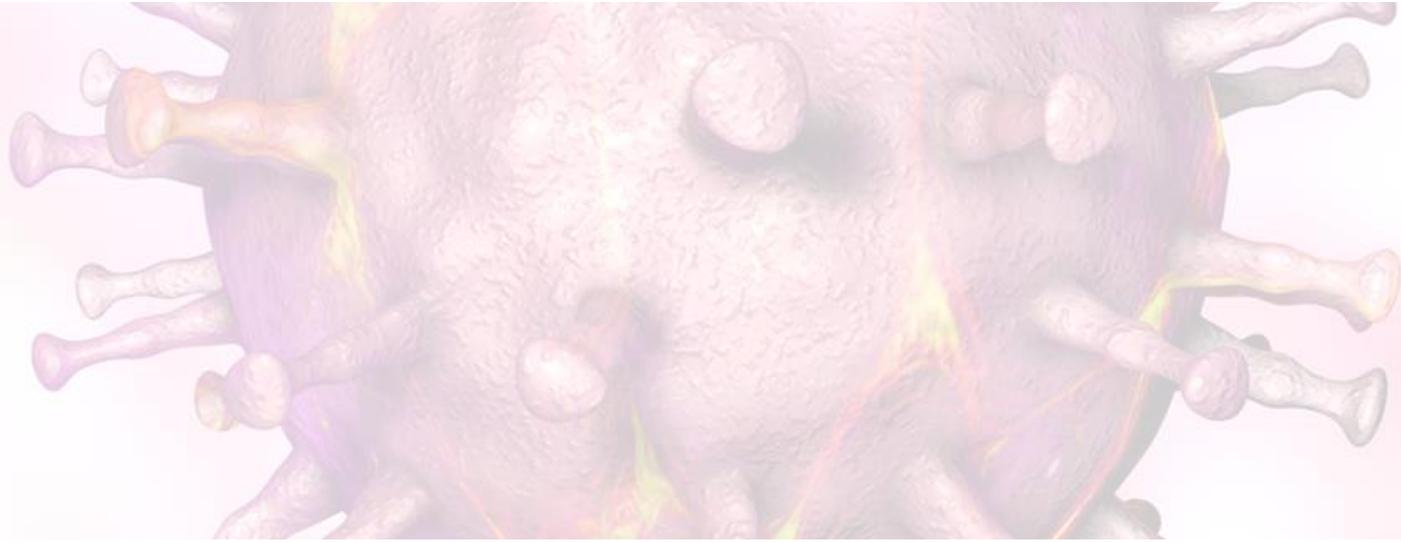


MOLECULAR CHARACTERIZATION OF AFRICAN SWINE FEVER VIRUS IN A BACKYARD SWINE FARM IN BULACAN PHILIPPINES

**Gianne May R. Gagan DVM, Erika Arellano,
RMT and Dennis V. Umali, DVM, PhD**

21 Feb 2020
SMX Convention Center
Lanang, Davao City



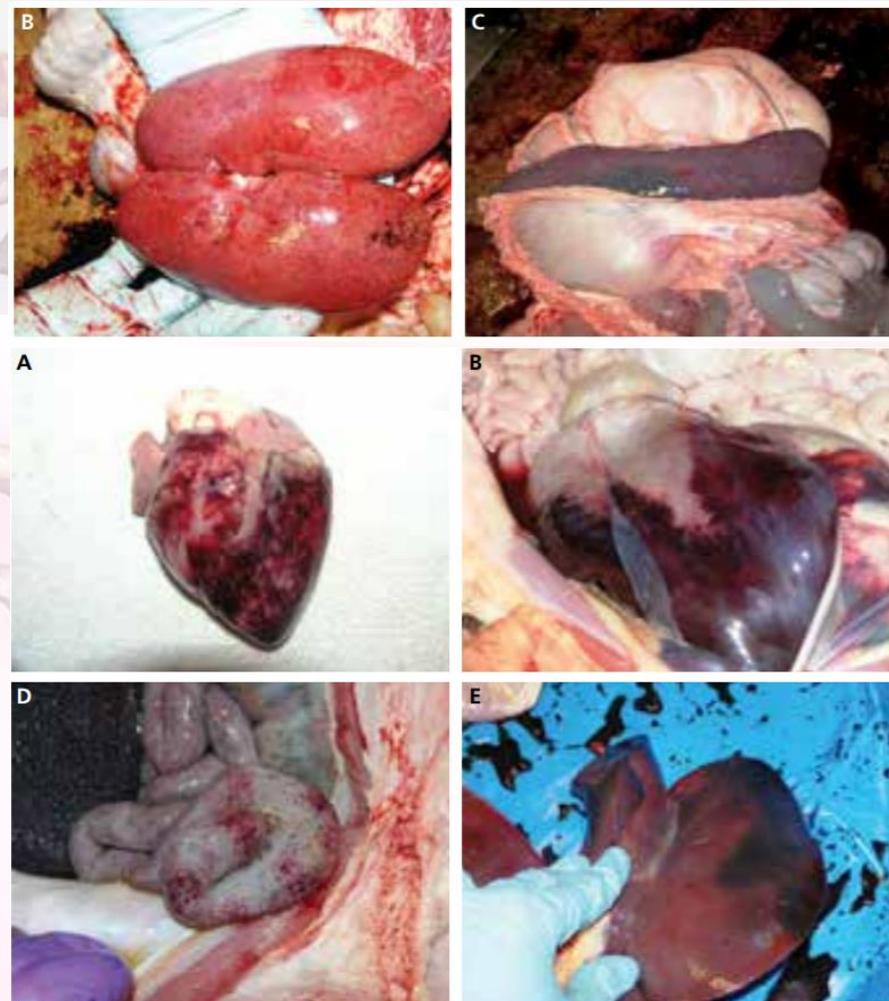


INTRODUCTION

African Swine Fever



2017, FAO, AFRICAN SWINE FEVER: DETECTION AND DIAGNOSIS: A manual for veterinarians



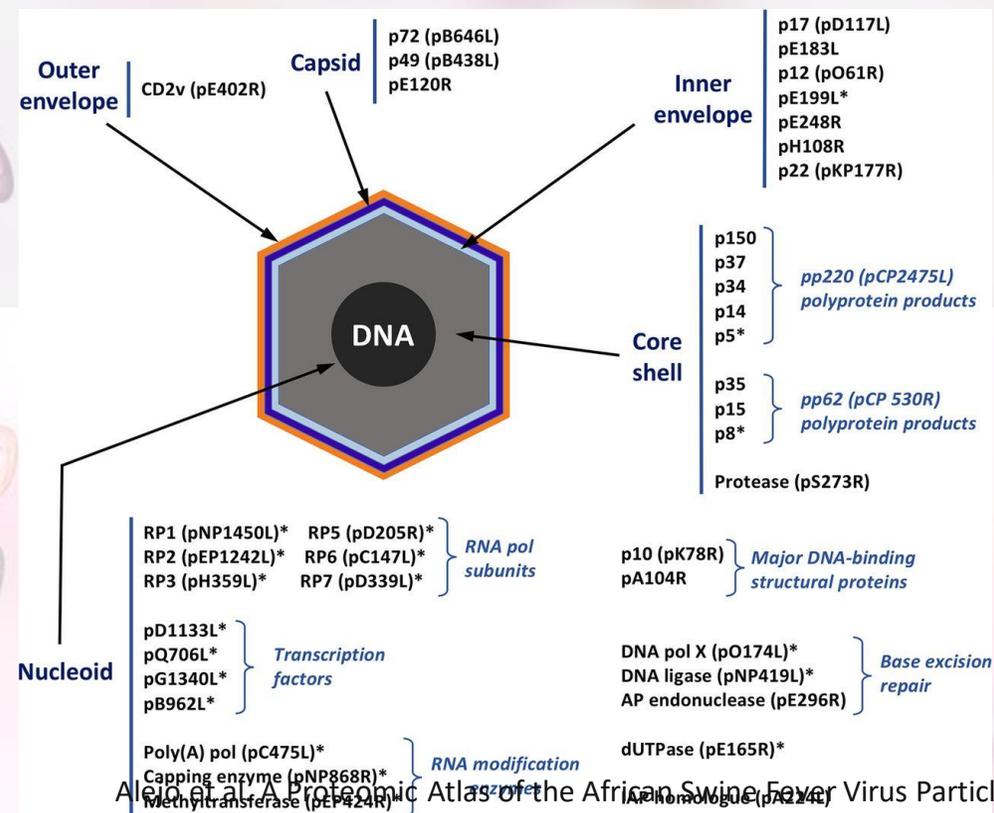
African Swine Fever

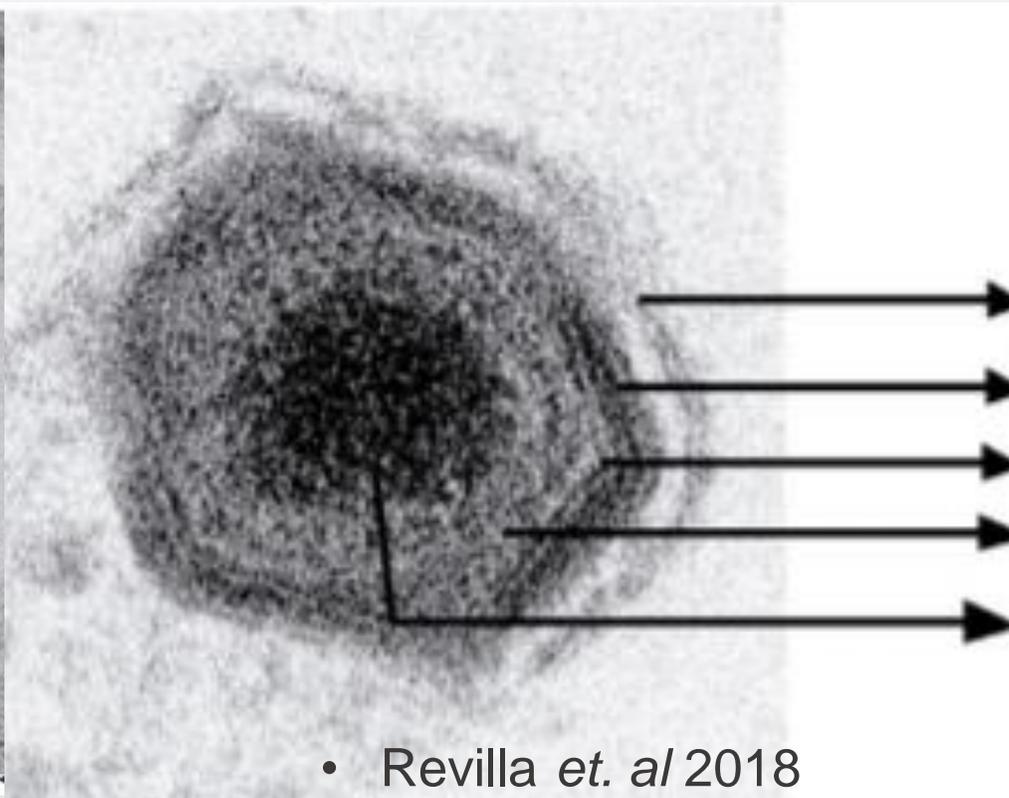
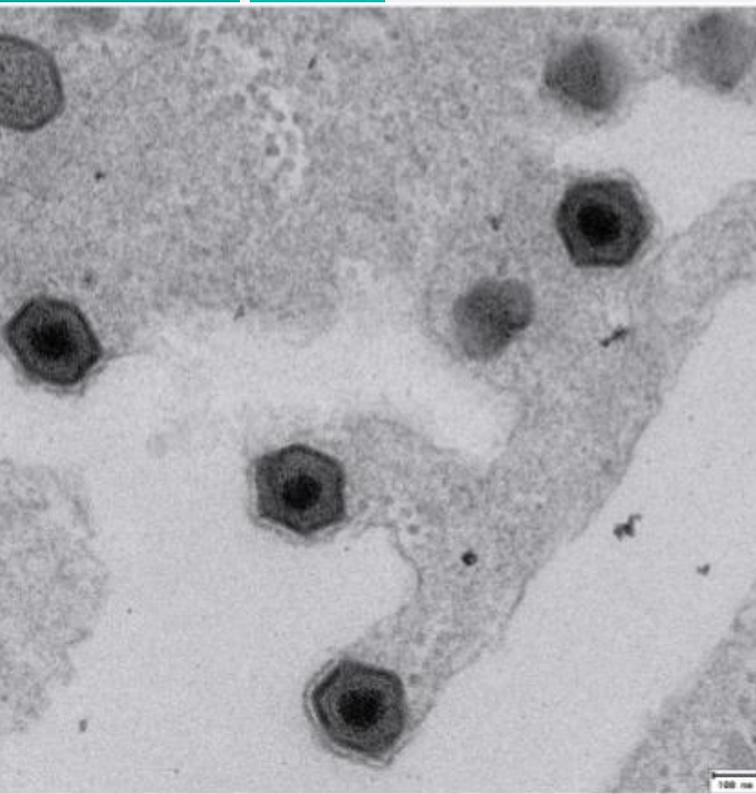


- Incubation period: 4-19 days
- Virus excretion: can begin up to two days prior to the appearance of clinical signs
- Viral shedding: saliva, tears, nasal secretions, urine, feces, and secretions from the genital tract

The ASF Virus

- Viral genome: 170 to 195kbp
 - encodes 150 to 200 proteins, around 50 of them structural
- Genotyping: partial sequence analysis of B646L gene encoding the viral protein72 (p72)
- Twenty-four ASFV genotypes (I-XXIV)





- External envelope (p12,p24)
- Viral capsid (p72)
- Inner envelope(p12, p17,p54)
- Core shell (pp220, pp60)
- Nucleoid (p10)

• Revilla *et. al* 2018

The use of the three regions of the ASFV DNA, p72, p54, and CVR, to characterize ASFV is much sufficient despite the presence of many other markers

p72

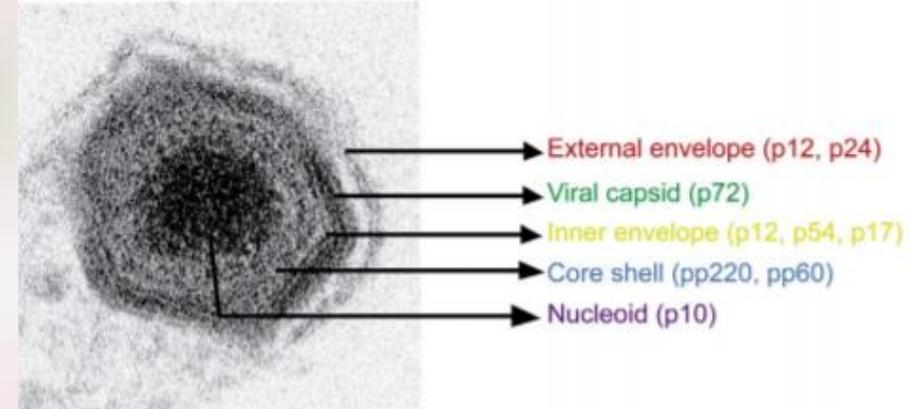
- B646L open reading frame encoding the p72 protein
- major capsid protein

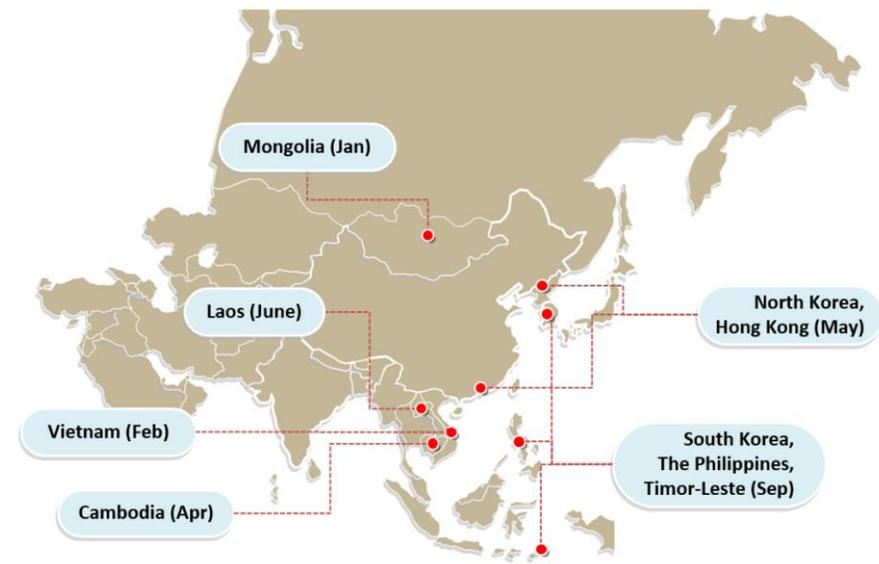
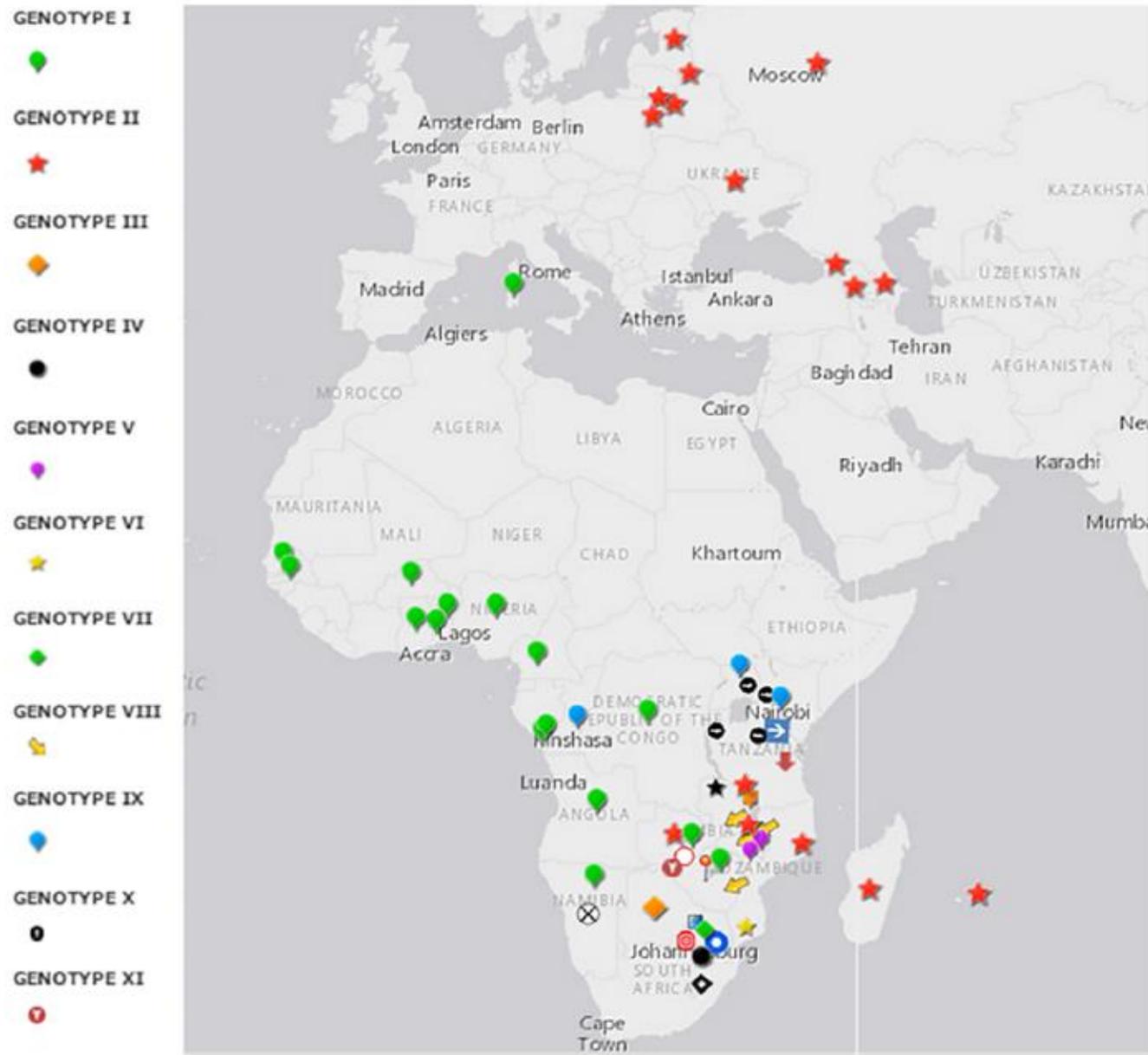
p54

- E183L gene that encodes the p54 ASFv protein essential in the recruitment of envelope precursors to the assembly site

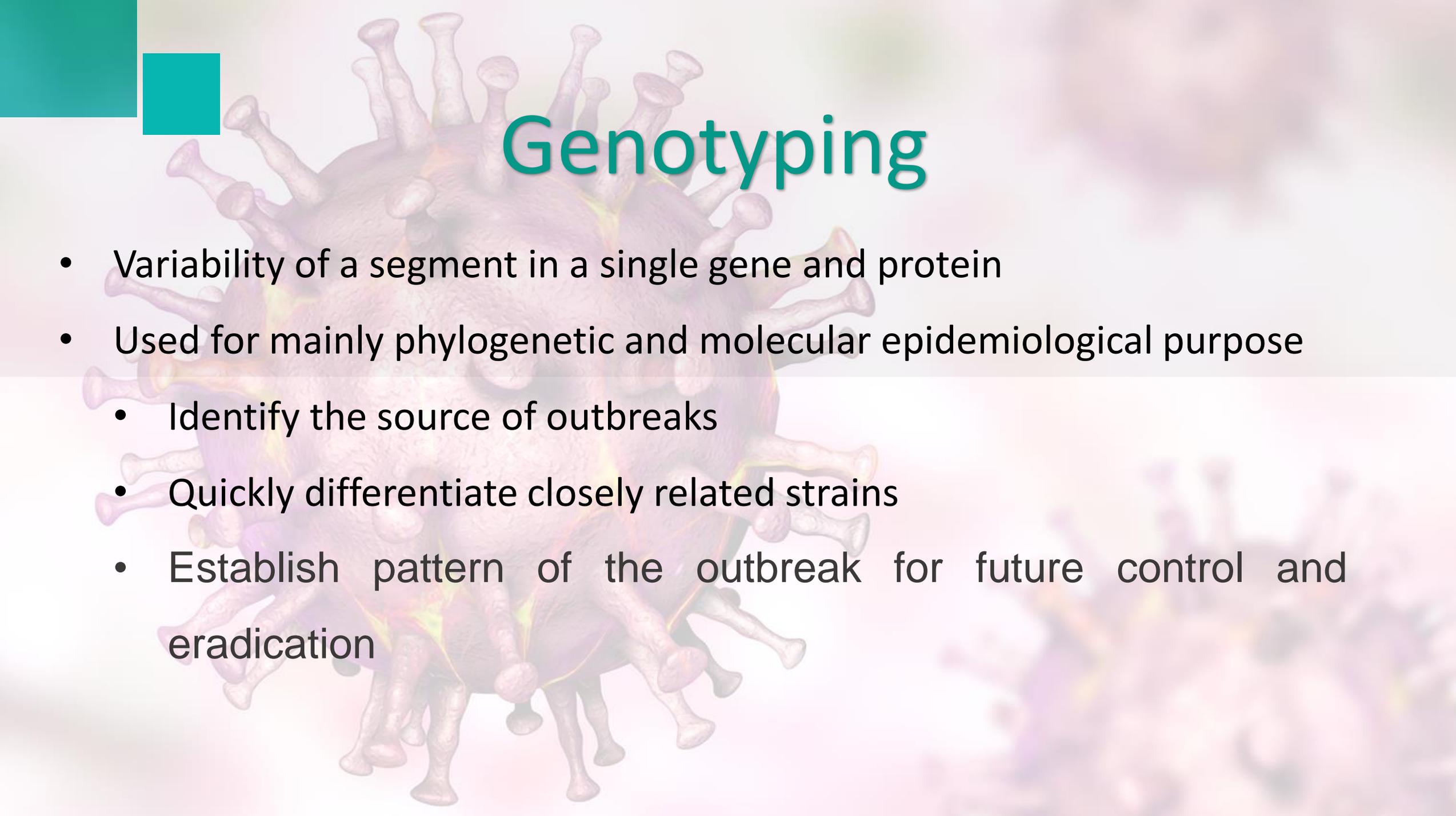
CVR

- The B620L gene encodes the central variable region where repeated amino acid tetramers that vary in number and type among ASFv isolates are located





2015, Gallarado et al. African swine fever: A global view of the current challenge

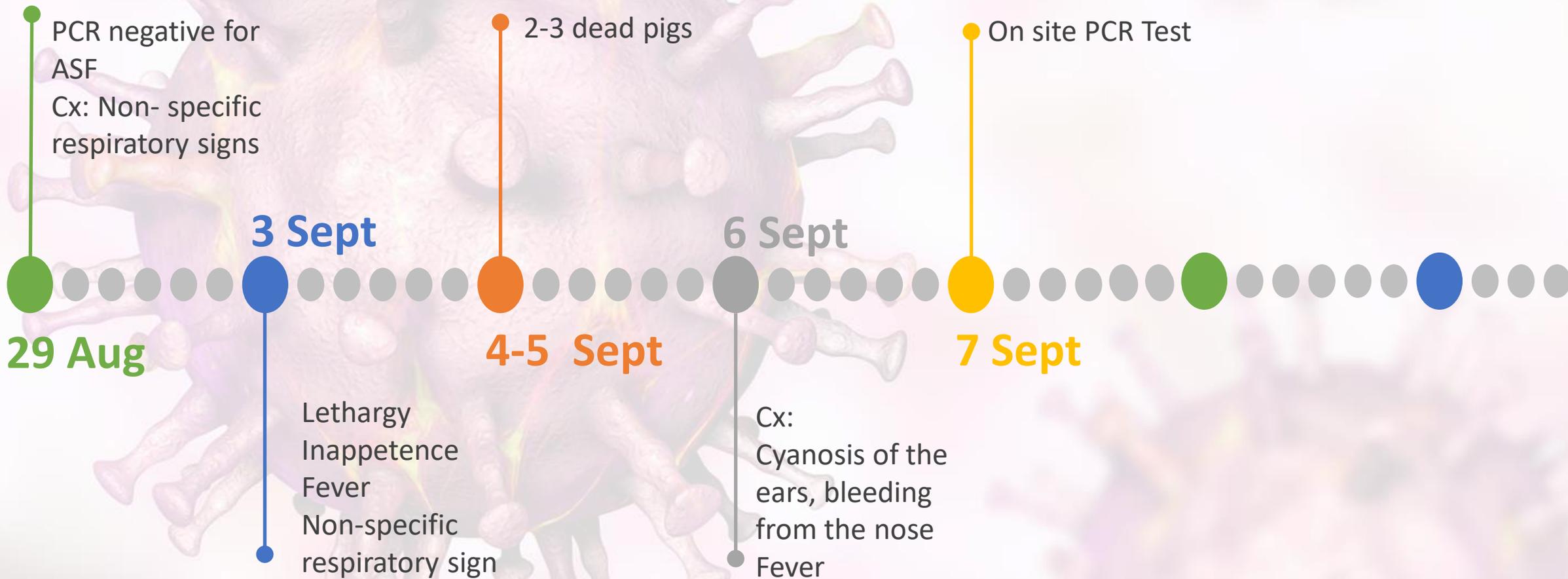


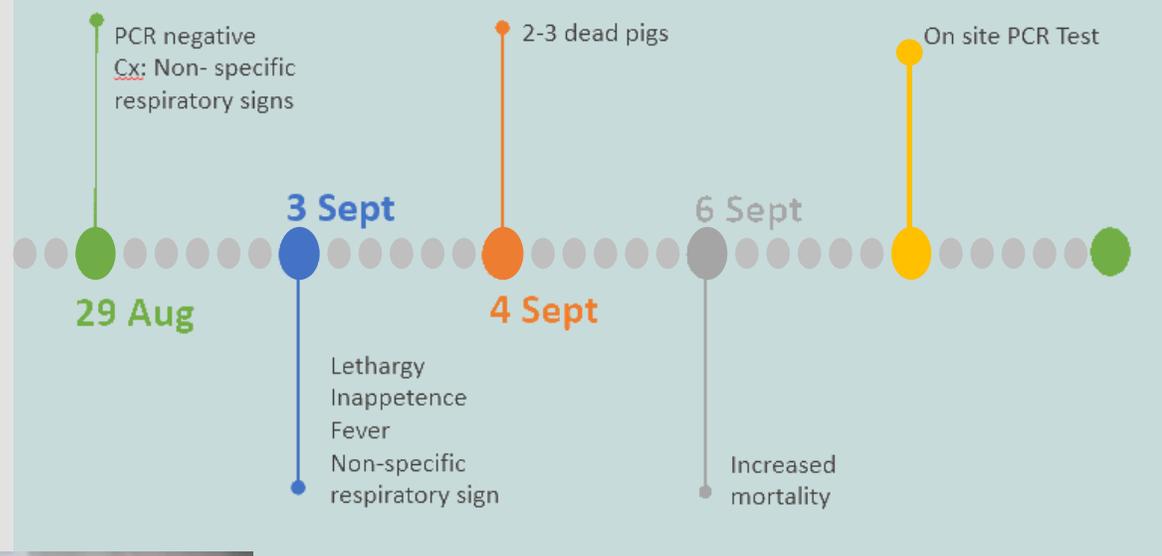
Genotyping

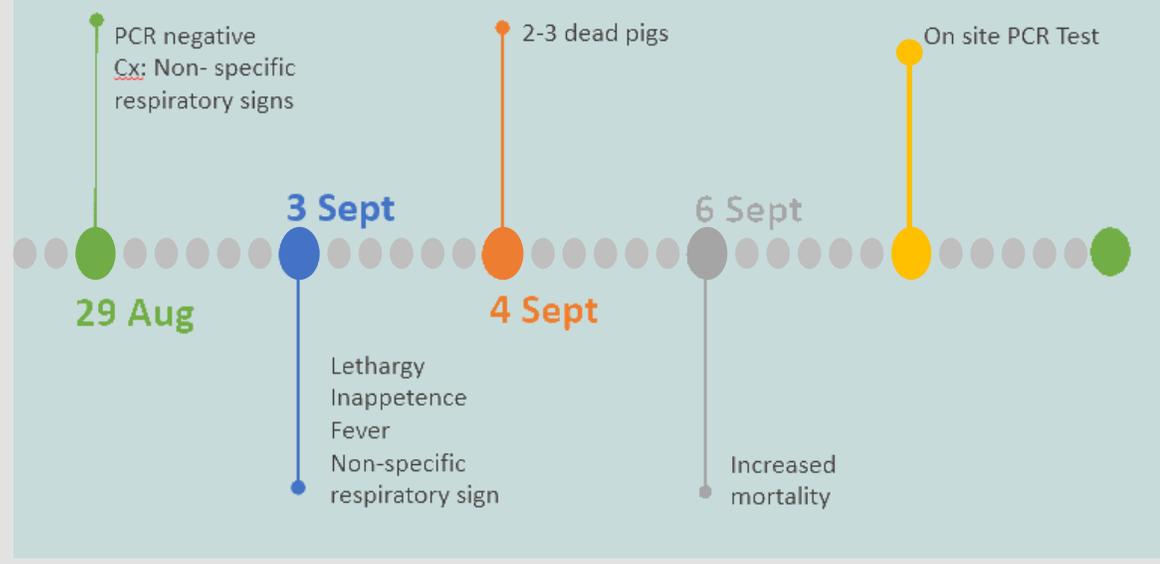
- Variability of a segment in a single gene and protein
- Used for mainly phylogenetic and molecular epidemiological purpose
 - Identify the source of outbreaks
 - Quickly differentiate closely related strains
 - Establish pattern of the outbreak for future control and eradication

CASE PRESENTATION

TIMELINE





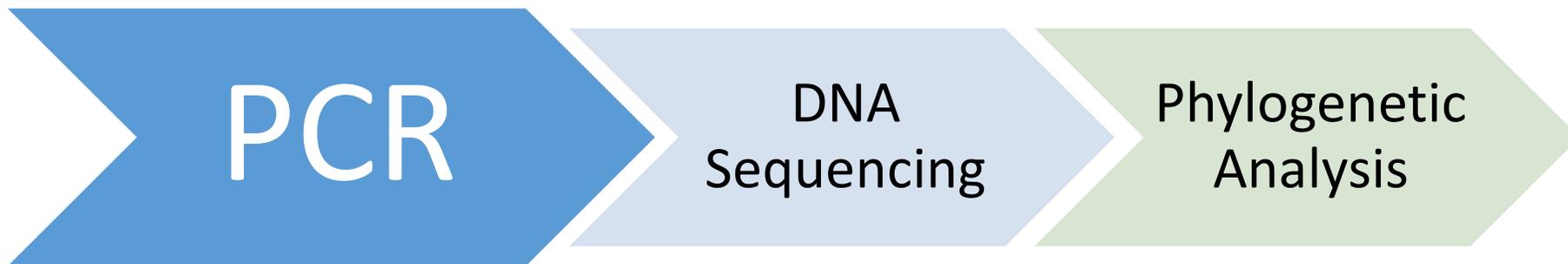


- 6 Blood**
- 4 from apparently healthy pigs
 - 2 from sick pigs

- 3 Feed Samples**
- From pens with sick pigs
 - From warehouse

- 1 Tissues**

- 5 Environmental Samples**
- Dirt/Mud
 - Water



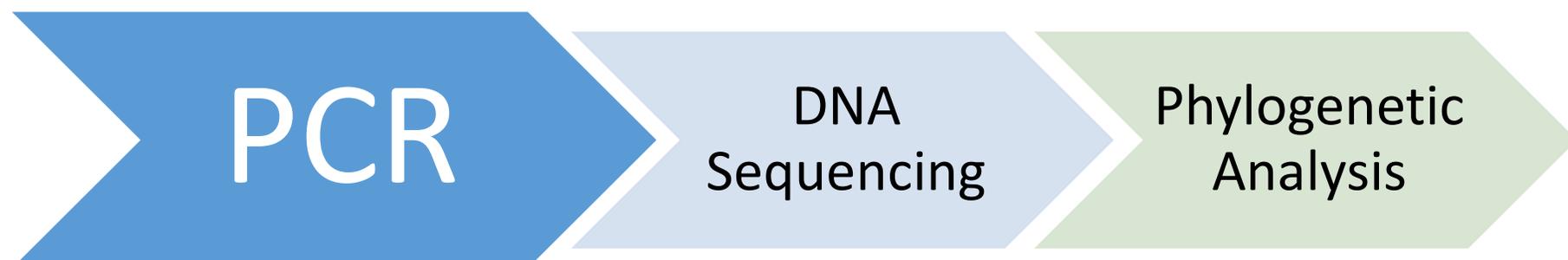
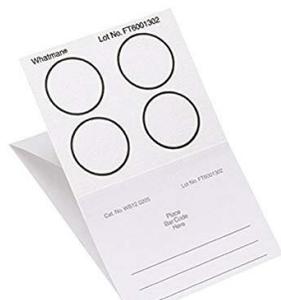
Primer Name	Primer Sequence	Thermoprofile	
Diag Primer p72-1	5- ATG-GAT-ACC-GAG-GGA-ATA-GC -3	Pre-warming	95°C for 3 mins (1 cycle)
Diag Primer p72-2	5- CTT-ACC-GAT-GAA-AAT-GAT-AC-3	Amplification	
		Denaturation	95°C for 30 sec
		Annealing	50°C for 30 sec
		Extension	50°C for 30 sec (35 cycles)
		Final Extension	72°C for 10 min (1 cycle)

- 6 Blood**
- 4 from apparently healthy pigs
 - 2 from sick pigs

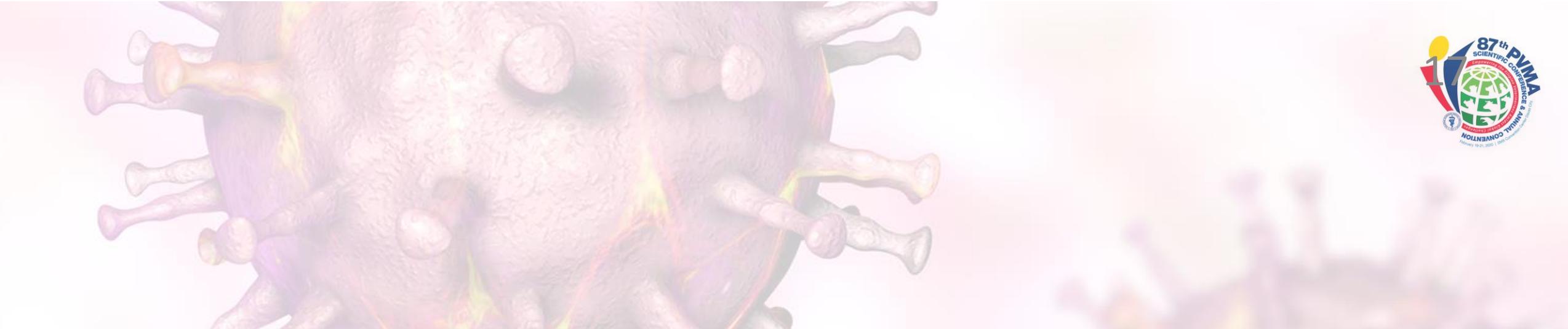
1 Tissues

- 3 Feed Samples**
- From pens with sick pigs
 - From warehouse

- 5 Environmental Samples**
- Dirt/Mud
 - Water



Primer Name	Target	Sequence	Band size
p72-U	C-terminal region of the p72 gene	5'-GGCACAAGTTCGGACATGT-3'	478bp
p72-D		5'-GTACTGTAACGCAGCACAG-3'	
PPA722	whole gene encoding the p54 protein	5'-CGAAGTGCATGTAATAAACGTC-3'	676bp
PPA89		5'-TGTAATTCATTGCGCCACAAC-3'	
CVR-FL1	B602L (CVR) gene	5'-TCGGCCTGAAGCTCATTAG-3'	Variable size
CVR -FL2		5'-CAGGAAACTAATGATGTTCC-3'	



RESULTS

Sample	
Blood (AH Pig 1)	Positive
Blood (AH Pig 2)	Negative
Blood (AH Pig 3)	Positive
Blood (AH Pig 4)	Positive
Blood (Sick Pig – Pooled Building 1)	Positive
Blood (Sick Pig – Pooled Building 1)	Positive
Dead Pig (Pooled Tissues)	Positive

Sample	
Feed from Warehouse	Negative
Water form Irrigation	Negative
Water from Entrance Gate	Negative
Water from Nearby Building	Negative
Canal Water	Negative
Feeds from Pens with Sick Pigs (Building1)	Negative
Feeds from Pens with Sick Pigs (Building 2)	Negative
Dirt/Mud from Connecting Pathway	Negative

TIMELINE

PCR negative
Cx: Non-specific
respiratory signs

3 Sept

2-3 dead pigs

4 Sept

6 Sept

On site PCR Test
PCR positive

DEPOPULATION

10 Sept

Seq
results

29 Aug

Lethargy
Inappetence
Fever
Non-specific
respiratory sign

Increased
mortality

7 Sept

Sent amplicons
to PGC

13 Sept

Direct nucleotide sequencing confirmed that the detected PCR products (n=6) contained nucleotide sequences of the p72 gene of African Swine Fever Virus.

Genetic analysis showed that the p72 gene of the ASFV from all samples were 100% similar to each other



Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. [Learn more](#)

Introducing: Magic-BLAST

Magic-BLAST is a new tool for mapping large sets of next-generation RNA or DNA sequencing runs against a whole genome or transcriptome. Wed, 24 Aug 2016 11:00:00 EST [More BLAST news...](#)

Web BLAST

Nucleotide BLAST
nucleotide → nucleotide

blastx
translated nucleotide → protein

tblastn
protein → translated nucleotide

Protein BLAST
protein → protein

BLAST Genomes

Enter organism common name, scientific name, or tax id

Human Mouse Rat Microbes

select all 82 sequences selected

[GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description
<input checked="" type="checkbox"/>	African swine fever virus strain Belgium/Etalle/wb/2018, complete genome
<input checked="" type="checkbox"/>	African swine fever virus isolate ASFV/Kyiv/2016/131, complete genome
<input checked="" type="checkbox"/>	African swine fever virus isolate ASFV-wbBS01, complete genome
<input checked="" type="checkbox"/>	African swine fever virus major capsid protein p72 gene, complete cds
<input checked="" type="checkbox"/>	African swine fever virus isolate ASFV Belgium 2018/1 genome assembly, complete genome
<input checked="" type="checkbox"/>	African swine fever virus isolate Pig/HLJ/2018, complete genome
<input checked="" type="checkbox"/>	African swine fever virus strain Georgia 2008/2, partial genome
<input checked="" type="checkbox"/>	African swine fever virus strain Georgia 2008/1, complete genome
<input checked="" type="checkbox"/>	African swine fever virus isolate ASFV-SY18 major capsid protein p72 gene, complete cds
<input checked="" type="checkbox"/>	African swine fever virus isolate China/2018/AnhuiXCGQ, complete genome
<input checked="" type="checkbox"/>	African swine fever virus isolate ASFV/POL/2015/Podlaskie, complete genome
<input checked="" type="checkbox"/>	African swine fever virus isolate Estonia 2014 genome assembly, complete genome: monox
<input checked="" type="checkbox"/>	African swine fever virus p72 (B646L) gene, complete cds
<input checked="" type="checkbox"/>	African swine fever virus Georgia 2007/1 complete genome
<input checked="" type="checkbox"/>	African swine fever virus isolate mk major capsid protein p72 (p72) gene, complete cds
<input checked="" type="checkbox"/>	African swine fever virus isolate Mkuzi 1979, complete genome
<input checked="" type="checkbox"/>	African swine fever virus isolate RSA/W/1/99 p72 gene, partial cds
<input checked="" type="checkbox"/>	African swine fever virus isolate DRC/624/89 p72 gene, partial cds
<input checked="" type="checkbox"/>	African swine fever virus isolate ANG/70 p72 gene, partial cds
<input checked="" type="checkbox"/>	African swine fever virus isolate wb major capsid protein p72 (p72) gene, complete cds
<input checked="" type="checkbox"/>	African swine fever virus isolate wart major capsid protein p72 (p72) gene, complete cds
<input checked="" type="checkbox"/>	African swine fever virus isolate Pr5 major capsid protein p72 (p72) gene, complete cds

Comparison of the partial sequence of the p72 gene showed that the ASFV strain from the samples were 97-100% closely related to:

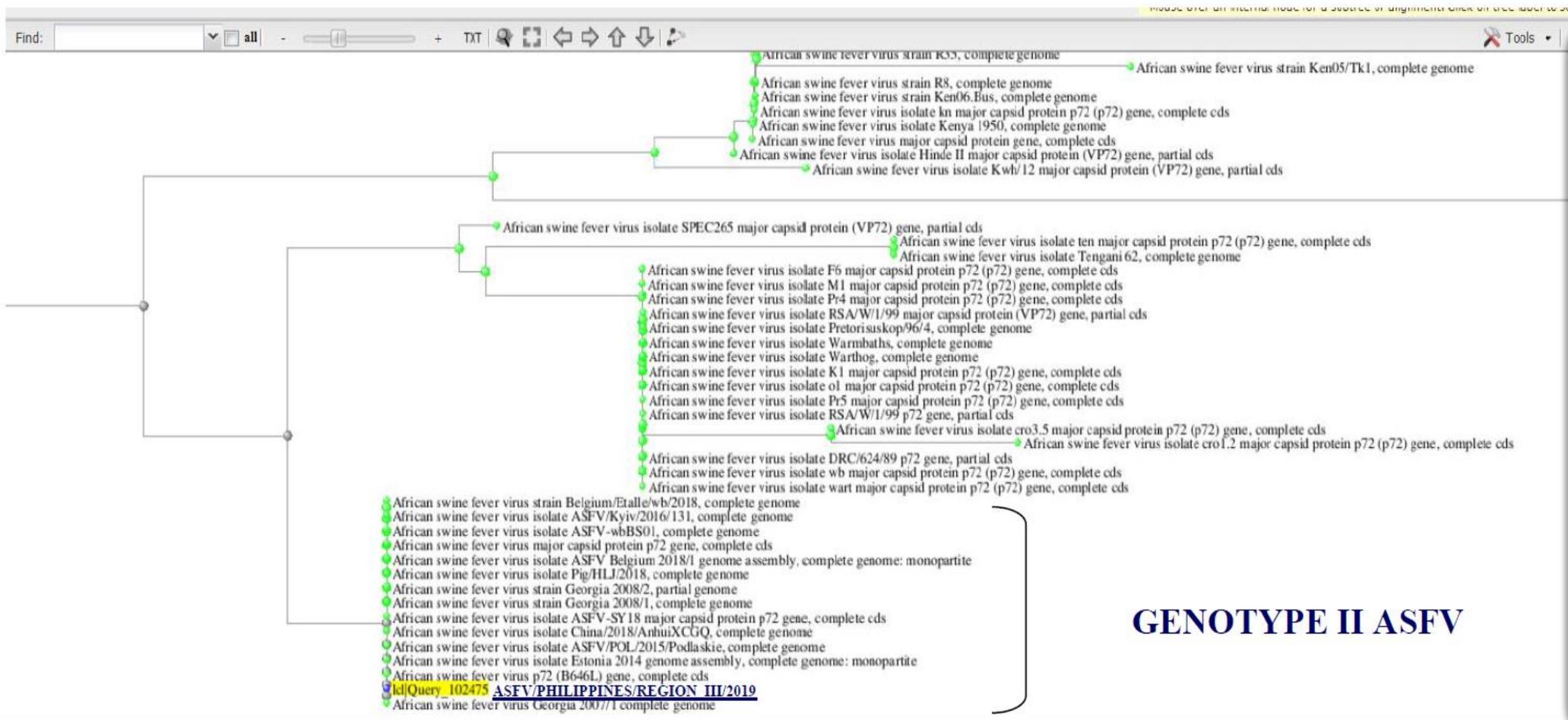
- Georgia ASFV (2007)
- Krasnodar, Russia ASFV (2012)
- Estonia ASFV (2014)
- Poland ASFV (2015)
- Belgium ASFV(2018)
- China ASFV (2018)
- Vietnam ASFV(2018)

MOLECULAR CHARACTERIZATION OF ECONOMICALLY IMPORTANT LIVESTOCK AND POULTRY PATHOGENS IN THE PHILIPPINES

(Funding Source: Poultry Products Quality Control (PPQC Co. Ltd.))



Figure 3. Phylogenetic analysis of the detected ASFV strain



Published information in the Genbank and phylogenetic analysis of the p72 gene showed that the detected ASFV from the samples were from Genotype II ASFVs.

GENOTYPE II ASFV

Information received on 30/10/2019 from Dr Ronnie Domingo, Officer-In-Charge, Chief Veterinary Officer, Bureau of Animal Industry, Department of Agriculture, Manila, Philippines

Summary

Report type	Follow-up report No. 1
Date of start of the event	25/07/2019
Date of confirmation of the event	30/08/2019
Report date	30/10/2019
Date submitted to OIE	30/10/2019
Reason for notification	First occurrence of a listed disease in the country
Causal agent	African swine fever virus (genotype II)
Nature of diagnosis	Clinical, Laboratory (advanced), Necropsy
This event pertains to	the whole country

Related reports	Immediate notification (09/09/2019) Follow-up report No. 1 (30/10/2019) Follow-up report No. 2 (04/11/2019) Follow-up report No. 3 (27/01/2020) Follow-up report No. 4 (28/01/2020) Follow-up report No. 5 (29/01/2020) Follow-up report No. 6 (29/01/2020)
-----------------	---

Epidemiology

Source of the outbreak(s) or origin of infection	Unknown or inconclusive Illegal movement of animals Swill feeding Fomites (humans, vehicles, feed, etc.)
Epidemiological comments	Suspected swill feeding, then spread through illegal movement of already sick pigs that were being sold at a lower price.

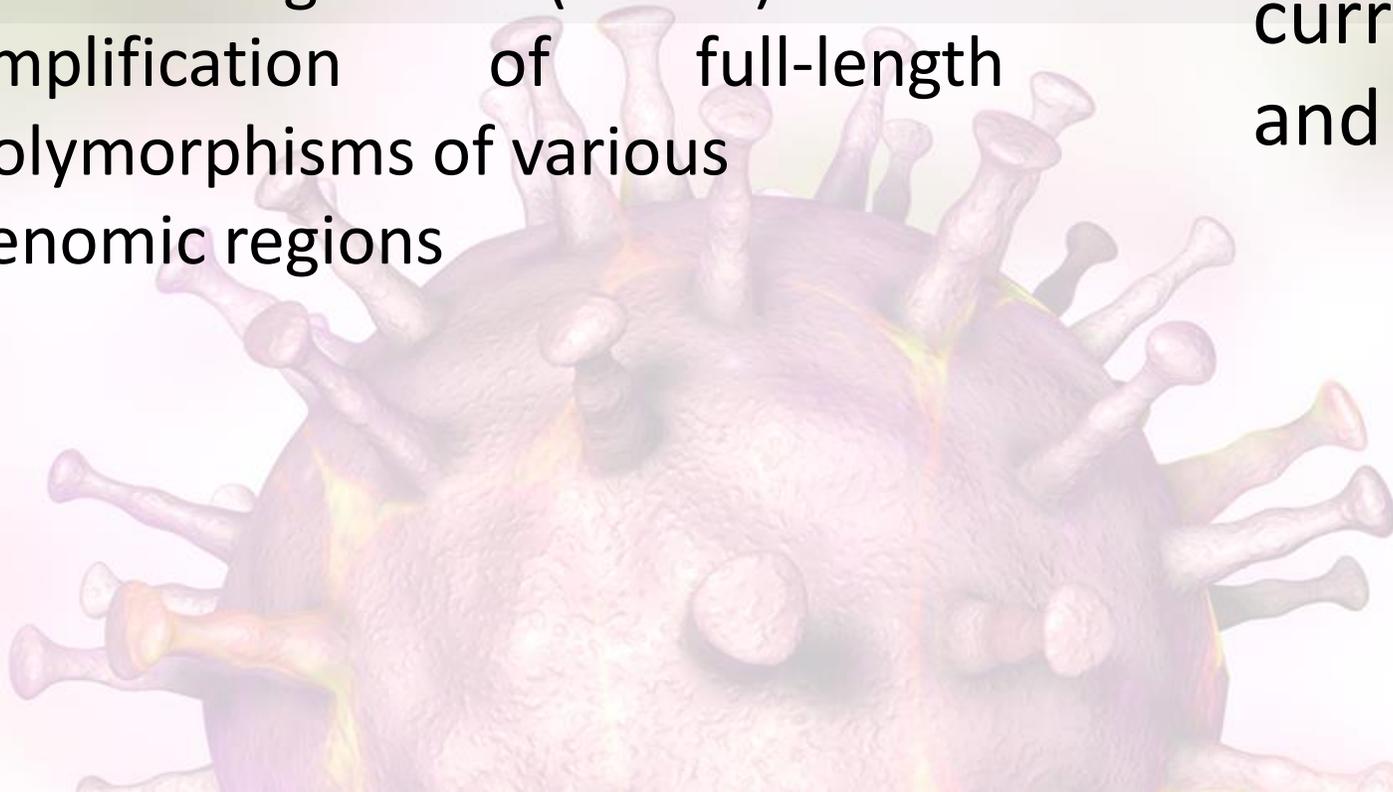
Diagnostic test results

Laboratory name and type	Species	Test	Test date	Result
Animal Disease Diagnostic and Reference Laboratory (ADDRL) (National laboratory)	Swine	real-time PCR	16/08/2019	Positive
The Pirbright Institute (OIE Reference Laboratory)	Swine	real-time PCR	30/08/2019	Positive

Laboratory name and type	Species	Test	Test date	Result
The Pirbright Institute (OIE Reference Laboratory)	Swine	virus isolation	11/09/2019	Positive
The Pirbright Institute (OIE Reference Laboratory)	Swine	virus sequencing	11/09/2019	Positive

Current genetic typing of ASFV isolates is based on nucleotide sequencing of the p72 capsid protein gene (B646L) and amplification of full-length polymorphisms of various genomic regions

- Genotype II
- Detected ASFV strain is 97-100% similar to the ASFV strain currently circulating in Europe and in Asia





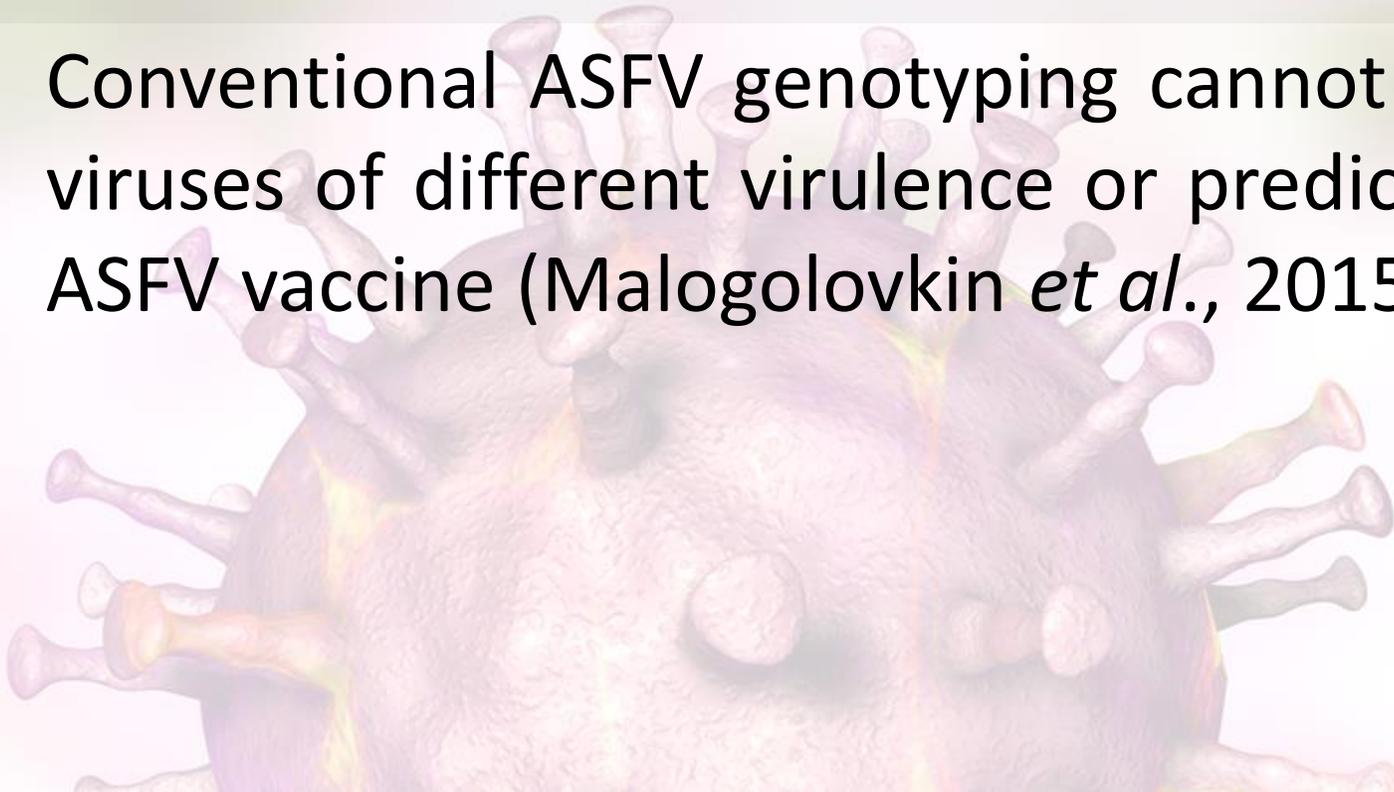
The Materials and Methods used in this study were from published references. Results obtained in this study are for scientific and research purposes only. Only ASFV Reference Laboratories are authorized to make an official ASF diagnosis following validated protocols by the OIE.



Thank you!

p72 ASFV phylogenetic analysis does not accurately define ASFV hemadsorption inhibition assay serogroups.

Conventional ASFV genotyping cannot discriminate between viruses of different virulence or predict efficacy of a specific ASFV vaccine (Malogolovkin *et al.*, 2015)



The results show that genotypic and serogroup diversity are greatest in a relatively limited area, mainly in southeastern Africa. In contrast, non-ASFV–endemic countries, where ASF outbreaks were caused by ASFV of a single genotype, exhibited low or no serogroup diversity. Single genotype clades of ASFV were observed (Malogolovkin, 2015)

