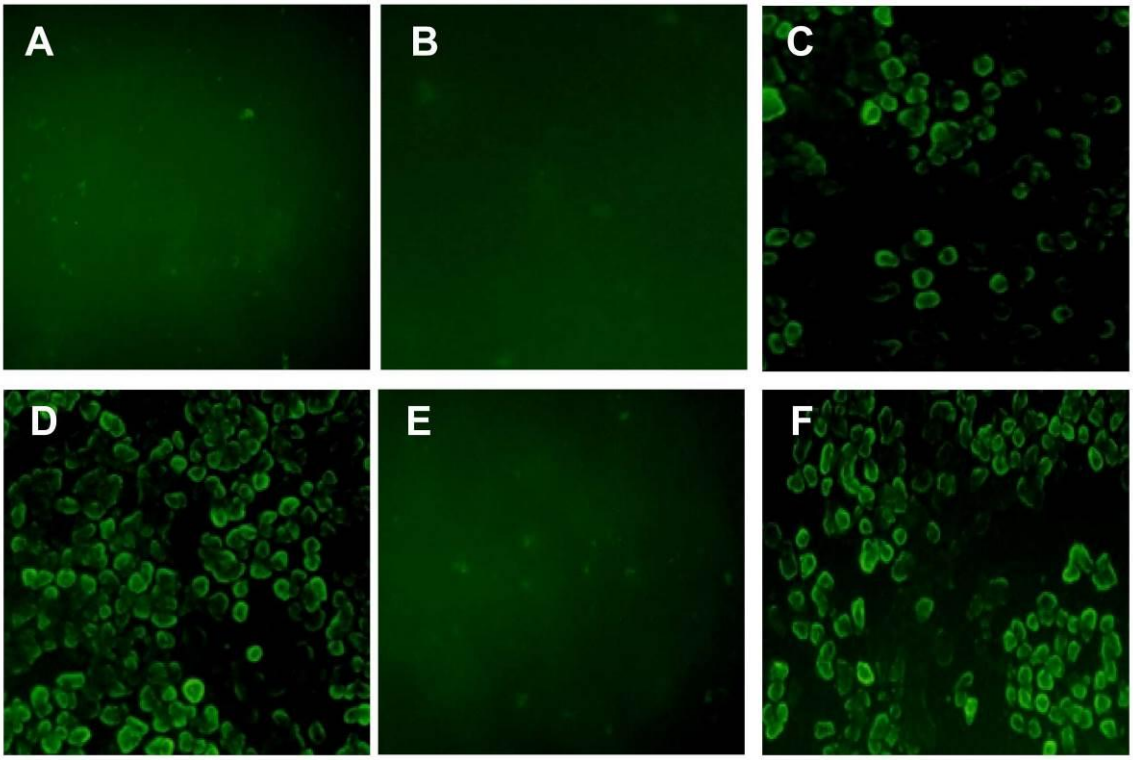

Figure 6 Indirect fluorescence antibody staining for CSFV C strain in splenic tissue (200×)
A) $10^{7.0}$ IFU
B) $10^{6.0}$ IFU (protected)
C) $10^{6.0}$ IFU (unprotected)
D) $10^{5.0}$ IFU
E) CSF spleen vaccine
F) PBS control
The filtered suspension of spleen was used to infect swine testis (ST) cells as described in Materials and Methods section 1.6. After culture, the cells were first incubated with pig CSFV antibody (1:300, IDEXX) and then fluorescein isothiocyanate (FITC)-labeled rabbit anti-pig IgG (1:500, Sigma). The plate was observed under a fluorescence microscope.
IFU, infectious units; CSFV, classical swine fever virus

Conclusion

Based on the stereotypical thermal response that occurs after infection with CSFV C strain in rabbits, the immunogenicity of rAd-E0-E2 vaccine expressing CSF virus E0-E2 gene was investigated in these animals. Our study not only established a rabbit model to evaluate the protective efficacy of rAd-E0-E2 vaccine, but also determined the minimum effective dose conferring immune protection. These results would help measure the quality of rAd-E0-E2, its efficacy and effectiveness, and determine the optimal parameters necessary for production of the vaccine in a large scale.

Competing interests: All authors declared that there were no conflict of interests involved

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บทคัดย่อ

สัตว์ทดลองกระต่ายสำหรับการประเมินประสิทธิภาพของวัคซีน Recombinant Adenovirus ที่แสดงแอนติเจน E0-E2 ของเชื้อไวรัส Classical Swine Fever

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การศึกษานี้เป็นการประสิทธิภาพของวัคซีน recombinant adenovirus ที่แสดงแอนติเจน E0-E2 (RAD-E0-E2) ของเชื้อไวรัส Classical Swine Fever (CSFV) และเพื่อหาความสามารถในการป้องกันโรคขั้นต่ำในกระต่าย โดยศึกษาในกระต่ายจำนวน 40 ตัว แบ่งออกเป็น 5 กลุ่ม ซึ่ง 3 กลุ่มได้รับวัคซีนที่มี RAD-E0-E2 ในขนาด 107.0, 106.0 และ 105.0 IFU เข้ากล้ามเนื้อ และกลุ่มควบคุมบวกได้รับเชื้อไวรัส CSFV สายพันธุ์ C และกลุ่มควบคุมลบได้รับน้ำเกลือ จากนั้น 14 วันหลังจากได้รับวัคซีน กระต่ายทุกกลุ่มได้รับเชื้อไวรัส CSFV โดยการฉีดเข้ากล้ามเนื้อ เพื่อประเมินความสามารถในการป้องกันโรคของวัคซีน จะตรวจการตอบสนองความร้อน (Stereotypical thermal responses) ผลการศึกษาพบว่า ในสัตว์กลุ่มที่ได้รับ 107.0 IFU และ CSFV ไม่พบการตอบสนองต่อความร้อน และมีค่าน้ำหนักม้ามต่อน้ำหนักตัวเท่ากับ 0.055% และ 0.05% ซึ่งแสดงถึง การปลอดภาวะเลือดคั่งในเนื้อเยื่อม้ามและไม่มีความเครียด และสามารถตรวจพบเชื้อไวรัส CSFV ในม้ามด้วยวิธี RT-PCR และวิธี indirect fluorescent antibody (IFA) ผลการศึกษาในกลุ่มที่ได้รับ 106.0 IFU พบว่ากระต่าย 6 ใน 8 ตัว แสดงการตอบสนองต่อความร้อนและมีค่าน้ำหนักม้ามต่อน้ำหนักตัวเท่ากับ 0.074% และพบ เนื้อเยื่อม้ามมีภาวะเลือดคั่ง และตรวจพบเชื้อไวรัสโดยวิธี IFA ส่วนในกลุ่มที่ได้รับ105.0 IFU และกลุ่มควบคุม พบสัตว์ทุกตัวมีอาการไข้ และมีน้ำหนักม้ามต่อน้ำหนักตัว เท่ากับ 0.099% และ 0.102% ตามลำดับ และพบภาวะเลือดคั่งในเนื้อเยื่อม้ามรุนแรง และตรวจพบเชื้อไวรัสในเนื้อเยื่อด้วยวิธี RT-PCR และ IFA โดยสรุปวัคซีนที่แสดงแอนติเจน RAD-E0-E2 สามารถป้องกันการติดเชื้อไวรัส CSFV สายพันธุ์ C โดยปริมาณแอนติเจนที่มีประสิทธิภาพต่ำสุดในกระต่ายในการศึกษาครั้งนี้คือ 107.0 IFU

คำสำคัญ: เชื้อไวรัส classical swine fever แอนติเจน E0-E2 วัคซีน recombinant adenovirus สัตว์ทดลองกระต่าย ประสิทธิภาพ

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