คณะสัตวแพทยศาสตร์
มหาวิทยาลัยเกษตรศาสตร์

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การประชุมวิชาการประจำปี สวทช. ครั้งที่ ๑๘

ทิศทางของการพัฒนางานวิจัยกับโจทย์ จากภาคสนามในการจัดการ ASFV

รศ.น.สพ.ดร. มานะกร สุขมาก
ภาควิชาเวชศาสตร์และทรัพยากรการผลิตสัตว์
คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์


เมื่อถามว่าใครอยากทำแลป

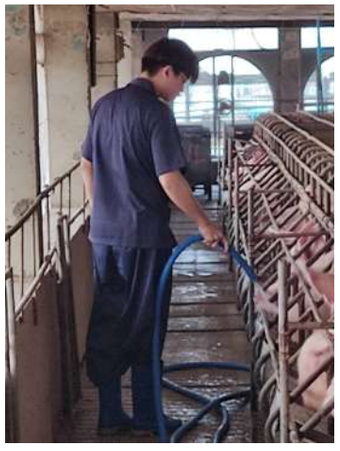
หน่วยงานชันสูตรโรคสัตว์กำแพงแสน ศูนย์วิจัยและบริการวิชาการทางสัตวแพทย์
คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์

Mission

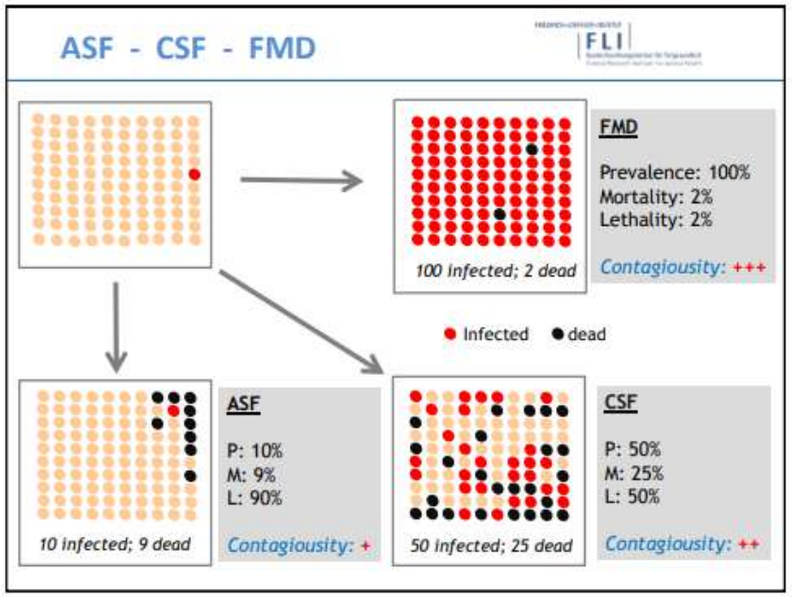
วินิจฉัย
&
บริการวิชาการ

วัคซีน
&
ชุดทดสอบ

ภาคสนาม
&
งานทดลอง



ASF outbreak (Past)



Clinical signs

- depress
- acute death
- panting
- high fever
- skin redness
- haemorrhage
- bloody discharge
- bloody diarrhea

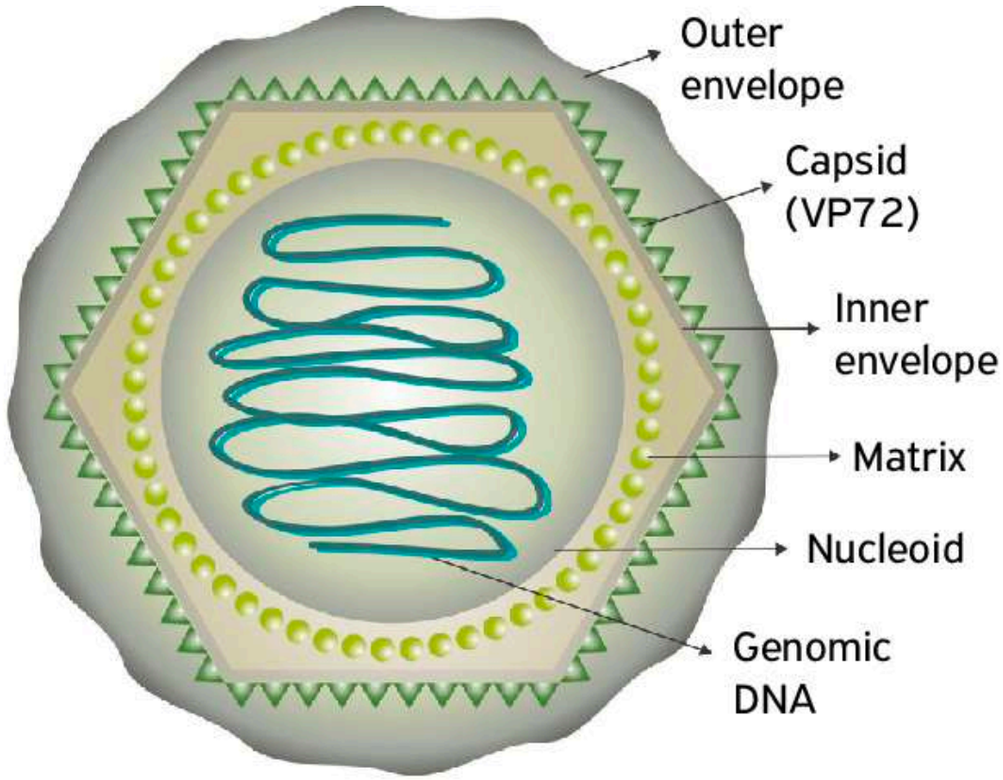
open system → **EVAP**

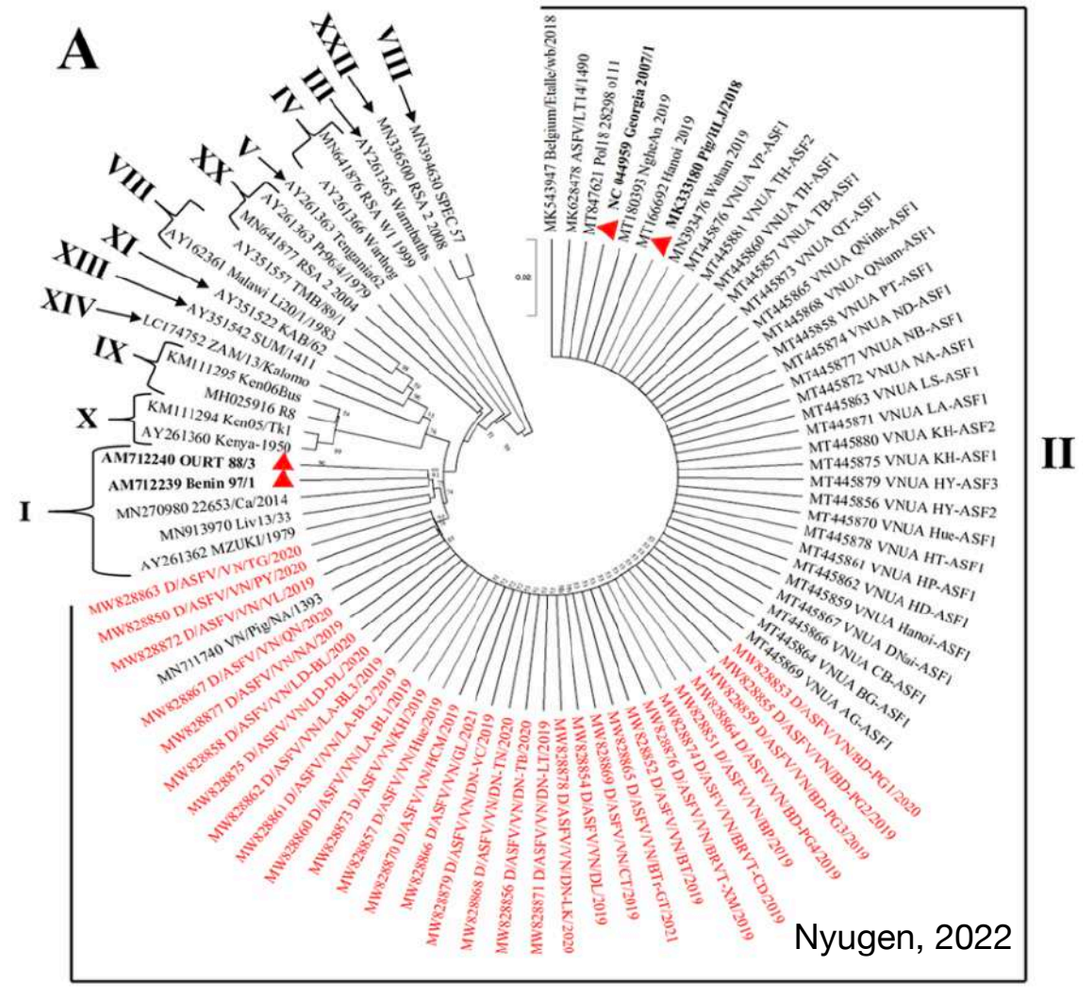
ASF outbreak (Present)

<u>Less clinical signs</u>

- **depress**
- **acute death**
- **haemorrhage ↓**
- **high fever ↓**
- **delay onset ↑**

“เชื้อเปลี่ยนไป หรือ อะไรที่เปลี่ยนแปลง ???”





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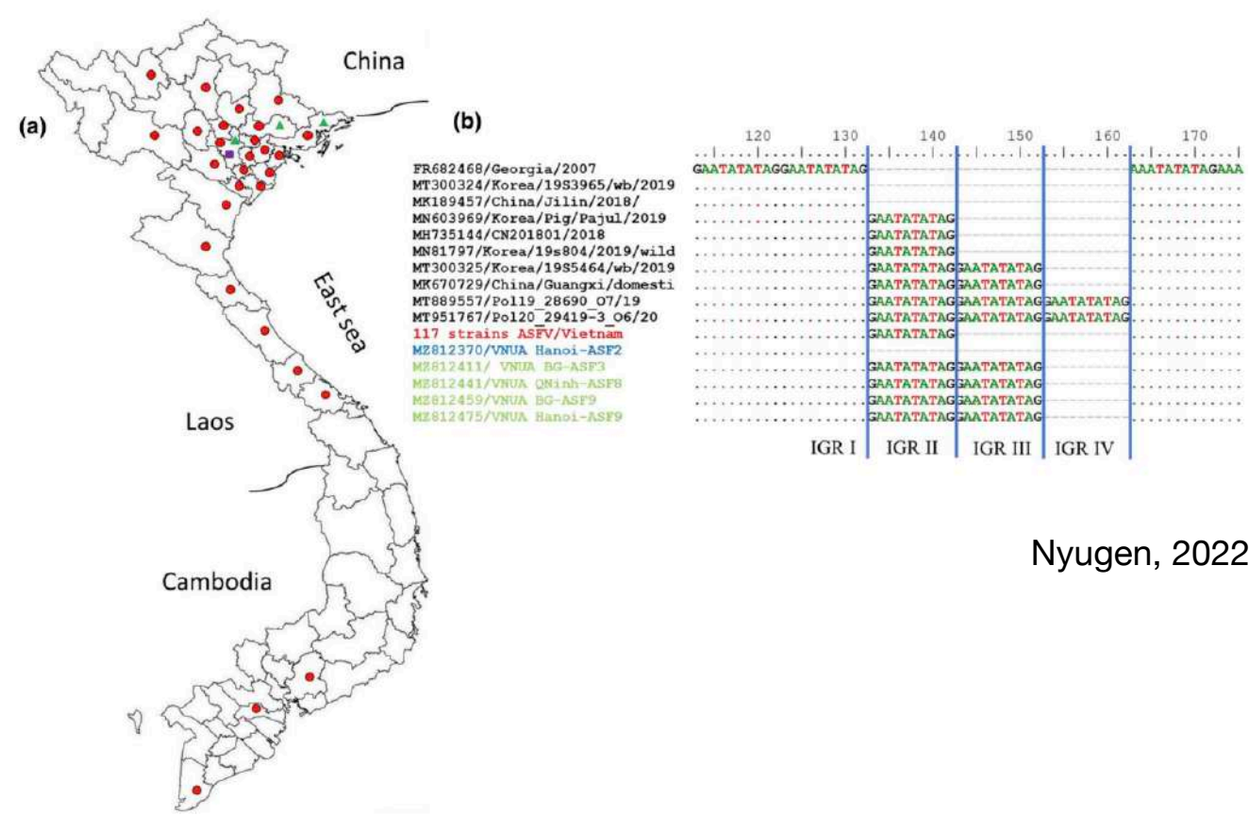
Genotype I African swine fever viruses emerged in domestic pigs in China and caused chronic infection

Emergence of genotype I ASFVs in China.

Encheng Sun, Lianyu Huang, Xianfeng Zhang, Jiwen Zhang, Dongdong Shen, Zhenjiang Zhang, Zilong Wang, Hong Huo, Wenqing Wang, Haoyue Huangfu, Wan Wang, Fang Li, Renqiang Liu, Jianhong Sun, Zhijun Tian, Wei Xia, Yuntao Guan, Xijun He, Yuanmao Zhu, Dongming Zhao & Zhigao Bu



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ORIGINAL ARTICLE

Transboundary and Emerging Diseases WILEY

Dynamics of African swine fever virus (ASFV) infection in domestic pigs infected with virulent, moderate virulent and attenuated genotype II ASFV European isolates

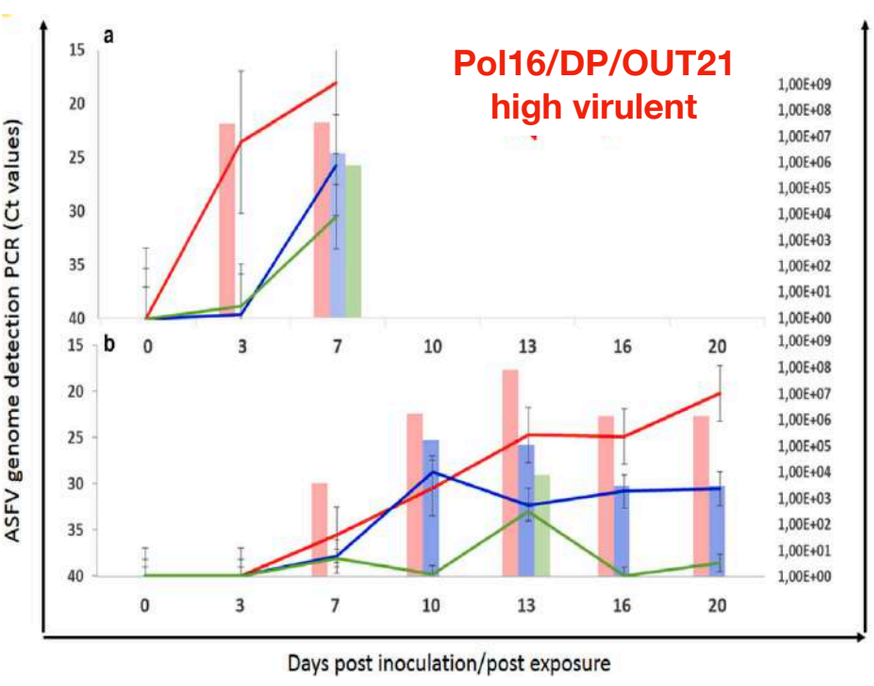
Carmina Gallardo[1] | Alejandro Soler[1] | Imbi Nurmoja[2] | Cristina Cano-Gómez[1] | Svetlana Cvetkova[3] | Maciej Frant[4] | Grzegorz Woźniakowski[4,5] | Alicia Simón[1] | Covadonga Pérez[1] | Raquel Nieto[1] | Marisa Arias[1]

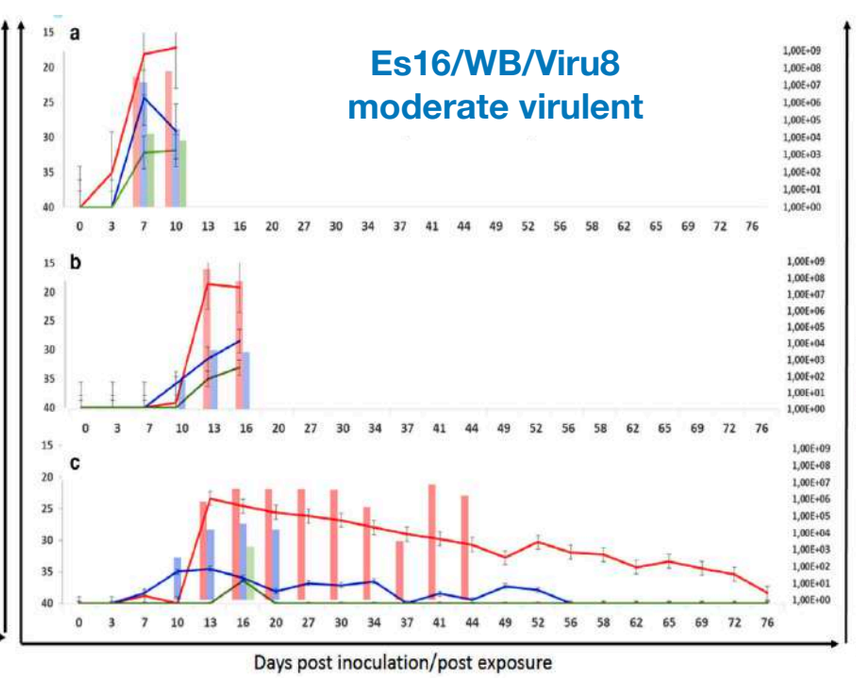
compare the infection dynamics of three genotype II ASFV circulating in Europe

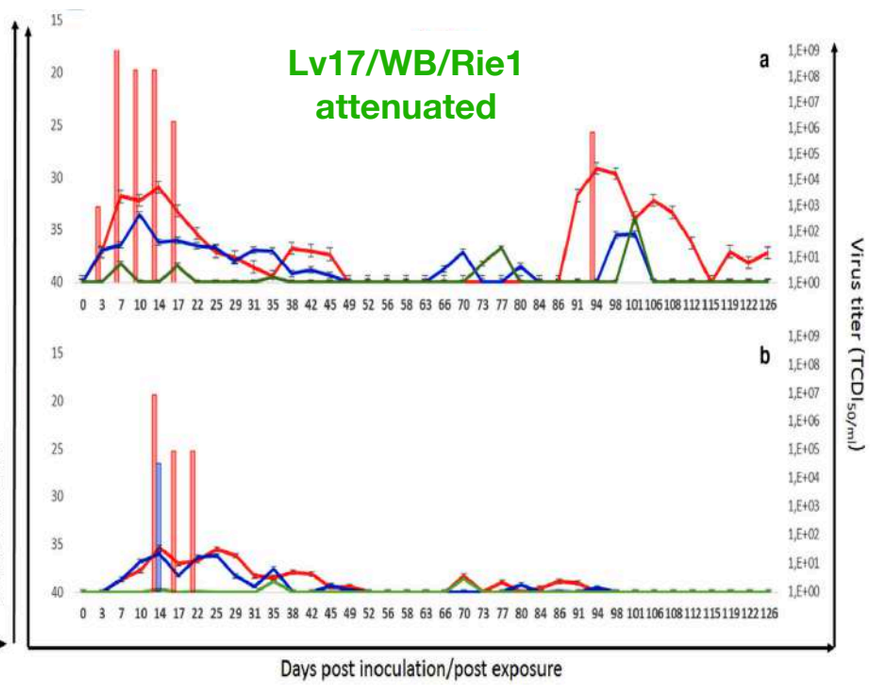
TABLE 2 Comparative results of the onset of the disease indicating the incubation period and survival rate in the domestic pigs infected with the HAD-Polish (Pol16/DP/OUT21), HAD-Estonia (Est16/WB/Viru8), or the non-HAD Latvian (Lv17/WB/Rie1) ASFVs

	Incubation period* (±SD)		Survival [Mean time to death days (±SD)]		
ASFV isolate	Inoculated	Contact	Inoculated	Contact	
Pol16/DP/OUT21	4.5 ± 0.7	9 ± 1	0% [8 ± 0]	0% [16.5 ± 2.4]	**high virulent**
Es16/WB/Viru8	5.5 ± 0.7	13 ± 0	0% [10 ± 0]	50% [18 ± 1.4]	**moderate virulent**
Lv17/WB/Rie1	8 ± 0	11.5 ± 0	100%	100%	**attenuated**

*Days from infection to onset of clinical signs; SD, standard deviation.







whole blood (–), oropharyngeal swabs (OPS) (–), and fecal swabs

viruses

MDPI

Article

Comparison of the Virulence of Korean African Swine Fever Isolates from Pig Farms during 2019–2021

Ki-Hyun Cho, Seong-Keun Hong, Min-Kyung Jang, Ji-Hyoung Ryu, Hyun-Jeong Kim, Yu-Ran Lee, In-Soon Roh, Hyun-Joo Sohn, Hae-Eun Kang and Jee-Yong Park *

Group	Inoculated ASFV Strain (Date of Outbreak)	Survival Period	Days to Onset of Fever	Clinical Signs		Viremia		Virus Shedding					
								Oral Shedding		Nasal Shedding		Rectal Shedding	
				Days to Onset	Max Score [3]	Days to Onset	Max Titer [3]	Days to Onset	Max Titer [3]	Days to Onset	Max Titer [3]	Days to Onset	Max Titer [3]
1	Korea/Pig/Paju1/2019 (16 September 2019)	8.7 ± 0.6	5.0 ± 1.0	5.0 ± 1.0	14.3 ± 2.1	3.3 ± 1.5	7.4 ± 0.1	4.7 ± 1.2	4.5 ± 1.0	5.3 ± 0.6	4.4 ± 1.0	5.3 ± 0.6	4.4 ± 0.5
2	Korea/Pig/Hwcheon1/2020 (8 October 2020)	6.7 ± 1.5	3.7 ± 0.6	4.0 [1]	13.0 [1]	2.3 ± 0.6	7.4 ± 0.1	3.7 ± 0.6	3.2 ± 1.2	4.0 ± 1.0	3.9 ± 0.6	4.0 ± 1.0	4.8 ± 0.6
3	Korea/Pig/Yeongwol/2021 (5 May 2021)	9.0 ± 0.0	4.3 ± 0.6	5.3 ± 1.5	14.3 ± 2.1	3.3 ± 0.6	7.6 ± 0.1	4.0 ± 0	4.8 ± 0.1	4.7 ± 0.6	4.8 ± 1.4	4.7 ± 0.6	4.3 ± 0.8
4	Korea/Pig/Inje2/2021 (8 October 2021)	7.0 ± 2.7	3.0 ± 0	5.0 [2]	14.5 [2]	2.0 ± 0	7.3 ± 0.2	3.0 ± 0	3.7 ± 1.4	3.0 ± 0	3.8 ± 2.0	3.3 ± 0.6	3.9 ± 2.4

[1] Pig no. 5, which died at 5 DPI without any clinical signs, was excluded. [2] Pig no. 10, which died at 4 DPI without any clinical signs, was excluded. [3] $\log_{10}$ genome copies.

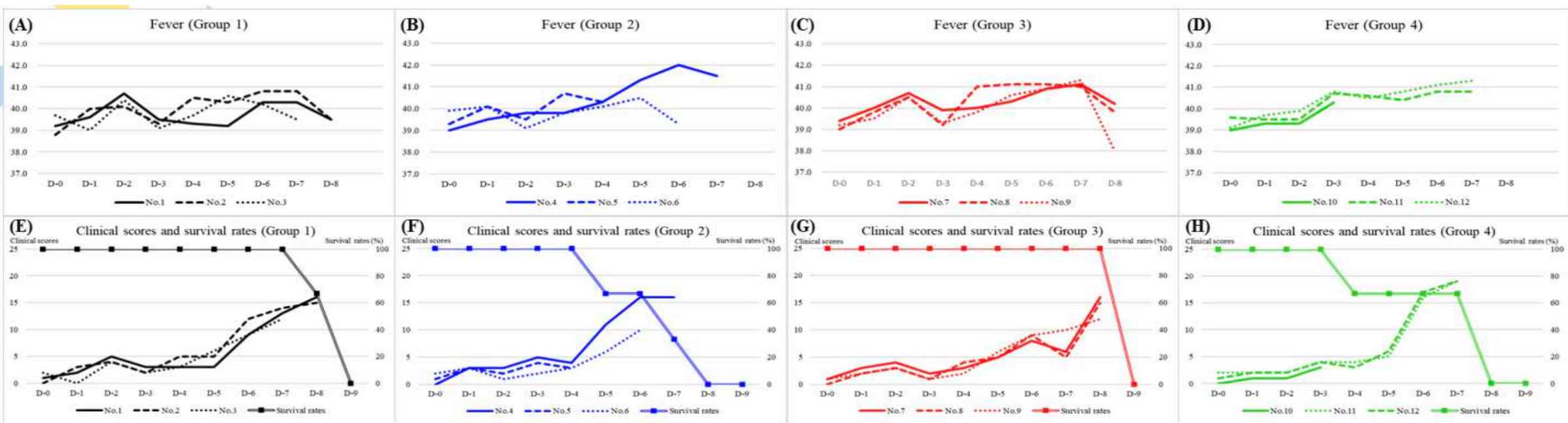


Figure 1. Results of rectal temperature, clinical score, and survival rate in four groups. Change of rectal temperature (**A**–**D**) and clinical scores and survival rates (**E**–**H**) of four groups.

Characterization of ASFV Genotype II

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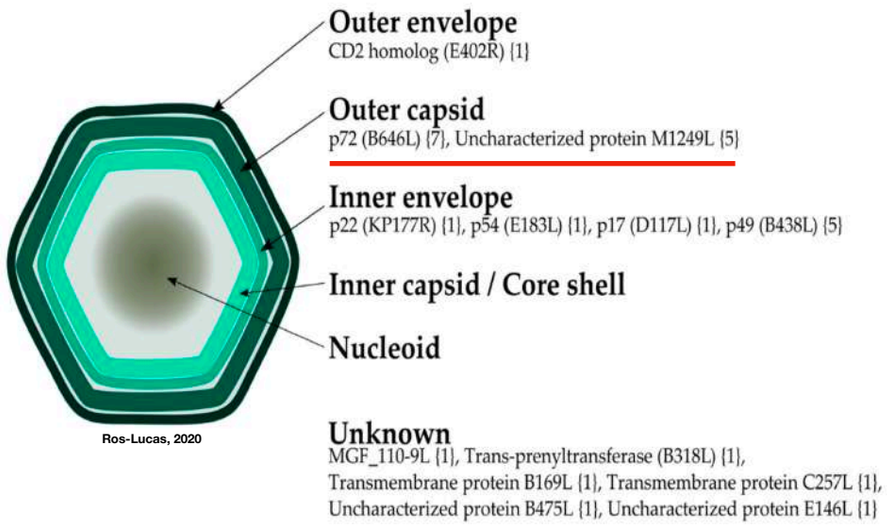
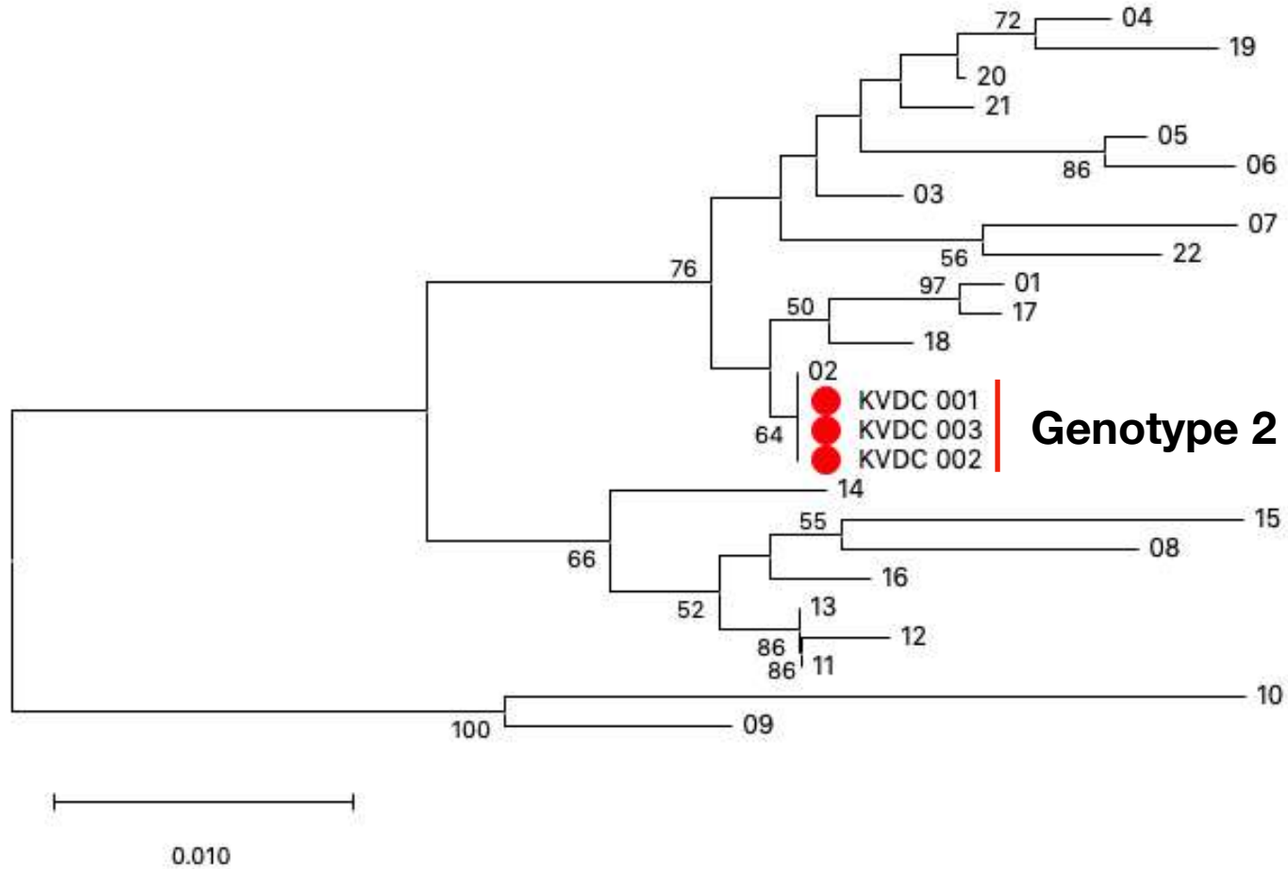
Country	Year	Genotyping	CVR subtyping	IGR I73R-I29L subtyping	IGR MGF subtyping
Georgia	2007	II	CVR-1	IGR-1	MGF-1
Estonia	2014	II	CVR-1	IGR-1	MGF-1
	2015-2018	II	CVR-1 + CVR-1SNP1 + CVR-2	IGR-1	MGF-1
Latvia	2019	II	CVR-1	IGR-2	MGF-1 + MGF-2
Lithuania	2017-2019	II	CVR-1 + CVR-1SNP3	IGR-2	MGF-1 + MGF-4 + MFG-5
Poland	2019	II	CVR-1	IGR-2 + IGR-4	MGF-1
Belgium	2018	II	CVR-1	IGR-2	MGF-1
Serbia	2019-2020	II	CVR-1	IGR-2	MGF-1
Greece	2020	II	CVR-1	IGR-2	MGF-1
Russia	2012-2017	II	CVR-1	IGR-1 + IGR-2	N/A
China	2019-2020	I + II	CVR-1	IGR-1 + IGR-2 + IGR-3	N/A
Vietnam	2019-2020	II	CVR-1	IGR-2 + IGR-3	N/A
South Korea	2019	II	CVR-1	IGR-1 + IGR-2 + IGR-3	N/A
Thailand	**2022**	**II**	**CVR-1**	**IGR-2**	**N/A**

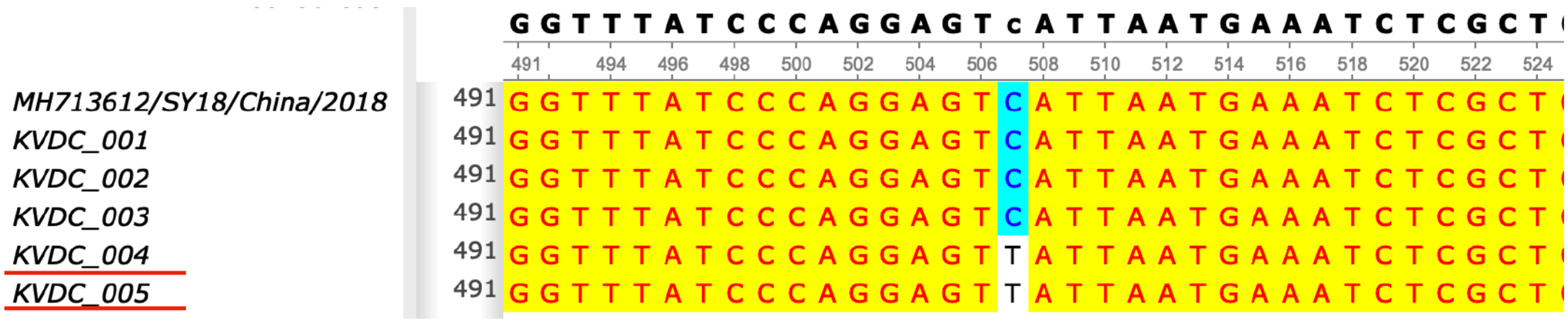
Source: NIAH

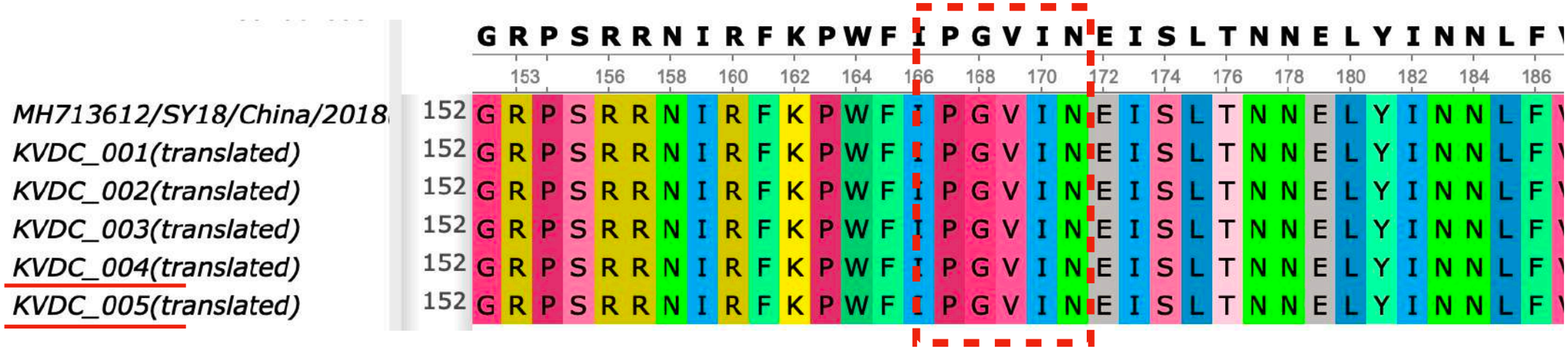


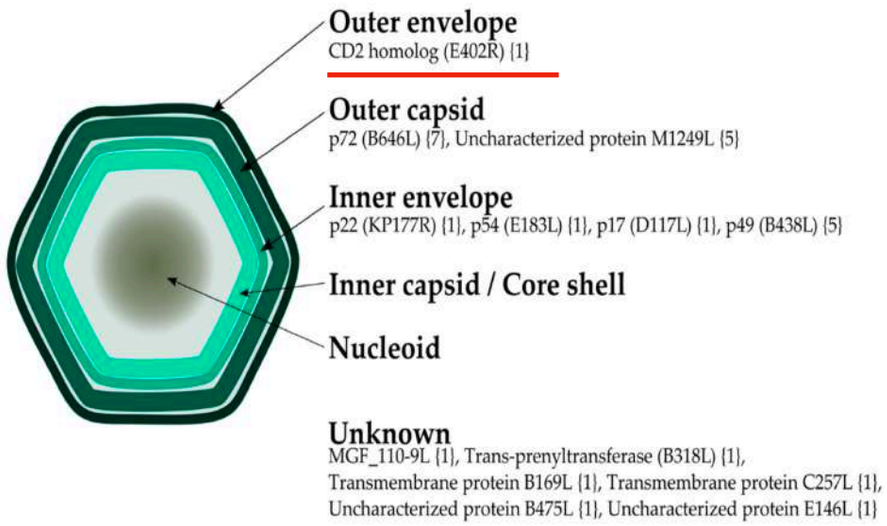
B646L (p72)



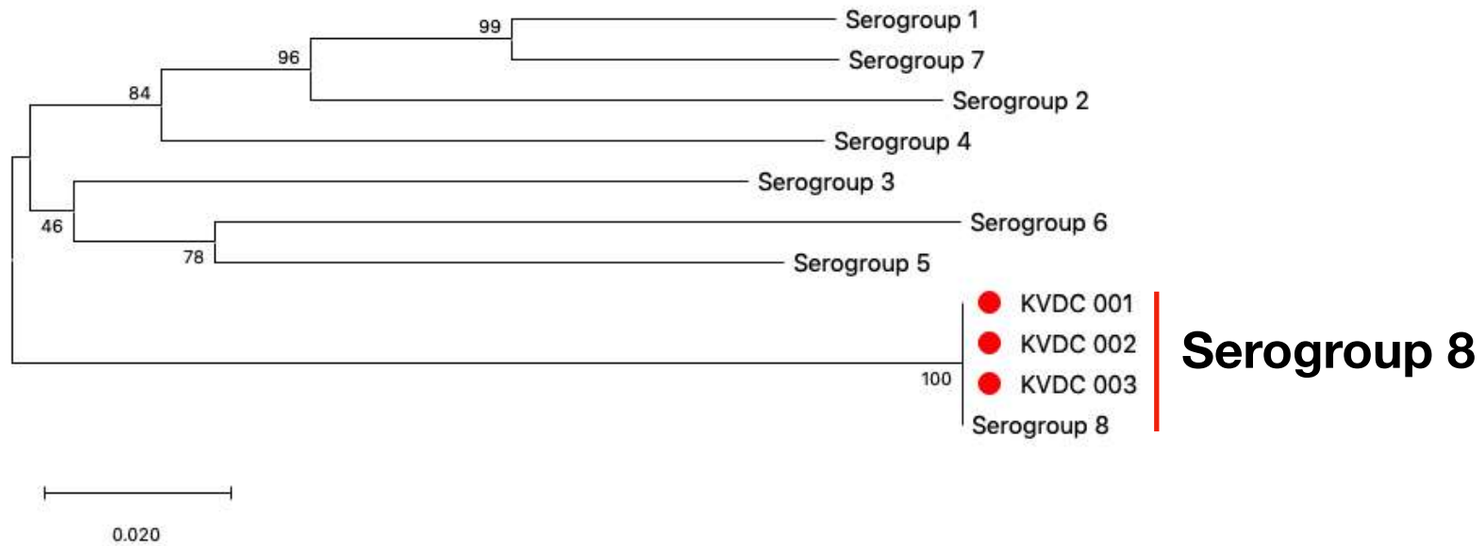
40 samples during 2020-2023



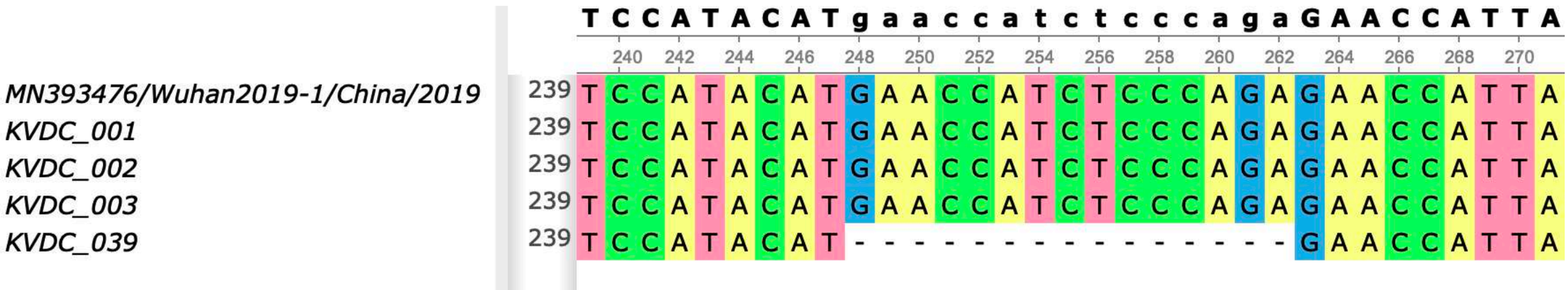


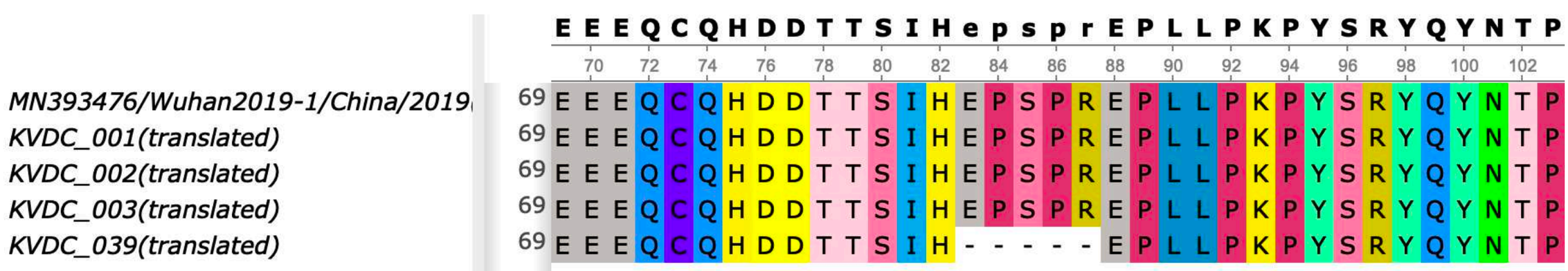


EP402R (CD2v)



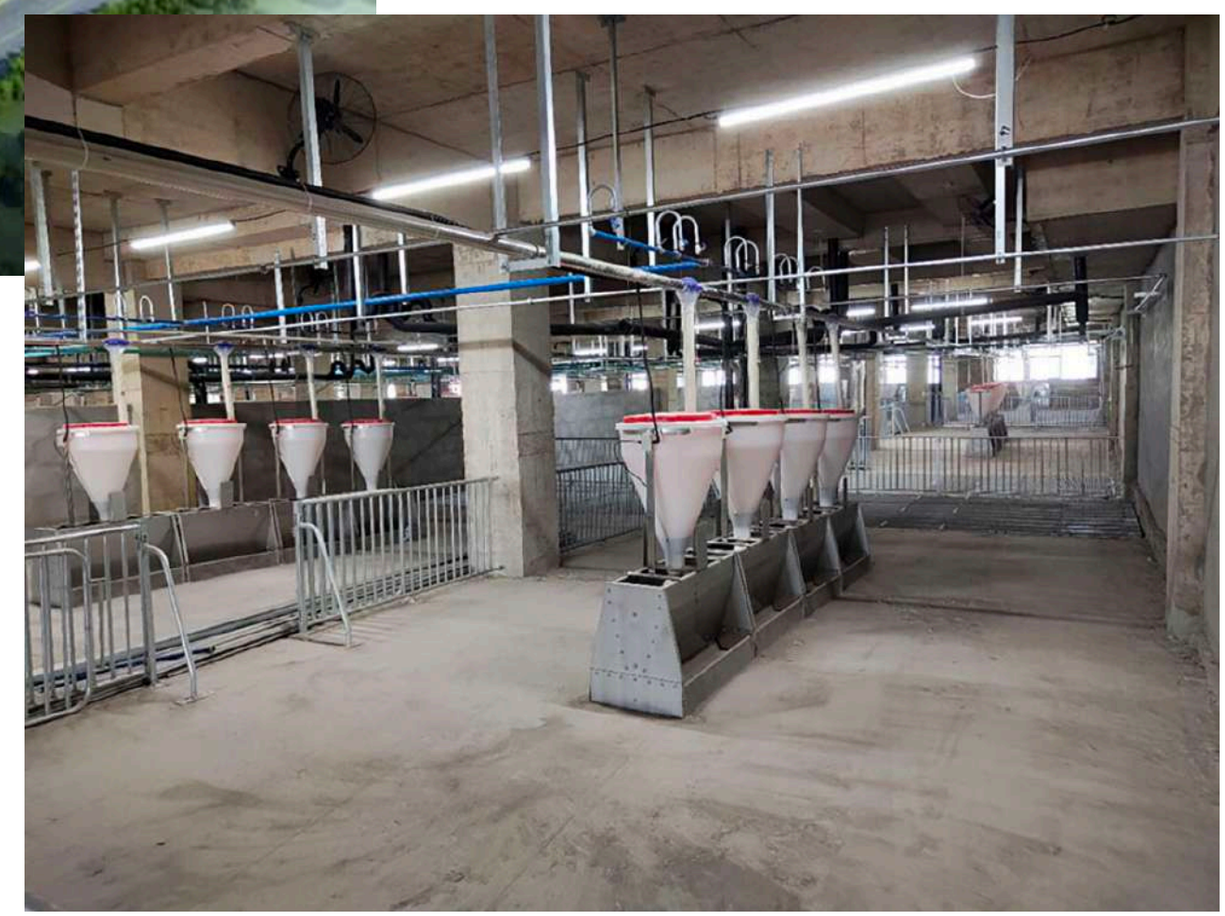
40 samples during 2020-2023





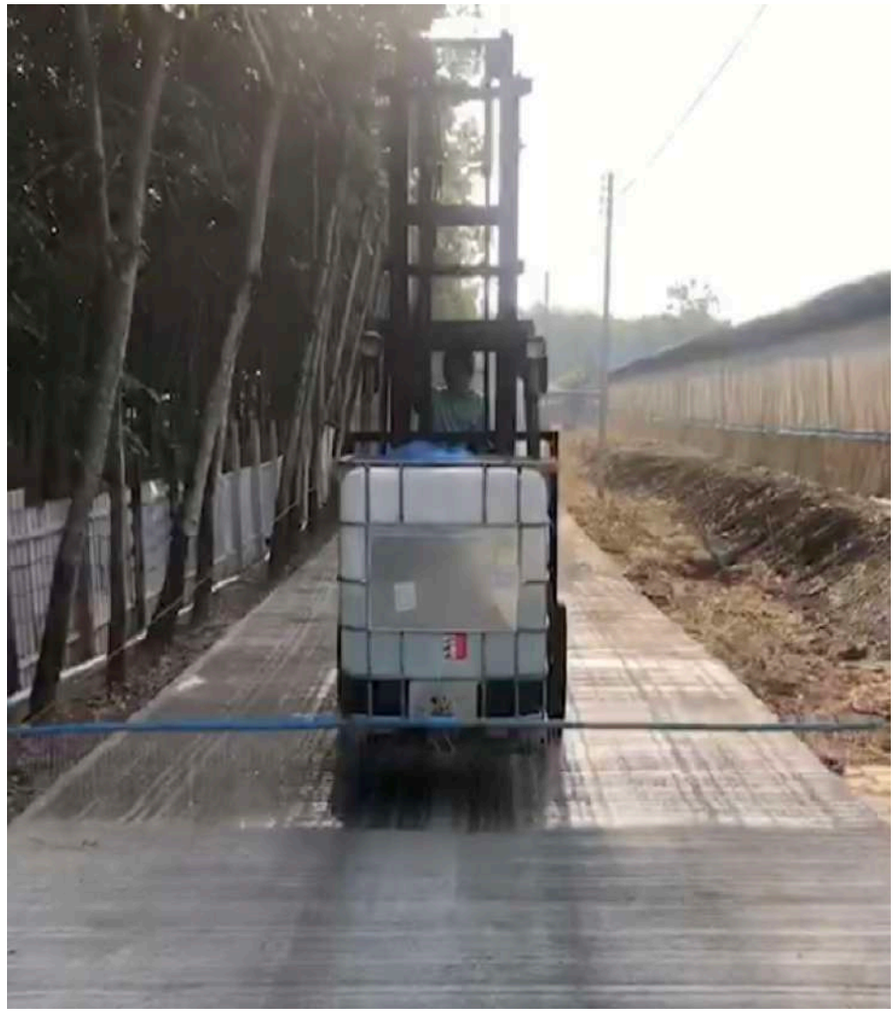
Under construction: A 26-storey pig house

source: pigprogress.net



Farm management >> increase biosecurity

Farm management >> apply a lot of disinfectant



high biosecurity/disinfectant → lower viral load ???

Onset of clinical sign/date of death related to infectious dose

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ASF titer	Test Animal I.D.	Day post-challenge exposure																				
		D 0	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14	D 15	D 16	D 17	D 18	D 19	D 20
ASFV 10^6	A1	100.1	104.2	105.0	104.2	105.2	Dead															
	A2	102.0	103.0	103.6	104.0	105.0	105.8	Dead														
ASFV 10^3	B1	104.0	100.6	104.0	104.0	104.0	104.0	105.4	105.8	Dead												
	B2	102.5	101.8	98.0	100.0	102.4	105.0	105.0	105.8	106.2	Dead											
	B3	103.8	102.4	101.0	100.8	103.0	101.4	102.0	103.0	103.6	104.0	105.0	105.8	Dead								
	B4	103.8	101.3	100.1	102.0	100.1	104.2	105.0	104.2	105.2	105.4	105.2	Dead									
ASFV 10^1	B13	100.8	103.0	102.6	103.1	103.5	103.6	104.1	104.2	104.2	105.0	105.2	105.1	105.4	Dead							
	B14	104.2	102.0	100.6	101.2	101.6	102.0	101.8	102.6	103.0	103.2	103.2	103.6	103.2	103.5	104.1	104.5	105.2	105.4	105.6	Dead	
	B15	102.5	101.8	102.2	101.6	101.8	102.3	102.6	102.5	103.0	103.4	103.6	102.2	102.5	102.4	102.5	104.5	105.2	105.8	105.8	Dead	
	B16	102.6	95.2	101.0	100.4	100.4	101.8	103.0	103.8	102.6	103.2	103.2	103.0	103.2	102.3	104.2	105.0	105.0	105.6	105.8	105.8	Dead

Source: NIAH

- ❖ Body temperature peak at day 1-2 prior dead
- ❖ Dead animal began to observe at day 5-6 post-challenge exposure
- ❖ Animals exposed with high titer of ASFV: 100% Die at day 6
- ❖ Animals exposed with moderate titer of ASFV: 100% Die at day 12
- ❖ Animals exposed with low titer of ASFV: 100% Die at day 20

Onset of clinical sign/date of death related to infectious dose

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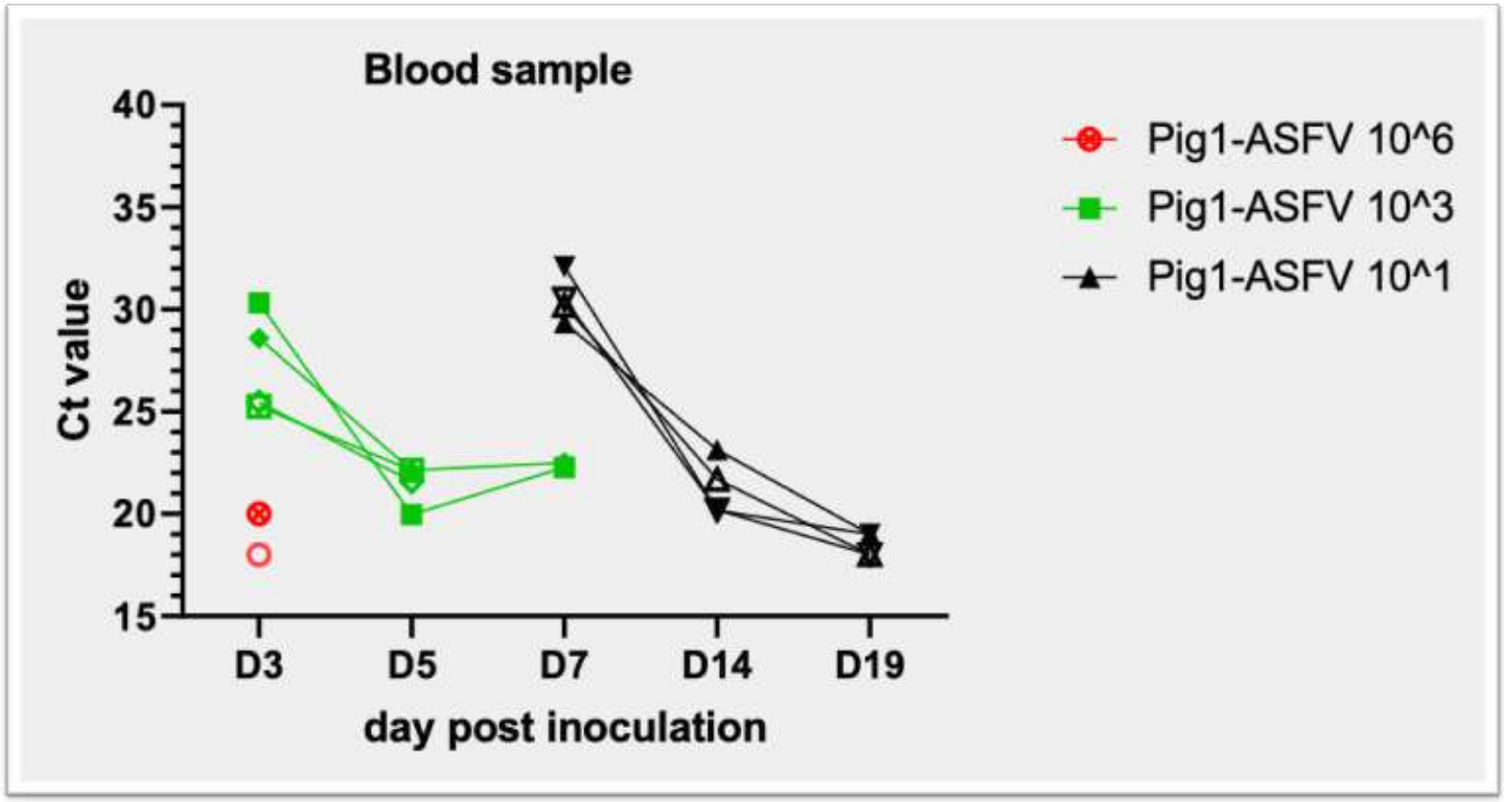
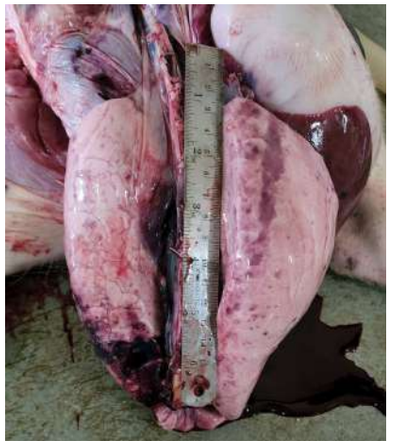
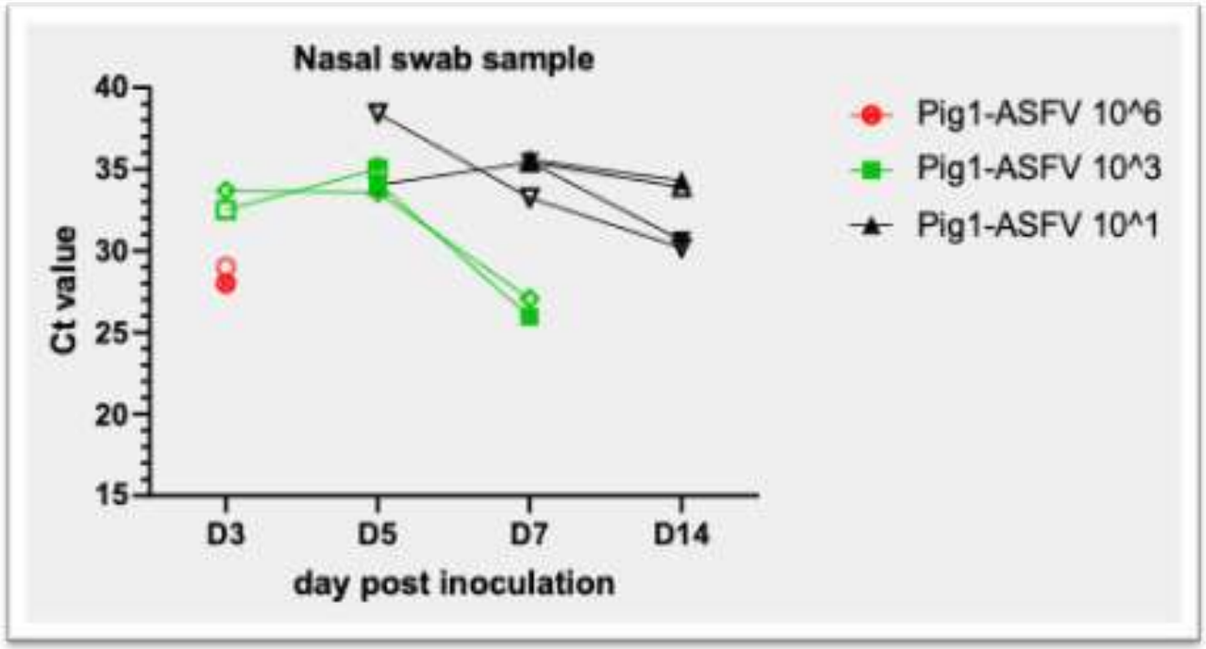
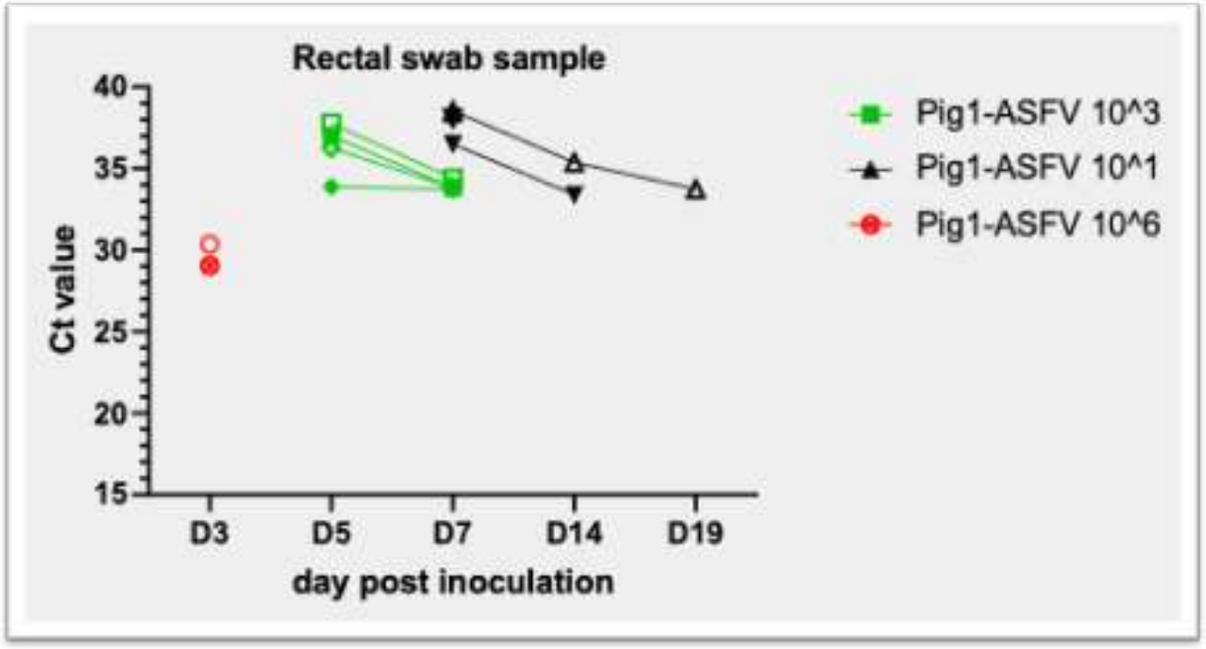


- blood samples showed higher viral load
- date of detection related to infectious dose

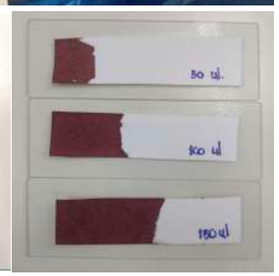


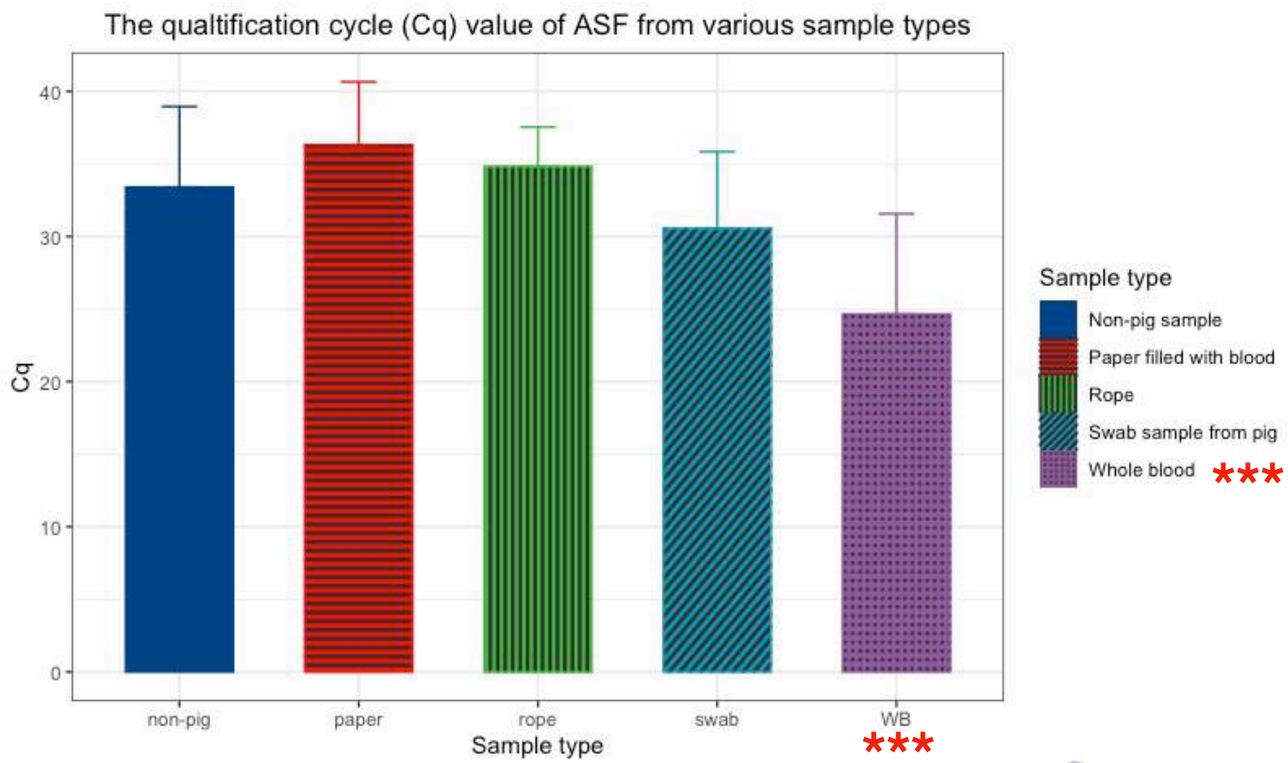




Source: NIAH

Samples collection





F5: Non-clinical finding	1	Oral fluids	0	Neg
	2	Oral fluids	0	Neg
	3	Oral fluids	33.14	Pos
	4	Oral fluids	0	Neg
	5	Oral fluids	0	Neg
F4: Non-clinical finding	1	Oral fluids	0	Neg
	2	Oral fluids	0	Neg
	3	Oral fluids	37.78	Pos
	4	Oral fluids	0	Neg
	5	Oral fluids	0	Neg
F8: Clinical finding	1	Oral fluids	29.9	Pos
	2	Oral fluids	32.36	Pos
	3	Oral fluids	33.7	Pos
	4	Oral fluids	36.26	Pos
	5	Oral fluids	37.38	Pos
	6	Oral fluids	36.34	Pos

Live animal

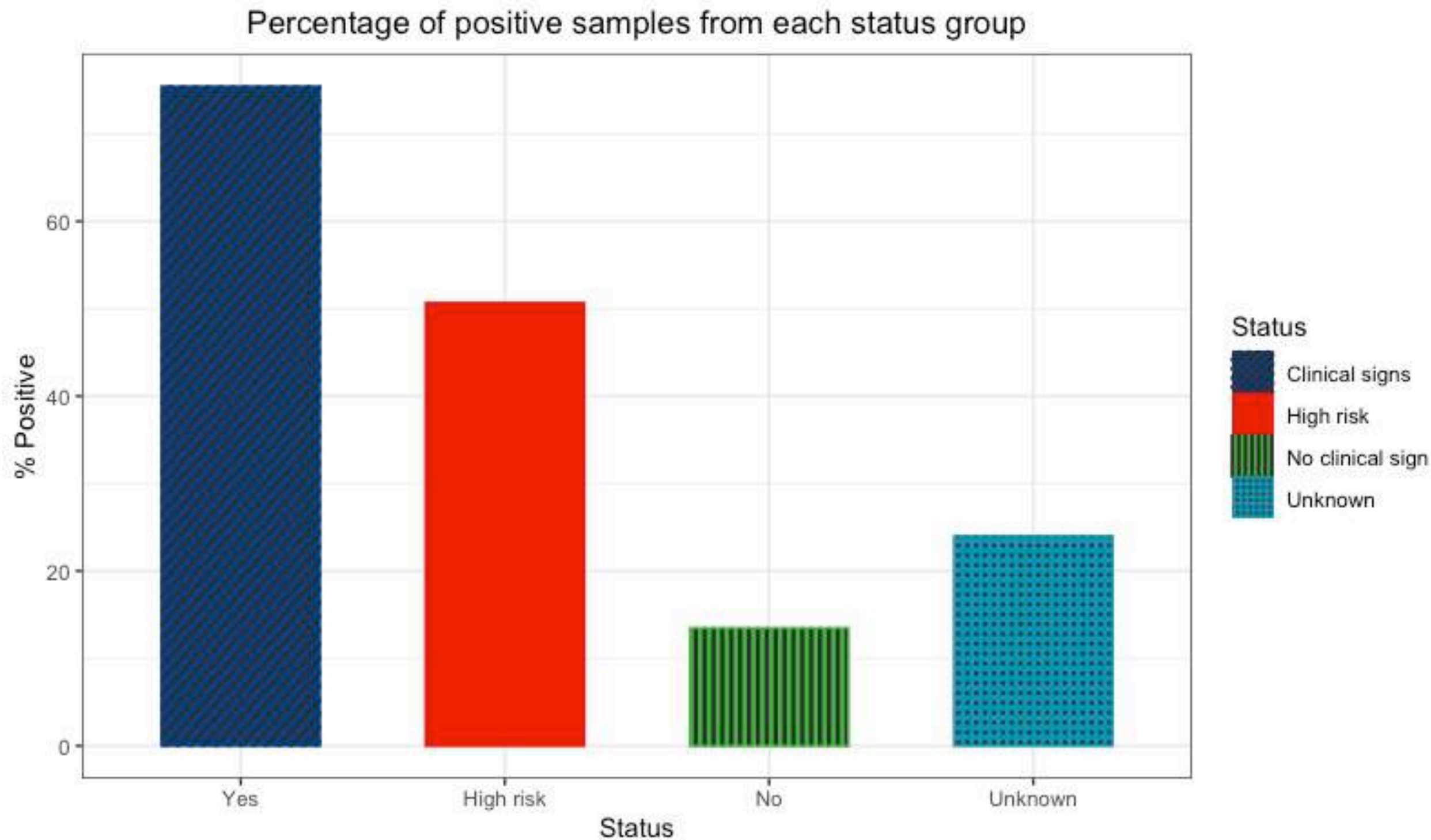
- **whole blood is the best**

Death animal

- **spleen**
- **lymph node**
- **tonsil**
- **lung**

*****increase the risk of ASFV spreading*****

Samples collection



Situation in Vietnam

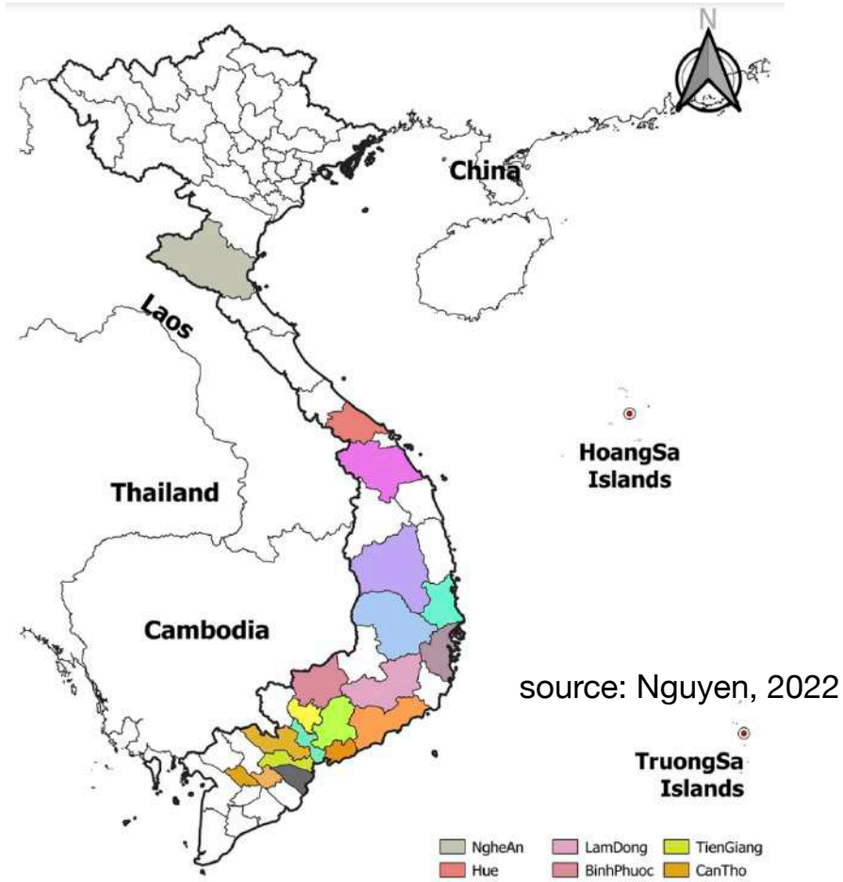
- ASFV clinical signs: Chronic > Acute

- New ASFV field variant strains

- Attenuated lived ASFV vaccine strains

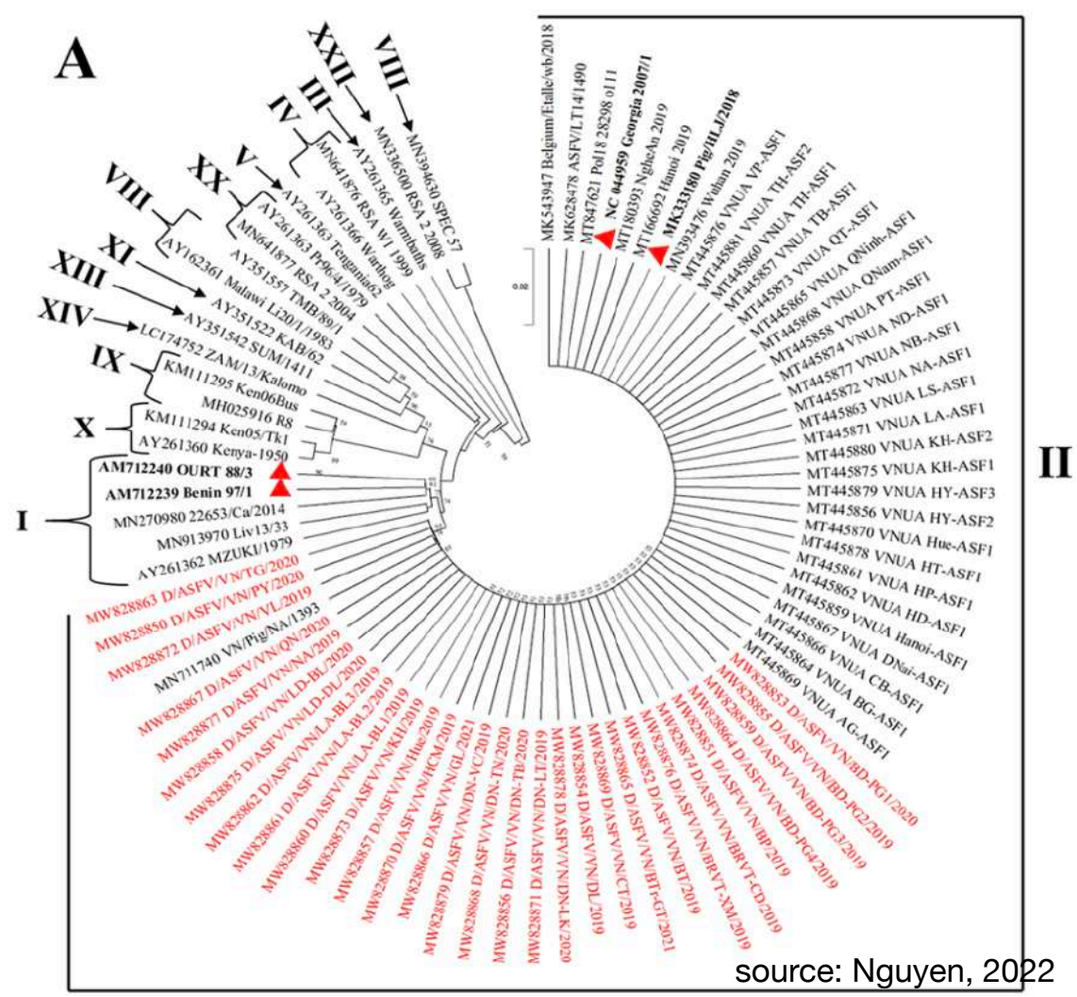
- ASFV persistant and latent infection

- Reproductive disorder ???

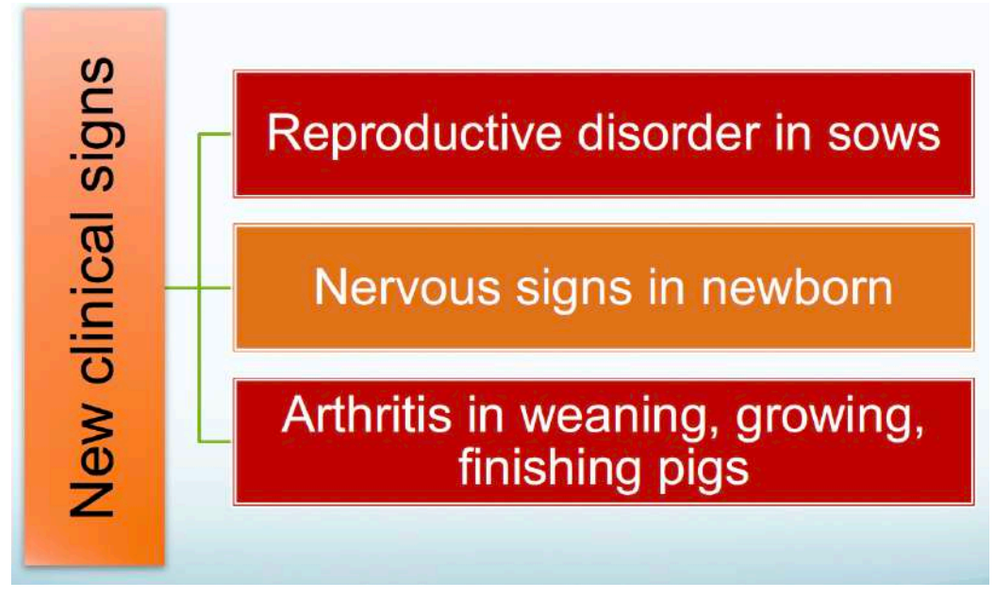


source: Nguyen, 2022

source: Hai, unpublished data



source: Nguyen, 2022



Sampling: Abortus, piglets with nervous signs…

Sample	Cerebrum	Lungs, spleen, lymp nodes, kidney
1 (N1/ M1)	(+)	(-)
2 (N2/ M2)	(-)	(+)
3 (N3/ M3)	(+)	(-)
4 (N4/ M4)	(+)	(-)
5 (N5/ M5)	(-)	(-)
6 (N6/ M6)	(-)	(+)
7 (N7/ M7)	(-)	(+)

Nguyen Ngoc Hai et al., unpublished data

Sows with reproductive disorder

Sow	Blood sample	ASFV	Sample	ASFV
259	Uncoagulated	-	Vulval fluid	-
06	Uncoagulated	-	Vulval fluid	+
290	Uncoagulated	-	Vulval fluid	-
05	Uncoagulated	-	Vulval fluid	+
29	Uncoagulated	-	Vulval fluid	-
48	Uncoagulated	-	Vulval fluid	-
305	Uncoagulated	-	Vulval fluid	-
319	Uncoagulated	-	Vulval fluid	-
62	Uncoagulated	-	Vulval fluid	-
405	Uncoagulated	-	Vulval fluid	+

source: Nguyen Ngoc Hai et al., unpublished data

Arthritis ???

Pigs	Sample	ASFV	HP-PRRSV	H. parasuis	S. suis type 2
Pool 2/ 8W	Anticoagulant blood	Neg.	Pos.	NA	NA
Pool 3/ 6W	Anticoagulant blood	Pos.	Pos.	NA	NA
Pool 2/ 8W	Lung, lympho node, spleen, kidney	Pos.	Pos.	NA	NA
Pool 3/ 6W	Lung, lympho node, spleen, kidney	Neg.	Pos.	NA	NA
Pool 2/ 8W	Brain	Pos.	NA	NA	Neg.
Pool 3/ 6W	Brain	Neg.	NA	NA	Neg.
Pool 2/ 8W	Joint fluid	Pos.	Neg.	Neg.	Neg.
Pool 3/ 6W	Joint fluid	Pos.	Neg.	Neg.	Neg.
Pool 2/ 8W	Pericadial fluid	NA	NA	Neg.	NA
Pool 3/ 6W	Pericadial fluid	NA	NA	Neg.	NA

Nguyễn Ngọc Hải et al., 2022.

FARM A

- outbreak in 2021
- stamp-out pigs that showed clinical signs or positive by qPCR
- keep healthy pig (negative by qPCR)

In-house indirect ASF ELISA: Colostrum samples (1-12-65)			
แม่คลอด	0.127	Negative	Colostrum
แม่คลอด	0.129	Negative	Colostrum
แม่คลอด	1.194	Positive	Colostrum
แม่คลอด	0.000	Negative	Colostrum
แม่คลอด	0.159	Negative	Colostrum
แม่คลอด	0.100	Negative	Colostrum
แม่คลอด	0.417	Positive	Colostrum
แม่คลอด	0.099	Negative	Colostrum
แม่คลอด	0.149	Negative	Colostrum
แม่คลอด	0.100	Negative	Colostrum
แม่คลอด	0.259	Negative	Colostrum
แม่คลอด	0.000	Negative	Colostrum

In-house indirect ASF ELISA: Colostrum samples (1-12-65)			
รายละเอียดตัวอย่าง	S/P ratio	ผล	Remark
แม่คลอด	0.062	Negative	Colostrum
แม่คลอด	0.106	Negative	Colostrum
แม่คลอด	0.014	Negative	Colostrum
แม่คลอด	0.102	Negative	Colostrum
แม่คลอด	0.000	Negative	Colostrum
แม่คลอด	1.138	Positive	Colostrum
แม่คลอด	0.185	Negative	Colostrum
แม่คลอด	1.719	Positive	Colostrum
แม่คลอด	0.103	Negative	Colostrum
แม่คลอด	0.253	Negative	Colostrum
แม่คลอด	0.000	Negative	Colostrum
แม่คลอด	0.876	Positive	Colostrum
แม่คลอด	1.003	Positive	Colostrum

Use ELISA to monitor seropositive pig then replace with negative pig

FARM A

Finding seropositive sow

หมูสาวทดแทนเล้าลอย 1	1.734	บวก
หมูสาวทดแทนเล้าลอย 2	2.170	บวก
หมูสาวทดแทนเล้าลอย 3	2.105	บวก
หมูสาวทดแทนเล้าลอย 4	1.687	บวก
หมูสาวทดแทนเล้าลอย 5	2.177	บวก
หมูสาวทดแทนเล้าลอย 6	1.883	บวก
หมูสาวทดแทนเล้าลอย 7	1.968	บวก
หมูสาวทดแทนเล้าลอย 8	2.313	บวก
53/5ไร่	0.370	ลบ
54/5ไร่	0.000	ลบ
54/5ไร่	0.000	ลบ
55/5ไร่	0.143	ลบ
56/5ไร่	0.000	ลบ
57/5ไร่	0.020	ลบ
57/5ไร่	0.128	ลบ
58/5ไร่	0.329	ลบ
58/5ไร่	0.347	ลบ
59/5ไร่	0.371	ลบ
811/เล้า7	0.018	ลบ
812/เล้า7	0.000	ลบ
822/เล้า7	1.883	บวก
720	1.382	บวก
722	0.039	ลบ
722	0.000	ลบ
723	0.000	ลบ
723	0.000	ลบ
724	0.000	ลบ

Replace with seronegative gilt

รายละเอียดตัวอย่าง	S/P ratio	ผล
สุกรสาวทดแทน	0.135	ลบ
สุกรสาวทดแทน	0.124	ลบ
สุกรสาวทดแทน	0.060	ลบ
สุกรสาวทดแทน	0.036	ลบ
สุกรสาวทดแทน	0.000	ลบ
สุกรสาวทดแทน	0.009	ลบ
สุกรสาวทดแทน	0.130	ลบ
สุกรสาวทดแทน	0.000	ลบ
สุกรสาวทดแทน	0.027	ลบ
สุกรสาวทดแทน	0.005	ลบ
สุกรสาวทดแทน	0.016	ลบ
สุกรสาวทดแทน	0.230	ลบ
สุกรสาวทดแทน	0.000	ลบ
สุกรสาวทดแทน	0.000	ลบ

Monitoring

ยูนิต	S/P ratio	ผล	Remark
Unit-6	0.011	ลบ	Colostrum
Unit-6	0.119	ลบ	Colostrum
Unit-6	0.129	ลบ	Colostrum
Unit-6	0.000	ลบ	Colostrum
Unit-6	0.351	ลบ	Colostrum
Unit-6	0.020	ลบ	Colostrum
Unit-6	0.085	ลบ	Colostrum
Unit-6	0.154	ลบ	Colostrum
Unit-6	0.122	ลบ	Colostrum
Unit-2	0.102	ลบ	Colostrum
Unit-2	0.034	ลบ	Colostrum
Unit-2	0.103	ลบ	Colostrum
Unit-2	0.000	ลบ	Colostrum
Unit-2	0.138	ลบ	Colostrum
Unit-2	0.000	ลบ	Colostrum

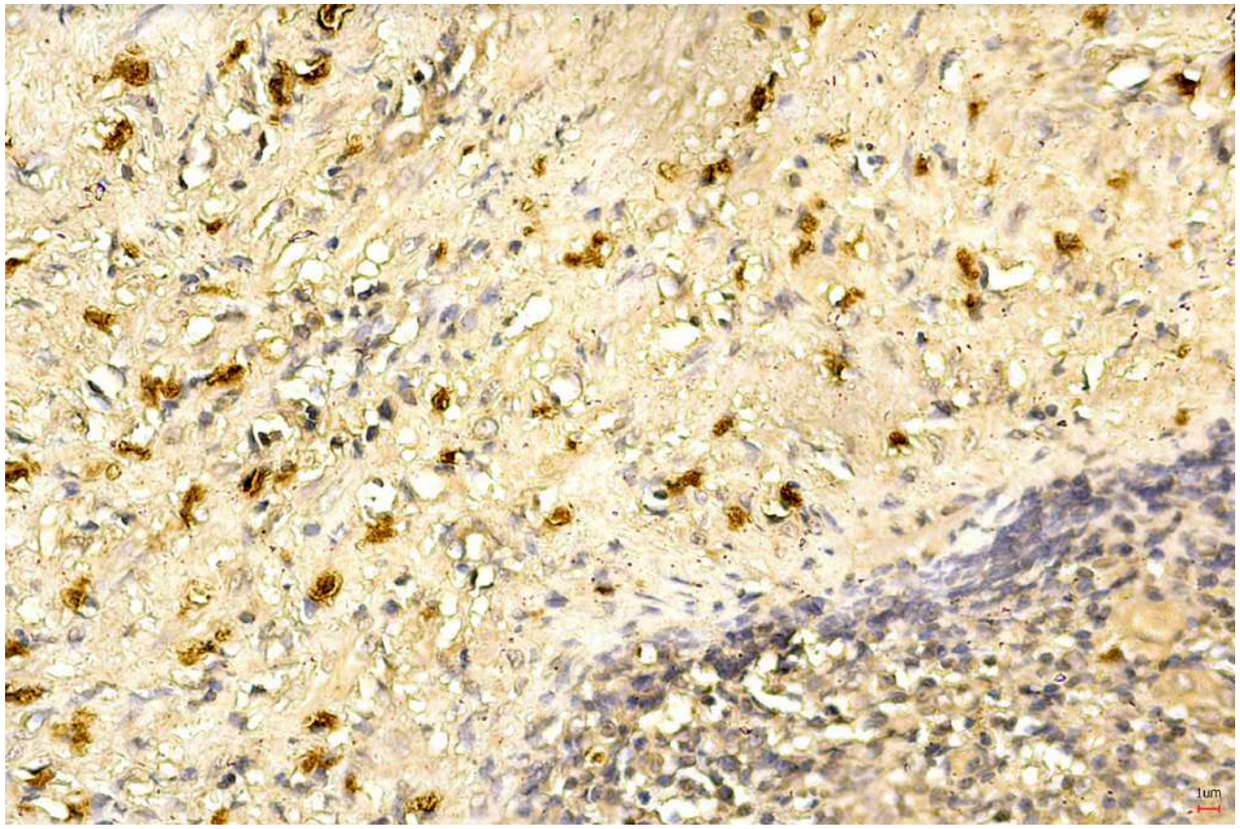
Stop outbreak for 13 months

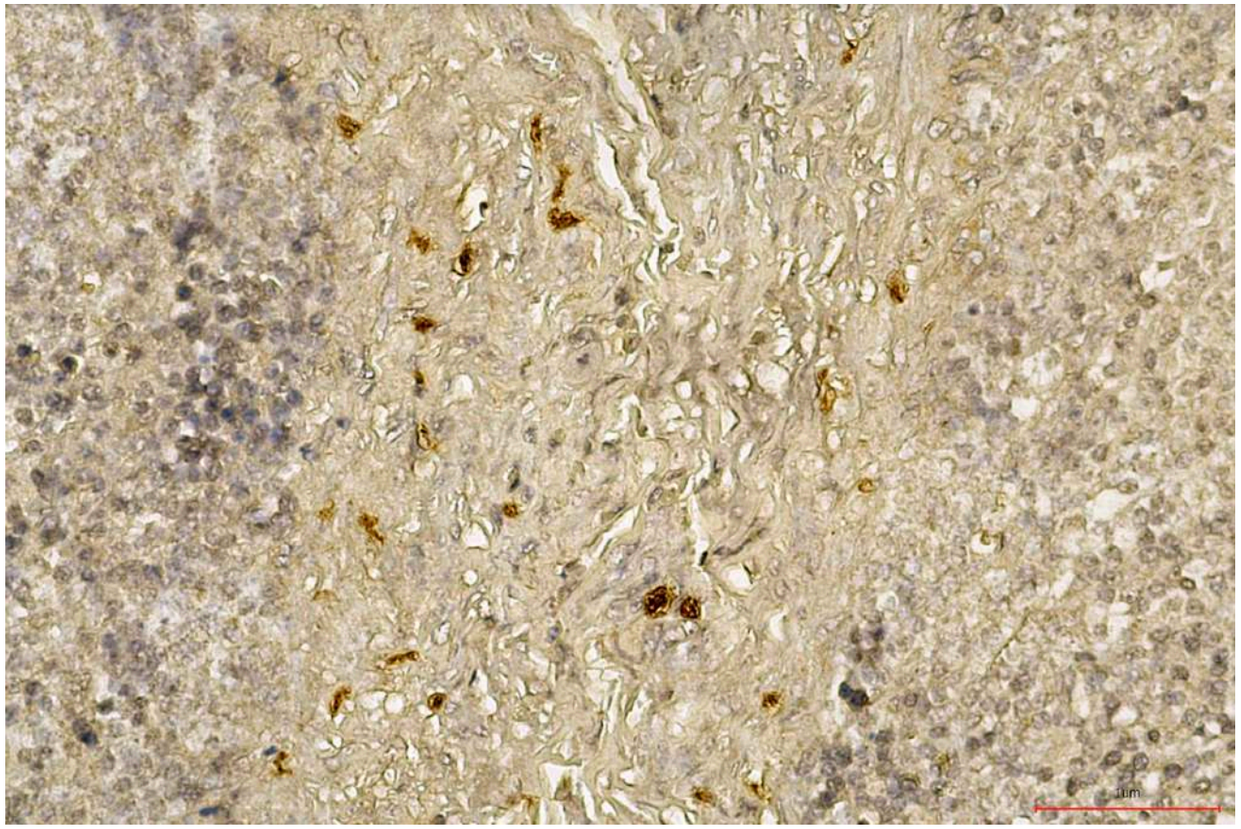
FARM B

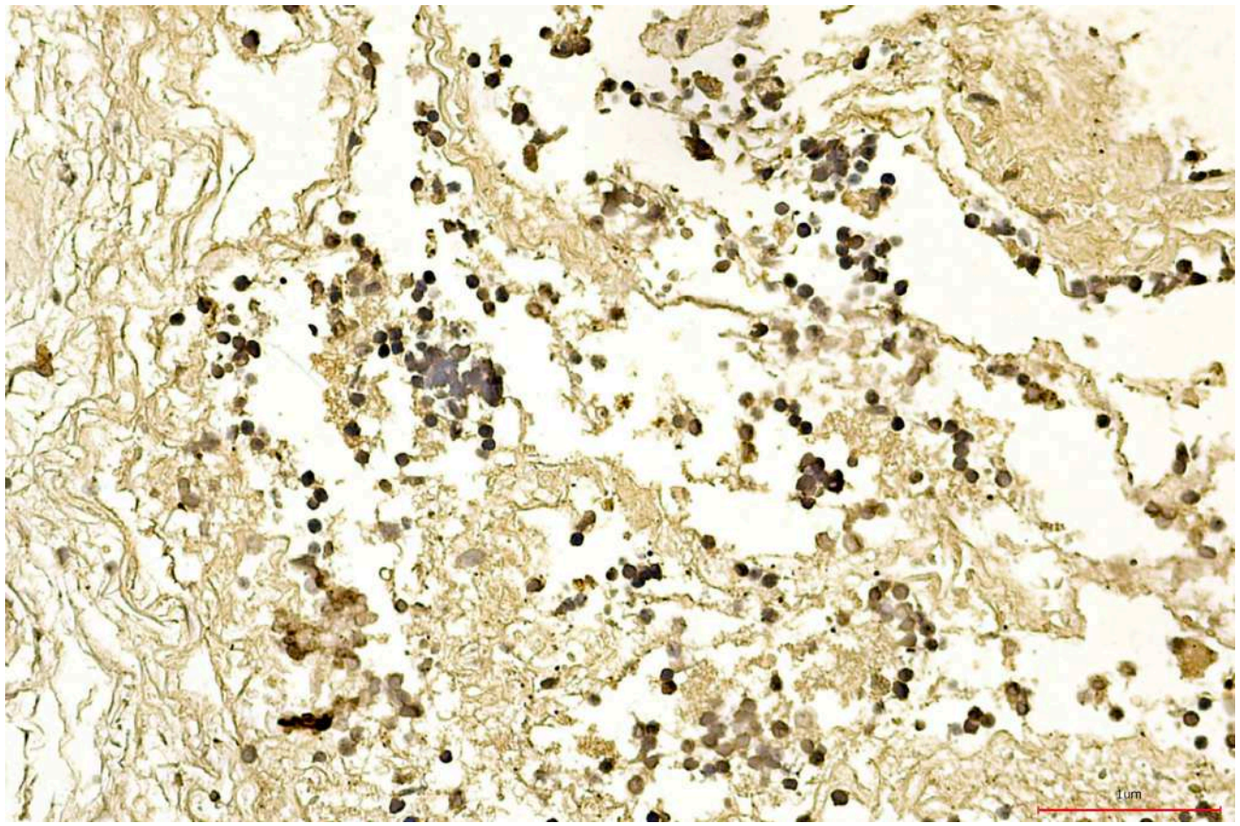
- outbreak in 2021
- stamp-out pigs that showed clinical signs or positive by qPCR
- keep healthy pig (negative by qPCR)
- ASFV seropositive
- no outbreak for 5 months
- sporadic in late 2022

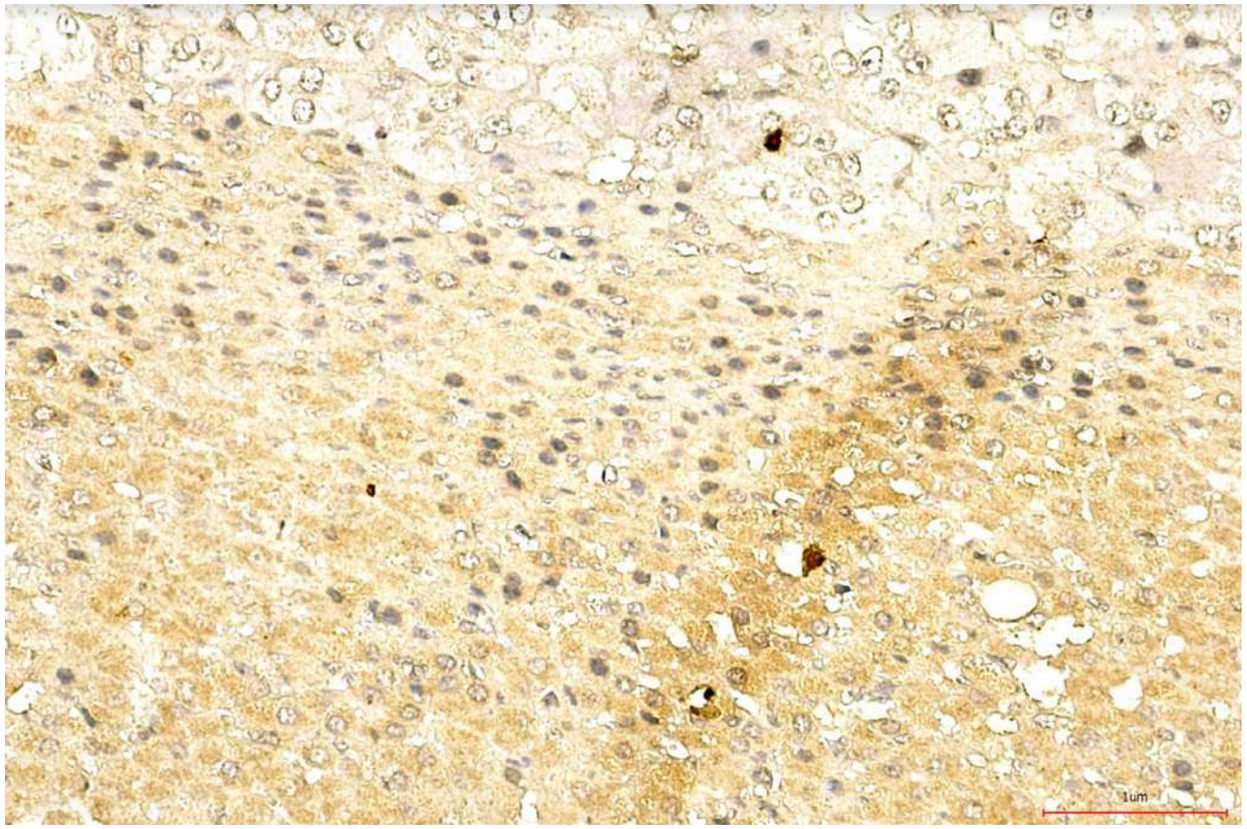
Organs	ASFV (ct.)	
	Pig 1	Pig 2
Whole blood	-	-
Tonsil 1	NA	40.12
Tonsil 2	NA	43.69
Eye ball	NA	43.69
Eye ball (optic N.)	NA	0
Eye ball (aqueous)	NA	0
Uterus (Mucosa)	NA	0
Uterus (Muscle)	43.03	0
Inguinal LN	0	0
Ovary	44.83	0
Ovary(fluid)	NA	0
Pancreas	0	0
Thymus	39.61	0
MSLN	0	0
Adrenal gl.	41.81	0
Spleen	0	0
Salivary gl.	38.42	0
ELISA	+	+

Immunohistochemistry









Vietnam

- Chronic and subclinical ASF
- ASFV persistence
- survival pig ???
- illegal attenuated vaccine ???

Thailand

- survival pig ???
- ASFV persistence ???
- Chronic and subclinical ASF ???

Farm management at present

- **Acclimatization** ↓

- Gilt management

- Re-infection/re-emerging of disease that normally used acclimatization for management e.g. *E. coli*, *Streptococcus suis*, Pasteurellosis, Glasser's disease. ↑

- Vaccination become one of important tool for management of herd immunity esp. bacteria ↑

Farm management at present

- **Sample collection** ↓

  - avoiding touching animal

  - avoiding blood collection

  - indirect sampling method e.g. oral fluid/ swab ↑

  - less invasive sampling method e.g. tail vein for blood droplet or needle aspiration for tissue ↑

Future

- Preventive surveillance/monitoring
  - Oral fluid ???
- less/non-invasive sample collection
  - multi-pathogen detection system
  - e.g. multiplex qPCR for enteric/respiratory disease
- Herd immunity in replacement sow
  - less vaccination with high immunization

Wild boar ?????

Food and Agriculture Organization of the United Nations