PCV2 DISEASE DIAGNOSTICS AND VIRUS EVOLUTION:

Predicting Vaccine Coverage Against Evolving PCV2 Field Strains

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tment of Veterinary Clinical Sciences, College of Veterinary Medicine, University of the Philippines Los Banos, College, Laguna 4031, Philippines
nter of the study

MOLECULAR CHARACTERIZATION OF ECONOMICALLY IMPORTANT VETERINARY PATHOGENS IN THE PHILIPPINES

2014-2023

FLUENZA AI)	NEWCASTLE DISEASE VIRUS	*Salmonella spp.*
TIOUS TIS VIRUS	INFECTIOUS BURSAL DISEASE VIRUS	MAREK'S DISEASE VIRUS
N BODY S (IBH)	AVIAN ENCEPHALOMYELITIS	SHS
ANEMIA US	CHICKEN ASTROVIRUS	AVIAN REOVIRUS
pticum/ oviae	AVIAN PATHOGENIC *E. coli (APEC)*	*ORT*
NE VIRUS	CANINE DISTEMPER VIRUS	*Faciola gigantica*

wton nd

SRC e for the future

2019

AFRICAN SWINE FEVER

PVMA, Feb 2020

PVMA, Feb 2021

2023-PRESE

AFRICAN FEV

zoetis

POR CIRCO

MOLECULAR DATABA ANIMAL DISEAS

DIAGNOSTIC KI

SURVEILLANCE SYST

DISEASE CONTROL PR

POLICY RECOMMEND

OUTLINE OF PRESENTATION

- Viral Structure of PCV
- PCV2-Associated Diseases
- PCV2 Evolution
- PCV2 Diagnostic and Monitoring Tools
- Clinical Applications

*tis

VIRAL STRUCTURE OF PCV

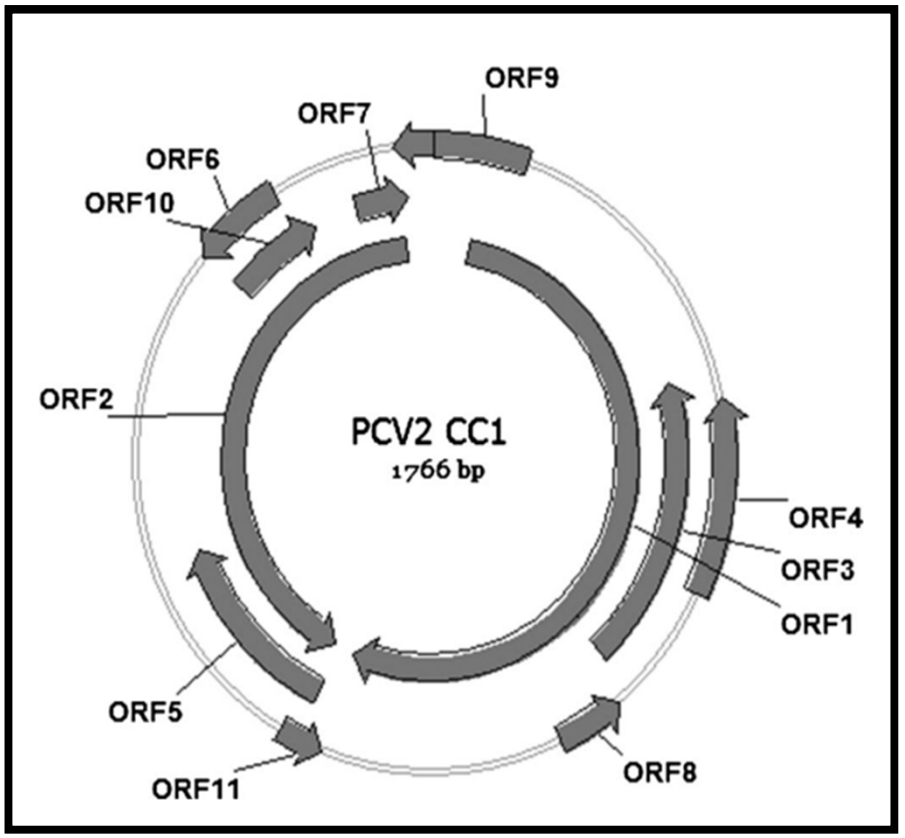
ly ***Circoviridae*****,** genus
ovirus

icosahedral, non-enveloped,
e stranded DNA virus

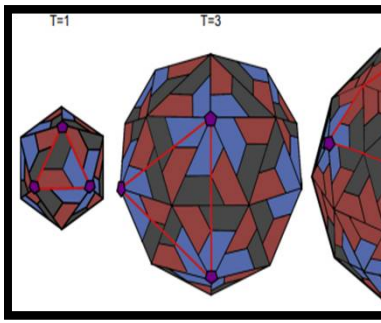
l ambisense circular genome
66 to 1768 bp)

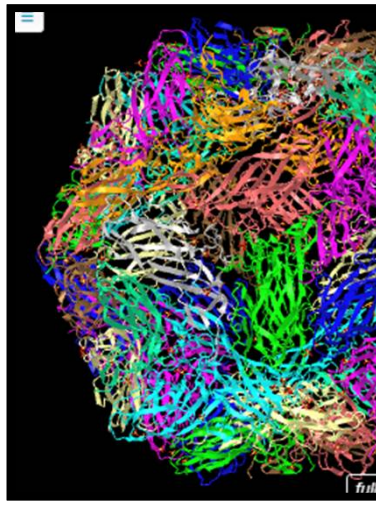
ast 11 Open Reading Frames
RF1 & ORF2: replicase (Rep and Rep')
id
RF3: apoptin
RF4: ORF4 protein
RF5: ORF5 protein

1, PCV2, PCV3 and PCV4



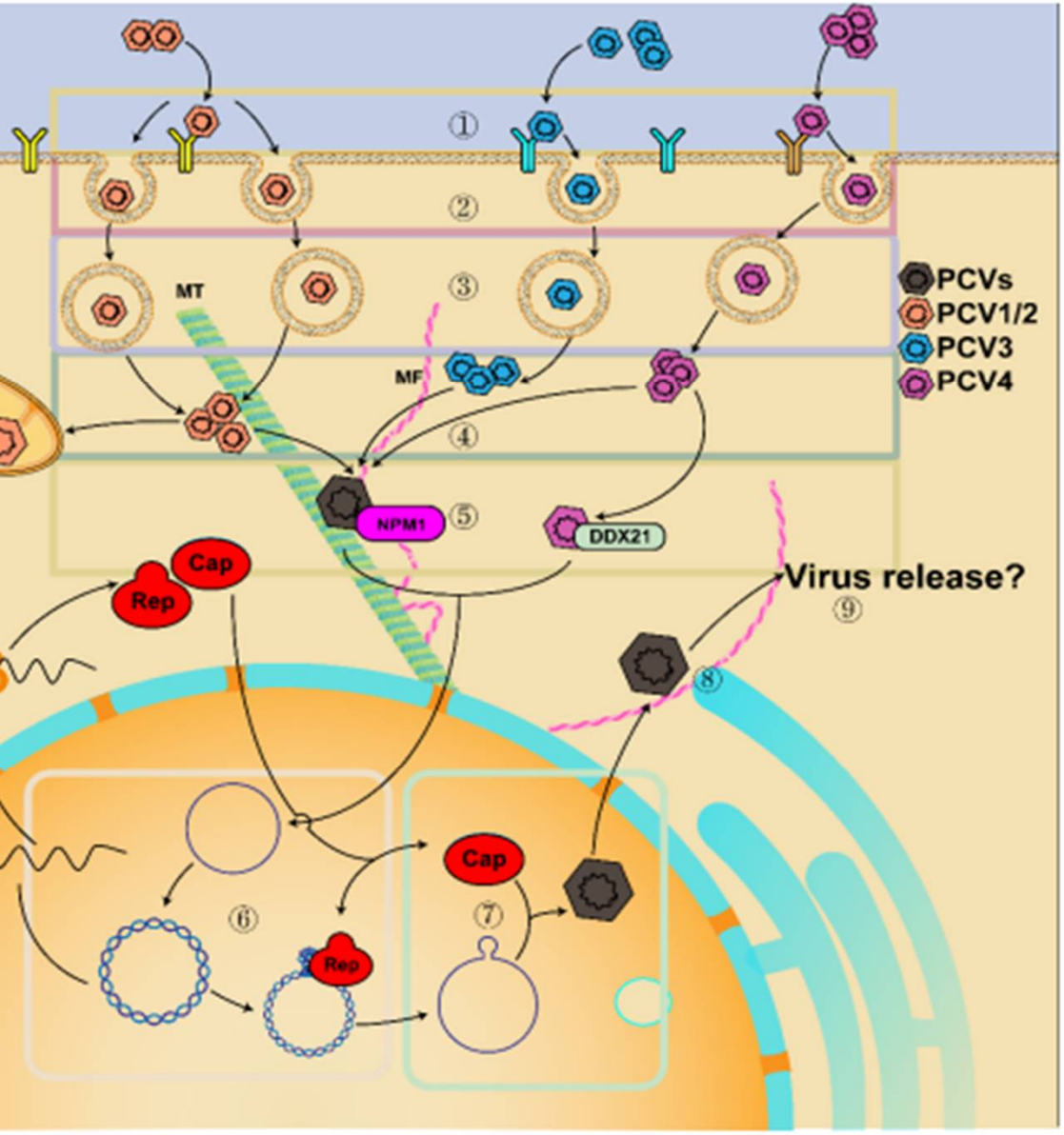
Ren *et al.*, 2016





www.ncbi.nlm.n

PCV REPLICATION CYCLE



Niu *et al., 2022*

VIRAL RECEPTOR
Heparin Sulfate
Chondroitin Sulfate B
Glycosaminoglycan

VIRAL LIGAND: Capsid

Clathrin-mediated Endocytosis

Macropinocytosis

Actin and Rho-GTPase dependent manner

Microtubules/ Microfibers
NPM1 and DDX21

dsDNA conversion & transcription

Rolling Circle Formation (Rep)

Viral Assembly (Cap)

Cell Lysis

Atta

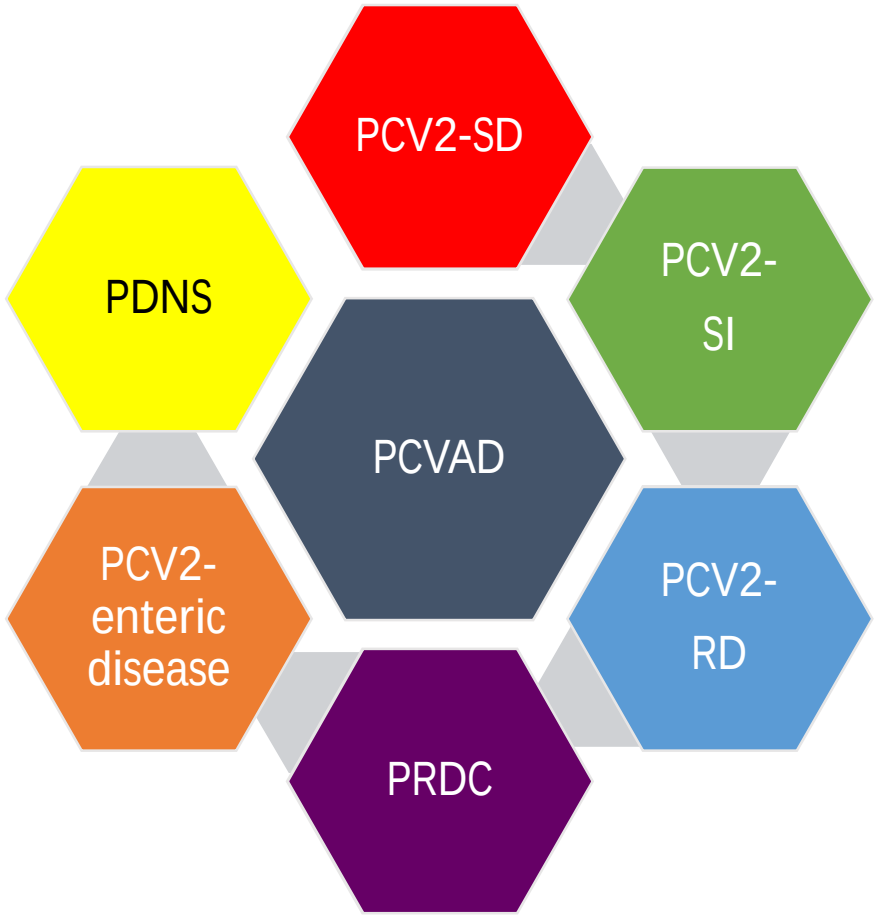
Vira

Tran

Rep
Tran
and T

Ass

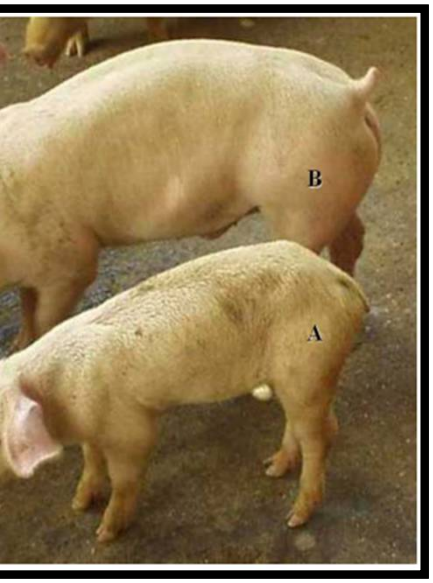
Vira



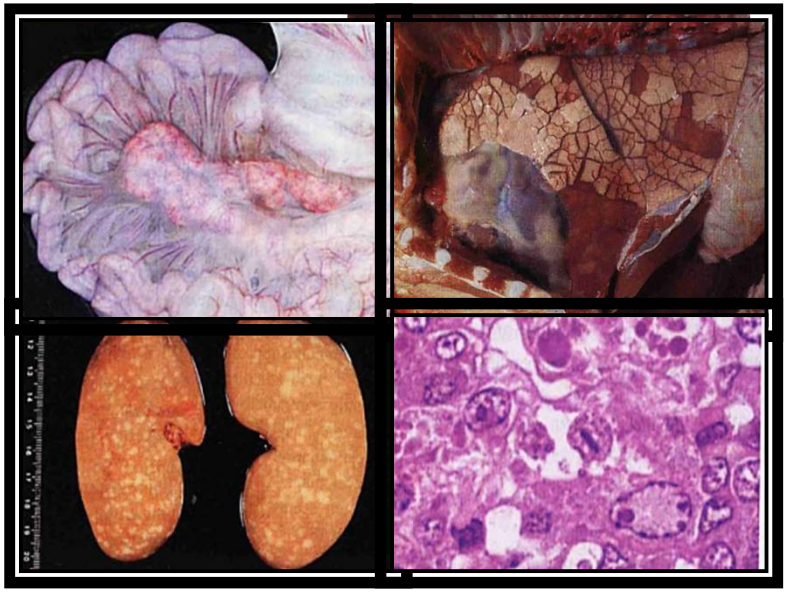
PCV2-ASSOCIATED DISEASES

etis

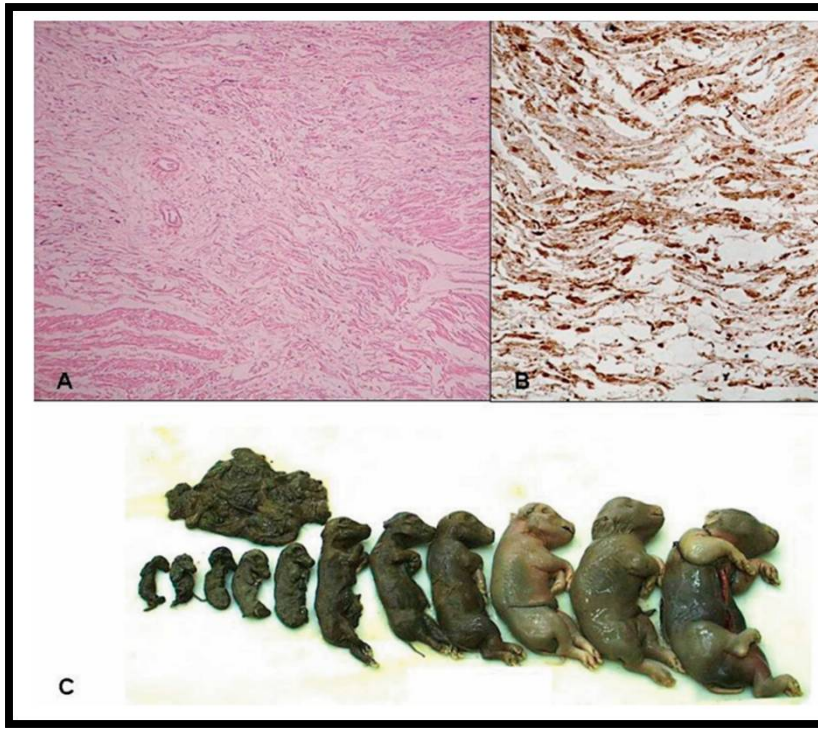
CV-2 ASSOCIATED DISEASES (PCVAD



Rodriguez-Carino, 2010



Segales & Domingo, 2002



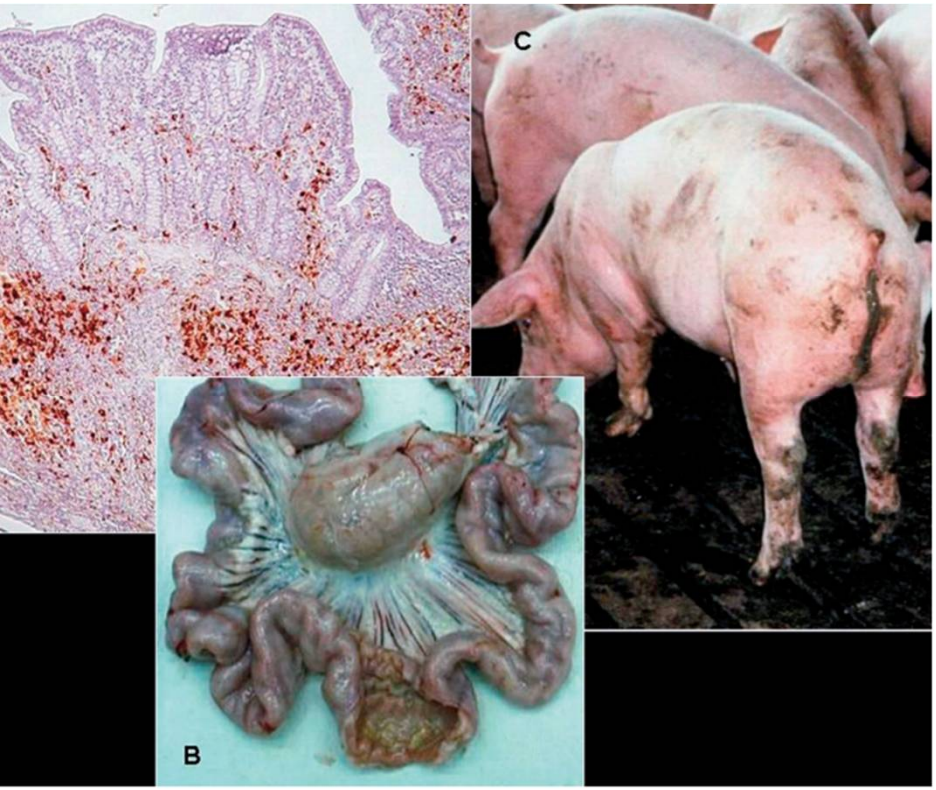
Opriessnig *et al.*, 2007

PCV2-systemic disease (PCV2-SD) (PMWS)

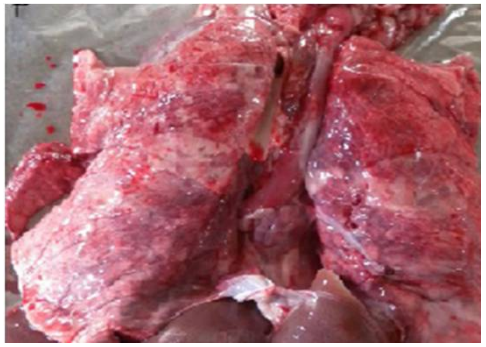
PCV2-reproductive disease (PCV2-RD)

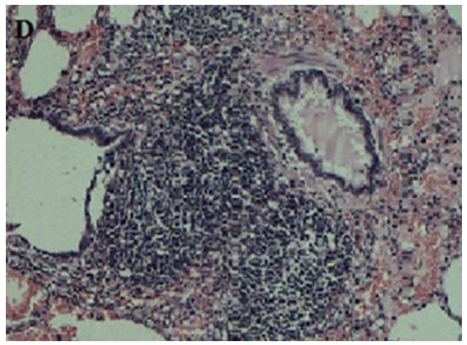
tis

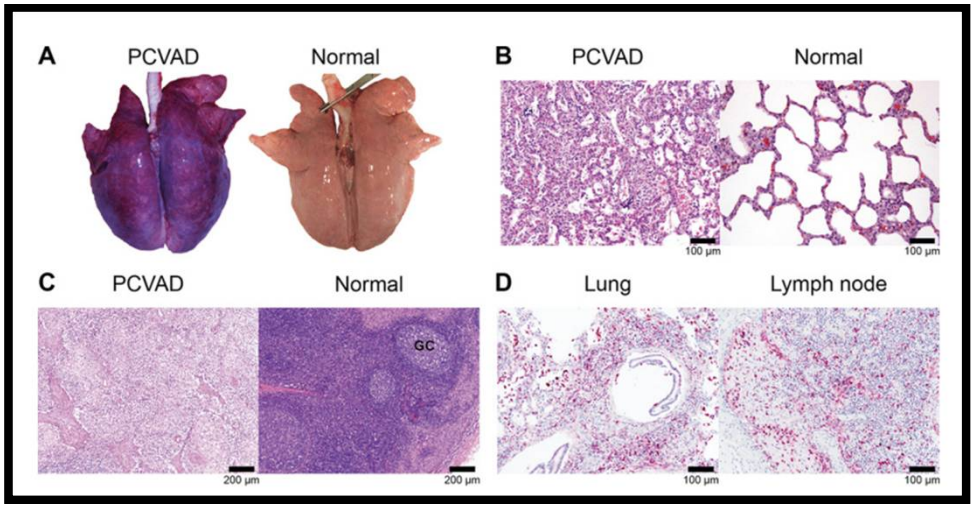
CV-2 ASSOCIATED DISEASES (PCVAD



Opriessnig *et al.*, 2007







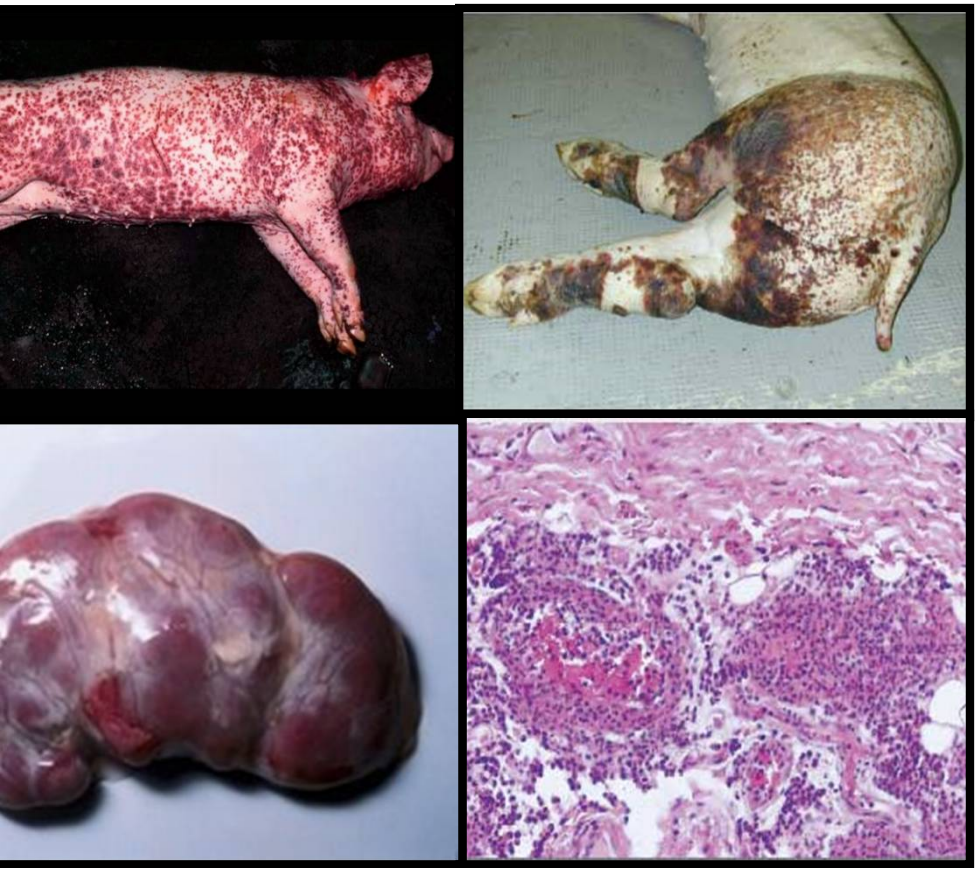
Niederwerder *et al.*, 2015/ Li *et al., 2016*

PCV2-enteric disease

PCV2-respiratory disease
(Porcine Respiratory Disease Complex) (P

tis

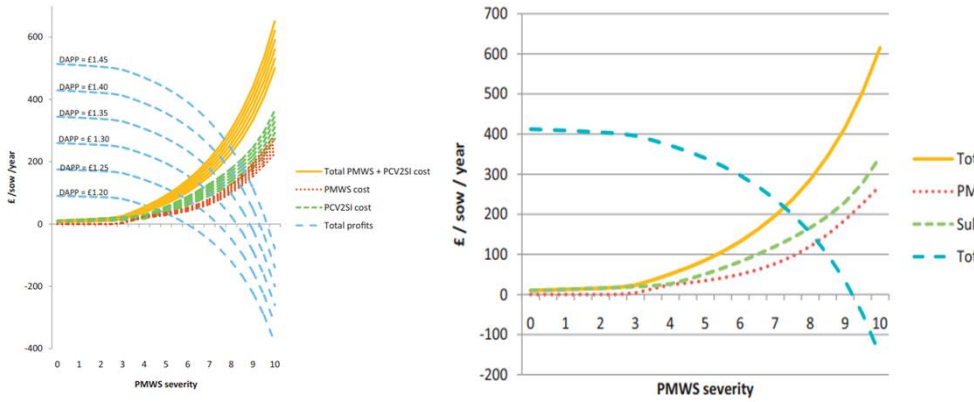
CV-2 ASSOCIATED DISEASES (PCVADS



Opriessnig *et al.*, 2007

Segales, 2002

Angulo, 2021

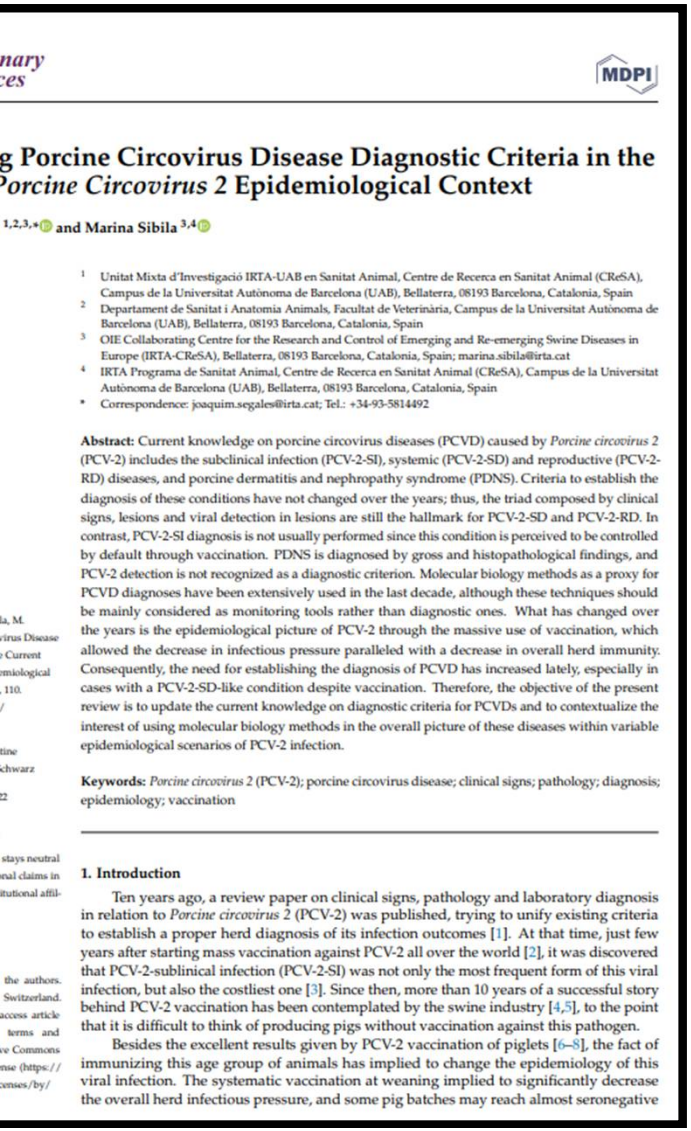


Alarcon *et al.*, 2013

cine Dermatitis and Nephropathy Syndrome (PDNS)

PCV2-Subclinical Infection (PCV2-SI)

SE DEFINITION OF THE DIFFERENT PCVA



nary
ces

MDPI

g Porcine Circovirus Disease Diagnostic Criteria in the
Porcine Circovirus 2 Epidemiological Context

1,2,3,* and Marina Sibila 3,4

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3 OIE Collaborating Centre for the Research and Control of Emerging and Re-emerging Swine Diseases in Europe (IRTA-CReSA), Bellaterra, 08193 Barcelona, Catalonia, Spain; marina.sibila@irta.cat
4 IRTA Programa de Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Catalonia, Spain
* Correspondence: joaquim.segales@irta.cat; Tel.: +34-93-5814492

Abstract: Current knowledge on porcine circovirus diseases (PCVD) caused by *Porcine circovirus 2* (PCV-2) includes the subclinical infection (PCV-2-SI), systemic (PCV-2-SD) and reproductive (PCV-2-RD) diseases, and porcine dermatitis and nephropathy syndrome (PDNS). Criteria to establish the diagnosis of these conditions have not changed over the years; thus, the triad composed by clinical signs, lesions and viral detection in lesions are still the hallmark for PCV-2-SD and PCV-2-RD. In contrast, PCV-2-SI diagnosis is not usually performed since this condition is perceived to be controlled by default through vaccination. PDNS is diagnosed by gross and histopathological findings, and PCV-2 detection is not recognized as a diagnostic criterion. Molecular biology methods as a proxy for PCVD diagnoses have been extensively used in the last decade, although these techniques should be mainly considered as monitoring tools rather than diagnostic ones. What has changed over the years is the epidemiological picture of PCV-2 through the massive use of vaccination, which allowed the decrease in infectious pressure paralleled with a decrease in overall herd immunity. Consequently, the need for establishing the diagnosis of PCVD has increased lately, especially in cases with a PCV-2-SD-like condition despite vaccination. Therefore, the objective of the present review is to update the current knowledge on diagnostic criteria for PCVDs and to contextualize the interest of using molecular biology methods in the overall picture of these diseases within variable epidemiological scenarios of PCV-2 infection.

Keywords: *Porcine circovirus 2* (PCV-2); porcine circovirus disease; clinical signs; pathology; diagnosis; epidemiology; vaccination

1. Introduction

Ten years ago, a review paper on clinical signs, pathology and laboratory diagnosis in relation to *Porcine circovirus 2* (PCV-2) was published, trying to unify existing criteria to establish a proper herd diagnosis of its infection outcomes [1]. At that time, just few years after starting mass vaccination against PCV-2 all over the world [2], it was discovered that PCV-2-sublinical infection (PCV-2-SI) was not only the most frequent form of this viral infection, but also the costliest one [3]. Since then, more than 10 years of a successful story behind PCV-2 vaccination has been contemplated by the swine industry [4,5], to the point that it is difficult to think of producing pigs without vaccination against this pathogen.

Besides the excellent results given by PCV-2 vaccination of piglets [6–8], the fact of immunizing this age group of animals has implied to change the epidemiology of this viral infection. The systematic vaccination at weaning implied to significantly decrease the overall herd infectious pressure, and some pig batches may reach almost seronegative

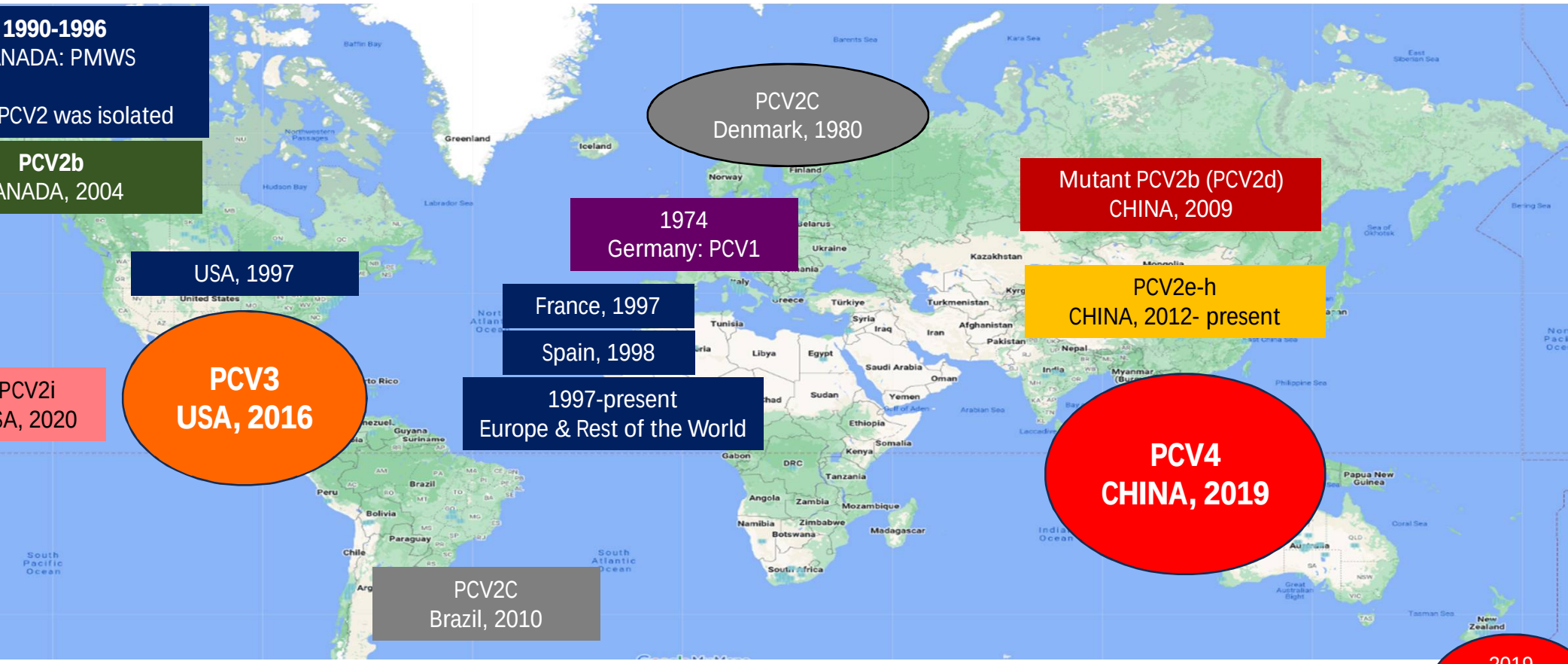
PCVD (Acronym)	Major Clinical Signs	Individual Diagnostic Criteria
PCV-2 subclinical infection (PCV-2-SI)	Decreased average daily gain (approx. 10–40 g/day) without any evident clinical sign	1. Lack of overt clinical signs 2. No or minimal histopathological lesions in tissues (lymphoid) 3. Low amount of PCV-2 in few (lymphoid) tissues, u in follicular areas Criteria 2 and 3 can potentially be substituted by PCV-2 detection techniques such as standard PCR
PCV-2 systemic disease (PCV-2-SD)	Wasting, weight loss, decreased rate of weight gain clinically evident, ill thrift or poor-doing animals, sometimes with respiratory and/or digestive disorders	1. Weight loss and paleness of skin (respiratory and/ digestive clinical signs may be present as well) 2. Moderate to severe lymphocyte depletion with granulomatous inflammation of lymphoid tissues granulomatous inflammation in other tissues) 3. Moderate to high amount of PCV-2 in lymphoid ti (the amount in the rest of affected tissues can be va
PCV2 reproductive disease (PCV-2-RD)	Abortions or mummifications	1. Reproductive failure at late gestation or SMEDI-lik condition * 2. Fibrous to necrotizing myocarditis of fetuses 3. Moderate to high amount of PCV-2 in the heart
	Regular return-to-estrus	1. Regular return-to-estrus/infertility 2. PCV-2 seroconversion following the return-to-estr and/or PCV-2 PCR/qPCR positivity around return-to-estrus occurrence
Porcine dermatitis and nephropathy syndrome (PDNS) **	Dark red papules and macules on skin, mainly in hind limbs and perineal area	1. Hemorrhagic and necrotizing skin lesions and/or s and pale kidneys with generalized cortical petechi 2. Systemic necrotizing vasculitis, and necrotizing an fibrinous glomerulonephritis

* SMEDI stands for stillbirth, mummification, embryonic death and infertility; infertility would apply to estrus scenarios. ** PCV-2 association with PDNS is still circumstantial, and detection of the virus is not c into its diagnostic case definition.

Segales and Sibila, 2022

ncbi.nlm.nih.gov/pmc/articles/PMC8953210/pdf/vetsci-09-00110.pdf

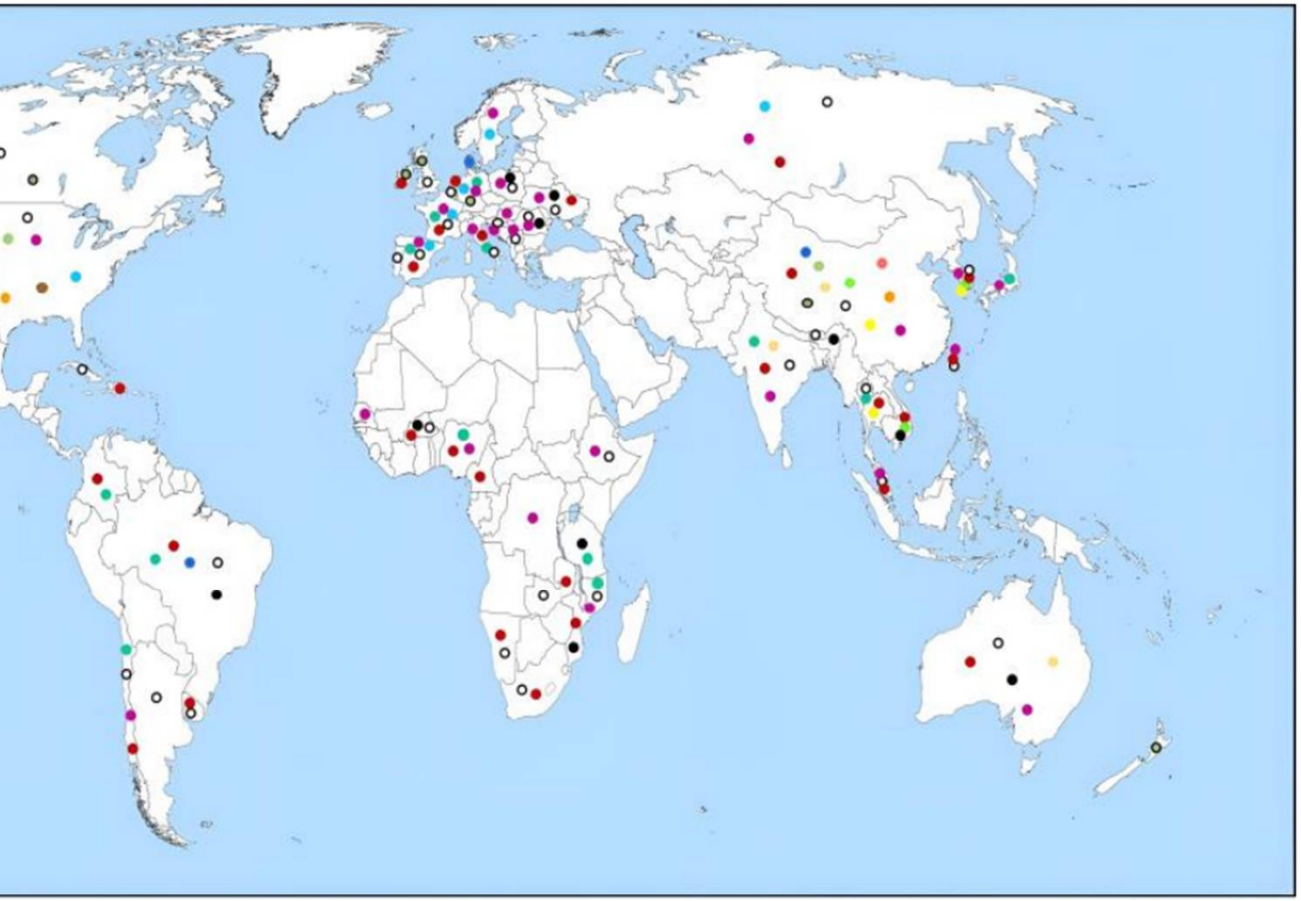
VOLUTION OF PORCINE CIRCOVIRUSE

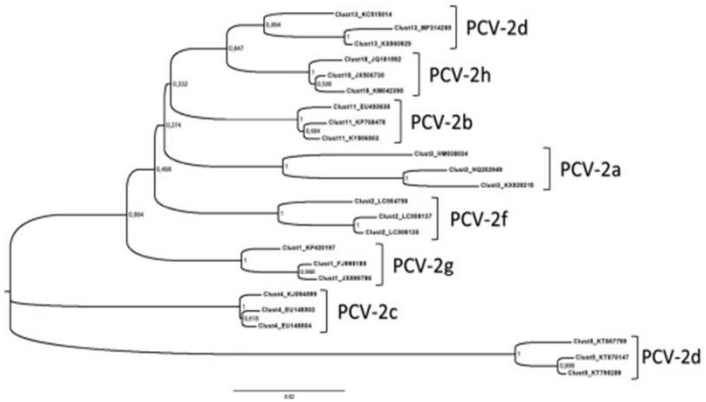


rmany | 1990-1996 PMWS - Canada | 1997 PCV2a – NA, Europe and Rest of the World | 2004 PCV2b - Canada | 2009-2013 PCV2d - China | 2012-present PCV2e-h - China | 2019 PCV4 - China

any | 1980s PCV2c - Denmark | 1998 PCV2d - Switzerland | Genotype Shift 2a/2b | Genotype Shift 2b/2d | 2010 PCV2c - Brazil | 2016 PCV3 - USA

VOLUTION OF PORCINE CIRCOVIRUS





Franzo and Segales, 2018

PCV1
PCV2a
PCV2b
PCV2c
PCV2d
PCV2e
PCV2f
PCV2g
PCV2h
PCV2i
PCV3a
PCV3b
PCV3c
PCV4a
PCV4b

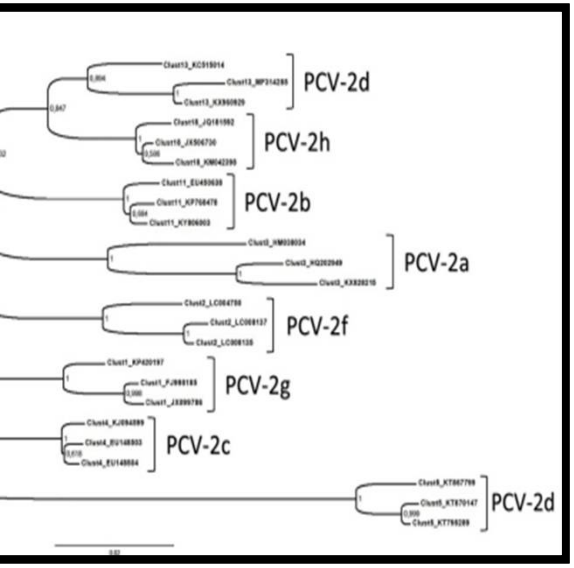
Table 2. Genomic similarities (%) among porcine circoviruses.

	PCV1–PCV2	PCV1–PCV3	PCV1–PCV4	PCV2–PCV3	PCV2–PCV4	PCV3–PCV4	References
e genome (nt)	68.0–76.0	43.5–44.0	50.3–51.6	42.7–48.0	51.5	42.9–45.0	[12,20–22,26,27]
icase (aa)	86.0	45.5–45.9	48.1–50.7	46.3–48.0	16.2–47.2	48.4–49.7	[20,22,24,26,27]
sid (aa)	65.0	24.0–25.2	43.1–44.4	25.9–37.0	12.7–45.0	23.2–24.8	[20,22–24,26,27]

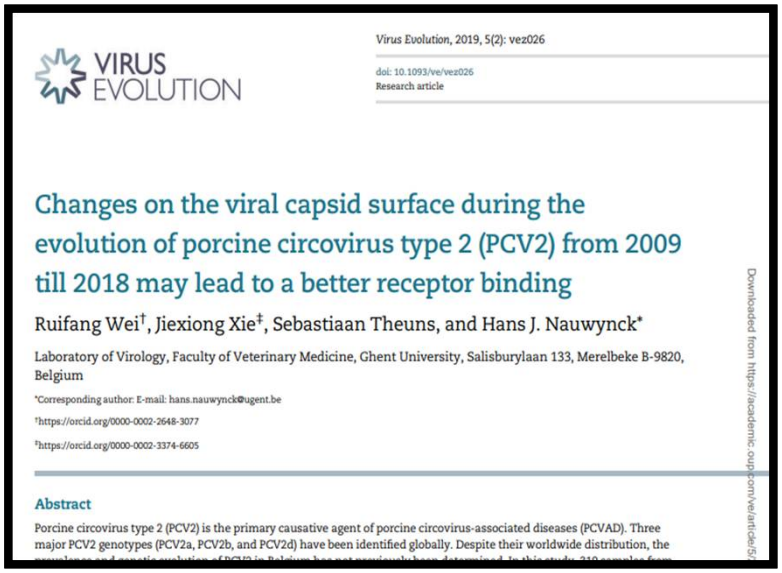
Maity *et al., 2023*

INICAL RELEVANCE OF DIFFERENT PCV2 GENOTYPE

NUMEROUS PCV-2 GENOTYPES BUT ALL BELONG TO ONLY ONE (1) SEROTYPE!



ranzo and Segales, 2018



VIRUS EVOLUTION

Virus Evolution, 2019, 5(2): vez026

doi: 10.1093/ve/vez026
Research article

Changes on the viral capsid surface during the evolution of porcine circovirus type 2 (PCV2) from 2009 till 2018 may lead to a better receptor binding

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Abstract

Porcine circovirus type 2 (PCV2) is the primary causative agent of porcine circovirus-associated diseases (PCVAD). Three major PCV2 genotypes (PCV2a, PCV2b, and PCV2d) have been identified globally. Despite their worldwide distribution, the

Wei *et al.*, 2019

- **Some degree of cross-protec across different PCV-2 genot**
- **Vaccination does not produc Sterilizing Immunity**
- **Changes on the viral capsid l a better receptor binding an fitness of certain PCV2 genot**

DIAGNOSTIC CRITERIA FOR PCV-2 INFECTIONS

nical signs and gross pathological lesions (i.e.
owth retardation, and wasting)

**esence of specific moderate to severe
ogical lesions in target tissues of affected**

mphoid tissues for PCV-2-SD,
eart for PCV-2-RD and
essels/glomeruli for PDNS; and

**esence of a moderate to high amount of PCV-
ese target tissues.**

tis

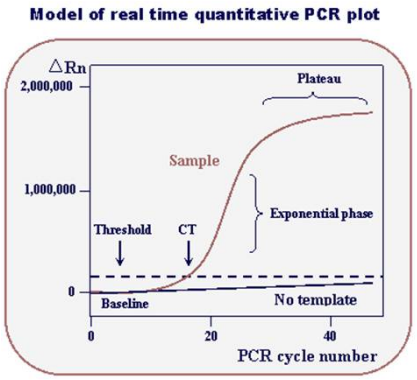
Table 1
Proposed terminology for porcine circovirus diseases (PCVDs) together with their case definition based on clinical and laboratorial findings.

PCVD proposed name (acronym)	Replaced terminology	Main clinical sign	Individ
PCV2 subclinical infection (PCV2-SI)	None	Decreased average daily gain without any evident clinical sign	1. Lack 2. No o lesions lymph 3. Low (lymph Criteria substit techniq
PCV2 systemic disease (PCV2-SD)	Postweaning multisystemic wasting syndrome (PMWS) Porcine circovirosis PCV2-associated systemic infection	Wasting, weight loss, decreased rate of weight gain clinically evident, ill thrift or poor-doer	1. Weig (respir clinical well) 2. Mod depleti inflam (plus g in a nu 3. Mod PCV2 i
PCV2 lung disease (PCV2-LD)	PCV2-associated respiratory disease Proliferative and necrotizing pneumonia (PNP)	Respiratory distress, dyspnea	1. Resp 2. Lym granul bronch peribr mild-t ulcerat prolife pneum lesions 3. Mod PCV2 i Lymph display (other scope
PCV2 enteric disease (PCV2-ED)	PCV2-associated enteritis	Diarrhea	1. Diar 2. Gran lymph granul Peyer's lymph 3. Mod PCV2 i patche Lymph patche micros would PCV2-S
PCV2 reproductive disease (PCV2-RD)	PCV2-associated reproductive failure	Abortions or mummifications	1. Repr gestati 2. Fibr myoca 3. Mod PCV2 i The us PCR on sensiti
		Regular return-to-estrus	1. Regu 2. PCV2 the ret PCR po return-
Porcine dermatitis and nephropathy syndrome (PDNS)[a]	None	Dark red papules and macules on skin, mainly in hind limbs and perineal area	1. Hem skin le pale ki cortica 2. Syst and ne glomer

[a] PDNS is considered an immune-complex disease of not yet demonstrated etiology; link with PCV2 is still circumstantial, and detection of t
its diagnostic case definition. Therefore, no novel terminology has been suggested for this particular syndrome.

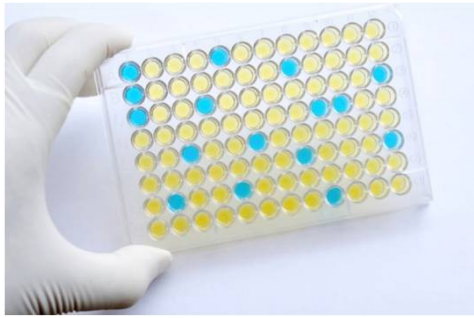
Segales, 2012

PCV-2 MONITORING TOOLS



qPCR THRESHOLDS	
erum	>10^7 PCV-2 DNA copies/ml
ungs or lymph node	2-3 log more than in serum
nummified and myocardium, spleen and liver)	>10^9 PCV-2 DNA copies/g tissue
	none

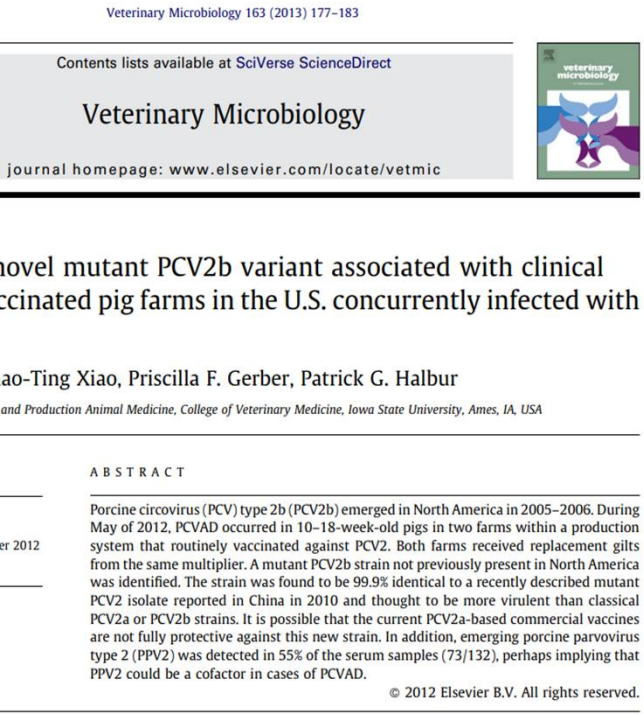
Segales and Sibila, 2022



qPCR

ELISA

PCVADs IN VACCINATED PIGS?



Veterinary Microbiology 163 (2013) 177–183

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

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

novel mutant PCV2b variant associated with clinical
ccinated pig farms in the U.S. concurrently infected with

ao-Ting Xiao, Priscilla F. Gerber, Patrick G. Halbur

and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

ABSTRACT

Porcine circovirus (PCV) type 2b (PCV2b) emerged in North America in 2005–2006. During May of 2012, PCVAD occurred in 10–18-week-old pigs in two farms within a production system that routinely vaccinated against PCV2. Both farms received replacement gilts from the same multiplier. A mutant PCV2b strain not previously present in North America was identified. The strain was found to be 99.9% identical to a recently described mutant PCV2 isolate reported in China in 2010 and thought to be more virulent than classical PCV2a or PCV2b strains. It is possible that the current PCV2a-based commercial vaccines are not fully protective against this new strain. In addition, emerging porcine parvovirus type 2 (PPV2) was detected in 55% of the serum samples (73/132), perhaps implying that PPV2 could be a cofactor in cases of PCVAD.

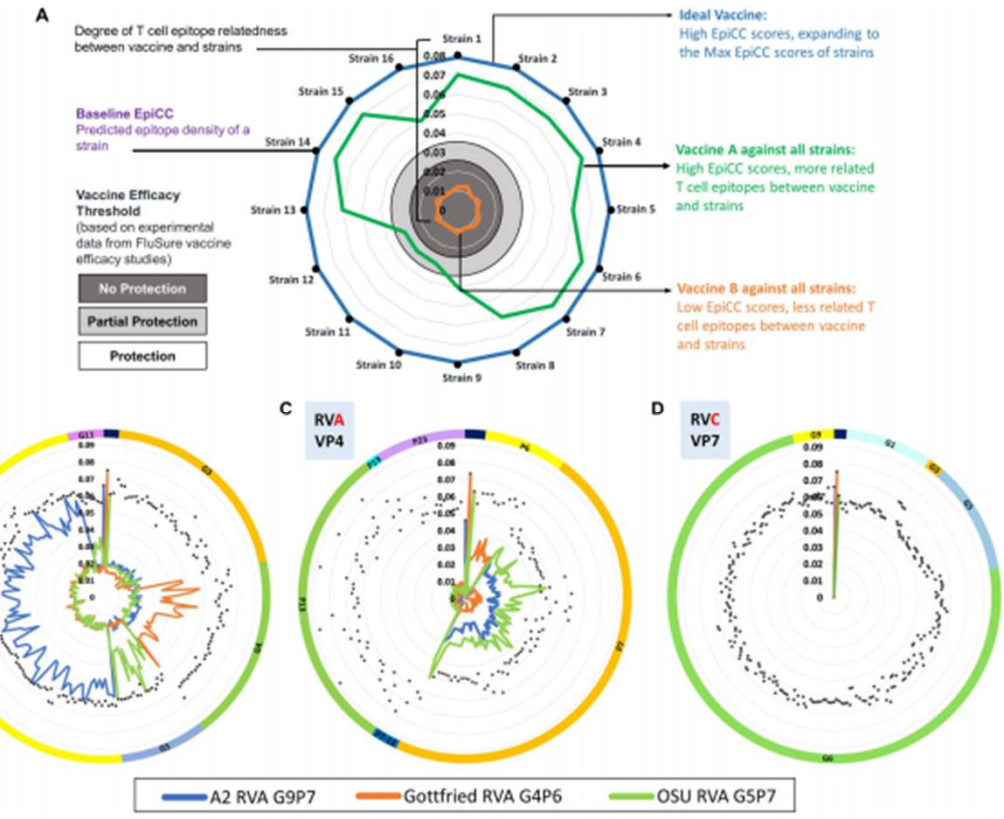
Opriessnig *et al.*, 2013

- **OCCURRENCE OF CLINICAL PCVADS IN VACCINATED P**
  - PCV2d (mutant PCV2b) is currently the most prevalent
  - Most of the commercially available PCV vaccines are prepared from Genotype 2A

- **DIFFERENTIAL EFFICACY of different vaccine platforn when animals are raised in suboptimum conditions?**
  - Non-sterilizing immunity provides opportunity for certain s to preferentially circulate in “challenging” environment

MINOR VARIATIONS IN B AND T CELL EPITOPES BETWEEN VACCINES AND FIELD PCV2 HAVE RESULTED TO DIFFERENCES IN IMMUNE RECOGNITION

Wei *et al.*, 2019



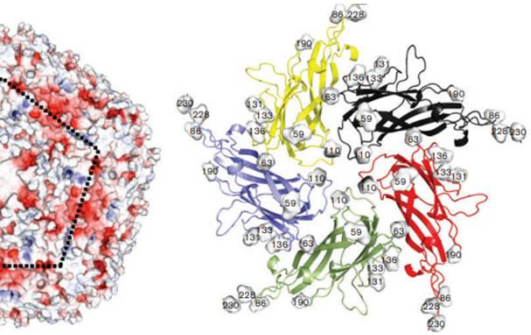
Moise *et al*, 2020

T-CELL EPITOPE CONTENT COMPARISON (EpiCC)

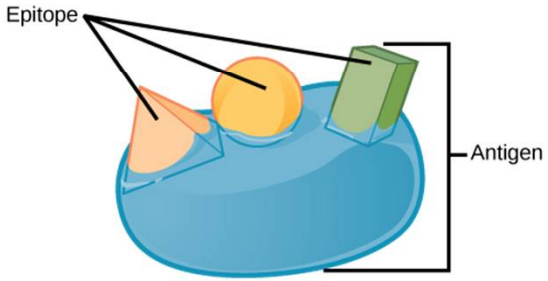
etis

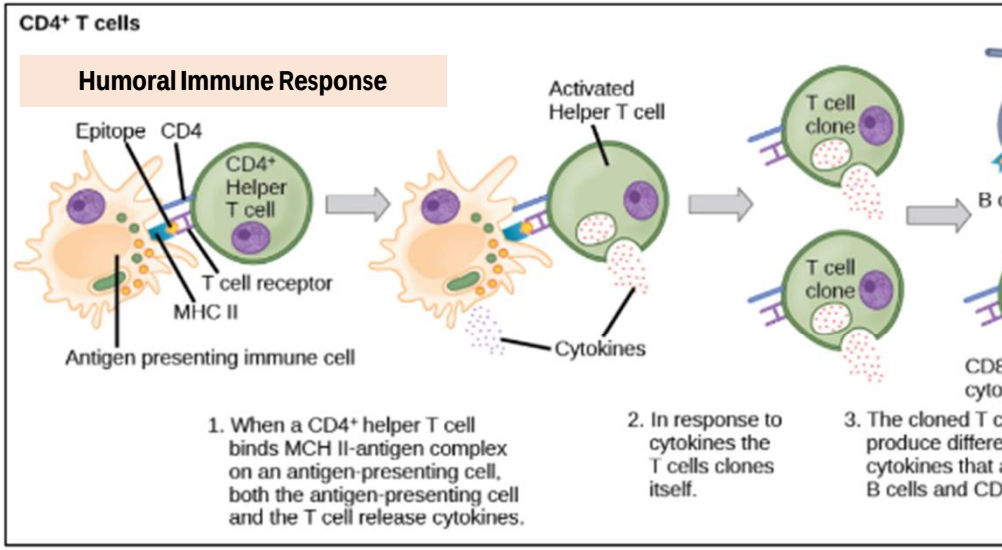
CELL EPITOPE CONTENT COMPARISON (EpiCC

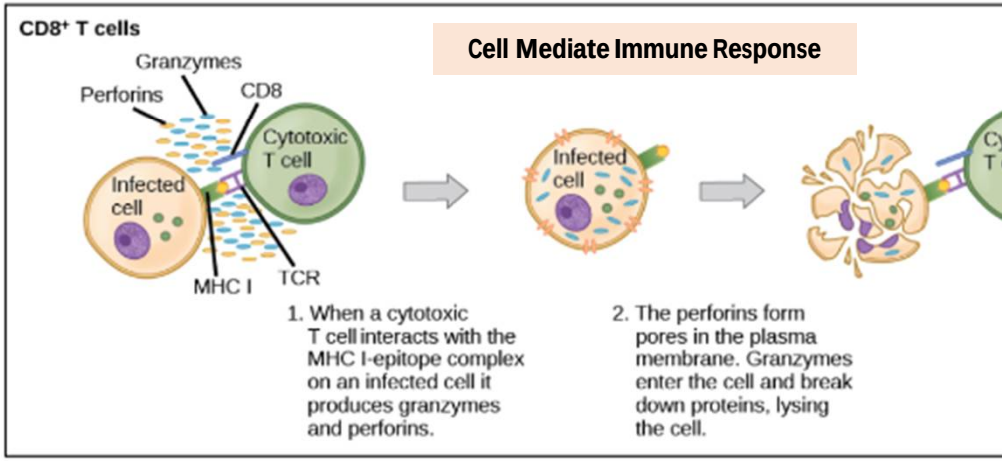
"AN EMERGING MUNOINFORMATICS TOOL T COMPARES T CELL EPITOPE CONTENT OF PCV2 AND OTHER VIRUSES"



with 60 sub-units of capsid proteins (5x12)
arranged in canonical viral jelly roll

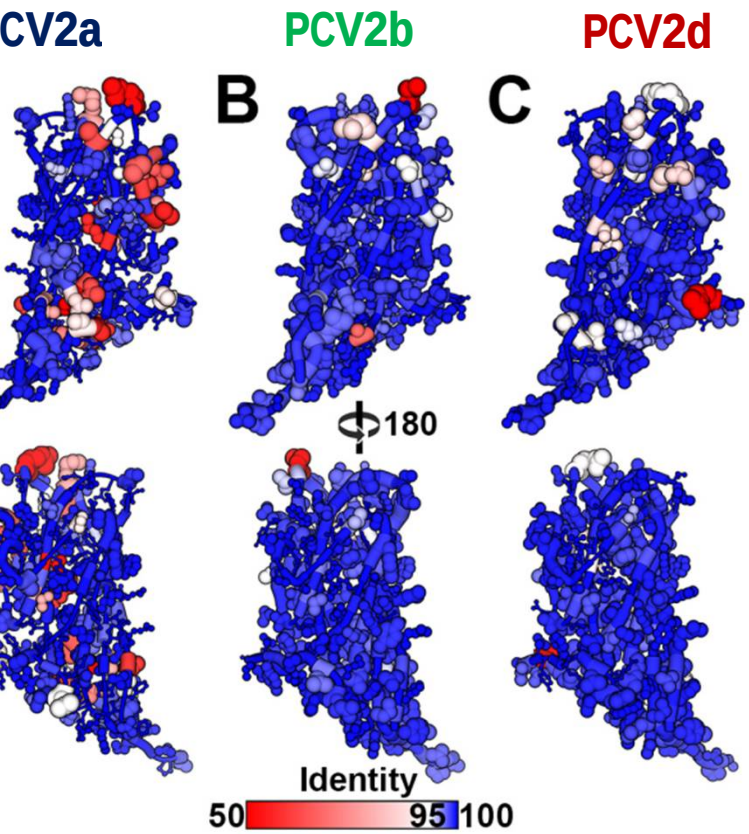






https://opentextbc.ca/

EPITOPES OF PCV-2



Khayat, *et al., 2011/* Kekarainen et al., 2014

Evolution Rate of PCV2:
1.2×10^{-3} substitution/site/year

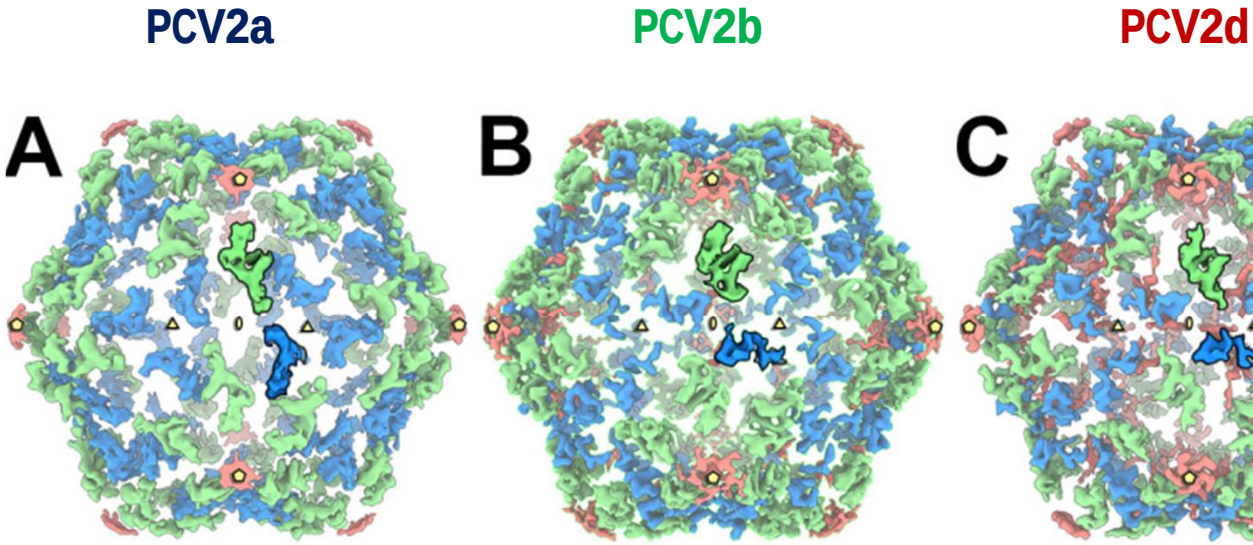


TABLE 1. Epitopes of PCV1 and PCV2

Epitope	Residues[a]	Aligned sequence[b]	MAb binding residue(s)
A	69–83	NV**NEL**RFNI**GQ**FLPP V**DMM**RFNI**ND**FLPPG	Asp70, Met71, Asn77, As
B[d]	113–127	T**SNQ**RGVGS**TV**VILD **QGD**RGVGS**SA**VILDD	Gln113, Asp115
C[d]	117–131	RGVGS**TV**VILD**A**NFV GVGS**SA**VILD**D**NFVT	Asp127
D	169–183	DQ**TID**W**FQPNNKRNQ** F**TIDY****FQPNNKRNQ**L	Thr170
E	193–207	NV**EH**TGLGY**ALQ**N**ATT** V**DH**VGLGT**AFE**N**SIY**	Glu203, Ile206, Tyr207
F	25–39	**RRPYLVHP**AFRN**RYRWR** **RRPWLVHP**--RH**RYRWR**	

Khayat, *et al., 2019*

CELL EPITOPE CONTENT COMPARISON (EpiCC

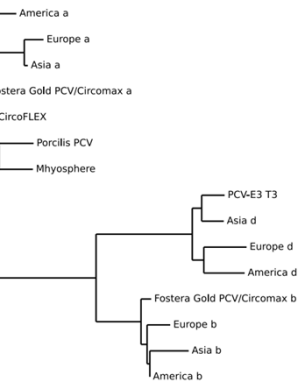
LD
ES OF
CTED
/2

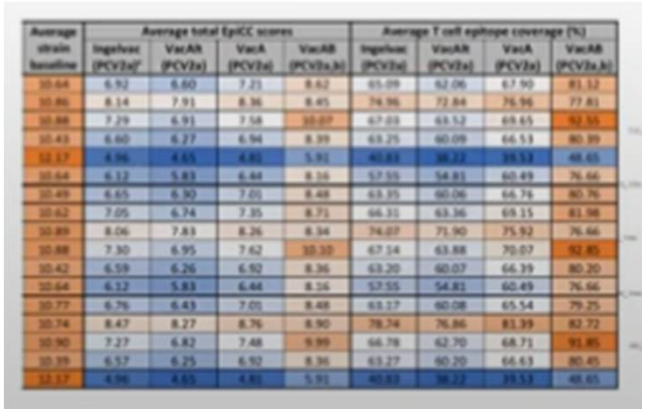
NUCLEOTIDE SEQUENCING OF ORF2 GENE OF PCV2

Input Sequence

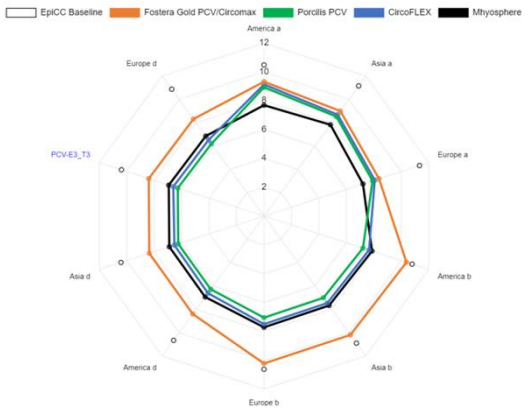
T-cell Epitope Prediction (Pig Matrix)

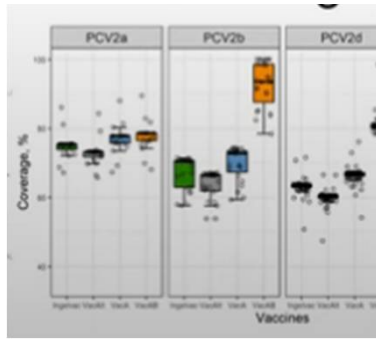
T Cell Epito
Content Compa
(EpiCC)





Average strain baseline	Average total EpiCC scores				Average T cell epitope coverage (%)			
	Ingelvac (PCV2a)*	VacAR (PCV2a)	VacA (PCV2a)	VacAB (PCV2a,b)	Ingelvac (PCV2a)	VacAR (PCV2a)	VacA (PCV2a)	VacAB (PCV2a,b)
10.64	6.92	6.60	7.21	8.62	65.09	62.06	67.90	81.12
10.86	8.14	7.91	8.36	8.45	74.96	72.84	76.96	77.81
10.88	7.29	6.91	7.58	10.07	67.03	63.52	69.65	92.55
10.43	6.60	6.27	6.94	8.39	63.25	60.09	66.53	80.39
12.17	4.96	4.65	4.81	5.91	40.83	38.22	39.53	48.65
10.64	6.12	5.83	6.44	8.16	57.55	54.81	60.49	76.66
10.49	6.65	6.30	7.01	8.48	63.35	60.06	66.76	80.76
10.62	7.05	6.74	7.35	8.71	66.31	63.36	69.15	81.98
10.89	8.06	7.83	8.26	8.34	74.07	71.90	75.92	76.66
10.88	7.30	6.95	7.62	10.10	67.14	63.88	70.07	92.85
10.42	6.59	6.26	6.92	8.36	63.20	60.07	66.39	80.20
10.64	6.12	5.83	6.44	8.16	57.55	54.81	60.49	76.66
10.77	6.76	6.43	7.01	8.48	63.17	60.08	65.54	79.25
10.74	8.47	8.27	8.76	8.90	78.74	76.86	81.39	82.72
10.90	7.27	6.82	7.48	9.99	66.78	62.70	68.71	91.85
10.39	6.57	6.25	6.92	8.36	63.27	60.20	66.63	80.45
12.17	4.96	4.65	4.81	5.91	40.83	38.22	39.53	48.65





OGENETIC ANALYSIS

EpiCC BASELINE

EpiCC SCORES

T-CELL EPITOPE CO

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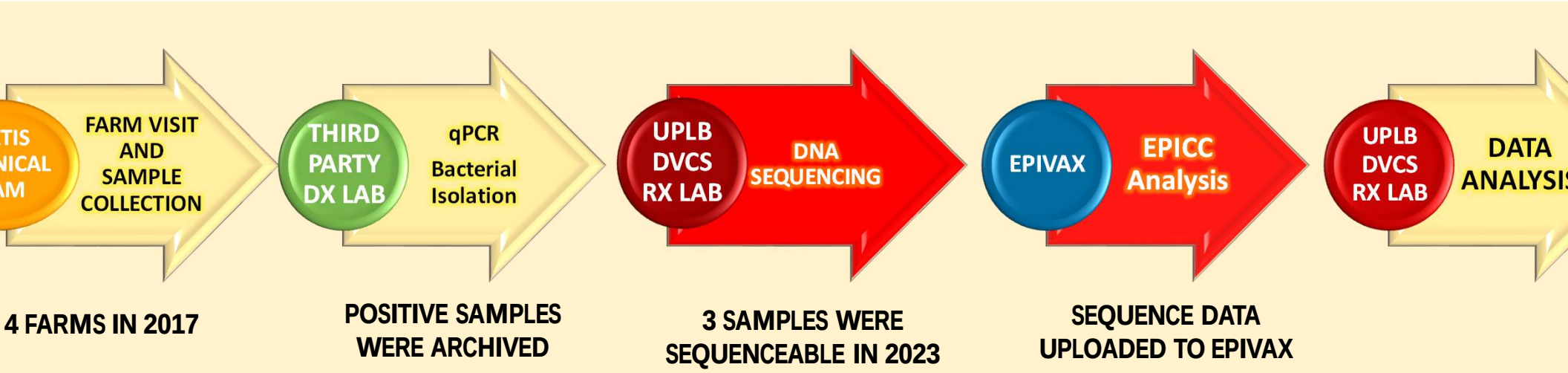
MOLECULAR CHARACTERIZATION OF PCV-2 IN THE PHILIPPINES: PILOT STUDY



hat Xuan Dinh *et al.* 2021. Porcine circovirus genotypes and their copathogens in pigs with respirato
ease in southern provinces of Vietnam. Archives of Virology 166:403-411.
ps://doi.org/10.1007/s00705-020-04878-y

RM 1 (1700 sow level farrow to finish farm, ndrace x Large White x Duroc three-way cros

1 (Module 1)

signs:

vasting, thumping, jaundice

levated mortality (8%) and morbidity (15-20%)

round 6-10 weeks old

psy signs:

nflamed lymph nodes,

nultifocal interstitial nephritis (white spotted kidneys),

dematous lungs.

ation: PCV2d whole cell inactivated vaccine at D21, 1mL IM

B. Isolate 2 (Module 2)

- **PCVAD signs:**
  - wasting, thumping, jaundice
  - elevated mortality 8% and morbidity (15-20%)
  - around 6-10 weeks old.
- **Necropsy signs:**
  - inflamed lymph nodes,
  - multifocal interstitial nephritis (white spotted kid
  - edematous lungs.

- **Vaccination:** PCV2d whole cell inactivated vaccine at D2

ULTS (from 10-weeks old tissue samples):

PCV2d PCR positive, Ct value of 10,

PRRS North American Strain, Ct value of 15.711

***Pasteurella multocida* positive in bacterial isolation**

LAB Results (from 10-weeks old tissue samp

1. **PCV2d PCR positive with Ct value of 10**
2. **PRRS North American Strain, Ct value of**
3. **PRRS EU strain with Ct value of 24.632**
4. **No significant bacteria isolated**

tis

Farm 2 (2200 sow level farrow to finish farm, ndrace x Large White x Duroc three-way cross)

AD signs

asting, thumping, jaundice
evated mortality (8%) and morbidity (40%)
round 9-10 weeks old.

opsy signs

flamed lymph nodes,
ultifocal interstitial nephritis (white spotted kidneys),
dematous lungs,
rthritis of the hind limbs.

cination:

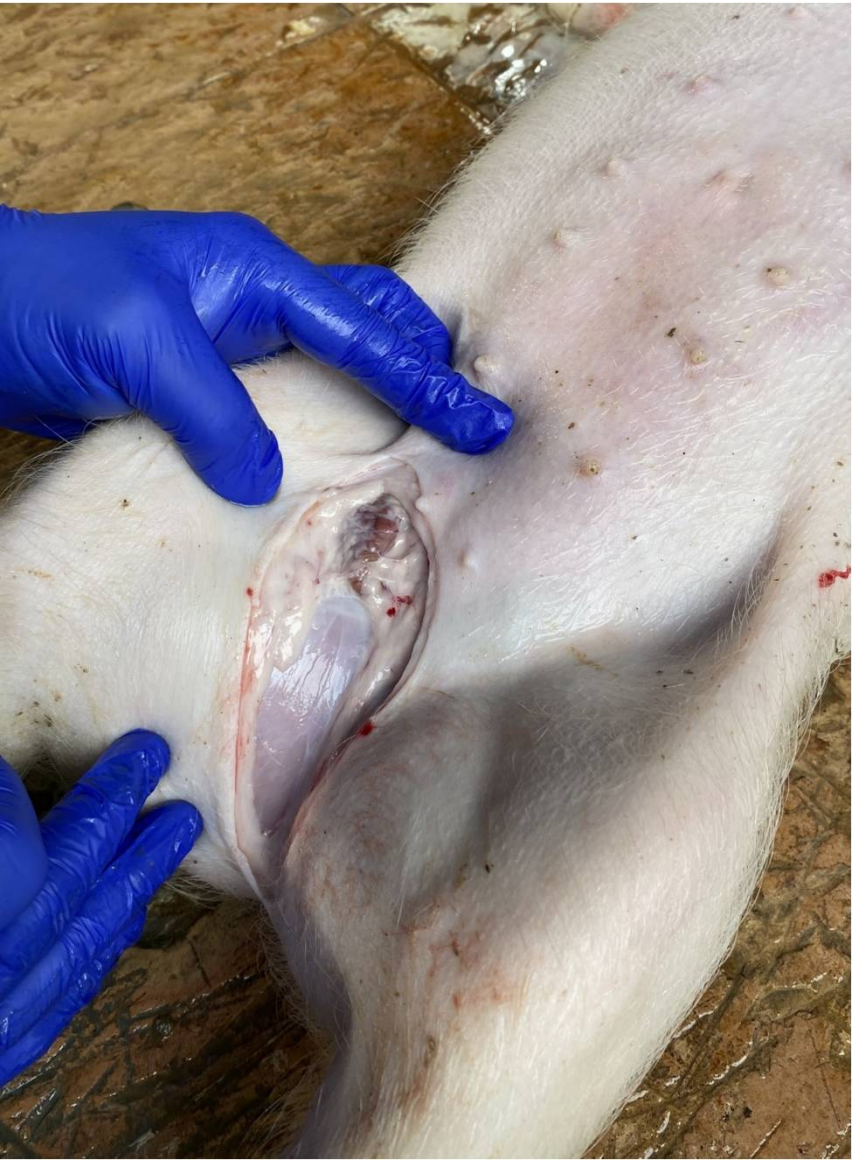
aculovirus expressed PCV2a vaccine, 1mL IM.

LAB Results (from 10-weeks old tissue samples):

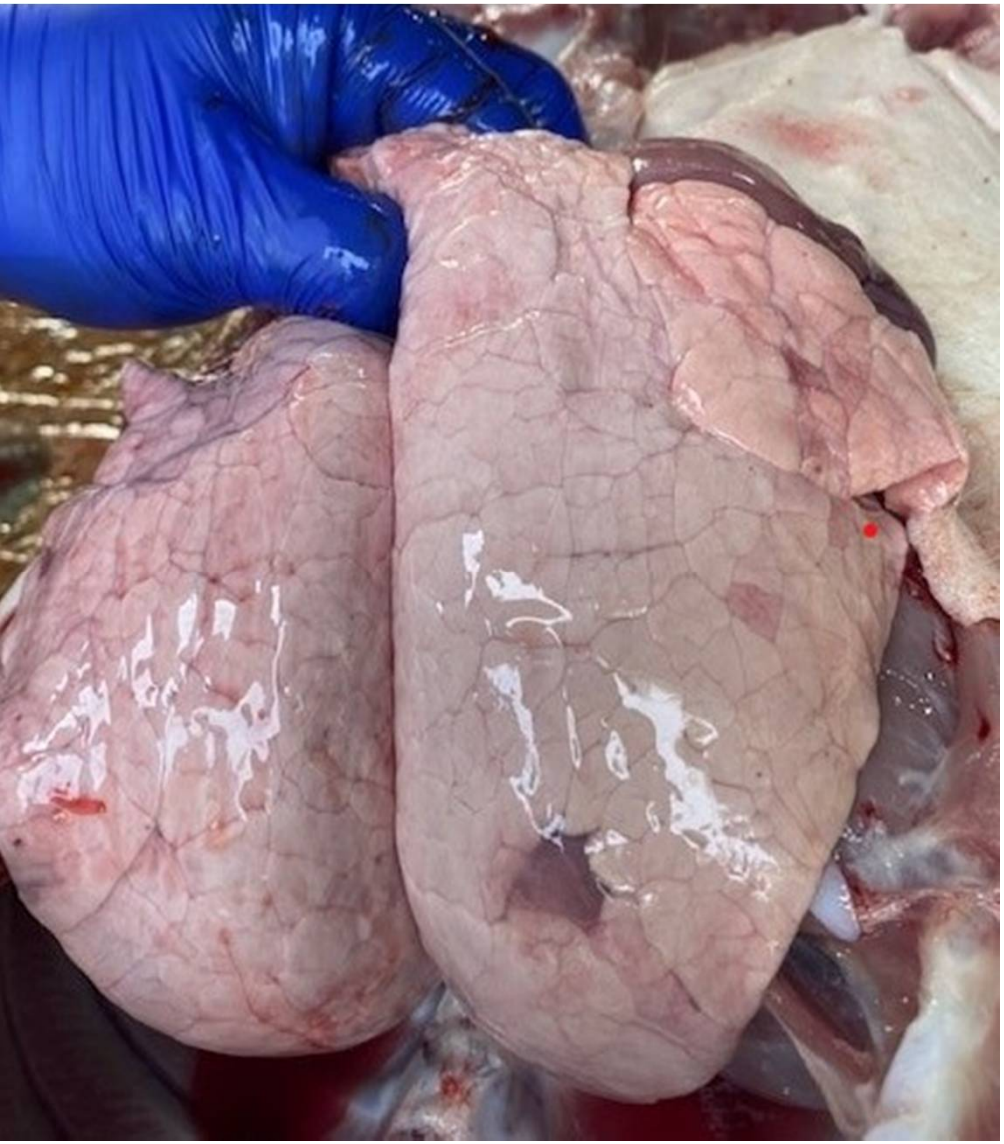
1. PCV2d PCR positive with Ct value of 5.
2. PRRS North American Strain, Ct value 13.338
3. *Hemophilus parasuis* serovar 6 and 14 positive in bacterial isolation and serotyping

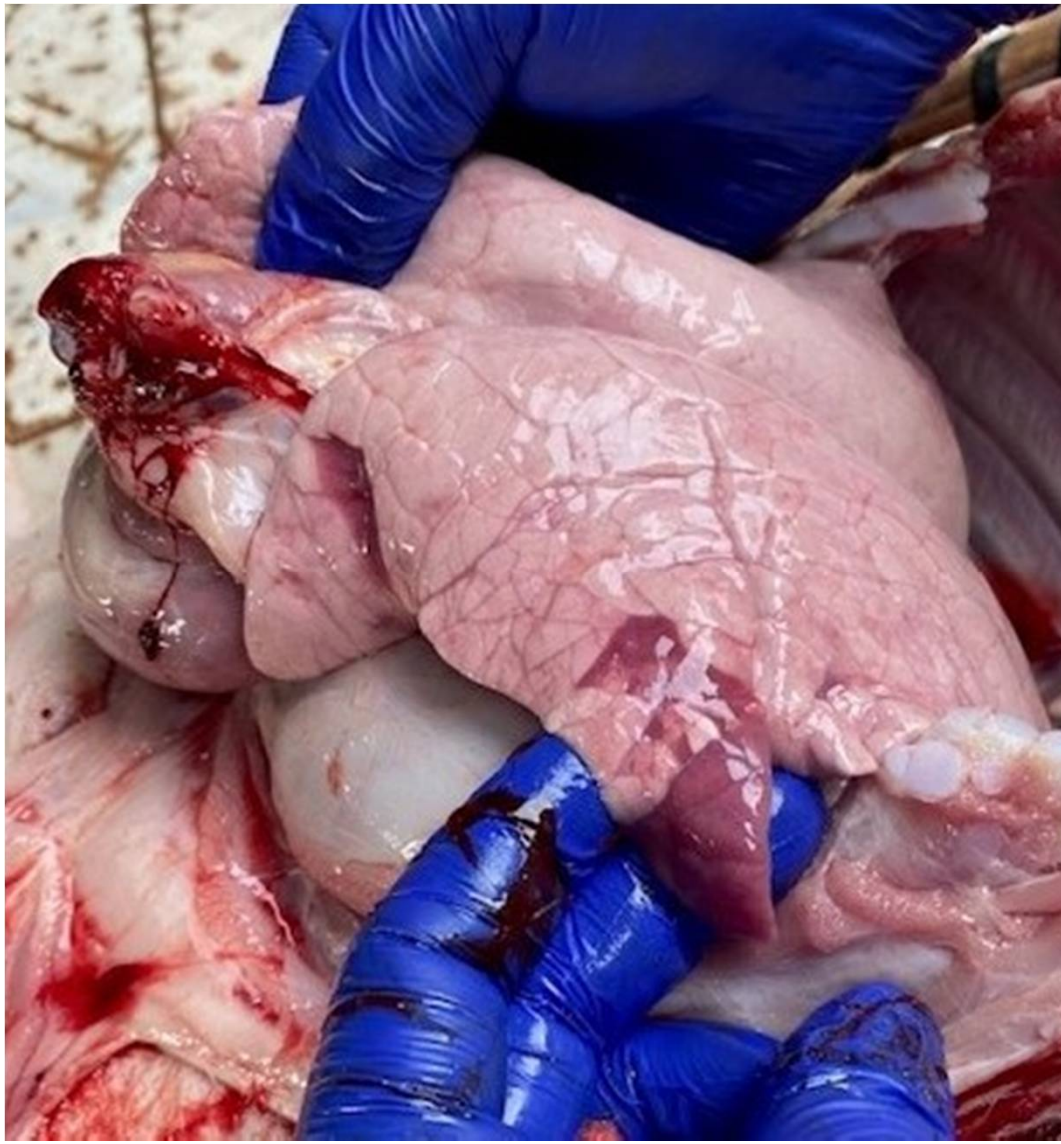
etis

GROSS MORPHOLOGICAL LESIONS



GROSS MORPHOLOGICAL LESIONS





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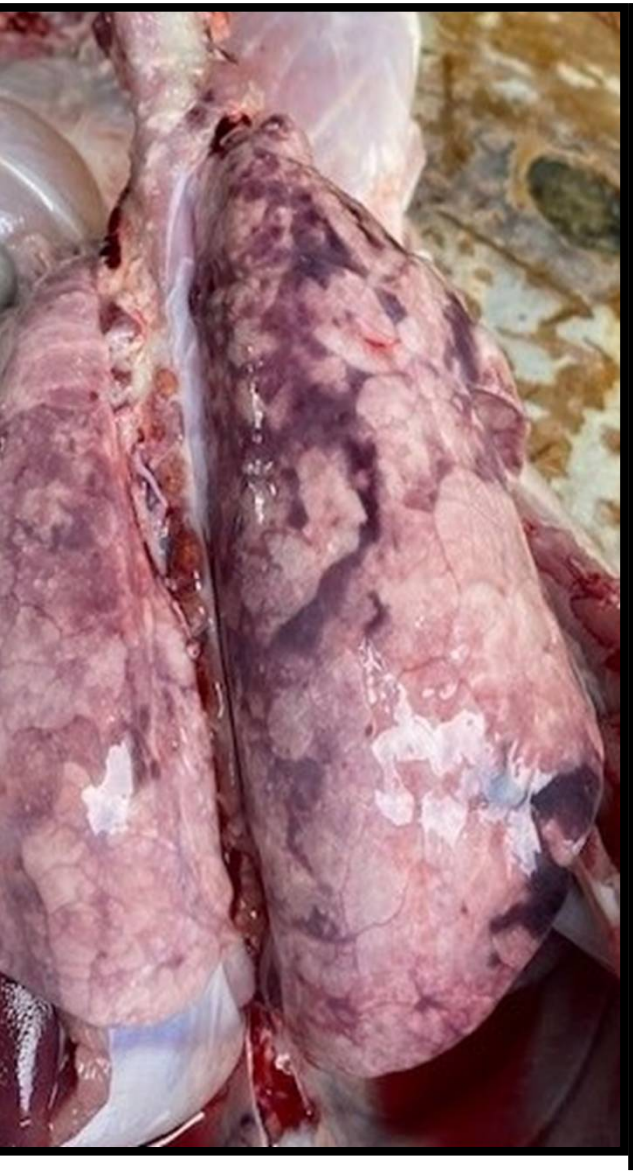
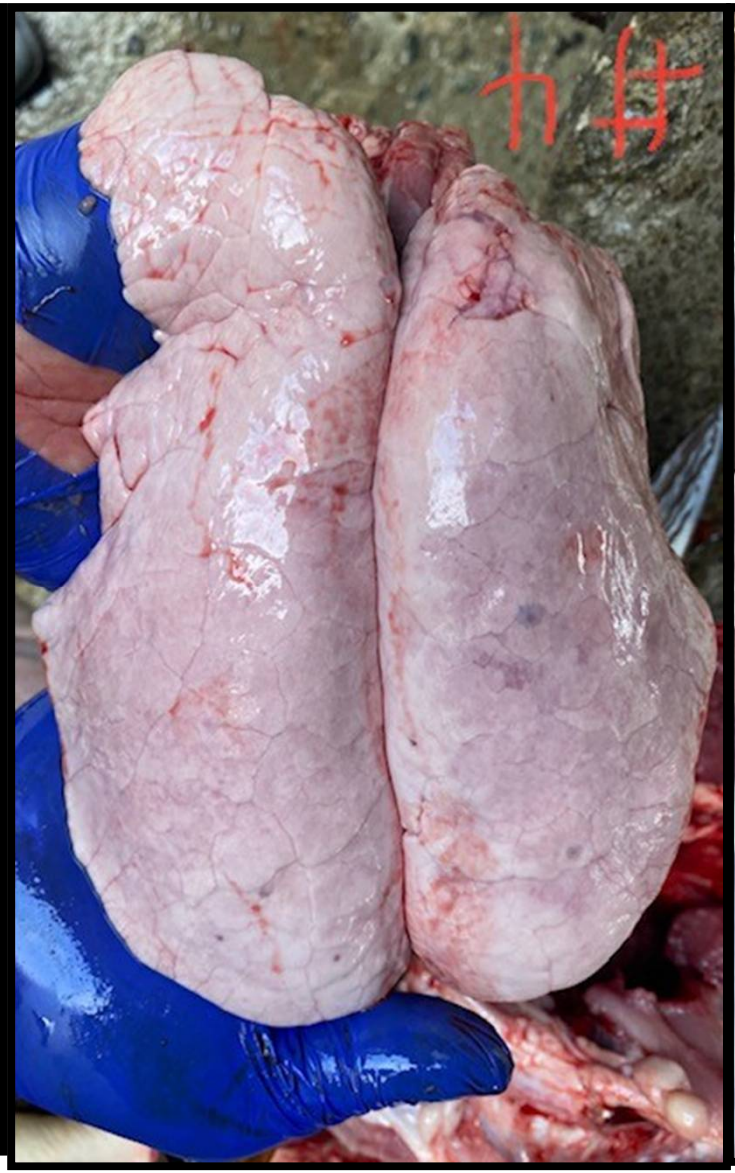
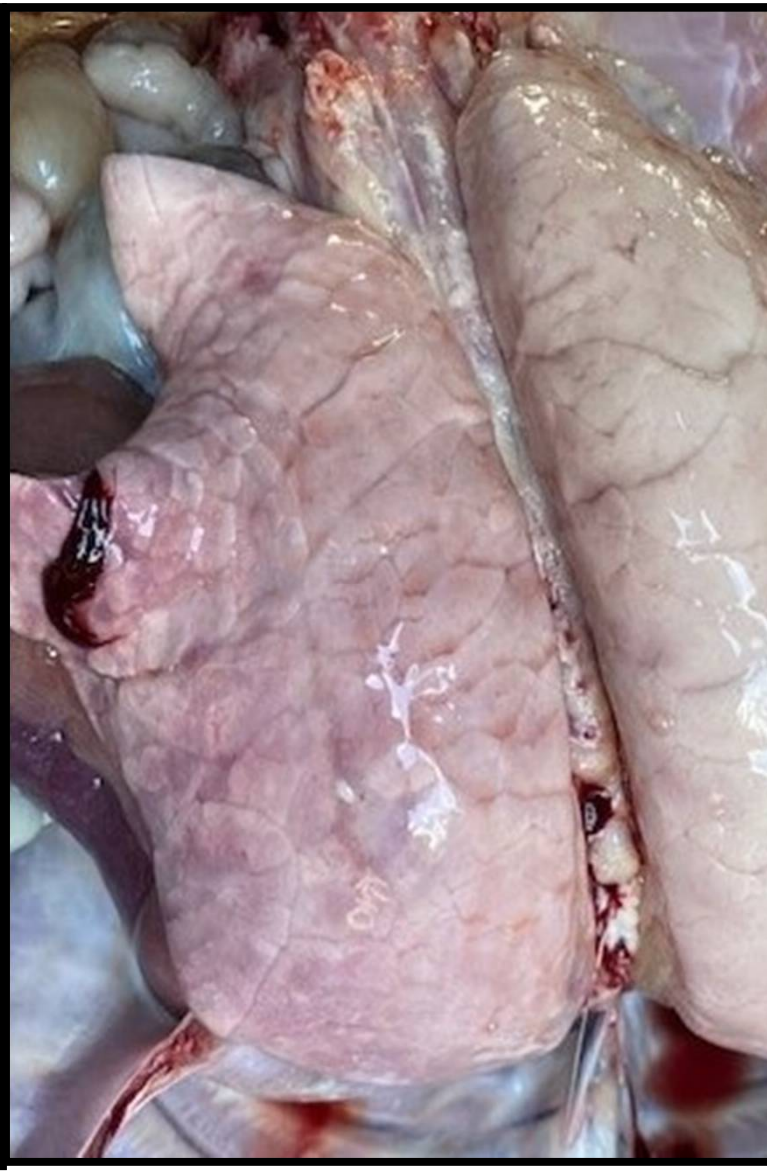
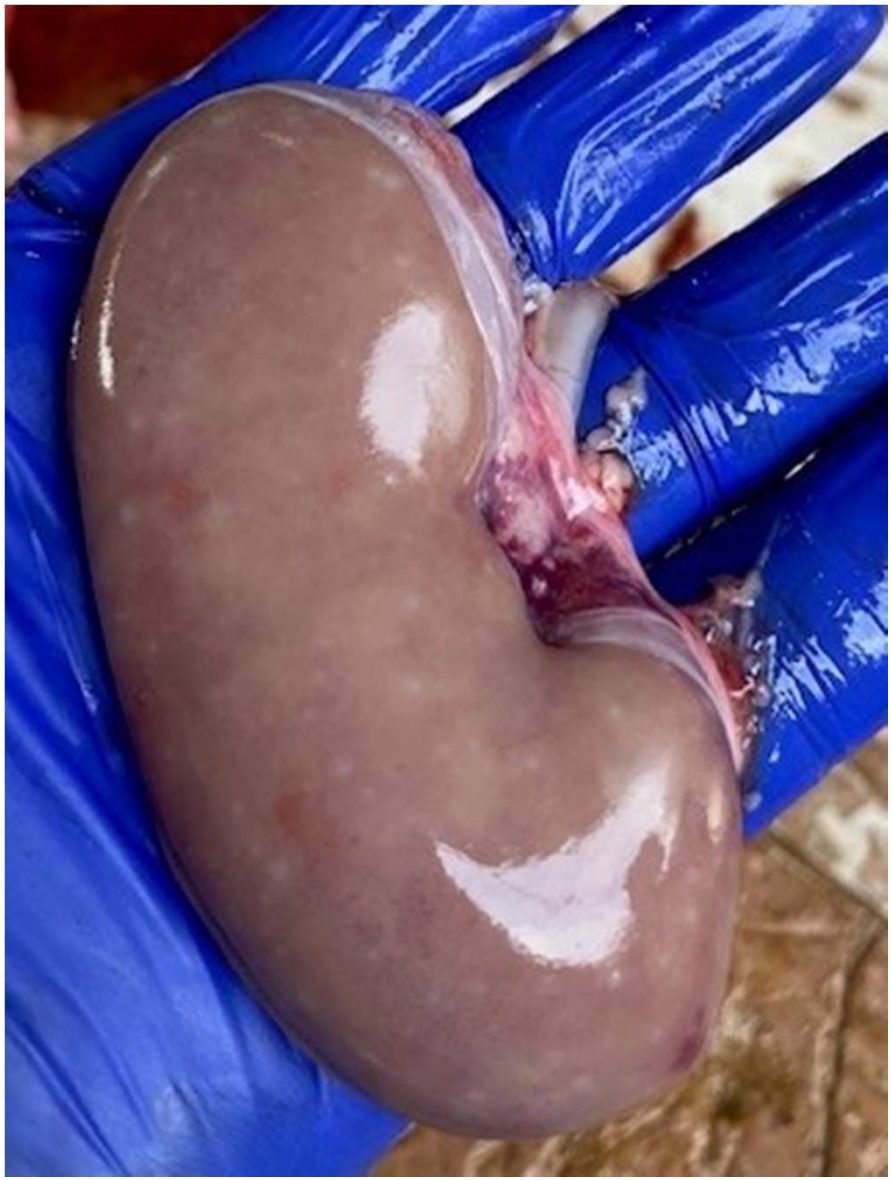
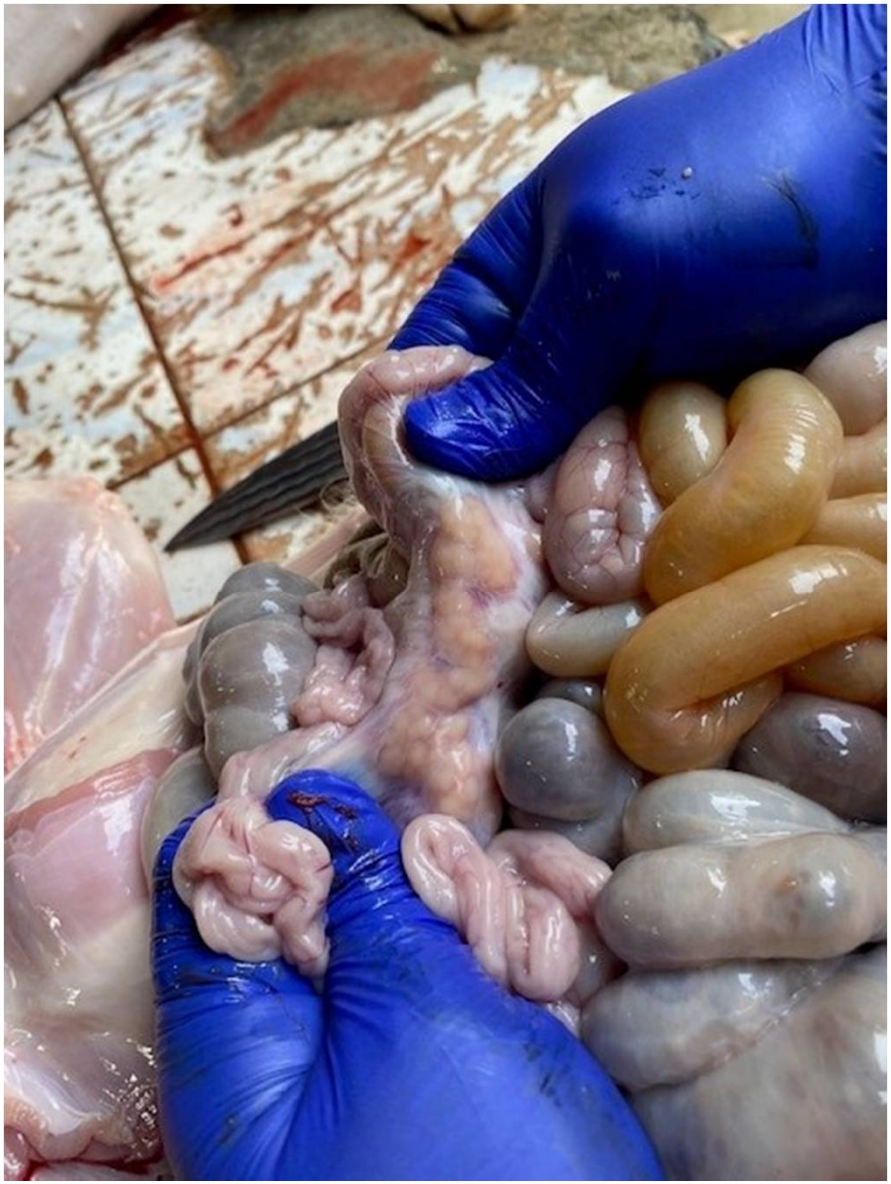






GROSS MORPHOLOGICAL LESIONS





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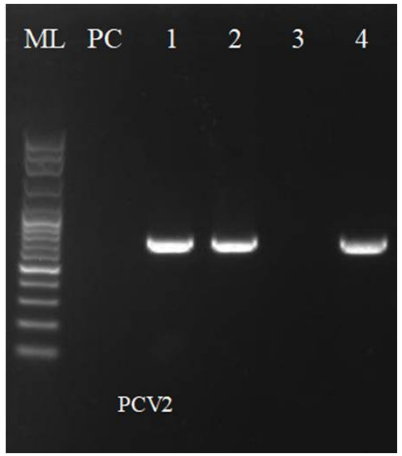
NUCLEOTIDE SEQUENCING OF ORF2 OF PCV-2 FIELD SAMPLES

AMPLE NAME	SAMPLE TYPE	RESULTS
Farm 1A	DNA extract	PCV-2 DNA Detected
Farm 1A	Tissue samples	PCV-2 DNA Detected
Farm 1B	DNA extract	PCV-2 DNA Detected
Farm 1B	Tissue samples	PCV-2 DNA Detected
Farm 3	DNA extract	PCV-2 DNA Not Detected
Farm 3	Tissue samples	PCV-2 DNA Not Detected
Farm 2	DNA extract	PCV-2 DNA Detected
Farm 2	Tissue samples	PCV-2 DNA Detected

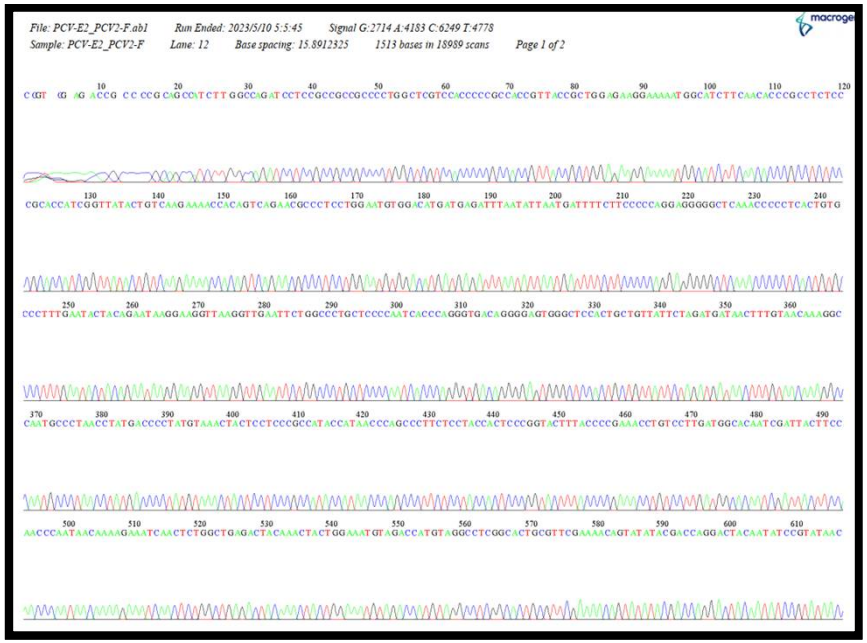


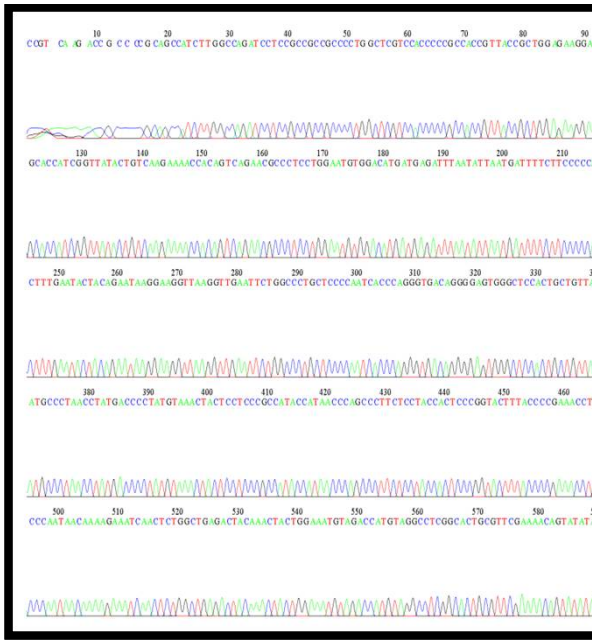
NUCLEOTIDE SEQUENC

674bp ORF2 gene

224 amino acid seque

Length of ORF2 of PCV2: 705bp
Total Amino Acid Sequence: 223-234 aa





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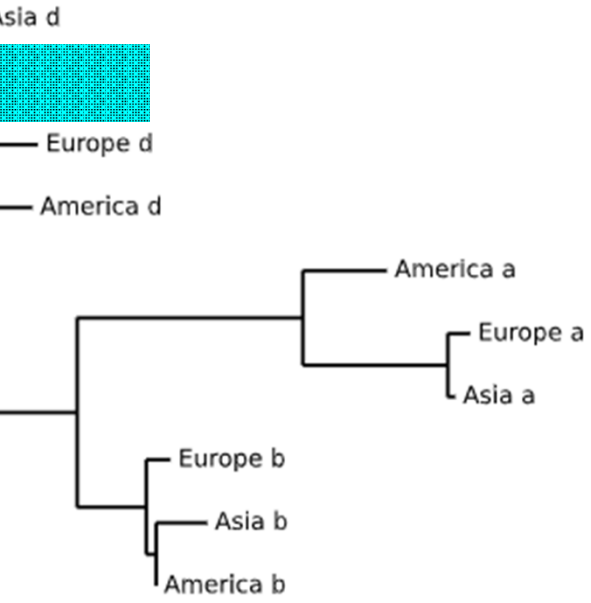
ANALYSIS 1. GENOTYPING AND PHYLOGENETIC ANALYSES OF FIELD PCV2 SAMPLES

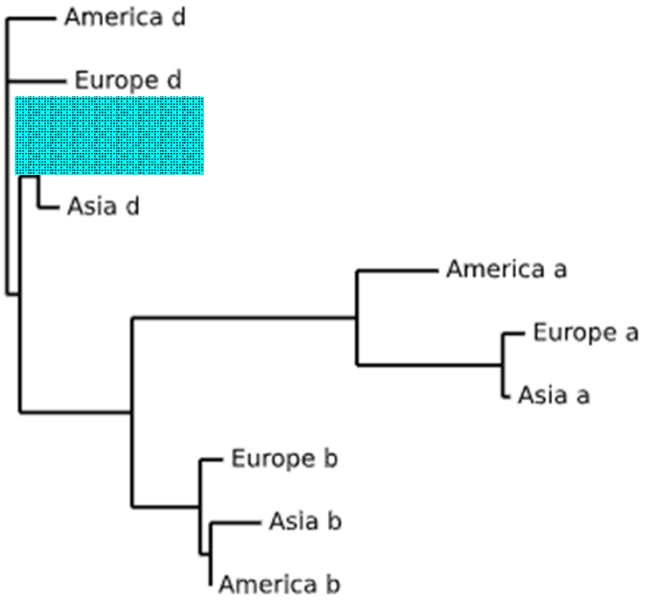
ID	Submission ID	Country	Genotype	Farm Name	Collection Date	Veterinarian Name	Sample Type	PCR Ct Values	PCR Vir
	ZTS-EpiVax-2023-51.1	Philippines	PCV2d	08222022 Farm 5	08/22/2022	Marlon Linatoc	Tissues (lungs+LN)	10 Ct	

ID	Submission ID	Country	Genotype	Farm Name	Collection Date	Veterinarian Name	Sample Type	PCR Ct Values	PCR Vir
	ZTS-EpiVax-2023-53.1	Philippines	PCV2d	08222022 Farm 5	08/22/2022	Marlon Linatoc	Tissues (lungs+LN)	10 Ct	

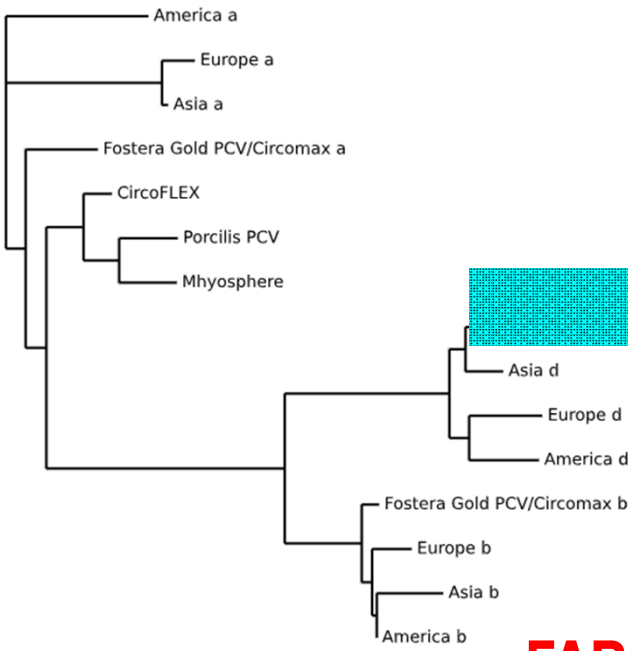
ID	Submission ID	Country	Genotype	Farm Name	Collection Date	Veterinarian Name	Sample Type	PCR Ct Values	PCR Vir
	ZTS-EpiVax-2023-46.1	Philippines	PCV2d	Farm 5 M3 020723	02/07/2023	Marlon Linatoc	Tissues (lungs+LN)	5.552 ct	



FARM 1A



FARM 1B

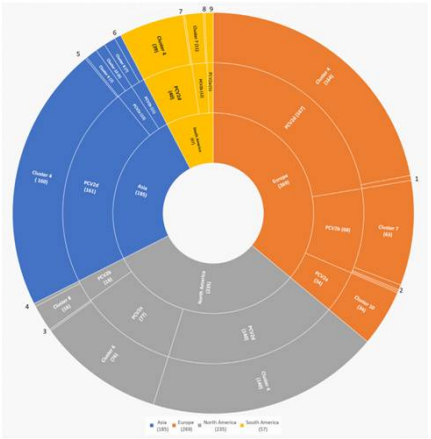


FAR

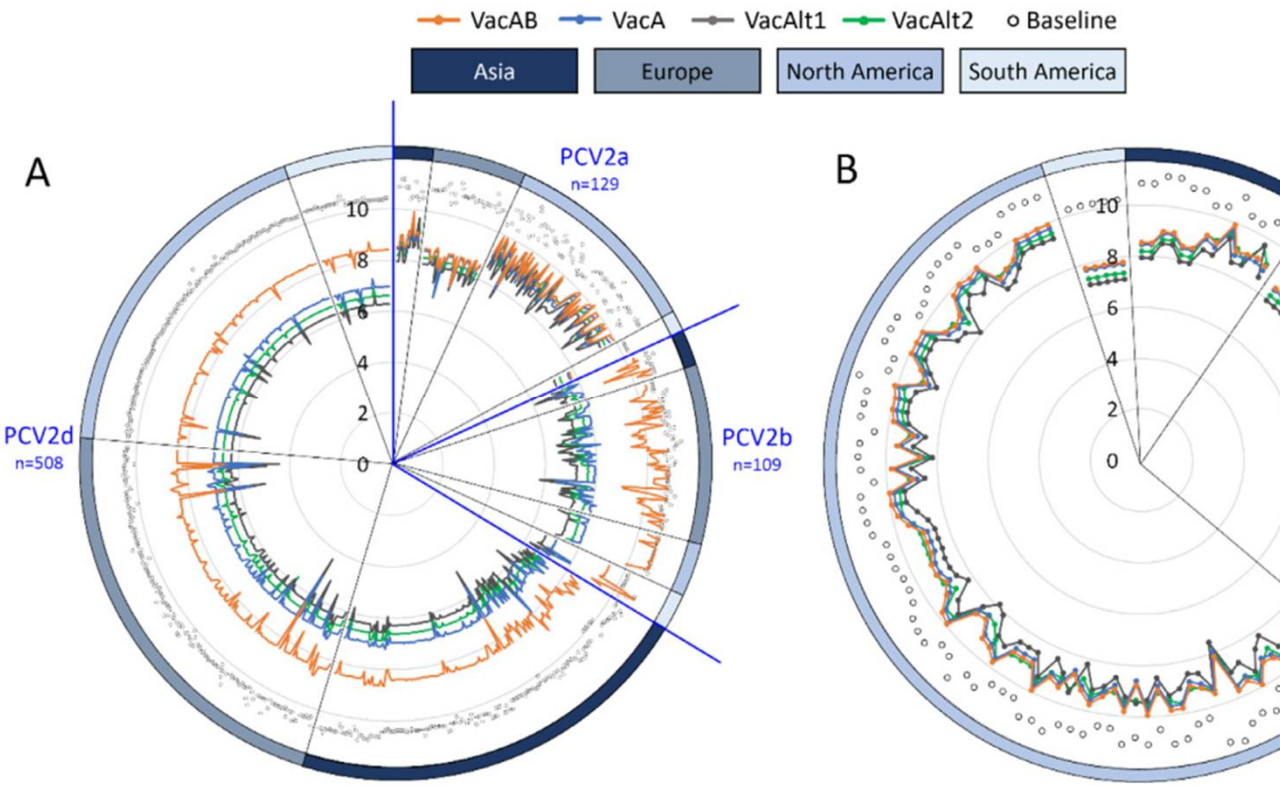
iCC BASELINE SCORES AND RADAR PLOT FOR PCV2 (GLOBAL STANDARDS

Field isolate genotype	America N=292; a=82, b=30, d=180 Average (min-max)	Asia N=185; a=13, b=11, d=161 Average (min-max)	Europe N=269; a=34, b=68, d=167 Average (min-max)
PCV2a	9.33 (7.80-10.10)	8.96 (8.54-9.93)	8.37 (7.34-8.65)
PCV2b	10.23 (9.00-10.87)	10.19 (8.90-10.76)	10.23 (9.10-10.87)
PCV2d	8.37 (7.37-8.74)	8.32 (7.02-8.91)	8.31 (6.34-8.78)
PCV2a	8.75 (7.13-9.48)	8.51 (7.94-9.71)	7.87 (7.16-8.18)
PCV2b	6.98 (6.21-7.41)	7.00 (6.06-7.40)	7.06 (5.80-7.48)
PCV2d	6.26 (5.25-6.63)	6.22 (4.90-6.94)	6.21 (4.37-6.67)
PCV2a	9.14 (7.38-9.71)	8.82 (8.19-9.66)	8.08 (7.19-8.34)
PCV2b	7.41 (6.65-7.69)	7.39 (6.50-7.69)	7.38 (6.25-7.76)
PCV2d	6.58 (5.58-6.95)	6.55 (5.23-7.47)	6.54 (4.73-6.95)
PCV2a	7.79 (6.43-8.49)	7.65 (7.24-8.46)	7.21 (6.59-7.97)
PCV2b	7.65 (6.87-7.95)	7.61 (6.76-7.95)	7.64 (6.51-8.18)
PCV2d	6.92 (5.91-7.56)	6.86 (5.56-7.60)	6.86 (5.03-7.33)

hum and Maximum EpiCC Scores of PCV isolates globally



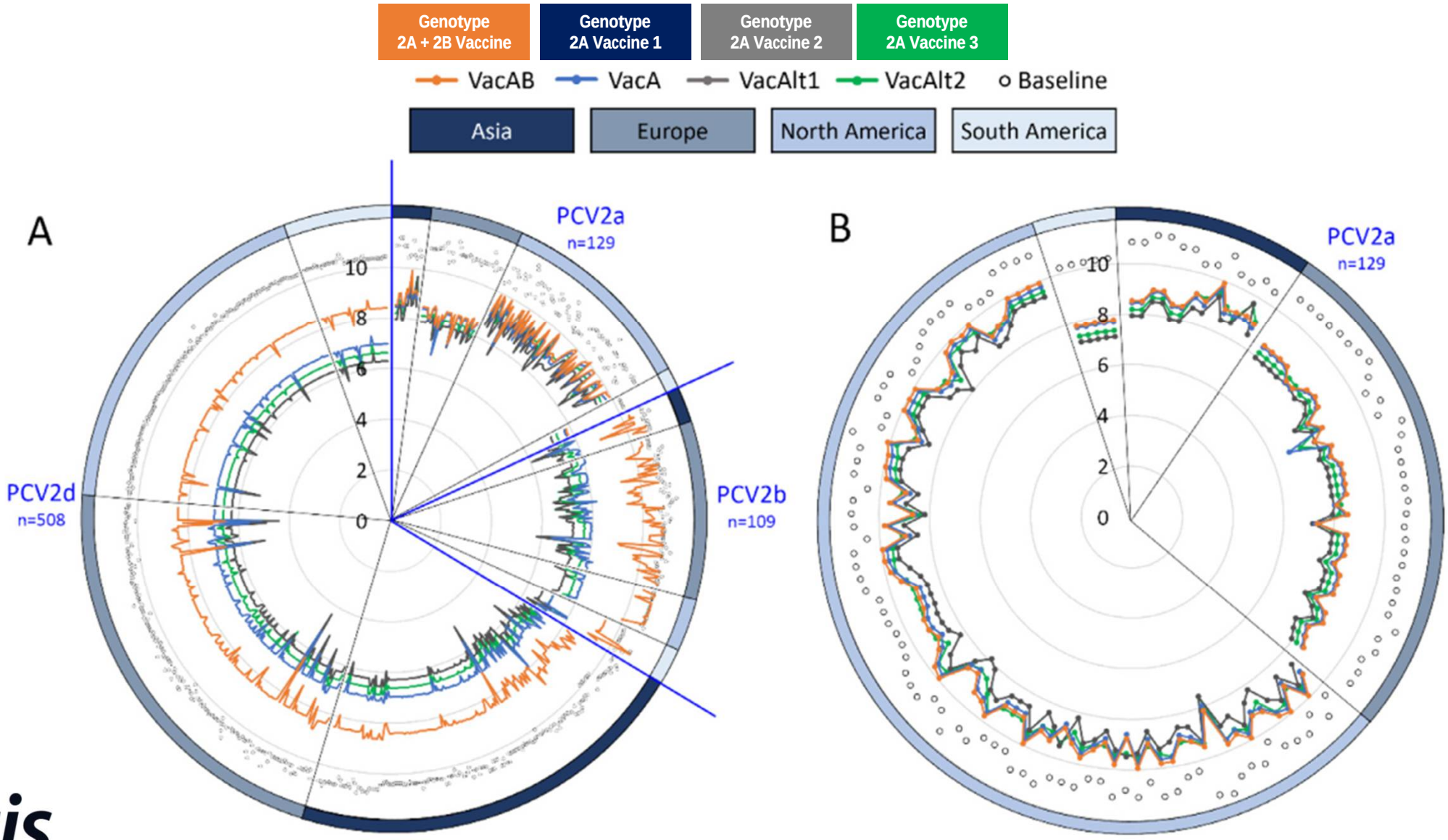
Distribution of tested PCV2 samples (N=746)



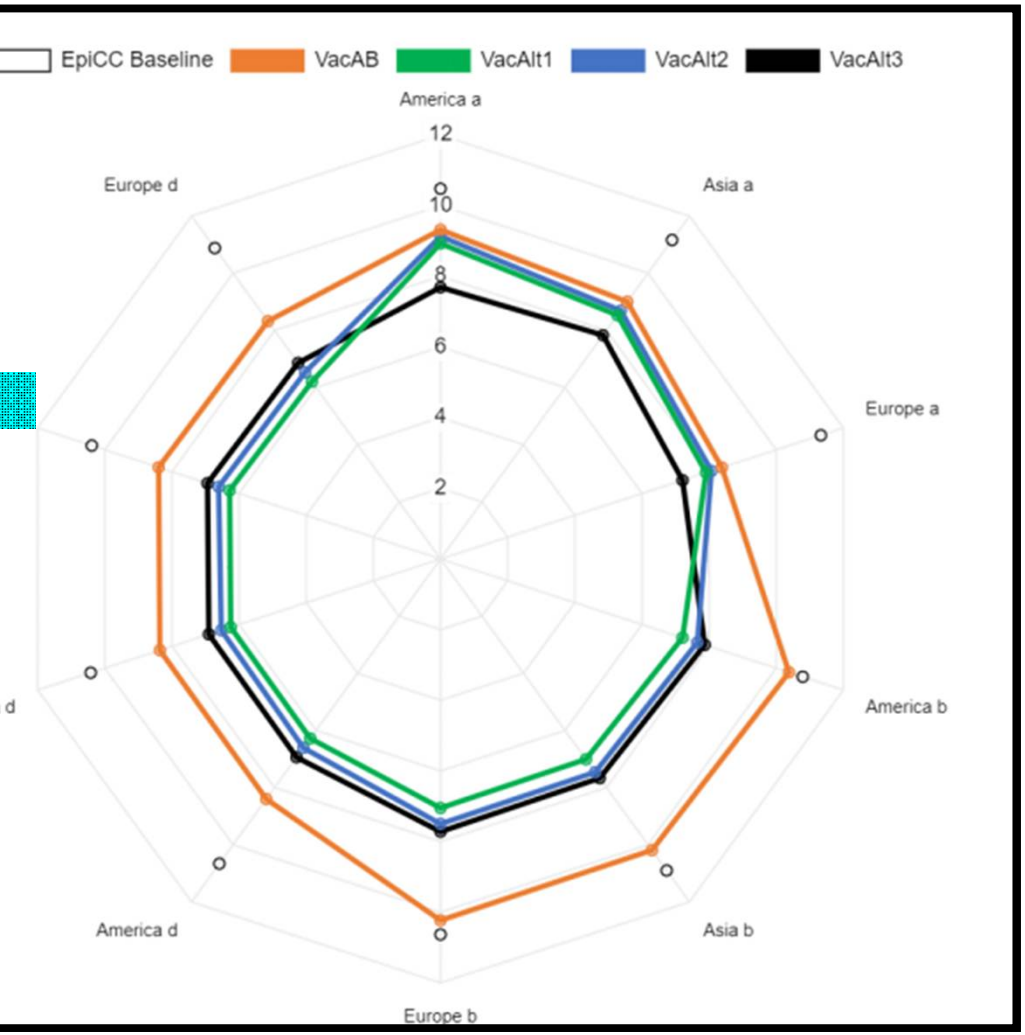
Capsid EpiCC scores of global PCV isolates and vaccines

Foss

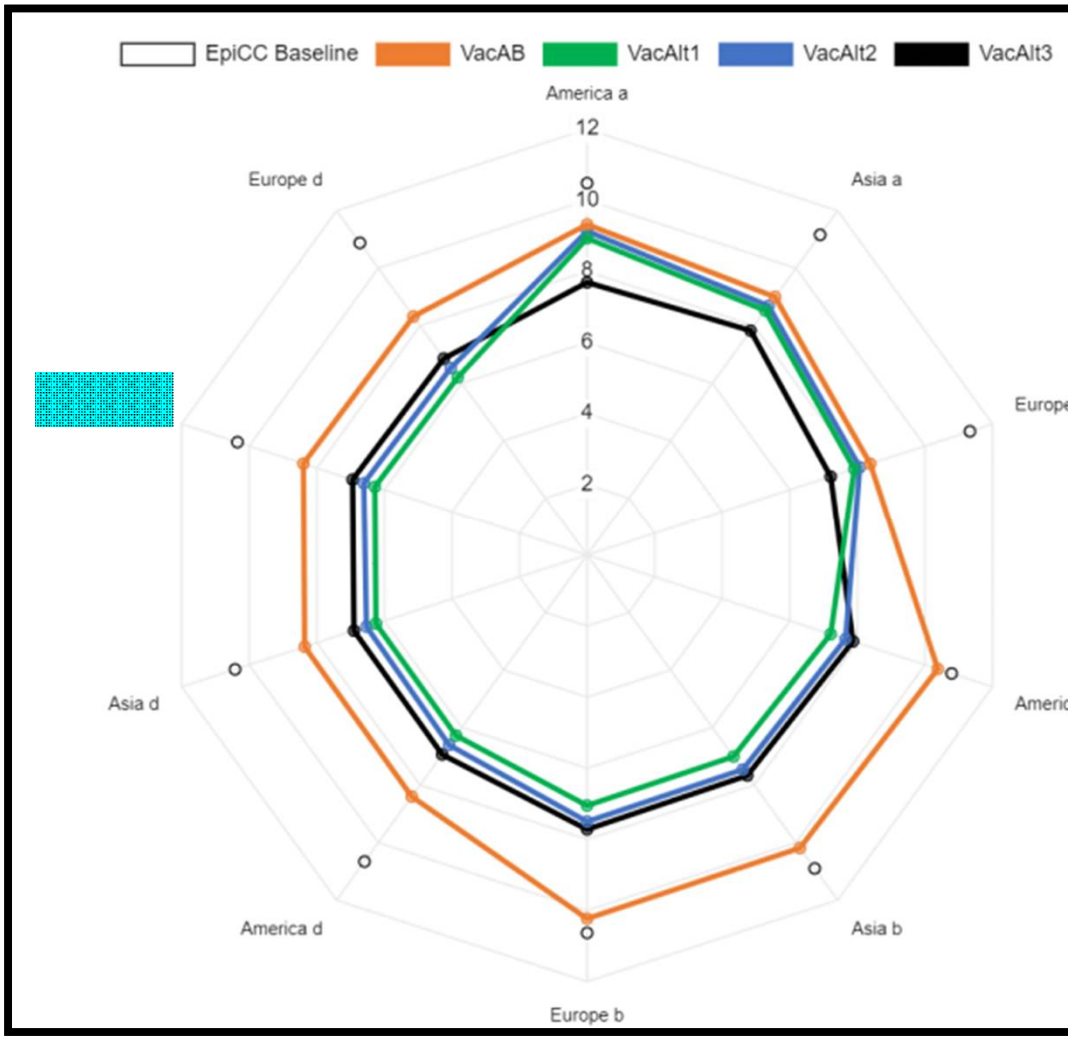
ANALYSIS 2. EpiCC BASELINE SCORES AND RADAR PLOT FOR PCV2 (GLOBAL STANDARDS)



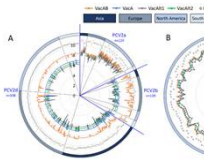
ANALYSIS 2. EpiCC SCORES AND RADAR PLOT OF FIELD PCV2 SAMPLES



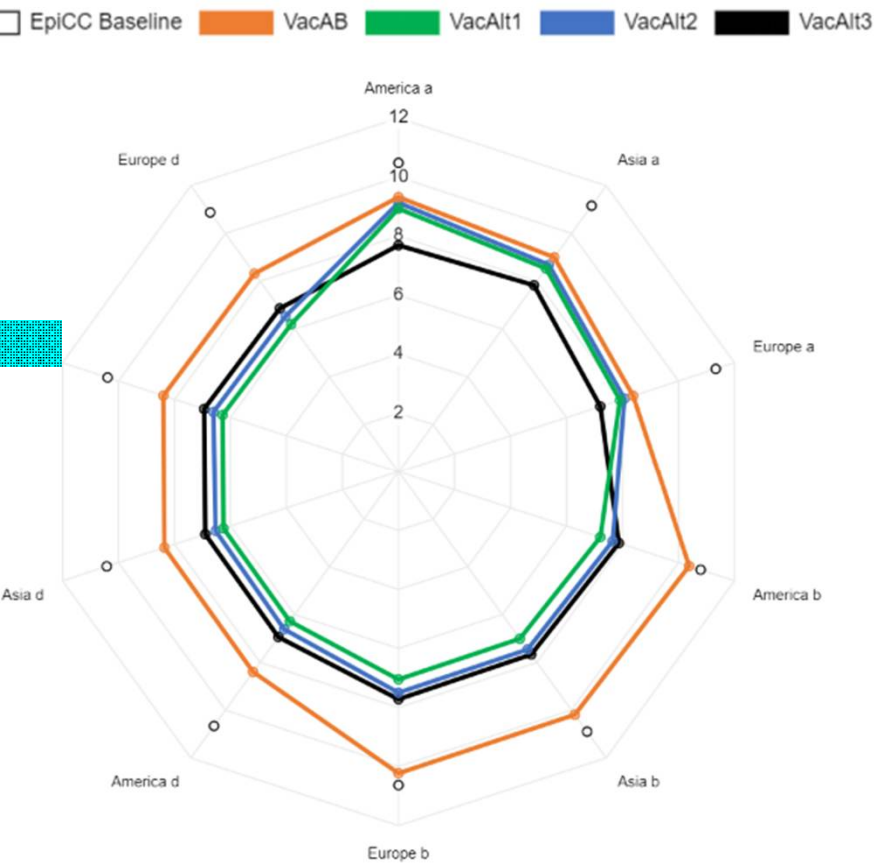
FARM 1A



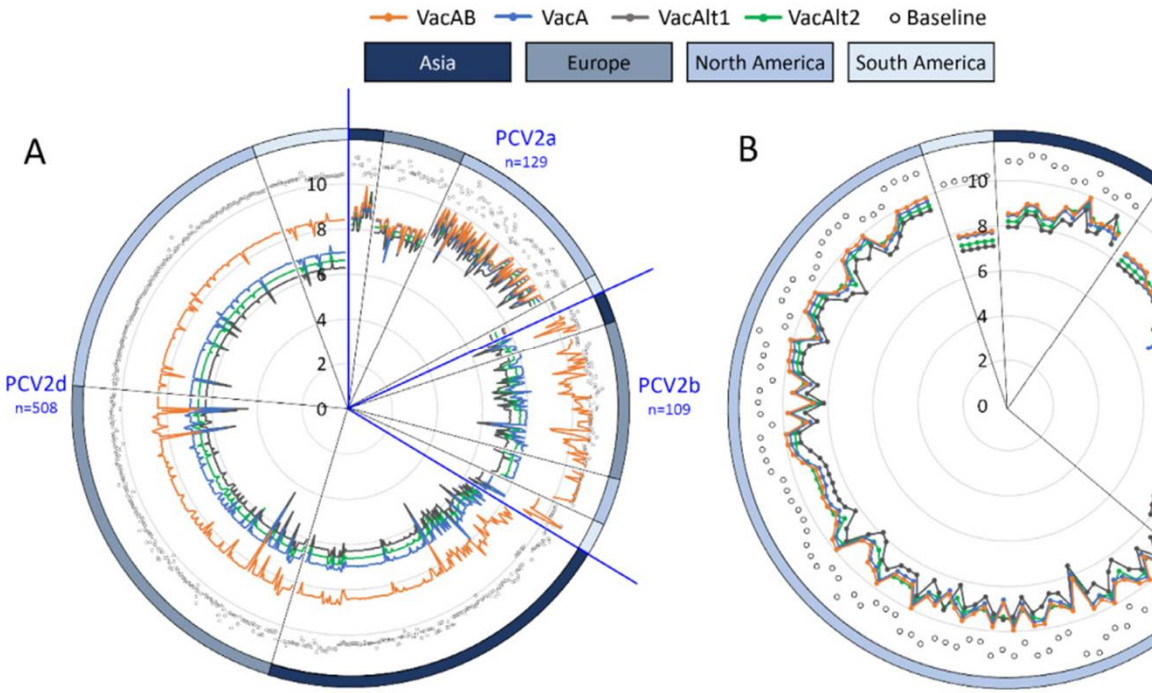
FARM 1B



ANALYSIS 2. EpiCC SCORES AND RADAR PLOT OF FIELD PCV2 SAMPLES



FARM 2



GLOBAL STANDARD

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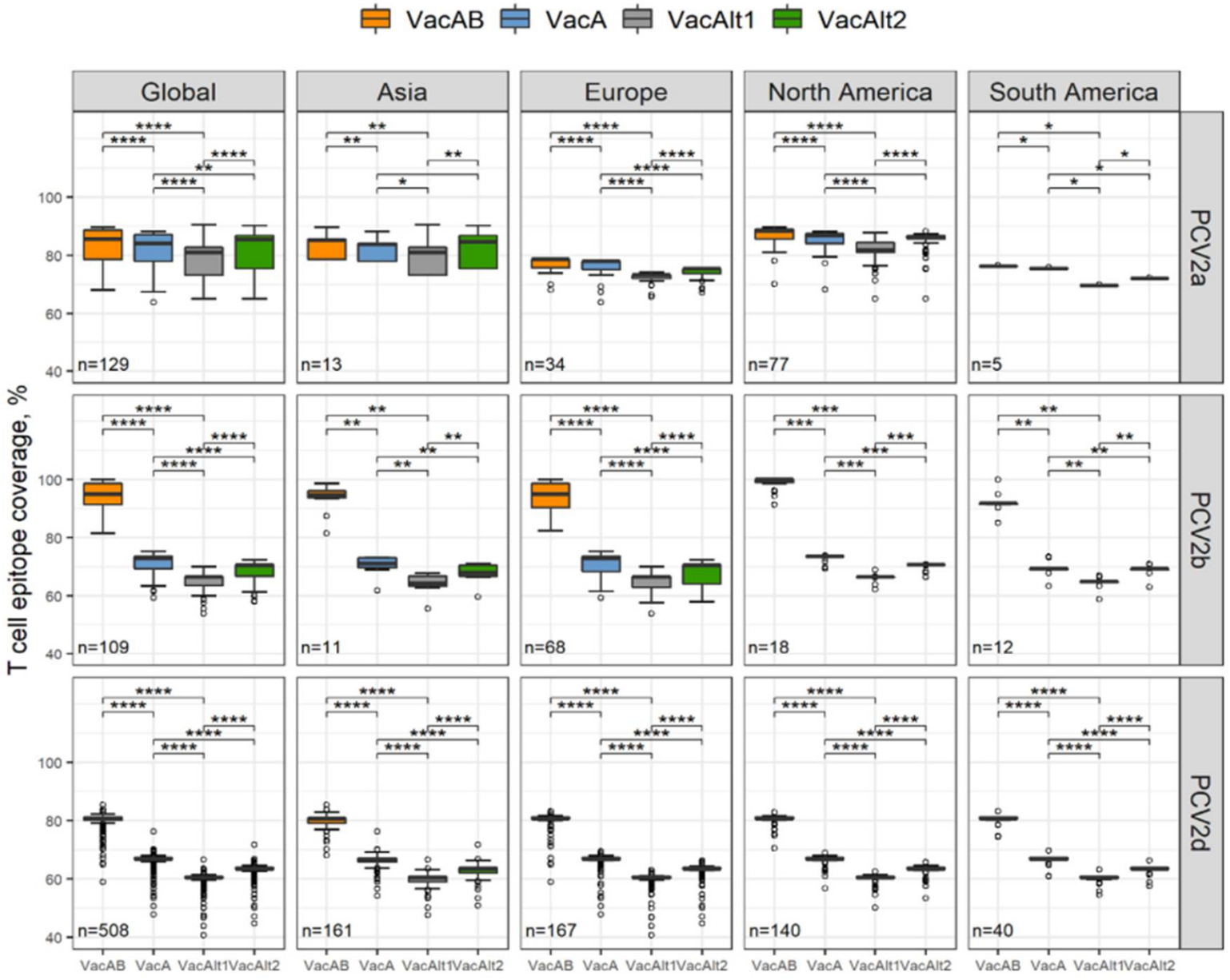
ANALYSIS 3. PERCENT T-CELL EPITOPE COVERAGE (GLOBAL STANDARDS)

type
Vaccine

type
cine 1

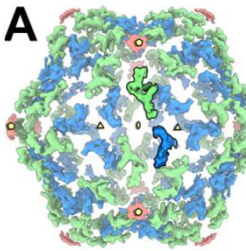
type
cine 2

type
cine 3

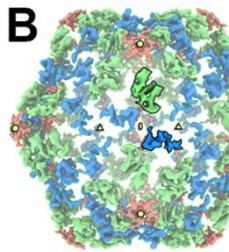


Foss *et al., 2022*

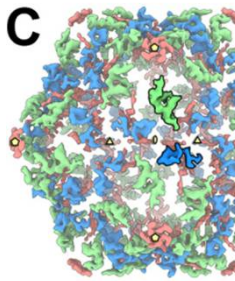
PCV2a



PCV2b

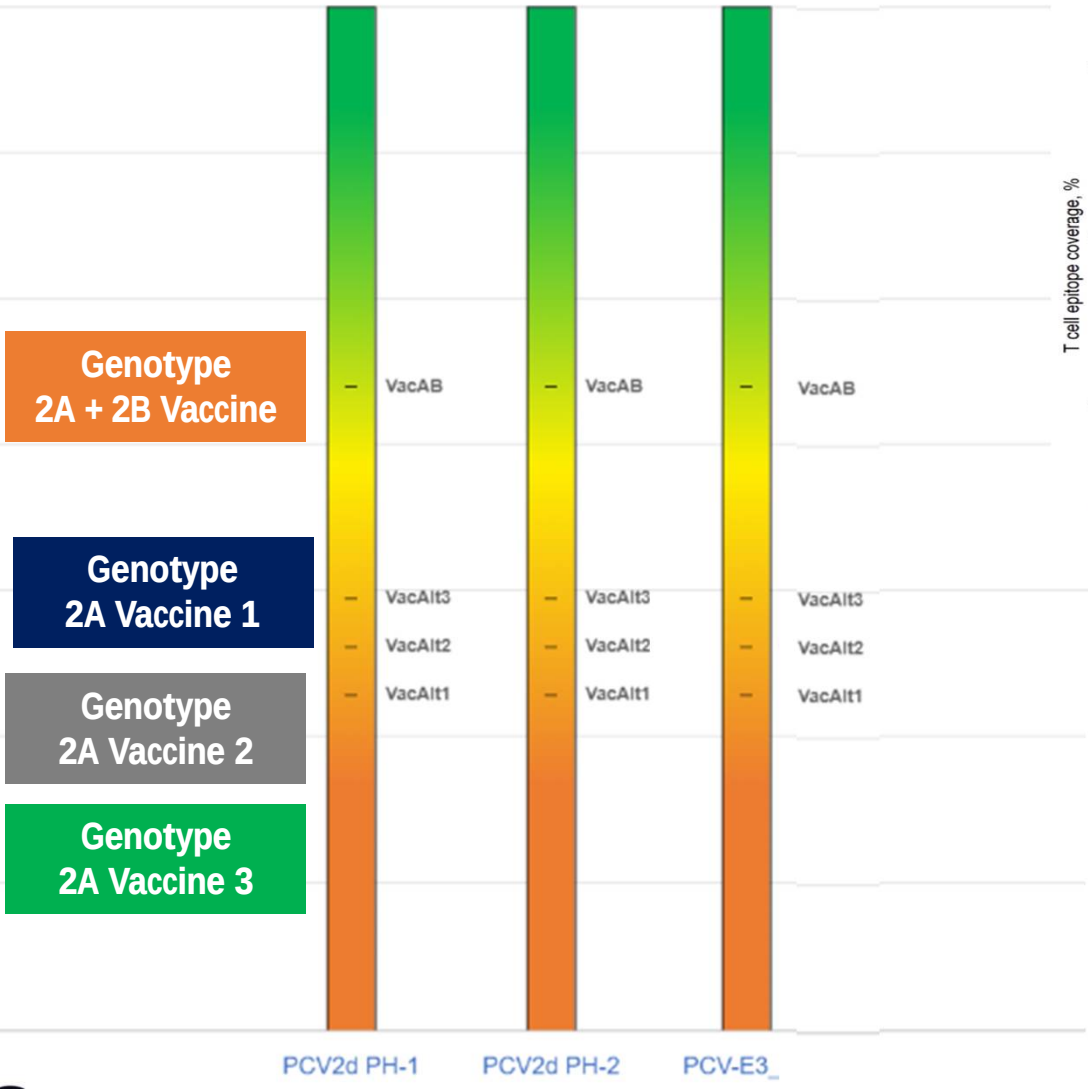


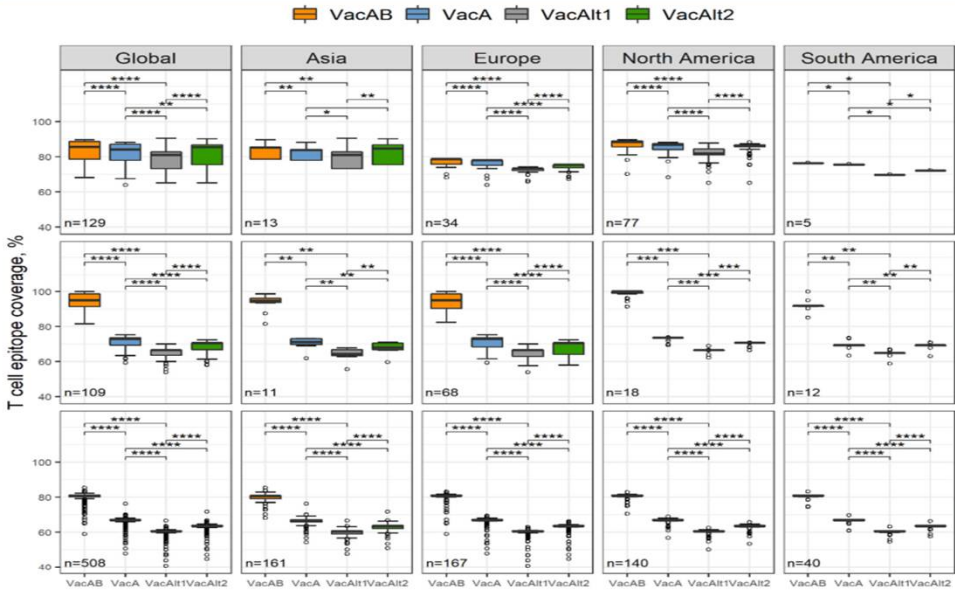
PCV2d



Khayat, *et al., 2*

NALYSIS 3. PERCENT T-CELL EPITOPE COVERAGE OF FIELD PCV2 SAMPLE





Foss *et al., 2022*

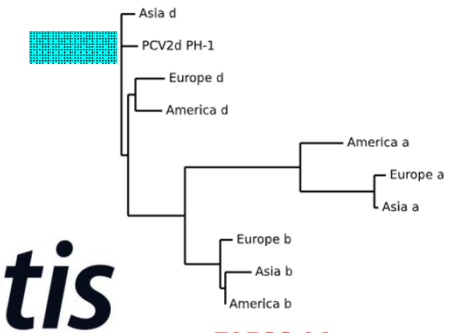
TABLE 1. Epitopes of PCV1 and PCV2

Epitope	Residues[a]	Aligned sequence[b]	MAb binding residue(s)
A	69–83	NV**NEL**RFNI**GQ**FLPP V**DMM**RFNI**ND**FLPPG	Asp70, Met71, Asn77, A
B[d]	113–127	T**SNQ**RGVGS**TV**VILD **QGD**RGVGS**SA**VILDD	Gln113, Asp115
C[d]	117–131	RGVGS**TV**VILD**A**NFV GVGS**SA**VILD**D**NFVT	Asp127
D	169–183	DQ**TID**W**FQPNNKRNQ** F**TID**Y**FQPNNKRNQ**L	Thr170
E	193–207	NV**EH**TGLGY**ALQNATT** V**DH**VGLGT**AFENSIY**	Glu203, Ile206, Tyr207
F	25–39	**RRPYLVHPAFRNRYRWR** **RRPWLVHP**--**RHRYRWR**	

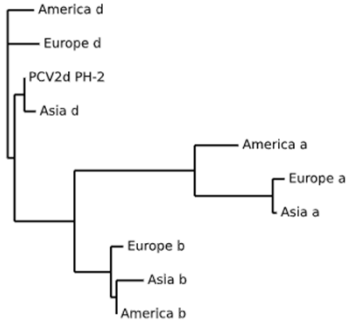
Khayat, *et al., 2019*

SUMMARY OF EpiCC RESULTS

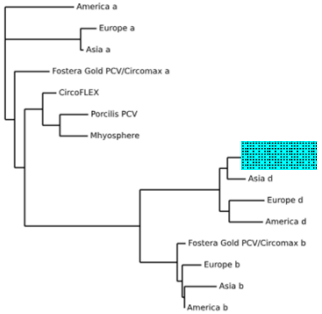
ID	Country	Genotype	EpiCC Baseline	Vaccines	Vaccine Genotypes	EpiCC Score[1]	T cell epitope Coverage %	Deficit relative to VacAB	VacAB coverage i ove each mon vacci
A	Philippines	PCV2d	10.388	VacAB	PCV2a, PCV2b	8.397	80.83%	-	-
				VacAlt1	PCV2a	6.282	60.47%	20.36%	33.67
				VacAlt2	PCV2a	6.61	63.63%	17.21%	27.05
				VacAlt3	PCV2a	6.941	66.81%	14.02%	20.99
B	Philippines	PCV2d	10.349	VacAB	PCV2a, PCV2b	8.397	81.14%	-	-
				VacAlt1	PCV2a	6.282	60.7%	20.44%	33.67
				VacAlt2	PCV2a	6.61	63.87%	17.27%	27.05
				VacAlt3	PCV2a	6.941	67.07%	14.07%	20.99
	Philippines	PCV2d	10.388	VacAB	PCV2a, PCV2b	8.397	80.83%	-	-
				VacAlt1	PCV2a	6.282	60.47%	20.36%	33.67
				VacAlt2	PCV2a	6.61	63.63%	17.21%	27.05
				VacAlt3	PCV2a	6.941	66.81%	14.02%	20.99



FARM 1A



FARM 1B



FARM 2

KEY TAKE AWAYS

V2 is an economically important pathogen that can lead to several **porcine covirus-associated diseases (PCVAD).**

V2's evolutionary rate is unusually high for DNA virus resulting to the **ergence of at least 8 genotypes.**

CV2 continues to evolve and EpiCC analysis provides a new tool to sess the possible impact of virus genetic divergence on T cell itope coverage of vaccine strains.

culating field PCV strains that share more T cell epitope content with cine strains will have higher EpiCC scores and higher vaccine coverage.

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PROJECT TITLE	DEVELOPMENT OF SURVEILLANCE SYSTEM FOR ASFV IN E ENVIRONMENT AND FOMITES OF ASF-AFFECTED SWINE FA AN ADDED TOOL FOR ASF SENTINEL, REPOPULATION AND RECOVERY PROGRAMS

General Objective

The general objective of the study is to op a surveillance system for ASFV that utilizes n environment and fomites as an added tool ng ASF sentinel, repopulation and recovery programs in the Philippines.

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UNIVERSITY OF THE PHILIPPINES 1908

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LOS BAÑ

MOLECULAR EPIDEMIOLOGICAL-BASED REPOPULATION SYSTE

RESEARCH TEAM

- ECULAR EPIDEMIOLOGIST
- VIROLOGIST
- OLECULAR BIOLOGIST
- MICROBIOLOGIST
- ANIMAL SCIENTIST
- BIOMATHEMATICIAN
- VETERINARIANS

MOLECULAR EPIDEMIOLOGY

- MOLECULAR BIOLOGY
- VETERINARY DIAGNOSTICS
- VETERINARY EPIDEMIOLOGY
- BIOMATHEMATICS AND STATISTICS

PHASE 1

- TOTAL OF 4,60 ENVIRONMENT SAMPLING POIN
- 30 HAULING TRU TRANSPORT VEHICLES
- 300 FEED SAMP

AREAS OF POSSIBLE COLLABORATIONS

ENTIAL COLLABORATORS

- SMALLHOLDER FARMS
- SEMI-COMMERCIAL FARMS
- COMMERCIAL FARMS
- ASSOCIATION-GROUPS
- LGUs

PLANNING TO (2023-2024):

Perform Sentinelling

Partial/Full Repopulation

Build-up of In-House Farm Laboratory Capabilities

PHASE 2 (FIELD VALIDATIO

FREEDOM FROM PREVIOUS INFECTION OF THE FARM ENVIRONMENT

BIOSECURITY ENHANCEMENTS - qPCR-BASED MOLECU SURVEILLANCE SYST

MOLECULAR EPIDEMIOLOGICAL-BASED REPOPULATION SYSTEM

RISK-BASED APPROACH TO SEGMENTAL DEPOPULATION

EARLY DETECTION REINFECTION THROU SYNDROMIC SURVEILL SYSTEM

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