PCV-2 and PCV-3 update and their role in reproduction problems

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World Organisation for Animal Health
Founded as OIE

Porcine circoviruses

Parameters	PCV-2	PCV-3
Year of discovery	1997	2016
Earliest detection	1962	1960s
First viral isolation	1997	2020
Distribution	Worldwide	Worldwide
Prevalence	High	High
Genotypes	Nine PCV-2a to PCV-2i	One PCV-3a
Disease reported in the field	Reproductive, Systemic, PDNS	Reproductive, Respiratory, Systemic, PDNS,

Adapted and updated from Opriessnig et al., 2020

PCVD

PCV-2 systemic disease (PCV-2-SD)

Pigs showing wasting

PCV-2 reproductive disease (PCV-2-RD)

Experimental PCV2-RD case: foetal mummification

PCV-2 subclinical Infection (PCV-2-SI)

PDNS

Disease associated to PCV-3

PCV-3 systemic disease (PCV-2-SD)

Pig showing wasting

PCV-3 reproductive disease (PCV-2-RD)

Stillborn

PDNS (??)

Saporiti et al., 2021

Reproductive problems associated to porcine circovirus

PCV-2-RD

PCV-3-RD

Clinical manifestations

PCV-2-RD

veterinary sciences

MDPI

Review

Revisiting Porcine Circovirus Disease Diagnostic Criteria in the Current *Porcine Circovirus 2* Epidemiological Context

Segales and Sibila et al., 2022

SMEDI-like condition

Late gestation problems:

Mummifications, stillbirths
abortions

Early gestation problems:

Embryonic death → Regurlar return-to-estrus/infertility

Viral replication in zona pellucida free morulae, early blastocysts and hatched blastocysts

PCV-3-RD

Transboundary and Emerging Diseases WILEY

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Porcine circovirus 3 (PCV-3) as a causal agent of disease in swine and a proposal of PCV-3 associated disease case definition

Viviane Saporiti[1] | Giovanni Franzo[2] | Marina Sibila[1,3] | Joaquim Segalés[3,4,5]

Late gestation problems:

Late-abortions, malformations, mummified and stillborn fetuses and weak-born piglets

Prevalence

PCV-2-RD

PCV2-RD seldom diagnosed (few cases). Mainly in start-up herds, with high proportion of **PCV-2** susceptible pigs.

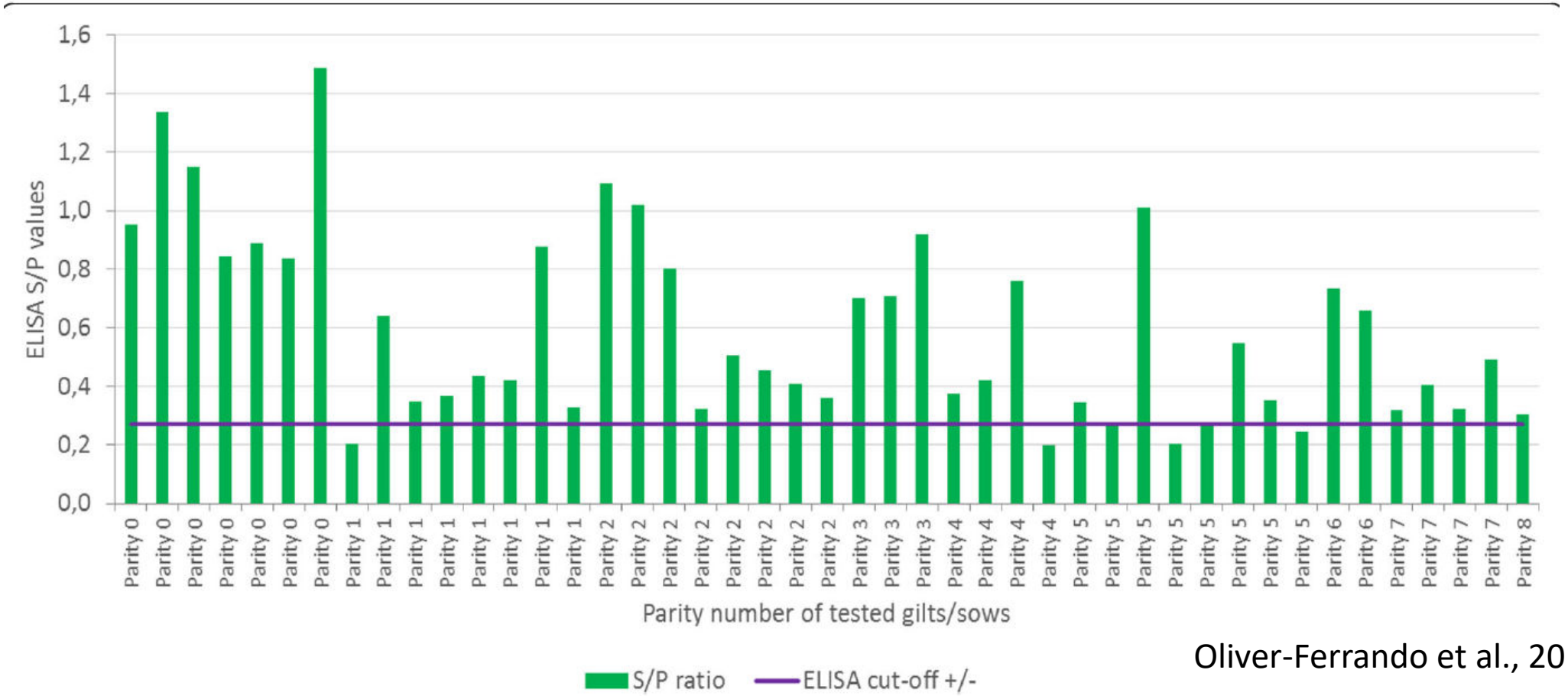
PCV2 reproductive disease (PCV2-RD): PCV2 has been linked to late term abortions and stillbirths (Brunborg et al., 2007; West et al., 1999) as well as mummification (Madson et al., 2009a) resembling the one caused by porcine parvovirus (Mengeling, 2006). However, PCV2-associated reproductive disease under field conditions is rare (Pensaert et al., 2004). This is probably due to the fact that the seroprevalence of PCV2 in adult pigs is high and, therefore, most breeding herds are not suffering from the clinical disease. Affected

Extracted from Segalés, 2012

PCV-3-RD

PCV2-RD prevalence after the continuous use of PCV2 vaccines

- ✓ The massive used of PCV-2 vaccine has changed the epidemiology of the disease
- ✓ The reduction of the infectious pressure may generate animals with minimal or no exposure to the virus
- ✓ Replacement stock with variable levels of PCV-2 antibodies



Oliver-Ferrando et al., 2018

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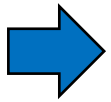
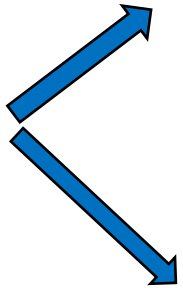
Presence of sows (mostly gilts) with variable antibody levels; even seronegative



Potential increased risk of intrauterine infections



Potential increase of **PCV2-RD (?)**

High prevalence of newborn subclinical infections

Segalés and Sibila , 2022

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Extracted from Segalés, 2012

PCV-3-RD

- ✓ Several cases reported from different countries
- ✓ Most of them reported as “sow population”
- ✓ No information on the antibody levels in sow population (but presumably it should be high)
- ✓ **PCV-3** is a ubiquitous virus
- ✓ In most of them, detection of the virus was done only by PCR/qPCR detection .
- ✓ Detection is not the same as causation (subclinical infections)

Prevalence

PCV-2-RD

PCV3 subclinical infections in sows/foetuses

Article

Pathogens **2020**, *9*, 533

Frequency of Detection and Phylogenetic Analysis of *Porcine circovirus 3* (PCV-3) in Healthy Primiparous and Multiparous Sows and Their Mummified Fetuses and Stillborn

Viviane Saporiti [1,2], Susanna Martorell [3], Taís F. Cruz [1,2,4], Francini Klaumann [1,2], Florencia Correa-Fiz [1,2], Mònica Balasch [3], Marina Sibila [1,2,†] and Joaquim Segalés [2,5,6,*,†]

Farm	Sampling Point	Primiparous Sows	Multiparous Sows	Total
A	S1	1/19 (5.3%)	0/25 (0.0%)	1/44 (2.3%)
	S2	8/19 (42.1%)	0/25 (0.0%)	8/44 (18.2%)
B	S1	0/17 (0.0%)	0/20 (0.0%)	0/37 (0.0%)
	S2	3/17 (17.6%)	0/20 (0.0%)	3/37 (8.1%)
C	S1	0/21 (0.0%)	0/19 (0.0%)	0/40 (0.0%)
	S2	7/21 (33.3%)	0/19 (0.0%)	7/40 (17.5%)

PCR positive

Primiparous 33,3% → Fetuses (73/91, 80.2%)

P<0,05

Multiparous 0% → Fetuses (13/164, 7.9%).

Prevalence

PCV2-RD

PCV3-RD

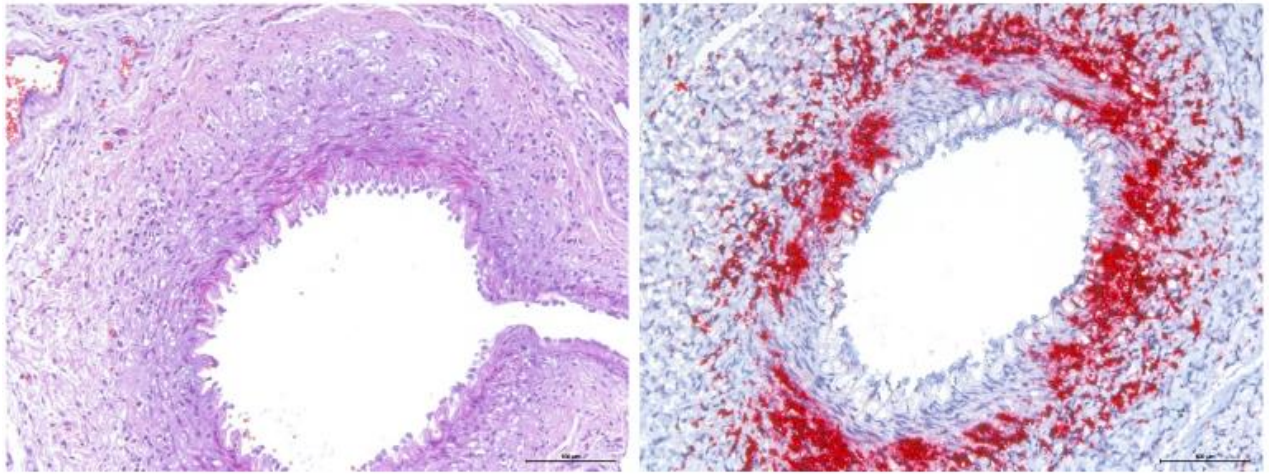
PCV-3 - 18/53 (34%) – 4 by ISH

PCV-2 - 5/53 (9.4%)

PRRSV - 4/53 (7.5%)

PPV - 0/53 (0.0%)

Moderate perivascular lymphocytic infiltration swelling



H&E Spleen ISH

PCV2-RD (late gestation)

Diagnosis

Courtesy of Dr. Darin Madson, Iowa State University, USA

Experimental PCV2-RD case: foetal mummification

Courtesy of Dr. Ellis (University of Saskatchewan, Canada)

Natural PCV2-RD case: foet

Natural PCV2-RD case: PCV2 immunohistochemistry

Best sample: heart from weak-born or stillborn animal

1-Clini anifestation

Reproduct t late gestati -like cond

aracteristic lesions

s to necrotizing ocarditis of foetuses

3-Detection of the pathogen within the lesions

Moderate to high amount of PCV-2 in the heart

Segalés and Sibila et al., 2022

PCV2-RD

Diagnosis

1-Clinical Manifestation

Regular retorn-to-estrus
Infertility

Early gestation problems

2-Detection of the pathogen within the lesions

PCV2 seroconversion following the return-to-estrus and/or PCV2 PCR/qPCR around return-to-estrus

SMEDI: stillbirths, mummification, embryonic death and infertility (Return-to-estrus)

Best sample:

Serum from sow

Diagnosis PCV-3-RD

1-Clini ifestation

Late r ms and higher pe

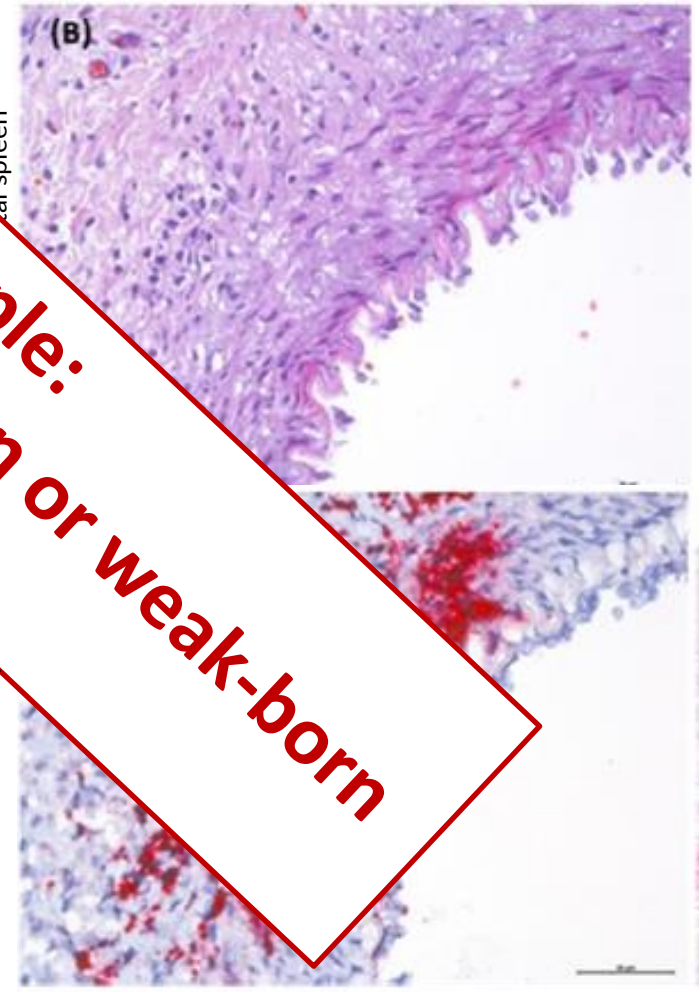
2-Character

Multisystemic lymph to lymphohistiocytic pe inflammation

3-Detection of the pathogen within the lesions

Moderate to high amount of **PCV-3** genome in damaged tissues

Best sample: Mesenterium from stillborn or weak-born piglets



PCV-2-RD

High to moderate genome amount by IHC/ISH

PCV-3-RD

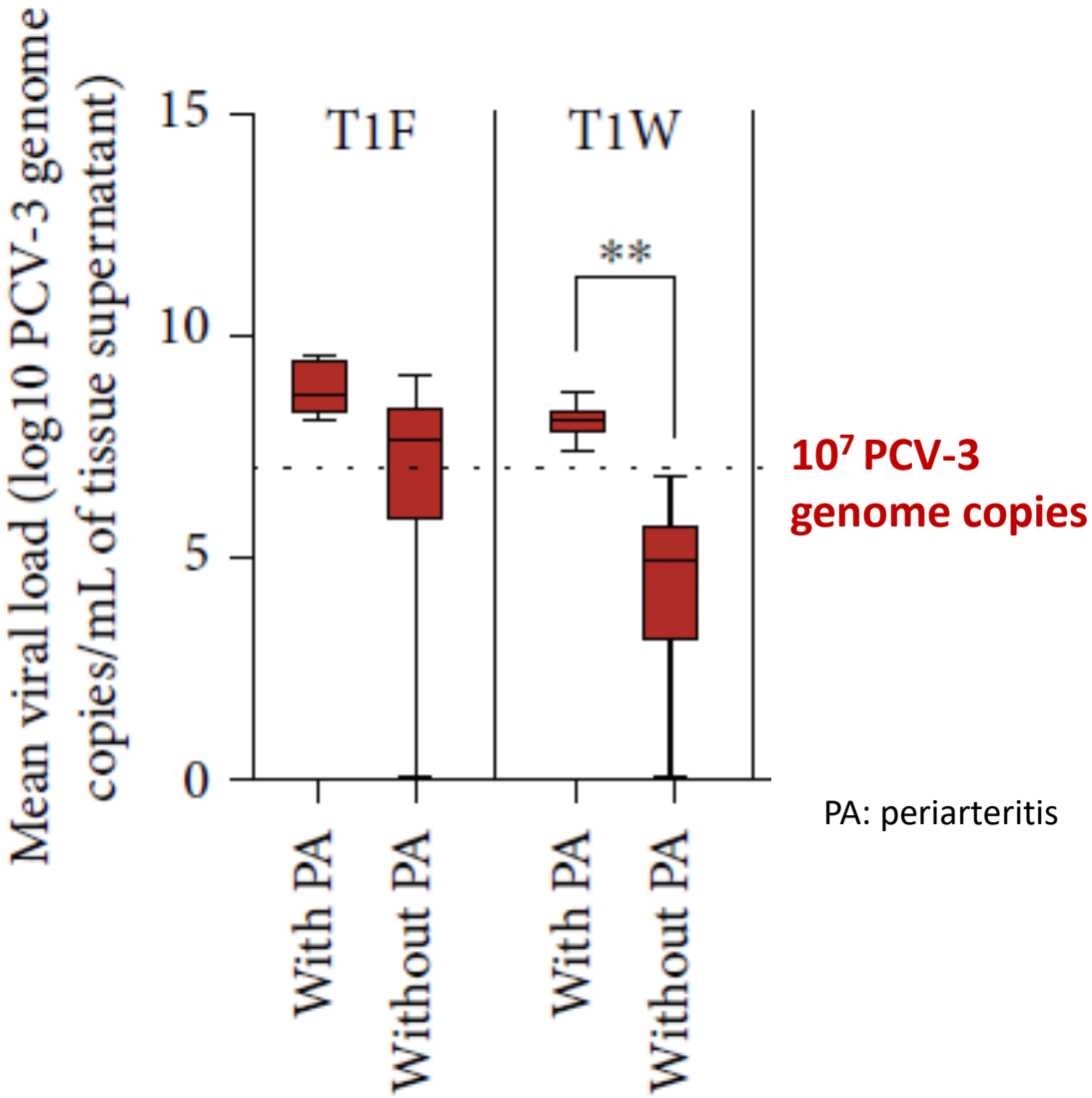
qPCR

mummified or stillborn

- >10^7 genome copies/500 ng DNA of tissue samples (Hansen et al., 2010)
- >10^9 genome copies /g of heart tissue (Unterwerger et al., 2021)

Newborn with miocarditis

- >10^5 genome copies /500 ng DNA of tissue samples (Hansen et al., 2010)



- ✓ Different qPCRs techniques
- ✓ Comparison of the current qPCR techniques not available

Cobos et al., 2023

Pathogenesis

Dissemination through semen?

PCV2-RD

- ✓ Boars can shed low amounts of infectious **PCV-2** in semen. However, this amount was not enough to infect sows artificially inseminated with this semen (Madson and Opriessnig, 2011)

PCV3-RD

- ✓ Although, **PCV-3** has been detected in semen, its detection rate in semen used for AI was low and with low viral load (Eddicks et al., 2022)

Pathogenesis

PCV2-RD

PCV3-RD

Through sow viremia and transplacental spread

- ✓ Intranasally inoculated sows showed abortion and delivered prematurely infected stillborn and liveborn piglets (Park et al., 2005)

- ✓ Intranasally and intramuscularly inoculated sows delivered infected mummified, stillborn foetuses and liveborn piglets (Cobos et al., 2023)

PCV2-RD

Pathogenesis

PCV3-RD

Time of infection vs outcome

- ✓ Outcome of the infection gestational age-dependent:
  - ✓ 1st third of gestation: embryonic death and return-to-estrus (Mayeusen et al., 2007)
  - ✓ 2nd third of gestation: fetal pathologic abnormalities (Sanchez et al., 2001)
  - ✓ 3rd third of gestation: variable outcome.

PCV2-RD

Outcome vs time of infection

- ✓ In-utero inoculation of 4 fetuses (from 2 sows) at 57, 75, 92 days of gestation
- ✓ 21 dpi, foetuses were collected

The outcome of PCV2 infections in porcine embryos and fetuses

Time of inoculation	Gross appearance	Presence of virus	Antibody response	Intra-uterine spread	Interruption of gestation
57 days	mummified	+	–	+ (restricted)	–
75 days	stillborn/ autolysed	+	+	–	–
92 days	normal	+	+	–	–

- ✓ The earlier the moment of infection, the higher number of infected tissues.
- ✓ Myocardium contained the highest amount of infectious virus

Adapted from Sanchez et al., 2001

Pathogenesis

PCV2-RD

PCV3-RD

Through sow viremia and transplacental spread

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- ✓ Outcome of the infection gestational age-dependent (Cobos et al., 2023)

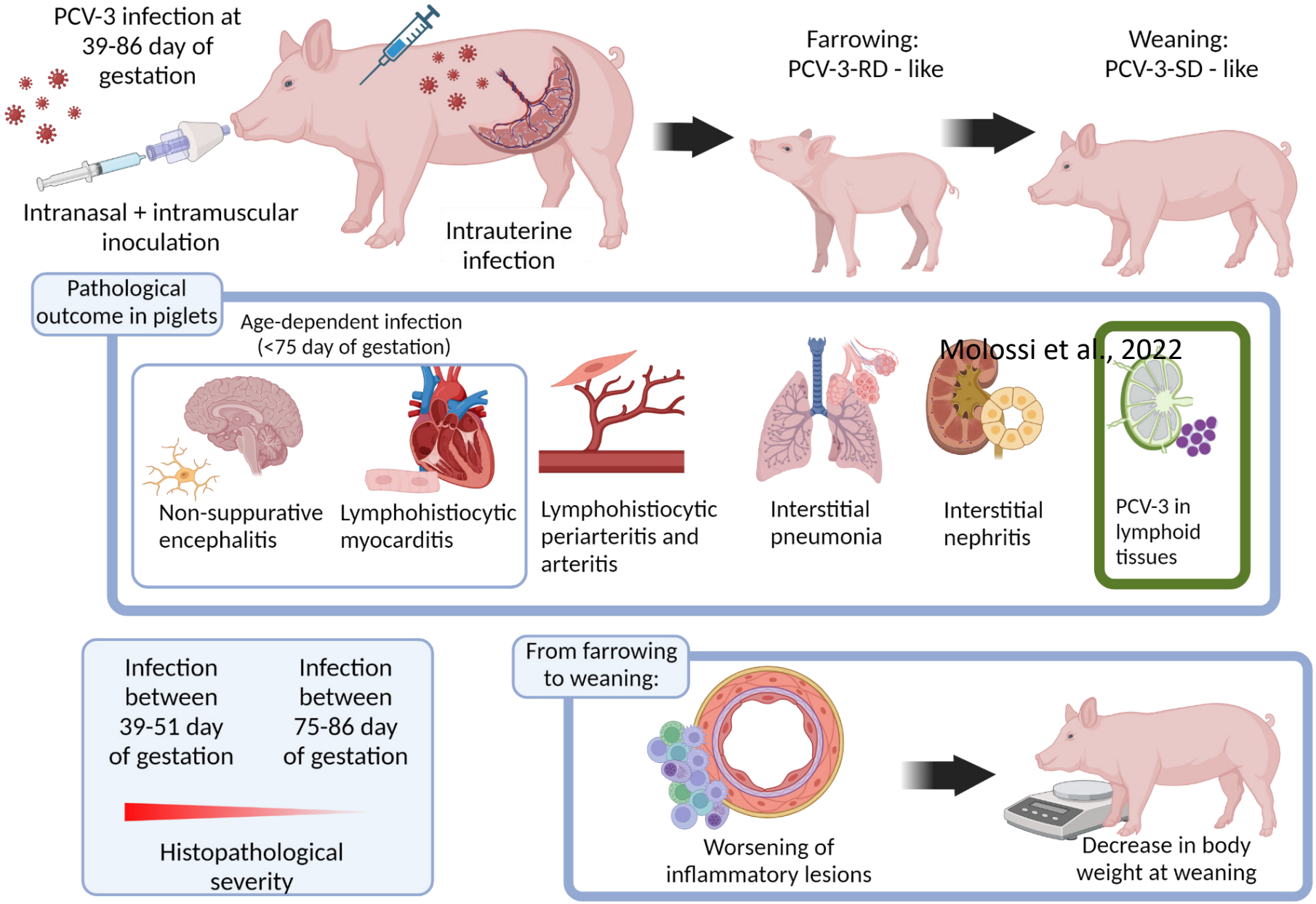
Experimental Inoculation of Porcine Circovirus 3 (PCV-3) in Pregnant Gilts Causes PCV-3-Associated Lesions in Newborn Piglets that Persist until Weaning

Àlex Cobos,[1,2,3,4] Albert Ruiz,[1,3,5] Mónica Pérez,[1,3,4] Anna Llorens,[1,3,4] Eva Huerta,[1,3,4] Florencia Correa-Fiz,[1,3,4] Robert Lohse,[6] Mònica Balasch,[5] Joaquim Segalés,[1,2,3] and Marina Sibila[1,3,4]

Transboundary and Emerging Diseases Volume 2023, Article ID 5270254, 14 pages

- Major results:
  - Prolonged viremia in gilts (subclinical infection)
  - Farrowing occurred normally
  - No statistically significant differences in reproductive parameters
  - Intrauterine infection in piglets coming from both groups of gilts
  - Non-suppurative arteritis and periarteritis as hallmark lesions in piglets
  - Correlation with viral load and lesion (periarteritis) severity
  - Pre-immunocompetent infected pigs showed more severe lesions, especially at weaning

Potential pathogenesis model for PCV-3



Cobos et al., 2023

Created with BioRender.com

PCV-2-RD

Vaccination

PCV-3-RD

- ✓ **Before Farrowing**:
  - ✓ Protection of newborn piglets
  - ✓ High levels of MDA causing potential interference with piglet vaccine efficacy
- ✓ **Before Artificial Insemination**:
  - ✓ Protection of sows during gestation
  - ✓ Moderate-to-high levels of MDA but not enough to cause interference with vaccine efficacy
- ✓ **Blanket vaccination**:
  - ✓ Benefits of both systems
  - ✓ Low proportion of sows vaccinated before farrowing: High MDA

Important enough to develop vaccines?

Table 3: Development of PCV3 vaccine.

Vaccine type	Description	Production system	Reference
VLP	Modified capsid protein of PCV3 adjuvanted with Montanide ISA27VG or 20% carbopol	Baculovirus vector system	[45]
Subunit	Chimeric PCV2d–PCV3 truncated capsid joined by GS-linker	*E. coli*	[46]
mRNA	Autogenous vaccine needs 8–12 weeks to custom	Synthetic RNA particle technology	Merck AH sequivity [47]

Vaccination

PCV-3-RD

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Will see in the next few years...

Pranoto et al., 2023

Take home message

PCV-2-RD

- ✓ **PCV-2-RD:** low prevalence and low impact for the sector
- ✓ Unkown frequency of early gestational problems
- ✓ Unkown effect of **PCV-2** subclinical infections on reproductive parameters
- ✓ Outcome of intra-uterine infection: gestational-age dependent
- ✓ Best sample for diagnosis: heart of stillborn or weak-born piglets
- ✓ Avoid seronegative subpopulations that can cause intra-uterine infections and newborn infections

PCV-3-RD

- ✓ **PCV-3** is considered spread worldwide
- ✓ No clue on the exact impact of **PCV-3** associated diseases... at least not yet!
- ✓ Unknown impact of **PCV-3** subclinical infections ?
- ✓ Outcome of intra-uterine infection: gestational-age dependent
- ✓ Case diagnostic criteria:
  - ✓ **PCV-3-RD**
  - ✓ **PCV-3-SD**

  "¿TWO FACES OF THE SAME COIN"? **PCV-3-AD**
- ✓ Do not confuse "viral detection" with "disease causality"- detection of virus in lesions compulsory

Many thanks for your attention!!