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QUALITY CONTROL OF A LIVE - ATTENUATED AFRICAN SWINE FEVER VACCINE IN VIET NAM

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National Center for Veterinary Medicine Control (VMC)

African Swine Fever

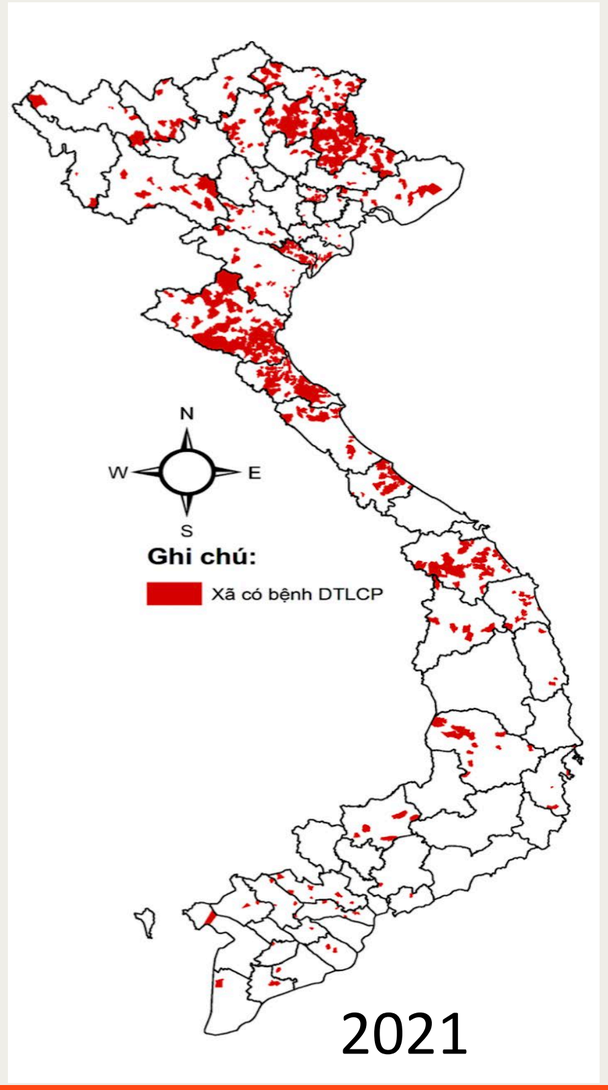
1. ASF was first reported in Kenya in 1921
2. Mortality up to 100%.
3. Cause economic losses and threat to swine industry
4. No vaccine and treatment are available
5. Control of ASF mainly relies on Biosecurity and destroy infected pigs and pigs in contact to infection and strictly movement

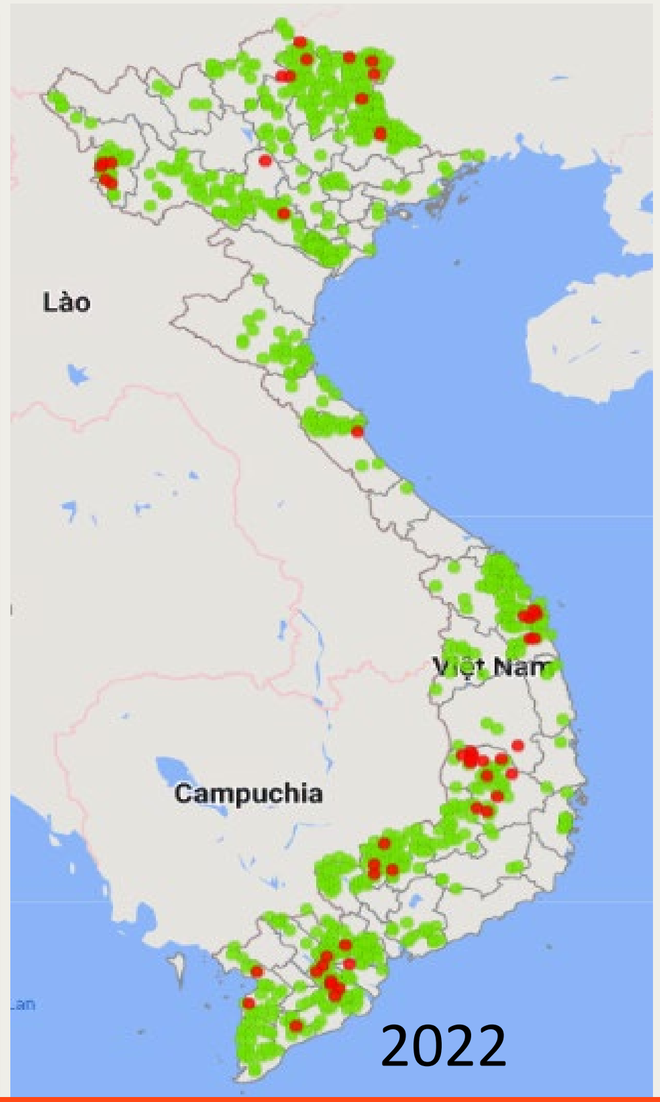
African Swine Fever

In Vietnam, the disease was first reported in February 2019 in the Northern provinces and became endemic afterward, causing serial outbreaks in domestic pigs across the country.

African Swine Fever in Vietnam 6/2020 – 6/2022

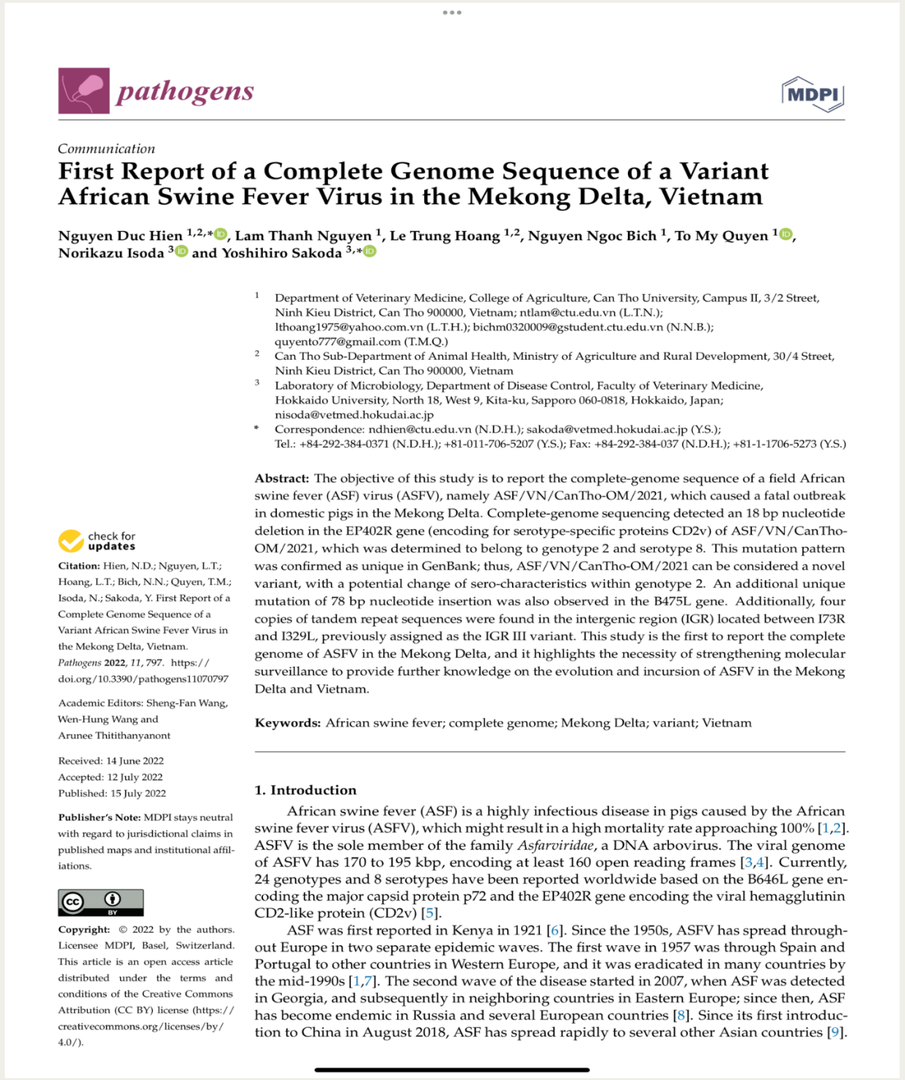
Content	From Jan-Jun/2020	From Jan-Jun/2021	From Jan-Jun/2022
Number of communes	**831**	**1140**	**753**
Number of districts	**223**	**226**	**225**
Number of provinces	**44**	**46**	**47**
Number of pigs infected and dead	**36.000**	**108.678**	**36.516**





African Swine Fever

Currently, at least three variants of genotype 2 based on the variation of tandem repeat sequences (TRS) in the intergenic region (IGR) located between I73R and I329L genes have been identified in North Vietnam



pathogens

MDPI

Communication

First Report of a Complete Genome Sequence of a Variant African Swine Fever Virus in the Mekong Delta, Vietnam

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Abstract: The objective of this study is to report the complete-genome sequence of a field African swine fever (ASF) virus (ASFV), namely ASF/VN/CanTho-OM/2021, which caused a fatal outbreak in domestic pigs in the Mekong Delta. Complete-genome sequencing detected an 18 bp nucleotide deletion in the EP402R gene (encoding for serotype-specific proteins CD2v) of ASF/VN/CanTho-OM/2021, which was determined to belong to genotype 2 and serotype 8. This mutation pattern was confirmed as unique in GenBank; thus, ASF/VN/CanTho-OM/2021 can be considered a novel variant, with a potential change of sero-characteristics within genotype 2. An additional unique mutation of 78 bp nucleotide insertion was also observed in the B475L gene. Additionally, four copies of tandem repeat sequences were found in the intergenic region (IGR) located between I73R and I329L, previously assigned as the IGR III variant. This study is the first to report the complete genome of ASFV in the Mekong Delta, and it highlights the necessity of strengthening molecular surveillance to provide further knowledge on the evolution and incursion of ASFV in the Mekong Delta and Vietnam.

Keywords: African swine fever; complete genome; Mekong Delta; variant; Vietnam

check for updates

Citation: Hien, N.D.; Nguyen, L.T.; Hoang, L.T.; Bich, N.N.; Quyen, T.M.; Isoda, N.; Sakoda, Y. First Report of a Complete Genome Sequence of a Variant African Swine Fever Virus in the Mekong Delta, Vietnam. *Pathogens* **2022**, *11*, 797. https://doi.org/10.3390/pathogens11070797

Academic Editors: Sheng-Fan Wang, Wen-Hung Wang and Arunee Thitithanyanont

Received: 14 June 2022
Accepted: 12 July 2022
Published: 15 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

African swine fever (ASF) is a highly infectious disease in pigs caused by the African swine fever virus (ASFV), which might result in a high mortality rate approaching 100% [1,2]. ASFV is the sole member of the family *Asfarviridae*, a DNA arbovirus. The viral genome of ASFV has 170 to 195 kbp, encoding at least 160 open reading frames [3,4]. Currently, 24 genotypes and 8 serotypes have been reported worldwide based on the B646L gene encoding the major capsid protein p72 and the EP402R gene encoding the viral hemagglutinin CD2-like protein (CD2v) [5].

ASF was first reported in Kenya in 1921 [6]. Since the 1950s, ASFV has spread throughout Europe in two separate epidemic waves. The first wave in 1957 was through Spain and Portugal to other countries in Western Europe, and it was eradicated in many countries by the mid-1990s [1,7]. The second wave of the disease started in 2007, when ASF was detected in Georgia, and subsequently in neighboring countries in Eastern Europe; since then, ASF has become endemic in Russia and several European countries [8]. Since its first introduction to China in August 2018, ASF has spread rapidly to several other Asian countries [9].

Vaccine research

Situation of ASF vaccine?

1. Not much information on this disease have been known, especially ASF vaccines.
2. Inactivated ASF vaccine is not possible.
3. Subunit vaccines have failed or give a modest protection rate
4. No live attenuated vaccine commercially available

Vaccine research

ASF vaccine project

1. Cooperation between Ministry of Agriculture and Rural Development (MARD) Vietnam and USDA
2. The Agreements signed between:

- NAVETCO, DABACO and Agricultural Research Service (ARS), USDA
- AVAC and Aptimmune Biologics Inc

Vaccine research

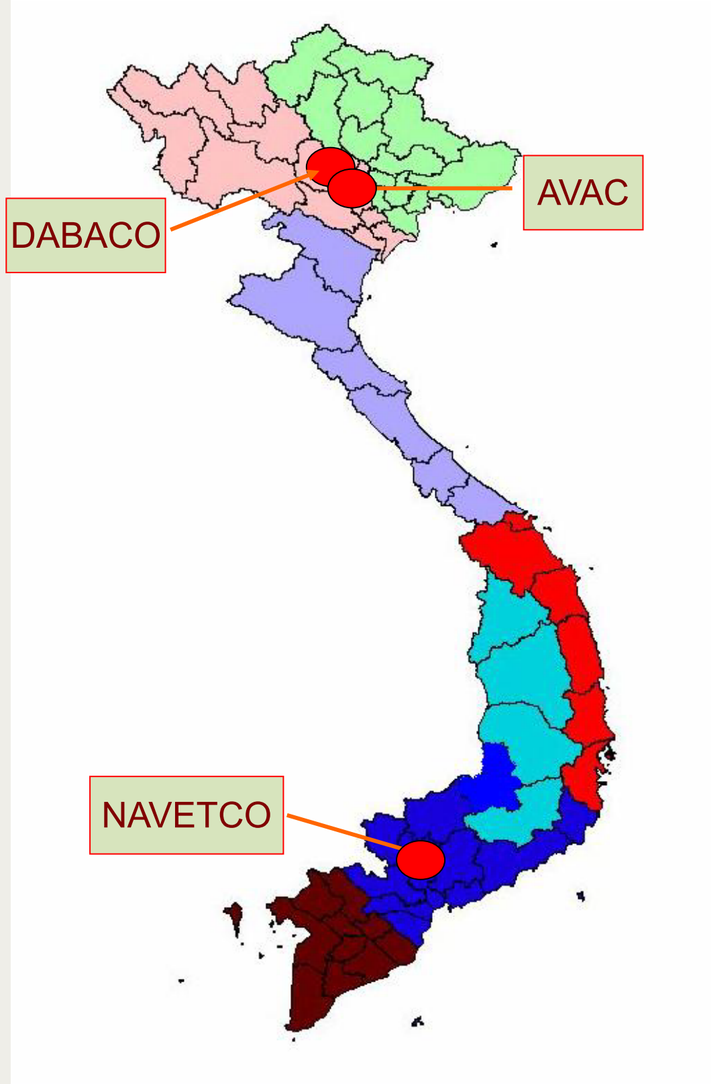
ASF vaccine project

3. Companies meets GMP-WHO standards for vaccines

4. Support from DAH:

* Supports to implement this project (legal procedures, international cooperation, and monitoring vaccination in local …);

* DAH appoints the National Center for Veterinary Medicine control No. 1 (VMC1), the National Center for Veterinary Diagnosis (NCVD) and the Regional Veterinary Sub-Department 1, 6 (RAOH) to directly participate in the research and production of vaccines.



AVAC Co., LTD (Hung Yen province)

ASF-G-ΔMGF

DABACO Group (Bac Ninh province)

ASFV-G- Δ I177L/ Δ LVR

NAVETCO (Binh Duong province)

Vaccine research

Purpose of research and production of vaccines

1. Research to produce vaccines as soon as possible, on the basis of prima cells.

2. Research on vaccine development on cloned cells.

Vaccine research

ASFV vaccine

1. NAVETCO

- Working seed: ASFV-G-ΔI177L Strain.
- Cell line: PBMC (Peripheral blood mononuclear cell)

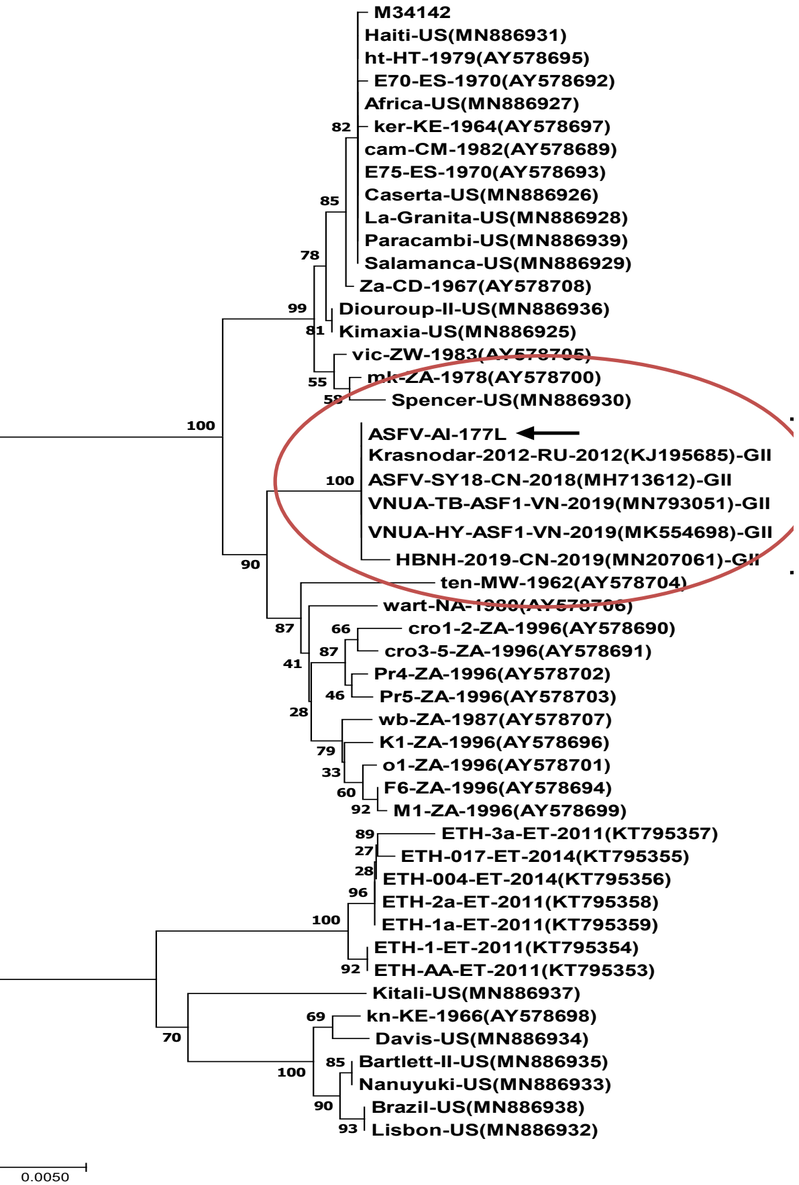
2. AVAC:

- Working seed: ASFV-G-ΔMGF Strain.
- Cell line: DMAC (Diep's Macrophage cell)

3. DABACO Group

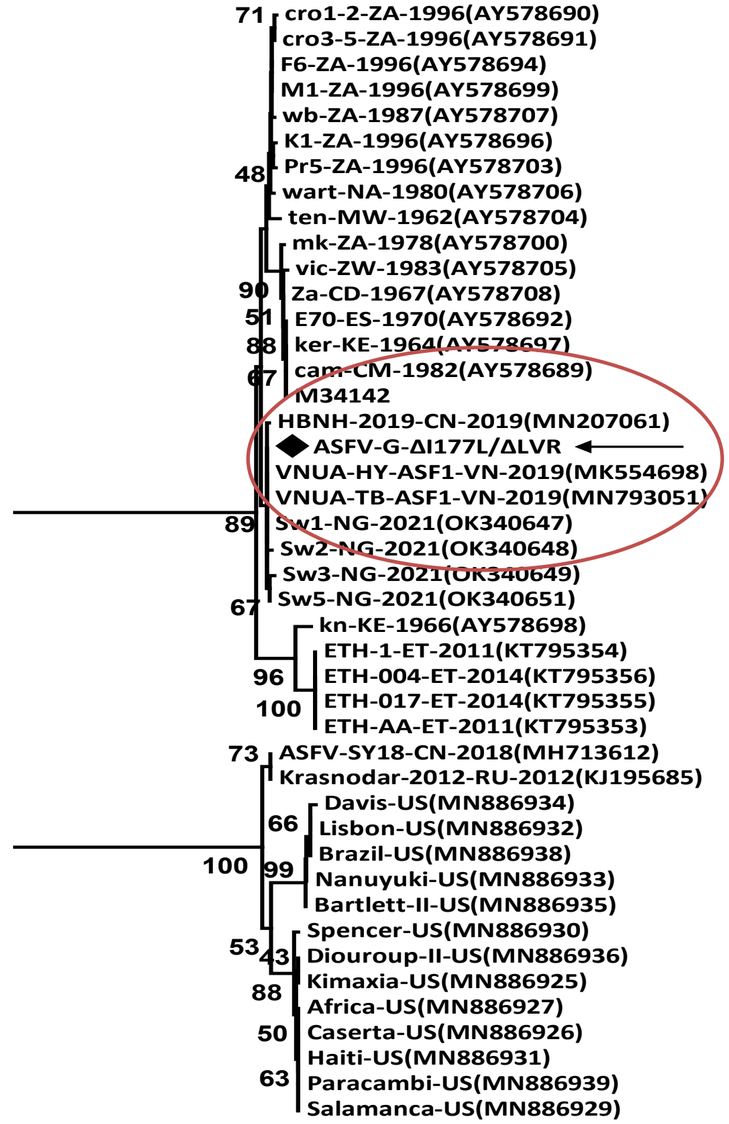
- Working seed: ASFV-G-ΔI177L/ΔLVR Strain.
- Cell line: PIPEC (Plum Island porcine epithelial cells)

ASFV-G-ΔI-177L NAVETCO



ASFV-G-ΔMGF(ASF-US01) AVAC

ASFV-G-ΔI177L/ΔLVR DABACO



African swine fever virus, ASFV-G-ΔMGF (effective January 27, 2020)

The ASFV-G-ΔMGF virus strain is an African swine fever virus (AFSV) mutant derived from AFSV Georgia strain. Attenuation is provided by the deliberate deletion of six multigene family 360 (MGF360) and MGF505 group of genes. The deletions contained in the attenuated virus prevented the development of clinical signs in host animals inoculated with increasing doses of the attenuated live virus.

African swine fever virus, ASFV-G-ΔI177L (effective April 3, 2020)

The ASFV-G-ΔI177L virus strain is an African swine fever virus (AFSV) deletion mutant derived from AFSV Georgia strain. Attenuation is provided by the deliberate deletion of the I177L gene from the ASFV Georgia strain. The deletion mutant virus attenuation prevented the development of clinical signs in host animals inoculated with increasing doses of the attenuated live virus.

African swine fever virus, ASFV-G-ΔI177LΔLVR (effective March 11, 2021)

The ASFV-G-ΔI177LΔLVR virus strain is an African swine fever virus (AFSV) deletion mutant derived from AFSV Georgia strain. Attenuation is provided by the deliberate deletion of the I177L gene and the Left Variable Region (LVR) which fully deletes the following 9 genes: MGF360-6L, X69R, MGF300-1L, MDF300-2R, MGF300-4L, MGF360-8L, MGF360-9L and MGF360-10L. The genomic modification also causes deletion of the N-terminal portion of the MGF360-4L gene, and the C-terminus portion of the MGF360-11L gene. The deletion mutant virus attenuation prevented the development of clinical signs in host animals inoculated with increasing doses of the attenuated live virus.

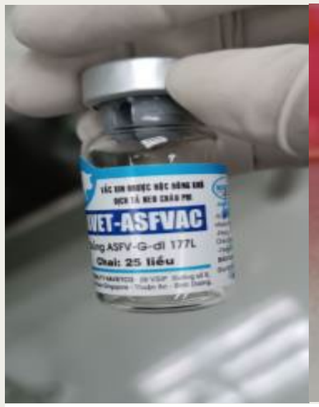
Quality control of Vaccine

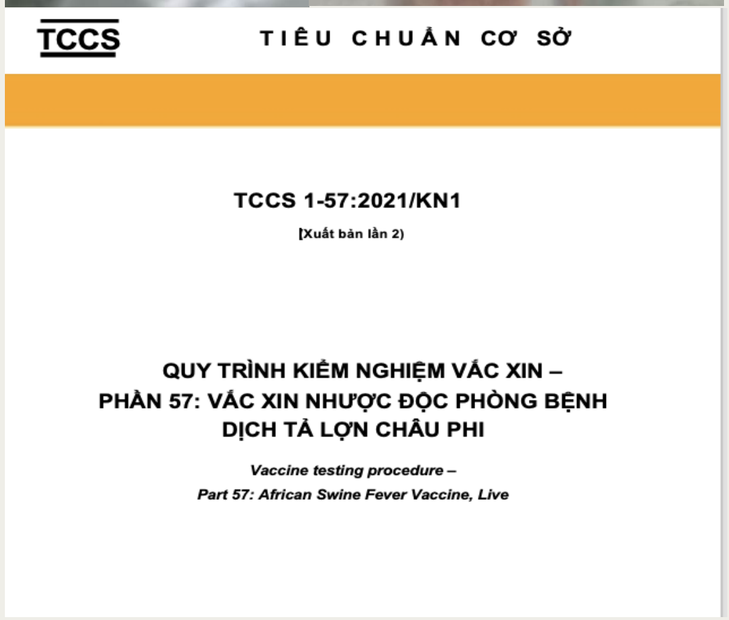
A. Quality control of vaccine conducted by National Centre for Veterinary Medicine Control I (VMC)

i. 03 batches of vaccine (NAVET-ASFVAC)

ii. Tests for purity, sterility, safety and potency

→ Pigs were tested negative for pathogens (CSFV, PRRSV, FMDV, PCV2, MH, ASFV) before experiment





TCCS TIÊU CHUẨN CƠ SỞ

TCCS 1-57:2021/KN1

[Xuất bản lần 2)

QUY TRÌNH KIỂM NGHIỆM VẮC XIN –
PHẦN 57: VẮC XIN NHƯỢC ĐỘC PHÒNG BỆNH
DỊCH TẢ LỢN CHÂU PHI

Vaccine testing procedure –
Part 57: African Swine Fever Vaccine, Live

+ The safety test of ASF vaccines

Lots	No of pigs	ELISA day 0	dose (X)	Re vaccination/ dose	Survivors/Total/ Status	Conclusion
QC-VR-22-00516	3	0/3	**10**	No	3/3, normal	Safety
QC-VR-22-00517	3	0/3	**10**	No	3/3, normal	Safety
QC-VR-22-00518	3	0/3	**10**	No	3/3, normal	Safety

+ Potency test for ASF vaccines

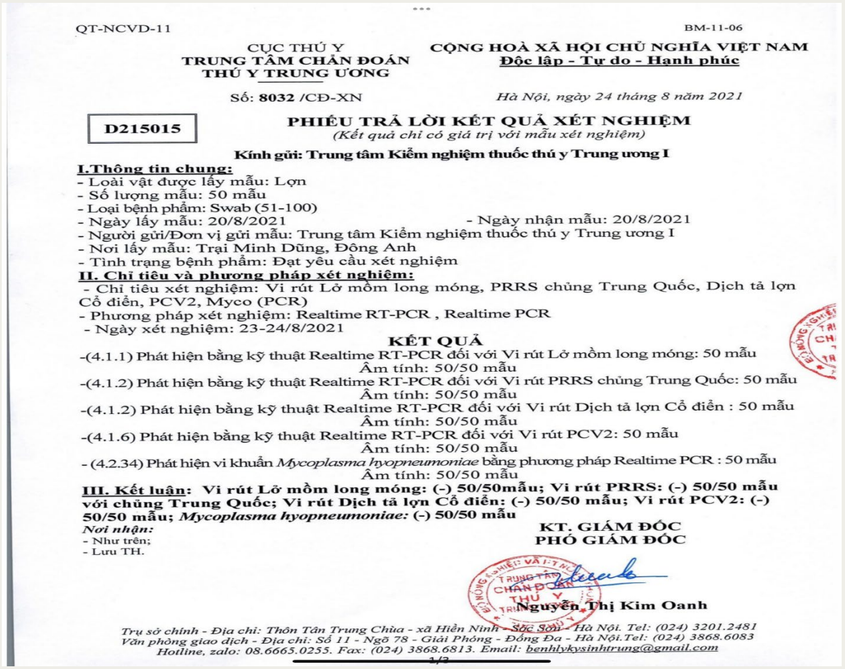
Group(s)	Exp. Group	No of pigs	ELISA d0	ELISA post vaccination (days)				Survival/total challenged		
				d14	d21	d28	X%	Survivor	Vac. group	Cont group
QC-VR-22-00516	Vac	5	**0/5**	**1/5**	**5/5**	**5/5**	**67.0±7.8**	5/5	**5**	
	Cont	3	0/3	0/3	0/3	0/3	-	0/3		**0/3**
QC-VR-22-00517	Vac	5	**0/5**	**5/5**	**5/5**	**5/5**	**69.6±2.9**	5/5	**5**	
	Cont	3	0/3	0/3	0/3	0/3	-	0/3		**0/3**
QC-VR-22-00518	Vac	5	**0/5**	**2/5**	**3/5**	**4/5**	**66.5±3.5**	5/5	**5**	
	Cont	3	0/3	0/3	0/3	0/3	-	0/3		**0/3**

Virulent ASFV challenge: TTKN/ASFV/ĐN/2019
Dose:10^2 HAD_{50}/pig

B. The field trials conducted by VMC, RAOH 1, RAOH 6 and NCVD-DAH

+ Experimental pig

All pigs were tested negative for pathogens: CSFV, PRRSV, FMDV, PCV2, MH, ASFV before experiment



QT-NCVD-11 BM-11-06

CỤC THÚ Y
TRUNG TÂM CHẨN ĐOÁN THÚ Y TRUNG ƯƠNG

CỘNG HOÀ XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập - Tự do - Hạnh phúc

Số: 8032 /CĐ-XN

Hà Nội, ngày 24 tháng 8 năm 2021

D215015

PHIẾU TRẢ LỜI KẾT QUẢ XÉT NGHIỆM
(Kết quả chỉ có giá trị với mẫu xét nghiệm)

Kính gửi: Trung tâm Kiểm nghiệm thuốc thú y Trung ương I

I.Thông tin chung:
- Loài vật được lấy mẫu: Lợn
- Số lượng mẫu: 50 mẫu
- Loại bệnh phẩm: Swab (51-100)
- Ngày lấy mẫu: 20/8/2021 - Ngày nhận mẫu: 20/8/2021
- Người gửi/Đơn vị gửi mẫu: Trung tâm Kiểm nghiệm thuốc thú y Trung ương I
- Nơi lấy mẫu: Trại Minh Dũng, Đông Anh
- Tình trạng bệnh phẩm: Đạt yêu cầu xét nghiệm

II. Chỉ tiêu và phương pháp xét nghiệm:
- Chỉ tiêu xét nghiệm: Vi rút Lở mồm long móng, PRRS chủng Trung Quốc, Dịch tả lợn Cổ điển, PCV2, Myco (PCR)
- Phương pháp xét nghiệm: Realtime RT-PCR , Realtime PCR
- Ngày xét nghiệm: 23-24/8/2021

KẾT QUẢ

-(4.1.1) Phát hiện bằng kỹ thuật Realtime RT-PCR đối với Vi rút Lở mồm long móng: 50 mẫu
Âm tính: 50/50 mẫu

-(4.1.2) Phát hiện bằng kỹ thuật Realtime RT-PCR đối với Vi rút PRRS chủng Trung Quốc: 50 mẫu
Âm tính: 50/50 mẫu

-(4.1.2) Phát hiện bằng kỹ thuật Realtime RT-PCR đối với Vi rút Dịch tả lợn Cổ điển : 50 mẫu
Âm tính: 50/50 mẫu

-(4.1.6) Phát hiện bằng kỹ thuật Realtime RT-PCR đối với Vi rút PCV2: 50 mẫu
Âm tính: 50/50 mẫu

- (4.2.34) Phát hiện vi khuẩn *Mycoplasma hyopneumonicae* bằng phương pháp Realtime PCR : 50 mẫu
Âm tính: 50/50 mẫu

III. Kết luận: Vi rút Lở mồm long móng: (-) 50/50mẫu; Vi rút PRRS: (-) 50/50 mẫu với chủng Trung Quốc; Vi rút Dịch tả lợn Cổ điển: (-) 50/50 mẫu; Vi rút PCV2: (-) 50/50 mẫu; *Mycoplasma hyopneumoniae:* (-) 50/50 mẫu

Nơi nhận:
- Như trên;
- Lưu TH.

KT. GIÁM ĐỐC
PHÓ GIÁM ĐỐC

Nguyễn Thị Kim Oanh

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Văn phòng giao dịch - Địa chỉ: Số 11 - Ngõ 78 - Giải Phóng - Đống Đa - Hà Nội.Tel: (024) 3868.6083
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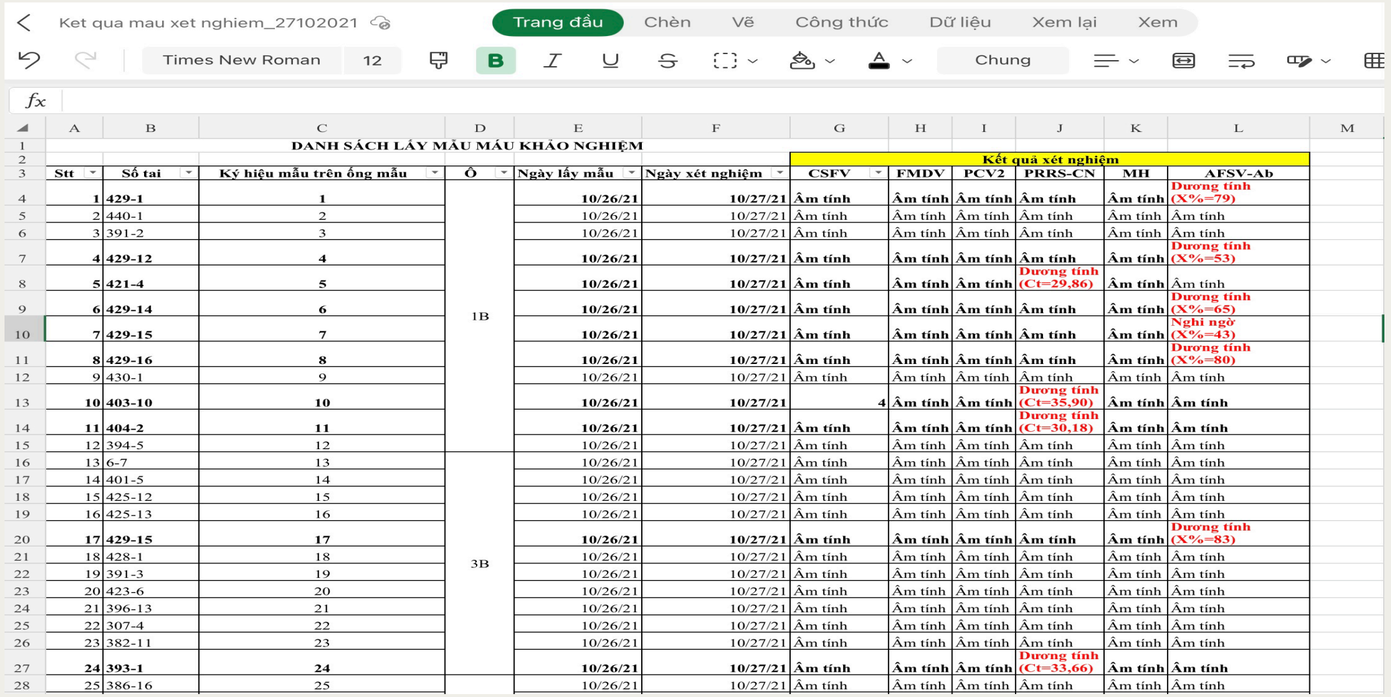


Ket qua mau xet nghiem_27102021

Trang đầu | Chèn | Vẽ | Công thức | Dữ liệu | Xem lại | Xem

Times New Roman 12 | Chung

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	DANH SÁCH LẤY MẪU MÁU KHẢO NGHIỆM												
2							Kết quả xét nghiệm						
3	Stt	Số tai	Ký hiệu mẫu trên ống mẫu	Ô	Ngày lấy mẫu	Ngày xét nghiệm	CSFV	FMDV	PCV2	PRRS-CN	MH	AFSV-Ab	
4	1	429-1	1		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Dương tính (X%=79)	
5	2	440-1	2		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
6	3	391-2	3		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
7	4	429-12	4		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Dương tính (X%=53)	
8	5	421-4	5		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Dương tính (Ct=29,86)	Âm tính	Âm tính	
9	6	429-14	6	1B	10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Dương tính (X%=65)	
10	7	429-15	7		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Nghi ngờ (X%=43)	
11	8	429-16	8		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Dương tính (X%=80)	
12	9	430-1	9		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
13	10	403-10	10		10/26/21	10/27/21	4	Âm tính	Âm tính	Dương tính (Ct=35,90)	Âm tính	Âm tính	
14	11	404-2	11		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Dương tính (Ct=30,18)	Âm tính	Âm tính	
15	12	394-5	12		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
16	13	6-7	13		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
17	14	401-5	14		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
18	15	425-12	15		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
19	16	425-13	16		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
20	17	429-15	17		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Dương tính (X%=83)	
21	18	428-1	18	3B	10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
22	19	391-3	19		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
23	20	423-6	20		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
24	21	396-13	21		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
25	22	307-4	22		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
26	23	382-11	23		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	
27	24	393-1	24		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Dương tính (Ct=33,66)	Âm tính	Âm tính	
28	25	386-16	25		10/26/21	10/27/21	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	Âm tính	

+ Safety of vaccine in pigs in the field

Farms	Exp. group	Number of pigs	Dose used	Results
Binh Minh Farm (South)	Safety group	10	10 times	Passed
	Efficacy group	30	2 shots	
	Control group	10	No	
Minh Dung Farm (North)	Safety group	10	10 times	Passed
	Efficacy group	30	2 shots	
	Control group	10	No	

+ Results of efficacy of ASF vaccine in the field

Farms	Number of pigs	Age	Type of pigs	Batch No	ELISA		Results
					Before vaccination	Post vaccination	
Binh Minh Farm	30	8-10 weeks	Yorkshire+ Landrace	08	30/30 Negative	30/30 Positive (100%)	Survival 5/5 (100%)
	Control				0/5		0/5 Survival
Minh Dung Farm	30	8-10 weeks	Yorkshire+ Landrace	08	30/30 Negative	**27/29 Positive (93,1%)**	**Survival 5/5 (100%)**
	Control				0/5		0/5 Survival
Total	**Vaccination**				60/60 Negative	**57/59 Positive (96,6%)**	**Survival 10/10 (100%)**
	Control				0/10 Negative		0/10 (0.0%)

Virulent ASFV challenge: TTKN/ASFV/ĐN/2019 (10^2 HAD_{50}/pig)

C. Approval

- Vaccine was controlled safety and potency by VMC1;

- NAVET-ASFVAC that was carefully evaluated in compliance with the current legislation and scientific requirements have been approved for use under a strict supervision

Marketing Authorization:

1. Trade name: NAVET-ASFVAC,
2. Live, attenuated, freeze-dried vaccines.
3. Storage conditions: 2-8^{0}C
4. Shelf life: 12 months
5. Duration of Immunity: 6 months under laboratory conditions
6. Animals: Pig of 8 weeks age
7. Protection received after vaccination is 21days and solid protection after 28 days.

BỘ NÔNG NGHIỆP VÀ PHÁT TRIỂN NÔNG THÔN
MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT
CỤC THÚ Y
DEPARTMENT OF ANIMAL HEALTH

CỘNG HOÀ XÃ HỘI CHỦ NGHĨA VIỆT NAM
SOCIALIST REPUBLIC OF VIETNAM
Độc lập - Tự do - Hạnh phúc
Independence- Freedom- Happiness

GIẤY CHỨNG NHẬN LƯU HÀNH THUỐC THÚ Y
MARKETING AUTHORIZATION
Số/*No:* 538/QLT-SX-22

Tên sản phẩm *Name of product:*	:	**NAVET-ASFVAC** (Vắc xin Dịch tả lợn Châu Phi nhược độc đông khô)
Số đăng ký *Registration number:*	:	TWII-171
Thành phần hoạt chất/ *Active Ingredients:*	:	Vi rút Dịch tả lợn Châu Phi nhược độc chủng ASFV-G-Delta-I177L.
Công dụng *Indications:*	:	Phòng bệnh Dịch tả lợn Châu Phi trên lợn từ 8 đến 10 tuần tuổi.
Dạng bào chế/ *Dosage form:*	:	Vắc xin nhược độc, đông khô.
Quy cách đóng gói/ *Packing Size:*	:	Chai, Lọ (10, 20, 25, 50, 100, 200, 250, 500 liều), kèm nước pha vắc xin tương ứng.
Hạn dùng/ *Shelf-life:*	:	12 tháng.
Tên cơ sở đăng ký lưu hành *Name of Marketing Authorization Holder:*	:	Công ty Cổ phần thuốc thú y Trung ương NAVETCO
Địa chỉ *Address:*	:	29A Nguyễn Đình Chiểu, phường Đakao, quận 1, Tp. Hồ Chí Minh
Số điện thoại/*Tel.:* +84-283-822-5063	:	Số Fax/*Fax. No:* +84-283-822-5060
Tên cơ sở sản xuất/ *Name of Manufacturer:*	:	Công ty Cổ phần thuốc thú y Trung ương NAVETCO
Địa chỉ *Address:*	:	28 VSIP Đường số 6, Khu Công nghiệp Việt Nam - Singapore, Tp. Thuận An, tỉnh Bình Dương
Số điện thoại/*Tel:* +84-274-376-3822		Số Fax/*Fax No:* +84-274-376-3824

Giấy chứng nhận lưu hành này có hiệu lực đến: 18/5/2027
This marketing authorisation is valid until

Hà Nội, ngày 18 tháng 5 năm 2022
Hanoi Date month year
Q. CỤC TRƯỞNG
DIRECTOR GENERAL
CỤC THÚ Y
Nguyễn Văn Long

During the initial phase, a license for using 600,000 doses

Post-vaccination surveillance

As of 26 Aug 2022, 20 provinces have used of a total of 23,344 doses including:

→ 4,494 doses were used under the supervision of veterinary agencies in compliance with the direction of MARD and belong to the guidance of DAH.

→ Up to now, vaccinated pigs have grown well. Only 27 pigs had adverse responses/died after injection (0.6%).

→17,750 doses were provided by Navetco to other localities, not monitored by veterinary agencies, or did not follow the direction and relevant guidelines (Binh Dinh and Phu Yen Provinces).

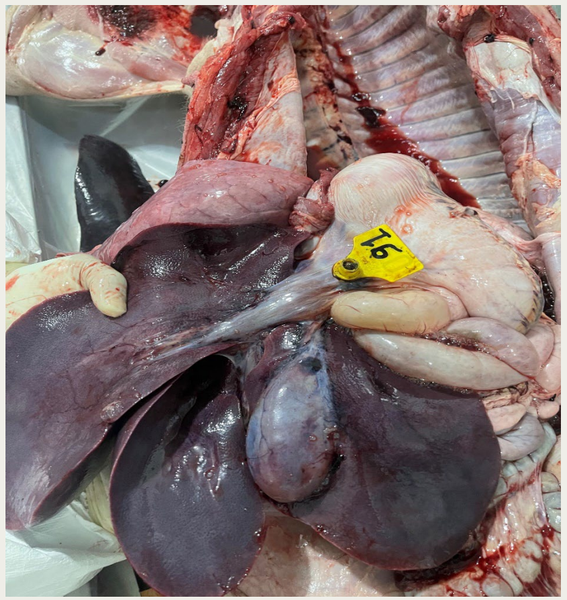
Post-vaccination surveillance

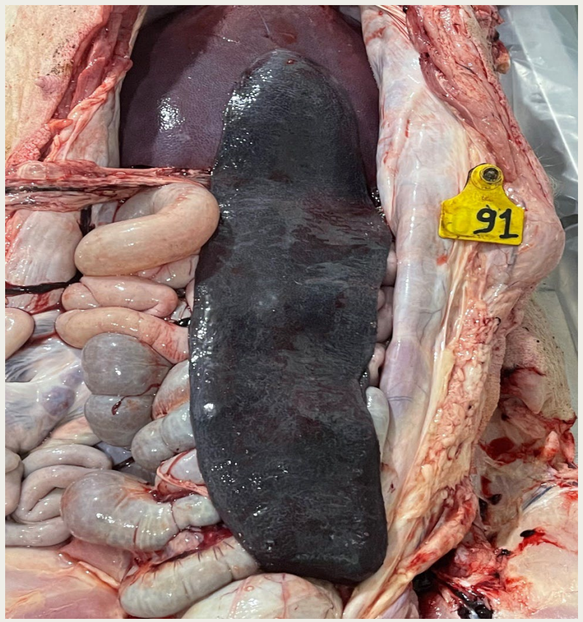
DAH: investigate and access the situation of dead and sick pigs after vaccination;

+ Sampling collection for diagnostic testing

+ Collecting the vaccine vial, Then, its was used for injecting to healthy pigs at VMC and NCVD’s Lab

+ Specimen testing for pathogens that can be cause sick and dead pig





Set up an experiment to find the cause of pig death (VMC, VNCD)

PURPOSES:

- Testing the response of pigs when receiving live attenuated vaccine NAVET-ASFVAC (as in field condition)
- Testing the response of pigs when injected with suspension samples taken from dead pigs after ASF vaccination

MATERIALS:

- Pigs (15 – 17 kg) (25 heads) purchased from commercial pig farm
- NAVET-ASFVAC bottles collected from Binh Dinh province
- Infectious specimens: 2 samples taken from dead pigs in Binh Dinh and Phu Yen provinces

EXPERIMENTAL DESIGN:

GROUP	Specimen injection (neat, Ct = 20)	Diluted specimen injection dilutled, Ct = 28)	Vaccine injection (1 dose, Ct = 28)	Vaccine diluent
Specimen from Binh Dinh	2 mL x 3 pigs	2 mL x 3 pigs		
Specimen from Phu Yen	2 mL x 3 pigs	2 mL x 3 pigs		
Vaccine			2 mL x 10 pigs	
Control				2 mL x 3 pigs

- *Groups: isolated in 3 rooms of ABSL-3, ensuring no cross-contamination*
- *Monitoring body temperature and health of pigs in 14 - 21 days after injection*
- *Collecting blood on 3, 7, 10, 14 & 21 dpi for viremia, DIVA and other pathogens detection*

RESULT

CLINICAL RECORDS:

Bình Định — Receiving specimen from Binh Dinh province

Phú Yên — Receiving specimen from Phu Yen province

Vắc xin — Receiving ASF vaccine

Đối chứng — Control, receiving vaccine diluent

GROUP	Fever/Total (>40.5 C)	Dead/Total (day-of-dead)
Specimen from Binh Dinh	06/06	01/06 (16 dpi)
Specimen from Phu Yen	05/06	01/06 (21 dpi)
Vaccine	07/10	01/10 (7 dpi)
Control	01/3	0/3

PIG DIED POST-INOCULATION (No. 21, Vaccination group, died at 7 dpi-NCVD)

Main lesions:

Hemorrhagic in lymph nodes
Acute, mild pneumonia
Multifocal hemorrhages in kidney
Inflammation pericarditis

Testing results:

ASF p72 gene: positive, Ct 32
ASF I177L gene: negative
PCV-2: negative
PRRS: positive, Ct 31
Mycoplasma: positive
Pasteurella: positive

PIG DIED POST-INOCULATION (No. 9, Phu Yen specimen group, died at 21 dpi-NCVD)

Main lesions:

Hmorrhagic in lymph nodes
Chronic, severe diffuse pseudopneumonia.
Multifocal hemorrhages in kidney
Inflammation pericarditis
Infarction in spleen

Testing results:

before test:

PCV2: positive, Ct 34.82
PRRS: positive, Ct 24.74

After 21 days:

ASF p72 gene: positive, Ct 30.07
PRRS positive, 22.4
Mycoplasma: positive
Pasteurella: positive

Viremia test after injection (NCVD)

	Realtime PCR for ASF p72 gene pos/total (mean Ct value)				
GROUP	**D0**	**D3**	**D7**	**D10**	**D14**
Specimen from Binh Dinh	Neg	01/06 (29.5)	03/06 (20.27)	03/06 (20.31)	04/06 (21.88)
Specimen from Phu Yen	Neg	02/06 (33.84)	04/06 (25.70)	04/06 (24.58)	05/06 (26.29)
Vaccine	Neg	Neg	02/09 (21.08)	04/09 (25.35)	06/09 (25.19)
Control	Neg	Neg	Neg	Neg	Neg

(All samples were negative for ASF I177L gene)

	Realtime PCR for PCV-2 pos/total (mean Ct value)				
GROUP	**D0**	**D3**	**D7**	**D10**	**D14**
Specimen from Binh Dinh	01/06 (34.72)	NT	NT	NT	03/06 (27.29)
Specimen from Phu Yen	01/06 (34.82)	NT	NT	NT	04/06 (25.57)
Vaccine	04/10 (31.02)	NT	NT	NT	02/09 (31.20)
Control	01/03 (28.21)	NT	NT	NT	01/03 (31.04)

NT = Not tested

	Realtime RT-PCR for PRRSV pos/total (mean Ct value)				
GROUP	**D0**	**D3**	**D7**	**D10**	**D14**
Specimen from Binh Dinh	04/06 (29.94)	NT	NT	NT	03/06 (25.44)
Specimen from Phu Yen	04/06 (27.07)	NT	NT	NT	01/06 (31.29)
Vaccine	04/10 (33.04)	NT	NT	NT	04/09 (30.13)
Control	02/03 (28.71)	NT	NT	NT	Neg

(Pigs received live attenuated PRRS vaccine 2.5 month before the experiment)

Antibody test after injection (NCVD)

	Detection of ASF Ab (Ingenasa ELISA kit) pos/total		
GROUP	**D0**	**D7**	**D14**
Specimen from Binh Dinh	Neg	Neg	04/06
Specimen from Phu Yen	Neg	Neg	05/06
Vaccine	Neg	Neg	05/09
Control	Neg	Neg	Neg

Summary of experimental results

Group	No	Observer up to 40 days	ASF p72	ASF I177L	PCV-2	PRRS-NA	*Myco. hyo.*	*Pas. mul.*
Binh Dinh dilution	1	nomal						
	2	nomal						
	14	nomal						
Binh Dinh specimen	3	Dead D16	20.14	Neg	Neg	32.13	Pos	Pos
	5	Nomal						
	8	Nomal						
Phu Yen dilution	9	Dead D21	30.07	Neg	Neg	22.4	Pos	Pos
	10	Nomal						
	12	Dead D40	25.73	Neg	12.71	34.28	Pos	Pos
Phu Yen specimen	6	Nomal						
	7	Nomal						
	11	Nomal						
Vaccine	4	Nomal						
	13	Nomal						
	15	Nomal						
	16	Nomal						
	17	Nomal						
	18	Nomal						
	19	Dead D36	34.23	Neg	27.55	19.16	Pos	Pos
	20	Nomal						
	21	Dead D7	32.22	Neg	Neg	31.25	Pos	Pos
	22	Dead D26	Neg	Neg	Neg	25.41	Pos	Pos
Control	23	Nomal						
	24	Nomal						
	25	Nomal						

Summary of experimental results

- ASFV vaccine appeared in the blood of some pigs from the 3rd day after vaccination in both the vaccine group and the specimen injection group.

- The percentage of pigs with vaccine virus in the blood increased gradually from day D3 to day D14. The group injected with the samples showed viremia earlier (from day D3) and the group vaccine (from day D7).

PCV-2 and PRRS virus concentrations a tendency to increase in some pigs of the same age, both the specimen injection group and the vaccine group.

However, the average Ct value reached about 20 - 21, equivalent to the Ct value commonly found in infected pigs, show that the vaccine virus have multiplied very strongly in the pig.

All blood samples positive for P72 gene were tested for I177L gene, the results were 100% negative for virulent virus and 100% positive for ASFV vaccine.

GENERAL FINDINGS: *(Based on the experimental results)*

- NAVET-ASFVAC vaccine for healthy pigs will not cause pig death. Pigs have an immune response.

- Some pigs have a mild fever reaction or a slight fatigue, which is a normal response to many attenuated vaccines.

- The number of pigs that died after vaccination with NAVET-ASFVAC in Binh Dinh and Phu Yen provinces was due to the vaccination of pigs infected with ASF or infection after vaccination with some diseases such as PCV2, PRRS, P.M, Myco Hyo...

→ vaccination with attenuated ASF vaccine, the vaccine virus multiplies in large numbers in macrophage cells, reducing the protective and immune function. Pre-existing or infectious pathogens are the cause of pig death

EXPERIENCE OF USING ASF VACCINE

1. Vaccinate the specified pigs according to the manufacturer's instructions

2. Epidemiological assessment, determine the herd of clinically healthy pigs before vaccination;

3. Not sure that the herd of pigs is clinically healthy, farmer should change the needle of syringe every time the pigs are vaccinated. Medium & large pig farms, needle of its should be changed after each barn.

4. Do not use ASF vaccine at the same time as other vaccines, at least 2 weeks apart.

THANK YOU

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