COUNTRY REPORT

The major swine diseases in Thailand and current diagnostic methods

Regional Workshop on Swine Disease Diagnosis

Beijing, P. R. China, 30 – 31 Oct 2019

WORLD ORGANISATION FOR ANIMAL HEALTH

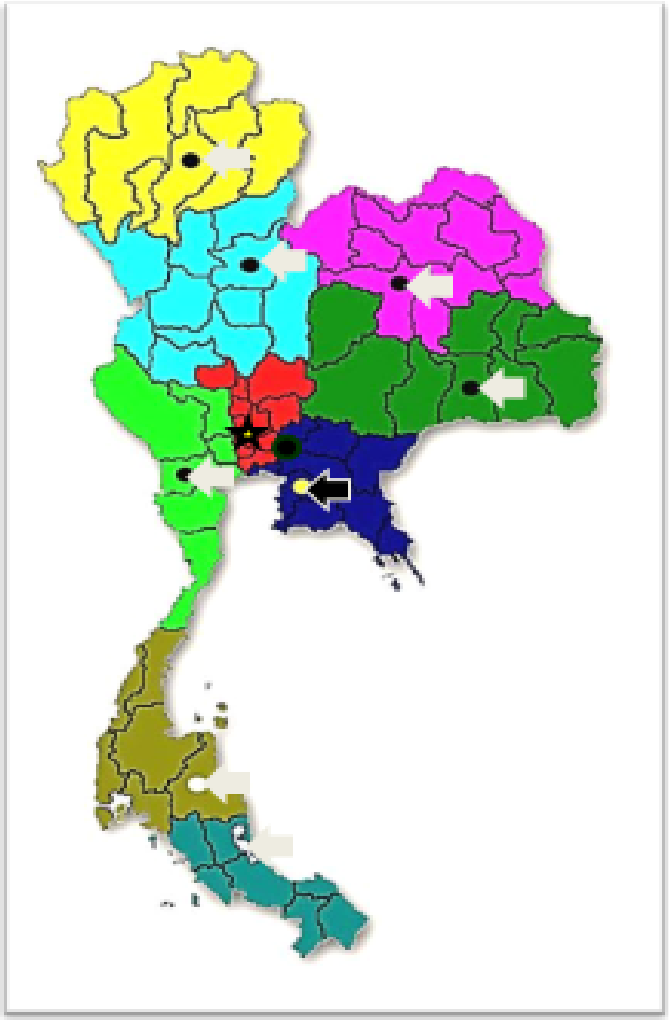
Protecting animals, preserving our future

Basic information

Basic information

Basic information

Laboratory Networks

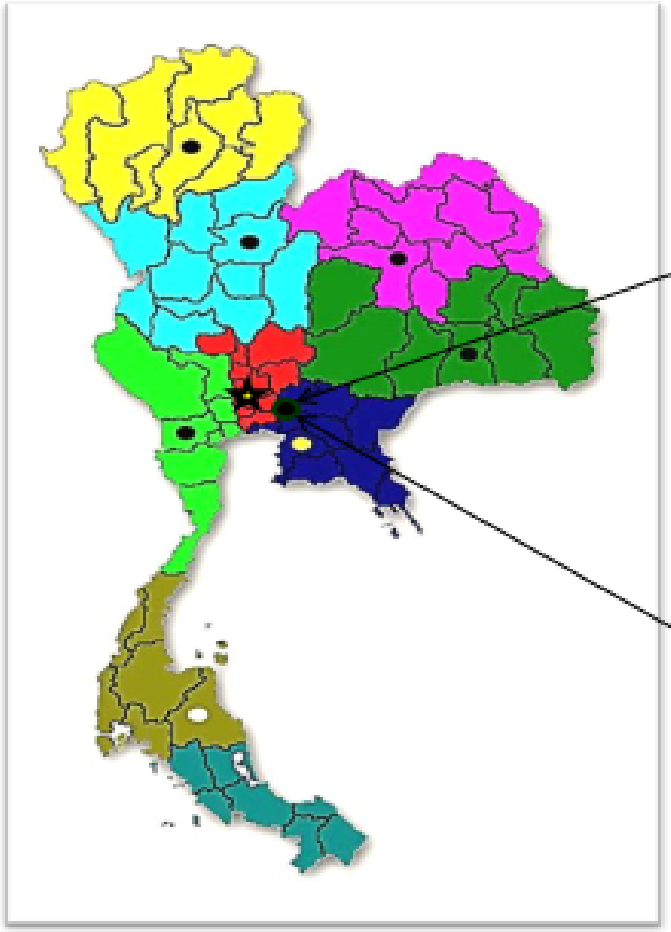


Veterinary Research and Development Center (VRDCs)

- Northern – Upper zone (Lampang)
- Northern – Lower zone (Phitsanulok)
- North East – Upper zone (Khon kaen)
- North East – Lower zone (Surin)
- Eastern – (Chonburi)
- Western – (Ratchaburi)
- Southern – Upper zone (Nakhon si thammarat)
- Southern – Lower zone (Songkhla)

Basic information

Laboratory Networks



Regional Reference Laboratory for Foot and Mouth Disease in South East Asia (RRL)

- Pakchong, Nakhon Ratchasrima

Veterinary Biologics Assay Division (VBAD)

- Pakchong, Nakhon Ratchasrima

Basic information

Backyard pig production system

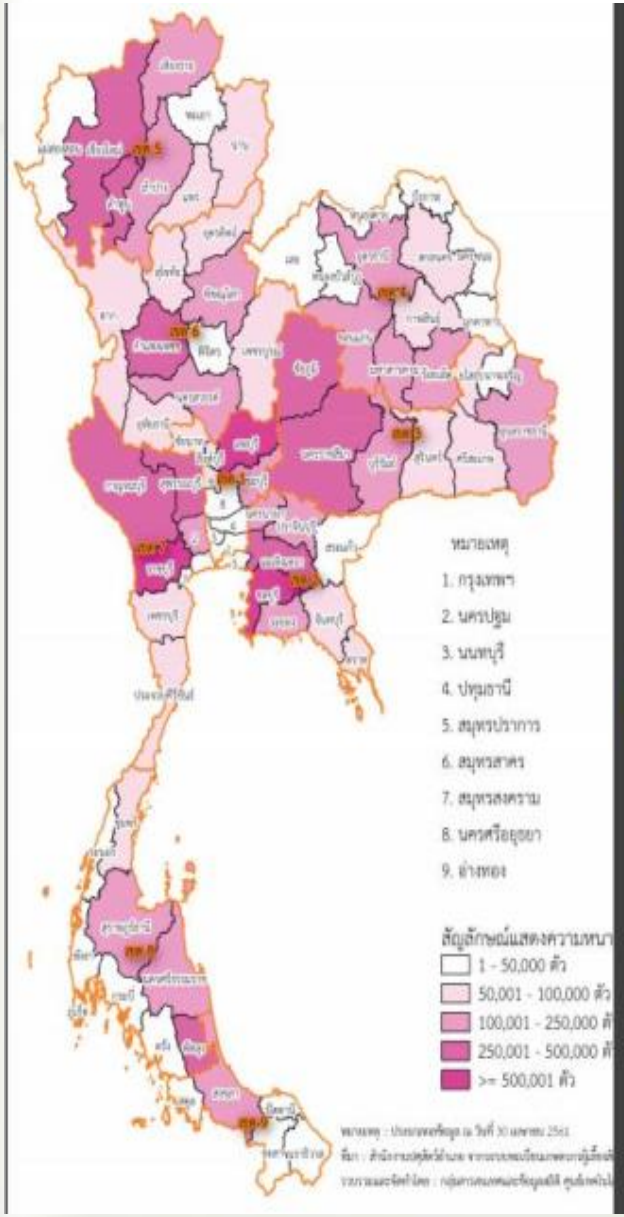
Basic information

Opened house pig production system

Basic information

Evaporative Cooling System (EVAP)

Basic information



Pig farm information

- 14.1 million heads
- 210,978 households

Farm size	Total no. of farm
1-50 pigs	172,735 (93.51%)
51-500 pigs	9,197 (4.98%)
501-5000 pigs	2,539 (1.37%)
>5000 pigs	246 (0.13%)

Source: DLD, 2019

Updates on disease situation

- Situation of major swine disease
  - Porcine Reproductive and Respiratory Syndrome (PRRS)
  - Classical Swine Fever (CSF)
  - Porcine Epidemic Diarrhea (PED)
  - African Swine Fever (ASF)

Porcine Reproductive and Respiratory Syndrome (PRRS)

Epidemiology of PRRS in Thailand

- Seropositive animals could be traced back to as early as 1989.
- In 1996, the first Thai PRRSV was isolated and was identified as Type 2 PRRSV (Damrongwatanapokin, S. et al 1996)
- A few years later, Type 1 PRRSV was also reported in Thailand, and some was found to be co-circulating with Type 2 PRRSV within the same herd.

  (Thanawongnuwech, R. et al 2004)

Virus Research 154 (2010) 7–17

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Review

Molecular epidemiology of PRRSV: A phylogenetic perspective

Mang Shi[a], Tommy Tsan-Yuk Lam[a], Chung-Chau Hon[a], Raymond Kin-Hei Hui[a], Kay S. Faaberg[b], Trevor Wennblom[c], Michael P. Murtaugh[d], Tomasz Stadejek[e], Frederick Chi-Ching Leung[a,*]

[a] *School of Biological Sciences, The University of Hong Kong, Hong Kong, China*
[b] *Virus and Prion Research Unit, National Animal Disease Center, USDA, ARS, Ames, IA, USA*
[c] *Supercomputing Institute, Minneapolis, University of Minnesota, MN, USA*
[d] *Department of Veterinary and Biomedical Sciences, St. Paul, University of Minnesota, MN, USA*
[e] *National Veterinary Research Institute, Department of Swine Diseases, OIE Reference Laboratory for PRRS, Pulawy, Poland*

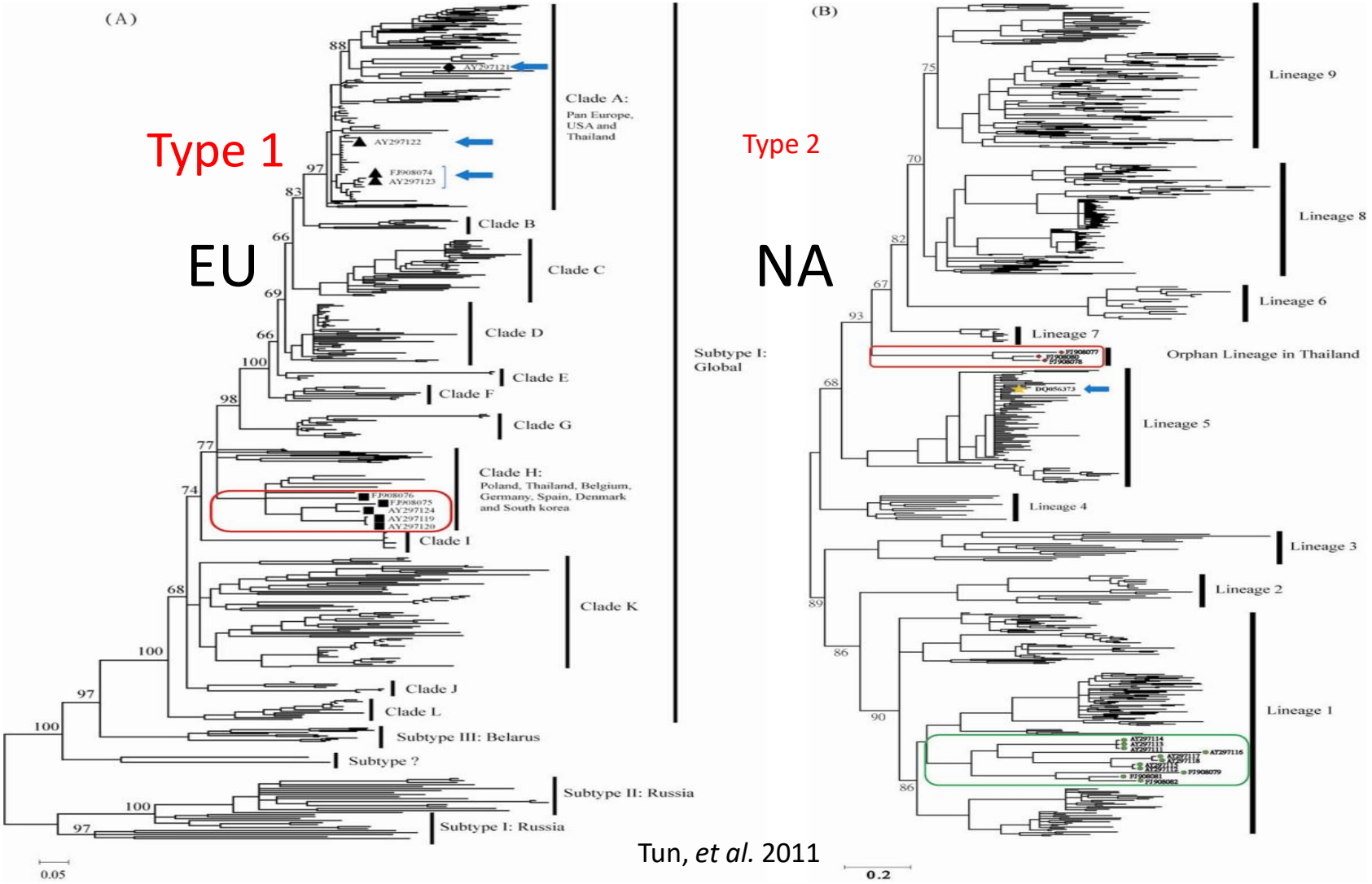
Tun *et al. Virology Journal* 2011, **8**:164
http://www.virologyj.com/content/8/1/164

VIROLOGY JOURNAL

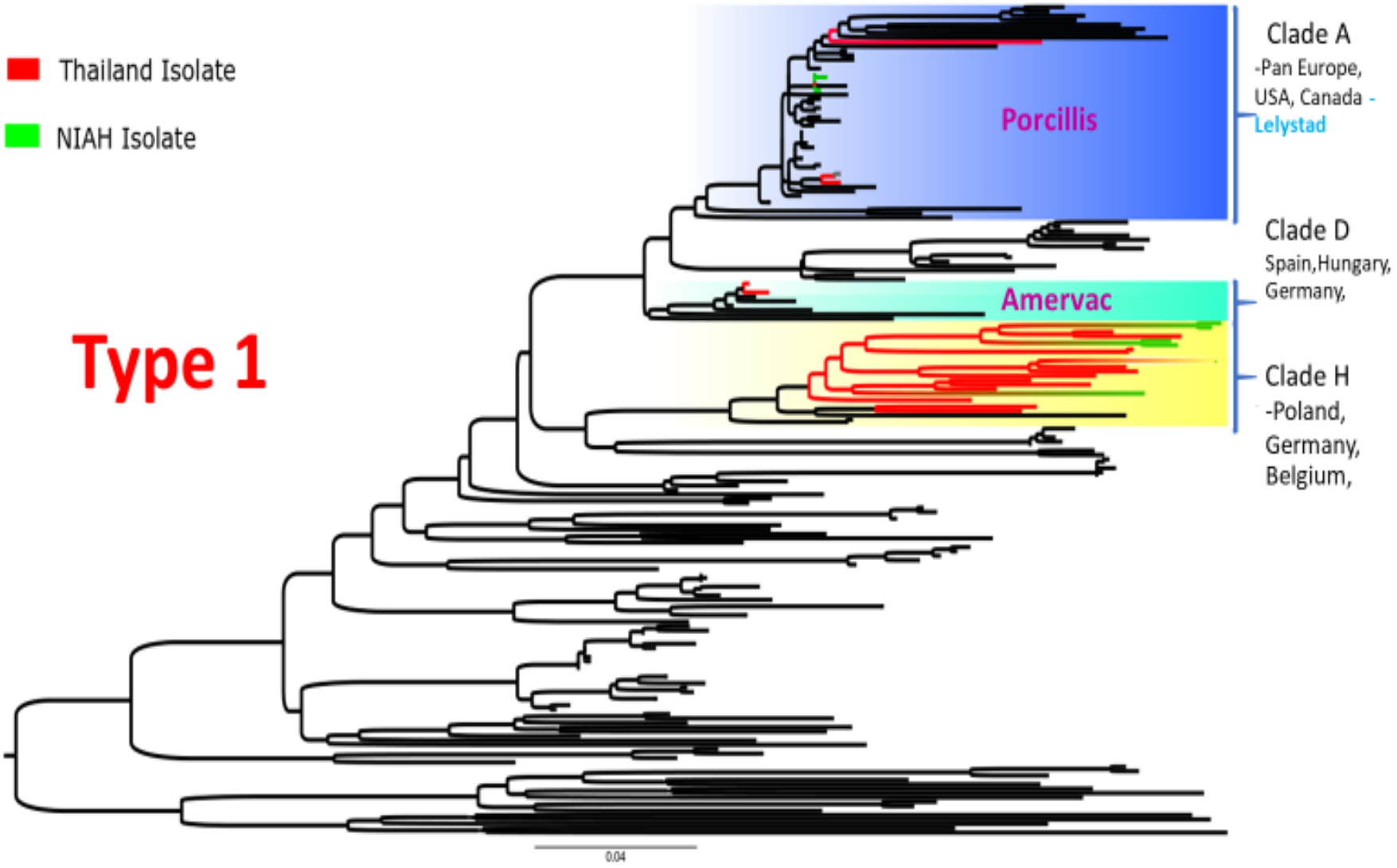
SHORT REPORT Open Access

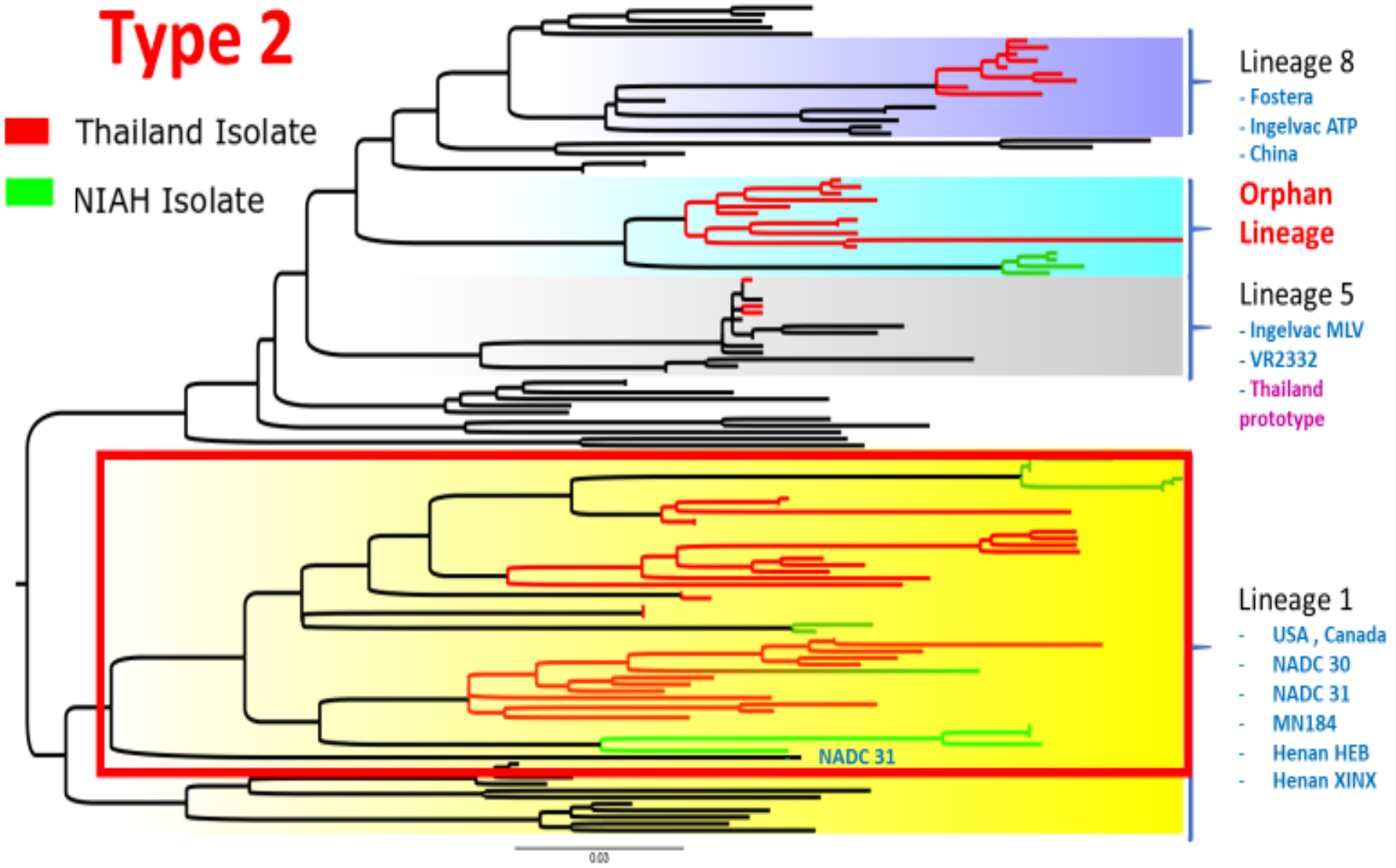
Genetic diversity and multiple introductions of porcine reproductive and respiratory syndrome viruses in Thailand

Hein M Tun[1], Mang Shi[1], Charles LY Wong[1], Suparlark NN Ayudhya[2], Alongkorn Amonsin[2], Roongroje Thanawonguwech[2] and Frederick CC Leung[1*]



Tun, *et al.* 2011


Thailand Isolate
NIAH Isolate
Type 1
Porcillis
Amervac
Clade A
-Pan Europe,
USA, Canada -
Lelystad
Clade D
Spain,Hungary,
Germany,
Clade H
-Poland,
Germany,
Belgium,
0.04


Type 2
Thailand Isolate
NIAH Isolate
Lineage 8
- Fostera
- Ingelvac ATP
- China
Orphan Lineage
Lineage 5
- Ingelvac MLV
- VR2332
- Thailand prototype
Lineage 1
- USA , Canada
- NADC 30
- NADC 31
- MN184
- Henan HEB
- Henan XINX
NADC 31
0.03

Porcine Reproductive and Respiratory Syndromes

Genotype	Clade/Lineage
Type 1 (European)	Clade A, Clade D, Clade H
Type 2 (North America)	L1, L5, L8, Orphanage Lineage

*Classification of PRRS isolate based on Shi et al. (2010) and Tun et al. (2011).

Laboratory diagnosis

- Multiplex qRT-PCR
- PRRSV primers and probes sequence

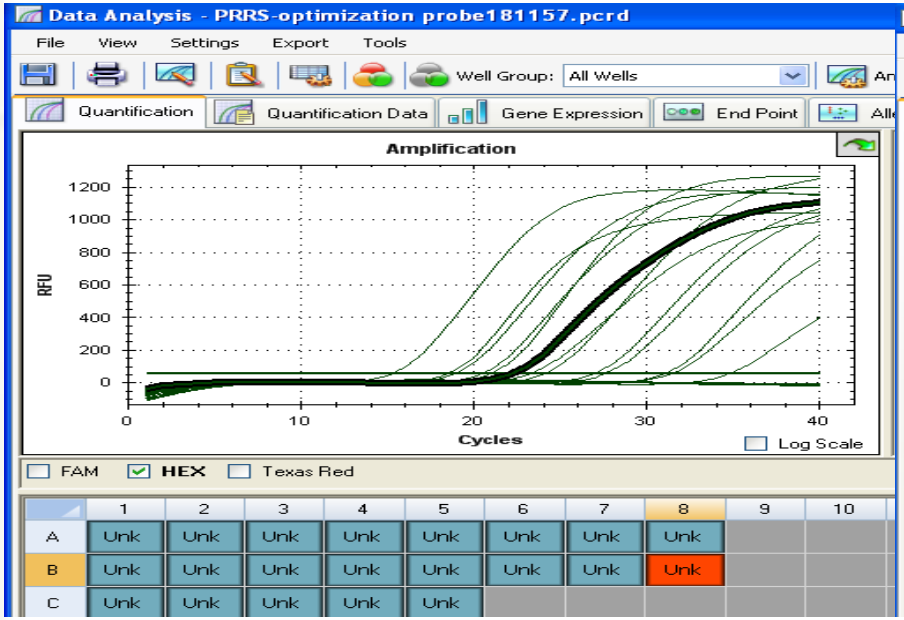
➢ Eu Fw 5’ GAT GAC RTC CGG CAY C 3’
➢ Eu Rev 5’ CAG TTC CTG CGC CTT GAT 3’
➢ Eu probe 5’ Fam-TGC AAT CGA TCC AGA CGG CTT-BHQ1

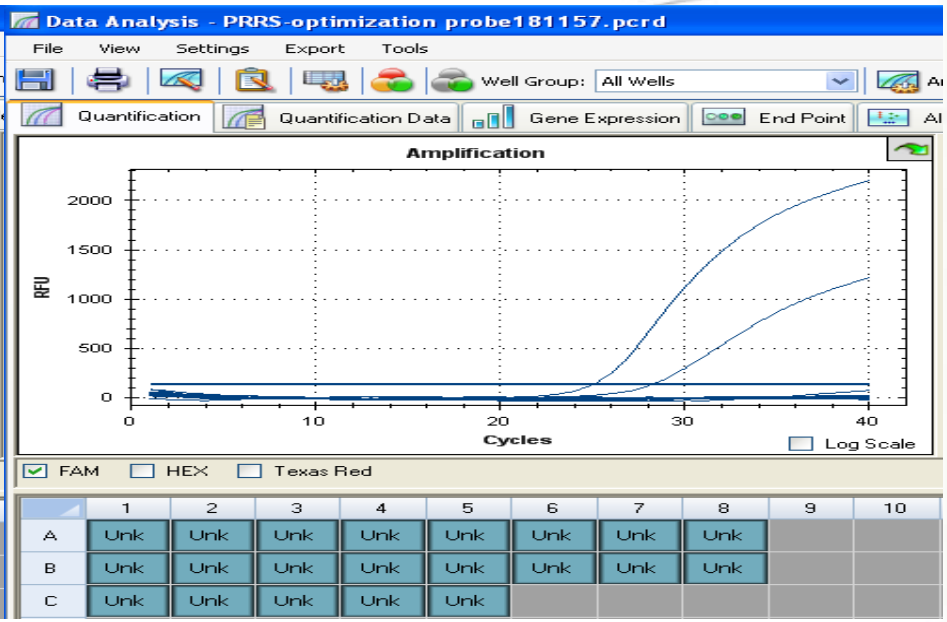
➢ NA_Fw 5' ATR ATG RGC TGG CAT TC 3'
➢ NA_Rev 5' ACA CGG TCG CCC TAA TTG 3'
➢ NA_probe Hex-TGT GGT GAA TGG CAC TGA TTG ACA-BHQ1

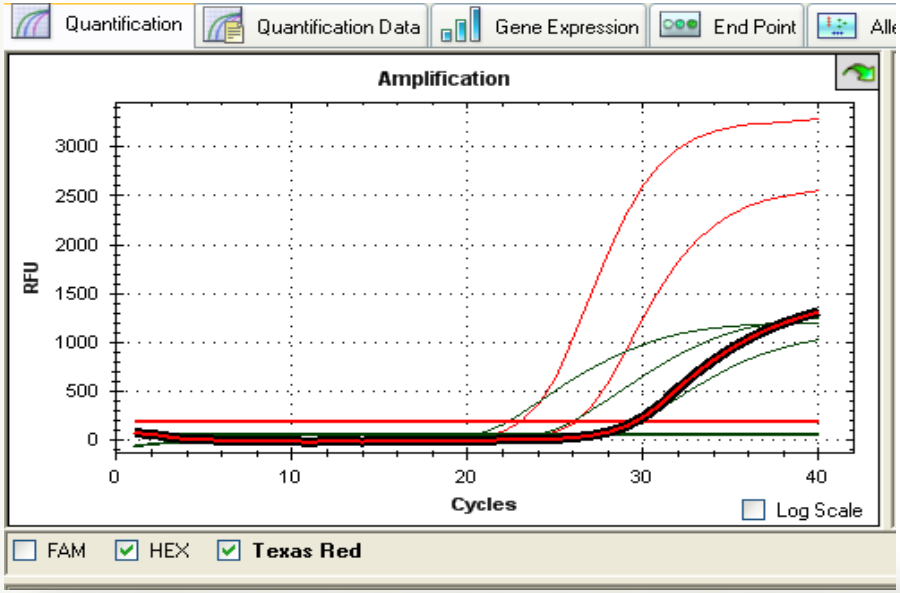
Kleiboeker SB, 2005

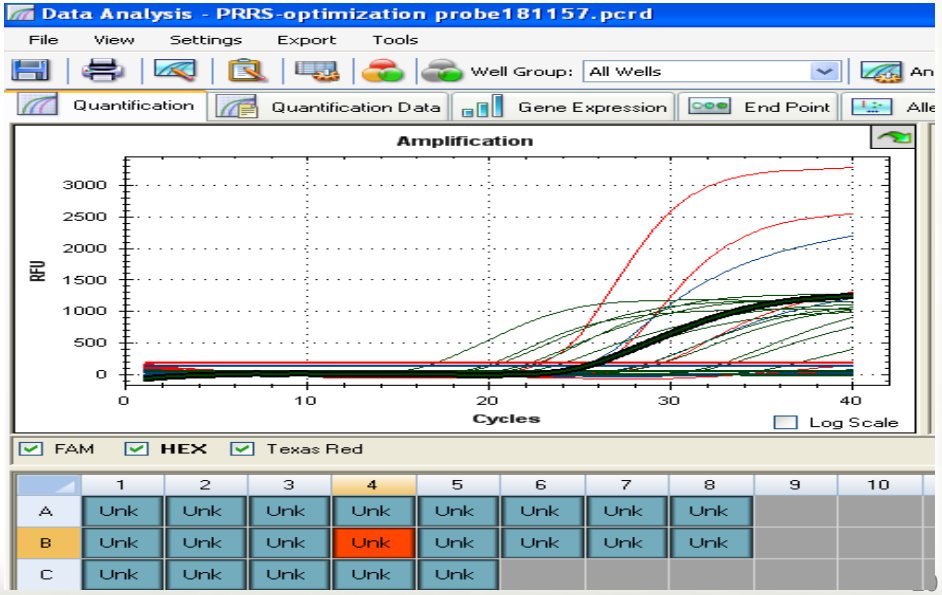
➢ CN_Fw 5’CCC AAG CTG ATG ACA CCT TTG3’
➢ CN_Rev 5’AAT CCA GAG GCT CAT CCT GGT3’
➢ CN_Probe Texas red-5’CGC GTA GAA CTG TGA CAA CAA CGC TGA3’-BHQ2

Laboratory diagnosis







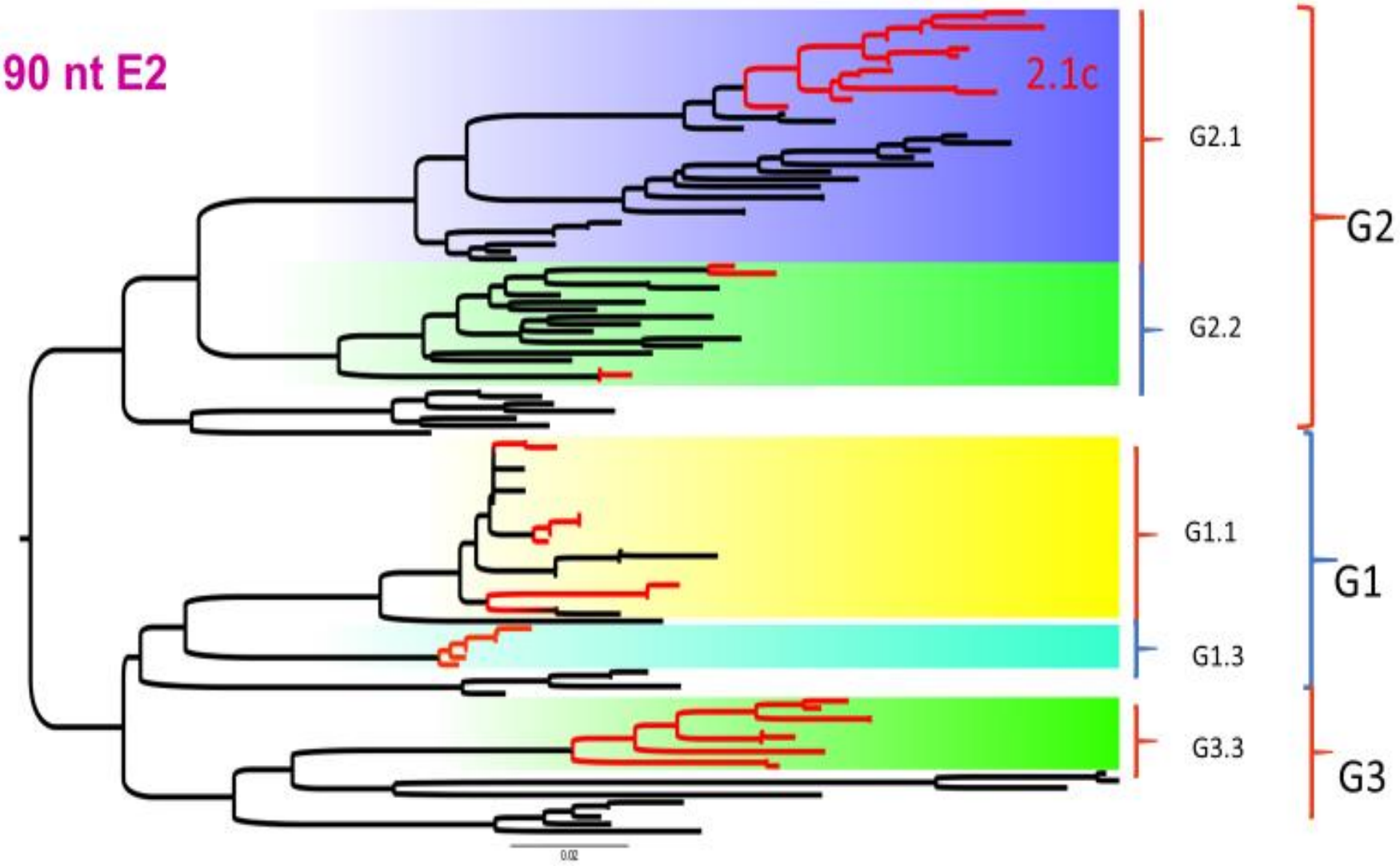


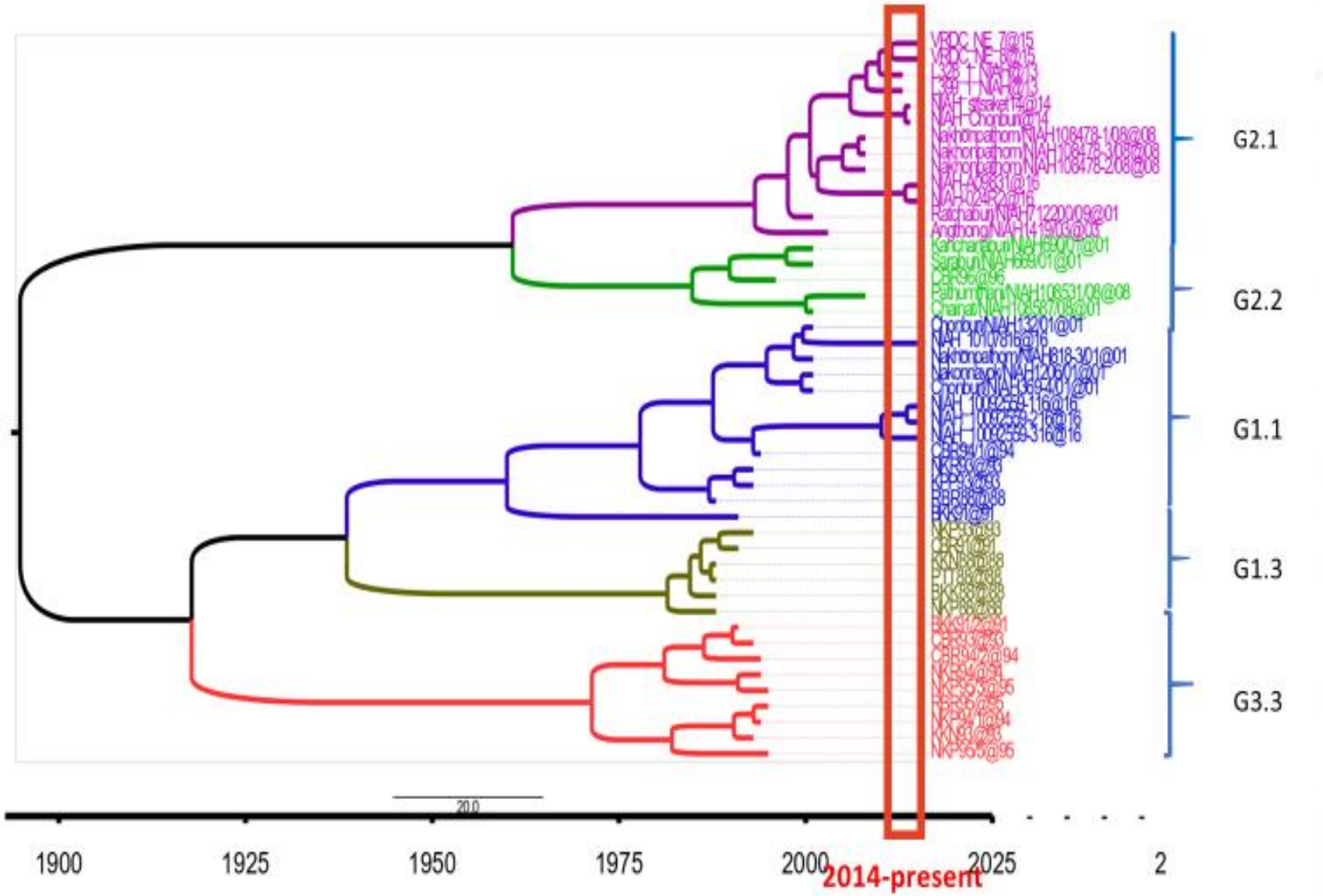
Classical Swine Fever (CSF)

Epidemiology of Classical Swine Fever

- The first report case in Thailand occurred in 1950 in Bang ken district, Bangkok.
- Thailand genogroup.

1. Genotype 1 (subgenotype 1.1 and 1.3)
2. Genotype 2 (subgenotype 2.1 and 2.2)
3. Genotype 3 ((subgenotype 3.3)


190 nt E2
2.1c
G2.1
G2.2
G1.1
G1.3
G3.3
G2
G1
G3
0.02


G2.1
G2.2
G1.1
G1.3
G3.3
20.0
1900
1925
1950
1975
2000
2025
2
2014-present

Classical Swine Fever

Genogroup	Period
1.1	**1988-present**
1.3	1988-1993
2.1	**2001- present**
2.2	1996-2008
3.3	1991-1996

Realtime PCR method

ELSEVIER

Journal of Virological Methods
Volume 130, Issues 1–2, December 2005, Pages 36-44

Validation of a real-time RT-PCR assay for sensitive and specific detection of classical swine fever

B. Hoffmann [a], M. Beer [a,*], C. Schelp [b], H. Schirrmeier [a], K. Depner [a]

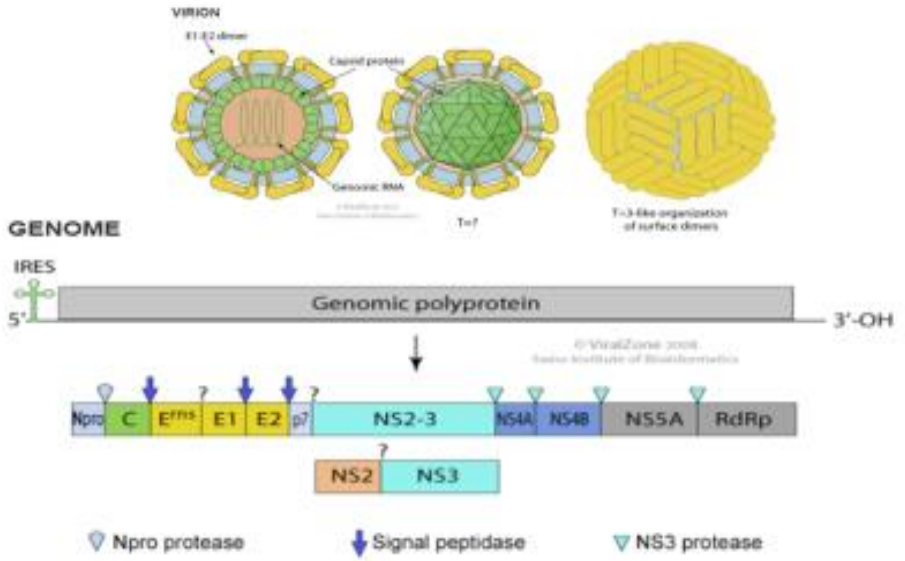
Show more

https://doi.org/10.1016/j.jviromet.2005.05.030

CHAPTER 3.8.3.

CLASSICAL SWINE FEVER (INFECTION WITH CLASSICAL SWINE FEVER VIRUS)

Classical Swine Fever (CSF)

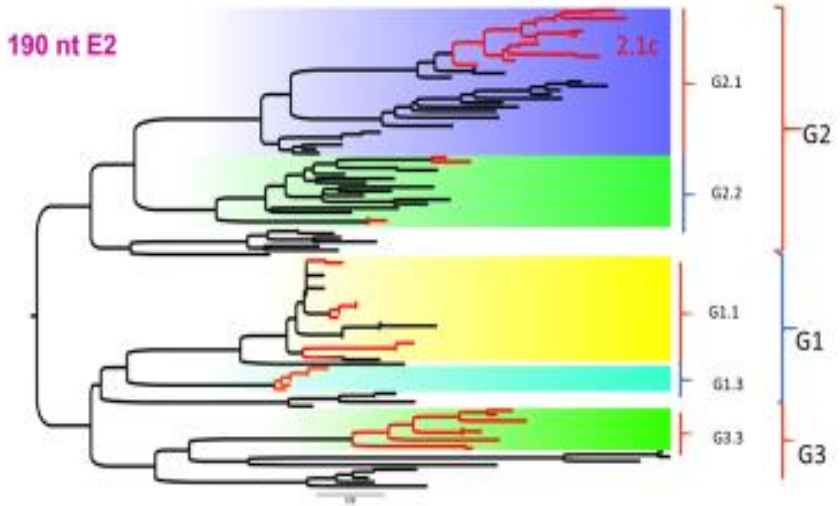


150 nt 5' UTR 190 nt E2 409 nt NS5B

Genetic typing of classical swine fever virus

D.J. Paton[a,*], A. McGoldrick[a], I. Greiser-Wilke[b], S. Parchariyanon[c], J.-Y. Song[d], P.P. Liou[e], T. Stadejek[f], J.P. Lowings[a], H. Björklund[g,1], S. Belák[g]

[a]Veterinary Laboratories Agency – Weybridge, Addlestone, Surrey KT15 3NB, UK
[b]Institute of Virology, Veterinary School Hannover, Buenteweg 17, 30559 Hannover, Germany
[c]National Institute of Animal Health, Bangkhen, Bangkok 109000, Thailand
[d]National Veterinary Research and Quarantine Service, 480 Anyang 6 Dong, Anyang 430-016, South Korea
[e]Taiwan Animal Health Research Institute, 376 Chung-Cheng Road, Tanzu, Taipei 25101, Taiwan
[f]National Veterinary Research Institute, P-24-100 Pulawy, Poland
[g]Department of Virology, National Veterinary Institute, Biomedical Center, PO Box 585, S-751 23 Uppsala, Sweden



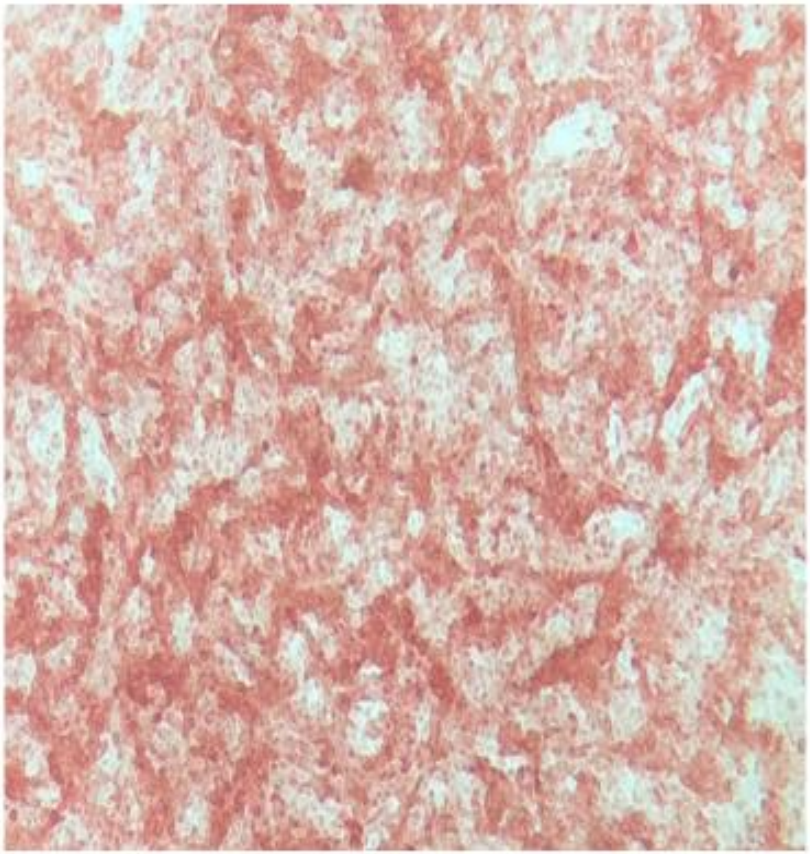
Molecular characterization

One serogroup

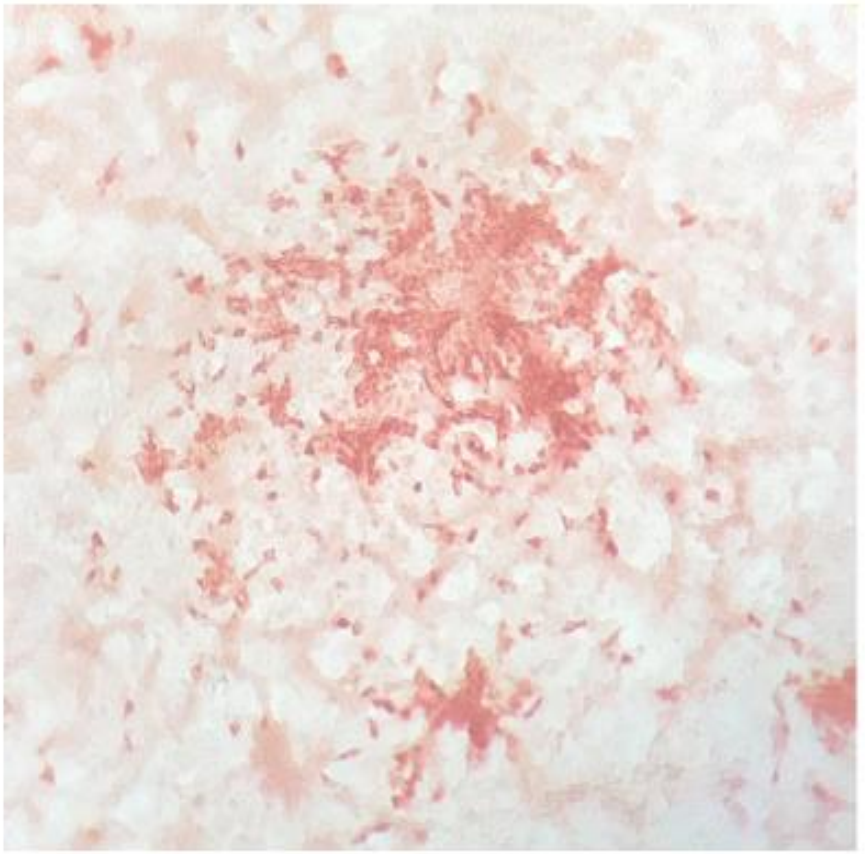
NPLA
IPMA

Immunoperoxidase monolayer assay (IPMA)

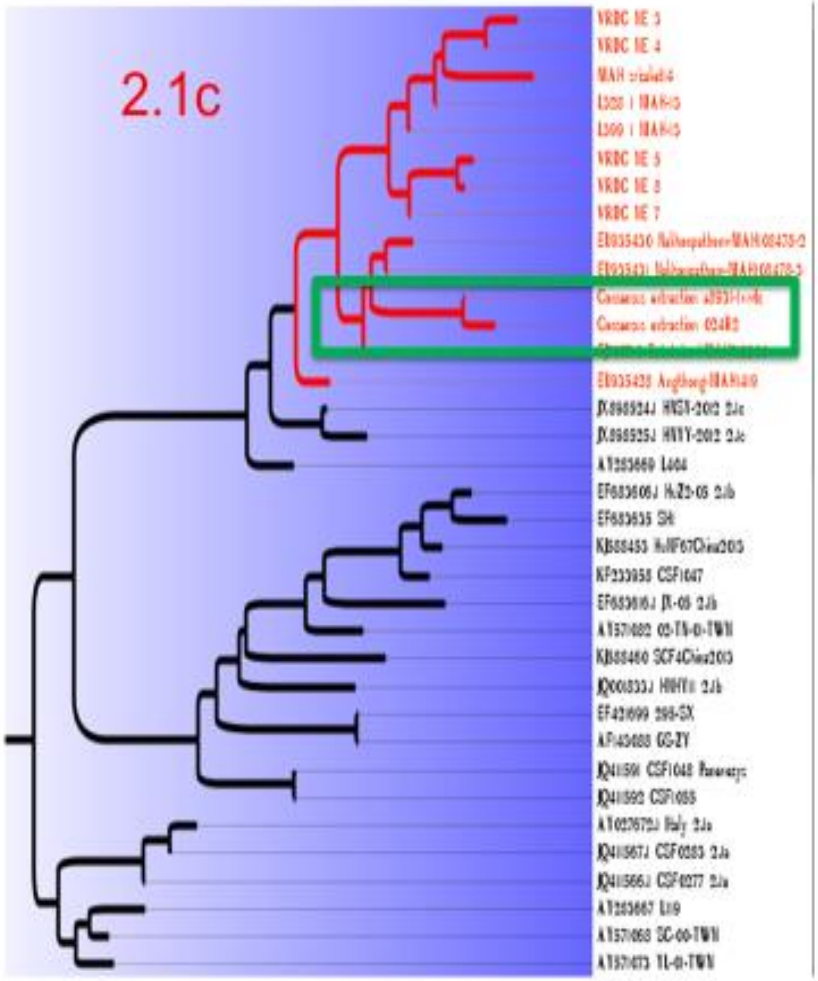
KAM PAENGPHET (1.1)

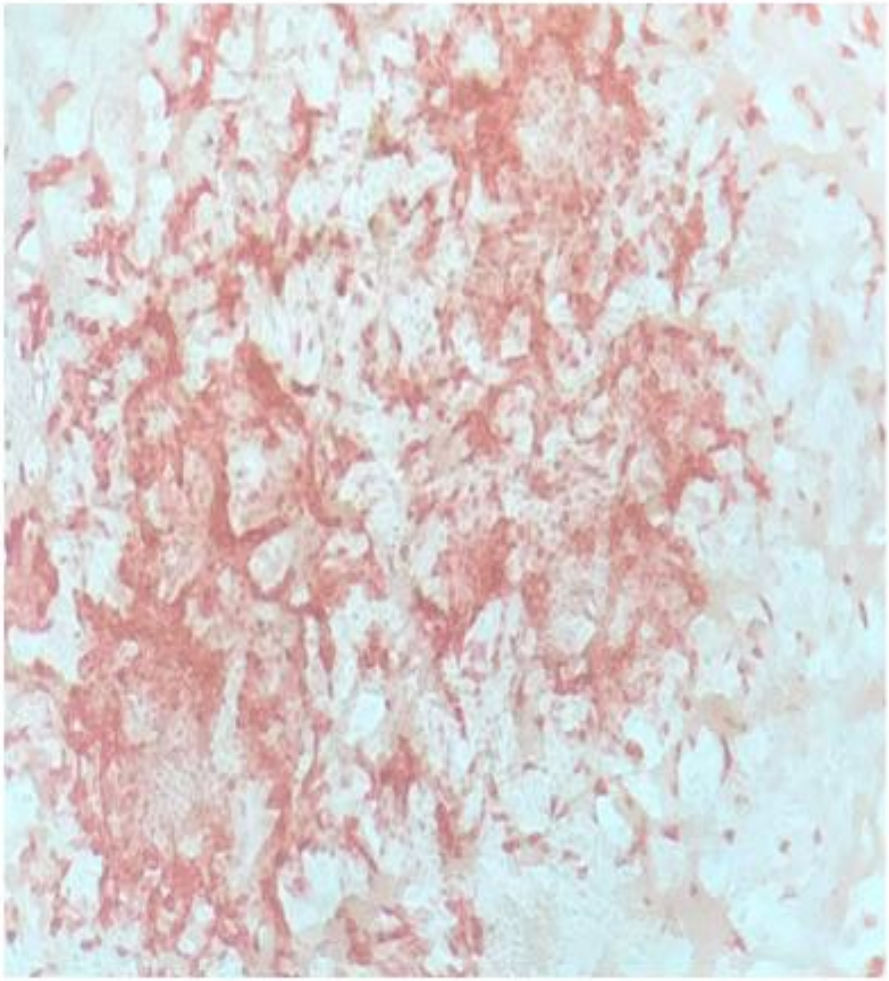


CBR 94/2 (3.3)



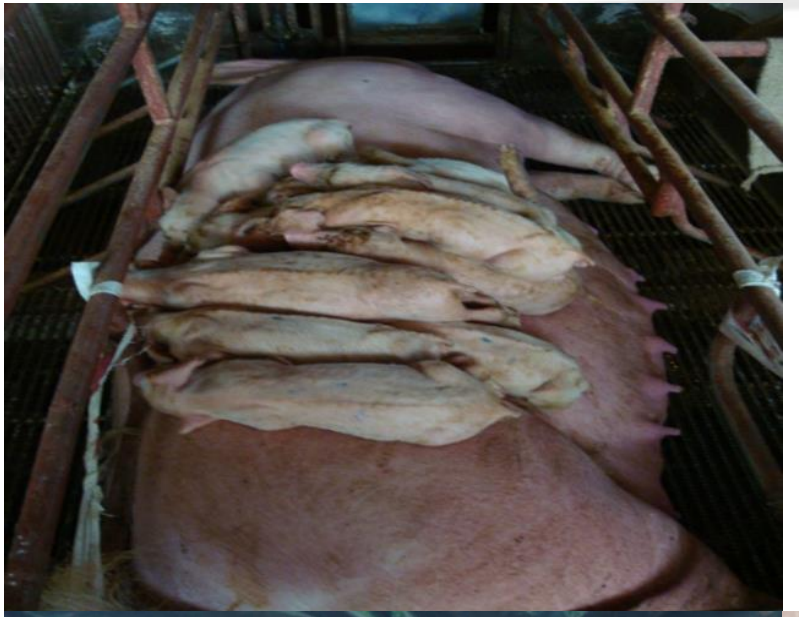
Immunoperoxidase monolayer assay (IPMA)

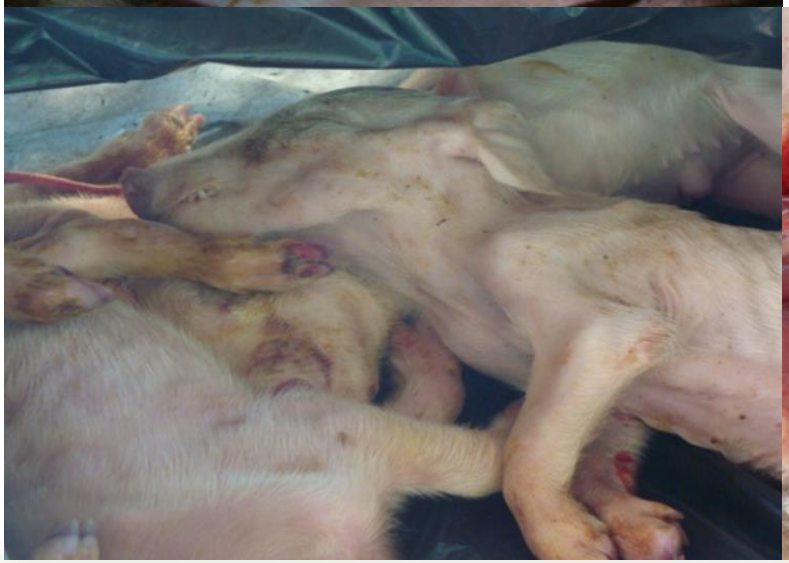


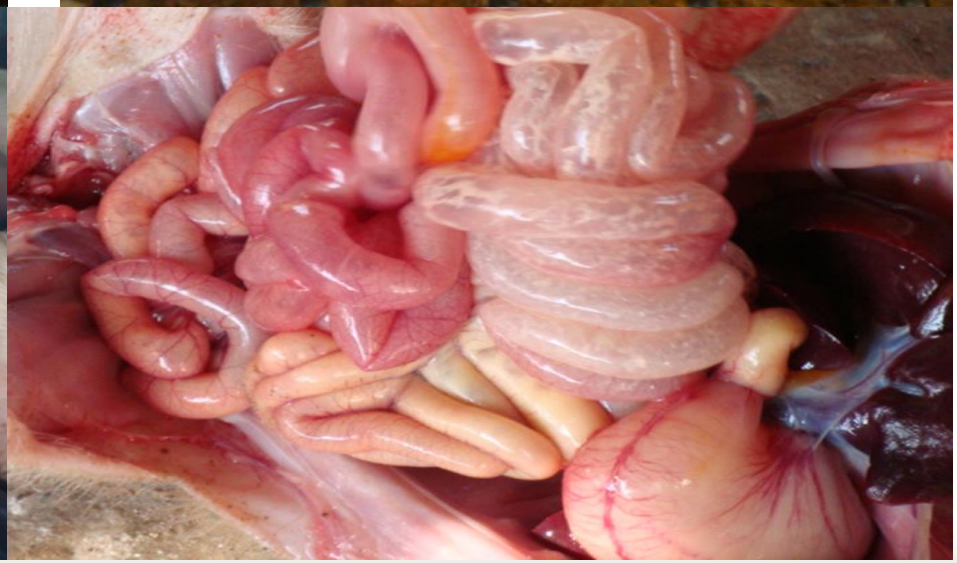


Porcine Epidemic Diarrhea (PED)

Porcine Epidemic Diarrhea







Laboratory diagnosis

- Real time PCR

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Development of a multiplex TaqMan probe-based real-time PCR for discrimination of variant and classical porcine epidemic diarrhea virus

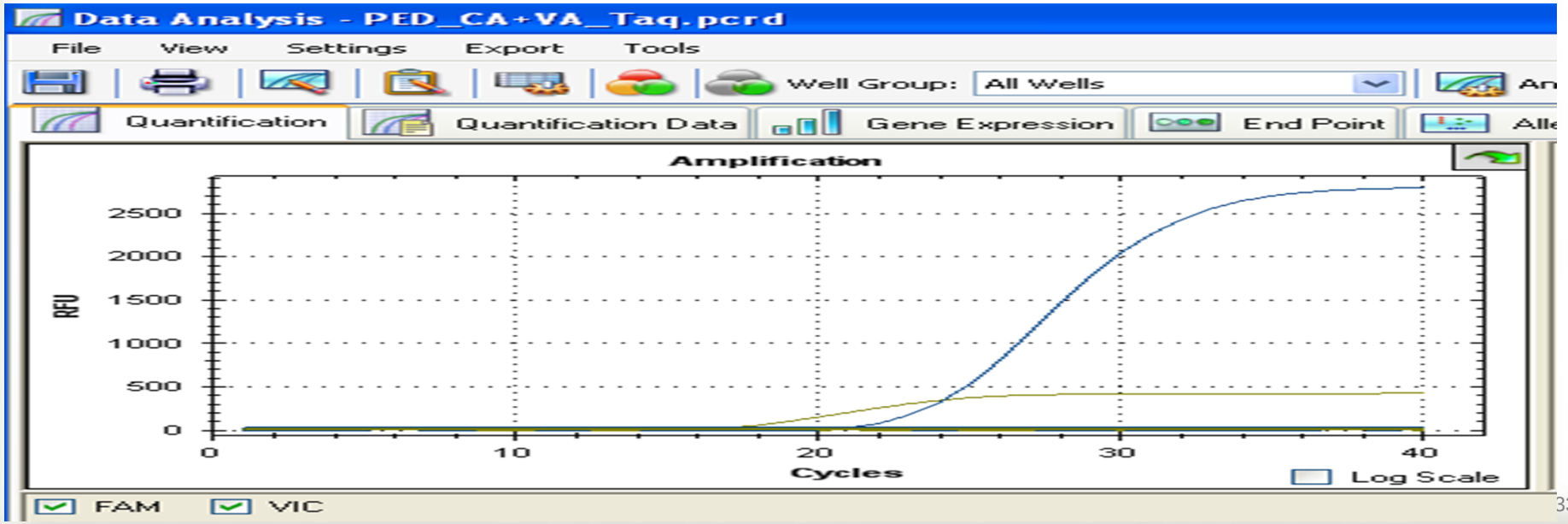
Pan-deng Zhao[a], Juan Bai[a], Ping Jiang[a,*], Tai-shan Tang[b], Yufeng Li[a], Chen Tan[a], Xiaoli Shi[a]

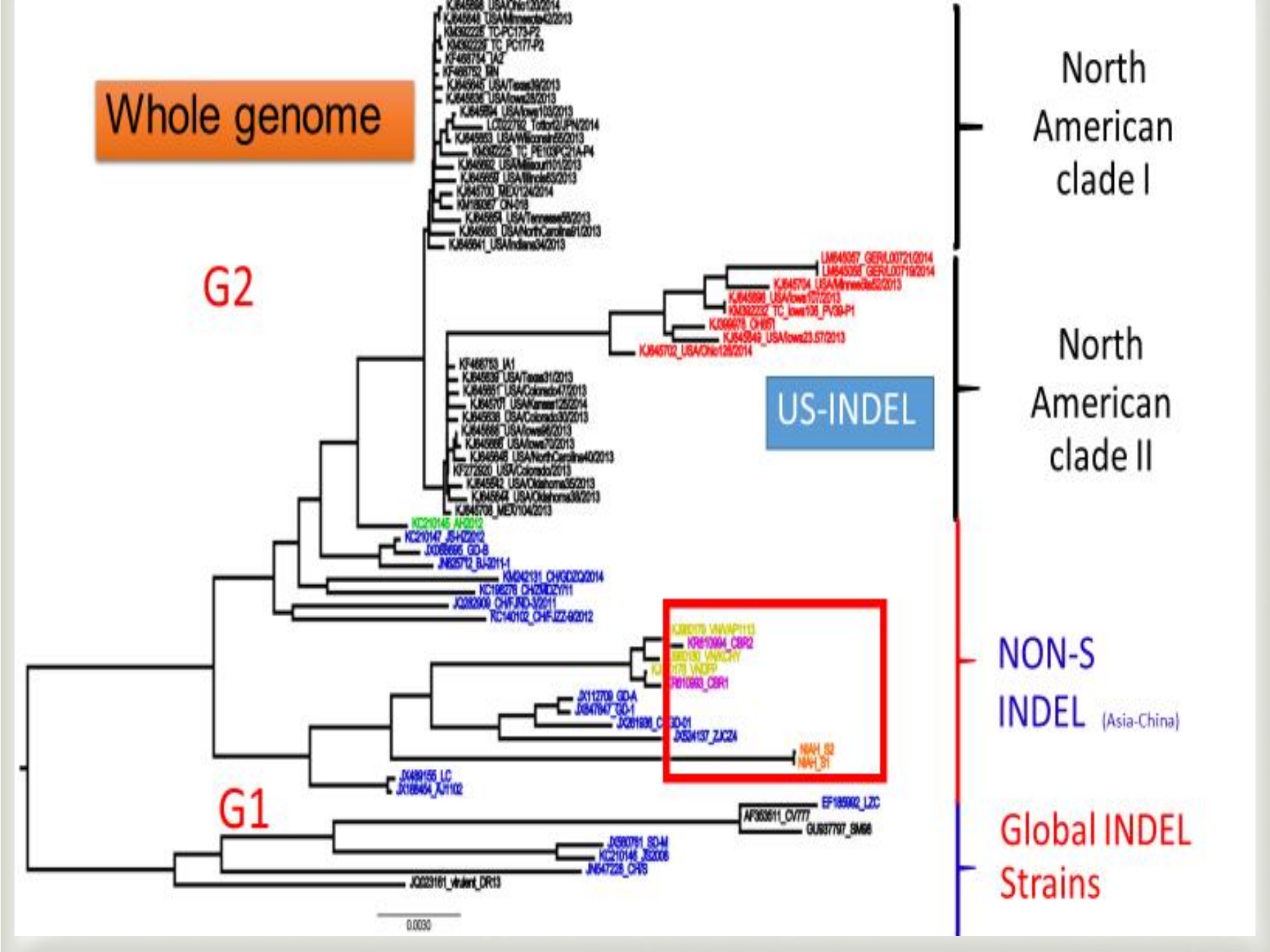
[a] *Key Laboratory of Animal Diseases Diagnostic and Immunology, Ministry of Agriculture, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China*

[b] *Jiangsu Entry–Exit Inspection and Quarantine Bureau, Nanjing 200001, China*

Primer and Probe

Primer name	Primer sequence (5′–3′)	Position	Application	Product size (bp)
F-V	GTTGTACTGGGCGGTTATCT	139–158[a]	TaqManRT-PCR	98
R-V	CCATGAACGCCACTAGCAGT	236–217[a]		
V-Probe	VIC-TGGTACTGTGCTGGCCAACATCCA-BHQ1	193–216[a]		
F-C	GTCGTTGTTTTGGGTGGTTA	136–155[b]		86
R-C	CCATGAACGCCACTATCAGT	224–205[b]		
C-Probe	FAM-TAGCTGGTACTGTGGCACAGGCATTG-BHQ1	177–202[b]		

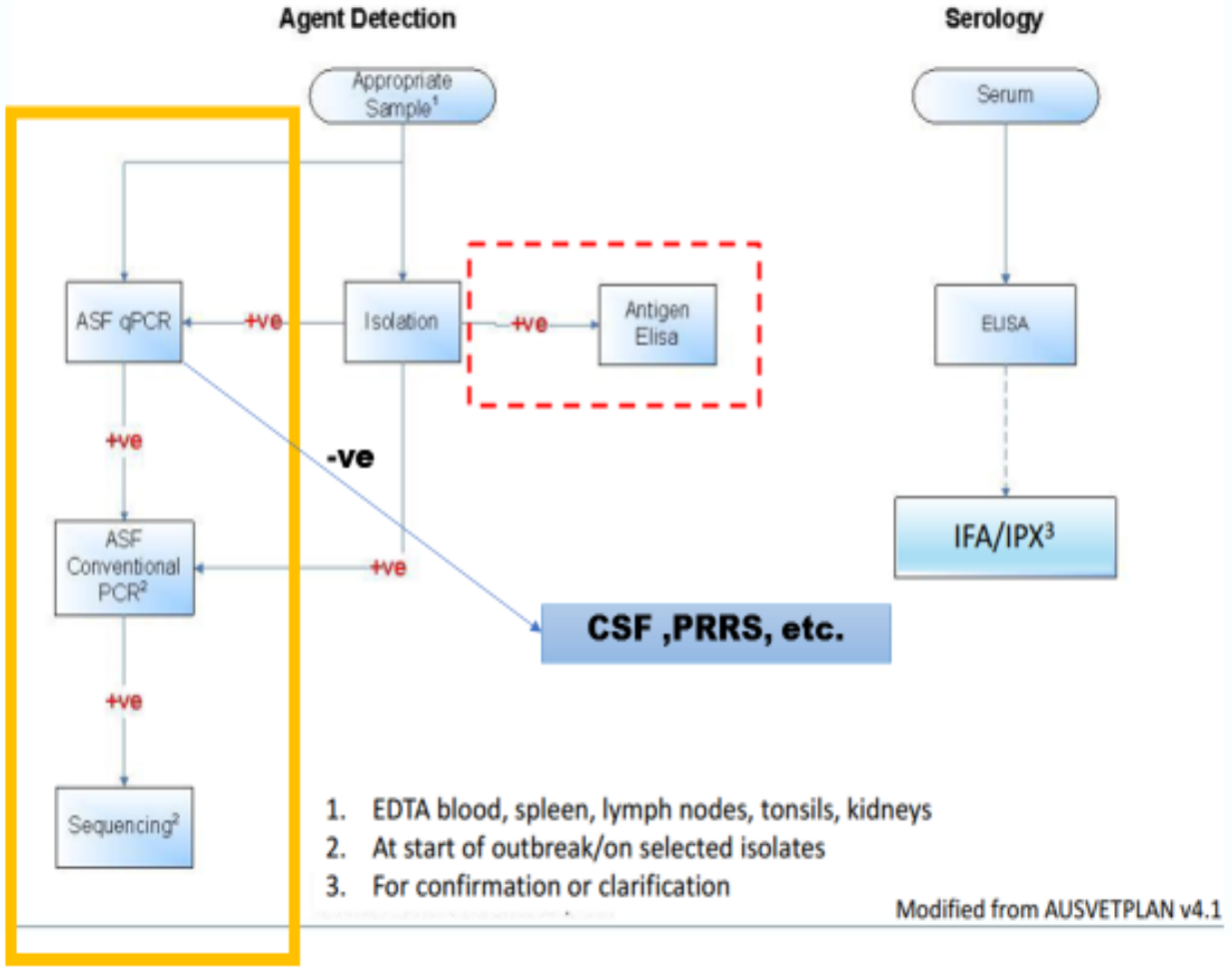


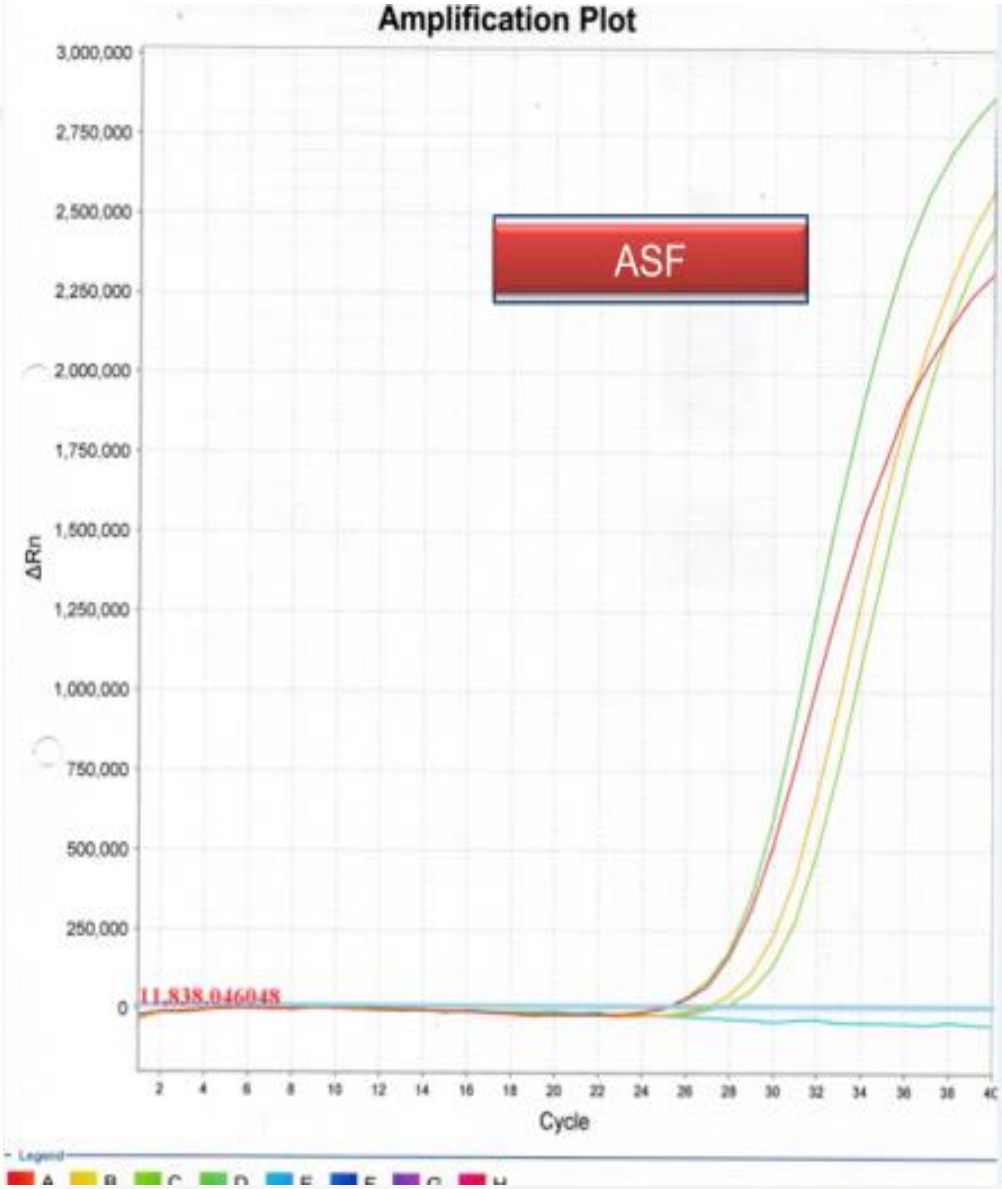

Whole genome
G2
G1
US-INDEL
North American clade I
North American clade II
NON-S INDEL (Asia-China)
Global INDEL Strains
0.0030

African Swine Fever (ASF)

Type of sample

Diagnostic algorithm





Primer and Probe (King *et al*,2003)

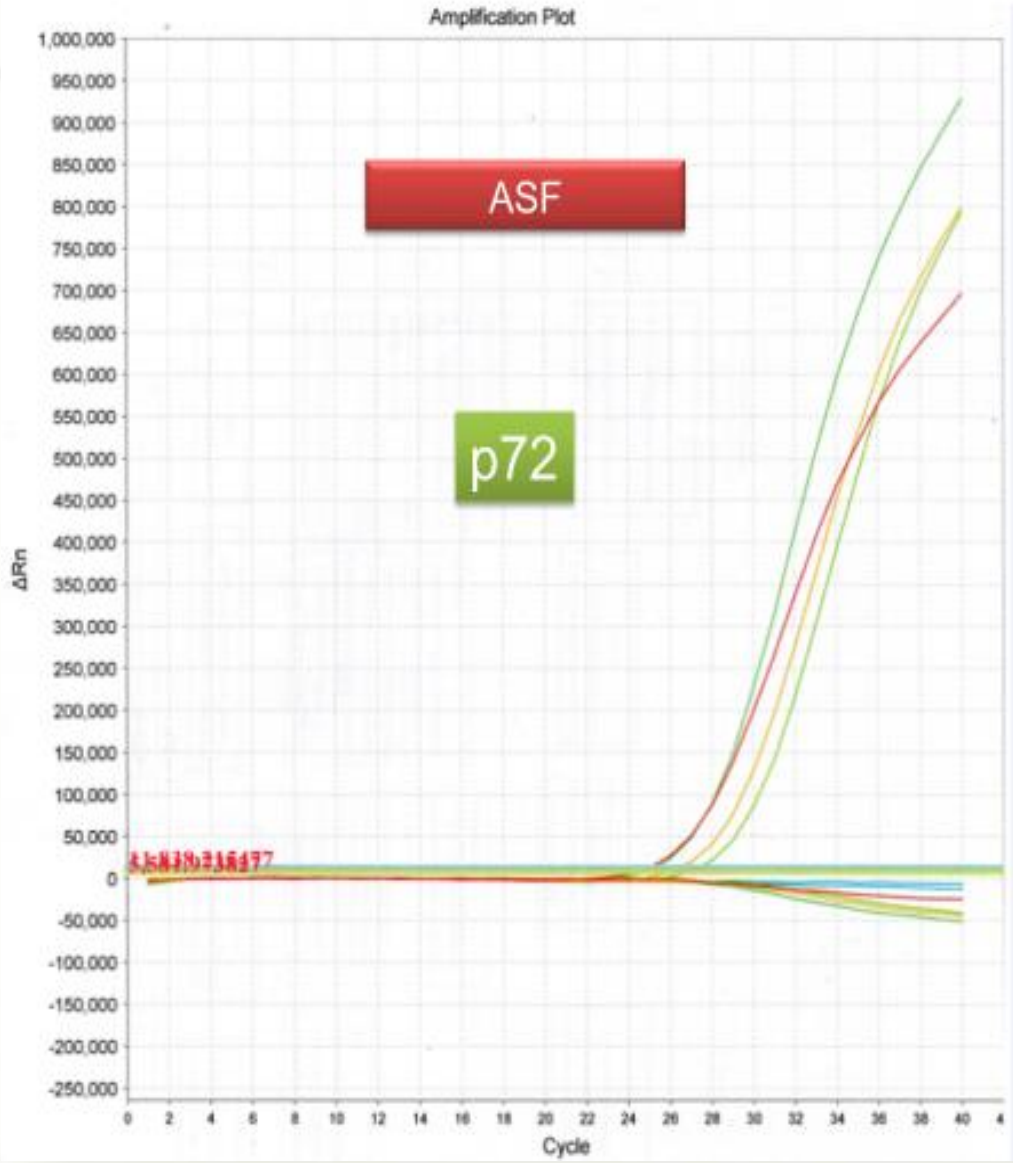
ASF Forward	5'-CTGCT-CATGG-TATCA-ATCTT-ATCGA-3'
ASF Reward	5'-GATAC-CACAA-GATC(AG)- GCCGT-3'
ASFV Probe	[FAM]- CCACG-GGAGG-AATAC-CAACC-CAGTG-3'-TAMRA

B646L gene

Product size = 250bp

p72

African swine fever-SY18



Transboundary and Emerging Diseases

Transboundary and Emerging Disease

ORIGINAL ARTICLE

Molecular Diagnosis of African Swine Fever by a New Real-Time PCR Using Universal Probe Library

J. Fernández-Pinero[1], C. Gallardo[1], M. Elizalde[1], A. Robles[1], C. Gómez[1], R. Bishop[2], L. Heath[3], E. Couacy-Hymann[4], F. O. Fasina[5], V. Pelayo[1], A. Soler[1] and M. Arias[1]

[1] Centro de Investigación en Sanidad Animal (CISA-INIA), Madrid, Spain

[2] International Livestock Research Institute (ILRI), Nairobi, Kenya

[3] ARC-Onderstepoort Veterinary Institute, Transboundary Animal Diseases Programme, Pretoria, South Africa

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Protocols

Sensitive detection of African swine fever virus using real-time PCR with a 5′ conjugated minor groove binder probe

John McKillen[a,*], Michael McMenamy[b], Bernt Hjertner[b], Francis McNeilly[a], Åse Uttenthal[c], Carmina Gallardo[d], Brian Adair[a], Gordon Allan[a]

[a] Veterinary Sciences Division, Agri-Food and Biosciences Institute, Stormont, Belfast BT4 3SD, United Kingdom
[b] Department of Veterinary Science, Queen's University of Belfast, Stormont, Belfast BT4 3SD, United Kingdom
[c] Department of Virology, National Veterinary Institute, DTU, Lindholm, DK-4771 Kalvehave, Denmark
[d] Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, Madrid 28130, Spain

CENTRO DE INVESTIGACION EN SANIDAD ANIMAL (CISA-INIA)

European Union Reference Laboratory for ASF, (EURL-ASF)
Centro de Investigación en Sanidad Animal
CISA-INIA, Valdeolmos 28130, Madrid, Spain.

Contact people
Dr. Carmina Gallardo
Raquel Nieto

E-mail: eurl.asf@inia.es

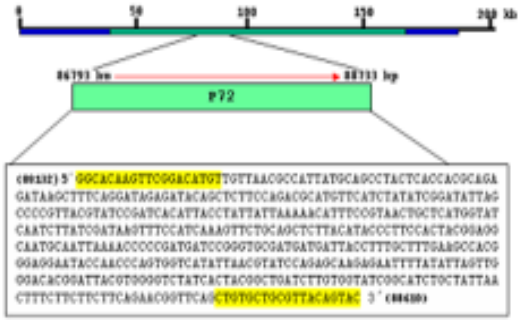
SOP/CISA/ASF/GENOTYPING/1

STANDARD OPERATING PROCEDURE FOR GENOTYPING OF AFRICAN SWINE FEVER VIRUS (ASFV) ISOLATES

CONTENTS

A. **PCR amplification of the <u>C-terminal region of p72 protein</u> using primers <u>p72- U</u> and <u>p72-D</u>.** These primers amplify 478 bp from the protein p72 of the Ba71V ASFV isolate (*GenBank accession no. ASU18466*- Figure 1) and have been previously described by Bastos et al., 2003.

Fig. 1: Sequence obtained using p72U/D primers set (marked in yellow) inside P72 protein.

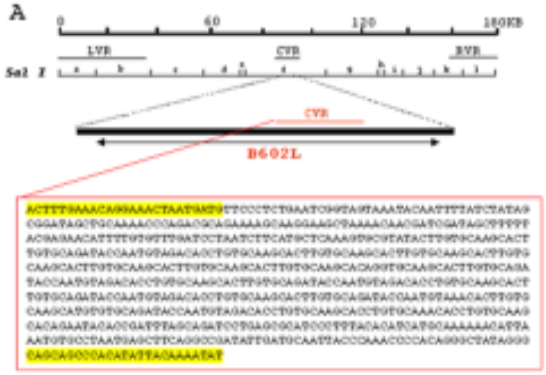


B. **PCR amplification of the <u>intergenic region located between the I73R and I329L genes.</u>** These primers amplify 356 bp located between the I73R and I329L genes and characterized by the presence of TRS of the Gergia ASFV isolate (*GenBank accession no. FR682468.1*- Figure 2) and have been previously described by Gallardo *et* al., 2014).

Fig. 2. Sequence obtained using Eco1A/B primers set (marked in yellow) in the Gergia ASFV strain.

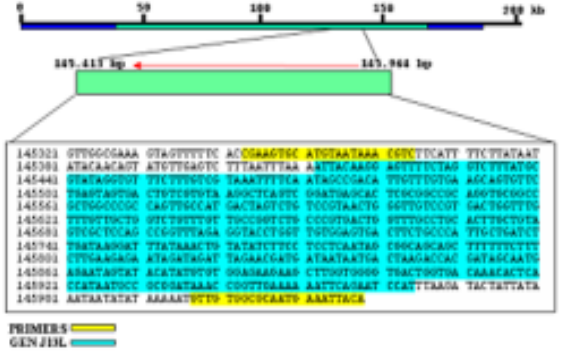
C. **PCR amplification of the CVR within the *B602L* gen using the primer set CVR1 and CVR2.** These primers amplify 665 bp of the Ba71V ASFV isolate (*GenBank accession no. ASU18466*- Figure 3) containing the amino acid tandem repeats and have been previously described by Gallardo *et* al., 2011.

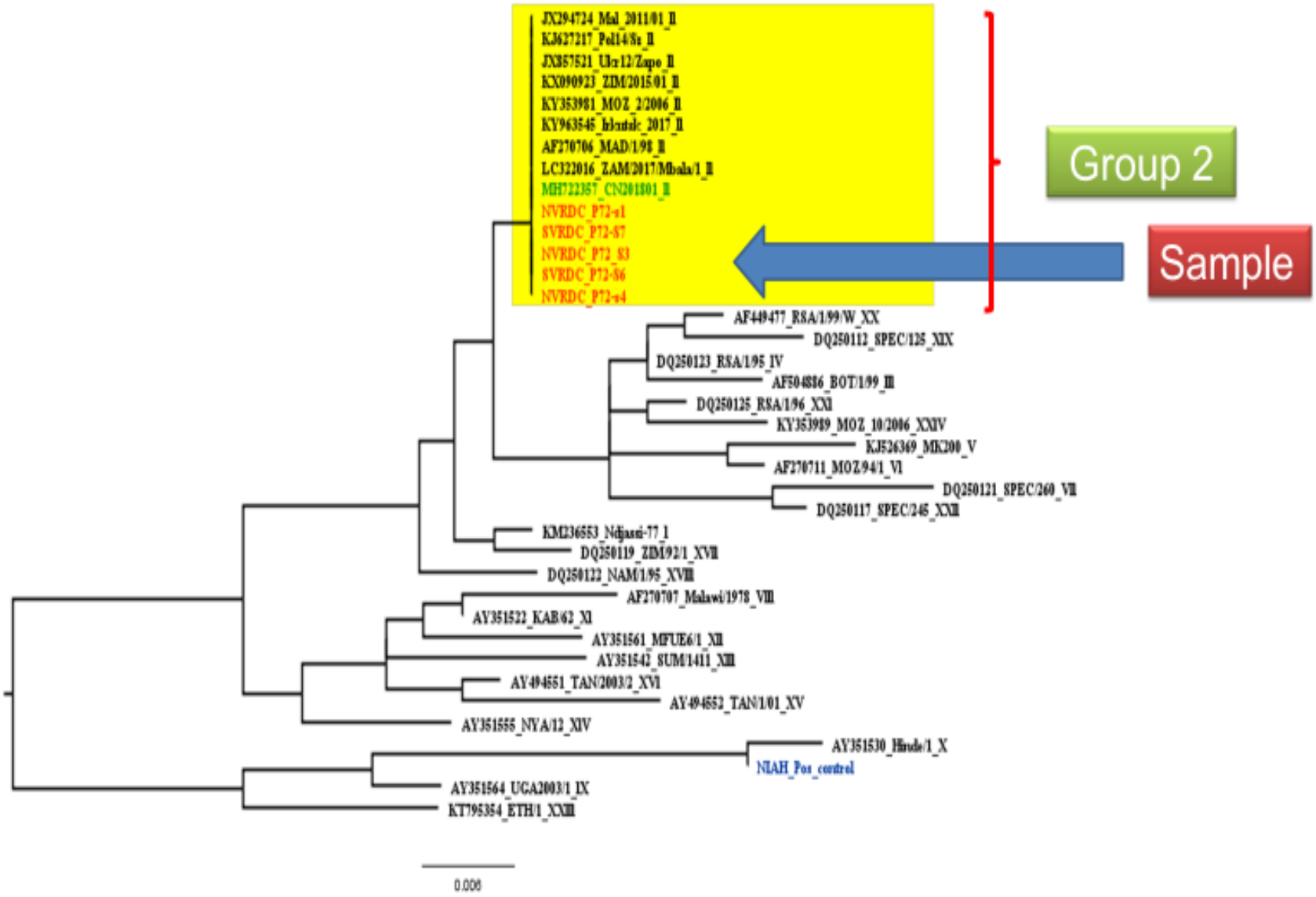
Fig. 3: Sequence obtained after PCR amplification of primers set ORF9L/9F (marked in yellow) within B602L gene.



D. **PCR amplification of the <u>full *E183L*-gene</u> encoding the p54 protein using primers <u>PPA89</u> and <u>PPA722</u>.** These primers amplify 676 bp flanking the complete VP54 sequence of the Ba71V ASFV isolate (*GenBank accession no. ASU18466*- Figure 4) and have been previously described by Gallardo *et* al., 2009.

Fig. 4: Sequence obtained using ASF89/722 primers set (marked in yellow) flanking the P54 protein




JX294724_Mal_2011/01_II
KJ627217_Pel14/8x_II
JX857521_Ukr12/Zapo_II
KX090923_ZIM/2015/01_II
KY353981_MOZ_2/2006_II
KY963545_Inkantsk_2017_II
AF270706_MAD/1/98_II
LC322016_ZAM/2017/Mbala/1_II
MH722357_CN201801_II
NVRDC_P72-s1
NVRDC_P72-S7
NVRDC_P72_S3
NVRDC_P72-S6
NVRDC_P72-s4
Group 2
Sample
AF449477_RSA/1/99/W_XX
DQ250112_SPEC/125_XIX
DQ250123_RSA/1/95_IV
AF504836_BOT/1/99_III
DQ250125_RSA/1/96_XXI
KY353989_MOZ_10/2006_XXIV
KJ526369_MK200_V
AF270711_MOZ/94/1_VI
DQ250121_SPEC/260_VII
DQ250117_SPEC/245_XXII
KM236553_Ndjasni-77_1
DQ250119_ZIM/92/1_XVII
DQ250122_NAM/1/95_XVIII
AF270707_Malawi/1978_VIII
AY351522_KAB/62_XI
AY351561_MFUE6/1_XII
AY351542_SUM/1411_XIII
AY494651_TAN/2003/2_XVI
AY494652_TAN/1/01_XV
AY351555_NYA/12_XIV
AY351530_Hinde/1_X
NIAH_Pos_control
AY351564_UGA2003/1_IX
KT795364_ETH/1_XXIII
0.006

What worked well

Proficiency testing for swine diseases

CSIRO AUSTRALIAN ANIMAL HEALTH LABORATORY
www.csiro.au
5 Portarlington Road, Geelong East, Victoria, 3219, AUSTRALIA
T +61 3 5227 5000 • F +61 3 5227 5555

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CENTRO DE INVESTIGACION EN SANIDAD ANIMAL (CISA-INIA)

Challenges and possible solution

- Lack of swine sample.
- Lack of collaboration between pig farmers and government agency.
- Have a good collaboration with laboratory network.

Thank you

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Protecting animals, preserving our future