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Leman China
October 2023

From PCV2 to PCV3: It Feels Like We Have Been Down This Road Before

History Lesson

Today we recognize PCV2 as an economically significant disease-causing pathogen that is important enough to vaccinate all growing pigs for within the modern swine production systems of the world.

For many years, however, the debate was active as to association and causality of PCV2 in disease processes.

Release of the first doses of commercial PCV2 vaccine in the U.S. in 2006 actually lead to a negative economic period on pork producers due to a sudden increase in supply, due to improved survivability.

But for over 10 years, we debated while pigs suffered from multi-systemic disease.

Will emerging strains of PCV2 and now PCV3 follow the same path?

Giving Credit Where Credit is Due

- There is a great deal of past and present work being done from which I have drawn and blended with my experiences in the field.
  - Ellis, Nauwynck, Segales, Palinski, Arruda, Madsen, Opriessnig, Harding

2023 GREECE May 31 - June 2
ESPHM 14th EUROPEAN SYMPOSIUM OF PORCINE HEALTH MANAGEMENT
Thessaloniki Greece

Causality

- Koch's postulates were introduced in the 19th century
  - First form of a rigid process to establish causality
  - Unfortunately with PCV2 those postulates are not possible to meet
    - Subclinical infection
    - PCV2 requires co-infection or immune stimulation
    - Not uncommon occurrence
    - Recognized as early as the 1930's when dealing with viral diseases (Rivers)
    - Others including Lilienfeld, Hill, Evans, Johnson in the 1960's and 1970's
  - More recently Fredricks and Relman in the 1990's looking at molecular/sequence-based guidelines

Evans' Unifying Concept

Henle-Koch Postulates

1. Not applicable to all pathogenic bacteria
2. May not be applicable to viruses, fungi, parasites
3. Do not include the following concepts:
   a. The asymptomatic carrier state
   b. The biologic spectrum of disease
   c. Epidemiologic elements of causation
   d. Immunologic elements of causation
   e. Prevention of disease by elimination of putative cause as element of causation
   f. Multiple causation
   g. One syndrome having different causes in different settings
   h. Reactivation of latent agents as cause of disease
   i. Immunologic processes as cause of diseases

Evans Unifying Concept

1. *Prevalence* of the disease should be significantly higher in those exposed to the putative cause than in controls not so exposed
2. *Exposure* to the putative cause should be present more commonly in those with the disease than in controls without the disease when all other risk factors are held constant
3. *Incidence* of the disease should be significantly higher in those exposed to the putative cause than in those not so exposed as shown in prospective studies
4. *Temporally*, the disease should *follow* exposure to the putative agent with a distribution of incubation periods on a bell-shaped curve
5. *A spectrum* of host responses should follow exposure to the putative agent along a logical biological gradient from mild to severe
6. *A measurable host response* following exposure to the putative cause should *regularly appear* in those lacking this before exposure (i.e., antibody, cancer cells) or should *increase* in magnitude if present before exposure; this pattern should not occur in those not so exposed
7. *Experimental reproduction* of the disease should occur in higher incidence in animals or man appropriately exposed to the putative cause than in those not so exposed; this exposure may be experimentally induced in the laboratory, or demonstrated in a controlled natural exposure (as with sentinel animals)
8. *Elimination or modification* of the putative cause or of the vector carrying it should decrease the incidence of the disease
9. *Prevention or modification* of the host's response on exposure to the putative cause should decrease or eliminate the disease (immunization, drugs)
10. The whole thing should make biological and epidemiological sense

Why Does This Matter?

Continued activity of PCV2

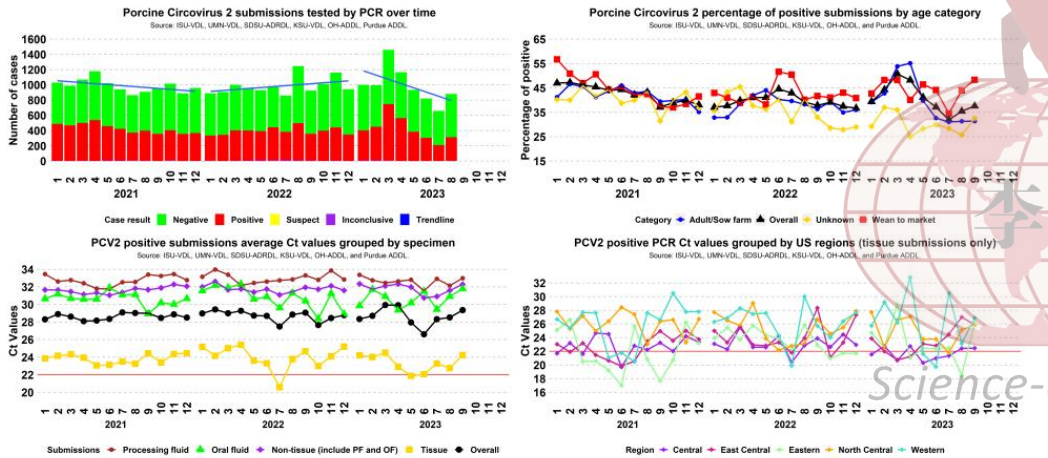


Figure 1. Top: *Left:* Results of PCV2 PCR cases over time; *Right:* PCV2 PCR-positive results, by category over time. **Bottom** *Left:* Average Ct values of PCV2 submissions by specimen; *Right:* Average Ct values of PCV2 tissue submissions by U.S. region; Central (IA), East Central (IL, IN, MO and WI), Eastern (AL, AR, CT, DE, FL, GA, KY, LA, MA, ME, MD, MI, MS, NC, NH, NJ, NY, OH, PA, RI, SC, TN VA, VT and WA), North Central (MN, ND and SD), Western (AK, AZ, CA, CO, HI, ID, KS, MT, NM, NV, OK, OR, TX, UT, WA and WY).

SDRS Advisory Group highlights:

- Overall, 37.6% of 492 cases tested PCV2-positive in September, a moderate increase from 35.43% of 923 in August;
  - Positivity in the adult/sow category in September was 31.37% (85 of 271), similar to 31.34% (147 of 469) in August;
  - Positivity in the wean-to-market category in September was 48.31% (86 of 178), a moderate increase from 44.06% (152 of 345) in August;
- In the month of September, the regions with the lowest PCV2 average Ct values was Central (25 submissions; average Ct 22.5), East Central (14 submissions; average Ct 25.9), North Central (5 submissions; average Ct 25.9), Eastern (10 submissions; average Ct 26.2), and Western (8 submissions; average Ct 26.9);

Emergence of PCV3

PORCINE CIRCOVIRUS 3

The mission of the Swine Health Information Center is to protect and enhance the health of the United States swine herd through coordinated global disease monitoring, targeted research investments that minimize the impact of future disease threats, and analysis of swine health data.

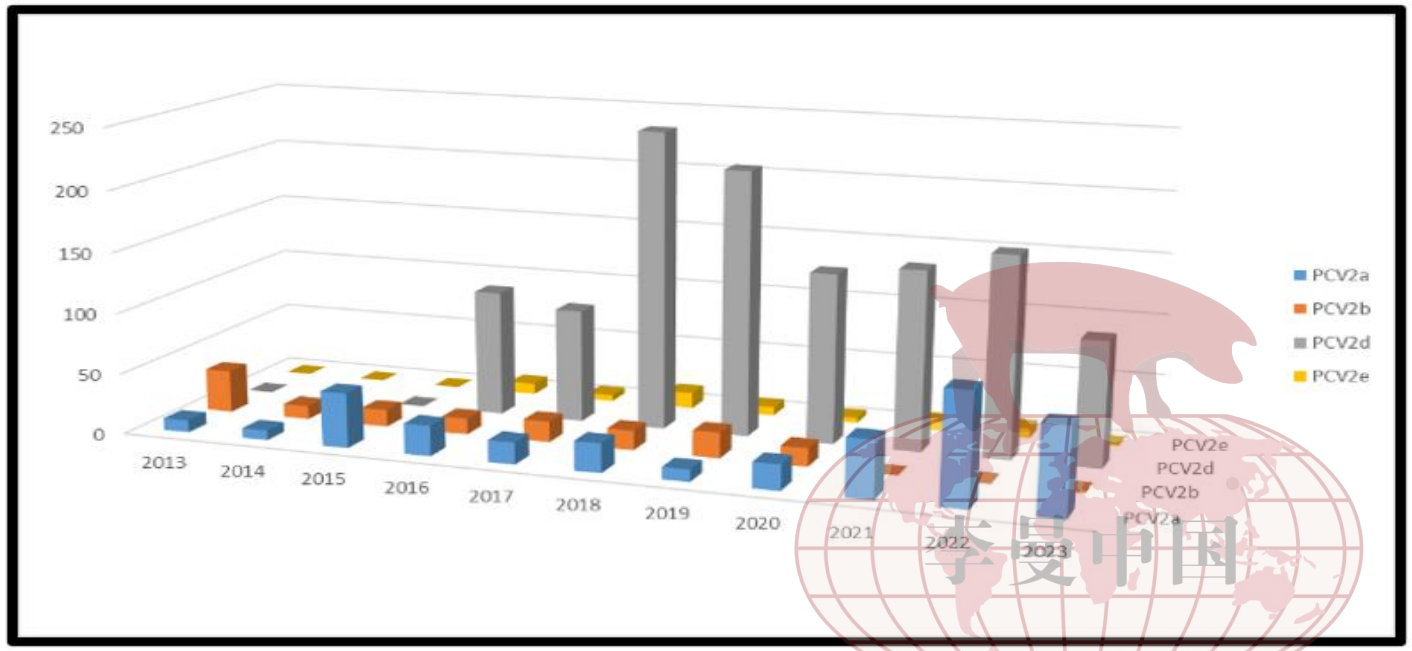
September 2016 | Updated July 2021

SUMMARY

IMPORTANCE

- Porcine circovirus 3 (PCV3) is a newly detected pathogen of swine. Although most cases are sublinical, PCV3 is also associated with clinical signs similar to those caused by porcine circovirus 2 (PCV2).
- Individual case definitions have been proposed for PCV3-associated reproductive disease and systemic disease to standardize diagnostic criteria (see *Infection in Swine*).

PCV2:Genotypes sequenced at ISU



Year	PCV2a	PCV2b	PCV2d	PCV2e	Grand Total
2013	10	35			45
2014	8	11			19
2015	45	14			59
2016	25	13	103	9	150
2017	18	17	93	5	133
2018	24	16	244	13	297
2019	10	21	217	7	255
2020	21	15	139	4	179
2021	47		147	7	201
2022	91	1	164	8	264
2023	71	1	102	1	175
Grand Total	**370**	**144**	**1209**	**54**	**1777**

- Credit: Dr. Darin Madsen, ISU

Could PCV2 Evolution Be Part of the Problem?

PCV2 Evolution

If not now, when will it be a problem?

There is no doubt that PCV2 has evolved over time.

That evolution has a lot to do with understanding viral replication

Inadequate vaccination programs (timing, frequency, dose)

Co-infections and immune activation are additive to the problem.

Scientific basics

Driving biological forces behind the evolution of PCV2 in swine

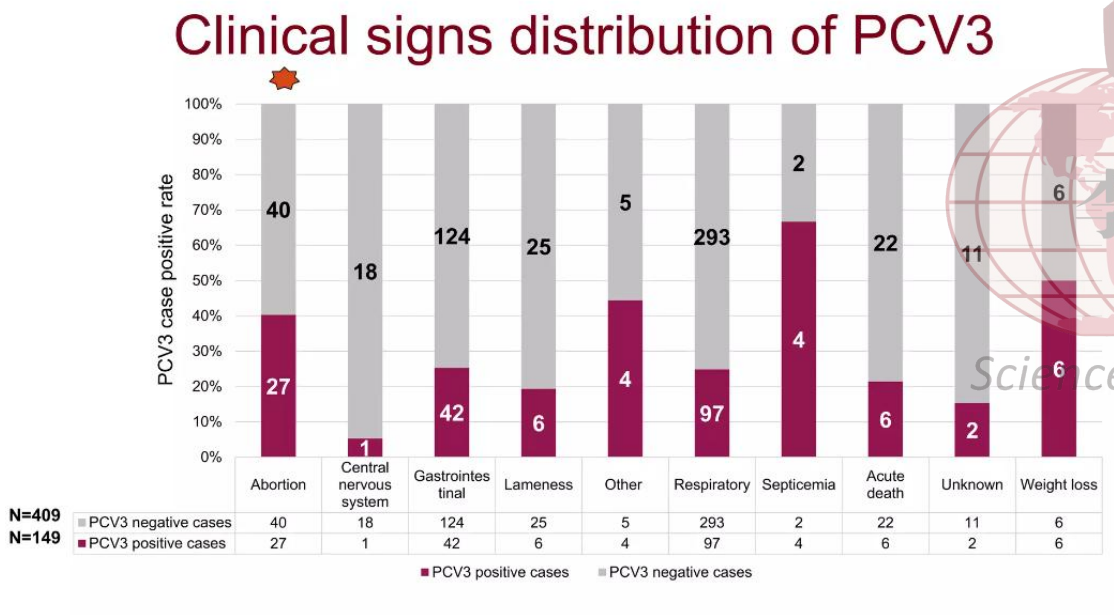
Hans Nauwynck

LABORATORY OF VIROLOGY

FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

GHENT UNIVERSITY

Emergence of PCV3



Full Scientific Report

Journal of Veterinary Diagnostic Investigation
2022, Vol. 34(4) 602–611

DOI: 10.1177/10406387221099538
jvdi.sagepub.com

Frequency of porcine circovirus 3 detection and histologic lesions in clinical samples from swine in the United States

Zhen Yang, Douglas G. Marthaler, Albert Rovira

Abstract. Porcine circovirus 3 (PCV3) is widespread in pigs worldwide. Diverse clinical signs and lesions have been associated with PCV3, but the role of PCV3 as a cause of disease in swine remains unclear. We investigated the association of PCV3 with clinical signs and histologic lesions in 730 diagnostic swine cases between February 2016 and January 2018. The cases contained 2,177 samples submitted from 474 sites located in 21 states in the United States. PCR assay results were positive for PCV3 for 577 of 2,177 (27%) samples, 255 of 730 (35%) cases, 181 of 474 (38%) sites, and 17 of 21 (81%) states. We detected PCV3 in 19 of 28 specimen types and in pigs of all ages and clinical presentations, including healthy pigs, with the highest detection rate in adult pigs. PCV3 detection was not associated with respiratory, gastrointestinal, or CNS signs, weight loss, or sudden death. Of 58 types of histologic lesions evaluated, PCV3 detection was associated with myocarditis, cardiac vasculitis, and interstitial pneumonia in growing pigs. A high PCV3 detection rate was observed in aborted fetuses.

More on Emergence of PCV3

Emerging Microbes & Infections
2019, VOL. 8
https://doi.org/10.1080/22221751.2019.1613176

EMi Taylor & Francis

OPEN ACCESS Check for updates

PCV3-associated disease in the United States swine herd

Bailey Arruda[a], Pablo Piñeyro[a], Rachel Derscheid[a], Ben Hause[b], Emily Byers[c], Kate Dion[d], Duane Long[e], Chris Sievers[f], Jon Tangen[d], Todd Williams[g] and Kent Schwartz[a]

[a]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA; [b]Cambridge Technologies, Worthington, MN, USA; [c]Prestage Farms, Inc, Clinton, NC, USA; [d]The Hanor Company of Wisconsin, LLC, Enid, OK, USA; [e]Swine Health Care, Mexico, IN, USA; [f]Swine Vet Center, St Peter, MN, USA; [g]Pipestone Veterinary Service, Ottumwa, IA, USA

ABSTRACT

Porcine circovirus-associated disease encompasses multiple disease syndromes including porcine circovirus 2 systemic diseases, reproductive failure, and porcine dermatitis and nephropathy syndrome. Until recently, porcine circovirus 2 was the only species associated with the porcine circovirus-associated disease. In this report, diagnostic investigations of thirty-six field cases submitted from multiple production systems, numerous sites and varied geographic locations demonstrated porcine circovirus 3 within lesions by *in situ* hybridization including fetuses with myocarditis, weak-born neonatal piglets with encephalitis and myocarditis, from cases of porcine dermatitis and nephropathy syndrome, and in weaned pigs with systemic periarteritis. Porcine circovirus 3 was detected by PCR in numerous fetuses and perinatal piglets at high viral loads (trillions of genome copies per mL of tissue homogenate). Samples from all cases in this study were assayed and found negative for porcine circovirus 2 by PCR. Metagenomic sequencing was performed on a subset of reproductive cases, consisting of sixteen fetuses/fetal sample pools. PCV3 was identified in all pools and the only virus identified in fourteen pools. Based on these data, porcine circovirus 3 is considered a putative cause of reproductive failure, encephalitis and myocarditis in perinatal piglets, porcine dermatitis and nephropathy syndrome, and periarteritis in swine in the United States.

ELSEVIER

Virus Research
Volume 314, June 2022, 198764

Review

Five years of porcine circovirus 3: What have we learned about the clinical disease, immune pathogenesis, and diagnosis

Molly Kroeger [a 1], Gun Temeeyasen [b 1], Pablo E Piñeyro [a]

But We are Still Divided

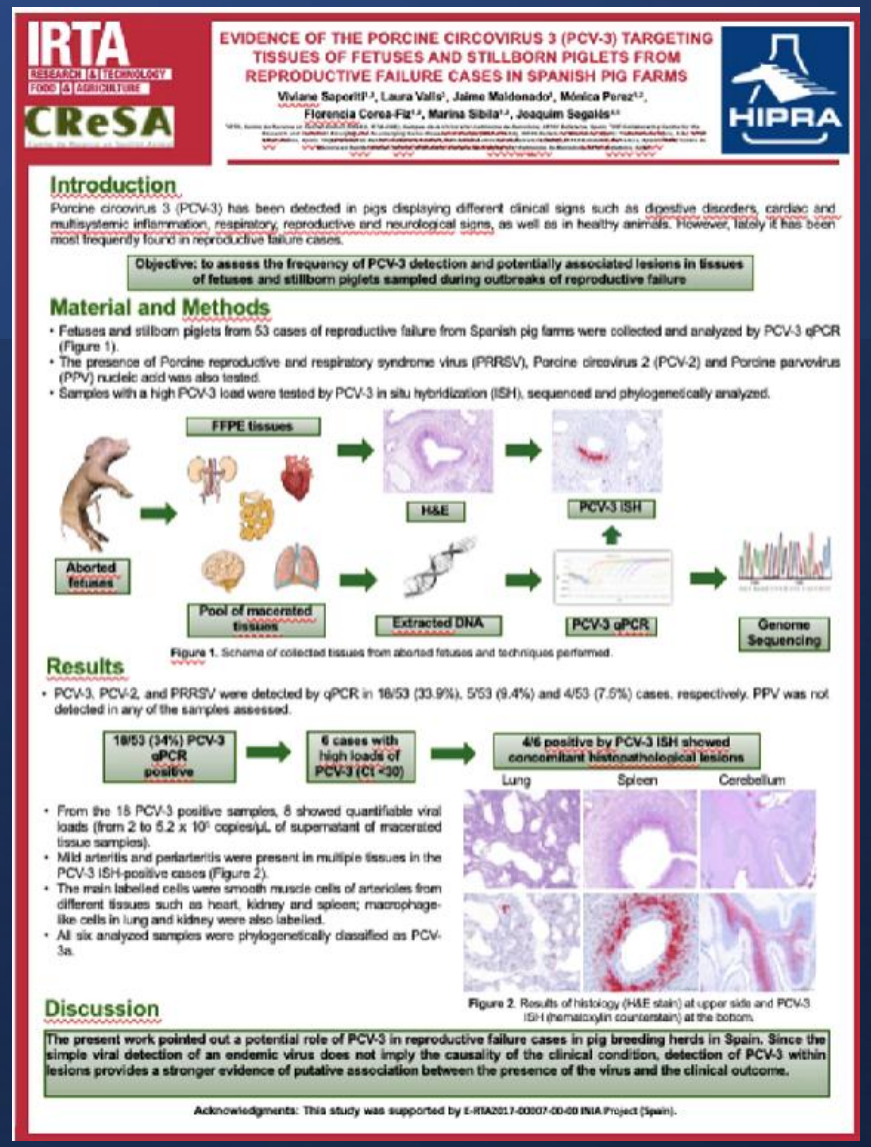
We've been here before!

- Reports of multi-systemic disease
- Oftentimes with co-infections
- Presence of PCV3 without obvious clinical signs
- Robust debate within the veterinary community

But some things are different!

- A clear memory of PCV2 and whether it was "circus" virus
- Higher levels and higher utilization of advanced diagnostics (NGS, RNAscope)
- Prescription platforms for vaccines and associated resolution of clinical signs

Scientific Basics in Academia



IRTA RESEARCH & TECHNOLOGY FOOD & AGRICULTURE

CReSA

HIPRA

EVIDENCE OF THE PORCINE CIRCOVIRUS 3 (PCV-3) TARGETING TISSUES OF FETUSES AND STILLBORN PIGLETS FROM REPRODUCTIVE FAILURE CASES IN SPANISH PIG FARMS

Viviane Saporiti[1,2], Laura Valls[1], Jaime Maldonado[1], Mónica Perez[1,2], Florencia Correa-Fiz[1,2], Marina Sibila[1,2], Joaquim Segalés[1,3]

Introduction

Porcine circovirus 3 (PCV-3) has been detected in pigs displaying different clinical signs such as digestive disorders, cardiac and multisystemic inflammation, respiratory, reproductive and neurological signs, as well as in healthy animals. However, lately it has been most frequently found in reproductive failure cases.

Objective: to assess the frequency of PCV-3 detection and potentially associated lesions in tissues of fetuses and stillborn piglets sampled during outbreaks of reproductive failure

Material and Methods

- Fetuses and stillborn piglets from 53 cases of reproductive failure from Spanish pig farms were collected and analyzed by PCV-3 qPCR (Figure 1).
- The presence of Porcine reproductive and respiratory syndrome virus (PRRSV), Porcine circovirus 2 (PCV-2) and Porcine parvovirus (PPV) nucleic acid was also tested.
- Samples with a high PCV-3 load were tested by PCV-3 in situ hybridization (ISH), sequenced and phylogenetically analyzed.

Figure 1. Schema of collected tissues from aborted fetuses and techniques performed.

Results

- PCV-3, PCV-2, and PRRSV were detected by qPCR in 18/53 (33.9%), 5/53 (9.4%) and 4/53 (7.5%) cases, respectively. PPV was not detected in any of the samples assessed.

- From the 18 PCV-3 positive samples, 8 showed quantifiable viral loads (from 2 to 5.2 x 10^6 copies/µL of supernatant of macerated tissue samples).
- Mild arteritis and periarteritis were present in multiple tissues in the PCV-3 ISH-positive cases (Figure 2).
- The main labelled cells were smooth muscle cells of arterioles from different tissues such as heart, kidney and spleen; macrophage-like cells in lung and kidney were also labelled.
- All six analyzed samples were phylogenetically classified as PCV-3a.

Figure 2. Results of histology (H&E stain) at upper side and PCV-3 ISH (hematoxylin counterstain) at the bottom.

Discussion

The present work pointed out a potential role of PCV-3 in reproductive failure cases in pig breeding herds in Spain. Since the simple viral detection of an endemic virus does not imply the causality of the clinical condition, detection of PCV-3 within lesions provides a stronger evidence of putative association between the presence of the virus and the clinical outcome.

Acknowledgments: This study was supported by E-RTA2017-00007-00-00 INIA Project (Spain).

PATHOLOGICAL OUTCOME OF PIGLETS WITH LOW-MEDIUM AND HIGH PCV-3 LOADS IN TISSUES COMING FROM PCV-3 INOCULATED PREGNANT GILTS

A. Cobos, M. Sibila, E. Huerta, A. Llorens, A. Ruiz, M. Pérez, A. Barceló, R. Lohse, M. Balasch, J. Segalés

VVD-OP-02

UAB Universitat Autònoma de Barcelona

IRTA CReSA Centre de Recerca en Sanitat Animal

World Organisation for Animal Health Founded as OIE

zoetis

Scientific Basics in the Field

Porcine Circovirus Type 3 RNAscope

Animal ID	Specimen	Target	Result	Comment
GA [1-3]	Assorted	PCV3	Positive	All 3 brains with abundant staining

Histopathology - With Interpretation

Animal ID	Specimen	Slides	Comments
GA [1-3]	Assorted	1	
1	Assorted	4	
2	Assorted	4	
3	Assorted	4	

Molecular Diagnostic

PCR - PRRSV Applied Biosystems

Animal ID	Specimen	PRRSV-2 (NA) Ct/Result	PRRSV-1 (EU) Ct/Result	Comment
1, Tube #1	Brain	>=37 / Negative	>=37 / Negative	
2, Tube #2	Brain	>=37 / Negative	>=37 / Negative	
3, Tube #3	Brain	>=37 / Negative	>=37 / Negative	

PCR - Porcine Circovirus 2 and 3

Animal ID	Specimen	PCV2 ct/ Result	PCV3 ct / Result	Comment
1, Tube #1	Brain	>=37 / Negative	16.0 / Positive	
2, Tube #2	Brain	>=37 / Negative	17.1 / Positive	
3, Tube #3	Brain	>=37 / Negative	16.8 / Positive	

Necropsy

Diagnostic Pathology Interpretation - Routine

Animal ID	Specimen	Slides	Comment
GA [1-3]	Assorted	1	
1	Assorted	12	

Bacteriology

Culture Summary

Animal ID	Specimen	Enrichment	Growth	Organism	Comment
1, Tube #1	Brain swab			No Significant Growth	
2, Tube #2	Brain swab			No Significant Growth	
3, Tube #3	Brain swab			No Significant Growth	

Scientific Basics in the Field

Image 1, below: Subgross PCV3 RNAscope including 1 section of brain from each pig. Red staining is positive signal. Fourth piece of tissue to the right of the slide is the positive control tissue.

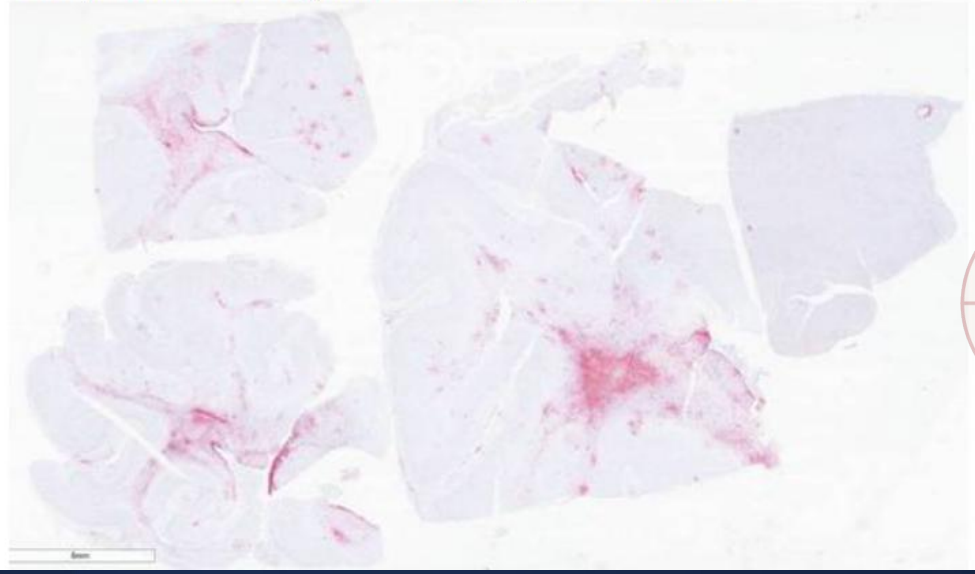
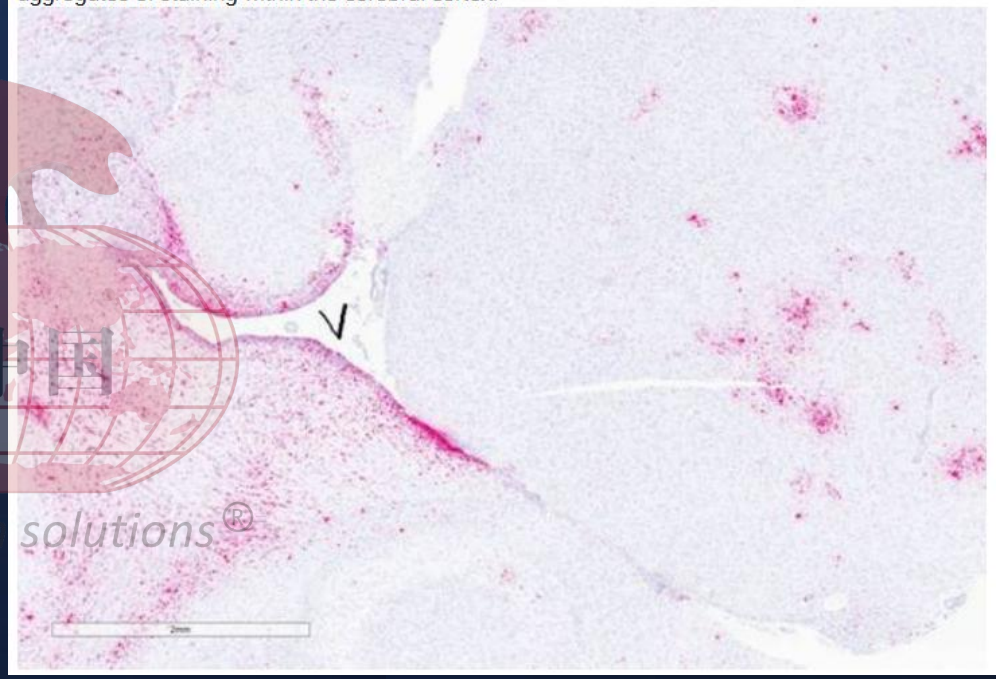


Image 2, below: 2X view of PCV3 RNAscope, showing the intense peri-ventricular staining and multifocal loose aggregates of staining within the cerebral cortex.



Scientific Basics in the Field

Laboratory Diagnosis:
Systemic PCV3

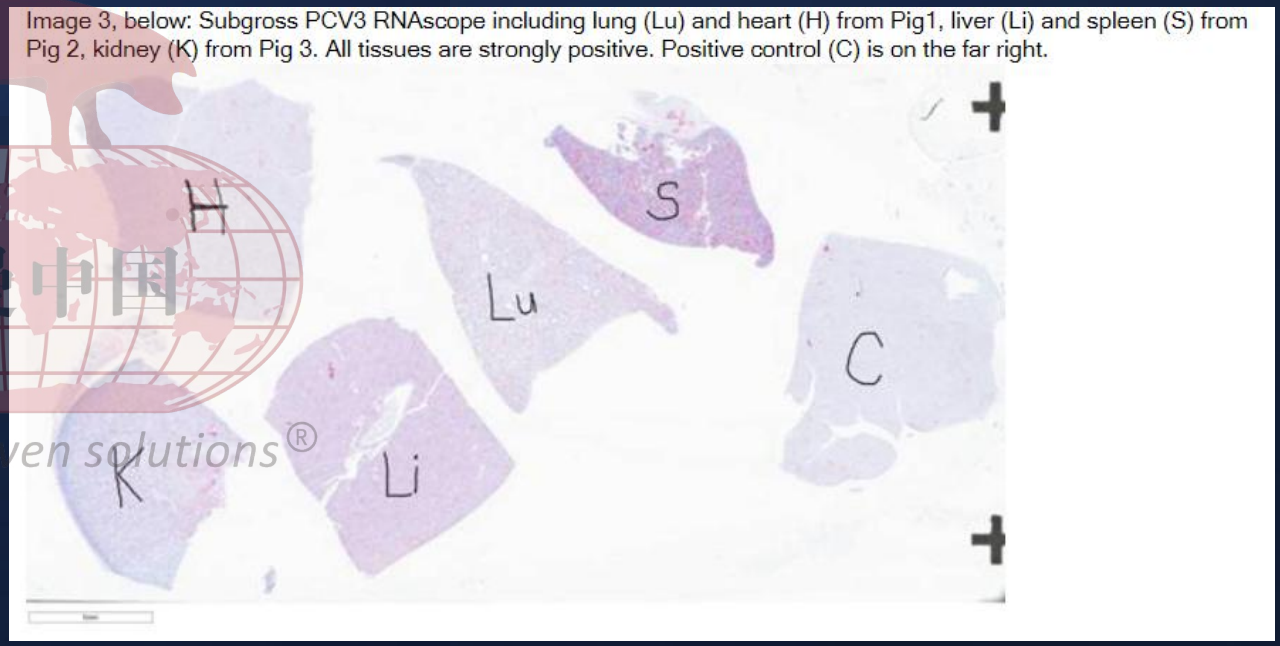
- Cerebrum: Encephalitis, lymphocytic, perivascular with multifocal gliosis
- Heart: Myocarditis and vasculitis, lymphocytic
- Spleen: Vasculitis and perivasculitis, lymphocytic
- Liver (Pigs 1 and 2): Vasculitis and perivasculitis, lymphocytic
- Kidney (Pig 3): Vasculitis and perivasculitis, lymphocytic

Comments:

- Interestingly, the only gross lesions you describe is large ears in an abnormal position. While heads are submitted, the ears are not.
- Specific testing is requested. Additional tests will not be performed unless you contact the laboratory to request or authorize them.
- Vasculitis and perivasculitis as well as myocarditis and encephalitis combined with detection of a moderate amount of genetic material of PCV3 in the brain, there is overall a diagnosis of PCV3. The described abnormal ears has not been something that has been described in pigs with PCV3.
- Additional H&E and RNAscope images shared with submitter, Dr. Petznick, via email.
- Please correlate clinically and contact the laboratory with questions or to request additional testing. (9/21/23 rd/np)

Rachel Derscheid, DVM, PhD, DACVP
Associate Professor
Diagnostic Pathologist

Image 3, below: Subgross PCV3 RNAscope including lung (Lu) and heart (H) from Pig1, liver (Li) and spleen (S) from Pig 2, kidney (K) from Pig 3. All tissues are strongly positive. Positive control (C) is on the far right.



Investigate and Collaborate

Review the Literature

PCV3-associated reproductive failure in pig herds in Brazil

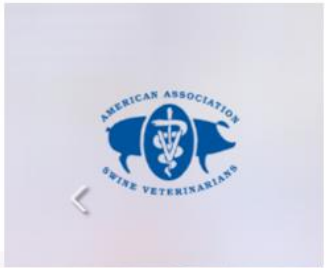
Franciéli Adriane Molossi, Bianca Santana de Cecco, Bruno Albuquerque de Almeida, Luan Cleber Henker, Mariana Soares da Silva, Ana Cristina Sbaraini Mósena, Cláudio Wageck Canal, Luciano Brandalise, Gustavo Manoel Rigueira Simão, Fabio Vanucci, Saulo P. Pavarini & David Driemeier

Tropical Animal Health and Production **54**, Article number: 293 (2022) | Cite this article

Abstract

Porcine circovirus type 3 (PCV3) has been widely detected worldwide in healthy and sick pigs. Recently its association with clinical disease and reproductive failure has been proven through the detection of intralesional viral mRNA in affected pigs. This study aims to describe the occurrence of PCV3-associated reproductive failure (abortions) in sow herds in southern Brazil. Eleven fetuses from five different litters from two herds were analyzed. These herds reported an increase in the rate of late-gestation abortions, stillbirths, and the percentage of mummified piglets. At gross examination, six of the fetuses had large caudally rotated ears and one fetus was mummified. Microscopically, multisystemic vasculitis, lymphocytic interstitial pneumonia, myocarditis, and encephalitis were observed. These six fetuses with gross and histological lesions were positive in qPCR analysis for PCV3, and PCV3 transcription was shown through in situ hybridization (ISH-RNA) within the histologic lesions. Samples from all 11 fetuses tested negative in PCR exam for Porcine Circovirus type 1 and 2, Porcine Reproductive and Respiratory Syndrome, Porcine Parvovirus, and Atypical Porcine Pestivirus. Furthermore, based on the ORF2 analysis, the PCV3a clade was identified. This is the first report of PCV3a-associated reproductive failure in pig herds in South America.

Attend the Meetings



NORTH AMERICAN PRRS SYMPOSIUM

Take Homes

- New pathogens and strains of pathogens continue to emerge throughout the pork industry worldwide
- Past history with a major pathogen, PCV2, is a history that we do not want to repeat
- Sensible causality criteria are needed to help that along
- Advanced disease diagnostics are helpful to correlate with clinical disease expression at the farm level
- Staying up to date on the written and spoken literature are key to moving forward with emerging pathogens.