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VIETNAM NATIONAL UNIVERSITY OF AGRICULTURE

Overview of ASF Vaccine Development in Vietnam

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Content

- I. Pig production in Vietnam**
- II. General information of ASF**
- III. ASF Status in Vietnam during the 2019-2022 period**
- IV. Overview of ASF vaccine development in Vietnam**



I. Pig production in Vietnam

❖ Pig population in 2019: Nearly 30 million

- Pork volume: 3.82 million tons
- Pork presents at 70% meals of Vietnamese

❖ Pig-raising households:

About 2.5 million

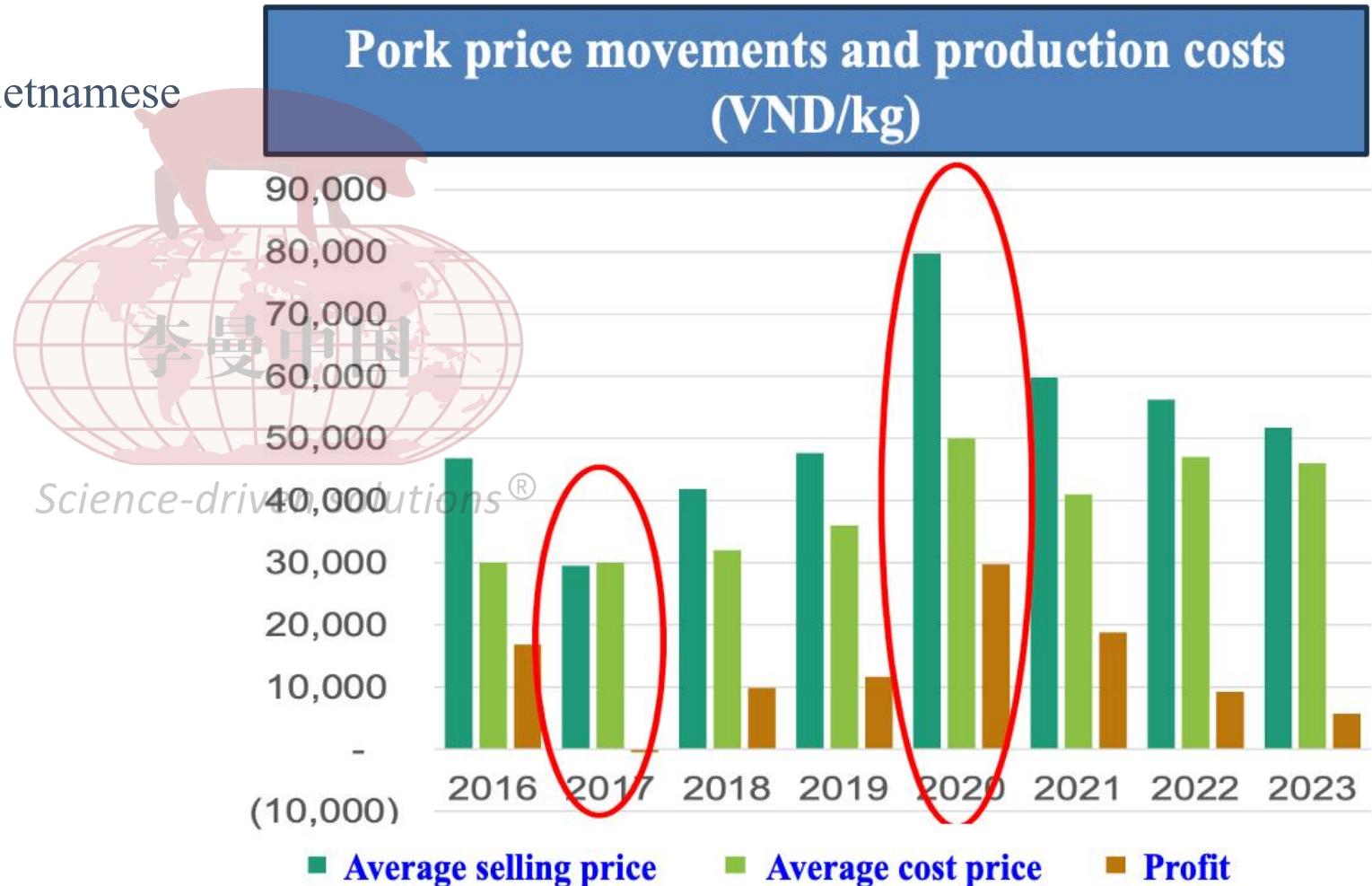
- 49% of the total pig population
- 40% of the total pork volume

❖ Large big farms: 10,000

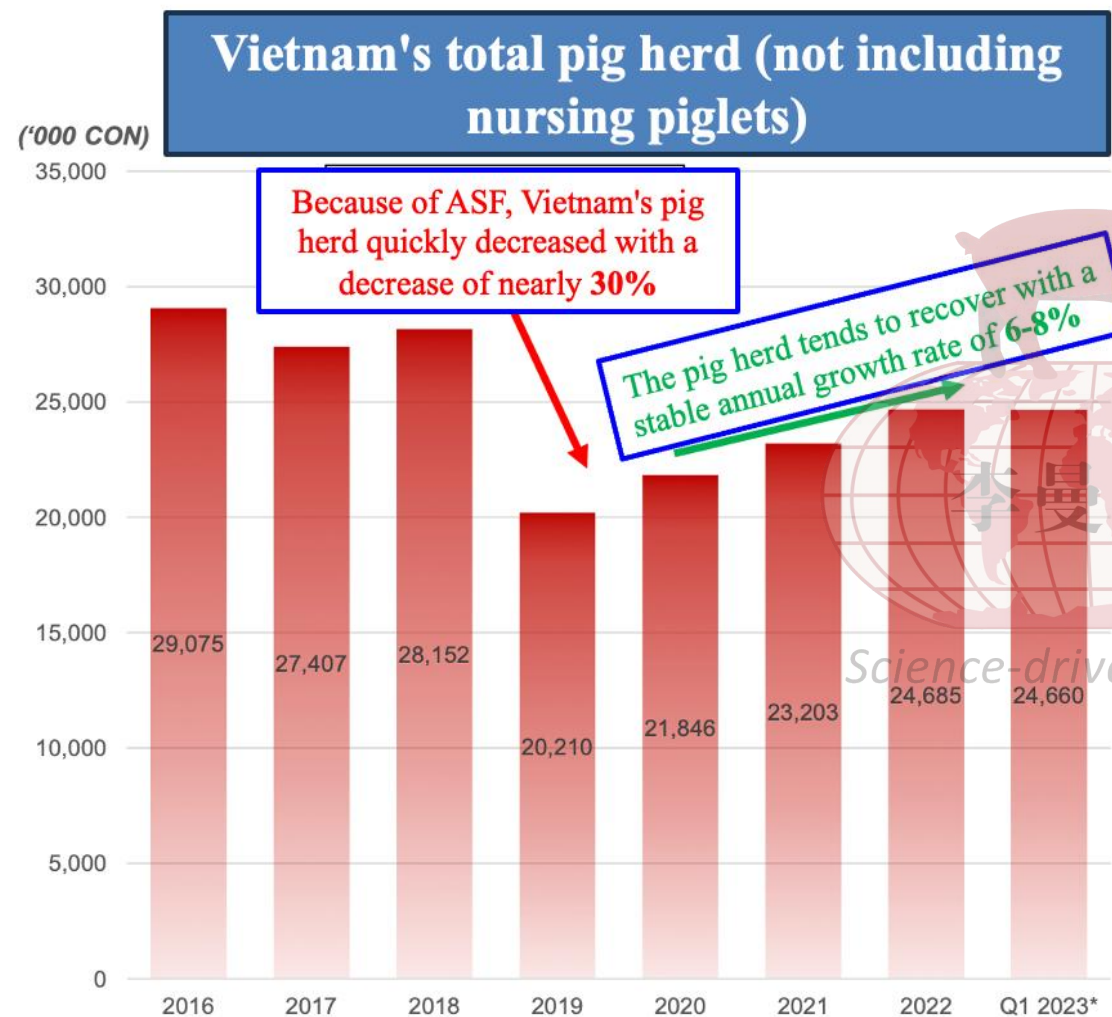
- 51% of the total pig population
- 60% of the total pork volume

❖ Breeding pigs:

- Sows: About 4.0 million
- Boars: 76,000



I. Pig production in Vietnam (*Continued*)

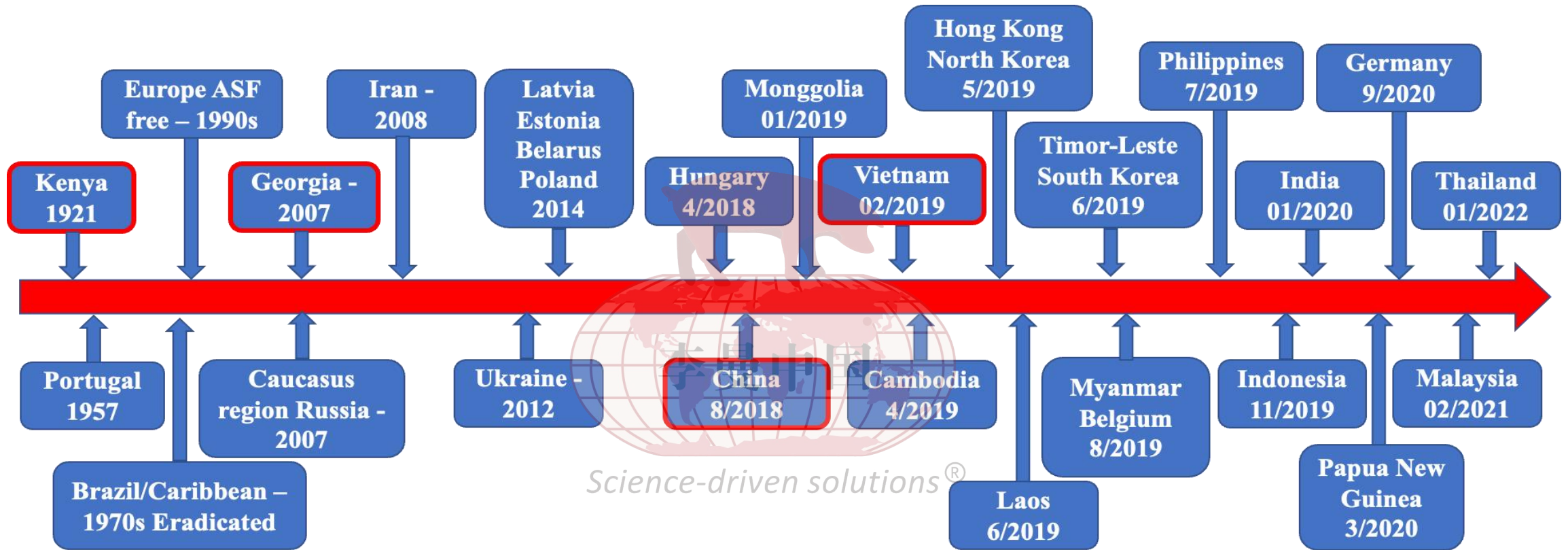


II. General information of ASF

- ❖ Asfarviridae: *Asfivirus*
 - ✓ Large, enveloped DNA virus
- ❖ Caused diseases in all pig species
 - ✓ Mortality (domestic pigs) up to 100%
- ❖ 24 genotypes
 - ✓ Vary in virulence
 - ✓ High virulence: up to 100% mortality
 - ✓ Low virulence: seroconversion
- ❖ Infects monocytes and macrophages



History of ASF



Persistence of ASFV across a variety of environmental conditions

Item.	Survival time
Salted meat	182 days
Dried meat	300 days
Smoked and deboned meat	30 days
Frozen meat	1000 days
Chilled meat	110 days
Offal	105 days
Blood stored at 4°C	18 months
Putrefied blood	15 weeks
Contaminated pig pens	1 month
Faeces at room temperature	11 days
Skin/Fat (even dried)	300 days



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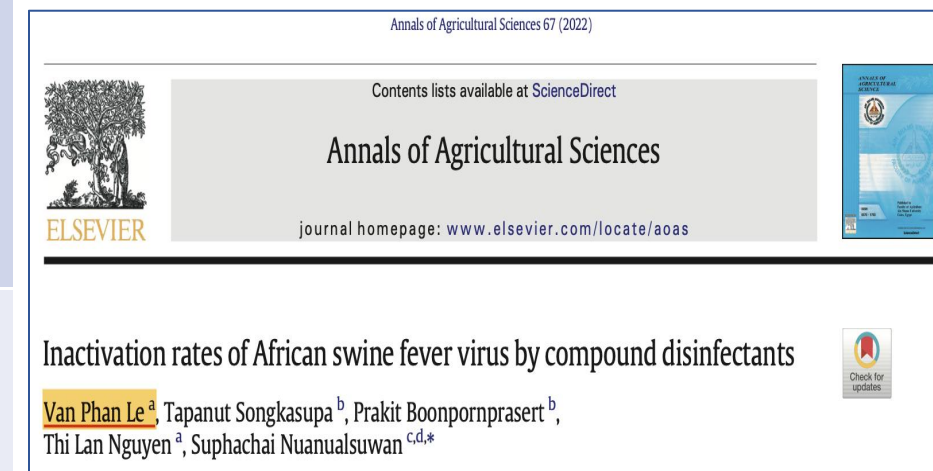


(<https://www.fao.org/3/i7228e/i7228e.pdf>)

Resistance of ASFV to physical and chemical action

Action	Resistance
Temperature	Highly resistant to low temperatures. Heat inactivated by 56°C/70 min; 60°C/20 min.
pH	Inactivated by pH <3.9 or >11.5 in serum-free medium. Serum increases the resistance of the virus, <i>e.g.</i> , at pH 13.4 , resistance lasts up to 21 h without serum and 7 days with serum.
Chemicals / Disinfectants	Susceptible to Ether and Chloroform. Inactivated by 8/1,000 sodium hydroxide (or NaOH) (30 min); Hypochlorites (ClO) as 2.3% chlorine (or clo) (3 min); 3/1,000 formalin (30 min) ; 3% ortho-phenylphenol (30 min) and iodine compounds.
Survival	Remains viable for long periods in blood, faeces, and tissues , especially infected uncooked or undercooked pork products.

(OIE. *Technical disease card for African swine fever*. 2009)



Clinical Disease

➤ Incubation period

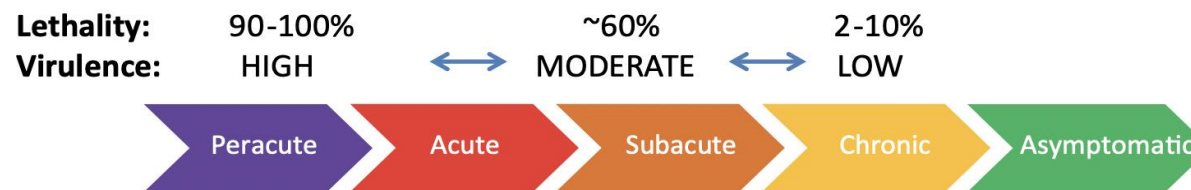
- ✓ 5-21 days following direct contact
- ✓ < 5 days after a tick bite

➤ Forms of disease

- ✓ Peracute – sudden death
- ✓ Acute
- ✓ Subacute
- ✓ Chronic



Clinical forms of African swine fever according to the virulence of the isolate involved



Source: FAO

III. ASF status in Vietnam during the 2019-2022 period

- First report: **February 1, 2019**, in Hung Yen Province, Northern Vietnam
- September 3 (after 7 months): ASF outbreaks were reported in **all 63/63** provinces of Vietnam
- At least **6 million pigs** were culled



[Emerg Infect Dis.](#) 2019 Jul; 25(7): 1433–1435.
doi: [10.3201/eid2507.190303](https://doi.org/10.3201/eid2507.190303)

PMCID: PMC6590755
PMID: [31075078](https://pubmed.ncbi.nlm.nih.gov/31075078/)

Outbreak of African Swine Fever, Vietnam, 2019

[Van Phan Le](#),¹ [Dae Gwin Jeong](#),¹ [Sun-Woo Yoon](#), [Hye-Min Kwon](#), [Thi Bich Ngoc Trinh](#), [Thi Lan Nguyen](#), [Thi To Nga Bui](#), [Jinsik Oh](#), [Joon Bae Kim](#), [Kwang Myun Cheong](#), [Nguyen Van Tuyen](#), [Eunhye Bae](#), [Thi Thu Hang Vu](#), [Minjoo Yeom](#), [Woonsung Na](#), and [Daesub Song](#)[✉]



ASF transmission in Vietnam



ASF transmission in Vietnam



Clinical sign investigation

Clinical signs	Type of pig			
	Boar (%) (n=3)	Sow (%) (n=178)	Fattening (%) (n=212)	Piglet (%) (n=93)
Fever	100	100	100	100
Loss of appetite	100	100	100	100
Vomiting	100	90	10	20
Foaming at mouth	0	40	55	80
Skin hemorrhage	33	40	100	50
Hemorrhagic discharge from nasal/anus	10	10	90	0
Abortion in pregnant sows	-	100	-	-
Leg problem	0	0	0	100

Clinical and Pathological Study of the First Outbreak Cases of African Swine Fever in Vietnam, 2019

Bui Thi To Nga¹, Bui Tran Anh Dao¹, Lan Nguyen Thi¹, Makoto Osaki², Kenji Kawashima², Daesub Song³, Francisco J. Salguero^{4†} and Van Phan Le^{1*†}

Basic reproduction number (R_0) in sow and fattening pig farms

Table 1. Mean, standard deviation of infected cases per day and R_0 values

Farm	Type of pig	Actual pig population	Mean	Standard Deviation	Basic reproduction number (R_0) (95% C.I)
HY1	Sow	384	4.5	2.78	1.78 (1.35 – 2.35)
	Fattening	1682	13.94	15.98	4.76 (4.18 – 5.38)
HY2	Sow	192	3.3	2.54	1.55 (1.08 – 2.18)
	Fattening	981	14.28	10.25	3.80 (3.33 – 4.28)

Note: C.I: Confident interval,

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> Front Vet Sci. 2022 Sep 29;9:918438. doi: 10.3389/fvets.2022.918438. eCollection 2022.

Estimation of basic reproduction number (R_0) of African swine fever (ASF) in mid-size commercial pig farms in Vietnam

Nguyen Tuan Anh Mai ¹, Thi Bich Ngoc Trinh ¹, Van Tam Nguyen ¹, Thi Ngoc Ha Lai ¹, Nam Phuong Le ¹, Thi Thu Huyen Nguyen ^{1,2}, Thi Lan Nguyen ¹, Aruna Ambagala ³, Duc Luc Do ⁴, Van Phan Le ¹



animals



Article

Estimation of a Within-Herd Transmission Rate for African Swine Fever in Vietnam

Van Phan Le ¹, Nguyen Thi Lan ¹, Jose Tobias Canevari ², Juan Pablo Villanueva-Cabezas ^{3,4,*}, Pawin Padungtod ⁵, Thi Bich Ngoc Trinh ⁶, Van Tam Nguyen ⁶, Caitlin N. Pfeiffer ², Madalene V. Oberin ², Simon M. Firestone ² and Mark A. Stevenson ²

Diagnosis of ASF

❖ Clinical diagnosis ???

- Clinical symptoms are very diverse, depending on the virus strains

❖ Laboratory diagnosis

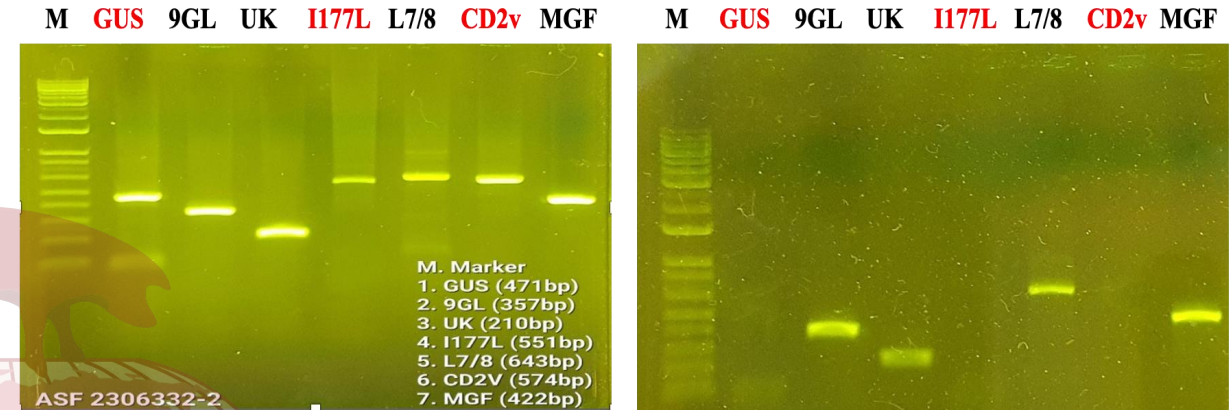
➤ Realtime PCR:

Samples: **Whole blood** or Serum, lymph node, spleen...

✓ High pathogenic strains: Ct = 16-25

✓ Low pathogenic strains: Ct = 29-35

- Serology assays: Elisa is used for disease detection (**antibody detection**)



> Vet Med Sci. 2021 Nov;7(6):2268–2272. doi: 10.1002/vms3.605. Epub 2021 Aug 13.

Development of a novel real-time PCR assay targeting p54 gene for rapid detection of African swine fever virus (ASFV) strains circulating in Vietnam[®]

Thi Bich Ngoc Trinh¹, Thang Truong², Van Tam Nguyen¹, Xuan Dang Vu¹, Le Anh Dao¹, Thi Lan Nguyen¹, Aruna Ambagala³, Shawn Babiuk³, Jinsik Oh⁴, Daesub Song⁵, Van Phan Le¹

> Transbound Emerg Dis. 2021 Jul;68(4):2595–2602. doi: 10.1111/tbed.13879. Epub 2020 Nov 4.

Direct colorimetric LAMP assay for rapid detection of African swine fever virus: A validation study during an outbreak in Vietnam

Diem Hong Tran¹, Hau Thi Tran¹, Uyen Phuong Le¹, Xuan Dang Vu², Thi Bich Ngoc Trinh², Hoang Dang Khoa Do¹, Van Thai Than^{3 4}, Le Minh Bui¹, Van Van Vu¹, Thi Lan Nguyen², Huong Thi Thu Phung¹, Van Phan Le²

ASFV distribution at different organ samples of pigs using Realtime PCR (Ct value)

Samples	Fattening pig 75	Fattening pig 79
Whole blood ➤ 10^8 HAD50/ml	19.2	15.56
Urine	31.43	25.89
Spleen	15.29	11.88
Kidney	22.86	17.11
Lung	20.28	14.56
Liver	18.86	14.48
Submandibular lymph nodes	16.91	13.61
Inguinal Lymph node	18.8	16.57
Mesenteric lymph node	19.54	15.86

Suggestion for sampling



> [Viruses](#). 2022 Jan 4;14(1):83. doi: 10.3390/v14010083.

Superficial Inguinal Lymph Nodes for Screening Dead Pigs for African Swine Fever

Kalhari Bandara Goonewardene ¹, Chukwunonso Onyilagha ¹, Melissa Goolia ¹, Van Phan Le ²,
Sandra Blome ³, Aruna Ambagala ^{1 4}

Pathological investigation of ASFV isolated in Vietnam

Experiment	Pig No.	Date of clinical symptom onset						Dead	Viremia
		Loss of appetite	Inactivity	Diarrhea	Cough	Fever	Hemorrhagic Skin		
10²HAD₅₀/pig (IM)	1	4	4	-	-	5	-	8	3
	2	4	5	-	5	4	-	8	3
	3	4	5	-	-	4	-	12	3
	4	4	4	10	5	3	-	13	3
	5	5	2	-	5	3	-	11	3
Mean		4.2±0.45	4.0±1.22		5	3.8±0.84		10.4±2.3	3

Note: (-): no clinical sign

Pathological investigation of ASFV isolated in Vietnam

Experiment	Pig No.	Date of clinical symptom onset						Dead	Viremia
		Loss of appetite	Inactivity	Diarrhea	Cough	Fever	Hemorrhagic Skin		
10³HAD₅₀/pig (Oral)	1	14	15	14	11	4	15	18	8
	2	14	16	--	-	5	-	21	12
	3	16	19	18	11	9	-	20	16
	4	22	23	-	-	19	-	25	16
	5	9	-	-	5	5	8	10	8
	6	18	19	-	-	15	20	22	12
	7	22	23	-	-	10	25	27	16
	8	15	-	-	7	8	16	18	8
	9	16	19	19	-	15	-	20	8
	10	14	15	-	15	11	15	17	12
Mean		16.0±3.92	18.63±3.2	17.0±2.65	9.8±3.9	10.1±4.9	16.5±5.68	19.8±4.7	11.6±3.5



pathogens



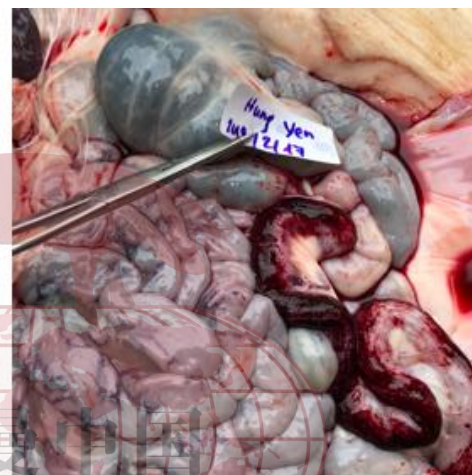
Article

Pathological Characteristics of Domestic Pigs Orally Infected with the Virus Strain Causing the First Reported African Swine Fever Outbreaks in Vietnam

Thi Thu Huyen Nguyen ^{1,2}, Van Tam Nguyen ³, Phuong Nam Le ³, Nguyen Tuan Anh Mai ³, Van Hieu Dong ¹, Tran Anh Dao Bui ¹, Thi Lan Nguyen ¹, Aruna Ambagala ⁴ and Van Phan Le ^{1,3,*}

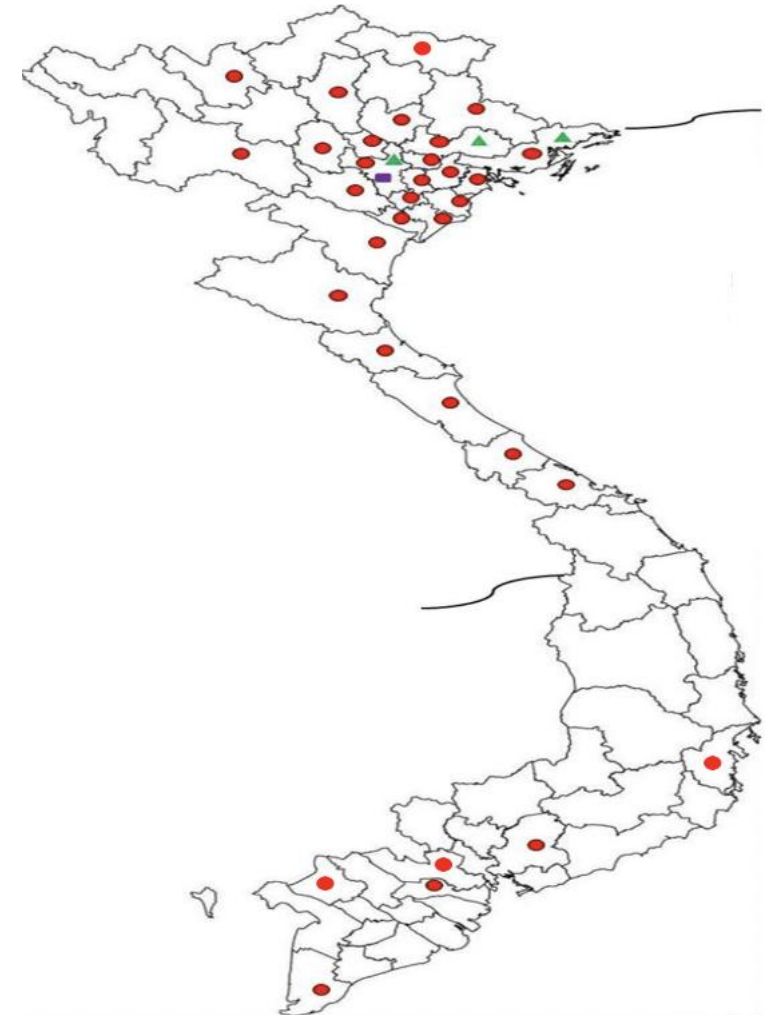
Note: (-): no clinical sign

Necropsy lesions of the ASFV- infected pigs



Genetic Analysis of ASF Viruses Circulating in Vietnam during the 2019-2022 period

- **319** ASFV-positive samples were collected for the study.
- From **Feb 2019 to Dec 2022**
- In **32 provinces**
- **p72** (B646L), full-length **p54** (E1803L), **CD2v** (EP402R), the central variable region (**CVR**) of pB602L, and the intergenic region (**IGR**) between the I73R and I329L genes of ASFV were used for genetic characterization.



Genetic Analysis of ASF Viruses Circulating in Vietnam during the 2019-2022 period

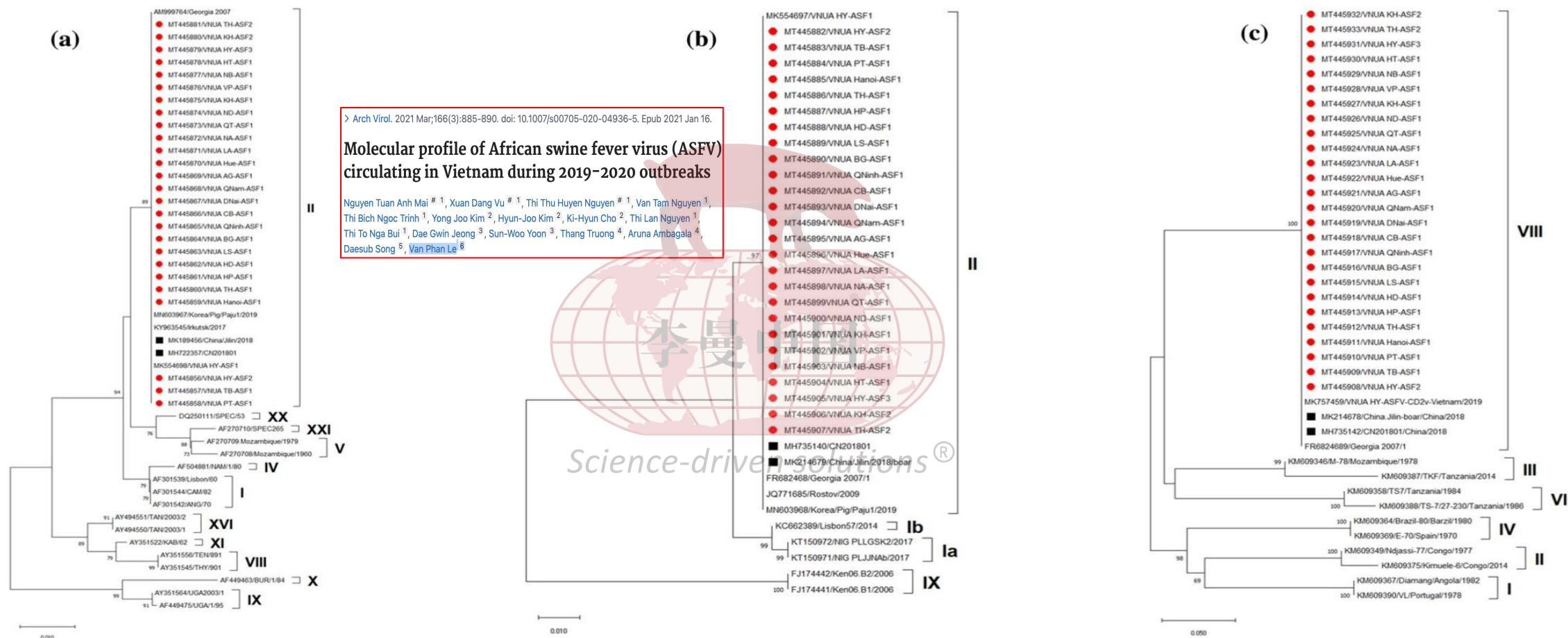


Fig. 2 P72-P54-CD2v phylogenetic trees based on nucleotide sequences. (A) P72 (neighbor-joining method); (B) P54 (neighbor-joining method); (C) CD2v (Kimura 2-parameter method). Bootstrap

analysis was performed with 1000 replicates. Only bootstrap values > 70% are shown.

Genetic Analysis of ASF Viruses Circulating in Vietnam during the 2019-2022 period

> Arch Virol. 2022 Apr;167(4):1137-1140. doi: 10.1007/s00705-022-05363-4. Epub 2022 Feb 21.

Multiple variants of African swine fever virus circulating in Vietnam

Van Tam Nguyen # ¹, Ki-Hyun Cho # ², Nguyen Tuan Anh Mai ¹, Jee-Yong Park ², Thi Bich Ngoc Trinh ¹, Min-Kyung Jang ², Thi Thu Huyen Nguyen ^{1, 3}, Xuan Dang Vu ¹, Thi Lan Nguyen ¹, Van Diep Nguyen ¹, Aruna Ambagala ⁴, Yong-Joo Kim ⁵, Van Phan Le ⁶

Veterinary Research Communications
https://doi.org/10.1007/s11259-022-10068-9

BRIEF REPORT

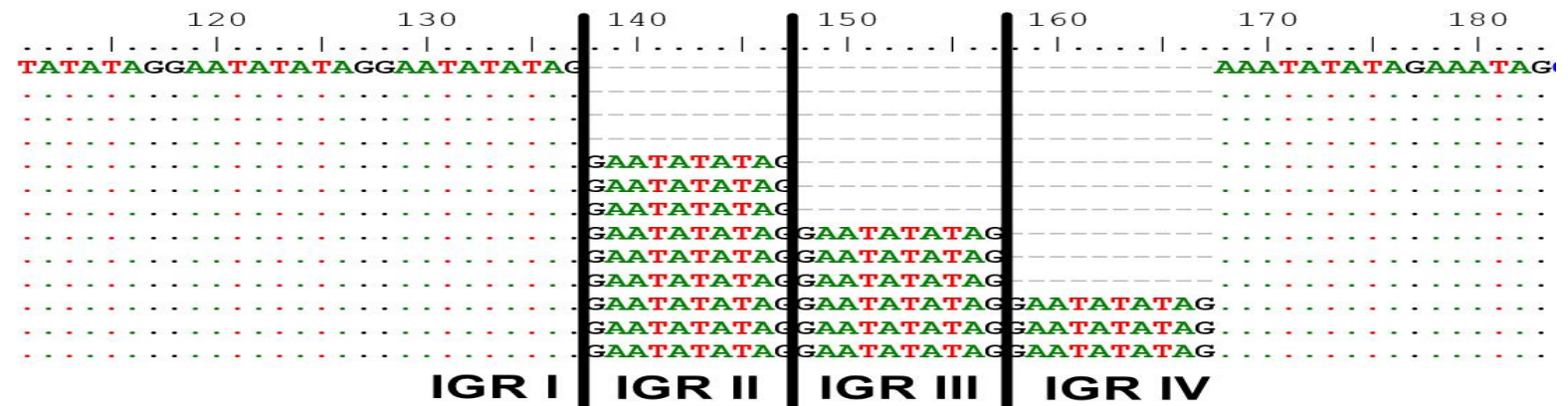
Emergence of a novel intergenic region (IGR) IV variant of african swine fever virus genotype II in domestic pigs in Vietnam

Nguyen Tuan Anh Mai¹ · Van Phai Dam¹ · Ki-Hyun Cho² · Van Tam Nguyen³ · Nguyen Van Tuyen⁴ · Thi Lan Nguyen¹ · Aruna Ambagala⁵ · Jee-Yong Park² · Van Phan Le^{1,3}

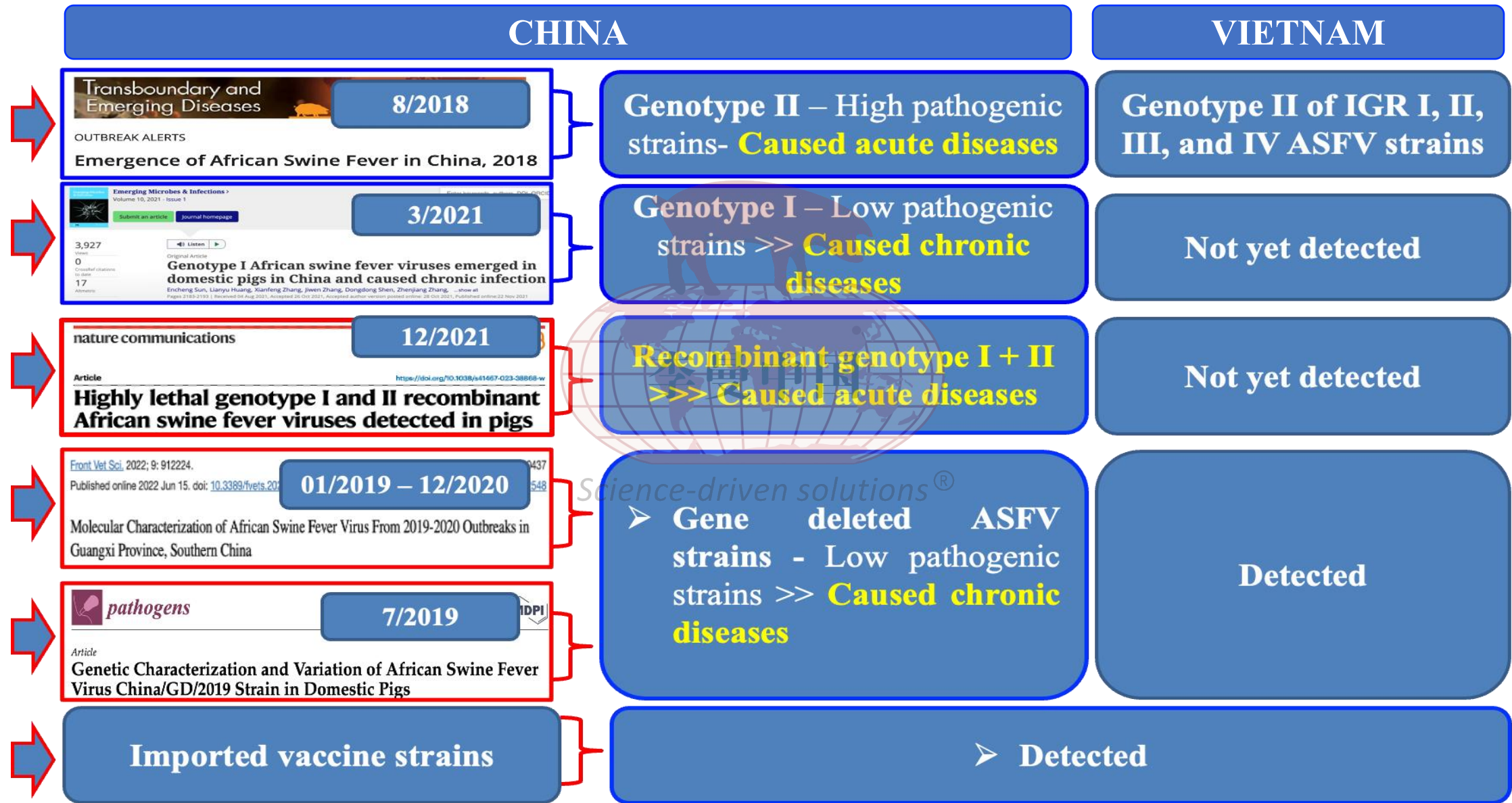
FR682468/Georgia-2007
ON053205 VNUA HY-ASF44
3 VN IGR I strains
MK189457/China/Jilin/2018
ON053204 VNUA TB-ASF3
154 VN IGR I strains
MH735144/CN201801
MZ812411 VNUA BG-ASF3
3 VN ASF IGR III strains
MK670729/China/Guangxi
ON053211 VNUA HB-ASF2
ON053216 VNUA VP-ASF7
MT889557/Pol119_28690_07/19

Based on the IGR gene region (Intergenic region) between the I73R and I329L genes

Year	IGR			
	I	II	III	IV
2019	1	75		
2020		87	3	
2021	3	28	1	2
2022	50	67	2	
Tổng	54	257	6	2
Rate (%)	16.9%	80.5%	1.9%	0.7%



What ASFV strains are circulating




Overview of ASF vaccine development

1. **Inactivated vaccine:** Not - less effective
2. **Recombinant and DNA vaccines:** Not yet effective
3. **Live vaccines:**
 - **Viruses attenuated by tissue culture passages:**
 - ✓ Caused chronic disease; High risk of reversion to high virulence.
 - ✓ Virus adaptation to grow in cell lines results in genomic changes that result in the inability of the attenuated virus to replicate in swine.
 - **Low virulence field isolates:**
 - ✓ All low virulent field isolates retain residual virulence → Causing chronic disease; Miscarriage)
 - ✓ Vaccines based on low virulent field isolates **NEED** further attenuation / safety measures.

3. Live vaccines (*continued*):

➤ Viruses attenuated by genetically engineered deletions

- ASFV-G-ΔMGF: Deletion of 6 MGF genes
- ASFV-G-Δ9GL/ΔUK: A double-gene-deletion recombinant virus
- ASFV-G-ΔI177L: Deletion of I177L gene
- ASFV-G-ΔI177L- LVR: The result of ASFV-G-ΔI177L strain after passaging on PIPEC cells
- HLJ/18-7GD– (ΔMGF ΔCD2): Using the ASFV-G-ΔMGF-USDA and deleting more CD2v gene.

 JVI
Journals.ASM.org

African Swine Fever Virus Georgia Isolate Harboring Deletions of MGF360 and MGF505 Genes Is Attenuated in Swine and Confers Protection against Challenge with Virulent Parental Virus

Vivian O'Donnell,^{a,b} Lauren G. Holinka,^a Douglas P. Gladue,^{a,b} Brenton Sanford,^a Peter W. Krug,^a Xiqiang Lu,^c Jonathan Arzt,^a Bo Reese,^d Consuelo Carrillo,^e Guillermo R. Risatti,^b Manuel V. Borca^a

Agricultural Research Service^a and APHIS^f USDA, Plum Island Animal Disease Center, Greenport, New York, USA; DHS, Plum Island Animal Disease Center, Greenport, New York, USA; Department of Pathobiology and Veterinary Science, CAHNR^g and Center for Genome Innovation,^h University of Connecticut, Storrs, Connecticut, USA

> J Virol. 2020 Mar 17;94(7):e02017–19. doi: 10.1128/JVI.02017–19. Print 2020 Mar 17.

Development of a Highly Effective African Swine Fever Virus Vaccine by Deletion of the I177L Gene Results in Sterile Immunity against the Current Epidemic Eurasia Strain

Manuel V Borca¹, Elizabeth Ramirez-Medina^{2 3}, Ediane Silva^{2 4}, Elizabeth Vuono^{2 5}, Ayushi Rai^{2 6}, Sarah Pruitt^{2 6}, Lauren G Holinka², Lauro Velazquez-Salinas^{2 4}, James Zhu², Douglas P Gladue¹

> J Virol. 2021 Jun 24;95(14):e0012321. doi: 10.1128/JVI.00123–21. Epub 2021 Jun 24.

A Cell Culture-Adapted Vaccine Virus against the Current African Swine Fever Virus Pandemic Strain

M V Borca¹, A Rai^{1 2}, E Ramirez-Medina^{1 3}, E Silva^{1 4}, L Velazquez-Salinas^{1 4}, E Vuono^{1 5}, S Pruitt¹, N Espinoza¹, D P Gladue¹

> Sci China Life Sci. 2020 May;63(5):623–634. doi: 10.1007/s11427-020-1657-9. Epub 2020 Mar 1.

A seven-gene-deleted African swine fever virus is safe and effective as a live attenuated vaccine in pigs

Weiyue Chen¹, Dongming Zhao¹, Xijun He², Renqiang Liu¹, Zilong Wang¹, Xianfeng Zhang², Fang Li¹, Dan Shan¹, Hefeng Chen¹, Jiwen Zhang¹, Lulu Wang¹, Zhiyuan Wen¹, Xijun Wang¹, Yuntao Guan², Jinxiong Liu¹, Zhigao Bu^{3 4}

ASF vaccine development projects in Vietnam

1. Ministry of Agriculture and Rural Development (MARD), Vietnam, and the USDA, USA, have a cooperation to develop ASF vaccines
2. DAH (Department of Animal Health), MARD, Vietnam, supports the ASF vaccine development projects in Vietnam (Legal procedures, international cooperation, and monitoring vaccination in local trials...).

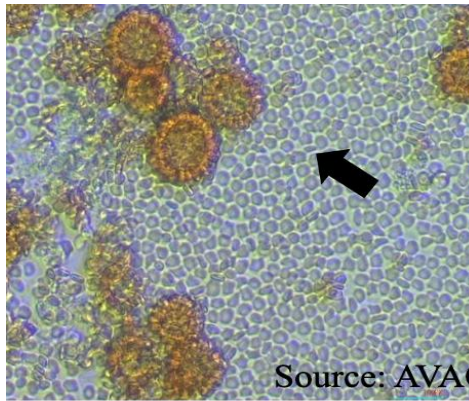
ASF vaccine development projects:

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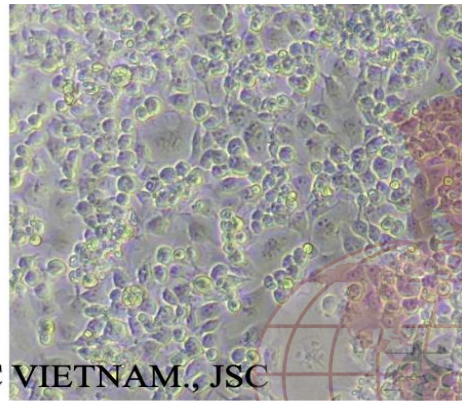
- AVAC Co., LTD and Aptimmune Biologics Inc, USA
- **DABACO Group** and Agricultural Research Service (ARS), USDA, USA
- **NAVETCO** company and Agricultural Research Service (ARS), USDA, USA

Manufacturing ASF Vaccine

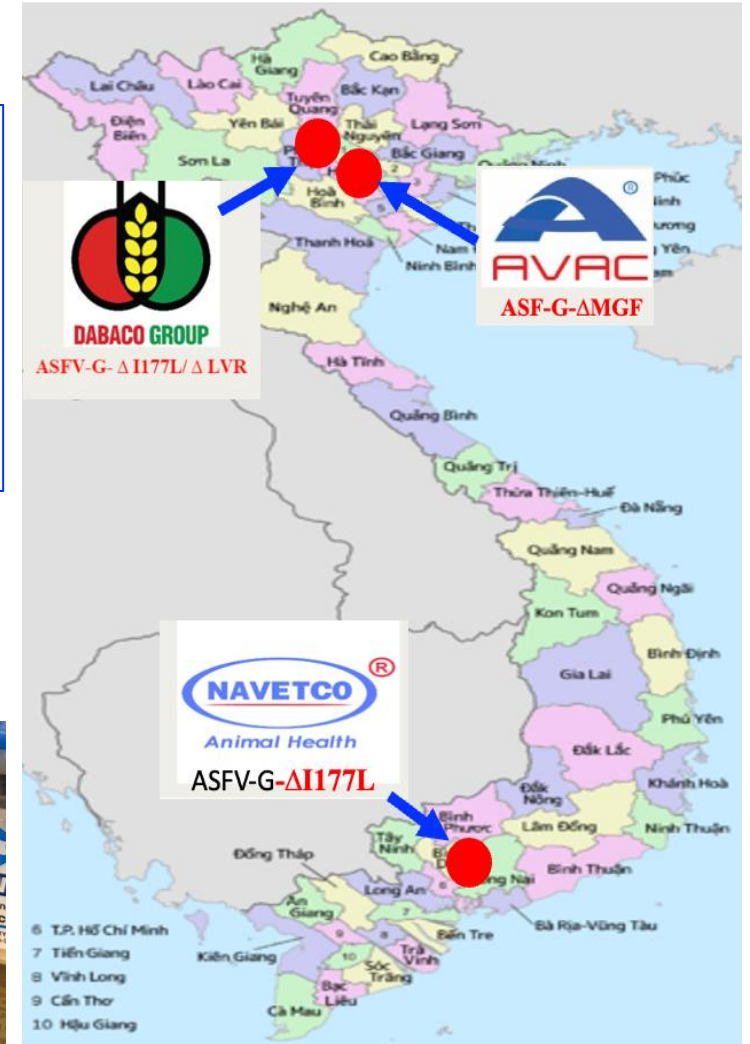
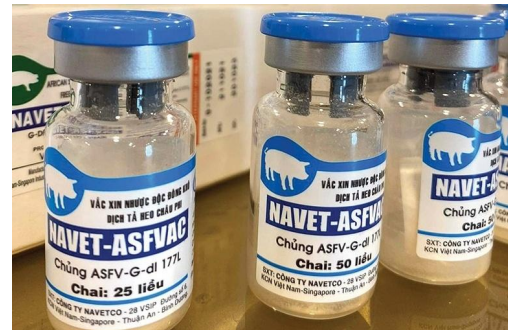
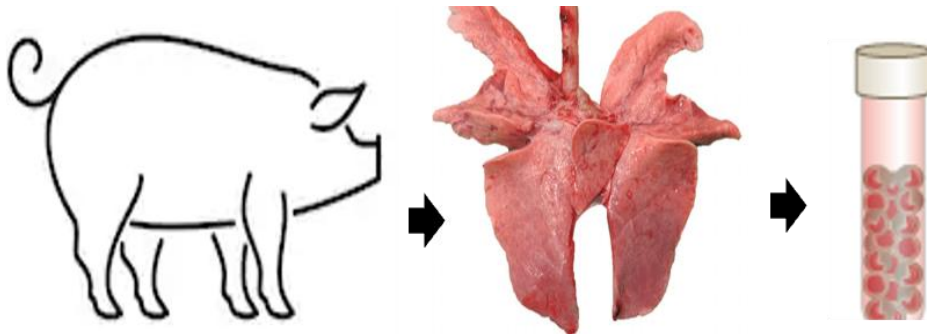
- AVAC Company used a cell line (DMAC)



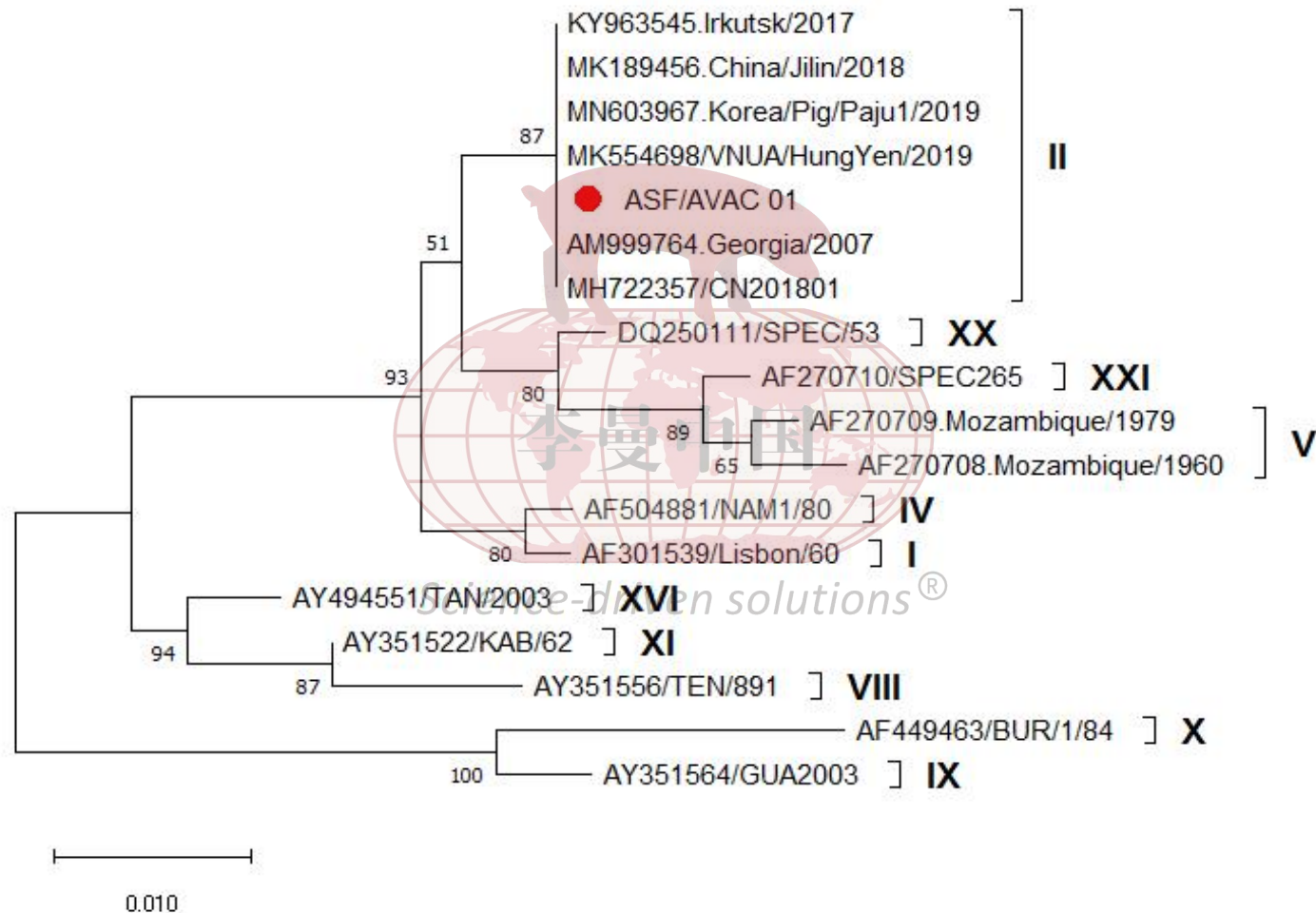
Source: AVAC VIETNAM., JSC



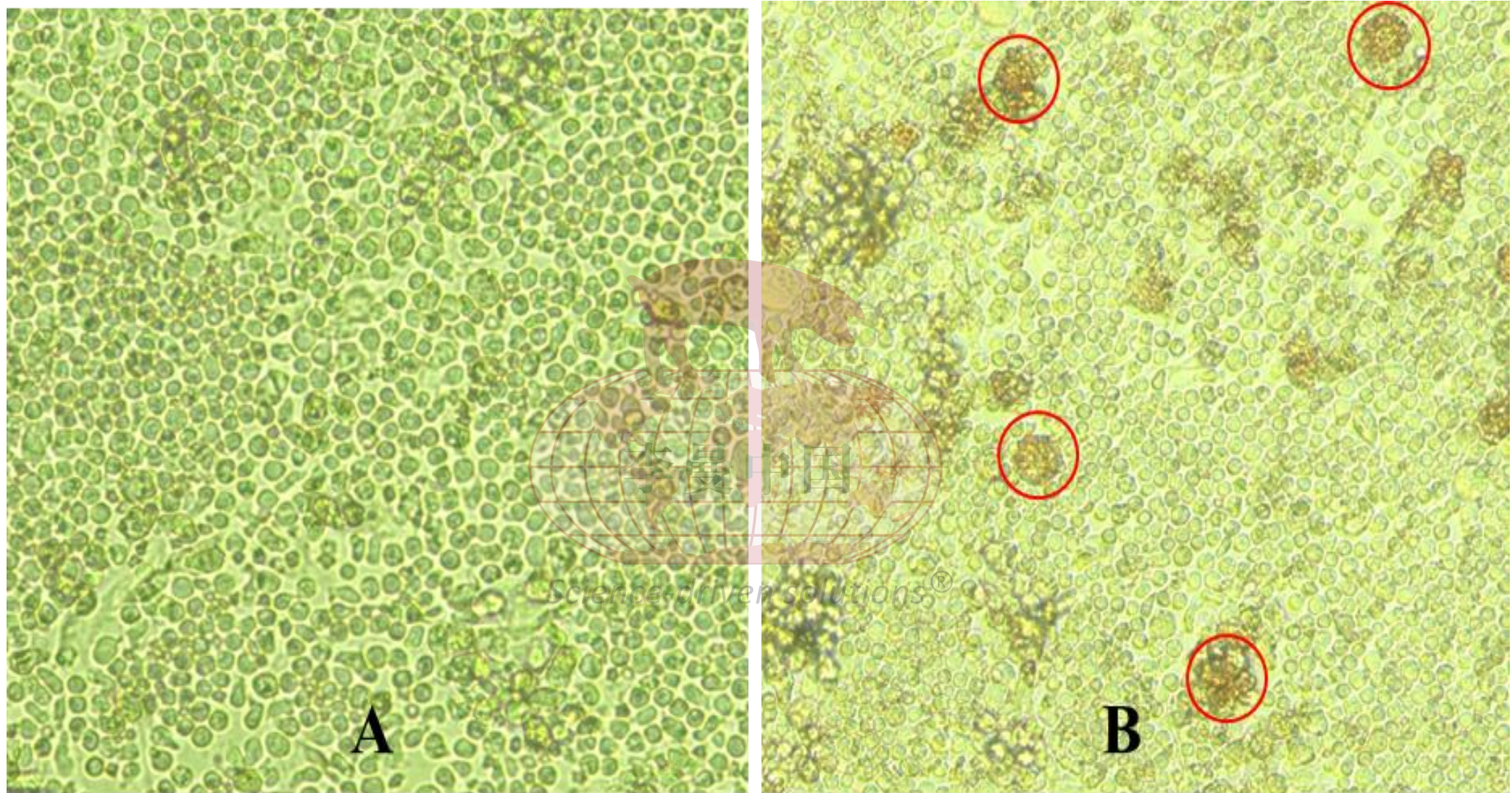
- DABACO company used PAM/PIPEC cells
- NAVETCO company used PAM cells



EVALUATION OF THE VACCINE



Phylogenetic tree based on the p72 gene of ASFV



Un-infected (A) and infected (B) DMAC cells with vaccine strain

RESULTS OF SAFETY TEST ON EXPERIMENTAL PIGS

	Age of pig	Number of pigs	Dose of vaccine
Experiment group	4 weeks old	10	10^5 HAD ₅₀ /pig/IM
Control group		5	1 ml PBS/pig/IM

Results of safety test on pigs

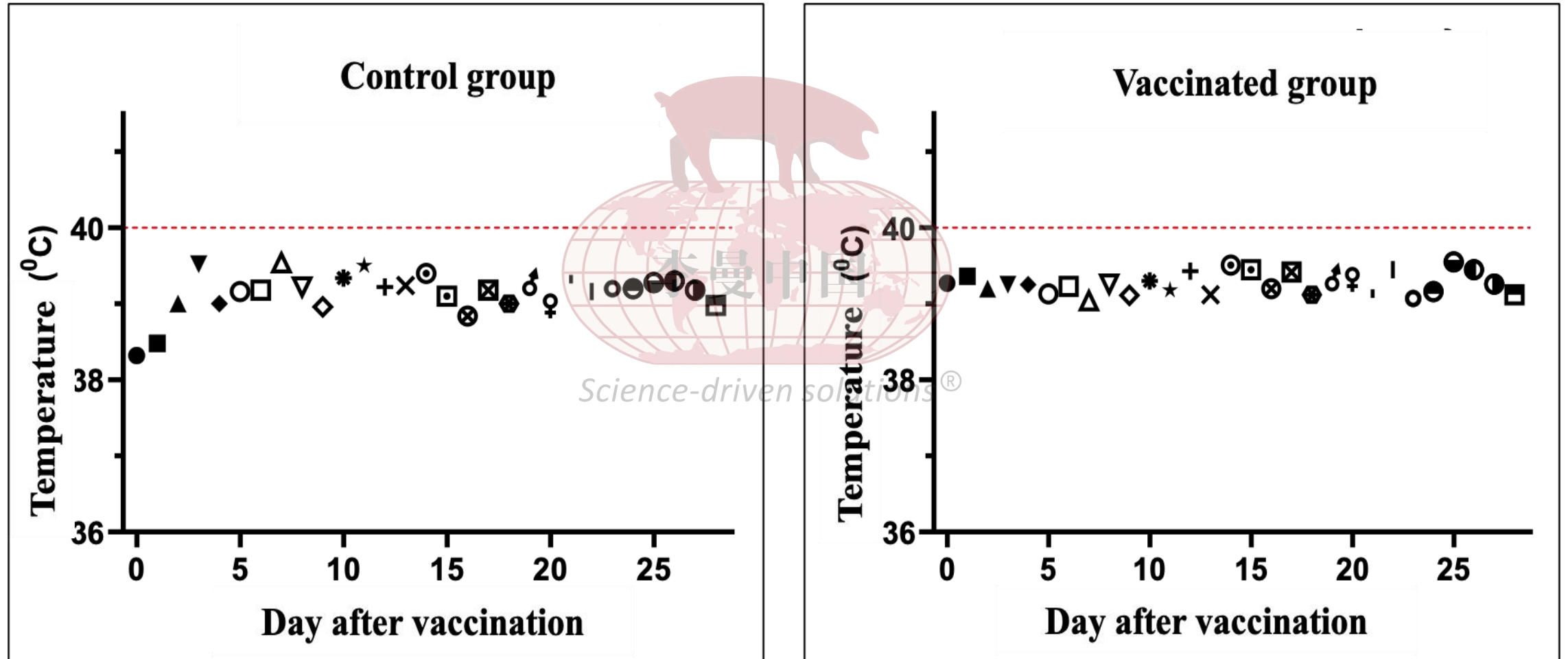
➤ Clinical sign investigation

Ngày	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
3	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0
4	0	0	6	0	0	0	6	0	0	0	0	0	0	0	0
5	0	0	6	0	0	0	0	0	0	0	0	6	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	4	0	0	6	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Clinical scores	
0	Normal
1	Lost appetite
2	Fever
3	Swollen joint
4	Cough
5	Haemorrhagic skin
6	Diarrhea
7	Unactive
8	Dead

Results of safety test on pigs

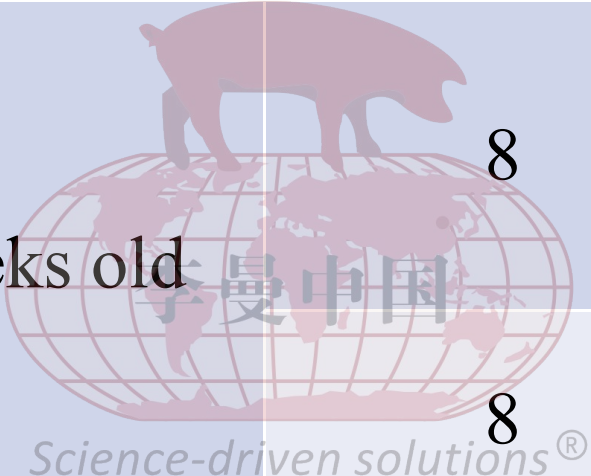
➤ Rectal temperature of experimental pigs



Results of safety test on pigs



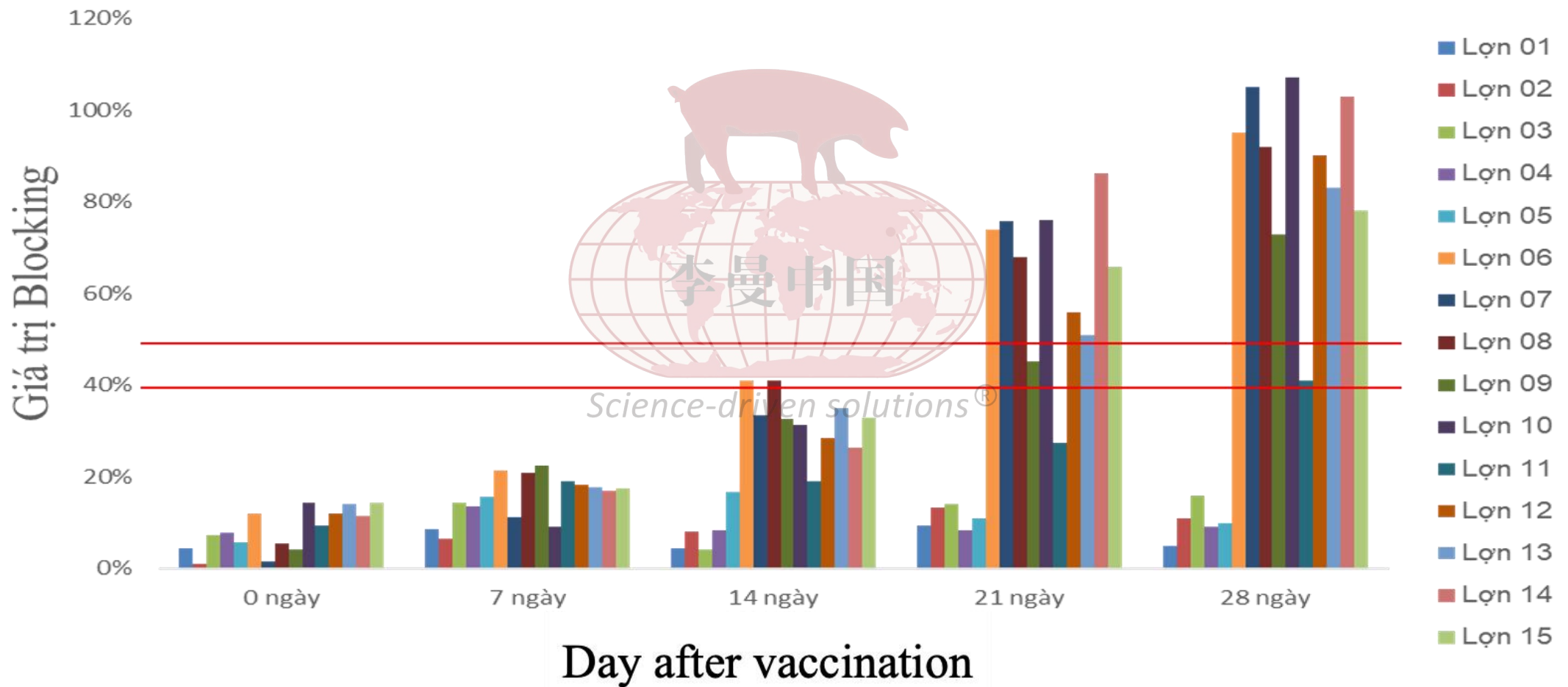
RESULTS OF EFFICACY TEST ON PIGS

	Age of pig	Number of pigs	Dose of vaccine
Experiment group	4 weeks old 	8	10⁴ HAD ₅₀ /pig/IM
Control group		8	1 ml PBS/pig/IM

After **28 days of immunizing** with the vaccine, the experimental and control groups of pigs were challenged with a virulent strain of ASFV isolated from the field (**10⁴ HAD₅₀/pig/IM**).

Results of efficacy test on pigs

➤ *The immune response of pigs after vaccination with ASF vaccine by ELISA*



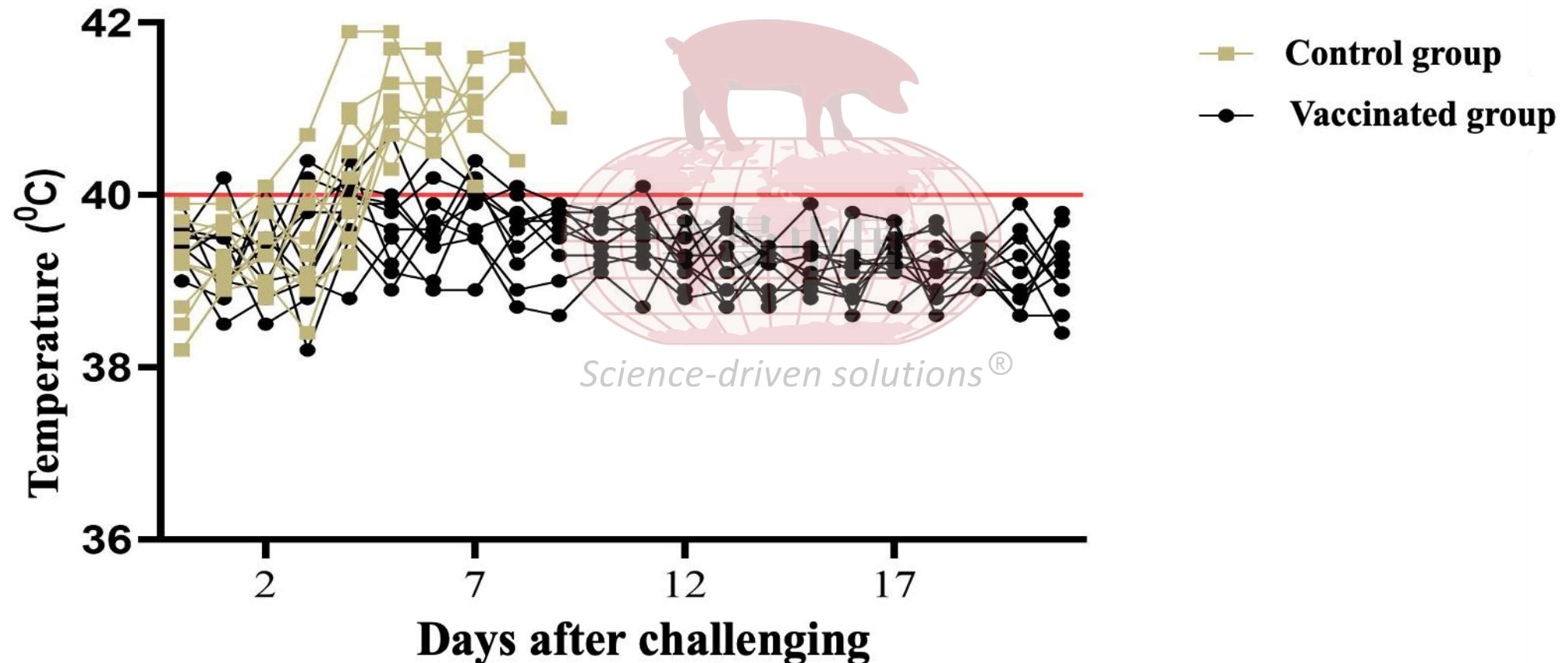
Results of the neutralization test

➤ *No neutralizing antibody detected*

Serum samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Results of challenging test on pigs

➤ Rectal temperature of experimental pigs after challenging



Results of challenging test on pigs

➤ Clinical sign investigation after challenging

Day	Control group								Vaccinated group							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	2	0	0	0	0	2	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
3	2	0	2	0	2	0	2	0	0	0	0	0	2	0	0	2
4	2	2	2	1,2	1,2	0	4	0	2	2	0	0	2	0	0	0
5	1,2	1,2	1,2	8	1,2	1,2	1,2	0	2	0	0	0	0	0	0	0
6	1,2,3,7	1,2,3,7	1,2,3,7		1,2,3,7	1,2,3	8	1,2	0	0	0	0	0	0	2	2
7	1,2,3,7	1,2,3	8		8	1,2,3,7		1,2,3,7	2	0	2	0	0	2	0	2
8	1,2,3	1,2,3				1,2,3,7		8	0	0	0	2	0	0	0	0
9	8	8				8			0	0	0	0	0	0	0	0
10									0	0	0	0	0	0	0	0
11									0	0	0	0	0	2	0	0
12									0	0	0	0	0	0	0	0
13									0	0	0	0	0	0	0	0
14									0	0	0	0	0	0	0	0
15									0	0	0	0	0	0	0	0
16									0	0	0	0	0	0	0	0
17									0	0	0	0	0	0	0	0
18									0	0	0	0	0	0	0	0
19									0	0	0	0	0	0	0	0
20									0	0	0	0	0	0	0	0
21									0	0	0	0	0	0	0	0

Note: 0: Normal 2: Fever (>40°C) 4: Cough 6: Diarrhea 8: Dead
 1: Lost appetite 3: Tired 5: Haemorrhagic skin 7: Unactive

Source: Avac company

Results of challenging test on pigs

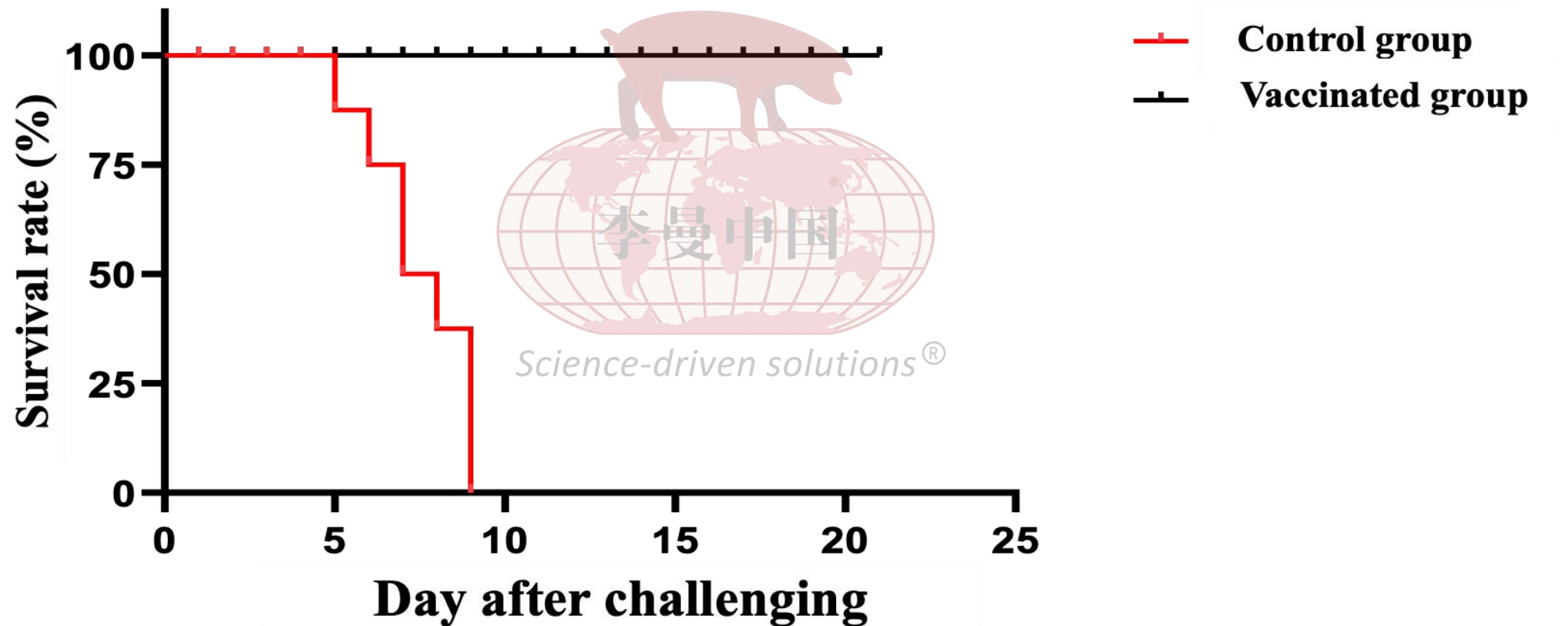
➤ Viremia of pigs after challenging

	Pig number	Day after challenging								
		1	3	5	6	7	8	9	14	21
Control group	1	-	31.56		16.81			16.5		
	2	-	32.47		17.95			16.58		
	3	-	28.55		17.08	19.2				
	4	-	22.47	20.12						
	5	-	22.33		17.68	20.15				
	6	-	31.58		17.36			18.58		
	7	-	27.32		19.77					
	8	-	28.32		20.12		17.32			
Vaccinated group	9	-	Science-driven solutions®					-	-	-
	10	-	-		-			-	-	-
	11	-	-		33.26			33.9	-	-
	12	-	-		-			-	-	-
	13	-	-		-			-	-	-
	14	-	-		-			-	-	-
	15	-	-		34.8			-	-	-
	16	-	-		-			-	-	-

Source: Avac company

Results of challenging test on pigs

- The survival rate of unvaccinated (red) and vaccinated (black) pigs



Pictures of gross pathological lesions



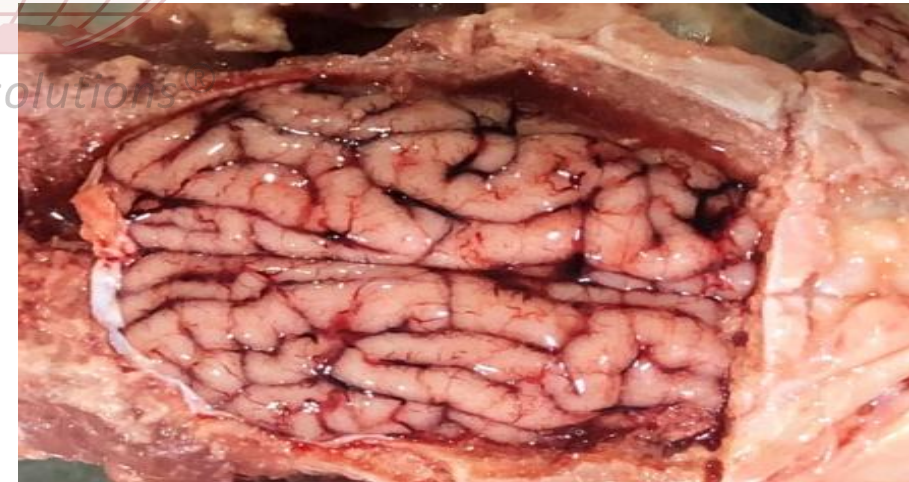
Congested and enlarged spleen



Hemorrhagic mesenteric lymph nodes



Hemorrhagic tonsils



Hemorrhagic meninges

Source: Avac company

Results of efficacy test on pigs



Control group



Vaccinated group

1 day after challenging

Results of efficacy test on pigs



Control group



Vaccinated group

4 days after challenging

Results of efficacy test on pigs



Control group

Vaccinated group

9 days after challenging

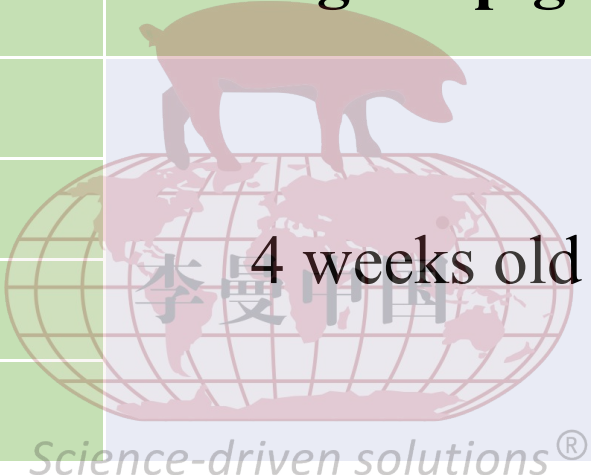
Results of efficacy test on pigs



20 days after challenging

Source: Avac company

EFFICACY OF ASF VACCINE USING DIFFERENT DOSES

Vaccine doses	Age of pig	Number of pigs
10^2 HAD ₅₀ /pig/IM	 4 weeks old	40 pigs /group
10^3 HAD ₅₀ /pig/IM		
10^4 HAD ₅₀ /pig/IM		
Control group		

- After 28 days of immunization with the vaccine, the experimental and control groups of pigs (5 pigs/group) were randomly selected and challenged at the vaccine manufacturer's animal house with a virulent strain of ASF virus isolated from the field (10^4 HAD₅₀/pig/IM).

Results of Elisa's test

Injection	Group	0 dpv			7 dpv			14 dpv			21 dpv			28 dpv		
		Pig no.	Median ver.2.0		Pig no.	Median ver.2.0		Pig no.	Median ver.2.0		Pig no.	Median ver.2.1		Pig no.	Median ver.2.1	
			S/P	Results		S/P	Results		S/P	Results		S/P	Results		S/P	Results
Pigs - 15kg - Control		N1. 1	0,04	Negative	Đc -1	0,04	Negative	DC1	0,04	Negative	ĐC 71	0,08	Negative	ĐC - 1	0,21	Negative
		N1. 2	0,03	Negative	Đc -2	0,04	Negative	DC2	0,04	Negative	ĐC 72	0,04	Negative	ĐC - 2	0,04	Negative
		N1. 3	0,04	Negative	Đc -3	0,03	Negative	DC3	0,04	Negative	ĐC 73	0,09	Negative	ĐC - 3	0,08	Negative
		N1. 4	0,03	Negative	Đc -4	0,05	Negative	DC4	0,04	Negative	ĐC 74	0,05	Negative	ĐC - 4	0,20	Negative
		N1. 5	0,05	Negative	Đc -5	0,04	Negative	DC5	0,05	Negative	ĐC 75	0,13	Negative	ĐC - 5	0,30	Positive
		N1. 6	0,03	Negative	Đc -6	0,02	Negative	DC6	0,04	Negative	ĐC 76	0,16	Negative	ĐC - 71	0,08	Negative
					Đc -7	0,03	Negative	DC7	0,08	Negative	ĐC 77	0,06	Negative	ĐC - 72	1,28	Positive
					Đc -8	0,04	Negative	DC 8	0,03	Negative	ĐC 78	0,04	Negative	ĐC - 73	0,10	Negative
					Đc -9	0,03	Negative	DC 9	0,04	Negative	ĐC 79	0,04	Negative	ĐC - 78	0,08	Negative
					Đc -10	0,03	Negative	DC 10	0,03	Negative	ĐC 80	0,07	Negative	ĐC - 79	0,37	Positive
10 ^{^2}	Pigs - 15kg - Group 1	N2. 1	0,03	Negative	N2 - 1	0,03	Negative	N2 - 1	1,03	Positive	N2 - 38	0,05	Negative	N2 - 1	1,13	Positive
		N2. 2	0,04	Negative	N2 - 2	0,03	Negative	N2 - 2	0,11	Negative	N2 - 41	0,81	Positive	N2 - 2	0,56	Positive
		N2. 3	0,04	Negative	N2 - 3	0,03	Negative	N2 - 3	0,03	Negative	N2 - 42	1,17	Positive	N2 - 3	1,46	Positive
		N2. 4	0,05	Negative	N2 - 4	0,03	Negative	N2 - 4	0,06	Negative	N2 - 43	1,50	Positive	N2 - 4	1,36	Positive
		N2. 5	0,04	Negative	N2 - 5	0,03	Negative	N2 - 5	0,83	Positive	N2 - 44	0,08	Negative	N2 - 5	0,09	Negative
					N2 - 6	0,03	Negative	N2 - 6	0,64	Positive	N2 - 46	0,74	Positive	N2 - 20	0,70	Positive
					N2 - 7	0,04	Negative	N2 - 7	0,78	Positive	N2 - 47	0,13	Negative	N2 - 21	0,35	Positive
					N2 - 8	0,03	Negative	N2 - 8	0,04	Negative	N2 - 48	0,77	Positive	N2 - 24	1,43	Positive
					N2 - 9	0,03	Negative	N2 - 9	0,05	Negative	N2 - 49	0,07	Negative	N2 - 25	1,43	Positive
					N2 - 10	0,03	Negative	N2 - 10	0,05	Negative	N2 - 50	0,06	Negative	N2 - 27	1,43	Positive
10 ^{^3}	Pigs - 15kg - Group 2	N3. 1	0,03	Negative	N3 - 1	0,04	Negative	N3 - 1	1,27	Positive	N3 - 51	1,20	Positive	N3 - 1	0,19	Negative
		N3. 2	0,04	Negative	N3 - 2	0,04	Negative	N3 - 2	0,96	Positive	N3 - 58	1,30	Positive	N3 - 2	1,40	Positive
		N3. 3	0,04	Negative	N3 - 3	0,04	Negative	N3 - 3	1,25	Positive	N3 - 52	0,95	Positive	N3 - 3	0,40	Positive
		N3. 4	0,04	Negative	N3 - 4	0,03	Negative	N3 - 4	0,24	Negative	N3 - 53	0,44	Positive	N3 - 4	1,41	Positive
		N3. 5	0,04	Negative	N3 - 5	0,03	Negative	N3 - 5	0,26	Positive	N3 - 54	1,43	Positive	N3 - 5	1,37	Positive
		N3. 6	0,04	Negative	N3 - 6	0,04	Negative	N3 - 6	0,24	Negative	N3 - 55	1,44	Positive	N3 - 6	0,12	Negative
		N3. 7	0,04	Negative	N3 - 7	0,03	Negative	N3 - 7	0,06	Negative	N3 - 56	1,00	Positive	N3 - 7	1,47	Positive
					N3 - 8	0,03	Negative	N3 - 8	0,71	Positive	N3 - 57	1,22	Positive	N3 - 8	1,02	Positive
					N3 - 9	0,03	Negative	N3 - 9	0,09	Negative	N3 - 59	0,55	Positive	N3 - 9	0,44	Positive
					N3 - 10	0,03	Negative	N3 - 10	0,75	Positive	N3 - 60	1,40	Positive	N3 - 10	1,47	Positive
10 ^{^4}	Pigs - 15kg - Group 3	N4. 1	0,04	Negative	N4 - 1	0,04	Negative	N4 - 1	0,95	Positive	N4 - 61	1,33	Positive	N4 - 1	1,07	Positive
		N4. 2	0,04	Negative	N4 - 2	0,05	Negative	N4 - 2	1,03	Positive	N4 - 62	0,48	Positive	N4 - 2	1,29	Positive
		N4. 3	0,04	Negative	N4 - 3	0,03	Negative	N4 - 3	0,53	Positive	N4 - 63	1,52	Positive	N4 - 3	1,40	Positive
		N4. 4	0,04	Negative	N4 - 4	0,04	Negative	N4 - 4	0,20	Negative	N4 - 64	1,45	Positive	N4 - 4	1,50	Positive
		N4. 5	0,03	Negative	N4 - 5	0,07	Negative	N4 - 5	1,61	Positive	N4 - 65	1,49	Positive	N4 - 5	1,47	Positive
		N4. 6	0,03	Negative	N4 - 6	0,03	Negative	N4 - 6	1,06	Positive	N4 - 66	1,38	Positive	N4 - 6	1,44	Positive
		N4. 7	0,06	Negative	N4 - 7	0,04	Negative	N4 - 7	1,59	Positive	N4 - 67	1,40	Positive	N4 - 7	1,51	Positive
					N4 - 8	0,05	Negative	N4 - 8	1,40	Positive	N4 - 68	1,48	Positive	N4 - 8	1,56	Positive
					N4 - 9	0,05	Negative	N4 - 9	1,35	Positive	N4 - 69	1,42	Positive	N4 - 9	1,41	Positive
					N4 - 10	0,03	Negative	N4 - 10	1,49	Positive	N4 - 70	1,42	Positive	N4 - 10	1,45	Positive

Results of challenging test on pigs

➤ Clinical sign investigation after challenging

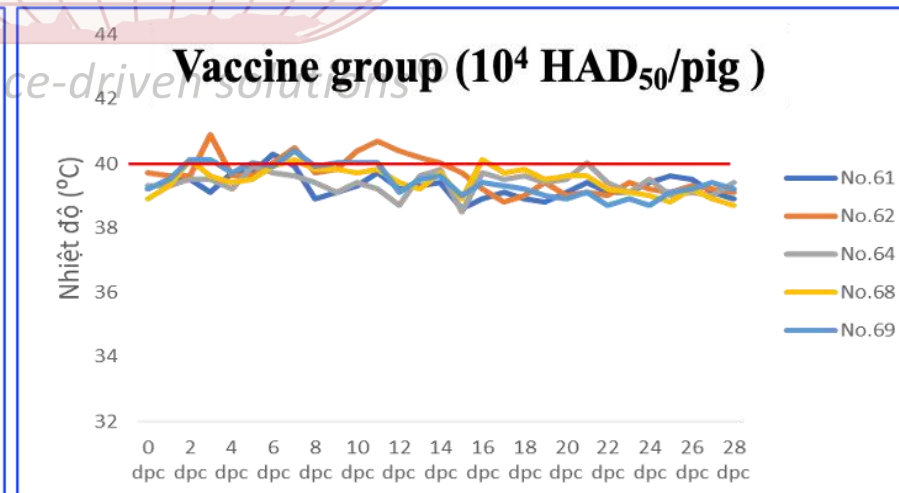
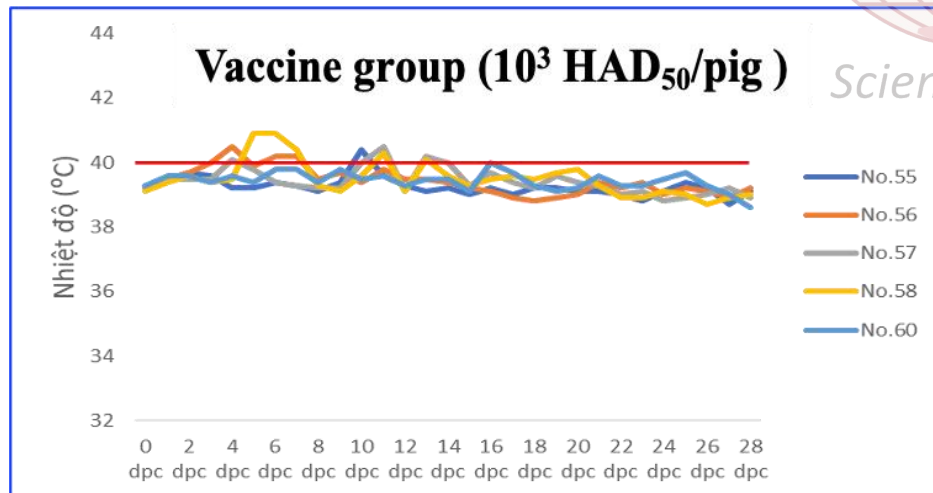
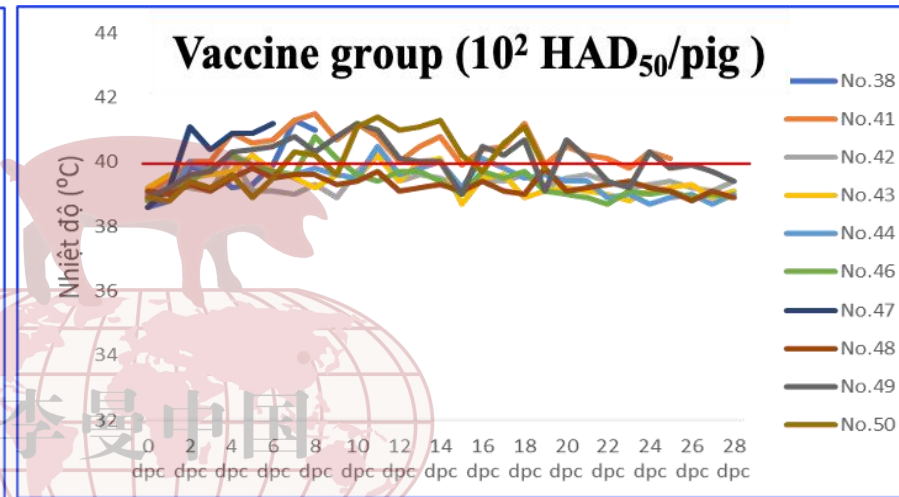
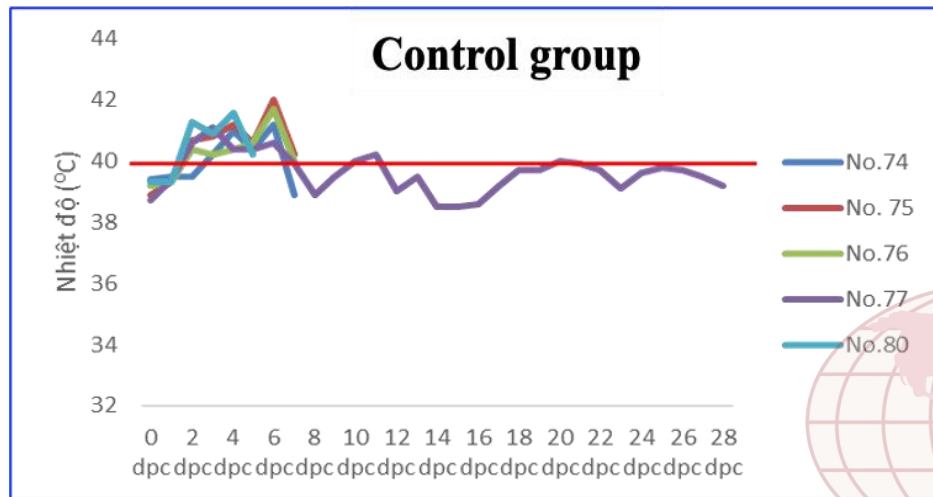
Day	Control group					Vaccine group (10 ² HAD ₅₀ /pig)										10 ³ HAD ₅₀ /pig					10 ⁴ HAD ₅₀ /pig				
	74	75	76	77	80	38	41	42	43	44	46	47	48	49	50	55	56	57	58	60	61	62	64	68	69
0 dpc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1 dpc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 dpc	0	2	2	2	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
3 dpc	2	2	2	2	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
4 dpc	2	2	2	2	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
5 dpc	2;1	2;1	2;1	2	2;1	0	0	0	0	0	0	2;1	0	0	0	0	0	0	0	0	0	0	0	0	0
6 dpc	2;1	2;1	2;1	2	8	0	2	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
7 dpc	8	2;1	8	0		2;1	2	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
8 dpc		8		0		2;1	2;1	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
9 dpc				2		8	2	0	0	2	2		0	2	0	0	0	0	0	0	0	0	0	0	2
10 dpc				2			2	0	0	2	2		0	2	0	0	0	0	0	0	0	0	0	0	0
11 dpc				2			2	0	0	2	0		0	2	2	2	0	2	0	0	0	2	0	0	2
12 dpc				2			2	0	0	2	0		0	2	2	0	0	0	0	0	0	0	0	0	0
13 dpc				0			0	0	0	2	0		0	0	0	0	0	0	0	0	0	0	0	0	0
14 dpc				2			2	2	2	2	0		0	2	2	0	0	2	2	0	0	2	0	0	2
15 dpc				2			2	0	0	2	0		0	2	2	0	0	0	0	0	0	2	0	0	0
16 dpc				2			2	0	0	2	0		0	2	2	0	0	0	0	0	0	0	0	0	0
17 dpc				2			2	0	2	2	0		0	2	2	0	0	2	2	0	0	2	0	0	0
18 dpc				2			2	0	0	2	0		0	2	2	0	0	0	0	0	0	0	0	0	0
19 dpc				0			2	0	2	2	0		0	2	2	0	0	2	0	0	0	2	0	0	0
20 dpc				0			2	0	0	0	0		0	0	8	0	0	0	0	0	0	0	0	0	0
21 dpc				0			0	0	0	0	0		0	0		0	0	0	0	0	0	0	0	0	0
22 dpc				0			0	0	0	0	0		0	0		0	0	0	0	0	0	0	0	0	0
23 dpc				0			2	0	0	0	0		0	2		0	0	0	0	2	0	0	0	2	0
24 dpc				0			2	0	0	0	0		0	2		0	0	0	0	0	0	0	0	0	0
25 dpc				0			2	0	0	0	0		0	2		0	0	0	0	0	0	0	0	0	0
26 dpc				2			8	0	0	0	0		0	2		0	0	0	0	0	0	0	0	0	0
27 dpc				0				0	0	0	0		0	2		0	0	0	0	0	0	0	0	0	0
28 dpc				0				0	0	0	0		0	2		0	0	0	0	0	0	0	0	0	0

Clinical scores

0	Normal
1	Lost appetite
2	Fever
3	Swollen joint
4	Cough
5	Haemorrhagic skin
6	Diarrhea
7	Unactive
8	Dead

Results of challenging test on pigs

➤ Rectal temperature of experimental pigs after challenging

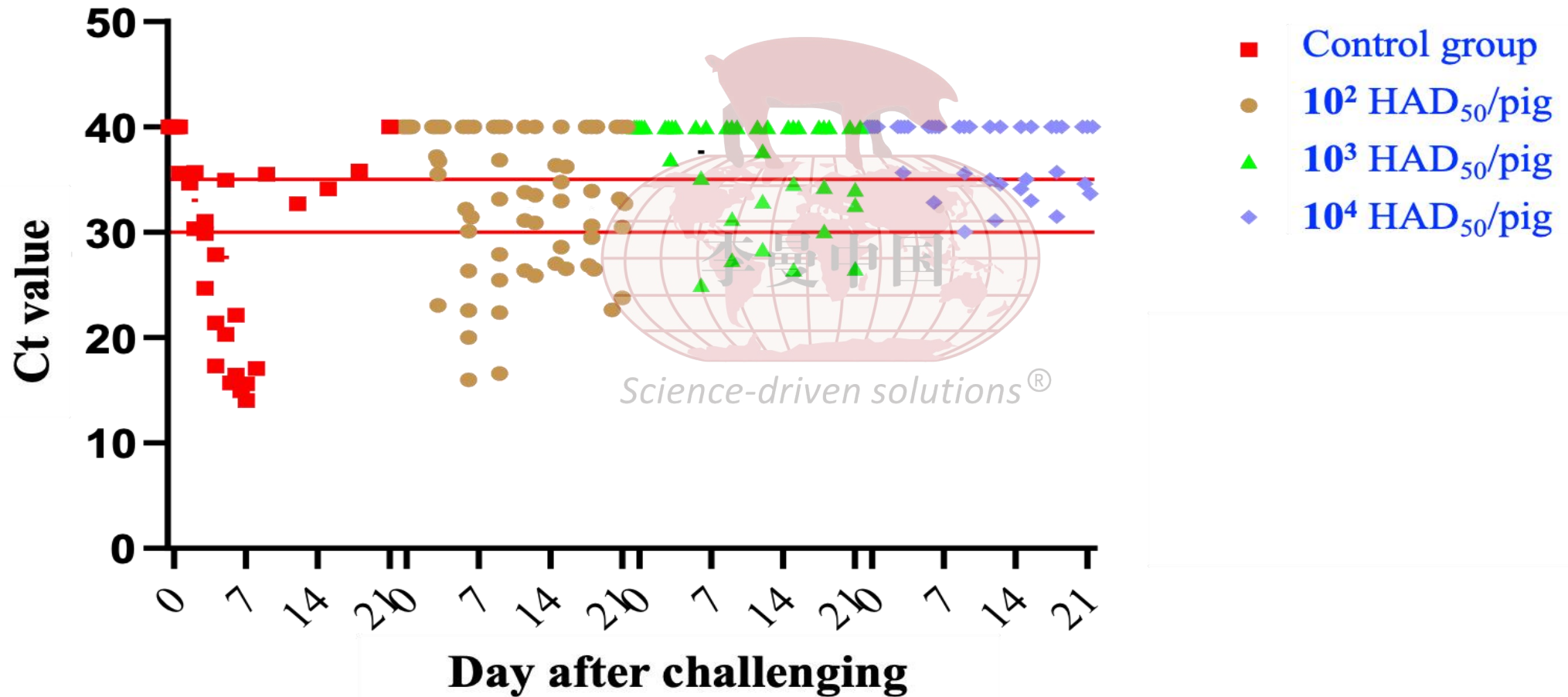


Temperature:

- Control: 100%
- Dose 10^2 : 50%
- Dose 10^3 : Normal
- Dose 10^4 : Normal

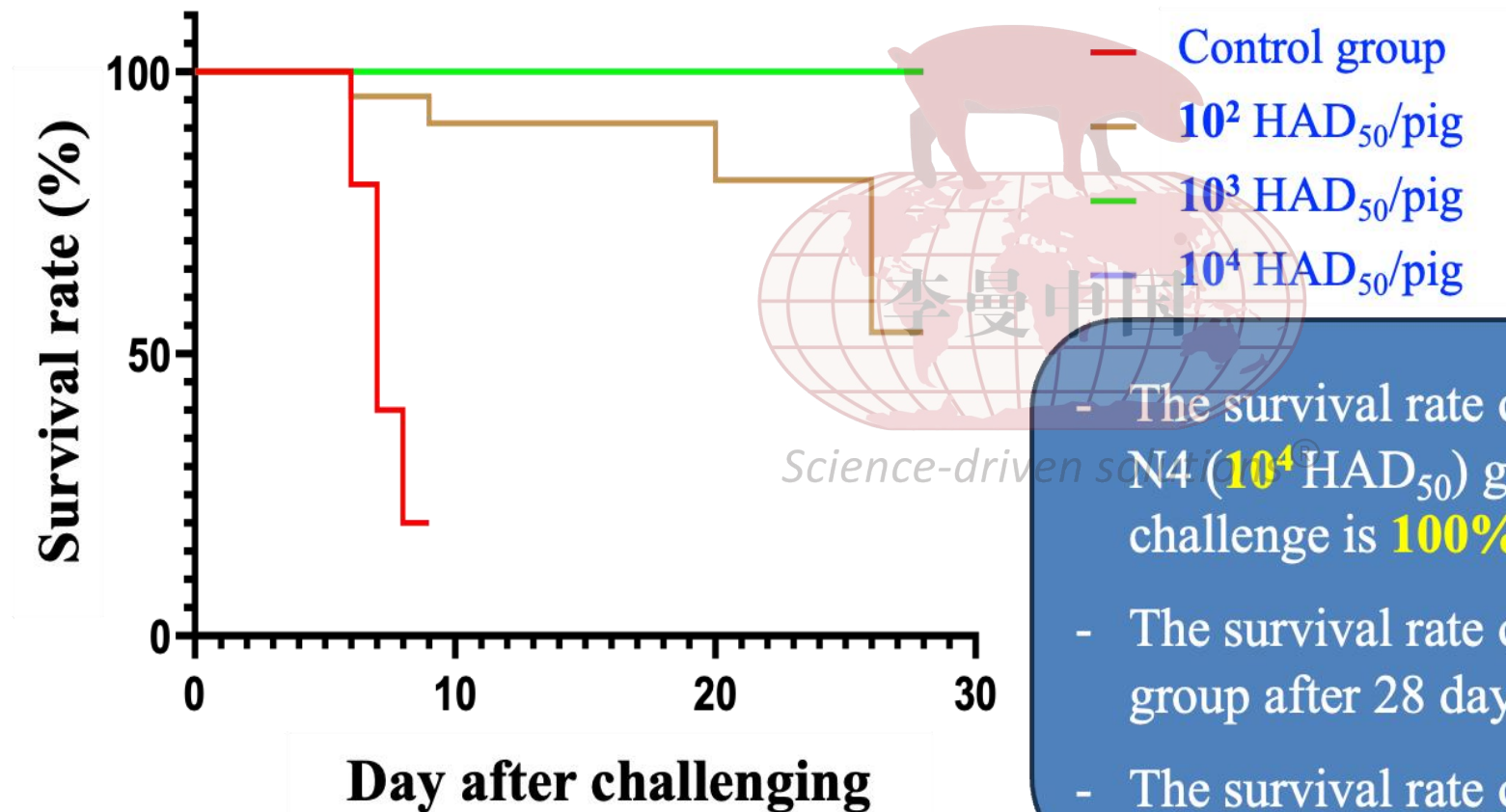
Results of challenging test on pigs

➤ Viremia of pigs after challenging



Results of challenging test on pigs

➤ The survival rate of unvaccinated (red) and vaccinated (black) pigs



- The survival rate of the N3 (10^3 HAD₅₀) and N4 (10^4 HAD₅₀) groups after 28 days of challenge is **100%**.
- The survival rate of the N2 (10^2 HAD₅₀) group after 28 days of challenge is **60%**.
- The survival rate of the control group is **20%**

What lessons learned from ASF outbreaks

Suggestion for outbreak investigation



- ❖ Vietnamese ASFV strains circulating in Vietnam belonged to **genotype II**.
- ❖ **Multiple variants of genotype II ASFV** strains, including gene-deleting strains, circulating in Vietnam
- ❖ Clinical signs were first observed in sows and then in fattening pigs and piglets → **Age sensitive**
- ❖ Clinical symptoms are very diverse, depending on the virus strains causing the disease.
- ❖ Clinical disease (**fever**) = Viral viremia
- ❖ No clinical disease (**no fever**) = No viral viremia
- ❖ All sera and tissue samples collected from infected pigs are strongly positive when tested by real-time PCR.
- ❖ ASF vaccine is protective to fattening pigs after vaccination

Acknowledgments



What are the opportunities for our collaboration?



Thanks for your attention



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