Nghiên cứu khoa học

ĐÁNH GIÁ TÍNH AN TOÀN VÀ HIỆU QUẢ BẢO HỘ CỦA VACXIN AVAC ASF LIVE PHÒNG BỆNH DỊCH TẢ LỢN CHÂU PHI TRONG ĐIỀU KIỆN THỰC ĐỊA

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EVALUATING THE SAFETY AND EFFECTIVENESS OF THE AVAC ASF LIVE VACCINE AGAINST AFRICAN SWINE FEVER UNDER FIELD CONDITIONS

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ABSTRACT

This article presents the results of evaluating the safety and effectiveness of the live-attenuated AVAC ASF LIVE vaccine against African swine fever (ASF) under field conditions. The vaccine was administered to 4,892 pigs at 4–13 weeks old (1 dose/pig) at four commercial pig farms. The studied results indicated that the vaccine was safe for pigs, with no adverse reactions post-vaccination, and effectively elicited a strong virus-specific antibody response. The average antibody-positive rate against ASF virus (ASFV) was 90.1% at 28 days post-vaccination. At this time point, 5 vaccinated pigs randomly selected from each farm and 5 unvaccinated controls were challenged with a highly virulent field strain of ASFV genotype II. After 21 days of observation, the vaccinated group exhibited an average of 95% (80.0–100%) of survival rates, lower viremia levels, and reduced viral shedding in oral fluids compared to the control group. These results confirm the safety, immunogenicity, and protective effectiveness of AVAC ASF LIVE against genotype II ASFV under field conditions. The trial outcomes provide a solid scientific basis for the wide spread adoption of AVAC ASF LIVE vaccination as part of a sustainable ASF control strategy in Vietnam's swine industry.

Keywords: African swine fever, vaccine, trials.

I. INTRODUCTION

African swine fever (ASF) is one of the most dangerous infectious diseases in pigs, caused by the African swine fever virus (ASFV). The disease has the capacity to spread rapidly, resulting in high mortality and severe economic consequences for the global swine industry (Zhang et al., 2023). Since its first identification more than a century ago, many countries have made considerable efforts to develop vaccines against ASF based on various technological platforms. Among these, inactivated vaccines have been considered safe but have failed to induce significant protective immunity. Subunit, DNA, and vector-based vaccines remain under investigation and have shown only limited efficacy. In contrast, live attenuated vaccines have demonstrated substantial protective potential against homologous ASFV strains, although concerns remain regarding residual virulence, reversion to virulence, or antigenic variation (Zhang et al., 2023).

Several gene-deleted live attenuated vaccine candidates have been demonstrated to be safe in pigs. Deutschmann et al. (2023) showed that the ASFV-G-ΔMGF strain, after five serial passages in pigs, gave rise to a variant capable of causing transient fever and replicating in vivo, but without significant reversion to virulence; all animals remained healthy at the end of the experiment. Diep et al. (2025) also reported the safety and protective efficacy of the AVAC ASF LIVE vaccine (containing the ASFV-G-ΔMGF strain serially passaged multiple times in DMAC cells) in four-week-old commercial pigs against genotype II ASFV challenge.

Regarding the ASFV-G-ΔI177L strain, multiple studies have reported that this strain maintains stable attenuation, does not induce clinical signs, and shows no evidence of reversion to virulence (Trần Xuân Hạnh et al., 2021a,b; Tran et al., 2022). Even when administered at a high dose (10^6 HAD_{50}) and monitored for an extended period of 90–180 days, pigs did not develop clinical symptoms or significant internal organ lesions, indicating that the strain had almost completely lost its virulence (Borca et al., 2023). However, van den Born et al. (2025) reported that ASFV-G-ΔI177L could revert to virulence by the third serial passage under experimental conditions and adversely affect reproduction when administered to sows in late gestation. Therefore, vaccines containing this strain are currently not recommended for use in pregnant sows.

Because ASFV can persist for long periods in the environment, and biosecurity measures alone have not been sufficient to effectively control the disease, vaccination is considered the most optimal strategy for its prevention and control (Zhang et al., 2023). Although concerns regarding the safety of live attenuated vaccines remain, this vaccine type has demonstrated strong and durable protective immunity and therefore continues to be widely applied in the control of many infectious diseases in both humans and animals. For ASF, efforts to develop inactivated, subunit, or recombinant vaccines have so far been unsuccessful, even when using the most promising specialized adjuvants. Consequently, live attenuated vaccines are regarded as a central strategy in the development of ASF vaccines (Kamboj et al., 2024). Importantly, optimization is required to ensure both safety and protective efficacy, while carefully balancing the benefits and risks associated with the use of live attenuated vaccines (Blome et al., 2025).

In Vietnam, ASF was first reported in 2019 (Le et al., 2019) and rapidly spread nationwide,

causing severe losses to the swine industry. Under the direction of the Government and with support from specialized agencies, several domestic companies proactively initiated research and development of ASF vaccines. Following a rigorous process of scientific and biosafety evaluation, three ASF vaccines, including AVAC ASF LIVE produced by AVAC Vietnam Joint Stock Company, were selected for field evaluation and licensing for commercial use.

In our previous publication, we reported the safety and efficacy of AVAC ASF LIVE under laboratory conditions (Diep et al., 2025). The present study reports the results of field evaluation of AVAC ASF LIVE, aiming to provide scientific evidence on the vaccine's safety and effectiveness under practical farming conditions.

II. RESEARCH CONTENT, MATERIALS, AND METHODS

2. MATERIALS AND METHODS

2.1. Research Objectives

- To evaluate the safety and the ability of the vaccine to induce an antibody-mediated immune response in vaccinated pigs under field conditions at four farms.
- To assess the protective effectiveness of the vaccine in vaccinated pigs following challenge infection.
- To evaluate the status of viremia and viral shedding in oral fluids after challenge infection.

2.2. Materials

2.2.1. Experimental vaccine

- The live attenuated lyophilized vaccine AVAC ASF LIVE for prevention of ASF was produced from the ASF-G-ΔMGF strain propagated in DMAC cell culture. The vaccine was administered as a single dose to pigs aged 4 weeks and older, with a minimum antigen content of $10^{3.5}$ HAD_{50} per dose.
- Manufacturer: AVAC Vietnam Joint Stock Company.

2.2.2. Experimental Animals

The study was conducted on healthy commercial pigs, aged 4–13 weeks, from four field farms that had never been vaccinated against ASF. All farms maintained stable husbandry conditions and appropriate veterinary hygiene standards, with different herd sizes as follows:

- **Farm 1**: Located in Trù Hựu commune, Lục Ngạn district, Bắc Giang province (currently Chũ ward, Bắc Ninh province), with a herd of 300 commercial pigs aged 4–7 weeks.
- **Farm 2**: Located in Phụng Châu commune, Chương Mỹ district, Hanoi (currently Chương Mỹ ward, Hanoi City), with a herd of 800 commercial pigs aged 8–10 weeks.
- **Farm 3**: Located in Tản Lĩnh commune, Ba Vì district, Hanoi (currently Suối Hai commune, Hanoi City), with a herd of 120 sows and 3,000 commercial pigs aged 12–13 weeks.
- **Farm 4**: Located in Long Sơn commune, Sơn Động district, Bắc Giang province (currently

Dương Hưu commune, Bắc Ninh province), with a herd of 4,220 sows and 15,700 commercial pigs aged 8–10 weeks.

2.2.3. Challenge virus

A highly virulent field isolate of ASFV genotype II was used as the challenge strain, supplied by the National Center for Veterinary Drug Testing No. I. The challenge inoculum was administered intramuscularly at a dose of 10^2 HAD_{50}/mL, with a volume of 1 mL per pig.

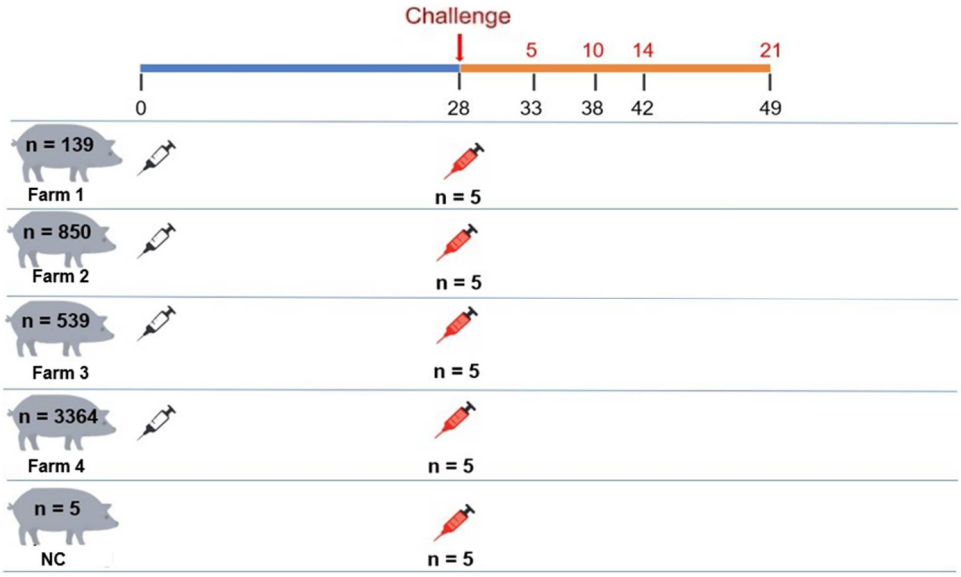
The challenge study was carried out at the National Center for Veterinary Diagnostics, located in Tân Trung Chùa hamlet, Hiền Ninh commune, Sóc Sơn district, Hanoi (currently Nội Bài commune, Hanoi City).

2.2.3. Challenge Virus Strain

2.3. Research Methods

2.3.1. Experimental Design

- After vaccination, pigs were monitored daily for health status. Random blood samples were collected from at least 30 pigs at each farm on day 28 post-vaccination for antibody testing, and five antibody-positive pigs from each farm were randomly selected for challenge. The pigs were challenged with a highly virulent ASFV genotype II at a dose of 10^2 HAD_{50} per pig.
- Following challenge, rectal temperature, clinical signs, morbidity, and mortality were monitored daily for 21 days. Blood samples and oral fluid samples (collected individually from each pig) were obtained on days 5, 10, 14, and 21 post-challenge for detection of ASFV DNA.
- Pigs that died during the monitoring period were subjected to necropsy examination for pathological lesion assessment.
- Sample collection, challenge inoculation, and laboratory testing were carried out at the National Center for Veterinary Diagnostics.



2.3.2. Methods

- Clinical signs of pigs were monitored after vaccination and observed daily during feeding.
- Rectal temperature was measured using an electronic thermometer.
- Oropharyngeal fluid samples were collected using swabs.
- Blood samples were collected from the auricular vein for viral DNA detection, and serum was separated for antibody testing.
- Antibodies were detected by ELISA using the INGENASA Ingezim PPA Compac kit (11.PPA.K.3, Spain), and interpreted according to the manufacturer's instructions.
- Virus detection was performed by real-time PCR according to TCVN 8400-41:2019.
- In positive samples, two separate qPCR assays were performed to differentiate viral strains based on the presence or absence of the *MGF360-12L* gene and the beta-glucuronidase (*β-GUS*) gene. The field virus strain (challenge strain) was characterized by the presence of the *MGF360-12L* gene, whereas the vaccine virus contained the *β-GUS* gene. The primers and probes used were as follows:

- **MGF360-12L Target:** Forward primer MGF360-12LF (5′-CATACCCTTCCCCTAAAGCTG-3′), reverse primer MGF360-12LR (5′-CTACTGCTATGTCCTGGGC-3′), and probe MGF360-12L (5′-FAM-ACCCTCTTCGAAAACATCAGCCCC-BHQ1-3′).

- **β-GUS Target:** Forward primer β-GUS-F (5′-TCTACTTTACTGGCTTTGGTCG-3′), reverse primer β-GUS-R (5′-CGTAAGGGTAATGCGAGGTAC-3′), and probe GUS (5′-FAM-AGGATTCGATAACGTGCTGATGGTGC-BHQ1-3′).

Each qPCR reaction consisted of 1 µL of DNA template, 0.4 µM of each primer, 0.2 µM probe, and 10 µL of GoTaq® Probe qPCR Master Mix from Promega. The reaction volume was adjusted to 20 µL with nuclease-free water.

The thermal cycling protocol included an initial denaturation step at 95°C for 2 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. All reactions were performed using the Bio-Rad CFX Opus 96 Real-Time PCR System (Diep et al., 2025).

A sample was considered positive when the Ct value of the corresponding target gene was below 38; samples with Ct ≥ 38 or with no detectable Ct value were recorded as negative.

The number of surviving/dead pigs was recorded after the challenge infection.

Vaccine effectiveness was calculated according to the following formula (Orenstein et al., 1985):

$$\text{Vaccine effectiveness (\%)} = \frac{\text{\% of infections in the control group} - \text{\% of infections in the vaccinated group}}{\text{\% of infections in the control group}}$$

2.4. Research ethics and supervision

Farm owners were fully informed about the experimental vaccination program and gave

consent for their pigs to be vaccinated. In the event that the vaccine caused any adverse effects in the animals, compensation would be provided in accordance with applicable regulations.

The trial program was conducted under the supervision of the Department of Livestock Production and Animal Health and the Regional Sub-Departments of Livestock Production and Animal Health (Region I and Region II).

III. RESULTS

3.1. Safety and immunogenicity of the vaccine in vaccinated pigs

Safety and the ability to induce protective immune responses are two important criteria for evaluating vaccine efficacy. These indicators were determined based on the health status of animals after vaccination and the development of specific antibodies against the pathogen in serum.

In this study, a total of 4,892 pigs from four farms were vaccinated with AVAC ASF LIVE according to the manufacturer's instructions. Clinical monitoring showed that all pigs remained healthy, with no abnormal reactions or clinical signs recorded after vaccination (Table 1).

To evaluate the immune response, serum samples from 141 pigs were randomly collected from the four farms on day 28 post-vaccination. Serological testing showed that the proportion of pigs positive for antibodies against ASFV ranged from 88.2% to 91.9%, with an average of 90.1% (Table 1).

Table 1. Immune response of pigs to the AVAC ASF LIVE vaccine

Farm	Post-vaccination reactions		Anti-ASFV antibodies at 28 days post-vaccination		
	No. of vaccinated pigs	No. of pigs with reactions	No. of tested samples	No. of positive samples	Seropositivity rate (%)
Luc Ngan (Chu, Bac Ninh)	139	0	37	34	91.9
Chuong My (Chuong My, Hanoi)	850	0	34	30	88.2
Ba Vi (Suoi Hai, Hanoi)	539	0	35	31	88.6
Son Dong (Duong Huu, Bac Ninh)	3364	0	35	32	91.4
Total / Mean	**4892**	**0**	**141**	**127**	**90.1**

3.2. Protective effectiveness in pigs following challenge

To evaluate the vaccine's ability to induce protective immunity, pigs were challenged with a highly virulent field strain of ASFV at 28 days post-vaccination and monitored continuously for 21 days. A total of 20 experimental pigs (five pigs from each farm) that tested seropositive for ASF-specific antibodies, together with five negative control pigs (unvaccinated and negative for both ASFV and ASF antibodies), were transferred to the National Center for Veterinary Diagnostics for challenge.

After challenge, the control pigs began to show characteristic clinical signs of ASF from days 2–3, including high fever, skin reddening, and reduced feed intake or anorexia over the following 3–5 days. The average body temperature reached 41°C (Figure 1).

From day 6 post-challenge, the control pigs began to die, and by day 9, all 5/5 pigs had died. Necropsy findings revealed typical ASF lesions, including generalized enlargement and severe hemorrhage of lymph nodes; enlarged spleens with dark purple-black discoloration; hemorrhagic pneumonia; and hemorrhages in the myocardium, endocardium, and renal pelvis. Laboratory examination of tissue samples from the dead control pigs confirmed ASFV infection, with high viral DNA loads (Ct = 16–17).

In contrast, the vaccinated pigs maintained a stable health status after challenge. Only a small number of individuals showed reduced feed intake for a few days or a slight transient increase in body temperature, which did not reach the fever threshold and returned to normal shortly thereafter (Figure 1).

Only one pig (C2.5 – Chương Mỹ farm) developed more severe clinical signs than the others, showing fever from days 5 to 7 and dying on day 8 post-challenge. Necropsy and laboratory examination revealed typical lesions of ASF. Tissue samples tested positive for the field ASFV strain (Ct = 18) and negative for the vaccine virus. The remaining 19 pigs survived and remained healthy throughout the 21-day observation period.

Thus, 100% of the control pigs (unvaccinated) died within 9 days after challenge. In contrast, vaccinated pigs at the Sơn Động, Lục Ngạn, and Ba Vì farms showed 100% survival, while pigs at the Chương Mỹ farm showed a survival rate of 80%. Based on these data and the applied formula for calculating protective effectiveness, the AVAC ASF LIVE vaccine demonstrated 80% at Chương Mỹ farm and 100% at the other three farms, with an overall average of 95% (Table 2).

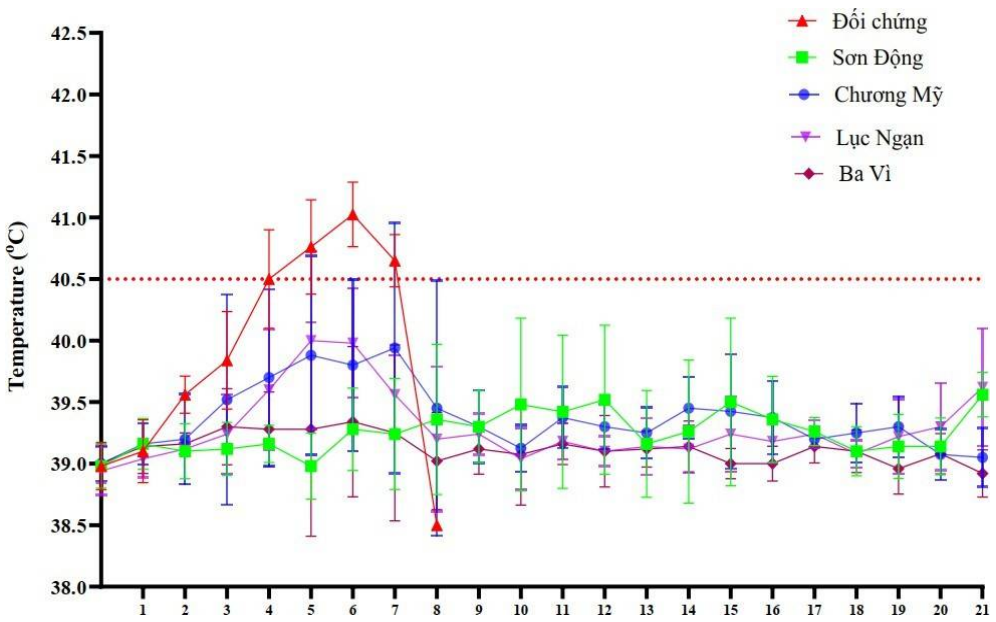


Figure 1. Average body temperature (±SD) of pigs at the farms following challenge

Table 2. Survival and mortality rates of vaccinated and control pigs

Farm	No. of surviving pigs / Total	Survival rate (%)	No. of dead pigs / Total	Mortality rate (%)	Vaccine efficacy (%)
Control	0/5	0	5/5	100	
Luc Ngan	5/5	100	0/5	0	100
Chuong My	4/5	80,0	1/5	20,0	80,0
Ba Vi	5/5	100	0/5	0	100
Son Dong	5/5	100	0/5	0	100

3.3. Results of viremia and viral shedding after challenge

To evaluate the replication and shedding of the field strain of ASFV in vaccinated pigs, blood and oral fluid samples were collected on days 5, 10, 14, and 21 post-challenge.

Viremia:

All 5/5 control pigs (unvaccinated) tested positive for viremia as early as day 5 post-challenge, with Ct values ranging from 15–16, indicating a very high viral load. This time point coincided with the onset of clear clinical signs, including high fever, anorexia, and cutaneous hemorrhages. All control pigs died before day 10 post-challenge.

In the vaccinated group, the virus was detected in blood samples from 1–3 pigs per group at different sampling time points. The proportion of pigs positive for viremia gradually decreased over time, reflecting the host's ability to control viral replication after vaccination. By day 21 post-challenge, only 3/19 pigs remained positive for viremia, and these animals were still clinically healthy (Table 3). The positive blood samples showed Ct values ranging from 23–32, indicating substantially lower viral DNA loads compared with the control group.

Table 3. Viremia results following challenge

Group	Farm	No. of viremia-positive pig's post-challenge			
		D5	D10	D14	D21
Control		5/5			
Vaccinated	Luc Ngan (Chu, Bac Ninh)	3/5	2/5	2/5	2/5
	Chuong My (Chuong My, Ha Noi)	1/5	0/4	1/4	0/4
	Ba Vi (Suoi Hai, Ha Noi)	1/5	1/5	1/5	1/5
	Son Dong (Duong Huu, Bac Ninh	0/5	0/5	1/5	0/5

Virus in oral fluids: At 5 days post-challenge, 4/5 pigs in the control group tested positive for the virulent ASFV in oral fluid samples (Table 4). This result indicates that oral fluid is an important route of viral shedding, contributing to the rapid spread of infection within unvaccinated pig populations.

In the vaccinated groups, only one oral fluid sample was detected positive for the virus at day 5 post-challenge. This sample belonged to pig C2.5 (from the Chương Mỹ farm), which was the only vaccinated animal that developed ASF and died 8 days after challenge. All remaining vaccinated pigs showed no detectable virus in oral fluid at any of the sampling time points after challenge (Table 4).

Thus, the study results demonstrated that AVAC ASF LIVE was clearly effective in reducing viremia levels and mitigating clinical signs in vaccinated pigs following challenge with the field strain of ASFV, compared with the unvaccinated control group.

In addition, the vaccine was shown to limit the shedding of virulent virus through the oral route, thereby contributing to a reduced risk of disease transmission within the herd.

Table 4. Results of viral detection in oral fluid samples post-challenge

Group	Farm	No. of ASFV-positive oral fluid samples post-challenge			
		D5	D10	D14	D21
Control		4/5			
Vaccinated	Luc Ngan (Chu, Bac Ninh)	0/5	0/5	0/5	0/5
	Chuong My (Chuong My, Ha Noi)	1/5	0/4	0/4	0/4
	Ba Vi (Suoi Hai, Ha Noi)	0/5	0/5	0/5	0/5
	Son Dong (Duong Huu, Bac Ninh	0/5	0/5	0/5	0/5

IV. DISCUSSION

To evaluate the safety and effectiveness of the live attenuated AVAC ASF LIVE vaccine for the prevention of ASF under field conditions, we vaccinated 4,892 pigs aged 4–13 weeks at four farms located in four districts of Hanoi and Bắc Giang province (now four communes/wards under Hanoi City and Bắc Ninh province).

The results showed that the vaccine was safe, causing no adverse post-vaccination reactions, while inducing a strong humoral immune response, with 88.2–91.9% (average 90.1%) of serum samples testing positive for antibodies against ASFV at 28 days post-vaccination.

Following challenge with a highly virulent genotype II field strain, 80–100% (average 95%) of vaccinated pigs survived and remained clinically healthy. In addition, the level of viremia was low, and viral shedding into the environment was markedly reduced. The immune response data and challenge results demonstrated that the protective effectiveness of AVAC ASF LIVE against ASF exceeded 80%, meaning that the vaccine reduced the risk of disease by more than 80%, even when pigs were exposed to a highly virulent genotype II field strain that caused 100% mortality in unvaccinated control pigs.

The antibody-positive rate and protective effectiveness under field conditions were lower than those observed under experimental conditions, where both the seropositive rate and protective effectiveness reached 100% (Diep et al., 2025). This difference is considered normal because field conditions differ substantially from laboratory settings, involving much larger pig populations and greater variation in individual immune responses (World Health Organization, 2025).

The results of the AVAC ASF LIVE field trial were also comparable to those of NAVET-ASFVAC, produced by NAVETCO from the ASFV-G-ΔI177L strain, which demonstrated 100% protection against virulent ASFV under laboratory conditions (Trần Xuân Hạnh et al., 2021a; Tran et al., 2022). In field trials involving 47 pigs aged 7–8 weeks at two farms, the antibody-positive rate against ASFV ranged from 84% to 90.9%, while protective effectiveness ranged from 80% to 100% (Trần Xuân Hạnh et al., 2021b).

Globally, many research groups have developed live attenuated ASF vaccine strains through targeted deletion of virulence-associated genes, showing high protective efficacy under laboratory conditions (Zhang et al., 2023). However, most of these studies remain at the small-scale experimental stage, without field validation, and have yet been licensed for commercial use. In this context, Vietnam became the first country to license two commercial ASF vaccines, produced by NAVETCO and AVAC Vietnam Joint Stock Company in 2022, followed more recently by Dacovac-ASF2, developed by DABACO Group in 2025.

To obtain more comprehensive data on the safety and effectiveness of AVAC ASF LIVE after its conditional licensing (from July 2022), the Department of Animal Health (now the Department of Livestock Production and Animal Health) required a large-scale field evaluation involving 600,000 doses across multiple regions of Vietnam and under different farming scales. The results of this large-scale evaluation were consistent with those obtained from the four farms in the present study. Based on these findings, the Department of Animal Health concluded that the vaccine met all requirements and granted approval for nationwide distribution and export from July 2023.

In addition to trials conducted in Vietnam, AVAC ASF LIVE was also evaluated in the Philippines and Indonesia. Trials in the Philippines demonstrated that the vaccine was safe and effective, with 100% of pigs developing ASFV-specific antibodies by 28 days post-vaccination, and no adverse reactions recorded (Fernandez-Colorado et al., 2024). These positive results were officially recognized and announced by the Philippine government, leading to plans for the importation and expansion of a controlled vaccination program using AVAC ASF LIVE nationwide (Marcos, 2024). In August 2024, the Philippine Department of Agriculture imported

160,000 doses, followed by an additional 340,000 doses in September 2025 to support the expanded vaccination campaign. In Indonesia, after completion of trial evaluations and independent assessments, the government officially granted commercial approval for AVAC ASF LIVE in April 2025. The first commercial batch of 120,000 doses was exported to the Indonesian market in June 2025.

Of particular concern at present is the emergence of a recombinant ASFV strain between genotype I and genotype II (rASFV I/II), which has been reported in Vietnam since late 2023 (Le et al., 2024). This strain has rapidly spread and become the main causative agent of most newly emerging outbreaks.

Studies have shown that the two ASF vaccines currently in use in Vietnam, AVAC ASF LIVE and NAVET-ASFVAC, do not provide complete protective immunity against this highly virulent recombinant strain when challenged at a dose of 10^3 HAD_{50}/pig (Diep et al., 2024). However, both vaccines have demonstrated strong homologous protection against the genotype II strain, which remains the predominant circulating strain in Vietnam (Trần Xuân Hạnh et al., 2021a,b; Diep et al., 2025).

These findings suggest that the continued use of currently available vaccines still plays an important role in ASF control strategies in areas where genotype II remains dominant, while they may also provide a certain level of partial protection against other variants.

In the future, further studies are needed to precisely determine the cross-protective capacity of commercial vaccines against the rASFV I/II strain under different conditions, such as lower challenge doses, alternative routes of infection, or booster vaccination regimens. In parallel, the development of next-generation ASF vaccines with broader protective spectrum will be essential for the effective control of both major ASFV strains currently circulating in Vietnam.

V. CONCLUSION

1. Under field conditions, the live attenuated AVAC ASF LIVE vaccine was demonstrated to be safe in commercial pigs, causing no adverse post-vaccination reactions, while inducing a strong humoral immune response, with an average ASFV antibody-positive rate of 90.1% at 28 days post-vaccination.
2. Pigs vaccinated with AVAC ASF LIVE achieved an average survival rate of 95% after challenge with a highly virulent genotype II field strain of ASFV, indicating that the vaccine provides effective protection against the genotype II strain currently circulating in Vietnam.
3. The vaccine also significantly reduced viremia and prevented the shedding of virulent virus in oral fluids, thereby contributing to a lower risk of disease transmission within the herd when exposure to the genotype II field strain occurs.

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