

COMMUNICATION

Inactivated African swine fever virus vaccine induces safe and efficient immune protection through mucosal immunity

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Abstract

African swine fever (ASF) is among the most devastating viral diseases of pigs. Despite nearly a century of research, there is still no safe and effective vaccine available. The current situation is that either vaccines are safe but not effective, or *vice versa*. The ASF vaccine prepared using the inactivation method with β -propiolactone provided 98.6% protection within 100 days after three intranasal immunizations spaced 7 days apart. In conclusion, an inactivated vaccine made from complete ASF virus particles using β -propiolactone can safely and effectively control ASF through mucosal immunity.

Keywords: African swine fever; Inactivated virus vaccine; Mucosal immunity; Safety; Efficacy; β -Propiolactone

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1. Introduction

African swine fever (ASF) is an acute, febrile, highly contagious animal infectious disease caused by ASF virus (ASFV), which can be transmitted through multiple routes such as the respiratory tract, digestive tract, and skin. With both morbidity and mortality rates similarly standing at 100%, ASF represents one of the most severe diseases threatening the pig farming industry.¹⁻³ Despite nearly a century of vaccine development efforts, due to certain reasons, no safe and satisfactory vaccine has been successfully developed.⁴ Although a messenger RNA vaccine against ASF has been designed and rolled out, its actual efficacy remains largely unknown.⁵ This has given rise to a perception that “vaccines that are safe may not be effective, whereas vaccines that are effective may pose safety risks.” Therefore, the development of a biologically safe and immunologically efficacious African swine fever (ASF) vaccine has emerged as a pivotal strategy for mitigating and controlling the endemic spread of this viral disease.^{6,7}

Inactivated vaccines, as the most classical type of vaccine, theoretically boast a high safety profile. However, after injection, inactivated vaccines for ASF can stimulate antibody production in only a subset of immunized pigs. Even with the aid of modern adjuvants, these antibodies cannot effectively neutralize the ASFV. The reason is that these antibodies are specific and capable only of identification rather than neutralization. Consequently, they fail to achieve the necessary neutralizing effect on the virus, resulting

in poor protective efficacy.⁸ Therefore, they can only serve to identify certain strains of the virus but cannot be used as a vaccine to protect susceptible animals. To date, other types of ASF vaccines, including live attenuated vaccines, recombinant vaccines, subunit vaccines, and DNA vaccines, have yet to be developed successfully.^{9,10}

ASFV particles primarily spread through mucosal tissues, such as the respiratory and digestive tracts, during natural infection.^{7,11} Although intramuscular injection of inactivated vaccines does not produce sufficient neutralizing antibodies to protect susceptible animals, our research indicates that mucosal exposure to inactivated whole virus particles can reduce viral load and even clear ASFV. This is likely due to the production of large amounts of secretory immunoglobulin A (SIgA), which can prevent further damage by viral particles at mucosal sites, thereby achieving a preventive effect.

In this investigation, we utilized the β -propiolactone inactivation technique, a method that ensures the maintenance of envelope protein conformational integrity to preserve immunogenicity, for the development of an African swine fever (ASF) vaccine. The vaccine formulation employs whole African swine fever virus (ASFV) particles as immunogens, with β -propiolactone treatment specifically designed to ablate viral infectivity while retaining the native structural architecture of surface envelope proteins. The β -propiolactone-fragmented viral DNA ensures safety. Our study demonstrated the vaccine's excellent anti-ASF prophylactic efficacy, evidenced by the reduction or clearance of ASFV loads in susceptible pigs that had been given three rounds of mucosal immunization.

2. Methods for preparing inactivated vaccines

2.1. Improved sample processing

Liver and spleen tissues from typical ASF-infected pigs were collected through sterile techniques. After removal of fat and connective tissue, the tissues were weighed, ground thoroughly, mixed with $\times 1$ phosphate-buffered saline (PBS) buffer solution at a ratio of 1:10, homogenized, freeze-thawed 3 times, and centrifuged at 5,000 r/min for 15 min. Following centrifugation, the supernatant was collected and filtered through a 0.45 μ m sterilizing filter (Millipore® SLGP033RB); the filtrate was set aside at 4°C.¹²

2.2. Viral load testing

The ASFV real-time polymerase chain reaction (PCR) Kit (Beijing MingRiDa Technology Development Co., Ltd., Beijing, China) was used according to the manufacturer's protocol. The test results showed that the cycle threshold (Ct) value of the prepared samples was <30 .

The primers and probes used in this study are as follows:

ASF-VP72-Forward: 5'-CCCAGGRGATAAAATGAC TG-3'

ASF-VP72-Reverse: 5'-CACTRGTTCCCTCCACC GATA-3'

ASF-VP72-Probe: 5'-(FAM)-TCCTGGCCRACCAA GTGCTT-3'-BHQ1.

Each qPCR reaction contained the following: qPCR Probe Master Mix ($\times 2$, 12.5 μ L), probe (10 pmol/ μ L, 0.5 μ L), upstream and downstream primers (10 pmol/ μ L, 0.5 μ L each), DNA (5 μ L), and ddH₂O to top up the volume to 25 μ L.

The cycling conditions for real-time PCR reactions were 95°C for 3 min, followed by 40 cycles of 95°C for 10 s and 60°C for 20 s.

2.3. Preparation of inactivated vaccines

Viral suspension with a Ct value of <30 was added with 0.1% β -propiolactone (Solarbio® Life Sciences, Beijing, China), followed by incubation at 4°C with shaking to inactivate the virus. After 24 h, another 0.1% β -propiolactone was added for 96 h, followed by hydrolysis of β -propiolactone at 37°C for 2 h and storage at 4°C.¹³

3. Safety testing

After streaking the inactivated vaccine on a blood agar medium and incubating it at 37°C for 72 h, no contaminants were detected. Subsequently, mucosal vaccination was performed on five healthy, non-immunized piglets, each of which had approximately 20 kg of weight. Each piglet was inoculated with 1 mL of vaccine nasally and 10 mL orally once every 7 days for three consecutive cycles. During the 30-day observation period, no adverse reactions or abnormal manifestations were noted in these piglets, highlighting the outstanding safety of the vaccine.¹⁵

4. Efficacy testing

A total of 150 ASFV-antigen-and-antibody-negative pigs, each weighing about 20 kg, were randomly divided into a 140-pig experimental group and a 10-pig control group. The experimental group received mucosal immunization with an inactivated vaccine: 1 mL intranasally and 3 mL orally per pig, administered once every 7 days for three consecutive cycles. Each pig in the control group was given 4 mL of PBS buffer, following the same immunization schedule. After three rounds of immunization, all 150 pigs were kept in an ASFV-infected pig farm, together with an additional 10 diseased pigs. They were randomly divided into 10 groups, and each group was composed of 14 experimental pigs, one control pig, and one pig that was suffering from the disease.

Three days later, regardless of whether the diseased pigs have died or not, they should all be harmlessly disposed of, leaving only the experimental pigs and the control pigs. This setup enabled natural ASFV exposure through cohabitation with infected pigs, simulating a precise cross-infection scenario. The 100-day experiment included randomly sampling five pigs from each group every 10 days. Blood was centrifuged to obtain serum, which was pooled separately for each group for analysis. Real-time PCR was used to detect the ASFV viral load in the serum, and pen-level mortality was closely monitored. As presented in Table 1, the experimental group's viral load decreased steadily and vanished by the 70th day. By the end of the experiment, 98.6% of the experimental pigs survived, with no adverse reactions or abnormalities detected. In contrast, the control group suffered 100% mortality within 25 days.

5. Discussion

This study primarily focuses on: (i) the preparation of an inactivated vaccine for ASF using whole virus particles as the antigen and β -propiolactone as the inactivating agent and (ii) the method of immunization involving stimulation of mucosal immunity through nasal spray and oral administration. After 3 times of immunization, experimental pigs were cohabited with diseased pigs, and serum viral loads were randomly sampled from the test pigs. The results showed that the viral load gradually decreased with the prolongation of the experiment until it was completely cleared at 70 days, suggesting that the β -propiolactone-inactivated ASF vaccine administered through mucosal immunity may induce high levels of SIgA. In precise cross-infections of ASF, the immune protection rate can reach 98.6% over a period of 100 days. It is significantly higher than the protection rate of the immunization method using the inactivated ASFV vaccine for injection³ and is similar to the result of the adenovirus-vectored vaccine.¹⁵

The results of the experiment indicate that mucosal immunization with ASFV β -propiolactone inactivated vaccine is feasible, safe, and can provide satisfactory

Table 1. African swine fever viral load in serum samples detected by real-time PCR test

Group	Day								
	20 d	30 d	40 d	50 d	60 d	70 d	80 d	90 d	100 d
Experimental group	23.60	25.16	28.07	28.24	30.01	-	-	-	-
Control group	29.99	time	●	●	●	●	●	●	●

Notes: “-” Negative; “•” No sample collected.
Abbreviation: PCR: Polymerase chain reaction.

immune protection. This study preliminarily demonstrates that mucosal immunization with inactivated ASF vaccine can provide safe and effective immune protection against ASF, evidenced by reduced mortality. We speculate that mucosal immunization with ASFV inactivated by binary ethylenimine or low-concentration formaldehyde may also achieve similar results, as long as it does not destroy the viral capsid proteins and envelope proteins but only fragments the viral DNA. It remains to be further verified whether these techniques are applicable to the generation of other inactivated virus vaccines that do not achieve protective effects through muscle injection.

The inactivated ASF vaccine once judged ineffective upon injection, might induce elevated levels of SIgA following mucosal immunization through means such as nasal spraying and oral intake. Mucosal immunization with this vaccine not only achieves outstanding results but is also straightforward and safe, offering a huge relief to this century-old problem.

6. Conclusion

Applying β -propiolactone to ASFV DNA alters the viral nucleic acid structure and leads to inactivation of the virus. Mucosal immunization, as opposed to injection-based immunization, can provide safe and effective prevention against the virus.

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Conflict of interest

The authors declare they have no competing interest.

Author contributions

Conceptualization: All authors

Formal analysis: JingXu Yang

Investigation: All authors

Methodology: Jinlong Yang

Writing-original draft: JingXu Yang

Writing-review & editing: Jinlong Yang

Ethics approval and consent to participate

The study was approved by the Ethical Review Board of Animal Experiments of Chongqing Jingshengtian Biotechnology Co., Ltd. (No.: CQJSTAE2023-02). The company provided laboratory swine, experimental facilities, and study protocols, and oversaw the execution

of the entire process to ensure compliance with regulatory requirements.

Consent for publication

Not applicable.

Availability of data

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Further disclosure

The paper has been deposited onto a preprint server (Preprints.org, doi: 10.20944/preprints202501.1545.v1).

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