

Brief Report

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Posted Date: 21 January 2025

doi: 10.20944/preprints202501.1545.v1

Keywords: African swine fever (ASF); inactivated virus vaccine; Mucosal immunity; safe; 21 effective;  $\beta$ -Propiolactone



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Brief Report

# Inactivated African Swine Fever Virus Vaccine Induces Safe and Efficient Immune Protection Through Mucosal Immunity

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**Abstract:** African swine fever (ASF), caused by the African swine fever virus (ASFV) discovered in 1921, is an acute, febrile and highly contagious animal disease. It poses one of the greatest threats to the pig farming industry [1,2]. A safe and effective ASF vaccine is crucial for preventing and controlling its spread [3,4]. However, due to various reasons, no such vaccine has been developed yet. Here we show that the existing inactivated ASF vaccine, ineffective in providing protection through injection immunization, can ensure both safety and offer satisfactory protection once the immunization approach is switched to mucosal immunization.

**Keywords:** African swine fever (ASF); inactivated virus vaccine; mucosal immunity; safe; effective

Inactivated vaccines, representing the most classical approach in vaccine development, are theoretically considered the safest type of vaccines. However, injecting pigs with inactivated ASF vaccine only elicits a partial antibody response. Even with modern adjuvants, these antibodies can't effectively neutralize ASFV. This is because they're specific antibodies that can only recognize, not neutralize, the virus. As a result, they fail to neutralize ASFV as needed, causing a loss of protective efficacy[5]. To date, other types of ASF vaccines, including live attenuated vaccines, recombinant vaccines, subunit vaccine, and DNA vaccines, have yet to be developed successfully.

How can we solve the century-old problem?

We have developed an ASF vaccine utilizing the  $\beta$ -propiolactone inactivation method. Employing the  $\beta$ -propiolactone inactivation technique, the developed ASF vaccine with whole ASFV particles as antigens preserves envelope protein integrity for immunogenicity. The  $\beta$ -propiolactone-fragmented viral DNA ensures safety. In experiments, susceptible pigs given three-round mucosal immunization and then exposed to ASF-infected pigs show the vaccine's excellent anti-ASF prophylactic efficacy, as ASFV loads are reduced or cleared.

## 1. Our Method for Preparing Inactivated Vaccines Is As Follows:

① Improved sample processing. Sterilely collect liver and spleen tissues from typical African swine fever-infected pigs, remove fat and connective tissue, weigh them, grind thoroughly, mix with  $1 \times$  PBS buffer solution at a ratio of 1:10, homogenize, freeze-thaw three times, centrifuge at 5000r/min for 15 minutes, take the supernatant, filter through a  $0.45 \mu\text{m}$  sterilizing filter, and set aside at  $4^\circ\text{C}$  [6].

② viral load testing. ASFV real-time PCR Kit (Beijing MingRiDa Technology Development Co., Ltd., Beijing, China) was used according to the manufacturer's protocol. The test results showed that the CT value of the prepared samples was less than 30.

③ Preparation of inactivated vaccines. Add 0.1%  $\beta$ -propiolactone to the viral suspension with a real-time PCR detection CT value less than 30, incubate at  $4^\circ\text{C}$  with shaking to inactivate, add another 0.1%  $\beta$ -propiolactone after 24 hours, inactivate for 96 hours, then hydrolyze  $\beta$ -propiolactone at  $37^\circ\text{C}$  for 2 hours, and store at  $4^\circ\text{C}$  [7].

## 2. Safety Testing:

After streaking the inactivated vaccine on a blood agar medium and incubating it at 37°C for 72 hours, no contaminants were detected. Subsequently, mucosal vaccination was performed on five healthy, non-immunized piglets. Each piglet was inoculated with 1 ml vaccine nasally and 10 ml orally every 7 days for 3 times. During the 30-day observation period, no adverse reactions or abnormal manifestations were noted in these piglets. This outcome demonstrates the outstanding safety of the vaccine [8].

## 3. Effective in Application:

One hundred and fifty 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 every 7 days for 3 times. The control group was given 4 ml of PBS buffer per pig, following the same immunization schedule. After the three-round immunization, all 150 pigs were placed in an ASFV-infected pig farm. They were randomly penned in groups of 10. This setup enabled natural ASFV exposure through cohabitation with infected pigs, simulating a precise cross-infection scenario. The 100-day experiment included randomly sampling 5 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 group pigs survived, with no adverse reactions or abnormalities. In contrast, the control group suffered 100% mortality within 25 days.

**Table 1.** Serum samples test results by real-time PCR for the viral load of ASFV.

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

Note: “-”: Negative; “●”: No sample can be collected.

## 4. Discussion

The inactivated ASF vaccine, once judged ineffective upon injection, might induce elevated levels of secretory immunoglobulin A (SIgA) following mucosal immunization via means like nasal spraying and oral intake. Mucosal immunization with this vaccine not only achieves outstanding results but is also straightforward and safe. The century-old problem has been effortlessly solved.

**Institutional Review Board Statement:** All methods were carried out in accordance with relevant guidelines and regulations Directive 2010/63/EU in Europe. Experiment was approved by Biomedical Ethics Committee of Chongqing Jingshengtian Biotechnology Co., Ltd, (No.): CQJSTAE2023-02.

**Data Availability Statement:** The datasets used and/or analysed during the current study available from the corresponding author on reasonable request. All data generated or analysed during this study are included in this published article [and its supplementary information files]. The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

**Acknowledgments:** I would like to thank all animal caretakers and technicians for their excellent work.

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