

KẾT QUẢ ĐÁNH GIÁ BƯỚC ĐẦU VỀ HIỆU QUẢ BẢO HỘ CHÉO CỦA VACCIN AVAC ASF LIVE ĐỐI VỚI CHỦNG TÁI TỔ HỢP rASFV I/II TRÊN LỢN THÍ NGHIỆM DƯỚI CÁC PHÁC ĐỒ TIÊM VÀ LIỀU CÔNG CƯỜNG ĐỘC KHÁC NHAU

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PRELIMINARY EVALUATION OF CROSS-PROTECTIVE EFFICACY OF THE AVAC ASF LIVE VACCINE AGAINST RECOMBINANT rASFV I/II STRAIN IN EXPERIMENTAL PIGS UNDER VARIOUS VACCINATION REGIMENS AND CHALLENGE DOSES

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ABSTRACT

The emergence and spread of recombinant African swine fever virus strains between genotypes I and II (rASFV I/II) present new challenges the live attenuated vaccines currently used in Viet Nam, which were developed from genotype II strains. This study aimed to evaluate the cross-protective efficacy of AVAC ASF LIVE vaccine against a highly virulent rASFV I/II strain isolated in Viet Nam. Experimental pigs were vaccinated according to two regimens, a single-dose regimen and a two-dose regimen, and were then challenged with the rASFV I/II strain at doses of 10, 20, 100, and 200 HAD₅₀. Protective efficacy was assessed based on clinical signs, viremia level, and survival rate after challenge. The results showed that all unvaccinated control pigs died at all challenge doses. In vaccinated pigs, challenge with 10-20 HAD₅₀ resulted in 100% survival without clinical signs. When the challenge dose increased to 100-200 HAD₅₀, survival rates decreased, ranging from 40.0% to 66.7%. The two-dose regimen produced a higher survival rate than the single-dose regimen and was also associated with lower viremia and milder disease after challenge. These findings indicate that the level of protective immunity induced by AVAC ASF LIVE against the rASFV I/II strain depends on both the vaccination regimen and the challenge dose.

Keywords: *African swine fever*; cross-protection; recombinant rASFV I/II strain; vaccine.

I. INTRODUCTION

The persistent circulation of African Swine Fever (ASF), caused by the African Swine Fever Virus (ASFV), continues to drive the need for safe and effective vaccine solutions to support disease control worldwide (Zhang et al., 2023). In this context, Viet Nam became the first country to commercialize live attenuated ASF vaccines, including AVAC ASF LIVE, NAVET-ASFVAC, and DACOVAC-ASF2. Although these vaccines differ in their gene-deletion strategies, all three were developed on the backbone of ASFV genotype II (GII), the viral genotype responsible for the first ASF outbreaks introduced into Viet Nam in 2019 (Le et al., 2019).

However, since 2023, the emergence of recombinant rASFV I/II strains between genotype I and genotype II (Le et al., 2023; Diep et al., 2024a) has posed a new challenge for disease prevention. A recent study showed that the protective efficacy of currently available vaccines decreased markedly when pigs were challenged with 10^3 HAD₅₀ of this recombinant strain (Diep et al., 2024b). Nevertheless, earlier evidence has demonstrated the possibility of cross-protection between genotypes, particularly between genotypes I and II (Monteagudo et al., 2017; Borca et al., 2025; Rathakrishnan et al., 2025).

These data raise important questions: can a vaccine developed from a GII strain induce cross-protection against the recombinant rASFV I/II strain when the challenge dose is lower and more reflective of mild exposure scenarios commonly encountered under field conditions? Can a booster vaccination enhance the immune response and thereby improve protective efficacy? To address these questions, the present study was designed with two main objectives: (i) to evaluate the cross-protective efficacy of AVAC ASF LIVE vaccine against the rASFV I/II strain when challenged at doses ranging from 10 to 200 HAD₅₀; and (ii) to determine the role of a booster vaccination in enhancing vaccine protection.

II. CONTENTS, MATERIALS, AND METHODS

2.1. Research contents

- Evaluate the cross-protective capacity of the AVAC ASF LIVE vaccine against the recombinant rASFV I/II strain at various challenge doses.
- Evaluate the impact of a booster dose on the level of cross-protection against the rASFV I/II strain.

2.2. Materials

2.2.1. *Experimental vaccine*

- **Vaccine name:** AVAC ASF LIVE, batch number: 0723 and 0424.
- **Dose:** 2 ml/pig (Each dose contains $>10^{3.5}$ HAD₅₀ vaccine virus).
- **Route of administration:** Intramuscular injection behind the ear (IM).

2.2.2. *Challenge virus strain*

- **Virus strain:** The rASFV 1/2-avac02 strain is a recombinant between genotype I and II, with its genome deposited in GenBank (PQ010732). The virus strain was isolated by AVAC Vietnam Joint Stock Company from an outbreak in Vinh Phuc in 2023.

- **Route of challenge:** Intramuscular injection behind the ear (IM).

- **Challenge dose:** 10 HAD₅₀, 20 HAD₅₀, 100 HAD₅₀, and 200 HAD₅₀ depending on the experimental group.

- **Time of challenge:** According to each experiment (Section 2.3.1).

2.2.3. *Experimental animals*

- Three-breed crossbred pigs (Yorkshire x Landrace x Duroc), 5 weeks old, healthy, and negative for ASFV, PRRSV, PCV2, CSFV, and FMDV.

- Pigs were quarantined for 5–7 days for adaptation, ear-tagged for identification, and monitored for baseline body temperature before the experiment.

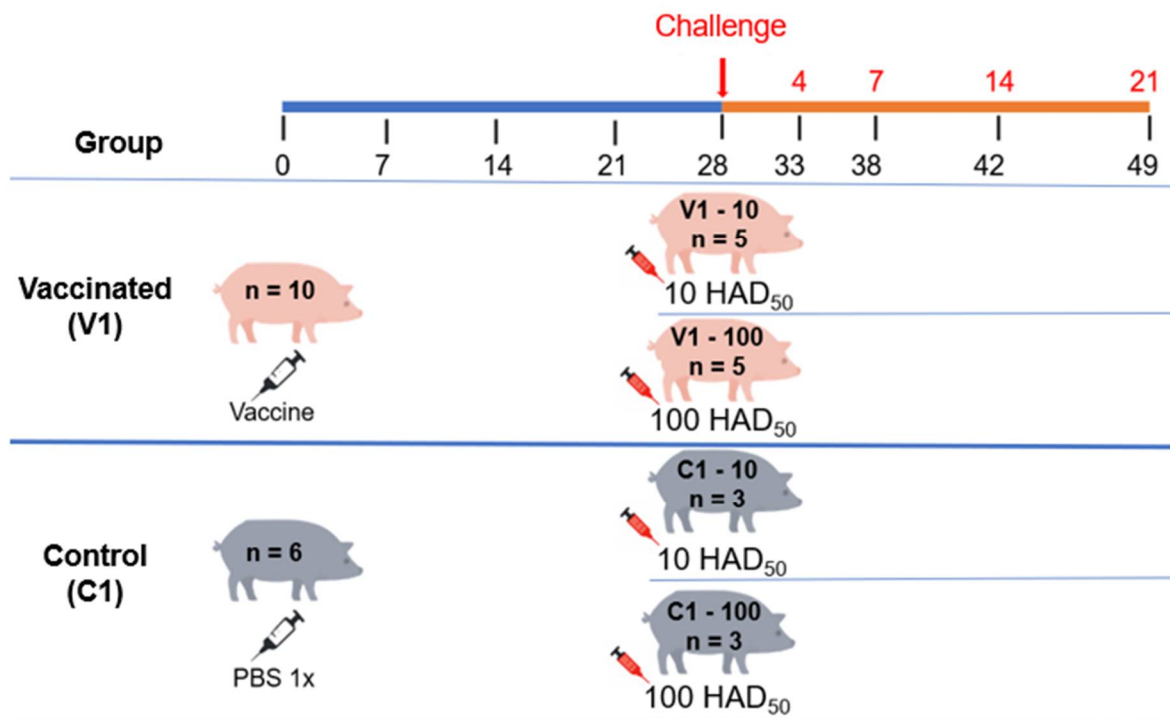
2.3. Methods

2.3.1. *Experimental design*

The study was conducted on 26 healthy 5-week-old pigs as described in section 2.2.3. Two independent experiments were performed to evaluate the protective efficacy of the vaccine following single-dose and two-dose regimen.

Experiment 1: Evaluation of protective efficacy with a single-dose vaccine regimen

A total of 16 pigs were randomly divided into two main groups: a vaccinated group (V1, 10 pigs) and a control group (C1, 6 pigs). At 28 days after vaccination, each main group was divided into two subgroups to conduct the challenge with different doses (10 and 100 HAD₅₀), including: groups V1-10 and V1-100 in the vaccinated group, and groups C1-10 and C1-100 in the control group.



- **Control group (C1-10 and C1-100):** 6 pigs (ear tags #1 to #6) were injected with 2mL of PBS 1x.

- **Vaccinated group (V1-10 and V1-100):** 10 pigs (ear tags #7 to #16) were administered a single dose of AVAC ASF LIVE vaccine according to the manufacturer's instructions.

Pigs in the vaccinated and control groups were housed in separate pens. They were provided with age-appropriate feed.

Pre-challenge sampling: Blood samples were collected at 7, 14, 21, and 28 days post-vaccination (D7, D14, D21, D28) for ASFV antibody testing.

Challenge: At D28, all pigs were challenged with the rASFV I/II strain via intramuscular injection behind the ear at various doses:

- Challenge with 10 HAD₅₀ dose: included 3 control pigs (group C1-10; #1, #2, #3) and 5 vaccinated pigs (group V1-10; pigs #7 to #11).

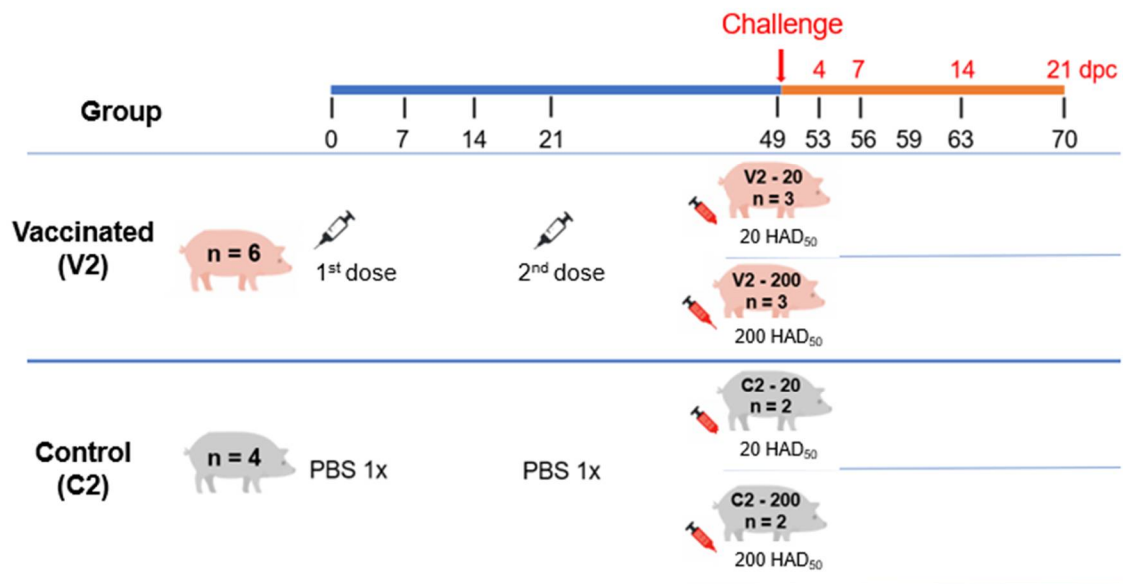
- Challenge with 100 HAD₅₀ dose: included 3 control pigs (group C1-100; #4, #5, #6) and 5 vaccinated pigs (group V1-100; pigs #12 to #16).

Post-challenge monitoring: Pigs were monitored continuously for 21 days. Rectal temperature, clinical scores, mortality rate, time of death, and necropsy findings in dead pigs were recorded.

Blood samples were collected at D4, D7, D14, and D21 post-challenge and at the time of death to test for ASFV DNA. All pigs that died during the monitoring period were necropsied, and samples were taken to determine the cause of death.

Experiment 2: Evaluation of protective efficacy with a two-dose vaccination regimen

10 pigs were randomly divided into two main groups: the vaccinated group (V2, 6 pigs) and the control group (C2, 4 pigs). Twenty-one days after the first vaccination (1st dose), the vaccinated group received a second dose (2nd dose), while the control group was injected with PBS 1x. Twenty-eight days after the second dose (49 days after the first dose), each main group was divided into two subgroups to conduct the challenge with various doses (20 and 200 HAD₅₀), including: V2-20 and V2-200 in the vaccinated group, and C2-20 and C2-200 in the control group.



- Control group (C2): 4 pigs (ear tags B1 to B4) were injected with two doses of PBS 1x following the same schedule as the vaccinated group.

- Vaccinated group (V2): 6 pigs (ear tags B5 to B10) were administered with two doses of the vaccine, 21 days apart.

Groups of pigs were raised in separate pens.

Post-vaccination sampling: Blood was collected at D7, D14, D21, D28, and D49 to test for ASFV antibodies.

Challenge: At D49, all pigs were challenged with the rASFV I/II strain via intramuscular injection behind the ear at various doses:

- Challenge with 20 HAD₅₀ dose: included 2 control pigs (B1, B2; group C2-20) and 3 vaccinated pigs (B5, B6, B7; group V2-20).
- Challenge with 200 HAD₅₀ dose: included 2 control pigs (B3, B4; group C2-200) and 3 vaccinated pigs (B8, B9, B10; group V2-200).

Pigs were monitored continuously for 21 days, for body temperature, clinical score, mortality rate, and time to death. Dead pigs were necropsied. Blood samples were collected on D4, D7, D14, and D21 after challenge, as well as at death, to detect ASFV DNA.

2.3.2. Laboratory methods

- Rectal temperature was measured in the morning using an electronic thermometer.
- Detection of ASFV DNA by real-time PCR
 - + DNA was extracted from blood, oropharyngeal swabs, feces, urine, and tissue using the DNeasy Blood & Tissue Kit (Qiagen, Germany). ASFV was detected using the VDX ASFV qPCR Kit (Median Diagnostics, South Korea).
 - o Ct < 38: Positive
 - o Ct ≥ 38 or no Ct: Negative
- Detection of ASFV antibodies by ELISA: using the VDPPro® ASFV Ab i-ELISA V2.0 kit (Median Diagnostics, South Korea).
 - o S/P ≥ 0.25: Positive
 - o S/P < 0.25: Negative

III. RESULTS

3.1. Single-dose vaccination regimen

3.1.1. Humoral immune response post-vaccination

ELISA results at 7, 14, 21, and 28 days after vaccination showed that ASFV-specific antibodies were not detected on day 7. However, from day 14 onward, all vaccinated pigs (10/10) were antibody-positive, reaching a rate of 100%. Antibody levels increased gradually over time, with

S/P values increasing from 0.84 $\pm$ 0.21 on day 14 to 1.14 $\pm$ 0.12 on day 28 (Figure 1). All control pigs (C1 group) remained negative for ASFV-specific antibodies.

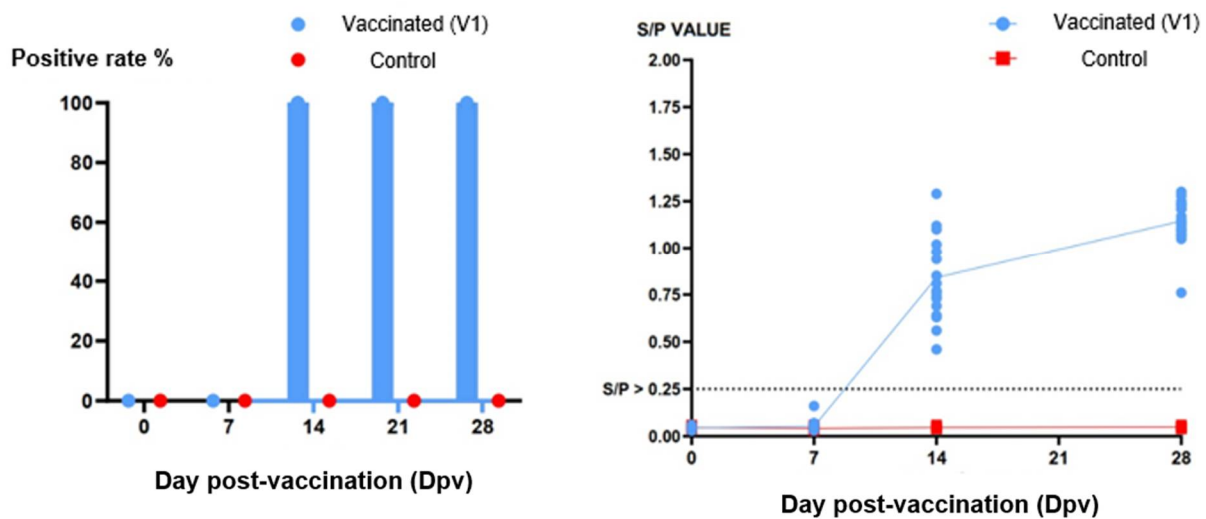


Figure 1. The seropositivity rate and S/P values in pigs following a single-dose vaccination

3.1.2. Clinical signs after vaccination and challenge

After vaccination, all pigs in both the vaccinated and control groups maintained normal appetite and body temperature. After challenge, control pigs developed clinical signs from day 5 onward, which became progressively more severe, including high fever ($>40.5^{\circ}\text{C}$), reduced appetite, and reduced movement. All control pigs died after challenge. The C1-100 group (100 HAD₅₀ challenge) died within 7-9 days, whereas the C1-10 group (10 HAD₅₀ challenge) died within 9-19 days. In the group vaccinated with one dose and challenged with 10 HAD₅₀ (V1-10), no major clinical signs were recorded. Some individuals developed mild fever and transiently reduced appetite for 1-2 days (Figure 2), after which body temperature returned to normal. All 5/5 pigs (100%) remained healthy after 21 days of monitoring (Figure 3).

In the group vaccinated with one dose and challenged with 100 HAD₅₀ (V1-100), three of five pigs (#12, #14, and #16) developed clinical signs from day 7 after challenge, with progressively severe disease (high fever and anorexia) and died between days 9 and 19. The two remaining pigs (#13 and #15) had only mild increases in body temperature from days 4 to 9 and then returned to normal. The survival rate in this group was 40% after 21 days of monitoring (Figure 3).

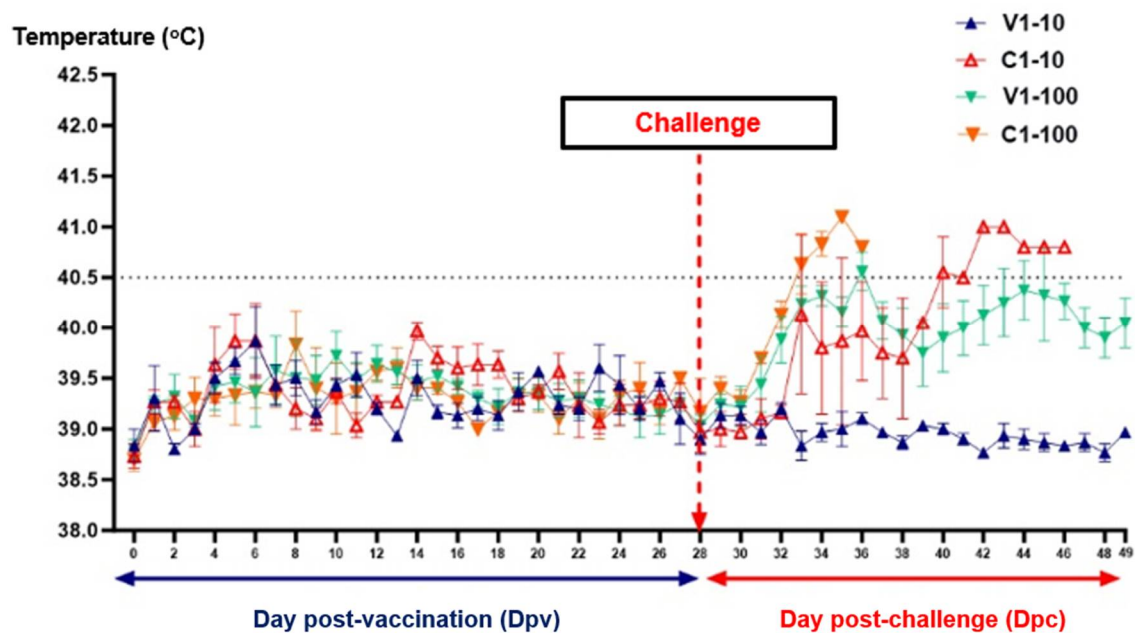


Figure 2. Body temperature of pigs in the single-dose vaccination regimen

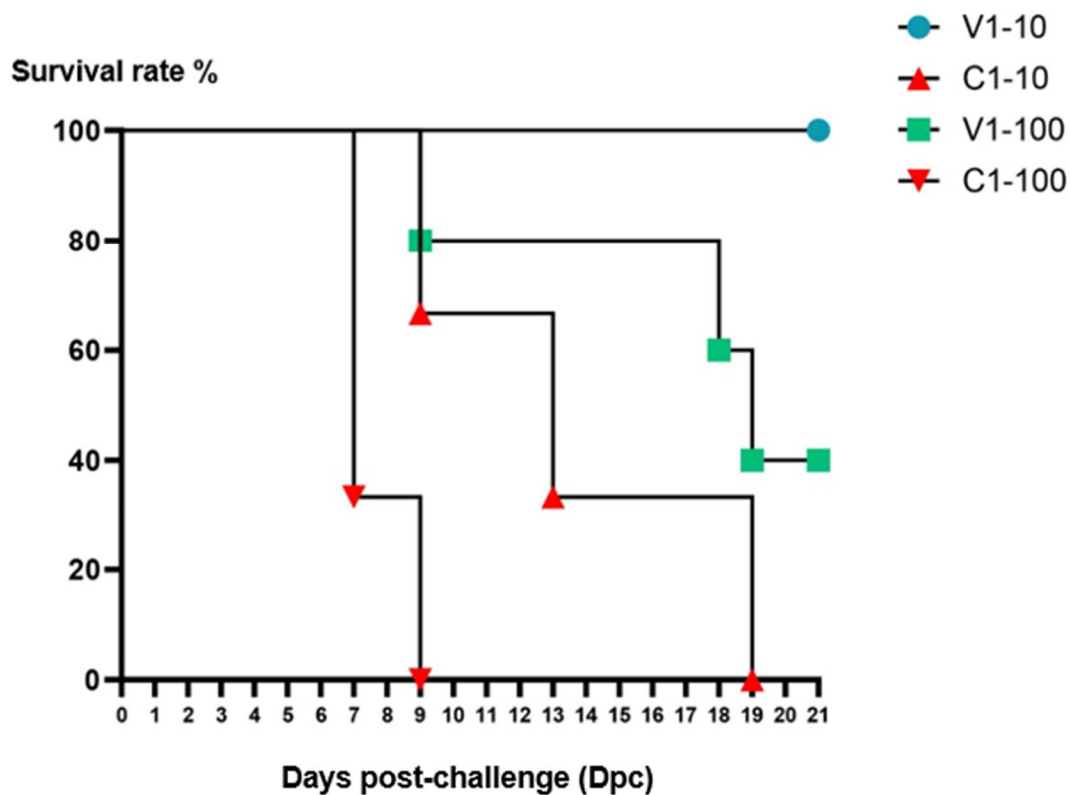


Figure 3. Survival rate of pigs during 21 days post-challenge following a single-dose vaccination

3.1.3. Post-challenge viremia

In the 10 HAD₅₀ challenge group: In the control group (C1-10), viremia was detected at varying levels: pig #2 had a very high viral load on day 7 (Ct = 14.21), while pigs #1 and #3 tested positive on day 10 (Ct = 25.71) and day 14 (Ct = 31.63), respectively. All dead pigs showed high viral loads in necropsy samples (Ct = 18.14-22.00). In contrast, in vaccinated pigs (V1-10), all samples were negative for ASFV DNA at 4, 7, 10, 14, and 21 days after challenge.

In the 100 HAD₅₀ challenge group: In the control group (C1-100), viremia was detected as early as day 4 post-challenge, showing strong replication of the highly virulent virus; Ct values during fever ranged from approximately 17.87–20.04.

In the vaccinated group (V1-100), pig #15 was negative for viremia at all sampling times. Pig #12 showed viremia on day 7 (Ct = 30.11) and died on day 9, with a high viral load at necropsy (Ct = 19.52). By day 14, viremia was detected in 2/4 pigs (#13 and #16), with Ct values of 36.84 and 35.06, respectively. During days 18-19, 2/4 pigs (#14 and #16) died with high viral loads in pathological samples (Ct = 20.09 and 20.41). At the end of the experiment (day 21), two pigs survived; pig #13 still had detectable viremia (Ct = 28.15) (Table 1).

Table 1. Viremia test results (Ct) in pigs post-challenge

Day post-challenge	10 HAD ₅₀ dose								100 HAD ₅₀ dose							
	Control C1-10			Vaccinated V1-10					Control C1-100			Vaccinated V1-100				
	#1	#2	#3	#7	#8	#9	#10	#11	#4	#5	#6	#12	#13	#14	#15	#16
0	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	31.81	-	-	-	-	-
6																
7	-	14.21	-	-	-	-	-	-	27.45	18.27	17.87	30.11	-	-	-	-
8																
9		18.14							20.04			19.52				
10	25.71		-	-	-	-	-	-	-				-	-	-	-
12																
13	20.74															
14			31.63	-	-	-	-	-	-				36.84	-	-	35.06
18														20.09		
19			22.00													20.41
21				-	-	-	-	-	-				28.15		-	

3.2. Experiment in pigs vaccinated with two doses

3.2.1. Humoral immune response post-vaccination

In pigs that received two vaccine doses, with the second dose administered 21 days after the first, serological results showed that the proportion of pigs positive for ASFV-specific antibodies increased over time. Antibodies were not detected at 7 days after the first dose; by day 14 after the first dose, 100% of pigs were antibody-positive, and this rate remained 100% at 21 days after the first dose. After booster vaccination, the antibody-positive rate continued to remain 100% on day 49 of the experiment, corresponding to 28 days after the second dose. The S/P index reached 0.92 $\pm$ 0.14 at 14 days after vaccination and increased to 1.23 $\pm$ 0.19 on day 49 (Figure 4). All control pigs were negative for ASFV-specific antibodies at all sampling times.

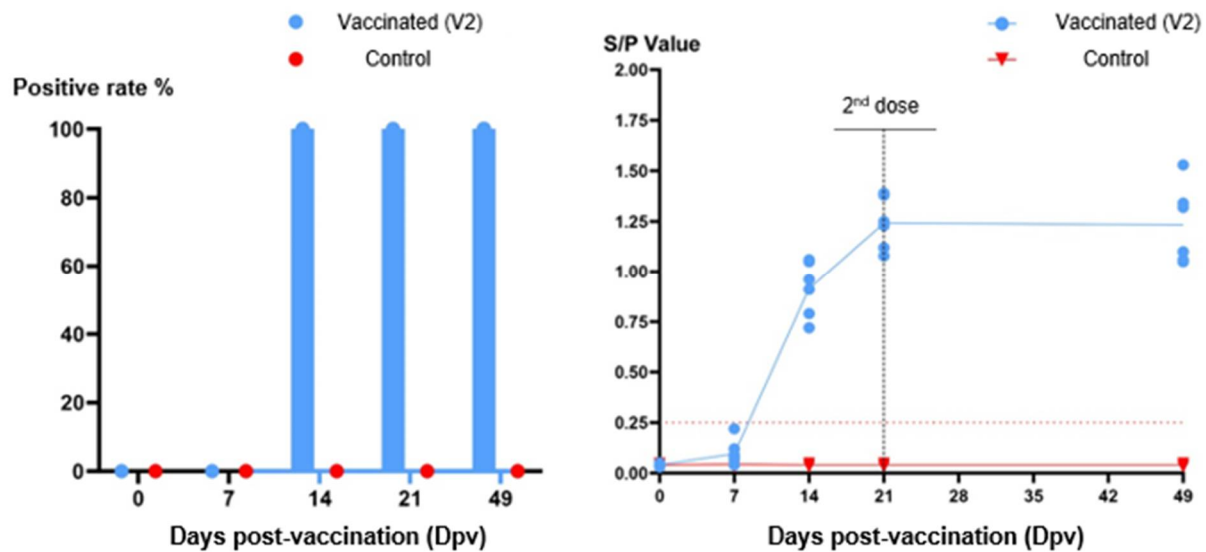


Figure 4. Antibody positive rate and S/P values in the two-dose vaccination regimen

3.2.2. Clinical signs of pigs before and after challenge

Before challenge, all experimental pigs were healthy, with no abnormal clinical signs; body temperature and feed intake remained within normal physiological ranges. After challenge, control pigs began to show characteristic clinical signs, including depression and high fever (>40.5 °C), which became progressively worse over time, with persistent fever, reduced appetite, and reduced movement. Both pigs in the C2-200 control group died 8 days after challenge with 200 HAD₅₀, while pigs in the C2-20 control group died 9-13 days after challenge with 20 HAD₅₀.

In contrast, among pigs vaccinated with two doses of AVAC ASF LIVE, challenge with 20 HAD₅₀ (V2-20) resulted in 100% survival (3/3), with no abnormal clinical signs throughout the 21-day post-challenge observation period (Figures 5 and 6). In the group challenged with 200 HAD₅₀ (V2-200), one of three pigs (B8) began showing clinical signs on day 8; signs became progressively severe, including high fever and anorexia, and the pig died on day 14 after challenge. The remaining two pigs in this group stayed healthy and maintained normal body temperature and appetite throughout the observation period. The survival rate of V2-200 was 66.7% after 21 days of monitoring (Figure 6; Table 2).

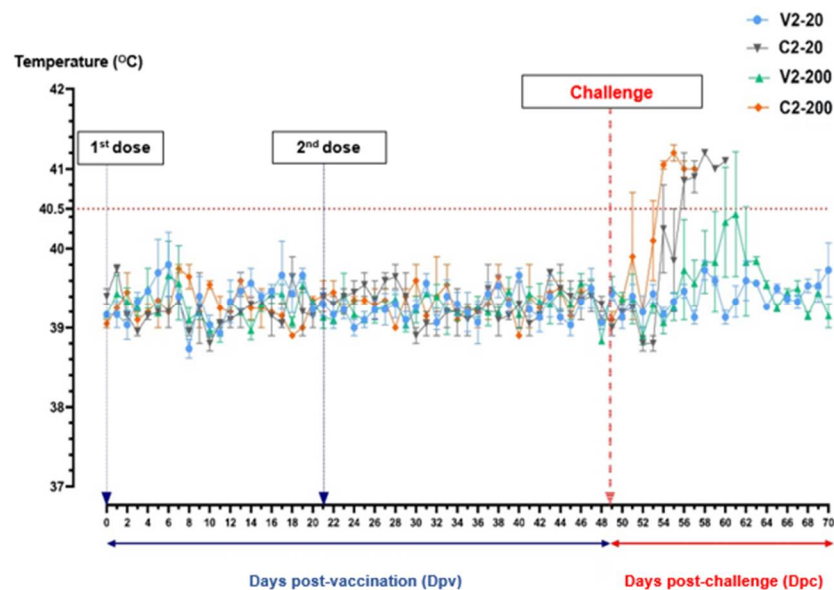


Figure 5. Body temperature of pigs in the two-dose vaccination regimen

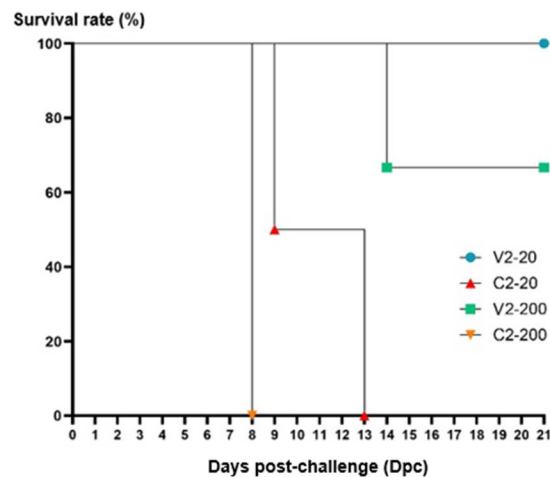


Figure 6. Survival rate of pigs post-challenge in the two-dose vaccination regimen

Table 2. Survival rates of pigs post-challenge in the experiments

Regimen	Challenge dose	Vaccinated Group	Control group
Single-dose	10 HAD ₅₀	5/5 (100%)	0/3
	100 HAD ₅₀	2/5 (40%)	0/3
Two-dose	20 HAD ₅₀	3/3 (100%)	0/2
	200 HAD ₅₀	2/3 (66,7%)	0/2

3.2.3. Viremia test results

The viremia test results for pigs post-challenge in the two-dose vaccination are presented in Table 3.

Table 3. Viremia test results in pigs post-challenge under the two-dose vaccination regimen

Pig group Dpc	20 HAD ₅₀ dose					200 HAD ₅₀ dose				
	Control C2-20		Vaccinated V2-20			Control C2-200		Vaccinated V2-200		
	B1	B2	B5	B6	B7	B3	B4	B8	B9	B10
0	-		-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	34.54	-	-	-
7	15.20	-	-	-	-	24.25	19.50	29.41	-	-
8						21.44	18.09			
9	18.83									
10		25.71	-	-	-			23.29	-	-
13		20.74								
14			-	-	-			19.58	-	-
19										
21			-	-	-				-	-

In the 20 HAD₅₀ challenge group: In the control group (C2-20), 1/2 pigs (#B1) tested positive for viremia on day 7 post-challenge with a very high viral load (Ct = 15.20), and died on day 9; the pathological sample collected at the time of death had a Ct = 18.83. The other control pig (B2) became viremic from day 10 after challenge (Ct = 25.71) and died on day 13; the pathological sample at death had a Ct value of 20.74. In contrast, in the V2-20 group vaccinated with two doses, all blood samples collected at all testing time points were negative, and no ASFV was detected in blood (Table 3).

In the 200 HAD₅₀ challenge group: In the control group (C2-200), viremia was detected early, starting from day 4 post-challenge, and replicated strongly; Ct values of blood samples collected on day 7 were 19.50 and 24.25. Both control pigs (B3 and B4) died on day 8 after challenge. In the V2-200 vaccinated group, 2/3 pigs had no detectable viremia in blood at any sampling time. Only one pig (B8) developed viremia on days 7 and 10 after challenge, with Ct values of 29.41 and 23.29, respectively; it died on day 14, and the pathological sample at death showed a high viral load (Ct = 19.58). Viremia was not detected in the two remaining vaccinated pigs (B9 and B10) at any sampling time (Table 3).

IV. DISCUSSION

The cross-protective capacity of a vaccine is closely dependent on the degree of antigenic similarity between the vaccine strain and the challenge virus strain. Many studies have reported different levels of cross-protection among ASFV strains. For example, the genotype I BA71ΔCD2 vaccine (CD2v-deleted) showed effective cross-protection against the highly virulent genotype II Georgia/2007 strain (Monteagudo et al., 2017). Conversely, the genotype II GΔDKE-Cmut strain could provide cross-protection against genotype I but was not effective against genotype IX (Rathakrishnan et al., 2025). Similarly, ASFV-G-ΔI177L induced durable immunity only against genotype II strains with high genetic similarity, whereas protective efficacy decreased when challenged with strains of greater genetic distance (Borca et al., 2025).

Based on studies showing bidirectional cross-protection between ASFV strains of genotypes I and II (Monteagudo et al., 2017; Rathakrishnan et al., 2025), vaccines developed from genotype II strains are expected to induce protective immunity against recombinant rASFV I/II strains. This recombinant strain has a hybrid genome structure, with approximately 44% of its genetic material derived from genotype I and 56% from genotype II. Notably, most genes related to virulence and immunogenicity belong to genotype II (Zhao et al., 2023). These characteristics provide a reasonable biological basis for explaining the potential cross-protective capacity of vaccines produced from genotype II viruses against the recombinant rASFV I/II strain.

The results of the present study showed that the live attenuated AVAC ASF LIVE vaccine (genotype II) induced a degree of cross-protection against the recombinant strain rASFV I/II-avac02. However, protective efficacy was clearly dependent on the challenge dose and vaccination regimen. When challenged with low doses (10-20 HAD₅₀), both the single-dose and two-dose regimens achieved complete protection, as shown by the absence of viremia and clinical signs in vaccinated

pigs. This suggests that the immune response induced by vaccination was strong enough to effectively control replication of the recombinant virus under low infection pressure.

In contrast, when the challenge dose increased to higher levels (100-200 HAD₅₀), vaccine efficacy declined, as reflected by the appearance of viremia, mild to moderate clinical signs, and some deaths. Compared with the single-dose regimen, the two-dose regimen resulted in a higher survival rate and lower viremia, even though pigs were challenged with a two-fold higher virulent dose (200 HAD₅₀ versus 100 HAD₅₀).

As with other infectious diseases, the virulence and pathogenicity of ASFV depend not only on viral genetic characteristics but also on infectious dose, route of entry, and host susceptibility (Karalova et al., 2015; Pietschmann et al., 2015; Olesen et al., 2017; Walczak et al., 2020). Several studies have shown that even very low infectious doses (5-10 HAU) can cause disease in susceptible pigs (Pietschmann et al., 2015; Walczak et al., 2020). Conversely, when the infectious dose is high, breakthrough infection may occur because the infection pressure exceeds the control capacity of vaccine-induced immunity (Plotkin, 2010; WHO, 2025). The study by Diep et al. (2024b), in which AVAC ASF LIVE and NAVET-ASFVAC failed to completely protect pigs against recombinant rASFV I/II when a 10³ HAD₅₀ challenge dose was used, clearly illustrates this phenomenon. The dependence of protective efficacy on challenge dose has also been reported for the genotype I BA71ΔCD2 vaccine, with clear protection at a challenge dose of 10₂ HAD₅₀ but a marked decrease in protection at higher challenge doses (Monteagudo et al., 2017).

Taken together, the present findings and published data indicate that the reduced cross-protection of AVAC ASF LIVE against the recombinant strain is associated with high challenge doses. At low exposure levels corresponding to many natural field infection scenarios, such as virus contamination on footwear, clothing, equipment, or transport vehicles, the vaccine still maintained complete protective efficacy. However, the clear decline in cross-protection at high challenge doses indicates that during intense outbreaks of recombinant strains, when diseased pigs shed large quantities of virus and exposure occurs continuously, the vaccine may not provide optimal protection. In addition, while one dose of AVAC ASF LIVE may be sufficient to induce protection against homologous genotype II strains (Diep et al., 2025), a two-dose regimen may be an option to enhance cross-protection against recombinant strains, particularly under conditions of high exposure risk. This regimen, however, requires confirmation in larger-scale studies.

Although important results were obtained, this study has several limitations. The sample size was small, the evaluation was limited to one recombinant strain currently circulating widely, and natural routes of infection such as oral or respiratory exposure were not simulated. In addition, virus shedding and the risk of transmission after challenge were not fully evaluated. Therefore, further studies should be conducted on a larger scale, include different recombinant strains, and perform more comprehensive analyses of viral shedding and horizontal transmission risk.

V. CONCLUSION

This preliminary study showed that the cross-protective efficacy of AVAC ASF LIVE vaccine against the recombinant rASFV I/II strain depended on the vaccination regimen and virulent challenge dose. Under experimental conditions, cross-protection tended to be higher with the two-dose regimen than with the single-dose regimen and decreased as the challenge dose increased. Further studies with larger sample sizes are needed to confirm these observations and provide a basis for applying vaccination regimens under field conditions to support ASF prevention and control when both genotype II strains and recombinant strains are co-circulating in Viet Nam.

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