VIET NAM'S EXPERIENCE ON THE DEPLOYMENT OF ASF VACCINES

Duc-Huy CHU, PhD., DVM

Veterinary Drug and Vaccine Management Division

Department of Animal Health

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I. Collaborate with Experts of the ARS/USDA

II. Carry out research of ASF vaccine

III. Assessment of ASF vaccine quality

IV. Field trials

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VI. Registration

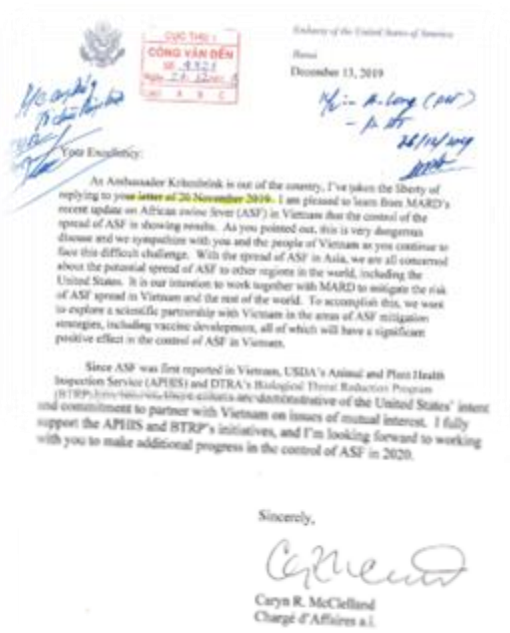
VII. Assessment of ASF vaccine at larger scale

VIII. Marketing authorization (free sale) in Viet Nam

I. Collaborate with Experts of the ARS/USDA

In November 2019, the US scientists published a research on ASF-G-Delta I177L strain

Letters between the Government of Vietnam and US for collaboration on research and development of ASF vaccines



I. Collaborate with Experts of the ARS/USDA

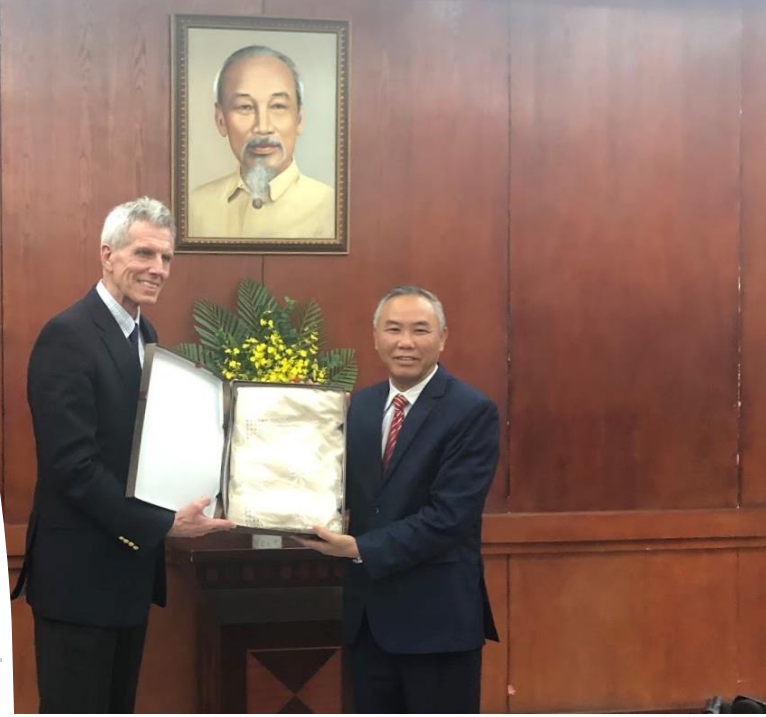
MEMORANDUM OF UNDERSTANDING
BETWEEN

THE UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
AND

MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT OF VIETNAI
(MARD)
THE DEPARTMENT OF ANIMAL HEALTH (DAH)

The United States Department of Agriculture, Agricultural Research Service, hereinafte referred to as 'ARS', and the Ministry of Agriculture and Rural Development of Vietnam, Department of Animal Health referred to as "MARD-DAH" hereinafter jointly referred to as 'Participants', hereby affirm their mutual interest in and desire to broaden cooperative research programs and exchanges.

Have reached the following understandings:



Agricultural Research Service

MTRA Accession no 436820

MATERIAL TRANSFER RESEARCH AGREEMENT FOR SELECT AGENT - OUT

Research conducted under this Agreement is authorized and governed by the terms of Cooperative Research Projects; Agreements with & Receipt of Funds from State & other Agencies (7 U.S.C. 3318a) & Material used in this Agreement is governed by the terms of the Federal Technology Transfer Act (15 U.S.C. 3710a (b) (3) (A)).

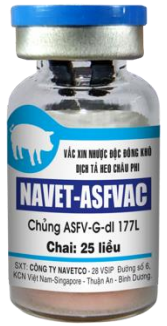
PARTIES:

ARS:	USDA, ARS, NEA
	Foreign Animal Disease Research Unit
	Plum Island Animal Disease Center
	40550 Route 25, Orient Point, NY 11957
	Scientists: Manuel Borca and Douglas Gladue
	Tel: 631-323-3019
	FAX: 631-323-3006
	E-mail: manuel.borca@usda.gov and douglas.gladue@usda.gov
Cooperator 1:	DEPARTMENT OF ANIMAL HEALTH (DAH)
	Ministry of Agriculture and Rural Development (MARD)
	No. 15, Lane 78 Giai Phong Road,
	Phuong Mai, Dong Da, Ha Noi
	Cooperator's Scientist: Nguyen Van Long
	Tel: 0903239918
	FAX: 024 38691311
	E-mail: long.dahvn@gmail.com

NAVETCO (Sept. 2020)

Working seed: ASFV-G-ΔI177L Strain.

Cell line: PBMC (Peripheral blood mononuclear cell)



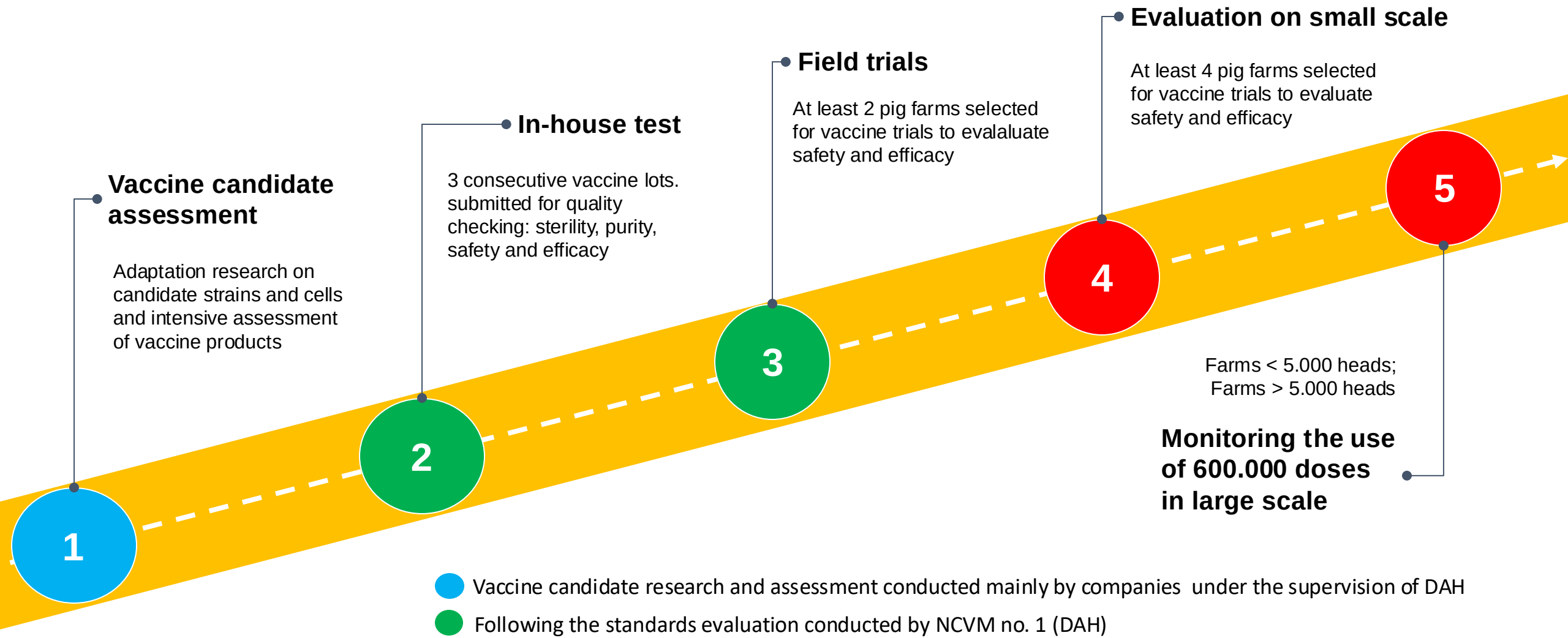
AVAC (Jan. 2021)

Working seed: ASFV-G-ΔMGF Strain.

Cell line: **DMAC** (Diep's Macrophage cell)

ASF vaccines available in Viet Nam

Procedure for ASF vaccine evaluation



- Vaccine candidate research and assessment conducted mainly by companies under the supervision of DAH
- Following the standards evaluation conducted by NCVM no. 1 (DAH)
- Additional evaluation conducted by NCVD (DAH)

Evaluation parameters

1 Vaccine candidate research and assessment

1. Back passage study
- Stability of candidate strains;
- Reversion to virulence study

2. Safety
- Clinical signs
- Virus shedding
- Viremia
- Horizontal transmission
- Overdose safety evaluation
- Growth performance and ability of the immunity response to other vaccines

3. Efficacy
- Antibody response and duration of protective immunity
- Challenge study

4. DIVA antigen development
Distinguishing vaccine viruses and wildtype strain

2 In-house test

3 consecutive vaccine lots. submitted for quality checking: sterility, purity, safety and efficacy

1. Visual inspection
The vaccine is ivory white, the lyophilized cake is spongy, intact, easy to separate from the wall of the vial, easy to dissolve with the vaccine diluent into a homogeneous solution.

2. Sterility
No bacteria, fungi growth

3. Purity
No cross-infection with FMDV, PRRSV, CSFV, Mycoplasma

4. Safety
Overdose safety evaluation

5. Efficacy
Challenge study, Antibody response

3 Field trials

At least 2 pig farms selected for vaccine trials to evaluate safety and efficacy

1. Screening test in field
No infection with FMDV, PRRSV, CSFV, Mycoplasma in pig herd

2. Safety
- Overdose safety evaluation
- Clinical signs
- Virus shedding
- Viremia

3. Efficacy
- Antibody response
- Challenge study

4 Evaluation on small-scale

At least 4 pig farms selected for vaccine trials to evaluate safety and efficacy

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No infection with FMDV, PRRSV, CSFV, Mycoplasma in pig herd

2. Safety
- Clinical signs
- Virus shedding
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- Antibody response
- Challenge study

5 Monitor 600.000 doses

Monitoring the use of 600.000 doses in large scale

Post vaccine monitoring: systemic and adverse reactions, clinical signs, antibody response and coverage

Evaluation parameters

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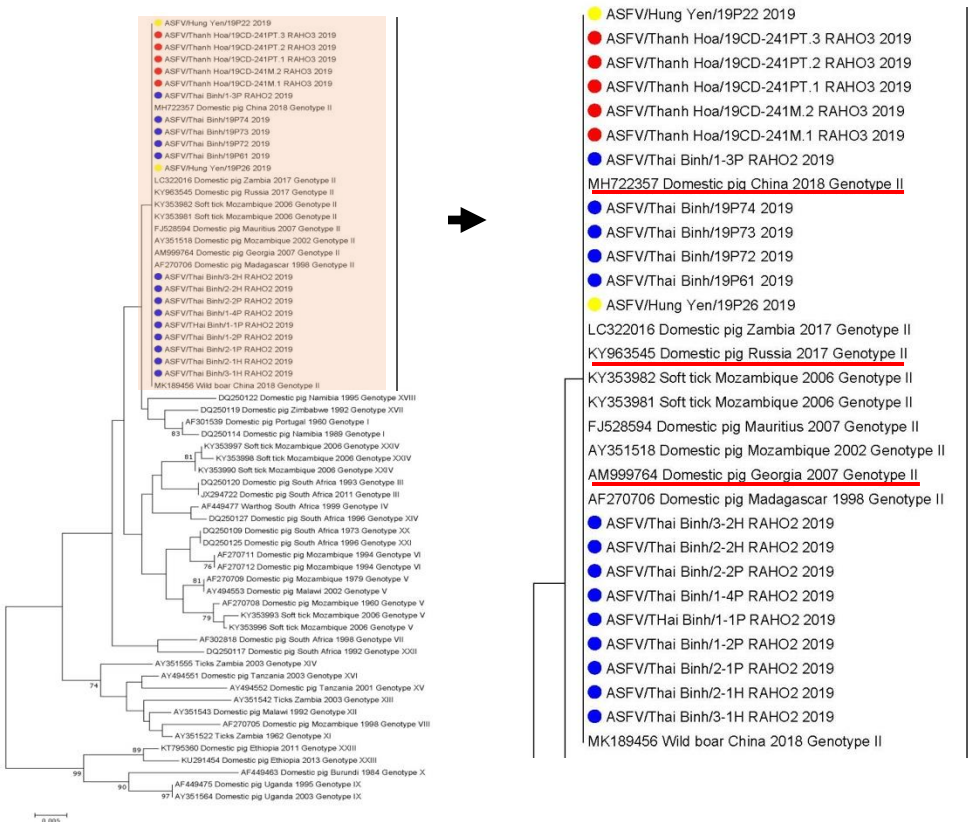
Monitoring the use of 600.000 doses in large scale

Post vaccine monitoring: systemic and adverse reactions, clinical signs, antibody response and coverage

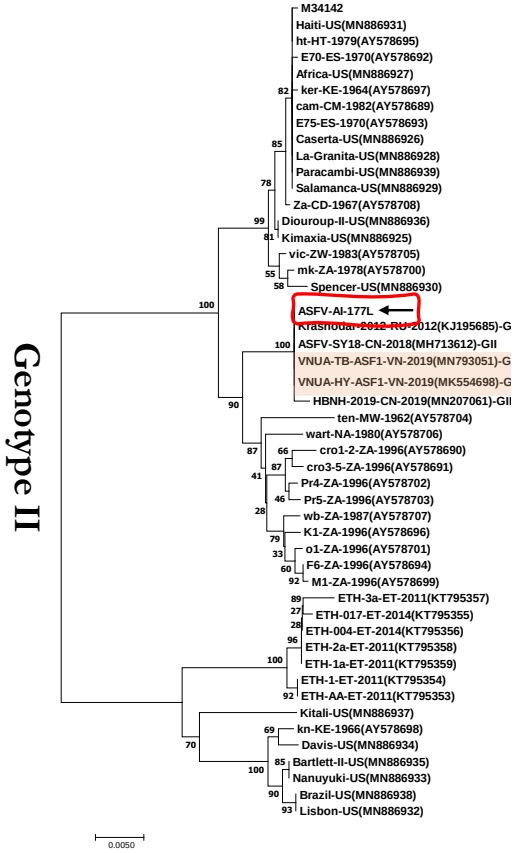
II. Carry out research of ASF vaccine

2.1. Genetic analysis for wildtypes and vaccine candidate strains

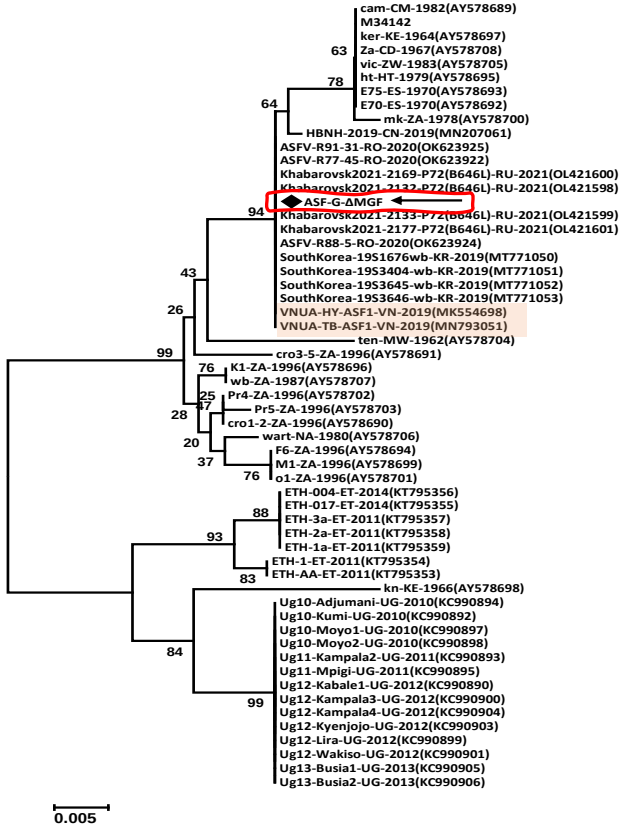
Wild type strains isolated from outbreaks occurred in Vienam in 2019



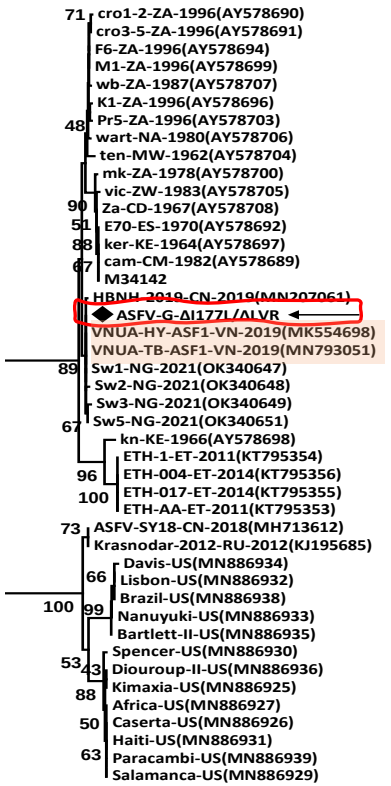
NAVETCO
ASFV-G-ΔI177L



AVAC
ASFV-G-ΔMGF



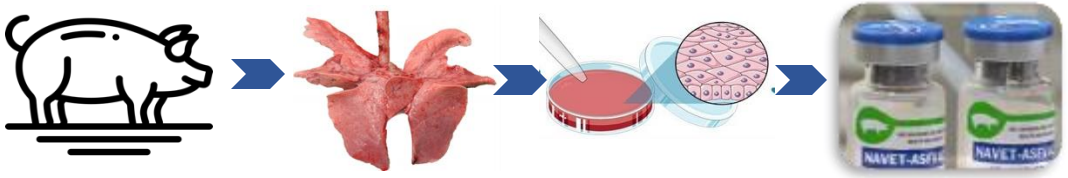
DABACO
ASFV-G-ΔI177L/ΔLVR

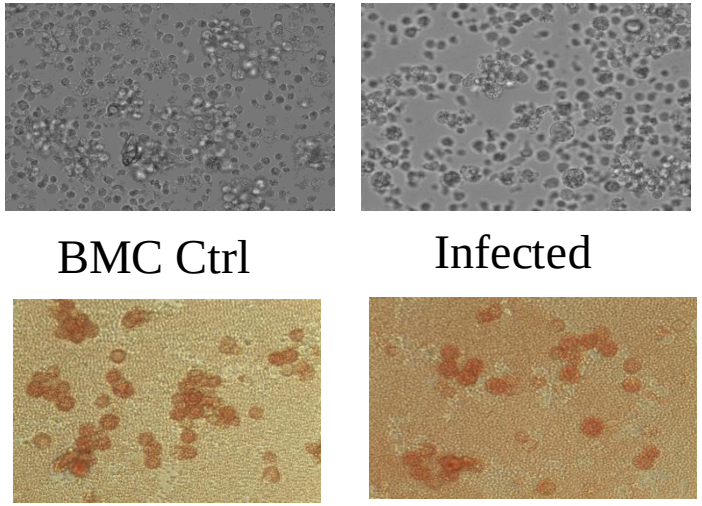


II. Carry out research of ASF vaccine

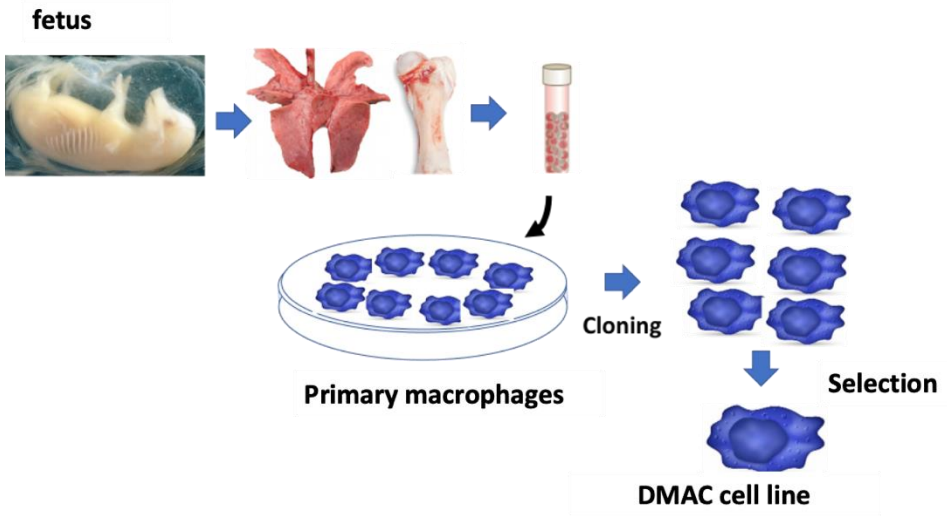
2.2. Cell culture and preparation

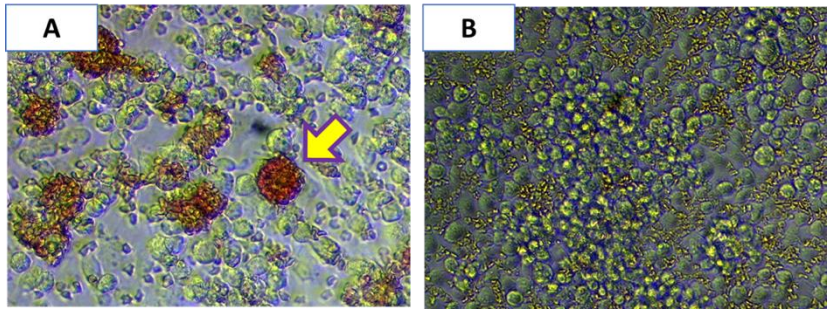
PBMC (Peripheral blood mononuclear cell)





DMAC cell line

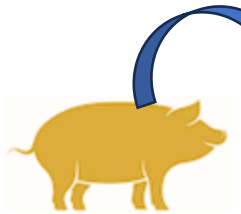




Cytopathic Effect (HAD)

II. Carry out research of ASF vaccine

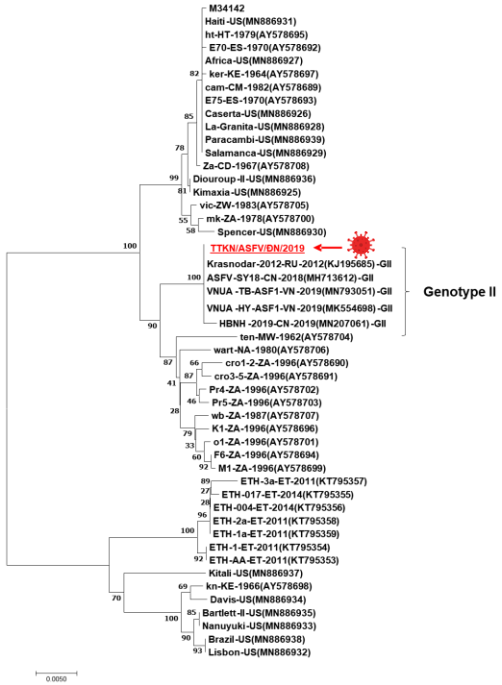
2.3. Characterization and determination of challenge dose for the field strain



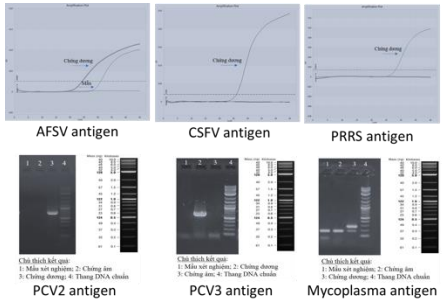
Infected sow from an ASF outbreak in Dong Nai in 2019

TTKN/ASFV/ĐN/2019

1 Genetic analysis

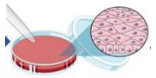


2 Screening for interspecies

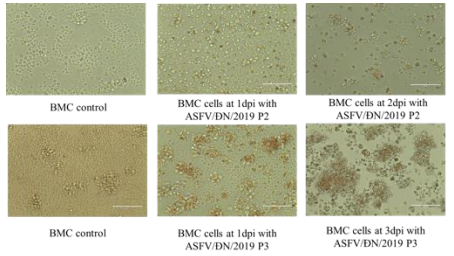


TT	Purity test	Threshold	Result
1	ASFV	Positive: Ct ≤ 35 Negative: Ct > 35	Positive
2	*CSF*	*Positive: Ct < 40* *Negative Ct ≥ 40*	*Negative*
2	*PRRSV*	*Positive: Ct ≤ 35* *Negative: Ct > 35*	*Negative*
3	*PCV2*	*Positive: line of approx. 760bp*	*Negative*
4	*PCV3*	*Positive: line of approx. 649bp*	*Negative*
5	*Mycoplasma hyopneumoniae*	*Positive: 2 line of approx. 180bp and 270bp* *Negative: line of approx. 180bp*	*Negative*

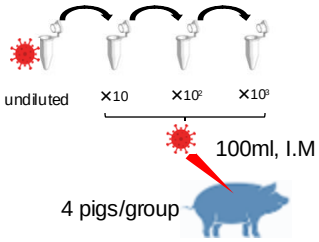
3 Culturing in BMC



	Virus titer log_{10} HAD_{50}/ml
P1	5,5
P2	6,5
P3	6,5



4 Animal experiment



Dilution	Survival	Sacrifice Date (d.p.c)
10^1	0/4	8-10
10^2	0/4	6-8*
10^3	0/4	6-7

Challenge dose = 10^2 HAD50

* Borca, M. V., Ramirez-Medina, E., Silva, E., Rai, A., Espinoza, N., Velazquez-Salinas, L., & Gladue, D. P. (2023). ASF Vaccine Candidate ASFV-G-ΔI177L Does Not Exhibit Residual Virulence in Long-Term Clinical Studies. *Pathogens (Basel, Switzerland)*, *12*(6), 805. https://doi.org/10.3390/pathogens1206080

II. Carry out research of ASF vaccine

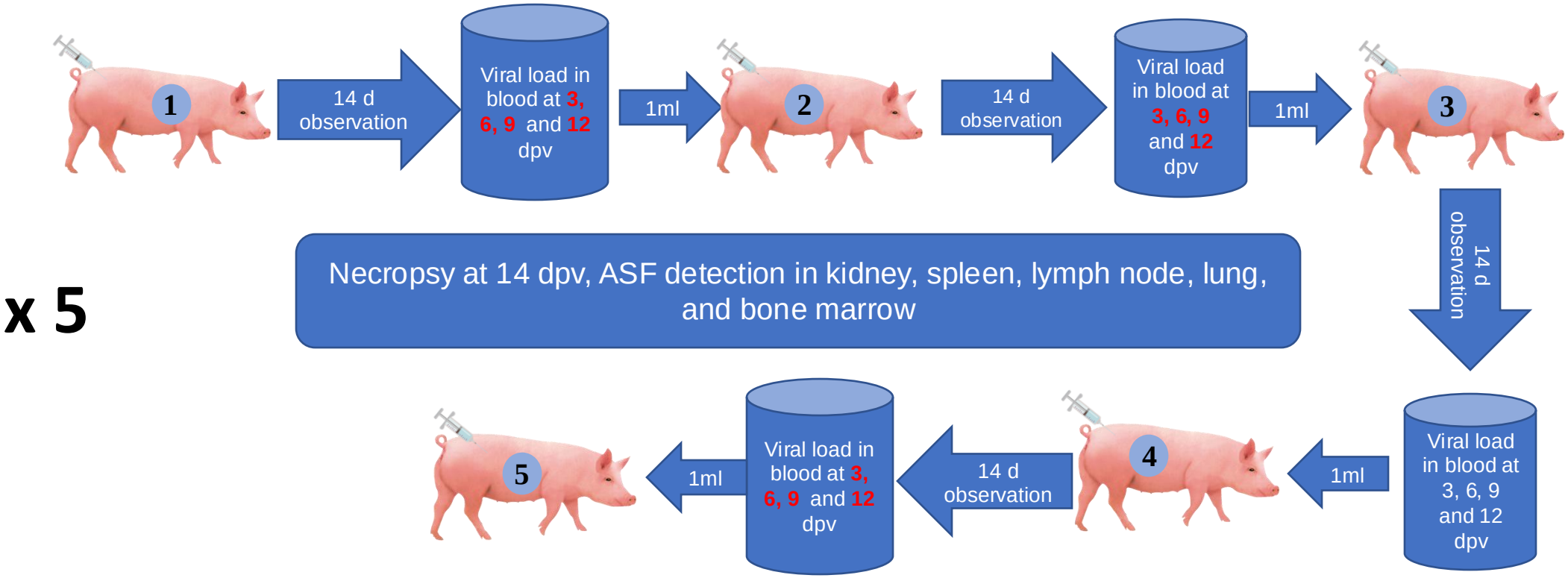
2.4. Differentiation of ASFV field strains and vaccine strains

p72	ASF-1 FP	CTGCTCATGGTATCAATCTTATCGA	King, 2003
	ASF-1 Probe	FAM-CCACGGGAGGAATACCAACCCAGTG-TAMRA	
	ASF-1 RP	GATACCACAAGATCRGCCGT	
I177L	SGRA FP	GAACTGGAAAAAACTTTAACGGC	Borca, 2020
	SGRA Probe	FAM-ACGGATCCCCCTTCGCATTTGA-MGBF	
	SGRA RP	CCATTACCGGCAAGCTAGG	
MGF360-12L	MGF360-12L FP	CATACCCTTCCCCTAAAGCTG	Velazquez-Salinas, 2021
	MGF360-12L Probe	FAM-ACCCTCTTCGAAAACATCAGCCCC-BHQ1	
	MGF360-12L RP	CTACTGCTATGTCCTGGGC	

ASFV strain	p72 gene	I177L gene	MGF360-12L gene
Genotype II, Field strain	Pos	Pos	Pos
Deleted I177L vaccine strain	Pos	Neg	Pos
Deleted MGF vaccine strain	Pos	Pos	Neg

II. Carry out research of ASF vaccine

2.5. Reversion to virulence study



Pigs at 5th passage (5 pigs/passage): healthy

II. Carry out research of ASF vaccine

2.5. Reversion to virulence study

No of passage	Pig No	Dose (ml blood)	Titration of virus (log HAD50) at different times (days)					Clinical signs			ELISA (X%)	Concl.
			7	11	14	21	28	Body Temp	Symptom	Sur/Total		
P1	1 S	$10^{2.8}$ HAD50	2.9±0.7	3.8 ± 0,5	5.2 ± 0,4	4.6 ± 0,3	N	39.1 ± 0.3	normal	3/3	3/3	Safety
	3 S							39.3 ± 0.2	normal		69 ± 14.6	
	4 S							39.6 ± 0.3	normal			
P2	24 S	2ml/head/IM ($10^{3.5}$ HAD_{50}/1 ml)	5.1±1.4	6.7 ± 1.8	6.2 ± 1,2	3.0 ± 1.9	N	39.1 ± 0.4	normal	3/3	3/3	Safety
	28 S							39.7 ± 0.3	normal		60 ± 10.6	
	32 S							39.6 ± 0.3	normal			
P3	25 S	2ml/head/IM ($10^{6.3}$ HAD_{50}/1 ml)	5.9±1,1	6.0 ± 1,4	5.1 ± 1,5	4.6 ± 1.5	4.5 ± 1.7	39.3 ± 0.3	normal	3/3	3/3	Safety
	34 S							39.3 ± 0.4	normal		62 ± 9.5	
	37 S							39.5 ± 0.3	normal			
P4	3 V	2ml/head/IM ($10^{5.7}$ HAD_{50}/1 ml)	6.1±0.3	6.0 ± 1.0	5.2 ± 1.0	5.8 ± 0.3	5.1 ± 0.6	39.7 ± 0.3	normal	3/3	3/3	Safety
	4 V							39.6 ± 0.3	normal		82 ± 5.1	
	5 V							39.3 ± 0.5	normal			
P5	10 V	2ml/head/IM ($10^{6.6}$ HAD_{50}/1 ml)	6.2±1.4	6.2 ± 1.8	6.4 ± 1.5	6.1 ± 1.2	5.6 ± 1.2	39.1 ± 0.4	normal	5/5	5/5	Safety
	11 V							39.5 ± 0.5	normal		88.8 ± 17.7	
	12 V							39.4 ± 0.4	normal			
	13 V							39.2 ± 0.4	normal			
	15 V							39.5 ± 0.3	normal			

NAVETCO® Animal Health

Pigs at 5th passage: healthy

Evaluation parameters

1 Vaccine candidate research and assessment

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At least 2 pig farms selected for vaccine trials to evaluate safety and efficacy

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- Antibody response
- Challenge study

5 Monitor 600.000 doses

Monitoring the use of 600.000 doses in large scale

Post vaccine monitoring: systemic and adverse reactions, clinical signs, antibody response and coverage

III. Assessment of ASF vaccine quality

3.1. Development of testing & evaluation standards for ASF vaccines

- No international or national standards for ASF vaccines before June 2021.
- Viet Nam, in June 2021, the Department of Animal Health issued the “Testing Procedures - Vaccines against ASF”.

CỤC THÚ Y

Số: 146 /QĐ-TY-KH

Hà Nội, ngày 04 tháng 8 năm 2021

QUYẾT ĐỊNH

Về việc chỉ định tạm thời bổ sung phạm vi thử nghiệm

CỤC TRƯỞNG CỤC THÚ Y

Căn cứ Luật chất lượng sản phẩm, hàng hóa ngày 21 tháng 11 năm 2007;

Căn cứ Quyết định số 1399/QĐ-BNN-TCCB ngày 13/4/2017 của Bộ trưởng Bộ Nông nghiệp và Phát triển nông thôn quy định chức năng nhiệm vụ, quyền hạn và cơ cấu tổ chức của Cục Thú y;

Căn cứ Nghị định số 132/2008/NĐ-CP ngày 31tháng 12 năm 2008 của Chính phủ quy định chi tiết thi hành một số điều Luật chất lượng sản phẩm, hàng hóa và Nghị định số 74/2018/NĐ-CP ngày 15 tháng 5 năm 2018 của Chính phủ sửa đổi, bổ sung một số điều của Nghị định số 132/2008/NĐ-CP ngày 31/12/2008 của Chính phủ quy định chi tiết thi hành một số điều Luật chất lượng sản phẩm, hàng hóa (sau đây gọi tắt là Nghị định số 74/2018/NĐ-CP);

Căn cứ Hồ sơ đăng ký bổ sung phạm vi chỉ định hoạt động đánh giá sự phù hợp của Trung tâm Kiểm nghiệm Thuốc thú y Trung ương I; Biên bản họp Hội đồng tổ chức ngày 17/5/2021 của Hội đồng thẩm định hồ sơ đăng ký pháp thử nghiệm đăng ký bổ sung hoạt động thử nghiệm và bổ sung phạm vi chỉ định của Cục Thú y (thành lập theo Quyết định số 119/QĐ-TT-KH ngày 17/3/2021 của Cục trưởng Cục Thú y) và Báo cáo khắc phục những điểm chưa phù hợp của Trung tâm Kiểm nghiệm Thuốc thú y Trung ương I ngày 28/5/2021;

Theo đề nghị của Trưởng phòng Kế hoạch,

QUYẾT ĐỊNH:

Điều 1. Chỉ định tạm thời bổ sung phạm vi thử nghiệm cho Trung tâm Kiểm nghiệm Thuốc Thú y Trung ương I (địa chỉ: Số 30 Ngõ 78, đường Giải Phóng, Phương Mai, Đống Đa, Hà Nội; điện thoại: 02438691236, fax: 02438691012) thực hiện việc thử nghiệm thuốc thú y: ***"Quy trình kiểm nghiệm vắc xin + Vắc xin phòng bệnh Dịch tả lợn Châu Phi"***.

Điều 2. Quyết định này có hiệu lực 01 năm kể từ ngày ký.

Điều 3. Trung tâm Kiểm nghiệm Thuốc Thú y Trung ương I có trách nhiệm thực hiện việc thử nghiệm phục vụ quản lý nhà nước khi có yêu cầu, phù hợp với quy định tại Điều 2 Quyết định này và các quy định, hướng dẫn của cơ quan nhà nước có thẩm quyền và chịu trách nhiệm về kết quả đánh giá sự phù hợp do đơn vị mình thực hiện.

Điều 4. Trung tâm Kiểm nghiệm Thuốc Thú y Trung ương I và các đơn vị liên quan chịu trách nhiệm thi hành Quyết định này./.

Nơi nhận:
- Như Điều 4;
- Bộ Nông nghiệp và PTNT (để b/c);
- Bộ KHCN, Tổng cục TDC (để biết);
- Lưu VT, KH.

CỤC TRƯỞNG

CỤC THÚ Y

2

Criteria	Characteristics
Visual inspection	The vaccine is ivory white, the lyophilized cake is spongy, intact, easy to separate from the wall of the vial, easy to dissolve with the vaccine diluent into a homogeneous solution.
Sterility	No bacterial growth, Mycoplasma and fungi on test media
Purity	No cross-infection with FMDV, PRRSV, CSFV, Mycoplasma
Safety	At least 3 susceptible pigs (4-10wks) for which the vaccine is intended for are each inoculated with 10 doses of vaccine by the recommended route and observed a minimum 21 days. No clinical signs or lesions of any disease attributable to the vaccine should occur in any of the pigs
Efficacy	5 susceptible pigs (4-10wks) are each with 1 dose of vaccine by the recommended route after 28 days the pigs together with 5 unvaccinated controls are challenged with at least 10^2HAD$_{50}$ of ASF virulent and observed for at least 21 days. At least the 4 controls must die and at least the 4 vaccinates survive and show no clinical signs of disease.

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

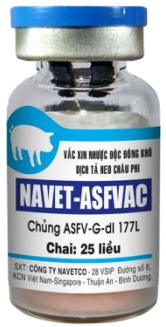
Vaccine testing procedure –

Part 57: African Swine Fever Vaccine, Live

III. Assessment of ASF vaccine quality

3.2. Results of NAVET-ASFVAC

- ❖ Vaccine strain: ASFV-G-ΔI177L Strain
- ❖ Cell line: PBMC (Peripheral blood mononuclear cell)
- ❖ ≥ $10^{2,6}HAD_{50}$/dose
- ❖ Storage conditions: 2-8oC
- ❖ Shelf life: 12 months (updating 20 months)
- ❖ Duration of Immunity: 6 months
- ❖ Animals: Pig 8-10 weeks of age (updating for 4 week-old and one shot of administration)



Assessment of **3 consecutive batches** of vaccine productions

No	Criteria	Test results		
		QC-VR-22-00516	QC-VR-22-00517	QC-VR-22-00518
1	**Visual inspection**	Pass	Pass	Pass
2	**Sterility test**	Pass	Pass	Pass
3	**Purity test**	Pass	Pass	Pass
4	**Safety test**	Pass	Pass	Pass
5	**Efficacy test**	Pass (100%)	Pass (100%)	Pass (100%)

III. Assessment of ASF vaccine quality

3.2. Results of NAVET-ASFVAC

Assessment of 3 consecutive batches of vaccine productions

Efficacy test

Group	Exp. Group	No of pigs	ELISA D 0	ELISA post vaccination (days)				Survival / total challenged		
				D 14	D 21	D 28	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	**5/5**	
	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	**5/5**	
	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	**5/5**	
	Cont	3	0/3	0/3	0/3	0/3	-	0/3		**0/3**

- Protocol of vaccination: 2 shots
- Virulent ASF challenge: code TTKN/ASFV/ĐN/2019
- Dose:10^2 HAD_{50}/pig


Vaccinated group


Control group

III. Assessment of ASF vaccine quality

3.3. Results of AVAC ASF LIVE

Assessment of **3 consecutive batches** of vaccine productions

Vaccine strain: ASFV-G-ΔMGF Strain

Cell line: DMAC (Diep's Macrophage cell)

≥ $10^{3,5}HAD_{50}$/dose

Storage conditions: 2-8oC

Shelf life: 12 months

Duration of Immunity: 5 months

Animals: Pig 4-6 weeks of age

Protection received after vaccination is 28 days

No	Criteria	Characteristics to test	Test results		
			Lot. 0121	Lot. 0221	Lot. 0321
1	**Visual inspection**	The vaccine is ivory white, the lyophilized cake is spongy, intact, easy to separate from the wall of the vial, easy to dissolve with the vaccine diluent into a homogeneous solution.	Pass	Pass	Pass
2	**Sterility test**	No bacterial growth, Mycoplasma and fungi on test media	Pass	Pass	Pass
3	**Purity test**	No cross-infection with FMDV, PRRSV), CSFV, Mycoplasma (MHP)	Pass	Pass	Pass
4	**Safety test**	At least 3 susceptible pigs (4-10w) for which the vaccine is intended for are each inoculated with 10 doses of vaccine by the recommended route and observed a minimum 21 days. No clinical signs or lesions of any disease attributable to the vaccine should occur in any of the pigs	Pass	Pass	Pass
5	**Efficacy test**	5 susceptible pigs (4-10w) are each with 1 dose of vaccine by the recommended route after 28 days the pigs together with 5 unvaccinated controls are challenged with at least 10^2HAD_{50} of ASF virulent and observed for at least 21 days. At least the 4 controls must die and at least the 4 vaccinates survive and show no clinical signs of disease.	Pass (100%)	Pass (80%)	Pass (100%)

III. Assessment of ASF vaccine quality

3.3. Results of AVAC ASF LIVE

Assessment of 3 consecutive batches of vaccine productions

Efficacy test

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
0121	Vac	5	**0/5**	**3/5**	**5/5**	**5/5**	**70.0±5.8**	5/5 (100%)	**5**	
	Cont	5	0/5	0/5	0/5	0/5	-	0/5		**0/5**
0221	Vac	5	**0/5**	**5/5**	**5/5**	**5/5**	**72.6±3.5**	5/5 (100%)	**5**	
	Cont	5	0/5	0/5	0/5	0/5	-	01/5 (20%)		**1/4**
0321	Vac	5	**0/5**	**2/5**	**3/5**	**4/5**	**69.5±3.5**	04/5 (80%)	**4**	
	Cont	5	0/5	0/5	0/5	0/5	-	0/5		**0/5**

- *Virulent ASF challenge: TTKN/ASFV/ĐN/2019 (10^2 HAD_{50}/pig)*
- *ELISA Kit: ID Screen African Swine Fever Indirect Screening Test*

Evaluation parameters

1 Vaccine candidate research and assessment

1. Back passage study
- Stability of candidate strains;
- Reversion to virulence study

2. Safety
- Clinical signs
- Virus shedding
- Viremia
- Horizontal transmission
- Overdose safety evaluation
- Growth performance and ability of the immunity response to other vaccines

3. Efficacy
- Antibody response and duration of protective immunity
- Challenge study

4. DIVA antigen development

Distinguishing vaccine viruses and wildtype strain

2 In-house test

3 consecutive vaccine lots. submitted for quality checking: sterility, purity, safety and efficacy

1. Visual inspection

The vaccine is ivory white, the lyophilized cake is spongy, intact, easy to separate from the wall of the vial, easy to dissolve with the vaccine diluent into a homogeneous solution.

2. Sterility

No bacteria, fungi growth

3. Purity

No cross-infection with FMDV, PRRSV, CSFV, Mycoplasma

4. Safety

Overdose safety evaluation

5. Efficacy

Challenge study, Antibody response

3 Field trials

At least 2 pig farms selected for vaccine trials to evaluate safety and efficacy

1. Screening test in field

No infection with FMDV, PRRSV, CSFV, Mycoplasma in pig herd

2. Safety
- Overdose safety evaluation
- Clinical signs
- Virus shedding
- Viremia

3. Efficacy
- Antibody response
- Challenge study

4 Evaluation on small-scale

At least 4 pig farms selected for vaccine trials to evaluate safety and efficacy

1. Screening test in field

No infection with FMDV, PRRSV, CSFV, Mycoplasma in pig herd

2. Safety
- Clinical signs
- Virus shedding
- Viremia

3. Efficacy
- Antibody response
- Challenge study

5 Monitor 600.000 doses

Monitoring the use of 600.000 doses in large scale

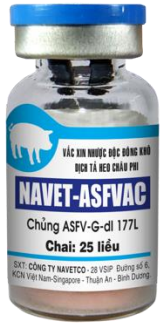
Post vaccine monitoring: systemic and adverse reactions, clinical signs, antibody response and coverage

IV. Field trials

4.1. Results of NAVET-ASFVAC

4.1.1. Safety test

Experimental pig: *Landrace+Yorshire (8-10 Weeks of age). All pigs were tested negative for pathogens: CSFV, PRRSV, FMDV, PCV2, MH, ASFV before experiment*



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

IV. Field trials

4.1. Results of NAVET-ASFVAC

4.1.2. Efficacy test

Farms	Number of pigs	Age	Type of pigs	Batch No	ELISA		Results
					Before vaccination	Post vaccination	
Binh Minh Farm	50	8-10 weeks old	Yorkshire+ Landrace	08	30/30 Negative	30/30 Positive (100%)	Survival 5/5 (100%)
	Control				0/10		0/5
Minh Dung Farm	50	8-10 weeks old	Yorkshire+ Landrace	08	30/30 Negative	27/29 Positive (93,1%)	Survival 5/5 (100%)
	Control				0/10		0/5
Total	**Vaccination**				60/60 Negative	57/59 Positive (96,6%)	Survival 10/10 (100%)
	Control				0/20 Negative		0/10 (0.0%)

- *Virulent ASF challenge: TTKN/ASFV/ĐN/2019 (10^2 HAD_{50}/pig)*
- *ELISA Kit: ID Screen African Swine Fever Indirect Screening Test*

IV. Field trials

4.2. Results of AVAC ASF LIVE

4.2.1. Safety test

Experimental pig: *Landrace+Yorshire (4 Weeks of age). All pigs were tested negative for pathogens: CSFV, PRRSV, FMDV, PCV2, MH, ASFV before experiment*

Farms	Exp. group	Number of pigs	Dose used	Results
Nguyễn Thành Hưng **Farm in Vĩnh Phúc** (Yorkshire+Landrace+Duroc)	Safety group	10	10 times	Passed
	Efficacy group	30	1 shots	
	Control group	10	No	
Bạch Đức Vượng **Farm in Bắc Giang** (Yorkshire+Landrace+Duroc)	Safety group	10	10 times	Passed
	Efficacy group	30	1 shots	
	Control group	10	No	

IV. Field trials

4.2.2. Efficacy test (AVAC ASF LIVE vaccine)

Farms	Number of pigs	Age	Type of pigs	Batch No	ELISA		Survival rate after the challenge
					Before vaccination	Post vaccination	
Nguyễn Thành Hưng Farm	50	>4 weeks old	Yorkshire+ Landrace+ Duroc	0121	30/30 Negative	30/30 Positive (100%)	Survival 5/5 (100%)
	Control				0/10		0/5
Bạch Đức Vượng Farm	50	>4 weeks old	Yorkshire+ Landrace+ Duroc	0121	30/30 Negative	28/30 Positive (97%)	Survival 5/5 (100%)
	Control				0/10		0/5
Total	**Vaccination group**				**60/60 Negative**	**58/60 Positive (97%)**	**Survival 10/10 (100%)**
	Control group				**0/20 Negative**		**0/10 (0.0%)**

- *ASF challenge: TTKN/ASFV/ĐN/2019 (10^2 HAD_{50}/pig)*
- *ELISA Kit: ID Screen African Swine Fever Indirect Screening Test*

Evaluation parameters

1 Vaccine candidate research and assessment

1. Back passage study
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2. Sterility
No bacteria, fungi growth

3. Purity
No cross-infection with FMDV, PRRSV, CSFV, Mycoplasma

4. Safety
Overdose safety evaluation

5. Efficacy
Challenge study, Antibody response

3 Field trials

At least 2 pig farms selected for vaccine trials to evaluate safety and efficacy

1. Screening test in field
No infection with FMDV, PRRSV, CSFV, Mycoplasma in pig herd

2. Safety
- Overdose safety evaluation
- Clinical signs
- Virus shedding
- Viremia

3. Efficacy
- Antibody response
- Challenge study

4 Evaluation on small-scale

At least 4 pig farms selected for vaccine trials to evaluate safety and efficacy

1. Screening test in field
No infection with FMDV, PRRSV, CSFV, Mycoplasma in pig herd

2. Safety
- Clinical signs
- Virus shedding
- Viremia

3. Efficacy
- Antibody response
- Challenge study

5 Monitor 600.000 doses

Monitoring the use of 600.000 doses in large scale

Post vaccine monitoring: systemic and adverse reactions, clinical signs, antibody response and coverage

V. Assessment of ASF vaccine at small scale

Results of AVAC ASF LIVE

No	Location	Farm size (head)	Age (week)	No of vaccination (head)
Farm 1	Luc Ngan – Bac Giang.	500	4-10	139
Farm 2	Chuong My- Ha Noi	2,700	8-10	850
Farm 3	Ba Vi – Ha Noi	3,000	12-13	539
Farm 4	Son Dong – Bac Giang	20,000	5-10	3364
	Sum			4766

V. Assessment of ASF vaccine at small scale

Results of AVAC ASF LIVE

Clinical observation

Criteria	Farm 01	Farm 02	Farm 03	Farm 04
Shock after vaccination	None	None	None	None
Growth rate	Normal	Normal	Normal	Normal
Health	Normal	Normal	Normal	Normal

Virus shedding

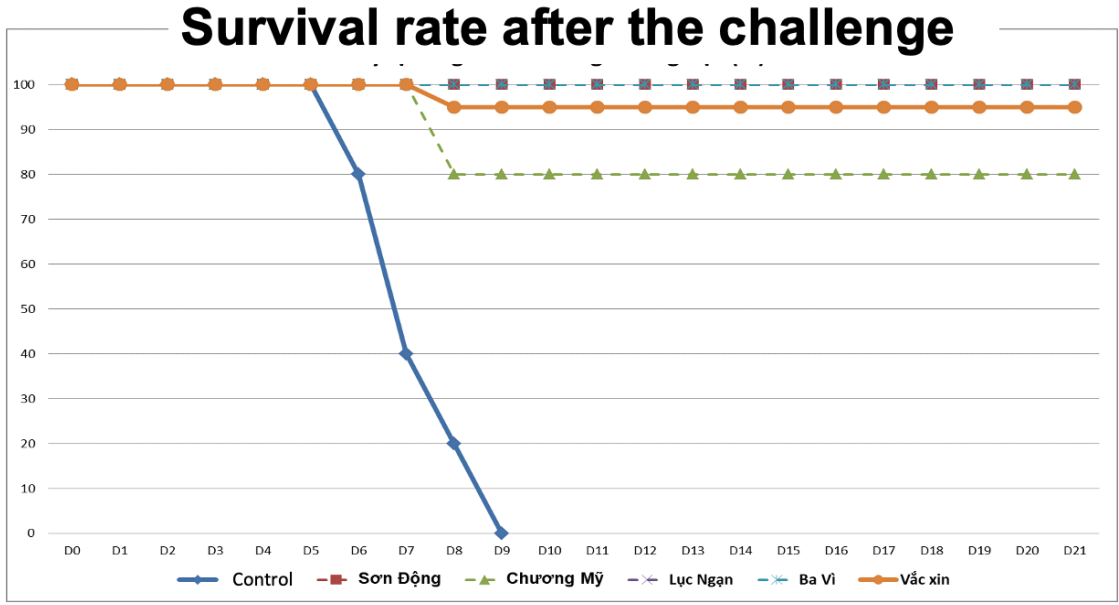
Sampling time\farm	Sample	Farm 01	Farm 02	Farm 03	Farm 04	Sum
14 dpv	Blood	–	–	–	–	0/143
	Oral swab	–	–	–	–	0/27
28 dpv	Blood	–	–	–	–	0/141
	Oral swab	–	–	–	–	0/20

V. Assessment of ASF vaccine at small scale

Results of AVAC ASF LIVE

Serological immunity

Trial farm	14 dpv			28 dpv		
	No of samples	Pos (+)	Percent (+)	No of samples	Pos (+)	Percent (+)
Total	143	81	56,6%	141	127	90,1%



Conclusions:

- Seroconversion 56,6%//14 dpv and 90,1% at 28 dpv
- No virus found in blood and excreates at 14, 28 dpv
- Pigs 4-13 week-old, one shot, protective rate: 95%.

Vaccine confers immunity enough to protect the pig and reduce virus shedding after the challenge.

VI. Registration

Registration and issuance of MA Certificate process (for common vaccine)

The steps according to regulations:

1 Submitting a dossier for veterinary vaccine testing registration

2 Establishing a specialized veterinary drug scientific council for review

3 Implementing testing according to the approved outline by the scientific council *

4 Submitting the dossier for product circulation registration along with test results that meet the requirements.

5 Establishing a specialized veterinary drug scientific council for approval

6 Issuing the Marketing Authorization (MA) Certificate

*: ***01 Trial Implementing Unit: NCVDC***
01 Trial Supervision Unit: RAHO
01 Farm for trial.

VI. Registration

1 Submitting a dossier for veterinary vaccine testing registration

2 ***The Science and Technology Council advises (Ministry level) on evaluating research and production results, as well as technical meetings.***

3 Establishing a specialized veterinary drug scientific council for review

4 Implementing testing according to the approved outline by the scientific council ***

5 Submitting the dossier for product circulation registration along with test results that meet the requirements.

6 ***Obtaining opinions from relevant units/ Establishing a specialized veterinary drug scientific council for reviewing the registration dossier for product circulation***

7 Issuing the Circulation Certificate (MA)

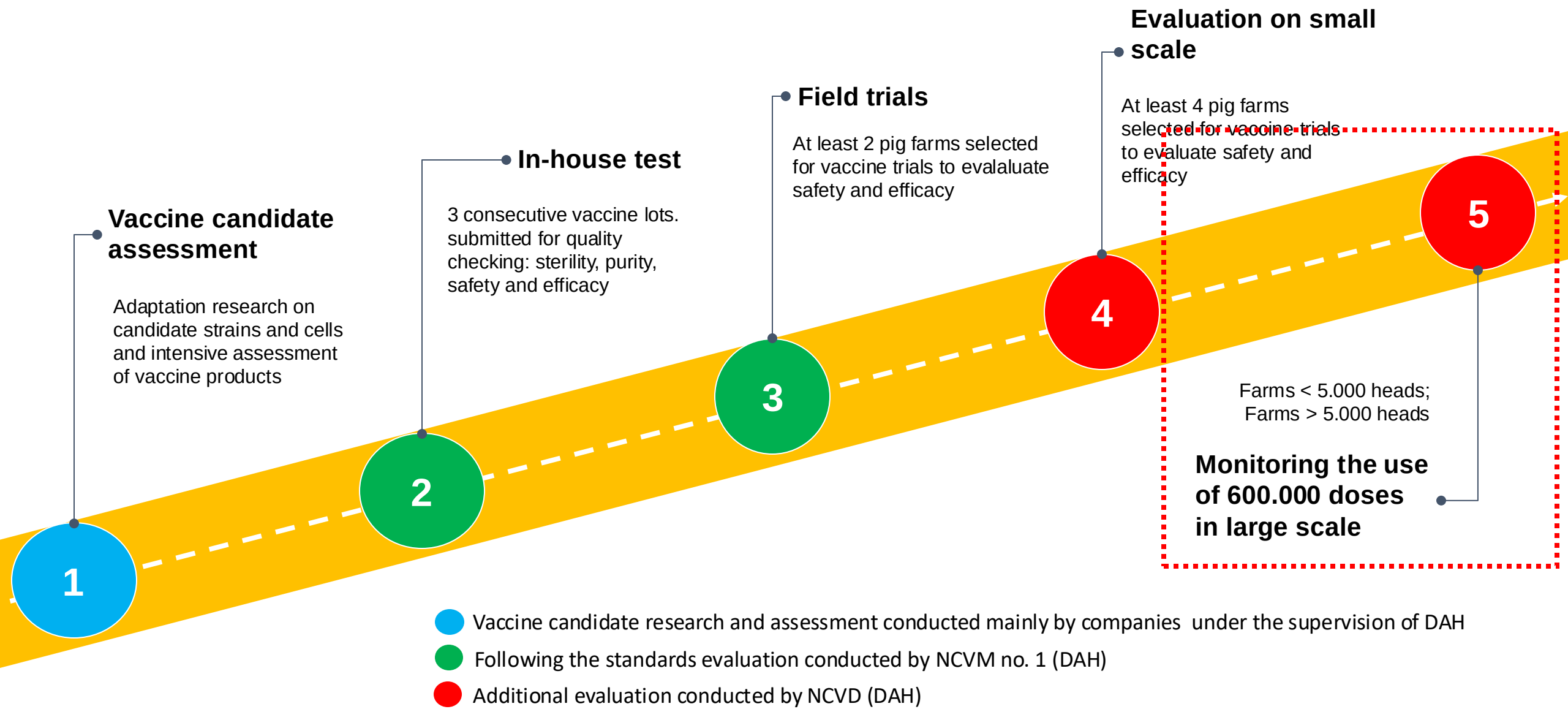
8 ***Monitoring the pilot use on a narrow scale of 600,000 doses ****

9 ***Evaluating the results of usage, the Department of Animal Health proposes, and reports to the Ministry for completed circulation approval***

***: *01 Trial Implementing Unit: NCVDC I; > 02 Trial Supervision Unit: RAHOs; 01 The coordinating unit evaluates the safety indicators of the vaccine: NCVD; > 02 Trial locations*

****: *Developing a plan for monitoring the quality of vaccine use on a narrow scale: Epidemiology-DAH; Coordinating and implementing monitoring activities: SDAH/RAHO/Reg. Company*

Procedure for ASF vaccine evaluation



Evaluation parameters

Monitor 600.000 doses

Monitoring the use of 600.000 doses in large scale

Post vaccine monitoring: systemic and adverse reactions, clinical signs, antibody response and coverage

VII. Assessment of ASF vaccine at large scale

Monitoring results of trial use for 600,000 doses in field

VII. Assessment of ASF vaccine at large scale

7.1. Results for using 600,000 doses of AVAC ASF LIVE vaccine at large scale

From October 2022 to June 2023, AVAC Company administered 605,211 doses of pork vaccine at 596 farms across 34 provinces and cities.

<u>< 5000 pig heads with strictly supervision apply:</u>
Supervised vaccinations were given to 1,819 pigs at 14 farms in 5 provinces, with a 94.4% antibody rate and no health issues.

<u>> 5000 pig heads without strictly supervision apply:</u>
Unsupervised vaccinations were done for 2,848 pigs at 37 farms in 8 provinces. All pigs remained healthy with no issues.

VII. Assessment of ASF vaccine at large scale

7.1. Results for using 600,000 doses of AVAC ASF LIVE vaccine at large scale

Vaccination at CP Vietnam farms (biggest pig producer in Vietnam):

As of 31 January 2023, CP Vietnam Livestock Corporation reported to the MARD and DAH:

- They've administered 600,544 doses of AVAC ASF LIVE vaccine to pigs at 545 farms (different scales) in 32 provinces and cities.

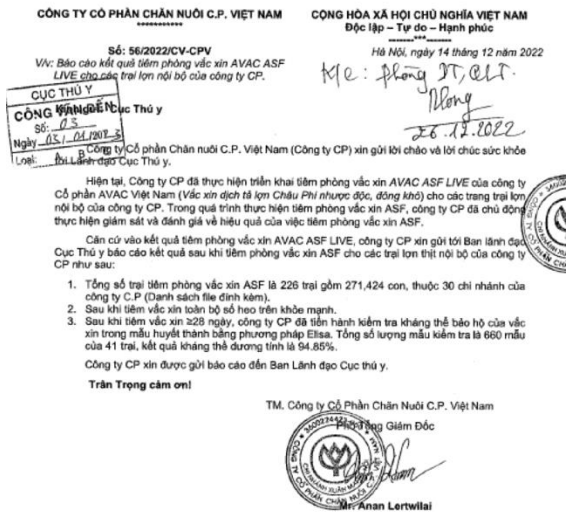
- More than 93% of tested samples (5,561 out of 5,958) had positive antibodies against ASF virus, indicating the vaccine's safety for all pigs at these farms.

STT	Vùng	Chi nhánh	Số lượng heo (con)	Số lượng trái
1	Miền Bắc	Hà Nội	56,873	93
2	Miền Bắc	Bắc Giang	12,384	21
3	Miền Bắc	Thái Nguyên	10,229	17
4	Miền Bắc	Phú Thọ	5,051	8
5	Miền Bắc	Hải Phòng	11,839	21
6	Miền Bắc	Thanh Hóa	9,500	15
7	Miền Bắc	Nghệ An	1,839	3
8	Miền Trung	Quảng Trị	9,806	9
9	Miền Trung	Đà Nẵng	3,947	3
10	Miền Trung	Quảng Nam	3,547	3
11	Miền Trung	Quảng Ngãi	5,558	7
12	Miền Trung	Bình Định	16,610	15
13	Miền Trung	Nha Trang	102,075	76
14	Miền Trung	Ninh Thuận	10,717	11
15	Miền Trung	Đắc Lắk	48,190	42
16	Miền Trung	Gia Lai	8,172	10
17	Miền Nam	Đồng Nai	33,465	19
18	Miền Nam	Bình Dương	34,610	19
19	Miền Nam	Bình Phước	48,271	33
20	Miền Nam	Đắk Nông	24,777	18
21	Miền Nam	Tay Ninh	11,750	6
22	Miền Nam	Vũng Tàu	7,431	6
23	Miền Nam	Lâm Đồng	28,057	23
24	Miền Nam	Long An	27,970	19
25	Miền Nam	Tiền Giang	18,155	16
26	Miền Nam	Vĩnh Long	10,805	7
27	Miền Nam	Đồng Tháp	12,561	7
28	Miền Nam	Bạc Liêu	1,600	1
29	Miền Nam	Hậu Giang	500	1
30	Miền Nam	Cần Thơ	10,550	6
31	Miền Nam	Cà Mau	3,000	2
32	Miền Nam	Kiên Giang	10,705	8
Tổng			**600,544**	**545**

VII. Assessment of ASF vaccine at large scale

7.1. Results for using 600,000 doses of AVAC ASF LIVE vaccine at large scale

In CP Viet Nam



CÔNG TY CỔ PHẦN CHĂN NUÔI C.P. VIỆT NAM

Số: 56/2022/CV-CPV

V/v: Báo cáo kết quả tiêm phòng vắc xin AVAC ASF LIVE cho các trại lợn nội bộ của công ty CP.

CỘNG HÒA XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập – Tự do – Hạnh phúc

Hà Nội, ngày 14 tháng 12 năm 2022

Kính gửi: Ban Lãnh đạo Cục Thú y

Công ty Cổ phần Chăn nuôi C.P. Việt Nam (Công ty CP) xin gửi lời chào và lời chúc sức khỏe tới Ban Lãnh đạo Cục Thú y.

Hiện tại, Công ty CP đã thực hiện triển khai tiêm phòng vắc xin *AVAC ASF LIVE* của công ty Cổ phần AVAC Việt Nam (*Vắc xin dịch tả lợn Châu Phi nhược độc, đông khô*) cho các trang trại lợn nội bộ của công ty CP. Trong quá trình thực hiện tiêm phòng vắc xin ASF, công ty CP đã chủ động thực hiện giám sát và đánh giá về hiệu quả của việc tiêm phòng vắc xin ASF.

Căn cứ vào kết quả tiêm phòng vắc xin AVAC ASF LIVE, công ty CP xin gửi tới Ban lãnh đạo Cục Thú y báo cáo kết quả sau khi tiêm phòng vắc xin ASF cho các trại lợn thịt nội bộ của công ty CP như sau:

1. Tổng số trại tiêm phòng vắc xin ASF là 226 trại gồm 271,424 con, thuộc 30 chi nhánh của công ty C.P (Danh sách file đính kèm).
2. Sau khi tiêm vắc xin toàn bộ số heo trên khỏe mạnh.
3. Sau khi tiêm vắc xin ≥28 ngày, công ty CP đã tiến hành kiểm tra kháng thể bảo hộ của vắc xin trong mẫu huyết thanh bằng phương pháp Elisa. Tổng số lượng mẫu kiểm tra là 660 mẫu của 41 trại, kết quả kháng thể dương tính là 94.85%.

Công ty CP xin được gửi báo cáo đến Ban Lãnh đạo Cục thú y.

Trân Trọng cảm ơn!

TM. Công ty Cổ Phần Chăn Nuôi C.P. Việt Nam
Phó Tổng Giám Đốc

Mr. Anan Lertwilai

VII. Assessment of ASF vaccine at large scale

7.2. Results for using 600,000 doses of NAVET – ASFVAC at large scale

- From July 2022 to June 2023, Navetco company supplied and collaborated with specialized monitoring agencies to vaccinate pigs against on 07 different scales, ranging from 50 pigs/household to 2,000 pigs/farm. This was done at 132 facilities across 23 provinces and cities, with a total of 47,435 doses administered, including 29,685 closely monitored doses.

- Out of the 29,685 monitored doses, 219 pigs (0.7%) exhibited reactions, and 42 pigs (0.1%) had to be culled. Common reactions, such as mild fever, reduced appetite, coughing, or diarrhea, occurred 2-4 days after vaccination, with subsequent return to normal. In conclusion, the vaccine is safe, and vaccinated pigs showed normal growth and development.

- Antibody test results after vaccination were 85.5% for the first dose and 97.4% for the second dose, with an average rate of 95.5% for both doses based on 1,488 serum samples, of which 1,421 tested positive for antibodies.

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VIII. Marketing authorization (free sale) in Viet Nam

ASF registration Process for Marketing Authorization

Evaluation, quality assessment of virus strains, cells

Stability, antigenic characteristics, immune response, and adaptability for cell culture propagation

NAVETCO: *Virus ΔI177L Strain; Cell: PBMC;*

AVAC: *Virus MGF ZMAC p2, D9GL/D UK, ΔI177L, ΔI177L/dLVR; Cell: ZMAC, PIPEC and DMAC;*

DABACO: *Virus ΔI177L/ΔLVR strain; cell: PIPEC (Plum Island porcine epithelial cells)*

Quality testing results

- Quality testing results from the manufacturer
- Testing results from the regulatory agency

MA reg.

Official licensing (MA)

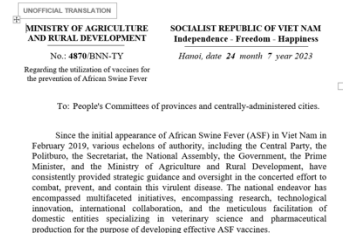
MARD has officially given permission for use nationwide and for export

Development of research, production, and evaluation protocols for product characteristics: Dosage determination; Shelf life; Safety, sterility, efficacy...

Research and production results

Sterility, safety, efficacy

Evaluation committee for Vaccine trial results



UNOFFICIAL TRANSLATION

MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT

No.: 4870/BNN-TY

Regarding the utilization of vaccines for the prevention of African Swine Fever

SOCIALIST REPUBLIC OF VIET NAM
Independence - Freedom - Happiness

Hanoi, date 24 month 7 year 2023

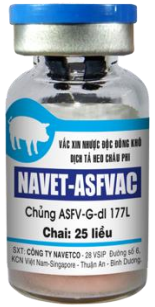
To: People's Committees of provinces and centrally-administered cities.

Since the initial appearance of African Swine Fever (ASF) in Viet Nam in February 2019, various echelons of authority, including the Central Party, the Politburo, the Secretariat, the National Assembly, the Government, the Prime Minister, and the Ministry of Agriculture and Rural Development, have consistently provided strategic guidance and oversight in the concerted effort to combat, prevent, and contain this virulent disease. The national endeavor has encompassed multifaceted initiatives, encompassing research, technological innovation, international collaboration, and the meticulous facilitation of domestic entities specializing in veterinary science and pharmaceutical production for the purpose of developing effective ASF vaccines.

Monitoring results of trial use for 600,000 doses in field

VIII. Marketing authorization (free sale) in Viet Nam

ASF registration Process for Marketing Authorization



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BỘ NÔNG NGHIỆP VÀ PHÁT TRIỂN NÔNG THÔN

Số: 4870 /BNN-TY

V/v sử dụng vắc xin phòng bệnh Dịch tả lợn Châu Phi

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

Hà Nội, ngày 24 tháng 7 năm 2023

Kính gửi: Ủy ban nhân dân các tỉnh, thành phố trực thuộc Trung ương.

Từ khi bệnh Dịch tả lợn Châu Phi (DTLCP) lần đầu tiên xuất hiện tại Việt Nam vào tháng 02 năm 2019 đến nay, Trung ương Đảng, Bộ Chính trị, Ban Bí thư, Quốc hội, Chính phủ, Thủ tướng Chính phủ, Bộ Nông nghiệp và Phát triển nông thôn đã chỉ đạo triển khai đồng bộ, quyết liệt các giải pháp phòng, chống và kiểm soát dịch bệnh; đẩy mạnh nghiên cứu, làm chủ công nghệ sản xuất vắc xin; tăng cường hợp tác quốc tế, hướng dẫn, hỗ trợ các cơ quan chuyên môn thú y, các doanh nghiệp trong nước tổ chức nghiên cứu, sản xuất vắc xin phòng bệnh DTLCP.

3. Cục Thú y

- Chỉ đạo, hướng dẫn, hỗ trợ kỹ thuật đối với các địa phương, các doanh nghiệp trong việc kiểm soát chất lượng, sử dụng vắc xin DTLCP.

- Phối hợp với các chuyên gia quốc tế, nhất là chuyên gia Hoa Kỳ để tiếp tục hỗ trợ các doanh nghiệp tổ chức nghiên cứu, sản xuất vắc xin DTLCP cho các đối tượng lợn khác (lợn nái, lợn đực giống) ở các lứa tuổi khác nhau; tổ chức nghiên cứu, đánh giá sử dụng vắc xin DTLCP tại thực địa.

Bộ Nông nghiệp và Phát triển nông thôn đề nghị đồng chí Chủ tịch Ủy ban nhân dân các tỉnh, thành phố trực thuộc Trung ương quan tâm chỉ đạo thực hiện các nội dung nêu trên; thường xuyên thông báo về Bộ Nông nghiệp và Phát triển nông thôn để phối hợp xử lý kịp thời các vấn đề phát sinh./.

Nơi nhận:
- Như trên;
- Thủ tướng Chính phủ (để b/c);
- Phó Thủ tướng Trần Lưu Quang (để b/c);
- Bộ trưởng Lê Minh Hoan (để b/c);
- Cục Thú y và các đơn vị thuộc Cục (để t/h);
- Các Vụ: Khoa học Công nghệ và Môi trường, Pháp chế;
- Thanh tra Bộ;
- Sở NN&PTNT, CCCN&TY các tỉnh, TP (để t/h);
- Công ty: NAVETCO, AVAC (để t/h);
- Lưu: VT, TY.

KT. BỘ TRƯỞNG
THỨ TRƯỞNG

Phùng Đức Tiến

Special thanks to

USDA United States Department of Agriculture

USDA ARS United States Department of Agriculture Agricultural Research Service

APHIS

Thank you for your attention!