An African Swine Fever Vaccine-Like Variant with Multiple Gene Deletions Caused Reproductive Failure in a Vietnamese Breeding Herd

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1 Introduction

African swine fever (ASF) is a transboundary and devastating disease affecting wild boars and domestic pigs caused by an etiological pathogen, ASF virus (ASFV). ASFV is a large, complex structure with a linear double-strand DNA virus belonging to the *Asfarviridae* family [1]. After emerging in China, it was introduced in Vietnam in February 2019, leading to at least 6 million animals being culled to control and prevent the spread of the disease in the country [2]. Vietnam is the first nation to successfully develop and commercialize two modified live-attenuated vaccines (LAVs), created through genetic engineering, against the highly lethal ASFV – genotype II [3]. The nationwide utilization of these LAVs in Vietnam for prophylaxis, coupled with unauthorized vaccine experiments, resulted in the improper release of master seeds. Therefore, our study aimed to identify and describe a novel ASF vaccine-like variant in unvaccinated Vietnamese breeders.

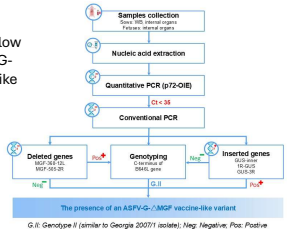
Key Points

- A novel ASFV vaccine-like variant
- Infected sows exhibited **atypical reproductive disorders** and **ulcerative dermatitis**
- Viral antigens are located in **macrophage-like cells** in sows' **reproductive organs.**

2 Materials and Methods

A comprehensive study was conducted in a non-ASF-vaccinated swine herd in Southern Vietnam, which has a capacity of 2,400 Landrace x Yorkshire sows. Samples were collected from breeding animals that exhibited atypical reproductive disorder (n=12) and/or skin-related lesions (n=8) and aborted fetuses during the herd's termination. Samples were extracted, and routine PCRs with specific primers, followed by a whole genome sequence (WGS), were performed to identify ASF virus variant (Fig. 1). Meanwhile, the formalin-fixed paraffin-embedded tissue samples from 6 animals underwent hematoxylin and eosin (HE) staining and immunohistochemistry (IHC) for histopathological examination and ASFV antigen localization. The mean score of the hallmarks is evaluated as standard previously reported [4].

Fig. 1 The workflow to detect ASFV-G-ΔMGF vaccine-like variant



3 Results

The pregnant sows suffered reproductive failure in late-term gestation, such as abortions, stillbirths, and mummies, with the incidence rate reaching up to 18%. Remarkably, approximately 25% of lactating sows manifested moderate-to-severe ulcerative dermatitis in the skin and udders (Fig. 2).

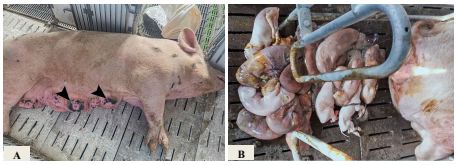
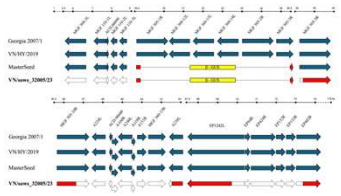


Fig. 2 The clinical manifestations in infected sows

The preliminary PCR and WGS results indicated the absence of six multiple gene families (MGFs) along MGF 360-12L, -13L, -14L, MGF 505-1R, -2R, -3R genes and inserted a beta-glucuronidase (B-GUS) marker gene. Notably, three other major deleted fragments were identified in the left and central regions (Fig. 3). These results are compatible with those reported for the live-attenuated recombinant strain (ASFV-G-ΔMGF). The sequence analysis of the C-terminal B646L gene, encoding a major capsid protein p72, classified this variant as genotype II. This ASFV-G-ΔMGF vaccine-like variant was homogenously detected in sows and aborted fetuses, potentially capable of vertical transmission.

Fig. 3 Schematic diagram of indel mutations among ASF strains



ASF-viral antigens were distributed in macrophage-like cells in sows' reproductive organs without typical macroscopic lesions and affected udders (Fig. 4 insets). Histopathological findings observed extensive fibrin-necrotizing vasculitis with marked inflammatory cell infiltration (neutrophils and lymphocytes) in the mammary gland and lymphoid follicles. Ulcerative dermatitis is characterized by widespread necrosis of the epidermal layers extending into the reticular dermis (Fig. 4). The average score of microscopic lesions ranged from mild to moderate (Table 1).

Fig. 4 HE staining and ASFV antigen localization by IHC (inset)

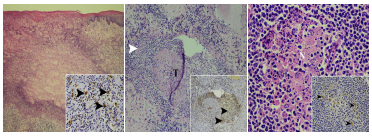


Table 1 The score evaluation of histological lesions (mean ± SD)

Organs	Hemorrhage	Necrosis	Inflammation	Vasculitis
Udders	2.00 ± 1.00	2.60 ± 0.89	2.20 ± 0.84	1.00 ± 0.71
Uterus	0.33 ± 0.52	0	0	0
Ovary	0	0	0	0
Oviduct	0	0	0	0
Lung	1.33 ± 0.52	1.17 ± 0.98	1.50 ± 0.84	0
Spleen	1.50 ± 1.05	1.17 ± 0.75	1.33 ± 0.82	0
Lymph nodes	1.33 ± 0,82	0.67 ± 0.52	0.50 ± 0.55	1.33 ± 0.82
Liver	0.83 ± 0.41	0.33 ± 0.52	0.83 ± 0.41	0
Kidney	0.67 ± 0.82	0.17 ± 0.41	0.33 ± 0.52	0

4 Discussion

After a nationwide vaccine implementation, an ASF vaccine-like variant was found in a Vietnamese breeding herd during herd replacements. Infected animals exhibited a high symptomatic incidence of abortions in multiparous sows and those in late-term gestation, speculating in chronic form due to animals' prolonged exposure to the virus. Lactating sows presented multiple udder ulcerations and dermatological lesions, primarily relevant to low-virulent infections [5].

The observed pathological changes frequently resembled lesions caused by immune complexes, suggesting that the tissue damage was primarily a result of dysregulated immune responses involving cytokines and activated monocytes or macrophages rather than direct harm caused by the virus [5].

Unlicensed vaccine uses must be controlled to reduce the negative impacts on ASFV genetic diversity [6]. Vaccine safety and efficacy are still unclear in field conditions that need more extensive research.

5 Conclusion

This study highlights the risks of genetically engineered ASF vaccines, emphasizing the critical need for stringent biosecurity, good husbandry practices, and cautious use of modified live vaccines with robust surveillance, especially during herd replenishment in ASF-free areas.

6 Reference and Acknowledgement

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