Nghiên cứu khoa học

ĐÁNH GIÁ SỰ TỒN LƯU CỦA VIRUS VACXIN AVAC ASF LIVE Ở LỢN SAU TIÊM PHÒNG

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EVALUATION OF THE PERSISTENCE OF AVAC ASF LIVE VACCINE VIRUS IN PIGS POST-VACCINATION

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ABSTRACT

This study aimed to evaluate the temporal persistence and distribution of the AVAC ASF LIVE vaccine virus in pigs post-vaccination, as well as the infectivity of residual vaccine virus for susceptible cells and pigs. The experiment involved 4- and 6-week-old pigs from two farms with different epidemiological conditions, with monitoring extended until the time of marketing (corresponding to 5 and 5.5 months post-vaccination). Blood and tissue samples were analyzed by real-time PCR and virus isolation on DMAC cells. In addition, experimental inoculation in pigs was performed to evaluate the infectivity of residual vaccine virus. The results indicated that viral DNA was primarily detected within the first 28 days post-vaccination at low levels. By the time of marketing, most pigs tested negative, with only 10–20% showing weak real-time PCR signals in specific tissues such as the spleen, lymph nodes, liver, and lungs. Blood and carcass samples from healthy pigs were negative, except for one stunted pig exhibiting a weak DNA signal. However, these PCR-positive samples did not contain virus capable of replication in susceptible DMAC cells and did not infect experimental pigs. Pigs inoculated with PCR-positive tissue or blood samples displayed no clinical signs, no viremia, and no specific antibody responses. These findings suggest that PCR-positive signals detected at the time of marketing reflect the persistence of vaccine viral DNA without the presence of infectious virus. Therefore, the AVAC ASF LIVE vaccine poses no food safety risk and does not result in the dissemination of infectious virus into the environment during slaughter. This study provides important experimental evidence confirming the safety of the AVAC ASF LIVE vaccine under field conditions and offers a scientific basis for supporting the effective control of African swine fever in Vietnam.

Keywords: African swine fever, AVAC ASF LIVE, viral persistence, live attenuated vaccine.

I. INTRODUCTION

African swine fever (ASF), caused by the African swine fever virus (ASFV), is one of the most dangerous infectious diseases in swine. It is characterized by high fever, multi-organ hemorrhages, and a mortality rate that can reach 90–100% in susceptible pig herds (Dixon *et al.*, 2019; WOAH, 2025). Since it was first recorded more than a century ago, ASF has caused severe and prolonged losses to the global swine industry, while continuing to threaten the livelihoods of farmers and food security in many countries (FAO, 2025). In Vietnam, the disease was first detected in 2019 and rapidly spread nationwide, resulting in particularly severe socioeconomic consequences (Le *et al.*, 2019; Nguyen-Thi *et al.*, 2021).

For more than a century, no commercial vaccine against ASF had been available worldwide (Zhang et al., 2023). In this context, Vietnam's authorization for the use of live attenuated vaccines, including AVAC ASF LIVE, NAVET-ASFVAC, and DACOVAC-ASF2, represents an important milestone in ASF prevention and control. However, because live attenuated vaccines are capable of replication, although limited, in the host, they always require strict biosafety monitoring, especially in relation to the risk of persistence and shedding of infectious vaccine virus (Coelho Cruz et al., 2023; Zhang et al., 2023). Therefore, in evaluating the safety of live attenuated vaccines, determining whether the residual vaccine virus remains infectious post-vaccination is of paramount importance. Viral DNA detection methods such as PCR or qPCR allow for the identification of viral genetic material but do not provide information regarding the replication capacity or infectivity of the virus. Consequently, to draw comprehensive and reliable conclusions regarding the biosafety level of live attenuated vaccines, it is essential to combine multiple complementary evaluation methods, including virus isolation on susceptible cell lines and experimental inoculation in susceptible animals.

Based on these requirements, the present study was conducted to: (i) determine the persistence level and distribution of AVAC ASF LIVE vaccine virus in blood and internal tissues of pigs after vaccination; (ii) evaluate the ability of virus from DNA-positive samples to replicate in susceptible cell culture; and (iii) examine the infectivity of virus from DNA-positive samples in susceptible animals. The results provide necessary scientific evidence for a comprehensive biosafety assessment of the vaccine and support guidance for the use of AVAC ASF LIVE in ASF control programs in Vietnam.

II. CONTENTS, MATERIALS, AND METHODS

2.1. Research contents

- Evaluate the persistence of vaccine virus in pig blood and organs at 2 and 4 weeks post-vaccination, as well as at the time of marketing using real-time PCR.

- Assess the replication potential of the virus from DNA-positive samples at the time of marketing on susceptible DMAC cells.

- Evaluate the infectivity of vaccine virus from tissues of DNA-positive pigs at the time of marketing via experimental inoculation in pigs through oral and intramuscular routes.

2.2. Materials

The vaccine used in this study was AVAC ASF LIVE (batch numbers 0723 and AAF0124) manufactured by AVAC Vietnam Joint Stock Company. The vaccine was developed from the African swine fever (ASF) virus strain ASF-G-ΔMGF in lyophilized form, which is reconstituted with a diluent before use.

2.3. Experimental animals

The experimental subjects were 4- and 6-week-old pigs, that had not been vaccinated against ASF. The experiments were performed at two farms with different production conditions and epidemiological characteristics, as described in Section 3.1. The pigs were sourced from breeding farms certified as ASF-free.

III. RESEARCH METHODS

3.1. Experimental design

The objective of this experiment was to evaluate the persistence of the vaccine virus from the time of vaccination until the time of marketing. The monitoring period was 5.5 months for the group vaccinated at 4 weeks of age and 5 months for the group vaccinated at 6 weeks of age. The experiment was conducted at two farms with different scales and health status, as follows.

- Farm I: 100 piglets (Yorkshire x Landrace), 4 weeks old, in good health, were carefully selected from a breeder farm. Before vaccination, all piglets were tested by RT-PCR / real-time PCR, confirming negative for ASFV, CSFV, FMDV, PRRSV, and Mycoplasma spp. The pigs were vaccinated intramuscularly with AVAC ASF LIVE, batch 0723, at a dose of 2 ml per pig.

- Farm II: A total of 500 clinically healthy 6-week-old three-breed pigs (Yorkshire x Landrace x Duroc) were used. The pigs were obtained from a sow farm with a history of positivity for PRRSV, PCV2, and *Haemophilus parasuis* (Glasser's disease). Immediately after arrival, blood and nasal swab samples from 15 randomly selected pigs were tested. The results were: 4/15 positive for PRRSV; 5/15 positive for PCV2; 9/15 positive for *Haemophilus parasuis*; 7/15 positive for *Streptococcus suis*; 15/15 negative for ASFV, CSFV, FMDV, and *Mycoplasma* spp.; and 15/15 negative for anti-ASFV antibodies. After 3-4 days of acclimatization, all pigs were vaccinated intramuscularly with AVAC ASF LIVE, batch 0124, at a dose of 2 ml per pig.

3.2. Sample collection and vaccine virus persistence testing

- Farm I: Blood and tissue samples (liver, spleen, kidney, lymph nodes, lung, heart, bone marrow, muscle) were collected at 14, 28, and 5.5 months post-vaccination to test for viral persistence by real-time PCR.

- Farm II: During the grow-out period, some pigs became stunted or showed clinical signs such as respiratory signs and diarrhea and required treatment. These pigs were moved to several separate pens at the end of the barn for intensive care. At the time of marketing (5 months after vaccination, corresponding to 6.5 months of age), 15 pigs with different health statuses were randomly selected from two groups: a healthy group of 10 pigs showing good growth, and a stunted/treatment group of 5 pigs that had shown clinical problems during the rearing period and had been treated with antibiotics or anti-inflammatory drugs.

Both groups were necropsied, and blood and internal tissue samples were collected to examine residual vaccine viral DNA by real-time PCR. Samples positive for viral DNA at necropsy were stored at -80°C and used to prepare pooled samples for virus isolation on DMAC cells and for experimental inoculation of six 4-week-old pigs. After inoculation, pigs were monitored clinically and blood and organ samples were collected at 14, 28, and 60 days. At the end of the observation period (day 60), all pigs were necropsied and tissue samples were collected. ASF viral DNA was analyzed by real-time PCR and specific antibodies were detected by ELISA to fully evaluate the infectivity of virus present in ASF viral DNA-positive samples.

3.3. Research methodology

3.3.1. Virus detection by real-time PCR

Total DNA was extracted from blood and tissue samples using the DNeasy Blood & Tissue Kit (Qiagen, Germany), then detected for ASF target genes using the VDx ASFV qPCR kit (Median Diagnostics, South Korea).

- Positive result: $Ct < 38$

- Negative result: $Ct \geq 38$.

3.3.2. ASF antibody detection by ELISA

Antibodies were determined using the VDPro® ASFV Ab i-ELISA V2.0 kit.

- Positive sample: $S/P \geq 0.25$

- Negative sample: $S/P < 0.25$.

3.3.3. Virus isolation on DMAC cells

Clinical samples were homogenized, diluted 1:10 in PBS, and centrifuged at 5000 rpm for 10 minutes. The supernatant was used to inoculate the DMAC cell line grown in 12-well plates at 37°C with 5% CO2, using 0.5 ml of virus suspension per well. After 48 hours, 1% pig red blood cells were added and the plates were monitored daily. A negative-control well and a positive-control well inoculated with ASF-G-DeltaMGF at 10^4 HAD50 were included in parallel for comparison. After 7 days of culture, samples that did not show cytopathic effect (CPE) or hemadsorption and in which cells grew normally as in the negative-control well were considered negative.

3.3.4. Assessment of vaccine virus infectivity persistence in susceptible animals

Blood and tissue samples positive for ASF viral DNA in the persistence experiment were pooled and treated with 10% gentamicin at 2-8°C for 8-12 hours. The pooled material was then used to inoculate six 4-week-old experimental pigs by two routes: intramuscular (IM), 2 ml per pig, and oral (PO), 2 ml per pig. Each inoculation route was applied to three pigs. The pigs were monitored clinically, and blood and internal organ samples were collected at 14, 28, and 60 days after inoculation to test for virus by real-time PCR.

IV. RESEARCH RESULTS

4.1. Persistence of the vaccine virus in pigs

4.1.1. Results at Farm I (monitoring up to 5.5 months post-vaccination)

At 14 days post-vaccination, three pigs (#1-#3) were necropsied and sampled for testing of vaccine viral DNA. The results showed that 2/3 pigs were weakly positive for ASF viral DNA, with Ct values ranging from 35.62 to 37.92. Pig #2 was positive in blood, lymph node, and lung samples, whereas pig #3 was positive only in the lymph node sample.

At 28 days post-vaccination, necropsy of three pigs (#4-#6) showed that viral DNA was detected only in the spleen sample of pig #4 (Ct = 37.28) and the lung sample of pig #5 (Ct = 36.23); all remaining samples were negative.

At the time of marketing (5.5 months after vaccination), Farm I had 98 healthy surviving pigs and two pigs that had died non-suddenly, without typical ASF lesions and negative for ASF viral DNA. Testing of blood samples from all 98 pigs at marketing showed that 100% were negative for ASF viral DNA. Ten pigs were randomly selected for necropsy and internal organ sampling to assess viral persistence. Necropsy showed that carcasses and internal organs were completely normal. Test results showed that only 1/10 pigs (individual #12) was weakly positive for ASF viral DNA in the spleen, with a very low viral load (Ct =

37.17). All other pigs were negative in all blood, carcass, internal organ samples (liver, kidney, lymph node, lung, heart), and bone marrow samples.

Table 1. Results of testing for residual ASF viral DNA in pigs at Farm I after vaccination to 5.5 months

Day	ID	CT value in samples								
		Blood	Liver	Spleen	Kidney	Lymph node	Lung	Heart	Bone marrow	Carcass
D14	#1	-	-	-	-	-	-	-	-	-
	#2	35.52	-	-	-	37.92	36.89	-	-	-
	#3	-	-	-	-	36.05	-	-	-	-
D28	#4	-	-	37.28	-	-	-	-	-	-
	#5	-	-	-	-	-	36.23	-	-	-
	#6	-	-	-	-	-	-	-	-	-
5,5 months	#10	-	-	-	-	-	-	-	-	-
	#11	-	-	-	-	-	-	-	-	-
	#12	-	-	37.17	-	-	-	-	-	-
	#15	-	-	-	-	-	-	-	-	-
	#16	-	-	-	-	-	-	-	-	-
	#17	-	-	-	-	-	-	-	-	-
	#18	-	-	-	-	-	-	-	-	-
	#25	-	-	-	-	-	-	-	-	-
	#45	-	-	-	-	-	-	-	-	-
	#48	-	-	-	-	-	-	-	-	-

4.1.2. Results at Farm II (monitoring up to 5 months post-vaccination)

After vaccination with AVAC ASF LIVE, the pig herd was generally healthy and grew normally. However, about 5% of pigs were weak or developed clinical signs such as respiratory signs and diarrhea. These pigs were separated and reared in several pens at the end of the barn (the treatment group). At the time of marketing, we randomly selected 10 healthy pigs from normal pens, weighing 115-130 kg (#K1-#K10), and five pigs from the treatment group (#D1-#D5), which were stunted or had previously shown clinical problems and had been treated, weighing approximately 95-110 kg. All pigs were necropsied and internal organ samples were collected to test for residual vaccine viral DNA. The results were as follows.

Healthy group: Vaccine viral DNA was not detected in blood, carcass, heart, or bone marrow samples from 10/10 pigs. In internal organ samples, viral DNA was detected in only 2/10 pigs at very low levels (Ct = 35.41-37.08): pig #K1 was positive in spleen and lung samples (Ct = 36.02 and 37.08), and pig #K6 was positive in liver, spleen, and lymph node samples (Ct = 37.05, 36.80, and 35.41, respectively).

Treatment group: ASF viral DNA was detected in 2/5 pigs at low levels. Pig #D1 was positive in blood (Ct = 34.94), carcass (Ct = 37.01), spleen (Ct = 34.88), and lung (Ct = 35.57) samples. Pig #D3 was positive only in internal organ samples: spleen (Ct = 33.12), lymph node (Ct = 35.22), and lung (Ct = 37.19).

Table 2. Results of testing for residual ASF viral DNA in pigs at Farm II 5 months after vaccination

Pig group	ID	CT value in samples								
		Blood	Liver	Spleen	Kidney	Lymph node	Lung	Heart	Bone marrow	Carcass
Healthy group	#K1	-	-	36.02	-	-	37.08	-	-	-
	#K2	-	-	-	-	-	-	-	-	-
	#K3	-	-	-	-	-	-	-	-	-
	#K4	-	-	-	-	-	-	-	-	-
	#K5	-	-	-	-	-	-	-	-	-
	#K6	-	37.05	36.8	-	35.41	-	-	-	-
	#K7	-	-	-	-	-	-	-	-	-
	#K8	-	-	-	-	-	-	-	-	-
	#K9	-	-	-	-	-	-	-	-	-
	#K10	-	-	-	-	-	-	-	-	-
Treatment group	#D1	34.94	-	34.88	-	-	35.75	-	-	37.01
	#D2	-	-	-	-	-	-	-	-	-
	#D3	-	-	33.12	-	35.22	37.19	-	-	-
	#D4	-	-	-	-	-	-	-	-	-
	#D5	-	-	-	-	-	-	-	-	-

4.2. Virus isolation on DMAC cells

To evaluate the infectivity of ASF viral DNA-positive samples collected at the time of marketing, we prepared five mixed samples, consisting of blood and internal organ tissues from individual pigs #12 from Farm I and #K1, #K6, #D1, and #D3 from Farm II. These samples were used for virus isolation on the DMAC cell line.

The results showed that, after 7 days of inoculation, 100% of wells treated with DNA-positive samples contained healthy DMAC cells and showed no hemadsorption (no rosette formation; Figure 1A), similar to the negative-control well (Figure 1B). In contrast, the positive-control well inoculated with ASF-G-ΔMGF vaccine virus at a dose of 10^4 HAD50 showed cell death and rosette formation after addition of 1% pig red blood cells as early as 3 days after inoculation (Figure 1C).

These results confirm that virus in the DNA-positive samples was unable to replicate in DMAC cells. In other words, these samples contained viral DNA but did not contain infectious viral particles.

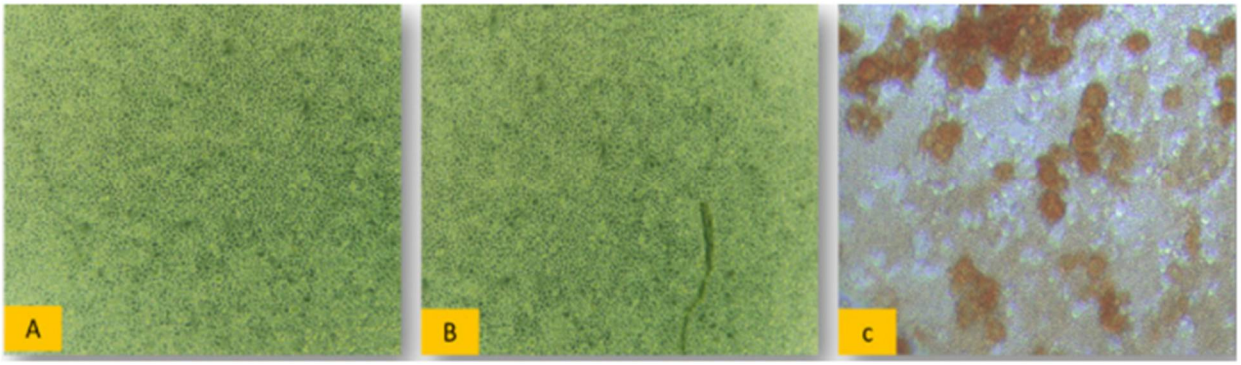

Figure 1. Results of ASF virus isolation on DMAC cells

A: *Well inoculated with an ASF viral DNA-positive pig sample: healthy cells, without cytopathic effects or hemadsorption;* ***B:*** *Negative-control well without inoculation: normal cell growth, no pathological changes. C: Positive-control well inoculated with infectious ASF virus: clear cell death and hemadsorption forming rosette structures.*

4.3. Assessment of the infectivity of residual vaccine virus in susceptible animals

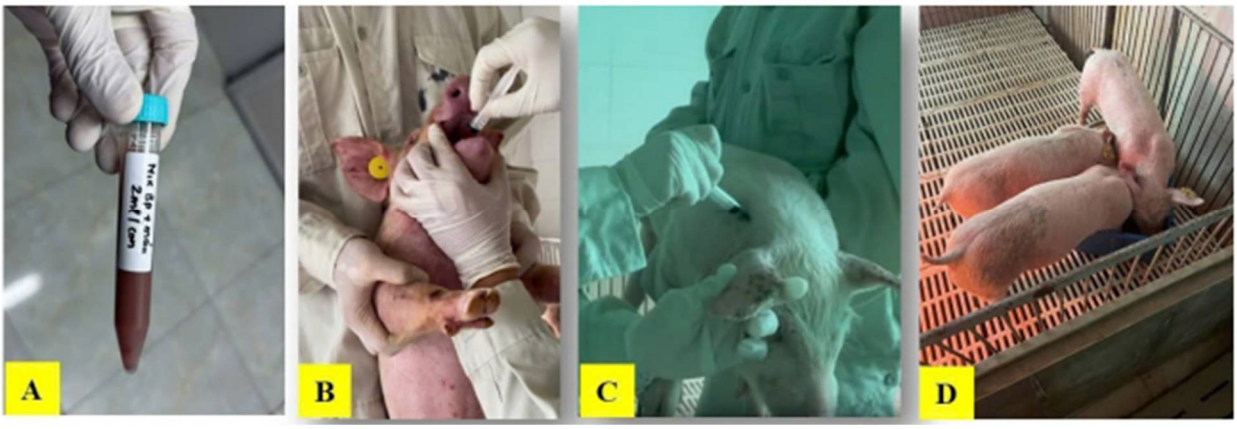

Figure 2. Experimental inoculation to evaluate infectivity in pigs

A: *Preparation of suspension samples from pigs positive for ASF viral DNA;* ***B:*** *Oral inoculation (PO), 2 ml per pig. C: Intramuscular inoculation (IM), 2 ml per pig. D: Pigs remained healthy and ate normally after inoculation.*

To determine the infectivity of residual vaccine virus in blood and tissues of pigs at the time of marketing, we pooled the five ASF viral DNA-positive samples, which were the same

samples used for the virus isolation test on DMAC cells, into one common suspension. This suspension was used to inoculate six experimental pigs: three by the intramuscular route and three by the oral route, at a dose of 2 ml of suspension per pig.

Throughout the 60-day observation period, all pigs remained healthy, ate normally, and showed no clinical signs. Real-time PCR testing of blood samples collected at 7, 14, 28, and 60 days after inoculation showed that 100% of samples were negative for ASF viral DNA.

At the end of the experiment (day 60), blood samples were collected from all six pigs and necropsy was performed to collect internal tissues, including liver, spleen, kidney, lymph node, lung, bone marrow, and carcass. ELISA and real-time PCR results showed that no anti-ASFV antibodies were detected in serum samples and no viral DNA was detected in any blood or tissue samples.

These results indicate that virus detected by real-time PCR in blood and tissues of pigs collected at the time of marketing was not infectious for pigs, consistent with the results of virus isolation on DMAC cells. This confirms that the detected signal represented only traces of DNA fragments, with no capacity for replication and no ability to stimulate an immune response.

V. DISCUSSION

The study results showed that in pigs vaccinated with AVAC ASF LIVE, ASF viral DNA was detected mainly in blood, spleen, lung, and lymph nodes during the first 28 days after vaccination, with low viral loads (high Ct values). By the time of marketing, most pigs were completely negative; only a small proportion (10-20%) still showed weak positive signals in some internal tissues such as liver, spleen, lung, or lymph nodes, whereas blood and carcass samples were negative. In stunted pigs or pigs with concurrent bacterial infections, the proportion of DNA-positive samples was higher (approximately 40%), including one case in which viral DNA was still detected in blood and carcass samples. However, all DNA-positive samples collected at the time of marketing were negative for virus isolation on DMAC cells and did not infect susceptible pigs, indicating that the vaccine virus no longer existed as infectious viral particles.

These findings are consistent with previous reports on the persistence and safety characteristics of live attenuated ASF vaccines. For the HLJ/18-7GD strain, viral DNA was detected only transiently in some lymph nodes, with no viremia or horizontal transmission (Chen et al., 2020; Wang et al., 2024). The MGF360/505-deleted Stavropol_01/08 vaccine caused viremia for approximately 7-14 days and became completely negative after day 21 (Koltsov et al., 2024). Similarly, the ASFV-G-DeltaI177L strain (NAVET-ASFVAC) showed low-level viremia, peaking during 11-21 days post-vaccination and becoming negative on day 28 (Tran et al., 2022), consistent with the 180-day follow-up reported by Borca et al. (2023). For AVAC ASF LIVE, Diep et al. (2025) also recorded viremia only up to days 7-14, with complete negativity from day 21 after vaccination.

A notable strength of this study is the long follow-up period of 5-5.5 months, corresponding to the time of marketing of commercial pigs. Most previous studies monitored pigs only for 4-8 weeks after vaccination (Chen et al., 2020; Tran et al., 2022; Wang et al., 2024; Koltsov et al., 2024; Choi et al., 2025), and therefore did not evaluate viral persistence at the end of the production cycle. An exception is the study by Borca et al. (2023), which followed pigs for 180 days but did not perform virus isolation. The combination of viral DNA detection, virus isolation on DMAC cells, and experimental inoculation in the present study provides direct evidence that AVAC ASF LIVE vaccine virus does not persist as infectious viral particles, although viral genetic material may still be detected at very low levels at the time of marketing.

The phenomenon of detecting vaccine viral DNA without isolating infectious virus has been reported for many attenuated vaccines. For example, in recipients of vaccinia vaccine, viral DNA can still be detected by real-time PCR for an extended period after vaccination, but live virus cannot be isolated from these samples. This indicates that a positive PCR result reflects the persistence of viral genetic material and does not necessarily mean the presence of infectious viral particles (Cohen et al., 2007).

From an immunological perspective, after a live attenuated vaccine is introduced into the body, the vaccine virus can replicate at a limited level for a short period, sufficient to induce long-lasting protective immunity against infection with the corresponding virulent field strain. Once adaptive immunity is established, vaccine virus replication is controlled and eliminated. Viral signals that remain detectable in tissues, especially lymphoid tissues or organs rich in macrophages, reflect persistence of vaccine viral genetic material rather than the presence of infectious particles and therefore do not indicate an epidemiological risk (Cohen et al., 2007; Carneiro et al., 2022; Tang et al., 2024).

From the perspective of disease management and control, according to Guideline No. 3708/HD-BNN-TY (2019), slaughter establishments are allowed to receive and process only healthy pigs that test negative for ASF virus. This regulation was issued when Vietnam had not yet introduced ASF vaccines; therefore, every PCR-positive case was assumed to represent infection with field virus. However, under conditions in which live attenuated vaccines have been licensed and used, the approach that "PCR positivity equals an outbreak" is no longer fully appropriate. Conventional PCR methods do not distinguish between vaccine-virus DNA and field-virus DNA; therefore, PCR-positive results in vaccinated pigs must be interpreted cautiously.

For PCR-positive cases detected at vaccinated farms or slaughter establishments, differential tests (DIVA) should be applied in combination with clinical assessment and evaluation of gross changes in carcasses and internal organs to determine the origin of the virus. This integrated approach allows differentiation between vaccine virus and field virus and enables an accurate epidemiological risk assessment.

The synchronized application of these measures will help avoid unnecessary control actions while ensuring the safe movement of vaccinated animals and animal products without reducing the effectiveness of ASF control.

VI. CONCLUSION

DNA of AVAC ASF LIVE vaccine virus can be detected in blood and some internal tissues of pigs at low levels at the time of marketing (5-5.5 months after vaccination). However, all DNA-positive samples were negative for virus isolation on DMAC cells and did not infect susceptible pigs, demonstrating that the virus had completely lost infectivity and persisted only as genetic material. These findings confirm the safety of AVAC ASF LIVE for pork products and rule out the risk of dissemination of infectious virus into the environment.

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